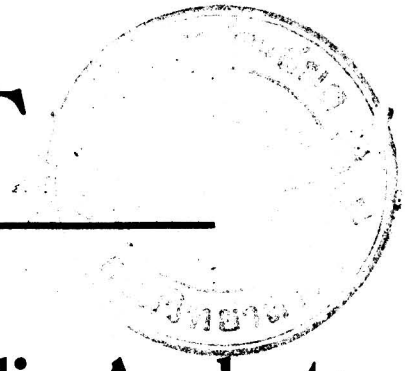
See Notices on p. 422

THE ANALYST



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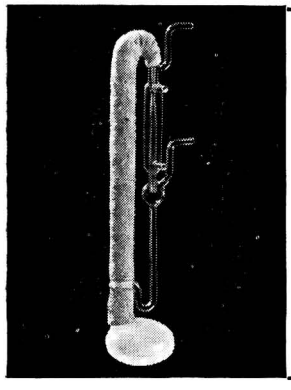

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THE ANALYST

PROCEEDINGS OF THE SOCIETY OF PUBLIC ANALYSTS AND OTHER ANALYTICAL CHEMISTS

MICROCHEMISTRY GROUP

Symposium on the Analysis of Traces and Ultra Micro Quantities

A meeting of the Microchemistry Group with the local sections of other chemical societies was held in Aberdeen on Tuesday and Wednesday, May 4th and 5th, 1948. On the morning of May 4th visits were paid to the Macaulay Institute for Soil Research, the Torry Research Station and the Research Laboratories of the Chemistry Department of the University. Lunch was provided to members of the Group at the invitation of the University Chemistry Department.

In the afternoon the following papers were read: "Ultra Micro Methods," by Dr. Cecil L. Wilson, Queen's University, Belfast; "Simultaneous Concentration of Trace Elements with Organic Precipitants," by Dr. R. L. Mitchell, Macaulay Institute for Soil Research, Aberdeen; "Trace Determinations by means of the Polarographic Method of Analysis," by Mr. G. W. C. Milner, Bragg Laboratory, Sheffield; "Micro-diffusion Analysis," by Dr. T. G. Brady, Department of Biochemistry, University College, Dublin.

By courtesy of Messrs. L. Oertling, Ltd., the latest type of semimicro-balance equipped with a multi-rider system and air damping was on show.

On Wednesday, May 5th, a further paper was read, entitled "The Microscope as a Chemical Tool," by Dr. Cecil L. Wilson, Queen's University, Belfast.

After the discussion on the above paper was completed, the members of the Group went by motor-coach through Deeside to Balmoral, returning to Aberdeen at 4.30 p.m., when the meeting finished.

Resignation of the Honorary Secretary

Owing to pressure of work, Mr. R. Belcher, who has been Hon. Secretary of the Microchemistry Group since its formation, in 1944, regrets that he is unable to continue in that office. Mr. Donald F. Phillips, A.R.I.C., who is a member of the Group Committee, has undertaken to serve as acting Hon. Secretary until the Annual General Meeting. Communications relating to the Group should be addressed to him at Central Research Laboratories, High Duty Alloys Ltd., Slough, Bucks. (Telephone: Slough 21201, Extension 45).

CHEMICAL COUNCIL

Conservation of Uranium Supplies

The Chemical Council has been informed by the Board of Trade that no further quantities of uranium compounds can be supplied to dealers for many months to come. Uranium compounds available will therefore be limited to the small stocks now held by dealers and users. In order to conserve these materials it is recommended that uranium compounds should only be used for research purposes and for sodium estimations, but not in phosphate determinations.

DEATH

We regret to have to record the death of John Edward Byles.

Analytical Methods Committee

Tragacanth Sub-Committee

REPORT No. 1

The Evaluation of Powdered Tragacanth

THE Analytical Methods Committee has received the following Report from the Tragacanth Sub-Committee and its publication has been duly authorised.

The Sub-Committee is composed as follows:—

Norman Evers, B.Sc., Ph.D., F.R.I.C. (*Chairman*).
C. A. Maunder Foster, M.Sc., Ph.D., F.R.I.C. (resigned, April, 1947)
D. C. Garratt, B.Sc., Ph.D., F.R.I.C.
C. A. Hallas, B.Sc., F.R.I.C.
J. S. Hamburger (*Hon. Secretary*).
R. S. Higginbotham, B.Sc. (appointed, April, 1947)
R. H. Marriott, D.Sc., F.R.I.C.
G. A. Mittler, Ph.D., F.R.I.C.
W. Mitchell, B.Sc., Ph.D., F.R.I.C. (appointed, June, 1946)
W. M. Seaber, B.Sc., F.R.I.C.
S. G. E. Stevens, B.Sc., F.R.I.C.

INTRODUCTION

The Sub-Committee was appointed in January, 1946, at the request of the British Standards Institution, to investigate the methods of analysis of tragacanth gum, with particular reference to the determination of the viscosity of the mucilage, volatile acidity, ash and moisture.

Tragacanth is imported in different grades of natural flake or ribbon, with large variations in colour, amount of extraneous vegetable tissue and viscosity of the mucilage. It is sold in this country either in its natural form or after grinding.

The present Report deals with the measurement of the viscosity of mucilages made from powdered gum. The treatment of unground gum prior to the determination of the viscosity presents further problems, owing to the effect of grinding on the viscosity. This matter, together with the determination of the volatile acidity and ash, will be the subject of a later report.

THE DETERMINATION OF VISCOSITY

Among the constants to be determined, the viscosity of the mucilage obtained by treatment of the gum with water is of paramount importance, since commercial evaluation of tragacanth largely depends upon this property.

The work on this problem has proved to be complicated and, as a considerable time has been given to it, much with negative findings, it was considered advisable to publish the complete results for the benefit of those interested in the work. A list of references pertinent to this investigation is given at the end of the Report.

Any method of examination recommended by the Sub-Committee has to satisfy two requirements:

(*a*) it must be capable of yielding reasonably reproducible results when used by different analysts,

(*b*) it must provide a basis of assessment of the gum in respect of viscosity.

The treatment of tragacanth with water causes swelling of the gum particles and partial solution with the production of a more or less viscous liquid. Since the mucilage formed is a non-Newtonian fluid the expression of its viscosity in absolute units has no validity. Where the term "viscosity" is used in this Report it should be taken to mean "apparent viscosity." For industrial purposes the flow properties of such mucilages are usually compared in an arbitrary and empirical manner by means of such instruments as the Ostwald, Stormer, Redwood, Technico, Searle, Falling Sphere and Rolling Ball viscometers.

Comparison of the results obtained is complicated by the very wide variation in gum concentration (0·1 to 4·0 per cent.) necessary to produce mucilages that can be used in one particular apparatus selected for the test. With the falling sphere type of apparatus, there is a wide variation in the size of spheres (1/16th to 5/16ths inch) which have to be selected according to the degree of viscosity so as to obtain convenient times of fall.

In view of the desirability of using an apparatus that is relatively simple in construction and manipulation, the Sub-Committee investigated three methods—

(1) the Falling Sphere method,
(2) the U-tube method,
(3) the Redwood viscometer method.

THE FALLING SPHERE METHOD

The Sub-Committee adopted in the first instance the following method for the preparation of the mucilage. One gram of the powdered tragacanth, weighed into a dry flask, was thoroughly wetted with 5 ml. of 95 per cent. alcohol, and then 95 ml. of distilled water were added as quickly as possible. The mixture was shaken at intervals to disperse any swollen masses of gum and left to stand for the time required.

Falling Sphere viscometers with a 1/8th-inch sphere and a distance of fall of 150 mm. were adopted as the standard apparatus for the tests, but it was found that a 1 per cent. concentration was not suitable for all grades of powdered gum. The concentration was therefore varied so as to obtain a time of fall of approximately 20 seconds at 20° C.

In Table I are recorded the extreme times of fall for two gums at different concentrations and times of standing after the addition of the water to the gum.

TABLE I

TESTS BY FALLING SPHERE METHOD

Gum concentration	Time of fall (seconds)	
	After standing 24 hours	After standing 72 hours
A, 1 per cent.	12 to 31	21 to 34
B, 2 per cent.	19 to 27	21 to 30

These results were extremely unsatisfactory and indicated that this method would be of little value in grading powdered tragacanth. With low grade gums difficulties arise from (*a*) the high concentration of powder required, which necessitates an increase in the amount of alcohol required to wet the gum, and (*b*) the opacity and colour of the mucilages, which interfere seriously with observation of the falling sphere and may even render it invisible. It was also evident that mucilages prepared from the higher grade gums are not necessarily homogeneous after 24 hours, for the sphere moves erratically between the masses of swollen gum instead of falling vertically.

From a consideration of these facts the Sub-Committee agreed that the falling-sphere method was not suitable for evaluating the viscosity of powdered tragacanth.

THE U-TUBE VISCOMETER*

The early experiments with the apparatus showed that there was no single U-tube viscometer suitable for one concentration of all types of powdered tragacanth, and tests were made with different viscometers and a standard concentration of gum after leaving the mucilage to stand for periods of 24 and 72 hours. In Table II are recorded the results obtained by individual members of the Sub-Committee working with three different grades of gum, all at 0·5 per cent. concentration.

These results indicate that with the method of preparation of the mucilage used it is impossible to define the time at which maximum viscosity is obtained. Some members report an apparent increase in viscosity with increase in time of standing, whereas others find only a slight variation.

To avoid the possible error arising from small differences of technique in the preparation of the mucilage two bulk mucilages were prepared and circulated to the members of the Sub-Committee; the results for viscosity after 72 and 96 hours standing are given in Table III. In this trial it was decided to express the results in centistokes.

* B.S. No. 188 (1937).

TABLE II

TIME OF FLOW OF 0·5 PER CENT. MUCILAGES OF POWDERED TRAGACANTH IN U-TUBE VISCOMETERS AT 20° C.

Gum	U-tube used	Member	Time of flow (seconds) After standing 24 hours	Time of flow (seconds) After standing 72 hours
A	No. 4	1	95	157
		2	70	98
		3	63	70
		4	86	—
		6	57	77
		7	131	131
		9	106	111
		10	84	—
		11	54	—
B	No. 3	1	120	138
		2	75	92
		3	72	77
		4	93	95
		6	37	47
		9	75	90
		10	57	—
		11	44	—
C	No. 2	1	69	64
		2	104	94
		3	79	75
		4	77	76
		6	58	55
		9	77	74
		10	56	—
		11	80	—

TABLE III

VISCOSITY OF MUCILAGES OF POWDERED TRAGACANTH AS DETERMINED BY THE U-TUBE METHOD AT 20° C.

Gum	U-tube used	Member	Viscosity (centistokes) After standing 72 hours	Viscosity (centistokes) After standing 96 hours
A1	No. 4	1	163 to 221	175 to 211
		2	211	211
		3	—	155
		4	—	157
		5	125	139
		6	151	156
		7	174	—
		9	178	171
		10	191	182
		11	280	237
B1	No. 3	1	17·5	16·2
		2	20	20
		3	18·4	18·5
		4	—	14
		5	13	15
		6	18·4	19·2
		7	15·2	—
		9	17	13
		10	14·4	15
		11	18	16

As these results were obtained on portions of the same mucilages it is reasonable to assume that the variations were due to differences in the U-tubes used. Table IV gives the constants, C, for the U-tubes used by each member, as determined with the same sample of liquid paraffin of known viscosity.

TABLE IV

THE DIFFERENCES IN CONSTANTS OF U-TUBES

Member	1	2	3	4	5	7	9	10
U-tube No. 4 ..	1·700	1·616	1·887	1·73	1·718	1·366	1·709	1·873
U-tube No. 3 ..	0·183	0·308	0·293	0·28	0·210	0·374	0·302	0·389

With such differences in the value of C it follows that, since we are dealing with a non-Newtonian liquid, the results for the viscosities of the mucilages would be expected to show wide differences.

To cover the variations in samples of powdered tragacanth for any fixed concentration of gum a number of U-tube viscometers would be required, and it would be extremely difficult to grade gums the viscosity of which had been determined in U-tubes of different bores.

HOMOGENISATION

The preparation of a uniform mucilage had always been a matter of some difficulty and, in an attempt to overcome this, mucilages were made as described above and passed through homogenisers of the domestic cream-making type, the effect of such treatment being examined by making determinations of times of flow. It became apparent that there were great differences in the efficiency of the various homogenisers used, but the action of these machines on the dispersion was unpredictable—some members finding a considerable increase and others a decrease in viscosity after the treatment. It was agreed that it would be impossible to obtain uniform results with any homogeniser of the cream-making type, even if only one model were recommended.

THE REDWOOD VISCOMETER

In the course of the experiments on the mucilage mentioned in Table II some members of the Sub-Committee had conducted parallel tests with the Redwood No. 1 viscometer, determining the efflux time of 50 ml. of mucilage at 20° C. The results were encouraging and it was decided to carry out further tests with this instrument, using the method of dispersion described in the British Pharmaceutical Codex, 1934, in which the mucilage is heated in a boiling water-bath. 200 ml. of a 0·5 per cent. mucilage were prepared, using 5 ml. of alcohol for the preliminary wetting, and heated for 1 hour in a boiling water-bath in a 500-ml. conical flask under a water-cooled reflux condenser, the flask being immersed so that the boiling water was 1 inch above the surface of the mucilage. The mucilage was swirled round occasionally during the heating. At the end of the period the flask was removed from the water-bath and allowed to cool to room temperature and the efflux time at 20° C. was measured in a Redwood No. 1 viscometer after standing periods of 24 and 48 hours.

It was found that the efflux time of 50 ml. of water varied in different instruments from 26·8 to 30·0 seconds. A correction was therefore applied to bring the results to a standard efflux time of 27 seconds by multiplying the result obtained by $27/x$, where x is the efflux time of 50 ml. of water at 20° C. in the particular instrument used, it being considered that this procedure gives a figure that, although approximate, is sufficiently accurate for practical purposes.

The results of this series of tests on two gums "F" and "G" are given in Table V.

TABLE V

EFFLUX TIMES OF 0·5 PER CENT. MUCILAGES OF POWDERED TRAGACANTH (CORRECTED TO A STANDARD WATER EFFLUX TIME OF 27 SECONDS)

	Efflux time (corrected), seconds			
	Gum "F"		Gum "G"	
Member	After standing 24 hours	After standing 48 hours	After standing 24 hours	After standing 48 hours
1	254 (91°)	263	54·4 (91°)	55·4
2	281 (95°)*	298	56 (95°)*	58
3	260 (92°)	269	54 (92°)	54
5	255 (85°)	255	59 (91°)	59
6	260	269	54	54
7	274 (92°)	—	59 (91°)	—

NOTES: (1) The figures in brackets indicate the temperature of the mucilage after removal of the flask from the water-bath.

(2) Member 2 (marked*) heated the mucilage to a temperature of 95° C. and maintained it at this temperature for 1 hour.

The results obtained showed a distinct improvement and two further samples were circulated, more precise conditions for the preparation of the mucilage being defined The efflux times after 24 hours are given in Table VI.

TABLE VI

EFFLUX TIMES OF 0·5 PER CENT. MUCILAGES OF POWDERED TRAGACANTH (CORRECTED TO A STANDARD WATER EFFLUX TIME OF 27 SECONDS)

	Efflux time (corrected), seconds	
Member	Gum "D" After standing 24 hours	Gum "E" After standing 24 hours
1	205	107
2	213	103
3	198	108
5	231	108
7	211	109
9	219	110

Good agreement was obtained with the grade of powdered tragacanth represented by sample "E" but the better grade of powder showed a wider variation in efflux times.

In the previous experiments the concentration of gum in the mucilage had been based on the air-dry material, but to avoid differences due to variations in moisture content it was agreed to calculate the concentration of gum used on a moisture-free basis. The method of determining the moisture content of the powdered gum is given in Appendix B. All the subsequent experiments were carried out on quantities calculated on a moisture-free basis.

Tests were then made on a high grade pharmaceutical powder at different concentrations of gum; the effect of heating in a boiling water-bath for 1 hour and 1½ hours was also studied. The results are given in Tables VII and VIII.

TABLE VII

EFFECT OF THE CONCENTRATION OF THE MUCILAGE ON THE EFFLUX TIME (CORRECTED) (MUCILAGE HEATED IN BOILING WATER-BATH FOR 1·5 HOURS)

	Efflux time (corrected), seconds Concentration of dry gum			
Member	0·25 per cent.	0·30 per cent.	0·40 per cent.	0·50 per cent.
1	101	141	423	1073
2	99	146	339	923
3	95	146	302	757
5	108	189	453	1238
6	101	145	396	1046
7	85	168	356	928
8	104	160	437	—
10	114	213	444	1327

TABLE VIII

EFFECT OF THE CONCENTRATION OF THE MUCILAGE ON THE EFFLUX TIME (CORRECTED) (MUCILAGE HEATED IN BOILING WATER-BATH FOR 1 HOUR)

	Efflux time (corrected), seconds Concentration of dry gum			
Member	0·25 per cent.	0·30 per cent.	0·40 per cent.	0·50 per cent.
1	110	149	414	1058
2	102	147	337	890
5	113	172	405	1063
6	99	119	271	823
7	111	174	444	1146
8	104	158	401	852
10	117	188	277	1190

These results demonstrated that the best agreement was obtained with a mucilage giving a corrected efflux time of about 100 seconds for 50 ml. and suggested that at higher concentrations incompletely dispersed swollen gum masses interfered with the steady efflux of the liquid or that equilibrium of the mixture had not been reached.

Bearing in mind the fact that the work of the Sub-Committee might lead to the introduction of a grading of powdered tragacanth in terms of viscosity the members considered the advisability of evaluating these gums *by determining the concentration of dry gum that would be required to produce a mucilage with a corrected efflux time of* 100 *seconds for* 50 *ml. of mucilage at* 20° *C.* In order to investigate the applicability of this scheme, nine samples of powdered tragacanth of different qualities were subjected to the test as described in detail in Appendix A. It was suggested that at least two results should be obtained on each gum at concentrations giving efflux times above and below 100 seconds and within the limits 75 to 125 seconds. The concentration giving an efflux time of 100 seconds should then be calculated by interpolation.

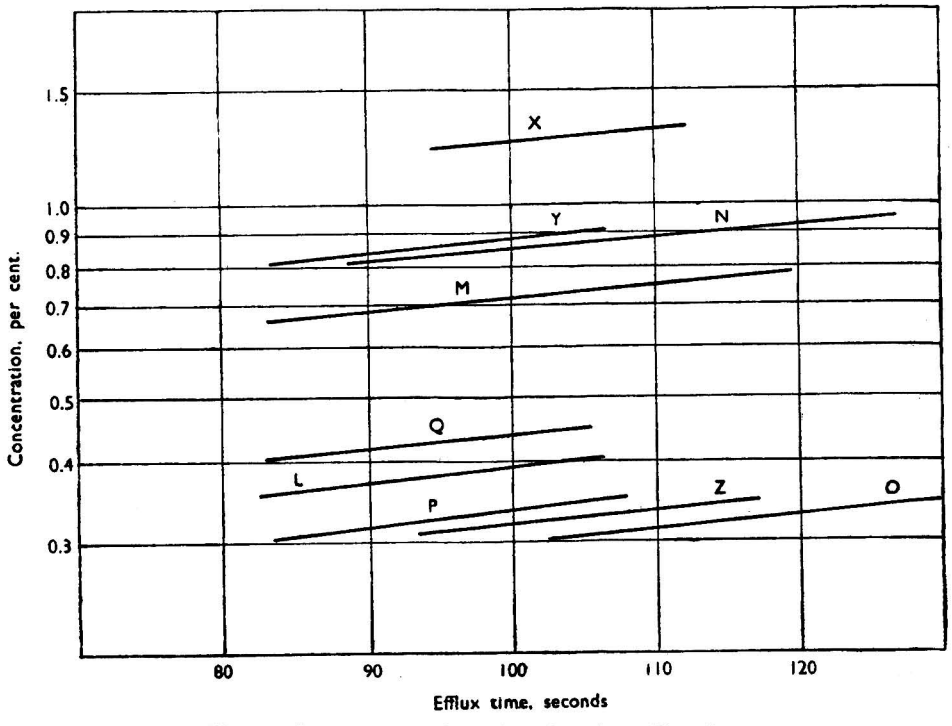

Fig. 1. Log concentrations plotted against efflux times.

It was found that when the efflux times were plotted against the logarithm of the concentration the slope of the curve between the efflux times of 75 and 125 seconds was approximately the same for all the gums tested (see Fig. 1). It is therefore possible to calculate from a single determination lying between these two values the approximate concentration required to give an efflux time of 100 seconds, the equation used being

$$\log C_Q = \log C_E - aE + 100a$$

where C_Q = concentration giving efflux time 100 seconds
C_E = concentration giving efflux time E
a = slope of the curve at E = 100 seconds

The mean value of a is approximately 0·002, so that the equation becomes

$$\log C_Q = \log C_E - 0{\cdot}002E + 0{\cdot}2$$

The results are given in Table IX. The column headed C_I shows the concentration giving an efflux time of 100 seconds calculated from two results by interpolation.

The standard deviation (s.d.) of C_Q for each gum has been calculated and indicates that the results show as good agreement as can be expected from a method of this kind. The wider variations found with gum "X" were due to the fact that this gum was of a crude type and the mucilage contained a considerable amount of undissolved matter.

The mean value of the slope a is 0·0022, with a standard deviation of ±0·0006. This is probably slightly high on account of one or two abnormally high results. An inspection

of the frequency distribution indicates that, if the mean value of a is taken as 0·002, this will be sufficiently accurate for the purpose.

Table IX

Results on powdered tragacanth by recommended method

C_E = concentration of dry gum giving efflux time, E seconds.
C_I = concentration of dry gum giving efflux time of 100 seconds calculated by interpolation.
C_Q = concentration of dry gum giving efflux time of 100 seconds calculated from equation $\log C_Q = \log C_E - 0{\cdot}002E + 0{\cdot}2$
a = slope of curve at E = 100 seconds.

Gum "L"

Member	C_E	E	C_I	C_Q	a
2	0·35 0·40	83 104	0·390	0·378 0·392	0·0028
6	0·40	107	—	0·387	—
7	0·39	99	—	0·392	—
8	0·40	109	—	0·384	—
Mean				0·387	
S.d.				±0·006	

Gum "M"

Member	C_E	E	C_I	C_Q	a
2(*a*)	0·65 0·75	73 107	0·730	0·736 0·726	0·0019
2(*b*)	0·65 0·75	89 124	0·681	0·684 0·671	0·0018
6	0·75	103	—	0·740	—
7	0·73 0·75 0·78	100 107 120	0·730	0·730 0·725 0·711	0·0015
8	0·70	94	—	0·719	—
Mean			0·714	0·716	
S.d.				±0·024	

Gum "N"

Member	C_E	E	C_I	C_Q	a
2(*a*)	0·80 0·95	86 127	0·851	0·853 0·840	0·0018
2(*b*)	0·80 0·95	89 139	0·833	0·841 0·794	0·0015
6	0·85	94	—	0·873	—
7	0·85	103	—	0·838	—
8	0·85	112	—	0·804	—
Mean			0·842	0·835	
S.d.				±0·025	

Gum "O"

Member	C_E	E	C_I	C_Q	a
1	0·25 0·30 0·35	65 100 135	0·300	0·292 0·300 0·295	0·0022 0·0019
3	0·25 0·35	66 124	0·309	0·292 0·313	0·0025
5	0·30 0·35	100 137	0·300	0·300 0·295	0·0018
9	0·30	106	—	0·292	—
Mean			0·303	0·297	
S.d.				±0·007	

TABLE IX—*continued*

Gum "P"

Member	C_E	E	C_I	C_Q	*a*
1	0·30 0·35	82 110	0·332	0·326 0·334	0·0024
3	0·35	99	—	0·350	—
5	0·30 0·33 0·35	85 100 109	0·330	0·321 0·330 0·336	0·0028 0·0028
9	0·33	108	—	0·316	—
Mean			0·331	0·330	
S.d.				±0·012	

Gum "Q"

Member	C_E	E	C_I	C_Q	*a*
1	0·40 0·45	80 104	0·440	0·439 0·442	0·0021
3	0·45	93	—	0·464	—
5	0·40 0·43 0·45	84 100 108	0·430	0·430 0·430 0·443	0·0019 0·0025
9	0·43	102	—	0·426	—
Mean			0·435	0·438	
S.d.				±0·012	

Gum "X"

Member	C_E	E	C_I	C_Q	*a*
1	1·2 1·3	90 111	1·25	1·26 1·24	0·0017
3	1·3 1·5	91 123	1·36	1·35 1·35	0·0019
5 (*a*)	1·2	90	—	1·26	
5 (*b*)	1·2	88	—	1·27	
5 (*c*)	1·2	95	—	1·23	
6	1·2 1·3	94 118	1·23	1·23 1·20	0·0015
8	1·2 1·4	90 106	1·33	1·26 1·36	0·0042
10	1·2 1·3	100 126	1·20	1·20 1·15	0·0013
Mean			1·27	1·258	
S.d.				±0·062	

Gum "Y"

Member	C_E	E	C_I	C_Q	*a*
1	0·90	106	—	0·875	
2	0·80 0·90	84 103	0·884	0·861 0·887	0·0027
3	0·90	98	—	0·908	
5 (*a*)	0·80 0·90	84 107	0·870	0·861 0·871	0·0022
5 (*b*)	0·80 0·90	85 113	0·853	0·857 0·847	0·0018
5 (*c*)	0·80 0·90	86 107	0·867	0·853 0·871	0·0024
7	0·80 0·90	79 100	0·900	0·881 0·900	0·0024
8	0·80 0·90	77 94		0·889 0·925	0·0024
10	0·80 0·90	94 128	0·818	0·822 0·791	0·0015
Mean			0·865	0·869	
S.d.			±0·028	±0·032	

TABLE IX—*continued*

	Gum "Z"				
Member	C_E	E	C_I	C_Q	*a*
1	0·30 0·35	85 116	0·324	0·321 0·325	0·0022
2	0·30 0·35	78 116	0·329	0·332 0·325	0·0017
3	0·30 0·35	85 118	0·322	0·321 0·322	0·0020
5 (*a*)	0·30 0·35	93 125	0·311	0·310 0·312	0·0021
5 (*b*)	0·30 0·35	92 126	0·312	0·311 0·310	0·0020
5 (*c*)	0·30 0·35	97 129	0·305	0·304 0·307	0·0021
6	0·30 0·35	87 109	0·330	0·318 0·336	0·0030
7	0·30 0·35	90 121	0·316	0·314 0·318	0·0022
8	0·30 0·35	84 102	0·336	0·323 0·347	0·0037
10	0·30	93	—	0·310	—
Mean			0·321	0·320	
S.d.			±0·010	±0·011	

APPENDIX A

RECOMMENDED METHOD FOR THE DETERMINATION OF THE VISCOSITY OF THE MUCILAGE

Place a quantity of the powdered gum equivalent to the required weight of dry gum* in a dry 500-ml. conical flask and add 5 ml. of alcohol (95 per cent.). Ensure that the gum is completely wetted and dispersed evenly over the inner surface of the flask. Add 195 ml. of cold distilled water as quickly as possible and shake. Connect the flask to a reflux condenser and place in a vigorously boiling water-bath, so that the surface of the water is about 1 inch above the surface of the mucilage. Continue the heating for 1 hour, gently swirling the mucilage at intervals of 15 minutes without removing the flask from the water-bath. At the end of 1 hour remove the flask from the water-bath, stopper it and allow it to cool naturally to room temperature.

After it has stood for 24 hours, determine the efflux time of 50 ml. in a Redwood No. 1 viscometer at 20° C. Record the mean of six readings. Correct to a water efflux time of 27 seconds by multiplying the result by $27/x$, where x is the efflux time of 50 ml. of water at 20° C. for the same instrument.

If the efflux time of the mucilage is not between 75 and 125 seconds repeat the test with a fresh mucilage of suitable concentration.

If a mucilage is found to give a higher efflux time than 125 seconds an approximate result may be obtained by diluting with water to bring the efflux time within the required range, but for accurate results it is recommended that a fresh mucilage be prepared.

Calculate the concentration of dry gum (C_Q) required to give an efflux time of 100 seconds from the equation

$$C_Q = \text{antilog } [\log C_E - 0{\cdot}002E + 0{\cdot}2]$$

in which C_E = concentration of dry gum giving an efflux time of E seconds, when E lies between 75 and 125 seconds.

APPENDIX B

THE DETERMINATION OF MOISTURE

Dry about 1 g. of the powder in an open dish to constant weight in a steam-heated oven.

* The dried gum must not be used for the preparation of the mucilage.

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The Volumetric Determination of Tin on a Small Scale and its Application to Non-Ferrous Alloys

BY CHRISTINA C. MILLER AND LESLIE R. CURRIE

VON MACK and Hecht[1] investigated the precipitation of about 1 to 7 mg. of tin^{IV} with phenylarsonic acid, ammonia and cupferron and converted the precipitates by high-temperature ignition into stannic oxide, obtaining good results with the last reagent. A method for the micro-analysis of bearing metals was suggested. We attempted to separate and weigh 10 mg. of tin as the complex tin phenylarsonate with a view to avoiding the ignition at 900° C. which is undesirable in micro-analysis. Although we succeeded in precipitating all the tin in a filterable form, the precipitate usually contained less than the theoretical amount of phenylarsonate. Moreover it was hygroscopic and lost weight on continued heating at 120° C.

Höltje[2] evolved a method for the volumetric determination of about 0·3 to 1 mg. of tin, which was based on the reduction of tin^{IV} with iron and the titration of tin^{II} with 0·01 *N* iodine. The absolute error was roughly $\pm 10\ \mu g$. Other metals, *e.g.*, zinc, aluminium and magnesium, when substituted for iron, gave unsatisfactory results. Alloys were not examined.

The object of our investigation was to devise for the volumetric determination of 1 mg. and less of tin a method that could be applied to a maximum of 10 mg. of a range of non-ferrous alloys containing from 1 to 90 per cent. of tin. We have achieved our end by using freshly cast antimony as the reductant for tin^{IV} and iodine as the titrant for tin^{II}. The element has been satisfactorily determined in brasses, bronzes, bearing metals, a fusible alloy and an aluminium alloy.

EXPERIMENTAL

Evans and Higgs[3] pointed out that iron is not a suitable reductant for tin in alloy solutions because any antimony and copper deposited carry down some tin. If, on the other hand, antimony powder, which causes no deposition of other metals, be used, too coarse particles give incomplete reduction and too fine particles consume some iodine, so that correct results betoken a balancing of errors. They found that nickel causes no co-precipitation of tin and reduces tin^{IV} quantitatively.

Nickel as reductant—In preliminary experiments made on a semimicro scale we failed to get complete reduction of tin^{IV}. Ten mg. of tin as stannic chloride were reduced in hydrochloric acid solution with coils of sheet nickel. Long-necked, round-bottomed 35-ml. flasks were used and an atmosphere of carbon dioxide was maintained during the reduction and the titration with iodine. The acid concentration was varied from 20 to 40 per cent. (v/v) for volumes of 15 to 25 ml. and the duration of boiling from ½ to 2 hours. Long boiling reduced the volume, necessitating addition of air-free water to restore it, and caused so much nickel to dissolve that the green colour obscured the indicator action of the starch. The surface area of the nickel, which was sometimes etched before use with a mixture of hydrochloric acid and sodium chloride or chlorate, ranged from 1 to 12 square inches. No set of conditions gave consistent results and there was a persistent negative error which occasionally reached 2½ per cent. There was evidence of a pronounced surface effect on the nickel, to which the irregularities were attributed.

Antimony as reductant—Cast sticks of this metal were eventually used with success. When a piece, 1 inch long and 0·3 inch in diameter was substituted for the nickel coil in the above method, full reduction of 10 to 20 mg. of tin^{IV} was effected in 40 per cent. hydrochloric acid in 1 hour, and the antimony itself showed no appreciable reaction with iodine at room

temperature. When the same piece of antimony had been used more than once it became covered in patches with a black amorphous deposit that hindered its reducing power. If the metal were cleaned with concentrated sulphuric acid, or by filing, it could be used again with improved, but not always fully quantitative, results. Next, the antimony was melted and recast into sticks of 0·2 inch diameter, and three 0·5-inch lengths were used in reductions, which once more gave improved results. A refinement of the method was to add a fourth piece shortly before the end of the reduction, in order to present a fresh surface for the reduction of the last traces of tin. After many meltings and recastings a comparatively small amount of antimony became not entirely reliable, and ultimately the originally recast antimony was used once only and then rejected. The results in Table I illustrate the above findings.

TABLE I

No.	Tin in mg. Taken	Found	Remarks
1	10·53	10·53	Fresh piece of antimony.
2	10·09	9·97	Antimony from No. 1 filed clean each time for these experiments.
3	2·45	2·33	
4	10·62	10·28	
5	10·70	8·92	Blackened antimony from No. 4 used uncleaned.
6	10·14	9·70	Used antimony cleaned with conc. H_2SO_4.
7	11·02	10·97	Recast antimony.
8	10·05	9·99	
9	10·78	10·77	As Nos. 7 and 8 with addition of more antimony near the end of the experiment.
10	10·26	10·23	

REDUCTION FLASKS AND PROCEDURE FOR EXPERIMENTS ON A MICRO SCALE

The long-necked apparatus shown in Fig. 1 (*cf.* Höltje[2]) and Fig. 2 were used. The first is suitable for short periods of reduction only. The second, with its ground-in reflux condenser, is to be recommended; it permits long periods of vigorous boiling without reduction in volume.

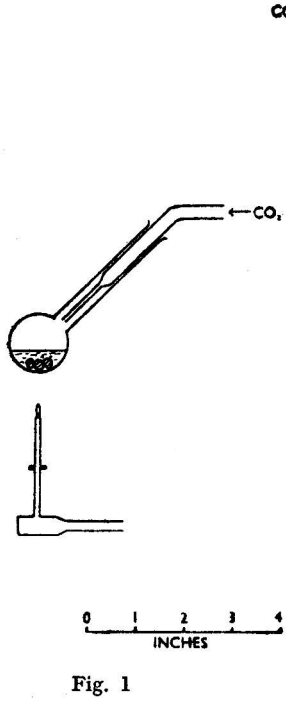

Fig. 1

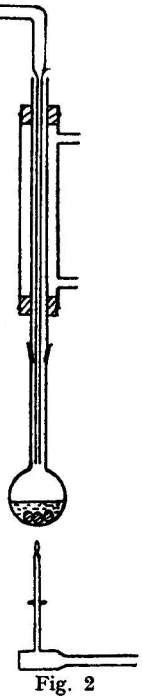

Fig. 2

The procedure for apparatus No. 2 is as follows. To 1 mg. or less of tin as tin chloride in 3 ml. of 40 per cent. hydrochloric acid (v/v) add three pieces of recast antimony, 0·2 inch in diameter and 0·3 inch long. Lubricate the ground connection with a drop of concentrated sulphuric acid, attach the condenser, and boil the solution briskly in an atmosphere of carbon dioxide, which is passed through a wash-bottle containing water, at the rate of 1 bubble per second. (If apparatus No. 1 is in use add a fourth piece of antimony within 10 minutes of the end of the reduction process.) When the reduction is finished, remove the flame and rinse the condenser, the neck of the flask and the capillary with 1 ml. of boiling air-free water. Allow the hot solution to stand for 5 minutes in order to reduce a trace of tin^{IV} that may have been accidently re-formed and then cool the flask on ice. Detach the flask from the condenser and, before it is clear of the capillary tube, insert another shorter capillary through which carbon dioxide is already passing. Take the apparatus to the burette and, maintaining the inflow of carbon dioxide through the short capillary, titrate with 0·1 *N* iodine solution until a full blue colour is reached in presence of a drop of 1 per cent. starch solution as indicator; then discontinue the flow of carbon dioxide and titrate back with 0·001 *N* sodium thiosulphate until the blue colour just disappears.

Notes on the titrimetric procedure—0·1 *N* iodine was preferred to a more dilute solution, the dissolved oxygen in which caused a small negative error (*cf.* Okell and Lumsden[4]). A micro-burette of 0·2-ml. capacity was constructed from capillary tubing of 1-mm. bore and the end drawn out to a long fine tip. A thermometer scale was attached and the burette calibrated by Benedetti-Pichler's[5] method. When in use it was mounted vertically and attached to Schilow's[6] pressure-controlling arrangement. It was filled afresh for every titration and washed out with water if the time interval between titrations exceeded 15 minutes. The sodium thiosulphate solution was delivered from a 2-ml. micro-burette provided with a tap. The iodine solution was standardised on the macro scale by means of arsenious oxide (U.S.A. Bureau of Standards quality); this could also be done successfully on a semi-micro or micro scale.

Preparation of Alloy Solutions for Reduction

Method 1, for alloys containing little or no copper (*e.g.*, solders, some bearing metals, fusible alloys)—Weigh out an amount (not more than 10 mg.) of alloy containing 0·2 to 1 mg. of tin. Dissolve solders and bearing metals in the reduction flask by warming gently with 1·2 ml. of hydrochloric acid (sp.gr. 1·16), and addition of a drop of perhydrol or 50 per cent. sodium chlorate solution as required. Dissolve fusible alloys in 0·2 ml. of concentrated sulphuric acid, dilute with water, and add the usual amount of hydrochloric acid Make up the solutions with water to 3 ml., proceed with the reduction for at least 1 hour, and cool and titrate as described.

Method 2, for alloys containing copper in excess of the limiting amount (*e.g.*, some bearing metals, brasses, bronzes, aluminium alloys)—Weigh 10 mg. or less of metal. Dissolve bearing metals and aluminium alloys as in method 1. Dissolve brasses and bronzes in not more than 0·5 ml. of *aqua regia* in a 5-ml. centrifuge tube, add 0·25 ml. of 1 per cent. lead acetate solution and, if lead chloride separates, dissolve it by heating. Convert copper into the soluble cuprammonium complex by adding 7 *N* ammonia until an excess of 0·1 to 0·15 ml. is present. Lead hydroxide assists the removal of small amounts of hydrated stannic oxide. Coagulate the precipitate by heating, cool and centrifuge. As a precautionary measure, filter through the filter stick designed by Miller[7] and wash the precipitate twice with 0·25-ml. portions of 0·4 *N* ammonia solution containing 5 per cent. of ammonium chloride. If much copper is present, dissolve the precipitate in 0·5 ml. of 6 *N* hydrochloric acid and repeat the precipitation. Finally dissolve the precipitate in 0·5 ml. of 6 *N* hydrochloric acid and draw the solution through the filter stick into the reduction flask. Wash the tube and filter stick with 6 *N* hydrochloric acid and prepare for the reduction as usual

Results

(i) *The reduction of stannic chloride*—Weighed portions of a standard solution prepared by dissolving Johnson, Matthey's "H.S." tin in cold hydrochloric acid and oxidising with sodium chlorate gave the following results, which are corrected for a small "blank" determined in absence of tin.

With apparatus 1—Period of reduction, ½ hour:
Error on 1 mg. of tin, 0, +5, −4, −3 and −7 μg.
Error on 0·2 mg. of tin, −3 and −7 μg.

With apparatus 2—Period of reduction, 1 hour:
Error on 1 mg. of tin, +2, −1, −4, −7, −4 and 0 μg.
Error on 0·2 mg. of tin, −1, +2, −1 and +2 μg.

Influence of copper—The presence of about 30 μg. of copper did not affect the results for 0·2 to 1 mg. of tin, provided that the period of reduction was at least 1 hour. Larger amounts had to be removed as the cuprammonium complex.

(ii) *Tin in alloys*—Since Benedetti-Pichler[8] points out that very small samples of a powder may not be homogeneous unless the particles are below a certain size, we have actually obtained our small amounts of alloys for analysis by aliquot partition of a standard solution made up from about 1 g. of the powdered metal, treated in accordance with the prescribed micro procedures, which had, of course, been worked out with mg. amounts of alloys. Results are shown in Table II.

The first two alloys were reduced for 1 hour in apparatus 1 and the others for 2 hours in apparatus 2. Increase in the time of reduction from 1 hour to 2 hours did not have a

very pronounced effect but, as Höltje[2] has pointed out, the reduction of the last few μg. of tin^{IV} is difficult.

Copper was not removed from white metal "A," lead-base bearing metal and the fusible alloy. In all the brasses and bronzes and the aluminium alloy it was removed by a double precipitation with ammonia. The "blank" correction has been deducted from all results.

TABLE II

	Approx. wt. taken (mg.)	Percentage composition							Percentage of tin found
		Pb	Sb	Cu	Zn	Al	Etc.	Sn	
*White metal "A"	10	82·6	12·04	0·33	0·08	—	0·15	4·64	4·58, 4·65
†Lead-base bearing metal	8–10	78·9	10·09	0·05	—	—	0·13	10·91	10·88, 10·93, 10·93
*White metal "B"	1	3·86	7·51	4·08	0·40	—	0·09	84·0	83·8, 84·6, 83·8, 83·8
†Tin-base bearing metal	1	0·21	7·32	3·75	—	—	0·10	88·6	88·9, 89·4, 88·8
*Bronze "A" ..	10	1·83	0·24	85·5	1·86	—	0·85	9·72§	9·57, 9·67, 9·61
*Bronze "C" ..	10	0·41	0·04	86·8	2·53	—	0·31	9·80	9·76, 9·77
*Manganese brass "B"	10	0·78	0·05	58·8	33·9	1·62	3·04	1·75	1·66, 1·67, 1·61
†Brass No. 37*b* ..	10	0·90	—	70·4	27·1	—	0·66	0·99	0·92, 0·98, 0·95
‡Aluminium alloy	5–6	—	—	3·14	0·19	82·3	5·3	9·09	8·98, 8·94
Fusible alloy (synthetic) ..	5–6	49·3	Bi 24·6	—	Cd 12·3	—	—	13·79	13·74, 13·69

* British Chemical Standards sample.
† U.S.A. Bureau of Standards sample.
‡ Tin added to a solution of a tin-free alloy.
§ Revised figure (*cf.* Schoeller and Holness[9] and Dunbar-Poole.[10])

CONCLUSION

The results in Table II show that the volumetric determination of tin in non-ferrous alloys is practicable on a small scale. In general there is a negative error which becomes appreciable when small amounts of tin are determined in brasses. The larger error there is attributed to loss of tin during the removal of copper and not to a faulty reduction of stannic chloride with antimony.

SUMMARY

A method has been evolved for the volumetric determination of 0·1 to 1 mg. of tin. Stannic chloride is reduced by boiling it vigorously under reflux with 40 per cent. hydrochloric acid (v/v) in presence of sticks of freshly recast antimony, and the stannous chloride formed is titrated at a temperature below 15° C. with 0·1 *N* iodine, which is contained in a micro-burette of 0·2-ml. capacity. The method has been applied to the determination of tin in bearing metals (1 to 10 mg.), a fusible alloy and an aluminium alloy (5 to 6 mg.), and brasses and bronzes (10 mg.), from which copper in excess of about 30 μg. has been removed as the cuprammonium complex.

We are indebted to Dr. A. Lacourt of the Centre de Microchimie of Brussels University for guidance on the use of micro-burettes, and gratefully acknowledge grants from the Trustees of the Moray Fund for the purchase of apparatus, and a maintenance grant to one of us (L. R. C.) from the Department of Scientific and Industrial Research.

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Some Analytical Applications of *m*-Nitrobenzoic Acid with Particular Reference to the Separation of Quadrivalent Elements from the Rare Earths (Lanthanides)

By G. H. OSBORN

The use of *m*-nitrobenzoic acid as a specific reagent for thorium in presence of rare earths was first recommended by Neish in 1904,[1] and it is of interest that this was one of the earliest reported applications of an organic reagent for the determination of inorganic radicals. It was claimed that thorium could be precipitated quantitatively from a neutral solution of the nitrate and that a complete separation from cerium, lanthanum and didymium (*i.e.*, neodymium and praseodymium), was obtainable. In this respect, Neish reported that *o*-, *m*-, and *p*-nitrobenzoic acids were equal, but that the *meta* isomer was to be preferred on account of its greater solubility in water. Apart from an occasional reference,[2,3,4] *m*-nitrobenzoic acid seems to have been largely neglected as an analytical reagent; however, in view of the recently increased interest in the analysis of the rare earths, it was thought that a further study of the procedure might be of value. The experimental work and the applications given below were the outcome of this investigation.

Experimental

The reagent solution was prepared by dissolving 4 g. of *m*-nitrobenzoic acid in 1000 ml. of water, heating to 80° C., allowing to stand overnight and filtering to remove excess and any impurities. The wash water used for all the precipitates was prepared by adding 5 ml. of the above solution to 95 ml. of water.

Thorium—

(*a*) *Determination*—A sample of $Th(NO_3)_4.12H_2O$ "Spec-pure" was used as a starting point. This was carefully analysed by classical methods and found to contain 83·5 per cent. of $Th(NO_3)_4$, approximating thus to $5\frac{1}{2}H_2O$. As the material contained 40·36 per cent. of thorium, 12·38 g. were dissolved in a litre of water to yield a solution containing 0·5 g. of thorium per 100 ml. 100-ml. portions were taken and the thorium was precipitated as the hydroxide by ammonia, filtered, burnt off and weighed; the solution was found to contain 0·500 g. per 100 ml.

Two 100-ml. portions were taken, 150 ml. of the reagent solution in water were added and the solutions heated to 80° C., allowed to stand for 1 hour and filtered; the precipitates were washed with 100-ml. of the wash solution, placed directly in platinum crucibles while wet, dried, ignited to ThO_2 and weighed when cool. The weights of thorium oxide obtained were 0·5690 g. and 0·5694 g. (theory, 0·5689 g.). It was found, however, that if one attempted to dry the filter paper before igniting, losses up to 10 per cent. occurred, as had been pointed out in the original paper. The author of the original paper[1] dried the complex at 115° C. and assumed it to be $Th(C_6H_4NO_2COO)_4.4H_2O$, but as there is some doubt about the hydration, it is better to ignite to the oxide.

(*b*) *Effect of acidity on the precipitation of thorium*—It had been stated that thorium was precipitated from a neutral solution, but no remarks were made about the effect of acidity, although at an early stage it was clear that it would have a very marked effect. Known weights of the thorium nitrate were dissolved in 100 ml. of 0·02 *N*, 0·05 *N*, 0·1 *N* and 1 *N* nitric acid, respectively. Complete precipitation of the thorium was obtained from the 0·02 *N* solution but only 60 per cent. from the 0·05 *N* solution. Thorium was not precipitated by the reagent from 0·1 *N*, 0·2 *N* and 1 *N* solutions.

Zirconium—

(*a*) *Determination*—In the original paper[1] it was stated that zirconium "gave a white opalescence and precipitate which developed on heating." Exactly 0·5 g. of a sample of commercial zirconium nitrate, found by classical methods of analysis to be equivalent to between 42 and 43 per cent. of ZrO_2, was dissolved in 100 ml. of water plus 1 ml. of nitric

acid, and 150 ml. of the reagent solution were added. A white precipitate occurred at once and became more pronounced on heating to 80° C. After standing for one hour it was filtered through a Whatman No. 44 filter paper, washed with 100 ml. of the wash solution, transferred to a crucible and ignited to the oxide. The residue weighed 0·2130 g., indicating that, under the conditions of the experiment, zirconium is quantitatively precipitated by *m*-nitrobenzoic acid and would, therefore, interfere with the determination of thorium in a mixture of salts of the two metals.

(*b*) *Effect of acidity on the precipitation of zirconium*—Known weights of commercial zirconium nitrate were severally dissolved in 100 ml. of 0·02 *N*, 0·05 *N*, 0·1 *N*, 0·12 *N* and 1 *N* nitric acid. Complete recovery was obtained by *m*-nitrobenzoic acid from all except the 1 *N* solution; from this, only 90 per cent. was recovered, the precipitate being too fine to filter easily without loss. In view of the above findings, it was decided to try to effect a separation of thorium and zirconium at an acidity of 0·2 *N*.

(*c*) *Determination of thorium and zirconium in presence of each other*—0·3 g. of thorium nitrate (45·92 per cent. ThO_2) and 0·3 g. of commercial zirconium nitrate (42–43 per cent. ZrO_2) were dissolved in 100 ml. of 0·2 *N* nitric acid, 150 ml. of the reagent were added and the solution was heated to 80° C., allowed to stand for 1 hour and filtered on a Whatman No. 44 paper; the precipitate was washed with the usual wash solution, burnt off and weighed. The weight found was 0·128 g. which is equivalent to the ZrO_2 known to be present. An excess of ammonia was added to the filtrate and the precipitate was washed, burnt off and weighed as ThO_2. The weight found was 0·138 g. which is that of the ThO_2 known to be present. *Thus it would appear that zirconium can be quantitatively separated and recovered in presence of thorium if the acidity is* 0·2 *N*.

HAFNIUM—

In view of the close relationship between zirconium and hafnium it appeared advisable to check the reactions of this quadrivalent element with the reagent. It proved unexpectedly difficult, however, to obtain a pure specimen of the salt and ultimately it was only possible to obtain 25 ml. of a solution stated to contain a 0·2 per cent. w/v of hafnium sulphate. It was stated that this was made from a mixture of the oxides of hafnium and zirconium believed from the specific gravity and from X-ray and spectrographic examination to contain 75 to 80 per cent. of hafnium oxide. Five ml. of this solution was taken in a beaker and diluted to 100 ml. with water. An excess of ammonia was added and the solution boiled, and the precipitated oxides were filtered, washed, dried and burnt off. The weight found, 0·0170 g., corresponds to just under 80 per cent. of HfO_2.

Two further 5-ml. portions of the hafnium sulphate solution were taken and diluted to about 20 ml.; 100 ml. of the reagent was added to each, and a faint precipitate developed. On heating as usual, the precipitates increased and after standing for an hour they were filtered, washed and treated as before and weighed. In both, 0·0170 g. was found, so both the hafnium and zirconium had been precipitated. Titration of 1 ml. of the original solution with 0·1 *N* sodium hydroxide from a micro-burette indicated an acidity of 0·78 *N*; thus the acidity in the 120 ml. of reaction mixture was of the order of 0·03 *N*.

It would have been desirable to find under what conditions, if any, the hafnium could have been separated from the zirconium, but shortage of material made this impossible. Further work will be carried out when more material is to hand.

CERIUM—

Neish states that cerium does not interfere with the precipitation of thorium, but Kolb and Ahle[2] found that quadrivalent cerium did give an insoluble compound with *m*-nitrobenzoic acid although, if the cerium was completely reduced to the tervalent form, no precipitation took place. In order to check these statements, solutions of ceric ammonium nitrate, $Ce(NH_4)_2(NO_3)_6$, and of cerous ammonium sulphate, $Ce_2(SO_4)_3(NH_4)_2SO_4$, were prepared and a portion of the reagent solution was added to each; with the ceric salt, there occurred a heavy white precipitate which, on further investigation, was found to be quantitative, while no precipitate whatever was formed in the solution of the cerous compound. If this latter solution was so treated as to oxidise the cerous salt to the ceric state an immediate quantitative precipitation took place. For strictly quantitative precipitation the solution of quadrivalent cerium should be allowed to stand at 80° C. for one hour after the addition of the reagent, as for thorium and zirconium. A complete separation of zirconium or thorium or both from cerium salts can always be obtained by first reducing the ceric to the cerous salt.

PLUTONIUM—

While this work was being carried out, there was published a paper by B. G. Harvey *et al.*,[5] who used *m*-nitrobenzoic acid as a reagent for plutonium and it was found that the quadrivalent ion was precipitated, but not the tervalent ion. It may be presumed, therefore, that *m*-nitrobenzoic will react with all the quadrivalent ions of the actinides series and it might possibly be a specific precipitant for quadrivalent elements.

REACTIONS WITH TER- AND BI-VALENT ELEMENTS—

Tests were made on solutions of aluminium nitrate, beryllium nitrate, lanthanum chloride, yttrium nitrate, uranyl nitrate, thallous nitrate, potassium chloropalladate, titanous sulphate, titanic sulphate, bismuth nitrate, lead nitrate, cadmium nitrate, lithium nitrate, manganous nitrate, magnesium cobalt nitrate and potassium antimonyl tartrate, and the spectroscopically pure oxides of gadolinium, neodymium, samarium and ytterbium were also used. With none could any reaction with *m*-nitrobenzoic acid be obtained and it seems probable that tervalent elements, as a whole, do not react with the reagent. This casts some doubt on the statement in the original paper that erbium, a tervalent rare-earth metal, reacts quantitatively. It has not been possible, even at the present time, to obtain a pure specimen of an erbium salt and it is quite probable that the sample believed in 1904 to be erbium was, in fact, a quadrivalent element.

REACTION WITH MERCURIC AND MERCUROUS SALTS—

In the original paper[1] there is a passing reference to precipitates given with stannous, stannic, mercurous and mercuric salts, and on investigation it was found that with the stannous and stannic salts the effects were, as expected, due merely to hydrolysis of the salts themselves and not to compounds with the *m*-nitrobenzoic acid. With the mercuric and mercurous salts, however, it was found to the author's surprise, in view of the valencies, that reactions did occur and that under the right conditions these were quantitative. In the original paper, the only remark made on mercury salts was that "the mercurous and mercuric nitrates gave very heavy precipitates which were white and curdy in the cold but dissolved on heating." Tests with $Hg(NO_3)_2.\frac{1}{2}H_2O$ and $HgNO_3.H_2O$ showed that in the cold there were heavy precipitates which, however, were not quantitative and that, on boiling, these redissolved. As with thorium, the correct condition of acidity was found to be critical; the solution should not be more acid than 0·03 *N* and should preferably be 0·02 *N*. When 0·5 g. of either salt was dissolved in 100 ml. of 0·02 *N* nitric acid and 150 ml. of the reagent were added in the cold, a heavy flocculent precipitate was formed, but this was not quantitative. If the solution was boiled and allowed to cool to room temperature, the precipitate reformed as a fine crystalline deposit which was entirely quantitative. If filtered and washed with a cold solution of the wash water, the mercury could be separated from other salts usually associated, and could then be redissolved and estimated by any of the normal methods for the estimation of this element. It will not interfere with the reactions for thorium, zirconium or cerium (apart from the fact that it is extremely unlikely to occur with them), since these may be filtered from a hot solution in which the mercury complex is soluble. It would, of course, have been possible to weigh the complex after drying at 110 to 115° C. but, as pointed out previously, the degree of hydration is somewhat doubtful and the temperature very critical, so that it was thought better to redissolve the complex and estimate the mercury by a standard procedure.

SUMMARY

(i) Some reactions of *m*-nitrobenzoic acid have been re-examined and it has been found that the reagent reacts quantitatively, under strictly controlled conditions, with thorium, zirconium, cerium^{IV}, hafnium and mercury.

(ii) Harvey *et al.*[5] have shown that quadrivalent plutonium reacts similarly.

(iii) The reagent does not react with the tervalent rare earths (lanthanides series) examined, and doubts are, therefore, cast on the reported reaction with erbium.

(iv) Apart from possible interference due to hydrolysis of certain tin salts and by the presence of mercury, the reagent appears to be a specific precipitant for all quadrivalent metals except titanium.

(v) Conditions are described under which most of the elements found to react can be separated from each other.

The author thanks the Directors of The British Drug Houses, Limited, for permission to publish these results. Thanks are also due to Mr. Noel L. Allport of this Company for very valuable assistance in the search of literature and for constructive criticism and advice.

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Internal Standards in Polarographic Analysis*

By R. Loofbourow and H. A. Frediani

Introduction

Considering the relatively early publication of the work of Forche[1] and the suggestions by Lingane,[4] Mueller and Petras,[5] it is surprising that there has been little application of the internal standard method to routine polarographic methods of analysis. A survey of some 26 articles appearing in the "*Analytical Edition of Industrial and Engineering Chemistry*" between 1943 and 1946 indicated that in one paper alone[6] was this method applied. Discussions at the Polarographic Symposium at Princeton, New Jersey (under auspices of the A.S.T.M. early in 1946) as well as at a private symposium of industrialists interested in the practical application of polarography (New Haven, Connecticut, May, 1946) indicated clearly that the technique was relatively unknown, though of great interest when mentioned.

There is little doubt in the authors' minds that the technique is being utilised to advantage by some industrial laboratories. It is with the thought of indicating the inherent advantages to other laboratories desirous of utilising polarographic measurements that this paper is being published. One definite instance of the satisfactory use of this technique[1] is in the determination of lead in gasoline, during the war years, by the Jeffersonville Quartermaster Depot at Jeffersonville, Indiana.[3]

Theoretical

The fundamental basis of the internal standard technique is as follows. A standard fixed amount of foreign ion, not normally present in the test material, is added to the test solution. The concentration of standard ion is so chosen as to be of approximately the same order of magnitude as the unknown (or unknowns). The diffusion currents of the known and unknown ions are then measured and from the ratio between that of the known and unknown the concentration of the unknown is calculated. Within the limits easily maintained in the laboratory by relatively untrained workers, normal variations in temperature, drop time, drop size, mercury height and capillary size have no effect upon the analysis. Furthermore, application of internal standard technique permits the use of different capillaries without tedious time-consuming recalibrations.

That the theory involved is sound may be ascertained by examining the equations involved.[2] Considering an ion A (variable concentration) and an ion B (fixed concentration) the equations for the diffusion currents obtaining are—

$$i_A = 605\, n\, D_A^{1/2}\, C_A\, M^{2/3}\, t^{1/6} \quad \ldots\ \ldots\ \ldots\ \ldots \quad (1)$$

$$i_B = 605\, n\, D_B^{1/2}\, C_B\, M^{2/3}\, t^{1/6} \quad \ldots\ \ldots\ \ldots\ \ldots \quad (2)$$

Dividing (1) by (2) and considering that in a single polarogram M and t will be maintained at fixed values one obtains—

$$\frac{i_A}{i_B} = \frac{D_A^{1/2}\, C_A}{D_B^{1/2}\, C_B} \quad \ldots\ \ldots\ \ldots\ \ldots\ \ldots \quad (3)$$

Since in routine work it is readily possible to maintain C_B at a known fixed value and since $D_A^{1/2}$ and $D_B^{1/2}$ may be considered constants (especially if C_B is kept constant, C_A permitted

* Presented at Atlantic City Meeting of the American Chemical Society in April, 1947.

to vary over a relatively small range and the ionic strength of the solution maintained constant by copious use of supporting electrolyte) equation (3) then reduces to:—

$$C_A = \frac{Ki_A}{i_B} \quad .. \quad .. \quad .. \quad .. \quad .. \quad (4)$$

Analogous reasoning will show that the same relationship would hold for other unknowns (C, D, etc.) that may be present, so that the diffusion current of a single added ion of known concentration may be used to ascertain the actual concentration of one or more unknowns.

The necessary conditions are—

(1) That the concentrations of the ions involved must be of relatively the same order of magnitude.
(2) That the half-wave potentials of the known and unknowns must be sufficiently separated to prevent overlapping.
(3) Since drop time varies somewhat with the potential it is essential that the half-wave potential of the internal standard be as close as possible to that of the substance sought. It is only when this condition is fulfilled that t_A will be approximately equivalent to t_B.

Experimental

To obtain actual data as to the efficacy of this technique, solutions were prepared wherein cadmium was considered as the "known" (maintained constant in concentration) and lead and zinc both as variables. The solutions prepared for this test had the following compositions—

	KCl	Pb	Zn	Cd
A	0·1 *M*	1×10^{-4} *M*	—	5×10^{-5} *M*
B	0·1 *M*	9×10^{-5} *M*	1×10^{-5} *M*	"
C	0·1 *M*	$7{\cdot}5 \times 10^{-5}$ *M*	$2{\cdot}5 \times 10^{-5}$ *M*	"
D	0·1 *M*	5×10^{-5} *M*	5×10^{-5} *M*	"
E	0·1 *M*	$2{\cdot}5 \times 10^{-5}$ *M*	$7{\cdot}5 \times 10^{-5}$ *M*	"
F	0·1 *M*	1×10^{-5} *M*	9×10^{-5} *M*	"
G	0·1 *M*	—	1×10^{-4} *M*	"

Nine polarograms were prepared for each of these solutions, using drop times of 2, 4 and 6 seconds at temperatures of 0°, 25° and 50° C. It is to be noted that the variation in both drop time and temperature were exaggerated so as to test the linearity of the functions indicated in equation (4). Under normal conditions these two conditions would seldom vary over such wide ranges.

Table I

All solutions contained 0·1 *N* KCl

Concentrations of Pb, Zn and Cd are expressed in 10^{-5} *M*

Drop time	A: Pb 10, Zn —, Cd 5		B: Pb 9, Zn 1, Cd 5		C: Pb 7·5, Zn 2·5, Cd 5		D: Pb 5, Zn 5, Cd 5		E: Pb 2·5, Zn 7·5, Cd 5		F: Pb 1, Zn 9, Cd 5		G: Pb —, Zn 10, Cd 5	
	Pb/Cd	Zn/Cd	Pb/Cd	Zn/Cd	Pb/Cd	Zn/Cd	Pb/Cd	Zn/Cd	Pb/Cd	Zn/Cd	Pb/Cd	Zn/Cd	Pb/Cd	Zn/Cd
Temperature 0° C.														
2 sec.	2·14	—	1·92	0·27	1·68	0·55	1·19	0·95	0·66	1·32	0·29	1·56	—	1·63
4 sec.	2·19	—	1·85	0·28	1·66	0·51	1·17	0·93	0·62	1·32	0·28	1·58	—	1·65
6 sec.	2·20	—	1·92	0·28	1·65	0·52	1·08	0·93	0·57	1·29	0·27	1·54	—	1·72
Temperature 25° C.														
2 sec.	2·20	—	1·91	0·29	1·62	0·51	1·09	0·96	0·65	1·39	0·30	1·55	—	1·73
4 sec.	2·10	—	1·90	0·26	1·63	0·50	1·12	0·93	0·61	1·31	0·30	1·60	—	1·68
6 sec.	2·06	—	1·92	0·27	1·63	0·50	1·09	0·94	0·56	1·30	0·26	1·58	—	1·77
Temperature 50° C.														
2 sec.	2·10	—	1·85	0·29	1·73	0·52	1·09	0·97	0·53	1·34	0·25	1·49	—	1·82
4 sec.	2·08	—	1·80	0·30	1·69	0·57	1·14	0·93	0·54	1·35	0·25	1·52	—	1·66
6 sec.	2·08	—	1·80	0·29	1·66	0·61	1·07	0·95	0·51	1·33	0·25	1·51	—	1·70
Averages														
—	2·13	—	1·88	0·28	1·66	0·53	1·12	0·94	0·58	1·33	0·27	1·55	—	1·70

The polarograms were recorded on a Sargent Model XX Polarograph. Nitrogen was used to remove oxygen and methyl red was added to each solution as maximum suppressor.

In collecting the data, three polarograms, representing three drop rates for the same solution at the temperature, were recorded on each chart. A typical set of polarograms made in this manner is shown in Fig. 1. The effect of change in drop time on wave height and amplitude of oscillations are clearly indicated (sensitivity and damping controls remained at fixed positions). It is also apparent that at the two-second drop rate the speed of the dropping has started to affect the regularity of the curve.

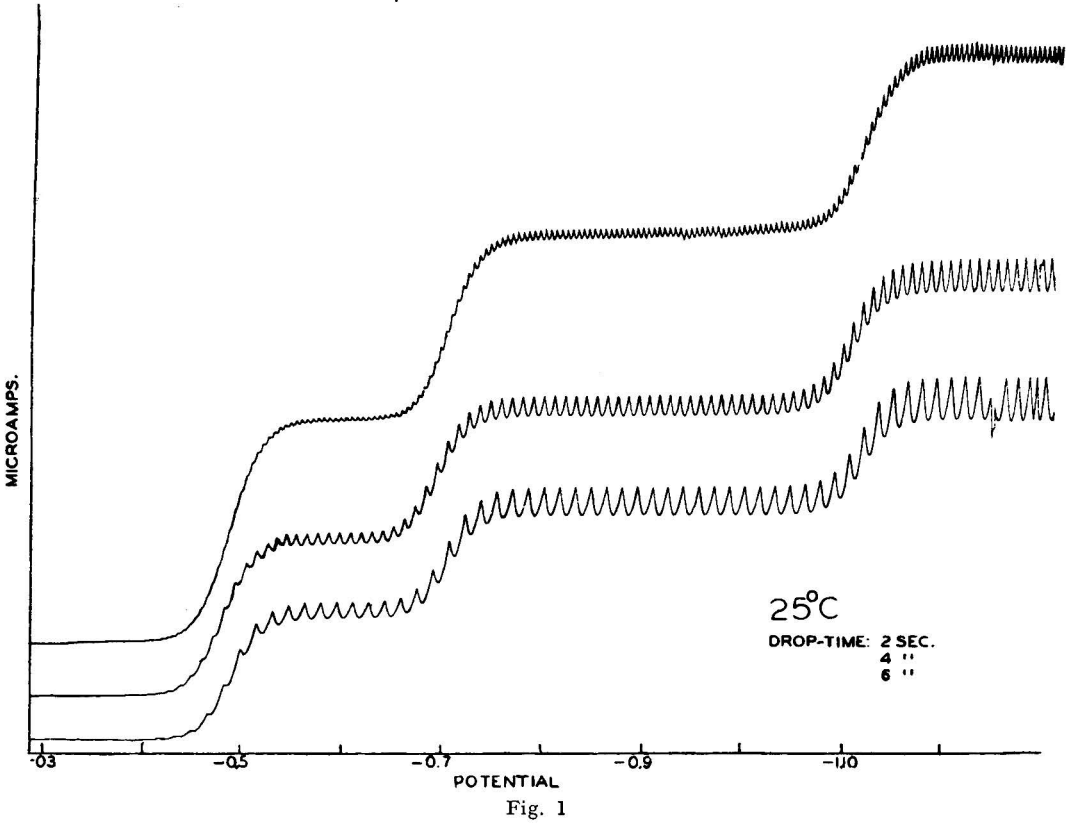

Fig. 1

Results

The data presented in Table I indicate that reasonable constancy of the i_{Pb}/i_{Cd} and i_{Zn}/i_{Cd} ratios was maintained over the wide ranges applied to the variables. Actual routine work with normal small variations in temperature and drop rate have permitted determinations with precision to 3 per cent. The graphical presentation of our data given in Fig. 2

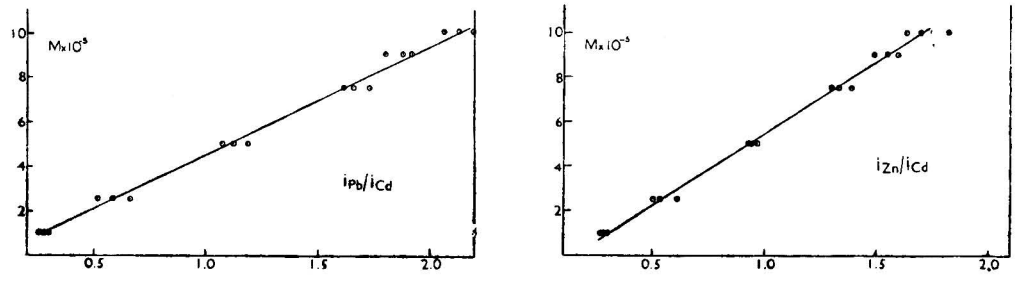

Fig. 2

shows that the straight-line relationship between concentration (variable element) and the element/standard ratio is maintained. In plotting these data the mean value for the nine ratios secured on each solution is given together with the maximum and minimum values.

SUMMARY

The use of absolute diffusion current measurements in quantitative polarography necessitates accurate control and measurements of capillary diameter, height of mercury, dropping rate, size of drop and temperature of measurement. By the use of an internal standard and ascertaining diffusion current ratios (of unknown to known ion) it is possible to neglect these factors for control work. This means that quantitative work may be carried out without rigid control of the aforementioned factors. This is of especial importance in routine analytical work since one need not recalibrate new capillaries when a replacement occurs.

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A Micro-Method for Molecular Weight Determination in Alcoholic Solutions

BY ROBERT WRIGHT

BARGER[1] has described a microscopical method of determining molecular weights, in which drops of two solutions—of different solutes in the same solvent—were placed close to each other in a capillary tube. If the drops differ in molar concentration, then the more concentrated solution will increase in volume owing to distillation of solvent from the more dilute drop; the change in drop size is observed by means of a microscope. The molar concentration of a solution containing a solute of unknown molecular weight, could be compared with a series of solutions of different known molar concentrations, and by this means the approximate molecular weight of the unknown determined. A certain amount of intermingling of the drops was found to be unavoidable, since the first drop drawn into the capillary wetted the walls of the tube on its passage, thus contaminating the following drop. Although this contamination does not prevent decision as to which of the two drops is the more dilute, it renders impossible any quantitative calculation of their relative concentrations.

In the present method, small quantities of two alcoholic solutions, held on filter paper, are left with their vapour phase in common until equilibrium has been reached, when the relationship holds:—

$$\frac{w}{m}\Big/\mathrm{W} = \frac{w'}{m'}\Big/\mathrm{W}'$$

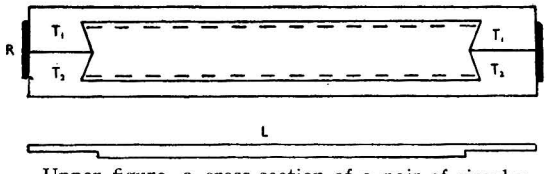
where W and W′ are the weights of solvent in the two solutions, w and w' the weights of the solutes, and m and m' the molecular weights of the solutes. Thus, if the composition of each solution is known, the molecular weight of the second solute may be calculated.

The apparatus employed consists of a pair of circular aluminium trays 70 mm. in diameter, cut from sheet 6 mm. thick. Each tray is recessed to a depth of 4 mm., the diameter of the recess being 55 mm. at the bottom and slightly less at the top. This undercutting of the edge of the recess allows a 55 mm. filter paper to be held in the recess even when the tray is inverted. The flat upper edges of the trays are faced so as to fit closely to one another, and when the trays are placed face to face in contact they are held in position by means of a rubber band passing round their circumference. Each tray is provided with a lid cut from 1·5-mm. aluminium sheet, stepped at the edge to half its thickness so as to fit the recess. Each tray and lid is appropriately marked.

The filter papers used to hold the solution films in position were of ordinary quality, but were extracted with alcohol before use in order to remove any alcohol-soluble material

that might be present; the papers were then dried and stored until required. As filter papers are seldom of uniform size it is necessary to make sure that at least one of the pair—that used in the inverted tray—should fit the tray recess snugly.

Since the estimation depends on attainment of equilibrium between the two solutions, experiments were made to ascertain the length of time necessary to reach that state. Two trays, fitted with papers and lids, were weighed and about 0·01 g. of benzoic acid was added to each tray and they were weighed again. Several drops of ethyl alcohol were then added to each tray so that the two lots of acid were completely dissolved. The pair of trays were then placed in contact face to face and held in position by means of the rubber band. They were then wrapped in cotton wool and placed in a small vessel of desiccator form containing a small quantity of alcohol, so that the air surrounding the trays was saturated with alcohol vapour. This precaution against loss of alcohol from the tray contents is necessary, the rubber band not being sufficient to prevent evaporation. After a suitable interval, one or two days, the trays were removed from the vapour chamber and the encircling rubber band slipped off. The pair of tray lids, placed back to back, were then slipped between the trays, one of which was replaced in the alcohol chamber while the other was being weighed. From the weights of the trays the weights of the two solutions on the papers could be found, and subtraction of the weight of solute gave, for each, the weight of solvent. If equilibrium had been attained, the ratio of solute to solvent should be the same for each tray, since the same solute was used in each solution. In these preliminary tests it was found that the time required to reach equilibrium never exceeded two days and one day often sufficed. It was therefore concluded that in general an interval of two days would be a period of waiting sufficient to give satisfactory results.



Upper figure, a cross section of a pair of circular aluminium trays, T_1 and T_2, held together by a rubber band, RR. The broken lines represent filter papers.
Lower figure, L, is the lid of a tray.

In comparing the molecular weights of two solutes the standard substance chosen was benzoic acid, as it is readily obtained pure and is very soluble in alcohol. The prepared papers and trays having been weighed, about 0·01 g. of benzoic acid was added to one tray and the weight determined. About the same weight of the second solute, ground as finely as possible, was weighed on to the second tray, and both solutes were dissolved by adding alcohol a drop at a time. Any solute employed should have a solubility of at least 10 g. per 100 g. of alcohol, and it should be made certain, by inspection of the filter paper with a lens, that the solute is completely dissolved. Care must also be taken that all the added solvent is held by the filter paper, so that none will flow off when the tray is inverted. The prepared trays, being fixed in contact, are left for two days in the alcohol chamber, after which they are removed and their contents estimated in the manner already described. From the results so obtained the molecular weight of the unknown solute may readily be calculated.

The results obtained for a number of solutes are shown in the Table. It will be noted that, in general, they do not deviate from the theoretical values by more than 10 per cent. The notable exception is tartaric acid which invariably gave too high a molecular weight. This is probably due to interaction between the acid and alcohol, producing ethyl tartrate and water. The presence of the water in the alcohol raises the vapour pressure, the maximum vapour pressure for the alcohol – water system occurring when about 4 per cent. of water is present. This greater volatility of the solvent containing tartaric acid, compared with the second solution, results in loss of solvent from the tartaric acid - alcohol system and a consequent increase in the calculated molecular weight of the tartaric acid. That some esterification had taken place was proved by hydrolysis of the solution with barium hydrate and was also indicated by the fact that the alcoholic solution of tartaric acid could not be evaporated to complete dryness owing to the presence of a trace of ethyl tartrate. It should be noted that tartaric acid is a relatively strong organic acid. Kohlrausch (*"Leitvermögen der Elektrolyte"*) gives the dissociation constant for tartaric acid as 97×10^{-5}, whilst the value for benzoic acid is $6{\cdot}6 \times 10^{-5}$ and that for acetic acid $1{\cdot}8 \times 10^{-5}$.

Apart from the length of time required for an estimation there are other drawbacks to the method just described. The concentrations of the solutions employed are high compared

with those used in the cryoscopic method. The size and weight of the trays necessitate the use of an ordinary analytical balance, and, since the sensitivity of such an instrument is usually about 0·1 mg., the weight of solute employed should not be less than 10 mg. The weight of solvent is limited by the amount that can be held by the filter paper, and cannot greatly exceed 0·2 g.; thus the concentration of solute is in the neighbourhood of 5 per cent., compared with 1 per cent. for a cryoscopic determination. Further, the molecular weights obtained are relative to that of the standard substance chosen and will be different if that standard is changed. Benzoic acid was employed as standard, but it would probably be more satisfactory if the solutes, standard and unknown, were of similar type. Thus a synthesised unknown might preferably be compared with a closely related intermediate.

TABLE

Solute and its mol. wt.	Wt. of solute g.	Wt. of EtOH g.	Wt. of enzoic acid g.	Wt. of EtOH g.	Mol wt.
Succinic acid	0·0064	0·1450	0·0101	0·2480	132
118	0·0098	0·2200	0·0099	0·2038	112
Camphoric acid	0·0098	0·1202	0·0120	0·2465	203
200	0·0091	0·1196	0·0107	0·2452	213
Benzophenone	0·0122	0·1623	0·0087	0·1662	176
182	0·0114	0·1310	0·0121	0·2266	198
Aspirin	0·0118	0·1593	0·0092	0·1879	184
180	0·0112	0·1867	0·0101	0·2326	169
Acetanilide	0·0116	0·1850	0·0117	0·1919	126
135	0·0103	0·1703	0·0101	0·1730	126
Cinnamic acid	0·0100	0·2638	0·0116	0·3588	143
148	0·0120	0·2898	0·0110	0·3558	163
Tartaric acid	0·0102	0·1936	0·0098	0·3564	234
150	0·0108	0·1253	0·0113	0·2418	224
	0·0053	0·1749	0·0051	0·3385	244

Ethyl alcohol was the only solvent employed, as it was not found possible to avoid the use of the encircling rubber band. When the band was omitted and the trays simply clamped in contact, vapour passed from the outer vessel, where the vapour pressure was that of the pure solvent, into the solutions and equilibrium could not be attained. Gas-tight connection between the trays should be possible, but undue clamping force must not be employed on account of the danger of alteration of the weight of the trays through scratching.

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PHYSICAL CHEMISTRY DEPARTMENT
GLASGOW UNIVERSITY

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The Colorimetric Determination of Diphenylamine in Nitrocellulose Powders

BY J. G. NORVALL, T. C. J. OVENSTON, AND C. A. PARKER

EXISTING procedures for the determination of "active" diphenylamine in nitrocellulose powders have recently been reviewed by Ellington,[1] who proposed a modification of the volumetric method of Ellington and Beard[2] as offering some advantages over previous methods both in ease of manipulation and in reliability of results. Of the other methods suitable for routine working, the commonly employed gravimetric procedure of Cook[3] has usually given good results.

In the methods of Ellington and of Cook, the first step in the prevention of interference by inactive derivatives of diphenylamine is the steam distillation of the "active" diphenylamine (consisting here of all the diphenylamine and most of the N-nitrosodiphenylamine). This process accounts for most of the man-power consumption, a factor of major importance in handling large numbers of samples.

The object of the present investigation has been the development of a more rapid routine

method for determining "active" diphenylamine without sacrifice of accuracy, and in the colorimetric procedure to be described it is believed that this object has been achieved.

Experimental

The intense blue colour produced by the oxidation of diphenylamine in strong sulphuric acid solution has been proposed from time to time as a basis for the colorimetric estimation of diphenylamine.[4,5] In the present investigation, potassium dichromate dissolved in dilute sulphuric acid is chosen as the oxidising medium, and extraction of the diphenylamine from the propellant is accomplished with 90 per cent. w/w acetic acid. The colour measurement is made with a Spekker photo-electric absorptiometer using 1-cm. cells which are 6·5 cm. high but standard in other respects. Details of procedure are as follows.

Dichromate – sulphuric acid reagent—Dilute 40 ml. of pure sulphuric acid with 160 ml. of water and cool to room temperature. Immediately before use add 2·0 ml. of a 0·2 *N* solution of potassium dichromate and mix. This will provide sufficient for twelve tests.

Procedure—Weigh out 0·1 g. of the propellant, reduced in size to pass a B.S. Test Sieve No. 18, into a screw-capped bottle. (Bottles 2½ inches high and 1 inch in diameter are convenient, a tight closure being ensured by fitting a polythene disc inside the Bakelite cap.) Add exactly 10 ml. of 90 per cent. w/w acetic acid (by pipette) and, after allowing to stand overnight, shake mechanically for 4 hours. Allow to stand for ½ hour and examine visually to ensure that the nitrocellulose particles have been fully extracted, as indicated by their colour; this is necessary when analysing artificially aged samples, and is always a wise precaution. If necessary, shake for further short periods until extraction is complete. Allow to settle.

Transfer exactly 1·00 ml. of the clear extract to a small beaker, and add (by pipette) 15 ml. of dichromate – sulphuric acid reagent with vigorous stirring. Allow 20 seconds for the pipette to discharge and drain, then pour immediately into a clean, dry, "tall form" Spekker 1 cm. cell.

Using Ilford filters No. 606 and a water-to-water setting of 1·00, take Spekker readings at exactly 3 minutes and 4 minutes after the first drop of reagent entered the acetic acid solution. The colour takes about a minute to develop fully, or a little longer with artificially aged samples. After 5 minutes the colour gradually fades. The 3-minute reading should be the same as, or very slightly lower than, the 4-minute reading. Irregular readings have been noted only very infrequently, and when this occurs the determination can be repeated on a fresh aliquot of the same extract. Carry out a blank determination, using 1 ml. of the 90 per cent. acetic acid. From the difference in drum readings between the same solution and the blank solution, at 4 minutes, estimate the amount of diphenylamine present from a graph prepared by using known weights of pure diphenylamine.

Notes on Procedure—With aged propellants a small quantity of nitrocellulose is dissolved by the 90 per cent. acetic acid, and this reprecipitates on addition of the oxidising reagent. The precipitated nitrocellulose takes 1½ to 2 minutes to collect at the surface of the liquid in the Spekker cell. The use of "tall form" cells ensures that this nitrocellulose is clear of the effective area of the light beam.

All glassware must be carefully cleaned, preferably by standing in contact with chromic-sulphuric acid mixture and then rinsing with distilled water, followed by pure acetone, and drying in warm air. When there are large numbers of determinations day after day, it is convenient to return the solutions after test to the original beakers, cover them and leave until the beakers are required for the next set of tests. They can then be rinsed and dried without further cleansing.

The necessary size reduction of the propellant can be conveniently accomplished with the standard cordite mill, using a slightly modified cutter.

This procedure is designed for powders containing up to 1·2 per cent. of diphenylamine.

Effect of variation in concentration of sulphuric acid—0·1-mg. quantities of diphenylamine dissolved in 1 ml. of 90 per cent. acetic acid were treated by the procedure described, but using various concentrations of sulphuric acid. The intensity of the colour developed in each case was measured as a function of the time elapsed after the initial addition of the reagent.

With increasing concentrations of acid it was found that the time for maximum colour development decreased rapidly. At concentrations of 25 per cent. w/w and over, the maximum intensity was reached within a minute. The maximum colour obtained also varied with the acid concentration, increasing slowly to about 30 per cent. w/w, remaining fairly steady between 30 and 50 per cent. w/w, and then falling rapidly to almost zero above 80 per cent. w/w.

The rate at which the colour faded increased with acid concentration, but was negligible in the first five minutes below 40 per cent. w/w.

The acid concentration finally chosen for the method corresponds to 31 per cent. w/w. The calibration graph prepared under these conditions shows a linear relationship between weight of diphenylamine and extinction reading.

Choice of extraction solvent—Acetic acid was chosen as the extraction solvent since it is unaffected by the oxidising agent and is not sufficiently volatile to prevent its convenient use in volumetric manipulations. The glacial acid completely extracts diphenylamine from nitrocellulose powders, but a considerable proportion of the nitrocellulose itself is also dissolved, to be reprecipitated on addition of the reagent. By diluting to 90 per cent. w/w, and using the tall Spekker cells, this objection is overcome, and the extraction, though slower, remains efficient.

Reproducibility—Table I illustrates the reproducibility obtainable with this method. Sixteen determinations were carried out on the same sample, the weights taken being varied slightly, and unknown to the two operators concerned.

TABLE I

REPRODUCIBILITY OF COLORIMETRIC METHOD

Analyst A. ..	1·02	1·01	1·03	1·03	1·03	1·03	1·03	1·03	} diphenylamine %.
Analyst B. ..	1·04	1·02	1·03	1·04	1·04	1·04	1·04	1·04	

Effect of the presence of other ingredients.—The presence of dibutyl phthalate and dinitrotoluene in the proportions existing in modern non-hygroscopic powders has no measurable influence upon the intensity of the colour developed.

Effect of the presence of transformation products of diphenylamine—Some nitro- and nitroso-derivatives of diphenylamine are present in all powders stabilised by diphenylamine and some of these compounds give colour reactions when treated by the prescribed method. In each case the intensity of the colour (as measured with use of the Ilford 606 filters) was found to be directly proportional to concentration. This colour may therefore be expressed as an "extinction per milligram," E_C, where

$$E_C = \frac{\text{drum difference reading}}{\text{weight of compound in mg.}}$$

For the present purpose it is more convenient to use a factor E_D, defined by

$$E_D = E_C \times \frac{\text{Mol. wt. of compound}}{\text{Mol wt. of diphenylamine.}}$$

E_D is thus the "extinction per milligram of equivalent diphenylamine."

Comparison of the factor, E_D, with that for diphenylamine shows to what extent each compound interferes in the colorimetric estimation of the latter. The values obtained by experiment are presented in Table II.

TABLE II

EFFECT OF TRANSFORMATION PRODUCTS OF DIPHENYLAMINE ON THE COLORIMETRIC MEASUREMENT

Compound	Colour produced	E_C	E_D	$\frac{E_D \text{ for compound}}{E_D \text{ for diphenylamine}} \times 100$
Diphenylamine (DPA)	Blue	8·65	8·65	100
N-Nitroso-DPA	Blue	6·3	7·4	85·5
4-Nitro-DPA	Wine red	4·7	5·95	69
N-Nitroso-4-nitro-DPA	Wine red	3·0	4·3	49·5
2-Nitro-DPA	Purple	2·3	2·9	33·5
N-Nitroso-2-nitro-DPA	Orange-red	1·0	1·45	17
More highly nitrated products.	No measurable colour with Ilford Filter No. 606	nil	nil	nil

THE MEANING AND EVALUATION OF "ACTIVE" DIPHENYLAMINE

The determination of diphenylamine in nitrocellulose powders by the methods of Ellington[1] and of Cook[3] results in a figure that is representative not only of the true diphenylamine present in the powder, but also of nearly all of the N-nitrosodiphenylamine. This is

fortunate, since N-nitrosodiphenylamine is almost as effective a stabiliser for nitrocellulose as diphenylamine, and hence the determination has long come to be regarded as a measure of the effective stabiliser, that is, "active" diphenylamine, present (or, in aged powders, remaining) in the powder. This measure is an approximation, since the mononitration products of diphenylamine and of N-nitrosodiphenylamine, which are formed during the ageing of these powders, also possess slight stabilising properties.[6]

By the present colorimetric method, the N-nitrosodiphenylamine contributes only 85·5 per cent. of its equivalent amount of diphenylamine to the final result, a proportion somewhat lower than that characteristic of the other methods. It is believed, however, that this figure bears a closer relation to the true relative stabilising powers of the two compounds, and this seems to be supported by the experiments of Becker and Hunold.[6] In addition, the mononitrated products of these two compounds contribute to smaller extents towards the colorimetric result (see Table II), which, therefore, recognises the proportionately lower stabilising power of these compounds. Hence it would appear that the colorimetric method gives the truest estimation of "active" diphenylamine.

Comparison of results

In a preliminary trial, 58 powders of various types were tested both by the present colorimetric method and by the gravimetric method of Cook. Determinations were made on the samples as taken from Service magazines and also after 350 hours' storage at 80° C. The means of all results can be summarised as follows:—

Colorimetric results	before hot storage	0·779 % diphenylamine.
	after hot storage	0·533 ,,
Gravimetric results	before hot storage	0·768 ,,
	after hot storage	0·531 ,,

The maximum systematic difference amounts therefore to only 0·01 per cent. (observed on the samples before storage). Statistical examination of the individual results indicates that for powders before hot storage, 95 per cent. of those obtained colorimetrically would be expected to fall within + 0·08 and − 0·06 per cent. of those obtained by the gravimetric method, whereas for powders after hot storage the corresponding range is ± 0·075 per cent.

As a result of a year's continual experience with the colorimetric method, it can be stated that the greater part of these errors arises from the gravimetric method, the results obtained by which are less reproducible.

In view of the remarks made on "active" diphenylamine evaluation it may seem surprising that the colorimetric and gravimetric results are so close. This is explained by the fact that, even after hot storage, the proportions of the nitrodiphenylamines are still very low. Examination of a number of powders of nominal diphenylamine content 1 per cent. has shown that after 350 hours' storage at 80° C. the N-nitrosodiphenylamine content may amount to 0·7 per cent. (calculated as diphenylamine), which would be recorded by the colorimetric method as about 0·6 per cent. In such powders the 4-nitrodiphenylamine and 2-nitrodiphenylamine are usually present to the extent of just over 0·1 per cent. each (also calculated as diphenylamine), the diphenylamine content having fallen to below 0·1 per cent., and other transformation products being present only in negligible quantities. These amounts of the 4-nitro- and 2-nitro-compounds will give by the colorimetric method figures of 0·07 and 0·03 per cent. diphenylamine respectively. Adding these colour contributions together it becomes clear how it happens in practice that even with hot-stored samples the colorimetric results agree closely with those obtained by the gravimetric method.

Conclusion

The colorimetric method described in this paper is considered to possess the following advantages over the previously employed methods of determining diphenylamine.

(i) It eliminates the laborious operation of steam distillation, and results in a saving of at least 60 per cent. in man-power.
(ii) It is quite as accurate as, and more reproducible than, the methods based on steam distillation.
(iii) It effects a saving of reagents.
(iv) There is a good indication that it gives a truer estimation of the "active" diphenylamine.

The work described in this paper was carried out in the Naval Ordnance Inspection Laboratory before its transfer from Holton Heath.

Thanks are due to the Admiralty for permission to publish this paper.

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Notes

A RAPID METHOD FOR THE CHEMICAL ESTIMATION OF PENICILLIN

An estimation of penicillin, based on the formation of an acidic group on treatment with hydrogen peroxide has been developed in these laboratories. It has subsequently been found that a similar method has been published in a C.P.S. Report.[1] The procedure given below is however more rapid and easier to operate.

Experimental

By analogy with the penicillinase method, the first procedure adopted was to adjust a solution of hydrogen peroxide to *p*H 8·00, using a *p*H meter, and then to add it to the penicillin solution, also previously adjusted to *p*H 8·00. It was found that there was a fall in *p*H owing to the production of an acid group. Sodium hydroxide was added to bring the *p*H back to 8·00 and the end of the reaction was shown by the *p*H remaining constant. The volume of sodium hydroxide used was a direct measure of the amount of penicillin originally present. The reaction was found to take about 20 mins. under these conditions.

It was noted that the rate of reaction was markedly dependent on the *p*H of the solution, which had to be kept above 7·5 at least. Some quantitative measurements on the effect of *p*H were made.

Hydrogen peroxide was added to a penicillin solution and a stop-watch started. The *p*H of this mixture was kept at a given value by the frequent addition of sodium hydroxide from a burette. A graph of burette readings against the corresponding time was plotted. The slope of this graph at zero time was taken as a measure of the rate of reaction. A graph of the rate of reaction, measured in this way, against *p*H is shown in Fig. 1.

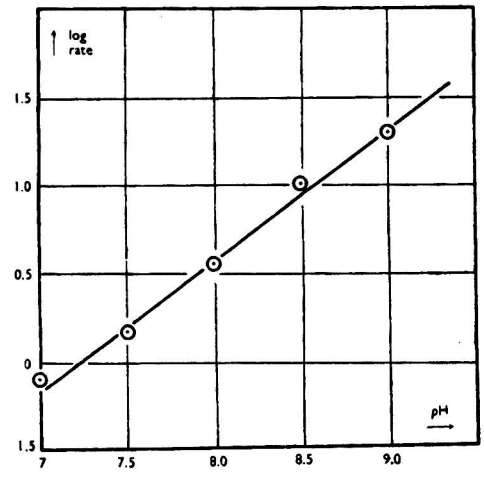

Fig. 1. Variation of initial rate of reaction with *p*H.

It is obvious from Fig. 1 that there is a considerable advantage in speed of working to be obtained by using a high *p*H. The method finally adopted was to add an excess of 0·05 *N* alkali together with hydrogen peroxide. Reaction was complete in a few seconds and the alkali remaining was titrated back. Under these conditions, direct hydrolysis of the penicillin by the alkali was found to be negligible and in any event would not greatly affect the estimation, as it would also release a carboxyl group.

It was found that the use of a *p*H meter was not necessary for these titrations; phenolphthalein or phenol red could be used as indicator. The colour change at the end-point was easily seen even in strongly yellow solutions. Hydrogen peroxide from different sources was used without any difference in action being found.

Method

Reagents—

Sodium hydroxide, approximately 0·05 *N*.

Hydrochloric acid, accurately 0·05 *N*.

Hydrogen peroxide, 100-volume solution diluted 10-fold. The *p*H of the reagent must be above 4.

Procedure—

Take a 10-ml. aliquot, containing about 30 to 60 mg. of penicillin (50,000 to 100,000 Oxford units), add the indicator and adjust to the end-point. Add 5·00 ml. of 0·05 *N* sodium hydroxide, 10 ml. of water and 5 ml. of the 10-volume hydrogen peroxide solution. Titrate back the excess of sodium hydroxide with 0·05 *N* acid from a micro-burette.

A blank titration on the peroxide need only be done periodically. Proceed as above, using an extra 10 ml. of water in place of the penicillin solution. The volume of 0·05 *N* acid used in the back-titration of the blank, minus that used in the back-titration of the sample, is equivalent to the penicillin.

One ml. of 0·05 *N* acid ≡ 17·8 mg. of sodium penicillin G, *i.e.*, 29,600 Oxford units.

This procedure can only be used on solutions of low buffering capacity.

Results—

This method has been compared with the iodimetric and penicillinase methods on a variety of samples. Typical results are shown in Table I. As will be seen, the agreement is satisfactory. It was thought that, with impure samples, oxidation of the impurities to acidic materials might occur and yield high results. Samples 1 to 5 were of comparatively low potency but the results are not significantly higher than those of the purer samples. The cation of the penicillin salt appears to have no influence on the analysis.

In speed of working, the hydrogen peroxide method is considerably quicker than the iodimetric method. The time of each analysis is about 5 minutes, apart from the blank. One limitation of the method is that, like the penicillinase method, it cannot be applied to buffered solutions.

With the above procedure, the limit of sensitivity is about 2000 units/ml.

Table I

No.	Penicillin salt	% Purity from iodimetric estimation	Hydrogen peroxide mg./ml.	Iodimetric mg./ml.	Penicillinase mg./ml.
1	Sodium	50	3·50	3·53	3·41
2	Sodium	47	1·15	1·17	1·13
3	Sodium	68	1·61	1·58	—
4	Calcium	44	1·78	1·77	—
5	Calcium	44	2·12	2·07	—
6	Calcium	95	1·90	2·01	—
7	N-ethyl piperidine ..	82	1·16	1·16	—
8	Ammonium	95	1·43	1·39	—
9	Potassium	87	2·60	2·58	2·57

Thanks are due to the Directors of the Distillers Company Limited for permission to publish this work.

Reference

1. C.P.S. Report, No. 602 by C. Pfizer and Co., Inc.

The Distillers Company Limited
Research and Development Department,
Great Burgh, Epsom

W. S. Wise
G. H. Twigg

October, 1947

THE DETECTION OF HYDROCYANIC ACID IN DECOMPOSED VISCERA

Hydrocyanic acid is difficult to detect in decomposed viscera. Chelle[1] states that it is converted into thiocyanic acid by the sulphur compounds formed during putrefaction. Thiocyanic acid is not formed in the ordinary process of putrefaction. Hydrocyanic acid may be recovered from the thiocyanic acid formed by treating it with an oxidising agent.[1] Magnin[2] states that in twelve cases of poisoning by hydrocyanic acid the viscera were examined after periods varying from 3 to 6 years by both (1) direct distillation and (2) Chelle's method; positive results were obtained with Chelle's method in every case, whilst the direct method gave negative results. The present investigation was undertaken to find out whether the conversion of hydrocyanic acid into thiocyanic acid in decomposed viscera is quantitative.

A test-paper modification of the Prussian blue test[3] was used to confirm the presence of hydrocyanic acid. The test renders distillation unnecessary. It consists in adding to one end of a strip of filter-paper a drop of 10 per cent. ferrous sulphate solution followed by a drop of 10 per cent. sodium hydroxide solution and suspending the paper in the flask containing the suspected material for 10 minutes. The paper is then

removed and immersed in hot dilute hydrochloric acid, whereupon a blue stain appears on the paper if hydrocyanic acid is present in the suspected material. The method is capable of detecting small amounts.

Experiments—Eighty g. of beef liver and 25 ml. of water were placed in each of nine air-tight bottles. To five of these were added 10 ml. of cyanide solution containing 9·7 mg. of potassium cyanide. To the remaining four bottles no cyanide was added—they served as controls. The bottles were allowed to stand for varying lengths of time and the contents were then steam-distilled, the distillation being carried out in two stages. (1) Direct distillation—the contents of the bottle were transferred to a distillation flask with about 50 ml. of water and acidified with about 40 ml. of dilute sulphuric acid and steam-distilled. (2) Distillation with dichromate—to the residue in the flask after the direct distillation, were added 65 ml. of 25 per cent. w/v sulphuric acid containing 10 g. of dissolved potassium dichromate and the material was steam-distilled again. Distillates from putrid viscera contain sulphides. These were precipitated by shaking with a little solid lead carbonate.[4] After filtration the hydrocyanic acid in an aliquot portion of the filtrate was determined by the alkaline titration method.

The results obtained are given below:—

Age of viscera, days	KCN added, mg.	HCN by direct method	HCN by Chelle's method ; in mg. KCN
6	9·7	nil	6·1
48	9·7	"	positive*
70	9·7	"	positive*
106	9·7	"	3·1
107	9·7	"	3·0
20	nil	"	nil
48	"	"	"
108	"	"	"
108	"	"	"

* The hydrocyanic acid was not estimated but tested for qualitatively by the modified Prussian blue test.

These results are in agreement with Magnin's observation that the conversion of hydrocyanic acid to thiocyanic acid is not quantitative.

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May, 1948

NOTE ON THE PREPARATION AND TESTING OF HYDROCARBON SOLVENTS FOR ABSORPTION SPECTROSCOPY

A NOTE by Ashmore recently published in this journal[1] described the removal of benzene from cyclohexane by passing the cyclohexane through a column of silica gel. The writer would like to direct attention to several examples of the use of this procedure for the removal of unsaturated and aromatic impurities from cyclohexane and other cycloparaffin or paraffin hydrocarbons.[2–5]

When a hydrocarbon solvent is to be used for absorption spectroscopy, its quality can be determined very conveniently spectrographically[4] or spectrophotometrically[5] with pure water as the reference solvent.

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NATIONAL BUREAU OF STANDARDS
WASHINGTON 25, D.C.

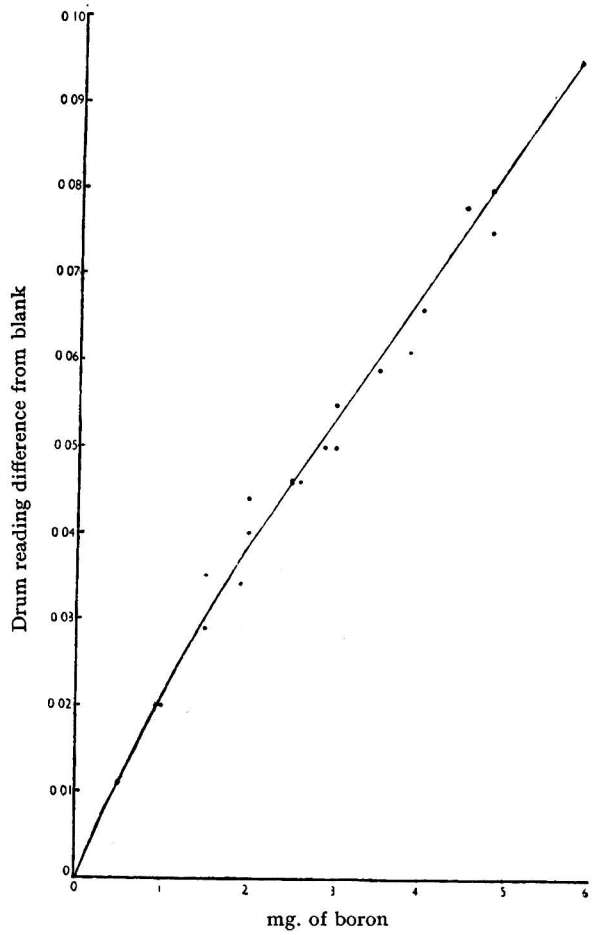
MARION MACLEAN DAVIS

September, 1947

ABSORPTIOMETRIC DETERMINATION OF BORON WITH SODIUM ALIZARIN-SULPHONATE

THE visual colorimetric method for the determination of quantities of boron up to 5×10^{-6} g. as previously described[1] has been adapted for use with the Spekker photo-electric absorptiometer. The procedure that appears to be most suitable is as follows. To calibrate the instrument, prepare a solution of sodium alizarin-sulphonate in concentrated sulphuric acid containing 0·04 g. of alizarin-S per litre and not less than 98 per cent. w/w of sulphuric acid. It is convenient to prepare this solution in quantities of 2 litres and to store it in a Winchester bottle fitted with a siphon and air-drying device similar to that described by Owen.[2] A series of standard solutions of boric acid containing up to 5 μg. of boron per ml. is also required. To 1 ml. of standard solution add 9 ml. of reagent solution, mix in a stoppered tube, cool and allow to stand for 30 min. Set the absorptiometer with distilled water on both sides, with the drum reading 0·8, and using blue-green filters (Ilford No. 603). Note the drum reading with each standard in turn in position, using 1-cm.

glass cells. A blank containing no added boron must be included in the series, and the setting of the instrument must be checked immediately before each standard is measured. Subtract the "blank" drum-reading—which should be about 0·15—from each standard reading and plot the differences against the boron contents. A calibration curve thus obtained is shown in the figure. It has much the same slope as those given by Owen[2] for quinalizarin, from which it appears that both reagents are of the same order of sensitivity; sodium alizarin-sulphonate is, however, much better suited for visual measurements than is quinalizarin.



This method of calibration is satisfactory, but there are variations in the actual drum readings for different series of standards for which no explanation can be offered. If, therefore, actual drum readings, uncorrected for the blank, are plotted against boron contents, calibrations carried out in this way on different occasions may give a series of parallel lines, even if the same batch of alizarin-S reagent is used throughout and all practicable precautions are taken to exclude moisture.

Sufficiently heavy traces of boron to impair the sensitivity of the method sometimes occur in sulphuric acid. Acid for use with this method should therefore be tested for freedom from boron (the blank determination will indicate this). Nitrate, which interferes, may also be present in the sulphuric acid. It is advisable to test for this radical by, for example, the brucine method; nothing more than a pale straw colour should be produced in this test if the acid is to be acceptably free from nitrate.

The work on which these recommendations are based was done at the University of Bristol Research Station, Campden.

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RESEARCH DEPARTMENT
SAML. HANSON & SON LTD.
TODDINGTON, GLOS.

DENIS DICKINSON
June, 1947

Medical Research Council

THE BACTERIOLOGY OF SPRAY-DRIED EGG WITH PARTICULAR REFERENCE TO FOOD POISONING

Medical Research Council Special Report, Series No. 260, 1947. By various Authors.* Price 1s.

THIS report contains a record of the bacteriological and epidemiological investigations carried out on imported spray-dried egg of American origin. This article of food was distributed to bakers, confectioners and caterers in the early years of the war but not until July, 1942, was there retail distribution to housewives and this lasted until January, 1946.

Between August 1942 and August 1945, about 9000 routine samples were examined at the laboratory of the Ministry of Food, at 13 laboratories of the Emergency Public Laboratory Service, and at Aberdeen University. The predominant bacteria in dried egg were found to be enterococci and aerobic spore-bearers. Coliform organisms were generally present in small numbers. Clostridia were present in 17 per cent. of samples, staphylococcus aureus (coagulase positive) in 3 to 4 per cent., and haemolytic streptococci, mostly group D, *i.e.*, enterococci, in 3 per cent.

Out of 7584 samples examined for Salmonella bacteria 9·9 per cent. were found to contain them. These were usually present only in very small numbers and never more than 30 per g. For this reason they were missed in many of the routine samples, when only 10 g. were taken, and in a side investigation when whole packets (5 oz.) were examined the percentage went up to 55 per cent.

Circumstantial evidence is brought to show that salmonella infection of dried egg was responsible for several outbreaks and large numbers of sporadic cases of food poisoning in persons consuming it. This may be summarised as follows:—

(*a*) From 1923 to 1939 the total number of outbreaks found to be due to salmonella was 428. In 1940 there was a sharp rise (thought to be due to communal feeding). In 1943 a sudden rise occurred and in 1944 the number of outbreaks reached a total of 450—greater than that of 17 pre-war years.

(*b*) Between 1923 and 1939 the strains of salmonella isolated from the 428 outbreaks belonged to 14 different species, many of which were indigenous in Great Britain and Ireland. In 1942, ten new species appeared, in 1943 eight further new species, and in 1944, six further new species. Of these new species the six commonest in order were oranienburg, montevideo, meleagridis, anatum, tennessee and bareilly—the same six, in fact, that were found to be commonest in the salomella strains isolated from the American dried egg samples.

(*c*) Retail distribution of dried egg began in July, 1942, and in the second half of 1942 22 strains of the ten new species were cultivated from cases of food poisoning out of a total of 25 for that year.

Taking (*a*), (*b*), and (*c*) together it is concluded that the great increase in the number of outbreaks of food poisoning was, almost certainly, due to infection conveyed by imported egg powder.

For various reasons direct evidence incriminating dried egg as the source of food poisoning was difficult to obtain. The article of food believed on subsequent investigation to have been responsible had always been completely consumed or destroyed. Nevertheless, a few outbreaks are recorded in which there was little doubt that the Salmonella isolated from the excreta of the patients was derived from dried egg. The following is particularly worthy of note. Within a period of four weeks in 1943, five strains of Salmonella from the excreta of five human cases of food poisoning, that occurred in five different public health districts in Lancashire and Cheshire, proved to be Salm. sundsvall, a species hitherto unknown in this country as a cause of illness and originally identified in Sweden. It was, however, isolated from a routine sample of American egg powder at about the time of the outbreak, showing that egg powder carrying this particular species was then in circulation. Salm. sundsvall has not been isolated before or since from any other article of food except dried egg. Investigation traced these widely separated cases to a common source, *viz.*, a trifle manufactured by X. and sold in 22 shops owned by X. and perhaps as many as 100 shops of other retailers. This outbreak forcibly illustrated the enormous value of the identification of Salmonella strains carried out at the Salmonella Reference Laboratory, Oxford, by Dr. Joan Taylor.

Numerous experiments on the effect of cooking on dried egg are reported. These showed that scrambled egg, omelette, and custard prepared from recently reconstituted dried egg were free from Salmonellae, but if the reconstituted egg was allowed to stand so as to allow bacterial multiplication, the normal cooking processes could not be relied upon to kill all the Salmonellae.

It is probable that this fault in technique was responsible for many outbreaks. In many cases matters were made very much worse by serving up again for tea what was left at breakfast, or for breakfast what was left the evening before.

In addition, investigations of food poisoning outbreaks of the toxin type with incubation periods of 2 to 6 hours and passing off within 24 hours are recorded, in which there was a strong suggestion that Salm. meleagridis was the cause, but these, though very interesting, were considered inconclusive.

D. R. WOOD

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* Including Professor G. S. Wilson, Dr. R. B. Haines, Dr. E. M. L. Elliott, Dr. Joan Taylor, Dr. R. L. Vollum, Dr. A. Q. Wells, Dr. W. H. Bradley, Lieut.-Col. E. C. G. Maddock, Dr. W. H. Brodie, Dr. J. Fanning, Dr. R. F. Edbrooke, Dr. V. Freeman, Dr. W. E. Hoffmann, Dr. M. Manson, Dr. J. Macmillan, Dr. W. Smith, and Dr. Elizabeth Topley.

The Tin Research Institute

THE SPECTROSCOPIC ANALYSIS OF TIN AND TIN - LEAD ALLOYS*

THIS new booklet by D. M. Smith is a critical summary of present-day knowledge of the quantitative spectroscopy of tin and tin – lead solders, part of which has not previously been published. It is designed to supply a reader with sufficient information on optical apparatus, electrical equipment and methods of analysis to enable him to select that most suited to a particular analytical problem. The subject-matter includes descriptions of the D.C. and intermittent A.C. arc and condensed spark methods of excitation, plate processing and a full discussion of the various methods that have been proposed and used for the quantitative interpretation of spectra. The procedures described for the analysis of tin and solder are detailed and are complete with analytical tables and plates showing the spectra obtained. A classified bibliography covers the period from 1922 to 1947. F. L. O.

British Standards Institution

CLEANLINESS OF FILLINGS AND STUFFING FOR BEDDING, ETC.

WE have been asked to give prominence to the following British Standard:

B.S. 1425: 1948. The Cleanliness of Fillings and Stuffing for Bedding, Upholstery, Perambulators, Cushions, Tea Cosies, Dolls and other domestic articles.†

The importance of clean bedding in relation to public health was acknowledged by the passing of the Rag Flock Act, 1911. This Act was intended to secure that all rags used in the manufacture of flock are adequately cleansed before being converted into flock for use in bedding, upholstery and cushions, but it has long been recognised that the chlorine test prescribed by the Rag Flock Regulations, 1912, does not achieve this object, and that the cleanliness of materials other than rag flock intended to be used for filling bedding, articles of furniture, toys, etc., should also be ensured.

Several years before the war the National Federation of Bedding and Allied Trades began exploring the possibility of setting up reasonable commercial standards of cleanliness for all types of fillings and stuffings, which would raise the standard of these materials and be accepted voluntarily by all sections of the bedding, upholstery and filling manufacturing trades. Much experimental work was carried out on behalf of the Federation, and in 1938 the British Standards Institution was asked to take over this investigation. Upon the outbreak of war the work had to be suspended, but it was restarted in 1943 at the request of the Amalgamated Union of Upholsterers.

A representative Technical Committee was set up, with Sub-Committees to deal with each type of filling and a panel of analysts and technologists to advise upon the various tests suggested. After careful consideration of the many reports of the work carried out by the National Federation of Bedding and others, and after discussions with textile analysts and all branches of the trades concerned, the following conclusions were reached.

(*a*) All filling materials, but in particular rag flocks, vary considerably in character and composition. For this reason chemical tests involving the determination of free and albuminoid ammonia, oxygen absorbed, chlorides, phosphates, etc., are by themselves unreliable and unsatisfactory.

(*b*) Bacteriological standards would be difficult if not impossible to apply in practice.

(*c*) The main consideration is whether the material is clean or dirty in the sense in which the housewife regards it as clean or dirty, and some form of washing test, precisely specified, that in practice gives concordant reproducible figures as a measure of the dirt extracted, is the best index of cleanliness.

(*d*) A washing test could not, of itself, effectively control the cleanliness of fillings because small proportions of excrement, sputum, etc., necessarily contribute but inappreciably to the matter extracted, and it was therefore essential to lay down the method by which each material must be cleaned.

On this basis the work proceeded. A washing test using water without a detergent proved unsatisfactory, since oil and grease prevented the material being thoroughly wetted, and the Fenton test, in which a detergent solution of ammonia, methyl alcohol, acetone and water is used, was finally adopted.

The Standard now issued contains twelve sections, as listed in the accompanying table.

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* Gratis from the Tin Research Institute, Fraser Road, Greenford, Middlesex.

† Obtainable from the Publications Department, British Standards Institution, 24–28, Victoria Street, London, S.W.1. Price 3s. net, post free.

Each specification details the cleaning operations in the process of manufacture, and a modified and standardised Fenton test, designated the "Cleanliness Test" (see this vol. p. 418), is used to assess the cleanliness of washed flock, jute wadding and jute flock, hair, fibre and hair-fibre mixtures, feathers and down, and of fillings falling in section 12. The limits fixed for these materials are considered to be such that fillings less clean than the best grades as marketed in 1939 will be eliminated.

For the other materials covered by this Standard the Fenton test is not satisfactory because the detergent solution dissolves appreciable amounts of some of their natural constituents. Cotton and kapok fillings when new are derived from raw materials that are inherently clean. For these, and for woollen flock mixtures, the trash content (seed, sand, etc.) as determined by the Shirley Analyser is adopted as the criterion of cleanliness. Washing is prescribed for used or second-hand cotton and kapok fillings, with an Analyser test on the washed material, and used or second-hand woollen flock is required to be boiled and dyed black, but for the cleanliness of these re-conditioned products, and also of woollen flock and woollen-flock mixtures, the processing requirement is relied upon.

Supplementary determinations of chlorine in water-soluble chlorides and/or of oil and soap are specified for certain articles.

Except for trash content, all results are to be calculated on the dry weight of the material. The trash content is based on the weight of the material after being conditioned for not less than 2 hours in a room maintained at a temperature not below 60° F. and at a humidity of 40 to 70 per cent. and these conditions must be maintained throughout the test.

The maximum limits permitted for the tests are set out in the accompanying table.

Article	Cleanliness test			Oil and soap %	Chlorine as soluble chlorides parts per 100,000	Trash %
	Soluble matter %	Insoluble matter %	Total impurities %			
1. Washed flock	1·5	—	—	5	30	—
2. Jute wadding and jute flock	1·5	—	—	5	30	—
3. Woollen flock (not less than 70 per cent. wool)	—	—	—	4	—	—
4. Woollen flock mixtures	—	—	—	4	—	15
5. Cotton flock and cotton millpuffs	—	—	—	Free from added oil	—	15
6. Cotton felt	—	—	—	Free from added oil	—	7½
7. Kapok	—	—	—	—	—	5
8. Kapok mixtures (Kapok/cotton; Kapok/Akund)	—	—	—	—	—	7½
9. Hair	—	—	1·0	—	—	—
10. Fibre and hair-fibre mixtures:						
Algerian fibre	—	0·5	4	—	—	—
Coir, undyed	—	—	1·25	—	—	—
Coir, dyed	—	—	1·5	—	—	—
Sisal	—	0·5	4	—	—	—
Mexican fibre (dyed and undyed)	—	0·5	4	—	—	—
Hair-fibre mixtures	—	0·5	2·5	—	—	—
11. Feathers and down	—	—	2·0	—	—	—
12. Other fillings	1·0	—	—	—	—	—

The methods of analysis, including the determination of wool in woollen flock, and the procedure to be adopted in sampling are detailed in appendixes. In the cleanliness test and the determination of trash the figure to be reported is the mean of the results obtained from three separate samples drawn from a gross sample.

Each specification requires that every bale or package of filling purporting to comply with this Standard shall be labelled, and the label must bear the name of the article (when made from used or second-hand material the name must be preceded by the word "re-conditioned"), the name or trade mark of the supplier and the registered certification mark of the B.S.I. Manufacturers will only be licensed to use this mark if the B.S.I. Mark Committee is satisfied, after inspection of the plant by an independent technical expert appointed by this Committee, that the applicant is equipped with plant capable of producing materials to this Standard and that he has in operation a method of production control that will enable him to comply with the specifications. Provision is made for periodical inspection and check sampling.

This scheme of standard specifications, licensing of manufacturers and certification marking of fillings, which has been developed by the trade through the medium of the B.S.I. and accepted generally by both users and manufacturers, allows of review and change where necessary, both to keep abreast of changes in process, plant, etc., as progress dictates and to provide for new fillings that may be brought into use.

DRAFT SPECIFICATIONS

A FEW copies of the following draft specifications, issued for comment only, are available to interested members of the Society and may be obtained on application to the Secretary, J. H. Lane, 7–8, Idol Lane, London, E.C.3.

Technical Committee OSC/24—British National Committee on Soaps and Fats, of the International Commission for the Study of Fats.

CJ(OSC)5111—Second Draft British Standard for Melting Point of Commercial Fats.

CJ(OSC)7177—Draft of Methods for the Analysis of Soaps.

Sub-Committee RDE/3/8—Testing of Road Mixtures.

CJ(RDE)7531—Methods for the Sampling and Examination of Bituminous Road Mixtures (Revision of B.S. 598).

Technical Committee LBC/4—Thermometers.

CJ(LBC)7621—Proposed Draft for Short-Range Short-Stem Thermometers.

ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

Food and Drugs

Polarographic Determination of Santonin. F. Sǎntavý (*Coll. Czech. Chem. Comm.*, **1947, 12**, 422–428)—Santonin and santonic acid give rise to well-defined polarographic reduction waves, which can be used for analytical purposes, lithium chloride or sulphate being used as supporting electrolyte. The wave-height is proportional to santonin concentration in solutions containing less than 20 mg. in 100 ml. The polarographic method is suitable for the determination of santonin in *Flores cinae*, or in tablets.

Procedure for Flores cinae—Extract 1·5 g. of the finely ground flowers with chloroform for 8 hr. in a small Soxhlet apparatus. Remove the chloroform by distillation and dissolve the residue in 50 ml. of ethyl alcohol in a volumetric flask. Add 1 ml. of this solution to 2 ml. of aqueous 0·2 *N* lithium sulphate in a polarographic cell, and remove dissolved oxygen by bubbling nitrogen through the solution. If any solid separates, filter the solution and pass more nitrogen through the liquid before recording a polarogram. The santonin content is calculated from a calibration curve.

Procedure for tablets—Santonin mixed with phenolphthalein, saccharose, lactose, or cocoa can be determined by this procedure. Weigh out 0·1 to 0·3 g. of the powder, or powdered tablet, add 10 ml. of 96 per cent. ethyl alcohol, and allow the mixture to stand for about 5 hr. with occasional shaking. Pipette 1 ml. of the supernatant liquid into a polarographic cell containing 2 ml. of 0·1 *N* lithium sulphate, and examine as before. If the solution is cloudy it should be filtered, but the milky turbidity obtained from samples containing cocoa does not interfere with the determination.

J. G. WALLER

Determination of Bismuth in Organic Bismuth Compounds. M. Langejan and J. A. C. van Pinxteren (*Pharm. Weekblad*, 1947, **82**, 651–657)—*Procedure—Bismuth tribromophenol*—Boil 1 g. of the compound gently for 10 min. with 15 ml. of water and 5 ml. of sodium hydroxide solution, add 30 ml. of water, and filter through a 5·5-cm. paper, washing the precipitate with warm water until the filtrate no longer reacts alkaline. Dissolve the precipitate in 10 ml. of dilute nitric acid, heat the solution to the boiling-point, and add a warm solution of 2 g. of ammonium phosphate in 30 ml. of water. After boiling for 5 min., filter and wash the precipitate with 100 ml. of warm water, and then with alcohol and ether. Dry the filter and precipitate, ignite it, and weigh the residue. The weight of bismuth phosphate multiplied by 0·6875 gives the amount of bismuth in 1 g. *Bismuth tetrabromopyrocatechol*—Boil 0·5 g. of the compound with 5 ml. of nitric acid until a light yellow solution is obtained, and add to this a warm solution of 1 g. of ammonium phosphate in 15 ml. of water. After boiling for 5 min. (frequent agitation is necessary to prevent bumping), filter, and wash and ignite the precipitate as described above.

G. MIDDLETON

Note on Paraldehyde. A. Brester (*Pharm. Weekblad*, 1948, **83**, 9–10)—For detecting peroxide in paraldehyde, the Dutch Pharmacopoeia uses a test with permanganate, which also responds to acetaldehyde. The results are unsatisfactory, apparently owing to the sulphuric acid in the test disturbing the equilibrium between paraldehyde and acetaldehyde. The test should be replaced by the potassium iodide test for peroxide of the Swiss Pharmacopoeia (Ed. V.), and the hydroxylamine method for acetaldehyde of the United States Pharmacopoeia (Ed. XIII).

G. MIDDLETON

Antimony Trichloride—Ethanol Precipitation for the Fluorimetric Determination of Riboflavine in Pork. J. R. Lewis and P. R. Gorham (*Canad. J. Res.*, 1947, **25, F**, 133–140)—A proposed study of the effect of various curing methods on the retention of several of the B-complex vitamins in pork muscle required a rapid riboflavine assay that would give results in good agreement with those of the slower micro-biological methods. The following procedure, which uses a portion of the papain - takadiastase extract used for assaying other members of the B-complex, was developed.

Procedure—To 5 to 15 g. of minced sample in a 250-ml. centrifuge tube add 100 ml. of acetate buffer at *p*H 4·6 containing 0·4 per cent. of a mixture of equal parts of papain and takadiastase, digest

at 37° C. with constant agitation for 16 hr., filter through Whatman No. 12 paper, adjust the *p*H of the filtrate to 6·8 with 4 to 5 ml. of *N* sodium hydroxide, and dilute to 200 ml. To a 25-ml. aliquot of this extract in a 100-ml. centrifuge tube add from automatic burettes, with constant swirling, 1 ml. of 1 per cent. antimony chloride in absolute ethanol and 24 ml. of 95 per cent. ethanol, stopper the tube and, after 15 min., centrifuge at 2000 r.p.m. for 5 min.

Determine the fluorescence of a 10-ml. aliquot of the supernatant liquid with a Coleman No. 12 photo-fluorimeter, using a 0·1 μg. per ml. sodium fluorescein solution to set the instrument at a reading of 50. Maintain all samples at room temperature when determining the fluorescence. For the blank correction add 0·25 ml. of a freshly prepared, ice-cold solution containing 2 g. of sodium dithionite ($Na_2S_2O_4.2H_2O$) and 0·7 g. of sodium bicarbonate in 20 ml. of water. Mix thoroughly, determine the residual fluorescence, and subtract this reading from that initially obtained. From a curve showing the relation between the concentration of riboflavine (μg. per ml.) in a 0·02 per cent. solution of antimony trichloride in 47·5 per cent. ethanol and the fluorimeter readings, ascertain the concentration of riboflavine in the sample solution and multiply by the dilution factor to obtain the amount in the sample.

The blank correction must be obtained with as little change in hydrogen ion concentration as possible. The use of strong alkali to increase the *p*H to 11 may destroy most of the fluorescence of riboflavine, may affect the fluorescence of the non-riboflavine material being assessed, and, with pork extract, causes turbidity that affects the precision of measurement. Decomposition of sodium dithionite in water with production of acid diminishes the fluorescence of non-riboflavine material in pork extract, but the recommended solution is stabilised somewhat by the sodium bicarbonate; it has a *p*H of 6·55, and the addition of 0·25 ml. to 10 ml. of extract at *p*H 6·8 does not appreciably alter the hydrogen ion concentration. Although riboflavine in antimony trichloride - ethanol solution appears to the eye to have its yellow-green fluorescence masked by the blue fluorescence of the solvent, the sharp cut-out of the filter transmitting fluorescent light (90 per cent. transmission at 540 mμ. to none at 510 mμ.) excludes measurement of the blue fluorescence.

As a rule, solvent precipitation as a means of removing interfering substances has proved untrustworthy because riboflavine may be adsorbed on the precipitate. This was confirmed by the low results obtained, in comparison with those of the micro-biological method, when ethanol and acetone were used for precipitation. With antimony trichloride and ethanol precipitation, however, good agreement with micro-biological assays was obtained, and also good recovery of added riboflavine. The presence of hydrochloric acid formed by interaction of antimony trichloride with water prevents adsorption of the riboflavine on the precipitate.

The good recoveries obtained with the use of an external reference curve show that antimony trichloride - ethanol does not alter the fluorescence of riboflavine. The external reference curve is so reproducible that routine calculations may be simplified by multiplying the corrected fluorimeter readings by the factor 0·005 to convert them to concentrations in μg. per ml. A. O. JONES

Biochemical

Chemical Method for the Estimation of Streptomycin in Blood and Spinal Fluid. G. E. Boxer and V. C. Jelinek (*J. Biol. Chem.*, 1947, **170**, 491–500)—The chemical methods of estimating streptomycin previously described have a lower limit of sensitivity of about 30 μg. of streptomycin. A new method for estimating streptomycin in blood at levels of 1 to 30 μg. per ml. is described. It depends on the formation of a hydrazone of streptomycin with 9*-hydrazinoacridine hydrochloride. The excess of reagent, together with the hydrazones of acidic, neutral and weakly basic compounds, is separated from the strongly basic streptomycin hydrazone by extraction from acid solution with benzyl alcohol. The streptomycin hydrazone is then estimated fluorimetrically.

Preparation of 9-hydrazinoacridine hydrochloride*—(German patent 364,031; see *Chem. Abstr.*, 1924, **18**, 1131)—Prepare 15 g. of 9*-chloroacridine from *N*-phenylanthranilic acid (*Organic Syntheses*, 1942, **22**, 6). Dissolve by heating in 400 ml. of 95 per cent. ethanol containing 30 ml. of 85 per cent. hydrazine hydrate. Heat under reflux for 6 hr. and then pour the mixture into 400 ml. of 6 *N* hydrochloric acid, stirring vigorously and cooling. Add 200 g. of cracked ice and refrigerate overnight. Wash the crude product three times with *N* hydrochloric acid, five times with a mixture (10 : 1) of ethanol and concentrated hydrochloric acid, three times with chloroform, and three times with ether saturated with concentrated hydrochloric acid. Crystallise the crude material, using half its weight of charcoal and 75 volumes of 50 per cent. ethanol. Wash the crystals with 50 per cent. ethanol and then with the ethanol - hydrochloric acid mixture until the washings remain straw-yellow. Wash finally with ether saturated with concentrated hydrochloric acid. Carry out one or two further recrystallisations with 90 to 100 volumes of 50 per cent. ethanol containing 1 ml. of concentrated hydrochloric acid per 100 ml. After each recrystallisation wash the product with the ethanol - acid mixture until the washings are straw-yellow in colour.

Prepare a solution of 9-hydrazinoacridine hydrochloride containing 5 mg. per ml. in a mixture of equal parts of water, methanol, and butanol. This reagent is stable for 1 week at 0° C.

Determination of streptomycin in aqueous solution and in spinal fluid—If the spinal fluid has a pathologically high protein content, first remove the

* The prefix 9- for these compounds accords with American practice; British nomenclature uses 5- for them.—*Editor.*

proteins by the procedure for blood given below. Take 14 ml. of a solution containing from 0·05 to 1·5 μg. of streptomycin per ml. and add 1 ml. of 3 *N* hydrochloric acid followed by 3 ml. of the 9-hydrazinoacridine reagent. Place in an oven at 37° ± 1° C. overnight for 16 hr. or, if a less accurate assay is sufficient, keep at 100° C. for 25 min. Extract the excess of hydrazine reagent and other acidic, neutral, or weakly basic hydrazones by means of a 10 : 1 mixture of benzyl alcohol and carbon tetrachloride (the latter facilitates phase separation). Extract first with 20 ml. of the benzyl alcohol mixture together with 2 ml. of concentrated hydrochloric acid (the concentrated hydrochloric acid is added separately because, when mixed with benzyl alcohol as one reagent, it forms fluorescent material on standing). Use 20 ml. of the benzyl alcohol mixture alone for the second extraction and then finally extract with 20 ml. of benzyl alcohol mixture and 2 ml. of concentrated hydrochloric acid.

Each extraction consists of shaking vigorously for 15 sec. Only one set of funnels is necessary, since the benzyl alcohol lower phase is discarded. The number of samples extracted at one time should be limited to four and the extractions should be rapidly completed in a dim light. The stoppers and mouths of the funnels must be well washed with the benzyl alcohol mixture as it is added for the second and third extractions. Cello-Seal, a lubricant containing only a small amount of fluorescent material, is used for the stop-cocks.

Centrifuge the water phase from the third extraction and pipette about 15 ml. of it into a 10- × 40-mm. cuvette. With the Pfaltz and Bauer fluorophotometer, use a 2-mm. Corning glass filter, No. 5113, in the path of the incident beam and a 1·5-mm. Corning filter, No. 3385, between the sample and the photo-cell. Standardise the fluorophotometer with a fluorescent glass block as used for the assay of riboflavine.

For calibration, weigh out two micro-samples of streptomycin trihydrochloride - calcium chloride double salt. Determine the moisture in one sample and use the other sample in the calibration. As a check, repeat the calibration on each new batch of 9-hydrazinoacridine hydrochloride.

Determination of streptomycin in blood samples—The above procedure is applicable after the removal of proteins. Take 1 ml. of plasma containing 1 to 30 μg. of streptomycin, dilute with 17 ml. of water, and add 1 ml. of 10 per cent. zinc sulphate solution followed by 1 ml. of 0·5 *N* sodium hydroxide. Take an aliquot containing not more than 20 μg. of streptomycin, if necessary diluting with water. Continue as above.

Eight per cent. of the streptomycin is lost by adsorption on the protein precipitate, and to correct for this the value from the calibration curve is divided by 0·92. No significant amounts of streptomycin are found in the erythrocytes and so, in ordinary circumstances, treat plasma or serum rather than whole blood. For maximal accuracy with streptomycin concentrations less than 5 μg. of plasma per ml., take a blood sample before streptomycin administration in order to obtain an accurate plasma blank. For normal use, the blank value for plasma or serum of different species (man, dog, cat, rat, and guinea-pig) is taken as constant.

In the above procedure, glucose, fructose, galactose, acetone, acetaldehyde, ethyl acetoacetate, pyruvic acid, salicylaldehyde, benzaldehyde, furfural, streptobiosamine, and *N*-methyl-L-glucosamine do not interfere. The reproducibility of the method is ±6 per cent. and the values obtained agree with the micro-biological assay and the chemical assay based on the formation of maltol.

W. S. WISE

Micro-determination of Manganese in Biological Materials by means of Catalysis. E. Kun (*J. Biol. Chem.*, 1947, **170**, 509–514)—The method is based on the catalysis by Mn^{++} ions of the oxidation of diethylaniline by potassium periodate to give a yellow dye. The speed of reaction, determined spectrophotometrically in buffered solutions, gives a measure of the Mn^{++} ion concentration with an accuracy to within about ±15 per cent. Only about 0·5 g. of sample is needed.

Reagents—(1) *Saturated diethylaniline solution*—Dilute 4 drops of diethylaniline base to 100 ml. with re-distilled water. Shake well and then allow to stand in the dark for 24 hr. Filter the solution immediately before use. (2) *Saturated potassium periodate solution*—Dissolve 3 g. of crystalline potassium periodate in 100 ml. of re-distilled water, then treat as for reagent (1). When a Beckman spectrophotometer is used, dilute reagents (1) and (2) five-fold. (3) *Standard manganese solution*—Use as a standard crystalline manganous sulphate dried for 3 hr. at 120° C.

Procedure—Wash all the vessels used with a mixture (1 : 1) of hydrochloric and nitric acids and then five times with re-distilled water. Into a platinum crucible measure a sample of 0·1 to 0·5 g. of tissue or 0·5 ml. of fluid. Add 10 drops of 0·1 *N* hydrochloric acid and ignite for 4 hr. at 850° C. in an electric oven. Cool and add 2 ml. of 0·1 *M* oxalic acid and 5 drops of 0·1 *N* hydrochloric acid. Keep at 80° to 90° C. for 20 min. to reduce the manganese to the manganous state, dilute to 100 ml. in a volumetric flask, take 2-ml. aliquots of this solution, and add to each 4 ml. of a phosphate - citrate - borate buffer of *p*H 7·0. Keep this mixture, the reagents, and the standard manganese solution at room temperature for 0·5 hr. Add 2 ml. of the diethylaniline solution and then 2 ml. of the periodate reagent and start a stop-watch immediately the latter reagent is added. Shake for 30 sec. and transfer to a photometer cell. Exactly 200 sec. after adding the periodate reagent measure the colour, using a spectrophotometer at 470 mμ., if available.

Prepare a calibration curve using the standard manganese solution. The range of the method is 0·0004 μg. to 0·04 μg. of Mn^{++} per ml. and the error ±15 per cent. Other heavy metals do not interfere in the above procedure. W. S. WISE

Colorimetric Procedure for the Determination of Small Quantities of Fatty Acid. W. R. Bloor (*J. Biol. Chem.*, 1947, **170**, 671–674)—The basis of the method is the well-known oxidation

of a fatty acid or cholesterol by a sulphuric acid - dichromate mixture. The colour change in the latter reagent is used for the estimation.

Dichromate reagent—Make separate solutions of 10 g. of silver nitrate and 10 g. of potassium dichromate. Mix the two solutions. Separate the precipitate by centrifuging, wash it with water, dissolve it in 500 ml. of concentrated sulphuric acid and keep the solution in a glass-stoppered bottle.

Procedure—Isolate the fatty acids by any of the usual procedures, then make a solution of about 0·1 mg. per ml. in either light petroleum or, better, a mixture (7 : 1) of light petroleum and chloroform. Place a 4 to 5 ml. aliquot in a 50-ml. beaker and add the same volume of pure solvent to another beaker. Evaporate the solvents by immersing the beakers in boiling water. Remove the beakers as soon as the solvent has evaporated to avoid volatilising or oxidising the residue. Add 3 ml. of dichromate reagent to each beaker and digest for 15 min. in a closed vessel immersed in boiling water. Then add 3 ml. of water, cool for 10 min., transfer to 5-cm. colorimeter cells, and take the reading within 10 min. using unfiltered white light. Prepare a calibration curve, using 1 to 8 ml. of a 0·1 mg. per ml. solution of palmitic, stearic, or oleic acid as standard. A separate calibration curve is necessary for cholesterol. The method is accurate to within ±5 per cent.

If no colorimeter cells over 2·5 cm. long are available, Duboscq colorimeter cups can be used. A simply constructed photo-electric colorimeter is described for use with these cells. W. S. WISE

Colorimetric Estimation of Small Quantities of Histamine. E. Havinga, L. Seekles, and T. Strengers (*Rec. Trav. Chim.*, 1947, **66**, 605–610) Many of the colorimetric methods of estimating histamine described in the literature are based on reactions in which it is coupled with diazo-compounds. These methods have been examined and found untrustworthy for amounts of histamine less than 5 μg. However, it is possible to estimate amounts of histamine down to 0·2 μg. by coupling it with diazosulphanilic acid (K. K. Koessler and M. T. Hanke, *J. Biol. Chem.*, 1919, **39**, 497) under controlled conditions.

Procedure—Make every solution with re-distilled water, free from traces of heavy metals, and cool each solution in ice before use. Add the reagents in the order given below.

Diazo-reagent—Place 1·5 ml. of a solution containing 9 g. of sulphanilic acid and 90 ml. of 37 per cent. hydrochloric acid per litre in a 50-ml. flask cooled in ice. Add 1·5 ml. of a 7 per cent. solution of sodium nitrite and, after 5 min., add 6 ml. more. Allow to stand for 5 min. and then dilute to 50 ml. with cooled, re-distilled water. Set aside the diazo-reagent for 15 min. before use.

Determination—Mix 1 ml. of the diazo-reagent with 0·5 ml. of the histamine solution (containing 0·2 to 5 μg. of histamine dihydrochloride). After 25 to 40 min., add 1·5 ml. of ice-cold, 1·83 per cent. sodium hydroxide solution. At 1-min. intervals measure the colour in a spectrophotometer at 500 mμ. against a blank. Read off the maximum colour, reached after several minutes. Beer's law is obeyed down to 1 μg. of histamine; below this, there are slight deviations. The method is accurate to within 3 per cent. on 1- to 5-μg. amounts of histamine and to within 6 to 7 per cent. on smaller amounts. W. S. WISE

Critical Considerations Regarding Nitrogen Determination by Kjeldahl Method in Amino-acids and Proteins. J. F. Reith and E. J. Wansink (*Chem. Weekblad*, 1948, **43**, 803–809)—The determination of nitrogen in histidine, lysine, and tryptophan, by the Kjeldahl method, is unsatisfactory when a selenium catalyst is used, as the results are always low. Satisfactory results are obtained with mercuric oxide as catalyst, provided that the heating is continued for a sufficient time after the mixture has become colourless. If this period is 30 min., then the error is not more than −0·6 per cent., which is sufficient for routine analyses; for accurate work, a longer period of heating is essential, and the exact time must be determined individually. G. MIDDLETON

Use of Grote's Reagent in the Determination of Methylthiouracil. E. C. M. J. Hollman and T. de Jonge (*Pharm. Weekblad*, 1948, **83**, 1–9)—For the determination of compounds of the type –CSH, Grote (*J. Biol. Chem.*, 1931, **93**, 25) used sodium nitroprusside at a *p*H of 8 to 9, at which reaction aldehydes and ketones do not interfere. If the nitroprusside solution is first exposed to light and filtered, it gives a blue coloration with thiourea derivatives and a red colour with –CSH compounds. As a substitute for exposing the solution to light, Grote gave the following formula: Dissolve 2 g. of sodium nitroprusside in 40 ml. of water in which are dissolved 2 g. of hydroxylamine hydrochloride; add, in successive portions, 4 g. of sodium bicarbonate and, after completion of the gas evolution, 0·1 ml. of bromine. Filter and dilute to 100 ml. This reagent can be kept for not more than a few days. It may be used for the colorimetric determination of a number of compounds, in particular, sulphapyridine, thiourea, thiouracil, and methylthiouracil. The reaction is carried out by mixing the reagent with the solution to be tested and allowing the mixture to stand, but it is important that the *p*H and time of action should be the same as for the standard solution. The method may be applied direct to blood serum and to urine without previous removal of proteins.
G. MIDDLETON

Determination of D(−)- and L(+)-Glutamic Acid in Protein Hydrolysates. M. S. Dunn, M. N. Camien, S. Shankman, and H. Block (*J. Biol. Chem.*, 1947, **168**, 43–49)—A microbiological technique is described whereby D(−)- and L(+)-glutamic acid can be estimated separately in mixtures by means of *Streptococcus faecalis* R (8043), which responds only to the L(+) isomeride, and any one of seven strains of lactic acid bacilli, *viz.*, *Leuconostoc dextranicum* (8086), *L. mesenteroides* (8293), *L. citrovorum* (797) and (7013), *Lactobacillus*

lycopersici and *L. dextranicum* (8358) and (8359), which respond to the total glutamic acid. *Leuconostoc citrovorum* (8082) could also be used to estimate the L(+) form, but eleven other strains that were tested showed variable, partial activity to the D(−) isomeride.

Method—The composition of the basal medium is given in the accompanying table.

COMPOSITION OF BASAL MEDIUM

Constituent	Milligrams per litre	Constituent	Milligrams per litre
DL-Alanine	667	Xanthine	12·0
Asparagine, natural	667	Dextrose	20,000
L(+)-Arginine.HCl	667	Sodium acetate	12,000
L(−)-Cysteine.HCl	667	Ammonium chloride	6,000
Glycine	667	KH_2PO_4	500
L(−)-Histidine.HCl.H_2O	667	K_2HPO_4	500
L(−)-Hydroxyproline	667	$MgSO_4.7H_2O$	200
DL-Isoleucine	667	$FeSO_4.7H_2O$	10
L(−)-Leucine	667	$MnSO_4.4H_2O$	10
DL-Lysine.HCl	667	NaCl	350
DL-Methionine	667	Aneurine.HCl	1·0
DL-Norleucine	667	Pyridoxine	1·6
DL-Norvaline	667	Pyridoxamine.2HCl	0·10
DL-Phenylalanine	667	Pyridoxal.HCl	0·10
L(−)-Proline	667	DL-Ca pantothenate	2·0
DL-Serine	667	Riboflavine	2·0
DL-Threonine	667	Nicotinic acid	2·0
DL-Tryptophan	667	Biotin	0·005
L(−)-Tyrosine	667	*p*-Aminobenzoic acid	0·10
DL-Valine	667	Folic acid	0·005
Adenine sulphate.2H_2O	13·8	Choline chloride	10·0
Guanine.HCl.2H_2O	13·0	Inositol	25·0
Uracil	12·0		

Separate solutions of basal medium, an amino acid test mixture, protein hydrolysates, standard amino acid, sodium chloride (to adjust all the solutions, except the basal medium, to the same sodium chloride concentration), and inocula were delivered by an automatic pipette into 3-inch test tubes to give a final volume of 1·5 ml. in each tube. Standards of D(−)-, L(+)-, and DL-glutamic acid were run in quadruplicate at eleven levels (0 to 25 μg.), and the test mixture and hydrolysates in duplicate at two to five levels. The tubes were incubated for 72 hr. at 35° C.

Results—An amino acid test mixture containing 1·27 per cent. of L(+)-glutamic acid and 0·424 per cent. of D(−)-glutamic acid was used to establish the specificity and accuracy of the procedure. The *S. faecalis* used to determine the L(+)-acid and all the seven organisms used for the total glutamic acid gave recoveries very close to 100 per cent. Assays on casein hydrolysate showed the presence of little, if any, D(−)-glutamic acid and 22 per cent. of total glutamic acid. A preparation of dry, de-fatted *Lactobacillus arabinosus* cells gave about 4 per cent. of each of the L(+) and D(−) isomerides. J. S. HARRISON

Apparatus for Quantitative Low-Temperature Vacuum Distillation of Millilitre Volumes. W. M. Grant (*Ind. Eng. Chem., Anal. Ed.*, 1946, **18**, 729)—An apparatus that provides a short distillation path for the rapid distillation of small samples, while minimising the chance of contamination from foaming, is described. It consists of a 25 × 150-mm. test tube, into the side of which is sealed a bulb of approximately 50-ml. capacity; this is considered to be the optimum size for the treatment of 1- to 3-ml. samples.

Method—Introduce the material to be distilled into the bulbous side-flask by means of a curved pipette, and close the apparatus by means of a silicone-greased rubber stopper carrying a stop-cock for connection to a vacuum pump. Freeze the sample completely by immersing the side-flask in a bath of "dry-ice" mixture, evacuate the apparatus to a pressure of about 1 mμ., and disconnect from the pump after closing the stop-cock. Remove the side-flask from the freezing-bath, immerse the test tube portion in its place, and allow the distillation to continue without any external heating. De-gassing of the sample can be achieved in part by allowing it to melt after evacuating the apparatus, re-freezing, and then evacuating a second time before immersing the tube in the cooling-bath. With whole blood samples, de-gassing is facilitated by addition of 1 drop of xylene. Figures indicating that quantitative separations of volatile substances from a variety of non-volatile materials can be achieved are quoted. Distillations must be carried to completion in order to obtain the same relative concentrations of volatile constituents in the distillate as in the original sample. J. ALLEN

Molecular Distillation as a Step in the Chemical Determination of Total and Gamma-Tocopherols. M. L. Quaife and P. L. Harris (*Ind. Eng. Chem., Anal. Ed.*, 1946, **18**, 707–708)—The quantitative separation of tocopherols from lipoids is possible by molecular distillation when only 60 μg. of tocopherols per gram of material are present.

Method—Weigh 1 g. or less of the sample, melted if necessary to form a thin film, into the aluminium

cup, and place in the still. Close the still with a large rubber stopper and de-gas the sample by reducing the pressure to less than 1 mμ. at room temperature. Restore the pressure to atmospheric, quickly insert the condenser, re-evacuate to less than 1 mμ. pressure and then fill the condenser with dry-ice and acetone. Adjust the surface of the oil in the heating-bath level with the bottom of the condenser, raise the temperature of the bath rapidly to 220° C., and maintain at 215° to 220° C. for 30 min. Remove the oil-bath, allow the still to cool to room temperature while still evacuated and then, after restoring the pressure to atmospheric,

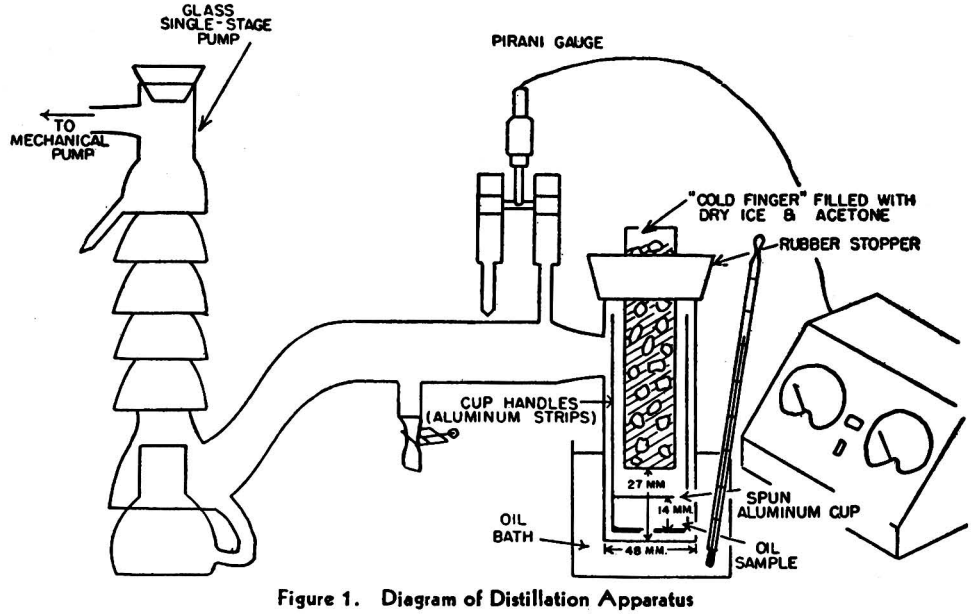


Figure 1. Diagram of Distillation Apparatus

carefully remove the condenser and wash off the distillate quantitatively with chloroform, finally transferring it to a known volume of absolute ethyl alcohol by evaporation under nitrogen. Assay separate portions of this solution for total tocopherols by the method of Emmerie and Engel (*Rec. Trav. Chim.*, 1938, **57**, 1351) and for γ-tocopherol by the procedure of Quaife (*J. Amer. Chem. Soc.*, 1944, **66**, 308).

Recoveries of tocopherols added to lard ranged from 92 to 102 per cent. Figures for the tocopherol content of various natural fats are given.

J. ALLEN

Agricultural

Arsenate-Displaceable Phosphate in Long-Fertilised and Unfertilised Plot Soils. C. J. Schollenberger (*Soil Sci.*, 1947, **64**, 371–378)—Analytical methods developed for extracting soils with arsenic acid - ammonium arsenate solutions in a range of *p*H values, and data on phosphoric acid thus displaced from long-fertilised and unfertilised soils are presented. Soil from superphosphated plots was higher in arsenate-displaceable phosphoric acid than was unfertilised soil, but the differences were small in comparison with the absolute amounts displaced. The marked effect of *p*H upon the displacement may indicate the presence of a ferric phosphate or similar complex in the soil. There was no significant displacement of phosphate by the acetate anion and the direct solvent effect of the hydrogen ion at *p*H 3 was slight. The results suggest that the observed effect of liming in increasing the utilisation of the native phosphoric acid of the soil as well as that added in the form of superphosphate is caused largely by increase of the *p*H of the soil.

Method—To prepare the extracting solution, which is an arsenic acid and ammonium arsenate solution *N* with respect to the arsenate content and of a definite *p*H, add the following amounts (ml.) of 2 *N* aqueous ammonia to 10 ml. of *M* arsenic acid (*infra*) and dilute the mixture to 30 ml.; the *p*H of the resulting solution is the figure in brackets—0 (1·3); 1·95 (2·0); 4·35 (3·0); 5·05 (4·0); 5·35 (5·0); 6·70 (6·0); 9·35 (7·0); 10·50 (8·0). Determine the phosphate colorimetrically by the Benedict - Theis procedure (*J. Biol. Chem.*, 1924, **61**, 63; ANALYST, 1924, **49**, 537). To prepare the molybdate solution add 20 g. of ammonia-free molybdic anhydride to 100 ml. of 5 per cent. sodium hydroxide solution prepared from sodium, shaking continuously and warming if necessary, dilute to 250 ml., and filter. To a portion for the day's use add an equal volume of sulphuric acid and cool. To prepare sodium bisulphite and hydroquinone solution dissolve 37·5 g. of sodium bisulphite in water, add 1·25 g. of hydroquinone, shake until all is dissolved, and dilute to 250 ml. If the solution is not clear after several days, filter. For the phosphate standards, dissolve 1·9165 g. of dried potassium dihydrogen phosphate in water

previously shaken with toluene and filtered, and dilute to 1 litre with toluene water. Dilute 5 ml. of this stock solution to 100 ml. with plain distilled water to provide the working standard (1 ml. ≡ 50 lb. of phosphoric anhydride per acre of 2 million pounds of soil when the aliquot of the extract taken represents 2 g. of soil and both sample and standard are diluted to the same volume for comparison).

Procedure—To 30 ml. of extracting solution in a 50-ml. stoppered Erlenmeyer flask, add 6 g. of finely ground sample, shake frequently during 45 min., and filter clear through dry, fluted paper. Evaporate two 10-ml. portions in similar flasks, add 1 ml. of concentrated nitric acid and 3 ml. of hydrochloric acid, evaporate again, and repeat the evaporation with 5 ml. of hydrochloric acid and finally with 5 ml. of hydrobromic acid (*infra*). To the residue add 8 ml. of water and 1 ml. of acidified molybdate solution. Shake until dissolution is complete, add 1 ml. of the sodium bisulphite - hydroquinone solution, stopper the flasks, place in boiling water for exactly 10 min., and cool quickly in running water. The colour is reasonably stable, but comparison with standards prepared simultaneously should not be unduly delayed. Without a range of standards, the use of a colorimetric curve (Yoe, "*Photometric Chemical Analysis. I. Colorimetry.*" John Wiley & Sons, Inc., New York, 1928) is necessary for high accuracy.

Purification of reagents—To prepare arsenious oxide practically free from antimony add 525 ml. of concentrated hydrochloric acid, with swirling, to 150 g. of powdered white arsenic in a litre boiling-flask connected by a rubber stopper and glass tubing of diameter 10 mm. to a vertically supported, inverted Liebig condenser with inner tube of similar width and with its expanded orifice dipping below the surface of 800 ml. of water in a beaker marked at 1200 ml. Distil 400 ml., with cold water circulating through the condenser. Coarse crystals of arsenious oxide separate in the end of the condenser and in the beaker. Wash the crystals with water until practically free from chloride and dry. About 60 g. should be obtained. The arsenic and antimony distil as chlorides and the arsenious chloride is hydrolysed, whereas the much smaller amount of antimony chloride dissolves in the acid liquid. Addition of metallic copper to the liquid to be distilled may improve the purification (Roark *et al.*, *J. Ind. Eng. Chem.*, 1916, **8**, 327; ANALYST, 1916, **41**, 217).

To prepare arsenic acid add not more than 10 ml. of concentrated nitric acid to 50 g. of purified arsenious oxide and 25 ml. of water in a litre Erlenmeyer flask, warm cautiously to prevent frothing, add more nitric acid as the reaction slackens until dissolution is complete and frothing ceases. The amount of nitric acid used should not exceed 50 ml. Evaporate the liquid rapidly until it becomes a syrup, then boil more cautiously until crystals appear. Bake the cooled, porcelain-like mass on a steam-plate to expel nitric acid, add 300 ml. of water, and boil to obtain a clear solution. Cool and dilute to 500 ml. to obtain *M* orthoarsenic acid.

Hydrobromic acid is sometimes badly contaminated by phosphoric acid. To prepare the acid free from phosphate distil the impure acid with a trap to scrub the vapours and prevent spray. Nearly all should be distilled, as the liquid in the flask at the end is the constant-boiling acid (125° C.) containing 52 per cent. of HBr. A. O. JONES

Water Analysis

Semi-micro-determination of Nitrogen in Sewage Sludges. L. Klein (*J. Soc. Chem. Ind.*, 1947, **66**, 376)—The micro-Kjeldahl apparatus used (Griffin and Tatlock, *Catalogue of Chemical Apparatus*, 1938, **15B**, 915) consists of a 100-ml. Kjeldahl flask, with a 50-ml. bulb serving both for digestion and distillation, connected to a West glass condenser of 18 cm. effective length by means of a ground-in joint with a steam delivery tube and trap, the joint being secured by two steel springs.

Weigh the finely ground sludge (0·1 g. or the equivalent of 3 to 7 mg. of nitrogen) in a micro-tube (5 mm. by 20 mm.) to within 0·1 mg. and drop the tube into the flask with 0·02 g. of selenium powder, 0·2 g. of hydrated copper sulphate, 1·3 g. of Analar potassium sulphate and, finally, 3 ml. of nitrogen-free, concentrated sulphuric acid. Boil gently until the liquid is very pale green in colour and continue heating for 5 to 10 min. Cool, add 16 ml. of water, a few drops of thymol blue indicator, and 40 per cent. sodium hydroxide solution from a pipette until the indicator colour is yellow. Add a fragment of porous pot, connect the flask to the ground-in joint, and run in a little more sodium hydroxide solution through the steam delivery tube until the indicator colour changes to green or blue (pH $>8·8$). Pass in steam generated from tap-water acidified with sulphuric acid, and heat the flask with a micro-burner. Distil for 5 min. with the end of the condenser dipping below 10 ml. of 2 per cent. boric acid solution containing a few drops of a mixture of 100 ml. of 0·2 per cent. alcoholic bromocresol green and 20 ml. of 0·2 per cent. alcoholic methyl red (Ma and Zuazaga, *Ind. Eng. Chem., Anal. Ed.*, 1942, **14**, 280) in a 100-ml. conical flask, and for a further 5 min. after lowering the flask so that the condenser-end is above the liquid. Regulate the heat and steam supply so that condensation occurs near the middle of the condenser tube. The beginning of the distillation is taken as the moment when the first trace of ammonia causes the colour of the indicator in the receiver to change to a brilliant greenish-blue. At the end of the distillation, wash the condenser tube with a small volume of water and titrate the distillate with 0·02 *N* hydrochloric acid from an ordinary 25-ml. burette until the blue colour just disappears and the liquid becomes grey or colourless. Deduct a reagent blank unless it does not exceed 0·05 ml. of 0·02 *N* hydrochloric acid.

Comparison of the results with those of macro-determinations in which 0·5- to 1-g. samples were used and the absorption and titration were made with 0·2 *N* hydrochloric acid and 0·2 *N* sodium hydroxide, respectively, showed that the accuracy of the semi-micro method is satisfactory.

A. O. JONES

Gas Analysis

Continuous Estimation of Oxygen in Gases by a Polarographic Method. W. S. Wise (*Chem. and Ind.*, 1948, 37–38)—The first polarographic wave due to the reduction of oxygen can be used to determine oxygen dissolved in a liquid, and has been adapted to the continuous estimation of oxygen in a gas. The current flowing between a dropping mercury cathode immersed in 0·01 *N* potassium chloride and a mercury pool anode with an applied potential of 0·7 v. is recorded while the gas to be examined is passed continuously through the solution. Irregularities in the current - time curve, caused by the gas bubbling past the dropping mercury electrode, are smoothed out by surrounding the capillary with a glass or metal gauze cylinder. The speed of response to a change in the oxygen content depends on the mesh-size of the cylinder, and 100-mesh gauze was found to be the best for general purposes. J. G. WALLER

Organic

Selectivity of Micro Methods for the Determination of Citric Acid as Pentabromoacetone. F. L. Breusch and R. Tulus (*Biochim. et Biophys. Acta*, 1947, **1**, 77–82)—The micro-colorimetric method of Pucher, Sherman, and Vickery (*J. Biol. Chem.*, 1936, **113**, 235; ANALYST, 1936, **61**, 267) was modified by Pucher (*J. Biol. Chem.*, 1944, **153**, 133; ANALYST, 1944, **69**, 322) to a micro-volumetric method because the light petroleum employed for extraction of the pentabromoacetone caused variations in colour when obtained from various sources. This difficulty was easily overcome by repeated use of the same light petroleum (b.p. 120° to 180° C.) after washing six times with water. The absorption curves then remained valid to within 5 per cent. for many months.

The behaviour of thirty-four acids in both methods has been investigated and the following have been found to give positive values: citraconic, itaconic, oxalocitramalic, glyoxylocitramalic, β-hydroxybutyric, acetoacetic, β-hydroxy-β-phenylpropionic, β,ε-diketohexoic, three α,γ-diketo acids, and tryptophan. Three tricarboxylic acids, tricarballylic, *iso*citric, and hydroxycitric, give no reaction; two others, oxalocitramalic and glyoxylocitramalic, give reactions by both methods, almost equivalent to that of citric acid, whilst most other reacting acids give much lower values, particularly by the colorimetric procedure, so that the latter is the more selective for citric acid.

Both methods are rendered more selective if a short boiling with alkali is introduced before, and in addition to, the boiling of the acid solution.

Procedure—Neutralise the trichloroacetic acid filtrate with 20 per cent. potassium hydroxide solution, add a further 2 ml., boil for 2 min., acidify, and continue according to the original technique.

The reactions of the same thirty-four acids have been repeated, following the modified procedure in both colorimetric and volumetric methods. Oxalocitramalic acid gives no reactions, whilst the reactions of the α,γ-diketo acids are almost entirely eliminated. The reactions of citric acid and of glyoxylocitramalic acid are not affected.

Diacetyl does not interfere in the colorimetric method but gives significant values in the volumetric method, and these values are not reduced by the modification described. W. C. JOHNSON

Polarographic Reduction of Aliphatic Aldehydes. VI. Polarographic Properties of Acetaldehyde. R. Bieber and G. Trümpler (*Helv. Chim. Acta*, 1947, **30**, 2000–2010)—Acetaldehyde gives rise to a polarographic reduction wave in solutions of *p*H values greater than 7·0, but at lower *p*H values the discharge of the supporting electrolyte occurs at a less negative potential than the reduction wave. The height of the step in buffered solutions increases with increasing *p*H value, the highest step being obtained in 0·1 *M* lithium hydroxide, and the height is proportional to concentration for solutions that are less than 0·001 *M* with respect to acetaldehyde. The half-wave potential with 0·1 *M* lithium hydroxide as supporting electrolyte is about −1·64 v. *versus* the mercury anode. Well-defined waves are also obtained with unbuffered 0·1 *M* lithium chloride as the supporting electrolyte.

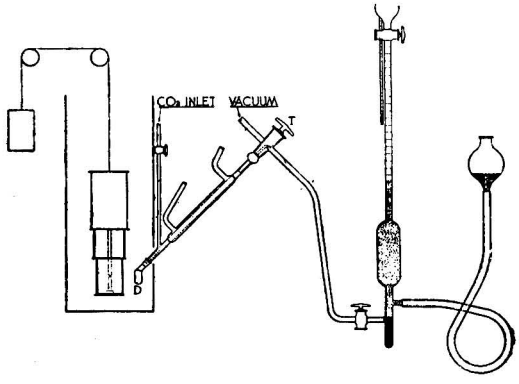
Since the half-wave potential of the acetaldehyde reduction is about 0·25 v. more negative than that of the formaldehyde reduction, it is possible to determine acetaldehyde in presence of formaldehyde, 0·1 *M* lithium chloride being used as the supporting electrolyte. J. G. WALLER

Determination of Diazo-compounds through Decomposition by Light. G. Spencer and F. J. Taylor (*J. Soc. Dyers and Colorists*, 1947, **63**, 394–395)—In general these compounds are determined by a coupling process that is widely applicable, fairly rapid, and moderately accurate. With compounds that couple slowly, *e.g.*, 1-diazo-2-naphthol-4-sulphonic acid and its 6-nitro derivative, back-titration after prolonged stirring with an excess of coupling reagent is necessary, and the method is slow and inaccurate. Measurement of the nitrogen evolved on boiling with alkaline arsenite solution is not always efficient.

Exposure to light results in evolution of nitrogen, and the use of a 3-kw. arc with 14-mm. carbons and the usual device for maintaining the arc resulted in quantitative replacement of the diazo group by hydroxyl. Fifty to 60 ml. of nitrogen were evolved in 1·5 hr., and correct results were obtained for the two diazo-compounds mentioned above. By reducing the volume of nitrogen to 5 or 6 ml. and collecting in an 8-ml. nitrometer calibrated to 0·01 ml., the time may be reduced to 10 to 15 min.

Method—The figure shows the arrangement of apparatus; the counterpoised arc-lamp is enclosed in an asbestos and angle-iron shield, and the front face is hinged to act as a door. The condenser is joined by a ground joint to the decomposition flask, D, a standard, flat cylindrical Pyrex culture flask (4·5 cm. diameter, 2 cm. thick, and 20 ml. capacity). A carbon dioxide inlet is sealed into the condenser tube which cools the gases and avoids heating of the

nitrometer and prevents condensation in it, and which projects through the screen. A 3-way tap, T, allows the decomposition flask to be connected to a vacuum line or to the nitrometer, which has a



thermometer fixed to its side, with the bulb opposite the gas space.

Procedure—Dissolve a sample of the material, and pipette an aliquot portion, 5 or 10 ml., sufficient to give 4 to 7 ml. of nitrogen, into the flask; add 1 ml. of *N* hydrochloric acid. Attach to the condenser and evacuate through the 3-way tap. Fill with carbon dioxide, and then repeat the evacuation and filling twice more. Turn T and connect the flask to the nitrometer and sweep out the tubes with carbon dioxide for a few seconds. Lower the arc into position and raise the nitrometer reservoir to fill the graduated portion with 30 per cent. potassium hydroxide solution. Close the tap, lower the reservoir, switch on the arc, and leave a slow stream of carbon dioxide passing. When the evolution of nitrogen ceases, switch off the arc, equalise the levels, and measure the volume.

Many compounds have been analysed by the method, which requires 30 to 35 min. and is accurate, the standard deviation being only ±0·07 per cent.

M. E. DALZIEL

Bromination of Phenols and Phenol Alcohols. I. W. Ruderman (*Ind. Eng. Chem., Anal. Ed.*, 1946, **18**, 753–759)—The quantitative nature of the procedure originally suggested by Koppeschaar (*Z. anal. Chem.*, 1876, **15**, 233) for the bromination of phenols has been investigated, particularly in respect of the anomalous results reported with certain substituted phenols (Fox and Barker, *J. Soc. Chem. Ind.*, 1918, **37**, 268; Francis and Hill, *J. Amer. Chem. Soc.*, 1924, **46**, 2498 and *Ind. Eng. Chem., Anal. Ed.*, 1941, **13**, 357; Day and Taggart, *Ind. Eng. Chem.*, 1928, **20**, 545; Sprung, *Ind. Eng. Chem., Anal. Ed.*, 1941, **13**, 35).

Method used—Dissolve an accurately weighed portion of the phenol in distilled water containing sufficient sodium hydroxide, about 1 g., to effect solution, and dilute to 1 litre. Transfer 25 ml. of this solution to a stoppered iodine flask, add 25 ml. of water, or more as indicated below, and 5 ml. of concentrated hydrochloric acid, and then run in enough 0·1 *N* bromate-bromide to give the desired excess of bromine. Quickly stopper the flask and shake intermittently for 5 min. Add 10 ml. of a 10 per cent. solution of potassium iodide, taking care to avoid loss of bromine on releasing the stopper, leave for 5 min., wash the stopper and sides of the flask with water, and titrate with 0·1 *N* sodium thiosulphate, using starch solution as indicator. Make a blank determination on 25 ml. of distilled water.

All the brominations were carried out at 25° C., except where the effect of temperature was being examined, and the acid concentration was maintained at 0·7 to 0·8 *N* by keeping the sum of the volumes of bromate - bromide solution and diluting water to about 50 ml. Where aqueous solutions of the phenols under examination could not be prepared because of the insolubility of their sodium salts, a weighed portion was dissolved in 600 ml. of pure, acetone-free methyl alcohol and the solution diluted to 1 litre with distilled water. Methyl alcohol was used for dilution prior to bromination instead of water to avoid precipitation. Under the conditions described, acetone-free methyl alcohol was found to be almost inert towards bromine. For highest accuracy, however, several blank determinations on aqueous solutions of methyl alcohol with various amounts of bromine in excess were made and a correction curve was drawn. Titration of the excess of bromine was always continued until the end-point was stable.

The results are presented in several tables and by means of a number of curves. They can be summarised as follows.

Phenol, saligenin, and p-*hydroxybenzyl alcohol*—Phenol brominates quantitatively at three positions whatever the amount of the excess of bromine. With large excess amounts of bromine, three reactive positions are found also for saligenin and for *p*-hydroxybenzyl alcohol.

Cresols—*m*-Cresol brominates quantitatively at three positions over a wide range of bromine concentrations, but the *o*- and *p*-isomerides absorb about 2 molecules of bromine when the excess is small. With larger excess amounts of bromine, these latter over-brominate to a considerable extent, the *o*-isomeride to a much greater degree than *p*-cresol.

Alkylated phenols and their hydroxymethyl derivatives—Of these, *p*-(1 : 1 : 3 : 3-tetramethylbutyl)-phenol, *p-cyclo*hexylphenol and 4-chloro-*m*-cresol show two reactive positions regardless of the size of the bromine excess. *p*-Cresol, *p*-ethylphenol, and *p-iso*propylphenol react abnormally; the larger is the excess, the greater is the number of reactive positions found. In general, phenols having primary or secondary alkyl groups in the *o*- or *p*-positions tend to over-brominate in presence of excess of bromine, the extent of over-bromination depending on the magnitude of the excess (*p-cyclo*-hexylphenol is exceptional). Phenols having tertiary alkyl groups in any position brominate quantitatively for all quantities of bromine in excess. Among the several phenolic dialcohols examined, only with 2-hydroxy-5-(1 : 1 : 3 : 3-tetramethyl butyl)-α^1 : α^3-xylenediol and 6-chloro-3-hydroxy-α^2 : α^4-*pseudo*cumenediol is quantitative

displacement of the hydroxymethyl groups effected by bromine and only then with a large excess; in general, it appears that *p*-alkylated phenol dialcohols whose alkyl groups are tertiary lose their two hydroxymethyl groups during bromination more easily than do those whose alkyl groups are primary or secondary.

Dihydroxydiphenylmethanes—The 4 : 4′-isomeride absorbs four molecules of bromine with a small excess of bromine and over-brominates with a large excess, whilst the 2 : 4′-compound requires a large excess to react similarly. The 2 : 2′-isomeride under-brominates even with a large excess of bromine.

The effects of acidity, temperature, and time of reaction on the bromination of phenols has also been studied. Reduction of the acidity from 0·75 *N* to 0·35 *N* decreases the amount of over-bromination of *p*-cresol, whilst with 2-hydroxy-α^1 : α^3-mesitylenediol, increasing the acidity increases the ease of displacement of the hydroxymethyl groups. The over-bromination of *p*-cresol can be inhibited by conducting the reaction at 10° C., even in presence of a large excess of bromine, whilst a smaller retardation in the replacement of the hydroxymethyl groups of 2-hydroxy-α^1 : α^3-mesitylenediol is observed at low temperatures. Reducing the time of reaction to 1 min. always significantly decreases the number of reactive positions found; increasing the time beyond 5 min. has no appreciable effect. J. ALLEN

Quantitative Separation of Methylated Sugars. F. Brown and J. K. N. Jones (*J. Chem. Soc.*, 1947, 1344–1347)—The mixtures of fully methylated and partly methylated sugars obtained in the end-group method for the investigation of carbohydrate structure (Haworth and Machemer, *Ibid.*, 1932, 2270) can be separated by chromatography or by extraction with light petroleum from aqueous solution.

Chromatographic separation—The column consisted of alumina from the British Aluminium Company, activated for 4 hr. at 360° C., length 18 in., cross section 1¼ in. The methylated sugars were dissolved in light petroleum, b.p. 40° to 60° C., or 60° to 80° C., washed with sulphuric acid and sodium hydroxide and distilled, and the eluent was chloroform dried by calcium chloride and distilled. Experiments with known mixtures of 2 : 3 : 4 : 6-tetramethyl methyl-D-glucoside and 2 : 3 : 6-trimethyl methyl-D-glucoside showed that the fully methylated sugar can be recovered in 93 per cent. yield from the eluate by evaporation; for example, 245 mg. of tetramethyl methylglucoside (n_D^{16} 1·4440) and 382 mg. of trimethyl methylglucoside (n_D^{16} 1·4572) dissolved in 50 ml. of light petroleum, b.p. 40° to 60° C., were separated by passing the solution through the column and eluting with six 100-ml. portions of chloroform, which yielded 226 mg. of the tetramethyl compound. Optical rotation measurements showed that a partial separation of 2 : 3 : 4 : 6-tetramethyl α- and β-methyl-D-glucosides was effected. The trimethyl methylglucoside can be recovered by eluting with methanol. When the method is applied to the methanolysis products from a methylated polysaccharide a preliminary enrichment of the tetra fraction is necessary to reduce the mixture to proportions similar to those employed in the standardising procedure. The methylated polysaccharide is hydrolysed by boiling with methanolic hydrogen chloride, neutralised with silver carbonate, diazomethane, or preferably *N* sodium hydroxide in slight excess, the solvent is removed on a water-bath and, finally, in a vacuum at 25° C. The residual syrup is dissolved in about 50 ml. of water and extracted continuously with re-distilled, sulphur-free, light petroleum for 4 hr. This removes all the fully methylated sugar and about an equal weight of trimethyl methylglucoside. The aqueous solution is again extracted for 4 hr. with light petroleum, b.p. 38° to 40° C. and the product from concentration of the combined extracts is separated on the column.

Separation by extraction—Two continuous extractors (Quickfit and Quartz/EX8/23) were used in series, the aqueous sugar solution (40 to 50 ml.) being placed in the top apparatus and 75 ml. of water in the other, the latter to wash the light petroleum extracts. In experiments with known mixtures of fully methylated glucose and trimethyl methylglucoside in approximately equal proportions, the former was recovered in 99 to 102 per cent. yield by extraction for about 4·5 hr. with light petroleum, b.p. 38° to 40° C. When the mixture contained 140 mg. of tetramethyl and 2577 mg. of trimethyl methylglucoside a repetition of the procedure was necessary, and similar mixtures containing also 2 : 3-dimethyl methylglucoside gave 98 to 100 per cent. yields of the fully methylated sugar by the double procedure.

Separation of tetramethyl methylgalactoside and 2 : 3 : 6-trimethyl methylglucoside—A solution of 199 mg. of heptamethyl methyl-lactoside in 25 ml. of 1 per cent. methanolic hydrogen chloride was boiled for 8 hr., neutralised with *N* sodium hydroxide, and concentrated in a vacuum. The syrup was dissolved in 50 ml. of water and the solution extracted for 9 hr. with light petroleum, b.p. 38° to 40° C. The extract yielded 105 mg. of tetramethyl methylgalactoside (n_D^{16} 1·4492) (96 per cent. of theory). The aqueous solution was further extracted with chloroform to yield 102 mg. (98 per cent. of theory) of 2 : 3 : 6-trimethyl methylglucoside.

The extraction method was also applied to the methanolysis products of methylated peanut araban. The results indicated that trimethyl, dimethyl, and monomethyl methyl-L-arabinoside were present in equimolecular proportions.

W. C. JOHNSON

Determination of Aconitic Acid in Sugarhouse Products. J. A. Ambler and E. J. Roberts (*Anal. Chem.*, 1947, **19**, 877–878)—One method for determining aconitic acid in sugarhouse products is by long extraction with ether, titration of the dried extractives with standard alkali, and correction for the oxalic acid that may be present (Balch *et al.*, *Sugar*, 1945, **40**, 32; 1946, **41**, 46; McCalip *et al.*, *Ind. Eng. Chem.*, 1941, **33**, 637;

ANALYST, 1941, **66**, 422; Yoder, *J. Ind. Eng. Chem.*, 1911, **3**, 640; ANALYST, 1911, **36**, 594). Ether-soluble waxy and fatty substances and pigments make the titration uncertain and sometimes impossible, and the method is too tedious for routine use.

The decarboxylation method of Roberts and Ambler (*Anal. Chem.*, 1947, **19**, 118; ANALYST, 1947, **72**, 453) is not subject to these difficulties and is accurate with solutions containing sucrose. Comparative determinations with identical products were made by these two essentially different methods and as a further check the titrated solutions of the ether extractives were dried and decarboxylated directly.

DECARBOXYLATION METHOD—No change in the decarboxylation procedure was necessary except when sulphur dioxide was present (*infra*). *Juices*—It is advantageous to clarify juice (Balch *et al.*, *loc. cit.*) with lime, and to heat before taking the sample. Add enough acetic acid to 500 ml. of the clarified juice to lower its *p*H to about 6, heat the liquid on the steam-bath, add 50 ml. of saturated lead acetate solution with stirring, and collect and decarboxylate the precipitate. *Syrups and molasses*—Weigh enough sample to contain 20 to 25 g. of Brix solids and dilute with water to about 200 ml. If necessary, adjust the *p*H to 5·5 to 6·2 with sodium hydroxide solution or acetic acid, heat the unfiltered solution on the steam-bath, and treat it with 50 ml. of saturated lead acetate solution. Collect and decarboxylate the precipitate. *Raw sugars*—Dissolve 20 g. of sugar in about 200 ml. of water, heat on the steam-bath, add 50 ml. of lead acetate solution, and collect and decarboxylate the resulting precipitate.

EXTRACTION METHOD—*Juices*—Acidify 100 ml. of clarified juice with 10 ml. of concentrated hydrochloric acid and extract with ether for 16 hr. Determine aconitic acid in the ether extract (dried *in vacuo* at 70° C.) by adding 5 ml. of benzene and about 50 ml. of water, titrating with standard alkali to phenolphthalein indicator, evaporating the titrated liquid to dryness on the steam-bath in a 250-ml. decarboxylation flask, and determining the aconitic acid in the dried salts by direct decarboxylation. *Syrups and molasses*—Dilute 15 g. of the sample with water to about 100 ml. and acidify with 15 ml. of concentrated hydrochloric acid. Extract the solution with ether and treat the extract as described for juices. *Raw sugars*—Dissolve 10 g. of sugar in water and dilute the solution to about 100 ml., acidify the solution with 10 ml. of hydrochloric acid, and extract with ether. Determine aconitic acid in the extract by titration as described for juices, but not by decarboxylation since the amount is small.

The only interfering substance encountered was sulphur dioxide in products such as edible and cooking molasses, the values for aconitic acid by decarboxylation of the lead precipitate from these samples being higher than those obtained by titration of the extractives. Lead sulphite precipitated with the aconitate reacted with the acetic acid and potassium acetate reagent to form free sulphur dioxide which passed into the alkaline absorbent, causing high values for the carbon dioxide. This interference can be prevented by using a saturated solution of potassium dichromate acidified with sulphuric acid for washing the gas evolved in the decarboxylation procedure. Interference by the "uronic acids" found in sugar cane (Browne and Phillips, *Intern. Sugar J.*, 1939, **41**, 430) was not encountered, nor was interference by the small amount of citric acid reported as present in sugar cane juices (Tanabe, *Rept. Govt. Expt. Sta., Tainan, Formosa*, 1937, **4**, 33) and molasses (Nelson, *J. Amer. Chem. Soc.*, 1929, **51**, 2808). Oxalic acid and oxalates are not decarboxylated in the aconitic acid procedure and therefore when present would cause the titration values of the extractives to be higher than both the decarboxylation values. In presence of ether-soluble pigments, waxes, and fats, re-extraction of the substrate may be necessary in the extraction method. A. O. JONES

Determination of Aconitic Acid in Mixtures with Citric Acid. J. A. Ambler and E. J. Roberts (*Anal. Chem.*, 1947, **19**, 879–880)—In the decarboxylation procedure for determining aconitic acid (see preceding abstract), citric acid sometimes gives considerable amounts of carbon dioxide, causing erratic results. The only variable in the procedure not under close control is the amount of water present during decarboxylation. Since as much as 5 g. of water per 100 ml. of acetic acid can be present without retarding decarboxylation of aconitic acid, it is not necessary, when determining aconitic acid in solution, to render the lead precipitate anhydrous or to use anhydrous reagents. The decarboxylation of citric acid in the potassium acetate and acetic acid reagent is very sensitive to water even in very small amounts and is quantitative in 1 hr. only when the reagent is completely anhydrous. This suggests that the decarboxylation of citric acid is not a reaction of the acid itself, but results from the formation and subsequent decomposition of one or more derivatives of citric acid that may be formed in the hot anhydrous mixture. As it has not been possible to isolate itaconic or citraconic acids from the reaction mixture, the principal reaction involved is not the withdrawal of water followed by decarboxylation of the aconitic acid formed. A possible sequence of reactions is the formation of acetylcitric acid

$$HO.C(COOH)(CH_2COOH)_2 + CH_3COOH = CH_3CO.O.C(COOH)(CH_2COOH)_2 + H_2O$$

followed by decarboxylation of the acetylcitric acid. Acetylcitric acid was found to be more rapidly and extensively decarboxylated than is citric acid in presence of like amounts of water. To prevent the production of carbon dioxide from citric acid it is therefore necessary to block or stabilise the hydroxyl group so that it cannot react with the hot anhydrous acetic acid.

When boric acid is added to the completely anhydrous reagent, the decarboxylation of aconitic acid is quantitative, but that of citric acid is effectively stopped.

Decarboxylation of citric acid in potassium

acetate and acetic acid reagent is very sensitive not only to traces of water but also to small amounts of acid anhydride. In preparing anhydrous reagents by fortifying glacial acetic acid with acetic, succinic, or phthalic anhydride, any appreciable excess of the anhydride over the amount required to react with the water in the acid always leads to excessive darkening during the decarboxylation and to evolution of more than the theoretical amount of carbon dioxide. Quantitative results were obtained only when the reagent was strictly anhydrous and contained no more than about 1 part of anhydride per thousand parts of acetic acid.

Method—To prepare 1 litre of the reagent dissolve 100 g. of dry potassium acetate crystals in 460 ml. of glacial acetic acid (99·6 per cent.). Dissolve by warming 10 g. of boric acid in another 460 ml. of glacial acetic acid. Mix the two solutions, add 30 ml. of acetic anhydride and heat under reflux for 1 hr.

If the sample is not thoroughly dry, dissolve 1 to 2 g. in about 100 ml. of water; if it is liquid, take the equivalent of at least 1 g. of total acids and dilute to 100 ml. Adjust to *p*H 6·0 to 6·2 with acetic acid or sodium hydroxide solution, dilute to about 150 ml., and heat on the steam-bath. Add, with stirring, 50 ml. of saturated lead acetate solution and cool to room temperature. Collect the precipitate as described in the procedure for aconitic acid (Roberts *et al.*, *Ibid.*, **1947**, **19**, 118; ANALYST, **1947**, **72**, 453), dry thoroughly at 120° C. and, using 100 ml. of the anhydrous boric acid and acetic acid reagent, decarboxylate for 1·5 hr. Calculate the carbon dioxide evolved as aconitic acid. If the sample to be analysed is dry, decarboxylate 1 to 2 g. with the boric acid and acetic acid reagent directly.

Oxalic, galacturonic, and mucic acids are slowly decarboxylated by the reagent. A. O. JONES

Determination of Minute Quantities of Phosphate, Calcium, and Magnesium in Rubber. G. J. van der Bie (*India Rubber J.*, **1947**, **113**, 499–500, 502, 541–542)—Standard procedures for ashing raw rubber are described after consideration of possible losses in the elements to be estimated. Methods for estimating phosphorus in quantities of the order of 10 μg., and less than milligram quantities of calcium and magnesium are investigated, and details of the recommended procedure, together with results on standard solutions, are given.

CALCIUM AND MAGNESIUM—*Procedure*—Moisten 2 g. of rubber in a porcelain crucible with 10 drops of nitric acid (sp.gr. 1·4) and, after allowing to stand for 1 hr., heat the crucible over a low flame until fuming ceases, and then transfer it to an electric muffle at 500° to 550° C. for 2 hr. Cool and weigh to obtain the total ash. Add 5 ml. of water and 1 drop of nitric acid (sp.gr. 1·2) for every 10 mg. of ash. Heat on a water-bath for 0·5 hr., transfer to a centrifuge tube, dilute to 8 ml., and centrifuge. Decant and add to the solution 0·5 ml. of 50 per cent. sodium acetate solution, 0·5 ml. of saturated ammonium oxalate solution, 1 drop of methyl red indicator, and then concentrated aqueous ammonia solution until just neutral (an excess of ammonia precipitates magnesium ammonium phosphate). Dilute to 10 ml. and centrifuge after allowing to stand for 0·5 to 1 hr. Remove 5 ml. of the clear liquid for the magnesium determination and decant the remainder. Wash the precipitate with 10 ml. of 2 per cent. aqueous ammonia solution, centrifuge, and decant the liquid. Dissolve the precipitate in dilute sulphuric acid and titrate hot with 0·01 *N* potassium permanganate in presence of 1 drop of manganous sulphate solution.

Take the 5 ml. of clear liquid set aside for the magnesium determination, add 1 ml. of a 5 per cent. solution of disodium hydrogen phosphate, add 10 drops of concentrated aqueous ammonia and centrifuge after allowing to stand for 1 hr. Wash the precipitate twice with 2 per cent. aqueous ammonia solution, dissolve it in diluted sulphuric acid (about 1 in 400) and dilute to 25 ml. in a standard flask. On aliquot parts of this solution determine phosphorus as described below.

PHOSPHORUS—A different method of ashing is given to precede the determination of phosphorus, an alkaline instead of an acid starting material being essential. Trials are reported with lecithin.

Procedure—For rubber, moisten 100 mg. in a porcelain crucible with 1 ml. of 15 per cent. sodium carbonate solution. Evaporate to dryness on the water-bath and then heat gently with a flame, continuing until the fuming that occurs ceases again. Place in an electric muffle at 500° C. for 2 hr. After cooling, add 1 ml. of 10 per cent. potassium nitrate solution and dry on the water-bath. When dry, heat gently until all carbon is destroyed. Heat the residue on the water-bath for 3 hr. with 5 ml. of diluted sulphuric (about 1 in 20) and eventually dilute to 25 ml. in a standard flask. Use aliquot portions for colorimetric determinations of phosphorus by the molybdenum blue method. Stannous chloride is used for reducing the ammonium molybdate, and a small amount of gum arabic is added as a stabiliser. Colour matching must be done immediately because of fading.

Results—Limits of concentration in which the Lambert - Beer law holds are given, errors due to washing the precipitates are evaluated, and results on standard solutions are compared with the theoretical values. W. C. WAKE

Inorganic

Quantitative Examination of Ammonium Iodide. C. G. van Arkel and J. J. M. van Sonsbeek (*Pharm. Weekblad*, **1947**, **82**, 530–534)—In the Dutch Pharmacopoeia process for the assay of potassium or sodium iodide, iodine is liberated by addition of iodate and tartaric acid, and is titrated with thiosulphate after addition of sodium phosphate. In this method, bromide and chloride do not affect the result of the titration. The same procedure is adopted in the Codex Medicamentorum Nederlandicus for ammonium iodide, but satisfactory results are not obtained, mainly because the correct *p*H is not obtained in the final titration. Before carrying out the assay it is better to remove

the ammonia as follows. Boil 10 ml. of solution (containing 1·208 g. of ammonium iodide in 100 ml.) with 15 ml. of 0·1 *N* sodium hydroxide to remove ammonia, cool, and neutralise to phenolphthalein with hydrochloric acid. Add 10 ml. of 0·2 *N* potassium iodate and 15 ml. of a 4 per cent. solution of tartaric acid; after allowing the solution to stand for 10 min., add 30 ml. of a 5 per cent. solution of sodium phosphate ($Na_2HPO_4,2H_2O$), shake, and titrate with 0·1 *N* sodium thiosulphate.

G. MIDDLETON

Micro-determination of Alkaline Earths as Normal Molybdates. R. Moser and R. J. Robinson (*Anal. Chem.*, 1947, **19**, 929–930)—The method described reduces the effect of weighing errors, as the conversion factors are favourable and the molybdates are not hygroscopic, and provides an effective separation from magnesium. Calcium, but not barium or strontium, has been previously determined by the method (Wiley, *Ind. Eng. Chem., Anal. Ed.*, 1931, **3**, 127).

Procedure—Pipette a solution containing between 0·2 and 1·0 mg. of the alkaline earth into a platinum dish of about 10-ml. capacity and 5-g. weight, and adjust the volume to 6 ml. Add 0·8 ml. of 5 per cent. sodium molybdate solution, adjusted to *p*H 4 by means of hydrochloric acid to reduce carbon dioxide absorption from the atmosphere. Adjust to *p*H 6 to 7 with aqueous ammonia solution, using methyl red as indicator. Boil gently for 2 or 3 min., and cool for at least 15 min. Filter off the supernatant liquid by means of a Pyrex glass filter-stick, and wash the precipitate with the minimum quantity of hot water. Ignite the dish and filter-stick for about 5 min. at 700° C. in a calibrated micro-muffle furnace, and weigh on a micro-balance.

Boiling with an eight-fold excess of the reagent is necessary for complete precipitation, *i.e.*, the solution is made about 0·06 *N* with respect to molybdate, agreeing with 0·07 and 0·1 *N* given by other authors. A larger excess has no harmful effect. Magnesium is co-precipitated when present in large quantity, but dissolution in hydrochloric acid and re-precipitation give accurate results. With a magnesium : calcium ratio of 5 : 1, dilution to a magnesium ion concentration of 0·1 *N* is also effective. At *p*H values less than 6, soluble polymolybdates are formed, but precipitation is complete at *p*H values above 6. The precipitates adhere to the vessel in which precipitation is carried out more firmly when the *p*H is near this lower limit. The method can be applied to limestone and to sea-water.

M. E. DALZIEL

Photometric Micro-determination of Calcium. H. A. DeLuca (*Canad. J. Res.*, 1947, **25**, 449–454)—Between 0·04 and 0·16 mg. of calcium can be determined by converting the precipitated oxalate to carbonate by heating for 1 hr. at 475° to 550° C., the carbonate being then dissolved in 1 ml. of 0·01 *N* hydrochloric acid, and the excess of acid determined by reaction with potassium iodide and iodate. The absorption of the resulting iodine solution is measured by means of an Evelyn colorimeter, and the calcium originally present is determined from calibration data.

Procedure—Put 5 ml. of a solution containing 0·04 to 0·16 mg. of calcium in a Pyrex centrifuge tube, and add 1 drop of bromophenol blue, sufficient 2 *N* hydrochloric acid to render the solution acid to the indicator, and 0·2 ml. of saturated ammonium oxalate solution. Adjust to *p*H 3·5, faintly blue, by adding drops of *N* aqueous ammonia solution. Allow to stand for 1 to 2 hr. with occasional shaking, and centrifuge at 3000 r.p.m. for 15 min. Decant the liquid and invert the tube on a blotter for 10 min., and wipe the mouth of the tube with a small piece of filter paper. Suspend the precipitate in 0·3 ml. of 0·5 per cent. ammonium oxalate solution and centrifuge for 15 min. more. Remove the liquid and invert the tube as before for 10 min. Place the tube in a cold muffle furnace and heat slowly to 475° to 550° C. and maintain at that temperature for at least 1 hr. Allow to cool, add exactly 1 ml. of 0·01 *N* hydrochloric acid and, with the aid of a stirring rod, dissolve the carbonate by heating in a water-bath at 85° to 95° C. for 15 min. with occasional stirring. Stopper the tube, the stirring rod being passed through a hole in the bung. Cool for 30 min., add 0·2 ml. of 0·5 per cent. potassium iodate solution and 0·2 ml. of 10 per cent. potassium iodide solution. Allow to stand for 15 min. and then add 10 ml. of a 10 per cent. potassium iodide solution in 0·002 *N* sodium hydroxide. Rinse the solution into a 25-ml. volumetric flask and dilute to the mark.

Treat 1 ml. of water in the same way as the solution of calcium chloride and dilute finally to 25 ml. as before. Prepare two such solutions and adjust the colorimeter to the mean of the two "centre-settings," *i.e.*, the galvanometer readings following the removal of the tubes from the colorimeter, the latter having been set to 100 with the tubes in place. Read the iodine solutions from the sample in the colorimeter and determine the calcium content from the calibration.

The conversion of oxalate to carbonate is effected at 475° to 550° C., as higher temperatures give samples almost incapable of neutralising the acid solution. Slight decomposition of the potassium iodide solution does not matter, as the blank corrects for this. The alkaline potassium iodide prevents evaporation of the liberated iodine, and intensifies the colour, but more strongly alkaline solutions appear to dissolve some of the free iodine. The hydrochloric acid used need not be standardised.

M. E. DALZIEL

Colorimetric Determination of Micro-Amounts of Silver and Silver Halides. S. Siggia (*Anal. Chem.*, 1947, **19**, 923–924)—The method is based on the reaction represented by the equation

$$AgX + K_2[Ni(CN)_4] \rightarrow K[Ag(CN)_2] + Ni(CN)_2 + KX$$

and is carried out in a pyridine - ammonia - water system containing dimethylglyoxime. The ammonia dissolves the nickel cyanide formed and pyridine prevents precipitation of the nickel dimethylglyoxime complex. The stable yellow colour produced obeys the Beer - Lambert law

closely, and the transmittance of the solution is measured at 400 mμ. For maximum accuracy, a solution containing a known amount of silver should be treated side by side with the unknown; reagent concentrations affect the colour slightly, and as they may vary from run to run the use of a calibration curve reduces precision.

Method—Weigh the sample into a volumetric flask of a size determined by the silver content of the sample, *viz.*, 0·002 to 0·02 mg. in a 2-ml., 0·02 to 0·5 mg. in a 10-ml., 0·5 to 2·5 mg. in a 50-ml., and 2·5 to 7·0 mg. in a 100-ml. flask. The final concentrations of the reagents are in the ratio 4 parts of diluted aqueous ammonia (1 + 3), 4 parts of pyridine, 0·6 part of a saturated solution of dimethylglyoxime in diluted aqueous ammonia (1 + 3), and 1 part of potassium nickelocyanide solution prepared by adding potassium cyanide to a suspension of 15 g. of nickel cyanide in 500 ml. of water until the nickel cyanide is almost all dissolved and stirring for 1 hr. to ensure that no free potassium cyanide is present before filtration.

If the sample is water-soluble, add an amount of the dilute ammonia solution based on the size of the flask and the reagent proportions given above; dissolve silver chloride or bromide in concentrated aqueous ammonia and then dilute with water to give a ratio of 1 to 3. If acid is necessary, use the minimum amount of nitric acid, added dropwise, and neutralise it with concentrated aqueous ammonia, then add diluted aqueous ammonia (1 + 3) until 40 per cent. of the volume of the flask is filled. Add pyridine, dimethylglyoxime solution, and potassium nickelocyanide solution in that order and in the proportions described above. After 3 hr. dilute with water to the calibration mark.

Use pipettes or burettes to measure all reagents and carry out a blank determination and, for accurate work, prepare a comparison standard. For the 0·002 to 0·02-mg. samples, micro-burettes are necessary, and the 2-ml. flask can be made from 7-mm. glass tubing, and calibrated by running in 2 ml. of water from a micro-burette. Measure the intensity of the colour by the usual methods.

The procedure was tested with metallic silver, and with silver nitrate, bromide, chloride, and iodide; the colour is independent of the anion present. Silver iodide, being only slightly soluble in ammonia, is more conveniently dissolved by adding it in the solid state to the solution containing potassium nickelocyanide. The colour is stable for more than 3 days.

The method is applicable to silver - silver halide mixtures, and to organic compounds prepared in presence of silver catalysts, in silver utensils, or in presence of silver electrical apparatus.

M. E. DALZIEL

Stable Colorimetric Reagent for Chromium. J. F. Ege, jun. and L. Silverman (*Anal. Chem.*, **1947, 19**, 693–694)—The reagent has been developed for use in a direct field method for the determination of chromic acid in air. Air-borne mists are collected by drawing them through a dry filter paper that has been impregnated with the reagent and a humectant, glycerol. A stain, proportional to the quantity of chromic acid, develops and the concentration is determined by comparison with a set of artificial colour standards.

Preparation of reagent—Add 4 g. of powdered phthalic anhydride to 0·25 g. of *s*-diphenylcarbazide in 100 ml. of 95 per cent. ethyl alcohol. Preserve in a brown-glass bottle and store in a refrigerator.

A. H. A. ABBOTT

Determination of Zinc in Metallic Cobalt by Dithizone. R. S. Young (*Metallurgia*, **1947, 36**, 347–348)—The method has been developed for cobalt containing 0·01 to 0·02 per cent. of zinc, with copper, lead, manganese, and silver in similar quantities, 0·6 per cent. of nickel, and rather less arsenic, antimony, bismuth, carbon, silica, iron, and sulphur. Potassium cyanide is added to inhibit the formation of cobalt and nickel complexes with dithizone, and sodium thiosulphate to inhibit the reaction of dithizone with bismuth, cadmium, copper, gold, lead, and mercury.

Procedure—Treat a 5-g. sample of the metal with nitric acid or *aqua regia*, cool, and dilute to 1 litre. For analysis, treat a 10-ml. portion in a 60-ml. separating funnel with 10 ml. of 5 per cent. potassium cyanide solution, and a few drops of universal indicator. Make slightly acid with dilute hydrochloric acid and adjust with sodium acetate to *p*H 4·5 to 5·5; add 0·2 ml. of 25 per cent. sodium thiosulphate solution. Add 1 ml. of standard dithizone solution from a burette and shake for 10 to 15 sec. or until the green solution turns red, and draw off the red solution in a test tube. Add successive quantities of the dithizone solution, and draw off each portion, until the lower carbon tetrachloride layer remains green.

Transfer the zinc solutions to a separating funnel, add 10 ml. of 0·2 *N* hydrochloric acid, and shake vigorously. Separate the layers in two test tubes, then re-shake the tetrachloride layer with 10 ml. of 0·02 *N* hydrochloric acid, and discard the tetrachloride layer. Combine the two aqueous extracts and adjust to *p*H 4·5 to 5·5 with saturated sodium acetate solution; add 0·2 ml. of 25 per cent. sodium thiosulphate solution, a few drops of 5 per cent. potassium cyanide solution, and 2 ml. of 5 per cent. hydroxylamine hydrochloride solution. Extract the zinc again by successive additions and withdrawals of standard dithizone. Since the dithizone is standardised against standard zinc under similar conditions, the amount of dithizone used is a measure of the zinc in the sample.

The dithizone solution should contain 0·2 g. per litre of carbon tetrachloride, 1 ml. of this solution being equivalent to about 2·5 μg. of zinc. Standardise daily against a zinc solution (0·0062 g. dissolved in 2 to 3 ml. of hydrochloric acid and diluted to 500 ml.) and store in the dark under sulphurous acid in a large separating funnel. All reagents should be tested for their zinc content by extraction with dithizone. Pyrex glassware must be used throughout.

The method is rapid and accurate to within ±5 per cent. in the range 0·005 to 0·05 per cent. of zinc, and can be used in presence of 10 to 1000

times as much aluminium, antimony, arsenic, bismuth, calcium, chromium, cobalt, iron, lead, magnesium, manganese, mercury, molybdenum, nickel, silver, tin, titanium, tungsten, uranium, vanadium, and zirconium. Large quantities of cadmium must be removed by washing the zinc dithizonate solution four or five times with 5-ml. portions of 40 per cent. sodium sulphide solution.

M. E. DALZIEL

Argentimetric Determination of Phosphate. R. Flatt and G. Brunisholz (*Analyt. Chim. Acta*, 1947, **1**, 124–134)—Phosphate can be determined by potentiometric titration with silver nitrate solution at *p*H 8·5 to 9·5. When the *p*H of the solution is lower no satisfactory end-point is obtained, whilst at higher *p*H values silver oxide is precipitated. An alternative method is to add an excess of silver nitrate solution to an acid solution of the phosphate, neutralise to precipitate silver phosphate, filter, and determine the excess of silver nitrate by titration. Complete precipitation of the silver phosphate is obtained by precipitating it at *p*H 7·5 in a solution buffered by an ammonium argentodiammine buffer.

Method for direct titration—The apparatus is that usually used for the potentiometric titration of chloride by silver nitrate solution.

Procedure—Add to the phosphate solution a few drops of phenolphthalein and then sodium hydroxide solution until the indicator is coloured a faint pink. After adding 30 ml. of a 0·1 *M* borax solution, dilute to 100 ml., and titrate slowly with 0·1 *N* silver nitrate. The phosphate solution should not contain any cations that form sparingly soluble phosphates, or anions that form sparingly soluble silver salts. The absence of a nearly vertical portion in the potential - titration figure curve at the point of inflexion limits the accuracy of the method.

Filtration method—The reagents used should be free from carbonate. *Procedure*—Place the weakly acid solution, containing not more than 100 mg. of phosphorus pentoxide, in a 250-ml. graduated flask and add 50 ml. of 0·1 *N* silver nitrate. Add a few drops of benzyl orange solution and 0·5 *N* sodium hydroxide until the colour of the indicator changes to yellow. Add a few drops of methyl red and continue the neutralisation, using 0·1 *N* borax, until the colour changes to yellow. Buffer the solution to *p*H 7·5 by adding 2·5 ml. of a solution containing 0·1 g.-mol. of ammonium nitrate and 0·2 g.-mol. of ammonia per litre or 7·5 ml. of 0·1 *N* ammonium nitrate to which 5 ml. of 0·1 *N* borax have been added. Dilute to the mark, shake, allow the precipitate to settle, and filter the solution through a dry filter paper. Acidify a 100-ml. portion of the filtrate with 1 ml. of diluted sulphuric acid and titrate with 0·1 *N* potassium bromide.

For amounts of phosphorus from 10 to 100 mg., results obtained by the filtration method are accurate to within ±0·2 mg. Calcium and magnesium ions do not interfere, but in presence of more than 0·1 mg.-mol. of ferric iron or aluminium ions the results are not satisfactory. If the amount of sulphate ion present exceeds 100 mg., silver sulphate is precipitated.

B. ATKINSON

Reduction and Determination of Arsenates in Presence of Catalysts, especially Rhenium. S. Tribalat (*Analyt. Chim. Acta*, 1947, **1**, 149–152)—Arsenate is slowly reduced to arsenite by stannous chloride in presence of 3 to 4 *N* sulphuric acid, no arsenic metal or arsine being formed. The reduction is very slow, but is catalysed by small amounts of potassium perrhenate, by ammonium molybdate, or by larger amounts of potassium iodide, the first being the most effective catalyst. In the determination proposed the arsenate is reduced by stannous chloride in presence of potassium perrhenate, the excess of stannous chloride oxidised by an equivalent amount of iodine added to the acid solution, and the arsenite titrated by iodine at *p*H 8·3. As the titration is not quantitative in presence of stannic hydroxide, tartaric acid is added to form a complex with the tin.

Procedure—Place in a 1000-ml. Erlenmeyer flask 25 ml. of the approximately 0·1 *N* arsenate solution, 7 to 8 ml. of 18 *N* sulphuric acid, 0·1 ml. of a 10^{-3} *M* solution of potassium perrhenate (or 0·5 g. of potassium iodide), 15 g. of tartaric acid, and 10 ml. of approximately 0·2 *N* stannous chloride made 0·5 *N* with respect to hydrochloric acid. Boil gently for 10 to 15 min., cool, and dilute with an equal volume of water. Add starch and titrate with 0·1 *N* iodine just until the blue colour appears. Neutralise to litmus paper with 6 *N* sodium hydroxide, dilute to 350 to 400 ml., saturate the solution with sodium bicarbonate, and titrate with 0·1 *N* iodine, preferably with the arsenite solution at 30° to 35° C. The results obtained are accurate to within about ±1 part in 500.

B. ATKINSON

Quantitative Determination of Potassium or Sodium Ferrocyanide. P. J. Hol and N. van der Burgh (*Chem. Weekblad*, 1947, **43**, 571–573)—In determining ferrocyanides by titration with a zinc solution, using ferric chloride as external indicator, very variable results were obtained by different analysts. This is due partly to the method of determining the end-point. When a drop of the reaction liquid is dropped on to filter paper, three zones are formed; the inner one holds the precipitate, the intermediate one the excess of ferrocyanide, and the outer one is of water only. When testing with a drop of ferric chloride solution, it is essential that this should come into contact with the intermediate zone, and not with the inner one, as the precipitate always gives a blue colour with ferric chloride. To avoid this, a filter paper should be folded in half, the mixture dropped on one half, and the test applied to the liquid that filters through to the other half. The result of the titration is also affected by the speed of addition of the zinc solution, since the zinc ferrocyanide first precipitated is only slowly converted into potassium zinc ferrocyanide by the excess of potassium ferrocyanide present. For consistent results, it is essential to maintain the same speed of stirring and the same rate of titration.

G. MIDDLETON

Deposition of Nickel and Cobalt by Chemical Reduction. A. Brenner and G. Riddell (*J. Res. Nat. Bur. Stand.*, 1947, **39**, 385)—Quantitative reduction of nickel salts to the metal in an ammoniacal hypophosphite and tartrate solution by the Paal and Friederici method (*Ber.*, 1931, **64**, 1766) is obtained by using a solution that is 5 per cent. with respect to sodium hypophosphite and 60 per cent. with respect to ammonium chloride; no catalyst is necessary. M. E. DALZIEL

Contamination of Silicate Samples Crushed in Steel Mortars. E. B. Sandell (*Anal. Chem.*, 1947, **19**, 652–653)—The extent of contamination of quartz and felspar by crushing in a hardened steel mortar of the Plattner type has been investigated. Pieces of clear quartz crystal, given 200 moderately heavy hammer blows and sifted through 0·7-mm. holes in paper after every 20 blows, were contaminated by iron 280, manganese 1·8, chromium 0·4, vanadium <0·1, nickel 0·25, cobalt <0·1, and copper 0·35 p.p.m. A piece of microcline treated similarly, but with only 60 hammer blows, was contaminated by iron, 170 p.p.m. Other similar samples of quartz and microcline crushed without the use of the close-fitting collar of the Plattner mortar were contaminated to a lesser extent. This contamination by iron, about 0·02 or 0·03 per cent., does not seriously affect the total iron content of a rock, but since 0·03 per cent. of metallic iron is equivalent to nearly 0·1 per cent. of ferrous iron, the ferrous iron content will be raised appreciably and the ferric iron content lowered. With the possible exception of chromium, the amounts of the minor constituents introduced from the mortar can be disregarded. By constructing the mortar of a steel selected for its low chromium, nickel, and copper contents, contamination of the sample can be kept small enough for present-day requirements in determining trace elements in silicate analysis. M. E. DALZIEL

Errata: June issue, p. 353, column 1, lines 13 and 11 from the bottom—
for "adl" *read* "add."
for "*p*H 7·d" *read* "*p*H 7·1."

Physical Methods, Apparatus, etc.

Spectrographic Analysis of Low-Alloy Steel. Statistical Examination of Sources of Error. H. T. Shirley, E. Elliott, and J. Meeds (*J. Iron and Steel Inst.*, 1947, **157**, 391–409)—Analytical results with low-alloy steels having indicated a lower order of accuracy than certain claims in the literature, notably those of Vincent and Sawyer (*J. Opt. Soc. Amer.*, 1942, **32**, 686), an extensive study was made with a view to obtaining quantitative indications of the magnitude of various contributions to the total variability.

Most of the work was carried out with a single sample of nickel - chromium - molybdenum steel having the composition: C 0·30, Si 0·25, Mn 0·58, Cr 0·77, Ni 2·75, Mo 0·50 per cent.

For the main investigation, this sample was used to prepare 31 plates, from each of which 30 spectrograms were read, involving some 60,000 readings from upwards of 9000 separate lines. These readings were so taken as to permit statistical examination for the separation of the variability into three portions, corresponding essentially to: (1) excitation response; (2) plate variability over small distances comparable with the length of line read; (3) microphotometry errors.

This separation was not absolute; for example, the excitation response figures would contain any contribution from plate variability over larger distances than those catered for under (2), but the analysis of the data has permitted a reasonably effective separation and indication of the relative magnitude of the contributions from these three main sources of variation.

All the work has been carried out in terms of log "intensity" ratios (Shirley and Elliott, *J. Iron and Steel Inst.*, 1943, No. 1, 299–337) and not individual line readings, since this is more closely related to working conditions. Silicon, manganese, chromium, nickel, and molybdenum were read, using line pairs listed in an earlier paper (*loc. cit.*).

Readings were carried out in triplicate. In certain selected cases, nine repeat readings permitted an estimate of residual reading error corrections applicable to the means of three readings. By reading each 1·7-mm. length line for two independent 0·5-mm. lengths, it was possible to estimate plate variability over such small distances.

The three most important standard deviations calculated were as follows:

σ_{TC}, corresponding to the total variability other than microphotometry error obtained from the differences of the 60 readings for a given element for any one plate and the mean for that plate, and corrected for reading error. Thus $\sigma_T = \sqrt{(\Sigma dT^2/59)}$ and $\sigma_{TC} = \sqrt{(\sigma_T^2 - \sigma_{RT}^2)}$, where σ_{RT} is the standard deviation corresponding to the estimated reading error still present in the means of three results used for the initial calculation.

σ_{pc}, the small-scale plate contribution described above, and based on the difference between the readings from two positions for each line pair. It was calculated from $\sigma_2 = \sqrt{(\Sigma dp^2/60)}$ with correction for residual reading error.

σ_{SC}, representing essentially the variability from sparking response, including contributions from both sample and excitation source, and calculated according to

$$\sigma_{SC} = \sqrt{(\sigma_{TC}^2 - \sigma_{PC}^2)}.$$

Finally, having obtained estimated mean values for these standard deviations for various conditions and types of plate, it was possible to recombine them to obtain comparative estimates for σ_T for various sets of conditions. Consideration of the data indicated the following conclusions.

Excitation response (σ_{SC})—Using the normal Hilger 15,000-v. transformer with 0·005-μF. condenser and no added inductance, a definite advantage was found for silver and graphite auxiliary electrodes over the use of a steel electrode cut from the sample. An 80° point, sparking against a flat surface on the sample, was employed in all cases. Pre-sparking for 15 sec. was also advantageous, but no significant

change was observed from the use of a synchronous motor generator feeding the excitation unit in place of the normal voltage regulator. The lowest variability in terms of logarithmic ratio was obtained with graphite auxiliary electrodes, but this was offset to some extent by a reduction in slope of the composition curve. In terms of logarithmic ratio, the standard deviations obtained from the means of the whole series for graphite auxiliary electrodes with pre-sparking ranged from 0·0050 for silicon, through 0·0045 for manganese and chromium to 0·0025 for nickel and molybdenum.

Plate variability (σ_{PG})—In this respect the Ilford Thin Film Half-Tone plate was easily the best of those tested, giving a standard deviation, in terms of logarithmic ratio, of 0·0020 as compared with 0·0030 for Kodak B.5 and B.10 and Eastman Kodak S.1 types. Kodak B.20 type gave a value of 0·0040 and Ilford Ordinary 0·0070. The variability over larger distances appeared to be least for the Thin Film and greatest for the Ordinary plates.

No significant difference was found between development with initial agitation only and continuous agitation with a Kodak type metal blade "squeegee."

Microphotometry (σ_R)—A mean estimate of 0·0030 in terms of logarithmic ratio was made for this under ordinary reading conditions, with a rather lower value of 0·0025 for the Ilford Thin Film plates.

Total variability (σ_T)—Calculated from appropriate values from the above standard deviations converted into terms of percentage of the element content, the following mean figures were obtained for the five elements read:

(*a*)	Original combination of silver auxiliary electrode with no pre-sparking and Kodak B.20 plates	2·5
(*b*)	Graphite auxiliary electrode with 15 sec. pre-sparking and Ilford Thin Film Half-Tone plates	1·7
(*c*)	Silver auxiliary electrode with 15 sec. pre-sparking and Ilford Thin Film Half-Tone plates	1·8

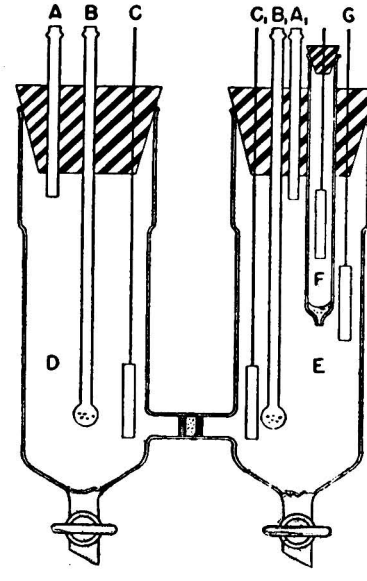
Comparison with data by Vincent and Sawyer—Considering the results for chromium under (*b*) above, a sparking response variability of 1·44 per cent. of content was obtained compared with a figure of 0·50 per cent. from the American data. Small-scale plate variability was 0·78 per cent. compared with a figure of 0·51 per cent. from the American figures after recalculation on the general lines used in the present work. Corresponding figures for microphotometry were 1·02 and 0·25 per cent.

Thus, for optimum conditions in the present work, a total deviation for chromium of 1·9 per cent. of the content corresponded to a figure of 0·76 per cent. calculated from the Vincent and Sawyer data.

B. S. COOPER

Titration of Acids by Electrolytically Generated Hydroxyl Ion. J. Epstein, H. A. Sober, and S. D. Silver (*Anal. Chem.*, 1947, **19**, 675–677)—Small amounts of acids can be titrated with hydroxyl ions generated by the electrolysis of sodium bromide solution in a special cell. The anode and cathode are in separate compartments connected by a glass tube into which is sealed a porous glass plate; the acid is introduced into the cathode compartment. The end-point is determined potentiometrically by means of two indicator electrodes in the cathode chamber.

The cell, shown in the figure, is designed for the determination of acids in air. AA_1 are air outlets, BB_1 are air inlets, CC_1 are the platinum electrolysis electrodes, D and E are the anode and cathode compartments, respectively, and G is the platinum



titration electrode. The reference electrode, F, is a glass tube drawn out to a fine tip and filled with citrate buffer, *p*H 4·3, into which is inserted a platinum wire. The tip is sealed with a 10 per cent. agar solution containing 5 per cent. of sodium bromide. The electrolysing electrodes are connected in series with a battery providing 3 to 6 v., a milliammeter reading to 20 ma., a 10,000-ohm variable resistance, and a switch. The indicator electrodes are short-circuited through a 15,000-ohm variable resistance and a galvanometer shunted with a 2000 ohm variable resistance. The galvanometer has a sensitivity of $0{\cdot}025 \times 10^{-6}$ microamp. per mm.

Procedure—Fill the cell with 0·3 *M* sodium bromide saturated with quinhydrone, and draw the air to be analysed slowly through the cathode compartment. Pass a constant, known current of about 10 milliamp. across the electrolysing electrodes and note the time for the potential across the indicator electrodes to fall to zero. The liquid in the cathode chamber is then at *p*H 4·3, and the amount of electricity that passes in the electrolysis circuit is proportional to the amount of acid originally present.

Owing to the use of *p*H 4·3 in the reference electrode, dilute solutions of strong acids give

slightly low results, but the use of this hydrogen ion concentration prevents interference from carbon dioxide. Results reproducible to within ±4 per cent. are obtained for 30 to 150 μg. of acid, the main sources of error being the measurement of current and time. The method can be adapted for determining gaseous substances that are oxidised by bromine, if use is made of the bromine liberated at the anode. J. G. WALLER

Some Applications of Inorganic Chromatography. G. Robinson (*Metallurgia*, 1947, **37**, 45–47, 107–108)—Since inorganic chromatography depends on stoicheiometric reaction, there is likely to be correlation between band-length and percentage composition. Absorption on alumina of metallic cations, ammine complexes, and tartrate complexes is in the order given below.

Metallic cations	Ammine complexes	Tartrate complexes
As	Co	Ni, Co, Zn, Cd, Mn
Sb	Zn	Pb
Bi	Cd	Cu
Cr, Fe^{III}, Hg	Ni	Bi
UO_2	Ag	Fe
Pb		Cr
Cu		
Ag		
Zn		
Fe^{II}, Ni, Cd, Co		
Tl		
Mn		

Adsorbed cations are identified by "developing," after thorough washing of the column, with a reagent producing coloured bands, ammonium sulphide and potassium ferrocyanide being particularly useful. Copper - nickel solutions can be analysed by adsorption on alumina and, on development of the column with ammoniacal dimethylglyoxime solution, the top copper layer is washed out as the copper ammine while the nickel is fixed as the red complex. When applied to nickel steels, some nickel is removed in the filtrate with iron as the alkaline tartrate complexes. Better results are obtained by using alumina pre-coated with dimethylglyoxime, and developing with alkaline ammonium tartrate; alumina coated with salicylaldoxime is probably better; 8-hydroxyquinoline cannot be used. In general, pre-coating with the reagent is found to give better results, especially when using sodium diethyldithiocarbamate for copper.

Application to brazing alloy—Adsorption on an 8-hydroxyquinoline column is known to give the following banded separation; VO_3' dark grey, WO_4'' yellow, Cu^{II} green, Bi^{III} yellow, Ni^{II} yellow, Co^{II} reddish, Zn^{II} yellow, Fe^{III} black, and UO_2^{II} orange.

It was required to find the variation of zinc content of the alloy during brazing, and whether the loss of zinc is constant with a given initial alloy receiving a constant heat-treatment procedure.

Reproducibility of bands was obtained by using an adsorbent of equal parts of 8-hydroxyquinoline and soluble starch, dried and ground to pass a 60-mesh and be retained by an 80-mesh sieve. The tubes were of 5-mm. internal diameter, and 15 to 20 cm. length. Solutions containing 2 mg. per ml. were satisfactory, but as little as 0·1 to 1 mg. per ml. could be used by controlling the *p*H at 5 to 6. To distinguish between the yellow complexes of nickel and zinc, the green fluorescence of zinc in ultra-violet light is employed.

Procedure—Dissolve 2 g. of the metal in nitric acid and evaporate to fuming with 10 ml. of concentrated sulphuric acid. Dilute to 1 litre and buffer the solution to *p*H 5 to 6 with sodium acetate.

After brazing, dissolve the 4 to 10 mg. of metal obtained by a similar method in a final volume of 10 ml. Prepare two adsorption tubes and, taking a volume of the referee solution containing the same weight of metal as the test, pass one solution through each column. Wash with equal volumes of distilled water in 4 or 5 portions. Determine the length of the zinc band in ultra-violet light, and calculate the variation by proportion.

Results show a constant variation after equal times in the furnace. As little as 5 μg. of zinc in the presence of 50 μg. of nickel and 2000 μg. of copper can be detected when a tube 2 mm. in internal diameter is used.

Violuric acid and 5-oxo-4-oximino-3-phenyl*iso*-oxazoline when diluted with equal quantities of starch, have each been used as adsorbents; the former gives a red band with sodium and the latter a yellow band with potassium.

Other possible reagents for separating the components of metallic mixtures are (*a*) sodium diethyldithiocarbamate: Cu—brown, Bi, Mn, Ni, Co, and Zn—greyish; (*b*) diphenylcarbazide, Cr^{VI}, and possibly Co, Cu, Pb, and Ni; (*c*) α-α′-dipyridyl: Mo; (*d*) rubeanic acid (dithio-oxamide): Cu, Co, Ni, and Ag; (*e*) salicylaldoxime: Cu and Ni; (*f*) toluene-3-4-dithiol: Ag, Hg, Cd, As, Sb, Pb - Cu, Ni, Co - Sn, Bi, Mo, W, Rh, and Pd; (*g*) dimercaptothiodiazole: Hg^{I}, Ag, Pb, Sn, Sb, Cu, Hg^{II}, Bi, Pd, Th, and Au.

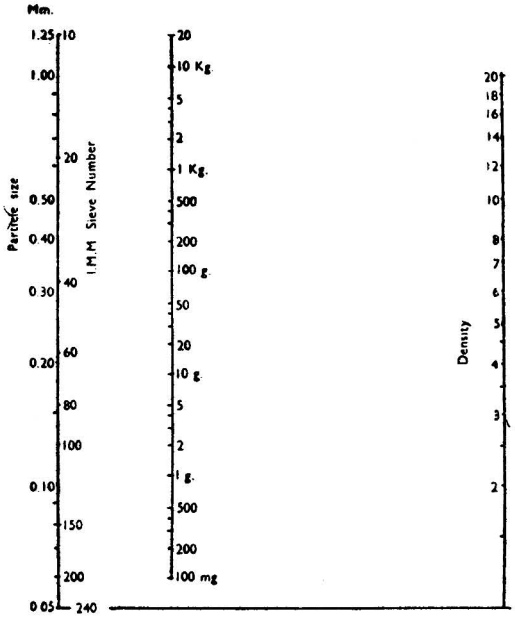
True adsorption of metallic organic complexes from solutions in organic liquids according to the original method of Tswett is suggested, and separation may be effected by elution.

Uniformity of packing, salt concentration, and *p*H must be carefully controlled for reproducible results; washing with solutions of differing *p*H may remove bands selectively.

Partition chromatography may be applicable using organic metal complexes in organic solvents and adsorption on a column of silica gel, cellulose, or starch, which holds the more polar phase of a pair of liquids, such as chloroform - water mixtures. Incorporation of an organic reagent in the adsorbent as an indicator for metallic ions would be advantageous, if practicable. M. E. DALZIEL

Sampling Problems. W. van Tongeren (*Chem. Weekblad*, 1947, **43**, 354–362)—A consideration of the factors involved in obtaining a representative sample from a large quantity of non-uniform material leads to the following conclusions. (1) The number of stages should be as small as possible. (2) In each stage, the smallest permissible quantity of material should be operated on. (3)

At each decisive stage, the portion taken should be equal to the minimum quantity required to give satisfactory results. This minimum value represents about one million particles. (4) The quantities operated on in successive stages should be related in powers of two, *i.e.*, the sample is



Nomogram for ascertaining minimum weight of sample to be taken

selected by successive halving. The minimum permissible sizes for samples for materials of different degrees of fineness and densities may be derived from the accompanying nomogram.

Owing to the irregularity of form of the powdered particles, these values are only approximate.

G. MIDDLETON

The Fenton Test for the Cleanliness of Fillings and Stuffings for Bedding, Upholstery, Etc. (*British Standard* 1425: 1948)—A modification of this test has been adopted by the British Standards Institution, and is included in the specification cited above (*cf.* ANALYST, this vol., p. 398).

Procedure—Place 25 g. of sample in a 2-litre Kilner-type jar, add, at 20° C., 500 ml. of a freshly prepared solution, made by mixing 415 volumes of distilled water with 12 volumes of aqueous ammonia (sp.gr. 0·880), 75 volumes of methyl alcohol, and 100 volumes of acetone. Shake in a Wagner-type machine for 20 min. at the rate of one revolution per second. Open the jar, and insert a plunger made by forming a flat spiral, 2¼ inches in diameter, and consisting of 3 turns, on the end of a length of steel wire, and loaded behind the spiral with a weight of 1 lb. Immediately the plunger comes to rest, pour off the liquid through an 8-in. diam. 100-mesh test sieve (B.S. 410) supported in a funnel delivering into a narrow-mouthed stoppered bottle.

For down or feathers, or mixtures of both, take 12·5 g. of sample and add 210 ml. of a washing solution prepared from 75 ml. of distilled water, 75 volumes of methyl alcohol and 100 volumes of acetone. Ensure that the sample is completely wetted by inserting a plunger with a flat metal end instead of a spiral for a few seconds. Remove the plunger, allow it to drain and add immediately 290 ml. of a solution prepared by mixing 340 volumes of distilled water, and 12 volumes of aqueous ammonia (sp.gr. 0·880). Loosen the mass by means of a wire hook, close the jar and shake in the machine for 20 min. at the maximum speed that allows the wetted mass to fall from end to end of the jar at each revolution. Continue as in the standard method above.

Soluble impurities—Mix the washings, transfer 200 ml. to a tall beaker and evaporate to 100 ml. Cool, make up to 200 ml. with water, and transfer to a 500-ml. conical flask. Add one 11-cm. No. 44 Whatman filter paper in small shreds or one 4·5-cm. by 4-mm. Whatman filter tablet, shake, and filter through a dry No. 1 Whatman filter paper without suction and re-filter the first runnings. Take 100 ml. of the filtrate in a tared dish, evaporate to dryness over steam and complete the drying in an oven at 98° to 105° C., cooling in a desiccator containing sulphuric acid before weighing; dry until 1 hour's further heating does not reduce the weight by more than 1 per cent.

Total impurities—Transfer 100 ml. of the original washings, after mixing well, to a tared dish and evaporate over steam, in small portions at a time. Complete the drying at 98° to 105° C. as for the soluble impurities.

Calculate the results for soluble and total impurities to a percentage of the dry sample. The difference between them represents the *insoluble impurities*.

F. L. OKELL

Reviews

MICRO-DIFFUSION ANALYSIS AND VOLUMETRIC ERROR. By EDWARD J. CONWAY. Second Edition. Pp. xi + 357. London: Crosby Lockwood & Son, Ltd. 1947. Price 21s.

It is only nine years since Professor Conway first published this book, but it already bids fair to become a classic of analytical chemistry. For much of that period, moreover, it has been unobtainable; stocks were destroyed during an air-raid and re-issue was for some time impossible owing to the conditions prevailing in the book-publishing business.

How is it that so young a book has, in such adverse circumstances, achieved a degree of authority not granted to many far longer established volumes by writers as authoritative in their own fields as Professor Conway in his, especially as he is not, in fact, an analytical chemist at all and would not call himself one? I believe there are two separate reasons for the recognised importance of "*Micro-diffusion Analysis and Volumetric Error*" and that they are indicated by the two distinct though connected subjects named in the title.

Many analysts—especially those approaching "the sere, the yellow leaf"—have something like a nostalgia for the kind of analytical chemistry that may rightly be regarded as both a craft and a science. Much though they respect the erudition and skill of their younger colleagues, who seem as much at home with spectroscope, polarograph, electrophoresis apparatus *et hoc genus omnes* as with air-damped balances and spot-tests, the reverent seniors still have a sneaking belief that the ability to manipulate relatively simple chemical glass-ware with an accuracy appropriate to the accuracy of its standardisation is the real bare bones of the matter. The elegance of the familiar simple burette and pipette has been, it may be, dimmed in our eyes by habituation: the complexity of many new methods and the armamentarium of physico-chemical apparatus, unavoidable as they undoubtedly are in an analytical world with increasingly complex problems to tackle, cannot ever yield quite the seductive bloom that was over the fruits of the older analysts' labours. Even the micro-analyst seems to them to have lost something of the earlier "*joie de titre.*" The invention of the Conway micro-diffusion unit, therefore, preceding by some years the appearance of Professor Conway's book, at once took by storm some analysts' imaginations. Here was something not only scientifically unimpeachable, but of the utmost elegance and accuracy withal. Here, moreover, was something that any analyst "skilled in the art" could learn to use, could use efficiently and could therefore revel in using. The ingenuity with which its inventor and his colleagues have extended micro-diffusion technique is witnessed by the list of 21 different determinations (p. 2) to which it has successfully been applied. Perhaps the appeal of the method has also been to some extent enhanced by the fact that no really expensive apparatus—at any rate, as prices go to-day—is required and that a large number of precise replicates can be carried out in a remarkably short time and at a cost that is low even when all allowance is made for capital outlay, that is, the price of units, micro-burette and test solutions, and depreciation, that is, breakages at the sink.

Parts I and II of this book are devoted, respectively, to "Apparatus and Principles Used in Micro-diffusion Analysis" (eight chapters) and "Description of Methods with the Standard 'Unit'" (twenty-five chapters). Thus, while Part I is about the same length as in the first edition (an increase of only 2 pages on 75), Part II has been increased by eleven chapters and 54 pages to 241. It is out of the question here to attempt even to name the new applications of Conway's technique now brought together for the first time in this edition, though most of the details have already appeared in *The Biochemical Journal* and elsewhere, but mention may perhaps be made of the rapidity with which blood dextrose can be determined thereby and the up-to-date application to carbonic anhydrase determination. It still remains the simplest, most rapid and probably most accurate method for determining urea in blood and urine. On the side of equipment, the most important innovation is certainly the introduction of the new smaller units, Nos. 2 and 2a.

The second cause that has led the more thoughtful analysts to put "Conway" on their shelves with many a standard work of far longer history lies in the subject-matter—and its treatment—named in the second half of the book's title and elaborated in Part III—"The Error of Volumetric Titration."

All elderly analysts have to a greater or less extent during the last two decades passed through a period of consciousness of sin and, let us hope, adequate expiation. The tacit, and perhaps smug, assumption that absolute quantitative truth is obtainable, provided only all the graduated apparatus involved has a "Class A" certificate, has given way to a tacit, and occasionally an expressed, acceptance of the fact that all analytical results are approximations. In the minds of many of them, moreover, the first doubts—should I say dawning of critical light?—came, or at least were put on a quantitative basis, by Part III of "Conway." To re-read it is good for the analyst's soul. It is not an easy form of penance, but its discussion of variable and constant errors, both glass and chemical, the distinction drawn between these four causes of imprecision or inaccuracy, the development of formulae that may be applied to their elimination—these are matters of the most fundamental concern to all the analyst fraternity and not solely to the little brothers of the micro technique, and must be read and pondered by all. When they have done this, few of them will fail to admit that the study, though perhaps arduous, especially for the less prehensile minds of middle-age, has been more than repaying to mind and soul.

I have been moved on a previous occasion to observe—and even on a subsequent one to repeat—that there is only one real difference between the analytical chemist and the biological assayist, for, whereas

the latter uses methods involving a large error, the extent of which he knows with considerable accuracy, the former uses methods involving a small error, of whose very existence he is as a rule completely ignorant. It is to Professor Conway in Part III of his book that analysts should render thanks because he has destroyed all excuse for this ignorance by the brilliant and convincing clarity with which he has expounded the whole subject of "Volumetric Error." Future generations of analysts will accept Conway's arguments and findings as an integral part of their training and present ones can merely offer their respectful gratitude and hope for many future new editions.

The next one might be made the opportunity for a few corrections. Thus "vaselin" should become "Vaseline" (it is a proprietary name), "c.c." should consistently become "ml." (in this edition it sometimes does—*e.g.*, p. 22—and it sometimes doesn't—*e.g.*, p. 9), the second sentence of par. 2 on p. 4 should be clarified—how can one avoid a micro-concentration?—and so on. No doubt Professor Conway has already noted most of these trivial defects himself. May we soon see them removed in a further enlarged and equally welcome third edition.

A. L. BACHARACH

A SURVEY OF DIAGNOSTIC AGENTS. By T. D. WHITTET, Ph.C., D.B.A. Pp. 32. London: The Pharmaceutical Press. 1947. Price 2s. 6d.

This book is concerned with the use of substances in the diagnosis of disease and in testing the efficiency of the various organs. As far as possible, the items are classified according to the part of the body to be examined and in each case a description is given of the mechanism of the test, the method of preparing the substance, the technique of administration, the results to be observed and the conclusions to be drawn. Thus, as an example, the second of the twenty-one sections into which the book is divided is entitled Examination of the Liver, and includes a description of Quick's hepatic function test (involving the administration of sodium benzoate and the determination of hippuric acid excreted in the urine), the laevulose and galactose tolerance tests, other tests severally using di-iodotetrachlorofluorescein, sulphobromophthalein, phenoltetrachlorophthalein and sodium salicylate, and concludes with an account relating to the use of colloidal thorium dioxide as a contrast medium in the X-ray examination of the liver and spleen. Other section headings, chosen at random, may be mentioned as follows: Cholecystography; Circulation Time; Diagnosis of Pregnancy; Allergy Tests; Biological Diagnostic Agents; The Tuberculins.

In a subsequent edition it might be helpful to allude under Renal Function Tests to the important researches of J. Trueta *et al* recently published in book form (*Studies of the Renal Circulation*: Blackwell Scientific Publications, 1947) which indicate that the interpretation of tests for renal efficiency needs more caution, since results may be due to temporary renal vascular dysfunction rather than to renal damage.

The work is essentially designed for the hospital pharmacist and for those studying for the Diploma in Biochemical Analysis. While allusion is frequently made to analytical procedures, no details are given as, presumably, it is intended that these shall be sought in treatises on chemical pathology. Nevertheless, the book provides valuable information for all analysts concerned with the examination of pharmaceutical products and it is well worth its modest cost.

N. L. ALLPORT

ORGANIC ANALYTICAL REAGENTS. By F. J. WELCHER, Ph.D. Vol. I, pp. xv + 442. Vol. II, pp. xi + 530. New York: D. Van Nostrand Company Inc. London: Macmillan & Co., Ltd. 1947. Price, each volume, 40s. net.

Each of these substantial volumes opens with an identical Preface wherein it is stated that: "The principal purpose in preparing this book has been to assemble in one place a description of all organic compounds used in the analysis of inorganic substances and to present a discussion of the methods employing these reagents." It is also stated that the treatment of the subject is intended to be complete but, apart from the obvious fact that the whole ground is not covered, there is nothing to indicate that more is to follow, although information derived elsewhere suggests that a four volume treatise is projected. The author applies a literal interpretation to the word "complete" and includes as reagents solvents employed in analytical procedures but, on the other hand, explains in an Introduction reproduced in the first volume that indicators used for the measurement of hydrogen ion concentration and oxidation-reduction reactions as well as adsorption indicators are not discussed.

The first forty-nine pages of Vol. I are occupied by an interesting disquisition touching on co-ordination, chelate compounds, the effect of structure on solubility and other theoretical considerations. The remaining twelve chapters describe the use of about one hundred and fifty organic reagents grouped according to their chemical class and, within each chapter, listed in alphabetical order; a further two hundred reagents are similarly dealt with in Vol. II. Thus, a start is made with acetylene under the general heading, hydrocarbons: after some physical and chemical properties there is a statement that it can be used for the detection and determination of copper and for the determination of gold, silver and palladium. Details for determining copper and silver follow, but only general outlines are given of the methods for dealing with gold and palladium. Together with the nineteen references this section occupies three and a half pages. Anthracene is the next reagent to be discussed and the method of applying it to the detection of bromine in chloroform solution is presented. Coming to benzene, its use for the detection of nitrates is described in fair detail; then half a page is occupied with allusions to its subsidiary role as a solvent for other tests as, for example, the colorimetric determination of bismuth by means of tetracetylammonium hydroxide, and the procedure

for this, together with a repetition of the references, is to be found in Vol. II in the mnographo dealing with this reagent. Again, heptane enjoys a section to itself and is stated to be used for the determination of ozone although, in fact, it is but the selected solvent for another reagent, butyraldehyde. Referring to the latter, it transpires that a better solvent for it is 2 : 2 : 4-trimethylpentane, and referring back to the chapter on hydrocarbons one finds nearly half a page devoted to this solvent, although it is employed only for this one purpose.

Following on the same plan, Vol. I includes chapters on phenols, aminophenols, ethers, aldehydes, and so on. Under alcohols one notices ethylene glycol monobutyl ether and ethylene glycol monomethyl ether described under the commercial names butyl cellosolve and methyl cellosolve respectively. The lengthy monograph on ethyl alcohol summarises its use as a solvent in the separation of the alkali metals and alkaline earth metals. Amyl alcohol is stated to be used for the detection of twenty-one metals and for the determination of fifteen, but only in the sense of being a solvent for a salt or for a colour produced by other reagents. Continuing the chemical classification, the chapter on amines occupies over two hundred and fifty pages, equal to half the descriptive text of Vol. II, a fact exemplifying the weakness of the plan adopted for this otherwise excellent treatise.

Consequent upon the aim to produce a comprehensive survey of the subject the author observes that "many obviously inferior reagents and methods are included." This policy is claimed to be justified on the grounds that time need not be lost in attempting procedures demonstrated to be unsatisfactory, and that inferior methods may, with suitable modifications, be made useful for certain purposes. However, indications of inferiority are not sufficiently emphasised and, unfortunately, imperfections in analytical processes are not always apparent from textual descriptions.

Wherever laboratory-scale synthesis of a reagent is practicable and likely to be useful a method of preparation is included in the appropriate monograph. This is a particularly valuable feature and the author, well aware that some of the instructions may not be altogether satisfactory, solicits the help of readers towards improving this aspect of his book.

Notwithstanding the claim that all relevant work mentioned in *Chemical Abstracts* up to the end of 1945 is covered, it would seem that there are omissions. Thus it is to be regretted that under *p*-aminodimethylaniline there is no mention of the interesting procedure developed by A. T. Palin (ANALYST, **1945, 70**, 203; *Chemical Abstracts*, 1945, **39**, 4172[8]) for the determination of free chlorine and chloramine in water. Again, although under *p*-methylaminophenol the use of this reagent in the form of its sulphate for the colorimetric determination of phosphates is mentioned (but not described in detail) there is no allusion to the work of Ernst and Emilio Tschopp (*Helv. Chim. Acta*, 1932, **15**, 793) who made the most important contribution towards the development of this widely used procedure.

In a work of this size a few omissions are, perhaps, inevitable and should not influence one's judgment; but the unusual approach to the subject, with the emphasis on the reagent rather than on the substance to be detected or determined, is more difficult to condone because it has resulted in much repetition with consequent waste of space that might otherwise have been utilised by including all working details for many of the more complicated separations instead of mere discussions of principles. However, direct reference to substances to be tested, or to reagents, is rendered possible by the provision of two indexes while, in justice to the author, it must be admitted that in certain instances the plan is vindicated, notably in the excellent chapter of sixty pages embodying over three hundred references, on 8-hydroxyquinoline and its derivatives. Doubtless, in a subsequent volume, there will be an equally useful monograph devoted to diphenylthiocarbazone.

It is a much simpler matter to review a book than to write one and, notwithstanding the above criticisms, these two handsomely bound and moderately priced volumes are to be highly commended; indeed, they deserve to find a place on the shelves of every analytical and chemical research laboratory throughout the world. The author is to be congratulated, not only for these volumes already published, but for his courage in undertaking a task of such monumental proportions. N. L. ALLPORT

NORTH OF ENGLAND SECTION AND PHYSICAL METHODS GROUP

A JOINT meeting of the North of England Section and the Physical Methods Group will be held at the Stork Hotel, Queen Square, Liverpool, 1, on Saturday, October 2nd, 1948, at 2.0 p.m. The following papers will be presented and discussed—

"Analysis of Rare Earth Oxides by Means of Emission Spectra."
By D. M. Smith, B.Sc., D.I.C., F.Inst.P., and G. M. Wiggins.

"Determination of Rare Earths, Using the Intermittent Arc."
By J. A. C. McClelland, B.Sc., Ph.D., A.R.I.C.

"The Chromatographic Estimation of Vitamin A in Whale Liver Oil."
By N. T. Gridgeman, B.Sc., A.R.I.C., G. P. Gibson, B.Sc., Ph.D., F.R.I.C., and J. P. Savage, B.Sc., A.R.I.C.

Further information on the arrangements for the meeting may be obtained from the Hon. Secretary of the North of England Section, Mr. Arnold Lees, F.R.I.C., 87, Marshside Road, Southport, Lancs.

PHYSICAL METHODS GROUP

THE following meetings have been arranged for the session 1948–49.

November 30th, 1948—At the Imperial College of Science and Technology, London. Annual General Meeting of the Group, followed by a lecture on "The Measurement of Colour," by Mr. R. Donaldson.

January 25th, 1949—At the Imperial College of Science and Technology, London, S.W.7. A symposium on theological methods.

April 1st, 1949—At University College, Nottingham. A symposium on electrophoretic analysis.

BRIGHTON CORPORATION WATERWORKS

Appointment of Chemist & Bacteriologist

Applications are invited for the above appointment at a salary in accordance with Grade VII (£635–£710) of the National Joint Council Scales for Administrative, Professional and Technical Staffs. Applicants should be qualified chemists and bacteriologists and have had practical experience in the examination and treatment of water for public water supplies.

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A form of application may be obtained from the Waterworks Engineer, 12, Bond Street, Brighton, and should be completed and returned together with copies of three recent testimonials in an envelope endorsed "Appointment of Chemist and Bacteriologist," so as to be received not later than 10 a.m. on Saturday, August 28th, 1948. Canvassing will disqualify.

J. G. DREW,
Town Clerk.

TOWN HALL,
BRIGHTON.
July, 1948.

HONOURS GRADUATE CHEMIST with personality and initiative required by Marks & Spencer Ltd. to assist in pursuing a progressive policy towards scientific control of food standards, storage and distribution. Applicant should have post-graduate experience of food analysis and/or applied food research. Some acquaintance with bacteriological technique would be an advantage. Age 25–35 years. The successful applicant will be expected to develop the food laboratory of which he will be in charge. Applications should be addressed to Marks & Spencer Ltd., Personnel Dept., 82, Baker Street, London, W.1.

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