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Editor: J. H. LANE, B.Sc., F.R.I.C.
7-8, IDOL LANE, LONDON, E.C.3
Telephone: MANsion House 6608

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THE ANALYST

PROCEEDINGS OF THE SOCIETY OF PUBLIC ANALYSTS AND OTHER ANALYTICAL CHEMISTS

A JOINT Meeting of the Society and the Food Group of the Society of Chemical Industry was held at 8.15 p.m. on Wednesday, December 6th, 1950, in the Meeting Room of the Chemical Society, Burlington House, London, W.1. The President, Mr. George Taylor, O.B.E., F.R.I.C., having opened the proceedings, invited Mr. A. L. Bacharach, M.A., F.R.I.C., Chairman of the Food Group, to occupy the chair for the remainder of the meeting.

The following paper was presented and discussed: "Applications of Paper Chromatographic Methods in the Sugar Industry," by H. C. S. de Whalley, F.R.I.C., M.I.Chem.E., N. Albon, B.Sc., A.R.I.C., and D. Gross, Dip.Ing., Ph.D.

NEW MEMBERS

ELECTED NOVEMBER 1ST, 1950

A. Claassen, Doctor of Chemistry; Glyn Davies, B.Sc. (Lond.), A.R.I.C.; Jack Whitehead, B.Sc. (Lond.).

ELECTED DECEMBER 6TH, 1950

Eric Morgan Hall, B.Sc. (Birm.), A.R.I.C.; Harry Gordon Ogden; Raymond Todd, B.Sc.; Arthur Israel Vogel, B.Sc., M.Sc., D.Sc. (Lond.), D.I.C., F.R.I.C.; Ronald Newall Woodward, A.M.C.T., A.R.I.C.

MINISTRY OF FOOD

APPOINTMENT OF SUB-COMMITTEE TO REVIEW THE PUBLIC HEALTH (PRESERVES, ETC. IN FOOD) REGULATIONS

As a result of a joint representation of the Food Group of the Society of Chemical Industry and this Society, the Food Standards Committee, appointed by the Minister of Food in January, 1948, has set up a Sub-Committee, under the chairmanship of Professor E. C. Dodds, M.V.O., M.D., D.Sc., F.R.C.P., F.R.I.C., F.R.S., Director of the Courtauld Institute of Biochemistry, with the following terms of reference—

> "To review the Public Health (Preserves, etc. in Food) Regulations and to make any recommendations the Sub-Committee may consider desirable for the amendment of the Regulations."

The Society is represented on this Sub-Committee by Dr. H. E. Cox, Ph.D., D.Sc., F.R.I.C.

Analytical Methods Committee

SUB-COMMITTEE ON VITAMIN ESTIMATIONS

Report from the Chemical Panel

THE Analytical Methods Committee has received the following Report from the Chemical Panel of the Sub-Committee on Vitamin Estimations, and its publication has been duly authorised.

The constitution of the Chemical Panel is: Professor W. H. Linnell (Chairman), Dr. A. J. Amos (Honorary Secretary), Mr. D. C. M. Adamson, Miss H. E. Davey, Dr. E. R. Dawson, Mr. J. King, Dr. F. W. Norris, Mr. T. L. Parkinson, Dr. H. G. Rees, Mr. W. Smith, Dr. S. Y. Thompson and Dr. F. Wokes.

REPORT

The Chemical Panel of the Sub-Committee on Vitamin Estimations was appointed for the purpose of ascertaining whether it could recommend a chemical method for the determination of nicotinic acid in a range of foodstuffs covering cereal products, yeast products and meat products. The Panel selected for study a published method; a method that was being used with success by a Panel member as a routine test on meat products; and a method that was proving satisfactory in the laboratories of another Panel member as a routine test on cereal products. Each of these methods involved the measurement of the colour developed by the interaction of nicotinic acid, cyanogen bromide and an amine. A long and detailed study of these three methods was undertaken and numerous modifications of them involving changes in the amine, the type of "blank" and the temperature of colour development were investigated. At no stage of the work, however, did the results returned by different laboratories show sufficiently satisfactory agreement to enable the Panel to recommend a particular technique as suitable for the complete range of foodstuffs being investigated. The conclusion was reached that a more fundamental approach to the problem is required before a standard chemical method for determining nicotinic acid can be devised.

The Evaluation of Liming Materials for Agricultural Purposes

BY A. M. SMITH, A. COMRIE AND K. SIMPSON

(*Presented at the meeting of the Society on Wednesday, October 4th*, 1950)

Experimental evidence is submitted to show that the value of a burnt lime for agricultural purposes may be seriously under-estimated by the method prescribed under the Fertiliser and Feeding Stuffs Act. Calcium silicates and magnesium oxide are able to neutralise soil acids, and the latter is also important as a source of magnesium in certain soils. Various methods of estimating the neutralising value have been examined and a simple technique, involving a short treatment with dilute acid and applicable to both carbonates and limes, has been found to give results in agreement with the effects produced on soil acidity in pot and field experiments.

THE question of the assessment of the agricultural value of a burnt lime has been raised many times in the last twenty years. The official method[1] makes use of the fact that a sugar solution dissolves calcium hydroxide only, and gives a value for the free calcium oxide in a sample. It is thus quite satisfactory for the analysis of a lime obtained from a properly-burned calcium carbonate limestone, where the calcium oxide is the only material of neutralising value. The method does not take into account any carbonate that may be left as a result of under-burning and it ignores almost entirely the presence of any magnesium

oxide, which has a greater neutralising value than calcium oxide and, incidentally, is invaluable as a source of magnesium in magnesium-deficient soils.

Many burnt limes contain other compounds that are of value in reducing soil acidity and increasing the amount of exchangeable calcium in the soil. These compounds are of the nature of calcium silicate and may be produced when limestones containing considerable amounts of siliceous material are burned. They are insoluble in sugar solution, but are so readily hydrolysed that they decompose ammonium salts and neutralise soil acids to an equivalent extent; they also dissolve easily in dilute acid and are, without doubt, of value as "liming material." The agricultural value of many burnt limes is, therefore, seriously under-estimated by the official method.

Attention was first directed to this question in 1931 in a communication to the Agricultural Education Association[2] concerning the behaviour of a waste carbonate after burning. It had long been appreciated that basic slag had a "lime value" greater than that corresponding to the small proportion of free calcium oxide it contained. Indeed it has frequently been claimed that a high-grade slag has the same lime value as an equal weight of limestone. This is an exaggeration, but a slag with a high solubility in citric acid does reduce soil acidity to the same extent as about half or two-thirds of its weight of calcium carbonate, because it contains compounds which readily provide an acid soil with exchangeable calcium.[3] A large proportion of the calcium in slag occurs in combination with phosphorus and silica as a result of the reaction at a high temperature between lime and the impurities in pig iron. Similar types of siliceous compounds are probably produced in a lime kiln and so it was not surprising to find that the lime value of certain burnt limes was indeed greater than indicated by their contents of free calcium oxide.

Methods of analysis

A new chemical method of evaluating an agricultural lime must be based upon convincing data obtained from experimental work with soils. It is difficult to obtain precise results on the effects of liming materials in the field on account of sampling errors, but it is nevertheless important that the accurate observations from laboratory and pot culture experiments should be supported by figures found under natural conditions.

In a preliminary approach to the question a number of burnt limes in use as liming materials were examined by five methods and the results are summarised in Table I. To measure the effect on soil acidity, a series of flasks each containing 20 g of soil, 50 ml of boiled distilled water and different quantities of the burnt lime were shaken at intervals

Table I

The evaluation of burnt lime by different methods

Burnt lime	Equivalent CaO by			CaO by	
	Soil titration	NH_4Cl distillation (*a*)	T.N.V. (*b*)	Conc. acid (*c*)	Sugar method (*d*)
	%	%	%	%	%
A	54	57	57	59	33
B	66	65	65	69	46
C	67	68	68	73	46
D	83	81	82	81	61
E	85	82	80	84	65
F	88	83	82	86	66
G	92	91	88	81	70
H	90	90	87	86	71
I	97	89	87	86	77

Methods employed—

(*a*) 0·2 g of material + 40 ml of *N* ammonium chloride + 700 ml of water boiled for 90 to 120 minutes, the distillate being collected in standard acid and the NH_3 expressed as CaO.

(*b*) *Total neutralising value*—0·5 g of material + 50 ml of 0·5 *N* hydrochloric acid boiled for 3 minutes, cooled and excess of acid titrated with 0·5 *N* sodium hydroxide with phenolphthalein as indicator; the result is expressed as equivalent CaO.

(*c*) Suitable quantity of material boiled with concentrated hydrochloric acid for 10 minutes and calcium in filtrate determined by standard method.

(*d*) The official method of determining caustic lime.

during several days until an approximate equilibrium was attained, and the final pH values of the suspensions were plotted against the amounts of lime added. A similar curve was obtained with a standard, usually precipitated calcium carbonate, and the relative value of the lime in reducing the acidity was estimated from the two curves. Each figure given in Table I is the average for three acid soils and represents the percentage efficiency or lime value of each material compared with a standard treatment. To take a particular example, 54 parts of lime (CaO) reduced the soil acidity to the same extent as 100 parts of sample A, at a point on the curve corresponding to an application of about 1 ton of lime per acre.

This method is a refinement and extension of that used in the routine estimation of the "lime requirement" of soils, where equal portions of a particular soil are shaken overnight with equal volumes but different concentrations of lime water and the final pH values are plotted against the amount of lime added.[4,5] The amount of lime required to bring the pH value of the soil to any level desired in practice can be interpolated on the curve and the dressing required in the field is calculated with or without a factor according to circumstances. Although the method is not suitable for the routine examination of liming materials it has been employed frequently in special cases and it has given valuable evidence in testing the reliability of the measurement of total neutralising value (T.N.V.). The method cannot be as precise as a chemical determination because the time required to reach equilibrium depends upon the nature of the soil and the physical and chemical properties of the liming material concerned. But it is possible to obtain smooth titration curves (some examples are shown in Fig. 1) on which interpolations at a particular pH value may be made.

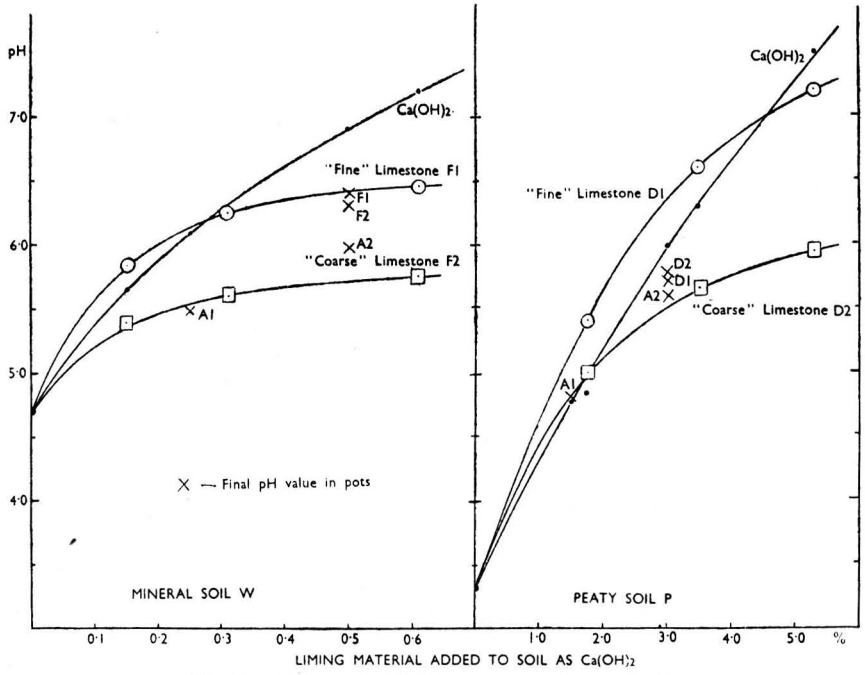

Fig. 1. Soil neutralisation curves and pot results

The results clearly indicated that the ability of the lime to reduce the acidity of different soils was equivalent to its power (*a*) to decompose a solution of an ammonium salt and (*b*) to neutralise dilute hydrochloric acid. These values were in turn only slightly less than (*c*) the percentage of calcium oxide extracted by boiling concentrated hydrochloric acid, but were always much greater than (*d*) the percentage of calcium oxide obtained by the official method. For many years, the values obtained by method (*d*) have, without exception, been low in comparison with those obtained by methods (*b*) and (*c*). For example, the last 30 samples of "lime" examined have given the results shown in Table II. Many of these samples were under-burned, but in the majority the amount of carbon dioxide was less than 3 per cent.

These results fully confirm the evidence in Table I, and show that on the average the official method has under-estimated the "liming" value or T.N.V. of the burnt lime by about

18 per cent., the range of differences being from 1 to as much as 30 per cent. However, the T.N.V. figure was always within 4 of the percentage of bases dissolved by boiling concentrated acid and expressed as CaO.

TABLE II

ANALYSES OF 30 SAMPLES OF BURNT LIME

	Fraction measured	Range	Average
(1)	Total CaO by concentrated acid	39·7 to 95·3	79·1
(2)	Total MgO by concentrated acid	0 to 3·1	1·1
(3)	CaO equivalent to (1 + 2)	41·4 to 95·3	80·7
(4)	Free CaO (official method)	8·7 to 91·1	60·6
(5)	CaO equivalent (T.N.V. method)	40·0 to 94·0	78·3
	Value (5) less value (4)	+1 to +30	—
	Value (3) less value (5)	+1 to +4	—

Of the various methods mentioned above, that giving the so-called total neutralising value is by far the simplest. It has been employed regularly by us during the past six years as a supplement to the other methods because it was felt that it gave a reliable estimate of the agricultural value of the liming material. It can equally well be used for samples of ground limestone or waste carbonate or shell sand, the result then being calculated in terms of equivalent $CaCO_3$ instead of CaO. For example, the last 30 samples of such materials examined have given the results shown in Table III.

TABLE III

ANALYSES OF 30 SAMPLES OF VARIOUS CARBONATES

	Fraction measured	Range	Average
(1)	Total CaO by concentrated acid	20·2 to 53·1	41·8
(2)	Total MgO by concentrated acid	0 to 9·0	1·1
(3)	$CaCO_3$ equivalent to (1 + 2)	36·0 to 94·8	77·5
(4)	$CaCO_3$ equivalent to CO_2	36·0 to 96·1	77·5
(5)	$CaCO_3$ equivalent (T.N.V. method)	43·4 to 96·5	77·7
	Value (3) less value (5)	−2·8 to +4·0	—

The agreement would seem to be adequate for this type of agricultural material, since the limit of variation permitted by the Fertiliser and Feeding Stuffs Act is, for limestone, 5 per cent. of the $CaCO_3$ stated, and for quicklime, 10 per cent. of the CaO.

The total neutralising value method is, therefore, applicable to both carbonates and limes and so gives a fair valuation for agricultural purposes of a badly burned or partially burned limestone; moreover, it takes account of the neutralising value of the magnesium oxide and other basic substances.

POT EXPERIMENTS

Having established that the total neutralising value was in fact a good measurement of the ability of a liming material to reduce soil acidity in the laboratory, it was used as the basis of comparison for different liming materials in pots and in the field. In one set of pot experiments, two highly unsaturated soils—a mineral soil, W, derived from glacial sand, with a pH value of 4·7 and a loss on ignition of 9 per cent., and a thin peaty soil, P, overlying acid andesite, with a pH value of 3·3 and a loss on ignition of over 80 per cent.—were each treated with calcium hydroxide, at two rates, and with fine and coarse fractions of three different limestones. The "fine" fraction consisted entirely of particles passing a 100-mesh sieve; the particles of the "coarse" fraction lay between 100-mesh and 1 mm in diameter. All the materials were used in equivalent amounts based upon the total neutralising values that are given in Table IV in terms of $CaCO_3$; the calcium hydroxide was also used at half that rate. The liming materials were thoroughly mixed with the air-dry soils before potting, and the treatments were replicated.

The pots were cropped in successive years with peas, barley and peas; the pH value of the soil in each pot was determined from time to time. For comparison, a range of treatments with each material was tested in soil suspensions under laboratory conditions to obtain titration curves. A selection of the results is presented in Table IV and a few typical curves are shown in Fig. 1.

TABLE IV

EFFECT OF DIFFERENT LIMING MATERIALS ON SOILS IN POTS

	Material	CaO	MgO	Equiv. $CaCO_3$	T.N.V. as $CaCO_3$	pH of soil W: Lab. expt.	pH of soil W: Weeks in pots 35	73	116	pH of soil P: Lab. expt.	pH of soil P: Weeks in pots 35	73	116
A1	$Ca(OH)_2$—half-rate ..	75·6	—	135·0	131·8	6·1	5·5	5·9	5·5	4·8	5·0	4·9	4·8
A2	$Ca(OH)_2$—full-rate ..	75·6	—	135·0	131·8	6·9	6·2	6·3	6·0	6·0	5·7	5·6	5·6
B1	Limestone—fine ..	40·2	6·5	87·8	90·5	6·7	6·5	6·4	6·3	6·1	5·7	5·6	5·7
B2	Limestone—coarse ..	44·6	5·2	92·8	92·0	6·2	5·9	6·3	6·4	5·3	5·7	5·7	5·8
D1	Limestone—fine ..	49·8	1·8	93·0	90·7	6·7	6·3	6·5	6·3	6·3	5·6	5·5	5·7
D2	Limestone—coarse ..	52·6	1·8	98·0	95·5	6·2	5·9	6·5	6·5	5·5	5·7	5·2	5·8
F1	Limestone—fine ..	31·1	20·3	106·4	105·3	6·4	6·4	6·6	6·4	6·2	5·8	5·8	6·1
F2	Limestone—coarse ..	31·7	20·1	106·8	107·0	5·7	5·7	6·1	6·3	5·3	5·5	5·4	5·9

The results show that—

(*a*) The T.N.V. figures were substantially the same as the equivalent $CaCO_3$ calculated from the total calcium and magnesium.

(*b*) With hydroxide and fine limestone, the soil pH values reached higher levels in the laboratory suspensions than in the pots; compare curves and points A, D1 and F1 in Fig. 1. This was possibly the result of leaching of the fine material from the pots that were kept outside throughout the experiment.

With the "coarse" fractions, however, the soils attained higher pH values in the pots than in the laboratory; see points D2 and F2 in Fig. 1. This was probably because the duration of the laboratory experiment was too short for complete reaction between soil and coarse particles. In the pots, the fine and coarse fractions ultimately gave the same result but more quickly in the peaty soil than in the mineral soil.

(*c*) All the materials, added in equivalent amounts according to their total neutralising values, produced essentially the same reduction in acidity as measured by the pH values of the soils.

FIELD EXPERIMENTS

It is easy to prepare an intimate mixture of soil and liming material for a pot experiment but it is very difficult to get an even distribution of a liming material to a particular depth in the field. Furthermore, sampling errors in the field are relatively large, so that a smooth curve depicting the change in pH value is not to be expected from a series of samples. The trouble may be particularly acute for an application of ground burnt lime; as a result of slaking and carbonation, small pockets of the material can form quite hard lumps, which do not disintegrate easily and remain very inefficient from the point of view of neutralising soil acidity. A limestone ground to pass a 100-mesh sieve may, therefore, provide a larger surface in contact with soil particles than a ground lime, and so reduce soil acidity more rapidly. Large particles of limestone have slower rates of reaction according to their size and hardness. Eventually, however, all forms will exert an effect depending upon their relative contents of available calcium and magnesium. Waste carbonates from paper works may contain some sodium hydroxide, but the amount is generally less than 1 per cent.

In recent field experiments, designed to compare different forms of liming materials, the dressings have been based on the total neutralising values of the materials used. The primary object of these experiments was to measure the response in crop yield, but it was sometimes possible to sample the soil of the individual plots at intervals during two or three seasons, and to compare the pH values with the results expected from the titration curve obtained in the estimation of the "lime requirement" of the original soil. A selection of average results from four experiments on different soil types is given in Table V. The letters L, LS and W represent respectively ground burnt lime, ground limestone and waste carbonate, and the dressings are equivalent amounts of CaO calculated from the total neutralising values (T.N.V.). For example, 25L means that ground burnt lime was added in amount sufficient to supply 25 cwt of CaO per acre according to its T.N.V.

The reduction in acidity was responsible for large increases in the yields of barley and roots in experiment 1, but the difference between equivalent amounts of lime and limestone

was not significant. Treatment did not produce a significant increase in roots in experiment 2 and only the smaller dressings produced a larger yield of beet tops and sugar in experiment 3. In experiment 4, equivalent amounts of lime and of two fractions of limestone produced the same yields. The highest pH values reached in experiments 1, 2 and 3 were quite in accordance with the dressings applied and with the calculated values. In experiment 4, equivalent amounts of lime and finely ground limestone had effected the same increases in pH value, but it was doubtful if equilibrium had been reached with the other materials in the period of less than 8 months.

TABLE V

EFFECT OF DIFFERENT LIMING MATERIALS ON SOIL AND CROP

1. NIDDRIE MAINS—Sandy loam, drainage good, original pH value 4·7.

Cwt of CaO per acre, 25.3.1947	nil	25L	50L	100L	50LS	S.E.
1947 *crop*—						
Barley grain (cwt/acre)	0·61	2·82	3·87	3·59	4·16	±0·43
Barley straw (cwt/acre)	0·89	2·87	3·56	3·21	4·13	±0·99
1948 *crop*—						
Turnips roots (tons/acre)	13·3	24·0	28·6	30·4	27·6	±1·17
Turnips tops (tons/acre)	2·27	3·82	4·54	5·22	4·36	±0·17
pH at 11.2.1948	5·0	5·1	5·2	5·3	5·2	
19.11.1948	4·8	5·3	5·8	5·8	5·6	
21.3.1949	4·7	5·3	5·8	6·4	5·9	
14.9.1949	4·7	5·2	5·9	6·6	5·9	
Calculated pH*	—	5·4	6·0	6·6	6·0	

Rainfall was low in 1947 and high in 1948.

2. CAMMERLAWS—Heavy loam, drainage poor, original pH value 5·8.

Cwt of CaO per acre, 18.3.1948	nil	20LS	52LS	52W	S.E.
Swedes roots (tons/acre)	22·1	23·5	21·0	20·7	±1·03
Swedes tops (tons/acre)	5·37	5·11	4·95	5·48	—
pH at 16.11.1948	5·7	6·0	6·3	6·6	
22.3.1949	5·6	6·0	6·8	6·7	
Calculated pH*	—	6·1	6·5	6·5	

3. AYTON LAW—Medium loam, drainage fair, original pH value 6·1.

Cwt of CaO per acre, 2.2.1948	nil	14LS	30LS	30W	S.E.
Beet roots (tons/acre	9·9	11·3	10·1	10·5	—
Beet tops (tons/acre)	10·4	12·3	10·8	11·2	±0·60
Beet sugar (cwt/acre)	33·8	38·8	34·5	36·5	±1·59
pH at 30.11.1948	6·2	6·5	6·9	6·9	
22.3.1949	6·1	6·5	6·8	7·1	
Calculated pH*	—	6·5	7·0	7·0	

4. CARSLOGIE—Light gravelly loam, drainage good, original pH value 5·4.

Cwt of CaO per acre, 29.3.1945	nil	31·0L	31·2LS (*a*)	31·5LS (*b*)	20·7LS (*c*)	20·4W (*d*)
Beet roots (tons/acre)	2·71	10·7	12·1	11·0	8·8	9·2
Beet tops (tons/acre)	4·89	17·5	20·3	19·4	15·1	17·1
pH at 18.11.1945	5·2	6·4	6·4	6·2	5·9	6·5
Calculated pH*	—	6·1	6·1	6·1	5·8	5·8

(*a*) 93 per cent. of 100 mesh. (*b*) 37 per cent. of 100 mesh, 88 per cent. of less than 2 mm. (*c*) Shell sand, 1 per cent. 100 mesh, 62 per cent. less than 2 mm. (*d*) Beet factory carbonate, 42 per cent. of water.

Standard error for roots ±0·86, for tops ±1·50.

L = ground burnt lime, LS = ground limestone, W = waste carbonate.

* Calculated pH value from the titration curve with the original soil.

The reliability of the final pH value calculated from the laboratory estimation of lime requirement depends, of course, upon the factor used to convert the laboratory figure to a field figure; this involves assumptions regarding the apparent density and depth of the soil concerned and the uniform incorporation of the liming material. For mineral soils with an apparent density of about 1 in the dry state, a factor of 1·7 has regularly been used to obtain an estimate of the lime required to bring the top 8 or 9 inches of soil to a particular pH value, 18 to 30 months after the application of this dressing. Special consideration must be given to soils containing more than about 10 or 12 per cent. of organic matter.

The agreement between the values found and those predicted is of secondary importance, however. More important for the present discussion is the fact that different liming materials applied in equivalent amounts calculated from the simple total neutralising value have produced the same effect on the pH value of these soils. Numerous data from a much larger range of soils and liming materials would be required to decide whether this is of general application. In principle, it would appear to be quite sound for limes from badly burned or from impure limestones, for ground limestone, for waste carbonates from paper works or sugar beet factories, and for certain types of blast furnace slags. The method might have to be modified for those less abundant waste materials that contain sulphides or other decomposable salts and are sometimes used locally for liming soils.

The virtue of the method lies in its simplicity and its ability to give a measure of all those compounds of calcium and magnesium which are of value in soil amelioration. It is of interest to observe, in this respect, that in the United States the Association of Official Agricultural Chemists defines a liming material as "any material whose calcium and magnesium content is capable of neutralising soil acidity."[6]

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1. "Fertilisers and Feeding Stuffs Regulations," 1932, p. 23.
2. Lauder, A., and Smith, A. M., *Agric. Prog.*, 1933, **10**, 172.
3. ——, ——, *Ibid.*, 1934, **11**, 93.
4. Smith, A. M., and Coull, R., *Soil Res.*, 1932, **3**, 10.
5. Smith, A. M., *Trans. 2nd Comm. I.S.S.S.*, 1933, A, 102.
6. "Official and Tentative Methods of Analysis," A.O.A.C., 6th Ed., Washington, 1948, p. 895.

Edinburgh and East of Scotland
College of Agriculture

Discussion

The President congratulated the authors on their paper, and mentioned that he had found high values by the Total Neutralising Value method for so-called "lime waste," which was essentially calcium silicate. He asked if the authors had tested such silicate materials in the course of their field work. He also asked whether, if the Total Neutralising Valuation be adopted, there should not be a statement about the magnesium content if any is present. He enquired whether the Total Neutralising Value method was efficient for very hard crystalline forms of carbonate.

Mr. J. G. Sherratt asked whether a fictitiously high value would not be given by the Total Neutralising Value in the analysis of waste industrial lime containing calcium sulphide. In many places throughout the country there were huge industrial dumps consisting of lime in various forms. Calcium carbonate predominated, but sulphate, sulphide and silica also were frequently present. Farmers were encouraged to use these wastes and the material was eligible for a Government grant on the basis of its lime content. In practice, a small amount of calcium sulphide did not seem to be harmful; indeed, it was claimed to be beneficial, although its value in neutralising the soil acidity was not apparent.

Mr. J. King asked whether limes of high silica content were as effective as the normal carbonate limes in flocculating clay soils.

Mr. W. F. Etheridge asked whether any experiments had been performed on tissue tests for calcium assimilation on the various liming materials mentioned.

Dr. J. H. Hamence said that he had listened to the paper with considerable interest, particularly in view of the fact that the Advisory Committee of the Ministry of Agriculture and Fisheries had been revived. He felt that many lime merchants had been unfairly penalised in the past, in view of the fact that lime in the form of chalk and also combined with silica had frequently not been taken into account when selling parcels of lime.

The problem was a very important one, since he had found that a very small amount of silica was capable of fixing a relatively large amount of lime, and of rendering it inert in respect of its solubility in the official sugar solution. It may be of interest to note in passing that it was his experience that roughly one molecule of silica was capable of fixing four molecules of calcium oxide. For many years he had held the view that lime loosely combined with silica in this manner was available for soil neutralisation, and it was therefore very pleasing to find that the authors had produced substantial experimental evidence to support this view.

Mr. C. J. Regan asked what was the relative value of calcium carbonate and calcium oxide from the aspect of the practical farmer. Was there any advantage in burning limestone to produce quicklime for agricultural use?

Dr. Smith replied to the President's questions that silicate materials had not been specially examined, the field experiments having been carried out only with liming materials in regular use. The Total Neutralising Value should be supplemented by a statement of the content of magnesium oxide or carbonate if that were more than, say, 10 per cent., because instances of magnesium deficiency in plants were becoming more frequent and the best method of correcting such acid soils was to use liming materials containing

magnesium. Hard crystalline forms of carbonate had not been tested, but the rate of reaction between soil and carbonate depended upon the size and hardness of the particles of the latter.

In reply to Mr. Sherratt, he said that the presence of sulphide presented a difficulty that might be overcome by a double titration, but the amounts of waste materials containing sulphides were relatively small and of local interest only, and farmers who used them were advised to leave the material exposed in the open until toxic substances had decomposed. The danger incurred was probably small if the waste by-product was incorporated in the soil some weeks before sowing the crop.

He informed Mr. King that no experiments had been made on the flocculating power of high-silica limes.

In reply to Mr. Etheridge, he said that the assimilation of calcium from different liming materials was not known. Of the several factors involved, the state of saturation of the soil with bases would probably be the most important and all the experimental evidence pointed to the fact that there was little ultimate difference between equivalent amounts of various liming materials as far as the reduction in soil acidity and the increase in exchangeable calcium were concerned.

In reply to Mr. Regan, he said that it did not matter whether quicklime or ground limestone was used provided the farmer appreciated what was meant by equivalent amounts. Contrary to the usual assumption, ground limestone might react more quickly than ground lime, which was liable to be carbonated as hard lumps on the surface of the soil and remain comparatively ineffective. There was little to be said in favour of the old practice of allowing heaps of burnt lime to slake on the field; subsequent spreading was laborious and irregular and might be very bad if rain made the heaps unworkable. Ground lime could be spread evenly, but it was a most unpleasant job. Ground limestone could be stored indefinitely and was easy to spread but, of course, was required in larger quantities than ground lime.

The Accurate Determination of "Phosphoric Anhydride" by Means of Quinoline Phosphomolybdate

By H. N. WILSON

(*Presented at the meeting of the Society on Wednesday, October 4th*, 1950)

Present methods of determining P_2O_5 are critically surveyed, and the requirements of a new method considered; it should be as accurate as the "official" method or better, and much quicker. As it appears that the phosphomolybdate reaction is the most suitable, previous work on this subject is summarised, and the reaction discussed in detail. It is then shown that by precipitating quinoline phosphomolybdate instead of the ammonium salt, very accurate results can be obtained by a volumetric method. Few substances present in fertilisers interfere except ammonium salts, which can be destroyed; a large excess of sulphuric acid also interferes.

Results by the "official" and the new method are compared and the respective standard deviations are 0·065 and 0·024 per cent. of P_2O_5. Full working details are given for the accurate determination of P_2O_5 in fertilisers.

There are two reactions, one or both of which enter into most analytical methods for the determination of phosphate—

(1) The precipitation of ammonium magnesium phosphate, which is subsequently converted to magnesium pyrophosphate and weighed.

(2) The precipitation of ammonium phosphomolybdate, which may be weighed as such, titrated, or converted to ammonium magnesium phosphate.

All procedures involving (1), as almost all accurate or official methods do, are slow, and none are of the highest order of accuracy. There are several reasons for this. The precipitation of magnesium ammonium phosphate is slow, and is not a simple reaction; the precipitate produced may be contaminated with $Mg_3(PO_4)_2$, $Mg(NH_4)_4(PO_4)_2$, or other substances.[1] No substances other than $MgNH_4PO_4$ will be quantitatively converted to the pyrophosphate on ignition, and it is only by purifying the original precipitate by solution and re-precipitation in presence of a small excess of reagent that absence of unconverted salts can be assured. The work of Epperson[2] conclusively confirms that double precipitation is essential if accurate results are to be obtained.

Accurate or referee methods in which the phosphate is first isolated as ammonium phosphomolybdate invariably continue by conversion of the isolated phosphate to magnesium

ammonium phosphate.[3] Prior precipitation as phosphomolybdate from acid solution has the great advantage of separating the phosphate from almost all substances that would interfere with the subsequent application of the magnesia process. Many of these "combined" procedures do not include a re-precipitation of the magnesium ammonium phosphate, and it is probable that a compensation of errors is involved.

Many attempts have been made to avoid the use of molybdates by precipitation of magnesium ammonium phosphate from a solution containing citric acid or ammonium citrate, which by formation of complexes prevents interference from calcium, iron or aluminium. It was stated by Epperson[2] that presence of citrate caused results to be slightly low and erratic, though only three results are quoted. The "citric magnesia" method is accurate, however. This is substantiated by Hoffmann and Lundell[4] who recommend it as a method for referee analysis. It has never been popular in the fertiliser trade, perhaps under the influence of the Fertiliser and Feeding Stuffs Act regulations, which prescribe molybdate precipitation and one subsequent precipitation as magnesium ammonium phosphate, and perhaps also because it tends to give lower results.

Numerous modifications of the volumetric molybdate procedure have been suggested, but none are sufficiently accurate for referee analyses, and all depend on an empirical standardisation of acid and alkali solutions using some supposedly pure phosphate as a standard substance. The "factor" obtained is neither stoicheiometric, nor constant. It is usually also considered that the weighing of ammonium phosphomolybdate is only suitable for small amounts of phosphate, as the errors caused by the variable composition of the precipitate are too great to allow the application of the method to major amounts. Nevertheless, it appeared to the author, following his experience with silicomolybdate,[5] that a phosphomolybdic acid method could be evolved that would be at least as accurate as the "official" method, and would take only a few hours.

Requirements of a new method

To add to the vast and unnecessary number of variants on the themes noted above, to produce yet another method of analysis for so familiar a substance as "P_2O_5", requires some justification, and the only real justification would be that the new method should have clear advantages over its predecessors. It should be—

(1) At least as accurate as the "standard" methods.
(2) Applicable to a large variety of samples without much modification, and free from interferences.
(3) Reasonably simple in manipulation.
(4) Quicker than "standard" methods.
(5) Truly stoicheiometric, and not dependent on empirical factors.

Requirement (4) at once cuts out any procedure in which there is a slow precipitation or re-precipitation, *i.e.*, magnesium ammonium phosphate is inadmissible. Requirement (2) also means that precipitation of magnesium ammonium phosphate is not suitable, as all substances which are precipitated as hydroxides or phosphates in ammoniacal solution must be absent. Requirement (3) suggests that double precipitation or the washing of precipitates with solvents other than water is undesirable. The only known reaction which fulfils requirement (2) is the phosphomolybdate reaction, and by use of quinoline as precipitant, requirements (1) and (5) have also been met.

The phosphomolybdate method

As at present carried out volumetrically, the phosphomolybdate method has not the qualities of a standard method; the composition of the precipitate varies with the temperature of precipitation, the concentration of ions other than phosphate and the amount precipitated. Usually standardisation is carried out against potassium di-hydrogen phosphate. This is not desirable; because of the influence of other ions present in the sample and not in the standard a bias is introduced (results tend to be high), the factors are empirical and vary with conditions, and the end-point is not very good.

The formula for ammonium phosphomolybdate is approximately—

$$(NH_4)_3[PO_4.12MoO_3].nH_2O,$$

but the precipitate tends to drag down and tenaciously hold other ions, or even acids. The older literature abounds in formulae for this salt, and it is significant that such formulae[6]

as $(NH_4)_3PO_4.12MoO_3.2HNO_3.H_2O$ are common. Sulphate ions have been said to have a very marked effect, and ammonium sulphate in particular influences the composition of the precipitate. Falk and Sugiura[7] considered that a definite compound was formed, to which they gave the formula $(NH_4)_{14}(PO_4)_4SO_4.53MoO_3$, which is unlikely, but indicates the extent of contamination under their conditions. Johnson[8] comments on the variable quality of the precipitate produced by molybdic acid reagents from different sources, which again points to variation in composition. Allen and Gault[9] found high results by the volumetric method for samples that had been digested with sulphuric acid to destroy organic matter. Lunge and Keane[10] summarise various modifications of procedure that lead to volumetric "factors" which, calculated in terms of 0·5 *N* sodium hydroxide solution, range from 1·421 to 1·540 mg of P_2O_5. "As regards fertilisers . . . which contain large percentages of phosphate, the liability of the precipitate to variation under different circumstances appears to the writer to make any modification of the (volumetric method) inadequate when really accurate results are required" (Bernard Dyer, *loc. cit.*[10]).

These undesirable features are explicable. The rather poor end-point is due to the presence of free ammonia in the solution, according to the equation—

$$(NH_4)_3[PO_4.12MoO_3] + 26NaOH = 12Na_2MoO_4 + Na_2HPO_4 + 3NH_3 + 14H_2O.$$

Molybdic acid is a weak acid, and ammonia is a sufficiently strong base to affect the indicator used (phenolphthalein): the extent of interference will vary with temperature and concentration. That the precipitate is a non-stoicheiometric compound is far less likely than that it is impure, as the conditions under which it is formed, *viz.*, simultaneous formation of the complex acid and precipitation of the salt from a strongly acid solution containing relatively high concentrations of other ions, are those most likely to yield an impure precipitate. Entangled nitric acid or other acid would be difficult to wash out, and the carrying down of excess molybdic acid would be even more likely. Unskilled operation can produce a precipitate visibly contaminated with molybdic acid, but who can say how often small but significant amounts of "free" molybdic oxide are carried down, and *perhaps* "allowed for" in the method of standardisation? A recent report by Bourdon and Cotte[11] states that the ammonium phosphomolybdate does not contain nitric acid, but that on precipitation in presence of excess of ammonium molybdate, the compound is always contaminated by molybdic acid, presumably adsorbed. Under standard conditions the extent of adsorption is more or less constant.

Finally, one must consider the solubility of the precipitate: probably even when it is allowed to stand for some time in contact with the mother liquor so that the crystals can grow, some may be lost in washing. This solubility is twofold, (*a*) the usual physical solubility in aqueous fluids and (*b*) the decomposition of the precipitate by hydroxyl ions. In gravimetric analysis this is prevented by washing the precipitate with very dilute nitric acid,[3] but in volumetric analysis a neutral wash liquid must be used. Potassium nitrate solution is popular for this purpose, but is probably no better than water.

So a method can only be based on the phosphomolybdate complex, with its favourable equivalent weight and freedom from interference, if a precipitate can be produced that is (*a*) less soluble than ammonium phosphomolybdate, (*b*) of constant composition, free from adsorbed or occluded impurities, and (*c*) free from cations that will interfere in the titration, if a volumetric process be used. The recent work[5] on silica indicates how these desiderata may be attained. The complex acid should first be formed in solution, not formed and precipitated simultaneously. This means that the solution must be free or practically free from ammonium salts before the molybdate reagent is added, and that ammonium molybdate must not be used as reagent. After formation, the phosphomolybdic acid must be slowly precipitated as a salt. Quinoline seems a suitable base; quinoline phosphomolybdate has a very low solubility and quinoline is a sufficiently weak base not to interfere in the titration.

The quinoline phosphomolybdate method

It was found that quinoline phosphomolybdate has almost all the desired properties. It contains exactly 12 moles of molybdic anhydride per mole of PO_4''', it is very insoluble, and a volumetric method of very great accuracy has been worked out. This does not depend on empirical standardisation but simply on the reaction—

$$(C_9H_7N)_3H_3[PO_4.12MoO_3] + 26NaOH = Na_2HPO_4 + 12Na_2MoO_4 + 3C_9H_7N + 14H_2O,$$

which is quantitatively achieved, so that in this reaction the equivalent weight of P_2O_5 is

142·08/52 = 2·732. The solubility of the quinoline phosphomolybdate is negligible and, as will be seen below, the whole procedure is of satisfactory accuracy. The quinoline phosphomolybdate produced is not absolutely pure; attempts to work out a gravimetric method were not quite successful. Whatever the contaminant, it is neutral and has no effect on the volumetric method; it is probably water retained in the crystal lattice, or it may be a minute amount of adsorbed sodium molybdate. For work on the usual analytical scale, the volumetric process is so good that there is little cause to regret the failure of the gravimetric process. The reactions are completely quantitative; by the weighing of aliquots of solutions instead of measuring them a considerable potential source of error has been avoided, and by recourse to weight burettes it is likely that a further advance would be made, but so far that has not been necessary.

Experimental

The description of the experimental work is divided into three parts, (*a*) the establishment of the quantitative nature of the proposed method, (*b*) attempts to establish a gravimetric method and (*c*) the effect of other elements and anions, and the application to fertiliser analysis.

The quantitative nature of the reaction—

The best analytical methods are only quantitative within a limited range of conditions; even as simple a matter as the precipitation of sulphate ion as $BaSO_4$ is only quantitative when properly carried out and hence it is to be expected that the method under investigation will only be accurate if the best conditions for precipitation, etc., can be found. A preliminary experiment was performed by precipitating the phosphate from a known amount of AnalaR potassium di-hydrogen phosphate under conditions similar to those used to precipitate quinoline silicomolybdate. On dissolving the precipitate in standard sodium hydroxide solution, titrating back and calculating the P_2O_5 present from the factor given above, 50·26 mg of P_2O_5 were found, 50·0 mg having been present. This preliminary result was regarded as most encouraging. A series of experiments was then made to ascertain the best conditions for complex formation, precipitation, etc., and a procedure was tentatively specified.

The next step was the preparation of standard solutions of phosphate, whose phosphate content should be known with the greatest possible accuracy. Two sources of P_2O_5 seemed most suitable, either dilute orthophosphoric acid or potassium di-hydrogen phosphate. Neither is ideal: phosphoric acid is not a very strong acid, hence its reference to standard substances such as sodium carbonate is not easy; potassium di-hydrogen phosphate is not easy to analyse for all possible impurities, and there is some evidence[12] for the existence of a more acid compound, $KH_2PO_4.H_3PO_4$, that would be very difficult to detect. On the other hand it has been much used by Sørensen and others as a standard salt for preparing buffer solutions. It was therefore decided to use both these chemicals as standards and to check the accuracy of the method against both.

Preparation of 0·5 M phosphoric acid solution—Dilute 56 ml of phosphoric acid (sp. gr. 1·740, and passing the AnalaR specification in every particular) to about 600 ml and boil gently for half an hour to ensure that any other phosphoric acids present shall be converted to orthophosphoric acid. Cool and dilute the solution to 2 litres.

Standardisation—The "end-point" for the neutralisation of the first hydrogen ion of H_3PO_4 occurs at pH 4·5, and is much sharper than the second end-point at pH 9 (see, *e.g.*, Smith[13]). Experiments with indicators showed that the most definite colour change at this pH was given by a mixed indicator containing 6 parts of bromo-cresol green solution (0·4 g per litre) and 1 part of methyl red solution (0·5 g per litre). This indicator is pink at pH 4·4, green at 4·6 and blue at pH 5. Partington[6] (*loc. cit.*, p. 623) states that the end-point is sharper at 55° C, so titrations were made at that temperature. As comparison solution an approximately 0·5 *M* solution of potassium di-hydrogen phosphate, containing the same amount of mixed indicator as the sample solution, was used.

The standard substance was sodium carbonate, freshly prepared by heating AnalaR sodium bicarbonate to 300° C in a platinum dish until constant in weight, and cooling it in a desiccator over fresh phosphoric anhydride; between 1·26 and 1·31-g portions were weighed out, giving titrations of about 50 ml. The usual precautions were taken to remove carbon dioxide at the end-point, and a calibrated chamber burette was used for the titrations.

The results were 47·40, 47·37, 47·39 and 47·34 g of H_3PO_4 per litre; average—47·375 g of H_3PO_4 per litre.

So that weight aliquots could be used as conveniently as volume aliquots, the density of this solution at 20° C was carefully determined by means of a Regnault's pyknometer, of approximately 135-ml capacity, very carefully calibrated at 20° C, a similar vessel being used as counterpoise throughout. As any weighings of the solution would be made in air, the density determined is apparent (*i.e.*, not corrected for the buoyancy of the air).

The mean result of two very closely agreeing determinations was—

Density at 20/4° = 1·0237.

Purification of potassium di-hydrogen phosphate—According to Pascal[14] this salt can be purified completely except for traces of aluminium, iron and calcium by recrystallisation from a boiling saturated solution. Accordingly, 200 g of AnalaR salt were dissolved in 400 ml of boiling distilled water, and the solution kept on a boiling water-bath for several hours. A faint turbidity appeared, so the solution was filtered through a pulp pad. The clear colourless solution was rapidly cooled with constant stirring, and allowed to stand overnight. The crystals were filtered with suction on a hardened filter-paper, washed twice with ice-cold water and once with 50 per cent. alcohol, and dried in an electric oven at 105° C. The dry salt was lightly ground in a Wedgewood mortar and dried for 8 hours at 105° C with occasional stirring. It was cooled over P_2O_5 and then regarded as pure. Even so, a 0·5 *M* solution after a few days deposited a few minute flocs of some white insoluble substance, possibly aluminium phosphate (compare Pascal, *loc. cit.*, p. 288). This substance was readily dispersed again by shaking, and must have been present in exceedingly small amounts. For most of the work, this pure salt was weighed as such on a semi-micro balance, so avoiding any errors of volumetric measurement.

The correct pH for the end-point of the titration—In the volumetric molybdate method one is titrating excess of sodium hydroxide and also hydroxyl ions from the dissociation of tri-sodium phosphate. The end-point for the phosphate part of the titration (Na_3PO_4 to Na_2HPO_4) is about pH 9; from the data of Travers and Malaprade[15] it can be seen that the end-point for the titration of molybdic acid is about pH 8, or rather higher. As there are 12 moles of molybdic acid present per mole of phosphoric acid, an indicator changing at about pH 8·5 is desirable. Thymol blue (pH range 8 to 9·6) is very satisfactory; it is found easier to avoid over-running the end-point by adding some phenolphthalein, which decolorises at about pH 9 and indicates the approach of neutralisation.

TABLE I

DETERMINATION OF KNOWN AMOUNTS OF PHOSPHATE

Phosphoric acid		Potassium di-hydrogen phosphate	
P_2O_5 present, mg	P_2O_5 found, mg	P_2O_5 present, mg	P_2O_5 found, mg
8·15	8·00	16·65	16·72
13·76	13·73	31·87	31·95
20·67	20·50	53·61	53·53
27·64	27·52	26·02	26·00
34·72	34·63	42·34	42·11
17·13	17·20		
30·68	30·65		
47·44	47·45		

NOTES—(1) In all the above experiments the amount present was unknown to the analyst.

(2) The first five results are low by an average amount of 0·1 mg. This was found to be due to a blank that was too high. In subsequent tests the volumetric solutions used for the blank were one-fifth as strong as those used in the test: it can be seen that the bias in subsequent results is negligible (−0·02 mg) and that the average error is ±0·07 mg.

Conditions of precipitation—By measuring known volumes of the 0·5 *M* phosphoric acid solution, precipitating under various conditions, washing and titrating the precipitate, the best acidity and temperature of precipitation were found. Acetic acid was shown to be unnecessary. The exact amount of hydrochloric acid present does not seem to be very important, as long as enough is present to prevent the precipitation of quinoline molybdate; 40 ml of diluted hydrochloric acid (1 + 1) per 100 ml of original solution is very satisfactory, and 50 ml will do no harm. By addition of 50 ml of concentrated acid to 100 ml of the

original solution prior to addition of the quinoline, results are somewhat low (*e.g.*, 24·04 mg of P_2O_5 recovered from 25 mg). The phosphomolybdate complex is readily formed at the concentration of 20 ml of concentrated hydrochloric acid per 100 ml of solution, especially when warm. Precipitation of the quinoline salt should take place *slowly* from boiling solution (see below under "Procedure" for details). The "blank," which is probably due to silica from the glassware, is important.

Results—Table I summarises the results on known amounts of phosphate. The aliquots of 0·5 *M* phosphoric acid solution were weighed in stoppered weighing bottles on an analytical balance, the aliquots of di-hydrogen phosphate on a semi-micro balance. It is considered that these results completely establish the validity of the volumetric method.

ATTEMPTED GRAVIMETRIC METHOD—

Numerous attempts to establish a gravimetric method were made, as this would avoid all errors associated with volumetric measurements. The insoluble salt was precipitated as in the volumetric process, washed several times with 1·5 *N* hydrochloric acid, then washed free from acid with cold water and dried to constant weight. It was ascertained that the precipitate was slightly hygroscopic, but could be dried to constant weight in 2 hours at 105° C; further drying at 120° C did not result in any further loss of weight. The results were rather disappointing; the precipitates were not quite pure enough, and results calculated on the theoretical molecular weight were high. In Table II the weight of precipitate obtained from various weights of P_2O_5 is given, also the "factor" (P_2O_5 per gram of precipitate) and the apparent molecular weight of each precipitate. By comparing this with the theoretical molecular weight, the degree of contamination can readily be seen.

TABLE II

GRAVIMETRIC RESULTS WITH QUINOLINE PHOSPHOMOLYBDATE

Weight of P_2O_5 taken, mg	Weight of precipitate obtained, mg	"Factor"	Apparent molecular weight of precipitate
11·31	353·6	0·03199	2219·8
21·41	668·7	0·03202	2218·2
31·76	992·4	0·03200	2219·1
13·99	440·0	0·03176	2233·6
20·54	642·7	0·03196	2222·2
14·78*	462·8	0·03194	2223·8
29·46*	917·8	0·03210	2212·4
10·51	332·3	0·03163	2244·5
17·21	540·3	0·03185	2229·6
27·51	864·8	0·03181	2232·5
15·06*	472·0	0·03191	2226·0
Average		0·03191	2225·6
Theoretical		0·03210	2212·8

* In these experiments, the source of P_2O_5 was potassium di-hydrogen phosphate, in the remainder it was 0·5 *M* phosphoric acid.

The nature of the contaminant was not discovered; it cannot be quinoline molybdate or molybdic acid, or the volumetric method would have been in error; it is not sodium chloride or hydrochloric acid as chloride could not be detected in the precipitates. It may be water very obstinately retained, or sodium molybdate, but the difficulties of analysing accurately for excess molybdate are very great.

Preliminary experiments have shown that the gravimetric method can be used in micro-chemical analysis; up to 4 mg of P_2O_5 the precipitates are of the correct composition. It is of interest that the precipitate is very readily soluble in acetone, but not in ether.

ATTEMPTS TO PREPARE CINCHONINE PHOSPHOMOLYBDATE—

By using the same technique, but substituting cinchonine for quinoline, a creamy-yellow precipitate is readily obtained. It is precipitated in a very finely divided state, filters badly and is difficult to remove from the sides of the beaker. The molecular weight of $3(C_{19}H_{22}N_2O)H_3PO_4.12MoO_5$ is 2708·2; in our experiments the molecular weight found

was 2352, 2365 and 2353. It is suggested that because of the peculiar shape of the cinchonine molecule, the crystal lattice may be unable to accommodate the theoretical amount of the base.

Pyridine was also unsatisfactory as a precipitant because pyridine phosphomolybdate is not sufficiently insoluble.

APPLICATIONS OF THE VOLUMETRIC METHOD—

Before using the method it is necessary to be sure that it is free from interference. Only those ions likely to be important in fertiliser analyses were investigated, *viz.*, (*a*) lime, (*b*) fluorine, (*c*) ammonia, (*d*) iron, (*e*) magnesia, (*f*) alkali salts, (*g*) citric acid, (*h*) ammonium citrate, (*i*) nitric acid and (*j*) sulphuric acid.

(*a*) *Lime*—The presence of 1 g of lime has no effect on the determination (50 mg of P_2O_5 present).

(*b*) *Fluorine*—In the presence of 2 ml of hydrofluoric acid, results were somewhat high (*e.g.*, 51·5 mg instead of 50 mg). This is due to attack on the beaker, bringing silica into solution, and the effect can be avoided by adding boric acid. For example, to 50 mg of P_2O_5 were added 3·5 g of calcium carbonate, 5 g of boric acid and then 2 ml of 40 per cent. hydrofluoric acid. Hydrochloric acid was then added until the calcium carbonate dissolved and the determination was continued as usual; 49·96 mg of P_2O_5 were found in one experiment and with a larger quantity of calcium carbonate, 50·08 mg.

(*c*) *Ammonia*—Ammonia, as might be expected, interferes. For example, in presence of 1 g of ammonium sulphate, only 47·83 mg of P_2O_5 were found. The ammonia can readily be destroyed by sodium nitrite, aqua regia or hypobromite. With sodium nitrite, a result of 49·96 mg was obtained; with aqua regia, 49·84 mg. In the method finally adopted, hypobromite was used because of convenience and speed.

(*d*) *Iron*—Iron is without effect. One gram of pure iron was dissolved in a few millilitres of nitric acid and added to 25 ml of a solution containing 50 mg of P_2O_5; the determined amount of P_2O_5 was unaltered.

(*e*) *Magnesia*—Two grams of $MgSO_4.7H_2O$ had no effect on the determination of 50 mg of P_2O_5.

(*f*) *Alkali salts*—Five grams of potassium chloride and 10 g of sodium sulphate added to a 50-mg aliquot of P_2O_5 have no effect on the determination.

(*g*) *Citric acid and ammonium citrate*—These salts are important in fertiliser analysis. Half a gram of each was dissolved in a 25-ml aliquot (that is equivalent to a 2 per cent. solution) so that it would represent the solution obtained by extraction of a sample with 2 per cent. citric acid or ammonium citrate solution. The 25-ml aliquot (of potassium di-hydrogen phosphate solution) contained 49·64 mg of P_2O_5, and the titrations of the solution, the citric acid and the ammonium citrate solutions were identical.

(*h*) *Nitric acid*—Substitution of an equivalent amount of nitric acid for hydrochloric acid in the analysis made no difference to the results.

(*j*) *Sulphuric acid*—With this present instead of an equivalent amount of hydrochloric acid results are erratic and high (*cf.* other workers[7,9]). Besides the normal yellow quinoline phosphomolybdate, a white solid is also precipitated. This was not analysed, as it was impossible to separate it from the yellow precipitate. Even in the absence of quinoline and phosphate, molybdic acid is slowly precipitated when sodium molybdate solution is heated with dilute sulphuric acid; this does not occur with hydrochloric acid, at least in the concentrations used in this analytical method. Precipitation occurs much more readily if some ammonium sulphate is also present. The amounts of SO_4'' and NH_4^+ in the precipitate are quite small, and adsorption seems more likely than formation of compounds.

If hydrochloric acid is present in amount slightly in excess of the sulphuric acid, the interference is prevented, but the total acidity should not be greatly in excess of 2 *N*. It is preferable to avoid large excesses of sulphuric acid, and until further investigation has been made, the volumetric molybdate method should not be applied, *e.g.*, to determining phosphorus present in organic substances that have been oxidised by Kjeldahl's method.

COMPARISON BETWEEN THE NEW METHOD AND OTHER METHODS

Various samples have been analysed by the new and older methods. Table III summarises the results.

Thirty-four samples of mixed fertiliser were analysed for water-soluble P_2O_5 by two

analysts working independently (but using the same volumetric solutions). The mean difference between the duplicates was 0·027, corresponding to a standard deviation of 0·024.

This may be compared with the standard deviation of the molybdate magnesia method of the Act; two analysts working independently analysed 27 samples of a similar mixture for water-soluble P_2O_5, and the standard deviation was found to be 0·065.

TABLE III

COMPARISON BETWEEN METHODS OF ANALYSIS

Sample	Method: Fertiliser and Feeding Stuffs Act, P_2O_5, %		Method: Quinoline phosphomolybdate, P_2O_5, %	Remarks
Morocco rock 1	33·1		33·29	Total P_2O_5
			33·29	
Morocco rock 2	33·0		32·87	Total P_2O_5
Superphosphate	20·15		20·29	Total P_2O_5
	20·12		20·23	
Mixed fertiliser sample No. 1	10·72		10·66	10·64 } by F. & F.S.
	10·72		10·66	10·66 } citrate method
	10·67			Water-soluble P_2O_5
	10·69			
Mixed fertiliser sample No. 2	11·61		11·52	Water-soluble P_2O_5
	11·64		11·49	
Solution containing N, P_2O_5 and K_2O	4·88		4·81	
	4·89		4·82	
Ditto	5·60	5·27	5·56	5·60 per cent. of P_2O_5 present
	5·57	5·28	5·57	
	5·56	5·29		
	5·59	5·42		
Calcium phosphate	44·48	44·51	44·24	
	44·45	44·38	44·24	Total P_2O_5
	44·52	44·38	44·24	

Finally, several samples of fertiliser were specially prepared by grinding to pass a 200-mesh sieve. Each was well mixed, and divided into six portions. Three analysts each received two portions of each sample, and made one analysis of each, with results as shown in Table IV.

TABLE IV

TESTS BY VARIOUS ANALYSTS, USING QUINOLINE PHOSPHOMOLYBDATE METHOD

Sample	Analyst: H. N. R. P_2O_5, %	J. S. P_2O_5, %	F. V. P_2O_5, %
Mixed fertiliser X	11·23	11·23	11·13
	11·19	11·18	11·14
Mixed fertiliser Y	15·47	15·35	15·32
	15·39	15·37	15·34
Mixed fertiliser Z	18·62	18·60	18·59
	18·63	18·65	18·60
Phosphate rock	33·20	33·38	33·23
	33·15	33·39	33·17

METHOD

REAGENTS—

Hydrochloric acid—Concentrated, and diluted to 1 + 9, 0·5 *N* and 0·1 *N* solutions.

Sodium hydroxide—0·5 *N* and 0·1 *N* solutions, both free from carbon dioxide, made from AnalaR pellets.

Sodium molybdate solution—A 15 per cent. solution of the hydrated salt in water. (See note 1 below.)

Quinoline hydrochloride solution—Add 20 ml of redistilled quinoline (synthetic quinoline has been found preferable to the usual coal tar product) to 800 ml of hot water acidified with 25 ml of concentrated hydrochloric acid, and stir well. Cool to room temperature, add paper

pulp and again stir well. Filter with suction through a pulp pad, but do not wash. Dilute to 1 litre with water.

Boric acid—Pure.

Bromine water—Distilled water *saturated* with bromine at room temperature.

Perchloric acid—AnalaR, 60 per cent.

Mixed indicator solution—Mix two volumes of 0·1 per cent. phenolphthalein solution with three volumes of 0·1 per cent. thymol blue solution (both in alcohol).

PROCEDURE—

A. *Total P_2O_5 in basic slag, phosphate rock, superphosphate, etc. (not containing ammonia)*—

(1) For samples of normal P_2O_5 content, weigh 2·500 g into a 150-ml beaker, add 20 ml of water and 10 ml of perchloric acid. (See note 2.) Warm until most of the sample is dissolved, then evaporate until fuming, and continue to heat while gently fuming with the beaker covered for at least 15 minutes, until attack is complete. Allow to cool, rinse the cover with a little distilled water, allowing the rinsings to run into the beaker, add 20 ml of diluted hydrochloric acid (1 + 9), warm carefully and then boil until all salts are in solution. Filter through a 9-cm Whatman No. 30 filter-paper in a 2-inch funnel into a stoppered conical 125-ml flask, which has been weighed to the nearest 0·5 mg, preferably with a similar flask as a counterpoise. Transfer the contents of the beaker to the filter with the minimum of water, using a wash bottle with a fine jet and a succession of small washes, allowing each to run through before adding the next to the filter. Wash the filter thoroughly with small washes of warm water in the same way. The total volume of washes and filtrate will be from 100 to 110 ml. Discard the filter-paper.

(2) Cool the flask, stopper it and shake to mix the contents thoroughly. Carefully dry the outside, and weigh to the nearest 0·5 mg. Call the weight of solution A grams. Weigh a dry stoppered weighing bottle, 6 × 3 cm diameter. Transfer to the weighing bottle, by means of a pipette, an aliquot that should contain about 50 mg of P_2O_5 (but not more than 60 mg), *e.g.*, if the material contains about 20 per cent. of P_2O_5, an aliquot of about 10 ml is desirable. This need not be accurately measured. Stopper the weighing bottle and weigh again, to the nearest 0·5 mg. Call the weight of the aliquot B grams; then the weight of the sample taken for analysis is $2{\cdot}5 \times B/A$ g. (See note 3.)

(3) Wash the stopper of the weighing bottle, collecting the washings in a 500-ml conical flask (this is precautionary as the stopper should not have become wet) and then quantitatively transfer the aliquot to the flask, washing the weighing bottle with about 90 ml of cold water. Add 20 ml of concentrated hydrochloric acid, then 30 ml of sodium molybdate solution. Raise the temperature to boiling, and from a burette with a coarse jet add a few drops of quinoline solution. Swirl the solution in the flask during the addition, again heat to boiling and add quinoline solution drop by drop with constant swirling until 1 or 2 ml have been added. Again boil, and to the gently boiling solution add the reagent a few millilitres at a time, with swirling, until 60 ml in all have been added. In this way a coarsely crystalline precipitate with good filtering properties is produced. Allow the solution to stand in a bath of boiling water or on the edge of a hot plate for 15 minutes, then cool to room temperature.

(4) Prepare a paper-pulp filter in a funnel fitted with a porcelain cone, and tamp well down. Decant the clear solution through the filter, and wash the precipitate twice by decantation with about 20 ml of hydrochloric acid (1 + 9). (See note 4.) Transfer the precipitate to the pad with cold water, washing the flask well, and wash the filter and precipitate with cold water, with small washes of about 25 to 30 ml, letting each wash run through before applying the next, until the washings are acid-free. (Test for acidity with litmus paper; 6 washes are usually sufficient). Transfer the pad and precipitate back to the original flask (now acid-free). Insert the funnel in the flask and wash it well with water to make sure that all traces of precipitate are transferred; use about 50 ml of water. Shake the flask well so that filter-paper and precipitate are completely broken up.

From a calibrated burette or pipette run in exactly 50·0 ml of 0·5 N sodium hydroxide solution, swirling the flask during the addition. Shake until the precipitate is *completely* dissolved. Add a few drops of indicator solution and titrate with 0·5 N hydrochloric acid. The end-point is very sharp; the solution becomes pale green and at the end-point suddenly changes to pale yellow. Record the volume of hydrochloric acid used to within 0·03 ml, and subtract from the volume of 0·5 N sodium hydroxide solution (50·0 ml).

Run a blank on all reagents, excluding only the aliquot of sample solution, but use 0·1 *N* acid and alkali solutions for the titration and calculate it to 0·5 *N* sodium hydroxide. Subtract this blank from the volume neutralised by the original precipitate (see note 5).

1 ml of 0·5 *N* sodium hydroxide solution ≡ 1·366 mg of P_2O_5

and $$\frac{\text{ml of 0·5 } N \text{ NaOH} \times 0·1366}{2·500\ B/A} = \text{percentage of } P_2O_5$$

B. *Total P_2O_5 in mixed fertilisers containing ammonium salts*—

(1) Weigh 2·500 g of sample into a 150-ml beaker, add 1 g of boric acid and 20 ml of water. Warm until the boric acid is dissolved. Add 10 ml of concentrated hydrochloric acid (see note 6), evaporate to dryness and bake gently for 15 minutes. Allow to cool, moisten the residue with 1 to 2 ml of concentrated hydrochloric acid and add 15 ml of hot water. Warm until the salts are dissolved and proceed as under A (1) from "Filter through a 9-cm Whatman No. 30 filter-paper . . . ."

(2) Proceed as A (2) above.

(3) Transfer the aliquot to a 500-ml flask as under A (3), but only use 70 ml of water. Add 2 g of sodium hydroxide (about 10 pellets) and swirl the solution until the pellets have dissolved. This amount of sodium hydroxide will normally be sufficient; enough should be present to liberate all the ammonia from its salts and leave an excess. Add from 20 to 40 ml of bromine water, according to the quantity of ammonia present (10 ml of bromine water destroys about 20 mg of ammonia). Mix the solution, allow it to stand for 5 minutes, acidify with concentrated hydrochloric acid added dropwise (normally about 6 ml of acid is required) and boil gently to remove excess of bromine. If necessary, adjust the volume to 100 ml, add 20 ml of concentrated hydrochloric acid, and from this point proceed as under A (3) above.

C. *"Water-soluble P_2O_5," ammonium salts absent*—

(1) Weigh 10 g of sample into a 500-ml volumetric flask (class A calibration) and add 400 ml of water at 20° C. Shake on a machine for exactly 30 minutes. (See note 7.) Dilute to the mark with water, mix well and filter through a dry Whatman No. 31 filter-paper. Reject the first 20 to 30 ml and then collect the filtrate. Adjust the temperature of the filtrate to 20° C. With a calibrated pipette (25 or 50 ml, according to the amount of soluble P_2O_5 expected), transfer a suitable sized aliquot to a 500-ml conical flask. Dilute to about 100 ml with water.

(2) Add 20 ml of concentrated hydrochloric acid and from this point proceed as under A (3) above.

D. *"Water-soluble P_2O_5," ammonium salts present*—

(1) Proceed as C (1) above.

(2) Proceed to the destruction of ammonium salts as in B (3) above, and complete the determination as in A (3) above.

NOTES ON THE PROCEDURE—

1. The sodium molybdate solution should not be kept too long, as it tends to dissolve silica from the glass bottles.

2. Some samples of phosphatic minerals have been found to be very resistant to attack by hydrochloric acid, but so far we have come across no samples that did not yield all their phosphate to an attack with perchloric acid. This procedure has also the advantage of completely removing fluorine, hence precautions to prevent later attack on beakers (addition of boric acid) are not necessary.

3. Volumetric measurements cannot be as accurate as weighing, so for work of great accuracy it seems desirable to eliminate measurement of volume as far as possible. It would be possible to weigh an aliquot of 100 mg or so direct on a semi-micro balance, but in non-homogeneous materials there is always some doubt as to how truly representative a very small sample may be. With a modern aperiodic balance taking a 200-g load, the procedure outlined here is quite speedy and reduces any error in taking the aliquot to negligible proportions. For referee work, it is probable that use of weight burettes for all "volumetric" work, the solutions being standardised on a kilogram basis instead of a litre basis, would

result in a further gain in accuracy at the cost of a very small loss in time and convenience. Errors due to changes in temperature would vanish.

4. The preliminary washes with acid remove most of the excess of quinoline and molybdate, and prevent errors through the precipitation of quinoline molybdate or molybdic acid in the pad or precipitate.

5. The "blank" is important; it is mostly due to silica, and must be carefully determined. It should not be more than about 0·4 or 0·5 ml; about 0·2 ml comes from the sodium molybdate and the remainder from the sodium hydroxide and the glass apparatus. Soft soda or potash glass must not be used. Flasks that become scratched or etched must be discarded; occasionally a flask that has been satisfactory may begin to yield appreciable quantities of silica.

6. This treatment is satisfactory in rendering silica insoluble; perchloric acid is not used, as the low solubility of ammonium perchlorate in water (and its rather unstable nature) may give rise to trouble. The addition of boric acid is essential if fluorides are present, and should precede acidification with hydrochloric acid.

7. "Water-soluble P_2O_5" has only one meaning in fertiliser analysis—as defined in the Fertiliser and Feeding Stuffs Act regulations. The regulations specify 20 g of sample and final dilution to 1 litre. Use of 10 g, as described, is satisfactory, and 500-ml flasks are easier to accommodate in shaking machines. The time of shaking must be adhered to, and filtration must take place immediately, because of possible slow reactions between the solid phase and the solution. We have not found that silica in appreciable quantities passes into solution during this treatment, but if a fertiliser of alkaline reaction were examined, silica might be dissolved.

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Imperial Chemical Industries Limited
Research Department
Billingham, Co. Durham

Discussion

Mr. L. Ganellin asked whether the author had had any experience in the determination of phosphate in basic slag by the quinoline method, and in particular, with citric acid soluble P_2O_5. It was his experience that silica soluble in the citric acid extract interfered in molybdic precipitates and had to be removed by the standard precipitation method.

Mr. Wilson replied that he had not had any experience with citric-soluble P_2O_5 in basic slag by this method, but he thought that silica, if extracted, might interfere. This interference could probably be avoided. The pH range over which silicomolybdic acid was formed was narrow and, at the acidity prescribed, conversion of silica to silicomolybdic acid would be far from complete. Moreover, it was only from fresh unpolymerised silicic acid that silicomolybdic acid was formed. If to the aliquot of citric acid solution taken for analysis, 20 ml of concentrated hydrochloric acid were added, as in the usual procedure, and the mixture was gently boiled for half an hour and then diluted to the proper volume before addition of the sodium molybdate solution, it was improbable that silica would interfere.

Mr. R. C. Chirnside complimented the author on a lucid account of an elegant piece of work. The author had pointed out the high standard that he had set himself, in particular in that he hoped the method would not embody any empirical factors. With this in mind, would it not have been more desirable to offer the volumetric procedure merely as a quicker alternative to the gravimetric procedure; if so, was

the constitution of the quinoline phosphomolybdate precipitate clearly known? Had the author considered the possible application of X-ray diffraction methods to a study of this precipitate? It might well be that a critical examination of the patterns so obtained would throw light on the nature of the attachment of the water that the author believed the precipitate contained. It would show whether the water was in the crystal lattice or was merely firmly adsorbed, and so enable conditions for its complete removal to be found.

Similarly, some light might be thrown on the nature of the cinchonine precipitates that the author mentioned earlier in the paper.

MR. WILSON replied that it was likely that X-ray diffraction methods would prove valuable in the investigation of the persistent moisture in the precipitate. He agreed that a gravimetric method would have been the most desirable but, as was clearly shown in Table II, the gravimetric results were not quite good enough, whereas the volumetric method gave acceptable results. Whilst it was still possible that a gravimetric procedure might be evolved, he thought that the investigation of weight titration was more likely to lead to a method of the highest possible accuracy.

MR. J. G. SHERRATT enquired whether the method was applicable to organic fertilisers and feeding stuffs, such as animal residues containing bone, and if so, what procedure was recommended for the destruction of organic matter. Hitherto he had invariably used sulphuric acid to oxidise organic matter prior to the determination of P_2O_5, but this involved the subsequent presence of ammonium sulphate and an excess of sulphuric acid in the final solution, both of which interfered with the proposed new method.

MR. WILSON replied that it was preferable not to use sulphuric acid, but if it were used as much as possible should be evaporated, and the remainder neutralised by sodium carbonate before the addition of hydrochloric acid. It was possible that a mixture of nitric and perchloric acids could be used. Although there might be hesitation about the use of perchloric acid with organic matter, this mixture had been used in the wet oxidation of organic compounds (see, *e.g.*, *J. Ass. Off. Agric. Chem.*, 1943, **22**, 182, or *Shirley Institute Memoirs*, April, 1949, Vol. **23**). Hamlin, in the latter of these papers, described the successful destruction of up to 5 g of cotton yarn with a mixture of 14 ml of perchloric acid, 20 ml of nitric acid and only 1 ml of sulphuric acid.

MR. C. G. DAUBNEY asked, in view of the reliance placed on AnalaR chemicals, whether the author would disclose the difference between AnalaR phosphate and the recrystallised phosphate used as his standard material to prepare the standard phosphate solution.

MR. WILSON said that in this investigation he had wished to neglect no possible precaution, and therefore had purified the material.

DR. J. H. HAMENCE said that workers in his laboratory had spent many years trying to evolve a really efficient volumetric method for the determination of P_2O_5, but without any real success, and he therefore would like to congratulate Mr. Wilson on his new method. In the absence of interfering substances, the St. Gobain method had been found to give very satisfactory results. The St. Gobain method was based on the well-known Pemberton method, with the difference that neutral formaldehyde was added for the final alkali titration; this gave a very much sharper end-point, since it removed the ammonia radical.

This procedure gave excellent recoveries with simple phosphates and with water solubles from superphosphate, but unfortunately the process broke down in the presence of fluorine and silica. Although many modifications of the method had been tried, he had never been successful in avoiding interference from silica other than by the somewhat lengthy process of removing silica prior to solution in acid. In view of this difference, he would be pleased if the author would amplify the procedure that he employed to avoid interference by fluorine and silica, particularly in substances like basic slag and apatite phosphate.

MR. WILSON said that, for compounds like apatites that were soluble only with difficulty, he thought the treatment with perchloric acid was best. He had come across no instance in which fuming a finely ground sample with perchloric acid did not bring into solution the whole of the P_2O_5 in reasonable time. This treatment also removed hydrofluoric acid. If treatment with hydrochloric acid was preferred, boric acid should first be added to combine with the fluorine. Mr. Wilson added that he thought the relative freedom from interference of his method as compared with the ammonium phosphomolybdate method was explained in part by the difference in crystal structure of the precipitates; whereas ammonium phosphomolybdate was cubic, the quinoline compound was of a much lower class of symmetry, and it might well be due to the different crystal lattice that the precipitate crystallised pure, without including or absorbing foreign ions from the solution.

The Microbiological Assay of Growth Factors after Separation by Paper Chromatography

By J. S. HARRISON

(*Presented at the meeting of the Biological Methods Group on Thursday, May 26th,* 1949)

A general method is described for the separation, identification and estimation of growth factors by means of paper partition chromatography.

The apparatus consists of two crystallisation dishes of different diameters, one placed within the other; the inner dish, containing a paper cylinder, is covered by a tall beaker or bell-jar.

On completion of the chromatographic separation and after removal of the solvent by evaporation, the positions of the factors on the chromatogram are located by placing strips cut from the paper on a synthetic agar medium that is deficient in the growth factor and inoculated with a suitable organism. The identified zones are then cut out and extracted with water, made up to known volume and aliquots submitted to microbiological assay.

The technique described has been applied with success to some of the growth factors in molasses.

Many natural products contain mixtures of growth factors that have similar stimulatory effects on micro-organisms; for instance, pyridoxine, pyridoxal and pyridoxamine all stimulate certain strains of *Saccharomyces carlsbergensis* to the same extent. These three compounds have been estimated in mixtures by Rabinowitz and Snell,[1] who used a differential microbiological assay with three organisms, and they have been separated qualitatively by Winsten and Eigen,[2] who used paper chromatography. We are not aware that any accurate quantitative determinations of growth factors have been carried out after separation,* although various workers have estimated amino acids[3,4] and other substances, including inorganic compounds,[5] by soaking the portion of the paper that contains the substance in water and carrying out chemical tests on the solution.

This general method has been developed to separate and estimate growth factors by microbiological assays. In particular, the technique has been applied to molasses for the quantitative separation of pantothenic acid from β-alanine and of biotin from desthiobiotin. After chromatographic separation, the growth factors were identified qualitatively by an agar plate method that fixed the positions on the chromatogram compared with those of the chemically pure factors, which were run separately on the same sheet. Microbiological assays were then carried out on the extracts from the portions of the chromatogram containing the separated factors.

Experimental

Separation—

Mixed growth factors in artificial mixtures or in natural products were separated by single-dimensional paper partition chromatography. The apparatus consisted of two crystallisation dishes of different diameters, the larger holding water saturated with the solvent and the smaller, which was placed inside this, containing the solvent saturated with water. A tall inverted beaker or a bell jar was placed over the smaller dish and fitted inside the larger. A sheet of Whatman No. 1 filter-paper, 15 inches square, treated with suitable quantities of the test sample, was rolled into a cylinder, which was held together with metal clips and stood in the smaller dish.

On account of the small amounts of growth factors present in some natural materials, it was often necessary to run on the chromatogram much more raw material than could be applied as one spot. This difficulty was overcome by applying the test material in a line along the lower edge of the filter-paper by means of a capillary pipette in such a way that the

* Since this paper was presented, various methods have been described in the literature, *e.g.*, J. P. Bowden and W. H. Peterson, *J. Biol. Chem.*, 1949, **178**, 533; J. T. Holden and E. E. Snell, *Ibid.*, 1949, **178**, 799; and H. Yacowitz, L. C. Norris and G. F. Heuser, *Proc. Soc. Exp. Biol. Med.*, 1949, **71**, 372.

total amount of solids deposited per inch was not greater than 2 mg, otherwise the concentration of solids was high enough to interfere with the running of the chromatogram by holding back part of the growth factors and so causing tailing. Beyond the end of the line of test substance were placed separate marker spots of the growth factors under examination, and the chromatogram was run with the chosen solvent. Tests with mixtures of amino acids, which gave easily identifiable colour reactions, showed that the line of substance being chromatographed travelled in a band parallel to the lower edge of the paper.

IDENTIFICATION—

After evaporation of the solvent, vertical strips above the marker spots were cut out and a narrow vertical strip of known width was cut from the centre of the portion of filter-paper holding the sample. These strips were placed on a large plate of synthetic agar medium deficient in the growth factor and inoculated with a suitable organism. The strips were removed after 5 minutes, by which time a proportion of the growth factors had been transferred to the medium. After incubation overnight, zones of growth marked the points to which the growth factors had travelled.

This step was necessary because the R_F values varied somewhat, and if the zones were near together it was important to locate them exactly. The zones given by the pure substances were used to identify the naturally occurring factors. If the R_F value for the natural factor and that of the pure substance agreed when several solvents were used, this was taken as evidence that the two were probably identical.

ESTIMATION—

Horizontal strips slightly wider than the zones of growth found with the sample under test were cut from the chromatogram. These were separately extracted with water, using a Waring blendor to homogenise the paper, which was then removed from the solution by filtering or centrifuging. The solution was made up to a known volume and aliquots were used to carry out a microbiological assay.

BIOTIN AND DESTHIOBIOTIN

The chromatographic method was first tested with biotin and desthiobiotin, because a reliable differential assay method was available to check the results. Most strains of yeast respond to both biotin and desthiobiotin, while a smaller number of yeasts, as well as certain other organisms, such as *Lactobacillus arabinosus*, respond specifically to biotin. By use of typical organisms in these two groups, the amount of biotin-like and desthiobiotin-like

TABLE I

R_F VALUES OF BIOTIN AND DESTHIOBIOTIN

Growth factor	R_F value		
	Phenol	Butanol	Collidine - lutidine
Biotin, pure	0·95	0·23	0·70 and 0·85
" in blackstrap molasses	0·95	0·33	—
" in high test molasses	—	0·23	—
Desthiobiotin, pure	0·86	0·48	0·83
" in blackstrap molasses	0·88	0·52	—
" in high test molasses	—	0·38	—
" in refiners' cane molasses	—	0·45	—

substances in natural products such as molasses can be estimated. There has naturally been some doubt as to the precise identity of these substances, particularly those that react as desthiobiotin, in the absence of any test more specific than the microbiological response. Chromatograms run in butanol, phenol and a 1 + 1 collidine - lutidine mixture showed that the biotin-like and desthiobiotin-like substances in several types of beet molasses behaved in a similar way to biotin and desthiobiotin respectively. Table I gives the R_F values obtained in these tests. It was found that both natural and synthetic biotin gave two distinct zones with R_F values of 0·70 and 0·85 in collidine or a mixture of collidine and lutidine. Two-dimensional chromatography with the same solvents showed that the leading fraction (R_F 0·85) changed progressively during the run to the slower fraction (R_F 0·70).

Quantitative microbiological assays using *S. carlsbergensis* or *L. arabinosus* as the test organism showed that biotin and desthiobiotin could be completely recovered from filter-paper after drying. After chromatography with butanol or phenol, the recovery was also 100 per cent. A sample of blackstrap molasses was tested after separation of the biotin and desthiobiotin, with the results shown in Table II. The values quoted are the means of three or more assays. It will be seen that the biotin and desthiobiotin contents after chromatography agree well with those obtained by differential assay.

TABLE II

BIOTIN AND DESTHIOBIOTIN CONTENT OF BLACKSTRAP MOLASSES

Growth factor	Growth factor content: Differential: Yeast, µg/g	Differential: *L. arabinosus*, µg/g	Chromatographic*: Phenol, µg/g	Chromatographic*: Butanol, µg/g
Biotin	1·4	1·1	1·4	1·3
Desthiobiotin	0·9	—	1·2	1·2

* Assayed microbiologically with yeast as test organism.

PANTOTHENIC ACID AND β-ALANINE

Substances that react microbiologically as pantothenic acid and as β-alanine occur in molasses, but although pantothenic acid can be estimated specifically by *L. arabinosus* and other organisms, a satisfactory method of assaying β-alanine has not been found. The reason is that α-amino acids interfere with the β-alanine activity,[6] and many natural substances contain amino acids. The chromatographic method has been used to separate and estimate the two growth factors.

Qualitative tests with three solvents showed that the growth factors in blackstrap cane molasses behaved chromatographically as β-alanine and pantothenic acid. Butanol was found to give the best separation of the two factors, the R_F value of β-alanine being 0·02 and that of pantothenic acid 0·57. Table III gives the values for various solvents.

TABLE III

R_F VALUES OF PANTOTHENIC ACID AND β-ALANINE

Growth factor	R_F value: Phenol	Butanol	Collidine - lutidine
Pantothenic acid, pure	0·73	0·57	0·67
" in molasses	0·74	—	—
β-Alanine, pure	0·63	0·02	0·21
" in molasses	0·63	0·02	—

Quantitative recoveries from paper before and after chromatography with butanol were close to 100 per cent., but this solvent was found to be unsatisfactory for the estimation of

TABLE IV

PANTOTHENIC ACID AND β-ALANINE IN BLACKSTRAP MOLASSES

Growth factor	Yeast assay direct, µg/g	Chromatographic: phenol, µg/g	Chromatographic: butanol, µg/g
Pantothenic acid	32	30	25
β-Alanine	c 70*	71	—†

* Obtained by correcting for inhibition due to α-amino acids.
† α-Amino acids invalidate this estimation.

β-alanine in natural materials, because many α-amino acids had low R_F values in butano and consequently the microbiological assay was invalidated. The most satisfactory results were obtained with phenol, which gave values for blackstrap molasses of 30·4 and 71·5 µg

per g for pantothenic acid and β-alanine respectively. The pantothenic acid content agreed with the value of 31·7 μg per g obtained by direct assay with *S. cerevisiae*, and the value for β-alanine was of the same order as that obtained by differential assays corrected for the inhibitory effect of α-amino acids. Table IV shows the amounts of pantothenic acid and β-alanine in molasses found by various methods. It should be noted that the growth factor measured is that available to the test organism. The total β-alanine content of this sample of blackstrap molasses, determined after alkaline hydrolysis, was 170 μg per g. The chromatographic behaviour of the bound form has not been investigated.

Preliminary tests show that pyridoxine, pyridoxal and pyridoxamine can be studied in a similar way, using butanol as the solvent.

Discussion of results

The technique described has already proved useful for the examination of some of the growth factors in molasses, and it is clear that modifications of the method could be used to clarify many points of a similar nature. For instance, it would be possible to examine cell contents by separating conjugates of growth factors, and to study the properties of these. If necessary, the information so obtained could be used to devise a suitable method of separating the factors on columns, when larger quantities would be available than in paper chromatography.

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The Distillers Company Limited
Research and Development Department
Epsom, Surrey

Discussion

Dr. G. E. Foster asked whether Mr. Harrison had tried any chemical methods for detecting growth factors on the chromatograms.

Mr. Harrison replied that he had used chemical methods only for β-alanine. With biotin the total amount of growth factor present was only about 2 to 3 mμg, and he doubted if so small an amount could be detected chemically.

The Spectrographic Determination of Linoleic and Linolenic Acids

By T. P. HILDITCH, C. B. PATEL and J. P. RILEY

The values of the extinction coefficients ($E^{1\%}_{1\,cm}$) for the ultra-violet absorption bands at 234 mμ and 268 mμ, which are developed when linoleic and linolenic acids are subjected to isomerisation with alkali under the conditions specified by Hilditch, Morton and Riley, have been re-investigated. Pure linoleic and linolenic acids that had been isolated from natural sources by physical methods alone were employed, in addition to specimens prepared respectively by debromination of tetrabromostearic and hexabromostearic acids. For linoleic acid prepared either by physical or chemical methods, and isomerised with alkali at 180° C for 60 minutes, the mean observed value of $E^{1\%}_{1\,cm}$ at 234 mμ was 908, in close agreement with the earlier value of 906. For linolenic acid prepared by debromination, the values were also usually close to those observed in the earlier work, but linolenic acid isolated by physical methods of separation gave somewhat different values, namely: after isomerisation at 170° C for 15 minutes, $E^{1\%}_{1\,cm}$ at 268 mμ = 555, and after isomerisation at 180° C for 60 minutes, $E^{1\%}_{1\,cm}$ at 234 mμ = 575. It is considered that these should be used in place of the earlier values of 532 (268 mμ) and 569 (234 mμ).

In 1945, Hilditch, Morton and Riley[1] recommended that, in the spectrophotometric determination of linoleic and linolenic acids after alkali isomerisation as originally proposed by Mitchell, Kraybill and Zscheile,[2] isomerisation to conjugated dienes should be carried out at 180° C for 60 minutes, and isomerisation to conjugated trienes (for linolenic acid) at 170° C for 15 minutes. Employing specimens of linoleic and linolenic acids prepared by debromination of the crystalline tetrabromo- or hexabromostearic acids obtained from the natural acids, these workers observed the following values of $E^{1\%}_{1\,cm}$ with a Hilger E3 Quartz Spectrograph with sector photometer for the pure acids—

	$E^{1\%}_{1\,cm}$	λ
Linolenic acid, alkali-isomerised at 170° for 15 minutes ..	532	268 mμ
Linolenic acid, alkali-isomerised at 180° for 60 minutes ..	569	234 mμ
Linoleic acid, alkali-isomerised at 180° for 60 minutes ..	906	234 mμ

In recent and current investigations a Beckman or a Unicam photo-electric spectrophotometer has been employed, and it is now possible to isolate natural linoleic and linolenic acids by solely physical means (crystallisation or chromatographic adsorption) without recourse to the chemical procedure of bromine addition and subsequent debromination. In view of the extensive use now made of the spectrophotometric methods for the determination of linoleic and linolenic acids in natural fats, it was considered desirable to re-investigate the above reference values for $E^{1\%}_{1\,cm}$. Accordingly, specimens of linoleic acid from sunflower, safflower and Niger seed oils, and of linolenic acid from linseed and conophor oils, have been prepared by both the older chemical and the newer physical procedures and, after the standard isomerisations with alkali, have been examined in a Beckman photo-electric spectrophotometer and (in one instance) in the Hilger E3 spectrograph.

Isolation of linoleic and linolenic acids

(*a*) By chemical methods—

The procedure used was that described in the earlier paper[1] (p. 69). Tetrabromostearic acid was prepared from concentrates of linoleic acid from sunflower or safflower seed oils and debrominated to methyl linoleate by the method of Rollett,[3] the crude ester being then fractionated in a vacuum. Hexabromostearic acid was prepared from unsaturated concentrates of the acids of linseed or conophor oils and purified by crystallisation from xylene followed by washing with boiling ether. The purified acid was debrominated to linolenic acid by the method of Kaufmann and Mestern.[4] The crude acid was methylated and the

methyl ester fractionally distilled. Six or seven fractions of distilled esters were obtained, those of maximum iodine value being used in the further investigation.

The characteristics of the specimens of esters finally obtained and subsequently used for alkali isomerisation and spectrophotometric examination are given in Tables I and II.

TABLE I

LINOLEIC ACID (METHYL LINOLEATE) PREPARED BY THE CHEMICAL METHOD

Source	Tetrabromostearic acid: Yield, % (w/w)	Tetrabromostearic acid: M.p., ° C	Methyl linoleate: Yield, % (w/w)	Methyl linoleate: Distilled fraction studied: No.	Methyl linoleate: Distilled fraction studied: Iodine value	Linoleic acid from fraction. Iodine value*
Safflower seed oil	55	115·5	33	3	171·4	180·1
Sunflower seed oil concentrates (I.V. 169)	84	115	41	4	171·0	179·2

TABLE II

LINOLENIC ACID PREPARED BY THE CHEMICAL METHOD

Source	Hexabromostearic acid: Crude: Yield, % (w/w)	Hexabromostearic acid: Crude: M.p., ° C	Hexabromostearic acid: Purified: Yield, % (w/w)	Hexabromostearic acid: Purified: M.p., ° C	Linolenic acid crude: Yield, % (w/w)	Linolenic acid crude: Iodine value*	Methyl linolenate. Distilled fraction studied: No.	Methyl linolenate. Distilled fraction studied: Iodine value*	Linolenic acid from fraction. Iodine value*
Linseed acid concentrates (I.V. 231) ..	65	178	35	181	33	265·3	3	259·4	270·6
Conophor mixed acids (I.V. 210) ..	50	178	29	181·4	33	264·0	4	259·6 258·0	270·7 269·8
Conophor mixed acids (I.V. 210) ..	44	178	33	181·2	32	264·2	4	259·9	271·0

* It was noticed (*cf.* McCutcheon[9]) that, at high concentrations of linolenic acid or ester, iodine values determined by the Wijs method required 3 hours contact with the Wijs reagent before a constant maximum absorption was reached. For this reason 3 hours (instead of the usual half-hour) contact with the reagent was employed for both the linoleic and the linolenic compounds dealt with in this paper when approximate purity was being approached. These 3-hour contacts are recorded as "iodine value*."

Theoretical iodine values: linoleic acid 181·4, linolenic acid 274·1.

(*b*) BY PHYSICAL METHODS—

Attempts were made to find conditions whereby linoleic or linolenic acids could be effectively separated from small proportions of accompanying unsaturated acids by crystallisation of the lithium salts from acetone, but without success. In other experiments the use of lithium or barium hydroxides in amounts sufficient only to neutralise a portion of the fatty acids present was attempted, but in none was the salt of linoleic (or linolenic) acid obtained in a condition approaching purity, whether from solutions in alcohol or acetone or from mixtures of the two solvents. The procedure, recommended by Nicholson and Formo,[5] of employing a mixture of two bases (*e.g.*, sodium and barium hydroxide) also failed to lead to the isolation of the pure acids.

Recourse was therefore had to crystallisation of the acids themselves from appropriate solvents at low temperatures. Following the general procedure recommended by Brown and Frankel[6] we were able by repeated crystallisation of acids rich in linoleic acid, first from acetone at −60° to −70° C, and finally from light petroleum at −65° to −70° C, to isolate specimens of linoleic acid of high purity; but we also confirmed the experiences of Shinowara and Brown[7] that linolenic acid cannot be prepared free from contamination by about 10 per cent. of linoleic acid by low-temperature crystallisation methods alone. Riemenschneider, Herb and Nichols,[8] however, have recently described an adsorption method for the isolation of pure linolenic acid, and we found that application of their procedure to concentrates rich in linolenic acid produced by preliminary use of low-temperature crystallisation enabled us to prepare almost pure specimens of natural linolenic acid.

Linoleic acid—Specimens of the pure acid were obtained from the mixed acids of sunflower, safflower and Niger seed oils by crystallisation from acetone and light petroleum (b.p. 40° to 60° C) at low temperatures. As an example, the isolation of linoleic acid from the mixed acids of Niger seed oil may be briefly described.

The mixed acids (100 g, iodine value 139·6) were first crystallised from 10 per cent. solution in acetone at −60° C, the deposited acids being further crystallised from acetone at −55° C, and this process was repeated twice at −55° C. The acids left in solution at each stage were: at −60° C, 17·9 g, iodine value 157·1; at −55° C (1), 17·8 g, iodine value 169·7; at −55° C (2), 17·0 g, iodine value 173·4; at −55° C (3), 10·0 g, iodine value 173·6. The first batch of acids contained most of the unsaponifiable matter from the original oil, but the three succeeding batches (44 g) were united and further crystallised as below; from this stage onwards linoleic acid was concentrated in the deposited solids and not in the acids left in solution. The acids deposited at each stage, and the conditions of crystallisation, were as follows—

Solvent	Crystallisation: Concentration of solute, %	Crystallisation: Temperature, °C	Deposited acids: g	Deposited acids: Iodine value*
Acetone	10	−70	38·0	173·5
Light petroleum	5	−60	33·1	176·2
Light petroleum	5	−60	28·2	177·1
Light petroleum	5	−60	23·2	177·7
Light petroleum	5	−60	17·1	180·0
Light petroleum	5	−60	11·7	179·9
Light petroleum	5	−60	5·5	179·5

The specimens with iodine values* 180 and 179·9 (theory 181·4) were used for spectrophotometric examination after alkali isomerisation.

The specimens of purified linoleic acid thus prepared and employed for spectrophotometric analysis are summarised in Table III.

TABLE III

SPECIMENS OF LINOLEIC ACID PREPARED BY LOW-TEMPERATURE CRYSTALLISATION

Source of acid	Iodine value* of acid studied
Sunflower seed oil	176·9
Safflower seed oil	179·3
Niger seed oil	180·0
Niger seed oil	179·9

Linolenic acid—Application of the crystallisation procedure used to isolate linoleic acid to the mixed acids of oils (linseed or conophor) rich in linolenic acid failed to give a pure product, a mixture of more or less constant composition (about 85 per cent. of linolenic and 15 per cent. of linoleic acid) being reached, which could not be further separated by crystallisation alone. Thus, the mixed acids (iodine value 215) of conophor oil (iodine value 205) left in solution in acetone at −70° C about 80 per cent. of fatty acids of iodine value 242. These were crystallised nine times from 5 per cent. solutions in light petroleum at −60° to −70° C, but the acids that separated from the last three recrystallisations remained at a practically constant iodine value* of 256.

Specimens of approximately pure linolenic acid were, however, obtained by submitting the methyl esters of polyethenoid concentrates of the acids of conophor or linseed oils obtained by low-temperature crystallisation to separation by chromatographic adsorption, employing the technique of Riemenschneider, Herb and Nichols.[8] The process may be illustrated with reference to fatty acids from linseed oil.

The mixed acids (188 g, iodine value 192) from linseed oil (iodine value 182·7) were first crystallised from 10 per cent. solutions in acetone at −60° C, when 132 g of acids (iodine value 235) were left in solution. These were converted to methyl esters (iodine value 226), which were fractionated in a vacuum through an electrically-heated and packed column: from 37·5 g of esters a distilled fraction (20·3 g, iodine value 231·0) was obtained for use in the separation over the silica gel adsorbent.

The adsorbent was prepared (*cf.* Riemenschneider, Herb and Nichols[8]) by thoroughly mixing 4 parts of silica gel (previously ground to pass a 30-mesh sieve and washed with aqueous hydrochloric acid and then with water until free from acid and fine particles of gel) with 1 part of Hyflo "Supercel" in a mortar and activating the mixture by heating for 2 hours at 220° C in a gentle current of carbon dioxide. The prepared adsorbent was cooled and at once transferred to the adsorption apparatus (a tube 4·3 cm in diameter filled to a height of 40 to 45 cm with a column of the adsorbent). The column was electrically heated and, after filling, was kept at 72° C for 3 hours in a current of carbon dioxide. It was then cooled in the stream of carbon dioxide and the packed column was wetted with light petroleum (b.p. 40° to 60° C). The apparatus was so designed that throughout the separation (which occupied several days) a slight positive pressure of carbon dioxide was maintained in the system. A solution of the unsaturated esters (19·5 g, iodine value 231·0) in light petroleum (97·5 ml, b.p. 40° to 60° C) was introduced into the column and, immediately after they had been adsorbed, elution with the same quality of light petroleum was begun, the rate of efflux of the solvent being about 250 ml per hour. This was an extremely lengthy process and, as shown in Table IV, resulted in a long series of fractions of esters being recovered, the first being of very low iodine value, and the final fractions approaching closely in iodine value to that of methyl linolenate (261·0). When this point was reached, the eluant was changed to a mixture of 98 per cent. of the light petroleum with 2 per cent. of ether, which rapidly removed the remaining methyl linolenate. This fraction (usually about 4 to 5 g) was then finally redistilled through a small vacuum-jacketed fractionation column, and the middle fractions of the distillate were converted to acid and used for the spectrophotometric analysis.

TABLE IV

ELUTION OF ADSORBED METHYL LINOLENATE FROM THE ADSORPTION COLUMN

(19·5 g of esters (iodine value 231·0) of unsaturated acid concentrates from linseed oil)

Elution with light petroleum

Fraction	Eluate, ml	Methyl esters		Fraction	Eluate, ml	Methyl esters	
		g	I.V.			g	I.V.
1	2500	0·10	66·3	12	1100	1·95	209·3
2	2900	0·05	83·6	13	1000	1·35	230·3
3	2500	0·05	—	14	1500	1·37	242·4
4	1500	0·06	31·2	15	1000	1·12	244·1
5	2300	0·05	—	16	850	0·46	246·6
6	2300	0·11	92·0	17	1250	0·51	246·6
7	2300	0·17	103·6	18	1000	0·38	—
8	2300	0·33	105·0	19	1000	0·28	250·0
9	1500	0·36	112·4	20	1000	0·23	—
10	2000	0·62	125·0	21	1500	0·29	253·5
11	1500	2·40	127·7	22	1000	0·17	252·3

Elution with 98% *of light petroleum* + 2% *of ether*

Fraction	Eluate, ml	Methyl esters	
		g	I.V.
23	450	0·09	255·0
24	700	0·12	
25	1000	4·98	256·7
26	1000	0·36	255·5

(Total weight recovered 18·0 g = 92·1%)

Fraction No. 25 had an iodine value* (Wijs, 3 hours contact) of 260·1 and was fractionally distilled. The third and fourth fractions of the distilled esters (1·68 and 1·73 g, iodine values* 259·8 and 261·0) were combined and hydrolysed, and the resulting acids (iodine value* 271·9) were used for spectrophotometric analysis.

From a similar sequence of operations conducted on the methyl esters (iodine value 200) of the mixed fatty acids of a specimen of conophor oil, there was finally obtained a distilled fraction of methyl linolenate, iodine value* 259·7, which yielded a specimen of linolenic acid with iodine value* 271·8.

Spectrophotometric examination of alkali-isomerised linoleic and linolenic acids

The specimens of highly-purified linoleic and linolenic acids isolated as described in the preceding pages by both chemical and physical methods were submitted to isomerisation with caustic potash in ethylene glycol solution, at 170° C for 15 minutes to develop triene

Table V

Extinction coefficients (at 234 mμ) for linoleic acid after alkali isomerisation at 180° C for 60 minutes

Isolation	Origin	Iodine value*	Extinction coefficient determinations: No.	$E^{1\%}_{1cm}$	Deviation: Mean	Deviation: Standard
Chemical (debromination)	Sunflower seed oil	179·2	6	906·3	10·1	13·1
	Safflower seed oil	180·1	6	915·5	9·7	12·5
Physical (crystallisation)	Sunflower seed oil	176·9	7	912·1	4·7	6·7
	Safflower seed oil	179·3	2	903·1	4·3	—
	Niger seed oil	180·0	2	906·3	1·3	—
		179·9	2	910·9	4·5	—

Table VI

Extinction coefficients for linolenic acid

Isolation	Origin	Iodine value*		Extinction coefficient determinations: No.	$E^{1\%}_{1cm}$	Deviation: Mean	Deviation: Standard
At 268 mμ, after alkali isomerisation at 170° C for 15 minutes							
Chemical (debromination)	Linseed oil	270·6		12	539·0	5·8	8·0
	Conophor oil	271·0		6	538·3	3·7	5·5
	Conophor oil	270·7		16	559·3	2·2	2·9
		269·8	Beckman:	8	555·0	6·2	7·4
			Hilger:	7	556·8	6·8	8·9
Physical (adsorption)	Linseed oil	271·9		9	558·9	2·1	2·8
	Conophor oil	271·8		6	552·0	3·2	5·5
At 234 mμ, after alkali isomerisation at 180° C for 60 minutes							
Chemical (debromination)	Linseed oil	270·6		6	560·7	3·1	4·4
	Conophor oil	271·0		6	570·3	3·6	4·6
	Conophor oil	270·7		8	589·7	8·0	10·0
Physical (adsorption)	Linseed oil	271·9		5	572·3	4·0	5·4
	Conophor oil	271·8		6	578·5	2·5	3·4

conjugation from linolenic acid, or at 180° C for 60 minutes to develop diene conjugation from either acid. The analytical details given in the earlier paper[1] (pp. 69, 70) for the preparation of the alkaline glycol reagent and for the conduct of the isomerisation were carefully followed. As therein recommended, all determinations were made on the free acids and not on their esters. In some instances we employed glycerol in place of ethylene glycol as the isomerisation solvent (*cf.* Brice and Swain[10]), but in our experience this led to less concordant values and we reached the conclusion that glycol is definitely preferable to glycerol for this purpose.

The spectrophotometric measurements were made throughout in a Beckman spectrophotometer, but in one series, for comparison, determinations were also made on a Hilger E3 Quartz Spectrograph (as used in the earlier work). Blank determinations with the alkaline glycol solution were carried out under exactly the same conditions as the actual determinations, the spectrographic observations of the latter being made in duplicate on each isomerised specimen. After the isomerisations at 170° or 180° C, the solutions were diluted with

purified absolute alcohol so that the values of log I_o/I recorded on the Beckman apparatus fell within the range 0·3 to 0·6.

Tables V and VI give a summary of the extinction coefficients recorded, respectively, for the specimens of linoleic and linolenic acid. In these tables are given the method of isolation of the acids (chemical or physical), the oils from which they were obtained, the observed iodine value of the specimen, the number of separate alkali isomerisations made in each instance, and the mean value of the extinction coefficients observed, together with the mean and standard deviations therefrom.

Discussion and conclusions

Linoleic acid—

The mean of all the observed values (Table V) for the extinction coefficient at 234 mμ of alkali-isomerised linoleic acid isolated by physical means from natural sources is $E^{1\%}_{1\,cm} = 908$. This value is within 0·3 per cent. of that formerly determined[1] (906), and we therefore consider that the latter may continue to be employed.

The final mean value for linoleic acid produced chemically by debromination of tetrabromostearic acid was very close to this figure, although the mean values for each series and the mean and standard deviations varied to a greater extent than the corresponding figures for the linoleic acids obtained by crystallisation.

Linolenic acid—

Extinction coefficients at 268 *m*μ (*triene conjugation*)—Here the values of $E^{1\%}_{1\,cm}$ for alkali-isomerised linolenic acid isolated by purely physical (adsorption) methods differed by about 4 per cent. from that (532) observed earlier,[1] the final mean value being 555 (*cf.* Table VI). The latter value should in our opinion now be adopted as the reference value for $E^{1\%}_{1\,cm}$ at 268 mμ for alkali-isomerised linolenic acid.

Of three specimens of linolenic acid prepared by the debromination procedure, one gave values of the same order as the above ($E^{1\%}_{1\,cm} = 555$ to 559), but the other two gave values similar to those observed in the earlier work for debrominated linolenic acid, namely, $E^{1\%}_{1\,cm} = 538$ to 539. Matthews, Brode and Brown,[11] who have contributed considerably to our knowledge of the chemical properties and behaviour of linoleic and linolenic acids alternatively prepared by debromination or isolated by low-temperature crystallisation, have concluded that the debrominated acids may contain 12 per cent. or more of unsaturated acids that are not the same as the natural linoleic or linolenic acids. Although some of the isomers produced will presumably be geometrical (*cis-trans*) isomers of the natural acids, and these may isomerise on treatment with alkali at 170° to 180° C more or less similarly to the natural (wholly *cis*-) acids, it seems probable that, at least when linolenic acid is concerned, some of the chemically regenerated acid does not react to alkali to the same extent as the natural acid. It is, nevertheless, curious that in one instance the acid obtained by debromination gave the same extinction coefficient at 268 mμ after isomerisation as that given by the linolenic acids prepared by physical means.

It is also evident that, although Brown *et al.*[11] have observed similar chemical differences between regenerated and recrystallised linoleic acid as with the linolenic acids, linoleic acids produced by either method give similar yields of conjugated diene acids when isomerised with alkali.

In one instance in Table VI it will be seen that a specimen of linolenic acid was examined, after alkali isomerisation, in both the Beckman and the Hilger instruments, with identical results. The differences in extinction coefficients that have been observed are accordingly due solely to the means employed to isolate linolenic acid, and not to any difference in the spectrographic technique.

Extinction coefficients at 234 *m*μ (*diene conjugation*)—The earlier figure found for the extinction coefficient at 234 mμ for linolenic acid after isomerisation with alkali at 180° C for 60 minutes was $E^{1\%}_{1\,cm} = 569$. The mean value determined in the present study from eleven determinations on linolenic acid isolated from linseed or conophor oils by the adsorption procedure was 575. Although this is only about 1 per cent. higher than the former figure, it is suggested that the value $E^{1\%}_{1\,cm} = 575$ at 234 mμ should be used for the contribution of linolenic acid to diene conjugation during alkali isomerisation.

The corresponding figures for linolenic acid specimens produced by debromination are less consistent but also lead to a final mean value of $E^{1\%}_{1\,cm} = 575$.

We recommend that the extinction coefficients for linoleic and linolenic acids to be used in spectrophotometric determinations of these acids should be as follows—

	$E^{1\%}_{1cm}$	λ
Linolenic acid, alkali-isomerised at 170° C for 15 minutes ..	555 (formerly 532)	268 mμ
Linolenic acid, alkali-isomerised at 180° C for 60 minutes ..	575 (formerly 568)	234 mμ
L noleic acid, alkali-isomerised at 180° C for 60 minutes ..	906 (as formerly)	234 mμ

On this basis, the figures based on spectrophotometric analyses recorded in our publications up to the present for linolenic acid in drying oils may be about 4 per cent. too high, *i.e.*, 2 to 3 units per cent. too high in linolenic-rich oils such as conophor or linseed, and about 1 unit per cent. high in candlenut or rubberseed oils, whilst in oils such as soya bean or hemp seed there is only a fractional difference. Similarly, the figures recorded hitherto for linoleic acid are about 1 per cent. low for oils rich in linolenic acid, but not sensibly different in other drying oils. The effect on the proportions of oleic and saturated acids is somewhat greater; in oils rich in linolenic acid (linseed, etc.) the oleic acid figures may be 4 to 5 units per cent. low, and the saturated acid figures 2 to 3 units per cent. high, as recorded on the basis of the $E^{1\%}_{1cm}$ values hitherto used. It is, however, only in the mixed acids of oils such as conophor or linseed that the differences involved exceed, at the most, about 1 unit per cent. in the component acid data.

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A Modification of the Elson and Morgan Method for the Estimation of Glucosamine

By J. P. JOHNSTON, A. G. OGSTON and J. E. STANIER

An investigation of the stages of the Elson and Morgan method for estimating glucosamine has led to modifications of the details of its stages. It has been found that 10 μg or more of free glucosamine or combined glucosamine of hyaluronic acid can be estimated with an accuracy of 3 per cent.

In spite of the satisfactory accuracy and sensitivity of the colorimetric method for the estimation of glucosamine described by Elson and Morgan,[1] a number of other workers have re-investigated and modified it: chief of these (using less than 3 mg of glucosamine) have been Palmer, Smyth and Meyer,[2] Sørensen[3] and Blix,[4] while others have modified it for use with larger quantities. The accuracies claimed range from 2 per cent. to 10 per cent. Ogston and Stanier[5] found the version of Palmer, Smyth and Meyer insufficiently accurate, in spite of minor modifications.

In order to test the last method further, we made a number of parallel determinations of free glucosamine, taken from the same batch of material; we found differences of up to 6 per cent. between estimations on samples of the same solution after treatment in bulk with acetylacetone, and even larger differences between estimations on lots separately treated with acetylacetone. Investigation of the stages of the method was therefore carried out, beginning with the final stage and working backwards. From this, it appeared that considerable errors might arise from the processes of transfer and dilution, from loss of acetylacetone during heating in open tubes and from variations in the temperatures and durations of the several stages. We therefore modified the method so as to eliminate the transfer of material from one vessel to another as far as possible. At the only remaining transfer, the whole sample is transferred and this has also the effect of reducing the amount of glucosamine that may be estimated to as little as 10 μg. Under the conditions chosen, the optimal times for some stages have been found to differ from those given by other authors. The procedure is given below, followed by comment on its stages.

Procedure

Hydrolysis (when the glucosamine is combined in hyaluronic acid)—Seal 1 ml of solution, containing 10 to 80 μg of glucosamine, and 1 ml of 8 *N* hydrochloric acid in a Pyrex tube 12 cm long and of 0·8 cm internal diameter. Mix the solution thoroughly and totally immerse the tube in a boiling water bath for 4 hours.

Removal of hydrochloric acid—Cool the tube, centrifuge to remove traces of solution from the upper end and open it. Remove the hydrochloric acid and evaporate the contents of the tube to dryness by placing the tube over solid sodium hydroxide in a desiccator evacuated by an oil pump.

Treatment with acetylacetone—Add 1 ml of distilled water to the tube to dissolve the contents, and then add 0·5 ml of freshly prepared acetylacetone solution (0·2 ml of acetylacetone in 10 ml of 0·5 *N* sodium carbonate). Re-seal the tube and totally immerse it in a boiling water bath for 60 minutes, then cool it in ice, centrifuge and open it. With free glucosamine, the estimation begins with the treatment with acetylacetone.

Treatment with Ehrlich's reagent—Transfer the contents of the tube quantitatively by Pasteur pipette to a 5-ml graduated flask. Wash the tube three times with a total of 2·9 ml of aldehyde-free ethanol and add the washings to the flask. Stopper the flask and mix the contents; warm the flask to 37° C by immersion in a thermostat for 3 minutes. Then add 0·5 ml of Ehrlich's reagent (0·8 g of *p*-dimethylaminobenzaldehyde, A.R., in 30 ml of aldehyde-free ethanol plus 30 ml of concentrated hydrochloric acid) and sufficient ethanol to make the solution up to 5 ml. After mixing, leave the flask in the thermostat for 60 minutes, then remove it and measure the extinction at 535 mμ with a spectrophotometer. Comparison is made with standard glucosamine introduced at the acetylacetone treatment stage.

Comments on procedure

Hydrolysis—The yield of estimated glucosamine (Fig. 1) from ox synovial-fluid mucin rose to a maximum after 4 hours and fell slowly again with longer hydrolysis to 88 per cent.

at 22 hours. If the hydrolysis and destruction are both unimolecular processes, the maximum yield at 4 hours would be 97 per cent. of the total glucosamine. No significant loss of pure glucosamine occurred under the same conditions up to 32 hours (Fig. 1); this allows the glucosamine standard to be introduced at the stage of the treatment with acetylacetone. The change of reactivity of glucosamine reported by Blix[4] to follow heating with 4 *N* hydrochloric acid was not observed. Evaporation of the hydrochloric acid *in vacuo* (Hadidian and Pirie[6]) avoids the need for transfer and neutralisation.

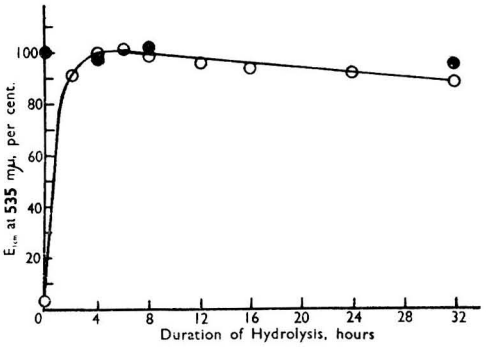

Fig. 1. Effect of duration of hydrolysis on extinction obtained with free glucosamine and with mucin. Ordinate: E_{1cm} at 535 mμ as percentage of value obtained at reference time.
● Extinction given by free glucosamine, as percentage of value at start of hydrolysis.
○ Extinction given by mucin glucosamine as percentage of value obtained at 4 hours

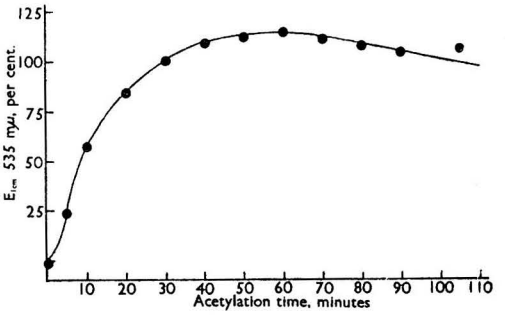

Fig. 2. Effect of time of heating with acetylacetone on final colour development. Ordinate: E_{1cm} at 535 mμ, as percentage of value obtained after 30 minutes

Treatment with acetylacetone—This is an intrinsically unsatisfactory reaction, since both the acetylacetone and the glucosamine undergo some destruction[7] during its course. Blix[4] has shown that a greater yield is obtainable by using larger amounts of acetylacetone and sodium carbonate and that, under his conditions, the yield is least sensitive to variations in the amounts of these reagents. We have used the amounts recommended by Palmer, Smyth and Meyer[2]; although these give a lower yield, the sensitivity of the reaction is sufficient, and we are satisfied that the ordinary errors of measuring the reagents will not introduce an error of more than 1 per cent. into the final estimate. We found it to be essential to carry out this reaction in sealed tubes, totally immersed at 100° C. Under these conditions, the maximum yield is at 60 minutes (Fig. 2); Blix[4] recommends this period, but other authors have used shorter periods (Sørensen[3] 20 minutes; Palmer, Smyth and Meyer[2] 15 minutes; Ogston and Stanier[5] 30 minutes).

Treatment with Ehrlich's reagent—Differences of up to 10 per cent. were found if the reagents were mixed at 0° C or room temperature and then brought to 37° C. Bringing to 37° C before mixing reduced this difference to 1·9 per cent. (standard deviation of eight determinations).

Accuracy—Fourteen parallel samples of glucosamine hydrochloride (each 30 μg of glucosamine) showed a standard deviation (single estimate) of 1·9 per cent. of the mean value, and seven parallel samples of ox synovial mucin (each containing 30 μg of glucosamine) a standard deviation of 3 per cent.

One of us (J. E. S.) is indebted to the Medical Research Council for a Training Studentship.

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Department of Biochemistry
Oxford

June, 1950

Some Qualitative and Quantitative Colour Reactions for the Lower Homologues of the Pyridine Series

By E. F. G. HERINGTON

Attention is drawn to the lack of specific colour reactions for bases of the pyridine series and to the desirability of developing methods for the detection and estimation of certain homologues. New qualitative tests based on the use of a phenol - chloroform - sodium ethoxide mixture are described. The colours obtained with this type of reagent when the phenol is phenol itself, *p*-chlorophenol or thiophenol are recorded. The presence of as little as 0·1 per cent. of γ-picoline in β-picoline or 2:6-lutidine can be detected with the phenol reagent, while 0·0005 ml of γ-picoline produces a detectable colour if other bases are absent. The reagent containing thiophenol yields particularly brilliant and characteristic colours suitable for the identification of single bases.

A reagent containing 1-chloro-2:4-dinitrobenzene, acetamide and 2:6-lutidine is described; this is suitable for the quantitative estimation of β- and γ-picoline in admixture with 2:6-lutidine.

THE formation of coloured compounds from bases of the pyridine series has been known for a long time but, although certain of these substances (*e.g.*, the cyanine dyes) have found widespread application in photography, few colour reactions specific for individual bases have been described. Recently, several bases of this series have become available commercially and it is likely that they will give rise to new and useful products with a variety of applications. Moreover, interest continues in the synthesis of pyridine bases (*cf.* Janz *et al.*[1,2,3,4]), so that the development of simple methods for the identification and estimation of these compounds appeared to be of interest.

Simple fractional distillation by modern distillation technique will readily separate α-picoline and pyridine from a mixture, but the homologues β-picoline, γ-picoline and 2:6-lutidine boil at so nearly the same temperature that other methods have to be used in their separation (see Coulson and Jones[5]). For this reason special attention is paid in this paper to the analysis of these close-boiling mixtures.

A large number of different types of reagent, for example, αβ-ethynyl ketone (Johnson[6]), were examined in preliminary experiments, but none appeared to be as suitable for analytical applications as those whose detailed use is now described.

PURITY OF BASE SAMPLES EMPLOYED—

To establish unambiguously the colours developed by any reagent it is necessary to use specimens of the pyridine bases of high and preferably known purity. The preparation of samples of these bases was undertaken for other purposes and will be described elsewhere, but the type of material used in the present investigation is indicated by the mole per cent. purity that was established by the freezing-point technique of Herington and Handley,[7] as follows: pyridine, 99·85 ± 0·07; α-picoline, 99·87 ± 0·06; β-picoline, 99·97 ± 0·02; γ-picoline, 99·75 ± 0·13; 2:6-lutidine, 99·93 ± 0·04.

QUALITATIVE COLOUR REACTIONS OF PYRIDINE BASES WITH A PHENOL - CHLOROFORM - SODIUM ETHOXIDE MIXTURE—

Certain phenols give strongly coloured products when allowed to react with chloroform and a pyridine base in the presence of sodium ethoxide. A number of phenolic substances have been tried in such mixtures; they include α- and β-naphthol, catechol, *o*-, *m*- and *p*-cresol, *p*-phenylphenol, 1:3:5-xylenol, tribromophenol, trichlorophenol, *o*-chlorophenol and *o*-hydroxyl diphenyl, but the strongest and most characteristic colours were obtained when phenol itself, *p*-chlorophenol or thiophenol were used. The behaviour of the reagents containing each of these three phenols is described because the reagent containing phenol provides a sensitive test for γ-picoline, that containing *p*-chlorophenol gives a characteristic colour with β-picoline and that containing thiophenol gives brilliant and characteristic colours

with the different bases. The colours produced by these reagents are modified by the addition of water and chloroform, and because these colour changes are in some instances characteristic of the base, the colours of the chloroform layers after the addition of water are recorded.

The colour observed when a mixture of bases reacts with one of these reagents is not the sum of the colours given by the constituent bases when they react singly, and as a result these reagents are not suitable for quantitative work. Nevertheless, the reagent containing phenol itself can be used to detect the presence of traces of γ-picoline in β-picoline and 2:6-lutidine, and its use for this purpose is described.

Colours produced by single bases

Phenolic reagent—

Take 1 ml of a solution of phenol in chloroform (25 g of phenol per litre), add 0·05 ml of the base and then 1 ml of sodium ethoxide (5 g of sodium in 1 litre of alcohol). Heat the mixture in an open test tube to 80° C for 10 minutes and then cool. The bases give the colours shown in Table I, column 3.

Add 1 ml of chloroform and 10 ml of water and shake. The colours so obtained are listed in Table I, column 4.

p-Chlorophenol reagent—

Use the same experimental conditions as described under the heading "phenolic reagent" above, but use a concentration of 20 g of *p*-chlorophenol per litre of chloroform. The colours obtained are recorded in Table I, columns 3 and 4.

Thiophenol reagent—

Take 1 ml of thiophenol solution (2 ml of thiophenol plus 10 ml of chloroform), add 0·1 ml of the base and then 1 ml of sodium ethoxide solution (50 g of sodium in 1 litre of alcohol). Allow the mixture to stand for 1 hour. Good colours can be obtained only if care is taken with the concentration of the reactants, and the solution must not be heated, otherwise certain colours, *e.g.*, that from β-picoline, will be destroyed. The colours observed are listed in Table I, as are also the colours after the addition of chloroform and water.

Table I

Colours observed with the phenol - chloroform - sodium ethoxide reagent

Phenolic constituent	Base	Colour	Colour of chloroform layer after addition of water
Phenol	pyridine	pale reddish orange	yellow
	α-picoline	pale reddish orange	colourless
	β-picoline	slightly orange	colourless
	γ-picoline	purple	bright blue
	2:6-lutidine	pale yellow	colourless
	reagent alone	pale yellow	colourless
p-Chlorophenol ..	pyridine	pink	pale yellow
	α-picoline	pale brown	colourless
	β-picoline	red	colourless
	γ-picoline	blue-violet, turning grey	grey-purple
	2:6-lutidine	yellow	pale yellow
	reagent alone	pale yellow	pale yellow
Thiophenol ..	pyridine	red	red
	α-picoline	reddish brown	red
	β-picoline	brilliant red	orange
	γ-picoline	brilliant blue-green	strong green
	2:6-lutidine	pale orange	yellow
	reagent alone	pale orange	colourless

Sensitivity of phenolic reagent for the detection of γ-picoline alone—

The phenol - chloroform - sodium ethoxide reagent, under the conditions described above, gives a colour with 0·0005 ml of γ-picoline that is distinguishable from that exhibited by the reagent alone.

Detection of γ-picoline in β-picoline or 2:6-lutidine

One-tenth of 1 per cent. of γ-picoline can be detected in β-picoline or 2:6-lutidine by means of the phenol - chloroform - sodium ethoxide reagent provided that the colours can be compared with those formed by pure samples of β-picoline or 2:6-lutidine. For the application of this test for the presence of γ-picoline in samples that consist chiefly of β-picoline or 2:6-lutidine, it was found to be advantageous to use more concentrated reagent solutions than those already described, and the following conditions are recommended.

Take 1 ml of a solution of phenol in chloroform (280 g of phenol per litre), add 0·5 ml of the base and then 1 ml of a sodium ethoxide solution (50 g of sodium per litre of alcohol). Heat the mixture to 80° C for 10 minutes. The single bases give the colours shown in Table II, column 2. Add 1 ml of chloroform and 10 ml of water and shake. Under these conditions 0·1 per cent. v/v of γ-picoline in 2:6-lutidine gives a purplish colour, but the same strength of γ-picoline in β-picoline yields a more scarlet shade than does β-picoline alone, and a brownish colour after adding chloroform and water. The colours obtained with a single base under these conditions are given in Table II, columns 2 and 3.

Table II

Colours observed with the more concentrated phenol - chloroform - sodium ethoxide reagent

Base	Colour	Colour of chloroform layer after addition of water
Pyridine	orange-red	yellow
α-Picoline	brown	brown
β-Picoline	red	pale yellow
γ-Picoline	deep purple	purple-blue
2:6-Lutidine	reddish orange	pale yellow
Reagent alone	pale yellow	pale yellow

Quantitative estimation of γ-picoline

Hamer and Rathbone[8] report that 1-chloro-2:4-dinitrobenzene does not combine with α-picoline or 2:6-lutidine to give a pyridinium salt. The behaviour of mixtures of this compound with each of the five bases was studied and as a result a method for estimating γ-picoline was devised. All five bases were found to give a purple colour when 0·001 ml of the base was heated to 100° C for 1 hour with 5 ml of a solution made by dissolving 50 g of 1-chloro-2:4-dinitrobenzene in 1 litre of alcohol; the colour produced by γ-picoline was the most intense. γ-Picoline was the only base that gave a purple colour under similar conditions if 40 g of acetamide were added to 1 litre of the reagent. The colours yielded by the other bases were pale and were as follows: pyridine, yellow-brown; α-picoline, brown-red; β-picoline, yellow; 2:6-lutidine, reddish brown.

The behaviour of the 1-chloro-2:4-dinitrobenzene - acetamide reagent when two bases, one of which was γ-picoline, were simultaneously present was observed by adding 0·050 ml of the other base to 0·001 ml of γ-picoline. The product from the mixtures containing pyridine or β-picoline as the additional base gave pale brown solutions, while the presence of α-picoline or 2:6-lutidine greatly enhanced the colour due to the γ-picoline, although these bases alone at the same concentration did not give a purple colour. The addition of this fifty-fold excess of α-picoline to γ-picoline gave a colour of twice the intensity of that produced by the γ-picoline alone, while the same quantity of 2:6-lutidine increased the intensity approximately eight-fold. Clearly it is impossible to use this simple 1-chloro-2:4-dinitrobenzene - acetamide reagent for the estimation of γ-picoline when unknown amounts of α-picoline and 2:6-lutidine are present in the mixture under analysis because of the enhancement of colour produced by these two α-substituted bases. However, this difficulty can be overcome by deliberately adding a large excess of pure 2:6-lutidine to the reagent so that any additional enhancement of the colour resulting from the presence of α-picoline or 2:6-lutidine in the sample under analysis is negligible. The following conditions were found to be satisfactory for the estimation of γ-picoline in mixtures.

Method for estimation of γ-picoline in mixtures

Reagent—

Dissolve 50 g of 1-chloro-2:4-dinitrobenzene, 40 g of acetamide and 10 ml of pure 2:6-lutidine in 1 litre of industrial methylated spirit.

Procedure—

Dilute 0·1 ml of the sample under investigation to 100 ml with alcohol and treat 0·1 ml of pure γ-picoline similarly. Run 5 ml of the reagent into each of three dry boiling tubes. Add 1 ml of the dilute solution of the unknown to one tube, add 1 ml of the dilute γ-picoline solution to the second and add 1 ml of alcohol to the third. Immerse the uncorked tubes simultaneously in boiling water and leave for 1 hour. Shake the tubes gently from time to time and ensure that water does not condense inside the tubes by placing them in a beaker of boiling water in such a way that the open ends protrude. At the end of the hour, cool the tubes in ice and dilute the product from each to 50 ml with alcohol. Measure the absorption immediately, because the colours have a tendency to fade. The Spekker absorptiometer is set with a full scale reading of 1·30 drum units with Ilford filter No. 606 and the blank solution containing 1 ml of alcohol in a 1-cm absorption cell. Measure the absorption of the other two solutions using the same cell. Calculate a first approximation to the volume percentage of γ-picoline in the unknown from the expression $100(1{\cdot}30 - a)/(1{\cdot}30 - b)$, where a and b are the drum readings for the unknown and the γ-picoline standard solutions respectively. The first approximate value of the γ-picoline content is used to calculate a fresh dilution of the unknown base mixture so that the new solution contains 0·1 ml of γ-picoline in 100 ml of alcohol, and the experiment is repeated using this fresh dilution. If this second experiment is correctly carried out the readings a and b should be nearly equal. The percentage of γ-picoline in the unknown is calculated in the same manner as described above, but taking cognisance of the new dilution factor. The mean of the first and second estimation is taken to be the γ-picoline content.

Typical results of analysis of synthetic mixtures expressed as volume percentages are shown in Table III. Pyridine, α-picoline and 2:6-lutidine do not interfere with this determination.

The dilutions recorded here may not be suitable for all absorption spectrophotometers, but the dilution should be chosen such that the reading $(1{\cdot}30 - b)$ may be made with a suitable accuracy, while the unknown should be diluted similarly. The rate of decay of the purple colour was found to be a function of the concentration of the coloured compound; for this reason a second determination is recommended, a dilution calculated on the results of the first experiment being used. In general, the results of the first approximation tend to be low (see Table III, column 4) and that of the second tend to be high (column 5), while the means of these values (column 6) are nearer the true figures than the individual determinations.

Table III

Determination of γ-picoline

Synthetic mixture			γ-Picoline found		
β-Picoline, %	2:6-Lutidine, %	γ-Picoline, %	First approximation, %	Second approximation, %	Mean, %
90	—	10	7·0	10·5	8·8
—	90	10	8·0	10·9	9·5
45	45	10	7·0	11·5	9·3
33·3	33·3	33·3	36·9	31·4	34·2
50	—	50	48·4	56·0	52·2
—	50	50	55·7	53·3	54·5
25	25	50	45·1	57·3	51·2

Quantitative estimation of β-picoline

The method recommended for the colorimetric estimation of β-picoline is based on the observation that if sodium ethoxide is added to the product obtained by heating the base with 1-chloro-2:4-dinitrobenzene - acetamide - 2:6-lutidine reagent, both β- and γ-picoline yield a purple coloured product but that produced by γ-picoline fades more rapidly. The

β-picoline yields a red-brown solution after 1 hour, while the colour of the γ-picoline solution differs little from that of the reagent. Under the conditions finally chosen, γ-picoline produces a colour whose intensity is approximately one-tenth of that generated by the same volume of β-picoline, and for this reason it is recommended that the blank and reference β-picoline solutions employed should be at approximately the same γ-picoline concentration as the unknown.

Method for estimation of β-picoline in mixtures

Reagents—

1-Chloro-2:4-dinitrobenzene reagent—Dissolve 50 g of 1-chloro-2:4-dinitrobenzene, 40 g of acetamide and 10 ml of pure 2:6-lutidine in 1 litre of industrial methylated spirit.

Acetamide solution—Dissolve 40 g of acetamide in 100 ml of water.

Sodium ethoxide solution—Allow 50 g of clean sodium to react with 1 litre of industrial methylated spirit. Prepare this solution just before use.

Procedure—

Dilute 0·1 ml of the solution under investigation to 100 ml with alcohol, and treat 0·1 ml of pure β-picoline and 0·1 ml of pure γ-picoline similarly. Run 5 ml of the 1-chloro-2:4-dinitrobenzene reagent into each of three dry boiling tubes. Add 1 ml of the dilute solution of the unknown to one tube, and to the second add x ml of the dilute γ-picoline solution (x is the volume fraction of γ-picoline in the unknown as measured by the method described above). Sufficient accuracy will be obtained if this value of x is adjusted to the nearest 0·05 ml, *e.g.*, if the volume percentage of γ-picoline had been found to be 34·1 per cent., then $x = 0{\cdot}35$ ml. Add to the third tube x ml of the dilute γ-picoline solution and $(1 - x)$ ml of the dilute β-picoline solution. Heat the three tubes for 1 hour in boiling water and take care that water does not condense inside the tubes. Shake the tubes gently from time to time. Dissolve the product from each tube in 10 ml of alcohol and cool the solutions to room temperature (*i.e.*, to between 10° and 20° C). Add 5 ml of the aqueous acetamide solution and then 1 ml of the sodium ethoxide solution. Shake the tubes and allow them to stand in a beaker of water at room temperature for 1 hour. Dilute the product from each tube to 100 ml with alcohol.

The following precautions must be observed: (i) the solutions must be cooled to room temperature before the sodium ethoxide solution is added, otherwise the reagent alone will give an intensely coloured solution; (ii) the specified concentration of sodium ethoxide must be adhered to, because it will be found that a smaller quantity than that recommended will fail to destroy the colour produced by γ-picoline, while a higher concentration will give a highly coloured solution with the reagent alone; (iii) the three solutions must be treated similarly in every way and the colours must be compared 1 hour after adding the sodium ethoxide, because if left too long the β-picoline solution also will lose its red colour.

The Spekker absorptiometer is set with a full scale reading of 1·30 drum units with Ilford filter No. 605 and with the solution made from the x ml of γ-picoline solution in the cell. The drum readings for the unknown, a, and for the synthetic mixture containing $(1 - x)$ ml of β-picoline solution, b, are recorded and the percentage of β-picoline in the unknown is calculated by the relationship $100(1 - x)(1{\cdot}30 - a)/(1{\cdot}30 - b)$.

Typical results obtained in this way are shown in Table IV, column 4.

Table IV

Determination of β-picoline

Synthetic mixture			Percentage β-picoline found	
β-Picoline, %	2:6-Lutidine, %	γ-Picoline, %	Exact value, %	Rough value, %
90	—	10	91·2	88·0
—	90	10	0	3·0
45	45	10	46·0	45·5
33·3	33·3	33·3	33·6	38·4
50	—	50	50	56·2
—	50	50	0	6·1
25	25	50	26·1	32·3

Rough values of the β-picoline content can, however, be found without first determining the γ-picoline concentration by employing 1 ml of alcohol in the blank and 0·5 ml of β-picoline as standard. The values shown in Table IV, column 5, were obtained in this way. Such values tend to be too high by approximately one-tenth of the γ-picoline concentration. α-Picoline and 2:6-lutidine do not interfere, but pyridine produces a colour having approximately 45 per cent. of the intensity of that given by an equal volume of β-picoline. Thus, if pyridine is present in a sample, the β-picoline concentration found will be too high by approximately $\frac{1}{2}y$ per cent. where the pyridine concentration is y per cent. For example, a concentration of 28·1 per cent. of β-picoline was found for a synthetic mixture containing 25 per cent. of β-picoline, 10 per cent. of pyridine, 50 per cent. of γ-picoline and 15 per cent. of 2:6-lutidine. In another example, 36·7 per cent. of β-picoline was found in a synthetic sample containing 25 per cent. of β-picoline, 25 per cent. of pyridine and 50 per cent. of γ-picoline. Clearly, if a considerable quantity of pyridine is present in a sample under analysis, the pyridine should either be removed before the analysis for β-picoline is undertaken or allowance should be made for its presence by determining the pyridine content by some independent method. The pyridine can readily be removed by fractional distillation with an efficient fractionating column (pyridine, b.p. 115·3° C; β-picoline, b.p. 144° C; γ-picoline, b.p. 145° C; 2:6-lutidine, b.p. 144° C). Pyridine can be estimated colorimetrically by the method due to Ploquin[9] or by the clearing point method described by Hamer, Pomfret and Stubbings.[10]

I wish to thank Dr. E. A. Coulsen, J. B. Ditcham, E. C. Holt and A. Sleven for supplying the pure base samples used, and R. Handley and A. J. Cook for the estimations of the purity of the bases.

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CHEMICAL RESEARCH LABORATORY
TEDDINGTON

May, 1950

The Arsenic Electrode as a pH Indicator

By A. A. MOUSA*

The preparation of arsenic electrodes by the deposition of sublimed arsenic on rotating platinum bases is described.

An investigation has shown that these electrodes may be used for electrometric recording of pH during acid-base titrations with a number of monobasic, dibasic and tribasic acids, and in particular with acids such as arsenious, arsenic and hydrocyanic that incapacitate the hydrogen and quinhydrone electrodes. The pk_a' values obtained with the arsenic electrode agree closely with hitherto accepted values.

The arsenic electrode is shown to be applicable also to the measurement of pH in precipitation reactions, and the results of such experiments with magnesium, zinc, cadmium, lead and aluminium are recorded.

THE behaviour of both massive arsenic and massive antimony electrodes in aqueous solutions at different pH values, as explained in the light of the theory of lattice defects, has recently been reported.[1,2] It was shown that these electrodes could legitimately be regarded as metal - metal oxide - oxygen electrodes; this necessarily implies a particular significance to the role played by oxygen. From several studies in this connection it was judged that the freshly deposited arsenic electrode could with advantage be used to replace the antimony electrode for pH measurements. This judgment was based chiefly on: (*a*) the readiness with which oxygen molecules accommodate themselves at fixed positions on the electrode surface, a fact that is reflected in the instantaneous establishment of steady potentials and also in the establishment of potential - pH relationships covering almost the whole pH range; and (*b*) the fact that the oxygen overvoltage effect at the electrode surface decays but slowly and that arsenious oxide, unlike antimonious oxide, dissociates in a simple manner,[3,4] so that marked deviations from a strictly rectilinear relationship, especially in the course of prolonged pH measurements, are not to be expected.

Before recording the experiments carried out to test the validity of using the arsenic electrode in pH measurements, it is of value to state the following facts to serve as a general guide when making measurements with that electrode.

(i) The electrode should be freshly deposited; aged electrodes or electrode systems involving the metal powder cannot be used.

(ii) Addition of arsenious oxide to the test solution must be avoided; a pre-immersion adsorbed oxide film with its oxygen doublets will do this with advantage.

(iii) Since the electrode process is subject to an oxygen overvoltage mechanism, the effect of temperature should not be overlooked.

(iv) The rate of the cathodic process, $O_2 + 2H_2O + 4e \rightarrow 4OH'$, that occurs at the electrode surface varies with the partial pressure of oxygen; measurements should, therefore, be made in the presence of a non-reacting gas such as nitrogen or hydrogen.

(v) As the electrode is a self-polarised one, the formal equation $E = E'_o - (RT/F)$ pH, derived on grounds of complete reversibility, should not be expected to hold. It is therefore essential to construct calibration graphs of the type $E = K - S.pH$, in which K stands for the term E'_o subject to the influence of the above-mentioned factors, and S for the slope of the potential - pH curves obtained experimentally.

Experimental

Preparation of electrodes—

As in the previous investigations,[1,4] freshly deposited arsenic electrodes were made by subliming and depositing the pure metal on platinum bases sealed into glass tubes; these, when furnished with a mercury contact, served as electrodes in the usual way. In the present investigation, however, it was found convenient to carry out the process of deposition while the electrode was rotating at a moderate speed; in this manner a compact homogeneous coating adhering firmly to the platinum base and the adjacent portion of the glass tube was always obtained. The apparatus used for this purpose is shown diagrammatically in Fig. 1.

* Present address: Department of Physical Chemistry, Free School Lane, Cambridge.

Crystals of arsenic metal were first placed in the sublimation vessel, V, made of Pyrex glass, and the electrode was then fitted in through the cup, C, of a mercury seal with the tip of the platinum base about half a centimetre above the crystals. A fine stream of oxygen-free nitrogen was allowed to circulate in the vessel, and the sublimation of the metal was carried out by heating with an ordinary bunsen burner while the electrode was rotated. The sublimation was continued for about 5 minutes, after which the electrode was left to cool in the circulating gas. Any oxide or unstable modification of arsenic metal that might have been deposited on the remote portions of the glass tube was carefully rubbed off with emery paper before the electrode was used.

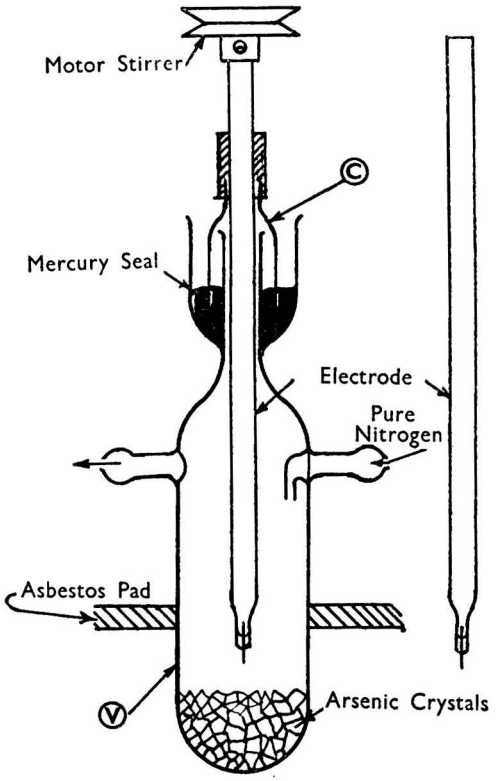


Fig. 1. Apparatus for deposition of arsenic on rotating electrode

TEST SOLUTIONS AND CALIBRATION GRAPH—

As the first object was to obtain a calibration graph on the basis of which the observed potentials might be converted to pH values, a modified form of the Prideaux - Ward universal buffer[5] was used. For this purpose, 0·12 *M* stock solutions of phosphoric, acetic and boric acids were prepared and standardised. A 100-ml mixture of the three acids was accurately diluted to 200 ml with redistilled water, then neutralised by gradual addition of exactly 0·20 *M* sodium hydroxide, which was prepared and kept out of contact with atmospheric carbon dioxide. The neutralisation was performed inside an air-bath, specially designed for the titrations, whose temperature was kept constant to within $\pm 0{\cdot}05°$ C. For the measurements carried out at temperatures above that of the laboratory, which varied from 18° to 20° C, the temperature of the mixture after each addition of alkali could be restored readily by means of a heating element supported by the titration vessel. The pH value of the mixture at each stage of neutralisation was determined electrometrically with a hydrogen electrode of the Hildebrand type used in conjunction with a saturated calomel electrode as a half-cell. Three sets of measurements carried out at 20°, 25° and 30° C are shown in Table I.

Similar acid mixtures were then neutralised with a stationary freshly deposited arsenic electrode as the indicator. This neutralisation was performed in the presence of oxygen-free

nitrogen, which was allowed to bubble through the solution. The electrode subjected in this way to varying pH was sensitive to variation in potential, and the instantaneous establishment of steady values was remarkable. In Table I are shown the potential values E_H as referred to the normal hydrogen electrode; these were recorded within 2 to 4 minutes after each addition of alkali.

TABLE I

RELATIONSHIP BETWEEN pH AND POTENTIAL *vs.* NORMAL HYDROGEN ELECTRODE AT VARIOUS TEMPERATURES

Alkali added, ml	20° C		25° C		30° C	
	pH	E_H	pH	E_H	pH	E_H
nil	2·24	+0·207	2·22	+0·190	2·25	+0·180
10·00	2·42	+0·202	2·40	+0·184	2·39	+0·176
20·00	3·11	+0·180	3·08	+0·168	3·06	+0·152
30·00	4·40	+0·107	4·37	+0·096	4·29	+0·090
40·00	5·44	+0·050	5·30	+0·041	5·29	+0·028
50·00	6·73	−0·024	6·66	−0·039	6·51	−0·048
60·00	7·52	−0·070	7·42	−0·083	7·38	−0·100
70·00	8·81	−0·144	8·77	−0·164	8·63	−0·172
80·00	9·76	−0·198	9·64	−0·222	9·56	−0·234
90·00	11·16	−0·281	11·02	−0·300	10·87	−0·310
100·00	11·59	−0·306	11·37	−0·317	11·24	−0·328

Except for acid solutions up to a pH of about 3, the potential - pH curves were almost straight lines that, when extrapolated to pH = 0, gave average values of K of 0·368, 0·355 and 0·344 volt at 20°, 25° and 30° C respectively. The corresponding average values for the term S ($\Delta E/\Delta pH$), as calculated from each pair of successive readings within the region of linearity, amounted to 0·0590, 0·0597 and 0·0602 respectively. Calibration graphs for the arsenic electrode may therefore be represented by the following equations—

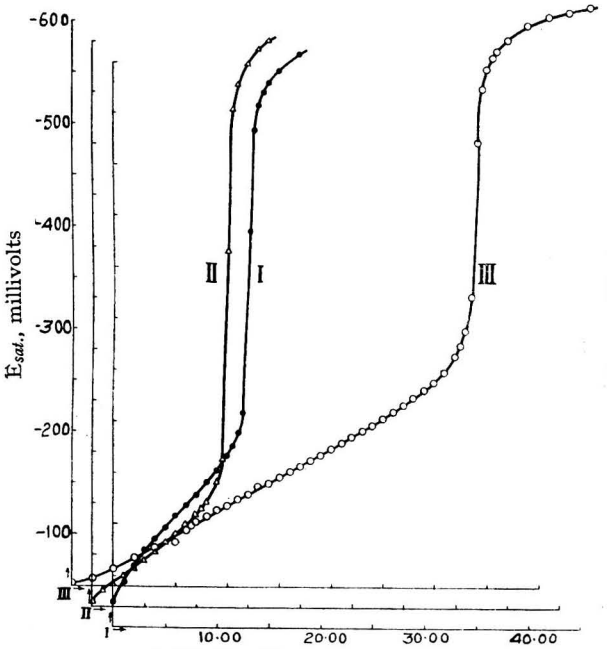
$$E_H \text{ (observed)} = 0{\cdot}368 - 0{\cdot}0590 \text{ pH at } 20° \text{ C}$$
$$E_H \text{ (observed)} = 0{\cdot}355 - 0{\cdot}0597 \text{ pH at } 25° \text{ C}$$
$$E_H \text{ (observed)} = 0{\cdot}344 - 0{\cdot}0606 \text{ pH at } 30° \text{ C}$$

where the observed potentials are referred to the normal hydrogen electrode. If the observed potentials are referred to the saturated calomel electrode the values of K in the above equations become 0·121, 0·112 and 0·104 volt respectively.

THE VALIDITY OF USING THE ARSENIC ELECTRODE IN ACID - BASE TITRATIONS—

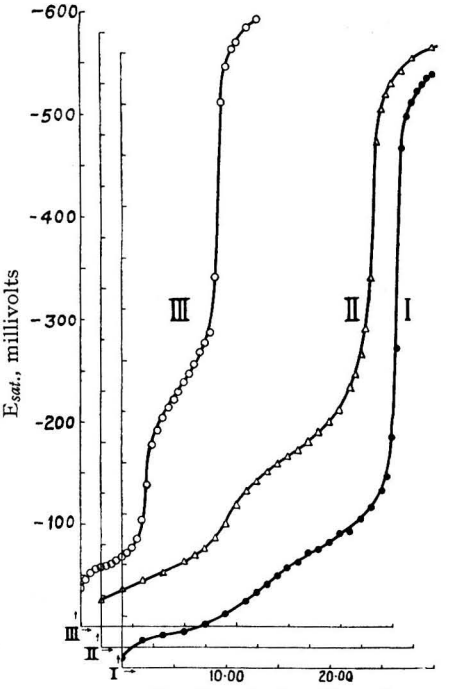
A number of monobasic acids, including acetic, boric, arsenious and hydrocyanic acids, some dibasic acids, including tartaric, oxalic, malonic, maleic and succinic acids, and some tribasic acids, including citric, arsenic and phosphoric acids, were each titrated against the arsenic electrode, to ascertain the extent to which it could be used in the determination of the titration end-points, and the dissociation constants were calculated from the neutralisation curves. In performing these titrations the experimental procedure described above was followed and the temperature of the bath was kept constant at 20° C. Fig. 2 shows representative curves obtained by plotting the $E_{sat.}$ values at the different stages of neutralisation against the amount of titrant added; the concentrations of the solutions examined are recorded below each diagram.

An inspection of the curves reveals that the inflexions marking the end-points are well defined and in good agreement with the theoretical amounts; further, that the most flattened portions of the curves possess slopes proportional in magnitude to the buffering capacity of each acid with its sodium salt. The arsenic electrode therefore provides a suitable and readily attainable method for the titration of those acids such as arsenious, arsenic and hydrocyanic, in particular, that incapacitate the hydrogen and the quinhydrone electrodes. The fifth column of Table II contains the mean pk_a' values for some of the acids; these were calculated on the basis of the familiar Henderson - Hasselbalch equation in the form $pH = pk_a' + \log b/(a - b)$, where a represents the original concentration of acid, b the concentration of added base and $a - b$ the concentration of unneutralised acid. The pH values used in the above equation were computed, for at least three different neutralisation stages in each example, from the relation $E_{sat.}$ (observed) = 0·121 − 0·0590 pH. The pk_a'



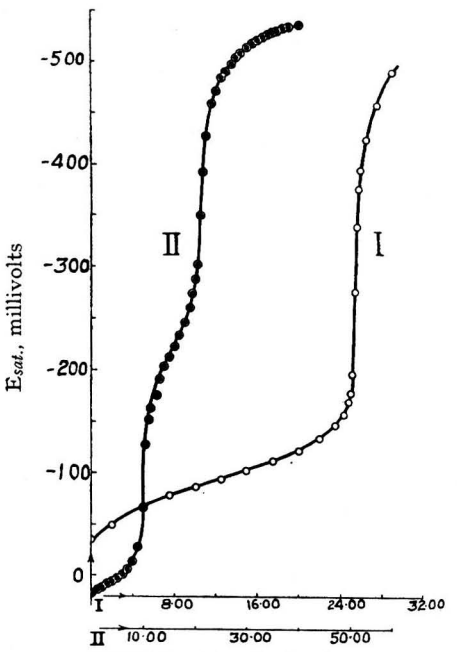
0·079 *N* sodium hydroxide, ml

I 10·0 ml of 0·050 *M* succinic acid
II 10·0 ml of 0·050 *M* tartaric acid
III 10·0 ml of 0·100 *M* citric acid



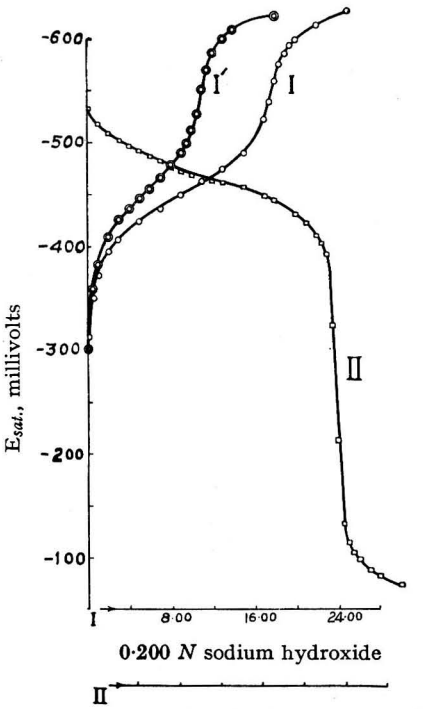
0·079 *N* sodium hydroxide, ml

I 10·0 ml of 0·100 *M* oxalic acid
II 10·0 ml of 0·100 *M* malonic acid
III 10·0 ml of 0·050 *M* maleic acid



0·039 *N* sodium hydroxide, ml

I 25·0 ml of 0·040 *M* acetic acid
II 10·0 ml of 0·040 *M* phosphoric acid



0·200 *N* sodium hydroxide

0·028 *N* hydrochloric acid

I 25·0 ml of 0·072 *M* As_2O_3 solution
I′ 50·0 ml of 0·022 *M* As_2O_3 solution
II 50·0 ml of 0·100 *M* potassium hydroxide

Fig. 2

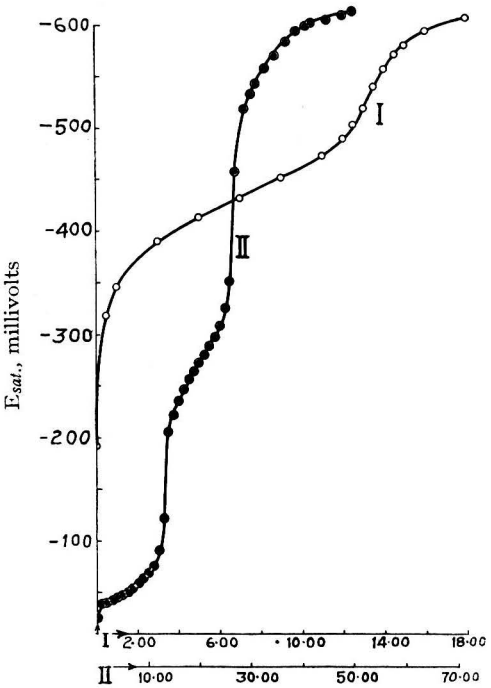


Fig. 2—*continued*

values obtained agree closely with the hitherto accepted values that have been obtained by different methods, and, as may be inferred from the data in columns 3 and 4, the fidelity of the arsenic electrode in responding to minute changes in pH is noteworthy.

THE VALIDITY OF USING THE ARSENIC ELECTRODE IN PRECIPITATION REACTIONS GOVERNED BY pH—

The importance of pH as a governing factor in some precipitation reactions has been further emphasised recently in connection with the behaviour of metal electrodes in aqueous solutions, where the separation of the metal hydroxide (or a basic salt) is often to be expected; hence the introduction of a new type of electrode reaction.[6,7] The separation of metal hydroxides from their salt solutions by the gradual addition of alkali was originally investigated by Hildebrand[8] and extended later by Britton[9] and Britton and Robinson.[10] According to the last-named authors, the precipitation pH value of a particular cation is essentially the same whether the phase separated is the pure hydroxide, which very seldom occurs, or a basic salt, and in general is but slightly affected by the concentration of the cation and the nature of anion combining with it. Nevertheless, it has been observed that in some instances, *e.g.*, the separation of zinc cations, the reported precipitation pH values are inconsistent among themselves, and that in some other instances, *e.g.*, the separation of lead cations, the nature of the phase separated as well as the course of its separation are not critically established. Therefore, it appeared necessary to test the validity of using the arsenic electrode in tracing pH during such separations, searching at the same time for any advantage of the electrode over those previously used and to which the anomaly observed has often been ascribed.

The first column of Table III shows particulars of the solutions examined. The separation of cations was effected through the gradual addition of exactly 0·111 *N* sodium hydroxide with a freshly deposited arsenic electrode serving as an indicator electrode. The curves in Fig. 3 show the different steps in the course of separation of each cation and were obtained by plotting the $E_{sat.}$ values against the amount of alkali added. By reasonable normalisation

of the curves, the number of equivalents of alkali corresponding to every step could be determined with fair accuracy; these are given in the second column of the table. Since it was

TABLE II

pk_a' VALUES CALCULATED FROM pH VALUES AS MEASURED BY THE ARSENIC ELECTRODE

Acid	Amount of acid neutralised, %	$E_{sat.}$	pH	pk_a'	Mean pk_a'	Published* pk_a'
Boric	40·0	−0·413	9·06	9·24		
	55·5	−0·432	9·38	9·28	9·27	
	71·4	−0·451	9·69	9·29		
Hydrocyanic	41·7	−0·468	9·98	9·83		
	50·0	−0·463	9·90	9·90	9·85	
	54·2	−0·462	9·88	9·81		
Arsenious Sol. 1	50·0	−0·450	9·68	9·68		
	61·1	−0·462	9·88	9·68		
	72·2	−0·474	10·09	9·68		
					9·69	9·26
Sol. 1′	45·5	−0·446	9·61	9·69		
	54·5	−0·456	9·78	9·70		
	63·6	−0·466	9·95	9·71		
Maleic	43·0	−0·222	5·80	5·92		
	60·1	−0·239	6·10	5·92	5·92	6·50
	75·2	−0·257	6·41	5·93		
Malonic (2nd step) ..	41·7	−0·187	5·22	5·37		
	49·6	−0·193	5·32	5·33	5·34	5·40
	57·5	−0·201	5·46	5·33		
Oxalic (2nd step)	41·7	−0·113	3·97	4·12		
	49·6	−0·117	4·03	4·04	4·05	3·90
	57·5	−0·123	4·13	4·00		
Arsenic (2nd step) ..	41·1	−0·273	6·68	6·84		
	48·0	−0·281	6·81	6·84	6·85	6·77
	54·9	−0·289	6·95	6·86		
(3rd step)	32·4	−0·558	11·51	11·83		
	46·5	−0·570	11·71	11·77	11·78	11·53
	60·7	−0·583	11·93	11·74		

* From H. T. S. Britton, "Hydrogen Ion Concentration," Volume I, Chapman and Hall, Ltd., London, 1942.

practically impossible to detect visually the initial formation of precipitates, it was considered justifiable to define the pH range within which precipitation occurs rather than to ascertain a particular pH value for incipient precipitation as has often been done. In the third column of the table are shown the pH ranges within which the different phases are precipitated (or otherwise); the values were computed from the relation $E_{sat.} = 0{\cdot}121 - 0{\cdot}0590$ pH, since the measurements were performed at 20° C and the potentials were referred to the saturated calomel electrode.

Except for lead cations, the pH values that might be considered to indicate incipient precipitation (points *a* on the curves) are in close agreement with those previously reported from hydrogen, oxygen or glass electrode measurements. For zinc, the value obtained in this investigation, 6·88, supports the values previously reported by Kolthoff[11] and Prytz[12] and is approximately one pH unit higher than that obtained by Britton and Robinson.[10] For lead cations, the value obtained cannot be compared with those previously reported as the course of their separation is found to be materially different, as shown below. As is inferred by the data in the second column of Table III, the phase separated from all the solutions examined is almost invariably a basic salt and not a true hydroxide. In agreement with the previous observations, the salt separated from each of the magnesium, zinc and cadmium solutions possesses the composition $MX_2.3M(OH)_2.[xH_2O]$, where X stands for a

TABLE III

USE OF THE ARSENIC ELECTRODE TO MEASURE pH IN PRECIPITATION REACTIONS

Solution (diluted to 200 ml)		Equivalents of NaOH	pH range
10·00 ml of 0·100 *M* $MgCl_2$ + 2·50 ml of 0·200 *N* HCl		1·66	10·53 to 10·86
10·00 ml of 0·103 *M* $ZnSO_4$ + 10·00 ml of 0·114 *N* H_2SO_4		1·56	6·88 to 7·47
10·00 ml of 0·096 *M* $CdCl_2$ + 5·00 ml of 0·200 *N* HCl		1·49	6·80 to 7·31
10·00 ml of 0·119 *M* $Pb(NO_3)_2$	1st step ..	0·98	5·51 to 5·92
10·00 ml of 0·106 *N* HNO_3	2nd step ..	0·45	7·47 to 8·66
10·00 ml of 0·101 *M* $Al_2(SO_4)_3$	1st step ..	4·57	4·00 to 4·32
+ 10·00 ml of 0·114 *N* H_2SO_4	2nd step ..	1·99	9·44 to 10·36

monovalent acid radical, since three-quarters only of the stoicheiometric amount of the alkali was consumed in every case. The curve for aluminium shows that within the range *ab* a basic salt is formed, and that further addition of alkali causes its decomposition as indicated by the gradual slope of the inflexion *bc*. The point *c* corresponds to exactly six equivalents

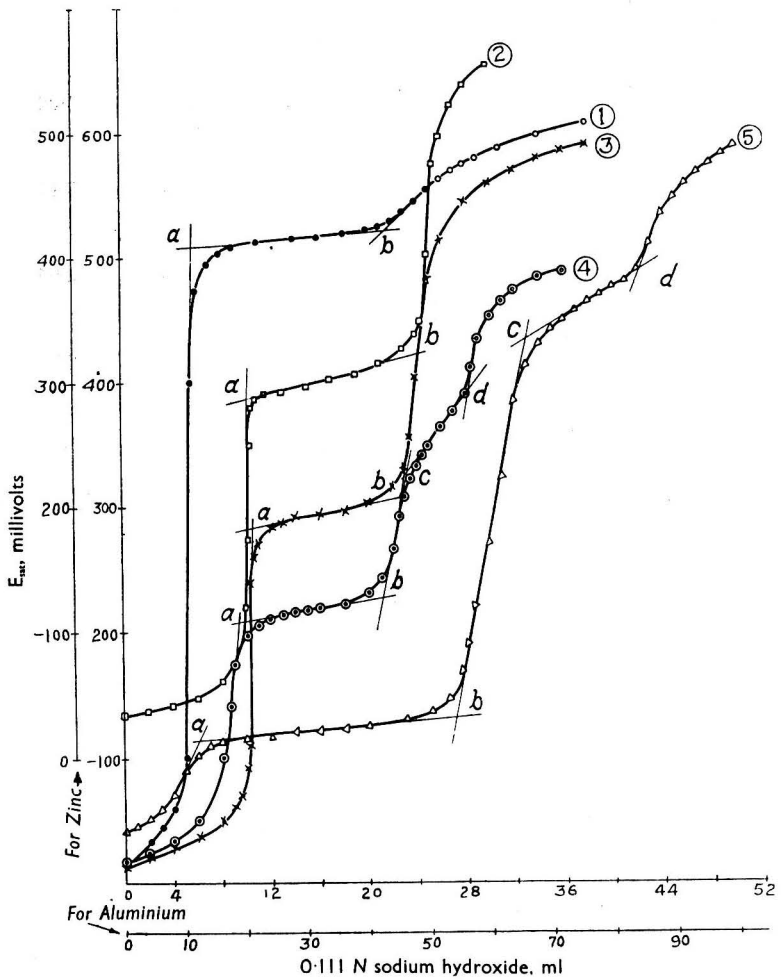


Fig. 3. Steps in the separation of each cation. Curve (1), magnesium chloride; curve (2), zinc chloride; curve (3), cadmium chloride; curve (4), lead nitrate; curve (5), aluminium sulphate

of sodium hydroxide; the composition of the phase at that point corresponds therefore to $Al_2O_3.[xH_2O]$. The portion *cd* on the curve corresponds to about two equivalents and indicates the dissolution of the phase to form aluminate. For the separation of lead cations, the curves that have been reported by previous investigators (Britton and Robinson[10] from oxygen electrode measurements, and Dorling[13] from glass electrode measurements) showed only one inflexion, which indicated the complete separation of a basic salt having the same composition as that separated from magnesium, zinc or cadmium solutions. For incipient separation of the salt, the former authors reported the pH value of 6·0. The curve obtained in this investigation, however, is peculiar as it shows two inflexions. The portion *ab* corresponds approximately to one equivalent of sodium hydroxide and so indicates the separation of a phase somewhat less basic than would correspond to $M(X)_2.M(OH)_2$ within the pH range 5·51 to 5·92. The portion *cd* corresponds to half an equivalent of sodium hydroxide and indicates the separation of the basic salt $M(X)_2.3M(OH)_2$ within the pH range 7·47 to 8·66. The separation of these two basic salts has recently been reported by Geloso and Faucherre[14]; the former phase within the pH range 5 to 7, and the latter phase within the range 7 to 8.

The above results show clearly that the freshly deposited arsenic electrode serves satisfactorily for tracing pH during the separation of metal cations; the limitation imposed, however, is to be anticipated from the position of arsenic metal in the electromotive series since the electrode cannot be used for solutions of nobler cations.

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Fouad I University
Faculty of Science
Abbassia, Cairo, Egypt

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The Micro-Estimation of Osmium in its Organic Compounds

By F. P. DWYER and N. A. GIBSON

A method is described for the colorimetric estimation of micro quantities of osmium in organic compounds. Fusion with sodium peroxide and sodium carbonate of an amount of substance equivalent to 4 mg of osmium metal is recommended. The sodium osmate resulting from the fusion is then converted to hydrogen hexabromosmate by means of concentrated hydrobromic acid and estimated colorimetrically with thiourea by a modification of Sandell's method. The over-all accuracy of the method is of the order of 1 per cent., and the reproducibility of the colorimetric part of the analysis is of the order of 0·3 per cent.

The estimation of osmium in its organic compounds is usually difficult owing to the ease of formation and the volatility of its oxide, OsO_4. This prevents the use of normal methods of oxidation of the organic matter by fuming in sulphuric acid and an oxidising agent in an open vessel and also direct ignition to the metal or its oxide. Up to the present, the methods used have all involved oxidation under wet conditions with simultaneous distillation of the tetroxide into a suitable reducing medium.[1,2,3,4,5] The osmium can then be estimated by several methods. These include the colorimetric estimation with thiourea,[3] titration with thiosulphate of the iodine released from potassium iodide,[1,6] precipitation and weighing as osmium dioxide[7] or titration with potassium permanganate.[7]

Despite claims that have been made for the completeness of the distillation of the osmium tetroxide, it has been the experience of the authors and also of others[8] that the recovery of osmium is often of the order of but 90 to 95 per cent. and hence it can scarcely be claimed that this method is sufficiently accurate for quantitative work.

Method

In the method used in the present work, the organic matter was destroyed under alkaline conditions by the gentle heating of an intimate mixture of the compound to be analysed with about forty times its weight of sodium peroxide. By this treatment the osmium was converted into sodium osmate^{VI}, which was then dissolved in water, acidified with concentrated hydrobromic acid and was thereby converted to hydrogen hexabromosmate^{IV}, which was estimated colorimetrically with thiourea. The results obtained rarely varied from the theoretically expected result by more than 2 per cent. and were usually of the order of 1 per cent. or better. The method has the advantage of simplicity of technique and apparatus; no special apparatus other than the colorimeter is required.

The colorimetric estimation of osmium with thiourea has already been described by Gilchrist,[3] and by Sandell.[8] The method used by Sandell was followed in general, with small modifications made necessary by the presence in the solution of a large concentration of hydrobromic acid as well as some free bromine. The most important modification was the use of a larger amount of stannous chloride to remove the bromine, which otherwise oxidised the thiourea with the formation of colloidal sulphur. In each determination, colorimetric comparison was made on three aliquot portions of the solution obtained after fusion. These did not differ from each other, on the average, by more than 0·3 per cent.

Reagents—

Thiourea solution—Dissolve pure B.D.H. thiourea in cold distilled water to form a 10 per cent. solution, filter through two thicknesses of Whatman No. 42 filter-paper. Prepare stannous chloride solution by dissolving C.P. stannous chloride in 2·5 *N* hydrochloric acid (A.R.) with warming to form a 10 per cent. solution, cool and filter through two thicknesses of Whatman No. 42 filter-paper.

Procedure—

Weigh sufficient of the compound to contain about 4 mg of osmium into a small silica crucible, mix thoroughly with about 0·8 g of sodium peroxide and cover with a layer of sodium

TABLE I

Compound	Osmium: Calc., %	Osmium: Found, %	Osmium: Mean, %	Percentage deviation from calc.
$(PhNH_3)_2OsBr_6$	22·17	22·08	22·35	+0·8
		22·61		
		22·37		
		21·62	21·68	−2·2
		21·73		
		22·20	22·23	+0·3
		22·25		
		21·72	21·88	−1·3
		22·02		
		21·80		
		21·97		
$(PhMe_2As)_3OsBr_3$	19·48	19·48	19·69	+1·1
		19·86		
		19·72		
		19·80	19·84	+1·8
		19·80		
		19·93		
$Os(dipy)_3Br_2.3H_2O$	21·80	21·60	21·63	−0·8
		21·69		
		21·60		
		21·46	21·43	−1·7
		21·46		
		21·36		
$Os(dipy)_3I_2.2Os(dipy)_3(SbOTart.)_2.12H_2O$	15·86	15·80	15·87	−0·1
		15·88		
		15·92		
		15·76	15·82	−0·3
		15·94		
		15·76		
$Os(phenan)_3(ClO_4)_2.H_2O$ (*d* form)	20·07	20·05	20·05	−0·2
		20·05		
		20·05		
		20·09	20·15	+0·4
		20·14		
		20·23		

TABLE II

Compound	Osmium: Calc., %	Osmium: Found, %	Average deviation from mean, %	Percentage deviation from calc.
$Os(dipy)_3Cl_2.6H_2O$	22·70	22·33	0·1	−2·1
		22·40	0·0	−1·3
$Os(dipy)_3I_2.3H_2O$	19·68	19·54	1·2	−0·7
$Os(dipy)_3(ClO_4)_2.H_2O$	21·72	21·35	0·6	−1·7
		21·36	0·3	−1·7
$Os(dipy)_3(Tart.).3H_2O$	22·09	21·49	0·4	−2·7
		21·78	0·6	−1·4
$Os(dipy)_3(SbOTart.)_2.2H_2O$	15·02	15·26	0·6	+1·9
		14·69	0·5	−1·9
$Os(dipy)_3(ClO_4)_3.H_2O$ (racemate)	19·51	19·13	0·3	−1·9
$Os(dipy)_3(ClO_4)_3.H_2O$ (*l* form)	19·51	19·63	0·3	+0·6
$(NH_4)_2OsBr_6$	26·99*	27·05	0·2	+0·2
		27·25	0·2	+1·0
$[Os(NH_3)_5Br](ClO_4)_2.H_2O$	33·24	33·64	0·2	+1·2
		32·90	0·3	−1·0
$Os(phenan)_3Cl_2.8H_2O$	20·11	19·98	0·4	−0·6
		20·17	0·0	+0·3
$Os(phenan)_3I_2.3H_2O$	18·32	17·97	0·3	−1·9
		17·93	0·0	−2·1
$Os(phenan)_3(SbOTart.)_2.3H_2O$ (*d* form)	14·03	13·92	0·1	−0·8
		13·93	0·5	−0·7

* Denotes gravimetric estimation by ignition to Os metal in ammonia.
Tart. = tartrate; dipy = dipyridyl; phenan = *o*-phenanthroline.

carbonate about one-tenth of an inch thick. Cover the crucible with a lid, place in a larger covered crucible and heat with a small flame for approximately one hour. Usually, after being heated for several minutes, the mixture emits a loud crackling sound and sometimes flashes of light are seen. The violent reaction with perchlorate compounds is moderated by mixing 25 to 30 per cent. of sodium carbonate with the sodium peroxide. When the fusion is complete, allow the small crucible and its contents to cool and transfer it to a wide-necked flask fitted with a ground-in reflux condenser. Add 20 ml of water down the condenser and dissolve the contents of the crucible by boiling under reflux. Cool the flask and contents to room temperature, and add 20 ml of 50 per cent. hydrobromic acid down the condenser, with swirling to produce complete mixing.

During the addition of the hydrobromic acid, the solution usually loses its light brown colour, becomes almost colourless and then changes to a deep red-brown. In some determinations a little flocculent silicic acid separates at this stage owing to attack on the crucible by the fusion mixture. This precipitate is removed by centrifugation before the colorimetric samples are withdrawn.

Allow to stand for a short time and transfer the acidified solution to a 100-ml graduated flask, washing in with normal hydrochloric acid. In the presence of a metal such as antimony, whose salts are liable to form a basic precipitate by hydrolysis, 2·5 *N* hydrochloric acid is used for the washing. Filter the solution through a sintered-glass filter of porosity 2, to remove traces of suspended impurities. Measure three 10-ml portions, by means of a pipette, into tubes that are ground to fit reflux condensers, add 1 ml of 10 per cent. thiourea solution and 1 ml of 10 per cent. stannous chloride in 2·5 *N* hydrochloric acid. Fit the tubes to reflux condensers and heat the mixture in boiling water for 15 minutes to develop the colour. Cool and make up each portion to 25 ml in a standard flask with either distilled water or, in presence of metals that form basic salts, with 2·5 *N* hydrochloric acid.

Calibration graph—

Prepare a calibration graph by treating measured quantities of a standard osmium solution, made from ammonium bromosmate and containing 0·1 mg of osmium per ml, with thiourea, stannous chloride and hydrobromic acid as described in the procedure above.

Measure the optical density by means of a photo-electric absorptiometer; with the Spekker absorptiometer use Ilford protective filters H503, blue-green spectrum filters No. 603 and 10-mm cells.

Results

Test analyses were carried out on a wide variety of compounds in which the osmium was present in anionic, cationic and neutral complexes and also on compounds in which arsenic and antimony were present with organic matter. The results of representative analyses are given in full in Table I, and others in an abridged form in Table II.

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Department of Chemistry
University of Sydney
N.S.W., Australia

May, 1950

Estimation and Separation of Zirconium by Use of Fumaric Acid

By M. VENKATARAMANIAH and BH. S. V. RAGHAVA RAO

Fumaric acid precipitates zirconium completely from solutions up to 0·35 *N* in hydrochloric acid. The precipitate, which has the approximate composition O=Zr=X (where X stands for the fumarate radical), is gelatinous; it is ignited to give the oxide.

From solutions 0·25 *N* in hydrochloric acid the reagent separates zirconium from aluminium, beryllium, uranium, nickel, barium, calcium, iron, manganese, thorium and the ceria earths in a single precipitation; vanadium, chromium, titanium, and tin are completely removed in a double precipitation. Zirconium has also been estimated in zircon.

Fumaric acid was recommended by Metzger[1] for the separation of thorium from rare earths in 40 per cent. ethanol solution, but in aqueous solution the precipitation of thorium is incomplete. Zirconium, however, is completely precipitated as a white gelatinous substance from solutions 0·30 to 0·40 *N* in hydrochloric acid. Under the same conditions many other elements including thorium are held in solution. The reagent thus has valuable properties for use in the analysis of zirconium minerals.

Experimental

Estimation of zirconium—

Twenty millilitres of a stock solution of zirconyl chloride in 0·10 *N* hydrochloric acid were used for the determination of zirconium by precipitation with mandelic acid[2] and ignition to the oxide: the weight of ZrO_2 found was 0·0923 g. By precipitation with fumaric acid by the procedure detailed below, the weight of ZrO_2 found was 0·0924 g, a value in excellent agreement with that found by the mandelic acid precipitation. Experiments with smaller quantities of zirconyl chloride solution indicated that as little as 0·0115 g of ZrO_2 could be accurately estimated. Smaller quantities may be determined by employing micro methods; work in this direction is in progress.

Effect of acid concentration—

Since thorium and other elements are partly precipitated at lower acid concentrations, their removal from zirconium depends on the control of acidity. The solubility of zirconium fumarate in hydrochloric acid was studied by determining the degree of precipitation in dilute acid of different concentrations. The results are shown in Table I.

Table I

Effect of hydrochloric acid

Concentration of acid in the final liquid, *N*	ZrO_2 taken, g	ZrO_2 found, g	Difference, mg
0·10	0·0924	0·0924	nil
0·15	0·0924	0·0923	−0·1
0·20	0·0924	0·0924	nil
0·20	0·0693	0·0694	+0·1
0·25	0·0693	0·0692	−0·1
0·30	0·0693	0·0693	nil
0·35	0·0462	0·0462	nil
0·35	0·0231	0·0230	−0·1
0·35	0·0115	0·0114	−0·1
0·40	0·0462	0·0460	−0·2
0·45	0·0462	0·0458	−0·4
0·50	0·0462	0·0452	−1·0

Composition of the precipitate—

The precipitate obtained in 0·30 *N* hydrochloric acid was washed and dried to constant weight at 105° C. Ignition tests showed this residue to contain 41 per cent. of zirconium.

This points to the empirical formula O = Zr = X (where X represents the fumarate radical) but the composition of the precipitate is subject to slight variation, so direct weighing of the dried precipitate is not possible.

Separation of zirconium from other elements

Procedure—

To the solution containing not more than 0·10 g of ZrO_2 add 5 g of solid ammonium nitrate and the calculated volume of 2·0 *N* hydrochloric acid to give a concentration of 0·25 *N* free acid in 200 ml. Dilute to 100 ml and boil. To the boiling solution add slowly and with continuous stirring 100 ml of a boiling 2 per cent. solution of fumaric acid. Continue to boil for 5 minutes and then set aside to cool. Filter the cold solution through an 11-cm Whatman No. 42 filter-paper. Wash first with a hot 0·20 per cent. solution of the reagent in 0·25 *N* hydrochloric acid and then with water. Ignite and weigh the residue as ZrO_2.

To reprecipitate—Dissolve the washed precipitate in hot diluted hydrochloric acid (1 + 1), collecting the filtrate in the original beaker. Add dilute ammonia until the concentration of free acid is reduced to 0·20 to 0·30 *N*, and then repeat the precipitation with fumaric acid as described above. Results by both single and double precipitation are shown in Table II. All impurities, added as chloride or nitrate, are calculated to oxide.

Interferences—

Aluminum, beryllium, uranium, nickel, barium, calcium, manganese and the ceria earths are not precipitated by the reagent, even from neutral solutions.

Thorium and iron are partly precipitated in neutral solution, but in 0·10 *N* hydrochloric acid no precipitation occurs.

Vanadium and chromium when present alone are not precipitated in neutral solution, but in the presence of zirconium small quantities are carried down even in 0·35 *N* hydrochloric acid.

Table II

Separation of zirconium from other elements

ZrO_2 taken = 0·0462 g. Concentration of free acid = 0·25 *N*

Impurity added,	g	ZrO_2 found, g	Difference, mg
U_3O_8	0·5110	0·0462	nil
Fe_2O_3	0·4332	0·0462	nil
MnO	0·1894	0·0463	+0·1
CaO	0·2548	0·0463	+0·1
BaO	0·4100	0·0462	nil
R_2O_3*	0·4300	0·0461	−0·1
BeO	0·4330	0·0464	+0·2
NiO	0·8220	0·0463	+0·1
ThO_2	0·0625	0·0462	nil
ThO_2	0·1250	0·0464	+0·2
ThO_2	0·1875	0·0463	+0·1
Cr_2O_3	0·2173	(*a*) 0·0466†	+0·4
		(*b*) 0·0461	−0·1
V_2O_4	0·2110	(*a*) 0·0474†	+1·2
		(*b*) 0·0463	+0·1
SnO	0·2380	(*a*) 0·0480	+1·8
		(*b*) 0·0462	nil
TiO_2	0·1143	(*a*) 0·0482	+2·0
		(*b*) 0·0463	+0·1
Al_2O_3	0·4210	0·0462	nil

(*a*) Single precipitation; (*b*) Double precipitation.

* Ceria earths; † The residue was slightly coloured.

Titanium and tin, which are not precipitated in 0·10 *N* hydrochloric acid, are, however, precipitated with zirconium even in 0·35 *N* hydrochloric acid. Titanium, tin, vanadium and chromium are completely removed by a second precipitation.

The fact that large quantities of nickel cause no interference is of importance in the analysis of zirconium minerals, since these are most easily decomposed by sodium peroxide fusion in a nickel crucible.

Analysis of zircon

Zircon from Travancore, India, was fused with borax[3] and, after removal of silica, the zirconium was determined by double precipitation in 0·25 *N* hydrochloric acid with fumaric acid. Qualitative analysis of the ore showed that it contained only titania and small amounts of ferric oxide in addition to silica. Zirconium was also determined for comparison by the standard cupferron method.[4] The results are shown in Table III.

Table III

Analysis of zircon

Cupferron method		Fumaric acid method	
Weight of sample, g	ZrO_2 found, %	Weight of sample, g	ZrO_2 found, %
0·1034	65·02	0·1249	65·06
0·1145	65·11	0·1048	65·12
—	—	0·0964	65·04
Mean .. =	65·06	Mean .. =	65·07

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Chemical Laboratories
Andhra University
Waltair, S. India

May, 1950

The Absorptiometric Estimation of Acenaphthylene

By M. Kaufman* and A. Fowler Williams

An absorptiometric method has been devised for the determination of acenaphthylene in pure material and works products. Satisfactory results were obtained from dilute toluene solutions of standards and test solutions by means of a mercury vapour lamp and an Ilford Spectrum Violet filter No. 601.

In presence of acenaphthene the results are accurate to within 1 per cent. but the effect of all possible impurities, such as naphthalene and α-methyl naphthalene has not been investigated.

The unsaturated hydrocarbon acenaphthylene has not yet been produced on a commercial scale, and as far as the writers are aware, details of a reliable method of estimation have not hitherto been reported. The trial manufacturing process consists of catalytic vapour-phase dehydrogenation of the hydrocarbon acenaphthene, which makes up about 1 per cent. of typical coke-oven tar. The loss of two hydrogen atoms converts the colourless acenaphthene (m.p. 95° C) to the yellow acenaphthylene (m.p. 92° to 93° C), but the reaction can also yield mono- and di-methyl naphthalenes as well as naphthalene. In addition, the product is usually contaminated with small amounts of highly condensed compounds of the chalcacene type. The possibilities inherent in the dehydrogenation reaction are illustrated in Fig. 1.

The relative amounts of these contaminants depend on the conditions of the conversion; the method described was designed to estimate the purity of a particular crude product that was to be used as the monomer in a process for producing high polymers from acenaphthylene.

There were several possible methods of estimating acenaphthylene, for example, measurement of the degree of unsaturation by the addition of hydrogen or halogens to the double

* Present address: The British Oxygen Co. Ltd., Research and Development Department, Morden Factory Estate, Morden Road, London, S.W.19.

bond, or use of the fact that acenaphthylene is an intensely coloured product amongst generally uncoloured impurities. Modifications of Wij's method were tried, but it was found that neither with iodine nor with bromine could consistent quantitative results be obtained. The values obtained were rather high, and it was concluded that, under the test conditions, a variable amount of substitution in, as well as addition to, the acenaphthylene molecule was taking place.

A colorimetric method making use of the Spekker photo-electric absorptiometer was then tried. Initially a tungsten filament lamp was used in conjunction with an Ilford No. 602 filter as the source of light, but with this white light it was not possible to obtain a straight line calibration curve as required by Beer's law, nor were the results obtained consistent. It appeared that the tungsten lamp and filter provided too wide a waveband (4400 to 5000 A) and too many possible absorptions.

A mercury vapour lamp was therefore used in conjunction with an Ilford Spectrum Violet filter, the principal wavelengths so provided being 2007·8, 4046·6, 4339·2 and 4358·4 A. This selectivity in wavelengths allowed a satisfactory method to be devised for the estimation of acenaphthylene in the crude products.

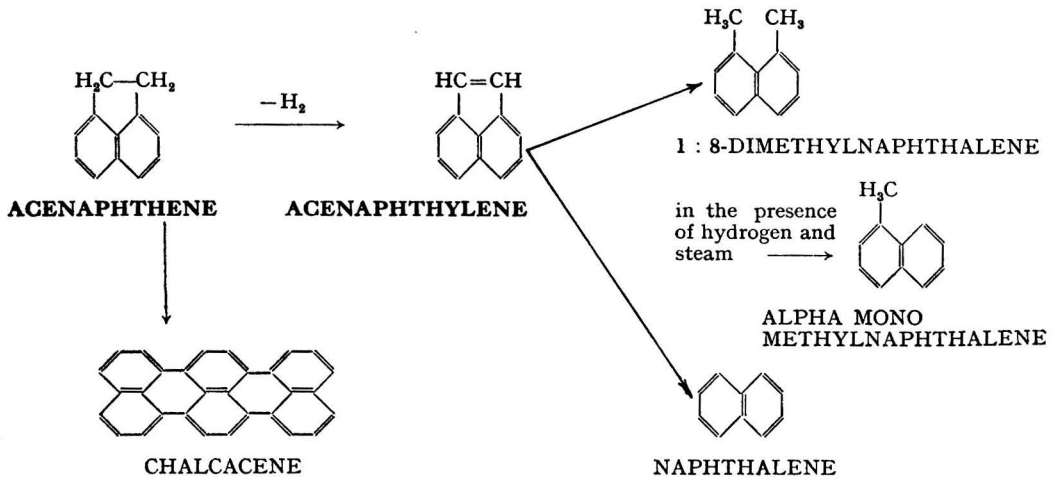

Fig. 1. Possible dehydrogenation reactions of acenaphthene

CONSTRUCTION OF THE CALIBRATION CURVE—

To establish the calibration curve, Fig. 2, pure acenaphthylene was prepared from the crude product in the following manner. The crude material (m.p. 87° to 88° C) was twice recrystallised from 2 parts by weight of alcohol and then treated in benzene solution with picric acid to form the hydrocarbon picrate. This was twice recrystallised from benzene to a final melting-point of 201° C, and the acenaphthylene was regenerated from the final picrate by treatment with dilute ammonia solution. The product was washed free from ammonia and picric acid with copious quantities of warm water and finally recrystallised from alcohol.

A solution of this purified material in light petroleum, boiling range 40° to 60° C, was poured on to a column of activated alumina measuring 40 cm × 4 cm, which was eluted to colourless washings with the same solvent. After removal of the light petroleum by evaporation, the acenaphthylene had a final melting-point of 92·5° C. From the sample used by the authors, two strongly adsorbed bands were left at the top of the column; one of these was yellow and was located above a colourless band that fluoresced blue in ultra-violet light.

For calibration and testing a mercury vapour lamp was used with an Ilford Spectrum Violet filter 601. It was necessary to ensure that the lamp was fixed in the correct position; none of the books on absorptiometric technique that were consulted mention this, but difficulties were encountered until this source of trouble was found.

With redistilled toluene (b.p. 110·5° C) as the solvent, the Spekker was found to be reasonably sensitive and it was possible to use solution concentrations of up to 0·25 per cent. with a "toluene setting" of 1·5 on the drum. The solutions were irradiated in glass cells 1 cm in width.

The curve (Fig. 2) is linear up to concentrations of about 160 mg per 100 ml and there is very close similarity with a curve drawn for "picrated" but unchromatographed acenaphthylene. Purification of standard acenaphthylene beyond the "picration" stage therefore seems to be unnecessary.

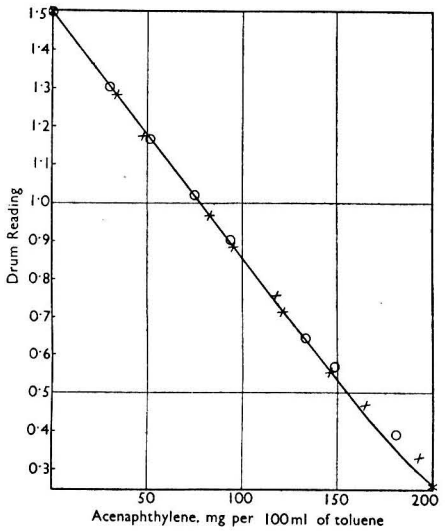

Fig. 2. Calibration curve for acenaphthylene, using the Hilger Spekker photo-electric absorptiometer. M.p. of acenaphthylene: 92° to 92·5° C (corr). Toluene setting: 1·500. Mercury lamp + Ilford Spectrum Violet filter 601. 1-cm glass cells. O = Unchromatographed, "picrate pure" sample; X = Chromatographed, "picrate pure" sample

The calibration curve was then tested in a number of ways. First the crude product was estimated in various concentrations and Table I shows the good agreement between results.

TABLE I

ESTIMATION OF CRUDE PRODUCT IN KNOWN CONCENTRATIONS

Acenaphthylene added, per 100 ml of toluene	0·1132	0·1454	0·1864	0·2187
Spekker reading	0·840	0·625	0·422	0·234
Acenaphthylene found, %	92·2	92·0	92·2	92·0

Synthetic mixtures of pure acenaphthylene and pure acenaphthene were then prepared and tested, and these results are shown in Table II.

TABLE II

ESTIMATION OF ACENAPHTHYLENE IN ARTIFICIAL MIXTURES WITH ACENAPHTHENE

Acenaphthylene added, per 100 ml of toluene	0·0403	0·1064	0·1259	0·1419	0·1560	0·1862
Acenaphthene added, per 100 ml of toluene	0·1653	0·0907	0·0851	0·0559	0·0485	0·0172
Acenaphthylene found by Spekker, g	0·0418	0·1172	0·1240	0·1408	0·1540	0·1835
Error, %	4·0	0·75	1·0	0·8	1·3	1·5

In the first experiment with artificial mixtures (Table II, column 1), although the percentage error appears large, the difference in weight between the acenaphthylene present and that determined experimentally is only 1·5 mg. The last two experiments (Table II, columns 5 and 6) gave readings that are off the linear portion of the calibration curve and hence may be neglected. These and many other determinations have shown that the error can be kept within 1 per cent.

With the Spekker absorptiometer, it is necessary to check the original calibration curve periodically, particularly when there is a lapse of a few months between determinations.

METHOD—

The method may obviously be applied when acenaphthylene is the only significant constituent of the test mixture that absorbs the relevant wavelengths. When the presence of impurities whose absorptions would interfere is suspected, they must first be removed chemically or by differential absorption on a column of alumina or silica gel.

The crude product of the particular dehydrogenation reaction under examination is coloured bright orange to deep red. Examination of the highly coloured impurities that were removed by adsorption on a column of alumina from light petroleum solution indicated that they were a mixture of rhodacene and chalcacene. However, quantitative estimation under the prescribed conditions of these compounds in the crude product, calculated as their acenaphthylene equivalents, gave a value of about 0·3 per cent. of a typical sample. Since this figure lies within the limits of experimental error, its contribution to the authentic acenaphthylene content was considered unimportant. Thus, for the material examined by the authors, preliminary chromatographic purification of the crude sample for analysis proved unnecessary, although it is conceivable that for acenaphthylene prepared under different conditions purification would be required.

The procedure to be adopted for routine analysis is very similar to that already described for the construction and testing of the calibration curve. Three solutions of the sample in toluene, of various concentrations up to 0·15 per cent., are prepared. A mercury lamp in conjunction with an Ilford Spectrum Violet filter 601 is used as the source of light, and measurements made on each solution contained in 1-cm glass cells.

Table III shows results obtained by the examination of the products from successive crystallisations of crude acenaphthylene from a pilot plant. The agreement between the individual determinations for the second and third crops is somewhat better than is the general rule.

TABLE III

EXAMINATION OF SUCCESSIVE CRYSTALLISATIONS OF CRUDE ACENAPHTHYLENE

Sample	Acenaphthylene in 100 ml of toluene, g	Drum reading	Acenaphthylene determined, %	
1st crop	0·1293	0·702	98·4	98·4
	0·1342	0·675	98·7	
	0·1096	0·820	98·0	
2nd crop	0·1238	0·752	95·3	95·2
	0·1312	0·712	95·1	
	0·1360	0·690	95·2	
3rd crop	0·1234	0·758	92·8	92·8
	0·1329	0·705	92·7	
	0·1200	0·792	92·8	

The authors wish to acknowledge the assistance of Mr. F. V. Bethell in the experimental work and to thank the Directors of the Company for permission to publish.

POWELL DUFFRYN RESEARCH LABORATORIES
140, BATTERSEA PARK ROAD
LONDON, S.W.11

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The Electrolytic Determination of Large Amounts of Lead as Lead Dioxide

By GEORGE NORWITZ

An electrolytic method is described for the determination of lead, in which the well-known tendency for the formation of non-adherent anodic deposits is overcome by using an electrolyte containing perchloric acid.

With the apparatus described, deposits of dioxide from as much as one gram of lead can be handled with ease.

The conversion factor for lead dioxide to lead is determined empirically.

Present methods for the electrolytic determination of lead as lead dioxide are not satisfactory for the determination of more than 0·10 to 0·12 g of lead[1,2,3,4] because of the difficulty of obtaining adherent deposits.

A method has been devised by the author for determining up to 1 g of lead as lead dioxide. A perchloric - nitric acid medium is used. The perchloric acid has the effect of making the deposit adhere firmly to the electrode. It has been stated by Willard and Furman[5] that the addition of a few drops of sulphuric acid will make lead dioxide more adherent; but the danger of precipitating lead as lead sulphate limits the use of sulphuric acid to the deposition of small amounts of lead dioxide.

The temperature used by the author to dry the lead dioxide was 120° C. This temperature is recommended by Kolthoff and Sandell,[3] Willard and Furman,[5] and the American Society for Testing Materials.[1] The factor used by the author to convert the lead dioxide to lead was the empirical factor 0·8611. This was the average factor obtained when several samples of very pure sheet lead, scraped clean of oxide, were carried through the determination. It is advisable for each laboratory to determine its own factor.

The method can be used for the determination of lead in high grade pig lead for which the specification requires a minimum of 99·90 per cent. of lead. The amount of tin, bismuth, arsenic and antimony that might be found in this type of material does not interfere with the method.

The addition of some copper nitrate before the electrolysis is recommended in order to prevent the deposition of metallic lead on the cathode.

Experimental

Accurately weighed portions of pure lead, contained in 300-ml electrolytic beakers, were dissolved in a mixture of 25 ml of perchloric acid, 25 ml of nitric acid and 25 ml of water

Table I

Lead present, g	Lead found, g
0·2000	0·1996
0·2000	0·2001
0·2000	0·1998
0·5000	0·4999
0·5000	0·5003
0·5000	0·4999
1·0000	1·0001
1·0000	0·9995
1·0000	0·9996

by heating on the hot plate. The solutions were diluted to 190 ml and 1 ml of a copper nitrate solution, prepared by dissolving 21·5 g of $Cu(NO_3)_2.5H_2O$ in 1 litre of water, was added. This amount of the copper nitrate solution is equivalent to 0·005 g of copper. The solutions were heated to about 70° C and electrolysed, with stirring, at 2 amperes for 1 hour. Large platinum gauze cylinders (50 mm high and 45 mm in diameter) were used as anodes and platinum spirals as cathodes. At the end of the electrolysis, the beaker containing the

electrolyte was quickly lowered and quickly replaced by a beaker containing water. The beaker containing the water was moved up and down a few times in order to wash the deposit thoroughly. The anodes were dipped in alcohol, dried at 120° C for 30 minutes, cooled and weighed. The deposits were stripped in nitric acid (1 + 1) containing a little hydrogen peroxide. The anodes were washed with water, dipped in alcohol, dried at 120° C for a few minutes, cooled and weighed again. The difference in weight was PbO_2. The factor for converting PbO_2 to Pb was 0·8611. The results obtained for lead are shown in Table I.

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3353, RIDGE AVENUE
PHILADELPHIA 32, PA.

July, 1950

ERRATA: December (1950) issue, p. 663. Under the heading "436 mμ.", *for* "Calorex (ON 3)" *read* "Calorex (ON 13)."

Ibid., p. 669, Table II. In column 5, opposite 405 mμ., *for* "0·2" *read* "0·1."

Notes

THE DETERMINATION OF ZINC OXIDE IN ZINC POWDER

ZINC powder always contains a small proportion of zinc oxide; several methods for the determination of the amount of this impurity are mentioned in the literature, but these are mostly based on the reducing power of the zinc. This may be measured by several reactions, *e.g.*, potassium bichromate, iodate, ferric sulphate and iodine. Fresenius proposed dissolving the zinc dust in dilute sulphuric acid and, after drying the gas, passing the resultant dry hydrogen over heated copper oxide in a combustion tube, absorbing the water formed in a calcium chloride tube. None of these methods is really satisfactory in that the results obtained by these different methods are not concordant and are not reproducible by the same method. The method most widely used in specialised laboratories is that based on the volume of hydrogen evolved when a sample of zinc dust is dissolved in dilute sulphuric acid; here again the zinc is measured, but the apparatus and technique is very complicated[1] and not suitable for the chemist who has only an occasional sample to examine. Another method has recently been described in America,[2] but again the apparatus and technique required are beyond the reach of any but the specialist.

Confronted by this problem, we have devised a simple but accurate method based on experimental evidence that showed that zinc oxide is soluble in ammonium acetate solution whilst zinc metal is not, and it may well prove of use to the chemist who has only an occasional determination to make.

Procedure—Take 2 g of the powder in a 250-ml beaker and add 100 ml of water containing 30 g of ammonium acetate. Stir well for 10 minutes, and then allow to stand for 2 hours. Filter on a sintered glass of No. 4 porosity with suction, and wash with cold water. If washed with absolute alcohol or sodium-dried ether, the zinc can be weighed after drying by suction and placing in a vacuum desiccator for 15 minutes. If a chemical finish is desired, dissolve the residue in hot dilute sulphuric acid, cool, add an excess of caustic soda and electrolyse the zinc, a copper-coated cathode being used. Alternatively, the zinc may be precipitated as the sulphide, and converted to the oxide in the well-known manner. This result gives the amount of zinc metal present.

The zinc oxide that has been dissolved out by the ammonium acetate can be determined by precipitation with hydrogen sulphide after addition of 10 ml of ammonia. When all the sulphide has precipitated, boil with a little paper pulp to help coagulation. Filter on sintered glass of No. 2 porosity with a paper-pulp pad and suction. Dissolve the residue in 5 *N* sulphuric acid and boil. Filter on sintered glass and wash well with hot water. Add an excess of caustic soda and, after cooling, electrolyse the solution on a copper-coated cathode in the usual manner. Calculate to ZnO.

The author thanks the Directors of the British Drug Houses Limited for permission to publish this note.

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ANALYTICAL DEPARTMENT
THE BRITISH DRUG HOUSES LIMITED
LABORATORY CHEMICALS GROUP
POOLE, DORSET

G. H. OSBORN
July, 1950

1-NITRO-2-NAPHTHOL AS A REAGENT FOR ESTIMATING COBALT

1-NITRO-2-NAPHTHOL is described in some modern textbooks[1,2] as a precipitant for cobalt that is superior to the more usual 1-nitroso-2-naphthol. We find that pure 1-nitro-2-naphthol does not give a precipitate with cobalt. This is in agreement with Mayr and Prodinger[3] who reported that the reagent used previously by Herfeld and Gerngross,[4] and by Mayr,[5] which actually precipitated cobalt, must have contained 1-nitroso-2-naphthol.

1-Nitro-2-naphthol is prepared by oxidising 1-nitroso-2-naphthol with nitric acid,[1] and the above-mentioned erroneous observations appear to be due to a difficulty in separating the nitro-compound from the nitroso-compound. We have found that 1-nitro-2-naphthol can be separated from the crude oxidation product by steam distillation and finally purified by crystallisation from alcohol. The steam distilled 1-nitro-2-naphthol melted at 101° to 103° C; after one crystallisation from alcohol the melting-point was 103° C, in agreement with the figure recorded in the literature.[6]

Acknowledgment is made to the Chief Scientist, Ministry of Supply, for permission to publish this note.

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MINISTRY OF SUPPLY,
EXPLOSIVES RESEARCH AND DEVELOPMENT ESTABLISHMENT
WALTHAM ABBEY
ESSEX

N. J. BLAY
L. A. WARREN
July, 1950

A COLOUR TEST FOR AMPHETAMINE

IN the test for cocaine previously described by the author,[1] amphetamine gives a purple colour similar to that of cocaine; the colour, however, changes in about 7 minutes to a beautiful violet colour.

A strong reaction is given by 0·1 mg of amphetamine. The test will detect 0·025 mg of amphetamine.

REFERENCE

1. Rathenasinkam, E., *Analyst*, 1950, **75**, 169.

GOVERNMENT ANALYST'S LABORATORY
COLOMBO
CEYLON

E. RATHENASINKAM
July, 1950

Apparatus

A MODIFIED APPARATUS FOR THE DETERMINATION OF ALCOHOL IN BLOOD AND URINE

THIS laboratory, like others engaged in forensic work, is experiencing an increasing demand for the determination of alcohol in urine or blood. Our practice, which we believe is common, was to use the modified form of the Southgate apparatus described by Evans and Jones.[1]

This apparatus has proved reliable as regards accuracy; it is, however, cumbersome and bulky. This becomes a noticeable disadvantage when series of estimations have to be made involving, as they do, duplicates and blanks that should be run concurrently. It is clear that the excessive bulk is due to an unnecessarily large evaporation chamber. With this in mind, we investigated the factors essential to accurate and reproducible results. From the experience gained, we designed the apparatus illustrated in the diagram, Fig. 1; its over-all dimensions are approximately half of those of the modified Southgate apparatus. The evaporation chamber is an enlargement, of approximately 2·5 ml capacity, of the inner U-tube. The apparatus as illustrated was made to our specification by Messrs. Quickfit and Quartz Ltd.

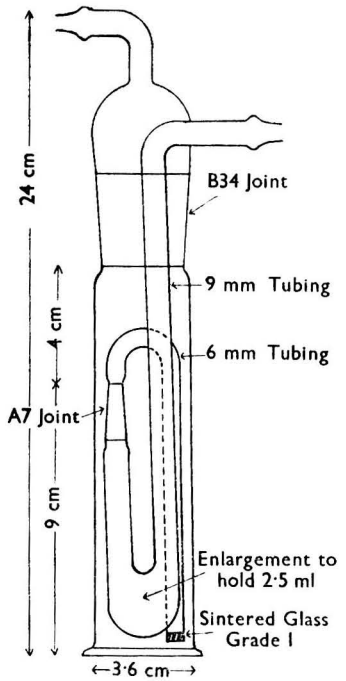

Fig. 1. Diagram of apparatus

Procedure—For urine, place 2 ml in the evaporation chamber by means of a pipette; for blood, weigh a comparable quantity into the chamber. Put 25 ml of 0·1 *N* potassium dichromate in 66 per cent. v/v sulphuric acid in the outer or absorption tube. Place the whole apparatus in a water-bath at 80° C. After completion of the test, the inside of the bubbler tube can be washed out easily with a wash bottle fitted with an A7 cone in place of the ordinary jet.

We use the apparatus for simultaneous duplicate and blank analyses. Identity of conditions between the duplicates and blanks is obtained by coupling in parallel to the same air dryer and purifier.

Over a period of 18 months this arrangement has given concurrent and accurate results.

REFERENCE

1. Evans, J., and Jones, A. O., *Analyst*, 1929, **54**, 134.

SOUTH-WESTERN FORENSIC SCIENCE LABORATORY
55, BROADMEAD
BRISTOL, 1

E. B. PARKES

June, 1950

AN ALL-PURPOSE EXTRACTOR

IT is frequently necessary in a biochemical laboratory to perform one or other of the following operations upon large quantities of material: (*a*) continuous extraction of a solid with a volatile solvent (percolation or Soxhlet extraction), (*b*) extraction of a liquid with a less dense immiscible liquid, or (*c*) extraction of a liquid with a more dense immiscible liquid.

Special types of apparatus have been designed for each of these operations (see, *e.g.*, Herzog[1]), but there is an advantage in the ability to adapt a single apparatus to any task required.

The apparatus described in this paper has been in use for some years in this laboratory. It is versatile, easily constructed, compact and robust, and it will operate automatically in all circumstances. The relative dimensions given below have been selected as the result of experience.

CONSTRUCTION—

The apparatus, Fig. 1, which is of Pyrex glass, consists of a main tubular body, E, of 5 cm diameter and 95 cm total length, with a tap, A, at the lower end and, at the upper end, a B34 socket to take a water-cooled condenser, F. The vapour tube, D, 22 mm in diameter, has a B24 cone at its lower end, and the upper end enters the body, E, 15 cm below the top. The solvent

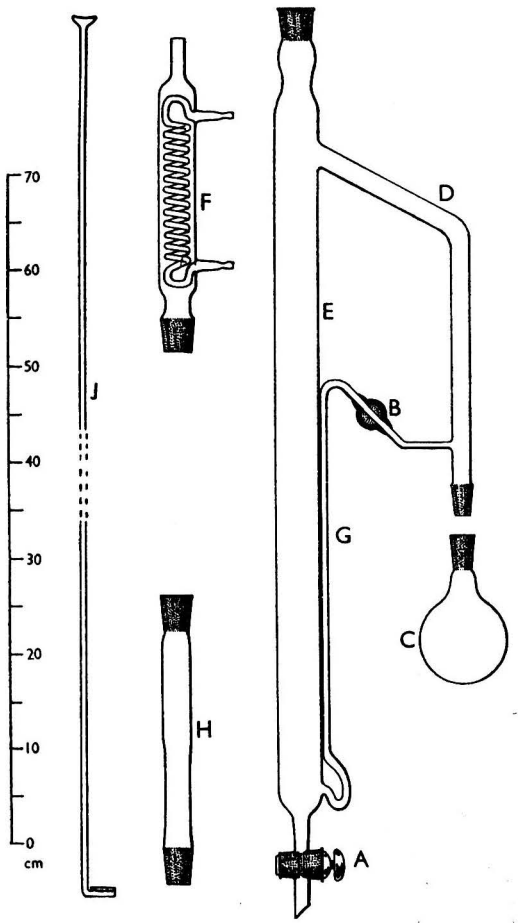

Fig. 1. Extractor

return tube, G, 7 mm in diameter, leaves E just above its lower constriction and, after rising vertically alongside E, bends downwards 44 cm above its exit from E; after passing through the tap, B, it enters the vapour tube, D, just above the B24 cone. It is advantageous to have tap B sloping downwards away from G, since it is not then continually charged with solvent during operation. C is a round-bottomed flask with a B24 socket in which the solvent may be boiled, H is an extension tube, 32 mm in diameter and 30 cm long, with B34 joints, and J is a conducting

funnel for light solvents, made of 7-mm tubing, 112 cm long and widened to 25 mm diameter at the top, this being the maximum diameter that allows it to pass through the B34 cones on H and F. It carries a coarse sintered plate, 20 mm in diameter (gas distribution tube, porosity No. 1) at the bottom. The whole apparatus is conveniently mounted on a wooden back-board or metal frame.

OPERATION—

As percolator—The column is packed with solid material, covered with solvent, and the rate of percolation regulated by tap A. Continuous percolation may be achieved by boiling the solvent in the flask, C (tap A closed), and allowing a suitable rate of return to C by regulating tap B. The vapour tube, D, acts as a safety overflow and prevents the overfilling of the column.

As a liquid - liquid extractor using a less dense extracting solvent—The extension tube, H, is fitted between the condenser, F, and the main tube, E, and the funnel, J, is inserted in the apparatus to rest on the bottom of E and arranged so that the solvent dropping from condenser, F, falls into its open end. Taps A and B are closed and the solvent is boiled in flask C, in which the extracted material collects. At the end of the experiment the extracted solution is run off through tap A and separated from the supernatant layer of lighter solvent.

As a liquid - liquid extractor using a more dense extracting solvent—The solvent is boiled in flask C as before, but no extension or conducting funnel is required. Tap A is closed and some solvent is poured into the main tube, E, before adding the less dense solution for extraction. This prevents fouling the solvent return tube, G, with the less dense solution. Tap B is opened to allow the heavy liquid to return to the boiling flask, C.

With an apparatus of the dimensions described, it is possible to extract 1 litre of aqueous solution, of density approximately 1, with chloroform or ether.

I wish to thank Professor C. Rimington for his interest and for suggestions relating to the publication of this note.

REFERENCE

1. Herzog, J., *in* Houben, J., "Die Methoden der organischen Chemie," Volume I, Thieme, Leipzig, 1925.

DEPARTMENT OF CHEMICAL PATHOLOGY
UNIVERSITY COLLEGE HOSPITAL MEDICAL SCHOOL
LONDON, W.C.1

A. W. HEMMINGS
June, 1950

Official Appointments

PUBLIC ANALYST APPOINTMENTS

NOTIFICATION of the following appointments has been received from the Ministry of Food since the last record in *The Analyst* (1951, **76**, 54).

Public Analyst	*Appointments*
GREEVES, William Francis (Deputy)	County Borough of Ipswich.
LYNE, Francis Arthur	County Borough of Oxford.
MCLACHLAN, Thomas (Additional)	County Borough of Oxford.
RYMER, Thomas Edward (Deputy)	Borough of Kingston-upon-Thames.

OFFICIAL AGRICULTURAL ANALYST APPOINTMENTS

NOTIFICATION of the following appointments has been received from the Ministry of Agriculture and Fisheries since the last record in *The Analyst* (1951, **76**, 54).

Agricultural Analyst	*Appointments*
GREEVES, William Francis (Deputy)	County Borough of Ipswich.
JONES, William Elwyn (Deputy)	County of Worcestershire.

Ministry of Food

STATUTORY INSTRUMENTS*

1950—No. 1764. The Meat Products and Canned Meat (Amendment) Order, 1950. Price 1d.

This Order, which came into operation on November 5th, 1950, provides that the Meat Products and Canned Meat (Control and Maximum Prices) Order, 1948 (S.I., 1948, No. 1509; Analyst, 1948, 73, 341), as amended by S.I., 1949, Nos. 782, 1303 and 2045, shall be further amended as follows—

(*a*) by substituting for Article 3 thereof the following Article:—

"3. A person shall not by way of trade prepare or manufacture or sell or have in his possession for sale any description of specified food mentioned in column 1 of the First Schedule to this Order the meat content of which is less than the minimum meat content prescribed as respects that description in column 2 of the said Schedule: provided that in any proceedings in which a person is charged with an infringement of this Article the Court may disregard any variation in the prescribed minimum meat content of beef sausages, beef sausage meat and beef slicing sausage if the meat content thereof is not less than 47½ per cent. or in the case of pork sausages, pork sausage meat and pork slicing sausage if the meat content is not less than 62½ per cent.: provided also that any fat of vegetable origin used in the manufacture of beef sausages, beef sausage meat, or beef slicing sausage shall be deemed to be meat for the purpose of assessing the meat content of any of those products, if the total quantity of such fat so used does not exceed 25 per cent. of the prescribed minimum meat content of the product";

(*b*) by substituting for the First Schedule thereto the First Schedule to this Order;

(*c*) by substituting for Part III of the Second Schedule thereto the Second Schedule to this Order.

THE FIRST SCHEDULE

(To be substituted for the First Schedule to the Meat Products and Canned Meat (Control and Maximum Prices) Order, 1948)

MINIMUM MEAT CONTENT OF SPECIFIED FOODS (NOT CANNED)

Column 1 Description of Specified Food	Column 2 Minimum Meat Content
Pork sausages, pork sausage meat, pork slicing sausage ..	**65** per cent., of which at least 80 per cent. shall consist of pork.
Beef sausages, beef sausage meat, beef slicing sausage ..	50 per cent.
Cooked sausages of the following descriptions:—	
Luncheon sausage, breakfast sausage and polony	30 per cent.
Meat roll or galantine	
Liver sausage	45 per cent.

(*The Second Schedule is a list of maximum prices.*)

—— **No. 1871. The Food Standards (Preserves) (Amendment) (Commencement) Order, 1950.** Price 1d.

This Order, which came into force on November 29th, 1950, provides that the Food Standards (Preserves) (Amendment) Order, 1950 (S.I., 1950, No. 1056; Analyst, 1950, 75, 503), shall come into force in respect of wholesale sales on January 25th, 1951, and in respect of retail sales on May 25th, 1951. The Order came into force on September 25th, 1950, in respect of sales by manufacturers.

—— **No. 1988. The Feeding Stuffs (Manufacture) Order, 1950.** Price 6d.

This Order, which came into operation on December 17th, 1950, replaces the Feeding Stuffs (Manufacture) Order, 1949, as amended (S.I., 1949, No. 1067 and S.I., 1950, No. 1540). The principal changes are—

(*i*) *The definition of "Animal protein rich substance" does not exclude dried blood.*

(*ii*) *The Order does not apply to food for any birds except pigeons, poultry and game-birds.*

(*iii*) *Formulae are prescribed for National Cattle Food No. 4 and National Poultry Food No. 3A, both of which may now be manufactured by any licensed compounder.*

(*iv*) *The proportions of the ingredients of some compounds have been changed, and the permitted use of certain ingredients in additional compounds has been extended.*

(*v*) *The method of expressing calcium in the analysis of National compounds has been changed; it is now expressed as CaO.*

(*vi*) *The permitted substitutes for cod-liver oil are added to.*

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

1951—No. 13. The Food Standards (Ice-Cream) Order, 1951. Price 2d.

This Order, which comes into operation on March 1st, 1951, should be read with the Food Standards (General Provisions) Order, 1944, as amended (S.R. & O., 1944, Nos. 42 and 654; Analyst, 1944, 69, 49 and 247). It prescribes standards for ice-cream, as follows—

STANDARD FOR ICE-CREAM

1. The standard for ice-cream shall be as follows:—

Ice-cream shall contain not less than 5 per cent. fat, 10 per cent. sugar and 7½ per cent. milk solids other than fat:

Provided that—

(i) ice-cream containing any fruit, fruit pulp or fruit puree shall either conform to the standard set forth above or, alternatively, the total content of fat, sugar and milk solids other than fat shall be not less than 25 per cent. of the ice-cream, including the fruit, fruit pulp or fruit puree, as the case may be, and such total content of fat, sugar and milk solids other than fat shall include not less than 7½ per cent. fat, 10 per cent. sugar and 2 per cent. milk solids other than fat;

(ii) "Parev" (kosher) ice sold, offered or exposed for sale under that description shall contain not less than 10 per cent. fat and not less than 14 per cent. sugar, and the standard for ice-cream set forth above shall not apply to this product.

2. For the purpose of the standards prescribed above "sugar" means sucrose, invert sugar or the solids of any sweetening material derived from starch so however that no ice-cream shall contain less than 7½ per cent. sucrose.

3. Each reference in this Schedule to any proportion or percentage means that proportion or percentage by weight.

CIRCULAR MF 1/51

This circular (price 1d.), dated January 5th, 1951, received with S.I., 1951, No. 13 (above), refers to it and points out inter alia *that—*

It is intended that the standard shall apply to any products (including those supplied in catering establishments) which are sold as "ice-cream" or "ices," including products where either of these descriptions is qualified by the mention of a flavour, such as "coffee ice." The standard is not intended to apply to water ices sold as such, or to "ice lollies."

CIRCULAR MF 2/51

This circular (price 2d.), dated January 18th, 1951, directs attention to the Defence Regulations (No. 8) Order, 1950, which came into operation on December 10th, 1950, and which revokes certain Regulations of the Defence (General) Regulations, 1938. Particular attention is directed to Article 4 of the Order which revokes the provisions affecting the use of borax as a preservative for imported bacon.

Under Regulation 60 CAA of the Defence (General) Regulations, 1939, certain departures from the Public Health (Preservatives, etc., in Food) Regulations were permitted, among them being the use of borax as a preserving agent in imported bacon. The effect of the present revocation is to restore the position as it was prior to the operation of Regulation 60 CAA, i.e., the matter is again governed by the provisions of the Public Health (Preservatives, etc., in Food) Regulations.

NOTICES

THE Ministry of Food have informed us of the following—

FOOD STANDARDS COMMITTEE

The Minister of Food has appointed Mr. Robert Norman Wright, B.Sc., A.R.C.S., to be a member of the Food Standards Committee in succession to Sir Harry Hague, M.P.S., who has resigned. The Minister has also appointed Dr. E. L. Sturdee, O.B.E., M.R.C.S., L.R.C.P., to be an additional member of the Food Standards Committee.

Mr. Wright, who was nominated by the Food Manufacturers' Federation is a Director of Crosse and Blackwell Limited; Dr. Sturdee is a Principal Medical Officer of the Ministry of Health.

REPORT OF THE MANUFACTURED MEAT PRODUCTS WORKING PARTY

The Minister of Food, in consultation with the Minister of Health and the Secretary of State for Scotland, has approved the publication of the Report of the Manufactured Meat Products Working Party. The Report, published on November 22nd, 1950, deals with the hygienic manufacture and with the wholesomeness of meat products. Copies may be obtained from H.M. Stationery Office or through any bookseller, price 1s. 3d.

British Standards Institution

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, Miss D. V. Wilson, 7–8, Idol Lane, London, E.C.3.

Draft Specifications prepared by Technical Committee LBC/1—Volumetric, Mouldblown and Lamp-blown Glassware.

CM(LBC) 6963—Draft Revision of B.S. 604, Graduated Measuring Cylinders.
CM(LBC) 6962—Draft Revision of B.S. 605, Crow Receivers.
CM(LBC) 6961—Draft B.S. for One-mark Graduated Flasks.
CM(LBC) 6960—Draft B.S. for Glass Stopcocks.
CM(LBC) 6959—Draft Revision of B.S. 846, Burettes and Bulb Burettes.

Draft Specification prepared by a technical committee of the British Disinfectant Manufacturers' Association and approved by Technical Committee DIC/10—Disinfectants.

CM(DIC) 7302—Draft Amendment to B.S. 541, Determining the Rideal - Walker Coefficient of Disinfectants, 1934 Edition, page 8, lines 3 to 20 *or* 1949 Reprint, page 9, line 28 to page 10, line 11.

Draft Specification prepared by a technical panel of the British Disinfectant Manufacturers' Association and circulated under the aegis of the Disinfectants Industry Standards Committee DIC/–.

CM(DIC) 7200—Draft B.S. for the Evaluation of Aerial Bactericides.

Draft Specification prepared by Technical Committee FCC/4—Solvents and Allied Products.

CM(FCC) 7422—Draft B.S. for Di-Ethylhexyl Phthalate.

Draft Specifications prepared by Technical Committee PVC/1—Pigments, Sub-Committee PVC/1/7—Iron Oxide Pigments.

CM(PVC) 7416—Draft Revision of B.S. 851, Manufactured Oxides of Iron (Marigold, Brown, Maroon and Yellow).
CM(PVC) 7418—Draft Revision of B.S. 306 and 339, Black and Purple Oxides of Iron for Paints.
CM(PVC) 7417—Draft Revision of B.S. 312, 313 and 337, Earth Colours for Paints.
CM(PVC) 7415—Draft Revision of B.S. 272, 305 and 370, Red Oxides of Iron for Paints.

Book Reviews

QUANTITATIVE ULTRAMICROANALYSIS. By PAUL L. KIRK, Ph.D. Pp. vii + 310. New York: John Wiley & Sons Inc. London: Chapman & Hall, Ltd. 1950. Price 40s.

Professor Kirk has published papers on microchemical investigations at frequent intervals during the last twenty years or so and his original work in this field is well known. To this experience must be added his close association with the applications of ultramicrochemistry by the Manhattan Project and the Atomic Energy Commission. It is with great interest, therefore, that one welcomes his recently published book. At first, the reader is disappointed to find that none of the atomic energy applications are mentioned because of the now familiar, and apparently inevitable, security restrictions. Further consideration and a closer examination of the book, however, lead to the conclusion that the text gives a very good account of the present state of the subject.

The book is most valuable because the author describes the various techniques and methods from personal practical experience of their operation; his statements are both critical and authoritative. This feature leads to somewhat uneven treatment; for example, the construction of a quartz microgram balance, the Cartesian Diver respirometer and the Beckman spectrophotometer are dealt with in greater detail than any other subjects. However, the first two of these are not discussed adequately in any other textbook, nor is the application of the spectrophotometer to 200 or 20-μl samples described so effectively elsewhere. Not only does the author detail clearly the methods he recommends, giving full precautions for accurate working, but he also points out their limitations and shows where satisfactory procedures have yet to be devised. The most obvious of these gaps is due to the paucity of gravimetric methods in the ultramicro range, so that most routine determinations are volumetric. The technique for these is carefully described and

specific examples are given in full. Besides the techniques mentioned earlier in this review, Prof. Kirk also includes chapters on volumetric gas analysis and selected physical determinations.

The book is recommended for providing sound instruction in existing ultramicrochemical methods and for showing the investigator where he may profitably direct his attention to expand the applications of the subject. At the end of each chapter are lists of critically selected literature references, which clearly reveal the neglect of the subject in this country: of more than three hundred references barely a score are to British journals. G. H. WYATT

ANALYSE QUALITATIVE MINÉRALE À L'AIDE DE STILLIRÉACTIONS. By R. DELABY and J. A. GAUTIER. Second Edition. Pp. 229. Paris: Masson et Cie. 1950. Price 820 fr.

In their opening chapter, the authors define *stillianalyse* as differing from classical inorganic analysis in two particulars; first, by a reduction in the scale of working and the employment of semi-micro techniques and, secondly, by the application of organic reagents for identification purposes wherever possible. The designation "stillireaction" or "stillianalysis" is derived from the Latin *stilla*, a small drop, and indicates both the use of drop reactions in solution on a plate or in a tube and spot tests on absorbent paper.

The layout of the book follows closely on the pattern associated with textbooks on qualitative inorganic analysis. After a brief discussion of the principles of "stillianalysis" and the problems of sensitivity and specificity, there follows a chapter dealing with technique and the reagents required. The analytical characters of the principal cations and anions are now enumerated in some detail and here, in addition to the more usual reactions, tests involving organic reagents are described wherever they are applicable.

The analysis of mixtures is dealt with fully; the separation of the cations into groups follows the classical procedure closely and each section is prefaced by a clear and concise statement of the theoretical principles involved. The elimination of phosphate before the separation of the iron - aluminium - chromium group is carefully described, preference being given to the ferric phosphate method of Villiers, although other procedures are mentioned, including the convenient and expeditious one using zirconium chloride as precipitant. The separation of the anions of a mixture is similarly described systematically, full notes on the theoretical principles being again given a prominent place. The various tests to be applied to the solid substance form the subject of the final chapter, which also includes instructions for the preparation of a solution. Werner's co-ordination theory of complex compounds is described in an addendum.

Although the present book cannot be said to present anything really new in the field of inorganic qualitative analysis, there is a direct quality about the method of presentation that is attractive. It is designed expressly from a practical viewpoint and the theoretical sections do not intrude upon this purpose, while still amply providing the necessary background for a clear understanding of the tests carried out. The confirmatory tests with organic reagents are, for the most part, limited to well-tried reactions, and the restrictions of sensitivity and specificity are discussed. It is pleasant to find that in a book of this character the authors have seen fit to introduce many references to the originators of familiar methods; this gives a useful historical background to the work, although it is somewhat disappointing to find that the bibliography does not give the full references needed for amplifying the bald statements of the text. This is a pity, as the majority of the books quoted in the bibliography are standard textbooks.

Printing and layout are good. The binding is in the French style. JOHN ALLEN

Publications Received

SYMPOSIUM ON RECENT ADVANCES IN THE FERMENTATION INDUSTRIES, held at the University of St. Andrews, July, 1949. Special Report. Pp. vii + 151. London: The Royal Institute of Chemistry. 1950. Price 10s.

NOTES ON THE LABORATORY USE OF ION EXCHANGE RESINS. Pp. 24. Poole, Dorset: The British Drug Houses Ltd. 1950. *Gratis*.

THE MODERN SOAP AND DETERGENT INDUSTRY. Volume I. THEORY AND PRACTICE OF SOAP-MAKING. Third Edition. By E. I. COOKE, M.A., B.Sc., A.R.I.C. Pp. xii + 384. London: The Technical Press Ltd. 1950. Price 50s.

International Chemical Meetings in the United States in September, 1951

THE British National Committee for Chemistry of the Royal Society wishes to draw the attention of British chemists to the following meetings—

Meeting of the American Chemical Society at New York: Monday, September 3rd to Friday, September 7th.

Meetings of the Executive Committee, the Council and Commissions of the Union at New York: Saturday and Sunday, September 8th and 9th.

Congress of Pure and Applied Chemistry at New York: Monday, September 10th to Thursday, September 13th.

Continuation of the Conference of the Union and Fiftieth Anniversary of the National Bureau of Standards at Washington: Friday and Saturday, September 14th and 15th (and if necessary Sunday, September 16th).

The headquarters of these meetings will be the Hotel Statler, New York. The Organising Secretary of the Congress is Dr. Harry L. Fisher, National Research Council, 2101, Constitution Avenue, Washington, 25, D.C.

Intending British participants in the Congress are invited to communicate with the Assistant Secretary of the Royal Society, from whom further information may be obtained.

The Universities Federation for Animal Welfare

THE Universities Federation for Animal Welfare (UFAW) propose to revise the 1947 Edition of the UFAW Handbook on the Care and Management of Laboratory Animals, edited by Prof. A. N. Worden, M.R.C.V.S. In consequence, they are appealing to all persons interested in laboratory animals for suggestions for the improvement of the Handbook and any additional information that has come to their notice.

Information may be forwarded to F. Jean Vinter, M.D., Technical Secretary, UFAW, 284, Regent's Park Road, Finchley, London, N.3, or to S. A. Price, Honorary Secretary of the Biological Methods Group of the Society, Walton Oaks Experimental Station, Vitamins Ltd., Dorking Road, Tadworth, Surrey.

Papers for Publication in THE ANALYST

THE Editor welcomes Papers and Notes for insertion in *The Analyst*, whether from members of the Society or non-members. They are submitted to the Publication Committee, who decide on their suitability for insertion or otherwise.

A copy of the current Notice to Authors, reprinted from *The Analyst*, 1950, **75**, 451, can be obtained on application to the Editor, *The Analyst*, 7–8, Idol Lane, London, E.C.3. All Papers submitted will be expected to conform to the recommendations there laid down and any that do not may be returned for amendment.

THE RESEARCH ORGANISATION of The Distillers Company Limited requires a CHEMIST, aged up to 26 years, for research and development work on the recovery of antibiotics and other fermentation products of pharmaceutical interest from fermented media. Applicants should possess a 1st or 2nd Class Honours B.Sc. degree, A.R.I.C. or equivalent, and preferably previous appropriate research experience. The appointment, which offers excellent opportunities with good prospects of advancement for a young chemist wishing to enter the antibiotics industry, will be tenable at Bromborough, Cheshire. Commencing salary will depend upon experience and qualifications. Non-contributory pension scheme. Apply to The Controller of Research and Development, Room 176, Distillers House, 21, St. James's Square, London, S.W.1.

THE RESEARCH and Development Department of the Distillers Company Limited, Great Burgh, Epsom, Surrey, requires a CHEMIST for training in antibiotics research work. The initial duties will be biochemical analyses. Applicants must possess a University degree or equivalent in chemistry but experience whilst desirable is not necessary. Age up to 26 years. This vacancy offers excellent opportunities for a young graduate wishing to enter the antibiotics research field, and good prospects of advancement. The commencing salary will depend upon experience and qualifications. Non-contributory pension scheme. Apply to The Controller of Research and Development.

WEST MIDLANDS GAS BOARD
BIRMINGHAM DISTRICT

VACANCIES ON CHEMICAL STAFF
CENTRAL LABORATORY, NECHELLS GAS WORKS

APPLICATIONS are invited for the following positions on the Chemical Staff of the Central Laboratory, Nechells Gas Works (Birmingham District):—

(1) Graduate Chemist to assist Senior Works Chemist in gasworks routine chemical control, including gas calorimetry in the Area of the West Midlands Gas Board.
SALARY—GRADE 11 (£595–£720).

(2) Graduate Chemist or Metallurgist to assist Senior Refractories Metallurgist in the control by testing and analysis of refractories, and to assist in the investigation of refractories problems in the Area of the West Midlands Gas Board. Knowledge of X-ray crystallography work useful.
SALARY—GRADE 11 (£595–£720).

(3) Graduate Analytical Chemist to assist Chemist in charge of Analytical Laboratories in analysis and development of analytical methods, etc.
SALARY—GRADE 12 (£645–£770).

(4) Graduate Chemical Engineer to assist in chemical work and supervision of plants such as benzole recovery, dephenolation, liquor concentration, water softening, etc., in the Area of the West Midlands Gas Board.
SALARY—GRADE 11 (£595–£720).

(5) Graduate and Analytical Chemist to assist in general analytical work, including work for assessing the effect of effluents on the filter beds of sewage works.
SALARY—GRADE 8 (Male £490–£570; Female £392–£456).

(6) Graduate and Analytical Chemist to assist in general analytical work, including gas analysis.
SALARY—GRADE 8 (Male £490–£570; Female £392–£456).

It will be necessary for candidates to indicate for which position or positions they are applying.

Applicants for posts in Grade 11 or over should have at least two or three years post-graduate experience.

The successful applicants may be required to pass a medical examination and will be subject to such Superannuation Regulations as the Board may, in due course, adopt.

Applications, stating nature of degree, age, experience, together with the names of two referees, should be addressed to Mr. J. E. Wakeford, Divisional Manager, West Midlands Gas Board, Birmingham District, Gas Offices, Edmund Street, Birmingham, 3, to reach him not later than 23rd February, 1951. Applicants are requested to quote ref. A when replying to this advertisement.

F. H. CURETON, *Secretary*.

CHEMISTS required for analytical laboratory in the Eastern Counties. The work involved will be principally organic analysis. Applicants possessing minimum of two years' experience in industrial analytical laboratories preferred. Salary according to qualifications and experience. Write stating age, full particulars and salary required to Box No. 3765, THE ANALYST, 47, Gresham Street, London, E.C.2.

CHEMICAL ASSISTANT for organic research laboratory in Eastern Counties. Man with experience of preparation of organic intermediates, not necessarily qualified. Salary from £400 per annum according to ability and experience. Write stating age and full particulars to Box No. 3766, THE ANALYST, 47, Gresham Street, London, E.C.2.

QUALIFIED CHEMISTS, male or female, required for laboratory of Midland Food Manufacturers. Previous experience desirable but not essential. Salary according to qualifications and experience. Write Box No. 3763, THE ANALYST, 47, Gresham Street, London, E.C.2.

CHEMIST and BACTERIOLOGIST required by a Brewery in the North-West. Applications giving age, qualifications and full particulars of experience should be sent to Box No. 3764, THE ANALYST, 47, Gresham Street, London, E.C.2.

A RAPIDLY expanding organisation in the Midlands invites applications from qualified chemists to undertake work into a variety of subjects, including lubricating oils and greases; cutting fluids; corrosion problems; the preparation and treatment of metallic surfaces by metal spraying, painting and enamelling; electroplating; adhesives. The nature of the posts demands that only applicants who are capable of taking responsibility and can show a high degree of initiative need apply. Send full details, including present salary, to Box No. 3767, THE ANALYST, 47, Gresham Street, London, E.C.2.

REPORTS OF THE ANALYTICAL METHODS COMMITTEE OBTAINABLE THROUGH THE EDITOR

The Reports of the Analytical Methods Committee listed below may be obtained direct from the Editor of THE ANALYST, 7-8, Idol Lane, London, E.C.3 (not through Trade Agents), at the price of 1s. 6d. to Members of the Society, and 2s. 6d. to non-Members. Remittances must accompany orders and be made payable to "Society of Public Analysts."

Milk Products Sub-Committee:

Reports Nos. 1 and 2. Analysis of Condensed Milks.
Report No. 3. Analysis of Sweetened Condensed Milk in which the Sucrose has altered during Storage. *Out of print.*
Report No. 4. Determination of Water, of Total Solids and of Fat in Dried Milk.

Sub-Committee on Dirt in Milk. Report. Determination of Dirt in Milk.

Report on the Determination of Total Solids in Fresh Liquid Milk.

Essential Oil Sub-Committee:

Report No. 1. Estimation of Cineole in Essential Oils. (1) Cajuput and Eucalyptus Oils.
Report No. 2. Physical Constants (1).
Report No. 3. Physical Constants (2).
Report No. 4. Interim Report on the Determination of Acetylisable Constituents in Essential Oils.
Report No. 5. Determination of Phenols in Essential Oils.
Report No. 6. Determination of Citral in Lemon Oil.
Report No. 7. Determination of Solubilities.
Report No. 8. Determination of Cineole in Essential Oils. (2) Camphor Oil. (3) Other Oils
Report No. 9. Determination of Carvone and Menthone.
Report No. 10. Determination of Citronellal.
Report No. 11. Determination of Aldehydes other than Citronellal.
Report No. 12. Determination of Ascaridole.
Report No. 13. Determination of Esters.
Report No. 14. Solubility Test for Ceylon Citronella Oil. (Gratis.)

Metallic Impurities in Foodstuffs Sub-Committee (formerly **Sub-Committee on the Determination of Arsenic, Lead, etc. in Food Colouring Materials**):

Report No. 1. Determination of Arsenic. *To be reprinted.*
Report No. 2. Determination of Lead.
Report No. 3. Determination of Copper.
Report No. 4. Determination of Zinc.

Sub-Committee on the Determination of Unsaponifiable Matter in Oils and Fats and of Unsaponified Fat in Soaps:

Report No. 1. Determination of Unsaponifiable matter in Oils and Fats.
Report No. 2. Determination of Unsaponified Fat in Soap. *Out of print.*
Report No. 3. Determination of Free Alkali in Soaps.
Report No. 4. Determination of Free Alkali and Silica in Silicated Soaps.
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Poisons Sub-Committee appointed to investigate Methods of Assay for Various Substances appearing in the Poisons Schedules of the Poisons Regulations, 1935:

Report No. 1. Assay of Lobelia (*Lobelia Inflata*).
Report No. 2. Assay of Gelsemium
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Report No. 4. Assay of Yohimba.
Report No. 5. Assay of Jaborandi.
Report No. 6. Assay of Ephedra and of Ephidrine in Nasal Sprays.

Fluorine in Foods Sub-Committee:

Report on the Determination of Fluorine in Foods.
Addendum to above Report. (Gratis.)

Sub-Committee on Vitamin Estimations. Microbiological Panel:

Report on the Microbiological Assay of Riboflavine and Nicotinic Acid.

Sub-Committee on Vitamin Estimations. Carotene Panel:

The Determination of Carotene in Green-Leaf Material. Part 1. Fresh Grass.

Tragacanth Sub-Committee:

Report No. 1. Evaluation of Powdered Tragacanth.
Report No. 2. Evaluation of Flake Tragacanth.

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