THE ANALYST

A Monthly Publication dealing with all branches of Analytical Chemistry: the Journal of the Society of Public Analysts and Other Analytical Chemists

Editor: F. L. OKELL, F.R.I.C.
7-8, IDOL LANE, LONDON, E.C 3
Telephone: MANsion House 6608

Published for the Society by
W. HEFFER & SONS, LTD., CAMBRIDGE, ENGLAND

Volume 77 Price 6s. 6d.
Subscription Rate, inclusive of Abstracts C, 80/- per annum, Post Free
No. 910, Pages 1-56 January, 1952

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Volume 77. No. 910, Pages 1-56 January, 1952

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THE JOURNAL OF
THE SOCIETY OF PUBLIC ANALYSTS AND OTHER ANALYTICAL CHEMISTS

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Printed and Published for the Society of Public Analysts and Other Analytical Chemists by W. Heffer & Sons Ltd., Cambridge, England. **Communications to be addressed to the Editor, F. L. Okell, 7 8, Idol Lane, London, E.C.3.** Enquiries about advertisements should be addressed to Walter Judd Ltd., 47, Gresham Street, London, E.C.2.

Entered as Second Class at New York, U.S.A., Post Office.

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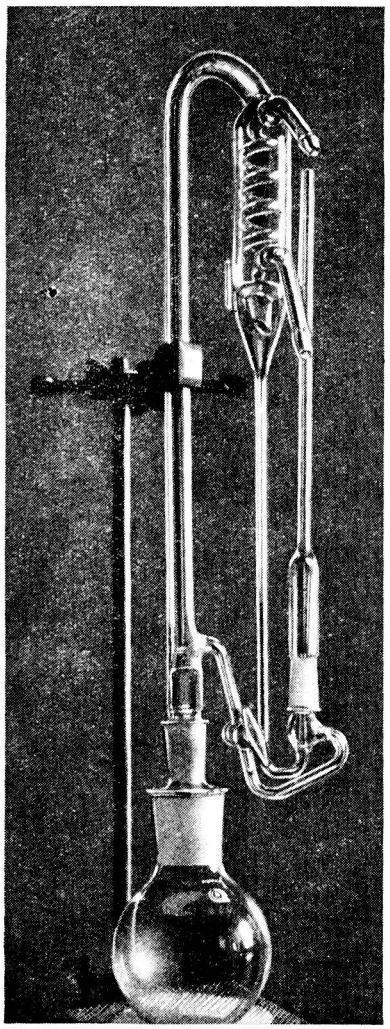
No. 911, Pages 57-110 February, 1952



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THE ANALYST

PROCEEDINGS OF THE SOCIETY OF PUBLIC ANALYSTS AND OTHER ANALYTICAL CHEMISTS

THE AMERICAN CHEMICAL SOCIETY

THE following letter has been received from the President of the American Chemical Society, Professor N. H. Furman, in acknowledgment of the address presented on the occasion of the 75th Anniversary Celebrations (*Analyst*, 1951, **76**, 503)—

Dear Doctor Nicholls:

The scroll presented by the Society of Public Analysts and Other Analytical Chemists to the American Chemical Society on the occasion of our Diamond Jubilee is one of the valued mementos of this event. The celebration has left happy memories in the minds of thousands. In the years to come re-examination of the scrolls and gracious letters from our sister societies, presented at this time, will bring back these recollections.

The analytical chemists of this country are served by our Division of Analytical Chemistry. It is one of our largest and most active groups. Contributions in this field are recognized by an annual award. Our Division has many similarities with the society of which you are president.

It is particularly gratifying to us to receive congratulatory messages from organizations such as yours representing a specialized field in the broad science served by the American Chemical Society. We know that members of the Division most closely related to yours will take particular pride in your greeting, but I can assure you that that of the parent society will be just as great.

We are confident that the past success of the Society of Public Analysts and Other Analytical Chemists will increase in the future and that from it will flow benefits to all chemists, not only in Great Britain but the entire world.

Cordially yours,

N. H. Furman

N. H. FURMAN,
President.

ORDINARY MEETING

AN Ordinary Meeting of the Society was held at 7 p.m. on Wednesday, November 7th, 1951, in the Meeting Room of the Chemical Society, Burlington House, London, W.1. The Chair was taken by the President, Dr. J. R. Nicholls, C.B.E., F.R.I.C.

The following papers were presented and discussed: "Has the Chemical Quality of Milk Deteriorated?" by J. G. Davis, D.Sc., Ph.D., F.R.I.C.; "Some Observations on the Determination of the Activity of Rennet," by N. J. Berridge, B.Sc., Ph.D.; "The Estimation of 2:4-Dichlorophenoxyacetic Acid," by S. W. Stroud, B.Sc.

NEW MEMBERS

Abdallah Said Beidas, B.Sc. (Lond.); Godfrey Jefford, B.Sc. (Wales), M.Sc. (Lond.); Eric Mather, A.R.I.C., A.R.T.C.S.; John Stanley Wragg, B.Sc. (Lond.), A.R.I.C.

DEATHS

WE regret to record the deaths of

Howard Alfred Caulkin
Nadhim Jaafar Chalabi
Harold Thomas Cranfield
Eric H. England.

SCOTTISH SECTION

AN Ordinary Meeting of the Section was held at 7.15 p.m. on Wednesday, November 14th, 1951, in the Central Hotel, Glasgow. Thirty members and guests were present.

The following papers were presented and discussed: "The Chromatographic Determination of the Acids in Bread," by H. C. Moir and A. Kerr; "The Micro-determination of Molecular Weights of Picrates by a Spectrophotometric Method," by K. G. Cunningham, W. Dawson and F. S. Spring. An informal discussion on "The Detection and Estimation of Small Amounts of Acetic Acid" followed.

A Critical Investigation of the Use of the Silver Reductor in the Micro-Volumetric Determination of Iron, especially in Silicate Rocks

BY CHRISTINA C. MILLER AND ROBERT A. CHALMERS

(*Presented at the meeting of the Society on Wednesday, October 3rd*, 1951)

Hydrogen peroxide is produced, even in small silver reductors, if air-free hydrochloric acid is not used, and this prevents complete reduction of ferric salts. The use of acid saturated with carbon dioxide reduces the error to negligible proportions.

Unstable end-points obtained in the titration of ferrous iron, subsequent to fusions with potassium bisulphate in platinum crucibles, are most probably caused by the presence of platinum[II], which reacts very slowly with ceric sulphate. In general, the error in the determination of iron is insignificant if, near the anticipated end-point, titration is not delayed and the *first* colour change of the indicator is noted. The use of gold crucibles eliminates all uncertainty. Results are shown for the determination of 0·03 to 0·6-mg amounts of iron, alone and in a number of silicate rocks.

THE silver reductor is a valuable means of reducing ferric iron, without interference from titanium[IV], prior to determination volumetrically with ceric sulphate. The simplicity of the process commends it for micro-analysis, but there are some sources of error.

In determining small amounts of iron with the large reductor of Walden, Hammett and Edmonds,[1] Fryling and Tooley[2] found that the production within the reducing column of various amounts of hydrogen peroxide caused large errors that could, however, be rendered insignificant if reductions were effected in an inert atmosphere and an appropriate correction was applied. Edmonds and Birnbaum[3] claimed to have almost eliminated the error merely by reducing the size of the reductor, and van Nieuwenburg and Blumendal[4] and Colson,[5] who reduced the size still further, made no mention of peroxide. If peroxide formation occurs and is ignored, negative errors arise in the determination of iron. Van Nieuwenburg attributed negative errors to partial oxidation of the reduced solutions by air; Colson excluded air in collecting and titrating reduced solutions. Wells[6] washed his small reductor thoroughly with acid in order to remove accumulated peroxide and determined iron potentiometrically without excluding air.

When large amounts of potassium bisulphate had been used to dissolve samples, Fryling and Tooley noted a tendency for the reduced form of the *o*-phenanthroline ferrous sulphate, used as an indicator, to return after the titration of ferrous iron. The addition of bromine to the solution before passage through the reductor, or the addition afterwards of an excess of cerous sulphate, which would lower the potential of the ceric - cerous system, was said to effect an improvement, but no adequate explanation was given.

In the microchemical laboratory we have been using small silver reductors for the determination of iron in the mixed oxide precipitates ($Fe_2O_3+Al_2O_3+TiO_2+Mn_3O_4+P_2O_5$) obtained from silicate rocks. Fusion of the oxides has been effected with potassium bisulphate and the acid extracts have been reduced, collected without exclusion of air, and titrated with ceric sulphate. Very variable "blank" determinations have been reported and unstable end-points have been general.

This investigation was to find the source of these irregularities and to evolve a satisfactory procedure for the determination of 1 to 15 per cent. of ferric oxide in 5-mg quantities of silicate rocks. We have found that, even in small reductors where air is not excluded from the columns, there is a variable peroxide error that may be virtually eliminated if acid saturated with carbon dioxide is used. Platinum dissolved from crucibles during fusions is not deposited on the silver of the reductor, as we had supposed; it is presumably reduced from the quadrivalent to the bivalent form, accompanies ferrous iron and is slowly re-oxidised by ceric sulphate after the oxidation of the iron. The substitution of gold for platinum crucibles eliminates all possibility of error, but if, after the use of platinum, titrations are not made too slowly near the equivalence-point for iron and the first colour change of the indicator is recorded, errors are frequently inappreciable.

Experimental

The reductor—

A reductor like Colson's larger model[5] was inserted into a suction apparatus, which was provided with inlet and outlet tubes for carbon dioxide and contained a 10-ml beaker as a receiver. The silver was prepared by the method of Walden *et al.*,[1] dried and sifted, and particles of appropriate size were added gradually with gentle stirring to *N* hydrochloric acid contained in the reductor. The glass-wool support for the silver was placed near the stopcock.

The peroxide effect—By means of Savage's test[7] about 0·5 μg of hydrogen peroxide was found in the effluent when 2 ml of *N* hydrochloric acid were passed through the reductor. When, immediately after, two portions of acid containing a little iron alum were successively passed through and the effluents tested, the first gave a very definite reaction for ferric iron because hydrogen peroxide initially present in the reductor passed into the receiver in advance of ferrous iron, a little of which it then oxidised. The second contained neither ferric iron nor peroxide, because the latter was immediately reduced in the reductor by ferrous iron, and reoxidised ferrous iron was reduced again by the silver. When, however, acid *follows* iron in the reductor, peroxide is formed and reaches the receiver before it is destroyed. Fryling and Tooley[2] corrected for this effect by *adding* to the titre for the ferrous iron solution the amount of oxidant required to oxidise hydrogen peroxide formed in the acid used for washing the silver after all the iron had entered the column. It is important to note that this addition is legitimate only if the acid is essentially iron-free. A 2-ml portion of the *N* hydrochloric acid used by us contained about 0·07 μg of iron.

Other reagents must also be examined for iron independently of the reductor, since, if they contain no iron, or less than the equivalent of the hydrogen peroxide found in the wash liquid, the effluent obtained from a full blank run ("reagent blank") will contain hydrogen peroxide and no ferrous iron, the respective amounts of the former being greater than, or less than that associated with the wash liquid alone. If, however, the reagent blank contains ferrous iron and the peroxide correction is constant, the titre for the reagent blank should be deducted directly from that for the solution under examination.

Earlier papers indicate that peroxide may be partly adsorbed on the surface of the silver, the effect being more marked in freshly prepared reductors. This was confirmed. When 2-ml portions of *N* hydrochloric acid were passed through such a reductor they used up successively 0·085, 0·008 and 0·004 ml of 0·01 *N* ceric sulphate. When a solution containing 500 μg of iron was passed through immediately after, and the reductor washed with 2 ml of acid, a deficiency of 8 μg of ferrous iron was noted, despite the fact that 0·004 ml was added

to the titre. In a second run with a solution containing iron no deficiency was indicated. Apparently most of the hydrogen peroxide first formed was removed mechanically by washing with acid, but a small residual amount remained adsorbed until removed by chemical action.

Reduction and stabilisation of the peroxide effect—Fryling and Tooley reduced peroxide formation to a minimum by using a somewhat complicated apparatus from which air was excluded by means of hydrogen. As a micro-adaptation did not seem practicable, we tried the simple expedient of substituting hydrochloric acid saturated with carbon dioxide for that normally used in the preparation of the reductor and in the washing. It was unnecessary to saturate solutions containing iron, since a trace of peroxide would be reduced by ferrous iron formed in the reductor. Blank runs made with successive 2-ml portions of *N* hydrochloric acid used 0·001 to 0·0015 ml of 0·01 *N* ceric sulphate, the variation in the course of a day being 0·0005 ml, which is equivalent to 0·3 μg of iron. When saturation with carbon dioxide was omitted 0·004 to 0·006 ml of ceric sulphate was generally required, but occasionally the amount exceeded 0·01 ml. All the above values have been corrected for the amount of ceric sulphate required to oxidise the indicator ("indicator correction").

RECOMMENDED PROCEDURE FOR THE REDUCTION AND DETERMINATION OF FERRIC IRON—

In preparing the reductor and for all rinsing, use essentially iron-free *N* hydrochloric acid saturated with carbon dioxide. When a reductor is freshly prepared, or has not been used for some time, pass through it 2 ml of acid containing a few milligrams of ferrous ammonium sulphate to remove adsorbed hydrogen peroxide, wash the column and carry out the reductor blank. Add to the reductor the ferric solution, made up in *N* hydrochloric acid, and rinse out the containing vessel, if required, with 0·5-ml portions of acid. Allow the solution to percolate at the rate of 0·05 ml in 8 seconds, but never expose the silver to the air. Finally, rinse the reductor with three 0·5-ml portions of acid. The reduced solution should be collected in an atmosphere of carbon dioxide. To the effluent add 0·02 ml of 0·001 *M* *o*-phenanthroline ferrous sulphate and titrate with 0·01 *N* ceric sulphate, while stirring the solution with a stream of carbon dioxide. Determine the indicator correction (about 0·002 ml) and deduct it. Carry out a blank run on 1·5 ml of acid, deduct the indicator correction and add the residuum to the titre for the iron. Leave the cup of the reductor filled with acid and cover it, preferably with a ground-glass cap.

Notes on the titrimetric procedure—A stock of approximately 0·01 *N* ceric sulphate in 0·5 *N* sulphuric acid was prepared and kept in the dark for some time before it was required, in order to ensure oxidation of traces of reducing impurities. It was standardised on the macro and micro scales by means of sodium oxalate[8] (U.S.A. Bureau of Standards quality) and also, as a further check, by means of a ferrous ammonium sulphate solution that was simultaneously compared with a permanganate solution standardised with sodium oxalate. There was close agreement between all the results. The ceric sulphate solution was afterwards checked at intervals. Great care had to be taken to avoid the presence of adventitious impurity (filter-paper fibre, grease and dust) in the small portion of the stock solution removed for a day's work.

Two micro-burettes, constructed from precision-bore tubing and with long tips, were calibrated by Benedetti-Pichler's method,[9] mounted vertically and attached to Schilow's pressure-controlling device.[10] The total capacities (1·1 and 0·17 ml) were found by determining the amounts of ceric sulphate delivered. The smaller burette served for the determination of 30 to 80 μg of iron.

THE SOURCE OF DISAPPEARING END-POINTS AFTER FUSIONS WITH POTASSIUM BISULPHATE—

The direct addition of 300 mg of potassium bisulphate to ferric chloride solutions did not influence the subsequent end-point behaviour, nor did bisulphate that had been fused in quartz. The end-points obtained in blank runs with bisulphate and hydrochloric acid were also stable. When, however, the bisulphate was fused in a platinum crucible, alone or in the presence of iron, and the extracts were reduced and titrated, the first colour change of the indicator was not permanent and further slow addition of about 0·04 ml of ceric sulphate during half an hour was required before a stable end-point was obtained. When iron was present the first end-point corresponded approximately to the amount taken. Potentiometric titrations were also done. Whereas with ferrous chloride alone a sharp increase in potential was recorded as expected, with a solution prepared from iron alum that had been fused in

platinum with potassium bisulphate before reduction, the potential near the equivalence-point for iron rose with each further small addition of ceric sulphate and then slowly fell. The equilibrium potential very gradually rose until oxidation was complete.

When a solution of chloroplatinic acid containing 100 μg of platinum was tested with potassium iodide[11] it soon gave a pink colour, whereas a similar solution that had been passed through the reductor gave a colour only after some time. Both solutions after evaporation and full oxidation were found to contain the same amount of platinum. A third solution, after passage through the reductor, required for full oxidation an amount of ceric sulphate corresponding to the conversion of about 40 μg of platinum^{II} into platinum^{IV}. The amount of platinum dissolved as a result of a bisulphate fusion was about 40 μg. It is probable that a good deal of this appears in the effluent as platinum^{II}, the rest being platinum^{IV}. The matter was not further investigated.

Attempts made to reduce platinum^{IV} to the elementary form, by means of formic acid, for example, before transferring solutions to the reductor, were not a success, so an alternative to platinum crucibles for fusions with potassium bisulphate was sought. A solution of gold chloride in *N* hydrochloric acid, after passage through the reductor, gave the customary blank value for the reductor and a stable end-point, the gold^{III} being presumably reduced to metallic gold and held on the reductor. Gold crucibles were therefore obtained and found to be excellent for the determination of iron in materials that had to be fused with potassium bisulphate in vessels that were unattacked by hydrofluoric acid. They were somewhat more heavily attacked by bisulphate than platinum crucibles but the attack was not severe.

PREPARATION OF SILICATE ROCKS FOR THE DETERMINATION OF IRON—

With the aid of a stoppered weighing stick weigh out about 5 mg of the dried material and transfer it to a 1-ml gold crucible. Add 0·1 ml of hydrofluoric acid (40 per cent.) and then, 3 minutes later, 0·05 ml of *N* sulphuric acid. Evaporate to dryness on a steam-bath and cautiously expel sulphuric acid over a micro-bunsen burner. Repeat the treatment, then fuse the residue with 0·1 g of potassium bisulphate and cool. If, as often happens, the melt has crept over the top of the crucible, place the latter in a 4-ml porcelain capsule containing 1 ml of *N* hydrochloric acid, warm until solution of the sulphates is complete, and lift out the crucible and rinse the exterior with 1 ml of *N* hydrochloric acid. By means of a short-stemmed pipette transfer the contents of basin and crucible to the reductor cup. Wash the crucible four times with 0·5 ml of *N* hydrochloric acid, transferring each washing to the basin and then to the reductor. Continue the flow of solution through the reductor until all has passed into the column of silver, then wash the latter with three 0·5-ml portions of acid. Except for the initial dissolution, use acid saturated with carbon dioxide. The final volume for titration is 5·5 ml, but should transference of the crucible to the capsule not be required, it can be reduced to 4 ml. Proceed with the determination of iron as already indicated. If the reagents contain iron, carry out a full blank run on them and deduct the titre found from that for the iron (see p. 3).

RESULTS

IRON IN FERRIC CHLORIDE SOLUTIONS—

A solution prepared from Hilger's "H.H.P." iron (impurities less than 0·08 per cent.) by dissolving it in hydrochloric acid and oxidising with chlorine, was standardised on the macro scale by reducing portions in a zinc reductor and titrating with ceric sulphate. Weighed aliquots were reduced in the silver reductor and iron determined as on p. 4. The results are shown in Table I.

TABLE I

DETERMINATION OF IRON IN FERRIC CHLORIDE SOLUTIONS

Weight of iron taken, μg	Error, μg	Weight of iron taken, μg	Error, μg	Weight of iron taken, μg	Error, μg
566·5	+0·5	274	−1	47·1	+0·1
559	−1	274	0	35·1	−0·2
558	+1·5	265	+0·5	30·1	−0·3
551	+1	250	0	28·1	0
556·5	+1·5	287·5	+1	39·8	−0·3
553	−1·5	247·5	0	45·8	0

In a few experiments on the determination of 500 μg of iron, in which the reduced solutions were neither collected in an atmosphere of carbon dioxide nor stirred with the gas during titration, the average result was 0·2 per cent. lower than those shown above.

Influence of other elements—Of elements that could interfere in the determination of iron only vanadium required to be considered in connection with the silicate rocks that were analysed here. Vanadium^{IV} that is formed in the silver reductor is slowly oxidised in *N* acid solutions by ceric sulphate.[12] Amounts of vanadium ranging from 50 to 250 μg, added to ferric solutions as ammonium vanadate, caused an error of only +3 μg in the determination of 300 to 400 μg of iron, and even this could be avoided by making the solutions to be titrated 5 *M* with respect to sulphuric acid.[1]

IRON IN SOLUTIONS AFTER FUSION WITH POTASSIUM BISULPHATE—

Weighed portions of the standard iron solution were evaporated with sulphuric acid in 1-ml gold crucibles, the residues fused with potassium bisulphate and the iron determined as on p. 5. The results, corrected for iron in the reagents, are shown in Table III. Those shown in parentheses refer to fusions done in platinum crucibles.

TABLE II

DETERMINATION OF IRON IN SOLUTIONS AFTER FUSION WITH POTASSIUM BISULPHATE

Weight of iron taken, μg	Error, μg	Weight of iron taken, μg	Error, μg	Weight of iron taken, μg	Error, μg
555·5	0	283·5	+1·5	33·1	−0·2
551·5	0	269	−0·5	33·6	−0·3
561	+0·5	269	−0·5	43·4	−0·3
(542)	(+1)	(263)	(−0·5)	(38·2)	(+2·0)

NOTE—Values in parentheses refer to fusions done in platinum crucibles.

When slow titration near the end-point is avoided and the *first* colour change of the indicator is noted, fusion in platinum need cause no significant error, except perhaps when a very small amount of iron is under consideration.

IRON IN SILICATE ROCKS AND A REFRACTORY—

In order to ensure homogeneity in the samples used, powders that passed through a 300-mesh sieve were analysed. The total iron content of each was also found on the macro

TABLE III

DETERMINATION OF IRON IN SILICATE ROCKS AND A REFRACTORY

Silicate	Approximate weight taken, mg	Fe_2O_3 by micro method, %	Fe_2O_3 by macro method, %
*Flint clay No. 97	4·0	0·93	0·94
	6·1	0·93	0·94
	(5·1)	(0·93)	
*Burnt refractory No. 76	4·7	2·23	2·18
	4·9	2·26	2·23
	(5·2)	(2·31)	
Phonolite	5·6	6·17	6·17
	6·0	6·10	6·14
	(5·8)	(6·16)	
Analcite syenite	5·5	8·76	8·83
	6·5	8·88	8·87
	(4·0)	(8·73)	
Kinkell tholeiite	4·2	15·66	15·75
	3·5	15·72	15·74
	4·1	15·79	
	3·5	15·64	
	(4·3)	(15·61)	

* U.S.A. Bureau of Standards sample.

NOTE—Values in parentheses refer to fusions done in platinum crucibles.

scale, the Bureau of Standards materials being analysed because only about 50 per cent. of the original samples passed through the fine sieve. As on the small scale the silicates (about 0·5 g) were freed from silica and fused with potassium bisulphate. Platinum crucibles were, however, used, and from the solutions of the melts platinum was removed by means of sulphuretted hydrogen, and ferric iron simultaneously reduced to ferrous iron,[13] which was subsequently determined with ceric sulphate.

In all the analyses allowance was made for iron in the reagents. All the samples, except the flint clay, which was dried at 140° C, were dried at 105° to 110° C before use. The results are shown in Table III. Those given in parentheses refer to fusions done in platinum crucibles.

Once more it is indicated that fusions in platinum need cause no significant error if titration near the equivalence-point for iron is not delayed. This might be of importance if high-temperature fusions with sodium carbonate, which could not be done in gold crucibles, were required before the determination of iron.

We gratefully acknowledge a maintenance grant to one of us (R. A. C.) from the Department of Scientific and Industrial Research, and grants from Imperial Chemical Industries Limited and the Trustees of the Ritchie Bequest. We are indebted to Dr. J. B. Simpson of H.M. Geological Survey for two of the rock samples.

References

1. Walden, G. H., Hammett, L. P., and Edmonds, S. M., *J. Amer. Chem. Soc.*, 1934, **56**, 350.
2. Fryling, C. F., and Tooley, F. V., *Ibid.*, 1936, **58**, 826.
3. Edmonds, S. M., and Birnbaum, N., *Ind. Eng. Chem., Anal. Ed.*, 1940, **12**, 60.
4. Van Nieuwenburg, C. J., and Blumendal, H. B., *Mikrochem.*, 1935, **18**, 39.
5. Colson, A. F., *Analyst*, 1945, **70**, 255.
6. Wells, I. C., *Anal. Chem.*, 1951, **23**, 511.
7. Savage, D. J., *Analyst*, 1951, **76**, 224.
8. Kirk, P. L., "Quantitative Ultramicro-analysis," Chapman & Hall, London, 1950, p. 131.
9. Benedetti-Pichler, A., "Microtechnique of Inorganic Analysis," Chapman & Hall, London, 1942, p. 258.
10. Schilow, E., *Z. angew. Chem.*, 1926, **39**, 232, 582.
11. Snell, F. D., and Snell, C. T., "Colorimetric Methods of Analysis," Vol. I, Chapman & Hall, London, 1936, p. 419.
12. Furman, N. H., *J. Amer. Chem. Soc.*, 1928, **50**, 1675.
13. Hillebrand, W. F., and Lundell, G. E. F., "Applied Inorganic Analysis," Chapman & Hall, London, 1929, p. 304.

Chemistry Department
The University, Edinburgh, 9

Discussion

The President thanked the authors for their paper and said that very often methods that were satisfactory on a macro scale could not be reduced to a micro scale. Clearly, this method had been studied with great care and very good results had been attained.

Mr. C. H. Price asked whether a method for the removal of platinum, if such were available, would not simplify the procedure. He had found that α-furildioxime quantitatively precipitated traces of platinum after sodium carbonate fusions.

Mr. Chalmers thanked Mr. Price for his suggested procedure. They had tried several techniques, but without success, and had had recourse to the use of gold crucibles. The results clearly showed that the presence of platinum was without significant effect, provided certain conditions were complied with in the titration.

Dr. K. A. Williams expressed his pleasure that Dr. Miller had been able to present her paper. He said that he used gold crucibles containing about 5 per cent. of platinum, as they did not fuse easily as would pure gold crucibles, and asked if the authors could say whether crucibles of that composition yielded an appreciable amount of platinum in this work.

Mr. Chalmers replied that he had no experience of 5 per cent. of platinum in gold. A gold crucible lost about 100 μg in weight per fusion with 0·1 g of potassium bisulphate; a platinum crucible lost about 40 μg per fusion. He would expect a small loss of platinum from the 5 per cent. alloy.

A Technique to Improve the Efficiency of Desiccators

By J. KING

(*Presented at the meeting of the Society on Wednesday, October 3rd, 1951*)

The inefficiency of the usual type of desiccator has been noted by several workers. After a study of the usual methods, the author has greatly increased the efficiency of the desiccator by a simple modification, namely, by placing a cylinder of perforated zinc in the upper compartment of a Scheibler desiccator and filling the annular space and the base with desiccant. The most suitable desiccant is calcium carbide in lumps about half an inch in diameter.

Figures showing the absorption of moisture by flour previously dried at 110° C, samples of which were placed in the modified calcium carbide desiccator and in the usual calcium chloride desiccator of Scheibler pattern, show the improvement in efficiency.

In a recent paper, Belcher and Mott[1] have again called attention to the inefficiency of the usual type of desiccator. It is surprising that this should have been necessary, in view of the previous publications by Bower[2] on the comparative efficiencies of various dehydrating agents and by Booth and McIntire[3] on the inefficiency of desiccators under the provocative title of "When is a desiccator?" Belcher and Mott in their paper appear to condemn the use of desiccators altogether and prefer to cool materials in the open air before weighing, but always in a covered dish of their own special design. It would, of course, be possible to evacuate the air rapidly from the desiccator immediately after introducing the apparatus, and then to allow dried air to enter before weighing, but this is not always convenient and introduces complications.

Some years ago I was faced with the problem of determining moisture, ash and so on, in circumstances in which the previously heated material might have to remain overnight or for some days between heating and weighing. Under these conditions the usual pattern of Scheibler desiccator was found to be unsuitable, owing to the absorption of moisture from the atmosphere of the desiccator. When a heated vessel containing a dry material is placed in a desiccator, moist air enters, and there is an immediate competition for this between the desiccant and the material to be subsequently weighed. As the desiccant is usually in the base of the desiccator it has a poor chance of competing with the material to be weighed. The amount of moisture that enters a desiccator is considerable. On the assumption that the air of the laboratory is at a temperature of 70° F, with a relative humidity of 70 per cent., each cubic inch will contain 0·2 mg of moisture. Experiments showed that in these conditions 10 to 20 mg of moisture could enter a 6-inch desiccator during laboratory manipulation involving the removal of the lid, placing a heated dish in the desiccator and replacing the lid as soon as possible. The Milk Products Sub-Committee of the Analytical Methods Committee of the Society[4] was aware of this difficulty in the determination of total solids of sweetened condensed milk, and prescribed the use of metal dishes having readily removable but close-fitting covers, in order to prevent exchange of atmosphere in the dish with consequent absorption of moisture from the partly dried atmosphere within the desiccator. These dishes are unsuitable for the determination of ash, for which they would need to be made of platinum or other equally resistant metal.

It was obvious from a study of the papers by Bower and by Booth and McIntire that even the most efficient desiccant known, barium oxide, would be of little use in a normal desiccator, and that the most that could be hoped for would be to increase the speed of removal of moisture to the maximum. This was accomplished, in the form of desiccator finally adopted, by placing a cylinder of perforated zinc sheet centrally in the upper compartment of a Scheibler desiccator and filling the annular space, which should be at least 1 inch, and the base with calcium carbide broken in pieces sifted to about half an inch in diameter (see Fig. 1). This allows free circulation of the atmosphere within the desiccator and hence a much more rapid removal of moisture than can be attained in the usual pattern. The acetylene evolved is less troublesome than the expansion of air caused by the introduction

of hot apparatus into the desiccator. The formation of calcium hydroxide necessitates brushing the zinc cylinder at regular intervals to prevent fine particles falling on the apparatus, and the whole of the carbide should be withdrawn, thoroughly sifted and replaced when necessary. The desiccants referred to by Bower,[2] as well as others, were considered, but calcium carbide was finally chosen as it has the following advantages: it is cheap and readily available, it has no tension of aqueous vapour either initially or during use and it allows free circulation of the internal atmosphere in the desiccator even after considerable use. In some determinations, the use of an open dish in this desiccator does not lead to any appreciable absorption of moisture; but, in general, apparatus furnished with a closure is necessary, as, for example, in the determination of an ash that is hygroscopic. Typical of this is the determination of the ash of acid casein; in this determination a measured volume of standard

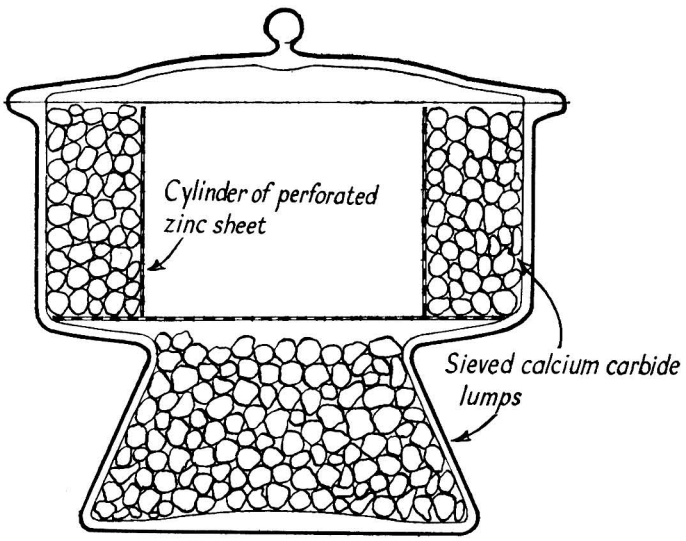

Fig. 1. Diagram of modified desiccator

calcium acetate is added and an excess of calcium oxide is left after ignition. The small amount of calcium hydroxide associated with the lumps of carbide is helpful in this instance in absorbing any carbon dioxide that may enter the desiccator. A normal type of platinum crucible furnished with a cover was found to be satisfactory for this operation, although the crucible was by no means hermetically sealed.

The efficiency of the desiccator compared with one of the Scheibler pattern charged with calcium chloride previously heated to 250° C for 2 hours can be judged from Table I. Wheat

TABLE I

ABSORPTION IN DESICCATORS OF MOISTURE BY 5 g OF FLOUR PREVIOUSLY DRIED AT 110° C

Time of storage in desiccator	Calcium carbide charged desiccator described in text		Calcium chloride desiccator of Scheibler pattern	
	Gain in weight during each interval in desiccator, mg	Total gain in weight, mg	Gain in weight during each interval in desiccator, mg	Total gain in weight, mg
30 minutes	1·5	1·5	5	5
additional 1 hour	nil	1·5	7·5	12·5
" 1 hour	nil	1·5	7·5	20·0
" 20 hours	−1·5	nil	12·5	32·5

flour of 72 per cent. extraction was dried at 110° C for 18 hours in 5-g portions in the metal dishes of the pattern recommended by the Analytical Methods Committee of the Society, the covers being adjusted before removing the dishes from the oven. Cooling before weighing was carried out in the type of desiccator described. After being weighed, each dish was placed in the test desiccator, the lid was removed for the minimum time possible and the cover was taken off the dish while it was in the desiccator. The dish cover was replaced each time before removing the dish from the desiccator for weighing, and the conditions for opening the two types of desiccator were made as nearly identical as was possible, so as to admit approximately the same amount of moisture. It will be seen that when the dish was allowed to remain in the carbide-charged desiccator for 30 minutes, 1·5 mg of moisture was absorbed, but after 1 hour or two separate hours, no further moisture was absorbed. After 20 hours in the desiccator, this 1·5 mg of moisture was removed by the desiccant. The flour in the calcium chloride desiccator showed progressive absorption of moisture with time. The experiments were repeated after the desiccators had been in normal laboratory use for six weeks. There was little difference from the previous results, which showed that recently heated calcium chloride had little advantage over the used material. Further experiments with 98 per cent. sulphuric acid and pieces of pumice gave results of the same order as those with calcium chloride.

These results obtained about fifteen years ago support the contention of Belcher and Mott that the desiccant in a normal laboratory desiccator is of little use. A simple modification in design, however, may greatly increase the efficiency.

The author desires to thank the Government Chemist for permission to publish this account.

References

1. Belcher, R., and Mott, R. A., *J. Appl. Chem.*, **1951**, **1**, 204.
2. Bower, J. H., *J. Res. Nat. Bur. Stand.*, 1934, **12**, 241.
3. Booth, H. S., and McIntire, L., *Ind. Eng. Chem., Anal. Ed.*, **1936**, **8**, 148.
4. "Report of the Milk Products Sub-Committee to the Standing Committee on Uniformity of Analytical Methods," Report No. 1, *Analyst*, **1927**, **52**, 402.

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Discussion

The President thanked the author for presenting his paper. He said that although small differences in moisture content might not matter, there were occasions when very hygroscopic substances had to be handled, and the simple type of desiccator described was then very effective.

Mr. C. H. Price pointed out the danger of evolution of acetylene and hydrogen sulphide from commercial calcium carbide, sometimes with the formation of acetylides.

Mr. King replied that the calcium carbide desiccant was not used for dehydrating wet materials, but only for removing moisture from the atmosphere of the desiccator.

If the very minute amounts of gas evolved from commercial calcium carbide by the action of the moisture in the atmosphere in the desiccator reacted with the substance being cooled, another desiccant would have to be substituted. Such a desiccant should maintain the advantages of calcium carbide, *viz.*, it should be cheap and obtainable in a suitable graded size, and the reaction with moisture should be irreversible, giving an end product having no tension of aqueous vapour. The use of lithium aluminium hydride had been suggested.

Dr. K. A. Williams, in confirming some of Mr. King's remarks, said that he thought it was not generally appreciated how slowly the atmosphere in an ordinary desiccator became dry. He could best illustrate this by reference to work carried out with desiccators used as humidifying chambers, in which the usual desiccant had been replaced by water. In these it was found that only a layer about an inch in depth immediately above the water surface acquired 100 per cent. relative humidity, even after several days; the upper layers of air in the chamber might not rise in humidity above 75 per cent. His experience was that one should not expect evenness of relative humidity, or total dryness, in a desiccator unless the atmosphere in it was stirred by mechanical means.

Mr. King agreed, and said that he knew of a desiccator that contained a fan for circulating the air; this desiccator had a small bath of sulphuric acid in the form of a ring on the top, which seemed most dangerous.

The President remarked that he had seen in America a circular oven, containing 10 or 12 dishes, which could be rotated to bring each dish to the front of the oven where it could be weighed while still in the oven. This was useful in routine work.

DR. A. M. MAIDEN asked why desiccators should not be made of metal, the dishes being put in by a slide arrangement (as in an automatic chocolate machine), thereby allowing only the minimum amount of laboratory atmosphere to enter.

DR. J. H. HAMENCE asked the author whether in his experience, except in special cases, there was any advantage to be gained by allowing a dish or a crucible to stand for half an hour in a desiccator before weighing, as opposed to the method, used in many laboratories, in which the dish was put into the empty desiccator on top of a metal block and then weighed immediately it was cold. He had tried many types of desiccator and had concluded that in many procedures their use was completely unnecessary.

MR. KING agreed with Dr. Hamence's conclusions provided the dish or crucible were fitted with an air-tight cover.

DR. WILLIAMS remarked that quick cooling could easily be achieved if one used a desiccator in which a cooling-water coil had been attached to the underside of the zinc platform. Leads to the coil could readily be passed through a side tubulure. An inert atmosphere, or vacuum, could easily be provided by means of appropriate leads through a tubulure in the cover of the desiccator.

MR. KING commented that for many years it had been customary to cool metal dishes on a large metal block, which dissipated the heat extremely rapidly.

THE PRESIDENT remarked that a similar effect was obtained when cooling dishes used for determining moisture in tobacco. A glass-fronted cubical metal desiccator was used; this had three water-cooled shelves and layers of desiccant at the top and bottom.

DR. WILLIAMS pointed out that these problems arose from the Society's Report on the Determination of Total Solids of Condensed Milk. In the prescribed method, the metal dish contained about 25 g of sand, and this took about 45 minutes to cool in an ordinary desiccator; without the use of artificial methods of cooling, this time could not be shortened.

THE PRESIDENT concluded the discussion by saying that even the commonest apparatus was worth an investigation from time to time.

Controlled Potential Electrolysis in the Analysis of Copper-Base Alloys

BY G. W. C. MILNER* AND R. N. WHITTEM†

(*Presented at the meeting of the Society on Wednesday, October 3rd, 1951*)

Full details are given of a simple electronic instrument that has been built for automatically controlling the potential of the cathode with respect to a standard reference electrode in electro-gravimetric determination of metals. With this instrument a scheme for determining the majority of the usual alloying constituents of copper-base alloys on a total sample weight of 1·0g has been successfully tested. In addition to the electrolytic equipment, an absorptiometer is necessary for the determination of iron, manganese and nickel. Practical details of this scheme of analysis are included.

THE advantages to be gained in the analysis of copper-base alloys by automatically controlling the potential of a platinum cathode at definite values with respect to a saturated calomel electrode were first noted by Diehl and Brouns.[1] Their electronic controlling apparatus, however, corrected for cathode voltage changes in one direction only.[2] Lingane[3] constructed an instrument that automatically corrected for positive and negative changes in the cathode potential. This instrument proved of great value in the analysis of copper-base alloys; for by maintaining the cathode at −0·36 volt with respect to the saturated calomel electrode, it removed copper and antimony simultaneously from a hydrochloric acid solution of the alloy and made possible the polarographic determination of lead and tin in the residual electrolyte. Similarly after electrolysing at −0·70 volt with respect to the saturated calomel electrode to remove copper, lead, tin and antimony simultaneously from solution, the polarographic determination of nickel and zinc was possible. As large amounts of copper seriously interfere in most polarographic determinations, this technique proved very valuable for removing it cleanly from solution without the addition of further chemicals. Lingane, however, did not use the deposited copper for the quantitative determination of this element in alloys. The

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work of the above authors showed that the controlled-potential electrolytic technique could be of some value to a laboratory engaged in the inspection analysis of copper-base alloys, and so we attempted to build suitable cheap and robust electronic equipment. Full details of our instrument, which has worked satisfactorily for several months, follow.

The construction and operation of a suitable instrument

A survey of the literature revealed several American papers[4,5,6,7,8] describing suitable instruments, but generally containing American components obtainable only with great difficulty in this country. Some of these instruments were simple in design but were of limited application, whereas others were versatile but were based on complicated electronic circuits. For the present purpose and for possible future applications we have, therefore, designed a simple yet widely applicable instrument having the following characteristics—

(*a*) Sensitivity to voltage changes of ± 5 millivolts over the range $+2$ to -2 volts, thereby enabling the cathode to be maintained throughout any electrolysis to within ± 5 millivolts of its pre-determined value.
(*b*) Stability of ± 5 millivolts over a period of at least one hour, assuming that this is the approximate time required for the completion of most electrolytic separations or determinations.
(*c*) An input resistance of 10 megohms to limit the current through the reference electrode so as to minimise polarisation and resistive effects.
(*d*) A speed of response suitable for correcting voltage changes within a few seconds without excessive hunting or overshooting.
(*e*) Ease of operation.
(*f*) Construction from locally available components, preferably standard radio parts.

Full details of the instrument are shown in Fig. 1. It incorporates some of the principles used by Diehl in the construction of his single-direction control instrument. In operation the cathode reference-cell potential is opposed by a precision potentiometer consisting of the network P1, P2, P3 and B2. If, therefore, the reference potential alters in any way during an electrolysis the difference between the two potentials is amplified by the three-stage D.C. amplifier V1, V2, V3, the output of which operates the relays Ry1 and Ry2. These relays in turn control the reversible motor driving the variable arm of the potentiometer, P7, supplying the current for the electrolysis, and hence the electrolysis current is automatically adjusted to correct the difference between the two potentials. In this manner, a slight positive or negative difference between the input potential (cathode reference-cell potential) and that of the precision potentiometer actuates the control circuit to increase or decrease the electrolysing current, thereby eliminating this difference.

Certain parts of the instrument are considered in greater detail below.

Amplifier—

The first stage of the amplifier contains a pentode, V1, having a very low grid current to satisfy condition (*c*) above. To satisfy the stability requirement (*b*), it proved necessary to supply the heater of this valve from a 6-volt accumulator. It was found that the smallest available accumulators (three 2-volt, 10-ampere hour types connected in series) could be used continuously throughout a five-day working week and then recharged each weekend. It should be noted that optimum values for the plate load and cathode resistors, R6 and R4, for this valve vary with individual valves and so must be determined empirically.

The second stage of the amplifier also contains a pentode, V2, giving a reasonably high gain. The output stage, V3, is a conventional "paraphase" circuit giving a push-pull output operating the Post Office type relays. The relays in turn control the reversible motor driving the variable arm of the potentiometer supplying the current for electrolysis, the contacts being connected so as to cause rotation of the motor in either direction, depending on which relay is operated. If neither or both relays are energised, the motor is open circuited; by adjusting the cathode resistor P5 the extent of the "dead space" can be reduced to a minimum. The condenser C1 is incorporated to minimise any relay chatter caused by mains-frequency pick-up and stray transients.

It was found necessary to stabilise the plate and screen voltage supplies to satisfy the stability requirement (*b*). A 1 per cent. change in the mains voltage is equivalent to a change of about 20 millivolts in the input and so the power supply and stabiliser (V4, V5 and V6) was constructed to meet this need. This is essentially a full-wave rectifier followed by a

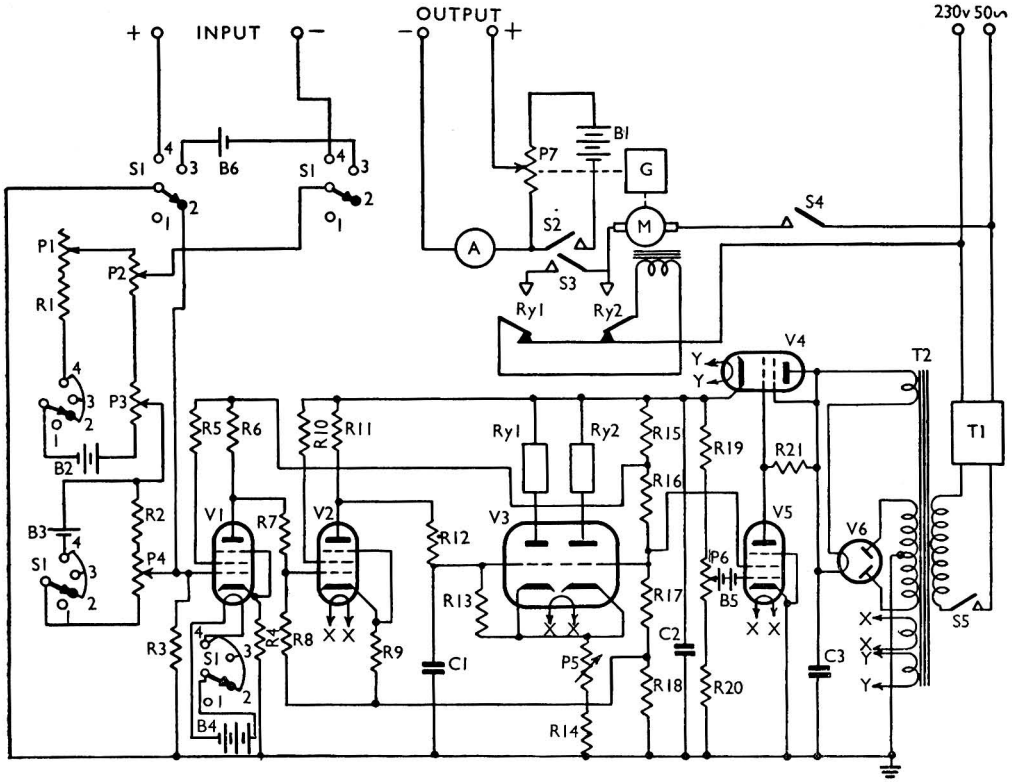

Fig. 1. Circuit of controller

KEY

Fixed Resistors: R1, 67 kΩ; R2, 100 kΩ; R3, 10 MΩ; R4, 2 kΩ; R5, 820 kΩ; R6, 300 kΩ; R7, 370kΩ; R8, 1·0 MΩ; R9, 2 kΩ; R10, 680 kΩ; R11, 560 kΩ; R12, 370 kΩ; R13, 1·0 MΩ; R14, 20 kΩ; R15, 10 kΩ; R16, 10 kΩ; R17, 10 kΩ; R18, 10 kΩ; R19, 30 kΩ; R20, 5 kΩ; R21, 390 kΩ.

NOTE—(1) All resistors are 1-watt except R19, which is 5-watt.
(2) The values of R4 and R6 are chosen to suit individual valves V1 and V2.

Condensers: C1, 0·25 μF, 400 v., paper; C2, 8 μF, 600 v., paper; C3, 8 μF, 600 v., paper.

Variable Resistors: P1, 25 kΩ; P2, 200 kΩ in 20 equal (±1%) steps; P3, 10 kΩ; P4, 50 kΩ "Helipot"; P5, 25 kΩ; P6, 5 kΩ set to give 400 v. across C2; P7, 15 Ω ("Variac" type 200 CMH).

Valves: V1, EF36; V2, 6SH7; V3, ECC32; V4, 6V6GT/G; V5, 6SJ7; V6, 5Z4G.

Transformers: T1, "Advance" constant-voltage transformer.
T2, Secondary tappings 600–0–600 v. at 40 milliamp., 2 × 6·3 v. at 3 amp., 5 v. at 2 amp.

Batteries: B1, 6-v. accumulator (150 ampere-hour); B2, 3 v., two Siemens type T cells; B3, 1·5 v., one Siemens type T cell; B4, 6-v. accumulator (10 ampere-hour); B5, 67·5 v. "Minimax" battery; B6, Weston Standard Cell.

Switches: S1 is a 5-bank 4-position Yaxley-type switch with the positions labelled (1) "Off," (2) "Zero," (3) "Calibrate," (4) "Use."
S2, S3, S4, S5 are single-pole single-throw toggle switches.

Miscellaneous: M is a G.E.C. motor type 497 DA70.
G is a 9000 to 1 reduction gear consisting of two "Meccano" worm drives and a friction drive.
A is an ammeter with the following ranges: 0 to 10, 0 to 1 and 0 to 0·1 amperes.
Ry1, Ry2 are 3000-type Post Office relays, 6500-ohm coil; fitted with tungsten change-over contacts.

conventional degenerative type regulator. In setting up, P6 is adjusted to give 400 volts across C2. Further stabilisation is afforded by the constant voltage transformer T1.

PRECISION POTENTIOMETER—

This is used for balancing the input voltage; P3 is a twenty-step potential divider with each step corresponding to 0·1 volt and P2 is a continuous control for fine adjustment from 0 to 0·1 volt. Before use the potentiometer is always calibrated against a Weston Standard cell (1·018 volts) by adjusting P1 and using the amplifier instead of the more usual galvanometer to indicate when balance is obtained. A lamp is connected to each of the relays to show when they are operated and with this arrangement balance is obtained either when both lamps are on or when both are off. Before using the amplifier, however, it is necessary to set it to zero by means of the potentiometer network B3, R2, P4 by again adjusting until both lamps are either on or off.

POTENTIOMETER DRIVE—

The drive for the main potentiometer supplying the current for electrolysis consists of two "Meccano" worm drives giving a ratio of about 2000 to 1, followed by a friction drive consisting of a 1-inch diameter rubber stopper driving a 5-inch diameter xylenite disc mounted on the knob of the "Variac" transformer. When S3 is closed the driving unit controls voltage changes in both directions, whereas when it is open a unidirectional control only is obtained.

For convenience the operating instructions for the instrument are summarised below—

Adjust P6 to give 400 volts across C2 and then adjust P5 to give conditions such that the voltage span over which both relays are simultaneously on or off is reduced to a minimum. Once set, these controls rarely need to be adjusted.

With S1 in No. 2 position, adjust P4 to balance the amplifier (*i.e.*, both relays operated or both released).

With S1 in No. 3 position and P2 and P3 accurately set to 1·02 volts, adjust P1 to again balance the amplifier (this step is required only occasionally, once or twice a week).

With S1 in No. 4 position, set P2 and P3 to the required operating potential. The instrument is now ready for use.

APPLICATION TO THE ANALYSIS OF COPPER-BASE ALLOYS

Preliminary investigations were directed to the determination of the major alloying constituent, copper. In agreement with Lingane,[9] the 1·0 to 1·5 *M* hydrochloric acid solution conditions recommended by Diehl and Brouns were found to give a somewhat patchy copper deposit, whereas this behaviour disappeared on keeping the hydrochloric acid concentration just high enough to prevent the hydrolysis of tin, but always less than 0·5 *M*. Even so, the copper deposits were generally not very satisfactory on electrolysing with the cathode potential at −0·36 volt with respect to the saturated calomel electrode. Some deposits were not adherent, being easily removed in the washing process after completion of the electrolysis, and often badly discoloured. However, by exercising very great care, reasonably quantitative deposits could be obtained, but the necessary precautions prevented the technique from being widely applicable to the inspection analysis of these alloys.

In recent work, Osborn[10] encountered sponginess in cadmium deposits in an electrolytic procedure for determining the percentage of this element in cadmium salts. Under the best conditions the deposits were always slightly powdery and easily detachable by rubbing. Osborn noticed, however, that cadmium deposited as a hard clean deposit from dilute sulphuric or perchloric acid solution in the presence of a small amount of a colloid, such as gelatin. This observation prompted us to add 10 mg of gelatin to the weak hydrochloric acid solutions of the alloys in an effort to improve the nature of the copper deposits. This modification proved most successful, giving good adherent deposits that were suitable for quantitative analysis. At a cathode potential of −0·36 volt with respect to the saturated calomel electrode, antimony deposited with the copper, and so for accurate copper figures for alloys containing more than trace amounts of antimony it proved necessary to correct the increase in weight of the cathode for this element.

In white metal analysis Lindsey and Sand[11] and later Torrance[12] determined the copper content of the deposit of copper and antimony by carrying out a further electrolytic separation from a fluoride electrolyte. In more recent work Lingane[13] achieved the same separation from an acidic tartrate solution with a pH of about 4·5. The relative proportion of antimony

and copper (of the order of 1 to 1000) in copper-base alloys is, however, such that it precludes the determination of antimony by subtraction of the re-plated copper content from the combined copper and antimony figure. For such deposits it was desirable to determine the antimony content of the sample chemically, on a separate sample weight, and then obtain the figure for copper by subtraction from the combined figure for copper plus antimony.

After removal of the copper, attempts were next made to determine the combined lead and tin contents directly by continuing the electrolysis with a copper-coated platinum cathode at −0·70 volt with respect to the saturated calomel electrode. The rate of deposition of the lead and tin was very slow, however, and too incomplete for quantitative purposes, but a great improvement resulted from increasing the hydrochloric acid content of the solution. Further difficulty was caused by the deposited metals redissolving during the washing process after the completion of the electrolysis, but this was overcome by using Lindsey's technique[14] of making the solution ammoniacal before washing. After determining the combined lead and tin percentage from the increase in weight of the cathode, the deposit was completely stripped off with nitric acid and the resulting solution evaporated to very low bulk to precipitate the tin completely as metastannic acid. After filtration, the lead content was determined by deposition as the peroxide on a small platinum-gauze anode from a nearly boiling solution of the filtrate. The increase in weight of the anode multiplied by the empirical factor[15] of 0·863 gave the weight and hence the percentage of lead in the alloy. The tin percentage was obtained by difference.

The solution remaining after the combined electrolysis of lead and tin was re-acidified with hydrochloric acid, diluted to a volume of 200 ml with water and suitable aliquots were taken for the determination of the other alloying constituents. In all alloys absorptiometric methods were used for the determination of iron, manganese and nickel, under the conditions and by use of the reagents recommended[16] in a scheme for the determination of these elements after the removal of copper from solution by deposition on pure aluminium wire. The percentage of zinc in tin bronzes was obtained, after its selective precipitation with 8-hydroxyquinoline along with magnesium as carrier, by titration with standard potassium ferrocyanide using naphthidine as indicator, whereas the percentage in brasses was obtained by difference after determining the total percentage of all other alloying constituents. Aluminium does not occur in tin bronzes, but the method chosen for its determination in brasses consisted of selectively precipitating with ammonium benzoate before finally estimating volumetrically.[18] By applying these techniques, the determination of all the usual occurring elements in copper-base alloys was made possible on a 1·0-g sample weight and the necessary working details follow.

DETERMINATION OF COPPER, LEAD AND TIN IN BRASSES AND BRONZES—

Transfer 1·0 g of sample to a covered 250-ml conical beaker and add 7·5 ml of hydrochloric acid, sp.gr. 1·16, and 5 ml of water. Warm gently on a hot-plate, adding the minimum amount of nitric acid, sp.gr. 1·42, dropwise to dissolve the sample. Then add 2 g of ammonium chloride and approximately 25 ml of water and boil for five minutes. Cool, transfer to a 400-ml squat beaker, add 2 g of hydrazine hydrochloride and about 25 ml of water containing 10 mg of gelatin and dilute to about 200 ml with water. Immerse the platinum-gauze electrodes (cathode 6 cm high, 6 cm in diameter; anode 3·5 cm high, 3·5 cm in diameter) in the solution, adjust an electrically-driven paddle-shaped stirrer to pass centrally through the electrodes and position a saturated calomel electrode so that the connecting bridge touches the outside surface of the cathode. Adjust the speed of the stirrer to give efficient stirring of the solution. Begin the electrolysis by controlling the cathode at −0·36 volt with respect to the saturated calomel electrode, using unidirectional control and also limiting the current in the first stages to a maximum of 4 amp. After the current has become constant (usually at about 20 milliamp., after electrolysing for 30 to 45 minutes) switch off the controller motor S4, remove the saturated calomel electrode and then lower the beaker, at the same time washing the cathode with a stream of water from a wash bottle. Switch off the stirrer, disconnect the electrolysis battery at S2 and remove the cathode. Rinse the cathode in ethyl alcohol, dry at not more than 105° C for five minutes and measure its increase in weight. If the alloy contains more than trace amounts of antimony, correct this weight for the co-deposited antimony and the final weight then corresponds to the copper content of the sample.

Add 10 ml of hydrochloric acid, sp.gr. 1·16, to the electrolyte remaining after the copper determination and immerse the electrodes as before, but this time use a copper-plated cathode.

Prepare this electrode by plating about 50 mg of copper from a conventional sulphuric - nitric acid solution, washing with water and with alcohol, drying at not more than 105° C and weighing. Add water to the solution until the cathode gauze is completely immersed in the solution and then electrolyse with the cathode maintained at −0·70 volt with respect to the saturated calomel electrode. Continue the electrolysis for 45 minutes, as this time has been found to be sufficient for the deposition of the lead and tin in most types of copper-base alloys with the above electrodes, whereas the final value of the current has proved an unreliable indication of the completion of the electrolytic process. Switch off the controller and neutralise the electrolyte by adding 30 ml of diluted (1 + 1) ammonium hydroxide solution and immediately lower the beaker whilst washing the electrodes with water. Rinse the cathode in alcohol, dry it and determine the increase in weight to give the combined percentage of lead and tin in the sample. Make the remaining electrolyte acid by adding hydrochloric acid, sp.gr. 1·16, transfer to a 1-litre conical beaker and boil down to a volume of less than 200 ml. Cool, accurately dilute to 200 ml in a volumetric flask and reserve for the determination of the remaining alloying elements.

Strip the deposit from the cathode with 25 ml of nitric acid, sp.gr. 1·20, in a 400-ml squat beaker and finally wash the cathode with water. Evaporate the resulting solution almost to dryness, then cool and add a further 25 ml of nitric acid, sp.gr. 1·20. Digest hot for a time and then filter the metastannic acid on a paper-pulp pad, and wash it about four times with hot water. Collect the filtrate and washings in a 400-ml squat beaker and dilute the resulting solution to about 100 ml with water. Heat the solution to boiling and electrolyse while it is as hot as possible with a small platinum-gauze anode and a current of 4 to 5 amp. Electrolyse until the deposition of the lead is complete, which generally takes about five minutes. Remove the anode, wash it with water and then dry and weigh it as before. Calculate the percentage of lead from the weight of lead dioxide by using the empirical factor of 0·863 and determine the tin content by subtraction from the combined tin and lead percentage.

DETERMINATION OF IRON, MANGANESE, NICKEL, ALUMINIUM AND ZINC IN BRASSES—

Thioglycollic acid reagent for iron—Prepare this reagent by diluting 25 ml of "Thiovanic acid"* (76 per cent.) to 100 ml with water. Then add 100 ml of ammonium hydroxide, sp.gr. 0·880, dilute to 500 ml with water and mix thoroughly.

Determination of iron—Filter a quantity of the reserved solution in dry apparatus and transfer 4 ml to a 125-ml conical beaker. Add 1 ml of 50 per cent. citric acid, make the solution just ammoniacal and proceed according to the iron content—

(*i*) For less than 1·20 per cent. of iron, dilute to 50 ml in a graduated flask, add 10 ml of the thioglycollic acid reagent and mix thoroughly.

(*ii*) For amounts of iron between 1·20 and 4·5 per cent., dilute to 100 ml in a graduated flask, add 10 ml of the thioglycollic acid reagent and mix thoroughly.

Measure the extinctions of the solutions with a Spekker absorptiometer with Ilford 604 filters, 4 and 2-cm cells and an instrument setting of 1·0. Calculate the percentage of iron by referring the extinction to calibration graphs.

Potassium periodate reagent for manganese—Prepare this reagent by dissolving 20 g of potassium periodate in 560 ml of diluted (1 + 3) sulphuric acid. Then add 240 ml of phosphoric acid, sp.gr. 1·75, and dilute to 1 litre with water.

Determination of manganese—With a pipette, transfer 10 ml of filtered solution to a 125-ml conical beaker, add 5 ml of diluted (1 + 1) sulphuric acid and evaporate to fumes of this acid to remove hydrochloric acid. Cool slightly, add a few millilitres of nitric acid, sp.gr. 1·42, to oxidise hydrazine hydrochloride, and fume again. Cool, add 20 ml of water and boil gently to give complete solution. Add 4 ml of potassium periodate reagent, boil gently until the permanganate colour starts to develop and continue boiling for a further three minutes. Then digest hot for a further five minutes to ensure the full development of the colour. Add 10 ml of water to prevent the crystallisation of salts and cool the solution to room temperature. Dilute to 50 ml with water in a graduated flask and mix thoroughly. Measure the extinction with the Spekker absorptiometer with Ilford 604 filters, a suitable size of cell and an instrument setting of 1·0. Add 1 or 2 drops of a 2 per cent. solution of sodium nitrite to the solution remaining in the flask to completely reduce the manganese

* "Thiovanic acid" is high-grade thioglycolic acid supplied by Evans Chemicals Ltd., Boreham Wood, Herts.

colour, and measure the extinction of this compensating solution in the same cell and with the same conditions as for the previous operation. Obtain the manganese content by referring the extinction difference reading to appropriate calibration graphs.

Dimethylglyoxime reagent for nickel—Prepare this reagent by dissolving 1 g of dimethylglyoxime in 1 litre of diluted (1 + 1) ammonium hydroxide, allow the solution to settle, and filter before use. Store in a tightly stoppered bottle.

Determination of nickel—Transfer 4 ml of filtered solution to a 125-ml conical beaker, add 1 ml of diluted (1 + 1) sulphuric acid and evaporate to fumes of this acid. Cool slightly, add a few millilitres of nitric acid, sp.gr. 1·42, to oxidise hydrazine hydrochloride and fume again. Cool, add 10 ml of water and re-cool. Then add, from burettes, 5 ml of 50 per cent. ammonium citrate solution, 5 ml of 0·1 *N* iodine and 20 ml of 0·1 per cent. dimethylglyoxime reagent, mixing thoroughly after each addition. Dilute the resulting solution to 50 ml with water. Measure the extinction of this solution in a suitable size of cell within 30 minutes of the addition of the reagents; use Ilford 604 filters and an instrument setting of 1·0. Prepare a blank solution by taking another 4-ml aliquot and applying the above procedure but adding 20 ml of diluted (1 + 1) ammonium hydroxide instead of the 20 ml of dimethylglyoxime reagent. Measure its extinction under the same conditions and obtain the percentage of nickel by referring the extinction difference reading to prepared calibration graphs.

Determination of aluminium—Take one of the following suitable aliquots of solution, depending upon the approximate aluminium content of the alloy: 100 ml for less than 2 per cent., 50 ml for between 2 and 4 per cent. and 20 ml for greater than 4 per cent. Adjust the volume of the aliquot taken to about 75 ml either by evaporation or by addition of water. Carefully add diluted (1 + 1) ammonium hydroxide until the solution is just alkaline and then add just sufficient diluted (1 + 4) hydrochloric acid dropwise to redissolve any precipitate. Add 70 ml of a pH 5 buffer (sodium acetate and hydrochloric acid) and 15 ml of 5 per cent. hydroxylamine hydrochloride solution and heat the solution to boiling. Boil for one minute, then remove from the hot-plate and add 20 ml of 10 per cent. ammonium benzoate solution to selectively precipitate the aluminium. Digest the solution hot for about 15 minutes to enable the aluminium benzoate precipitate to settle and the supernatant liquid to become perfectly clear. Filter the precipitate on a paper-pulp pad, redissolve in hot ammoniacal tartrate solution and complete the determination volumetrically after precipitating the aluminium with 8-hydroxyquinoline. Refer to the original paper for further details.[18]

Determination of zinc—Determine the percentage of this element by difference.

DETERMINATION OF IRON, NICKEL AND ZINC IN BRONZES—

Iron and nickel—Determine the percentage of these elements by using the same procedures as those outlined for brasses.

Zinc—With a pipette, transfer a 100-ml aliquot into a 500-ml conical beaker and add 1 ml of 4 per cent. magnesium sulphate solution and 20 ml of 30 per cent. sodium potassium tartrate solution. Then add 10 per cent. sodium hydroxide solution until the change point of phenol red is reached, followed by 15 ml in excess. Add 20 ml of 2 per cent. 8-hydroxyquinoline solution and boil to give selective precipitation of the zinc and magnesium. Filter the precipitate on a Whatman No. 40 filter-paper, dissolve in hot diluted (1 + 1) hydrochloric acid and separate the zinc from the magnesium oxinate by adjusting the solution to pH 5 and adding a quantity of an acetate buffer of this pH. Filter the zinc oxinate, dissolve it in hot 25 per cent. sulphuric acid, evaporate to fumes of this acid, and oxidise the organic matter with nitric acid, sp.gr. 1·42. Titrate the zinc with a standard potassium ferrocyanide solution by a back-titration procedure with naphthidine as the indicator. Refer to the original paper for full details.[17]

RESULTS AND DISCUSSION

The above procedures were thoroughly tested by applying them to the analysis of 1·0-g portions of standard copper-base alloys. The three British Chemical Standard alloys, Bronzes A and C and Brass B, were chosen for this work and the analytical results were tabulated to enable an easy comparison with the accepted figures for these alloys. An examination of Table I showed that a fairly close agreement existed between the two sets of results, thereby establishing the suitability of the procedures recommended above for the inspection analysis of these types of alloys.

By using automatically controlled potential electrolysis, satisfactory procedures have been made possible for the complete accurate analysis of copper-base alloys, especially those

available in limited supply. Moreover, they have assisted in the general speeding up of the analysis of these alloys by eliminating certain familiar waiting periods necessary in the chemical methods to allow complete precipitation of some alloy constituents before proceeding with the determination of the other constituents. As the control of the electrolysis is fully automatic, time is available during the first 90 minutes of any analysis for devoting to other work, because it is only necessary to prepare, weigh and change electrodes during this period.

TABLE I

COMPARISON OF RESULTS BY THE RECOMMENDED PROCEDURES WITH THE ACCEPTED RESULTS

	Percentage composition										
Alloy	Cu+Sb	Cu	Mn	Fe	Ni	Al	Pb+Sn	Pb	Sn	Zn	Sb
B.C.S. "A."		By diff.							By diff.		
Analyst A	85·82	85·58	—	0·06	0·03	—	11·53	1·85	9·68	1·82	—
Analyst B ..	85·77	85·53	—	0·07	0·03	—	11·54	1·81	9·73	1·79	—
Standard analysis	—	85·5	—	0·07	trace	—	—	1·83	9·70*	1·86	0·24
B.C.S. "C."											
Analyst A	86·90	86·86	—	0·07	0·10	—	10·24	0·38	9·86	2·50	—
Analyst B	86·89	86·85	—	0·065	0·095	—	10·25	0·37	9·88	2·50	—
Standard analysis	—	86·85	—	0·06	0·09	—	—	0·41	9·80	2·53	0·04
B.C.S. "B."										By diff.	
Analyst A	58·81	58·76	1·05	0·88	1·00	1·54	2·56	0·75	1·81	34·16	—
Analyst B	58·82	58·77	1·06	0·89	1·01	1·54	2·57	0·77	1·80	34·11	—
Standard analysis	—	58·8	1·03	0·91	1·01	1·62	—	0·78	1·75	33·9	0·05

* Figure reported by Schoeller, W. R., and Holness, H., *Analyst*, 1946, **71**, 217.

An exception to this rule arises, however, when a preliminary spectrographic examination has shown the presence of antimony in more than trace amounts. This time is then most advantageously utilised in chemically determining the percentage of this element on a separate portion of the sample. After the completion of the second electrolysis at −0·70 volt with respect to the saturated calomel electrode, it is possible for a competent analyst to carry out the determination of the remaining elements simultaneously. For example, whilst precipitates in the aluminium and zinc determinations are digesting, progress can be made with the determination of iron, manganese and nickel, since relatively simple and rapid techniques are involved in the determination of these elements.

The absorptiometric method was chosen for the determination of as many elements as possible after the electrolysis in preference to the polarographic method because nearly every laboratory has an absorptiometer available, whereas polarographs are still generally limited to the larger laboratories. Therefore, after building the unit for controlling the electrolysis, the majority of metallurgical laboratories should be able to put these procedures into operation by using normally available equipment. If a laboratory already possesses polarographic equipment, however, it should be able to use this technique to improve and simplify the determination of zinc in bronzes by incorporating the ammonia - ammonium chloride base-electrolyte recommended by Lingane[9] for the determination of this element. When all the necessary reagent solutions have been previously prepared, the time needed for a full analysis is from 4 to 5 hours. This is a great saving over the time required for the full analysis of scarce samples by accepted chemical procedures, but only a small saving in time when unlimited amounts of samples are available. In the former case it is impossible to proceed with the determination of the majority of the alloying constituents until lead has been completely removed as lead sulphate by allowing the solution of the alloy to stand for several hours. These circumstances do not arise in the latter case, however, since whilst the lead is precipitating it is possible to continue with the determination of iron, manganese, nickel, aluminium, etc., on other portions of sample by precipitating and filtering away interfering alloy constituents.

Although the electronic controlling instrument described in this paper was primarily built to assist in the electrogravimetric determination of certain constituents of non-ferrous alloys, it should also be applicable to controlling mercury-cathode electrolyses for reducing the concentrations of interfering substances in inorganic analytical problems to reasonable proportions prior to determinations by the polarographic technique. Moreover, its operational

characteristics are such that it should be applicable to the types of problems listed by Lingane,[19] including the identification of the oxidation states corresponding to polarographic steps, the electrolytic preparation of organic and inorganic compounds, coulometric analysis and automatic potentiometric titrations.[6]

References

1. Diehl, H., "Electrochemical Analysis with Graded Cathode Potential Control," G. F. Smith Chemical Co., Columbus, Ohio, 1948.
2. Caldwell, C. W., Parker, R. C., and Diehl, H., *Ind. Eng. Chem., Anal. Ed.*, 1944, **16**, 532.
3. Lingane, J. J., *Ibid.*, 1945, **17**, 332.
4. Allen, M. J., *Anal. Chem.*, 1950, **22**, 804.
5. Lamphere, R. W., and Rogers, L. B., *Ibid.*, 1950, **22**, 463.
6. Lingane, J. J., *Ibid.*, 1949, **21**, 497.
7. Lingane, J. J., and Jones, S. L., *Ibid.*, 1950, **22**, 1169.
8. Penther, C. J., and Pompeo, D. J., *Ibid.*, 1949, **21**, 178.
9. Lingane, J. J., *Ind. Eng. Chem., Anal. Ed.*, 1946, **18**, 429.
10. Osborn, G. H., *Metallurgia*, 1949, **40**, 111.
11. Lindsey, A. J., and Sand, H. J. S., *Analyst*, 1934, **59**, 335.
12. Torrance, S., *Ibid.*, 1937, **62**, 719.
13. Lingane, J. J., *Ind. Eng. Chem., Anal. Ed.*, 1945, **17**, 640.
14. Lindsey, A. J., *Analyst*, 1950, **75**, 104.
15. Sand, H. J. S., "Electrochemistry and Electrochemical Analysis," Blackie & Son Ltd., London, 1940, Volume 2, p. 74.
16. Milner, G. W. C., and Groom, H., *Metallurgia*, 1951, **44**, 271.
17. Milner, G. W. C., *Anal. Chim. Acta*, in the press.
18. Milner, G. W. C., and Townend, J., *Analyst*, 1951, **76**, 424.
19. Lingane, J. J., *Anal. Chim. Acta*, 1948, **2**, 584.

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Radioactive Tracer - Paper Chromatography Techniques*

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(*Presented at the meeting of the Physical Methods Group on Tuesday, May 22nd*, 1951)

The uses and potentialities of combined radiochemical and paper chromatography techniques are discussed. The principle of the methods is to associate one or more radioactive isotopes with one or more substances separated on a paper chromatogram. The labelled substances can then be located and estimated by scanning the paper radiometrically. A simple device for doing this automatically and its use in quantitative work is described.

The use of the techniques of paper chromatography as a micro-analytical tool is well established. In many instances paper chromatography is capable of separating substances in amounts below the limits of chemical detection. At other times the resolving powers of paper chromatography may be undiscovered or unexploited because of the lack of suitable chemical methods of detection.

The application of radioactive tracer techniques to paper chromatography may permit the separated components not only to be located and characterised, but also to be estimated quantitatively. In the combined technique the principle is to associate one or more suitable radioactive isotopes with one or more of the components of the mixture, either before or after chromatography. The components separated on the chromatogram are then located and

* An abbreviated version of this paper was read to the Isotope Techniques Conference in Oxford on July 17th, 1951.

After this paper was presented to the Physical Methods Group, a paper by R. R. Williams and R. E. Smith describing a method for automatically scanning and recording radioactive paper chromatograms appeared in *Proc. Soc. Exp. Biol. Med.*, 1951, **77**, 169.

estimated by their associated radioactivity. Sometimes the radioactivity can be used for characterising or identifying a particular component when its association can be made to depend upon a specific chemical reaction. Location and estimation are carried out by systematically scanning the paper chromatogram with a Geiger - Müller counter, for example, or by clamping the whole or part of the paper chromatogram to a photographic plate in the dark. In the photographic method the radioactive zones, if sufficiently intense, will have "auto-radiographed" themselves on the finished plate. The scanning method is capable of great sensitivity and is quantitative, but the photographic method is sometimes more convenient in qualitative work.

Preparation of labelled paper chromatograms

The association of a suitable radioactive isotope with one or more bands of the resolved components of the paper chromatogram can be brought about in three ways.

Labelling of mixture before paper chromatography—

The mixture for chromatography may already contain one or more labelled components, as in tracer experiments. For example, Benson, Bassham, Calvin, Goodale, Haas and

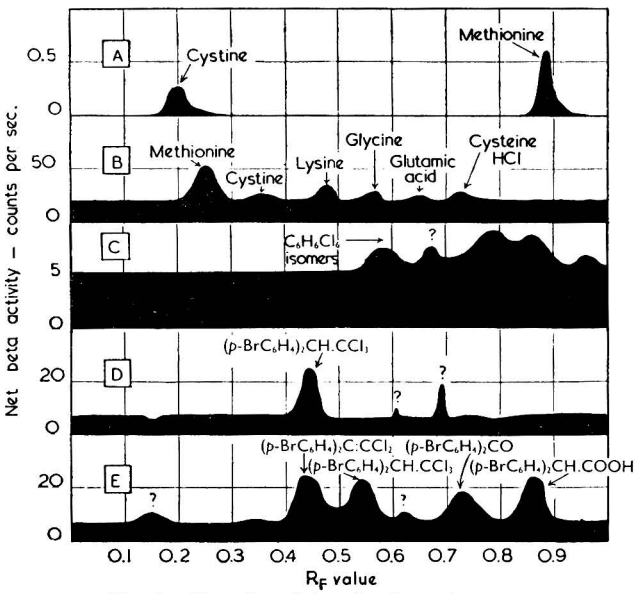

Fig. 1. Experimental radio-chromatograms

A Chromatogram of protein hydrolysate from wheat grown on ^{35}S.
B Strip spotted with known amino acids and exposed to ^{131}I-labelled CH_3I.
C Neutron activated chromatogram of $C_6H_6Cl_6$ isomers.
D Neutron activated chromatogram of a bromine analogue of DDT.
E Neutron activated chromatogram of bromine analogues of DDT derivatives

Stepka[1] have studied the path of radioactive carbon in the photo-synthetic incorporation of this element by respiring plant cells. Labelled intermediates were located and estimated on paper chromatograms by auto-radiographic and counting techniques. The metabolism of a radioactive bromine analogue of DDT absorbed by susceptible and DDT-resistant houseflies has been studied[2,3] by the combined techniques, in which paper chromatograms were scanned by the methods to be described. Keston, Udenfriend and Levy[4] have analysed an unlabelled mixture of amino-acids by treating the mixture with a labelled reagent before applying paper chromatography. The paper chromatograms were cut up into small sections

that were mounted in turn below a Geiger - Müller tube and the measured rate of counting plotted against distance along the strip. In this way they were able to determine quantitatively glutamic acid, serine, glycine and alanine in a mixture of amino-acids. Another example in which the mixture already contained labelled components is illustrated in Fig. 1A. A paper chromatogram was made from a protein hydrolysate prepared from some wheat grown on a solution containing radioactive sulphur-35. The paper was scanned radiometrically and the "radiochromatogram" shown was obtained by plotting the ^{35}S-activity against R_F value. The two peaks correspond respectively to cystine and methionine biosynthesised in the plant.

TREATMENT OF THE PAPER CHROMATOGRAM WITH A LABELLED REAGENT—

Little attention seems to have been paid as yet to the possibilities of this method. We have made experiments on the possible location of certain amino-acid groups by methylation with ^{131}I-labelled methyl iodide. No attempt has been made to apply this particular reaction as a quantitative tool and its description here is merely included to illustrate the principles of the method, which, it is believed, has considerable potentialities. The important condition for the successful application of the method is that a labelled reagent must be chosen that will react selectively with one or more of the compounds separated on the paper chromatogram but not with the paper material itself.

A strip of Whatman No. 1 filter-paper was spotted with solutions of different amino-acids, dried and then exposed to ^{131}I-labelled methyl iodide vapour at room temperature. Methylation of the amino-acids resulted in the liberation of radioactive iodine in the amino-acid zones and particularly in the methionine zone, probably as a result of the formation of the methyl methionine sulphonium iodide. After pumping away the excess of methyl iodide, the paper was scanned radiometrically (see below) and the plotted radiochromatogram is shown in Fig. 1B. The high background was apparently due to some methylation of the paper.

NEUTRON ACTIVATION OF THE PAPER CHROMATOGRAM—

In this method the finished chromatogram undergoes neutron irradiation in an atomic pile after evaporation of residual solvent, the pile probably being the most convenient method of radioactivation. For the success of this method, the separated components must contain or be associated with an element of suitable activation cross-section so that it can be readily assayed against any background radioactivity of the paper chromatogram itself. Fortunately the carbon, hydrogen and oxygen of cellulose did not possess significant neutron activation cross-sections in this respect, and it was found that the wide range of trace metals almost certainly present in washed Whatman No. 1 filter-paper did not give rise to any serious effects under the moderate conditions of some experimental irradiations in the Harwell pile. This method has successfully been applied to the radioactivation of bromine-containing organic compounds by taking advantage of the $^{81}Br \xrightarrow{n,\gamma} {}^{82}Br$ reaction. For example, an inactive bromine analogue of DDT and three derivatives were separated on a reversed-phase paper chromatogram.[3] The chromatogram was then irradiated for 3 days in the Harwell pile at a flux of 10^{10} neutrons per sq. cm per second. The chromatogram was scanned a few days later and the resultant radiochromatogram is shown in Fig. 1E. Only the DDT analogue was present on the scanned chromatogram reproduced in Fig. 1D. The small unidentified additional peaks may have been due to activated chromatographed impurities or to contamination. In the method of neutron activation one important point must be borne in mind. The energy of recoil from the gamma emission in the neutron-gamma reaction is usually more than sufficient to rupture any chemical bond between the target atom and the rest of the molecule. The induced radioactivity can therefore be associated only with the component and cannot be used as a tracer in further tests with the eluted substance. Another complication may be due to the volatile nature of the recoil-freed isotope resulting in loss of activity and contamination of other parts of the chromatogram. For example, in the neutron activation of organic bromine compounds, the recoil-freed bromine is probably present in part as hydrogen bromide. It was found, however, that if the paper strip were rolled up with a second plain strip of paper, contamination was negligible. There seems to be no reason why chlorine compounds could not be estimated on a paper chromatogram by taking advantage of the $^{35}Cl \xrightarrow{n,p} {}^{35}S$ reaction, and many applications could be suggested. One

possible application is to the analysis of a mixture of the isomers of hexachloro*cyclo*hexane, the gamma isomer of which is a well-known insecticide. A mixture containing 10 μg of the α, β, γ and δ isomers was chromatographed under the conditions used for the DDT analogues.[3] The chromatogram was then irradiated at a flux of 10^{11} neutrons per sq. cm per second for one week and scanned a fortnight later. The radiochromatogram is shown in Fig. 1C; the peaks are due to sulphur-35 arising from the chlorine of the partly separated isomers by the $^{35}Cl \xrightarrow{n,p} {}^{35}S$ reaction. This particular application is being further investigated.

Radiometric scanning

When the paper chromatogram has been prepared so that the resolved components are labelled or associated with a suitable radioactive tracer, the distribution of the tracer must be determined quantitatively. Fink, Dent and Fink[5] have located ^{131}I-labelled compounds by placing the chromatogram against an X-ray plate in the dark so that the radioactive zones could be located auto-radiographically after fixing and developing the exposed plate. This method does not easily lend itself to quantitative work and there is reason to believe that it is far less sensitive than counting techniques. It has been estimated[6] that, in order to obtain a reasonable contact auto-radiograph of a ^{82}Br-labelled compound, a radioactive disintegration density of the order 10^9 disintegrations per sq. cm (of the paper chromatogram) would be required. If this figure were obtained, say, in a 24-hour exposure, it would correspond initially to about 0·5 microcuries of bromine-82. One-hundredth of this activity, or 5×10^{-3} microcuries, could be determined to within ± 5 per cent. by Geiger counting for 1 minute with typical apparatus.

A simple method of scanning a paper chromatogram is to cut it up into small equal sections, which are mounted in turn below an end-window Geiger tube. This method is very tedious and, unless each section is cut exactly and precisely mounted, errors will result.

Automatic scanning device—

In Figs. 2 and 3, respectively, are shown the front elevation and plan of a device for automatically scanning $1\frac{1}{8}$-inch unidimensional paper chromatograms. Whatman No. 1 filter-paper is conveniently available in rolls of this width. Two-dimensional chromatograms are scanned by cutting the sheet into the equivalent number of suitable strips. The strip, O, is wound around the 5·05-cm drum, H, and passes over the 5·05-cm idler roller, M, under tension from the 100-g weight, Q, which is fastened to the free end of the strip by means of a "bull-dog" clip, P. Each drum has a 3-mm flange on both sides. The other end of the strip is held to the drum by the spring steel wire, Y, passing through the drum slot, K. The wire is anchored to the drum at J and screwed on the opposite side at Z. The drum, H, drives an eight-bladed escapement wheel, D, by a 4 to 1 gear, F - R, so that one-eighth of one revolution of D allows the strip, O, to advance, under tension, almost exactly 0·5 cm. The diameter of the drum, H, is such that the changing thickness of paper on the unwinding drum has a negligible effect on the distance the paper moves with each escapement movement, and the mean value of this distance is 0·5 cm for the average chromatogram. The roller, M, is mounted exactly below a $1\frac{1}{8}$-inch by 0·5-cm slot, L, cut in the platform, N, so that a transverse 0·5-cm section of the paper strip is exposed through the slot. The effect is that, for every escapement movement (see below), consecutive 0·5-cm sections of the paper strip are exposed to the thin end-window, WI, of the Geiger - Müller tube, GM (Fig. 4), which is housed inside the lead castle, LC. The milled nuts, E, allow the platform to be removed for cleaning and inspection. The heavy frame, B, is screwed to the edge of the laboratory bench, A, through which a suitable hole is cut for the descending strip, O. The drum and roller shafts run in phosphor-bronze bearings mounted in the bearing plate, C. So far, scanning has been limited to beta counting, the Geiger - Müller tube being relatively insensitive to the gamma-rays from bromine-82, iodine-131 and so on. The platform, N, must be sufficiently thick to eliminate all the beta particles from neighbouring but unexposed sections of the strip, an important factor in the resolving powers of the scanner. Steel plate one-eighth of an inch thick has been found to be satisfactory. The platform and frame are constructed in stainless steel, the drums in aluminium. The left prong, V, of the escapement rocker, U, normally engages a blade of the escapement wheel, D, under the tension of a small spring, T, attached to the rocker and frame. The armature of the solenoid, X, momentarily pulls back the rocker via the connecting rod, W, against the spring, the right

prong engaging the opposite-but-one blade of the escapement wheel, so that when the rocker returns to the normal position, D rotates one-eighth of a complete revolution, but no more. The friction clutch, S, can be disengaged by relaxing the milled nut, I, so that the drum, H, is free of R and hence of the escapement mechanism. New strips can then be wound on by means of the handle, G. The lead castle (Fig. 4) is in two parts to facilitate handling. In assembling, the Geiger - Müller tube, GM, is placed exactly over L. The lower half of LC is then lowered into position so that the prongs, PR, engage the flange of GM. The top half of LC is then placed in position so that the contact disc, CD, engages the anode, AN. EHT and earth connections are then made at AL and ET. The insulating glass tube, GL, is flared at the lower end to guide AN on to CD. The solenoid, X, is the electro-mechanical

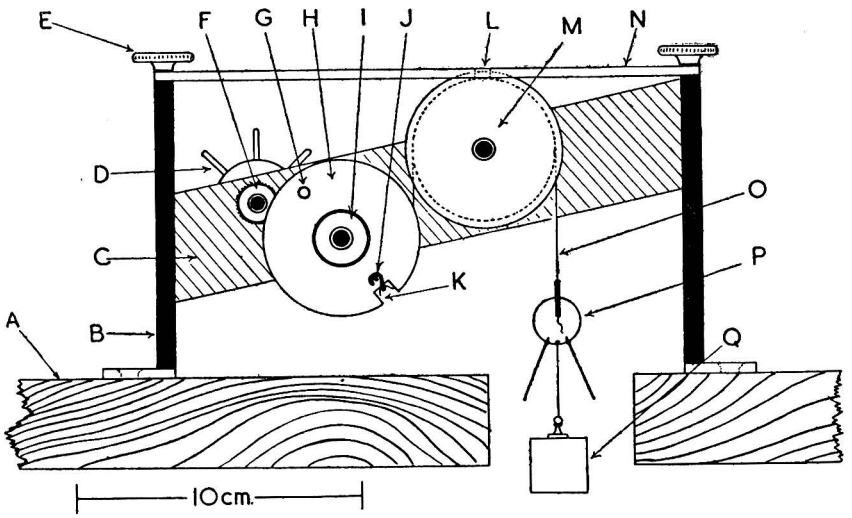

Fig. 2. Timer-controlled device for scanning paper chromatograms radiometrically—front elevation

register unit taken from a timing unit type 1003, which is available in many laboratories equipped for beta counting. Its operation requires 50-volt D.C. pulses, which can be drawn from the timing unit at ½-second, 1-second, ½-minute or 1-minute intervals. By inserting additional scale-of-two units, as shown in Fig. 5, the solenoid can be made to operate at 2-minute, 4-minute or 8-minute intervals, and so on. The probe unit amplifies pulses from the Geiger - Müller tube and feeds them to a rate-meter type 1138A. The 0 to 100-millivolt potential difference across the output potentiometer is proportional to the mean rate of counting; alternatively the rate-meter output can be obtained as a 0 to 5-milliamp. current in the same circuit. This output is fed to a pen-recording milliammeter or to a self-balancing potentiometer. In this way the rate of counting is automatically plotted against distance along the strip, which gives the required radiochromatogram. In our experience the milliammeter recorder was more suitable for this type of work, because the tendency of a self-balancing potentiometer to over-balance gave rise to fluctuations greater than the intrinsic statistical fluctuations of particle counting. The statistical fluctuations of the rate-meter output for a given mean rate of counting can be modified by the variable capacitance of the integrating circuit of the rate-meter. It is important that the corresponding integrating time is less than the time intervals of the scanner, otherwise the rate-meter will be unable to keep pace. On the other hand the integrating time must be sufficiently large for the statistical fluctuations in the counting rate to be small compared with the radioactivity peaks of the chromatogram.

The essential features of this apparatus are: (*a*) the paper chromatogram is automatically scanned in a geometrically uniform manner, (*b*) the movement of the paper is controlled by a simple escapement mechanism operated by conventional timing equipment, (*c*) provision is made for plotting the radiochromatogram automatically and (*d*) all parts of the scanning unit are easily accessible for cleaning purposes, decontamination and so on.

QUANTITATIVE INTERPRETATION OF RADIOCHROMATOGRAMS

A separated component will normally be distributed over several sections of the paper chromatogram. Ideally the counting geometry will be the same for each section. The weight of component is invariably small compared with the weight of paper, so that self-absorption of the beta particles assayed will depend only upon the density, or weight per unit area, of the paper strip, which for practical purposes can be taken to be constant. On this basis the total weight, w, of the labelled component is proportional to the sum total of the net rates of counting (corrected for background, radioactive decay, dead-time losses and so on) of the relevant sections, *i.e.*,

$$w = K \Sigma a \qquad (1)$$

where a is the corrected net rate of counting of each relevant section. The constant K depends on the specific radioactivity of the original component and on the over-all counting efficiency. In practice it has been found convenient to scan a chromatogram made with a

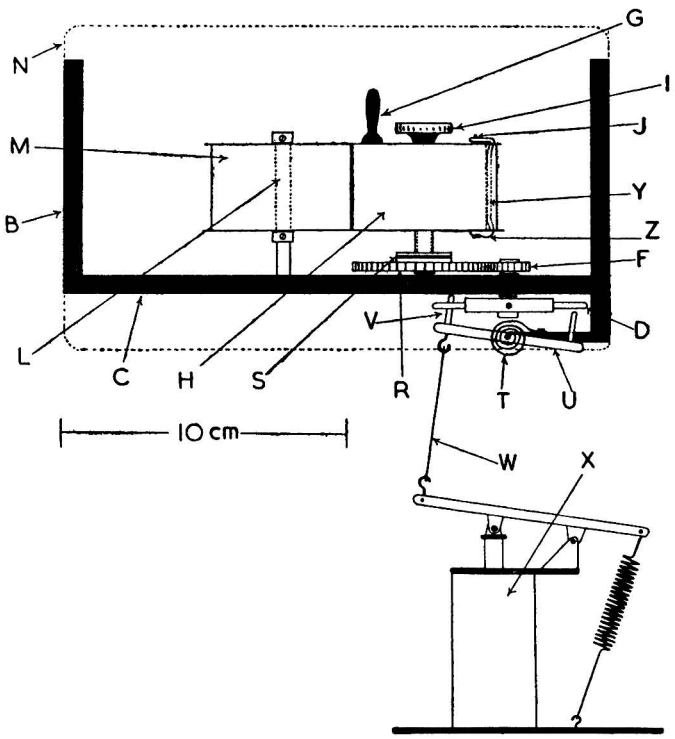

Fig. 3. Timer-controlled device for scanning paper chromatograms radiometrically—plan

known weight of compound of known specific activity. The position of this compound also serves to identify the peaks of the original chromatogram.

When the radiochromatogram is plotted as net rate of counting against distance along the strip, w is proportional to the area enclosed by the relevant part of the curve (see below). With the assembly described above, it has been found convenient to measure these areas as plotted on the recorder by means of a planimeter. In comparing different radiochromatograms quantitatively it is important that the radiochromatogram is plotted on this basis, as the total radioactivity is the product of the mean rate of counting (over the relevant sections) and the distance in an absolute sense over which the radioactivity is spread. For the purposes of identification, however, the R_F value is the important property, and the radiochromatogram should be then plotted as net rate of counting against R_F value. Other factors of significance in quantitative work are discussed below.

Decay corrections—Usually the time taken to scan a unidimensional paper chromatogram is short compared with the half-life of the isotope being assayed, so that corrections for decay can be applied to the measured areas as a whole and based on the mean time of scanning. When the time of scanning is not relatively short, the time scale corresponding to each part of the strip must be recorded and different corrections applied according to position on the strip.

Dead-time corrections—Correction for counter dead-time or quench-time losses for every section of a radiochromatogram would be tedious. For this reason it is recommended that quench and rate-meter-paralysis times be kept to a minimum. For example, if a Geiger

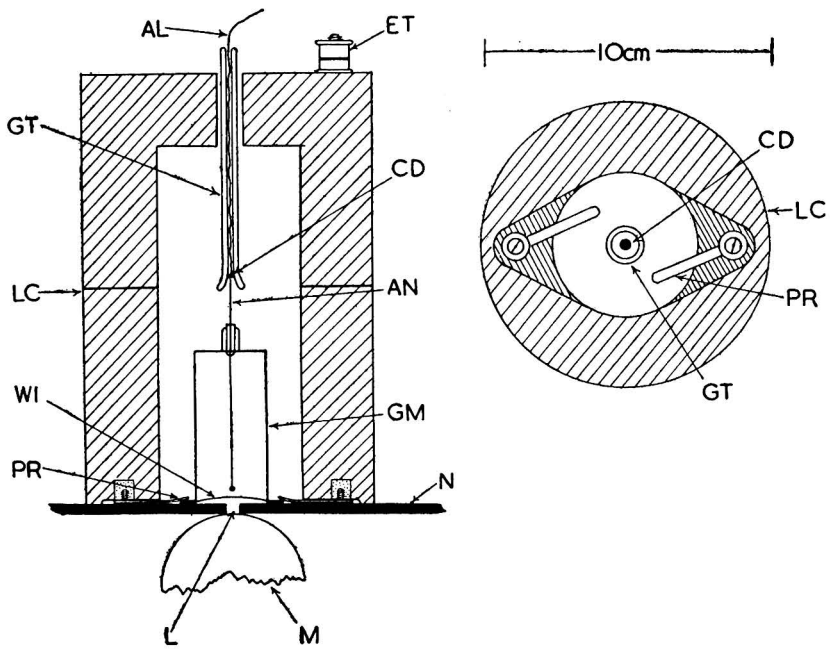

Fig. 4. Timer-controlled device for scanning paper chromatograms radiometrically—cross section through Geiger-Müller tube and lead castle and worm's eye view of lead castle

counter is effectively quenched for 100 microseconds after each pulse, this correction for a maximum rate as high as 100 counts per second will be only 1 per cent. As the rate of count necessarily varies from zero to zero through the maximum for a resolved component, the dead-time error in its estimation will be less than 1 per cent.

Geometry effects—Ideally the geometrical distribution of radioactivity would be the same for every exposed section of the paper chromatogram. In practice variations occur, especially in strips cut from a two-dimensional sheet, when the radioactive zone would not be expected to be in the middle region of the section, except by chance. The beta-particle sensitivity of an end-window type of Geiger tube varies according to the position of the source below the window, becoming less as the source becomes more off-centred. For this reason Boursnell[7] has used a large-area window tube for scanning. In unidimensional chromatograms, however, the radioactive zones appear to be sufficiently similar to permit assays based on adequate controls with known amounts of radioactive material. It is possible that back-scattering from the sides of the platform slot partly compensates for any geometrical effects due to zones being off-centre. For two-dimensional chromatograms a tube of larger window area than the one depicted in Fig. 4 might be desirable. However, a simpler alternative is to cut the two-dimensional sheet into strips sufficiently narrow to eliminate significant geometrical effects. Strips 1 cm wide have been found to satisfy this condition. The narrow strips are then wound round the middle of the winding drum, and the unwinding strip keeps to the middle of the idler drum without relying on the flanges for guidance.

Self-absorption—Self-absorption of a constant source will vary according to the variations in paper density. Corrections for this factor in quantitative comparisons along a unidimensional strip are tedious, but the following calculations indicate that the effect is small. For the purpose of the calculation it was assumed that a resolved component would be spread over about 5 sq. cm of paper. The coefficients of variation in mean density of sections

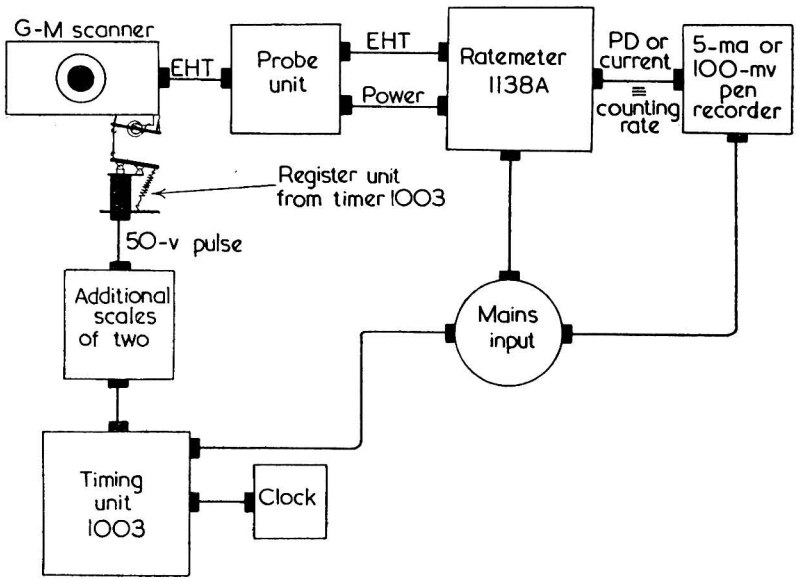

Fig. 5. Timer-controlled assembly for radiometric scanning of paper chromatograms with automatic recording—unit to unit connections

measuring 5 sq. cm cut from a sheet of Whatman No. 1 filter-paper chosen at random was ±3·9 per cent. The mean density was 8·785 mg per sq. cm. The effect of a variation of ±10 per cent. on the self-absorption and on the corresponding corrections in the assay of some typical isotopes was calculated by means of Libby's self-absorption equation.[8] The results are summarised in Table I.

TABLE I

EFFECT OF PAPER DENSITY VARIATION ON SELF-ABSORPTION

Isotope	Proportion of beta particles unabsorbed by paper of mean density 8·785 mg/sq. cm	Variation of self-absorption correction factor corresponding to a variation of ±10% in paper density
^{14}C	0·366	±7·4%
^{35}S	0·431	±6·5%
^{82}Br	0·787	±2·4%
^{131}I	0·828	±1·9%
^{36}Cl	0·843	±1·7%
^{32}P	0·957	±0·5%

It will be seen that, even for soft-beta emitters, this variation is unlikely seriously to impair quantitative work.

Resolution—When two or more labelled components are incompletely resolved, an arbitrary decision must be made on the way to divide the area of the plotted radiochromatogram. Alternatively, an ingenious technique developed by Keston, Udenfriend and Levy[4] may be used. In this method one of the unresolved components is labelled by means of a second isotope of sufficiently different radiation characteristics to enable its boundary on the chromatogram to be determined by selective scanning.

Corrections for recorder-drum speed, for rate of scanning and for background—The radiochromatogram is plotted by the recorder as counting rate (ordinate) against distance along the strip (abscissa). In equation (1) the activity or rate of counting, a, will be constant, if statistical fluctuations and decay are neglected, for each exposed section; Σa is therefore proportional to the area A enclosed by each radioactivity peak of the radiochromatogram, so that $w = K'\mathrm{A}$, where K' depends on the over-all counting efficiency (*i.e.*, observed rate of counting divided by absolute disintegration rate) and upon the units in which A is measured. In practice it is convenient to adjust the recorder-drum speed (provision is made for this in the majority of recorders) so that radiochromatograms plotted from $\mathrm{R_F} = 0$ to $\mathrm{R_F} = 1$ at different scanning rates will be represented by equal or comparable distances along the recorder paper. The scanning rate should be varied by altering the timer-controlled intervals between pulses fed to the scanner solenoid, according to the level of activity on the paper strip. For example, low activities will require a lower scanning rate than will high activities for the same statistical accuracy (see below). Corrections for different recorder-drum speeds and scanning rates in quantitative work are then made in the following manner.

Suppose, in the calibration of the apparatus for quantitative work, a known weight, w, of a labelled substance is scanned. Let s be the rate of scanning, d the drum speed and r the rate of counting corresponding to unit scale (ordinate) of the recorder. Let A be the area enclosed by the radioactivity peak corresponding to the labelled substance on the radiochromatogram. This area on the recorder paper can be measured in any convenient units (to suit a particular planimeter, for example). The value of A must be corrected for decay in the usual manner, and also for background, which is simply the observed counting rate over the part of the strip that is free from labelled material. Then K' can be derived from the expression $K' = w/\mathrm{A}$.

A second strip containing an unknown weight w' of the same labelled substance is now scanned at a rate s' and recorder drum speed d'. Let A' be the corresponding net area on the radiochromatogram and r' the rate of counting equivalent to unit scale of the recorder. It follows that—

$$w' = K'\mathrm{A}'\frac{d\,s'r'}{d's\,r} \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad (2)$$

In the apparatus described above, r'/r will be $10^{\pm n}$ where n is 0, 1, 2, 3 and so on, corresponding to the sensitivity ranges of the ratemeter. The fraction s'/s may be more conveniently replaced by t/t', where t is the timer interval or time of exposure of each section and will therefore be a simple multiple of 1 second or of 1 minute.

Precision in quantitative work—In radiometric assays it is rare to attempt to determine a labelled substance by calculating the absolute disintegration rate from the observed counting rate. This involves accurate data on the effects of geometry, counter efficiency and self-absorption and on the specific radioactivity of the substance itself. Assays are usually based on the rate of counting observed with a known weight of the substance under standardised conditions. In the methods described in this paper, the assays are similarly based on radiochromatograms made with known weights of labelled material. In these circumstances the mean recoveries are complete, and precision or reproducibility becomes the important point. The principal factors affecting precision, apart from manipulative errors such as those involved in transfering material by micro-pipette to the paper strip, are self-absorption, statistical errors[9] inherent in all random particle counting, and geometry. The effects of self-absorption have been discussed. The statistical coefficient of variation of a net count obtained as the difference between the total count $\mathrm{N_T}$ and the background count $\mathrm{N_B}$ over the same interval of time is $\pm 100\sqrt{\mathrm{N_T} + \mathrm{N_B}}/(\mathrm{N_T} - \mathrm{N_B})$. It can be shown that the coefficient of variation of an assay by scanning is $\pm 100\sqrt{d(\mathrm{A_T} + \mathrm{A_B})}/(\mathrm{A_T} - \mathrm{A_B})$, where $\mathrm{A_T}$ and $\mathrm{A_B}$ are the total and background areas in cm-counts per second and d is the recorder-drum speed in cm per second. The particular contribution of geometrical factors to variation, such as an asymmetrical distribution of active material over an exposed section, has not been investigated. Data are available, however, on the total variation due to all errors, including those due to manipulation, incomplete chromatographic resolution and so on, as a result of some resolution experiments with ^{82}Br-labelled derivatives. Single labelled substances or simple mixtures were resolved on reversed-phase paper chromatograms[3] and scanned. In one experiment the separated components were activated after chromatography (see above). All recoveries were estimated in terms of a reference chromatogram

made with a known weight of pure substance and scanned. The results are shown in Table II. They are not intended to demonstrate the sensitivity of the methods. In all experiments fairly active samples were used and weights of the order of one-hundredth or one-thousandth of those given could have been assayed with comparable accuracy.

TABLE II

RADIOMETRIC ASSAY OF ^{82}Br-LABELLED DERIVATIVES SEPARATED ON PAPER CHROMATOGRAMS

Compound or mixture	Weight applied to chromatogram, μg	Recovery by scanning, %	Remarks
$(p\text{-BrC}_6\text{H}_4)_2\text{CH.CCl}_3$	0·2	109	Labelled compound applied singly
"	1·0	104	" " " "
"	1·0	111	" " " "
"	2·0	105	" " " "
"	5·0	85	" " " "
"	5·0	92	" " " "
$(p\text{-BrC}_6\text{H}_4)_2\text{CH.CCl}_3$	1·0	111	Applied as a labelled mixture
$+(p\text{-BrC}_6\text{H}_4)_2\text{C:CCl}_2$	0·5	97	
$(p\text{-BrC}_6\text{H}_4)_2\text{CH.CCl}_3$	50	107	Applied as a labelled mixture
$+(p\text{-BrC}_6\text{H}_4)_2\text{C:CCl}_2$	50	118	
$+(p\text{-BrC}_6\text{H}_4)_2\text{CH.COOH}$ ..	50	100	
$(p\text{-BrC}_6\text{H}_4)_2\text{C:CCl}_2$	20	126	Applied as a mixture. Activated after chromatography
$+(p\text{-BrC}_6\text{H}_4)_2\text{CO}$	20	62	
$+(p\text{-BrC}_6\text{H}_4)_2\text{CH.COOH}$ ..	20	90	
Average recovery		101·2	

The authors are indebted to Mr. W. K. Cordaroy for the construction of the escapement mechanism and scanning frame in the instrument shop of the Laboratory, and to the E.R.D. Engineering Company for constructing the specially designed lead castle shown in Fig. 4. This paper is published by permission of the Department of Scientific and Industrial Research.

REFERENCES

1. Benson, A. A., Bassham, J. A., Calvin, M., Goodale, T. C., Haas, V. A., and Stepka, W., *J. Amer. Chem. Soc.*, 1950, **72**, 1710.
2. Winteringham, F. P. W., Loveday, P. M., and Harrison, A., *Nature*, 1951, **167**, 106.
3. Winteringham, F. P. W., Harrison, A., and Bridges, R. G., *Ibid.*, 1950, **166**, 999.
4. Keston, A. S., Udenfriend, S., and Levy, M., *J. Amer. Chem. Soc.*, 1947, **69**, 3151.
5. Fink, R. M., Dent, C. E., and Fink, K., *Nature*, 1947, **160**, 801.
6. Winteringham, F. P. W., *J. Chem. Soc.*, 1949, S416.
7. Boursnell, J. C., *Nature*, 1950, **165**, 399.
8. Libby, W. F., *Anal. Chem.*, 1947, **19**, 2.
9. Rainwater, L. J., and Wu, C. S., *Nucleonics*, 1947, **1**, 60.

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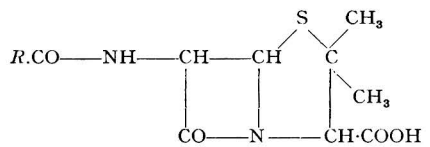
Paper Chromatography of Radioactive Penicillin

By E. LESTER SMITH and D. ALLISON

(*Presented at the meeting of the Physical Methods Group on Tuesday, May 22nd, 1951*)

Biosynthetic ^{35}S-penicillin of very high specific activity has been chromatographed on buffered paper strips. The distribution of the penicillins along the strips was quantitatively assessed by the Goodall and Levi bio-autographic method on agar plates inoculated with *B. subtilis* and by two radiometric methods; sections containing individual penicillins were cut from the paper chromatograms, radio-autographs being used as guides, and either they were "counted" directly under a thin end-window Geiger-Müller tube or aqueous extracts were evaporated on planchettes for counting. The good agreement between the three methods confirms the validity of the Goodall and Levi method.

At least five varieties of penicillin, having the structures shown in Fig. 1, are produced by current fermentation techniques and may be present in commercial penicillin.



Penicillin		*R*
Benzyl	(G)	$-CH_2 \cdot C_6H_5$
p-Hydroxybenzyl	(X)	$-CH_2 \cdot C_6H_4OH$
Δ^2-Pentenyl	(F)	$-CH_2 \cdot CH:CH \cdot CH_2 \cdot CH_3$
Amyl		$-CH_2 \cdot (CH_2)_3 \cdot CH_3$
Heptyl	(K)	$-CH_2 \cdot (CH_2)_5 \cdot CH_3$

Fig. 1. Structure of penicillins

Many methods are available—biological, chemical and physical—for the assay of total penicillins.[1] However, it is now usually required to determine benzylpenicillin (penicillin G) specifically, and this is much more difficult.[1] The need has arisen partly because it is common manufacturing practice to add to the fermentation medium precursors containing the benzyl group and these tend to give a product consisting largely of benzylpenicillin.[2,3] Moreover, the clinical efficacy of benzylpenicillin has been investigated more thoroughly than that of other penicillins, except heptylpenicillin (penicillin K), which is known to be less effective.

n-Ethyl piperidine was originally claimed to precipitate exclusively benzylpenicillin as a crystalline salt,[4] but it has been shown that the method is neither fully quantitative nor specific; that it seems to give results of the right order of magnitude is probably due to compensating errors.[5,6] Similarly it was claimed that only benzylpenicillin would crystallise in the form of the *iso*propyl ether complex of the free acid.[7] The yield is too low for this to be adaptable as an analytical method; in any event, as will be shown later, other penicillins co-crystallise with this *iso*propyl ether complex.

Chromatographic separation—

Chromatography is the only means known for separating the penicillins completely from one another, and the only chromatographic methods suitable for routine use involve separation on buffered filter-paper strips, followed by bio-autographic delineation of the zones due to the various penicillins. This technique was originally devised by Goodall and Levi[8]; a number of modified methods have subsequently been described.[9,10,11]

Provided careful attention is given to various points of technique, in particular to control of humidity, this micro-chromatographic method gives good separation of the penicillins. However, the zones of inhibition arising from the developed strips are usually somewhat elliptical, which makes quantitative assessment difficult. Goodall and Levi

applied an arbitrary factor of 1·2 in calculating the results, while others have preferred to induce a similar degree of ellipticity in the standards by developing strips spotted with pure standard penicillins alongside the unknowns. Another approach—too laborious for routine use—has been to use a narrow strip cut into numerous small squares after development; the penicillin in each square was assessed separately by applying it to a seeded agar plate.[12]

ISOTOPIC TRACER TECHNIQUES—

Radioactive penicillin of very high specific activity (up to 600 millicuries per gram) has been prepared for other purposes by fermentation of a synthetic medium containing sulphur-35 as sulphate. Concentrations up to 1 curie per litre have caused no obvious damage to the mould. It was desired to know the composition of this material[13,14] in terms

TABLE I

PAPER CHROMATOGRAPHIC ANALYSIS OF RADIOACTIVE PENICILLIN

Crystalline sodium salt

Penicillin species		Total penicillins: By biological method: Average of 3 strips, % w/w	Total penicillins: By biological method: Average of 3 strips, % w/w	Total penicillins: By tracer method: 1st strip, % w/w	Total penicillins: By tracer method: 2nd strip, % w/w	Total penicillins: By tracer method: Eluates from 1st strip, % w/w
p-Hydroxybenzyl	(X)	0·1	0·2	—	—	—
Benzyl (including X_1)	(G)	84·7	83·8	87·5	88·2	89·1
Δ^2-Pentenyl	(F)	2·8	3·5	2·5	2·4	2·8
n-Amyl	(A)	5·0	4·9	4·7	4·6	3·6†
n-Heptyl	(K)	7·4	7·6	5·3	4·8	4·5
Non-penicillin *S (from paper chromatograms)		—	—	13	24	14
Non-penicillin *S (by direct ether extraction)		—	—	5		—

† Taking to dryness inadvertently during extraction may have caused loss.

of individual penicillins, and the opportunity was taken to check the bio-autographic method by tracer techniques. A preliminary experiment has already been described.[15] Accordingly, the radioactive penicillin was spotted out on buffered strips, which were developed with ether in the special apparatus described by Goodall and Levi.[8] The developed strips were left in contact with X-ray film in a cassette for several days and the film was then developed. With these radio-autographs as guides, the corresponding paper strips were cut into squares or, where necessary, rectangles, in such a way as to avoid having parts of different penicillin zones on the same square. The squares were then fitted into planchettes and measured radiometrically in a thin end-window Geiger - Müller counter. From the total counts for each penicillin species, the proportions by weight (more strictly, the molar proportions) could readily be calculated.

Alternatively, again using the radio-autographs as a guide, a strip was cut into sections each containing the whole of one penicillin species. Each section was then extracted by boiling for a few minutes with very dilute phosphate buffer. An aliquot of each extract was evaporated down on a planchette and the radioactivity measured.

Corresponding strips developed with ether at the same time were plated out on agar. The results were calculated, against developed benzylpenicillin standards, in terms of proportions by weight of the penicillin species. The results of the biological and the two tracer techniques are recorded in Tables I and II. In view of the errors inherent in methods that involve micro-chromatography of labile substances, the agreement between the various techniques is satisfactory. Clearly the tracer methods are not available for routine use with non-radioactive penicillin; their value lies in providing independent evidence for the validity of the biological method, which in some quarters has been accepted only reluctantly.

The tracer experiments also brought to light an interesting phenomenon. The radio-autographs and the radiometric assays revealed that an appreciable part of the radioactivity

remained in the position of the original spot, where there was no corresponding zone of biological activity. At first we supposed that this spot was due to sulphur-containing impurities or to penicillin decomposition products in the penicillin preparations. These explanations appeared doubtful when it was found that the phenomenon persisted with

TABLE II

PAPER CHROMATOGRAPHIC ANALYSIS OF RADIOACTIVE PENICILLIN

Impure sodium salt

Penicillin species		Total penicillins: By biological method: Average of 3 strips, % w/w	Total penicillins: By biological method: Average of 3 strips, % w/w	Total penicillins: By tracer method: 1st strip, % w/w	Total penicillins: By tracer method: Eluates from 2nd strip, % w/w
p-Hydroxybenzyl	(X) ..	0·1	0·1	—	
Benzyl	(G) ..	85·8	87·5	88·5	84·2
Δ^2-Pentenyl	(F) ..	3·2	3·0	2·5	[5·1?]
n-Amyl	(A) ..	3·9	3·6	4·2	4·9
n-Heptyl	(K) ..	7·0	5·8	4·8	5·8
Non-penicillin *S (from paper chromatograms)	.. ..	—	—	32	32·5

purified crystalline penicillin and that the amount of radioactivity in the initial spot differed in replicate experiments. It then seemed more likely that this spot arose through decomposition of penicillin after application to the strip. This suggestion was confirmed by an independent assessment of the non-penicillin radioactive substances present in the crystalline penicillin. A dilute solution of this penicillin was acidified and rapidly extracted at ice temperature with three 1-ml portions of ether. The total counts in the three extracts and aqueous residue are shown in Table III. It is clear from the trend that practically all the

TABLE III

ESTIMATION OF NON-PENICILLIN SULPHUR COMPOUNDS BY ETHER EXTRACTION

	Radioactivity as percentage of Initial Value: Experiment 1	Experiment 2
1st Extract	87·0	83·0
2nd "	8·0	12·3
3rd "	1·5	2·0
Aqueous residue	3·5	2·7

penicillin is extracted, leaving about 3 per cent. of other sulphur-containing substances. It was confirmed that penicilloic acid, produced by inactivation of radioactive penicillin with penicillinase, was not readily extracted from acid solution with ether and remained substantially at the origin after chromatography on buffered filter-paper.

Destruction of penicillin on the paper was confirmed in further experiments in which the strips were left for various periods between spotting and development. It is clear from the results in Table IV that destruction increases with time of standing and is more severe on strips kept in a dry laboratory atmosphere than on those maintained in a humid condition and kept in a refrigerator. It had been shown in earlier experiments that penicillin is highly unstable in concentrated salt solutions, and these are the conditions that obtain when the penicillin spot dries out on the strip, since this has been previously soaked in 20 per cent. phosphate buffer. It is more difficult to explain why so much penicillin is inactivated when the minimum time elapses between spotting and development, and also why destruction does not continue during development with ether. If it did, then clearly there would be a radioactive streak right down the strip, whereas in fact very little radioactivity is detected between the penicillin zones. It seems probable that evaporation of ether from the strips in a moist atmosphere causes water to condense on them, so diluting the buffer solution and rendering it less destructive towards penicillin; the limp appearance of the strips on removal from the developing tank would support this suggestion.

The bearing of these observations on the conduct and validity of the chromatographic assays needs to be considered. It is clearly desirable to minimise destruction of penicillin by maintaining the strips in a humid condition and avoiding delay between spotting and developing. Nevertheless, destruction of penicillin will not invalidate the assay, which

TABLE IV

PERCENTAGE OF RADIOACTIVITY LEFT IN INITIAL SPOT

Time between spotting and development	Experiment	Residual radioactivity, %	
		After storage in humid refrigerator	After storage in dry laboratory
½ hour	A		24, 23
	B	15, 17	19, 18
2¼ hours	A		23, 28
			32, 33
2½ hours	B	14	32
5 hours	B	20, 25	51, 49
23 hours	A		42, 62

measures only the *proportions* of the penicillin species, unless inactivation of the different penicillins occurs at different rates. The phenomenon does, however, throw doubt on the wisdom of using developed standards.

NOTES ON CHROMATOGRAPHIC PROCEDURE—

In the course of several years' experience, a number of modifications of the paper chromatography technique have been tried from time to time. The following represent the main features in which our current method differs from that described by Goodall and Levi. We use Whatman No. 1 filter-paper already cut into strips 1·8 cm by 30 cm when purchased. The strips are immersed in 20 per cent. potassium phosphate buffer at pH 6·2, pressed between blotting-paper and kept in a blotting-paper folder until used a few hours later.

Numerous methods have been tried for the control of humidity, but none have been found to give absolutely dependable results. Our present practice is to spot the strips in a large refrigerator at about 4° C. The test solutions and standards are measured from Agla micrometer syringes. To prevent the developed heptylpenicillin zone passing right off the strip, we have sometimes found it necessary to cover only about the top three-quarters of the central drum with wet bandage. Quantitative assessments are made with two benzylpenicillin standards differing in concentration by a factor of 10; these solutions are spotted on to buffered strips and developed alongside the test strips.

ISOTOPE DILUTION ASSAYS FOR BENZYLPENICILLIN—

It was hoped to utilise the ^{35}S-penicillin in an isotope dilution assay for benzylpenicillin specifically. The method would be to add to the sample of penicillin or fermentation broth

TABLE V

*iso*PROPYL - ETHER COMPLEX OF PENICILLIN ACID

Penicillin species		Original penicillins			Recrystallised *iso*propyl ether complexes from		
		Crystalline sodium salt A	Yellow calcium salt B	*Cyclo*hexylamine salt C	A	B	C
p-Hydroxybenzyl	(X) ..	0·2	0·3	trace	0·1	0·1	trace
?	(X_1) ..	0·7	1·0	0·3	0·5	0·9	0·5
Benzyl	(G) ..	87·6	77·1	56·6	97·2	94·7	92·0
Δ^2-Pentenyl	(F) ..	1·8	3·8	6·9	0·7	1·6	2·4
n-Amyl	(A) ..	4·8	6·9	13·8	0·9	1·1	2·3
n-Heptyl	(K) ..	4·9	10·9	22·4	0·6	1·6	2·8

a small known weight of ^{35}S-penicillin G of known specific activity. From the mixture a specimen of pure penicillin G would be isolated (not quantitatively) and its specific activity

measured. It was expected that the isolation could be effected through the *iso*propyl - ether complex. The accuracy of the method clearly depends on the ability to free the standard and the final specimen completely from other penicillins. Unfortunately, as Table V shows, the *iso*propyl - ether method is in fact far from adequate as a means of purification.

This experience serves to re-emphasise the fact that isotope dilution methods can give misleading results unless one has avilable a dependable method for purifying (in however poor yield) the substance to be determined. An isotope dilution method for benzylpenicillin that partly avoids this difficulty has recently been published.[16] The marker penicillin is labelled with the stable isotope carbon-13 in the phenylacetyl group (radioactive carbon-14 could be used alternatively). The final specimen need not then be purified rigorously, because only benzylpenicillin carries the benzyl group, and the final measurement is, in any event, made on the phenylacetic acid liberated on hydrolysis. It is still imperative, however, that either the labelled standard or a reference standard of purest benzylpenicillin should be available.

Grateful acknowledgments are made to Miss Hazel Thorpe and Mr. M. Robson for assistance with the radiometric assays.

References

1. "Symposium on Methods of Penicillin Assay: their Purpose, Scope and Validity," W. Heffer & Sons Ltd., Cambridge, 1948; reprinted from *Analyst*, 1948, **73**, 197–216; 244–257.
2. Lester Smith, E., and Bide, A. E., *Biochem. J.*, 1948, **42**, xvii.
3. "The Chemistry of Penicillin," Princeton University Press, Princeton, New Jersey, 1949, p. 9.
4. Sheehan, J. C., Mader, W. J., and Cram, D. J., *J. Amer. Chem. Soc.*, 1946, **68**, 2407.
5. Boon, W. R., "Symposium on Methods of Penicillin Assay: their Purpose, Scope and Validity," W. Heffer & Sons Ltd., Cambridge, 1948, p. 9; *Analyst*, 1948, **73**, 202.
6. "Report of the Analysts' Sub-Committee of the Ministry of Health Conference on the Differential Assay of Penicillin," *Analyst*, 1949, **74**, 79.
7. Trenner, N. R., and Buhs, R. P., *J. Amer. Chem. Soc.*, 1948, **70**, 2897.
8. Goodall, R. R., and Levi, A. A., *Analyst*, 1947, **72**, 277.
9. Winsten, W. A., and Spark, A. H., *Science*, 1947, **106**, 192.
10. Fording, O. B., and Breed, N. D., *Fed. Proc.*, 1949, **8**, 197.
11. Glister, G. A., and Grainger, A., *Analyst*, 1950, **75**, 310.
12. Karnovsky, M. L., and Johnson, M. J., *Anal. Chem.*, 1949, **21**, 1125.
13. Rowley, D., Miller, J., Rowlands, S., and Lester Smith, E., *Nature*, 1948, **161**, 1009.
14. Rowlands, S., Rowley, D., and Lester Smith, E., *J. Chem. Soc.*, 1949, *Supplement No.* 2, p. S405.
15. Lester Smith, E., "Partition Chromatography," *Biochem. Soc. Symposia No.* 3, 1950, p. 89.
16. Craig, J. T., Tindall, J. B., and Senkus, M., *Anal. Chem.*, 1951, **23**, 332.

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Discussion

Mr. C. R. Bond asked whether there was any possibility that a radioactive sample might inactivate more quickly than an ordinary sample of penicillin when spotted out on the paper strip.

Dr. Lester Smith replied that it was improbable that the radioactivity of these penicillin samples would be intense enough to cause inactivation.

Mr. N. Strafford asked whether the authors considered that the radiochemical method gave more accurate results than the Goodall and Levi method and whether it was being used for routine control.

Dr. Lester Smith replied that the radiochemical method was probably more accurate than the original Goodall and Levi method, but unfortunately it was only applicable to very highly radioactive samples of penicillin.

Mr. R. C. Chirnside asked if the decomposition of the penicillin that had been detected in the course of the work on the radioactive assay minimised the value of the Goodall and Levi method. He had understood Dr. Lester Smith to say that such decomposition could be appreciable in the course of an hour or two, whereas in the Goodall and Levi method the tests were allowed to stand overnight. Might the amount of decomposition therefore be sufficient to affect the results adversely?

Dr. Lester Smith replied that the decomposition of penicillin on the paper strips was certainly a disturbing feature. However, it would only invalidate the Goodall and Levi method if the various penicillin species were decomposed at different rates. The inactivation did not appear to continue during development with ether, and could therefore be minimised by transferring the strips to the developing tank as soon as possible after the test spots had dried.

A Rapid Clinical Method for the Estimation and Fractionation of Urinary 17-Ketosteroids

By E. R. COOK

Total 17-ketosteroids in urine can be rapidly estimated, but further partitioning into α- and β-17-ketosteroids requires a considerable amount of work. This paper describes attempts to reduce this work to a minimum by separating smaller quantities of material and by a direct estimation of the β-ketosteroid component as its digitonide. Considerable time is saved without loss of accuracy. Results obtained with pure 17-ketosteroids and with pure 17-ketosteroids added to urine extracts indicate recoveries of above 93 per cent.

Although many methods have been proposed for the determination and fractionation of urinary 17-ketosteroids, they are all essentially similar, involving acid hydrolysis of the water-soluble ketosteroid conjugates and extraction of the crude total 17-ketosteroids with a lipoid solvent, removal of non-specific chromogenic material by a chemical separation into non-ketonic and ketonic fractions, with a further separation of the ketonic fraction into 3(α)-hydroxy- and 3(β)-hydroxy-17-ketosteroids. The 17-ketosteroids in the various extracts are estimated by colorimetric or polarographic methods.

For clinical purposes, crude total 17-ketosteroids can be rapidly estimated in 10 ml of urine by the method of Drekter, Pearson, Bartczak and McGavack,[1] but few non-specialist clinical laboratories could possibly carry out the lengthy and detailed procedures necessary for further fractionation. It is thought, however, that the increasing significance of steroid metabolism, especially with the prospect of increasing availability for clinical use of adrenocorticotrophic hormone, requires the development of rapid, simple fractionation procedures.

A method is described, based on the routine procedure of Reiss, Hemphill, Gordon and Cook,[2] by which the crude extract from 250 ml of urine may be rapidly purified and partitioned into 3(α)-hydroxy- and 3(β)-hydroxy-17-ketosteroids, the results being available within 30 hours of receiving the urine specimen.

Method

Reagents—

Acetic acid—Heat AnalaR glacial acetic acid under reflux for 1 hour with 2 g of chromic acid per litre and then fractionally distil (see Orton and Bradfield[3]).

Benzene—AnalaR benzene has proved satisfactory, and the benzene distilled from extracts may be used again if washed with water, dried over anhydrous sodium sulphate and fractionally distilled.

Alcoholic m-dinitrobenzene—Make an accurate 2 per cent. w/v solution with purified alcohol and *m*-dinitrobenzene purified by the method of Callow, Callow and Emmens.[4] The solution should be kept in the dark and discarded after 4 to 5 days.

Ethanol—Dry commercial absolute alcohol by heating under reflux with calcium oxide for 1 to 2 hours, then fractionally distil through a 6-pear column. Heat the distillate under reflux for 1 hour with 10 g of semicarbazide acetate per litre and again fractionally distil.

Alcoholic 2·5 N potassium hydroxide—Shake the appropriate weight of potassium hydroxide pellets with pure alcohol and centrifuge to remove potassium carbonate. Titrate an aliquot of the clear supernatant liquid against 0·25 *N* hydrochloric acid and adjust the normality to 2·5 ± 0·01 *N* by addition of alcohol. This reagent must be kept in a refrigerator and discarded after 4 days.

Girard's reagent T[5]—On account of its hygroscopic nature, the solid reagent should be kept over silica gel in a desiccator. Make the solution in acetic acid just before use.

The hydrolysis and extraction of urinary 17-ketosteroids—

An examination of the many procedures for the hydrolysis and extraction of 17-ketosteroids reveals that most investigators prefer either independent acid hydrolysis followed

by extraction with ether or benzene, or simultaneous hydrolysis and extraction with benzene. Bitman and Cohen[6] have shown that destruction of 3(β)-hydroxy-17-ketosteroids may occur during the usual heating under reflux for 10 minutes with 15 per cent. v/v of concentrated hydrochloric acid, and we have accordingly made use of the simultaneous hydrolysis and extraction procedure of Hamburger,[7] modified to deal with 250 ml of urine.

Procedure—Acidify 250 ml of urine with 25 ml of 40 per cent. v/v sulphuric acid, add 50 ml of benzene and heat the mixture under reflux on an electric hot-plate for 30 minutes. Cool rapidly and remove the benzene extract in a separating funnel. Repeat the extraction under reflux with 50 ml and then with 40 ml of benzene.

Wash the combined extracts with 30 ml of water, followed by four 30-ml portions of 2 *N* sodium hydroxide and three 30-ml portions of water, and remove the final water wash as completely as possible. Adjust the volume of benzene to 150 ml and evaporate two 5-ml aliquots to dryness in an oil-bath at 100° to 105° C; remove the final traces of benzene in a vacuum desiccator. The binary azeotrope between water (91 per cent.) and benzene (9 per cent.) boils at 69·3° C and eliminates the need for chemical drying of the wet extracts. Estimate the total 17-ketosteroids in the dried residue by the Zimmermann reaction.

THE COLORIMETRIC DETERMINATION OF 17-KETOSTEROIDS—

The original method of Zimmermann[8] as modified by Callow, Callow and Emmens[4] has proved satisfactory for routine purposes. However, traces of water in the reacting solution may depress the final colour density considerably (see Fig. 1), and care must be taken to store the reagents under anhydrous conditions.

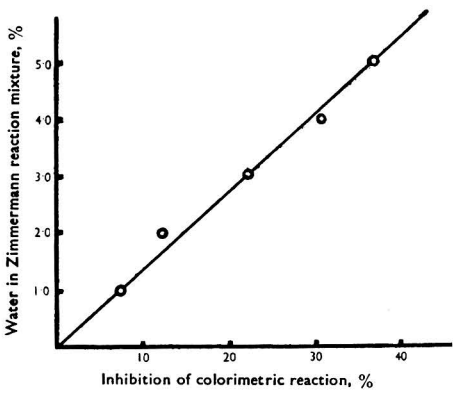

Fig. 1. The influence of water on the colour produced in the Zimmermann reaction

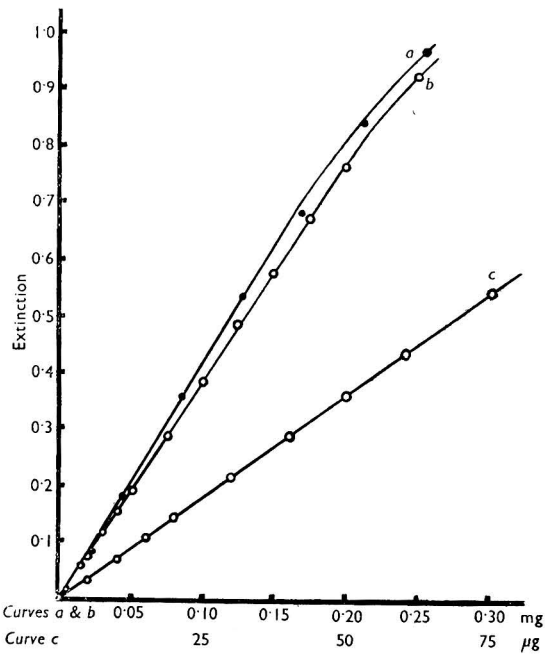

Fig. 2. Standard curves prepared with crystalline 17-ketosteroids. Curve (*a*) androsterone, reaction mixture diluted with 10 ml of alcohol B.P.; curve (*b*) dehydro*iso*androsterone, reaction mixture diluted with 10 ml of 85 per cent. alcohol; curve (*c*) dehydro*iso*androsterone, reaction mixture diluted with 5 ml of 85 per cent. alcohol

Procedure—Add 0·2 ml of pure alcohol to the dried 17-ketosteroid residue, followed by 0·2 ml of alcoholic 2 per cent. *m*-dinitrobenzene solution and 0·2 ml of alcoholic 2·5 *N* potassium hydroxide. Determine the reagent blank on 0·2 ml of pure alcohol. Use pipettes

of 1 ml capacity, graduated in 0·01 ml, with the tip drawn out to a fine jet inclined sideways; this device ensures that the liquid that invariably creeps around the tip is taken off by the wall of the tube. Shake the tubes thoroughly, firmly stopper them and incubate them in the dark at 25° ± 0·1° C for 1 hour. Add 10 ml of absolute alcohol and measure the colour density against the reagent blank in a photo-electric absorptiometer, using Ilford green filters with a maximum transmission at 5200 A.

Perform duplicate determinations, which must be repeated if the absorptiometer readings are not within 2 per cent. Read the 17-ketosteroid content from a standard graph (see Fig. 2) prepared with androsterone and checked at frequent intervals. No correction is made for the non-specific chromogenic material present in the crude benzene extract,[9] as the true 17-ketosteroid content is obtained after the Girard-T separation.

SEPARATION OF KETONIC MATERIAL—

The removal of the ketonic fraction from the crude extract by the use of Girard's reagent T follows along conventional lines. By using smaller amounts of crude steroid, and consequently smaller volumes of reagents, it is possible to carry out the separation very quickly, a further factor introduced in the present investigation being the increased rate of hydrolysis of the Girard T - 17-ketosteroid complex on raising the temperature of hydrolysis from 50° to 100° C. Experiments with pure 17-ketosteroids and urine extracts containing added known quantities of 17-ketosteroids indicate recoveries between 95 and 105 per cent. (Table I).

TABLE I

RECOVERY OF 17-KETOSTEROIDS AFTER THE MICRO GIRARD-T SEPARATION

Experiment	Crude steroid, mg	Pure steroid added, mg	Total pure steroid found, mg	Added steroid recovered, mg	Recovery, %
Dehydro*iso*androsterone ..		0·98	0·92		93·9
Androsterone		1·40	1·38		98·6
		1·00	0·97		97·0
		1·00	1·00		100·0
Urine extract A	1·8	—	1·54		
Urine extract A	1·0	0·74	1·60	0·74	100·0
Urine extract B	1·8	—	1·18		
Urine extract B	1·0	0·74	1·41	0·75	101·4
Urine extract C	1·8	—	1·09		
Urine extract C	1·0	0·74	1·33	0·72	97·3

Procedure—While the estimation of crude 17-ketosteroids is proceeding, distil 135 ml of the wet benzene extract down to 2 to 3 ml, quantitatively transfer the distillate to a test tube with pure benzene and evaporate to dryness; remove the last traces by suction. Add 9 ml of pure benzene and dissolve the residue by gentle warming. Take an aliquot of this solution containing 1·8 mg of crude 17-ketosteroids, calculated from the result of the Zimmermann reaction, and evaporate to dryness by heating in an oil-bath, followed by suction. With a pipette, add 0·1 ml of glacial acetic acid containing 5 mg of Girard's reagent T, lightly stopper the tube and incubate in a bath of boiling water for 4 minutes. Cool rapidly, add 4 ml of chilled 2·1 per cent. sodium carbonate solution and gently shake the tube to remove as much carbon dioxide as possible.

Pour the aqueous contents into a 20-ml separating funnel and wash the tube with 4 ml of ether; put this into the funnel, which should then be shaken vigorously. Complete the removal of the non-ketonic fraction by two further extractions with 4 ml of ether. Wash the combined ether extractions with 2 ml of water and add the washing to the aqueous extract containing the Girard T - ketosteroid complex. With a pipette, add 0·5 ml of concentrated hydrochloric acid to the aqueous solution, and then pour this back into the separating funnel and shake the funnel to remove the ether - water emulsion adhering to the walls; finally transfer the solution back to the test tube. Rinse the funnel with 2 ml of water and drain the washings into the aqueous extract, which should now be about 8 ml in volume. Lightly stopper the tube, preferably by means of a glass bulb with a sealed tail, and place it in a small water-bath at 50° to 60° C for 3 to 5 minutes to boil off most of the dissolved ether; finally incubate the tube in a bath of boiling water for 5 minutes. Girard's reagent T does

not decompose under these conditions. Cool and pour the aqueous extract into the funnel, and remove the free 17-ketosteroids with 4 ml of ether followed by three 3-ml portions of ether, washing the test tube with the ether each time.

Wash the combined ethereal extracts with 2 ml of water followed by 2 ml of 2·1 per cent. sodium carbonate and three 2-ml portions of water, and make the final separation as sharp as possible. Wash the funnel with 2 ml of ether, and add this washing to the extract; then add 2 ml of benzene and a small chip of porcelain. Evaporate the wet extract to dryness in an oil-bath; the ether boils off first, followed by the water - benzene azeotrope and finally by the remaining benzene; remove the last traces by suction. Add 2 ml of pure alcohol, gently warm the tube to promote solution of the 17-ketosteroid, then cool and take 0·2-ml aliquots for the Zimmermann reaction.

PRECIPITATION OF THE 3(β)-HYDROXY-17-KETOSTEROID DIGITONIDE—

Under the appropriate conditions, digitonin combines with β-17-ketosteroids to form a complex insoluble in ether. Earlier methods involving this reaction to separate urinary 17-ketosteroids into α- and β-fractions have been criticised. The determination proposed by Talbot, Butler and MacLachlan[10] suffers from the obvious defect that the β-17-ketosteroid is not directly estimated, while the method of Baumann and Metzger[11] gives variable results, depending upon the initial concentration of total 17-ketosteroids. The procedure of Frame[12] requires a minimum of 15 mg of total steroid, although modifications permit this separation to be applied to micro quantities of total 17-ketosteroid.[2,13] The determination is comparatively lengthy and necessitates decomposition of the β-17-ketosteroid digitonide by pyridine, extraction with ether, removal of pyridine from the ether extract and subsequent estimation of the 17-ketosteroid by colorimetric or polarographic[13] methods.

Preliminary investigation showed that an excess of digitonin has only a slight inhibitory action upon the Zimmermann reaction, and a method was developed whereby, after quantitative precipitation, a modified Zimmermann colour reaction could be carried out directly on the β-ketosteroid digitonide. By this process 30 to 450 μg of β-17-ketosteroid can be recovered in yields generally above 95 per cent. from 800 to 1000 μg of total 17-ketosteroid, as shown in Table II. Below 30 μg, equivalent to 3 per cent., yields are occasionally low. This is not a disadvantage for clinical purposes, as the normal values lie between 0 and 18 per cent.

TABLE II

RECOVERY OF KETOSTEROID BY THE PROPOSED PROCEDURE

Experiment	Total ketosteroid present, μg	Dehydro*iso*androsterone added, μg	Dehydro*iso*androsterone found, μg	Recovery, %
Solutions of dehydro*iso*androsterone		400	382	95·5
		300	279	93·0
		200	197	98·5
		100	100	100·0
		50	49	98·0
		40	38	95·0
		30	29	96·7
Solutions of androsterone and dehydro*iso*androsterone	1000	128	129	100·8
	1000	86	93	108·1
	1000	43	44	102·3
	900	427	400	93·6
	850	342	338	98·8
	800	256	251	98·1
	800	171	169	98·8
	400	171	166	97·1
	400	128	122	95·3
	400	86	81	94·2
Ketonic extracts of patients' urines with added dehydro*iso*androsterone	1000	nil	32	
	1000	40	73	102·5
	1000	nil	40	
	1000	80	118	97·5
	900	nil	90	
	900	40	132	105·0

Procedure—Take an aliquot of the alcoholic solution from the Girard-T separation containing 800 to 1000 μg of ketosteroid and evaporate it to dryness in a 15-ml tapered centrifuge tube. Add 0·2 ml of pure alcohol and 0·2 ml of a 2 per cent. solution of digitonin in 80 per cent. alcohol, and place the lightly stoppered tube in a water-bath at 70° to 75° C for 4 minutes. Cool to room temperature, add 0·1 ml of ether, firmly stopper the tube and leave it in a refrigerator overnight. Precipitation of the β-ketosteroid - digitonin complex occurs within an hour, but is not quantitative unless allowed to stand at 0° C for at least 12 hours.

Centrifuge the flocculent precipitate at 2500 r.p.m. (radius 14 cm) for 3 minutes and pour off the supernatant liquid carefully; then invert the tube and allow it to drain for 2 minutes. Wipe the lip with filter-paper, add 1 ml of ether and break up the precipitate with a fine glass rod. Use a further 1 ml of ether to wash the glass rod and the sides of the tube and centrifuge it at 2500 r.p.m. for 2 minutes. Pour off the supernatant ether, but do not allow the tube to drain, as the precipitate dries very quickly and easily flakes off the tube. Wash the residue with a further 2 ml of ether, centrifuge and pour off the supernatant ether as before. Add 0·5 ml of pure alcohol to the partly dried digitonide complex, together with a small porcelain chip, and evaporate the mixture to dryness in a boiling water-bath and follow by suction. This alcohol stage, introduced to break up and distribute the precipitate around the bottom of the centrifuge tube, has improved the recovery of β-17-ketosteroids by several per cent.

Colorimetric estimation of the β-17-ketosteroids—

The presence of digitonin has little effect on the Zimmermann reaction, but an opalescent precipitate forms in a few moments if absolute alcohol is added to the reaction mixture. This may be prevented by dilution with 85 per cent. alcohol, although a slight quenching of the colour is observed.

Procedure—Add 0·2 ml of pure alcohol and 0·2 ml of alcoholic 2 per cent. *m*-dinitrobenzene solution to the dried β-17-ketosteroid - digitonin precipitate in the tube, mix well, bring rapidly to incipient boiling over a bunsen burner and then plunge the tube into a bath of cold water. Little, if any, alcohol is lost by this seemingly drastic treatment, which is necessary to bring all the residue into solution. Then add 0·2 ml of alcoholic 2·5 *N* potassium hydroxide, shake the tube well, firmly stopper it and incubate at 25° $\pm$ 0·1° C for 1 hour. Similarly incubate a reagent blank consisting of 0·2 ml of pure alcohol, 0·2 ml of 2 per cent. *m*-dinitrobenzene solution and 0·2 ml of alcoholic 2·5 *N* potassium hydroxide.

Add 10 ml of 85 per cent. alcohol and measure the colour developed as before. However, owing to the slight quenching effect of the aqueous alcohol used as the diluting medium, it is necessary to draw a standard curve for these conditions (see Fig. 2, curve B). We have found it convenient to construct a further standard curve for quantities of steroid less than 100 μg, in which the absorptiometer readings are considerably increased by the addition of only half the usual amount of 85 per cent. alcohol (see Fig. 2, curve C).

Summary

A method has been described whereby the estimation of total 17-ketosteroids in 250 ml of urine, and the determination of the separated β-fraction can be completed in 30 hours.

A new feature of the method is the direct determination of the β-fraction in the form of its digitonide; this avoids the usual decomposition stage.

Standard quantities of pure 17-ketosteroids added to urine gave recoveries of at least 93 per cent.

The procedure is recommended as a rapid and satisfactory routine determination for use when the estimation of fractional 17-ketosteroids may be of diagnostic value.

The author wishes to express his thanks to Dr. Max Reiss, Dr. J. J. Gordon, Miss M. Rooks and Miss J. Pelly for their advice and assistance in this work. Thanks are also due to Dr. C. L. Hewett of Organon Laboratories Ltd., Glasgow, for supplies of pure dehydro*iso*-androsterone.

REFERENCES

1. Drekter, I. J., Pearson, S. J., Bartczak, E., and McGavack, T. H., *J. Clin. Endocrinol.*, 1947, **7**, 795.
2. Reiss, M., Hemphill, R. E., Gordon, J. J., and Cook, E. R., *Biochem. J.*, 1949, **44**, 632.
3. Orton, K. J. P., and Bradfield, A. E., *J. Chem. Soc.*, 1927, 983.
4. Callow, N. H., Callow, R. K., and Emmens, C. W., *Biochem. J.*, 1938, **32**, 1312.
5. Girard, A., and Sandulesco, G., *Helv. Chim. Acta*, 1936, **19**, 1095.
6. Bitman, J., and Cohen, S. L., *J. Biol. Chem.*, 1949, **179**, 455.
7. Hamburger, C., *Acta Endocrinol.*, 1948, **1**, 375.
8. Zimmermann, W., *Hoppe-Seyl. Z.*, 1936, **245**, 47.
9. Gibson, J. G., and Evelyn, K. A., *J. Clin. Invest.*, 1938, **17**, 153.
10. Talbot, N. B., Butler, A. M., and MacLachlan, E., *J. Biol. Chem.*, 1940, **132**, 595.
11. Baumann, E. J., and Metzger, N., *Endocrinology*, 1940, **27**, 664.
12. Frame, E., *Ibid.*, 1944, **34**, 175.
13. Butt, W. R., Henley, A. A., and Morris, C. J. O. R., *Biochem. J.*, 1948, **42**, 447.

THE BIOCHEMICAL AND ENDOCRINOLOGICAL RESEARCH DEPARTMENT
THE BRISTOL MENTAL HOSPITALS
BARROW GURNEY, BRISTOL

November, 1950

Porcelain Microchemical Apparatus for Gravimetric Analysis

BY C. WHALLEY AND G. H. WYATT

An investigation into the behaviour of porcelain micro-apparatus has been made by members of a British Standards Institution Sub-Committee. The behaviour both on heating and on acid treatment was investigated in order to provide standard methods of testing and to suggest limits to the changes in weight that could be allowed. The results showed that on heating to temperatures in excess of 950° C softening and discoloration of the glaze occurs and is accompanied by increase in weight. Acid treatment of ordinary micro-crucibles showed very little attack, but with filter crucibles incorporating an unglazed porcelain filtering base the losses were large and inconsistent. In view of the results of these tests, the authors would welcome information from other sources as to the behaviour of porcelain apparatus used in gravimetric micro-analysis and the tests applied in deciding whether the apparatus is suitable for this purpose.

THE British Standards Institution is producing a series of standards for microchemical apparatus (B.S. 1428), and a sub-committee, of which one of us is the present chairman, was established in January, 1948, to consider accessory apparatus connected with gravimetric micro-analysis. Included in this committee's considerations were the following: combustion boats, crucibles, filter crucibles and filtersticks. These items are available in several materials, including porcelain. It was considered desirable that the standard should control the quality of the materials used, but the only available data for porcelain appeared to be those in the draft revision of B.S. 914:1940, "Tests for Laboratory Porcelain," the title of which has been changed to "Quality of Laboratory Porcelain." This draft describes the methods of testing glazed porcelain recommended for laboratory apparatus (*i.e.*, essentially macro-apparatus) and specifies the performance of such items when so tested, but it does not include filtration apparatus incorporating porous porcelain. The committee therefore undertook an examination of porcelain micro combustion boats, crucibles and filter crucibles.

The following members of the British Standards Institution Sub-Committee were responsible for the experimental investigations: Miss I. H. Hadfield, Prof. H. B. Nisbet, Messrs. A. Bennett and G. Ingram and the authors.

It was thought that it would be useful to publish the results, most of which were obtained on experimental batches. The main object of the committee was to devise a form of treatment that would bring the articles to a constant weight when used for further experiments, but with the treatment described below it was usually not found possible to achieve stability. The members of the committee do not feel that they can make any recommendations as to the use of porcelain for gravimetric micro-analysis without further work and would welcome any information or suggestions about the use or testing of porcelain microchemical apparatus

during the last ten years. Comments should be addressed to the authors, care of the Editor of *The Analyst*.

Combustion boats and crucibles

It was understood that these two items are customarily manufactured from similar materials. Therefore the boats were tested for constancy of weight on ignition and the crucibles for resistance to acid attack; both methods were derived from B.S. 914 (draft revision) and are described below.

(*a*) "*Method of test for constancy of weight on ignition* (carried out on complete articles or broken pieces). Wash the articles or pieces in cold 10 per cent. hydrochloric acid followed by distilled water, dry and ignite them at a dull red heat. Allow to cool and weigh, and repeat the ignition until a constant weight is obtained. Then heat for 2 hours in a muffle furnace at a temperature of 950° to 1000° C, allow to cool and weigh again."

It was found that a second ignition of the combustion boats for 2 hours at 650° to 800° C resulted in changes of weight ranging from a loss of 11 μg to a gain of 30 μg; 60 per cent. of the boats gained in weight (mean loss = 6 μg, mean gain = 11 μg). Further ignition, however, rapidly led to constancy of weight to within 2 μg. When the boats were ignited at the higher temperature (950° to 1000° C) there was a large gain in weight of the majority of those tested and the glaze became appreciably discoloured, as if by oxidation of ferrous to ferric oxide. Of 16 boats tested only one lost weight (2 μg); the remainder gained weight by amounts ranging from 30 μg to 1100 μg (mean 387 μg). Subsequent ignition at 950° to 1000° C resulted in reduced gains in weight, but the increases remained highly significant and there was no indication that a constant weight could be expected after a reasonably small number of ignitions. The initial weights of the boats were about 0·7 g, so that the mean gain was 5·5 mg per 10 g of total weight; B.S. 914 (draft revision) specifies a maximum of 0·1 mg per 10 g and the committee had considered that a limit of 0·01 mg per 10 g should be attained for microchemical apparatus.

The above tests were also applied to micro crucibles of 3-ml capacity and it was found that at 950° to 1000° C the glaze softened.

(*b*) "*Method of test for resistance of glaze to acid* (carried out on complete dishes). Wash the dish in cold 10 per cent. hydrochloric acid followed by distilled water, dry to constant weight at 120° C and, when cold, tare it against a similar dish. Fill it to three-quarters of its total capacity with constant boiling-point hydrochloric acid, cover with a clock glass and heat on a steam-bath for four hours. Then wash with distilled water and dry to constant weight at 120° C taring against the same dish as before."

Micro-crucibles of 3-ml capacity subjected to this test changed in weight by amounts ranging from a loss of 20 μg to a gain of 6 μg. Calculation from measurements of the crucibles showed that the average loss in weight was about 0·05 mg per square decimetre of surface. It should be noted, however, that the distribution of the weight changes showed that they were probably controlled by non-uniformity of surface condition during weighing rather than by loss of substance. It is to be concluded that the glaze is resistant to hydrochloric acid attack and that the limiting loss of 0·1 mg per square decimetre of surface considered necessary by the committee could be attained by the manufacturers (B.S. 914, draft revision, specifies a maximum loss of 1 mg per square decimetre).

Filter crucibles

Filtersticks and filter crucibles are manufactured from similar materials and the committee's attention has been confined to the latter in the belief that the findings would also be applicable to filtersticks.

Resistance to hydrochloric acid—A series of tests was devised by the committee as follows—

(*i*) Immerse the filter crucibles in concentrated hydrochloric acid, sp.gr. 1·16, on a steam-bath for 6 hours; suck through the filter one crucible-full of acid and wash with distilled water; dry, ignite at 650° C, cool and weigh.

(*ii*), (*iii*) Repeat treatment (*i*) twice, but each time reduce the treatment with concentrated acid to 1 hour.

(*iv*), (*v*) Repeat the treatment twice, but each time use 2 *N* hydrochloric acid for 1 hour.

A set of 18 porcelain filter crucibles, prepared for experimental purposes only, was supplied in three separate groups, and samples from each group were tested by three independent observers. The behaviour of all the groups was found to be similar by all observers; the changes of weight are summarised in Table I (crucibles only were tested, *i.e.*, without lids).

TABLE I

LOSS OF WEIGHT OF SET NO. 1 OF PORCELAIN FILTER CRUCIBLES AFTER TREATMENT WITH HYDROCHLORIC ACID

18 crucibles examined

Treatment	Loss in weight, μg	
	Range	Mean
(*i*)	400 to 2700*	1500*
(*ii*)	12 to 192	77
(*iii*)	42 to 426	162
(*iii*) repeated†	87 to 627	299
(*iii*) again repeated‡	5 to 12	9
(*iv*)	61 to 533	214
(*v*)‡	109 to 242	146

* Excluding one abnormal loss of 4200 μg.
† Test by two investigators only.
‡ Test by one investigator only.

Further treatments of various kinds failed to effect any improvement in the performance of these crucibles.

Another set of porcelain crucibles from another experimental batch was tested in a similar manner. The results were more regular than those shown in Table I and the losses of weight were less, but they were still too great to permit use of the filter crucibles for gravimetric micro-analysis. It was also evident from the results of these tests that the attack of 2 *N* hydrochloric acid was greater than that of the concentrated acid.

A third experimental set of 12 porcelain filter crucibles embodying a modified filter base was then tested by two observers in the manner described above, but including additional treatments with concentrated nitric acid and with distilled water. The new reagents were included lest hydrochloric acid should exert an unexpected specific action. The results are

TABLE II

LOSS OF WEIGHT IN μg OF SET NO. 3 OF PORCELAIN FILTER CRUCIBLES AFTER TREATMENT WITH ACIDS AND WITH DISTILLED WATER

Crucible No.	Duration and nature of treatment					
	1 hour, conc. HCl	1 hour, conc. HCl	1 hour, conc. HCl	1 hour, conc. HCl	1 hour, conc. HCl	1 hour, conc. HCl
1	793	62	51	13		
2	815	60	40	0	69	69
	1 hour, 2 *N* HCl	1 hour, 2 *N* HCl	1 hour, 2 *N* HCl	1 hour, 2 *N* HCl	1 hour, 2 *N* HCl	1 hour, 2 *N* HCl
3	1644	72	54	56	68	77
4	1067	45	25	63	60	76
	1 hour, conc. HNO_3	1 hour, conc. HNO_3	1 hour, conc. HNO_3	1 hour, conc. HNO_3	1 hour, conc. HNO_3	1 hour, conc. HNO_3
5	407	30	10	25	21	5
6	448	24	17	5	15	7
	46 hours, 2 *N* HCl*	1 hour, 2 *N* HCl	1 hour, 2 *N* HCl	3 hours, 2 *N* HCl	1 hour, water	2 hours, water
7	376	100	274	1029	71	32
8	367	336	167	914	35	38
9	934	599	187	1263	53	76
10	312	253	378	1207	120	58
11	329	261	138	922	51	120
12	339	265	232	973	121	63

* This acid was at room temperature; all other treatments were on a steam-bath.

shown in Table II. It is evident that hydrochloric acid was not specific in its attack and these results confirmed that the dilute acid removed more material than did the concentrated acid. The losses of weight after heating with water are surprising, especially when it is noted that this treatment followed repeated extractions with dilute acid. Finally three crucibles, Nos. 7, 8 and 9, were placed in a Pyrex beaker containing distilled water that was alternately heated nearly to boiling and cooled. The water was hot for a total of $8\frac{1}{2}$ hours and at room temperature for a total of 32 hours. Another similar beaker containing distilled water only was treated in the same manner to serve as a control. The crucibles lost in weight 92, 92 and 115 μg respectively, and the water was found to have extracted from the three crucibles about 130 μg silica and a trace of alkali.

The results of the tests on ordinary micro-crucibles as described above show that glazed porcelain supplied by the same manufacturer withstands treatment with hydrochloric acid; it must, therefore, be concluded that with the filter crucibles it is primarily the porous-porcelain filter base that is attacked.

Committee members also examined the behaviour of transparent *silica* filter crucibles and the results are given here for comparison with those obtained with porcelain filter crucibles of approximately the same size and weight. During the initial 6-hour treatment with concentrated acid, the 17 crucibles and lids tested lost weight, the amounts varying from 13 to 600 μg (mean 131 μg). These losses did not appear to be important, because the crucibles were used in the condition in which they were received from the makers and it was to be expected that drastic treatment would remove foreign matter and loose particles that might be present. After treatments (*ii*) and (*iii*) the three investigators obtained rather different results, which are, therefore, shown in full in Table III.

TABLE III

CHANGE IN WEIGHT IN μg OF SILICA FILTER CRUCIBLES AFTER AN INITIAL TREATMENT WITH CONCENTRATED HYDROCHLORIC ACID

	Treatment					
	(*ii*)	(*iii*)	(*iv*)	(*v*)	(*ii*) repeated	(*iii*) repeated
Observer A—						
Crucible No. 1 ..	−11	−16	−17	−15		
2 ..	0	−37	+ 7	−13		
3 ..	−31	−27	−26	−25		
4 ..	− 5	−36	+ 7	−19		
5 ..	+29	−66	+ 4	−15		
Observer B—						
Crucible No. 1 ..	−55	−51	−128		+2	−6
2 ..	− 2	−90	−79		−5	−6
3 ..	−72	−70	−30		+7	+2
4 ..	−128	−18	−54		−8	+4
5 ..	−47	−232	−38		+6	−8
6 ..	− 9	−43	−37		−5	+7
Observer C—						
Crucible No. 1 ..	−36	− 6	+ 2			
2 ..	−11	− 7	+ 1			
3 ..	−25	− 7	− 1			
4 ..	−35	−10	+12			
5 ..	−15	−11	− 6			
6 ..	−11	− 2	+ 4			

NOTES—Observer A treated crucibles and lids separately; the algebraic sums of the changes in weight are shown.

Observer B repeated the 6-hour and two 1-hour treatments with concentrated hydrochloric acid on the same filter crucibles.

The test was severe, but after sufficient treatment with acid the silica filter crucibles would attain reasonably constant weights. Some of the irregularity in the results may have been due to difficulty in bringing the surface of the silica to the same condition before each weighing.

c/o THE EDITOR, *The Analyst*
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The Vibrating Electrode in Polarographic Determinations of Alkyl Peroxides

BY E. R. ROBERTS AND J. S. MEEK

(*Presented at the meeting of the Polarographic Discussion Panel of the Physical Methods Group on Friday, November 16th, 1951*)

The construction and use of a form of vibrating platinum micro-electrode, for use in polarographic analysis, is described. Its application to the analysis of alkyl peroxides is discussed, and some results for ethyl hydroperoxide are given.

THE vibrating electrode, first described by Harris and Lindsey[1] and later used by them in amperometric titrations,[2,3] is now described in a modified form for straightforward polarographic investigations.

The following work was undertaken as part of an attempt to determine polarographically very small quantities of unstable alkyl peroxides, such as occur in the products of fuel combustion under experimental conditions. These compounds tend to decompose very rapidly

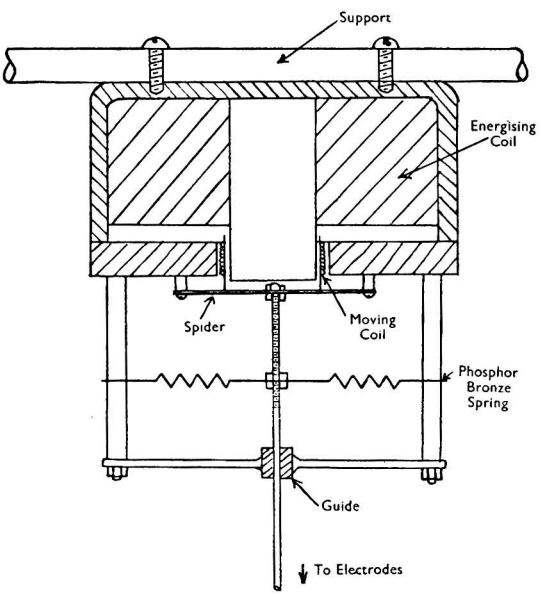

Fig. 1. Vibrator unit

in the presence of mercury, forming an oxide layer on the surface of an ordinary mercury-pool anode within a few minutes of being placed in the polarograph cell. It was therefore decided to use a platinum micro-electrode, since bright platinum does not catalyse the peroxide decomposition to any marked extent. The vibrating type was chosen, after communication with Dr. Lindsey, as steady readings are obtained much more rapidly with this form than with the stationary electrode, and it appeared easier to construct than the rotating electrode.

The vibrator mechanism was constructed from a moving-coil loudspeaker unit. This consisted of a light cylindrical plastic former carrying the "moving" coil, suspended by means of a flexible "spider" in the annular space between the poles of an electro-magnet, round which was wound the "energising" coil. When this energising coil was supplied with

110 volts D.C. with a suitable series resistance, and the moving coil with 6 volts A.C. at 50 cycles per second, the latter coil vibrated with a frequency of 50 cycles per second and an amplitude of several millimetres. The motion of the plastic former carrying the moving coil was transmitted by means of a light brass rod to the electrode. This brass rod was bolted to a thin phosphor-bronze spring, which supported most of the weight of the electrode, and then passed through a guiding collar, which ensured that all the vibration was in the vertical plane. The whole vibrator unit was bolted to massive iron supports, as shown in Fig. 1.

The electrode unit consisted of two identical electrodes, mounted side by side on the same support to ensure that each vibrated at the same speed and in phase. Of these electrodes,

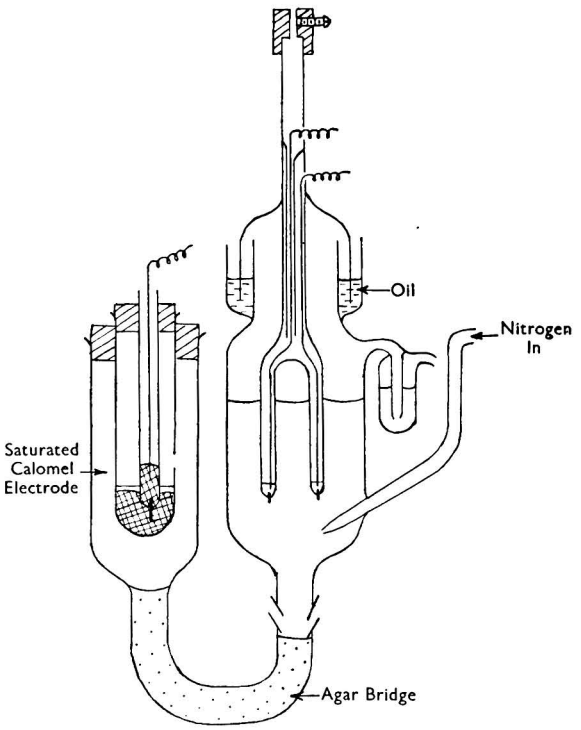

Fig. 2. Vibrating electrode and electrolytic cell

one was at any given time acting as the cathode of the polarographic cell, while the other was short-circuited to the calomel electrode, which was used as the permanent external anode. By means of a multivibrator circuit operating two miniature relays the electrode connections could be changed over at regular intervals, with the result that each electrode was being alternately polarised and depolarised for equal periods of time. The multivibrator design allowed the change-over period to be varied from 0·2 to 5 seconds, while keeping the mark - space ratio constant. It was hoped that by this method, suggested by Müller[4] and quoted by Morris,[5] one of the main disadvantages of the solid micro-electrode, *viz.*, the building up of reduction products at the electrode surface, could be at least partly removed, with a consequent improvement in the wave-form and reproducibility of the curves.

The electrode design, together with that of the cell, is shown in Fig. 2. The supporting glass tube was cemented into a brass collar, which was screwed to the moving brass rod of the vibrator unit. The leads to the platinum electrodes were insulated by fine glass tubes. The lengths of the exposed platinum tips were varied from 0·5 to 2 mm, 1·0 mm giving the most satisfactory results, and the wires were cut to the correct lengths under a microscope. This method of electrode preparation was apparently quite satisfactory, as variations in

current on the change-over from one cathode to the other were almost undetectable, except in the neighbourhood of the half-wave potential.

To make an air-tight seal where the electrode entered the cell, the supporting tube carried a glass collar that fitted inside a corresponding collar joined to the neck of the cell. When the space between the collars was filled with oil of a suitable viscosity, no gas could enter the cell, nor did the oil escape into it. Even with the damping that this additional load entailed, the amplitude of vibration of the electrodes in the cell was approximately 2 mm.

A manually-operated polarising unit of normal design was used, and gave an applied voltage range of 0 to 2 volts against the saturated calomel electrode.

A series of experiments was carried out with this apparatus, cadmium sulphate being used as the test solution with potassium chloride as the supporting electrolyte, to determine

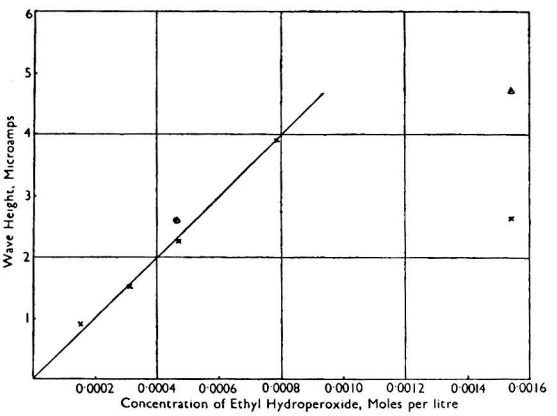

Fig. 3. Typical results for ethyl hydroperoxide. Points plotted × are measurements made in 0·01 *M* lithium chloride; ○ in 0·015 *M* LiCl; △ in 0·05 *M* LiCl.

whether the reproducibility and general shape of the curves obtained were in fact superior to those that had been found previously with a single, permanently polarised, vibrating electrode. Whereas with the single electrode a steady diffusion current was very difficult to obtain, owing to the deposition of cadmium metal on the electrode above the reduction potential, with the double electrode a large proportion of the cadmium deposited was redissolved during the depolarising half-cycle, and this resulted in a much flatter top to the reduction wave. The addition of a little gelatin to the electrolyte was helpful, perhaps by causing smoother plating of the cadmium on to the platinum and hence its speedier dissolution. Reproducibility was also greatly improved with the double electrode system, and a reasonably linear relationship was obtained between the concentration and the wave-height for the range 0·0002 to 0·002 *M*.

The reduction of alkyl peroxides being an irreversible process, the polarographic waves of these substances are always very flat and drawn out. The curves obtained with the double vibrating electrode are at least as good as those obtained with the dropping-mercury electrode, and there is the advantage that the solution may be left in the cell for some time without appreciable decomposition taking place.

Some results obtained with ethyl hydroperoxide may be taken as an example. This substance was polarographed in dilute aqueous solution over a concentration range of 0·0001 to 0·0015 *M*, using 0·01 *M* lithium chloride as the supporting electrolyte. In spite of the unsatisfactory nature of the waves, reproducibility of wave height was good, and a linear concentration - wave height curve was obtained between 0·0001 and 0·001 *M*, as shown in Fig. 3. At higher concentrations the wave height is considerably lower than that required for linearity. This may be because the concentration of peroxide is approaching that of the supporting electrolyte. This view is borne out by the observation that when the lithium chloride concentration was increased from 0·01 *M* to 0·05 *M*, the wave height obtained for

a 0·00153 *M* peroxide solution was increased by more than 2 micro-amp. A similar increase in wave height was observed when 0·00046 *M* peroxide was polarographed first in 0·01 *M* and then in 0·015 *M* lithium chloride. It would obviously be preferable to use 0·1 *M* lithium chloride throughout, but unfortunately at such concentrations the lithium reduction wave starts at a sufficiently low potential to interfere with the peroxide wave, and causes the flat diffusion current portion of the latter to disappear entirely.

REFERENCES

1. Lindsey, A. J., and Harris, E. D., *Nature*, 1948, **162**, 413.
2. Harris, E. D., and Lindsey, A. J., *Analyst*, 1951, **76**, 647.
3. ——, ——, *Ibid.*, 1951, **76**, 650.
4. Müller, S. D., *Trans. All-Union Conf. Anal. Chem.*, 1943, **2**, 551.
5. Morris, C. J. O. R., *Analyst*, 1947, **72**, 298.

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Notes

THE MICRO-GRAVIMETRIC DETERMINATION OF PALLADIUM WITH 1:10-PHENANTHROLINE AND THE SEPARATION OF PALLADIUM FROM PLATINUM

IN previous papers[1,2] it has been shown that 1:10-phenanthroline can be used to precipitate palladium quantitatively as the complex $PdC_{12}H_8N_2Cl_2$ and to separate it from platinum, iridium and rhodium; a single precipitation permits the separation from rhodium, but a second precipitation is necessary for platinum and iridium. The complex has a low palladium content, is of constant composition, and is a suitable form in which to weigh palladium. Further investigation now shows (*a*) that palladium can be determined micro-gravimetrically with 1:10-phenanthroline, (*b*) that the extreme stability of the complex allows palladium to be separated from platinum without destroying the first precipitate and (*c*) that the dinitrate compound is hydrolysed, in aqueous solution, to give three ions.

MICRO-GRAVIMETRIC DETERMINATION—

When micro-beakers and filter-sticks are used, 0·2 to 2 mg of palladium can be determined if the precipitate with 1:10-phenanthroline is allowed to stand for 2 hours before filtering. Smaller amounts can be accurately determined if the precipitates are set aside for a longer period. More than 2 mg of palladium is handled with difficulty by this method on account of the bulky precipitate; for this amount the beakers and volumes of solution previously specified[2] must be used instead of micro-beakers and filter-sticks.

PROCEDURE FOR DETERMINING BETWEEN 0·2 AND 2 mg OF PALLADIUM—

Weigh a clean micro-beaker (5 to 10 ml) and a porcelain filter-stick on a micro-balance. Take by pipette not more than 5 ml of a chloride, sulphate, or nitrate solution containing up to 2 mg of palladium metal. Make the solution 5 to 10 per cent. v/v with respect to concentrated hydrochloric acid and add 1 ml of a 0·5 per cent. aqueous solution of phenanthroline. Set aside at room temperature for 2 hours. Filter through the filter-stick, wash the precipitate three or four times with a few drops of diluted hydrochloric acid (1 + 49), dry the beaker and filter-stick at 110° C for 1 hour, wipe and weigh. Results given by this procedure are recorded in Table I.

TABLE I

MICRO-GRAVIMETRIC DETERMINATION OF PALLADIUM WITH 1:10-PHENANTHROLINE

Pd taken, mg	Pd found, mg	Weight of complex, mg	Time before filtration
0·863	0·865	2·90	2 hours
0·432	0·430	1·43	2 hours
0·218	0·220; 0·216 0·217; 0·219	0·738; 0·723 0·729; 0·735	2 hours
0·145	0·138	0·462	2 hours
0·145	0·145	0·486	overnight
0·086	0·066	0·222	2 hours
0·086	0·088	0·295	overnight

SEPARATION FROM PLATINUM—

In the earlier work,[1,2] with milligram quantities of palladium, the small amounts of platinum and iridium that contaminated the phenanthroline palladous chloride precipitate were eliminated by igniting it to the metal, dissolving this in aqua regia, filtering and re-precipitating the palladium by 1:10-phenanthroline. It has now been found that the platinum can be eliminated by taking advantage of the great stability of the complex, which can be dissolved in concentrated sulphuric acid and re-precipitated, apparently unchanged, by adding the solution to dilute hydrochloric acid or to water.

PROCEDURE FOR DETERMINING MILLIGRAM AMOUNTS OF PALLADIUM IN THE PRESENCE OF PLATINUM—

Precipitate palladium by 1:10-phenanthroline, in the presence of platinum, in the usual manner.[2] Filter the precipitate through a No. 4 filter-crucible and wash with diluted hydrochloric acid (1 + 49). Dissolve the precipitate by passing 10 to 15 ml of concentrated sulphuric acid through the filter-crucible and collect the filtrate in a clean test tube. Re-precipitate the dichloride by slowly adding the sulphuric acid solution to 100 ml of 0·1 *N* hydrochloric acid, set aside for 2 hours, filter, wash the precipitate several times with the diluted hydrochloric acid to remove completely any sulphuric acid, and dry at 110° C until the weight is constant. The complex is re-precipitated from dilute hydrochloric acid to ensure the presence of sufficient chloride ion for complete precipitation of the dichloride. Results by this method are recorded in Table II.

TABLE II

DETERMINATION AND SEPARATION OF PALLADIUM BY RE-PRECIPITATION FROM CONCENTRATED SULPHURIC ACID

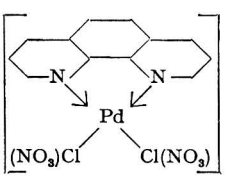
Pd taken, mg	Pd found, mg	Pt present, mg	Volume for first pptn., ml
4·31	4·29	—	150
4·31	4·28	6·5	150
4·31	4·31	13	150
4·31	4·27	20	200
4·31	4·30	40	200
4·31	4·31	60	200
4·54	4·54	—	125
4·54	4·53	10	125
4·54	4·54	20	125
4·54	4·18*	50	125

* Orange-yellow residue not completely dissolved by sulphuric acid.

The amount of platinum that can be tolerated depends on the volume of solution used for the initial precipitation; if the concentration of platinum is greater than 0·3 mg per ml the contaminated palladium precipitate does not completely dissolve in sulphuric acid. The compound obtained on adding 1:10-phenanthroline to a chloroplatinic acid solution is not readily soluble in concentrated sulphuric acid and it is not re-precipitated when the sulphuric acid solution is added to 0·1 *N* hydrochloric acid and set aside for 2 hours.

COMPOSITION OF THE COMPLEX—

The empirical formula of the palladous chloride complex with 1:10-phenanthroline is $PdC_{12}H_8N_2Cl_2$ and that of the nitrate complex is $PdC_{12}H_8N_2(NO_3)_2$.[1] Because of the insolubility of the dichloride in most solvents the molecular complexity cannot be determined by physical methods. Comparison with similar compounds, however, suggests that the structure should be designated as in formula I.



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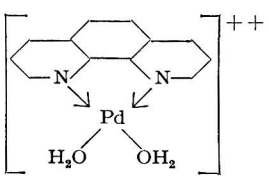

II

Such a compound should be a non-electrolyte.

Measurements on aqueous solutions of the dinitrate, however, showed that the solutions conducted electricity, the molecular conductance being 194 mho-cm² on the assumption that $PdC_{12}H_8N_2(NO_3)_2$ is the molecular formula. This result suggests that there are three ions in the solution; each nitrate ion would contribute 71 mho-cm² per equivalent while the conductance of large organic and complex inorganic ions decreases with increasing size to a limiting value of 28 mho-cm² at 25° C. Thus, if $[PdC_{12}H_8N_2(NO_3)_2]$ were completely hydrolysed in solution to give a stable ion of the type $[PdC_{12}H_8N_2(H_2O)_2^{++}]$ (see formula II), the theoretical conductivity would be 198 mho-cm² per mole, a value that agrees satisfactorily with the experimental result.

Similar results have been found for the amino[3] and aminopyridine[4] complexes of palladous chloride. It seems probable that these compounds, which should be non-electrolytes, carry a current of electricity and give positive tests for the anion because they hydrolyse rapidly, probably by replacement of the anion by a water molecule.

Appreciation and thanks are expressed to Mr. L. S. Theobald, in whose laboratory this work was carried out, for his encouragement and help. The author takes this opportunity to thank Lord Beaverbrook for providing the scholarship, through the Beaverbrook Overseas Scholarship Fund, that enabled this work to be done.

References

1. Ryan, D. E., and Fainer, P., *Canad. J. Res,*. 1949, *B*, **27**, 67.
2. Ryan, D. E., *Ibid.*, 1949, *B*, **27**, 938.
3. Grinberg, A. A., Shulman, V. M., and Khorunzhenkov, S. I., *Ann. Inst. platine* (USSR), 1935, **12**, 119.
4. Rubinshtein, A. M., *Doklady Acad. Nauk* (USSR), 1941, **30**, 223.

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THE DETERMINATION OF SOLUBLE PHOSPHATE BY TITRATION WITH A STANDARD BISMUTH SOLUTION

Rathje[1] stated that bismuthyl perchlorate, $BiOClO_4$, is the only soluble bismuth compound not hydrolysed by water and recommended a 0·5 *M* solution as a volumetric reagent for determining phosphate in perchloric acid solution at the boiling-point. Potassium iodide was used as the indicator. The end-point (completion of precipitation of white bismuth phosphate) is marked by the appearance of the slightly more soluble red bismuthyl iodide—

$$BiOClO_4 + H_3PO_4 \rightarrow BiPO_4 \downarrow + HClO_4 + H_2O$$
$$BiOClO_4 + KI \rightarrow BiOI \downarrow + KClO_4$$

It was recommended that the concentration of perchloric acid in the *titrated* solution should be between 0·1 and 0·01 normal.

As 0·5 *M* is rather too concentrated for the convenient determination of small amounts of phosphate, the method was re-examined on a scale one-tenth of the original.

Experimental

Reagents—

Standard solution of bismuthyl perchlorate—A concentrated solution was prepared by dissolving pure bismuth carbonate in 9 *N* perchloric acid, filtering, crystallising, drying *in vacuo* and dissolving in water with the addition of about 2 ml of 9 *N* perchloric acid per 100 ml. (The acid was necessary to keep the reagent in solution, which shows that even bismuthyl perchlorate is subject to hydrolysis.) This solution was diluted to about 0·05 *M*, assayed by precipitation with ammonium carbonate and ignition to Bi_2O_3; the concentration of this solution was 0·0518 *M* ± 0·00007.

Standard solution of potassium dihydrogen phosphate—The AnalaR salt was dried for 3 hours at 110° C and a standard 0·0518 *M* solution prepared by dissolving 3·5259 g in 500 ml of water; 10 ml of this solution ≡ 10·00 ml ± 0·015 of this bismuthyl perchlorate solution.

Perchloric acid solution—9 *N* (60 per cent. w/v).

Potassium iodide solution—10 per cent. w/v.

Ten ml of potassium dihydrogen phosphate solution were pipetted into a 250-ml conical flask, diluted to 50 ml and small amounts (see below) of perchloric acid and potassium iodide added. The solution was heated to boiling and titrated slowly with standard bismuthyl perchlorate

solution. Near the end it was re-heated to boiling. Rathje[1] stated that at the end-point the white precipitate acquires a pink tinge, but it was found that about 1 drop before this point is reached the solution acquires a *permanent* yellow colour. From the results shown in Table I it appears that this is the best indication of the true end-point. Presumably the bismuthyl iodide formed at this point requires a slight excess of reagent to cause precipitation.

TABLE I

TITRATION OF PHOSPHATE ION WITH BISMUTHYL PERCHLORATE

All test solutions contained 10 ml of standard potassium dihydrogen phosphate solution, equal to 10 ml of standard bismuthyl perchlorate solution

Series	Perchloric acid, ml	Potassium iodide, ml	Bismuthyl perchlorate, ml	Mean	Remarks
1	0·4	0·2		9·92	Low. The volume of KI solution was decreased in subsequent tests
2	0·4	0·1	10·07, 10·03, 10·05, 10·04, 10·00, 10·03, 10·03, 10·04, 10·03, 10·03	10·04	Satisfactory
3	0·5	0·1		10·04	Rather slow disappearance of the temporary yellow colour formed at each addition of reagent solution
4	0·8	0·1	10·04, 10·05	10·05	
5	1·1	0·1		10·01	End-point difficult to detect
6	0·3	0·1	9·99, 10·07, 10·00, 10·06	10·03	Satisfactory
7	0·2	0·1	10·05, 10·01, 10·04	10·03	Satisfactory
8	0·075	0·1		9·78	Titration extremely slow

CONCLUSIONS—

The method is satisfactory in practice and of reasonable accuracy and precision provided the recommended end-point is used.

The optimum concentration of perchloric acid is about 0·4 ml of 9 *N* solution in a volume of about 60 ml (after titration), *i.e.*, 0·06 *N*. On the scale used in these experiments the limits are, however, narrower than in Rathje's method,[1] say from 0·2 to 0·5 ml, *i.e.*, 0·03 to 0·075 normal in the titrated solution (compared with 0·01 to 0·1 normal). Even within these limits there may be some falling-off in precision, see series 6, Table I.

The most interesting conclusion is that the mean readings within this range (all based on the "pink precipitate" end-point) are about 1 drop (0·04 ml) above the theoretical. This suggests that the appearance of a permanent yellow colour in solution is the true end-point.

REFERENCE

1. Rathje, W., *Z. angew. Chem.*, 1938, **51**, 256.

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June, 1951

FURTHER NOTES ON THE ISOLATION OF THE LINES OF THE MERCURY ARC BY FILTERS

THIS note supplements a previous publication.[1] New combinations, which replace those previously given, are described for the 405 and 492 mμ lines and are necessitated by changes in component filters introduced by the manufacturers. Alternative combinations are given for other lines and these take into account the possibility of difficulty in obtaining supplies of certain gelatin filters. The revised 405 mμ combination is of markedly lower density than that previously recommended.

ALTERNATIVE GELATIN FILTERS—

In the earlier communication we described a series of filters for isolating the lines of the mercury arc for photo-electric absorptiometry. Since this work was undertaken the two manufacturers of gelatin filters in this country have made a number of changes in their products, and

have not always made these changes clear to purchasers. Ilford Ltd. have changed the composition of Ilford 603 filters. The filters we first investigated consisted of two components, one dark blue and the other bright green, whereas later deliveries were yellow and deep blue. The

TABLE I

ALTERNATIVE FILTER COMBINATIONS

Wavelength, mμ	Recommended combination	Individual densities*	Calculated over-all density (cemented)	Effective red and infra-red transmission, %	Air-to-air setting on Spekker for full deflection†	Remarks
365	OB2, 1 mm	0·551				
	Ilford 84/50	0·652				
	OX1, 3 mm	0·150	1·283	0·03	1·37	
405	OV1, 4 mm	1·171				Recommended to replace previously described filter.
	Wratten 2B	0·579				
	OB10, 1·43 mm	0·242	1·922	0·03	0·624	Sensitive galvanometer not now essential
436	Wratten 2B (double thickness)	0·258				
	Ilford 809 (f)	0·213				
	Wratten 36	0·862				
	OB2, 2 mm	0·338	1·566	0·03	1·115	
492	Ilford 603	1·039				Composition of Ilford 603 changed by makers since previous paper. Requires sensitive galvanometer
	Wratten 5	0·324				
	Ilford 302	0·092				
	Ilford 804	0·115				
	Ilford 803	0·087	1·517	0·1	—	
546	Ilford 111 (double thickness) (f)	0·258				
	Wratten 74	1·090				
	Ilford 804	0·251	1·494	0·03	1·60	
"607"	Wratten 25 (double thickness) (f)	0·373				Includes 607–623 mμ lines. Requires sensitive galvanometer. Note that cemented filter is denser than expected (mixed light error)
	Ilford 804	1·319	1·766	—	—	

NOTES—Filters marked (f) are fluorescent.
Filters not marked Wratten or Ilford are made by Chance Bros.
Filters are listed in the order that light should traverse them.

* These figures differ from Table II of the earlier paper[1] because different individual filters were used
† These figures are higher than those in Table II of the earlier paper[1] probably because of replacement of the Spekker lamp during the intervening period.

makers, in a personal communication, state that the transmission curve of this filter remains unaltered. Kodak Ltd. have changed the nature of Wratten 86C as mentioned in a footnote to the previous paper; they have replaced Wratten 2A by 2B, which is stated to be of identical transmission but of greater stability. Further, they have discontinued the manufacture in England (though not elsewhere) of Wratten 16, 17 and 26. A batch of Wratten 2B purchased shortly after its introduction was very satisfactory for cutting on the short-wave side of the 436 mμ line, and had densities at 405 and 436 mμ of 1·74 and 0·145 respectively. This batch is not now obtainable, and we are informed by the manufacturers that the standard to which they are now working has densities of 0·52 and 0·15, from which variations of up to 15 per cent. are possible. With the present type of Wratten 2B it has been possible to produce a more satisfactory 405 mμ filter than that previously described.

We are informed that stocks of discontinued Wratten filters are at present still available, with the exception of the earlier types of 86C and 2B. It was, nevertheless, thought advisable to examine the modified filters and to seek freely available alternatives to those that have been discontinued. In addition to those commercially available we also examined three filters specially made by Ilford Ltd., one of which, Number 84/50, is incorporated in the alternative 365 mμ combination. This filter is now obtainable from the manufacturers to special order.

The spectrographic and photographic techniques used were the same as described in the previous paper, but a new batch of photographic plates necessitated increased exposure times. The original combinations were taken on the same plates for comparison and no difference was visible in the spectrograms of the new and old combinations for 365, 436, 492, 546 and "607" mμ. The new 405 mμ combination shows slightly greater transmission of background on the short-wave side of the required line. Perceptible blackening from the line and background on both sides extended symmetrically over a range of 16 A with the new combination; with the old combination the blackening extended over 10 A. The increased background transmission was of low intensity in relation to the required lines and was tolerated with the object of securing a lower density at 405 mμ. The density is now such that the combination is usable with the Cambridge Spot galvanometer. Effective red and infra-red transmission by all combinations was measured in the same way as before. There was no change in this transmission except in the 492 mμ combination, where it was found to be substantially increased if the new Ilford 603 was simply substituted for the old. The addition of Ilford 803 to the combination restored the red and infra-red transmission to its original value.

Table I gives the recommended alternative combinations. Of these the 405 and 492 mμ should be regarded as entirely replacing the earlier ones, which used filters not now obtainable.

In view of the many uses to which these filters are put, it would be helpful if changes in their composition were indicated by the manufacturers by the use of a new number or a suitable suffix attached to the original number. The quotation of batch numbers would also assist users.

PREPARATION OF FILTERS—

The technique of cementing filters has been discussed elsewhere.[2] One further difficulty, however, remains. It has been found that Chance OX1 filters are sometimes supplied with areas opaque to ultra-violet radiation. These areas cannot readily be detected by simple inspection, but become obvious if the filter is held in a collimated beam from a mercury arc with a strongly fluorescent filter (*e.g.*, Wratten 2 or Ilford 809) immediately after it. It is recommended that all OX1 filters be inspected in this manner and that those found faulty be rejected.

REFERENCES

1. Nicholas, J. W., and Pollak, F. F., *Analyst*, 1950, **75**, 662.
2. Pollak, F. F., and Nicholas, J. W., *Metallurgia*, 1951, **44**, 329.

J. W. NICHOLAS
F. F. POLLAK
SILVER END
WITHAM, ESSEX

First submitted, *April*, 1951
Amended, *August*, 1951

Apparatus

AN AUTOMATIC CLOTTING-TIME RECORDER

(*Demonstrated at the meeting of the Biological Methods Group on Friday, June 1st*, 1951)

ONE of the usual methods, described by Adams,[1] for the assay of heparin is to invert a tube containing the coagulating system every 15 seconds until coagulation occurs. The disadvantages of this method are that the fibril formation is disturbed by the frequent agitation, giving erratic results, and that it is necessary for the operator to be present the whole time. Mechanisation of the method of inverting the tube does not overcome these objections, although the second is avoided with the recently described apparatus[2] made by Techne (Cambridge) Ltd. for the determination of the gelation time of oils and resins. In the apparatus to be described, a fresh portion of the coagulating system is always presented to the detecting device and, when once set up, the operator is free for other duties, as the coagulation time can be read at any convenient moment after the coagulation has taken place.

METHOD

APPARATUS—

This is shown in Fig. 1 and consists of a 20-ml glass container, A, detachable for cleaning, mounted on a 4·5-inch diameter disc, B, which has a graduation mark at every 3·6° on its periphery. The distance between these graduations represents 0·1 minute when the disc is driven at 0·1 r.p.m. by the clockwise synchronous electric motor, C (Sangamo Weston Ltd., Model S7), through the split-collet chuck, D. This chuck allows the disc to be re-set to the zero mark.

A 3·5-inch long by 0·375-inch wide by 0·06-inch thick paddle-shaped member, E, of Perspex is suspended from the pivots, F, and dips into the coagulating system along a radius from the

centre of rotation. At 1·25 inches from the pivot a short length (0·25 inch) of platinum wire, G, is mounted opposite and at right angles to a similar piece of platinum wire on a fixed member, to constitute a contact breaker, and at 0·5 inch from the pivot there is an adjustable counterweight, H. Personal contact with the pivots and contacts, which are electrically alive, is prevented by an insulating box. The whole is suspended by an arm, I, from a vertical rod, J, on which it is reproducibly placed in position by the adjustable block, K, with a pin that engages with a slot in the arm.

The electrical circuit consists of a switch, L, to start the motor and a neon lamp, M, in parallel with the latter. These are in series with leads from the contacts, G, which, when opened, switch off the motor and neon light. The time the motor has been running is shown on the disc by the fixed pointer, N.

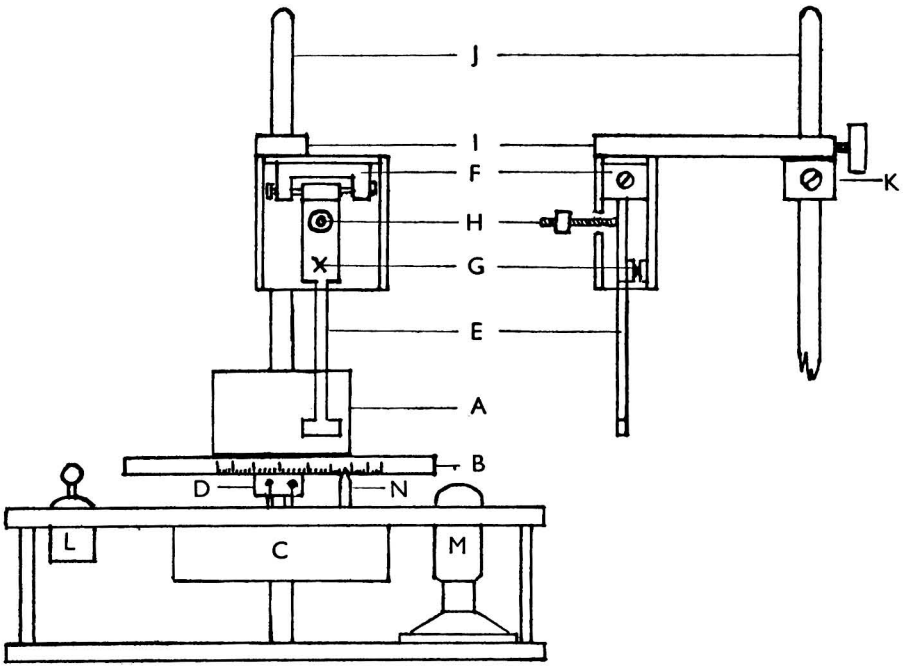

Fig. 1. Diagram of recorder

A relay can be wired in parallel with the motor to switch on a second circuit to operate an alarm signal when the coagulation is complete. If two or more recorders are in use, they could be arranged to give equal coagulation times by adjusting the counterweights on the paddles.

MATERIALS—

Sulphated blood and thrombokinase solution as described by Adams[1] were used and a series of solutions of heparin containing 0·0 to 0·8 i.u. per ml were made up.

OPERATION—

The sliding block on the vertical rod is adjusted until the paddle just clears the bottom and one side of the container when the latter is rotated through 360°. The apparatus is connected to the mains and the disc is set to the zero mark. The paddle is then raised clear of the container, into which are placed, by means of a pipette, 2·0 ml of heparin solution and a previously determined volume of thrombokinase solution to give a suitable coagulation time. A 2·0-ml portion of sulphated blood is quickly added, with a swirling motion to mix the contents, and the main switch is switched on at the instant that half the blood is added. The paddle is then gently lowered until it is located in the correct position, after which the apparatus can be left.

When the blood coagulates, the rapid increase in viscosity causes the paddle to follow the rotation of the container and so to open the contacts to stop the motor and extinguish the neon lamp. The time taken for the blood to clot is then read from the edge of the disc.

Results

With 2·0 ml of a 0·6 i.u. per ml solution of heparin, 0·4 ml of thrombokinase solution and 2·0 ml of sulphated blood, replicate clotting-times of 5·1, 4·8, 4·9 and 4·8 minutes were obtained.

The relationship between the time of clotting and heparin concentration is given in Fig. 2.

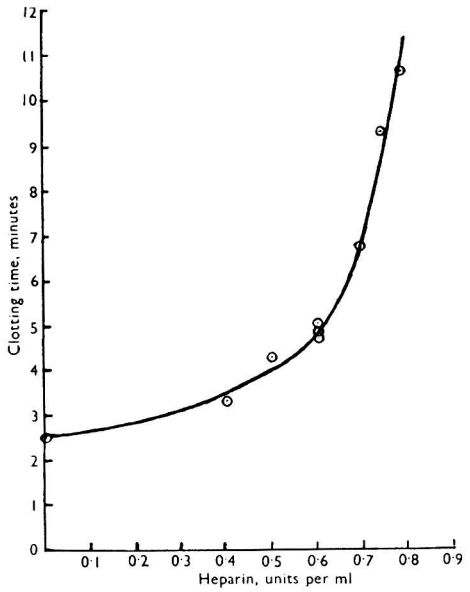

Fig. 2. Typical results

It was found that if the clotting-time exceeded 10 minutes, a rather soft clot was obtained and larger variations in the end-point occurred.

The apparatus described is the subject of patent application No. 12751/51.

The author wishes to thank Sir Jack Drummond, F.R.S., Director of Research, Boots Pure Drug Co., for his encouragement.

References

1. Adams, S. S., *J. Pharm. Pharmacol.*, 1950, **2**, 836.
2. Anon., *Brit. J. Indust. Finish.*, 1951, **3**, 513.

Research Department
Physical Chemistry Section
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Nottingham

S. S. Randall

TWO-PIECE FILTRATION FUNNEL

Hall, Lee, Ormerod and Williams[1] have described shortcomings in the method for fibre determination prescribed by the Fertilisers and Feeding Stuffs Regulations, 1932. For National Flour a modification of the method has been described as a result of the work of the Analytical Methods Committee of the Society of Public Analysts and Other Analytical Chemists.[2] The method still remains highly empirical, and in such a method it is desirable that details of technique and apparatus should be as standardised as possible. This new design of funnel has been found extremely useful in fibre tests and has many other applications in the laboratory.

Filtration of acid digest of fibre—

The acid digest is usually filtered through a conventional Büchner funnel fitted with a Whatman No. 54 filter-paper. The result of this operation is that the fibre particles are distributed not only on the paper, but also round the periphery of the base of the funnel. Some particles also find their way on to the under edges of the paper. The subsequent removal of this fibre to the vessel for

the second digestion therefore involves washing both sides of the paper, and also the funnel itself, with a limited amount of alkali (200 ml). The special funnel,* the construction of which is shown in the sectional drawing, Fig. 1, simplifies this part of the operation for the following reasons—

The particles of fibre are localised on a circular patch in the middle of the paper, leaving a clear margin of uncontaminated paper, which facilitates its handling.

There is no possibility of any fibre particles reaching the under side of the paper, or even passing through to the filtrate, which may occur with a badly made Büchner funnel.

Removal of the fibre with alkali is a much simpler and quicker operation with this funnel, as a negligible amount of fibre remains on the flanged ring if this is first rinsed with the aid of a wash-bottle. The base of the funnel need not be washed, and therefore only one side of the paper has to be considered. The fibre is best removed by placing the paper on the sloping side of a 5-inch funnel and washing downwards with a jet from a pressure-operated wash-bottle containing boiling sodium hydroxide.

It is believed that these funnels have applications in operations other than those connected with fibre determination. They can be used with advantage wherever a rapid-filtering precipitate or residue has to be transferred quantitatively to another vessel. They are also suitable for filtering organic preparations in a clean and efficient manner. In filtering large volumes of liquid, such as milk, water or oil, when it is desired to examine any extraneous residue microscopically, they have advantages, as the liquid must pass through a disc of filter-paper of known area.

In using the funnel, a filter-paper having a diameter the same as the full width of the funnel is first wetted with the liquid to be used, in order to avoid crinkles, and placed on the lower component.

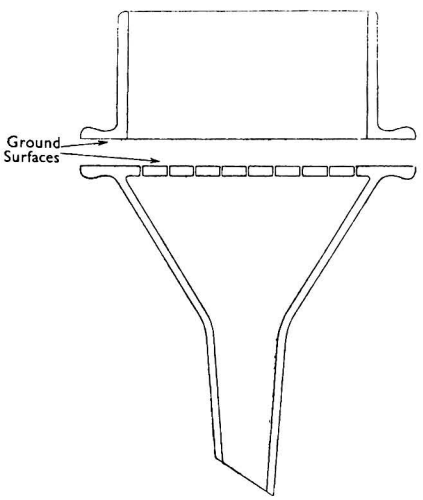

Fig. 1. Section of two-piece funnel

The flanged ring is then placed on the paper, and usually the weight of this ring makes the joint leak-proof, but small Terry clips can be used to keep the two parts together and effectively to clamp the paper round its edges.

To avoid leaks it is necessary that the ground surfaces of the funnel shall be as flat and smooth as possible. It is preferable to apply suction before the funnel is filled with liquid.

Thanks are due to the Directors of Spillers Limited for permission to publish this note.

REFERENCES

1. Hall, E. M., Lee, W. V., Ormerod, O., and Williams, E. T., *Analyst*, 1949, **74**, 438.
2. Analytical Methods Committee, *Ibid.*, 1943, **68**, 276.

SPILLERS LIMITED
CENTRAL LABORATORY
CAMBRIDGE

A. W. HARTLEY
July, 1951

* These funnels are being manufactured by the Worcester Royal Porcelain Co. Ltd.

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, Society of Public Analysts and Other Analytical Chemists, 7–8 Idol Lane, London, E.C.3.

Draft Specification prepared by Technical Committee OSC/12—Vegetable Oils.

CN(OSC) 6660—Draft B.S. for Sunflower Seed Oil.

Draft Specifications prepared by Technical Committee LBC/11—Microchemical Apparatus.

CN(LBC) 6676—Draft B.S. for Heating and Cooling Blocks for Microchemical Purposes (Part G.1 of B.S. 1428—Microchemical Apparatus).

CN(LBC) 6666—Draft B.S. for Micro-Zeisel Apparatus for the Determination of Alkoxyl Groups (Part C.1 of B.S. 1428—Microchemical Apparatus).

CN(LBC) 6667—Draft B.S. for Micro-Beakers (Part E.2 of B.S. 1428—Microchemical Apparatus).

CN(LBC) 6668—Draft B.S. for Micro-Centrifuge Accessories (Part E.3 of B.S. 1428—Microchemical Apparatus).

Book Reviews

SIX-MEMBERED HETEROCYCLIC NITROGEN COMPOUNDS WITH FOUR CONDENSED RINGS. By C. F. H. ALLEN, in collaboration with D. M. BURNESS, JEAN V. CRAWFORD, F. W. SPANGLER, ELEANOR R. WEBSTER and C. V. WILSON. Pp. xiii + 345. New York and London: Interscience Publishers Inc. 1951. Price $10.00; 80s. Subscription price, $9.00; 72s.

It has been estimated that more than half of the types of organic compounds that occur in nature belong to the heterocyclic group. This alone would be sufficient to account for the unremitting attention given to compounds of this group by organic chemists. In view of these and other circumstances it is surprising that up-to-date comprehensive treatises on the heterocyclic compounds have been entirely lacking. To remedy this deficiency two major works are in course of preparation, and in fact two volumes of each have now been published. One of these works, edited by R. C. Elderfield, is planned on orthodox lines and deals systematically with the various classes of heterocyclic compounds arranged in ascending order of complexity. The other, edited by A. Weissberger, of which the volume under review is the second to be issued, is rather different in conception and seems more in the nature of a series of monographs on selected topics within the field of heterocyclic chemistry. The first volume, by Dr. F. G. Mann, appeared in 1950 and was concerned with all classes of compounds in which phosphorus, arsenic, antimony, bismuth or silicon forms part of a ring.

The present volume is very much a corporate effort, as is indicated by the list of collaborators. There are seven chapters, dealing respectively with nitrogen analogues ("azalogues") of the tetracyclic aromatic hydrocarbons, naphthacene, 1 : 2-benzanthracene, 3 : 4-benzphenanthrene, chrysene, triphenylene, benzanthrene and pyrene. There are interesting accounts of the aporphine alkaloids and the chelidonium alkaloids, the ring-systems of which fall within the range selected for this volume, but in some ways these seem out of place here, especially as it is stated that there is to be a separate volume in the series on alkaloids. Apart from these groups, the substances dealt with in the present volume are mostly dyestuffs. Chapter II is largely concerned with anthraquinone dyes, and Chapter VI, which is much the largest chapter, as well as the most attractive one, includes an account of the mono- and di-azabenzanthrones, which are more familiarly known as anthrapyridines, anthrapyridones, anthrapyrimidines and anthrapyrimidones. It is for the surveys of the chemistry of these dyestuffs that the book will be of most value. The information, much of which is taken from the patent literature, has never before been assembled in such an accessible form. There are also descriptions of compounds prepared in the course of the programme of antimalarial research, of others obtained in studies of new analgesics, and of yet others prepared on account of their relationship to the carcinogenic hydrocarbons.

With such an array of authors the chapters are inevitably lacking in uniformity of treatment. Some of them are little more than classified lists of compounds, with résumés of their principal properties. There is a certain amount of repetition, the same material being dealt with in different chapters by different collaborators. There is even some internal inconsistency, as, for example, in regard to the structure of Ciba Yellow 3G, where the conclusions in one chapter are at variance with those in two others. Even within the scope set by the chapter headings there has been some

arbitrary choice in the subject-matter. Chapter II deals with azalogues of 1 : 2-benzanthracene, but no attempt has been made to survey the derivatives of the corresponding benzphenazine or the two isomeric benzacridines, and it is stated that these have been omitted because the numbers of derivatives are so great. This is a very curious reason, and it might well be argued that they should have been included for this very reason. As regards the benzacridines, it is suggested that these will more appropriately come in a special volume on acridines, but other acridine derivatives have in fact been included in the present volume.

In spite of these criticisms, and others of a minor character that might also be made, the book will serve a very useful purpose and may be commended to all who are interested in nitrogen-ring compounds as well as those specifically concerned with vat dyes and related colouring matters.

J. W. Cook

Heterocyclic Compounds. Volume I. Three-, Four-, Five- and Six-Membered Monocyclic Compounds Containing One O, N and S Atom. Edited by R. C. Elderfield. Pp. ix + 703. New York: John Wiley & Sons Inc. London: Chapman & Hall Ltd. 1950. Price $15.00; 120s.

The past quarter of a century has seen a great increase in the technical applications of heterocyclic compounds, so it is not altogether surprising that two publishing houses should each have begun almost simultaneously a series of books devoted to this subject. This is the first volume of one of these series.

It may be stated at once that the various topics are dealt with from a purely chemical angle; practical uses and even physical properties are not mentioned, unless it be incidentally. Further, no detailed treatment of the alkaloids is to be given, as the information is already available elsewhere, nor is it claimed that the literature has been exhaustively covered; attention is drawn to previous reviews and, generally, emphasis is laid on work of more recent times.

The first two chapters deal with ethylene and trimethylene oxides and with ethylene-imine. The four-membered system with nitrogen had been neglected until a few years ago, but the discovery that the penicillins contained a β-lactam ring gave much impetus to its study. There is some discussion on unsaturated γ-lactones (though not of the saturated compounds) under the heading of the furan group. In this and the thiophen and pyrrole series, the space devoted to the hydrogenated derivatives is rather sparse, most being allowed for the sulphur system, possibly because a number of compounds of this type have been isolated from natural sources; the pyrrollines and pyrrollidines are dismissed in three pages.

The pyrans, pyrones, thiapyrans and thiapyrones form a compact group. The pyridine bases were among the earliest known heterocyclic compounds and they have been the subject of many investigations. It is not altogether surprising, therefore, that the articles on these bases and their hydrogenated derivatives occupy the largest section—nearly one-half—of the book even though some topics, in particular the chemical aspects that this ring system has in common with the homocyclic series, which are available in other books or reviews, have here been condensed.

As an example of the style of treatment, the furan group may be cited. Following an introductory note on nomenclature, the production of furan derivatives from carbohydrates and by synthesis is discussed. Then come addition and direct substitution reactions in general terms, the carboxylic acids, aldehydes and ketones, amino- and hydroxy-compounds being dealt with more individually later. Hydrogenation, pyrolysis and ring-opening are then reviewed, followed by sections on certain unsaturated lactones and naturally occurring furan derivatives; the reference to ascorbic acid is intentionally brief, as wider information is already available elsewhere.

The thiophen section includes a useful survey of biotin, whilst the pyrrole group covers porphyrins and some synthetic analogues of the bile pigments, and the pyrone group introduces meconic and kojic acids and maltol.

There are a few gaps, but it must be admitted that chemical reviews of this kind to deal with β-lactones (*e.g.*, propiolactone), thia*cyclo*propane, thia*cyclo*butane and thialactones would be rather slim at the present time.

B. A. Ellis

Publications Received

The Barker Index of Crystals. Volume I. Crystals of the Tetragonal, Hexagonal, Trigonal and Orthorhombic Systems. By M. W. Porter, D.Sc., and R. C. Spiller, M.A. Pp. 500 + 1000. Cambridge : W. Heffer & Sons Ltd. 1952. Price 120s.

The first part, which includes a General Introduction, is also sold separately, price 30s.

H.M. STATIONERY OFFICE: ASSISTANT EXAMINER OF PAPER AND OFFICE REQUISITES. The Civil Service Commissioners invite applications for three pensionable appointments. Candidates must be at least 22 and under 32 years of age on 1st September, 1951, with extension for regular service in H.M. Forces and, up to two years, for established civil service. Candidates must have had such training in Chemistry and Physics as will enable them to investigate faults in paper of all kinds and stationers' sundries and to carry out tests appropriate to the examination of supplies for the Public Service. A practical knowledge of some or all of these materials is desirable. Salary scale (London) £430–£700 (men) and £430–£575 (women); somewhat lower in the Provinces. Prospects of promotion. Full particulars and application forms from Secretary, Civil Service Commission, 6, Burlington Gardens, London, W.1, quoting No. 205/52. Completed application forms must be returned by 31st January, 1952.

CHEMISTS required by Ministry of Supply in London and the provinces on Inspection, Production, Research and Development and Experimental work connected with aircraft, atomic energy, armaments, ammunition and associated equipment. *Qualifications:* British, of British parentage; appropriate industrial experience in addition to one of the following:—Honours degree in metallurgy, engineering, chemistry, physics or chemical engineering. Associateship of the Royal Institute of Chemistry, or Institute of Metallurgists or Institute of Physics. Corporate membership of the Institute of Chemical Engineers. Exceptionally, evidence of high professional attainments may be accepted. *Duties:* cover a wide range, including the following:—Testing, manufacture and procurement of (*a*) explosives propellants, ammunition filling, pyrotechnics and associated chemicals; (*b*) fuels, lubricants, oil coolants, detergents, preservatives, etc.; (*c*) anti-gas equipment (respirators, etc.). Application of insecticides, smoke screens, etc., and physiological research on clothing and equipment. Planning, installation and maintenance of chemical and radio-active plant. Analysis, testing (destructive and non-destructive), manufacture and procurement of metallic and non-metallic materials (including high temperature materials). *Salaries:* Within the range £600–£1,200 p.a. (London) dependent on age, qualifications and experience. Rates in provinces and for women slightly lower. Not established, periodical competitions for established pensionable posts. Application forms from Ministry of Labour and National Service, Technical and Scientific Register, (K), Almack House, 26, King Street, London, S.W.1, quoting reference F.917/51/A. Closing date 30 January, 1952.

SURREY COUNTY COUNCIL

APPLICATIONS are invited for the post of TECHNICAL ASSISTANT in the CENTRAL PURCHASING DEPARTMENT. The Officer appointed who should possess a Degree in chemistry or an equivalent qualification, will be required, under the general direction of the Chief Supplies Officer, to be responsible for the examination and testing of various commodities purchased, and to assist in the formulation of specifications and in other technical matters.

Preference will be given to applicants who have had a general analytical experience.

Salary on National Scale Grade V (£570 × £15/£20–£620 per annum), plus London Weighting Allowance, the commencing salary depending upon the qualifications and experience of the person appointed.

The appointment is subject to the staffing regulations of the Council and to the provisions of the Local Government Superannuation Act, 1937. The person appointed will be required to pass a medical examination.

Applications, stating age, qualifications and details of experience accompanied by copies of not more than three recent testimonials, must be addressed, to the undersigned the envelope endorsed "Technical Assistant," so as to reach me not later than the 23rd January, 1952. Canvassing either directly or indirectly will lead to disqualification.

T. W. W. GOODERIDGE,
Clerk of the Council.

County Hall,
Kingston upon Thames.
28th December, 1951.

CHEMIST (Male or Female) required for laboratory of large engineering works in West London area. Work involves analysis of ferrous and non-ferrous alloys and investigation of various problems in connection with metal finishing and corrosion. 5-day week. Pension fund. Good prospects for advancement. Write Box No. 3797, THE ANALYST, 47, Gresham Street, London, E.C.2.

CHEMIST with bacteriological experience required at Cambridge Factory or Bacteriologist prepared to work on Industrial Effluent disposal. Salary according to qualifications and experience. Write (quoting Ad. 574) stating age, experience and salary required to Personnel Manager, Pest Control Limited, Bourn, Cambridge.

A VACANCY occurs for an Analytical Chemist in the field of ferrous and non-ferrous metals, oils and allied substances. Applicant should possess B.Sc. or A.R.I.C. and have had at least two years' laboratory experience. The post is one of responsibility and offers excellent prospects. Commencing salary commensurate with experience and qualifications; superannuation under F.S.S.U. Applications to the Secretary, Production Engineering Research Association, Melton Mowbray, Leics.

ALBRIGHT & WILSON LTD. have a vacancy at their Merseyside Works for a chemist for analytical duties and for research into analytical methods. A degree is necessary, and there are good prospects of advancement. Starting salary according to age, qualifications and experience. Bonus is normally payable and a non-contributory pension scheme is in operation. Apply to The Appointments Officer, Albright & Wilson Ltd., Oldbury, Birmingham.
