THE ANALYST

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No. 15 November, 1953

THE SOCIETY OF PUBLIC ANALYSTS AND OTHER ANALYTICAL CHEMISTS

BULLETIN

FORTHCOMING MEETINGS

Ordinary Meeting of the Society, December 2nd, 1953

AN Ordinary Meeting of the Society will be held at 7 p.m. on Wednesday, December 2nd, 1953, in the Meeting Room of the Chemical Society, Burlington House, London, W.1.

A Lecture on "Recent Advances in Medical Chemistry" will be given by Professor C. H. Gray, M.D., D.Sc., M.R.C.P., F.R.I.C.

Meeting of the North of England Section, November 28th, 1953

AN Ordinary Meeting of the North of England Section will be held at 2 p.m. on Saturday, November 28th, 1953, at the City Laboratories, Liverpool.

At this meeting a paper on "Chromatography" will be presented by Dr. R. L. M. Synge, F.R.S.

Joint Meeting of the Scottish Section with the Stirlingshire and District Sections of the Society of Chemical Industry and the Royal Institute of Chemistry, December 16th, 1953

A JOINT Meeting of the Scottish Section with the above bodies will be held at 7.30 p.m. on Wednesday, December 16th, 1953, at the Lea Park Restaurant, Callendar Road, **Falkirk.**

A Lecture on "Principles of Chromatography" will be given by Dr. R. L. M. Synge, F.R.S.

Annual General Meeting of the Physical Methods Group, November 24th, 1953

THE Ninth Annual General Meeting of the Physical Methods Group will be held at 6 p.m. on Tuesday, November 24th, 1953, in the Meeting Room of the Chemical Society, Burlington House, London, W.1.

The Business Meeting will be followed at 6.30 p.m. by an Ordinary Meeting of the Group, open to non-members, at which the retiring Chairman, J. Haslam, D.Sc., F.R.I.C., will deliver an address on "Physical Methods in the Analysis of Plastic Materials—Some Observations of an Analytical Chemist."

Meeting of the Biological Methods Group, November 19th, 1953

A SPECIAL Meeting of the Group will be held at 8 p.m. on Thursday, November 19th, 1953, at the Royal Society of Medicine, 1, Wimpole Street, London, W.1, at which Dr. Chester I. Bliss (*Connecticut Agricultural Experimental Station, Newhaven, U.S.A.*) will give an address entitled "The Standardisation of a Drug in Production as Illustrated by Adrenal Cortical Extract."

Visitors will be welcome at the meeting.

Joint Meeting of the Biological Methods Group with the Pharmaceutical Society, December 11th, 1953

A JOINT Meeting of the Biological Methods Group with the Pharmaceutical Society will be held in two sessions on Friday, December 11th, 1953, in the Main Lecture Theatre, University College Hospital, London. The subject of the meeting will be "The Assay and Detection of Pyrogens."

The afternoon session will begin at 2.30 p.m., and the following papers will be presented and followed by a discussion—

"The Occurrence and Importance of Pyrogens," by T. D. Whittet.

"Routine Pyrogen Testing," by K. L. Smith.

A paper on the Leucocyte Response, by Miss Mary Dawson.

At 4.20 p.m. the **Annual General Meeting of the Group** will be held and an interval for tea will follow.

The evening session will begin at 5.10 p.m., and the following papers will be presented and followed by a discussion—

"Determination of Minimum Pyrogenic Dose for Man and Animals," by J. G. Dare.

"Standards of Pyrogenic Activity," by W. L. M. Perry.

THE CHEMICAL SOCIETY

THE Chemical Society has announced two new services to be available, from January, 1954, to Fellows of the Chemical Society and others.

Current Chemical Papers

The Chemical Society will publish under the title *Current Chemical Papers* a monthly classified world list of new papers in pure chemistry, the first issue appearing in January, 1954. The publication date will be mid-month, and each issue will normally contain the titles of relevant papers contained in all the journals received during the whole of the previous month. Titles that do not convey adequately the contents of the paper will be expanded and the entries will be classified into the principal branches of chemistry under some twenty different headings. Names of authors and references to the original journal will be given.

It is emphasised that this publication is not intended to become a substitute for an abstracting service, but it will advise research workers of newly published papers more rapidly than would be possible in a journal publishing full abstracts.

Current Chemical Papers will be available to Fellows at a price of 25s. per annum. The price to outside subscribers will be 50s. ($7.50) post free, or by Air Mail 85s. ($12.75) per annum. Subscriptions can be forwarded through the usual Booksellers, or directly to the **Chemical Society**, Burlington House, London, W.1, from whom an order form can be obtained.

Journal Reprint Service

Original papers published in the *Journal of the Chemical Society*, from January, 1954, will be available from the Chemical Society in reprint form. Single reprints can be obtained at 5s. ($0.75) each, or books of vouchers can be purchased at 10s. ($1.50) per book or 90s. ($13.50) for 10 books. Each book contains five reprint vouchers, valid for three years, which can be exchanged for reprints of any five scientific communications published in the *Journal*.

Every paper published in the *Journal* will carry a Reprint Order Number to be used in connection with this service, and the *Proceedings* (issued free each month with the *Journal*) will contain lists of papers accepted for publication. The papers so listed will normally appear in the *Journal* during the next succeeding two months, and if reprints are ordered promptly after the announcement of the title they will be distributed to subscribers at approximately the same time as the issue of the *Journal* in which the papers are published.

Reprint voucher books can be obtained from The General Secretary, The Chemical Society, Burlington House, London, W.1.

THE SOCIETY OF CHEMICAL INDUSTRY

Applied Chemistry Abstracts

THE Society of Chemical Industry has, since 1885, provided its members with abstracts of publications on applied chemistry. For some years this service has been provided through *British Abstracts*, which, in their present form, will no longer be published after December, 1953. The Society of Chemical Industry has therefore decided to resume the publication of abstracts on applied chemistry, and further details are given in a Leaflet enclosed with this issue of *The Analyst*.

PAPERS ACCEPTED FOR PUBLICATION IN *THE ANALYST*

THE following papers have been accepted for publication in *The Analyst*, and are expected to appear in the near future. It is not possible to enter into correspondence about any of them.

"Conductimetric Determination of Carbon in Metals," by J. E. Still, L. A. Dauncey and R. C. Chirnside.

> The metal sample is burnt in oxygen in a normal combustion tube and resistance furnace. The carbon dioxide evolved is absorbed in sodium hydroxide or barium hydroxide solution and measured by the change in electrical conductance of this solution.
>
> An improved loading device allows introduction of the sample and gives a good view of the combustion process, without admission of atmospheric carbon dioxide. A conductivity cell made of Perspex provides for continuous circulation of the absorbing solution and for measurement of the conductance at any time without stoppage of the oxygen flow. Except when the highest precision is required for extremely low carbon contents, sodium hydroxide solution is preferred to barium hydroxide solution. A special conductivity bridge, ordinary 50-cycle A.C. and a vibration galvanometer give high precision of measurement over a wide range; provision is made to compensate for the capacitance of the cell. The apparatus requires the minimum of maintenance, both in preparation for a series of analyses and in use.
>
> The method gives adequate precision and accuracy on 1-g samples of standard steels and cast irons containing from 0·03 to 3 per cent. of carbon, the only change necessary over this range being an alteration in solution concentration. A wide variety of samples of metals and alloys, weighing from 0·1 to 3 g and containing from 8 per cent. down to 0·01 per cent. or less of carbon, have been analysed satisfactorily.

"The Determination of Silicon in Tungsten and Titanium Metal Powders, Carbide Sintering Alloys, Tungstic Oxide and Tungstates," by B. Bagshawe and R. J. Truman.

> A method is described for the determination of silicon in such tungsten and titanium based compounds and materials as tungstic oxide, ammonium tungstate, tungsten metal and carbide, titanium metal and carbide and mixtures of them with molybdenum carbide, tantalum and cobalt. It depends upon calcination to oxide, fusion with sodium carbonate, extraction of the fusion product under conditions that prevent absorptive and hydrolysis losses of silicic acid and determination by an adaptation of the molybdisilicic acid - molybdenum blue reaction. Optimum conditions for the application of this reaction to the materials under review are given, and results are shown that establish the validity of the method for a series of synthetic mixtures.

"The Fractionation of Urinary Neutral 17-Ketosteroids by Adsorption and Partition Chromatography," by E. R. Cook, S. R. Stitch, A. E. Hall and Mary P. Feldman.

> The fractionation of urinary neutral 17-ketosteroids by adsorption and by partition chromatography is described, and the close agreement of certain fractions obtained by both methods is illustrated. Despite the influence of the method of hydrolysis of the 17-ketosteroid conjugates upon the chromatographic pattern, the constancy of the patterns for the urines from some subjects over a period of weeks is shown by each fractionation procedure.

"The Determination of Lithium in Magnesium-Lithium Alloys by Internal-Standard Flame Photometry," by (the late) A. M. Robinson and T. C. J. Ovenston.

The use of a double-beam flame photometer of simple design for the determination of 11 to 14 per cent. of lithium in magnesium - lithium alloys is described. The internal-standard technique is used, and its general application to the determination of alkali metals as major constituents is discussed. In the example given, the internal standard selected is potassium, which, when added in amounts to give a comparatively large concentration, minimises errors caused by variations in alloy composition. Accuracy is to within ± 1 per cent. for 11 to 14 per cent. of lithium.

Sodium and small amounts of potassium in the alloys do not interfere, and silver, zinc and cadmium, if totalling not more than 10 per cent. of the alloy, also have no effect.

"A Semi-micro Wet Combustion Method for the Determination of Carbon," by E. E. Archer.

The method involves digesting the sample with a wet combustion mixture, aspirating the evolved gases by a slow stream of air through a heated silica tube containing a silver spiral into an evacuated Büchner flask containing barium hydroxide solution, neutralising the excess of barium hydroxide to thymolphthalein and titrating the precipitated barium carbonate directly by means of standard acid with bromophenol blue as indicator. A correction is made for the amount of acid needed to change the acidity of the solution from that required for the colour change of thymolphthalein to that for the change of bromophenol blue. Thus the carbon dioxide evolved is directly estimated, without isolation of the precipitated barium carbonate, and errors caused by volatile mineral acids are avoided.

The method is applicable to hydrocarbons, aliphatic carboxylic acids, alcohols, ketones and various sulphur and chlorine compounds. Results are quantitative for glucose. Dry sucrose give low and irregular results, but results are quantitative with aqueous solutions of sucrose. Aqueous solutions, in general, can be successfully analysed when they contain not less than 0·5 per cent. of carbon.

"The Spectrophotometric Determination of Hydrazine in Dilute Solutions," by J. P. Riley.

A method is described for the determination of hydrazine at concentrations as low as 10^{-5} M with a coefficient of variation of about 1·0 per cent. Hydrazine solution is treated with a solution of picryl chloride in chloroform and, after addition of an alcoholic solution of potassium acetate, the intensity of the resultant brown colour is measured spectrophotometrically. Beer's law is obeyed for up to 40 parts of hydrazine per million. The interference of hydroxylamine and a number of inorganic anions and cations has been investigated.

NOTICE

The Physical Society Colour Group

THE next meeting of the Physical Society Colour Group will take place at 2.30 p.m. on Wednesday, December 9th, 1953, at the Institute of Electrical Engineers, when contributions to a symposium on the colour-rendering properties of fluorescent lamps will be made by Dr. B. H. Crawford, by Dr. S. T. Henderson and Mr. D. T. Waigh, and by Miss B. M. Young and Mr. G. T. Winch.

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APPLIED CHEMISTRY ABSTRACTS

The Society of Chemical Industry has, since 1885, provided its members with abstracts of publications on applied chemistry. For some considerable time this service has been provided through British Abstracts, published under the direction of the Bureau of Abstracts. This organization will cease to exist at the end of this year and British Abstracts, in their present form, will no longer be published after December 31st next.

The Council of the Society, faced with this position, has decided to resume the publication of abstracts on applied chemistry on its own account and to revert to the position as it was before the formation of the Bureau.

The Abstracts will appear monthly, separately paged, in the Journal of Applied Chemistry and the Journal of the Science of Food and Agriculture, whichever is relevant to the subject of the paper abstracted. Broadly speaking, Abstracts hitherto covered by B I and B II will appear in the Journal of Applied Chemistry and those covered by B III in the Journal of the Science of Food and Agriculture.

Abstracts cannot be supplied separately from either Journal.

Application should be made to the Society at the following address.

Society of Chemical Industry,
9/10 Savile Row,
London, W.1.

November, 1953

THE ANALYST

EDITORIAL

ANALYTICAL ABSTRACTS

THE Society of Public Analysts and Other Analytical Chemists has provided abstracts of the literature on analytical chemistry for readers of *The Analyst* ever since the journal was first published in 1876. For the last four years these abstracts were prepared by the Bureau of Abstracts and supplied to members of the Society and to subscribers to *The Analyst* under the title *British Abstracts C*. The Bureau of Abstracts will cease to exist at the end of 1953, and British Abstracts will not appear in their present form thereafter.

In these circumstances, the Council of the Society has decided to resume publication of abstracts of analytical literature. *British Abstracts C* will be replaced in the new year by a new publication to be called *Analytical Abstracts*. This will be edited by Norman Evers, B.Sc., Ph.D., F.R.I.C., and published by W. Heffer & Sons Ltd. for the Society, and it will cover the whole range of analytical literature in the same way as did *Abstracts C*. It is expected that it will be possible to extend its usefulness to readers as time passes.

Analytical Abstracts will appear each month bound separately from *The Analyst* and in a format similar to that of the abstracts included in *The Analyst* prior to 1950. The two journals, *The Analyst* and *Analytical Abstracts*, will be available in 1954 to members of the Society and to subscribers on the same terms as obtained for *The Analyst* and *Abstracts C* in 1953.

PROCEEDINGS OF THE SOCIETY OF PUBLIC ANALYSTS
AND OTHER ANALYTICAL CHEMISTS

A Simple Volumetric Method for the Routine Determination of Glycerol

By J. W. B. ERSKINE, C. R. N. STROUTS, G. WALLEY and W. LAZARUS

Glycerol is oxidised at room temperature by a solution of sodium metaperiodate to give formaldehyde and formic acid. The formic acid, after the excess of periodate has been destroyed with ethylene glycol, is titrated with standard alkali, with phenol red as indicator, to give a measure of the glycerol present. Carbon dioxide must be excluded, and the oxidation must be carried out in the dark to minimise side reactions between sodium metaperiodate and the formaldehyde and formic acid. A number of other polyhydric alcohols and sugars interfere, but these are not likely to be present in the glycerol-containing products of soap-making and fat-splitting, such as crude glycerine, lye and soap. The most likely organic impurities in these products are trimethylene glycol, which does not react, and polyglycerols, which do not yield formic acid; these substances, therefore, do not interfere as they do in the acetin and dichromate methods. Glycols with adjacent hydroxyl groups, like ethylene glycol, react, but produce only formaldehyde.

The method has been checked against distilled glycerine, and against soap-lye crude glycerine, soap lyes and soaps containing known added amounts of glycerol, and also on commercial crudes, soap lyes and soaps by comparison with the acetin and dichromate methods.

During the last forty years two standard methods* have been available for the determination of glycerol in crude glycerine, namely, the dichromate and the acetin methods. The former measures the total oxidisable matter and is, therefore, unsuitable when the glycerine contains other oxidisable matter not easily separated from the glycerol. The acetin method is more specific and measures the hydroxyl content of the product. The most likely impurities in commercial glycerine that would be returned as glycerol by this method are polyglycerols, for which a correction can be made, and trimethylene glycol,† for which no correction is usually made, although a method[1] exists for its determination.

In recent years new methods have been suggested for the determination of glycerol, and of these the most important is that based on oxidation by periodate. Glycerol is oxidised by periodates to give formaldehyde and formic acid; under certain conditions the amount of formic acid produced gives a measure of the glycerol present. Other hydroxy bodies that may be present in glycerine include glycols and polyglycerols, but it has been shown that these compounds do not react with periodates to give formic acid. Polyhydric alcohols with three or more adjacent hydroxyl groups, *e.g.*, sorbitol and sugars, yield formic acid, which would interfere with the determination. They are not likely to be present in crude glycerine, soap lyes or soaps. For practical purposes the periodate oxidation is specific for glycerol in these products.

Methods based on oxidation with sodium metaperiodate have been developed for glycerine by Bradford, Pohle, Gunther and Mehlenbacher[2] and the American Oil Chemists' Society (A.O.C.S.).[3,4] Although the latest A.O.C.S. method (Ea. 6–51) represents a notable advance, it has certain disadvantages, the most important being the need to titrate to two different end-points, the dependence of the result on the weight of sample used in the determination and (for some laboratories) the necessity of using a pH meter. These disadvantages are absent from the method now described, which, based on extensive work carried out in the laboratories of Imperial Chemical Industries Ltd. (Nobel Division) and of Unilever Limited, consists in a simple acidimetric titration.

* Analysis of Crude Glycerine, International Standard Methods, 1911, and Supplement No. 1 (I.S.M., 1911) British Executive Committee, 1914. Referred to in this paper as "standard methods."

† One per cent. of trimethylene glycol is returned as 0·8 per cent. of glycerol by the acetin method.

Experimental

Investigation of the conditions affecting the determination of glycerol by sodium metaperiodate oxidation—

The effect of light and temperature—In early experiments with the A.O.C.S. method, erratic results were sometimes obtained, especially with distilled glycerine; this was attributed to the various degrees of exposure to light between different experiments. It was also found in the course of experiments on a modification of the Newburger and Bruening method,[5] which was ultimately developed into the method described later, that, in daylight, the result was affected by the temperature at which the reaction was carried out. A series of experiments was then carried out in which a distilled glycerine* was oxidised with sodium metaperiodate according to the procedure described on p. 635, under various conditions of temperature and illumination. The results are shown in Table I and indicate that (*i*) the glycerol content was accurately determined when the oxidation was carried out in the dark either at 0° C or at 25° C, (*ii*) that the amount of exposure to light had an appreciable effect on the amount of glycerol found, a result over 5 per cent. too high being recorded in bright sunlight at 25° C, and (*iii*) that at 0° C the effect of light, although not so marked, was still appreciable.

Table I

The effect of light on the determination of glycerol by sodium metaperiodate

Separate weighings of distilled glycerine (90·25 per cent. of glycerol) were used in each determination by the method described on p. 635
Time of oxidation = 1 hour

Glycerol found				
In the dark at:		In daylight at:		
0° C, %	25° C, %	0° C, %	25° C, %	Daylight conditions
90·30	90·30	90·86	91·76	Sunny, indirect
90·24	90·40	90·31	90·74	Dull, indirect
90·32	90·26	90·94	95·68	Sunny, direct
90·20	90·46	90·16	90·52	Dull, indirect
90·12	90·36	—	—	—
90·32	90·16	—	—	—
Mean 90·25	90·32			

Experiments were also carried out to study the action of sodium metaperiodate on the products of the glycerol oxidation, formic acid and formaldehyde, as it was probable that these side reactions caused the anomalous results found in daylight. Portions of solutions containing known amounts of formic acid and formaldehyde were treated with sodium metaperiodate under various conditions of light and temperature, and the formic acid present at the end of the reactions was measured. The amounts of formic acid and formaldehyde taken were roughly equivalent to those that would be present at the end of a determination in which the amounts of sample and reagents recommended in the method on p. 635 were used. The amount of sodium periodate was approximately equal to the excess of oxidant remaining at the end of such a determination. The results are shown in Table II and indicate that (*i*) sodium metaperiodate reacts with both formic acid and formaldehyde, (*ii*) the reactions proceed slowly in the dark but are greatly accelerated by daylight, and (*iii*) between 0° and 20° C temperature has no appreciable effect when the oxidation takes place in the dark. Head and Hughes,[6] who also carried out experiments on the action of sodium periodate on formic acid and formaldehyde, report similar findings.

Experiments 17 to 21 show that the presence of sodium iodate reduces the oxidation of formic acid and formaldehyde. As iodate is formed in the oxidation of glycerol, the main reaction, the influence of these side reactions is in practice less than would be expected from the earlier results shown in this table.

* A multiple-distilled glycerine, containing no impurity but water, whose glycerol content had been accurately determined from specific gravity and moisture determinations.

TABLE II

THE OXIDATION OF FORMIC ACID AND FORMALDEHYDE BY SODIUM METAPERIODATE IN DAYLIGHT AND DARKNESS AT 0° AND 20° C

Mixture titrated with 0·1 *N* sodium hydroxide after destruction of excess of periodate with ethylene glycol

Time of oxidation = 1 hour

Expt. No.	Temperature, °C	Lighting conditions	*A* HCHO added, g	*B* HCOOH added, g	*C* $NaIO_4$ added, g	*D* $NaIO_3$ added, g	*E* HCOOH found, g	*F* HCOOH found, as glycerol, g	Difference *E*–*B*, g	Glycerol error, %
1	20		Nil	0·4257	Nil	Nil	0·4257	0·8514	—	—
2	20		0·6	Nil	Nil	Nil	0·0003	0·0006	+0·0003	+0·07
3	0	In the	Nil	0·4257	1·5	Nil	0·4264	0·8524	+0·0005	+0·12
4	0	dark	0·6	0·4257	1·5	Nil	0·4259	0·8518	+0·0002	+0·05
5	20		Nil	0·4257	1·5	Nil	0·4258	0·8516	+0·0001	+0·02
6	20		0·6	Nil	1·5	Nil	0·0002	0·0004	+0·0002	+0·05
7	20		0·6	0·4257	1·5	Nil	0·4263	0·8526	+0·0006	+0·14
8	20	Dull	0·6	Nil	1·5	Nil	0·0127	0·0254	+0·0127	+2·98
9	20	daylight	Nil	0·4257	1·5	Nil	0·4093	0·8186	−0·0164	−3·85
10	20		0·6	0·4257	1·5	Nil	0·4354	0·8708	+0·0097	+2·28
11	0	In the	0·6	Nil	1·5	Nil	Nil	Nil	Nil	Nil
12	0	dark	Nil	0·4174	1·5	Nil	0·4187	0·8374	+0·0013	+0·31
13	0		0·6	0·4174	1·5	Nil	0·4194	0·8388	+0·0020	+0·48
14	20		0·6	Nil	1·5	Nil	0·0360	0·0720	+0·0360	+8·47
15	20		Nil	0·4174	1·5	Nil	0·3667	0·7334	−0·0507	−12·15
16	20	Bright	0·6	0·4174	1·5	Nil	0·4407	0·8814	+0·0233	+5·58
17	20	daylight	0·6	Nil	1·5	4·0	0·0090	0·0180	+0·0090	+2·12
18	20		Nil	0·4174	1·5	4·0	0·4149	0·8258	−0·0025	−0·60
19	20		0·6	0·4174	1·5	4·0	0·4214	0·8428	+0·0040	+0·96
20	20	In the	0·6	0·4256	1·5	4·0	0·4255	0·8510	−0·0001	−0·02
21	20	dark	0·6	0·4256	1·5	4·0	0·4254	0·8508	−0·0002	−0·04

NOTE—In experiments 2, 6, 8, 14 and 17 the glycerol error has been calculated on the assumption that a sample weight of 0·85 g of glycerol was taken.

TABLE III

TIME OF OXIDATION AND EFFECT OF SODIUM CHLORIDE

Separate weights (about 0·8 g) of glycerine taken for each test and determination carried out by procedure given on p. 635

	Time of oxidation, minutes	Sodium chloride added, g	Glycerol found, %
Distilled glycerol, 90·25 per cent. by specific gravity measurement	60	Nil	90·20
	60	Nil	90·36
	30	Nil	90·35
	60	1	90·24
	60	1	90·24
	30	1	90·38
	30	1	90·40
	60	2	90·28
	30	2	90·24
Soap lye crude 1	60	Nil	84·27
	30	Nil	84·30
Soap lye crude 2	60	Nil	82·47
	30	Nil	82·44

THE TIME OF OXIDATION AND THE EFFECT OF SODIUM CHLORIDE—

A study was made of the time required for the oxidation to go to completion. Weighed quantities of distilled glycerine were treated with sodium metaperiodate according to the method described on p. 635, the oxidation being allowed to proceed for 30 minutes or 1 hour. Bradford and his co-workers[2] have stated that sodium chloride retards the oxidation, so,

as soap-lye crudes contain about 10 per cent. of sodium chloride, some tests were also carried out in which large quantities of sodium chloride (relative to the amount of the glycerol) were added. Some results for crude glycerines are also shown. The results, shown in Table III, indicate that under the conditions used, an oxidation time of 30 minutes is sufficient, even in the presence of much sodium chloride.

THE EFFECT OF TRIMETHYLENE GLYCOL—

According to Bradford *et al.*[2] the periodate oxidation gives low figures for glycerol in the presence of trimethylene glycol. Several determinations were made with trimethylene glycol added, but it was found that it had no effect under the test conditions.

THE EFFECT OF CARBON DIOXIDE—

It was found that the titration was affected by atmospheric carbon dioxide (or other acidic gases) and that the sharpness of the end-point was considerably increased by passing a slow stream of nitrogen (freed from carbon dioxide) through the solution being titrated. It was also found that the passage of nitrogen through the solution caused no volatilisation of formic acid, as shown by the figures in Table IV.

TABLE IV

NON-VOLATILITY OF FORMIC ACID UNDER THE CONDITIONS OF THE TEST

Formic acid titrated with 0·1 *N* alkali. Volume of solution at the start: 200 ml

Total time of passage of nitrogen, minutes	*A* Formic acid present, g	*B* Formic acid found, g	*C* Loss of formic acid (*A* − *B*), g	Loss of formic acid, %
0	0·4632	0·4633	Nil	Nil
10	0·4632	0·4632	Nil	Nil
30	0·4632	0·4631	0·0001	0·02
0	0·4404	0·4404	Nil	Nil
10	0·4404	0·4404	Nil	Nil
30	0·4404	0·4402	0·0002	0·05

THE DETERMINATION OF GLYCEROL IN PURE GLYCERINES, CRUDES, SOAP LYES AND SOAP BY THE PERIODATE PROCEDURE—

Pure glycerines—Tests that were carried out on a pure multiple-distilled glycerine (see Table I, columns 1 and 2) show excellent agreement between the figures for glycerol by the periodate method and those from specific gravity and moisture determinations. Similar results were attained with other samples of pure distilled glycerine. Experiments were also carried out on glyceryl sesquicarbonate, a crystalline derivative that was prepared in a state of high purity; the results for glycerol, after hydrolysis, were in excellent agreement with elementary analysis.

Mixtures containing known amounts of glycerine—In order to check the accuracy of the periodate method for glycerol in soap-lye crudes and to show whether the method could be used for soap lye and soap, it was tested on made-up soap-lye crudes, soap lyes and soaps containing known amounts of glycerol. The glycerol was determined by the procedure described on p. 635. As the glycerol content of soap is low, some tests were carried out in which the amount of glycerol to be determined was reduced to one-tenth, with a corresponding reduction in the reagents and volumes; a 10-ml micro-burette was used for the titration. The satisfactory recoveries of glycerol shown in Table V indicate (*i*) that the glycerol in crudes is accurately determined with no interference from trimethylene glycol and polyglycerol, and (*ii*) that the method can be applied to soap lyes and soaps (after acidifying, cooling and filtering to remove fatty acids). When the amounts taken for the determination are reduced to one-tenth, the precision is reduced but the results are still satisfactory.

Commercial products—Table VI shows results by the periodate method for glycerol in industrial soap-lye crudes, saponification crudes, soap lyes and soap, together with comparison results by the standard acetin and dichromate methods. The figures indicate that there is good agreement between the periodate method and the standard methods.

TABLE V

REPLICATE ANALYSES OF MADE-UP SOAP-LYE CRUDES, SOAP LYES AND SOAPS BY THE PERIODATE METHOD

Type of product	Glycerol taken, g	Poly-glycerol taken, g	Tri-methylene glycol taken, g	Sodium chloride taken, g	Soap taken, g	Glycerol found, g	Recovery, %
Soap-lye crude	0·9210	0·005	0·013	0·1	Nil	0·9195	99·84
	0·9238	0·005	0·013	0·1	Nil	0·9245	100·08
	0·8413	0·005	0·013	0·1	Nil	0·8406	99·92
	0·8999	0·005	0·013	0·1	Nil	0·8999	100·00
	0·8904	0·005	0·013	0·1	Nil	0·8899	99·94
	0·9276	0·005	0·013	0·1	Nil	0·9249	99·71
Soap lye	0·8694	Nil	Nil	1·79	0·14	0·8673	99·76
	0·8642	Nil	Nil	1·79	0·14	0·8650	100·10
	0·9174	Nil	Nil	1·64	0·13	0·9144	99·70
	0·8575	Nil	Nil	1·64	0·13	0·8542	99·62
	0·8956	Nil	Nil	1·64	0·13	0·8910	99·50
	0·8320	Nil	Nil	1·64	0·13	0·8320	100·00
Soap (fatty acids removed before determination)	0·8928	Nil	Nil	Nil	160·0	0·8775	98·3
	0·9333	Nil	Nil	Nil	160·0	0·9284	99·5
	0·8979	Nil	Nil	Nil	160·0	0·8877	98·9
	0·9490	Nil	Nil	Nil	160·0	0·9310	98·1
	0·9286	Nil	Nil	Nil	160·0	0·9140	98·4
	0·9327	Nil	Nil	Nil	160·0	0·9220	98·9
	0·0968	Nil	Nil	Nil	10·0	0·0962	99·4
	0·0968	Nil	Nil	Nil	10·0	0·0960	99·2
	0·0888	Nil	Nil	Nil	10·0	0·0891	100·3
	0·0888	Nil	Nil	Nil	10·0	0·0904	101·8
	0·0888	Nil	Nil	Nil	10·0	0·0893	100·6

TABLE VI

ANALYSES OF COMMERCIAL SOAP-LYE CRUDES, SAPONIFICATION CRUDES, SOAP LYES AND SOAPS BY THE PERIODATE AND STANDARD METHODS

Type of product	Glycerol, %		
	Acetin method	Dichromate method	Periodate method
Soap-lye crude* 1	84·29	—	84·27
" 2	82·44	—	82·53
" 3	83·83	—	83·83
" 4	82·36	—	82·47
" 5	82·16	—	81·92
" 6	83·23	—	83·09
Saponification crude* 1	91·94	—	91·90
" 2	91·80	—	92·09
" 3	86·62	—	86·53
" 4	93·03	—	93·06
" 5	89·93	—	90·00
" 6	89·17	—	89·03
Soap lye 1	6·30	6·40	6·35
" 2	6·18	—	6·19
" 3	6·10	6·37	6·29
" 4	—	9·50	9·48
" 5	—	8·99	8·84
" 6	—	9·09	8·92
Soap† 1	—	0·33	0·28
" 2	—	0·46	0·41
" 3	—	0·42	0·40

* Corrected for total acetyl value of residue.
† Glycerol expressed on total fatty matter.

NOTE—The figures for soap-lye crudes and saponification crudes have been analysed statistically, and the results show that there is no significant difference between results by the two methods.

Method

Preparation and standardisation of reagents—

*Sodium metaperiodate**—Dissolve 228 g of pure periodic acid, which may conveniently be prepared electrolytically,[7] in 1 litre of water contained in a 3-litre beaker. Add slowly with constant stirring 1 litre of a *M* solution of sodium hydroxide. Transfer the contents of the beaker to a 3-litre round-bottomed flask, place it in a water-bath, and distil off the water under reduced pressure until the volume of liquid that remains is about 250 ml. Allow the solution to cool, filter off the sodium metaperiodate on a sintered-glass funnel, porosity grade 3, and dry the salt overnight at 100° to 110° C. The yield is 80 per cent., calculated on the periodic acid, and its solubility in water is 10 g per 100 ml at 20° C.

Determine the composition of the material as follows. Dissolve 0·36 g of the salt in 100 ml of water contained in a flask with a ground-glass stopper. Add 7·5 g of analytical reagent grade sodium bicarbonate followed by 3 g of analytical reagent grade potassium iodide. Stopper the flask and set the mixture aside for exactly 15 minutes.

Titrate the solution with 0·1 *N* sodium arsenite solution that has been standardised against 0·1 *N* iodine immediately before use.

1 ml of 0·1 *N* sodium arsenite ≡ 0·010696 g of $NaIO_4$.

Material made in the manner described will have a purity of 99·8 per cent. or more.

Sodium metaperiodate solution, 3·33 per cent. w/v—Measure 150 ml of water into a 1-litre conical flask and boil to expel carbon dioxide. Add 5 g of sodium metaperiodate. Close the flask with a rubber bung carrying a soda-lime guard tube, swirl the contents of the flask to dissolve the salt, and cool the flask in running water. Larger quantities of the reagent may be made up *pro rata*, but the solution is not stable and must be freshly prepared each day.

Sodium hydroxide solution, 0·1 N—In order to avoid indicator errors it is essential that the sodium hydroxide used to titrate the formic acid shall be standardised against 0·1 *N* hydrochloric acid itself standardised against pure anhydrous sodium carbonate with phenol red as indicator. Full details of the standardisation of the hydrochloric acid are given in the literature.[8] In this titration it is necessary to add an excess of acid to the alkali, then to boil to expel carbon dioxide and finally to titrate the residual acid.

From a 105-ml bulb-burette, measure into a hard-glass flask, containing 50 ml of water, 85 ml of 0·1 *N* hydrochloric acid standardised against pure anhydrous sodium carbonate with phenol red indicator. Boil the solution for a few minutes to expel carbon dioxide. Close the flask with a rubber stopper carrying a soda-lime guard tube and cool to room temperature. Wash down the sides of the flask with a little water free from carbon dioxide, add 0·2 ml of phenol red indicator and titrate the acid with the 0·1 *N* sodium hydroxide solution to a pale pink end-point while passing a slow stream of nitrogen free from carbon dioxide through the solution. The flow of gas should be regulated to avoid loss of solution as spray. From the corrected volumes of acid and alkali, calculate the factor for the 0·1 *N* sodium hydroxide. Determine the glycerol equivalent of the reagent at intervals (or for each batch of reagent) by means of standard glycerol, the concentration of which is known from its specific gravity[9] and its moisture content.

Determination of glycerol—

Weigh into a dry 500-ml conical flask, a sample portion containing 0·7 to 0·8 g of glycerol.† Wash down the sides of the flask with water and then add 50 ml of water and 1 ml of *M* hydrochloric acid. Boil the solution for 3 minutes to expel carbon dioxide, close the flask with a rubber stopper carrying a soda-lime guard tube and cool in running water to room temperature. Add 0·2 ml of phenol red indicator and neutralise the solution with 0·1 *N* sodium hydroxide solution standardised to the same indicator.‡ Add 150 ml of a 3·33 per cent. w/v solution of sodium metaperiodate, swirl, stopper the flask again and allow it to stand in the dark for

* We are indebted to Messrs. K. Sporek and J. Templeton for the preparation and analysis of pure sodium metaperiodate. At the time this work was carried out the material available commercially was not sufficiently pure, but supplies at the requisite standard of purity can now be obtained.

† For the determination of glycerol in soaps (after removal of fatty acids), a sample containing one-tenth of this amount may be taken, with a corresponding reduction in reagents and volumes. Use a 5 or 10-ml micro-burette for the titration.

‡ The colour at the approach to the end-point is a pale reddish-brown; the titration must therefore be continued until the definite pink colour of the indicator is seen.

30 minutes. Wash down the sides of the flask with carbon dioxide-free water, add 5 ml of redistilled ethylene glycol and set the mixture aside in the dark for 20 minutes. Titrate the liberated formic acid from a 90-ml bulbed burette with 0·1 *N* sodium hydroxide standardised as described above; pass a stream of nitrogen free from carbon dioxide through the solution at such a rate that no liquid is lost by spraying. The end-point is indicated by a definite pink colour.* Carry out a blank titration at the same time.

1 ml of 0·1 *N* NaOH = 9·209 mg of $C_3H_8O_3$.

A determination can be completed in an hour and a half.

Since this work was submitted for publication a paper by L. Hartman has appeared (*J. Appl. Chem.*, 1953, **3**, 303) describing a "Rapid Determination of Glycerol by the Potassium Periodate Method."

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The Chromatographic Determination of Glutamic Acid in Wheat Gluten and Gluten Hydrolysates

BY P. MORRIES AND R. E. STUCKEY

A method has been devised for the determination of glutamic acid in gluten and in gluten hydrolysates. Total dicarboxylic acids (glutamic acid and aspartic acid) are determined by adsorption on Amberlite IR–4B resin at a pH value of 3 to 4 and subsequent elution with *N* hydrochloric acid; the amount of aspartic acid present is then found by means of descending paper-strip chromatography with a phenol - water system. The paper chromatography of gluten hydrolysates has been investigated with phenol, collidine and *iso*butyric acid as the mobile phase; the precision of the estimation of a single spot on a water - *iso*butyric acid chromatogram was found to be ±12 per cent. (P = 0·95).

MANY of the available data relating to the glutamic acid content of proteins are based on the work of Jones and Moeller,[1] which involves the precipitation of the calcium or barium salts of glutamic acid from strong aqueous alcohol. This procedure is somewhat lengthy, however, and cannot readily be adapted for routine use; in addition, Chibnall, Rees, Bailey and Williams,[2] who subjected the method to an extensive critical examination, stressed the necessity for solubility corrections. It was thought that chromatographic procedures for the estimation of glutamic acid were worth investigating in the hope of evolving a method more convenient for routine evaluation of gluten hydrolysates.

The methods available for the chromatography of the dicarboxylic amino-acids fall into two groups: first, the traditional chromatographic techniques on alumina or, more recently, on ion-exchange materials, and, secondly, partition chromatography on columns of starch or silica gel or on paper strips. Both methods were investigated for their suitability as quantitative analytical procedures and to find a satisfactory combination of chromatographic methods.

* The colour at the approach to the end-point is a pale reddish-brown; the titration must therefore be continued until the definite pink colour of the indicator is seen.

Quantitative paper chromatography

Many recent methods for the analysis of mixtures of amino-acids have involved the paper chromatographic techniques developed by Consden, Gordon and Martin.[3] We have used their method for some time for the routine separation and examination of amino-acids and consider it possible to determine glutamic acid in wheat gluten hydrolysates by means of a similar technique.

The method used in the work reported here is based on that suggested by Atkinson, Stuart and Stuckey.[4] Developing solvents were removed from the papers by washing with ether rather than by oven-drying, as Fowden and Penny[5] have shown that drying at elevated temperatures causes serious losses of amino-acids. The hydrolysates to be examined were produced by hydrochloric acid digestion of wheat gluten; the excess of acid was removed by treatment with silver oxide. The amounts of gluten hydrolysates used were adjusted to contain 20 to 40 μg of glutamic acid for each chromatogram.

Procedure—

Weigh about 1 g of wheat gluten and digest it with 10 ml of concentrated hydrochloric acid on a bath of boiling water for 4 hours under a reflux condenser. Cool, dilute to 25 ml and neutralise the excess of acid by adding dry silver oxide a little at a time, mixing thoroughly after each addition; when neutralised to a pH value of approximately 7, as determined by indicator papers, allow the precipitate to settle and place 2 μl of the supernatant liquid on a strip of Whatman No. 1 filter-paper by means of an "Agla" micrometer syringe. Place the filter-paper strip in a chromatograph chamber containing an atmosphere saturated with water and the solvent used (usually *iso*butyric acid or phenol), develop the chromatogram (descending) overnight and remove the developing solvent by washing with ether and drying at room temperature. Spray the chromatogram with a 1 per cent. solution of ninhydrin in *n*-butanol containing 10 per cent. of pyridine and heat in an oven at 80° C for 30 minutes. Cut out the appropriate spots and elute with 5 ml of a 20 per cent. solution of pyridine in water. Gentle swirling in a test tube is usually sufficient to extract the colour within 5 minutes; if necessary, remove fibres by centrifugation. Measure the absorption at 570 mμ on a suitable spectrophotometer and compare the results with those obtained from extracts prepared similarly from standard solutions of glutamic acid run side by side on the same sheet of paper.

Results—

In the first series of quantitative experiments a mixture of *iso*butyric acid and water was used to attain separation. The spot corresponding to the R_F value of glutamic acid (0·38 in *iso*butyric acid by comparison) was assumed to contain glutamic acid only, in order to determine the precision of the estimation. The results shown in Table I were obtained on a single gluten hydrolysate by comparison with a solution of glutamic acid as standard.

Table I

The precision of glutamic acid estimation by paper chromatography

Material	Number of results	Maximum, %	Minimum, %	Average, %	Standard deviation	Error, %
Gluten hydrolysates (single spot)	16	14·5	11·4	12·5	0·737	(P = 0·95) 12
Solution of amino-acids (double spots)	12	1·34	1·23	1·25	0·036	(P = 0·98) 7 (P = 0·96) 6

A study of the R_F values indicated that phenol was the most likely solvent to effect separation of glutamic acid from other amino-acids. But the determination of glutamic acid by the above method in a synthetic mixture of amino-acids approximating in composition to a wheat gluten hydrolysate gave results about 5 per cent. too high. This interference was thought to be caused chiefly by serine; this was confirmed with a two-dimensional chromatogram and phenol and collidine - lutidine solutions as the solvents. It is possible that this

interference could be eliminated in a one-dimensional chromatogram with a phenol - ammonium hydroxide solvent, but with this solvent high paper-blanks were obtained for the ninhydrin reaction. Although the two-dimensional paper chromatogram was reasonably satisfactory, it was hoped that a simpler method would be found for routine use. Fowden[6] published a method that avoids this paper blank, but these difficulties and the doubtful accuracy of the procedure for quantitative work encouraged a search for other methods.

Ion-exchange resin techniques

Glutamic acid is one of the few dicarboxylic amino-acids present in protein hydrolysates, the only other of importance being aspartic acid. A study of the chromatogram with phenol and collidine - lutidine showed that aspartic acid gave a well-defined spot not contaminated by other amino-acids when phenol was used as solvent in a one-dimensional chromatogram. Ion-exchange resins have been used by a number of workers for the separation of the dicarboxylic amino-acids. Therefore, if a procedure were available for the determination of total dibasic acids (glutamic acid plus aspartic acid), the aspartic acid could then be determined by paper chromatography and the glutamic acid found by difference.

Several schemes for the separation of amino-acids by ion-exchange materials have been published; those for the separation of the dicarboxylic amino-acids depend on the use of a weakly basic anion-exchanger. Cannan[7] determined the dicarboxylic acids in several proteins by treating them repeatedly with Amberlite IR–4 resin in a flask. Tiselius, Drake and Hagdahl[8] used a column technique and estimated the amino-acids in the eluates by means of a Kjeldahl nitrogen determination. Partridge and Brimley[9] separated glutamic acid from mixtures of other amino-acids with De-Acidite B, their yield of pure glutamic acid being about 82 per cent. of the theoretical. Drake[10] effected the separation of glutamic acid and aspartic acid on columns of Amberlite IR–4 resin, using a Kjeldahl nitrogen estimation to determine the glutamic acid in the eluates. Consden, Gordon and Martin,[11] also using IR–4 resin, described a method of determining glutamic acid and aspartic acids in proteins and their subsequent separation; they determined amino-nitrogen in the eluates by the copper method of Pope and Stevens,[12] but failed to obtain quantitative recoveries of amino-acids from synthetic mixtures, and attributed this to the presence of formaldehyde from the resin in the eluates. In general, either recoveries were not quantitative or the methods could not easily be adapted to routine analytical estimations.

In the separation of the dicarboxylic amino-acids by means of a weakly basic anion-exchange resin, the hydrolysate is run down a column of the chloride form of the resin; the dicarboxylic amino-acids are retained on the column and the mono-amino mono-carboxylic acids and the more basic amino-acids pass through. The analytical grade of Amberlite IR–4B was used in this work (the nearest equivalent in the Zeocarb series is De-Acidite E), the principle of the method being that of Consden, Gordon and Martin,[11] who used IR–4. The excess of hydrochloric acid in the gluten hydrolysate was removed by treatment with silver oxide and filtration instead of repeated evaporation *in vacuo*. No difficulties from formaldehyde liberated from the resin were encountered, but in order to attain quantitative recovery it was essential to have the particle size of the resin sufficiently small for the concentrations used, otherwise the rate of flow through the column would need to be inconveniently slow.

Procedure—

Reduce Amberlite IR–4B resin, which is usually supplied as the base with a B.S.S. mesh size of 16 to 50, to 40 to 60 mesh by grinding gently under water. With it still under water, add dilute hydrochloric acid until the pH of the liquid is less than 2 and wash with water by decantation until the pH of the supernatant liquid is greater than 3. Transfer the resin as a slurry to a glass column of 1 cm internal diameter to a height of 15 cm and wash it with sufficient water to ensure that the pH of the washings is between 3 and 4. Do not allow the column to become dry at any stage during the procedure or channels will form.

Dilute a suitable amount of the gluten hydrolysate to a measured volume so that it contains about 1 per cent. of the dicarboxylic amino-acids. Neutralise the solution to a pH of 3 to 4 with silver oxide and filter through a dry filter-paper. Transfer 5 ml of the filtrate by means of a pipette to the top of the resin column and allow it to run through at a rate of 1 to 2 ml per minute. Wash the column with water until a portion of the washings no longer gives any reaction for amino-acids with ninhydrin. This usually occurs after about

40 ml of washings have been collected. Elute the column with about 100 ml of *N* hydrochloric acid and evaporate the eluate to small bulk *in vacuo*. Determine the amino-nitrogen by the copper method or the total nitrogen by a normal Kjeldahl estimation.

When working with the liquors derived from gluten hydrolysates after the extraction of glutamic acid—they usually contain considerable quantities of ammonium chloride—it was found necessary to use a smaller quantity of sample, otherwise separation of amino-acids on the column was not clean.

A paper chromatogram was run concurrently with the resin separation, and the aspartic acid was estimated by the method referred to above (p. 637). The amino-nitrogen from the aspartic acid is subtracted from the total dicarboxylic amino-nitrogen and the result calculated to glutamic acid.

RESULTS—

The method was first applied to the analysis of known amino-acid mixtures approximating in composition to gluten hydrolysates and containing glutamic acid, aspartic acid, cystine, alanine, serine, glycine, threonine, lysine, arginine, histidine, methionine, tryptophan, tyrosine, valine, leucine and phenyl alanine. Table II shows the results obtained.

TABLE II

RECOVERY OF TOTAL DICARBOXYLIC ACIDS FROM SYNTHETIC AMINO-ACID MIXTURES

Glutamic acid and aspartic acid taken (calculated as glutamic acid), mg	Glutamic acid and aspartic acid recovered (calculated as glutamic acid)		
	By amino-nitrogen method, mg	Total nitrogen, mg	Recovery, %
53·6	54·7	—	102·2
54·6	54·0	—	98·8
53·4	52·5 53·6	—	98·4 100·4
50·8	—	51·1	100·6

Recoveries of the combined glutamic acid and aspartic acid were good both by amino-nitrogen and by total nitrogen determinations. A blank determination of total nitrogen was negligible.

TABLE III

DETERMINATION OF TOTAL DICARBOXYLIC ACIDS IN GLUTEN HYDROLYSATES

Material	Glutamic acid added, %	Pope and Stevens amino-nitrogen method		Glutamic acid and aspartic acid calculated as glutamic acid from total nitrogen, %
		Glutamic acid and aspartic acid calculated as glutamic acid, %	Recovery of added glutamic acid, %	
Gluten hydrolysate (1) ..	—	7·85	—	9·7
	3·94	11·85	101·5	—
	8·06	15·59	100·5	—
Gluten hydrolysate (2) ..	—	9·05	—	9·9
	2·17	11·15	97·0	—
	6·15	15·1	98·4	—
Glutamic acid liquors ..	—	3·3	—	6·9
	6·12	9·35	98·5	—

Table III shows results obtained with gluten hydrolysates and with glutamic acid liquors. Recoveries of added glutamic acid were good, the results in each instance being for total dibasic acids calculated as glutamic acid. The total nitrogen retained on IR–4B resin always exceeded the amino-nitrogen with actual hydrolysates and liquors, indicating the value of a method for determining glutamic acid by an α-amino-nitrogen estimation in the eluates, rather than by determining total nitrogen, as some workers do. In this connection the comments of Fromageot and Colas[13] are of interest; they point out that the de-amination

of certain amino-acids, particularly tryptophan, results in the formation of acidic nitrogenous substances that would be retained on IR–4B resin.

TABLE IV

GLUTAMIC ACID AND ASPARTIC ACID CONTENT OF GLUTENS

Material	Total dicarboxylic amino-acids (calculated as glutamic acid), %	Aspartic acid in gluten, %	Glutamic acid in gluten, %	Protein (N × 5·7), %	Glutamic acid in protein, %
Maize gluten ..	21·7	1·5	20·0	70·5	28·3
Wheat gluten (1) ..	29·2	2·5	26·4	68·8	38·4
Wheat gluten (2) ..	32·4	1·9	30·2	79·5	38·0
Wheat gluten (3) ..	32·4	2·2	30·4	71·8	42·3
Wheat gluten (4) ..	25·5	2·3	23·2	64·2	36·1
Wheat gluten (5) ..	29·5	2·3	27·2	65·3	41·6

Table IV shows some results for glutens. That the estimation of aspartic acid by paper chromatography should suffer from greater inaccuracies than the estimation of total dicarboxylic amino-acids is not a serious disadvantage in dealing with proteins like those in gluten, in which the amount of aspartic acid is fairly small compared with the amount of glutamic acid. For comparison with previous figures the results of Jones and Moeller may be quoted; these workers found 25 per cent. of glutamic acid in glutenin and 44 per cent. in gliadin, these being the chief proteins of gluten. More recently, Miller, Leiffe, Shellenberger and Miller,[14] Rice and Ramstead[15] and Pence, Mecham, Edder, Lewis, Snell and Alcott,[16] using microbiological methods, have reported glutamic acid contents of wheat gluten from 30·4 to 35·5 per cent., calculated to a theoretical gluten containing 17·5 per cent. of nitrogen (corresponding to a nitrogen to protein conversion factor of 5·7). The results quoted in Table IV are somewhat higher than these, although the samples examined were reputed to have a high gliadin content.

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THE BRITISH DRUG HOUSES LTD.
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The Identification and Determination of the Lower Straight-chain Fatty Acids by Paper Partition Chromatography

By R. E. B. DUNCAN and J. W. PORTEOUS*

An improved method of detecting and determining the lower (C_2–C_6) straight-chain fatty acids is described; it involves running paper chromatograms in *n*-butanol - ammonia mixtures and spraying with a mixture of methyl red - bromothymol blue indicators in formalin. The critical factors for obtaining reproducible chromatograms applicable to the range 5 to 80 μg of each acid are described. A variation has been noted in the relationship between spot area and the acid concentration with the indicator used. The method has been applied to two problems, volatile fatty acid production in grass fermentations, and the metabolism of *Actinomyces israeli*.

The difficulties underlying the chromatographic separation and identification of the lower volatile fatty acids have been summarised by Elsden.[1] Since that date several paper partition chromatographic separations of the volatile fatty acids have been devised by Brown,[2] Brown and Hall,[3] Hiscox and Berridge,[4] Kennedy and Barker,[5] Miettinen and Virtanen[6] and Reid and Lederer.[7] All these methods are similar in principle: 2 to 10-μl spots containing 5 to 100 μg of each of the fatty acids as their sodium or ammonium salts are applied to the chromatographic paper; the solvent system is *n*-butanol, ethanol or both, equilibrated with ammonium hydroxide; the spots are subsequently revealed by spraying the dried chromatogram with an acid - base indicator solution; bromothymol blue, bromophenol blue, bromocresol green and bromocresol purple solutions adjusted to various pH values have been used. The fatty acid hydroxamates have also been used (Fink and Fink,[8] and Thompson[9]).

Of the methods available we have found that of Reid and Lederer[7] to be the most satisfactory because of its simplicity and quantitative nature. We could not, however, obtain consistent results with less than 20 μg of the individual acids, so this paper is concerned with the systematic elaboration of the critical factors involved in a more reliable technique.

Method

Reagents—

n-*Butanol*—Redistil as required through a 12-inch fractionating column, collecting the fraction boiling between 116° and 118° C.

Ethanol—Reagent grade.

Ammonium hydroxide solutions—(*a*) 1·5 *N* ammonium hydroxide. Distil reagent grade ammonium hydroxide, sp.gr. 0·880, into distilled water at 2° C to give an approximately 10 *N* solution. Dilute this as required to 1·5 *N* with freshly distilled water.

(*b*) 3 per cent. ammonium hydroxide. Prepare by diluting reagent grade ammonium hydroxide, sp.gr. 0·880, with tap water.

Formaldehyde solution, 40 per cent.—Made from formalin, B.P.

Indicator spray—Dissolve 200 mg each of methyl red and bromothymol blue in a mixture of 100 ml of formalin and 400 ml of ethanol. Add 0·1 *N* sodium hydroxide until a reading of 5·2 is obtained on a glass-electrode pH meter.

Fatty acids—Acetic, propionic, *n*-butyric, *n*-valeric, *n*-hexanoic and DL-lactic acids. Acetic and DL-lactic acids were of analytical reagent quality and the others of reagent quality in the work described here.

Procedure—

Standard acid solutions—Prepare separate aqueous solutions of each member of the homologous series, acetic to hexanoic acid, and titrate against the pH meter to a pH value

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of 8·0 with freshly prepared 1·5 *N* ammonium hydroxide. Mix these stock solutions and dilute them as required to give solutions in which the individual acid concentrations range from 5 to 80 μg per 5 μl. Apply 5-μl spots of the desired mixed acid solution to the chromatographic paper as described below.

The solvent system—Equilibrate *n*-butanol and 1·5 *N* ammonium hydroxide by shaking equal volumes of them together. Use 15-ml aliquots of the upper (butanol) layer as the mobile phase for each chromatogram; place an equal volume of the lower (aqueous) layer separately in the tank to maintain equilibrium conditions.

Preparation of the chromatographic paper—Wash 11·25-inch by 9·25-inch sheets of Whatman No. 54 chromatographic paper by serial transfer in pairs through three shallow photographic dishes each containing 400 ml of the butanol layer of a 1 + 1 butanol and 1·5 *N* ammonium hydroxide system to which 5 per cent. v/v of ethanol has been added after separation of the phases. Allow 10 minutes for each wash. Hang the papers individually in a fume cupboard, keeping them out of contact with any metal, until dry, and then weigh them to the nearest 100 mg.

Running the chromatogram—Arrange the starting points for the chromatograms at intervals of 2·5 cm along a line 6 cm from the bottom of the sheet, with the outer points not less than 5 cm from the sides. Apply spots of standard and unknown solutions from a 5-μl pipette. Transfer the sheets to the mobile (butanol) phase in Petri dishes in the tank, and run the chromatograms by upward displacement for 15 hours at 18° to 20° C, during which time the solvent reaches the top of the paper.

Drying and spraying the papers—Remove individual papers from the tank at 15-minute intervals, weigh them immediately, and hang them to dry at room temperature in still air in a fume cupboard. Reweigh at 10-minute intervals until the papers return to their original dry weight; 10 minutes later spray the paper with the solution of methyl red and bromothymol blue indicator. Deliver the indicator as evenly as possible from an all-glass "atomiser," with a rotary pump supplying the air blast. At the first even tinting of the paper faint yellow spots usually show on a pink background. Continue to spray until these spots are obliterated and the paper is evenly damped through to the back. About 30 ml of indicator solution, delivered in approximately 90 seconds, is required for each sheet.

Colour development on the chromatogram—Acid spots are shown on the sprayed chromatogram, which has an intense red colour, by dipping the paper (in cylindrical form) immediately into ammonia vapour contained in a tall cylinder 6 inches in diameter at the bottom of which is a layer of 3 per cent. v/v ammonium hydroxide. It is necessary to expose the paper to the ammonia vapour as evenly as possible, and to develop the alkaline (dark green) colour of the indicator rapidly on the paper. The acids show as orange or red spots. After the paper has been exposed for 2 to 3 seconds to the ammonia vapour, remove the paper, open it out and hold it away from the ammonia for about 1 minute, during which time the acid (red) colour of the indicator returns evenly to the whole paper. Coil the paper in the reverse direction, invert it and again hold it in the ammonia vapour for a few seconds until the alkaline colour reappears as an even background to the acid spots, after which remove the paper and allow the acid colour to return evenly to the whole paper. Repeat this procedure several times, gradually increasing the length of exposure to the ammonia vapour to 10 to 15 seconds as the time taken for the alkaline colour to develop in the ammonia vapour increases. After four or five exposures acid spots fail to appear at all, but reappear with increased intensity in later exposures to the ammonia, and at the same time the green background becomes more and more stable between exposures. Finally, intense red spots persist on a green background. The chromatograms can be marked at any time within the next 20 minutes. Thereafter the background returns slowly to an even pale brick-red colour; the acid spots remain visible as dark red areas for some time, but over periods ranging from 1 to 24 hours, depending on the concentration of the acid in the spot, the acid spots merge completely into the background.

Quantitative analysis—Trace the acid spots on graph paper and measure the areas. The standard acid solutions chromatographed along with the unknown solutions are those containing 20 and 50 μg per 5 μl of each acid. Estimations are made from the mean of four results from quadruplicate chromatograms. A linear relationship exists between spot area and the acid content of the spot; the spot areas of standard acids are plotted against the concentration of each acid to give regression lines from which the concentrations of acids in experimental solutions are estimated.

Experimental

The method described above gives the optimum conditions for the chromatographic identification and determination of the normal C_2 to C_6 fatty acids; it was elaborated only after exhaustive investigation of each of the possible variable factors involved.

Reid and Lederer[7] used bromocresol purple as an indicator spray; acid spots on the chromatograms showed as yellow spots on a purple background. Using this method we found that acid spots often failed to appear in several of eight replicate chromatograms treated identically. When acid spots did appear they were often indefinite and the background, instead of being entirely purple, presented a purple and yellow mottled appearance. The identification of "unknown" spots was then difficult. With standard fatty acid solutions, acid spots could be identified fairly consistently at concentrations of 20 μg per 5 μl; at 10 μg per 5 μl identification was possible in about 75 per cent., and at 5 μg per 5 μl in about 50 per cent. of the chromatograms.

To increase sensitivity and reproducibility it was necessary to eliminate this mottling effect and to improve spot definition and colour contrast.

Optimum conditions for each variable factor in the present technique were found. These conditions are described and discussed for each successive step in the method.

Pre-treatment of chromatographic paper—

Whatman Nos. 1, 4 and 54 filter-papers have been used, all three giving satisfactory results after suitable pre-treatment. Untreated papers and those saturated with vapour from concentrated ammonium hydroxide, sp.gr. 0·880 (Reid and Lederer[7]) showed marked mottling of the final chromatogram, as did papers washed with 1·5 *N* ammonium hydroxide. Washing with redistilled *n*-butanol prevented some of the mottling in the final chromatogram. Washing with the butanol phase of a system of equal volumes of butanol and 1·5 *N* ammonium hydroxide almost completely prevented mottling. During the washing procedure an aqueous phase sometimes separated out and on these occasions results were poor, presumably owing to uneven washing of the paper. Ethanol (5 per cent. v/v) added to the butanol phase after separation from the equilibrated system prevented the subsequent formation of an aqueous phase. R_F values of the acids varied with the paper and the pre-treatment, but were constant for any one set of conditions (see Table I). Lactic acid and acetic acid have the same R_F value and are indistinguishable at low concentrations. When each of these two acids was present to the extent of 30 μg per 5 μl or more, it was found that lactic acid gave a darker and more permanent spot within the acetic acid spot, so that the two could be observed simultaneously.

Table I

R_F values of the normal C_2 to C_6 fatty acids on various chromatographic papers after upward development with *n*-butanol saturated with 1·5 *N* ammonium hydroxide

	R_F values				
Paper*	Acetic acid	Propionic acid	Butyric acid	Valeric acid	Hexanoic acid
1 (W)	0·13	0·21	0·31	0·41	0·52
1 (U)	0·13	0·22	0·35	0·49	0·63
4 (W)	0·10	0·19	0·30	0·42	0·54
4 (U)	0·11	0·20	0·33	0·45	0·60
54 (W)	0·09	0·16	0·25	0·33	0·44
54 (U)	0·09	0·15	0·27	0·40	0·52

Notes—(W), washed with butanol saturated with 1·5 *N* ammonium hydroxide (see text); (U), untreated papers.

* Numbers refer to Whatman chromatographic papers.

Application of fatty acids to the paper—

The volatility of the lower fatty acids and of their ammonium salts has been the cause of one of the difficulties in the elaboration of a chromatographic technique (Elsden[1] and Reid and Lederer[7]). In the present work, however, it has been possible to obtain satisfactory

qualitative results over the range 5 to 50 μg per 5 μl by spotting the papers with the sodium, barium or ammonium salts of the acids 30 minutes before transferring the papers to the chromatographic tanks. Room temperature did not exceed 20° C. The sodium or the barium spots did not overlap the acetic acid spot, provided the total cation on the paper did not exceed approximately 0·5 micro-equivalents. At higher concentrations, the cation spots tended to obscure the acetic and even the propionic acid spots. Consequently the ammonium salts have been used in routine work (*cf.* Reid and Lederer[7]).

In qualitative experiments up to 40 μl of the ammonium salts have been applied to each starting point, 5 μl at a time, by the usual intermittent drying technique, without appreciably affecting the final result. For quantitative work, however, it is preferable to make only one 5-μl application to each starting point and to transfer the paper immediately to the tank.

PURIFICATION OF SOLVENTS—

Redistilled *n*-butanol was used throughout the investigations. In initial experiments a broad acidic band extending 3 cm up from the foot of the paper was apparent in the final chromatogram. This band was eventually traced to acidic material absorbed from the atmosphere by the stock ammonium hydroxide solutions. The use of 1·5 *N* ammonium hydroxide made by dilution of freshly distilled ammonia eliminated this acidic band and decreased mottling in the final chromatogram.

DRYING THE CHROMATOGRAM—

It is undesirable to spray damp chromatograms, as movement and spreading of spots can take place, and in the present instance residual ammonia in the paper tends to swamp the indicator. Papers dried over long periods at room temperature (18° to 20° C) in still air also gave poor results, presumably because the ammonium salts of the acids volatilised. Results were best on spraying the papers 10 minutes after they had returned to their original dry weight in still air at room temperature. Table II shows typical drying times. Considerable variation in drying times is apparent for identically treated papers.

TABLE II

THE LOSS OF MOBILE PHASE (*n*-BUTANOL SATURATED WITH 1·5 *N* AMMONIUM HYDROXIDE) FROM VARIOUS CHROMATOGRAPHIC PAPERS, 11·25-INCH BY 9·25-INCH, WHEN DRIED IN STILL AIR AT 18° TO 20° C

	Mobile phase on the paper at various times after removing the paper from the chromatographic tank, g						
Paper*	0 mins.	10 mins.	20 mins.	30 mins.	40 mins.	50 mins.	60 mins.
1 (U)	6·8	4·3	2·6	0·9	0·0	0·0	0·0
1 (U)	7·3	4·8	3·3	1·8	0·6	0·0	0·0
1 (W)	7·1	4·6	3·0	1·5	0·5	0·0	0·0
1 (W)	7·2	5·5	3·8	2·0	1·0	0·3	0·1
54 (U)	5·8	4·0	2·8	1·4	0·6	0·1	0·0
54 (U)	5·2	2·8	1·4	0·3	0·2	0·0	0·0
54 (U)	4·3	1·5	0·1	0·0	0·0	0·0	0·0
54 (W)	6·1	3·9	1·9	0·3	0·1	0·0	0·0
54 (W)	5·8	2·5	1·0	0·3	0·0	0·0	0·0
54 (W)	6·3	4·2	2·5	1·1	0·5	0·1	0·0

NOTES—(U), Untreated; (W), washed with *n*-butanol saturated with 1·5 *N* ammonium hydroxide.
* Numbers refer to Whatman chromatographic papers.

INDICATOR SPRAY—

Theoretically an indicator with a pH range of between 4 and 9 should be used to reveal the fatty acids. Accordingly, a number of indicators within this range and just outside it were selected for trial. These indicators, with their pH ranges, were: bromophenol blue (3·1 to 4·4), bromocresol green (4·0 to 5·6), methyl red (4·2 to 6·3), bromocresol purple (5·2 to 6·8), bromothymol blue (6·0 to 7·6), phenol red (6·8 to 8·4), cresol red (7·2 to 8·8), thymol blue (8·0 to 9·6), phenolphthalein (8·3 to 10·5) and thymolphthalein (9·3 to 10·5).

There was no colour differentiation between background and acid spots with bromophenol blue, thymol blue, phenolphthalein or thymolphthalein. The remainder of the

indicators were taken singly (0·04 per cent. w/v in a mixture of ethanol and formalin) and in pairs (0·02 per cent. w/v of each) to give 21 indicators, all of which were put through comparative tests with unwashed papers. Of these 21 indicators, those containing bromocresol green were poorest owing to lack of good colour contrast, those containing cresol red or bromocresol purple gave good colour contrast, whilst those containing bromothymol blue, phenol red or methyl red gave good contrast and improved spot definition. In this last group, one mixture, bromothymol blue and methyl red, was markedly superior to the rest and was adopted for the method used; the concentration of each component indicator was increased to 0·04 per cent. w/v to intensify the colours.

COLOUR DEVELOPMENT IN THE FINAL CHROMATOGRAM—

Successful colour development depended on intermittent, but even, exposure of the sprayed chromatogram to the optimum concentration of ammonia vapour in the manner described. Attempts to develop the colour in an "ammonia tank" of large cross-sectional area with either continuous or intermittent exposure of the paper to the ammonia vapour were not successful, presumably because of uneven loss of vapour when the tank was opened to insert the paper. In the present work a tall cylinder, at least 12 inches deep and of just sufficient diameter (6 inches) to accommodate the roll of paper, has been used; whenever the paper was removed the cylinder was closed by a glass plate to maintain a uniform concentration of ammonia.

In the present technique there was a tendency for a strip round the edge of the paper to develop more rapidly than the rest of the paper. For this reason, particularly in quantitative work, spots were not applied within 5 cm of the edge of the paper.

QUANTITATIVE ANALYSIS—

Using bromocresol purple as the spray reagent and Whatman No. 1 paper, Reid and Lederer[7] obtained a linear relationship between spot area and the logarithm of the acid concentration. With Whatman No. 4 paper and the technique described above, but with Reid and Lederer's spray reagent, this relationship has been confirmed. When, however, the mixed indicator spray of methyl red and bromothymol blue was used, a linear relationship between spot area and concentration of acid was observed over the range 16 to 80 μg per 5 μl for each acid. With standards solutions containing 16 and 80 μg per 5 μl of each acid, regression lines from the means of spot areas in quadruplicate runs have been drawn and the spot areas for other concentrations of acids predicted. The deviations of the predicted from the actual concentrations are shown in Table III.

TABLE III

DEVIATIONS OF PREDICTED ACID CONCENTRATIONS FROM ACTUAL ACID CONCENTRATIONS WHEN SPOT AREA IS PROPORTIONAL TO ACID CONCENTRATION FOR THE NORMAL C_2 TO C_6 FATTY ACIDS

Acid	Range of concentration, μg per 5 μl	Number of concentrations tested	Mean deviation, μg per 5 μl	Bias	Standard deviation, μg per 5 μl	Error at mid-point of range, %
Acetic	8 to 80	6	3·16	+0·278	3·75	7·8
Propionic	"	"	3·16	+1·58	3·55	7·4
Butyric	"	"	3·0	+0·417	3·60	7·5
Valeric	"	"	3·71	+0·958	4·32	9·0
Hexanoic	"	"	2·88	+1·04	3·48	7·25

It will be noted that the bias, *i.e.*, the "mean deviations taking acount of sign," is persistently positive (Table III). With the logarithmic relationship, Reid and Lederer obtained negative figures and suggested these might be partly caused by personal errors in over estimating spot areas of the acids at lower concentrations.

The perimeter of the coloured spot observed in the sprayed and developed chromatogram marks the minimum concentration contour at which the indicator in the spray reagent will show the acid in the spot. The lower the detectable concentration contour, *i.e.*, the more sensitive the reagent, the larger the spot and the less well-defined is the spot boundary.

Visual appreciation of the coloured spot boundary, *i.e.*, the precision with which the spot area can be estimated, will depend on the colour contrast between the spot and the background. Both of these possible sources of variation in the estimated size of the visible spot are inherent in the method and could vary with the indicator used. It is not surprising, therefore, that the use of two different indicators should give two different relationships between visible spot area and acid concentration, nor is it surprising that the experimental deviations from these relationships are of opposite sign in the two methods. Personal errors in tracing spot boundaries and estimating spot areas add a further complication. It is clear that experimental conditions should be rigidly standardised if the method is to be used quantitatively.

With the optimum conditions described above, mottling was completely eliminated from the chromatograms; colour contrast and spot definition were excellent. The normal C_2 to C_6 fatty acids at concentrations between 5 and 80 μg per 5 μl (0·1 and 0·8 per cent. w/v) were readily handled simultaneously in one application to give reproducible chromatograms without tailing or overlapping of spots. Identification over the range stated and determination over the range 16 to 80 μg per 5 μl were consistent for each acid. Two assistants, untrained in any form of chromatographic technique, were immediately able to attain results conforming with the authors'. Fig. 1 shows a typical chromatogram.

Applications

One of us (R. E. B. D.) has successfully applied the technique described to the identification and determination of the C_2 to C_6 fatty acids in laboratory grass - water fermentation mixtures and in field silages. In both instances it was necessary to have available a method for detecting and estimating the C_5 and C_6 acids at small concentration in the presence of relatively large concentrations (7 to 10 times as much) of the C_2 to C_4 acids as a preliminary to fuller investigation by gas-phase partition chromatography (James and Martin[10]).

One of us (J. W. P.) has used the method to discover whether or not the slow-growing anaerobic pathogen *Actinomyces israeli* produced any of the C_2 to C_6 fatty acids. It has been shown by Erikson and Porteous[11] that the organism produced lactic acid when grown in a glucose broth medium containing lactic acid and sometimes also acetic, propionic and butyric acids; a particularly sensitive method applicable to low concentrations of fatty acids was therefore essential.

We wish to acknowledge the receipt by one of us (R. E. B. D.) of a D.S.I.R. grant and by the other (J. W. P.) of a M.R.C. personal grant.

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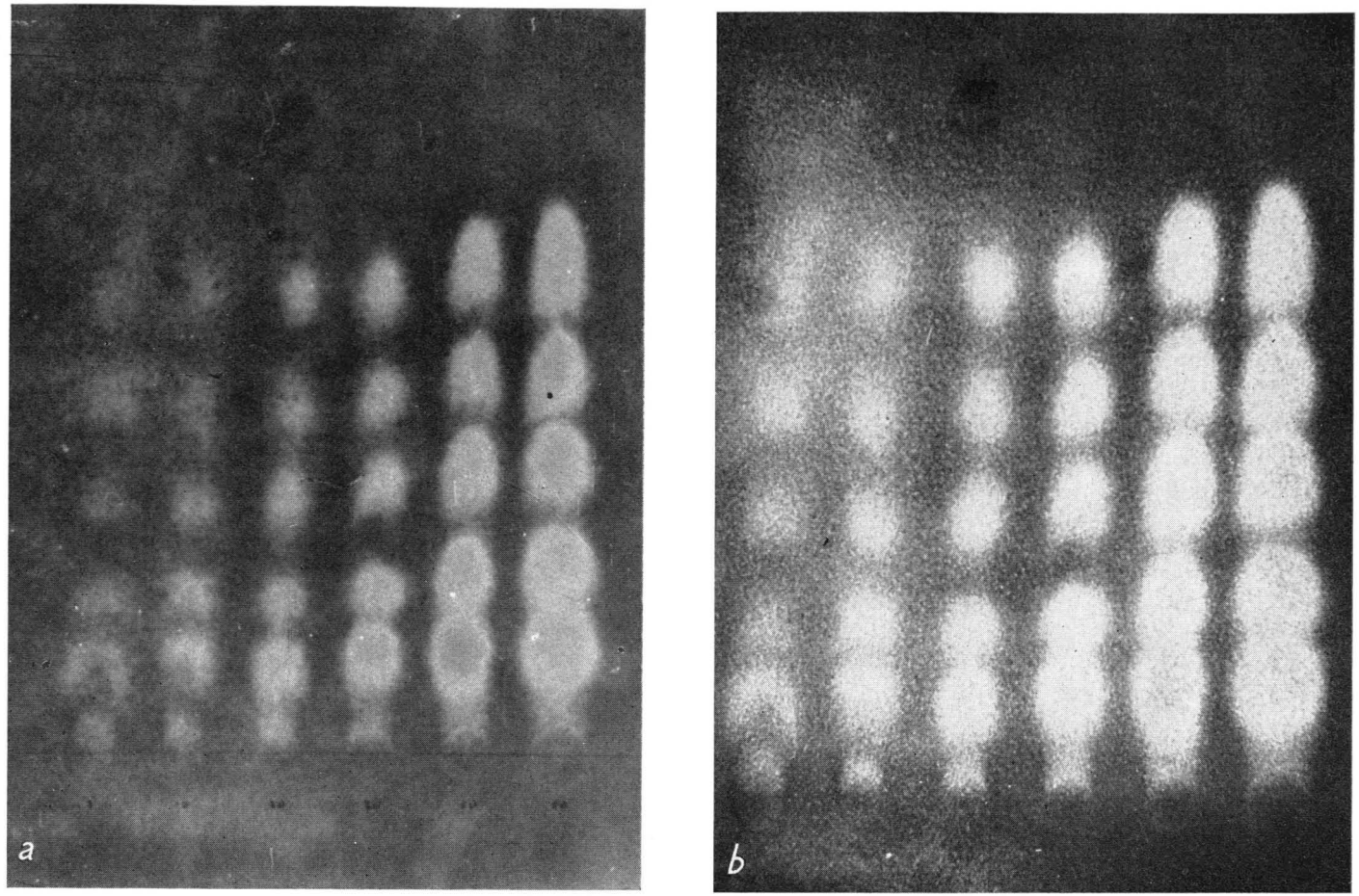

Fig. 1. Separation of the C_2 to C_6 volatile fatty acids. The same chromatogram photographed by (*a*) reflected and (*b*) transmitted light. Concentrations of each acid (μg per 5 μl) from left to right: 5, 10, 20, 30, 50, 80. In (*b*) the grain of the chromatographic paper is enhanced when photographed by transmitted light

The Determination of *iso*Butyraldehyde in *n*-Butyraldehyde

By G. R. PRIMAVESI

*iso*Butyraldehyde and *n*-butyraldehyde are reduced to the corresponding alcohols by sodium borohydride. The *iso*butyl alcohol is then estimated absorptiometrically by means of a modified Komarowsky reaction for higher alcohols, of improved reproducibility, carried out under controlled conditions.

In Komarowsky's method[1] for the determination of higher alcohols by means of the colour produced on heating with concentrated sulphuric acid and an alcoholic solution of salicylaldehyde, *iso*butyl alcohol gives a colour from 100 to 150 times deeper than that produced by an equal amount of *n*-butyl alcohol.

This difference in the behaviour of the two alcohols forms, after a reduction by sodium borohydride, the basis of a proposed method for the absorptiometric determination of *iso*-butyraldehyde in *n*-butyraldehyde.

Many modifications have been made to Komarowsky's method; these include the use of substituted benzaldehydes other than salicylaldehyde or furfural, variations in the methods of cooling or heating the reaction mixture, and variations in the method and order of adding the reagents and test samples.[2,3,4,5,6,7,8,9] Most of these modifications deal with considerably larger amounts of higher alcohols than the method now described. Some authors have had trouble with high blanks.

It has been found that the following variables affect the degree of colour developed: the proportions of sulphuric acid, ethanol, water, salicylaldehyde and *n*-butyl alcohol present in the reaction mixture; the technique of adding the reagents and mixing the reaction solution; the temperature at which the reaction is carried out; the duration of heating and the time of standing after cooling the mixture. Of these variables the addition of the reagents and mixing the reaction mixture need the closest attention; the others are important, but are merely a matter of ordinary control. The total amount of ethyl alcohol plus water is more critical than slight variations in the amount of either.

The blank gave no difficulty with the ethyl alcohol used; this was P.I. rectified spirit (96·1 per cent.) supplied by The Distillers Co. Ltd. This spirit is free from aldehydes and contains less than 1 p.p.m. of higher alcohols as *iso*butyl alcohol, which figure represents the whole of the yellow colour produced by the reagents and sulphuric acid. The amount of *iso*butyl alcohol that gives a reading on the Spekker photo-electric absorptiometer of 0·1 higher than the blank under the conditions described is 6 μg; the corresponding figure for *n*-butyl alcohol is 830 μg.

The object of the technique now described is to prevent, as far as possible, any heating of the reaction mixture during the mixing process and subsequently to control accurately the duration and temperature of heating. If this is done carefully the standard error of a single determination is equal to a Spekker drum reading of about 0·010 anywhere on the scale.

As the object was to estimate *iso*butyraldehyde in *n*-butyraldehyde, all series of experiments were made with a standard concentration of total butanol and various ratios of *iso*- to *n*-butyl alcohol. A blank on ethyl alcohol was included in each series. The conditions described were determined by means of many statistically planned experiments and were chosen to give the maximum difference between *iso*- and *n*-butyl alcohol, combined with reasonable insensitivity to slight variations in timing and dilution.

Method

Reagents—

Ethyl alcohol—This must be free from aldehydes and higher alcohols. P.I. rectified alcohol (96·1 per cent.) supplied by The Distillers Co. Ltd. is satisfactory.

Salicylaldehyde reagent—Dissolve 0·167 g of laboratory grade salicylaldehyde in 100 ml of ethyl alcohol. The concentration is arbitrary, but for use with a standard graph it must be the same as that used in the preparation of the graph. Accordingly, the aldehyde content

should be determined by the hydroxylamine[10] or other suitable method and the strength of reagent should be adjusted if necessary.

Concentrated sulphuric acid—This should be of recognised analytical purity.

PREPARATION OF THE SAMPLE—

Prepare an ethanolic solution of the sample, by successive dilution if necessary, to give the required total butyl alcohol content. Of the calibration series given below, one was done with a total ethyl alcohol volume of 6·5 ml by using a 3-ml sample of a 0·045 per cent. v/v solution of total butyl alcohol in ethyl alcohol, the *iso*butyl alcohol ranging from 0 to 4 per cent. v/v of the total butyl alcohol. The other series was done with a total ethyl alcohol volume of 6 ml by using a 3-ml sample of a solution of 0·02 g of total butyl alcohol in 100 ml of ethyl alcohol, the *iso*butyl alcohol ranging from 0 to 7·84 per cent. w/w of the total butyl alcohol.

PROCEDURE—

Measure from a burette 10 ml of concentrated sulphuric acid into a 7 × 1-inch test tube and add from a pipette 3 ml of the salicylaldehyde reagent; the tube should be held vertically, resting on the bench, and the solution run carefully down the side to form a layer; without moving the tube, add, similarly, from a pipette 3 ml of the sample solution. Immediately immerse the end of the tube in solid carbon dioxide - ethyl alcohol mixture (temperature about −78° C) and swirl vigorously for 15 seconds. Remove the tube and mix the contents thoroughly. This is best done by first twirling the tube held almost horizontally, so as to wet the entire inner surface, and then swirling the tube held vertically. Repeat the mixing at least once more. It is most important to get the two liquids of widely differing densities, viscosities and volatilities completely homogeneous without undue heating; the mixture should be at or slightly below room temperature when mixed, and, in order to attain this end, the time of cooling in the carbon dioxide bath should be varied to suit the mode of action of the operator. When its contents are homogeneous, place the tube in a thermostat bath at 80° C for exactly 30 minutes; remove it and cool it in tap water. Exactly 10 minutes after removal from the bath, read the optical absorption on the Spekker absorptiometer, fitted with a 2-cm cell and Ilford No. 605 filters, against distilled water in a 2-cm cell.

For reproducible results the greatest care must be taken to ensure that the addition and mixing of the sample and reagents are done in a reproducible manner.

THE DETERMINATION OF *iso*BUTYRALDEHYDE IN *n*-BUTYRALDEHYDE

PRELIMINARY TREATMENT AND REDUCTION OF SAMPLE—

Take 1 ml of sample in 50 ml of water and flash distil into an ice-cooled 100-ml standard flask until no "tears" are visible in the condenser and for 3 or 4 minutes further. The volume taken need not be exact as the final aliquot is adjusted later, but should be within ±10 per cent. of 1 ml. Make the distillate up to 100 ml with water and immediately take two 10-ml aliquots. Analyse one aliquot for total aldehyde by the hydroxylamine method; to the other in a 50-ml calibrated flask add 10 ml of a freshly prepared solution of 0·15 g of sodium borohydride in 100 ml of water; the amount is not critical but it must be an excess to ensure complete reduction of all the butyraldehyde (not merely the *iso*butyraldehyde). The reagent is unstable, but keeps for at least a day in aqueous solution. Allow the solution to stand in the stoppered flask for at least 10 minutes and then make it up to 50 ml with water. The time of standing can, if necessary, be prolonged indefinitely.

From the titre given by the hydroxylamine method calculate the volume of reduced aldehyde solution required to give exactly 15 mg of total butyl alcohol; take this volume and make it up to 50 ml with pure ethyl alcohol. This total butyl alcohol concentration of 0·030 g per 100 ml in the final solution was chosen for estimating about 4 per cent. of *iso*butyraldehyde in *n*-butyraldehyde. If higher or lower concentrations are expected, the total butyl alcohol content of the final solution and the standard graph should be modified accordingly.

The reduction of the aldehyde by sodium borohydride was based on work by Chaikin and Brown.[11] They state the reaction to be—

$$4RCHO + NaBH_4 + 2NaOH + H_2O \rightarrow 4RCH_2OH + Na_3BO_3$$

But results of better reproducibility were attained by omitting the alkali, the reaction presumably being—

$$4RCHO + NaBH_4 + 2H_2O \rightarrow 4RCH_2OH + NaBO_2$$

The reagent solution seems to be stable for at least a day, provided that the solution is not acid. Acid causes immediate evolution of hydrogen—

$$NaBH_4 + HCl + 3H_2O \rightarrow NaCl + H_3BO_3 + 4H_2.$$

Aldehydes react in the Komarowsky test to give a colour. Mole for mole, *n*-butyraldehyde gives about one-fifteenth and *iso*butyraldehyde about one-fifth as much colour as *iso*butyl alcohol. It is therefore essential to ensure that no aldehyde or potential aldehyde is present at the end of the reduction stage. Polymers of the aldol type would probably give a colour even if reduced to the corresponding hydroxy-alcohols, and paraldehyde-type polymers are probably not reduced by the neutral or slightly alkaline reagent. It is in order to remove interfering substances of this kind that the sample is flash distilled immediately before analysis. The distillation is carried out in a large excess of water to save manipulation and unnecessary oxidation of the distillate. It is assumed that any oxidation that does take place affects the two aldehydes proportionately. Neither *iso*- nor *n*-butyric acid give a colour in the test.

ABSORPTIOMETRIC ESTIMATION OF THE *iso*BUTANOL—

Proceed with the Komarowsky test exactly as described above, using 3 ml of the final solution in ethyl alcohol as sample. Read the proportion of *iso*- to *n*-butyraldehyde from a standard graph prepared as described below.

PREPARATION OF THE STANDARD GRAPH—

Synthetic mixtures were made by weighing the two aldehydes direct into a small stoppered weighing flask. Each aldehyde was flash distilled immediately before making up any mixture and each mixture was analysed immediately after making up. These precautions were taken to ensure that a minimum quantity of butyric acid was present in the standard samples.

The two aldehydes used as samples were obtained from a careful fractionation of the ordinary laboratory stock of each aldehyde. The *iso*butyraldehyde fraction used had b.p. 64·6° C at 760 mm of mercury and n_D^{20} of 1·3728 and the *n*-butyraldehyde fraction b.p. 74·9° C at 760 mm of mercury and n_D^{20} of 1·3795.

RESULTS

Four replicate determinations were made on 1 ml of each mixture. The final colorimetric estimation was made in duplicate in each determination. Tables I and II show results for standard series of solutions. In Table III the volume of the aliquot used in making the final dilution is given to show the kind of variation that can occur without greatly affecting the result.

STANDARD SERIES—

TABLE I

RESULTS FOR STANDARD SERIES: 0 TO 4 PER CENT. V/V OF *iso*BUTYL ALCOHOL IN *n*-BUTYL ALCOHOL

Reaction mixture: 10 ml of concentrated sulphuric acid, 3·5 ml of 0·143 per cent. v/v solution of salicylaldehyde in ethyl alcohol, 3 ml of 0·045 per cent. v/v solution of total butyl alcohol in ethyl alcohol

	Blank on ethyl alcohol	*Iso*- in *n*-butyl alcohol, per cent. v/v				
		0	1	2	3	4
Spekker readings	0·193	0·297	0·539	0·738	0·938	1·095
	0·174	0·298	0·535	0·738	0·931	1·110
	0·183	0·287	0·541	0·748	0·939	1·094
	0·184	0·278	0·525	0·748	0·922	1·076
		0·306		0·732		
		0·293		0·735		
		0·299		0·732		
		0·309		0·739		
Mean	0·184	0·296	0·535	0·739	0·933	1·094
Colour due to *iso*butyl alcohol, *i.e.*, mean − "0" figure	—	—	0·239	0·443	0·637	0·798
Standard error	0·008	0·010	0·007	0·006	0·008	0·014
Ditto as a percentage of "*iso*" colour	—	—	3	1·4	1·2	1·8

TABLE II

RESULTS FOR STANDARD SERIES: 0 TO 8 PER CENT. V/V OF *iso*BUTYL ALCOHOL IN *n*-BUTYL ALCOHOL

Reaction mixture: 10 ml of concentrated sulphuric acid, 3 ml of a solution of 0·167 g of salicylaldehyde in 100 ml of ethyl alcohol, 3 ml of a solution of 0·020 g of total butyl alcohol per 100 ml of 10 per cent. v/v water in ethyl alcohol

	Blank on ethanol	*iso*Butanol in *n*-butanol, per cent. w/w								
		0	0·98	1·96	2·94	3·92	4·90	5·88	6·86	7·84
Spekker readings ..	0·111	0·190	0·308	0·403	0·528	0·618	0·718	0·815	0·939	1·012
	0·114	0·186	0·293	0·405	0·512	0·631	0·719	0·826	0·909	1·018
	0·113	0·194	0·294	0·409	0·514	0·633	0·732	0·829	0·934	0·997
		0·179		0·386		0·595		0·827		1·000
		0·178		0·393		0·610		0·817		0·998
		0·182		0·384		0·612		0·812		0·992
Mean	0·113	0·185	0·298	0·397	0·518	0·617	0·723	0·821	0·927	1·003
Colour due to *iso*butyl alcohol, *i.e.*, mean — "0" figure ..	—	—	0·113	0·212	0·333	0·432	0·538	0·636	0·742	0·818
Standard error	—	0·006	0·008	0·010	0·009	0·014	0·008	0·007	0·016	0·010
Ditto as a percentage of "*iso*" colour	—	—	7	5	3	3	2	1	2	1

TABLE III

APPLICATION OF PROCEDURE TO KNOWN MIXTURES OF BUTYRALDEHYDE

	*iso*Butyraldehyde in *n*-butyraldehyde, per cent. w/w									
	0		0·94		2·33		3·47		4·38	
	1*	2†	Col. 1	Col. 2	Col. 1	Col. 2	Col. 1	Col. 2	Col. 1	Col. 2
	10·78	0·319	10·39	0·496	10·81	0·700	11·15	0·882	10·72	1·000
		0·329		0·488		0·684		0·863		0·999
	9·84	0·337	10·92	0·468	11·32	0·679	10·34	0·891	10·52	1·035
		0·322		0·495		0·676		0·936		1·006
	9·97	0·343	10·59	0·514	12·45	0·663	10·76	0·898	10·95	0·956
		0·353		0·479		0·681		0·880		1·010
	10·45	0·332	10·45	0·483	10·39	0·714	11·26	0·881	11·85	1·020
		0·319		0·476		0·693		0·841		1·009
Mean absorptiometer readings	0·332		0·487		0·686		0·884		1·007	
Colour due to *iso*butyraldehyde, *i.e.*, mean — "0" figure	—		0·155		0·354		0·552		0·675	
Standard error	0·012		0·015		0·016		0·028		0·023	
Ditto as a percentage of "*iso*" colour	—		10		5		5		3	

NOTE—Subsequent work has shown that the *n*-butyraldehyde used as a standard contained 0·9 per cent. of *iso*butyraldehyde, so all Spekker absorptiometer readings in Table III should be reduced by 0·15 unit.

* Columns headed 1 show the volume of reduced aldehyde solution used in the final dilution to 50 ml.
† Columns headed 2 show the corresponding absorptiometer readings.

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The Quantitative Separation of Copper, Lead and Tin by Cathodic Deposition

BY G. H. AYLWARD AND A. BRYSON

A method is described for the quantitative separation of copper and lead from tin by cathodic deposition from phosphoric acid solution. The tin forms an anionic complex that is not reduced at the cathode and the copper is separated from the lead by deposition at a controlled potential. The lead is deposited in a metallic form and the tin is determined volumetrically. The difficulties associated with the quantitative cathodic deposition of lead have been investigated and optimum working conditions have been established to overcome these difficulties.

IN recent years increasing attention has been given to the problem of separating copper, lead and tin by controlled-potential deposition. Several of these methods[1,2,3,4] depend on the deposition of copper by controlled cathode-potential followed by simultaneous cathodic deposition of tin and lead. The tin and lead deposit is then dissolved and the metals are determined separately. It is possible, however, to increase the difference between the deposition potentials of these two metals by forming stable anionic stannic complexes. Lassieur[5] used this method to separate copper and lead dioxide simultaneously from tin in a solution containing hydrofluoric and nitric acids. Boric acid, sodium oxalate, hydroxylamine and sodium hydroxide were then added and the tin was deposited cathodically from the hot solution. His results reported to the nearest milligram show errors in each estimation of up to 1 mg. Lingane and Jones[6] made a complex of tin with tartrate and separated copper from lead in slightly acid solution in the presence of hydrazine dihydrochloride. After deposition of lead, hydrochloric acid was added and the tin was deposited. The lead figures show losses of from 0·3 to 2·1 mg, whilst five tin figures have an average deviation of 0·7 mg. Norwitz[7] used phosphoric acid to keep tin in solution in a nitric acid electrolyte and thus avoided the tedious separation of metastannic acid before electrolytically depositing copper. The stability of tin phosphate complexes is shown by the distillation of antimony chloride at 160° C without interference from stannic chloride if phosphoric acid is present.[8]

Polarograms were taken for copper, lead and tin in phosphoric acid and revealed little change in the half-wave potentials of copper and lead, whereas no step could be found from the tin in acid or ammoniacal solutions. These observations prompted the present investigation, which gives a method for the successive cathodic deposition of copper and lead from phosphoric acid, followed by tin if desired. We believe that the volumetric method for tin is more suitable and therefore have used this method throughout our work.

The cathodic deposition of lead presents several difficulties, which have been well recognised in the past and which have led many to believe that quantitative electro-deposition of the metal is impossible. Among these difficulties are the unsatisfactory nature of the deposit and the tendency to re-solution during the washing process. During the progress of this research, these and other difficulties were met and systematically investigated. As a result it has been possible to formulate a method that reduces these errors and allows a quantitative determination of the element.

In the procedure adopted, the alloy containing copper, lead and tin is dissolved in hydrochloric and nitric acids, phosphoric acid is added and the solution acids are expelled by

fuming. The solution is diluted to 120 ml and copper is deposited at −0·35 volt with respect to the saturated calomel electrode (S.C.E.) by controlled-potential electrolysis. After weighing, the copper-covered cathode is replaced in the solution and the lead is deposited, without control, as the tin phosphate complex is not decomposed in this electrolyte. When lead deposition is complete, the solution is neutralised with ammonium hydroxide. The electrolysis beaker is withdrawn and the plated electrode is washed with three successive solutions of ammonium sulphate and then with distilled water, alcohol and ether. The cathode is dried and weighed. Hydrochloric acid and nickel shot are added to the electrolyte and, after boiling, the reduced tin is titrated with iodine.

Experimental

Apparatus—

The apparatus comprised a Griffin and Tatlock electrolysis unit adapted for manual potential control by wiring a 240-volt "Variac" transformer into the electrode input circuit. The e.m.f. to the cell could be controlled by varying the alternating voltage applied to the transformer - rectifier circuit of the instrument. With this circuit the cathode potential could be held to within 20 mV of the required value. The smaller platinum gauze electrode, 75 sq. cm in area, was generally used as the cathode, as investigation showed that the lead deposition area should be as small as possible. Stirring was automatic and it was found that the design of the stirrer had some effect on the nature of the lead deposit. The most satisfactory stirrer was one that forced the flow of the electrolyte evenly through the mesh of the platinum gauze. Under these conditions the deposit is more uniform and finer in texture.

Electro-deposition of copper—

If the initial current-density does not exceed 1·5 amperes per sq. dm, copper will be deposited from phosphoric acid electrolyte as a bright salmon-coloured plate. If the cathode potential is not allowed to become more negative than −0·35 volt with respect to the S.C.E., separation of the copper from the lead and tin is quantitative. Four hundred milligrams of copper are deposited completely in 35 minutes on a platinum cathode 125 sq. cm in surface area.

Electro-deposition of lead—

Lead is deposited quantitatively from this electrolyte, but gives a dark powdery non-adherent deposit. This trouble was encountered in the early days of lead electroplating and was overcome by Betts,[9] who added gelatin to the plating bath. By the addition of 1 ml of a 0·5 per cent. gelatin solution to the electrolyte a bright grey metallic deposit is obtained.

Re-solution of lead during washing—

Schoch and Brown,[1] in their method for depositing lead from hydrochloric acid, washed the lead by substituting for the electrolyte successive beakers of distilled water, alcohol and ether. The greatest error in ten results was 0·7 mg with an average deviation of 0·3 mg. They recommended this method in preference to the anodic deposition of lead dioxide. Sand[10] preferred to deposit the dioxide, owing to the oxidisability of the lead deposit. Lingane and Jones,[6] who deposited metallic lead from tartrate solutions, recognised the loss of lead during washing and commented: "This loss averages 1·5 ± 0·6 mg and is sufficiently reproducible so that it can be added as a correction to obtain lead results which generally will be correct to well within 1 mg."

The technique of washing described by Schoch and Brown gave wash solutions that invariably contained lead, although none remained in the electrolyte. This finding was reported by Kny-Jones, Lindsey and Penney,[11] in connection with the re-solution of tin deposits from hydrochloric acid. These workers recognised that the loss was due to solution of the deposit in the film of electrolyte left in contact with the electrode on removing the electrolysis beaker. The procedure suggested by these authors of neutralising the electrolyte with ammonium hydroxide before washing was adopted for the present problem. But polarographic investigations showed that copper, equivalent to several tenths of a milligram, was dissolved from those parts of the copper-plated electrode exposed to the slightly ammoniacal

solution during the washing. This difficulty was overcome by ensuring that the lead deposit completely covered the copper. By this washing technique, loss by solution caused by the electrolytic action between the lead deposit and the platinum electrode is negligible.

ADSORPTION OF PHOSPHATE ON THE LEAD PLATE—

The weights of lead found by using this washing technique were consistently high. It was considered that the increase of weight was not caused by oxidation during drying of the deposit because a quick low-temperature method was used. It was proved, after numerous tests, that the high figures were caused by adsorbed phosphate ions. The modified washing technique described below was adopted; it removed the adsorbed phosphate film and simultaneously reduced the amount of re-solution of the lead deposit.

THE WASHING TECHNIQUE—

Experiments were carried out on lead-plated electrodes after deposition on copper-plated platinum from solutions of the analytical reagent grade metal. Distilled water, ammonium nitrate, ammonium sulphate, ammonium chloride and hydroxylamine wash solutions were tried. A fresh lead-plated electrode was immersed three times in 120 ml of each solution by moving the beaker up and down with the stirrer running. Each solution was tested polarographically for lead.

The only solution to offer any advantages over distilled water was 0·1 per cent. ammonium sulphate. Less lead was present in this solution and the deposit did not tarnish during drying.

In a second set of experiments, separate plated electrodes were treated with various numbers of ammonium sulphate washings and the lead deposit was weighed each time. The deposits were dissolved in nitric acid and the weight of adsorbed phosphate determined by the phosphomolybdate method. Each solution was tested for dissolved lead and its phosphate content was determined. The results are shown in Tables I and II.

TABLE I

THE REMOVAL OF PHOSPHORIC ACID FROM LEAD DEPOSITS BY INCREASING THE NUMBER OF WASH SOLUTIONS

	Wash method	Amount of lead taken, mg	Amount of lead found, mg	Difference, mg	Amount of phosphate on lead plate, mg
(*a*)	1 beaker of 0·1% $(NH_4)_2SO_4$ ..	50·0	51·0	1·0	0·80
(*b*)	2 beakers of 0·1% $(NH_4)_2SO_4$ ..	50·0	50·3	0·3	0·18
(*c*)	2 beakers of 0·1% $(NH_4)_2SO_4$ and 1 beaker of distilled water ..	50·0	49·9	0·1	0·03
(*d*)	3 beakers of 0·1% $(NH_4)_2SO_4$ and 1 beaker of distilled water ..	50·0	49·9	0·1	negligible

The results in Table I indicate that it is necessary to wash the lead deposit with at least three separate volumes of wash solution to reduce the weight of adsorbed phosphate to less than 0·05 mg.

TABLE II

PHOSPHATE AND LEAD TESTS ON WASH SOLUTIONS OF (*d*) IN TABLE I

Wash beaker	Phosphate content, mg	Polarographic test for lead, mg
First	171·6	0·12
Second	6·4	less than 0·05
Third	0·46	negligible
Fourth	0·12	negligible

From the results in Table II it is evident that ammonium sulphate effectively prevents re-solution of the lead during the washing procedure. A small amount of lead is present in the first wash beaker but not in the other three. Results are good for as little as 10 mg

by plating on a smaller surface area and using the four-beaker wash and finally an alcohol and then an ether rinse.

Method for determining the tin

The tin can now be quantitatively deposited if hydrochloric acid and hydrazine dihydrochloride are added, but we have used the iodimetric method after reducing the tin with nickel shot.

Procedure—

To the weighed sample, containing not more than 400 mg of copper, add 5 ml of 10 *N* hydrochloric acid and then 5 ml of 15 *N* nitric acid. When solution is complete, add 10 ml of phosphoric acid, sp.gr. 1·75, and evaporate the phosphoric acid to a syrupy consistency to remove the solution acids. Cool, add 120 ml of distilled water and electrolyse, using platinum electrodes, with the smaller gauze electrode as the cathode. Control the cathode potential at −0·35 volt with respect to the S.C.E. For the best copper deposit the initial current density should not exceed 1·5 amperes per sq. dm. When the current becomes constant at the control potential, after approximately 35 minutes, switch off the stirrer and lower the electrolysis beaker, washing the electrodes, calomel electrode and stirrer with distilled water. Switch off the current and remove the cathode. Rinse it in alcohol, dry at 105° C, cool and weigh.

Replace the weighed copper-plated electrode in the circuit. Add 1 ml of 0·5 per cent. gelatin solution to the electrolyte and adjust the electrolyte level so that all the copper is covered by the solution (approximately 200 ml). Electrolyse at a current density that causes no hydrogen gas to be liberated during the deposition of the lead. This is approximately 0·4 amperes per sq. dm, or at a cathode potential not lower than −0·8 volt with respect to the S.C.E. When electrolysis is complete, after 75 to 90 minutes, neutralise the electrolyte with 15 *N* ammonium hydroxide. Switch off the stirrer and quickly replace the electrolysis beaker by a 150-ml beaker containing 120 ml of 0·1 per cent. ammonium sulphate solution. With the stirrer running, immerse the electrodes three times by quickly moving the wash beaker up and down. Repeat this procedure with two fresh sulphate solutions and then wash the beaker successively with distilled water, alcohol and ether. Switch off the current and remove the cathode. Quickly dry the deposit at 100° C, cool and weigh.

After the removal of the copper and the lead, transfer the solution to a 500-ml conical flask and add to this the first lead wash solution. Add 75 ml of 10 *N* hydrochloric acid, reduce the tin with nickel shot and determine the tin iodimetrically using starch solution to indicate the end-point.

Results

Analyses of mixtures of copper, lead and tin are shown in Table III. Solutions for all the separations were made up from analytical reagent grade metals.

Table III

Results of copper, lead and tin determinations for synthetic alloys

Amount of copper		Amount of lead		Amount of tin	
taken, mg	found, mg	taken, mg	found, mg	taken, mg	found, mg
385·0	383·2	97·5	97·5	15·6	15·4
386·2	386·0	76·0	76·2	38·9	38·8
400·9	401·1	49·8	49·6	51·0	50·9
358·2	358·2	25·0	24·9	103·8	103·6
275·0	275·0	10·0	9·8	—	—
245·2	245·1	10·0	9·9	—	—
75·0	75·0	—	—	198·2	198·0
45·2	45·0	—	—	195·5	195·4
Higher range for lead ..		150·0	149·8		
		200·6	200·3		
		350·2	350·0		
		400·3	400·5		

Applications

The separation can be applied, with equally good results, to the determination of the three metals in copper-based alloys and to the determination of copper and tin in white metals. In 10 ml of phosphoric acid no more than 450 mg of copper and 60 mg of bismuth will remain in solution. Nevertheless, more than 500 mg of each of the following metals, tin, antimony, zinc, nickel, iron, lead, cadmium, aluminium, chromium and manganese, can be present in the solution without the formation of insoluble phosphates.

If bismuth is present it will be deposited before all the copper is removed. Copper is separated from antimony, which is partly reduced to the metal at approximately −0·76 volt with respect to the S.C.E. The antimony, however, is deposited simultaneously, but not quantitatively, with the lead. The problem of removing arsenic and antimony before electrolysing the copper is at present under investigation.

On neutralising the electrolyte with ammonium hydroxide to wash the lead deposit, the phosphates present prevent the precipitation of iron and aluminium. The cathode potential must not be more negative than the deposition potential of nickel, iron or zinc, for they will, if present, be deposited from slightly ammoniacal phosphate solution. Nickel is deposited first at −1·1 volts with respect to the S.C.E., if 200 mg of the metal are present in 200 ml of solution at room temperature. If the initial conditions of applied voltage are adhered to for the lead deposition, on neutralising the electrolyte the cathode potential will be more positive than this value and nickel will not be deposited.

This method enables the determination of copper, lead and tin in an alloy to be completed within 3½ hours. It avoids the tedious separation of the tin as metastannic acid and the separation of large amounts of lead by fuming with sulphuric acid. The method has two further advantages: it avoids filtering and, if the solution of the alloy and the electrolytic procedures are carried out in the same beaker, only one transfer of the analysis solution from vessel to vessel is necessary in the course of the determination.

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Diethylammonium Diethyldithiocarbamate for the Separation and Determination of Small Amounts of Metals

Part I. The Successive Determination of Small Amounts of Copper, Manganese and Iron in Organic Compounds

By P. F. WYATT

A scheme for the successive separation and absorptiometric determination of copper, manganese and iron is described, whereby iron is isolated as cupferrate, and copper and manganese separately as diethyldithiocarbamates. Methods are described for the absorptiometric determination of all three elements as their coloured diethyldithiocarbamate complexes in chloroform, and alternative absorptiometric procedures are indicated.

Tests on random mixtures of the three metals over the ranges 20 to 1000 μg of iron, 5 to 70 μg of copper and 10 to 200 μg of manganese show good recoveries.

THE determination of small amounts of copper and manganese in dyestuffs and other organic compounds used in the rubber industry and in rubber-proofed fabrics is often required, because these metals accelerate ageing of the rubber. In the method described here, iron, copper and manganese are successively separated and determined absorptiometrically. Separation of iron is necessary to avoid interference with the copper and manganese determinations, and provision is made for its determination if required.

The method depends on—

(*i*) the quantitative separation of ferric iron from copper and manganese by extraction of its cupferron complex with chloroform, provided the reaction is carried out at sufficiently high acidity,

(*ii*) the complete separation of copper from manganese by extraction of its diethyldithiocarbamate complex from mineral acid solution, and

(*iii*) the formation of a highly coloured (purplish-brown) complex of manganese with diethylammonium diethyldithiocarbamate in acetate-buffered solution, which is extractable with chloroform.

METHOD

REAGENTS—

All reagents must be of recognised analytical quality.

Aqueous cupferron solution, 5 per cent.—Prepare freshly from Hopkin and Williams' AnalaR reagent and filter.

Diethylammonium diethyldithiocarbamate solution—Dissolve 2 g of Hopkin and Williams' AnalaR reagent in 200 ml of redistilled chloroform and preserve the reagent in the dark in an amber-coloured bottle. Reject the solution when it begins to show any yellow discoloration.

Standard ferric iron solution—Dissolve 0·7023 g of ferrous ammonium sulphate, $(NH_4)_2SO_4.FeSO_4.6H_2O$, in about 100 ml of water containing 5 ml of concentrated sulphuric acid, add a slight excess of bromine water, boil off the excess of bromine, cool, and dilute to 1 litre at 20° C with water in a graduated flask. One millilitre is equivalent to 100 μg of iron.

Standard copper solution—Dissolve 0·3928 g of copper sulphate, $CuSO_4.5H_2O$, in water and dilute to 1 litre at 20° C with water in a graduated flask. One millilitre is equivalent to 100 μg of copper.

Dilute 10·0 ml of this solution to 100 ml at 20° C with water in a graduated flask as required. One millilitre of this diluted solution contains 10 μg of copper.

Standard manganese solution—Measure 45·5 ml of 0·1 *N* potassium permanganate into a 250-ml beaker. Alternatively dissolve 0·144 g of potassium permanganate in about 50 ml

of water. To either of these solutions add 5 ml of diluted sulphuric acid (1 + 1) and then a saturated aqueous solution of sulphur dioxide, about 1 ml at a time, until the solution is just decolorised. Dilute to about 150 ml with water and boil gently for 15 minutes. Cool, transfer to a 500-ml graduated flask and dilute to the mark at 20° C with water. One millilitre contains 100 μg of manganese.

Dilute 10·0 ml of this solution to 100 ml at 20° C with water to give a solution containing 10 μg of manganese per ml, as required.

PREPARATION OF SOLUTION—

Weigh accurately 2 to 5 g of prepared sample into a 100-ml Kjeldahl flask and decompose the sample with sulphuric and nitric acids, using 4 to 6 ml of concentrated sulphuric acid, depending on the weight of sample taken. Finally clean up the solution with a little perchloric acid.[1]

Evaporate twice to fuming with 5 to 10-ml portions of water to remove all nitric acid, and allow to cool. Add 10 ml of water and 10 ml of diluted hydrochloric acid (1 + 1), and boil gently for 3 to 5 minutes. Cool, filter through a small filter if necessary, washing with the minimum amount of water, transfer to a 50-ml separating funnel and dilute to 30 to 35 ml with water.

With some materials it is preferable to reduce the sample to ash. Weigh 2 to 5 g of it in a 25 to 30-ml platinum crucible, support the crucible in a hole of suitable diameter cut in asbestos board, and reduce the contents to ash at as low a temperature as possible, finally igniting in a muffle furnace controlled at 500° $\pm$ 50° C, until all carbon is removed. Fuse the ash with 2 g of potassium pyrosulphate, cool, heat with 10 ml of diluted hydrochloric acid (1 + 1) and 10 ml of water until the melt is dissolved and add 5 ml of diluted sulphuric acid (1 + 1). Transfer the solution to a small beaker, add 2 ml of bromine water to ensure oxidation of all the iron, and boil off the excess of bromine. Cool, filter unless clear, transfer to a 50-ml separating funnel and dilute to 30 to 35 ml with water.

Carry out a blank test on the reagents simultaneously with the test.

SEPARATION AND DETERMINATION OF IRON—

Add 1 ml of cupferron solution, mix well, add 5 ml of chloroform and shake the mixture for 30 seconds. Run the chloroform layer into a dry 25-ml graduated flask, wash with a few drops of chloroform, without mixing, to remove any drops of highly coloured extract, and transfer the wash to the flask. Add a further 0·5 ml of cupferron solution to the contents of the separating funnel and mix. If all the iron is removed the turbidity will be almost white. Extract with two or three further 5-ml portions of chloroform, adding the extract to the flask and taking care to introduce no water into the flask. Dilute to the mark with chloroform, mix, and immediately place in the dark. Transfer the aqueous layer to a 100-ml conical flask, add 1 ml of 60 per cent. perchloric acid and evaporate to fuming. Continue to heat, adding a few drops of nitric acid if necessary, until the residue is colourless, add 5 ml of water and evaporate to fuming. Reserve for the determination of copper and manganese.

Measure the optical density of the chloroform extract in a 1-cm cell by means of a Spekker absorptiometer, using Ilford No. 601 violet filters with H503 heat filters and the solution obtained from the blank test as reference solution. Calculate by means of a calibration graph, prepared as described below, the weight of iron corresponding to the optical density. Calculate the amount of iron in parts per million in the sample.

Preparation of calibration graph—Add known amounts of standard ferric iron solution containing 100 μg of iron per ml, equivalent to, say, 0, 100, 200, 300, 400 and 500 μg of iron, to a mixture of 5 ml of diluted sulphuric acid (1 + 1), 10 ml of water and 10 ml of diluted hydrochloric acid (1 + 1) and cool. Transfer each standard in turn to a 50-ml separating funnel, dilute to 30 to 35 ml with water, add 1 ml of cupferron solution and continue as described for the test solution. Determine the optical density of the solutions to which standard iron solution has been added, using the solution to which no standard iron solution has been added as reference solution. Plot a graph relating optical density to micrograms of iron.

NOTE—Measurement of the colour of the cupferron complex has the disadvantage that the excess of cupferron is apt to cause a green colour to develop, which increases rapidly in intensity, particularly if the extract is exposed to light, and this leads to incorrect results. For accurate work it is therefore safer to transfer the cupferron extract to a 100-ml conical

flask, add 2 ml of diluted sulphuric acid (1 + 1), evaporate the chloroform, and decompose the residue with small amounts of nitric acid, followed by 0·5-ml of perchloric acid. Then complete the determination of iron by one of the well-known methods, *e.g.*, by the thioglycollate or *o*-phenanthroline method.

Alternative determination of iron—Absorptiometric determination of the iron can be effected by means of diethylammonium diethyldithiocarbamate, as follows.

After decomposing the chloroform extract of the cupferron complex, add 5 ml of water and evaporate to fuming. Add 15 ml of water, boil for 1 minute, cool and dilute the solution to 25·0 ml in a measuring flask. Transfer the whole or a suitable aliquot portion of this solution, containing up to 250 μg of iron, to a 50 or 100-ml graduated separating funnel, and add 20 ml of 4 *M* sodium acetate solution. Immediately add 10 ml of diethylammonium diethyldithiocarbamate reagent, shake vigorously for 1 minute, allow to separate, and carefully transfer the chloroform layer to a dry 25-ml graduated flask. Wash with 1 or 2 ml of chloroform, without mixing, and run the washes into the flask. Extract with two further 5-ml portions of carbamate reagent, shaking for 30 seconds each time, transfer the extracts to the flask, and dilute to the 25-ml mark with chloroform. Mix and set aside in the dark for 15 minutes, and measure the optical density within the next hour, in a 1-cm cell, using Ilford No. 601 violet filters with H503 heat filters. If the solution shows the slightest turbidity, run it through a dry 9-cm Whatman No. 1 filter-paper before measuring the optical density.

Preparation of calibration graph—Add known amounts of standard ferric iron solution containing 100 μg of iron per ml, equivalent to 0, 50, 100, 150, 200, 250 μg of iron, to 2 ml of diluted sulphuric acid (1 + 1) contained in a 50 or 100-ml graduated separating funnel, dilute to 25 ml, add 20 ml of 4 *M* sodium acetate solution and 10 ml of diethylammonium diethyldithiocarbamate reagent, and extract the iron exactly as described for the test solution, diluting the combined extracts to 25·0 ml with chloroform in a graduated flask. Measure the optical densities of the solutions to which standard iron solution has been added against the solution containing no added iron as reference solution. Plot a graph relating optical density to amount of iron.

SEPARATION AND DETERMINATION OF COPPER—

Add 15 ml of water to the reserved solution (p. 657), boil for 1 minute, cool and add 1 ml of 5 per cent. sodium metabisulphite solution. Transfer to a 50-ml separating funnel and dilute to 25 ml with the water used for rinsing. Add from a burette 10·0 ml of diethylammonium diethyldithiocarbamate solution and shake vigorously for 40 seconds. Allow to separate, dry the stem of the funnel with filter-paper, and run sufficient of the chloroform layer into a 1-cm cell, filtering through a dry filter-paper unless the extract is perfectly clear. Cover the cell with a small sheet of glass and set it aside in the dark for 15 minutes.

Reject the remainder of the chloroform extract and wash the aqueous layer with small amounts of chloroform with brief shaking until the chloroform washes are colourless. Transfer the aqueous layer to the original 100-ml flask, washing with a little water, and heat until occluded chloroform is evaporated. Continue to evaporate until the volume is reduced to 25 to 30 ml. Cool and reserve the solution for the determination of manganese.

Measure the optical density of the chloroform extract of the copper in a 1-cm cell by means of a Spekker absorptiometer, using Ilford No. 601 violet filters with H503 heat filters and the solution obtained from the blank test as reference solution. Calculate from a calibration graph, prepared as described below, the weight of copper corresponding to the optical density, and hence find the amount of copper in parts per million in the test solution.

Preparation of calibration graph—Add 0, 1·0, 2·0, 3·0, 4·0 and 5·0 ml of standard copper solution containing 10 μg of copper per ml to 5·0 ml of diluted sulphuric acid (1 + 1) contained in 50-ml conical flasks, dilute to about 15 ml with water and cool. Transfer each solution in turn to a 50-ml separating funnel, dilute to 25 ml with water and extract with 10·0 ml of diethylammonium diethyldithiocarbamate solution as described for the test. Measure the optical density of the solutions to which standard copper solution has been added using the solution to which no standard copper solution has been added as reference solution. Plot a graph relating optical density to amount of copper.

SEPARATION AND DETERMINATION OF MANGANESE—

Add a small piece of litmus paper to the reserved solution (above), make just alkaline with ammonium hydroxide, then make just acid with diluted hydrochloric acid (1 + 1), and

add 0·5 ml in excess. Add 0·5 ml of 5 per cent. sodium metabisulphite solution and cool. The solution at this stage should be colourless. Transfer it to a 100-ml separating funnel, add 10 ml of 4 *M* sodium acetate solution and dilute to 50 to 60 ml with water. Extract by shaking for 30 seconds with 10 ml and then 5 ml of diethylammonium diethyldithiocarbamate solution, washing with a little chloroform, without mixing, between each extraction. Transfer the extracts and washings to a dry 25-ml measuring flask and dilute to the mark with chloroform. Filter through a dry 9-cm Whatman No. 1 filter-paper into a dry 50-ml conical flask, close the flask with a cork or glass bulb, and set it aside in the dark for 5 minutes. Measure the optical density within the next half hour in a 4-cm cell by means of a Spekker absorptiometer, using Ilford No. 601 violet filters with H503 heat filters and the solution obtained from the blank test as reference solution. Find from a calibration graph, prepared as described below, the weight of manganese corresponding to the optical density and calculate the amount of manganese in parts per million in the test sample.

Preparation of calibration graph—Add 0, 2·0, 4·0, 6·0, 8·0 and 10·0 ml of standard manganese solution containing 10 μg of manganese per ml to 5·0 ml of diluted sulphuric acid (1 + 1) contained in a 100-ml conical flask, dilute to 25 to 30 ml with water and continue as described for the test solution. Determine the optical density of the solutions to which standard manganese solution has been added, using the solution to which no standard manganese solution has been added as reference solution. Plot a curve relating optical density to weight of manganese in micrograms.

NOTE—If desired, the diethyldithiocarbamate extraction may be used merely to separate the manganese, and the determination can be completed by the periodate method. This further step is always necessary if nickel and cobalt are present, as these remain with the manganese and form coloured complexes with the reagent. Proceed as follows.

Transfer the combined chloroform extracts to a 50-ml conical flask, add 2 ml of diluted sulphuric acid (1 + 1), evaporate the chloroform and continue to evaporate to fuming. Decompose the diethyldithiocarbamate by heating with small amounts of nitric acid, until a clear colourless digest is obtained. Add 10 ml of water and again evaporate to fuming. Dilute with 20 ml of water, add 2 ml of diluted phosphoric acid (1 + 1) and about 0·2 g of potassium periodate and boil gently for 1 minute. Set aside at just below boiling temperature until the permanganate colour is fully developed, cool well and dilute to 25 ml in a graduated flask. Measure the optical density in a 4-cm cell, using Ilford No. 604 green filters with H503 heat filters. Establish the calibration graph by adding known amounts of standard manganese solution (over the range 0 to 200 μg of manganese) to 2 ml of diluted sulphuric acid in 50-ml conical flasks and proceeding as described for the test solution. Measure the optical densities and plot a graph relating optical density to amount of manganese.

DISCUSSION OF METHOD

IRON—

The cupferron extraction affords a most complete separation of iron.[2] If the amount of iron present is abnormal, say, above 500 μg, it will be necessary to extract with further 0·5-ml portions of cupferron solution and 5-ml portions of chloroform, until the last extract is free from iron colour, but a large excess of cupferron should be avoided. The measurement of the optical density will then be carried out at a suitable higher dilution. Provided pure reagent is used, the extracts are not exposed to bright light and the measurement of the optical density is not unduly delayed, good results are obtainable by direct measurement of the colour of the ferric cupferrate complex. If a number of tests are done, it is best to measure the optical densities of the blank value of the reagents and each test as soon as they are extracted, and to correct for the reagent blank reading.

If it is decided to adopt the safer course and decompose the cupferron extract, final absorptiometric determination of the iron by means of thioglycollic acid or *o*-phenanthroline is quite satisfactory. Determination by extraction with diethylammonium diethyldithiocarbamate in chloroform from sodium acetate buffered solution as the dark brown ferric complex provides a simple, sensitive and accurate method of determination, as the cupferron separation will have isolated the iron from other interfering elements.

COPPER—

Although copper forms a stable complex with cupferron, it remains in the acid solution provided the acidity is sufficiently great. Before proceeding with the separation of the copper,

it is essential to destroy the copper cupferrate and any free cupferron in the solution. Although it is customary to extract copper as its diethyldithiocarbamate complex from ammoniacal citrate solution, extraction from acid solution is equally complete, provided that a chloroform-soluble diethyldithiocarbamate is used, so that an excess of the reagent is maintained in the chloroform extract.[1] The procedure has the advantage of making the separation more selective, bismuth being the only element to interfere under the conditions described. If bismuth is present, the copper can be separated and re-extracted as follows. Shake the combined chloroform extracts with 2 ml of 10 per cent. potassium cyanide solution for 15 seconds. Add 8 ml of water and transfer the chloroform layer to another separating funnel. Again shake it with 2 ml of potassium cyanide solution, add 8 ml of water and reject the chloroform layer, washing the potassium cyanide extracts with a little chloroform. Combine the potassium cyanide extracts in a 100-ml conical flask, add 2 ml of diluted sulphuric acid (1 + 1) under a hood, and boil down to half its volume to destroy the potassium cyanide. Cool, dilute to about 25 ml and extract directly with 10·0 ml of diethylammonium diethyldithiocarbamate reagent, measuring the optical density as before.

MANGANESE—

Manganese forms a purplish-brown compound with diethyldithiocarbamates, but this does not appear to be as stable as those formed by other metals. Although it can be extracted from slightly ammoniacal solution containing a little citrate or tartrate, it is better to extract manganese from acetate-buffered solution under such conditions that a reasonably high concentration of diethyldithiocarbamate is maintained in the chloroform. By this means full recovery of manganese is obtained and the colour remains reasonably stable.

SENSITIVITY—

The sensitivity of the methods can be gauged from the observed optical densities shown in Table I, obtained with a Spekker photo-electric absorptiometer, Ilford filters and a tungsten-filament lamp.

TABLE I

SENSITIVITY OF THE METHODS

	Optical densities with Ilford colour filters					
	Iron			Copper,	Manganese	
Amount, μg	Cupferron	Thioglycollic acid	Diethyldi-thiocarbamate	Diethyldi-thiocarbamate	Diethyldi-thiocarbamate	Periodate
10	—	—	—	0·165	0·092	—
20	—	0·105	—	0·33	0·185	—
30	—	—	—	0·50	—	—
40	—	0·215	—	0·665	0·38	—
50	—	—	0·153	0·83	—	0·30
60	—	0·32	—	—	0·565	—
80	—	0·43	—	—	0·77	—
100	0·23	0·535	0·305	—	0·95	0·595
150	—	0·80	0·46	—	—	0·89
200	0·45	1·06	0·615	—	—	1·19
250	—	—	0·77	—	—	—
300	0·67	—	—	—	—	—
400	0·88	—	—	—	—	—
500	1·11	—	—	—	—	—
Dilution, ml—	25	50	25	10	25	25
Cell size, cm—	1	4	1	1	4	4
Filter No.—	601	604	601	601	601	604
Filter colour—	Violet	Green	Violet	Violet	Violet	Green

RECOVERIES—

Recoveries obtained from random mixtures of known amounts of the three metals are shown in Table II.

TABLE II

RECOVERY EXPERIMENTS

Amount of metal added			Amount of metal recovered and reagent used		
Fe, μg	Cu, μg	Mn, μg	Fe, μg	Cu, μg	Mn, μg
500	20	20	490·0 (*a*)	20·0 (*c*)	18·5 (*c*)
100	50	50	102·0 (*a*)	49·5 (*c*)	48·0 (*c*)
300	70	100	295·0 (*a*)	68·0 (*c*)	100·0 (*c*)
400	50	80	400·0 (*b*)	49·9 (*c*)	78·0 (*c*)
200	20	20	203·0 (*b*)	20·6 (*c*)	21·0 (*c*)
50	70	50	52·0 (*b*)	69·0 (*c*)	47·0 (*c*)
300	10	10	291·0 (*b*)	10·6 (*c*)	10·3 (*c*)
1000	50	50	980·0 (*b*)	51·0 (*c*)	51·5 (*c*)
20	5	200	19·5 (*b*)	4·8 (*c*)	198·0 (*d*)
250	10	150	247·0 (*c*)	9·9 (*c*)	148·0 (*d*)
150	30	50	150·0 (*c*)	30·0 (*c*)	48·0 (*d*)

Reagents—(*a*) Cupferron, (*b*) thioglycollic acid, (*c*) diethyldithiocarbamate, (*d*) periodate.

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The Photometric Determination of Phosphorus in Copper-based Alloys Containing Tin

BY H. K. LUTWAK

The method of the A.S.T.M. (E62–50T) for the determination of phosphorus in copper-based alloys has several shortcomings. An investigation has been made to obviate them and to simplify the method.

Calibration graphs that obeyed Beer's law were obtained by (*a*) determining the sensitivity of the phosphovanadomolybdate complex to acidity and dilution changes and choosing the most favourable conditions, and (*b*) increasing the rate of colour development by heating the coloured solution before dilution. Colour intensities that reach their final value at room temperature are stable; colours obtained by heating fade slowly.

Separation of metastannic acid is prevented by the use of a suitable mixture and amount of nitric and hydrochloric acids in the final solution. The conditions chosen also provide optimum acidity for colour development.

The same procedure is recommended for development of calibration graphs both for alloys of low and of high phosphorus contents.

IN the determination of phosphorus in copper-based alloys containing much tin by the photometric procedure given in "A.S.T.M. Designation E62–50T, A.S.T.M. Methods," it was found that there were several limitations: (*i*) only the lower parts of the calibration graphs obeyed Beer's law, (*ii*) the hydrochloric acid concentration was sometimes insufficient to prevent the formation of interfering metastannic acid, and (*iii*) it was inconvenient to follow different procedures for preparing the solutions for each of the two calibration graphs. An investigation was therefore made with the object of overcoming these difficulties.

Preliminary experiments showed that—

(*a*) it was unnecessary to use hydrogen peroxide after dissolving the sample in acid. This is consistent both with the observation of Kurakawa and Sakamoto[1] that the oxidation of phosphorus to phosphate is catalysed by copper nitrate, and with our experience over many years that the oxidation of phosphorus in the conventional

types of bronze is complete with hot mixed acids, *e.g.*, 320 ml of nitric and 120 ml of hydrochloric acid made up to 1 litre, as in the A.S.T.M. method[2];

(*b*) it was best to use 10 ml of each colouring reagent solution (ammonium molybdate and ammonium vanadate) for all alloys irrespective of their phosphorus contents.

Experimental

Apparatus—

A Beckman quartz spectrophotometer, model DU, was set at wavelength 4700 A.

Time and temperature effects on colour development—

Various directions for the time and temperature requirements for colour development have been given in the literature. The A.S.T.M. recommends 5 minutes,[2] Zischkau[3] 10 minutes (20 minutes in presence of hydrochloric acid) and Kitson and Mellon[4] either 30 minutes at room temperature or 10 minutes in boiling water.

Solutions containing little phosphorus prepared by the A.S.T.M. method[2] (without hydrogen peroxide) for preparation of the calibration graph were measured after 10, 20, 30 and 60 minutes and 24 hours at room temperature. The intensity of the colour reached a maximum after 10 minutes and did not change further. A similar result was obtained with the calibration graph for solutions containing much phosphorus, except that, for solutions containing 4 to 6 mg of phosphorus, the colour intensified on standing without reaching the value required by Beer's law. In accordance with directions by Kitson and Mellon[4] and Harrison,[5] all solutions for the high-concentration phosphorus graph were heated (after initial colour formation) to 80° to 90° C both before and after dilution to final volume; the measurements of optical density were made at room temperature. An improved graph was obtained, but it still departed from the linearity required by Beer's law.

Stability—

The literature contains conflicting reports on the stability of the phosphovanadomolybdate colour. Three groups of investigators, Harrison and Fisher,[6] Murray and Ashley[7] and Kitson and Mellon,[4] state that the colour is permanently stable. The A.S.T.M.,[2] on the other hand, says that the colour is stable for at least 1 hour, whilst Koenig and Johnson[8] give the time of stability as 12 to 24 hours.

It was found experimentally that the colours developed at room temperature were stable if they reached values required by Beer's law. Colours developed by heating faded in a few hours, and the rate of fading increased when the acid concentration was more than about 0·5 *N*.

Dilution of solution and acid concentration—

The influence of dilution on the phosphorus complex was investigated. Solutions containing 0·2 mg and 6 mg of phosphorus were prepared in accordance with the A.S.T.M. method, in which these concentrations are stated to be the extreme limits. These solutions were prepared with 10, 15, 20 and 25 ml of mixed acids, and were diluted to 100 ml (in accordance with the A.S.T.M. method), and also to 200 and 500 ml. The optical densities, which were measured in 1, 2 and 5-cm absorption cells, would have remained constant if the dilution had not affected the light-absorbing compound. The results of these measurements are shown in Table I.

Table I

Influence of dilution and acid concentration on optical density

Phosphorus present, mg	Amount of acids, ml	Optical density: Dilution to 100 ml in 1-cm cell	Optical density: Dilution to 200 ml in 2-cm cell	Optical density: Dilution to 500 ml in 5-cm cell
0·2	10	0·050	0·050	0·055
0·2	15	0·045	0·045	0·050
0·2	20	0·045	0·045	0·045
0·2	25	0·040	0·040	0·040
6·0	10	1·13	1·14	1·14
6·0	15	1·09	1·12	1·13
6·0	20	0·98	1·07	1·08
6·0	25	0·82	1·02	1·02

It can be seen that for low phosphorus concentrations the optical density is not sensitive to changes in concentration, but solutions with high phosphorus contents have an increased optical density on dilution from 100 to 200 ml; further dilution to 500 ml has little effect.

An increase in the acid concentration of solutions of low phosphorus content causes a decrease in the optical density that is independent of the volume of the solutions. For high phosphorus contents the optical density also decreases with increased acid concentration, but the difference varies with dilution. In 100-ml solutions the difference between extreme values is approximately 38 per cent., whereas in 200 and 500-ml solutions this difference is about 10 per cent.

The difference between optical densities of solutions containing much phosphorus is smallest for solutions containing 10 and 15 ml of mixed acids. As some acid is lost on dissolving the alloy, the resulting error in optical density is least if 15 ml of mixed acids are used and the sample is diluted to a volume of more than 200 ml. For convenience, 250-ml calibrated flasks were chosen.

BLANKS—

Blank solutions containing all reagents but molybdate were used for setting the instruments, and the test solutions were compared with the blank solutions by substitution. This eliminated the effects of interfering ions, especially copper, and of temperature.

INTERFERENCE BY TIN—

When the A.S.T.M. method with the optimum conditions as established above was applied to the analysis of a number of tin bronzes, it was found that metastannic acid separated some time after dilution. When the tin content was low, there was enough time for measurement. When the tin content was high (approximately 10 per cent.) metastannic acid formed a colloidal solution too soon and caused erroneous readings. If the particle size of the colloid was allowed to increase by ageing, the metastannic acid could be removed by filtration and correct values were obtained for the filtrate. Nevertheless, this procedure was undesirable, and means were sought to obviate filtration.

Kitson and Mellon[4] mention that tin interferes with their method, but give no details. Attempts to apply Iron and Steel Institute's[9] or Harrison and Fisher's[6] methods for removing the tin by means of hydrobromic acid or brominated hydrochloric acid showed that both are too lengthy for use.

Prevention of metastannic acid separation—Fifteen millilitres of mixed acids were sufficient to dissolve the sample and to develop the colour, but this amount did not prevent the formation of metastannic acid in a final volume of solution of 250 ml. It seemed likely that an increase in the hydrochloric acid concentration would keep the tin in solution. The optimum ratio of nitric to hydrochloric acid to prevent interference by tin and the effect of the total acid concentration on the development of colour intensity were therefore investigated concurrently.

Synthetic alloys containing 0·45 g of copper and 0·05 g of tin were dissolved in various amounts of the mixed-acid solution recommended by the A.S.T.M. After the metals had dissolved, hydrochloric acid was added so that the final solutions contained 50 ml of hydrochloric acid (120 ml of concentrated acid per litre) and 5, 10, 15, 20, 25 or 50 ml of nitric acid (320 ml per litre). The acidities of the final solutions were 0·3, 0·4, 0·5, 0·6, 0·7 and 1·1 *N*.

After development of the colour, measurements of optical density were made immediately after diluting to the final volume of 250 ml, after 10 minutes, and after leaving overnight. In another series the colours were developed by heating to incipient boiling, and the optical densities were measured as before at room temperature.

These results showed that—

(*a*) The colours developed at high acidity are less stable than those obtained at lower acidity.
(*b*) Colours developed at medium acidity and room temperature gave almost linear calibration graphs.
(*c*) At low acidity (about 0·3 *N*) Beer's law was obeyed.
(*d*) The optimum acidity was between 0·3 and 0·4 *N*.
(*e*) Slightly better graphs for high phosphorus contents were obtained at all acidities if the colour was developed by heating.

Kitson and Mellon's[4] value of approximately 0·5 *N* acid for most suitable colour development is therefore confirmed, but Barton's[10] value of 0·8 *N* acid is too high under the present conditions. Kitson and Mellon[4] and Zischkau[3] state that hydrochloric acid interferes with their methods. The above-mentioned experiments showed that hydrochloric acid is necessary, that it does not interfere with the method in the presence of tin, and that a decrease in nitric acid concentration does not cause any deterioration in colour development or stability.

Method

The following modification of the A.S.T.M. Designation E62–50T is recommended, as a result of the work already described.

Reagents—

Tin—Not less than 99·98 per cent. pure.

Hydrochloric acid—Dilute 300 ml of concentrated acid to 1 litre.

Other reagents—Copper, phosphate solutions, mixed acids, ammonium vanadate and molybdate solutions are as in the A.S.T.M. method,[2] but hydrogen peroxide is not required.

Apparatus—

A Beckman quartz spectrophotometer, model DU, set at wavelength 4700 A. A photoelectric absorptiometer is also suitable (the author used a Hilger Spekker absorptiometer equipped with a mercury-vapour lamp and Ilford No. 601 and H503 filters).

Preparation of the calibration graphs—

Dissolve 21 samples of 0·45 g of fine drillings of copper and 0·05 g of tin in 5-ml portions of mixed acids by heating, taking care to prevent loss of acid by boiling. Tall-form 100-ml or 150-ml beakers are most suitable.

Add phosphate solutions to give solutions containing 0·05, 0·10, 0·15, 0·20, 0·25, 0·40, 0·60, 0·80 and 1·00 mg of phosphorus for establishing the calibration graph for low phosphorus concentrations.

Add phosphate solutions to give solutions containing 0·2, 0·4, 0·6, 1·0, 2·0, 3·2, 4·0, 4·8, 5·2, 5·6 and 6·0 mg of phosphorus for establishing the calibration graph for high phosphorus concentrations.

Keep the beakers covered and the solutions at just below their boiling-point for 20 to 30 minutes on a hot-plate. Add 20 ml of hydrochloric acid, 10 ml of ammonium vanadate and 10 ml of ammonium molybdate. Place the beakers on the hot-plate and rinse their sides thoroughly with water. Heat the solutions until the first air bubbles appear. Cool the solutions to room temperature and dilute them to 250 ml. Use one sample as the blank.

Measure the optical densities of solutions containing 0·05 to 1·00 mg of phosphorus in 5-cm cells when using the Beckman spectrophotometer, and in 4-cm cells when using the Spekker absorptiometer at a blank - water setting of 1·3. The change of optical density per mg of phosphorus is 0·375; the change of drum reading per mg of phosphorus is 0·64.

Measure the optical densities of solutions containing 0·2 to 6·0 mg of phosphorus in 1-cm cells when using either instrument. The Spekker absorptiometer setting is 1. The change of optical density per mg of phosphorus is 0·072; the change of drum reading per mg of phosphorus is 0·15.

Procedure—

Dissolve two samples of 0·5 g of fine drillings of the alloy as described earlier under preparation of the calibration graphs. Develop the phosphovanadomolybdate colour in one sample. Add all reagents but the molybdate to the other sample and use it as the blank.

Measure the optical density with the Beckman spectrophotometer in 1-cm absorption cells, or, if the optical density is less than 0·050, in 5-cm cells. Alternatively, measure the solutions in the Spekker absorptiometer in 1-cm cells and at a blank - water setting of 1. If the drum reading is more than 0·9, use 4-cm cells and a setting of 1·3.

Results—

A comparison of results obtained on a number of copper-based alloys by chemical and photometric methods is shown in Table II, from which it can be seen that the results agree satisfactorily. The chemical (volumetric) method referred to has been used successfully

at Defence Research Laboratories for a number of years, and its accuracy has been checked at frequent intervals against certified standard samples.

TABLE II

COMPARISON OF CHEMICAL AND PHOTOMETRIC PHOSPHORUS DETERMINATION

Phosphorus content		
	By photometric method	
By chemical method, %	With a Beckman spectrophotometer, %	With a Spekker absorptiometer, %
0·10	0·11, 0·10	0·11, 0·10, 0·12
0·21	0·21, 0·20	0·20, 0·21
0·22	0·21, 0·21	0·21, 0·21
0·24	0·23, 0·23	0·24, 0·23
0·32	0·32, 0·34, 0·33	—
0·33	0·31, 0·32	0·33, 0·34
0·42	0·42, 0·40, 0·43	—
0·42	—	0·42
0·73	0·74, 0·73	0·75
0·83	—	0·82, 0·83, 0·84
1·01	1·00, 1·00	1·01, 1·02
1·17	1·16, 1·16	—

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The Indirect Polarographic Determination of Calcium by Chloranilic Acid

BY B. BREYER AND J. MCPHILLIPS

Calcium reacts quantitatively with chloranilic acid, precipitating calcium chloranilate. The concentration of the calcium can be determined, without removing the precipitate, by measuring the polarographic diffusion current of the residual chloranilic acid and referring the value obtained to a calibration graph. Calcium can thus be determined within a concentration range of $1{\cdot}3 \times 10^{-4} M$ to $1{\cdot}2 \times 10^{-2} M$ with a maximum error of ± 3 per cent.

The determination cannot be carried out in the presence of cobalt, zinc, lead, manganese, cadmium, nickel, aluminium, copper and silver ions, because they precipitate the reagent. Sodium, potassium, ammonium, magnesium, iron and mercury ions do not cause precipitation, but ferric and mercuric ions interfere if their concentrations are greater than $4{\cdot}8 \times 10^{-4} M$ and $5{\cdot}9 \times 10^{-4} M$, respectively. Magnesium does not interfere if present at concentrations of less than $2{\cdot}4 \times 10^{-3} M$, nor do lithium, barium and chromium when their concentrations are below $5{\cdot}9 \times 10^{-4} M$. Strontium does not affect the determination at concentrations up to $3{\cdot}6 \times 10^{-4} M$.

Calcium has been determined in blood serum and in milk.

STANDARD methods for the determination of calcium frequently involve lengthy procedures, owing to the necessity for removing other ions, such as magnesium and iron, that commonly occur with calcium. Also inherent in some of these methods are errors caused by faulty filtration techniques or by solution of part of the precipitate. Many of these disadvantages can be overcome by polarography.

The direct polarographic determination of calcium, however, presents difficulties arising from poorly defined diffusion currents[1] and interference from other ions, especially magnesium. Cohn and Kolthoff[2] therefore determined calcium by an indirect polarographic method based on the reaction between calcium and picrolonic acid. The calcium was determined by polarographic measurement of the residual picrolonic acid after the reaction was complete. This method requires a long reaction time and the calcium determination is limited to a concentration range of $10^{-3} M$ to $10^{-2} M$.

Chloranilic acid has been used by Tyner[3] for the colorimetric determination of calcium in plant ash, and by Gammon and Forbes[4] for similar work with plant ash and soil extracts. Preliminary polarographic experiments revealed that chloranilic acid was much more sensitive than picrolonic acid and that it gave well-defined polarographic waves in several buffer solutions over a wide range of pH values. In addition it was found that chloranilic acid could be used for the indirect polarographic determination of cobalt, nickel, cadmium, manganese, zinc and lead.

EXPERIMENTAL

POLAROGRAPHIC BEHAVIOUR OF CHLORANILIC ACID—

The polarograms of chloranilic acid were well defined in a concentration range of $10^{-3} M$ to $3{\cdot}3 \times 10^{-4} M$. Maxima on the polarographic step were observed when the concentration was greater than $1{\cdot}25 \times 10^{-3} M$. Good results were obtained both in universal buffer (pH 2 to 11) as well as in a sodium acetate and acetic acid buffer mixture (pH 4 to 6). The concentration of the buffer had little effect on the diffusion current, but in unbuffered electrolyte, *e.g.*, $0{\cdot}1 M$ potassium chloride, disturbing maxima appeared.

Chloranilic acid decomposes if exposed to light over long periods of time, so stock solutions should be kept in the dark. A small progressive drop in the diffusion current of a solution stored in darkness was observed, but this was caused by ageing.

Solutions with an acid reaction were found to be more stable than neutral or alkaline solutions. However, chloranilic acid solutions should be regularly checked and new solutions prepared when a marked fall in the diffusion current occurs.

The diffusion current of chloranilic acid increased with increasing temperature to the extent of 2 per cent. per ° C. The half-step potential of the wave is $-0{\cdot}21$ V as measured against the saturated calomel electrode.

THE REACTION OF CHLORANILIC ACID WITH CALCIUM—

Chloranilic acid reacted with calcium quantitatively, forming slightly soluble calcium chloranilate. The reaction went to completion more rapidly when the reagent was dissolved in water than when dissolved in buffer solution. The precipitated calcium chloranilate was found to be least soluble at a pH value of about 4·4. Table I shows the change in solubility of calcium chloranilate, as exemplified by change in diffusion current, with the pH value.

TABLE I

DIFFUSION CURRENTS OF SATURATED CALCIUM CHLORANILATE SOLUTIONS IN UNIVERSAL BUFFER AT DIFFERENT pH VALUES

Capillary characteristics: $m^{\frac{2}{3}}t^{\frac{1}{6}} = 0{\cdot}01576$ (open circuit)

pH value	2·4	4·4	6·3	8·2
Current, μA	3·09	0·96	1·16	2·06

THE REACTION OF CHLORANILIC ACID WITH IONS OTHER THAN CALCIUM—

Sodium, potassium, ammonium and magnesium ions did not precipitate a $2 \times 10^{-3}\,M$ solution of chloranilic acid when they were present at concentrations of less than $2{\cdot}4 \times 10^{-3}\,M$. A small reduction in the diffusion current was observed for strontium, barium, chromium and lithium, whilst aluminium, copper and silver reacted strongly. Cobalt, cadmium, zinc, manganese and lead ions reacted quantitatively in the same way as calcium. Nickel also reacted, but the calibration graph was not linear. Table II shows the diffusion currents obtained when 0·5 ml of $10^{-2}\,M$ solutions were added to 7·5 ml of $2 \times 10^{-3}\,M$ chloranilic acid. The solutions were made up to a final volume of 20 ml with universal buffer at a pH value of 4·5 after the reaction was complete.

TABLE II

DIFFUSION CURRENTS OF $7{\cdot}5 \times 10^{-4}\,M$ SOLUTIONS OF CHLORANILIC ACID AFTER THE ADDITION OF VARIOUS IONS

Capillary characteristics: $m^{\frac{2}{3}}t^{\frac{1}{6}} = 0{\cdot}01576$ (open circuit)

Diffusion current of $7{\cdot}5 \times 10^{-4}\,M$ chloranilic acid: 4·51 μA

Concentration of added ion: $6{\cdot}25 \times 10^{-4}\,M$

Ion	Current, μA	Ion	Current, μA	Ion	Current, μA	Ion	Current, μA
Li^{+}	4·40	Ca^{++}	3·10	Cu^{++}	3·83	Mn^{++}	2·98
Na^{+}	4·51	Sr^{++}	4·35	Ag^{+}	4·29	Cr^{+++}	4·38
K^{+}	4·48	Ba^{++}	4·48	Zn^{++}	3·32	Fe^{+++}	4·48
NH_4^{+}	4·44	Al^{+++}	3·99	Cd^{++}	3·01	Co^{++}	3·12
Mg^{++}	4·38	Pb^{++}	3·05	Hg^{++}	4·46	Ni^{++}	2·97

ION INTERFERENCE IN THE DETERMINATION OF CALCIUM—

Ferric and mercuric ions interfered with the determination when present at concentrations greater than $4{\cdot}8 \times 10^{-4}\,M$ and $5{\cdot}9 \times 10^{-4}\,M$, respectively. Magnesium did not interfere unless its concentration was higher than $2{\cdot}4 \times 10^{-4}\,M$. Lithium, barium and chromium at concentrations of $5{\cdot}9 \times 10^{-4}\,M$ or less did not affect the determination, but strontium interfered when present at concentrations greater than $3{\cdot}6 \times 10^{-4}\,M$. The determination could not be carried out in the presence of aluminium, copper, silver, cobalt, cadmium, zinc, manganese or lead. Phosphate or sulphate ions did not interfere.

A test solution containing sodium, calcium, magnesium and iron in roughly the same concentrations as in blood serum was analysed and the calcium was recovered with an error of ±3 per cent. No interference was observed.

METHOD

APPARATUS—

A thermostatically controlled water-bath (25° C) with an aluminium cover accommodating six polarographic cells, a water-trap for nitrogen, a thermometer, the heating element fixture, the stirrer shaft and the mercury - toluene thermo-regulator. The cells

were connected in series, so allowing nitrogen to be passed through all six cells simultaneously. After the first polarogram had been recorded, it was necessary to pass nitrogen for only 2 minutes before recording the polarogram of the next solution.

A manual polarograph.

REAGENTS—

Chloranilic acid—Prepare chloranilic acid (3:6-dichloro-2:5-dihydroxyquinone) according to Graebe[5] as follows.

To 10 g of chloranil (tetrachloroquinone) wetted with alcohol, add a solution prepared by dissolving 9 g of sodium hydroxide in 210 ml of water and heat to 70° to 80° C. After 2 hours add 20 g of sodium chloride and allow the sodium chloranilate to separate. Filter after several hours and wash the filter with 10 per cent. w/v sodium chloride solution. Wash the precipitate with a little hot water and dissolve it in 1·5 to 2 litres of water. Filter whilst hot and precipitate the chloranilic acid with concentrated hydrochloric acid. The precipitate should be in the form of red, shiny leaflets, m.p. 283° to 284° C. Recrystallisation is unnecessary. Prepare a $2 \times 10^{-3}\,M$ solution by dissolving 0·418 g of the acid in a litre of water at room temperature.

Universal buffer, pH 4·5 (Prideaux and Ward[6])—Prepare this by adding 56·25 ml of N sodium hydroxide solution to a mixture containing 2·5 ml of glacial acetic acid, 2·6 ml of syrupy phosphoric acid and 2·472 g of boric acid and make up to a litre with water.

PROCEDURE—

To establish calibration graphs, add various amounts of a 0·01 M calcium solution to a series of polarographic cells each containing 7·5 ml of $2 \times 10^{-3}\,M$ chloranilic acid. The addition of calcium should cover the concentration range from $1{\cdot}3 \times 10^{-4}\,M$ to $1{\cdot}2 \times 10^{-2}\,M$. Neutralise the solution to Congo-red paper with solid sodium bicarbonate and set it aside for 10 minutes. Bring the volume to 20 ml by adding universal buffer of pH 4·5. Record polarograms and plot diffusion current against concentration.

When solutions containing an unknown amount of calcium are analysed, it is first necessary to estimate the calcium concentration roughly by adding 0·5 ml of the solution to 7·5 ml of $2 \times 10^{-3}\,M$ chloranilic acid; the reagent should not be decolorised within 10 minutes of the addition of the solution.

APPLICATIONS

DETERMINATION OF CALCIUM IN MILK—

To 2 ml of well mixed whole milk add a drop of glacial acetic acid to prevent formation of a skin and evaporate to dryness at 105° C in a platinum crucible. Heat the residue to

TABLE III

CALCIUM CONCENTRATIONS IN TWO MILKS AND RECOVERY OF ADDED CALCIUM

Milk	Calcium in milk, mg per 100 ml	Calcium added, mg per 100 ml	Calcium recovered, mg per 100 ml	Calcium expected, mg per 100 ml	Error, %
A1	124	20	147	144	+2·0
A2	120	20	143	140	+2·1
B1	124	20	141	144	−2·0
B2	123	20	140	143	−2·0

ash over a bunsen flame. Dissolve the ash in 0·4 ml of N hydrochloric acid and make up to 5 ml with distilled water. Add 0·5 ml of this solution to 7·5 ml of $2 \times 10^{-3}\,M$ chloranilic acid in a polarographic cell. Neutralise the contents of the cell to Congo-red paper with solid sodium bicarbonate and make up the volume to 20 ml with universal buffer of pH 4·5. Pass nitrogen for 10 minutes and record a polarogram.

Recovery experiments were carried out on two milks by adding 1 ml of $10^{-2}\,M$ calcium chloride to a duplicate sample so as to increase the calcium concentration of the original milk by 20 mg per 100 ml. Two sets of determinations were made for each milk. Table III shows the calcium concentrations of the two milks and the recovery of the added calcium.

DETERMINATION OF CALCIUM IN BLOOD SERUM—

Evaporate 2 ml of blood serum in a platinum crucible at 105° C and ignite to ash over a bunsen flame. Dissolve the ash in 0·2 ml of *N* hydrochloric acid and make the volume up to 5 ml with distilled water. Add 2 ml of this solution to 7·5 ml of 2×10^{-3} M chloranilic acid in a polarographic cell. Neutralise the contents of the cell to Congo-red paper with solid sodium bicarbonate and make up the volume to 20 ml with universal buffer of pH 4·5. Pass nitrogen for 10 minutes and record a polarogram.

For clinical investigations a polarographic microcell is best used. The amount of blood required is then only 0·3 ml, from which 0·1 ml of serum is recovered. After treatment as set out above and with proportionately smaller amounts of reagents, the solution is brought finally to 1 ml and a polarogram is recorded.

Calcium determinations were made on blood sera from four different persons. A comparison of the results obtained by the method described above with those obtained by the oxalate - permanganate method is shown in Table IV.

TABLE IV

A COMPARISON OF THE RESULTS OBTAINED BY THE INDIRECT POLAROGRAPHIC METHOD AND THE OXALATE - PERMANGANATE METHOD FOR CALCIUM IN BLOOD SERUM

Sample	By polarograph, mg per 100 ml	By oxalate - permanganate, mg per 100 ml	Difference, %
A	9·5	9·75	− 3
B	9·9	9·0	+10
C	9·0	9·0	0
D	11·0	10·0	+10

A recovery experiment was carried out on blood sera from four sheep. One millilitre of 0·01 *M* calcium chloride was added to 2 ml of serum so as to increase the concentration of the calcium in the original serum by 20 mg per 100 ml. Table V shows the concentration of the serum calcium and the recovery of the added calcium.

TABLE V

RECOVERY OF CALCIUM ADDED TO SERUM

Sheep	Calcium in serum, mg per 100 ml	Calcium added, mg per 100 ml	Calcium recovered, mg per 100 ml	Calcium expected, mg per 100 ml	Error, %
A	10·7	20	29·7	30·7	−3
B	11·0	20	31·2	31·0	+1
C	12·7	20	31·5	32·7	−4
D	12·0	20	32·0	32·0	0

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The Analysis of Rosin Size

By D. E. DAVIS and K. LINKE

Methods are suggested for the determination of all the constituents of rosin size. They include methods for free alkali and for free acid in the presence of alkali. Results are given that indicate that the proportions of free acid and alkali in rosin size may be large.

Application of the results obtained by the methods for the complete analysis of rosin size suggested here has led to greater efficiency in the production of size. No new analytical techniques are described, but as far as the authors are aware none of the methods previously suggested for the determination of free rosin acid in size takes into consideration the fact that alkali carbonate will also be present, and that its presence will affect the result obtained for the quantity of free rosin acid. In none of the previous methods is a suitable procedure suggested for the determination of free carbonate alkali.

That the proportion of both free alkali and free acid in size can be high is illustrated by the results shown; these are representative of many analyses.

Carbon dioxide displaces rosin acid from aqueous and from alcoholic solutions of rosin soap. For rosin acid to displace carbon dioxide from solutions of sodium carbonate, it is necessary to raise the temperature of the mixture almost to boiling point. The reaction is reversible and thus alkali carbonate can, and usually does, exist in the presence of free rosin acid in size.

Procedure—

For each determination in the analytical procedures that follow, 25 ml of liquid size containing approximately 1 g of total solids are used for convenience. For the analysis of paste size it is desirable to use a weight of material corresponding to approximately 1 g of total solids.

It should be noted that here the term "unsaponifiable" means paper-makers' unsaponifiable, or that part of the rosin which does not react with boiling sodium carbonate solution or with cold potassium hydroxide solution.

(*A*). *Unsaponifiable (including wax)*—To 25 ml of rosin size at room temperature add 15 ml of 0·5 *N* potassium hydroxide solution. Add sufficient ethyl alcohol or industrial spirit for the resulting solution to contain one volume of alcohol for every two volumes of water. Without heating, extract the unsaponifiable material by the British Standard method[1] using ethyl ether as solvent.

The material extracted will consist of the non-acidic part of the rosin, together with any wax or other similar materials that may be present in the size.

(*B*). *Total rosin and other ether-soluble material*—To 25 ml of the size, add a known and sufficient amount of standard sulphuric acid to make the resulting solution acid to methyl orange. An excess equivalent to 5 ml of 0·1 *N* acid is sufficient. Extract the total rosin with ethyl ether and dry the extract at a temperature not exceeding 80° C. The extracted material contains the rosin acid, the neutral rosin and any other material soluble in ethyl ether that is present in the size.

(*C*). *Total rosin acid*—Subtract the result obtained under section (A) from that found under section (B) to obtain the total rosin acid. If the proportion of unsaponifiable in the rosin used in the size production is known, then the proportion of non-rosin unsaponifiable, for example, wax, can be calculated from the results obtained in sections (A) and (B).

(*D*). *Equivalent weight of rosin acid*—Dissolve the total rosin obtained in section (B) in 10 ml of neutral alcohol and titrate the solution with standard alcoholic potassium hydroxide to the phenolphthalein end-point. Calculate the equivalent weight from the weight of total rosin acid found in section (C).

It should be noted that the equivalent weight of the rosin acid thus derived is usually a few units higher than that obtained by titrating raw rosin of a quality identical to that separated from the size.

(*E*). *Total alkali*—After the total rosin has been extracted (section B), boil the combined aqueous solution and washings to expel ether, cool to room temperature and titrate to the phenolphthalein end-point with standard aqueous potassium hydroxide solution. The standard acid absorbed is a measure of the total alkali present.

(*F*). *Free sodium hydroxide*—Except for a very small amount that may be due to hydrolysis, sodium hydroxide cannot be present unless—

(*i*) sodium hydroxide has been used in the preparation of the size, and

(*ii*) no free rosin acid is present in the size.

Add 150 ml of neutral alcohol containing phenolphthalein to 25 ml of size. Then add an excess of neutral barium chloride solution. If the mixture remains pink, sodium hydroxide is present; titrate it to the phenolphthalein end-point with standard hydrochloric acid.

(*G*). *Carbonate alkali*—Add an excess of sulphuric acid to 25 ml of size in a closed flask. Boil the mixture and at the same time, by means of air washed free from carbon dioxide, draw the liberated carbon dioxide through a known quantity of aqueous baryta solution previously standardised with hydrochloric acid of known strength. After the carbon dioxide has been absorbed, titrate the baryta solution with the standard hydrochloric acid. The difference between the amounts of acid used in the two titrations gives a measure of the carbonate alkali present.

(*H*). *Apparent free rosin acid*—This is the free acidity as determined by direct titration. Add 100 ml of neutral alcohol to 25 ml of size at room temperature, mix and immediately titrate the apparent free acid to the phenolphthalein end-point with standard potassium hydroxide solution.

(*K*). *Net free rosin acid*—This is the free acidity remaining after the free alkalinity present has been neutralised by its equivalent amount of free rosin acid present in the size. Add 100 ml of neutral alcohol to 25 ml of size. Boil under a reflux condenser for at least 30 minutes. Cool rapidly to room temperature and titrate the net free acid to the phenolphthalein end-point with standard potassium hydroxide solution.

If the solution remains pink after heating under the reflux condenser, there is no excess of free rosin acid, but there is an excess of alkali. Estimate this by adding an excess of standard sulphuric acid and, after refluxing as above, titrating the remaining acid with standard potassium hydroxide solution.

(*L*). *Actual free rosin acid*—Actual free rosin acid can be calculated from the following formulae; acid "freed" by virtue of hydrolysis of rosin soap is not included—

$$(L) = 2(H) - (K),$$
$$(L) = (K) + (G)$$
or $$(L) = (D) + (G) - (E),$$

where (L) = Amount of 0·1 *N* alkali in millilitres equivalent to actual free rosin acid in 25 ml of size,

(H) = Amount of 0·1 *N* alkali in millilitres equivalent to apparent free rosin acid in 25 ml of size,

(K) = Amount of 0·1 *N* alkali in millilitres equivalent to net free rosin acid in 25 ml of size,

(G) = Amount of 0·1 *N* acid in millilitres equivalent to carbonate alkali in 25 ml of size,

(D) = Amount of 0·1 *N* alkali in millilitres equivalent to the total rosin acid present in 25 ml of size

and (E) = Amount of 0·1 *N* acid in millilitres equivalent to total alkali present in 25 ml of size.

(*M*). *Water*—If it should be deemed necessary to determine water, the Dean and Stark method is suitable. But, as rosin soap forms a gel in toluene, it is advisable to select amounts such that the proportion of total solids to toluene does not exceed 1 part in 30.

RESULTS—

Some of the results obtained from the analysis of rosin size of different kinds are shown in Tables I, II and III.

TABLE I

TYPICAL ANALYSES OF 25-ml SAMPLES OF ROSIN SIZES

Letters at tops of columns refer to procedure section headings

	Weight found,			Volume of 0·1 *N* acid or alkali required					(L) calculated		
Sample	(A), g	(B), g	(C), g	(D), ml	(E), ml	(G), ml	(H), ml	(K), ml	2(H)−(K), ml	(K)+(G), ml	(D)+(G)−(E), ml
1	0·044	0·858	0·814	25·2	22·3	7·4	6·7	2·9	10·5	10·3	10·3
2	0·095	0·982	0·887	27·9	29·8	7·8	2·1	−2·0*	6·2	5·8	5·9
3	0·060	0·923	0·863	26·9	19·1	1·1	8·6	8·0	9·2	9·1	8·9
4	0·062	0·932	0·870	27·2	22·7	1·1	5·3	4·7	5·9	5·8	5·6
5	0·101	0·944	0·843	25·5	21·7	3·2	5·6	3·9	7·3	7·1	7·0
6	0·059	0·914	0·855	26·7	17·5	0·53	9·45	9·2	9·7	9·73	9·73
7	0·059	0·880	0·821	25·6	16·9	0·60	9·0	8·7	9·3	9·3	9·3

* Solution alkaline after heating under reflux. An excess of acid was added and the solution was heated under reflux again.

TABLE II

CALCULATIONS FROM RESULTS IN TABLE I

Sample	Equivalent weight of rosin acid	Carbonate alkali (Na_2O) in total alkali (Na_2O), %	Net free acid in total rosin, %	Actual free acid in total rosin, %	Total alkali (Na_2CO_3) per ton of total rosin, lb
1	323	33·2	10·9	39·1	309
2	318	26·2	—	19·4	360
3	321	5·75	27·8	31·6	246
4	320	4·85	16·1	19·9	289
5	331	14·8	13·7	24·9	273
6	321	3·03	32·4	34·2	227
7	321	3·55	31·7	33·9	228

TABLE III

ANALYSIS OF A PROPRIETARY BRAND OF PASTE SIZE

Unsaponifiable and neutral rosin, %	5·7	
Actual free rosin acid, %	16·3	
Combined rosin anhydride, %	40·3	(41·5 of acid)
Total rosin, %	63·5	
Alkali combined with rosin (Na_2O), %	3·9	
Carbonate alkali (Na_2CO_3), %	2·1	(1·23 of Na_2O)
Water, %	31·0	
	99·3	

Equivalent weight of rosin acid	=	326
Proportion of carbonate alkali (Na_2O) in total alkali (Na_2O), %	=	23·8
Proportion of actual free rosin acid in total rosin, %	=	25·7
Total alkali in (Na_2CO_3) per ton of total rosin	=	308 lb

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Determination of Borate in Presence of Silver

BY S. Z. HAIDER

A modification of the usual volumetric procedure for the determination of boric acid in presence of silver ions is described. The interfering effect of the silver ion is first nullified by masking it as a soluble thiosulphate complex, after which the usual procedure is applicable. The proportions of free acid and thiosulphate have to be carefully controlled.

The investigation covered a range of boric acid from 0·0805 to 0·2480 g and sodium pyroborate from 0·0560 to 0·1505 g in mixtures with different proportions of silver nitrate. Results were found to vary within an error of ±0·7 per cent.

DURING a study of the properties of metallic borates it became necessary to analyse silver borate prepared by different methods, and a simple procedure for the determination of boric acid in presence of silver was required. The method of titration with alkali in presence of a polyhydric alcohol has to be modified when heavy-metal ions are present. Usually the metallic ions are first removed by precipitation as hydroxides.[1] This is time consuming and is apt to give low values for boric acid. More recently, separation of metallic ions by ion exchange[2] before determining the borate has been suggested. On the other hand, the effects of the metallic ions can be nullified without their removal from the titration samples being necessary. For example, with nickel a cyanide complex[3] can be formed, and borate can be accurately determined in presence of the nickel cyanide complex. Similarly, copper[4] can be masked by the formation of an iodide complex. The present paper reports the use of a soluble thiosulphate complex for silver.

If to a solution containing a suspension of silver chloride, sodium thiosulphate solution is added, a soluble thiosulphate is formed as follows—

$$AgCl + Na_2S_2O_3 \rightarrow NaAgS_2O_3 + NaCl$$

$$2AgCl + 3Na_2S_2O_3 \rightarrow Na_4Ag_2(S_2O_3)_3 + 2NaCl$$

Higher complexes are also possible, but from a consideration of the stability, the above representation is thought to be near to the truth. An excess of sodium thiosulphate must be avoided, or it may react with the mineral acid (or even with the boric acid) and vitiate the determination. The solution during addition of thiosulphate should be only slightly acid. The concentration of the finally diluted titration solution should not be above 0·1 *N*, otherwise decomposition of the complex occurs during subsequent heating of the liberated boric acid according to the equation—

$$2NaAgS_2O_3 + 2HCl \rightarrow 2NaCl + Ag_2S + SO_2 + H_2O.$$

METHOD

REAGENTS—

Silver nitrate solution, 0·1 N.
Boric acid solution, 0·1 N.
Sodium pyroborate solution, 0·1 N.
Sodium chloride solution, 5 *per cent. w/v.*
Sodium hydroxide solution, 0·1 N.
Sodium thiosulphate solution, 0·1 N.
Hydrochloric acid, dilute (1 + 60, *v/v*).
Glycerin—A diluted aqueous solution (1 + 1, w/v) made neutral to phenolphthalein with a drop or two of sodium hydroxide solution.

All the above reagents were AnalaR grade B.D.H. chemicals, except sodium thiosulphate and hydrochloric acid, which were B.D.H. pure chemicals. All solutions were prepared with re-distilled water free from carbon dioxide. Sodium hydroxide solution was prepared from freshly prepared sodium amalgam with carbon dioxide-free distilled water, care being taken to prevent the formation of any carbonate.

PROCEDURE—

For mixtures of sodium pyroborate and silver nitrate—First, mix together the desired proportions of solutions of sodium pyroborate and silver nitrate in a 250-ml conical flask; a precipitate of silver borate forms. Add a drop or two of methyl red and then, depending on the amount of borate taken, add dropwise 2 to 8 ml of the dilute hydrochloric acid solution until the solution is just acid to methyl red. A slight excess, up to 1 ml, but not more, may be added. Silver borate dissolves and silver chloride is precipitated. Dilute all of the solution to about 100 ml with distilled water free from carbon dioxide and gently warm under a reflux condenser to coagulate the silver chloride precipitate, which then conveniently falls to the bottom of the flask. Then add thiosulphate solution dropwise from the burette, shaking frequently, while the silver chloride precipitate goes into solution. If a turbid suspension of sulphur is formed, too much hydrochloric acid has been added, and the determination must be begun again with a fresh solution and a smaller proportion of hydrochloric acid. Addition of sodium thiosulphate solution must be stopped when the silver chloride precipitate has just disappeared. In fact, it is preferable to stop adding the thiosulphate while one or two grains of silver chloride precipitate still remain undissolved. The solution must be clear at this stage. A somewhat greyish precipitate indicates the addition of an excess of thiosulphate, which gives erroneous results. To the clear solution, add a drop or two of methyl red and neutralise the mineral acid accurately. The boric acid can then be conveniently titrated with phenolphthalein as indicator in presence of a sufficient amount of glycerin.

Boric acid and silver nitrate—For mixtures of boric acid and silver nitrate, precipitate the silver chloride with a solution of sodium chloride instead of hydrochloric acid. The rest of the procedure is the same as described above.

RESULTS

TABLE I

DETERMINATION OF BORIC ACID FROM A MIXTURE WITH SILVER NITRATE, WITHOUT THE FORMATION OF THIOSULPHATE COMPLEX

Silver taken, g	Boric acid taken, g	Boric acid found, g	Error, %
0·0123	0·0922	0·0940	+1·9
0·0369	0·1120	0·1138	+1·6
0·0615	0·1378	0·1410	+2·3
0·0861	0·1232	0·1262	+2·4
0·1230	0·1354	0·1392	+3·0
0·2460	0·1512	0·1563	+3·4

TABLE II

DETERMINATION OF BORIC ACID IN PRESENCE OF SILVER AFTER MASKING THE LATTER AS A SOLUBLE THIOSULPHATE COMPLEX

Silver taken, g	Boric acid taken, g	Boric acid found, g	Error, %
0·0123	0·0805	0·0799	−0·7
0·0246	0·1000	0·1002	+0·2
0·0369	0·1272	0·1268	−0·3
0·0492	0·1360	0·1367	+0·6
0·0615	0·1570	0·1571	+0·1
0·0738	0·1624	0·1620	−0·3
0·0861	0·1755	0·1760	+0·4
0·0984	0·1884	0·1874	−0·5
0·1107	0·2000	0·2004	+0·2
0·1230	0·2480	0·2492	+0·5

The error involved in estimating boric acid in presence of silver (without masking) is shown by the results in Table I. The error is always positive and increases with increasing proportions of silver. In Table II, analyses of boric acid from similar mixtures with silver nitrate are shown, but in these, before the boric acid was titrated, the silver was converted, with proper precautions, to the soluble complex of silver thiosulphate. Observed values

of boric acid were found to lie always within $\pm 0{\cdot}7$ per cent. of the true values. This is considered to be well within the experimental error in the usual method of boric acid determination. Table III shows results of determination of boric acid from mixtures of sodium pyroborate and silver nitrate.

TABLE III

DETERMINATION OF BORATE FROM A MIXTURE WITH SILVER NITRATE AFTER MASKING THE LATTER AS THE SOLUBLE THIOSULPHATE COMPLEX

Silver taken, g	Sodium pyroborate taken, g	Sodium borate found, g	Error, %
0·0123	0·0560	0·0559	−0·3
0·0246	0·0771	0·0765	−0·8
0·0369	0·0926	0·0931	+0·5
0·0492	0·1020	0·1022	+0·2
0·0615	0·1072	0·1077	+0·5
0·0738	0·1251	0·1243	−0·6
0·0984	0·1286	0·1289	+0·3
0·1230	0·1505	0·1515	+0·7

Results with sodium pyroborate show similar accuracies. It is therefore considered possible by the method presented to determine boric acid and borate in mixtures with silver nitrate.

The author wishes to thank Dr. M. H. Khundkar for kindly suggesting the problem and taking keen interest during the progress of work, and also Dr. H. Marsh for his help.

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DEPARTMENT OF CHEMISTRY
UNIVERSITY OF DACCA
EAST PAKISTAN

March 27th, 1953

The Analysis of Acid Chlorides

BY C. C. T. CHINNICK AND P. A. LINCOLN

A method is described for the analysis of samples of organic acid chlorides containing as impurities free organic acid, anhydride and hydrochloric acid.

The procedure consists in (*i*) plotting a titration graph against a pH meter, from which the points corresponding to the neutralisation of hydrochloric acid and organic acid are read and (*ii*) determining the ester by hydrolysis with an excess of standard alkali.

Special provision is made for determining anhydride in the presence of hydrochloric acid and details of an alternative, but less convenient, titration procedure, by means of a double indicator, are also given.

Formulae for the calculation of results are included.

IN comparing the relative efficiency of various chlorinating agents used for the preparation of acid chlorides, or in estimating the yields in reactions in which impure acid chlorides have been used, a method of determining acid chlorides and the impurities likely to be present is necessary. In addition to the acid chloride there may be present free acid, hydrogen chloride and sometimes acid anhydride. The method of analysis suggested here was primarily designed for the determination of acid chloride, free acid and hydrogen chloride, but the results can be interpreted to give a determination of acid anhydride if this is present.

Several methods have been suggested in the literature for the analysis of acid chlorides that make use of the reaction of an acid chloride with ammonia or an amine to form an amide.[1,2,3] After conversion of the acid chloride to amide the free acid is determined by titration with alkali. A determination of total acid by hydrolysing a second sample with alkali enables the amount of acid chloride to be calculated by difference. No analytical application of the reaction of acid chlorides with alcohols to form esters appears to have been made, although the converse application of this reaction has long been used as a method for the determination of hydroxyl groups. It has been found that this reaction has several advantages over the amide reaction; a more complete analysis can be made, only a single sample is required and the results can be interpreted to determine the presence of acid anhydride.

METHOD

To 25 ml of a 33 per cent. v/v solution of dry pyridine in dry butanol add the acid chloride (0·025 moles approximately). Then heat on a steam-bath under a reflux condenser (an air condenser is satisfactory) to complete the esterification.* Cool the sample, dilute with ethanol and titrate with *N* potassium hydroxide, using a pH meter.

Two of the curves plotted in this way are shown in Figs. 1 and 2. If free acid or acid

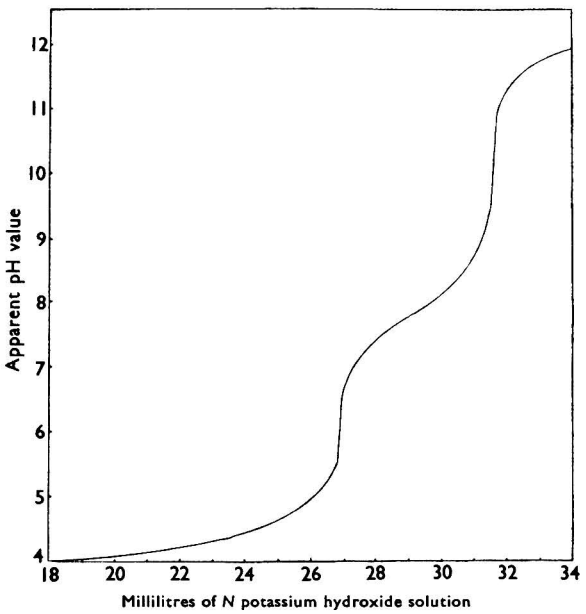

Fig. 1. Titration graph for sample of lauroyl chloride

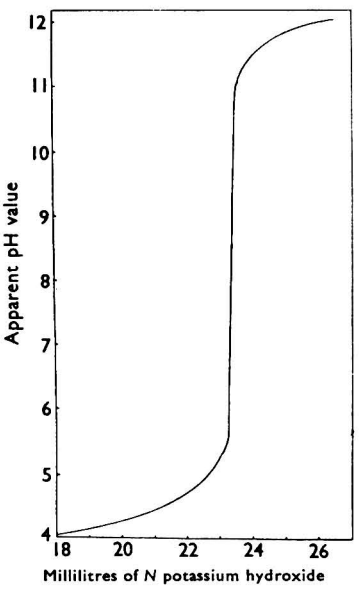

Fig. 2. Titration graph for sample of benzoyl chloride

anhydride is present, two points of inflection will be found on the pH - titration curve. The first point of inflection at about pH 6 corresponds to the neutralisation of the hydrochloric acid and the second, at about pH 10, to that of the organic acid. After reaching the second point of inflection, add a known excess, about 50 ml, of *N* potassium hydroxide and boil the solution under a reflux condenser for 2 to 3 hours to hydrolyse the ester. If the ester is difficult to hydrolyse, it may be found advantageous to remove some of the ethanol by distillation to increase the concentration of the alkali and hence also the rate of hydrolysis. When the hydrolysis of the ester is complete, titrate the excess of alkali with *N* hydrochloric acid.

ALTERNATIVE METHOD—

If a pH meter is not available, the hydrochloric and organic acids may be differentiated by other means. Attempts were made to show the end-points at pH 6 and pH 10 in the

* The time required depends on the reactivity of the acid chloride. Acetyl chloride reacted almost instantaneously at room temperature, benzoyl chloride required heating for 5 minutes and lauroyl chloride about 30 minutes.

potassium hydroxide titration by means of a double-indicator method. Although methyl red and thymolphthalein were the best pair of indicators found, there was a considerable loss of accuracy in the determination. A better differentiation between the hydrochloric and organic acids can be obtained by modifying the method so that the former is determined by titration with silver nitrate. After esterifying the acid chloride, determine the total acid, hydrochloric and organic, by titrating with *N* potassium hydroxide to a phenolphthalein or thymolphthalein end-point. Then hydrolyse the ester with alkali in the usual way and titrate the excess of alkali with *N* nitric acid to the same end-point. Evaporate the solution to about 50 ml, add 300 ml of water and boil until the solution is free from pyridine. Titrate an aliquot portion of this solution with 0·1 *N* silver nitrate to a dichlorofluorescein end-point.

In some admixtures the organic acid interferes with the silver nitrate titration and must be removed, *e.g.*, by evaporating to dryness and ashing, before the titration can be carried out. This alternative method is less convenient than the electrometric method and should be avoided if possible.

Interpretation of results

Let T_1 = millilitres of *N* alkali equivalent to hydrochloric acid.
T_2 = millilitres of *N* alkali equivalent to organic acid.
T_3 = millilitres of *N* alkali used to hydrolyse the ester.

The four components of the mixture may be acid chloride, free acid, acid anhydride and hydrochloric acid, and these will react as follows—

$$\mathrm{RCOCl} + \mathrm{BuOH} \rightarrow \mathrm{RCOOBu} + \mathrm{HCl}$$
$$(\mathrm{RCO})_2\mathrm{O} + \mathrm{BuOH} \rightarrow \mathrm{RCOOBu} + \mathrm{RCOOH}$$
$$\left.\begin{array}{l}\mathrm{RCOOH} \rightarrow \mathrm{RCOOH} \\ \mathrm{HCl} \rightarrow \mathrm{HCl}\end{array}\right\} \text{ no reaction.}$$

From this it can easily be seen that—

$$T_1 \equiv \mathrm{RCOCl} + \mathrm{HCl}$$
$$T_2 \equiv (\mathrm{RCO})_2\mathrm{O} + \mathrm{RCOOH}$$
$$T_3 \equiv \mathrm{RCOCl} + (\mathrm{RCO})_2\mathrm{O}$$

These simultaneous equations can be solved only if one of the four variables is equal to zero. In most mixtures no anhydride is present and the results can therefore be interpreted to give the amounts of acid chloride, free acid and hydrochloric acid present. If the presence of anhydride is indicated (*i.e.*, when T_1 is less than T_3) or suspected, one of the other variables must be made zero. This is most conveniently carried out by removing any free hydrochloric acid present in the sample by passing a stream of dry air through it. With one of the four variables made equal to zero, the amounts of the remaining three components may be calculated as under—

If W = weight of sample taken,
M_1 = molecular weight of acid chloride,
M_2 = molecular weight of organic acid,
and M_3 = molecular weight of acid anhydride,

then either $\dfrac{T_3 \times M_1}{10\,W}$ = percentage of acid chloride,

$\dfrac{T_2 \times M_2}{10\,W}$ = percentage of free organic acid

and $\dfrac{(T_1 - T_3) \times 36{\cdot}46}{10\,W}$ = percentage of free hydrochloric acid

or $\dfrac{T_1 \times M_1}{10\,W}$ = percentage of acid chloride,

$\dfrac{(T_3 - T_1)M_3}{10\,W}$ = percentage of acid anhydride

and $\dfrac{(T_1 + T_2 - T_3)M_2}{10\,W}$ = percentage of free organic acid.

Some results obtained by this method of analysis are shown in Tables I, II and III.

TABLE I

THE ANALYSIS OF CRUDE LAUROYL CHLORIDE, ALONE AND MIXED WITH LAURIC ACID

Lauroyl chloride taken, g	Lauric acid taken, g	T_1	T_2	T_3	Lauroyl chloride Found, %	Lauroyl chloride Calcd., %	Lauric acid Found, %	Lauric acid Calcd., %
6·2732	0	26·5	2·3	26·5	92·55	—	7·35	—
5·8728	0·6711	24·8	5·5	24·8	83·01	83·13	16·86	16·82
5·2248	1·2036	22·0	7·9	22·0	75·00	75·31	24·66	24·71

(The molecular weight of acid used was determined by titration in neutralised alcohol as 200·6; $C_{11}H_{23}COOH$ = 200·3.)

TABLE II

THE ANALYSIS OF ACID CHLORIDES

Acid chloride taken, g	T_1	T_2	T_3	Acid chloride found, %	Free acid found, %
Acetyl chloride, 2·2938	27·9	1·9	27·7	94·80	4·97
Acetyl chloride, 2·3629	28·2	2·4	28·2	93·70	6·10
Benzoyl chloride, 3·3025	23·4	0	23·4	99·60	0

TABLE III

THE ANALYSIS OF A MIXTURE OF ACETYL CHLORIDE AND ACETIC ANHYDRIDE

Acetyl chloride taken, g	Acetic anhydride taken, g	T_1	T_2	T_3	Acetyl chloride Found, %	Acetyl chloride Calcd., %	Acetic anhydride Found, %	Acetic anhydride Calcd., %	Free acid Found, %	Free acid Calcd., %
1·9212	0·7763	23·0	10·5	30·5	66·94	66·73	28·40	28·78	4·45	4·34

Table I shows the analysis of crude lauroyl chloride made by the action of 1·2 moles of thionyl chloride on 1 mole of lauric acid (*cf.* the results of Ackley and Tesoro[2]) and samples of the same material to which known additions of lauric acid had been made. In all these samples free hydrochloric acid had been removed by passing dry air through the material; it can be seen that $T_1 = T_3$ in all these analyses, which shows the absence of lauric anhydride.

Table II shows the results of some analyses of acetyl and benzoyl chlorides. In the first analysis of the acetyl chloride, T_1 is less than T_3, which indicates the presence of some free hydrochloric acid. Dry air was then passed through this material, which was then analysed again. It was now found that $T_1 = T_3$ but that the acid chloride content of the sample had decreased slightly owing to the volatility of acetyl chloride. To illustrate the analysis of a sample containing an anhydride, a known amount of acetic anhydride was added to this acetyl chloride, and the result of analysing the mixture is shown in Table III.

The authors thank the Directors of Milton Antiseptic Ltd. for their permission to publish this article.

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MILTON ANTISEPTIC LTD.
LONDON, N.7

May 12th, 1953

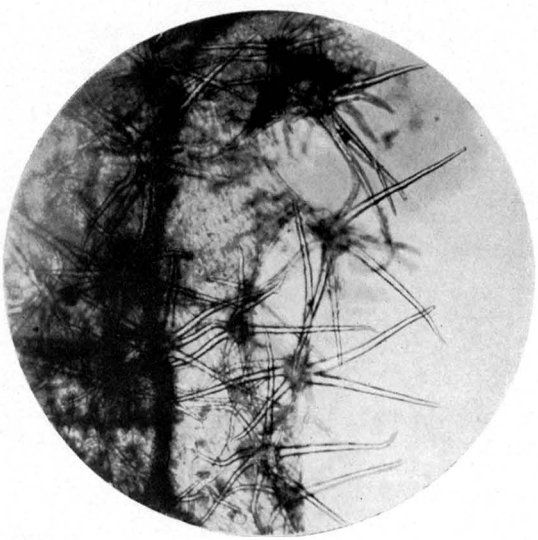

Fig. 1. Cistus ($\times 87$)

Notes

AN ADULTERANT OF DRIED SAGE

SEVERAL samples submitted as dried sage and recently examined in this laboratory have been found to contain from 10 to 20 per cent. of a foreign leaf.

This leaf is believed to be that of a species of *Cistus*, a genus of flowering shrubs growing commonly in the Mediterranean region. Some, at least, of these samples were known to contain sage of French origin, and as the two plants are superficially similar in appearance, the admixture may be due to carelessness in picking the leaves. The presence of *Cistus* in marjoram has previously been reported.

The foreign leaf is difficult to differentiate from sage macroscopically, but can be distinguished under the microscope by the stellate hairs (see Fig. 1) and the presence of calcium oxalate crystals.

I am indebted to the Director of the Royal Botanic Gardens, Kew, for assistance in identifying the adulterant.

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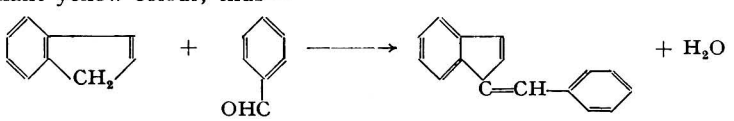
WALWORTH ROAD, LONDON, S.E.17

First submitted, *January 8th*, 1953
Amended, *June 24th*, 1953

THE COLORIMETRIC DETERMINATION OF INDENE

UHRIG, Lynch and Becker[1] used the yellow colour of a fulvene produced from *cyclo*pentadiene or *cyclo*pentadiene homologues and an aldehyde or ketone for the colorimetric determination of *cyclo*pentadiene. They found that benzaldehyde gives the most stable colour with *cyclo*pentadiene.

It has now been found that indene can be determined in the same way, as its methylene group is capable of reacting with benzaldehyde in alkaline solution to give a fulvene derivative with a stable and brilliant yellow colour, thus—



*cyclo*Pentadiene and most other hydrocarbons having a reactive methylene group boil at a temperature very different from that at which indene boils and can, therefore, be easily separated from it by distillation. The present method based on the formation of a fulvene permits the determination of small concentrations of indene (down to as little as 0·2 per cent.) with sufficient accuracy when a Spekker absorptiometer standardised as described below is used, so that the full scale of the drum corresponds to an indene concentration of 10 per cent. Higher concentrations can be measured after suitable dilution with toluene.

It is important that all conditions laid down below are strictly adhered to, especially the order of mixing the reagents, as erratic results may otherwise occur.

METHOD

REAGENTS—

Benzaldehyde—A 20 per cent. solution of distilled benzaldehyde in ethyl alcohol.

Potassium hydroxide—A 5 per cent. solution in ethyl alcohol.

Acetic acid—A 4 per cent. v/v solution in water.

Toluene—Nitration grade.

Anhydrous sodium sulphate—Dry by heating over a bunsen burner for an hour, cool and keep in a desiccator.

Ethyl alcohol—Pure ethyl alcohol is required for the preparation of the benzaldehyde and potassium hydroxide solutions. Prepare it by slowly adding 10 g of metallic calcium to 500 ml of absolute alcohol, heating under a reflux condenser for 3 hours and distilling.

APPARATUS—

A Hilger Spekker absorptiometer with 1-cm cells, Ilford No. 603 filter and an H503 heat filter.

PROCEDURE—

To two 250-ml conical flasks with ground-in stoppers, A and B, add 10 ml of sample (diluted with toluene, if necessary, so that the indene concentration is between 1 and 10 per cent.) and 5 ml of benzaldehyde solution. Add 10 ml of acetic acid solution to flask B, which serves as blank, and then 5 ml of alcoholic potassium hydroxide solution to both flasks. Note the time, shake the flasks well by hand and set them aside for exactly 30 minutes from the time of adding the potassium hydroxide. Then add 10 ml of acetic acid solution to flask A.

Decant the two hydrocarbon layers into test tubes each containing about 1 g of anhydrous sodium sulphate. Stopper the tubes and shake them thoroughly. After 5 minutes, pour the solutions into the absorptiometer cells, and determine the optical densities. Standardise the absorptiometer for sensitivity with the blank at a drum reading of 1·00. The drum reading for the sample gives the direct percentage of indene from a graph constructed from the figures shown in Table I.

NOTES ON THE PROCEDURE—

If alkali is added to the sample at the same time as the benzaldehyde solution or mixed with the benzaldehyde solution before the sample is added, the colour that develops interferes with the indene determination, as the intensity of this colour is constant only if the stated conditions are strictly adhered to.

Errors are also introduced if the final hydrocarbon solutions are not perfectly dry. Sufficient desiccant must be added for some fine dry salt to be present after the drying of the solution is complete.

The indene sample used for analysis should be dissolved in toluene or a similar hydrocarbon solvent.

STANDARDISATION—

Commercially pure indene was fractionated on a 2-foot Dixon column under reduced pressure (100 mm of mercury); hydroquinone was used as the inhibitor and the distillate was collected in a receiver cooled with solid carbon dioxide. The fraction boiling at 114° C, the equivalent of 181° to 182° C at a pressure of 760 mm of mercury, having a bromine value of 132·0 and a refractive index $n_D^{20} = 1{\cdot}5721$, was taken and various amounts diluted with toluene and treated as described in the procedure above. The results are shown in Table I; from these figures a standardisation graph can be drawn.

TABLE I

STANDARDISATION OF SPEKKER ABSORPTIOMETER FOR INDENE DETERMINATION

Indene concentration, %	Drum reading	Indene concentration, %	Drum reading
1	0·862	6	0·471
2	0·755	7	0·417
3	0·662	8	0·391
4	0·590	9	0·351
5	0·535	10	0·326

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PETROCARBON LTD.
TWINING ROAD, TRAFFORD PARK
MANCHESTER, 17

W. ROMAN
M. SMITH
April 16th, 1953

THE "ALBUMINOID AMMONIA VALUE" IN THE ANALYSIS OF FRUIT JUICES, SQUASHES AND CORDIALS

As reported earlier,[1] the determination of albuminoid ammonia has been used in the analysis of fruit drinks such as fruit juices, squashes and cordials.

PROCEDURE—

The procedure for the determination of albuminoid ammonia in vinegar has been applied to soft drinks with the following modifications.

Neutralise exactly 5 g of sample instead of 5 ml as taken for vinegar. Unlike that of vinegar, the specific gravities of soft drinks are distinctly above 1·0 and the results have been expressed in parts per million w/w.

After the first distillation and the addition of 50 ml of alkaline potassium permanganate reagent, add 4 g of solid potassium permanganate and continue distillation, inserting a trap between the distilling flask and the condenser. This excess of permanganate is required to counteract the reducing action of sugars present in these products.

In all other respects the method is exactly the same as in the analysis of vinegar. Calculate the result in parts per million w/w.

RESULTS—

By the above procedure, a broad correlation was observed between the figures for total and albuminoid ammoniacal nitrogen. Different categories of soft drinks analysed gave the results shown in Table I.

TABLE I

TOTAL AND ALBUMINOID NITROGEN IN SOFT DRINKS

Type of drink	Range of albuminoid ammoniacal nitrogen, p.p.m. w/w	Range of total nitrogen, p.p.m. w/w
Pure undiluted lemon juice (15 samples)	294 to 456	800 to 1090
Pure undiluted orange juice (15 samples)	337 to 557	807 to 1170
Pure undiluted tomato juice (15 samples)	333 to 570	806 to 1200
Lemon squash conforming to the standards laid down in the Fruit Products Order, Government of India (7 samples)*	72 to 150	205 to 420
Orange squash conforming to the above standards (7 samples)	100 to 220	200 to 616
Lime juice cordial conforming to the above standards (7 samples)	80 to 100	185 to 315
Purely factitious products, and those containing very little fruit juice and not conforming to the above standards (10 samples)	nil to 40	7 to 81
Pure undiluted mango juice (35 samples)	290 to 1000	750 to 2000
Pure undiluted grape juice (7 samples)	250 to 360	600 to 790
Pure undiluted pineapple juice (14 samples)	260 to 520	540 to 1036
Pure undiluted orange ("Mossambic" variety) juice (8 samples)	520 to 700	1162 to 1530
Pure undiluted apple juice (4 samples)	130 to 210	365 to 650

* Under the Fruit Products Order, Government of India, squashes and cordials must contain not less than 25 per cent. of fruit juice.

It will be seen that there is a significant difference between the albuminoid ammonia values of soft drinks of good quality, that is, conforming to the above Government standards, and those of purely artificial products or of those containing very little fruit juice. This determination provides an approximate measure of the percentage of fruit juice and serves as an excellent routine sorting test. In the examination of these soft drinks, the determination of total nitrogen by the long Kjeldahl method may be dispensed with, just as for vinegar.

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WEST BENGAL PUBLIC HEALTH LABORATORY
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S. N. MITRA
S. C. ROY

April 7th, 1953

Apparatus

AN APPARATUS FOR DEAD-STOP END-POINT TITRATIONS WITH ACOUSTIC INDICATION OF THE END-POINT

THE apparatus, the circuit diagram of which is shown in Fig. 1, is designed to provide an audible indication of the end-point by means of a continuous note in "dead-stop"[1,2] titrations with adequate warning of the approach to the end-point, when local and transitory excesses of titrant produce an intermittent note.

The apparatus was exhibited at the First International Congress on Analytical Chemistry, Oxford, 1952.

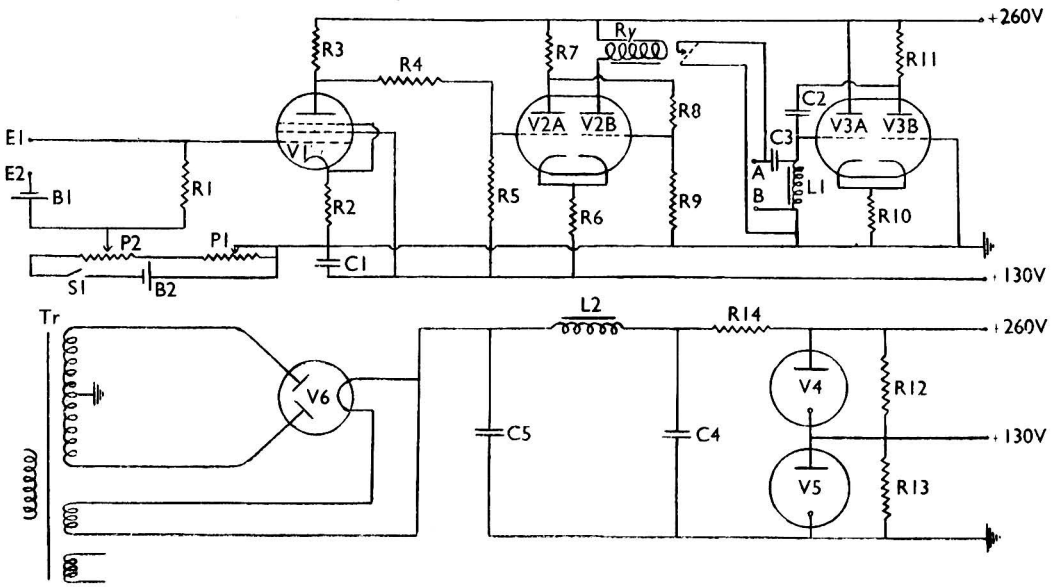

Fig. 1. Circuit diagram of apparatus for giving acoustic indication of dead-stop end-points

DESCRIPTION AND MODE OF USE—

The apparatus, used with a pair of platinum electrodes, E_1 and E_2, polarised by battery B_1, consists of a direct-current amplifier, V_1, a double triode Schmitt trigger[3] circuit, V_{2A}, V_{2B}, a double triode cathode-coupled audio frequency oscillator,[4] V_{3A}, V_{3B}, and a conventional neon-stabilised power supply.

The grid bias of V_1 is adjustable by means of P_1 and P_2, which act as coarse and vernier controls, respectively, to control the standing anode voltage of the valve and therefore the voltage at the grid of V_{2A}, the control grid of the trigger circuit. If P_1 and P_2 are adjusted to increase the negative bias of V_1 until V_{2A} and V_{2B} trigger, V_{2B} cuts off, the relay, Ry, opens, switching on the output of the oscillator, and a note is heard in headphones or a loudspeaker connected across A and B. If P_2, the vernier control, is then turned back until the relay closes and short-circuits the oscillator output, the note ceases until the additional voltage that appears at the grid of V_1 at the end-point of a direct titration again switches on the oscillator by the action of the trigger. A direct titration here is one in which a solution that polarises one or both electrodes is titrated with the appropriate depolarising reagent.

The common cathode resistance, R_6, to V_{2A}, V_{2B}, which controls the sensitivity of the trigger circuit, has a value such that the apparatus does not respond to external electrical interference, but is sensitive, *e.g.*, in the titration of thiosulphate with iodine, to 0·05 ml of 0·1 *N* iodine solution in 50 ml of solution.

The apparatus is applicable to any other dead-stop end-point titration, *e.g.*, the titration of water with Karl Fischer's reagent, but is not suitable for titrations with other electrode systems without the provision of a high-impedance input stage of the cathode-follower type.

COMPONENT VALUES—

V_1 = 6SJ7.	R_6 = 250 ohms.	C_1 = 2 μF.
V_2 = 6SN7.	R_7 = 100KΩ.	C_2 = 0·1 μF.
V_3 = 6SN7.	R_8 = 470KΩ.	C_3 = 0·01 μF.
V_4 = S130.	R_9 = 2MΩ.	C_4 = 8 μF.
V_5 = S130.	R_{10} = 3KΩ.	C_5 = 8 μF.
V_6 = 5U4G.	R_{11} = 100KΩ.	L_1 = 4 henries.
R_1 = 500KΩ.	R_{12} = 250KΩ.	L_2 = 10 henries.
R_2 = 5KΩ.	R_{13} = 250KΩ.	Tr. = 350–0–350 V, 5 V, 6·3 V transformer.
R_3 = 100KΩ.	R_{14} = 10KΩ.	S_1 = Single pole on–off switch.
R_4 = 100KΩ.	P_1 = 1MΩ.	B_1 = 1·5-V cell.
R_5 = 5MΩ.	P_2 = 100KΩ.	B_2 = 18-V battery.

The author wishes to thank the Directors of May & Baker Limited for permission to publish this note.

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THE ANALYTICAL CONTROL LABORATORIES
MAY AND BAKER LIMITED
DAGENHAM, ESSEX

H. A. GLASTONBURY
April 20th, 1953

Ministry of Food

CURRENT STATUTORY INSTRUMENTS AND STATUTORY RULES AND ORDERS RELATING TO FOOD

The Index of Current Statutory Instruments and Statutory Rules and Orders, Sectional List No. **33**, *has been revised to June 30th*, 1953, *and can be obtained from H.M. Stationery Office at cost of postage. See Analyst*, 1953, **78**, 257.

British Standards Institution

DRAFT SPECIFICATIONS

A FEW copies of the following draft specifications, issued for comment only, are available to members of the Society, and can be obtained from the Secretary, Society of Public Analysts and Other Analytical Chemists, 7–8, Idol Lane, London, E.C.3.

Draft Specifications prepared by Technical Committee TPC/2—Cresylic Acid and Phenol.
- CR(TPC)5988—Draft B.S. for Refined Cresylic Acid. (Revision of B.S. 524.)
- CR(TPC)6315—Draft B.S. for Cresylic Acid of Specified Orthocresol Content. (Revision of B.S. 517.)

Draft Specification prepared by Technical Committee LBC/15—Laboratory Autoclaves.
- CR(LBC)6264—Draft B.S. for Copper Laboratory Autoclaves.

Draft Specifications prepared by Technical Committee FCC/4—Solvents and Allied Products.
- CR(FCC)6266—Third Draft B.S. for Di-2-Ethylhexyl Sebacate.
- CR(FCC)6267—Third Draft B.S. for Ethanediol (Ethylene Glycol).
- CR(FCC)6268—Third Draft B.S. for Monochlorobenzene.
- CR(FCC)6269—Third Draft B.S. for Di-*n*-Butyl Sebacate.

Draft Specifications prepared by Technical Committee LBC/11—Microchemical Apparatus.
- CR(LBC)6265—Draft B.S. for Syringe Pattern Micro-Pipette (Part D5 of B.S. 1428, Microchemical Apparatus).
- CR(LBC)6285—Draft B.S. for Micrometer-Operated Burette (Part D6 of B.S. 1428, Microchemical Apparatus).

Draft Specifications prepared by Technical Committee HCC/2—Desiccants.
- CR(HCC)5921—Second Draft B.S. for Activated Alumina for use as Desiccant for Packages.
- CR(HCC)5922—Fourth Draft B.S. for Silica Gel for use as Desiccant for Packages.

Infra-red Spectra

THE Chemical Society has accepted a report from a Joint Committee on the Reproduction of Infra-red Absorption Data on which were represented the Royal Society, the Chemical Society, the Physical Society, the Faraday Society, the Institute of Petroleum and the Society of Public Analysts and Other Analytical Chemists, as well as other interested bodies. The conditions governing acceptance of infra-red absorption curves for publication by the Chemical Society were laid down in the *Proceedings of the Chemical Society* for December, 1952, p. 164. The relevant part of the report that the Publication Committee has agreed to apply to *The Analyst* is as follows—

The format in which spectra are published must accord with the following conventions:

(*i*) absorption should be plotted with 100 per cent. at the top;
(*ii*) the low-frequency (long wavelength) end of the spectra should be on the right;
(*iii*) spectra should be linear in wave-numbers;
(*iv*) in regions where absorption rises above about 80 per cent., or where significant weaker bands are not made evident, the spectra of more than one thickness should be recorded when possible; and
(*v*) scales of different parts of the curves may be different so as to prevent undue spreading or bunching of the maxima.

Examples of infra-red absorption spectra presented in this format appear in *The Analyst*, 1950, **75**, 68–69; 1951, **76**, 35; 1952, **77**, 738–739.

The full recommendations of the Joint Committee place considerable limitations on the publication of infra-red spectra. To permit their application without impeding the progress of science a recording and indexing system must be instituted, and it is only the possibility of such an organisation being established that has enabled the full recommendations to be framed.

As an interim measure until a full library scheme is in operation, the Chemical Society will accept for deposit copies of infra-red spectra referred to in new papers in *The Analyst*, whether such spectra are published or not. Spectra so deposited must be drawn on forms obtainable from the General Secretary of the Chemical Society (Burlington House, Piccadilly, London, W.1), and the Chemical Society will make copies available to others on request. Copies of spectra intended for deposit must be sent direct to the Chemical Society; spectra submitted for reproduction in *The Analyst* should accompany manuscripts in the usual way.

Photocopies of spectra deposited with the Chemical Society will be made available to the public on request to the General Secretary of the Chemical Society. Applications must quote the C.S. numbers of the spectra desired and must be accompanied by a remittance of 3s. per copy per spectrum (post free). Lists of spectra available will be published quarterly in the *Proceedings of the Chemical Society* and annual lists will be published in the Index Volumes of the *Journal of the Chemical Society*.

Publications Received

PRACTICAL CHROMATOGRAPHY. By R. C. BRIMLEY and F. C. BARRETT. Pp. 128. London: Chapman & Hall Ltd. 1953. Price 15s.

ION EXCHANGERS IN ANALYTICAL CHEMISTRY. By OLOF SAMUELSON. Pp. xviii + 291. New York: John Wiley & Sons Inc.; Stockholm: Almquist & Wiksell; London: Chapman & Hall Ltd. 1953. Price $6.50; 52s.

FLUORESCENCE OF SOLUTIONS. By E. J. BOWEN, M.A., D.Sc., F.R.S., and FRANK WOKES, Ph.D., B.Sc., F.R.I.C., Ph.C. Pp. viii + 91. London and New York: Longmans, Green & Co. Ltd. 1953. Price 25s.; $4.00.

THE FURANS. American Chemical Society Monograph Series No. 119. By A. P. DUNLOP and F. N. PETERS. Pp. xx + 867. New York: Reinhold Publishing Corp.; London: Chapman & Hall Ltd. 1953. Price $18.00; 144s.

ORGANIC REACTIONS. Volume VII. Editor-in-Chief, ROGER ADAMS. Pp. viii + 440. New York: John Wiley & Sons Inc.; London: Chapman & Hall Ltd. 1953. Price $9.00; 72s.

AIDS TO QUALITATIVE PHARMACEUTICAL ANALYSIS. By J. PRIESTMAN, Ph.D., B.Sc., M.P.S., and F. C. G. EDWARDS, Ph.C. Pp. vii + 144. London: Baillière, Tindall & Cox Ltd. 1953. Price 6s.

STRUCTURE AND MECHANISM IN ORGANIC CHEMISTRY. By C. K. INGOLD, D.Sc., F.R.S. Pp. x + 828. London: G. Bell & Sons Ltd. 1953. Price 77s. 6d.

IMPERIAL CHEMICAL INDUSTRIES LIMITED, Wilton Works, near Redcar, has vacancies for MALE LABORATORY ASSISTANTS. Candidates should be aged 19 or over and passed five subjects at Ordinary level in the General Certificate of Education examination or be of equivalent standard. Experience in an Analytical Laboratory would be an advantage. Write for application form to Staff Officer, Imperial Chemical Industries Limited, Wilton Works, Middlesbrough, quoting advertisement reference ICI/X/148/F.

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CHEMIST REQUIRED to assist in laboratory dealing with members' day-to-day production problems. Initial salary up to £650. Superannuation (F.S.S.U.). Five-day week. Application forms from Printing, Packaging and Allied Trades Research Association, Randalls Road, Leatherhead, Surrey.

QUALIFIED CHEMIST REQUIRED. Must have good experience in cereal chemistry. Age about 35 years. Capable of controlling staff, directing research and running the analytical side of laboratories. Salary according to experience. Box No. 3851, THE ANALYST, 17, Gresham Street, London, E.C.2.

S.P.A. member wishes to borrow January, 1918, *Analyst* for photostat copying to complete set. Anybody oblige? Grant, 107, Fenchurch Street, London, E.C.3.

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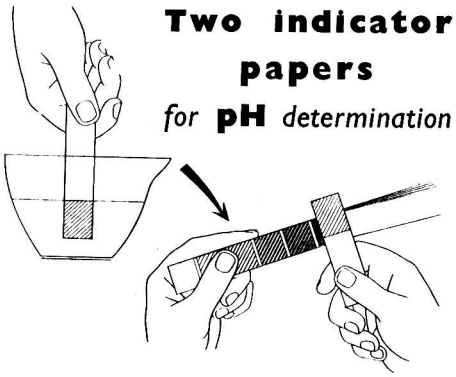

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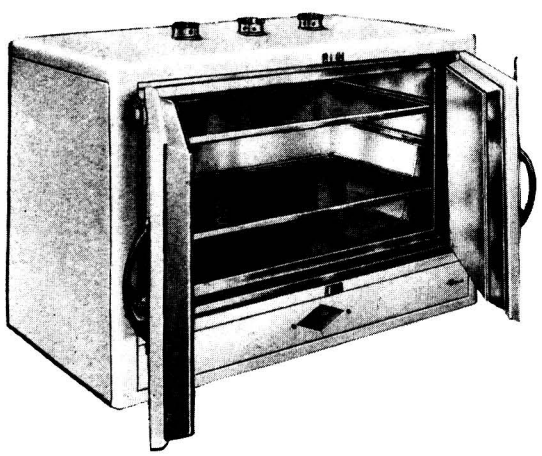

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