THE ANALYST

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Editor: J. B. ATTRILL, M.A.
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No. 18 February, 1954

THE SOCIETY FOR ANALYTICAL CHEMISTRY

BULLETIN

FORTHCOMING MEETINGS

Annual General Meeting of the Society, March 3rd, 1954

THE Annual General Meeting of the Society will be held at 4.30 p.m. on Wednesday, March 3rd,* 1954, in the Meeting Room of the Royal Society, Burlington House, Piccadilly, London, W.1.

This will be followed at about 5 p.m. by the Bernard Dyer Memorial Lecture, entitled "The Contribution of Public Analysts and Other Analytical Chemists to Public Welfare," to be given by E. B. Hughes, D.Sc., F.R.I.C.

* The date of this meeting has been altered since the Programme of Meetings for the Session was printed.

Informal Dinner of the Society

THE Society will hold an Informal Dinner at the Trocadero Restaurant, Piccadilly Circus, London, W.1, on Wednesday, March 3rd, 1954, at 7 p.m. for 7.30 p.m. Guests, particularly ladies, will be welcome.

Ordinary Meeting of the Scottish Section, March 11th, 1954

AN Ordinary Meeting of the Scottish Section will be held at 7.15 p.m. on Thursday, March 11th, 1954, at the George Hotel, George Street, Edinburgh, 2.

At this meeting a lecture entitled "Applications of Infra-red Spectroscopy" will be given by H. A. Willis, B.Sc.

Ordinary Meeting of the Physical Methods Group, March 9th, 1954

THE Forty-fourth Ordinary Meeting of the Physical Methods Group will be held at 6.30 p.m. on Tuesday, March 9th, 1954, in the Meeting Room of the Chemical Society, Burlington House, Piccadilly, London, W.1.

The subject of the meeting will be "Refractometry and Interferometry," and the following papers will be presented—

"Differential Refractometers: Theory and Construction," by G. H. F. Seiflow, M.A., A.Inst.P.

"An Application of Differential Refractometry," by R. Hill, B.Sc., A.R.I.C.

"Interferometric Refractometry: a Survey of the Methods," by H. G. Kuhn, M.A., D.Phil.

"The Use of a Rayleigh Interferometer for Estimating Trichloroethylene," by R. E. Jahn, M.A.

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.

"The Polarographic Determination of Fluoride. Part I: Basic Principles of the Method: Application to the Cathode-ray Polarograph," by B. J. MacNulty, G. F. Reynolds and E. A. Terry.

> Modern interest in traces of fluoride has revealed the need of more sensitive methods of fluoride determination. In this paper a method of great sensitivity for determining fluoride polarographically is described. The method is based on the depression by fluoride of the polarographic step given by the reduction of the aluminium - Solochrome Violet R.S. complex. The step depression is shown to be linearly related to amount of fluoride down to 0·001 μg per ml. The use of this method with the cathode-ray polarograph is described.

"The Determination of Glucosamine," by R. Belcher, A. J. Nutten and Miss C. M. Sambrook.

> The colorimetric method for the determination of glucosamine based on the reactions with acetylacetone and *p*-dimethylaminobenzaldehyde has been systematically examined. Several improvements have been made in the method, which has been applied to the determination of glucosamine in N-acetyl-α-methylglucosaminide and heparin. The method has been adapted for use with commercially available instruments.

"Colorimetric Determination of Iridium and Rhodium," by A. D. Maynes and W. A. E. McBryde.

> A procedure for the colorimetric determination of iridium has been developed in which the sulphate is oxidised by cerium^{IV} sulphate to produce a red solution. The tin^{II} chloride procedure for the colorimetric determination of rhodium has been examined with special attention to the effect of the presence of iridium. A procedure has been worked out for the application of these methods in sequence to the analysis of solutions containing iridium and rhodium together, without the necessity of a prior separation. Some data are presented to show that the mixed perchloric - phosphoric acid procedure for the colorimetric determination of iridium may lack precision.

"The Spectrophotometric Determination of Magnesium with Thiazol Yellow Dyes," by T. A. Mitchell.

> A critical examination of the Thiazol yellow method for the colorimetric determination of magnesium has shown the following: (*i*) The fading of the Thiazol yellow - magnesium hydroxide complex is caused by "ageing" of the magnesium hydroxide. This change in the structure of the colloid, which takes place both in the presence and the absence of Thiazol yellow, is inhibited by the addition of glycerol and concentrated sodium hydroxide. (*ii*) The solubility of magnesium hydroxide and hence of the coloured complex is greatly increased by the colloid protectors, starch and polyvinyl alcohol. Starch, however, is preferable to polyvinyl alcohol for this purpose, because, unlike the alcohol, it does not itself affect the colour of the dye or dye-complex. (*iii*) Numerous cations and organic compounds interfere, and neither their removal by precipitation nor the use of "compensating solutions" satisfactorily controls this effect.
>
> A method is accordingly proposed in which the magnesium itself is precipitated from solution as magnesium ammonium phosphate, the precipitate is redissolved and the colorimetric determination is carried out on the resulting solution, starch being used as the protective colloid and glycerol as the colour stabiliser. The absorptions of the solutions are measured at a fixed brief interval after colour development.

"An Improved Apparatus for the Micro-determination of Unsaturation in Organic Compounds by Catalytic Hydrogenation," by A. F. Colson.

A description is given of an improved form of the apparatus devised by Johns and Seiferle for the micro-determination of unsaturation by catalytic hydrogenation. The improved apparatus can be used for the hydrogenation of solid or volatile liquid samples at temperatures ranging from 0° C (or lower) to about +50° C. The final measurement of the volume of hydrogen absorbed has been made more accurate and precise by simple modification of the original apparatus, and by housing the entire assembly in a constant temperature cabinet.

The procedure for making a determination is described in detail, and some results for pure organic compounds are given. The maximum relative error to be expected in routine determinations is about ±2 per cent.

"The Assay of Penicillin in Compound Feeding Stuffs," by S. A. Price and Kay A. Boucher.

A large-plate method is described for the assay of penicillin in feeding stuffs. *Bacillus subtilis* is used as the test organism.

The primary standard solution is prepared by dilution in methanol of a buffered aqueous solution of crystalline sodium penicillin G. Final dilutions for assay are made in an unfortified extract of the feeding stuff under test. Both sodium penicillin G and procaine penicillin are found to be unstable when dissolved in aqueous methanol, unless phosphate is also present.

Samples are extracted with methanol and the methanolic solutions are taken up on paper discs essentially as described by Esposito and Williams. The dried discs are applied to the medium in a randomised Latin square assay design. With the method as described, the limits of error ($P = 0{\cdot}95$) are of the order of 90 to 111 per cent. in an 8×8 assay of two samples with 8 replicates per dose, or 85 to 117 per cent. if four samples are accommodated and the number of replicates is reduced to 4.

"The Absorptiometric Determination of Traces of Copper in Highly Purified Water," by E. N. Jenkins.

A method is described for the determination of copper in highly purified water at the low levels significant in aluminium corrosion. Traces of copper down to 0·001 p.p.m. can be measured absorptiometrically as cupric diethyldithiocarbamate after a single extraction from a 500-ml sample into 10 ml of chloroform in the presence of a citrate buffer and of disodium ethylenediaminetetra-acetic acid. No interference results from the presence of 1 p.p.m. of common cations or of the sulphide or cyanide anions.

A simple modification is described to eliminate the interference of bismuth and antimony.

"The Absorptiometric Determination of Dissolved Oxygen," by J. Banks.

The use of 3:3′-dimethylnaphthidine as a colorimetric reagent for dissolved oxygen in boiler feed-water is described; the range of concentrations covered is 0 to 0·1 ml per litre. The Winkler reaction is first applied to convert oxygen to its equivalent amount of iodine. The literature covering 3:3′-dimethylnaphthidine is briefly summarised.

"Micro-determination of Acetaldehyde as its 2:4-Dinitrophenylhydrazone," by G. R. A. Johnson and G. Scholes.

A colorimetric method for the determination of acetaldehyde is described. Formation of the 2:4-dinitrophenylhydrazone in aqueous solution is followed by quantitative extraction into carbon tetrachloride. It has been found that direct addition of ethanolic sodium hydroxide to the carbon tetrachloride extract produces a strong red colour, which can be measured absorptiometrically. Perchloric acid has been used in the preparation of the 2:4-dinitrophenylhydrazine reagent; this has the advantage that the hydrazine is much more soluble and also that carbon tetrachloride extracts less of the unchanged reagent. Quantities down to 5 μg per 20-ml sample can be estimated with satisfactory precision. Acetaldehyde can be determined in the presence of pyruvic acid.

"The Simultaneous Determination of Cadmium and Magnesium with Disodium Ethylenediaminetetra-acetate," by E. G. Brown and T. J. Hayes.

The simultaneous determination of cadmium and magnesium by titration with a solution of disodium dihydrogen ethylenediaminetetra-acetate containing zinc sulphate is described. Selective control of the pH at 6·8 permits cadmium alone to be titrated, and magnesium is subsequently titrated at pH 10 in the same solution. Solochrome Black is used as indicator for both titrations. The molecular ratio of magnesium to cadmium must not be greater than unity for quantitative results, but a large excess of cadmium in the presence of magnesium can be satisfactorily determined. A theory is postulated for the reaction.

NOTICES

Vth International Spectroscopy Colloquium

UNDER the chairmanship of Prof. F. X. Mayer, the Spectrochemistry and Colorimetry Group of the Society of Austrian Chemists has undertaken the organisation of the next international Colloquium, which will be held at Gmunden, in Upper Austria, from August 30th to September 3rd, 1954.

As in previous Colloquia, both emission and molecular spectroscopy will be discussed and the following topics are proposed—

Emission spectroscopy—Analysis of non-conductors and of base metals, evaluation of spectrograms and experiences with methods not involving standard samples (*e.g.*, Harvey, Addink).

Molecular spectroscopy—Studies of artificial fibres, non-dispersive infra-red spectroscopy, Raman spectroscopy and a critical comparison of photo-electric and photographic methods of analysis.

As an innovation, it is proposed to devote the last hour of the afternoon session to correlated reviews of selected topics by one or more speakers relating their *personal* experiences. This will be followed by informal discussions, again stressing personal experiences.

Groups in each country have been asked to circulate invitations to their members and each country is asked not to submit more than five contributions. The final selection of papers and arrangement of the programme will rest with the Austrian Group. Comments on the Austrian proposals are invited, and those intending to take part in the Colloquium are requested to communicate as soon as possible with Mr. J. R. Stansfield, Hilger & Watts Ltd., 98, St. Pancras Way, Camden Road, London, N.W.1.

Symposium on Analytical Chemistry, 1954

THE Midlands Society for Analytical Chemistry has made a final announcement on the Symposium on Analytical Chemistry being held at the University of Birmingham from August 25th to September 1st, 1954.

The programme includes original papers by 23 authors, lectures on recent advances in industrial application and special techniques by 17 lecturers, and three plenary lectures by speakers of international repute.

There will also be an exhibition of apparatus, reagents and literature, and new techniques will be demonstrated.

In addition to visits and social functions, including special visits for lady visitors, there will be a Library Exhibition of historical chemical literature, including the Joseph Priestley collection.

Registration forms and further information can be obtained from the Symposium Secretary, J. W. Robinson, B.Sc., Ph.D., A.R.I.C., 139, Stourport Road, Kidderminster, Worcs.

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THE ANALYST

PROCEEDINGS OF THE SOCIETY OF PUBLIC ANALYSTS AND OTHER ANALYTICAL CHEMISTS

DEATHS

WE regret to record the deaths of

May Badger Craven
Percy George Terry Hand
Edward Oscar Heinrich.

NORTH OF ENGLAND SECTION AND MICROCHEMISTRY GROUP

A JOINT Meeting of the North of England Section, the Microchemistry Group and the Liverpool and North-Western Section of the Royal Institute of Chemistry was held in Southport on Saturday, September 26th, 1953.

On the afternoon preceding the meeting a visit was made to Simpson's Gold Thread Works in Preston. Visits to the Victoria Colliery, near Wigan, and to the Southport Gas Works were made on the Saturday morning. After this an informal lunch was enjoyed at Woodhead's Café, Lord Street, Southport.

In the afternoon about sixty members of the two Societies were welcomed by His Worship the Mayor of Southport, Alderman Tattershall, in the Council Chamber of the Town Hall. A symposium on the Training and Education of Microchemists followed, in which Dr. Cecil L. Wilson dealt with "An Academic Approach," Mr. Gerald Ingram put the "Technical Aspects" and Mr. Rudolph Rothwell spoke on "Industrial Requirements." A discussion followed these papers.

The meeting was under the Chairmanship of Dr. A. M. Ward.

SCOTTISH SECTION

AN Ordinary Meeting of the Section was held at 7.15 p.m. on Tuesday, November 10th, 1953, in the Central Station Hotel, Glasgow.

The following papers were presented and discussed: "Rapid Determination of Glycerol in Fermentation Solutions: A New Chromatographic Procedure," by K. Sporek, M.A., and A. F. Williams, B.Sc., F.R.I.C. (see p. 63); "Field Analysis in Connection with Water Treatment Problems," by I. A. Heald, B.Sc. (see summary below).

FIELD ANALYSIS IN CONNECTION WITH WATER TREATMENT PROBLEMS

MR. I. A. HEALD emphasised the necessity of an immediate analysis rather than one subjected to the delay entailed by sending samples to a central laboratory for the determination of some constituents of both raw and treated waters. Owing to the wide variation of solids content, from 1 to 2 p.p.m. for condensates to 10,000 p.p.m. for low-pressure boiler waters, analytical methods must be chosen accordingly. For field tests, rapidity of determination was essential, which ruled out many methods suitable for laboratory use. Examples of test kits were shown in which the use of polythene instead of glass for reagent bottles and apparatus had effected a welcome reduction in weight. Metal hydrometers for total-solid determination had reduced the probability of breakage.

A prototype protected hydrometer with coloured plastic rings to indicate approximate solids within 1000 p.p.m. was exhibited.

The uncertainty of the blank correction and the difficult end-point when total hardness was determined with Wanklyn's solution in the presence of high salt concentrations was cc sted with the greater accuracy of determinations with ethylenediaminetetra-acetic ...d.

The history of the discovery of the use of ethylenediaminetetra-acetic acid in Switzerland and its development in the United States and later in Great Britain was related. The role of murexide and Solochrome Black as indicators and the effects of interfering metals were discussed. Copper to the extent of 0·2 p.p.m. completely suppressed the end-point, but could be eliminated by sodium sulphide.

Iron led to a somewhat indefinite end-point, but could usually be removed by filtration. Ethylenediaminetetra-acetic acid could also be used for the volumetric estimation of sulphate hardness.

The problem of caustic embrittlement in boilers was also discussed, and the use of sodium nitrate instead of sodium sulphate had presented a difficult problem for rapid field analysis.

Many laboratory methods were too long, but some success had been achieved by a colorimetric brucine method, a tintometer disc being used as the standard.

BIOLOGICAL METHODS GROUP

A MEETING of the Group was held at 8 p.m. on Thursday, November 19th, 1953, at the Royal Society of Medicine, 1, Wimpole Street, London, W.1. The Chair was taken by Dr. H. O. J. Collier.

An address entitled "The Standardisation of a Drug in Production as Illustrated by Adrenal Cortical Extract" was given by Dr. Chester I. Bliss (see summary below).

THE STANDARDISATION OF A DRUG IN PRODUCTION AS ILLUSTRATED BY ADRENAL CORTEX EXTRACT

DR. C. I. BLISS described the experience gained over several years in standardising adrenal cortex extract from the liver glycogen of the rat as an illustration of the problems arising in the biological control of quality. The assay on each day was self-contained, with two dose levels in the ratio of 3 to 5 of the reference standard and of each test preparation or unknown. As rats were assigned in equal numbers and at random to the two dose levels of any one preparation, the potency of each unknown and its standard error could be estimated by simplified equations for a self-contained assay, as illustrated numerically.

As the assay procedure was repetitive, stable laboratory estimates of the error variance or standard deviation and of the slope of the dose - response curve would further shorten the calculation of potency and increase its reliability. The range from groups of five equivalent rats was plotted against the sum of each group to test the independence of the mean and variance, and its distribution was compared with that expected for a normal variate with a stable variance. Both tests and a study of the time relation showed adequate stability above a mean of 0·56 per cent. of liver glycogen, with a uniform laboratory variance from 1947 to May, 1950, that dropped to a lower stable value in October, 1950. The slope also proved independent of the mean response, stable in time, and normally distributed, confirming the linearity of the dose - response relation. The advantages of computing potency and its standard error with stable laboratory estimates have been exemplified.

The validity of the assay technique was tested from the agreement of two to four independent assays of each of 15 lots of extracts. Their potencies agreed well within the limits expected from the standard errors. Eight other assays compared two different concentrations of each of three preparations, which were assayed against each other. Their assayed relative potencies agreed with their known true values to the extent predicted by their standard errors.

The suitability of the percentage of liver glycogen as the response metameter was examined by co-variance against initial body weight and liver weight. The first had no effect, but the second was statistically highly significant, indicating that liver glycogen was not proportional to liver weight. An alternative simpler response metameter, the liver glycogen per rat, performed as well or better than the percentage of liver glycogen and gave promise of more precise estimates of adrenal cortex extract.

Chromatographic Determination of Glycerol in Fermentation Solutions

By K. SPOREK and A. F. WILLIAMS

(*Presented at the meeting of the Scottish Section on Tuesday, November 10th, 1953*)

A new chromatographic procedure is described for the rapid determination of glycerol in its mixtures with sugars and the constituents of molasses. It has been applied to the analysis of fermentation solutions derived from molasses. Alumina is used as adsorbent in conjunction with a solvent consisting of acetone containing 5 per cent. v/v of water and 0·05 per cent. v/v of glacial acetic acid. Sodium sulphite and sodium acetate are added to the sample solution to assist in the retention of sugars by the adsorbent. Glycerol is then determined in the column eluate, after removal of the solvent, by direct titration of the formic acid produced by oxidation with sodium metaperiodate.

At the beginning of this work[1] there appeared to be no rapid or completely reliable procedure for the determination of glycerol in solutions derived from the sulphite fermentation of molasses.[2] The main difficulty in devising a suitable method was the extreme complexity of such solutions. Owing to the presence of complex hydroxy compounds, such as fermentable and unfermentable sugars, which may undergo reactions similar to glycerol, procedures such as those based on acetylation or oxidation with sodium metaperiodate could not be readily applied. Indirect methods have previously been used for the analysis; they are usually based on some form of entrainment distillation of the glycerol with an organic solvent such as kerosene or *cyclo*hexane,[3] but in this laboratory these methods have not proved satisfactory and the procedure involved is usually lengthy and tedious.

Experiments were begun to develop a chromatographic procedure for the separation of glycerol from sucrose and its inversion products, glucose and fructose, which are the major constituents of the molasses. A simple method has now been devised for this separation and it has been applied to fermentation solutions derived from the "sulphite" process. It has also been used in this laboratory for solutions from the alkaline fermentation process of Eoff, Linder and Beyer.[4]

A chromatographic procedure has previously been described by Neish[5] for separating milligram amounts of glycerol from small amounts of fermentation solutions. He used a column of Celite and organic solvents for extraction, the eluted glycerol being finally determined by a colorimetric procedure. The method was not considered to be suitable for rapid routine determinations in this laboratory, particularly as it was desired to use much larger amounts of sample.

Qualitative chromatographic procedures involving partition chromatography on paper strips have been widely used in the separation of sugars and polyhydric alcohols.[6] Experiments in this laboratory showed that glycerol could be readily separated from sucrose, glucose, fructose and mannitol with acetone, *iso*propanol and *n*-butanol solvents. The simple glycols (ethylene, propylene and butylene) gave higher R_F values than glycerol. The results for the three different solvents are shown in Table I.

These results indicated that in any simple quantitative chromatographic procedure developed for glycerol and based on the use of an adsorbent such as cellulose, it was unlikely that a separation from the glycols would be effected, and, as such compounds often occurred with glycerol, it was important that allowance should be made for their presence. It was known that, of the direct procedures available for the determination of glycerol, the one that appeared to be most selective involved oxidation with sodium metaperiodate and titration of the formic acid produced.[7] The glycols do not produce formic acid in this oxidation. It was clear that, provided the periodate procedure was used after chromatography, the possible presence of glycols could be ignored. Throughout the work described, therefore, the periodate oxidation procedure was used for the glycerol determination. This had the further advantage that there was no interference by common organic solvents, such as alcohols or ketones, which might be used in a chromatographic method.

It was considered that, for rapid routine work, it was a pre-requisite of a chromatographic method that the solvent used should be easily removed from the eluate without danger of loss of glycerol. For this reason, initial experiments were restricted to low-boiling solvents such as acetone and *iso*propanol.

With the technique described in the method (p. 65) but with columns of cellulose powder and aqueous sample solutions containing only glycerol, it was impossible to extract more than 80 to 90 per cent. of the glycerol in a reasonable volume (250 ml) of pure acetone solvent. This figure was increased to 95 per cent. by using acetone to which 5 per cent. of water had been added. When the same conditions were used for artificial mixtures of sucrose, glucose, fructose and mannitol, there was a high degree of extraction of these substances, which increased with the amount of water present in the solvent. With *iso*propanol instead of acetone as solvent, this extraction was still greater. Extraction of the constituents of molasses also took place under the same conditions.

TABLE I

R_F VALUES IN VARIOUS SOLVENTS ON PAPER STRIPS

Compound	Solvent		
	Acetone (b.p. 57° C)	*iso*Propanol (b.p. 82° C)	*n*-Butanol (b.p. 118° C)
Sucrose	0·2	—	0·04
Glucose	0·18	0·01	0·07
Fructose	0·20	0·02	0·10
Mannitol	0·18	0·02	0·07
Glycerol	0·47	0·54	0·33
2:3-Butanediol	0·77	0·82	0·72
1:2-Propanediol	0·70	0·77	0·62
Ethanediol	0·59	0·69	0·50

These experiments were repeated with the use, as adsorbent, of alumina supported on a short column of cellulose (see Method, p. 65), and it was found that extraction of the substances other than glycerol was considerably reduced, so further work was carried out with alumina as adsorbent. Samples (1 g) of Cuban blackstrap molasses (used for the production of glycerol by the "sulphite" fermentation process) were dissolved in 5 ml of water and extractions were made in the manner described. These molasses contained about 50 per cent. of fermentable sugars together with unfermentable sugars and hydroxylated substances of relatively high molecular weight, such as polysaccharides. Extractions were made both with and without added glycerol. It was found necessary to add a small amount of acetic acid to both the sample solution and to the solvent (see Method) in order to prevent strong retention of the glycerol by the constituents of the molasses; by using 250 ml of acetone containing 5 per cent. of water and 0·05 per cent. of glacial acetic acid, over 95 per cent. of the glycerol could then be extracted. Whereas the constituents of the synthetic sugar mixture were extracted in appreciable amount, the corresponding extraction from the molasses was only slight (see experiments 4 and 12, Table II). The exact cause of this difference in behaviour is not readily explained, but it may partly be connected with the ratio between the amount of sucrose and its inversion products in the molasses, for sucrose is more strongly held on alumina than is glucose or fructose (see experiments 6, 7, 8 and 9, Table II).

As the fermentation solutions, for which the method was primarily required, would contain sodium sulphite, it was considered desirable to examine the effect of this salt on the extraction of glycerol and on other possible constituents of fermented liquors. As there was no obvious way of preparing such solutions free from glycerol, but identical in all other respects, the experiments were made on the original molasses, which in some respects presented a more complex sample owing to the higher concentration of fermentable sugars present in addition to all the unfermentable material. Addition of 0·8 g of sodium sulphite to the sample solutions (1 g of molasses in 5 ml of water, with and without added glycerol) increased the extraction of glycerol to 97 per cent., and the retention of constituents of the molasses was increased. Because of this beneficial effect of sodium sulphite, a further series of experiments was made with artificial sugar mixtures to which sodium sulphite was added, and the same increased retention was observed. It was found that, if 1 g of sodium acetate was also added, extraction of sugars was negligible (see experiment 5, Table II).

The method for the determination of glycerol in sugar solutions and in its mixtures with substances of the type present in molasses and also in fermentation solutions is described in detail below. It involves addition of 0·8 g of anhydrous sodium sulphite and 1 g of sodium acetate trihydrate to an aqueous solution of the sample of volume about 5 ml (provided the total volume of sample solution is about 5 ml the actual amount of water present is not critical so long as it exceeds about 2 ml) and then 0·1 ml of glacial acetic acid. The glycerol present is next extracted from the alumina adsorbent (supported on cellulose) in a volume of 250 ml of acetone containing 5 per cent. v/v of water and 0·05 per cent. v/v of glacial acetic acid. After removal of acetone the glycerol is determined by a periodate procedure.[7] Under the conditions described, about 3 per cent. of the glycerol present in the original sample is retained on the column. For this reason the procedure is standardised by the use of a known amount of glycerol together with 1 g of molasses as sample.

About eight determinations can be completed in a working day, and it is believed that the method may be applicable to many complex mixtures containing glycerol.

Method for the determination of glycerol in complex sugar mixtures and fermentation solutions

Preparation of solvent—Prepare a mixture of 950 ml of pure dry acetone, 50 ml of water and 0·5 ml of glacial acetic acid.

Chromatographic column[8]—

The tube used for the adsorbents was 1·8 to 2·0 cm in diameter and about 25 cm long, with a funnel at the top to facilitate introduction of the sample. It terminated in a short length of glass tubing, 6 mm in diameter. A number of indentations were made in the glass where the narrow tube joined the main column and the whole column was then made water-repellent by treating with a silicone fluid.

Weigh 2·5 g of Whatman coarse-grade cellulose into a 250-ml beaker and suspend it in 100 ml of acetone. After swirling the beaker, pour the mixture into the chromatographic tube, the outlet of which has been closed with a small rubber stopper. When the cellulose has partially settled, remove the stopper and allow the acetone to flow out of the tube; return any entrained cellulose to the column by pouring the acetone back through the funnel. When the cellulose has settled to form a smooth column, add a further amount of acetone and sprinkle 5 g of chromatographic alumina (Peter Spence, type H) into the tube. When the last of the visible acetone has drained from the column, pass 100 ml of solvent (see above) through the column, and, when the solvent layer has reached the surface of the alumina, replace the rubber stopper in the outlet of the tube. The column is then ready for use.

Preparation of sample solution—

Weigh or measure a convenient amount of sample into a 150-ml beaker. With fermentation solutions, a volume of 5 ml is usually taken. In the experiments on molasses, samples of which were relatively dry, about 1 g was taken and 4 to 5 ml of water were added. In all determinations the final volume was approximately 5 ml of aqueous solution, of which not less than about 50 per cent. was water; the exact amount of water in the sample solution was not critical. Then add sufficient anhydrous sodium sulphite to the contents of the beaker to bring the total amount present to 0·8 g.

Add 1 g of sodium acetate, $CH_3COONa.3H_2O$, and 0·1 ml of glacial acetic acid, and allow the mixture to stand for about 5 minutes with intermittent stirring. With samples derived from the alkaline fermentation process of Eoff, Linder and Beyer, which usually contain sodium carbonate, the amount of acetic acid to be used must be increased to 0·2 to 0·3 ml.

Chromatographic extraction of glycerol—

Add 15 g of alumina to the sample solution and stir the whole with a stout glass rod to produce a homogeneous friable mass. Transfer the mixture as completely as possible to the top of the prepared column. Add about 50 ml of solvent to the 150-ml beaker and, after stirring, transfer the solvent to the column and gently beat the "wad" with a stout glass plunger to form a smooth continuation to the main column. Remove the rubber

stopper from the base of the column and allow the solvent to fall to the level of the top of the alumina; collect the eluate in a 750-ml conical flask. Pour more solvent first into the beaker and then into the column until a total of 250 ml has been used and collected in the conical flask. Evaporate the acetone from the flask on a steam-bath in a well-ventilated fume cupboard until the residual aqueous volume is about 20 ml.

DETERMINATION OF GLYCEROL—

Dilute the residual aqueous solution to a volume of 250 to 300 ml with water and boil the solution for 1 minute to expel carbon dioxide. Then cool it thoroughly in running water and determine the glycerol by titrating the formic acid produced by oxidation with sodium metaperiodate, using a procedure similar to that described by Erskine, Strouts, Walley and Lazarus.[7] It was found that 7 minutes was sufficient time of oxidation for the reaction with sodium metaperiodate and 5 minutes for the reaction of the excess of periodate with ethylene glycol (short times of reaction have also been recommended by Hartman[9]). Titrate the formic acid with 0·1 *N* sodium hydroxide standardised by carrying out the entire chromatographic procedure on 1 g molasses to which a known amount, about 0·5 g, of glycerol has been added. Determine the blank on the same weight of molasses. In this way any hold-up of glycerol by the adsorbent (about 3 per cent. occurs) is allowed for. The factor found for the standard alkali solution was usually of the order of 0·0095 g of glycerol per ml of 0·1 *N* sodium hydroxide, and the blank was about 0·3 to 0·5 ml.

THE EXTRACTION OF SUGARS AND CONSTITUENTS OF MOLASSES FROM CELLULOSE AND ALUMINA UNDER CONDITIONS USED FOR GLYCEROL AND THE EFFECT OF SODIUM SULPHITE AND SODIUM ACETATE

The best solvent mixture for extracting glycerol was found to be acetone containing 5 per cent. v/v of water and 0·05 per cent. v/v of glacial acetic acid, the acetic acid preventing strong adsorption of glycerol by the constituents of molasses. With this solvent, incorporation of sodium sulphite or sodium acetate in the "wad" led to decreased extraction of sucrose, glucose and other sugars, and the other constituents of molasses. With only cellulose as adsorbent and with sodium sulphite and sodium acetate together in the sample solution, experiment 2 (Table II) shows the decrease in extraction (material dried at 110° C) from a mixture of sucrose, glucose and mannitol, compared with experiment 1, in which these salts were absent. When alumina was used as adsorbent under the conditions of extraction described in the method (above), the corresponding figures for artificial mixtures in experiments 5 and 4 were much lower. Experiment 5 shows that the extraction is reduced to less than 2 mg when sodium sulphite and acetate are present (*cf.* experiment 2), and alumina is shown to have a distinct advantage over cellulose. With alumina adsorbent and a sample of molasses, the results found in the presence and absence of sodium salts are shown by experiments 14 and 12, and the corresponding titrations after periodate oxidation are shown by experiments 15 and 13. These experiments show that the effect of adding salts to the sample of molasses extracted (see experiments 14 and 12) is less marked than when an artificial mixture is used (see experiments 5 and 4). It is possible that this difference can be partly explained by the higher concentration of sucrose in the molasses compared with that of invert sugar, for with sucrose alone there is much greater retention in the absence of salts than when corresponding amounts of glucose and fructose are taken alone (experiments 6, 8 and 9).

The results shown in Table II demonstrate the value of alumina as an adsorbent for sugars and the constituents of molasses when sodium sulphite and acetate are present in the sample solution.

In all experiments under the conditions laid down in the method (p. 65), extraction of glycerol was about 97 per cent. No advantage was gained by increasing the amount of water in the acetone solvent, for although the amount of glycerol extracted was increased, extraction of other constituents was also greater.

With the artificial mixture of sucrose, glucose, fructose and mannitol, a decrease in the amount of sodium sulphite from 0·8 to 0·5 g caused a slight increase in the extraction of the sugars, whereas an increase in sodium sulphite made no appreciable difference. The part played by sodium sulphite and sodium acetate in promoting retention of sugars is not clear, but it is possible that the sodium sulphite forms a complex with the sugars, whereas the sodium acetate acts as a buffer salt.

TABLE II

EXTRACTION OF SUGARS AND MOLASSES FROM CELLULOSE AND ALUMINA AND THE EFFECT OF SODIUM SULPHITE AND ACETATE

Expt.	Sample	Adsorbent	Sodium sulphite, g	Sodium acetate, g	Weight extracted (dried at 110° C), mg	Final titration after periodate oxidation, 0·1 *N* NaOH, ml
1	0·3 g each of sucrose, glucose and mannitol	Cellulose	Nil	Nil	488	—
2	"	"	0·8	1·0	48, 32, 80	—
3	1 g of molasses	"	Nil	Nil	215	—
4	0·3 g each of sucrose, glucose and mannitol	Alumina	Nil	Nil	192	—
5	"	"	0·8	1·0	<2	—
6	0·5 g of sucrose	"	Nil	Nil	5	—
7	0·5 g of mannitol	"	"	"	80	—
8	0·5 g of glucose	"	"	"	103	—
9	0·3 g of fructose	"	"	"	50	—
10	0·3 g of sucrose, 0·1 g each of glucose, mannitol and fructose	"	0·8	1·0	2	—
11	"	"	0·8	1·0	—	0·35
12	1 g of molasses	"	Nil	Nil	20	—
13	"	"	"	"	—	1·10
14	"	"	0·8	1·0	10	—
15	"	"	0·8	1·0	—	0·35

THE QUANTITATIVE EXTRACTION AND DETERMINATION OF GLYCEROL IN AQUEOUS SOLUTION AND ITS MIXTURES WITH MOLASSES

By the procedure described in the method (p. 65), a series of determinations was carried out on pure glycerol solutions and on 1-g samples of Cuban blackstrap molasses to which glycerol had been added to cover a range of concentrations. The results are shown in Table III. The accuracy of the method is reflected in the value and constancy of the factor found for each series of experiments, and it will be seen that, provided the procedure is standardised, a high degree of accuracy can be attained. The factor for the standard sodium hydroxide solution is slightly lower (nearer to the theoretical value) for glycerol in the presence of molasses than for its aqueous solutions.

TABLE III

CHROMATOGRAPHIC SEPARATION AND DETERMINATION OF GLYCEROL IN AQUEOUS SOLUTION AND IN MIXTURES WITH MOLASSES

	Glycerol taken, g	Titration, 0·1 *N* NaOH, ml	Factor,* grams of glycerol per ml of 0·1 *N* NaOH
Aqueous solution	Blank	0·1	—
	0·4167	42·6	0·0098
	0·3333	34·9	0·0096
	0·2499	25·9	0·0097
	0·2080	21·5	0·0097
	0·0833	8·5	0·0099
Sugars present	0·4149†	43·45	0·0096
Aqueous solution with 1 g of molasses	Blank	0·4	—
	0·0833	9·1	0·0096
	0·2499	27·0	0·0094
	0·3333	35·8	0·0094
	0·3818	40·6	0·0095
	0·4167‡	44·8	0·0094
	0·7500	80·4	0·0094

* Factor for direct periodate determination on glycerol solution = 0·0092.
† Sample containing 0·3 g of sucrose, 0·1 g each of glucose, mannitol and fructose.
‡ 0·5 g of molasses taken.

Application of the chromatographic procedure to the determination of glycerol in sulphite fermentation liquors

Solutions derived by sulphite fermentation of Cuban blackstrap molasses contained approximately 4 per cent. of glycerol, and a volume of 5 ml gave a reasonable titration with the 0·1 *N* sodium hydroxide solution at the final periodate stage of the analysis. The original solution contained approximately 0·3 g of sodium sulphite (free or fixed as the bisulphite compound of acetaldehyde produced in the process) in 5 ml of solution, so that a further

Table IV

Determination of glycerol in fermentation solutions (sulphite process): comparison between chromatographic procedure and existing kerosene method

Sample	Chromatographic method			Kerosene method	
	Amount taken, ml	Titration, 0·1 *N* NaOH, ml	Glycerol found,* % w/v	Glycerol found, % w/v	Corrected value for glycerol, % w/v
1	5	22·7	4·3	4·0	4·3
2	5	20·1	3·9	3·5	3·7
3	5	20·9	4·0	3·8	4·0
4	5	22·9	4·4	4·0	4·3
5	5	23·0	4·4	4·0	4·3
6	5	22·3	4·3	3·8	4·1
7	5	22·1	4·2	4·0	4·3
8	5	22·0	4·2	3·8	4·1
9	5	11·1	2·1	2·1	2·2

* Factor taken = 0·0095 g of glycerol per ml of 0·1 *N* sodium hydroxide (see Method).

0·5 g was added together with 1 g of sodium acetate and 0·1 ml of acetic acid. The solution was then ready for chromatography on alumina. The method was tested on a range of nine fermentation solutions, which were also analysed by a longer procedure that was the only alternative available. It consisted of three stages, namely—

(*i*) Extraction of a weighed amount of sample, mixed with sodium sulphate, by means of hot acetone.

(*ii*) Removal of acetone and separation of the glycerol by distillation with kerosene.

(*iii*) Extraction of glycerol from kerosene by washing with water, followed by titration of glycerol by an oxidation procedure making use of potassium dichromate.

This kerosene method was known to give results that were low to the extent of 7 per cent. when applied to fermentation solutions. Results by this procedure and by the chromatographic method are shown in Table IV.

Table V

Determination of sodium hydroxide factors by addition of glycerol to fermentation solutions and concentrates

Type of sample	Volume or weight taken	Glycerol added, g	Titration, 0·1 *N* NaOH, ml	Factor found for NaOH	Glycerol found in sample (factor used = 0·0094),* g	Probable error, %
Fermentation liquor ..	5 ml	Nil	23·5	—	4·4 (w/v)	
„	5 ml	0·3818	63·4	0·0096	—	−2
„	5 ml	Nil	27·1		5·1 (w/v)	
„		0·3818	68·1	0·0093	—	+1
„	5 ml	Nil	27·2	—	5·1 (w/v)	
„	5 ml	0·3054	60·2	0·0093	—	+1
Fermentation concentrate ..	2·494 g	Nil	40·9		15·4	
„	2·500 g	0·3818	81·4	0·0094	—	Nil

* See Method, p. 65, for standardisation of sodium hydroxide.

The results by the chromatographic procedure were completed during a working day, whereas determinations made by the kerosene procedure entailed one week of work.

Table V shows the factors for the 0·1 *N* sodium hydroxide in grams of glycerol per ml obtained by chromatographic determinations made before and after the addition of a known weight of glycerol to samples of fermentation solution.

The factors derived from determinations made before and after addition of glycerol to samples of fermentation solutions are seen to be in reasonable agreement with those obtained from molasses (Table III).

Conclusions

The chromatographic method described is both simple and rapid. It provides a means for the determination of glycerol with a reasonable degree of accuracy in complex mixtures of sucrose, invert sugar, unfermentable sugars, polysaccharides and so on. The precise composition of fermentation solutions is not known, but it is considered that the chromatographic procedure, used in conjunction with the selective periodate technique involving oxidation of the extracted glycerol to formic acid, gives an accurate measure of the amount of glycerol present in such samples.

The mechanism by which the addition of sodium sulphite and sodium acetate prevents the movement of glucose, fructose and mannitol is not easily understood, and it is possible that the complexing action of the added salts may be extended to other substances.

The method is in use in this laboratory for the analysis of fermentation solutions derived from the sulphite process,[2] and has been applied to the alkaline fermentation process of Eoff, Linder and Beyer.[4]

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Research Department
Imperial Chemical Industries Limited
Nobel Division
Stevenston, Ayrshire

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Discussion

Dr. I. C. Willox asked whether Mr. Williams had any experience of the direct estimation of glycerol after separation on a paper strip.

Mr. Williams replied that work on these lines had been considered and that a paper-strip procedure might be developed, but it was doubtful whether a high accuracy would be obtained. It might be possible to cut off the portion of the paper containing the glycerol and to apply a colorimetric method.

Mr. R. Kerr wanted to know if there was any development of the chromatographic method for the determination of the individual sugars present in fermentation solutions.

Mr. Williams said, in reply, that he had not done any work involving the use of the chromatographic technique for the separation of individual sugars present in fermentation solutions, but in view of the amount of work that had been carried out by other workers on the separation of sugars generally, it seemed reasonable to suppose that similar techniques might be applied to fermentation solutions.

Mr. A. Carey asked whether the time allowed for the oxidation of glycerol by sodium periodate was critical.

Mr. Williams replied that the paper by Erskine, Strouts, Walley and Lazarus in *The Analyst* described the procedure, but its use in the chromatographic method had shown that the time allowed for the reaction could vary over a wide range without any adverse effect.

Mr. J. A. Eggleston asked about the availability of sodium periodate of a suitable degree of purity.

Mr. Williams replied that the authors made their own periodate by an electrolytic procedure and the purity of the reagent was such that a blank of less than 0·1 ml of 0·1 *N* sodium hydroxide was obtained at the titration stage in a determination of glycerol. He understood that supplies were now available from certain manufacturers.

The Determination of Hydroxyl, Ketone and Ester Groups in Autoxidised Fatty Esters and Related Compounds by Infra-red Spectroscopy

BY N. H. E. AHLERS AND N. G. MCTAGGART

Infra-red spectroscopic methods have been devised for the quantitative determination of hydroxyl, ketone and ester groups in autoxidised or co-polymerised fatty esters and related compounds. The technique consists in relating the intensity measurements in solution at the wavelengths of the characteristic absorption bands to those of suitable reference compounds. Provision is made for the elimination of errors arising from scattered radiation, finite slit width, association phenomena and so on. The methods are simple and rapid in operation. They require only small amounts of sample, about 20 mg, which can be recovered unchanged after the examination. The accuracy of each determination is similar to that of the corresponding conventional chemical method.

STUDIES of the oxidation of fatty esters require accurate analytical methods for the determination of the variety of oxygen-containing groups introduced during oxidation. These groups include hydroxyl and carbonyl, and this paper is concerned with their determination.

Of the several methods in use for the determination of the hydroxyl group, the most widely used is the acetic anhydride - pyridine method,[1] by which only primary and secondary alcoholic hydroxyl groups are measured.

Another method in common use, that of Zerevitinov,[2] requires correction for carboxylic hydroxyl groups, since it determines those as well as the alcoholic hydroxyl groups.

Most of the methods proposed for the determination of ketone groups in fatty esters and related compounds depend on the reaction with either phenylhydrazine[3] or hydroxylamine.[4,5] These methods are not reliable in the presence of organic peroxides, which are known to be present in autoxidised fatty ester systems.

A more recent method[6] is based on the oxidation of aluminium *iso*propoxide to acetone, which is then determined as its dinitrophenylhydrazone.

The normal chemical methods of determining esters are based on direct saponification. There are, however, a number of compounds for which chemical saponification methods are not completely satisfactory. Alcohol-insoluble polymers of low ester content, *e.g.*, certain styrene - fatty ester copolymers, are difficult to saponify. Moreover, for some autoxidised fatty ester systems there is evidence that saponification leads to chemical changes other than simple hydrolysis of the esters present.

For compounds unsuitable for saponification this paper describes methods, dependent on infra-red spectroscopy, that can be carried out rapidly and require but small amounts of sample, about 20 mg, which can, if required, be recovered unchanged at the end of the examination. These methods are applicable to a wide range of materials and appear to be as accurate as the more lengthy chemical methods, which require from 0·25 to 1·0 g of sample.

THEORETICAL CONSIDERATIONS

The theoretical aspects of infra-red absorption spectroscopy and the techniques involved in its use for both qualitative and quantitative determinations on organic molecules have already been reviewed.[7]

Vapours and dilute solutions of compounds containing an alcoholic hydroxyl group all show an absorption band in the infra-red region near 3600 cm^{-1}, which is attributed to the OH-stretching vibration.

Hence the infra-red spectrum of a hydroxy fatty ester, methyl ricinoleate, in dilute carbon tetrachloride solution, shows a sharp absorption band at 3625 cm^{-1}. With an increase in the concentration of the solution, the intensity of this band decreases and a new broad band appears at 3460 cm^{-1}. This new band is ascribed to the presence of associated hydroxyl groups.

It is not practicable to use the associated hydroxyl band to assess the hydroxyl content of a sample with any precision because the degree of association, and hence the intensity of absorption, varies markedly with the concentration and the nature of the sample. If, however, very dilute solutions of the sample are used, the degree of association is negligible and measurements of the intensity of the unassociated or "free" hydroxyl absorption band serve to provide the specific extinction coefficient[8] (defined on p. 73) of the hydroxyl group and hence permit the determination of the hydroxyl content of the sample.

The general procedure is to use a pure substance of known hydroxyl content, *e.g.*, methyl ricinoleate, to determine the reference specific extinction coefficient. This can then be used to determine the hydroxyl content of other similar samples by comparison of the relative intensities of the unassociated hydroxyl absorption bands.

All compounds containing a carbonyl group show a characteristic absorption band in the 1700 cm^{-1} region of the infra-red spectrum, which arises from the CO-stretching vibration

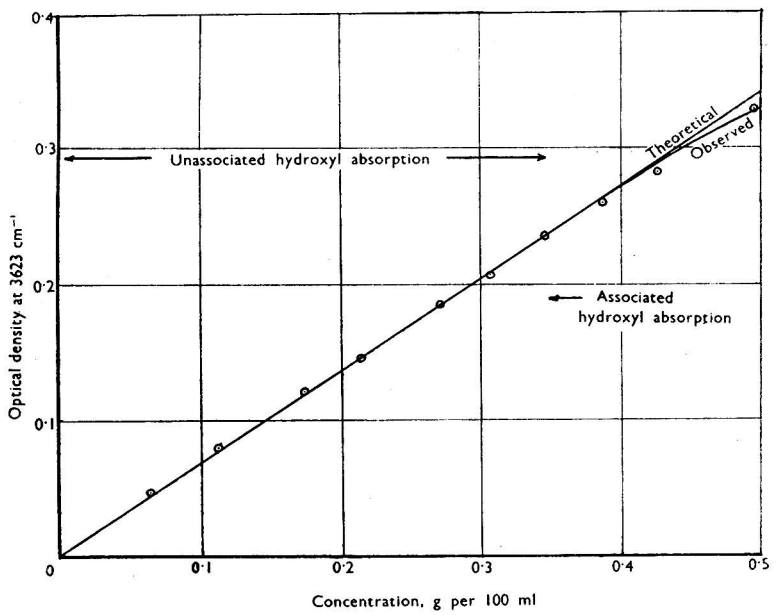

Fig. 1. Beer - Lambert relationship for methyl ricinoleate in carbon tetrachloride solution. Theoretical hydroxyl content of methyl ricinoleate = 5·45 per cent.; k = 0·068; path length = 1 cm

of the system. The exact location of the band depends on whether the group is present in the molecule as ester, ketone, aldehyde or other configuration, and it is also affected by the nature of adjacent groupings,[9] particularly if the group is conjugated with a double bond.

Previous work on the analysis of autoxidised fatty ester systems by infra-red spectroscopy has shown the necessity for using reference standards with a molecular structure similar to that of the samples under investigation. The infra-red spectra of methyl stearate and methyl ketostearate show bands characteristic of the ester group at 1742 cm^{-1} and the spectrum of the keto compound shows a band arising from the ketone valence vibration at 1718 cm^{-1}. These compounds form suitable standards for ester and ketone determinations.

At first sight it would appear that the ketone content of an unknown sample could be determined directly by comparing the intensity of the ketone absorption band with that of methyl ketostearate; but this method is complicated by the presence of the ester group, which shows considerable general absorption near the ketone absorption wavelength. Unless a correction is made for this general absorption, the results will be inaccurate, particularly if the ketone content is low. The reverse effect is negligible and the ester content can be calculated from a direct comparison of the absorption intensity at the ester wavelength with that of methyl stearate.

The procedure found to be satisfactory involves measurement of the absorption intensity

at the specific ketone wavelength, determination of the ester content, and correction of the ketone absorption from the determined ester content.

EXPERIMENTAL

In the determination of hydroxyl the measurements must be confined to the range of concentrations within which no appreciable association of the hydroxyl group occurs. This is readily effected by diluting the solution until no absorption characteristic of the associated hydroxyl is observed. It is also necessary to check the validity of the Beer - Lambert law for solutions of the material to be examined. Data establishing this relationship for methyl ricinoleate at 3623 cm^{-1} in a 1-cm cell in carbon tetrachloride solution are shown in Fig. 1. Similar linear relationships are observed for methyl ketostearate at 1718 cm^{-1} and for methyl stearate at 1742 cm^{-1}.

METHODS

The results in this paper were obtained with a Perkin Elmer Model 12C infra-red spectrometer[10] equipped with a rock-salt optical system.

The sample under investigation was weighed and dissolved in purified carbon tetrachloride[10] contained in a calibrated flask. The concentration of the solution was then adjusted so that the measured optical density in a 1-cm cell lay within the range of 0·4 to 0·8.

In making absorption measurements the practice was to record the reference spectrum of the cell filled with carbon tetrachloride. The cell was then carefully washed out and filled with the solution and the spectrum was recorded again. The intensity of the characteristic absorption band was determined by difference between the two sets of spectra.

Scattered radiation was eliminated by the use of a glass shutter in the 1700 cm^{-1} region.

The effect of slit width[11] on intensity measurements in the infra-red spectrum is important, especially in dealing with sharp absorption bands. The true intensity of an absorption band can only be found by extrapolating a series of measurements to zero slit width.

In Fig. 2 the effect of variation of slit width on optical density is shown for a solution

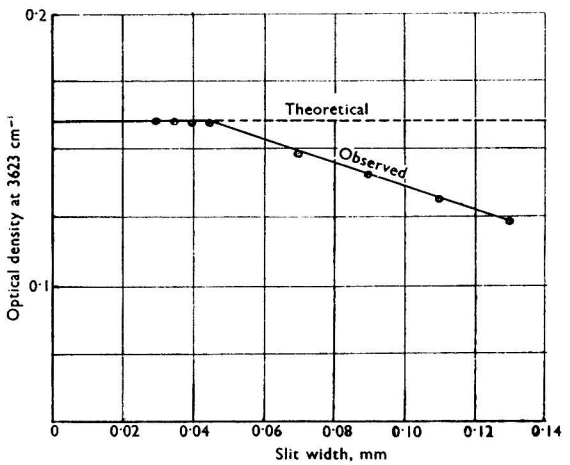

Fig. 2. Effect of variation of slit width on optical density for a solution of methyl ricinoleate in carbon tetrachloride. Concentration = 0·233 g per 100 ml; path length = 1 cm

of methyl ricinoleate in carbon tetrachloride. For the relative measurements in this work a constant slit width suffices and consequently all determinations were made with an aperture of 0·02 mm for the hydroxyl and 0·06 mm for the carbonyl determinations.

DETERMINATION OF HYDROXYL—

The slope of the line in Fig. 1 provides the reference specific extinction coefficient for the calculated theoretical hydroxyl content (5·45 per cent.) of pure methyl ricinoleate.

Now, $D = kcl$ from the Beer - Lambert relationship,
where D is the optical density of the hydroxyl band,
k is the specific extinction coefficient,
c is the concentration in g per litre and
l is the path length in cm.

The reference specific extinction coefficient then becomes 0·068. The specific extinction coefficient of the sample, k', is then measured and the hydroxyl content of the sample is obtained from the relation—

$$\text{hydroxyl content} = \frac{k' \times 5{\cdot}45}{0{\cdot}068} \text{ per cent.}$$

Determinations of the hydroxyl content of many autoxidised fatty esters and related compounds by both the acetic anhydride and the infra-red methods have shown satisfactory agreement. A selection of the results is shown in Table I.

TABLE I

A COMPARISON OF THE HYDROXYL VALUES OBTAINED BY THE ACETIC ANHYDRIDE AND INFRA-RED METHODS

Sample	Hydroxyl value, per cent.	
	Acetic anhydride method	Infra-red method
Linseed oil monoglyceride	7·52	7·70
Dimethylstearyl alcohol	5·70	5·87
Methyl hydroxystearate	5·42	5·34
Methyl dihydroxystearate	10·30	10·10
Castor oil	4·80	4·74
Dehydrated castor oil	1·27	1·65

DETERMINATION OF ESTER—

The reference specific extinction coefficient, k, at 1742 cm^{-1} for the calculated ester-group content ($-COOCH_3$ = 19·8 per cent.) for pure methyl stearate, *i.e.*, the slope of the graph representing the Beer - Lambert relationship, is 1·48.

The specific extinction coefficient of the sample, k', is then measured and the ester content calculated from the relation—

$$\text{ester content} = \frac{k' \times 19{\cdot}8}{1{\cdot}48} \text{ per cent.}$$

$$\text{or saponification value} = \frac{k' \times 198}{1{\cdot}48} \text{ mg of KOH per g.}$$

When the method is to be used for a large number of routine analyses a direct calibration curve may be used. Either ester content or saponification value can be plotted against the observed specific extinction coefficient for the reference materials. Such a graph prepared from data for methyl stearate is shown in Fig. 3. Saponification values can then be read directly from the graph.

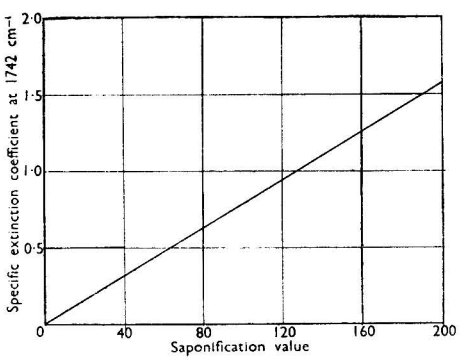

Fig. 3. Relation between saponification value and specific extinction coefficient for methyl stearate

Comparison of the results for a number of samples with those obtained by the normal saponification method are given in Table II and show that the spectroscopic method gives, in general, results in good agreement with the chemical procedure.

TABLE II

COMPARISON OF THE ESTER CONTENTS OBTAINED BY THE SAPONIFICATION AND INFRA-RED METHODS

	Ester content, per cent.		Saponification value, mg of KOH per g	
	Infra-red method	Chemical method*	Infra-red method†	Chemical method
Methyl azelate	55·0	55·7	523	528
Methyl myristate	24·1	24·4	229	231
Methyl ketostearate	19·1	18·9	182	180
Methyl dihydroxystearate	17·9	17·9	170	170
Autoxidised methyl elaeostearate fraction	17·7	23·7	168	225
Hydrogenated autoxidised methyl elaeostearate fraction	18·8	18·3	178	175
Methyl ester fraction from autoxidised linseed oil	17·8	23·7	169	225
Linseed oil	—	—	197	195
Impatiens oil	—	—	231	237
Styrene copolymer	2·85	3·06	27·1	29·1

* Calculated from the saponification value, measured chemically.
† Calculated from the ester content, measured spectroscopically.

The tabulated results indicate satisfactory agreement between the spectroscopic and chemical procedure with all samples except the autoxidised methyl elaeostearate and linseed oil fractions. For these, the chemical method gives a higher value. On hydrogenation of the autoxidised methyl elaeostearate sample, the chemical saponification value falls and agreement with the spectroscopic method is attained. It appears that certain autoxidised materials contain reactive groupings that lead to high saponification values. Hydrogenation removes these groupings and the true ester content is given by saponification of the hydrogenated material.

DETERMINATION OF KETONE—

This analysis depends, in the first place, on the determination of the ester content of the sample relative to methyl stearate. From this datum, the true intensity of the ketone absorption in fatty esters can be deduced and compared directly with that of the reference material, methyl ketostearate. The specific extinction coefficient, k_3, for methyl ketostearate at 1718 cm^{-1} in carbon tetrachloride solution, *i.e.*, the slope of the graph representing the Beer - Lambert relationship, is 0·921.

Let k_1 be the observed extinction coefficient for methyl stearate in carbon tetrachloride solution at 1742 cm^{-1},
k_2 be the observed extinction coefficient for methyl stearate in carbon tetrachloride solution at 1718 cm^{-1},
k_3 be the observed extinction coefficient for methyl ketostearate in carbon tetrachloride solution at 1718 cm^{-1} and
k_4 be the observed extinction coefficient for methyl ketostearate in carbon tetrachloride solution at 1718 cm^{-1} corrected for the absorption at this wavelength due to its ester content.

For methyl stearate the ester content is 19·8 per cent. and for methyl ketostearate the ester content is 18·9 per cent. and the ketone content is 8·95 per cent. For methyl ketostearate, the contribution of the ester content to absorption at 1718 cm^{-1} is given by—

$$k_2 \times \frac{18{\cdot}9}{19{\cdot}8},$$

whence

$$k_4 = k_3 - \left(k_2 \times \frac{18{\cdot}9}{19{\cdot}8}\right) \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (1)$$

Let k_5 be the observed extinction coefficient for the sample in carbon tetrachloride solution at 1742 cm^{-1}; then the ester content of the sample is given by—

$$\frac{k_5}{k_1} \times 19{\cdot}8 \text{ per cent.} \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad (2)$$

Let k_6 be the observed extinction coefficient for the sample in carbon tetrachloride solution at 1718 cm^{-1}. This extinction coefficient is then corrected for the contribution at this wavelength due to the ester content given by equation (2).

Then k_7, the observed extinction coefficient for the sample in carbon tetrachloride solution at 1718 cm^{-1}, corrected for the "interference" of the ester content is given by—

$$k_7 = k_6 - \left[\left(\frac{k_5}{k_1} \times 19{\cdot}8\right)\left(\frac{k_2}{19{\cdot}8}\right)\right]$$

or

$$k_7 = k_6 - \left(\frac{k_2 \times k_5}{k_1}\right) \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad (3)$$

The expression for the ketone content of the sample can then be obtained from equations (3) and (1).

$$\text{Ketone content} = \frac{k_7}{k_4} = \frac{\left[k_6 - \left(\frac{k_2 \times k_5}{k_1}\right)\right]}{k_3 - \left(k_2 \times \frac{18{\cdot}9}{19{\cdot}8}\right)} \text{ per cent.}$$

With the reference data obtained—

$$\text{ketone content} = \frac{k_6 \times 0{\cdot}085\, k_5}{0{\cdot}089} \text{ per cent.} \quad .. \quad .. \quad .. \quad (4)$$

The infra-red method for the determination of ketone content has been tested on a number of mixtures of methyl stearate and methyl ketostearate made up to give known ketone contents (see Table III) and various fatty oil samples on which ketone determinations had been made by the hydroxylamine method[4,5] (see Table IV). The results show that the spectroscopic method gives results in good agreement with the chemical procedure.

TABLE III

KETONE CONTENTS OF VARIOUS MIXTURES OF METHYL STEARATE AND METHYL KETOSTEARATE

Ketone content, per cent.	
Theoretical	Infra-red method
0·70	0·75
0·90	1·00
2·10	2·20
2·90	2·60
3·80	3·40
5·55	5·48

TABLE IV

COMPARISON OF THE KETONE CONTENTS DETERMINED BY THE HYDROXYLAMINE AND INFRA-RED METHODS

Sample	Ketone content, per cent.	
	Hydroxylamine method	Infra-red method
Oiticica oil	6·00	6·20
Hydrogenated peroxidised methyl elaeostearate:		
(Fraction A)	0·70	0·82
(Fraction B)	0·45	0·47

The authors wish to thank the Council and Director of the Research Association of British Paint, Colour and Varnish Manufacturers for permission to publish this paper, which is based on work carried out at the Paint Research Station.

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PAINT RESEARCH STATION
TEDDINGTON, MIDDLESEX

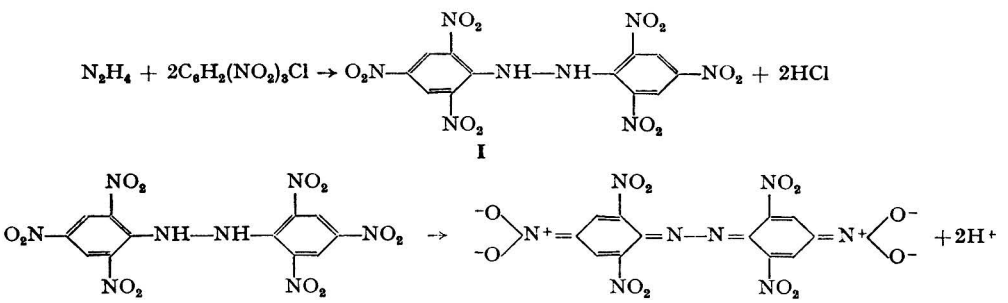
June 5th, 1953

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.

THE normal methods of determining hydrazine by making use of its reducing power,[1] although accurate, are not suitable for the analysis of extremely dilute solutions. Two procedures have been described for the colorimetric determination of hydrazine at low concentrations. Pesez and Petit[2] made use of the orange azine formed when hydrazine reacts with *p*-dimethylaminobenzaldehyde in the presence of hydrochloric acid. The same method was used by Watt and Chrisp,[3] who reported a coefficient of variation of 1 per cent. for hydrazine concentrations ranging from 0·06 to 0·47 parts per million. Kul'berg and Cherkesov[4] treated the test solution with an ethanolic solution of picryl chloride, forming bistrinitrophenylhydrazine, **I**, which, when buffered with borate, developed a red colour, whose intensity (measured after excess of reagent had been removed by filtration) was proportional to the concentration of hydrazine.



I

In the course of work on the oxidation of hydrazine in dilute aqueous solution by either oxygen or nitrate ion, a method was required for the determination of hydrazine at concentrations as low as 10^{-5} *M*. The method of Kul'berg and Cherkesov[4] gave erratic results (about ± 20 per cent.). The error was attributed to—

(*i*) the rapidity with which picryl chloride is hydrolysed in water,
(*ii*) the instability of the alcoholic solution of the reagent,[5]

(*iii*) the difficulty of obtaining absolutely clear solutions after removal of excess of reagent and

(*iv*) the effect of light on the initial reaction between hydrazine and picryl chloride.

To minimise the last of these difficulties all subsequent work was carried out in artificial light only.

Experimental

In unsuccessful attempts to improve the accuracy of the procedure, a number of water-soluble solvents for picryl chloride other than alcohol were tested. When a solution of

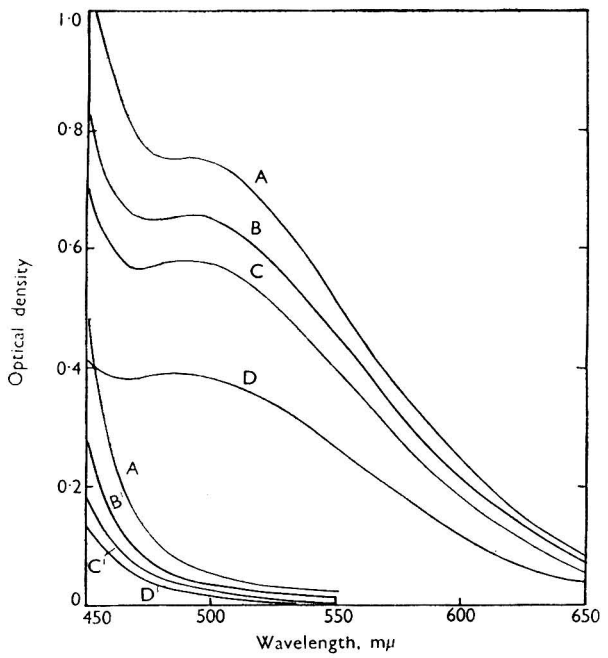


Fig. 1. Influence of concentration of picryl chloride on absorption spectra, 2·5 ml of 0·001 per cent. hydrazine and 1 ml of picryl chloride reagent and 5 ml of 0·02 per cent. potassium acetate made up to 25 ml with absolute alcohol.

Concentration of picryl chloride: curve A, 4 per cent.; B, 2 per cent.; C, 1 per cent.; D, 0·5 per cent. Reagent blank solutions with various concentrations of picryl chloride; curve A′, 4 per cent., B′, 2 per cent.; C′, 1 per cent.; D′, 0·5 per cent.

picryl chloride in chloroform was used as a reagent and an alcoholic solution of potassium acetate was subsequently added, reproducibility was improved. The effect of concentration of picryl chloride on the absorption spectrum of the reaction mixture with these reagents is shown in Fig. 1; the absorption maximum of the red colour is at 494 mμ and not, as stated by Kul'berg and Cherkesov, at 530 mμ.

To establish the optimum conditions for the determination of hydrazine, 2·5-ml samples of hydrazine sulphate solution (0·001 per cent. with respect to hydrazine) were treated with 1-ml portions of picryl chloride at several concentrations. After 1 minute various amounts of a 0·05 per cent. alcoholic solution of potassium acetate were added and the mixtures were diluted to 25 ml with ethanol. After 1 hour the optical densities of the solutions were measured at 530 mμ and gave the results shown in Fig. 2. On consideration of Figs. 1 and 2 and on taking into account the desirability of having low reagent blanks, it was evident that the most suitable amounts of reagents were 1 ml of 2 per cent. picryl chloride and 5 ml of 0·05 per cent. potassium acetate solution; these amounts were used in all subsequent work.

On allowing the picryl chloride solution to stand in contact with the hydrazine solution for various periods before adding the potassium acetate reagent and making up to volume, it was found that the initial reaction was complete within 15 seconds and that no change took place within at least 15 minutes. The development of the red colour after the addition of the potassium acetate solution was complete after 40 minutes and the colour remained stable for at least a further 4 hours.

EFFECT OF TEMPERATURE—

When the determination was carried out at 30° C, it was found that although the maximum colour intensity developed in approximately 15 minutes, the extinction coefficient was only 0·9 per cent. higher than that found at 20° C.

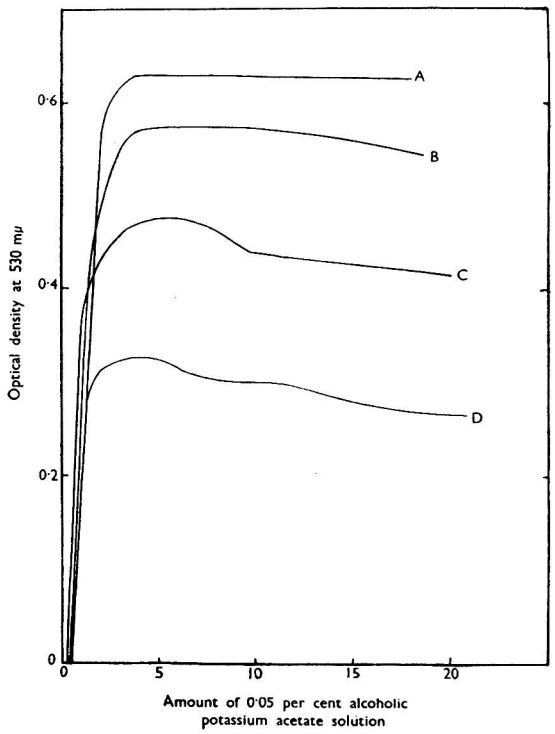


Fig. 2. Effect of amount of potassium acetate solution on optical density for various concentrations of picryl chloride, 2·5 ml of 0·001 per cent. hydrazine and 1 ml of picryl chloride + x ml of 0·05 per cent. alcoholic potassium acetate solution made up to 25 ml with alcohol. Concentration of picryl chloride: curve A, 4 per cent.; B, 2 per cent.; C, 1 per cent.; D, 0·5 per cent.

INFLUENCE OF SAMPLE VOLUME—

As the position of equilibrium of the initial reaction is a function of the amount of water present, the flasks in which the determination is carried out should be dry, and the same volume of test solution should always be taken. With 25-μg samples of hydrazine in various volumes of water the following results were obtained—

Volume of sample, ml	1·0	1·5	2·0	2·5	3·0	4·0	5·0
Optical density at 494 mμ (1-cm cell)	0·801	0·750	0·720	0·663	0·620	0·535	0·450

EFFECT OF pH—

Solutions of hydrazine sulphate (containing 0·001 per cent. as hydrazine), adjusted to cover a range of pH values by addition of hydrochloric acid or sodium hydroxide were

examined, and gave the results shown in Fig. 3. Over the pH range 3·2 to 5·00 there was an increase in optical density of 0·7 per cent. Above a pH value of 5 there was a more rapid increase up to pH 11·5, and solutions more alkaline than this absorbed strongly owing to the

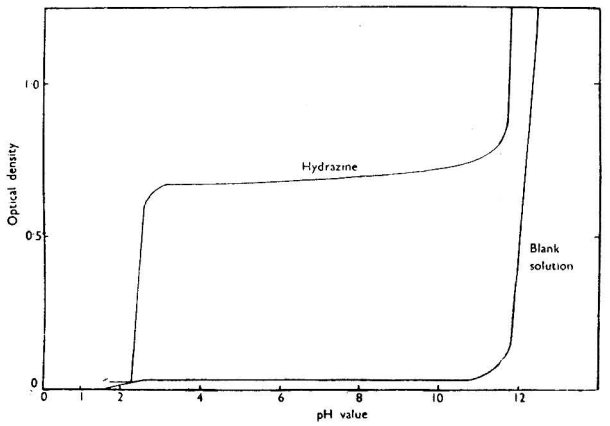


Fig. 3. Effect of pH on optical density measured for 2·5 ml of 0·001 per cent. hydrazine solution.

reaction of picryl chloride itself with the buffer. No colour was formed below a pH of 2·3. To avoid any risk of oxidation of hydrazine from the air, the pH value of test solutions should lie between 3·3 and 5·0.

Method

All measurements of optical density were made with a Unicam SP500 spectrophotometer and 2-mm, 1-cm or 7·5-cm cells, as appropriate.

Reagents—

Hydrazine sulphate stock solution—A solution containing 0·4062 g of hydrazine sulphate (assaying at 99·3 per cent. of hydrazine sulphate) per litre.

Hydrazine sulphate dilute solution—Dilute 100 ml of the stock solution to 1 litre. This solution, which contains 10 mg of hydrazine base per litre, must be prepared fresh daily.

Picryl chloride reagent—Dissolve 0·5 g of picryl chloride in 15 ml of chloroform, filter the solution into a dry 25-ml calibrated flask and dilute to volume with chloroform.

Potassium acetate solution, 0·05 per cent.—Dissolve 0·125 g of anhydrous potassium acetate in 250 ml of absolute ethyl alcohol.

Procedure—

Place, by means of a pipette, 2·5 ml of the test solution (pH value 3·3 to 5) in a dry 25-ml calibrated flask, and add from a micro-burette 1 ml of 2 per cent. picryl chloride reagent. Shake the flask and set it aside for at least 40 seconds. Add 5 ml of 0·05 per cent. potassium acetate solution, mix well and dilute to 25 ml with absolute alcohol. Measure the optical density of the solution at 494 mμ (or at 530 mμ if very low concentrations of hydrazine are being determined or if hydroxylamine is present). Determine the blank value for the reagents in the same manner, using 2·5 ml of distilled water. Calibrate the method with the dilute hydrazine sulphate solution.

Results

Replicate determinations were made over a considerable range of hydrazine concentrations to test the accuracy of the method. For the determination of less than 1 part of hydrazine per million the optical density was measured in a 3-inch cell at 530 mμ instead of 494 mμ; this reduced the reagent blank. The figures in Tables I and II indicate that Beer's law is obeyed for up to about 30 parts of hydrazine per million with a coefficient of variation of 1 per cent.; above this concentration the deviation from linearity increases rapidly.

TABLE I

DETERMINATION OF HYDRAZINE

Amount of hydrazine, μg per ml	Mean optical density less blank at 494 mμ	Number of determinations	Standard deviation	Deviation from linearity, %
0·5	0·033	4	4·0	+0·6
1	0·067	5	1·2	+2·1
2	0·133	4	0·7	+1·3
4	0·258	6	1·1	−1·4
6	0·388	6	0·2	−1·1
8	0·518	4	0·4	−0·9
10	0·650	6	0·4	−0·9
12	0·784	3	0·6	−0·4
14	0·924*	8	0·2	+0·6
16	1·055*	8	0·7	+0·4
18	1·200*	6	0·3	+0·6
20	1·298*	12	0·4	−1·0
25	1·642*	8	0·5	+0·2
30†	1·964*	6	0·2	−0·2
40†	2·694*	4	0·2	+2·6‡
50†	3·414*	3	0·8	+4·0‡
60†	4·164*	6	0·4	+5·7‡
80†	5·620*	3	0·8	+6·9‡

* Measured in 2-mm cell and calculated to 1-cm cell.
† Ten millilitres of 0·05 per cent. potassium acetate added.
‡ Excluded from calculation of mean slope and standard deviation from linearity.

NOTE—Amount of hydrazine, p.p.m. $= \frac{\text{Optical density}}{0{\cdot}0656}$

TABLE II

DETERMINATION OF LOWER CONCENTRATIONS OF HYDRAZINE

Amount of hydrazine, μg per ml	Mean optical density less blank at 530 mμ*	Number of determinations	Standard deviation, %	Deviation from linearity, %
0·04	0·018	4	5·5	+15·0
0·1	0·034	4	0·6	−12·9
0·2	0·078	3	0·0	±0·0
0·4	0·156	5	0·6	±0·0
0·6	0·230	4	0·3	−1·8
0·8	0·307	4	0·2	−1·5
1·2	0·471	4	0·3	+0·6
1·6	0·629	4	0·3	+0·6

* Three-inch cell.

NOTE—Amount of hydrazine, p.p.m. $= \frac{\text{Optical density}}{0{\cdot}390}$

INTERFERENCE OF FOREIGN ANIONS AND CATIONS—

Twenty-five-millilitre portions of 0·2 per cent. and 2 per cent. solutions of a number of salts were treated with 5 ml of hydrazine sulphate solution (0·01 per cent. as hydrazine) and diluted to 50 ml. The diluted solutions were analysed for hydrazine, any precipitate formed being removed by centrifugation before the optical density was measured. None of the salt solutions tested in the absence of hydrazine showed greater absorption than the reagent blank. The results shown in Table III are expressed in terms of the percentage reduction in optical density compared with the optical density in the absence of the salt. Chloride ion causes a much greater reduction in colour than other anions (except azide) probably because of its influence on the equilibrium in the reaction of hydrazine with picryl chloride.

EFFECT OF HYDROXYLAMINE—

Preliminary experiments showed that hydroxylamine hydrochloride when treated with picryl chloride and potassium acetate exhibited appreciable light absorption at 494 mμ,

but absorbed only slightly at 530 mμ. Solutions of hydroxylamine hydrochloride containing 0·0001, 0·001 and 0·005 per cent. of hydroxylamine and various concentrations of hydrazine were examined by the proposed method, all solutions being measured at 530 mμ. The figures shown in Table IV indicate that interference at a concentration of 0·0001 per cent. of hydroxylamine is negligible; with 0·001 and 0·005 per cent. hydroxylamine the optical density is reduced by 5·1 and 9·9 per cent., respectively. Hence, hydrazine can be determined with reasonable accuracy in the presence of at least its own weight of hydroxylamine.

Table III

Influence of foreign ions on determination of 0·001 per cent. hydrazine

Compound	Present as	Reduction in optical density at 494 mμ for 0·1 per cent. solutions, %	Reduction in optical density at 494 mμ for 1 per cent. solutions, %
Sodium chloride	NaCl	8·8	27·6
Sodium azide	NaN_3	22·1	—
Potassium chloride	KCl	9·8	24·6
Potassium sulphate	K_2SO_4	—	0·0
Potassium nitrate	KNO_3	2·5	19·1
Ammonium chloride	NH_4Cl	20·0	60·0
Ammonium sulphate	$(NH_4)_2SO_4$	2·0	25·0
Magnesium chloride	$MgCl_2.6H_2O$	20·4	72·0
Calcium chloride	$CaCl_2.6H_2O$	15·0	52·0
Barium chloride	$BaCl_2.2H_2O$	7·0	—
Zinc sulphate	$ZnSO_4.7H_2O$	40·0	—
Cadmium chloride	$CdCl_2.6H_2O$	21·0	—
Lead acetate	$Pb(OOC.CH_3)_2.2H_2O$	0·0	21·2
Manganese chloride	$MnCl_2.6H_2O$	31·0	—

Table IV

Optical densities measured at 530 mμ of solutions containing hydroxylamine and hydrazine after reaction with picryl chloride

Concentration of hydroxylamine, %	Optical densities of solutions containing various concentrations of hydrazine: 0	0·0002 per cent.	0·0005 per cent.	0·001 per cent.	0·0015 per cent.	0·002 per cent.
0	0·011	0·114	0·270	0·528	0·786	1·045
0·0001	0·014	0·114	0·270	0·533	0·810	1·050
0·001	0·018	0·110	0·256	0·500	0·760	1·020
0·005	0·021	0·105	0·245	0·474	0·720	0·980

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Department of Oceanography
The University
Liverpool, 3

July 6th, 1953

Experiments on the Hydrolysis of 610, 66 and 6 Nylon

By J. HASLAM and Miss S. D. SWIFT

Comparative tests have been carried out on the hydrolysis with 26 per cent. w/v hydrochloric acid in sealed tubes of samples of 66, 610 and 6 nylon. The extent of the hydrolysis has been determined by potentiometric titration of the hydrolysis products with standard alkali.

In the original work[1] on the analysis of nylon samples it was the practice to hydrolyse the sample in the first place with 20 per cent. v/v hydrochloric acid solution (100 ml of this solution contained 20 ml of hydrochloric acid, sp.gr. 1·18) for extended periods of time. This caused difficulties where samples of 610 nylon were concerned, and it was shown that it was desirable to prepare the 610 nylon samples by re-precipitation in a finely divided form before hydrolysis. Since this early work, however, it has been shown that the hydrolysis of 66 and 610 nylons and copolymers can be carried out much more effectively with 50 per cent. v/v hydrochloric acid solution (100 ml of this solution contained 50 ml of hydrochloric acid, sp.gr. 1·18). With this strength of acid it is no longer necessary to prepare a re-precipitated specimen of 610 nylon before hydrolysis.

Other workers have used rather different procedures. Zahn and Wolf,[2] who have carried out a considerable amount of work on the chromatographic examination of nylon hydrolysis products, hydrolyse 50 mg of perlon L or nylon in a sealed tube for 24 hours at 110° C with 0·5 ml of 6 *N* hydrochloric acid. For samples of perlon U, the hydrolysis is carried out with 0·5 ml of 12 *N* hydrochloric acid.

Ecochard and Duveau,[3] in their work on the examination of interpolyamides, hydrolyse a mixture of 610 and 6 polyamides by heating 1 g of the mixture with 2 ml of hydrochloric acid (260 g of hydrogen chloride per litre) in a sealed tube at 130° C for about 6 hours. Subsequently the hydrolysis product is dissolved in water and titrated potentiometrically. From the result of this titration the proportions of 610 and 6 polymers in the mixture are deduced. Incidentally, the example these authors give of the result of the hydrolysis of a mixture of 0·71 g of 6 polymer and 0·29 g of 610 polymer on p. 154 of their paper is erroneous. For such a mixture the amount of alkali used between the first and second end-points of the potentiometric titration should be 8·34 ml of *N* sodium hydroxide, and not 9·50 ml as stated by them. This 8·34 ml is made up of (*a*) 6·28 ml of *N* sodium hydroxide corresponding to the hydrochloric acid in the ϵ-aminocaproic acid hydrochloride derived from 0·71 g of polymer 6 and (*b*) 2·06 ml of *N* sodium hydroxide corresponding with the sebacic acid derived from 0·29 g of polymer 610.

Nevertheless it seemed to us that the principle of the method used by Ecochard and Duveau might be very usefully extended to a study of the hydrolysis of various nylons and related polymers with hydrochloric acid solution. For example, after a known amount of 66 nylon polymer had been heated with hydrochloric acid, it would be possible on completion of the hydrolysis to carry out a potentiometric titration with alkali of the hydrolysis products. The first end-point would give the free hydrochloric acid, and the adipic acid resulting from the hydrolysis would be titrated between the first and second end-points. This difference between the first and second end-points would, therefore, give a simple measure of the extent of the hydrolysis and it was hoped, therefore, that considerable information could be quickly obtained by means of this comparatively simple test.

Such tests have been carried out on the hydrolysis of samples of nylon 66, 610 and 6, a ratio of 4 ml of 26 per cent. w/v hydrochloric acid solution (100 ml of this solution contained 26 g of hydrogen chloride) to 2 g of polymer being used and the hydrolyses being carried out for varying periods of time. No advantage was found in increasing the amount or concentration of the acid.

Experimental

Nylon 66—

A 2-g sample of nylon 66 was weighed into a glass tube, 10 inches by $\frac{3}{8}$-inch diameter, and 4 ml of 26 per cent. w/v hydrochloric acid were added. The tube was then sealed and placed in an oven at 130° C. After 1 hour the tube was shaken gently to assist solution,

and again shaken at half-hourly intervals until solution of the nylon was complete. This usually occurred after 2 hours. It was noticed that the solution became light brown in colour during the hydrolysis.

After a definite time the tube was removed and allowed to cool. When cool it was opened and the contents were washed with distilled water into a 500-ml beaker. To dissolve all the adipic acid the beaker and contents were warmed gently and then allowed to cool to room temperature. The final volume of solution was approximately 150 ml.

POTENTIOMETRIC TITRATION OF THE HYDROLYSIS PRODUCT—

The initial pH of the solution was usually 1·3. A 10 per cent. solution of sodium hydroxide was run in until a pH of 2 was reached. At this point *N* sodium hydroxide was used and the additions were made in 0·2-ml portions. The burette readings and the corresponding pH values were noted and recorded until it was apparent that the first end-point had been passed. This first end-point, which represents the amount of free hydrochloric acid in the solution, occurred at about pH 3.

N sodium hydroxide was then run in until pH 6 was reached. Small additions of 0·2 ml were again made, the burette reading and pH values for each addition being noted as before until the second end-point had been passed. This second end-point occurred at or near pH 8·3.

For deducing the end-points, the method of Gran[4] was used, *i.e.*, $dV/d\text{pH}$ was plotted against $V + \frac{1}{2}dV$. The graphs obtained were similar to that shown in Fig. 1.

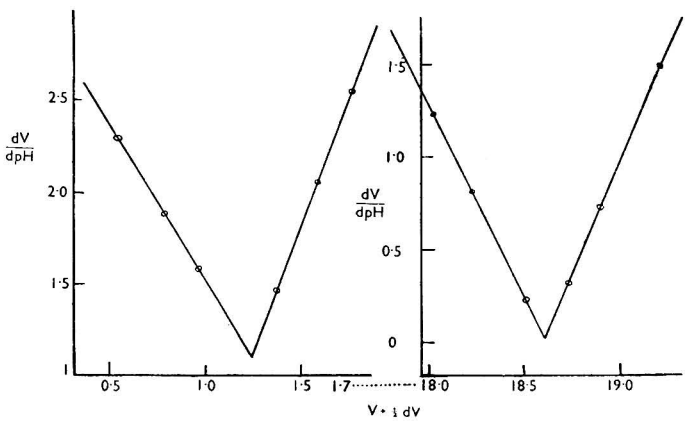

Fig. 1. Typical graph of end-points, plotted by Gran's method[4]

The amount of alkali used between the first and second end-points is equivalent to the adipic acid in the solution and hence is a measure of the amount of hydrolysis that has taken place.

Table I shows how the degree of hydrolysis attained varies with the time of heating. The sample of nylon 66 used in these experiments had a moisture content of 2·4 per cent., which has not been allowed for in the percentages quoted.

TABLE I

DEGREE OF HYDROLYSIS OF NYLON 66

Time of heating at 130° C, hours	2	4	6	8	12	16
Degree of hydrolysis, per cent.	73·6	76·9	84·9	96·4	97·6	98·2
	66·3	82·5	88·6	95·8	96·9	98·4

NYLON 610—

The procedure for hydrolysing nylon 66 was first followed, *i.e.*, 2 g of the polymer was heated in 4 ml of 26 per cent. w/v hydrochloric acid at 130° C. It was found that the polymer was slow to hydrolyse; after heating for 6 hours only approximately 16 per cent. had hydrolysed and after 12 hours less than 70 per cent.

It was then decided to fit an apparatus for shaking the tubes in the oven in the hope that continuous shaking would promote hydrolysis. The apparatus, shown in Fig. 2, consisted of a horizontal rod to which the tubes were attached and which was driven by an electric motor placed outside the oven.

The results attained with this method of shaking showed a marked improvement, after 6 hours from 16 per cent. (without shaking) to 63 per cent. (with shaking) and after 12 hours from less than 70 per cent. (without shaking) to 92 per cent. (with shaking). Nylon 610, however, was still much slower to hydrolyse than nylon 66, the highest figure attained being 97·7 per cent. hydrolysis after 24 hours.

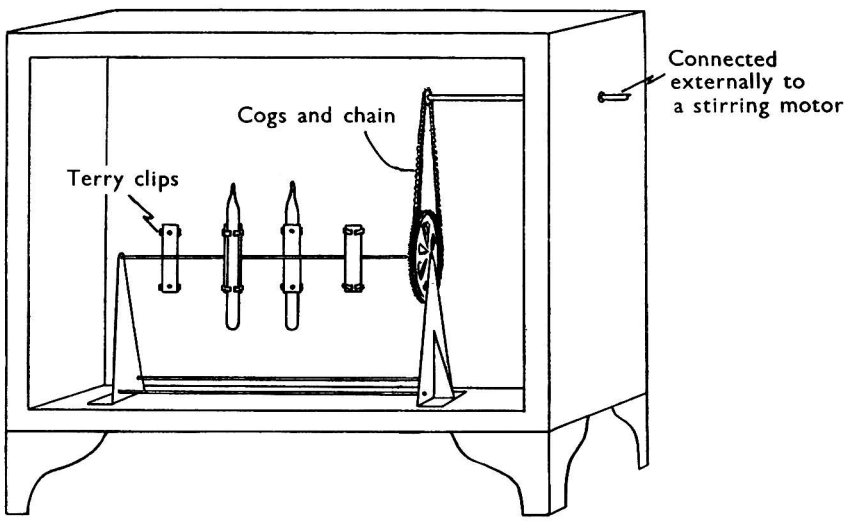

Fig. 2. Continuous-shaking apparatus

The potentiometric titration for the hydrolysis products of nylon 610 was similar in principle to that of nylon 66, the end-points being finally deduced by the method of Gran.[4]

In order to facilitate solution of the sebacic acid formed in the hydrolysis, ethyl alcohol was used to wash out the reaction tubes. The final solution for titration consisted of approximately 100 ml of ethyl alcohol and 50 ml of water. The initial pH of the solution was usually 1·3.

The first end-point in the solution occurred at pH 3·8 and the second end-point at pH 8·2 approximately. The difference between the first and second end-points corresponded to the sebacic acid in the solution and hence to the degree of hydrolysis achieved.

Table II shows the degree of hydrolysis of nylon 610 attained by varying the time of heating. Shaking was used in these experiments. The moisture content of the nylon 610 was 1·3 per cent.

TABLE II

DEGREE OF HYDROLYSIS OF NYLON 610

Time of heating at 130° C, hours ..	4	6	8	12	14	16	24
Degree of hydrolysis, per cent. ..	58·5	63·1	81·9	92·0	91·2	91·2	94·6
		57·1	90·0	90·2	85·4	93·6	96·4
				88·4		89·8	
				86·0		92·7	

It should be noted that with the hydrolysis products from 66 and 610 nylon, the hexamethylenediamine dihydrochloride produced plays no part in the potentiometric titration.

NYLON 6 (CAPROLACTAM POLYMER)—

Nylon 6 shows great ease of hydrolysis when submitted to the conditions outlined for nylon 66 and 610.

A 2-g sample of polymer and 4 ml of 26 per cent. w/v hydrochloric acid were sealed in a reaction tube and heated, with shaking, for various lengths of time. The polymer dissolved after 15 minutes, and after 2 hours as much as 91·8 per cent. had hydrolysed, as compared with 66 to 73 per cent. of nylon 66 and less than 10 per cent. of nylon 610.

To determine the degree of hydrolysis of nylon 6, it is necessary to determine the amount of ϵ-aminocaproic acid hydrochloride present in the reaction product. The procedure is similar to that followed in determining the amounts of adipic and sebacic acids from nylon 66 and 610, *i.e.*, potentiometric titration. The first end-point gives the free hydrochloric acid present and the difference between the first and second end-points gives the ϵ-aminocaproic acid hydrochloride.

The initial pH of the reaction product was 0·4 to 0·5. The first end-point occurred at pH 2·6 and the second between pH 7·4 and 8·7.

Table III shows the degree of hydrolysis of nylon 6 for various times of heating. The moisture content of the polymer was found to be 2·17 per cent.

TABLE III

DEGREE OF HYDROLYSIS OF NYLON 6

Time of heating at 130° C, hours	2	4	6	8	10	12
Degree of hydrolysis, per cent.	91·5	94·9	96·7	96·3	97·0	97·8
	91·8	95·9	96·9	97·4	97·8	

These experiments indicate that 6 nylon is readily and completely hydrolysed by 26 per cent. w/v hydrochloric acid, that the hydrolysis of 66 nylon proceeds to completion with greater difficulty and that 610 nylon is most difficult to hydrolyse and in our experience hydrolysis is not quite complete under the most rigorous conditions. In general, the hydrolysis of 610 nylon gives rather variable results.

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IMPERIAL CHEMICAL INDUSTRIES LIMITED
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WELWYN GARDEN CITY, HERTS.

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A Modified Iodimetric Determination of Organic Peroxides

By B. DUDLEY SULLY

A modified iodimetric method for the estimation of organic peroxides, including those present in rancid fats, is described. The procedure is similar to the method of Lea, but the necessity for de-aerating the reagents and for working in a current of inert gas is avoided by mixing all the reactants in a boiling acetic acid - chloroform solution.

Organic peroxides such as benzoyl peroxide, ethylbenzene hydroperoxide and the peroxides in rancid fats are most conveniently determined by the iodimetric method. Recent work has shown that the weight of iodine liberated when an organic peroxide is added to an acidified solution of potassium iodide is not always the stoicheiometrical equivalent. Various factors operate, some tending to reduce the amount of iodine liberated, as, for example, the choice of an unsuitable reaction medium or the use of an insufficient excess of iodide, whilst other factors, such as contamination by atmospheric oxygen, tend to increase the amount of iodine liberated.

Perhaps the most frequent cause of erratic results is contamination by atmospheric oxygen. At one time it was thought that this could be allowed for completely by a blank determination, but it is now known that the liberated excess of iodine is related, not to the partial pressure of the oxygen during the reaction, but to the quantity of peroxide decomposed. This was first observed by Lea,[1] who showed that the ferric thiocyanate method of peroxide estimation described by Lips, Chapman and McFarlane[2] gave peroxide values that were four times as high as those obtained under similar conditions but with rigorous exclusion of oxygen. Lea[3] has described a method in which a specially designed flask is used so that it is possible to mix the reactants in an inert atmosphere. The degree of de-aeration necessary is shown by the fact that 100 ml per minute of cylinder nitrogen (purest "oxygen-free" grade) must be passed through a 100-ml reaction flask for a period of 15 minutes to ensure consistent results; a period of 5 minutes is definitely inadequate. A blank titration of between 0·1 and 0·3 ml of 0·002 *N* sodium thiosulphate is obtained when the acetic acid, chloroform and potassium iodide solutions are mixed only 2 to 3 minutes before the de-aeration is begun. According to Lea[3] the blank titration can be reduced, usually to zero, by de-aeration of the acetic acid - chloroform solvent for 5 minutes before adding the potassium iodide. The modern explanation is that the reaction of the peroxide with the iodide produces radicles that admittedly have only a short life, but which nevertheless have time enough to react with gaseous oxygen and so produce more peroxide. Such an effect cannot be estimated by a blank determination.

In the proposed method the acetic acid - chloroform solvent chosen for the reaction is boiled in a flask fitted with a plain tube about 75 cm long, which acts as a fractionating column. The top of the tube is cooled with a water jacket to prevent the escape of solvent. If acetic acid - chloroform is boiled to the top of the column and all the reactants are added to the flask down through the fractionating column it is possible to ensure that they are de-aerated before reaction to a degree that is not possible by the usual inert-gas techniques. By working in the proposed way it is possible to add potassium iodide solution to a boiling acetic acid - chloroform solution and then to continue the boiling for a further 30 minutes or more without liberating any iodine. With freshly made potassium iodide solution and analytical grade acetic acid, the blank value should always be zero. Measurable values have been traced to the acetic acid, and it would appear that the so-called reducing substances that it sometimes contains, and which are detected by their reaction with potassium permanganate, are in reality peroxides. The effect of varying the ratio of acetic acid to chloroform in the solvent mixture has been studied by various earlier workers. The original peroxide method of Taffel and Revis[4] makes use of acetic acid only, and considerable shaking or boiling is needed because it is not a solvent for the oil. Wheeler[5] added sufficient chloroform to form a homogeneous solution and claimed that under such conditions it was not necessary

to boil or to use an inert atmosphere. Stuffins and Weatherall[6] showed that low values are obtained when the solvent contains more chloroform than acetic acid and the reaction takes place at room temperature. These authors also showed the importance of excluding all oxygen. Other workers, including Stansby[7] and Risbey and Nisbet,[8] have observed discrepancies when the ratio of the chloroform to acetic acid is varied. Skellon and Wills[9] pointed out that an ionising solvent is necessary and that no more chloroform or carbon tetrachloride should be added than is necessary to dissolve the fat. From the present work it is believed that the discrepancies observed are caused by incomplete reaction owing to the use of chloroform in an amount sufficient to depress the ionisation of the solvent, leading in some methods to precipitation of the potassium iodide. In the method recommended it is considered undesirable to use less than 50 per cent. of chloroform in the solvent mixture because a lesser proportion would raise the boiling point and so needlessly increase the rate of reaction. It is believed that the solvent plays only a minor role, but the reaction should clearly not be too rapid by comparison with the speed of the mixing of the reactants. With the proposed technique it has been found that chloroform can be replaced by ether without influencing the result significantly, although the boiling point is lower. Ether is not recommended, however, because it may contain peroxides and its lower boiling point makes it less easy to manipulate without loss.

Certain techniques, as for example that of Stuffins and Weatherall,[6] make use of a reaction mixture that contains insufficient water to dissolve all the potassium iodide. This is not theoretically desirable, but it may not do harm if the rate of solution of the solid potassium iodide exceeds the rate at which it is consumed in the reaction. In the present technique the reaction is very rapid and it is considered highly desirable that the whole of the potassium iodide should remain in solution. Precipitation of the potassium iodide when the solution is added to the boiling acetic acid - chloroform mixture indicates that insufficient water is present and it is necessary to add water, 0·1 ml at a time, until the potassium iodide dissolves. The amount required is readily determined within 0·1 ml because the solubility changes very rapidly with water content. The acetic acid usually contains water and on such occasions a corresponding reduction may be necessary in the amount of water used to dissolve the potassium iodide. It may be noted, however, that in practice there is not usually any difference between the peroxide value found with a solvent mixture containing either 1·0 or 1·5 ml of water, although the lesser amount precipitates much of the potassium iodide. This is in agreement with the findings of Lea.[3]

Method

Procedure—

Fit a 100-ml round-bottomed flask with a ground-glass joint to a plain reflux tube about 75 cm long and 9 mm internal diameter, the upper 15 cm of which are cooled with a water jacket. Add 10 ml of chloroform and 10 ml of acetic acid to the flask and boil the mixture to the top of the tube, where it is condensed by the water jacket. Use a micro gas flame close to the glass to ensure steady boiling. The plain portion of the tube acts as a fractionating column to remove the trace of dissolved oxygen and, further, by adding the potassium iodide solution and the sample through the column it is possible to ensure that they too are de-aerated before a reaction with the reagents in the flask. When the acetic acid - chloroform mixture is refluxing steadily, pour a solution of 1 g of potassium iodide dissolved in 1·3 ml of water sufficiently slowly down through the column for refluxing from the water jacket to continue without interruption. Redissolve any precipitated potassium iodide by the addition of not more than about 6 drops of water. The solution can be boiled under these conditions for long periods, *e.g.*, 30 minutes, without causing the liberation of iodine, provided that the solvents are free from peroxides and the potassium iodide is free from iodate. Then add the organic peroxide to be determined down through the column without interrupting the steady refluxing and turn off the cooling water in order to raise the condensation level and so ensure that all the sample is washed into the flask. After boiling the solution for a further 3 to 5 minutes, rapidly cool the contents of the flask, dilute the contents with 50 ml of water and titrate the liberated iodine with 0·01 *N* sodium thiosulphate in the usual way.

Comparison with other methods

The method described has been used in routine analysis for about four years, mainly in the estimation of peroxides present in oxidised hydrocarbons and aldehydes. Even

relatively unstable hydroperoxides, such as ethylbenzene hydroperoxide, appear to react quantitatively. Dibenzoyl peroxide and peracids also react quantitatively. More recently a comparison has been made with the peroxide values obtained for rancid oils by the so-called "hot" method of Lea[3] and the "cold" method of Stuffins and Weatherall,[6] and the results are shown in Tables I to IV.

Discussion of results

It is believed that both the "hot" method of Lea and the "cold" method of Stuffins and Weatherall give results that are comparable with those obtained by the proposed method. The proposed method, however, has the advantage that no inert gas is required and that it is quicker and more suitable for routine operation. Tables I and II show that there is a tendency for the proposed method to give slightly lower values than the comparison methods, but this may be explained by oxygen contamination, as the commercial cylinder nitrogen used was not specially purified to remove the last trace of oxygen. It is believed, therefore, that the results by the proposed method are nearer estimates of the peroxide contents.

Table I

Comparison of the proposed method with the "hot" Lea method

Type of oil	Peroxide value, milli-equiv. per kg	
	"Hot" Lea method	Proposed method
Sunflower oil containing vitamin concentrate, sample 1	44·2	42·7
	45·2	41·7
	43·7	
Ditto, sample 2	38·1	33·2
	36·8	32·8
Olive oil	48·6	47·9
	48·6	
Cottonseed oil	13·3	11·7
	12·8	
Soya-bean oil	59·2	57·8
	59·2	
Linseed oil	4·9	2·9
	3·4	
	3·9	

Table II

Comparison of proposed method with Stuffins and Weatherall's method

Type of oil	Peroxide value, milli-equiv. per kg	
	Stuffins and Weatherall's method	Proposed method
Sunflower oil containing vitamin concentrate, sample 1	47·8	42·7
	47·4	41·7
Ditto, sample 2	36·9	33·2
	37·1	32·8
Olive oil	58·6	55·2
	59·8	55·8
Cottonseed oil	15·3	13·1
	14·9	12·3
Soya-bean oil	65·2	59·7
	65·1	58·3
Linseed oil	3·7	2·9
	3·0	

Tables III and IV are considered to be more absolute tests of the method. Table III shows that the sample size can be varied over a ratio of 16 to 1 without causing any significant difference in the measured peroxide value. This method of checking has been previously used by earlier workers, including Lea[3] and Stansby.[7] Usually there is a tendency for the apparent peroxide value to increase as the size of the sample is reduced. The variations

shown in Table III are caused in part by the difficulties in observing the starch end-point, and an electrometric method is advised for more accurate results. Table IV shows that the peroxide value is unchanged after boiling the solution for half an hour instead of the usual 5 minutes and that ether gives results similar to those given with chloroform. In all these experiments the blank determinations showed no liberation of iodine.

TABLE III

VARIATION OF PEROXIDE VALUE WITH SAMPLE SIZE

Oil	Approximate weight of sample, g	Peroxide value, milli-equiv. per kg
Olive oil	0·25	48·4
	0·5	46·7
	1·0	47·9
	2·0	47·2
	4·0	45·9*
Refined cottonseed oil	0·25	12·8
	0·5	13·2
	1·0	11·65
	2·0	11·8*
	4·0	11·2*
Soya-bean oil	0·25	57·0
	0·5	57·8
	1·0	56·9
	2·0	57·0*
	4·0	52·1*
Linseed oil	0·5	3·6
	1·0	2·9
	2·0	2·8*
	4·0	2·6*
Sunflower oil containing vitamin concentrate	0·25	48·6
	0·5	48·3
	1·0	48·6
	2·0	47·7
	4·0	43·3*

* Separates into two phases.

TABLE IV

EFFECT OF VARIATION IN SOLVENT

Oil	Time of heating, minutes	Solvent mixture: Equal volumes of ether and acetic acid	Solvent mixture: Equal volumes of chloroform and acetic acid
Sunflower oil containing vitamin concentrate	5	41·7 43·2	43·7 42·7
	30	43·9	43·2
Olive oil	5	55·2	55·2
	30	56·1	55·8
Cottonseed oil	5	11·9	13·1
	30	12·8	12·3
Soya-bean oil	5	58·7	59·7
	30	58·9	58·3
Linseed oil	5	2·0	2·9
	30	3·3	2·9

The mixture separated into two layers during the boiling when acetic acid ether - mixture was used as the solvent.

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RESEARCH LABORATORY
A. BOAKE, ROBERTS & CO. LTD.
LONDON, E.15

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The Absorptiometric Determination of Perchloric and Chloric Acids in the Electrolyte of Lead - Acid Secondary Cells after Reduction by Titanous Sulphate

BY C. F. FORSTER

A rapid and accurate method has been devised for the determination of a few parts per million of perchloric and chloric acids in the sulphuric acid electrolyte of secondary cells. The total acids are determined by reduction to hydrochloric acid with nascent hydrogen in the presence of titanous sulphate. The resulting chloride is precipitated by silver nitrate at 45° C and the opalescence is measured by means of a Spekker photo-electric absorptiometer. A separate determination, titanous sulphate being omitted, is made for chlorate and subtracted to give the perchloric acid by difference.

THE presence of perchloric acid in the electrolyte of a lead - acid secondary cell leads to premature failure of the battery owing to growth and swelling of the paste in the positive plates. Some perchloric acid may be present in the plates initially, as it is used in their preparation, but it is more usually derived from chloride in the tap water used for topping up the cells to replace losses due to evaporation. This chloride is converted to perchloric acid by the vigorous oxidising action of nascent oxygen at the positive plates when the cells are gassing. This transformation is more prevalent in charge - discharge working, when overcharging is more likely to occur, than in float working. The acid, once formed, remains permanently in the electrolyte.

In order to find at what level the perchloric acid concentration becomes dangerous and to keep a check on batteries in operation, it was necessary to determine with reasonable accuracy quantities of perchloric acid of less than 100 parts per million in sulphuric acid of specific gravity 1·20. The method of Meldrum, Clarke, Kouba and Becker,[1] whereby the perchloric acid is reduced while hot by an excess of standard titanous chloride and titrated back with ferric alum, was found to be rather tedious and liable to error in semi-skilled hands because of the instability of the reagent and the very small difference in the titration for such small amounts. Other oxidising substances that might be present, such as chloric acid, are determined by the same procedure in the cold, under which conditions perchloric acid does not react. Although concentrated perchloric acid is a powerful oxidising substance and can in certain circumstances be dangerous, in dilute solutions it is difficult to reduce and resists the action of such strong reducing agents as sulphur dioxide and nascent hydrogen. Titanous salts are among the few reagents that will reduce it, but even they have no action in the cold. On heating, titanous salts reduce perchloric acid to hydrochloric acid. It was considered that it might be simpler to reduce the perchloric acid with an excess of a titanous salt and to determine the hydrochloric acid formed by precipitating it with silver nitrate and measuring the opacity on a photo-electric absorptiometer. Titanous chloride was obviously useless for this purpose, so titanous sulphate was tried instead. The work of Vil'yanovich[2] suggested that titanous sulphate was not as satisfactory as titanous chloride,

but it was found that complete reduction could be achieved in half an hour if the strength of the sulphuric acid was not less than 40 per cent. w/w. Chloric acid, on the other hand, reduces more readily under weaker acid conditions. Only commercial quality titanous sulphate was available. This is made from the chloride and must be purified before use. For the determination of large amounts of perchloric acid a gravimetric finish is suitable, but for the very small quantities usually present in electrolytes the absorptiometric method is preferable. The determination includes all other chlorine compounds, such as hydrochloric and chloric acids, that might be present in the electrolyte, and separate determinations must be made for these.

Experimental

In the method as originally devised and used in this laboratory, the reduction of the total acids was effected by boiling the electrolyte under reflux in the presence of a sufficient amount of titanous sulphate. This had the disadvantage that the titanium salts present in the solution gave rise to a blank amounting to about 30 divisions on the Spekker absorptiometer drum when a 4-cm cell was used. This disadvantage has been overcome by using a very small amount of titanous sulphate, which is kept in the reduced state by the evolution of nascent hydrogen generated by dissolving metallic zinc in the solution during the reduction procedure. By this means the blank reading is reduced to 3 drum divisions or less.

The chief factors governing the precipitation of silver chloride are (*a*) acid concentration, (*b*) temperature at the moment of precipitation and (*c*) time of standing.

The strength of the sulphuric acid at the moment of precipitation is 4 per cent. This is not critical, and the solution may be further diluted when the perchloric acid concentration is outside the range prescribed in the method without introducing any additional error.

The results of variation in temperature of precipitation and the effect of time of standing are shown graphically in Fig. 1. At 20° C the maximum optical density is not attained until 20 minutes after precipitation. This time is subject to variation when the room temperature is above or below 20° C. The time taken to reach the maximum optical density progressively increases as the temperature of precipitation is lowered. When the silver nitrate is added to solutions at temperatures above 20° C, the time required to reach the maximum optical density is reduced and the value of the maximum is itself increased. This increase is at its greatest between 40° and 50° C, and the time required to reach the peak is very short; a decline sets in within 1 minute of precipitation. At temperatures above 50° C, the height of the initial peak becomes progressively lower owing to rapid agglomeration of the precipitate.

These conditions are only valid for concentrations of perchloric acid up to 15 parts per million in the test solution. At higher concentrations the maximum is only reached if the precipitation temperature is higher than 50° C. However, the results are less reliable and there is a danger of cracking the absorptiometer cells, so that dilution of the sample is recommended whenever the amount of perchloric acid present exceeds the range prescribed in the method.

In the original method the precipitation was carried out at 20° C and 20 to 30 minutes were allowed to elapse before a reading was taken on the absorptiometer. It has now been found that the reproducibility of results by precipitating at 45° C and taking the reading without delay is superior and that the increase of optical density, which amounts to nearly 50 per cent. of that at 20° C, gives increased sensitivity.

The procedure as described below can be completed in 1 hour. Special care is necessary to ensure that all the apparatus used is free from chloride; and the determination should be carried out in a room that is free from fumes of hydrochloric acid.

A blank determination on the reagents should be made with each set of determinations.

The optical absorption of the opalescent solution is measured by the Spekker photoelectric absorptiometer with deep-violet filters (Kodak No. 1 colour filter with a peak transmission at 420 mμ) and a 4-cm cell.

Method

Apparatus—

A 250-ml flask with a ground-glass joint and a single-walled straight-bore condenser similarly fitted should be used, as they are more easily kept clean and free from contamination by extraneous chloride.

REAGENTS—

Sulphuric acid, 40 per cent. w/w—Dilute 35 ml of sulphuric acid, sp.gr. 1·84, with 100 ml of distilled water.

Titanous sulphate, 15 per cent. w/w in 23 per cent. sulphuric acid—Purify this by adding an equal volume of distilled water and evaporating down to low bulk in a narrow-necked flask. Repeat the procedure until a test on a few millilitres diluted with distilled water shows no detectable turbidity with silver nitrate. Finally, dilute to twice the original volume and store for use.

Silver nitrate, 5 per cent. in 1 per cent. nitric acid.

Copperised granulated zinc—Immerse zinc granules in dilute copper sulphate solution for a few seconds and then rinse them in distilled water.

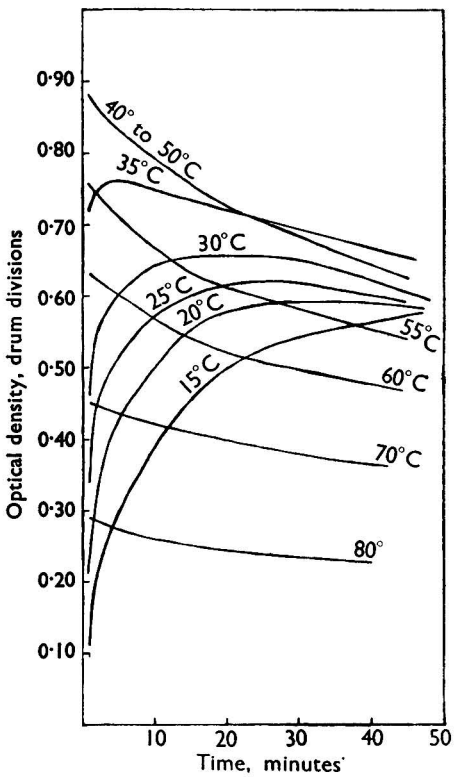

Fig. 1. Effect of time of standing on the optical density of solutions containing 10 ml of 0·001 *N* hydrochloric acid per 100 ml of solution for various temperatures of precipitation of silver chloride

CALIBRATION PROCEDURE—

Accurately measure out portions of 0·001 *N* hydrochloric acid into 250-ml flat-bottomed flasks; 0, 1, 2, 4, 6, 8, 10 and 12 ml are suitable volumes. Add to each flask 10 ml of pure 40 per cent. sulphuric acid, sp.gr. 1·30, and sufficient distilled water to bring the volumes up to 100 ml. Warm the flasks to 45 $\pm$ 1° C one at a time on a hot-plate and immediately add 1 ml of 5 per cent. silver nitrate reagent with rapid agitation. Quickly fill a 4-cm cell and measure the optical density as rapidly as possible on a Spekker absorptiometer that has been set against distilled water at 1·00 with violet Kodak No. 1 colour filters, or their equivalent, with a peak transmission as near as possible to 420 mμ. After correcting for any blank, plot the drum readings against concentration expressed as millilitres of 0·001 *N* acid per 100 ml of sulphuric acid, sp.gr. 1·30.

NOTE—As the equivalent weight of perchloric acid is 100·47, this graph can be used to read direct as parts of perchloric acid per million of electrolyte (w/v) if 50 ml of sample acid diluted to 500 ml are used for the determination.

PROCEDURE FOR THE DETERMINATION OF TOTAL CHLORINE ACIDS—

Reduction—Measure 50 ml of the electrolyte into a 250-ml flask. If the specific gravity of the acid is below 1·3, add sufficient concentrated sulphuric acid to bring the specific gravity to this figure. Add 1 ml of titanous sulphate reagent and about 5 g of copperised granulated zinc. Attach the flask to a water-cooled reflux condenser and heat it over a small flame for half an hour. (A longer period, up to 1 hour, is necessary for concentrations greater than 100 parts per million.) Pour 75 ml of distilled water down the condenser into the flask and continue the heating for a further quarter of an hour. (This step can be omitted if chloric acid is absent.) Rinse down the condenser and detach the flask.

Precipitation—Decant direct (or filter through glass wool) into a 500-ml measuring flask and dilute to the mark with distilled water. Measure two 100-ml portions into clean 250-ml flasks and warm to 45° C on a hot plate. Add 1 ml of 5 per cent. silver nitrate reagent with rapid swirling and measure the optical density in a 4-cm cell without delay, having previously adjusted the instrument against distilled water at 1·00 with a Kodak No. 1 deep-violet colour filter, or its equivalent, having a peak transmission at 420 mμ. If the optical densities of duplicates do not agree to within 0·02, another portion should be taken.

Calculation—Correct the drum reading for the blank and calculate the total amount of chlorine acids present as millilitres of 0·001 *N* acid per 100 ml of sample.

PROCEDURE FOR THE DETERMINATION OF CHLORIC AND HYDROCHLORIC ACIDS TOGETHER—

Reduction—Measure 50 ml of electrolyte into a 250-ml flask and add 75 ml of distilled water and approximately 5 g of copperised granulated zinc. Heat under reflux for 20 to 30 minutes. Rinse down the condenser, detach the flask and complete the determination as described under "Precipitation," above.

Calculation—Correct the drum reading for the blank and determine the result as millilitres of 0·001 *N* acid per 100 ml of sample from the calibration graph.

PROCEDURE FOR THE DETERMINATION OF HYDROCHLORIC ACID—

Measure 50 ml of electrolyte into a 500-ml graduated flask and make up to the mark with distilled water. Complete the determination as described under "Precipitation," above.

Calculation—Determine the result as millilitres of 0·001 *N* acid per 100 ml of sample from the calibration graph. To express the result as parts per million w/v of hydrochloric acid, multiply by 0·363.

EXPRESSION OF RESULTS AS PERCHLORIC ACID AND CHLORIC ACID—

The difference between the results of the first two determinations is the amount of perchloric acid in the electrolyte as millilitres of 0·001 *N* acid per 100 ml of sample. To express the result as parts per million w/v, multiply by 1·0047.

The difference between the results of the second and third determinations is the amount of chloric acid in the electrolyte as millilitres of 0·001 *N* acid per 100 ml of sample. To express the result as parts per million w/v, multiply by 0·841.

RESULTS

To test the validity of the methods, solutions were made up to contain known quantities of perchloric or chloric acid in sulphuric acid of specific gravity 1·30.

The basic calibration graph was prepared by adding various amounts of 0·001 *N* hydrochloric acid to pure sulphuric acid, as described above.

In addition, two further series were prepared, (*a*) by the addition of various amounts of 0·001 *N* perchloric acid and (*b*) by the addition of various amounts of 0·001 *N* potassium chlorate.

These series were treated by the procedure for total chlorine acids and by the procedure for chloric and hydrochloric acids together, respectively, and the results were plotted graphically. Both curves coincided with the curve derived from hydrochloric acid within the limits of experimental error. This was sufficient evidence that the reduction to hydrochloric acid was complete. Some results of the determination of known amounts of perchloric and chloric acids are shown in Table I.

TABLE I

ADDITION OF PERCHLORIC ACID OR POTASSIUM CHLORATE TO SULPHURIC ACID, SP.GR. 1·30

0·001 *N* acid added per 100 ml of sulphuric acid,		0·001 *N* acid found,		
ClO_4', ml	ClO_3', ml	ClO_4', ml	ClO_3', ml	Error, ml
5	0	7		+2
18	0	17		−1
20	0	19		−1
40	0	42		+2
60	0	60		nil
63	0	62		−1
90	0	92		+2
90	0	87		−3
0	20		18	−2
0	40		42	+2
0	60		60	nil
0	80		79	−1

To test the accuracy of the methods for solutions containing perchloric and chloric acids together, synthetic electrolytes were prepared to contain both acids in various amounts. These were treated by the procedures for total chlorine acids and for chloric and hydrochloric acids together, and the results are shown in Table II. The mean error is 1·4 ml of 0·001 *N* acid per 100 ml of sample, which is approximately the same as ±1·4 parts per million for perchloric acid.

TABLE II

ADDITION OF PERCHLORIC ACID AND POTASSIUM CHLORATE TO SULPHURIC ACID, SP.GR. 1·30

0·001 *N* acid added per 100 ml of sulphuric acid			0·001 *N* acid found			Error	
ClO_4', ml	ClO_3', ml	Total, ml	Total, ml	ClO_3', ml	ClO_4' (by diff.), ml	Total, ml	Chlorate, ml
80	30	110	108	30	78	−2	nil
60	30	90	92	30	62	+2	nil
40	70	110	108	69	39	−2	−1
40	40	80	80	42	38	nil	+2
40	30	70	70	30	40	nil	nil
30	20	50	53	20	33	+3	nil
20	70	90	93	68	25	+3	−2
20	20	40	43	22	21	+3	+2

The method as described covers the range 1 to 130 parts per million of perchloric acid. Amounts in excess of 130 parts per million can be determined by diluting the solution to a greater volume. Smaller concentrations down to 0·2 part per million can be estimated by diluting the reduced solution to 100 ml instead of to 500 ml, but a new calibration graph is necessary because of the effect of the greater concentration of sulphuric acid on the optical density of the solution.

INTERFERING IONS—

Free hydrochloric acid is the substance most likely to interfere with the determination of perchloric acid in sulphuric acid. If it does not exceed about 200 parts per million and is not more than 5 times the amount of perchloric acid present it can be tolerated, and is accounted for in the method. Larger quantities of free hydrochloric acid can be removed by distillation of the electrolyte down to one-third of its original volume without loss of perchloric acid. Any hydrochloric acid still remaining is accounted for in the method.

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TEST AND INSPECTION BRANCH
POST OFFICE ENGINEERING DEPARTMENT
STUDD STREET, ISLINGTON, N.1

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The Quantitative Analysis of Iron-stones Containing Small Amounts of Titanium, Vanadium, Manganese, Chromium and Phosphorus

BY D. N. GRINDLEY, E. H. W. J. BURDEN AND A. H. ZAKI

A routine method is described for the analysis of iron-stones containing small quantities of titanium, vanadium, chromium, manganese and phosphorus. After bringing the ore into solution, the iron is extracted from acid solution by ether, and the titanium, vanadium and manganese are determined colorimetrically, the chromium volumetrically and the phosphorus gravimetrically on aliquot parts of the iron-free solution.

WE have been called upon in recent months to examine a number of samples of iron-stones occurring in the Sudan, mainly in the Red Sea Hills. These ores usually contain about 85 per cent. of iron as ferric oxide, together with small amounts of titanium, vanadium and manganese. Phosphorus, if present, must also be determined, as it is a very undesirable constituent. A simple and rapid routine method was therefore devised to avoid the rather lengthy classical procedures and for use in place of spectrophotometric methods, which are not available in the Sudan.

EXPERIMENTAL

SEPARATION AND ESTIMATION OF IRON—

It is well known[1,2,3,4,5,6,7,8] that ferric chloride can be extracted with ether from solutions that are 6 *N* with respect to hydrochloric acid, and it was decided to simplify the subsequent analysis by removing as much as possible of the iron in this way. The efficiency of the extraction was determined by extracting a known quantity of iron by the procedure described below and determining the iron extracted in each ether layer separately; the iron remaining in the aqueous phase was determined colorimetrically with ammonium thiocyanate. The following results were obtained—

Iron taken as Fe_2O_3, g	0·7146
Iron removed in first ether extract, g	0·6847
Iron removed in second ether extract, g	0·0246
Iron removed in third ether extract, g	0·0039
Iron remaining in aqueous phase, g	0·0008

It is therefore evident that, for this purpose, the iron is effectively removed by three ether extractions and the separations are clean and rapid. The other elements stated[8] to be extracted in appreciable amounts under these conditions are gallium, germanium, arsenic^{III}, molybdenum^{VI}, tin, antimony, tellurium, iridium, gold^{III} and thallium^{III}; these are unlikely to be found in the minerals examined, and do not at present concern us.

*iso*Propyl ether has been recommended[7] for this extraction in preference to ethyl ether because of its greater efficiency over a wider range of hydrochloric acid concentration. As *iso*propyl ether also extracts considerable quantities of vanadate, molybdate and phosphate[7] in the presence of iron, it was important to know whether diethyl ether also would extract vanadate and phosphate under the conditions used for the extraction of iron. Accordingly, (*a*) a mixture of ferric chloride and sodium phosphate, (*b*) a solution of ammonium vanadate

and (*c*) a solution of quadrivalent vanadium were extracted by the procedure described on p. 98 for the extraction of iron, with the results shown in Table I.

TABLE I

ETHER EXTRACTION OF PHOSPHATE, VANADATE AND QUADRIVALENT VANADIUM FROM 6 *N* HYDROCHLORIC ACID SOLUTIONS

Radical taken	Amount taken, mg	Amount extracted, mg	Extracted, %
(*a*) $Fe^{\cdots}$	505	504·3	99·88
(*a*) $PO_4^{\prime\prime\prime}$	76	nil	nil
(*b*) $VO_3^{\prime}$	78	26	32
(*c*) $V^{\cdots\cdot}$	20	trace	—

These results indicate that the removal of iron by extraction will not interfere with a subsequent phosphate determination, and that it will only interfere to a slight extent with a vanadium determination, as experience has shown that the vanadium does not occur in the quinquivalent state at this stage of the analysis of the ore.

Dichloroethyl ether has also been shown[9] to extract iron efficiently, but its use has not been considered here.

DETERMINATION OF TITANIUM, VANADIUM, MANGANESE, CHROMIUM AND PHOSPHORUS

The aqueous solution may contain, among other ions, titanium, vanadium, manganese, chromium and phosphorus. After evaporation to remove chloride and dilution to a standard volume, aliquots are taken for the individual determinations. Manganese is estimated by the periodate method of Willard and Greathouse.[10] Phosphorus is usually absent, and rarely present in more than minor amounts. If present, it is estimated gravimetrically as ammonium phosphomolybdate by the well-known method of Finkener,[11] provided that vanadium and titanium are absent. If vanadium is present it may be co-precipitated as vanado-molybdate with the phosphomolybdate to give an orange-coloured precipitate, but if the precipitate is converted to ammonium magnesium phosphate by the well-known method of Schmitz,[12] it is freed from vanadium, as shown by the following figures—

Taken	Found
76·4 mg of P_2O_5	76·0 mg of P_2O_5
20·0 mg of V_2O_5	—

If titanium is present, it must be separated before phosphorus can be determined by pouring the solution into hot *N* sodium hydroxide solution, filtering and washing with hot dilute sodium hydroxide solution. The phosphate can then be determined in the filtrate as described above. Although chromium has so far not been detected in these ores, the possibility of its presence must be borne in mind, for if present in appreciable quantities it necessitates a modification of the procedure for the determination of vanadium and titanium.

Chromium may be detected with diphenylcarbazide[13] and, if required, oxidised and then determined with ferrous ammonium sulphate.[14] It cannot be determined colorimetrically by the diphenylcarbazide method[15] or iodometrically because vanadium interferes with both methods.

DETERMINATION OF TITANIUM AND VANADIUM (*a*) IN THE ABSENCE OF CHROMIUM—

The separation of titanium and vanadium was attempted by the precipitation of titanium dioxide (*a*) in the presence of ammonia and ammonium chloride and (*b*) in the presence of acetic acid and ammonium acetate. Titanium dioxide precipitated under these conditions is easily re-dissolved in hot dilute sulphuric acid. Vanadium, in the absence of other elements, is not precipitated, but it is co-precipitated in large amounts in the presence of titanium.

Both of these elements give strong colours with hydrogen peroxide in acid solution. Titanium gives a yellowish-brown colour that is destroyed by hydrofluoric acid and is unaffected by an excess of hydrogen peroxide; vanadium gives a reddish-brown colour that is partially suppressed by an excess of hydrogen peroxide, and hydrofluoric acid restores most of the colour that is lost in the presence of an excess of hydrogen peroxide without itself affecting the colour when it is present in excess. This provides the basis for the colorimetric determination of these elements.

The method described below can conveniently be used to determine vanadium in the range 0·1 to 2·5 mg as V_2O_5 and titanium in the range 0·1 to 2 mg as TiO_2. The results obtained with mixtures of titanium and vanadium are shown in Table II.

TABLE II

DETERMINATION OF TITANIUM AND VANADIUM IN MIXTURES

Titanium dioxide taken,* mg per 100 ml	Vanadium pentoxide taken,† mg per 100 ml	Titanium dioxide found, mg per 100 ml	Vanadium pentoxide found, mg per 100 ml
20	5	20	4·9
10	5	10	4·9
40	5	40	4·9
10	10	10	10·0

* A 5-ml aliquot was taken for each determination.
† A 20-ml aliquot was taken for each determination.

In all the minerals of this type so far examined the amount of titanium has greatly exceeded that of the vanadium, *e.g.*, a typical ore contained 3 per cent. of titanium dioxide and 0·4 per cent. of vanadium pentoxide. For these amounts the method works admirably, as the correction to be applied for vanadium in the titanium determination is comparatively small. If, however, the amount of vanadium exceeds that of the titanium, the method requires modification, as the colour produced by the vanadium alone does not differ appreciably from that given by the two elements together. To overcome this difficulty, advantage is taken of the bleaching effect of an excess of hydrogen peroxide on the colour due to vanadium. Vanadium is determined as before, and in order to determine the titanium, the colour due to the vanadium is suppressed as much as possible by greatly increasing the concentration of hydrogen peroxide. By this modification an amount of titanium can be satisfactorily determined in the presence of ten times its weight of vanadium. Results obtained are shown in Table III.

TABLE III

DETERMINATION OF TITANIUM DIOXIDE IN THE PRESENCE OF LARGE AMOUNTS OF VANADIUM PENTOXIDE

Titanium dioxide taken,* mg per 100 ml	Vanadium pentoxide taken, mg per 100 ml	Titanium dioxide found, mg per 100 ml
10	50	9·8
10	100	10·1
30	100	29·9

* A 5-ml aliquot was taken for each determination.

Care should be taken to rinse out the Nessler cylinders used for the vanadium determination as soon as possible after use, on account of the effect of hydrofluoric acid on the glass.

DETERMINATION OF TITANIUM AND VANADIUM (*b*) IN THE PRESENCE OF CHROMIUM—

If chromium is present in the original ore, it will be found in the solution after the extraction of iron as the chromic salt, as any chromate present is reduced by the strong hydrochloric acid. It will show its presence by its characteristic greenish-blue colour, or it may be detected in traces by diphenylcarbazide, after oxidation with alkaline hydrogen peroxide and re-acidification.

If chromium is present in amounts of the same order as the vanadium or titanium, the colour is insufficiently dark to interfere to an appreciable extent with the determination of these elements by the procedure described. However, if chromium is present to the order of ten times or more, the colour of the chromic ion masks the colours produced by the action of hydrogen peroxide on titanium and vanadium, and matching is impaired; chromium must therefore be removed from the solution. To achieve this, an aliquot of the solution after removal of iron is oxidised to convert the chromic salts into chromates. Attempts were

made to bring this about by boiling the solution with potassium bromate, but although this oxidised the chromium satisfactorily, it also precipitated manganese as manganese dioxide (although not quantitatively in the absence of iron), and with it appreciable amounts of vanadium, although this last is not precipitated if manganese is absent. The problem of removing the excess of bromate also proved a difficulty, so its use was abandoned in favour of alkaline hydrogen peroxide. The solution is made just alkaline with sodium hydroxide and hydrogen peroxide is added. On boiling, the chromium is oxidised, boiling being continued until effervescence ceases when the removal of excess hydrogen peroxide is complete. Titanic acid is precipitated, but it readily re-dissolves on acidification with dilute sulphuric acid.

The solution will now have the characteristic orange-red coloration of dichromate. The solution is cooled in ice and put in a separating funnel with an equal volume of ether that has also been cooled in ice. Hydrogen peroxide is added dropwise with vigorous shaking after each addition, any excess being avoided; this converts the chromate into the deep blue "perchromic acid," which is very soluble in ether and comparatively stable in ethereal solution. Provided that shaking is begun with the first addition of hydrogen peroxide, nearly all the "perchromic acid" formed can be removed from the aqueous layer before it has time to decompose into chromic salts. If one addition of a large excess of hydrogen peroxide is made, a large proportion of the chromium present is reduced to chromic salts that cannot be extracted, and the maximum extraction of chromium is not attained. The volume of hydrogen peroxide added must be noted. Titanium and vanadium are not extracted by ether under these conditions, and it has been shown, by using known weights of these two elements, that they can readily be determined in the separated aqueous layer by the procedure described, even when large amounts of chromium were originally present. The extraction of the chromium is not sufficiently complete to permit this to be made the basis of its determination, as only 90 to 95 per cent. is extracted.

Preparation of solution and removal and determination of silica

Reagents—

Hydrochloric acid, concentrated.
Potassium hydrogen sulphate.
Fusion mixture—An equimolecular mixture of sodium and potassium carbonates.
All reagents must be of analytical reagent grade.

Procedure—

Weigh 0·5 g of the finely powdered ore into a 200-ml beaker and heat it with 10 ml of hydrochloric acid for several hours on a bath of water, replacing any acid lost from time to time. Filter off any insoluble matter and wash, ignite and fuse this with potassium hydrogen sulphate, avoiding any excess. Lixiviate the melt with dilute hydrochloric acid, filter and wash any insoluble matter. Add the filtrate to the original filtrate and fuse the insoluble matter with fusion mixture. Lixiviate again, filter, and add the filtrate to the main solution. Evaporate this solution to dryness and heat at 130° C for 30 minutes. Extract the residue with water containing a few drops of hydrochloric acid and filter off any silica present; wash, ignite and weigh it as SiO_2.

If silica is present, as it is quite frequently, treat it with hydrofluoric acid and sulphuric acid and volatilise it by the usual procedure. Fuse any residue, which might contain a trace of titanium, with potassium hydrogen sulphate, and add it to the main filtrate.

Separation and determination of iron

Reagents—

Hydrochloric acid, concentrated.
Ether.
Both reagents must be of analytical reagent grade.

Procedure—

Evaporate the filtrate from the silica determination to 40 ml and add 60 ml of concentrated hydrochloric acid. Cool and extract the mixture with three successive 100-ml

portions of ether. Combine the ether extracts and pour them into a beaker containing 50 ml of water. Evaporate the ether on a bath of water and determine the iron volumetrically in the aqueous solution.

Determination of manganese, chromium, phosphorus, vanadium and titanium

Reagent—

Sulphuric acid, 50 per cent. v/v.

Preparation of solution—

To the aqueous layer after the removal of iron, add 6 ml of 50 per cent. sulphuric acid, and evaporate on a hot-plate until gentle fumes are evolved, to remove the last traces of hydrochloric acid. After cooling, dilute to exactly 100 ml, when the solution will be approximately normal with respect to sulphuric acid. Aliquots of this solution are taken for the determination of manganese, chromium, phosphorus, vanadium and titanium.

Determination of manganese—

Procedure—Take an aliquot of the solution containing not more than 1 mg of manganese and determine manganese colorimetrically after oxidation with potassium periodate.

Determination of phosphorus—

In the absence of titanium and vanadium—Take an aliquot of the solution and determine phosphorus by Finkener's method,[11] weighing it as ammonium phosphomolybdate.

In the presence of vanadium—Proceed as in the absence of titanium and vanadium, then re-dissolve the ammonium phosphomolybdate and re-precipitate the phosphorus as magnesium ammonium phosphate by the method of Schmitz.[12]

In the presence of titanium—Separate the titanium by pouring an aliquot of the solution into hot *N* sodium hydroxide, filtering and washing the precipitate with hot 0·5 *N* sodium hydroxide. Determine the phosphate in the filtrate by Finkener's method.[11]

Determination of chromium—

If chromium is present, determine it volumetrically with standard ferrous ammonium sulphate solution.

Determination of titanium and vanadium (*a*) in the absence of, or in the presence of small amounts of, chromium—

Reagents—

Hydrogen peroxide, 10-volume.

Sulphuric acid, approximately N.

Hydrofluoric acid, concentrated, analytical reagent grade.

Standard vanadium solution (1 ml ≡ 1 mg V_2O_5)—Take 0·10 g of V_2O_5, add 5 ml of concentrated H_2SO_4, heat until dissolved, cool and dilute to 100 ml with water.

Standard titanium solution (1 ml ≡ 1 mg TiO_2)—Take 0·10 g of TiO_2, fuse with $KHSO_4$, and dissolve and dilute to 100 ml with *N* H_2SO_4.

Determination of vanadium—Take an aliquot of the solution containing about 1 mg of vanadium expressed as pentoxide, V_2O_5, and add 1 ml of hydrogen peroxide. Dilute to 50 ml with *N* sulphuric acid in a Nessler cylinder, add 1 ml of hydrofluoric acid from a waxed glass tube and mix. Prepare a blank containing 1 ml of hydrogen peroxide and 1 ml of hydrofluoric acid diluted to 50 ml with *N* sulphuric acid in a Nessler cylinder. Titrate the blank with standard vanadium solution until the red colour exactly matches that in the other cylinder.

Determination of titanium—Take another aliquot of the solution containing about 1 mg of titanium expressed as dioxide, TiO_2, add 5 ml of hydrogen peroxide* and dilute to 50 ml with *N* sulphuric acid. Prepare a blank containing 5 ml of hydrogen peroxide* and add sufficient of the standard vanadium solution so that the amount of V_2O_5 added is equal to that

* If the amount of vanadium is greatly in excess of that of the titanium, add instead 40 ml of hydrogen peroxide. The hydrogen peroxide must be accurately measured.

present in the aliquot taken for the determination. Titrate the blank with standard titanium solution until the colours of the two cylinders are matched.

DETERMINATION OF TITANIUM AND VANADIUM (*b*) IN THE PRESENCE OF LARGE QUANTITIES OF CHROMIUM—

REAGENTS—

Sodium hydroxide, 2 N.
Hydrogen peroxide, 10-*volume*.
Ether—Analytical reagent grade.
Sulphuric acid, 2 N.

SEPARATION OF CHROMIUM—

Procedure—Take a 20-ml aliquot in a 250-ml beaker, make just alkaline with 2 *N* sodium hydroxide solution, add an excess of hydrogen peroxide and boil until effervescence ceases. Make just acid with 2 *N* sulphuric acid, when any precipitate should re-dissolve, and cool the solution in ice. Transfer the ice-cold solution to a separating funnel and add an equal volume of ice-cold ether. Add hydrogen peroxide dropwise, shaking the solution vigorously after each addition until no further extraction of the blue colour due to chromium occurs (should the end-point be difficult to observe, separate the ether layer, and continue separation with a further portion of ice-cold ether). Wash the aqueous layer with two 50-ml portions of ice-cold ether and reject the ether extracts. Dilute the aqueous layer to 50 ml and determine vanadium and titanium in aliquots of this solution by the procedure described above.

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WELLCOME CHEMICAL LABORATORIES
MEDICAL SERVICES, MINISTRY OF HEALTH
KHARTOUM, SUDAN

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Notes

THE DETECTION OF SERINE AND THREONINE BY THE 1:2-DINITROBENZENE - ENEDIOL REACTION

COMPOUNDS, whose molecules contain adjacent hydroxy and amino groups, such as serine and threonine, are de-aminated to enediols by alkaline hypochlorite, and these enediols yield a violet colour with 1:2-dinitrobenzene.

METHOD

REAGENTS—

1:2-*Dinitrobenzene solution, approximately* 0·02 *per cent.*—A saturated solution of analytical reagent grade 1:2-dinitrobenzene in copper-free distilled water. This solution keeps indefinitely.

Sodium hypochlorite solution, 1 *per cent.*—The solution must not be strongly alkaline, and should have its available chlorine content checked occasionally. The proprietary product known as "Milton" is a convenient form of the reagent.

PROCEDURE—

To 1 ml of a neutral solution containing at least 1 mg of the amino-acid, add 2 ml of the dinitrobenzene reagent, 5 drops of 20 per cent. sodium hydroxide solution, and 3 to 6 drops of 1 per cent. hypochlorite solution. Mix well and maintain the solution at room temperature. In a positive test a violet colour develops slowly within 10 minutes. A control containing no hypochlorite should be tested at the same time, to detect interference from preformed enediols.

RESULTS

DELICACY—

In the absence of interfering solutes, the test will detect free serine or threonine at concentrations as low as about 1 in 5000. An excess of hypochlorite must be avoided in testing dilute solutions, as it tends to bleach the violet pigment.

Serine is more reactive with hypochlorite than threonine, and gives an enediol reaction within 30 minutes in 0·1 *N* sodium hydroxide solution at room temperature. Under similar conditions, threonine gives no appreciable colour.

Sensitivity is diminished by excess of other amino-acids, amines, ammonium ions, or other compounds capable of competing for the hypochlorite. This interference can be overcome by increasing the concentrations of hypochlorite and dinitrobenzene in the mixture.

SELECTIVITY—

The test is negative with the other common amino-acids, including alanine, aspartic acid, asparagine, canavanine, citrulline, cysteine, cystine, glycine, glutamic, acid, histidine, hydroxyproline, the leucines, lysine, ornithine, phenylalanine, proline, tryptophan, tyrosine and valine. It is negative also with ammonium salts, amides, carbamides, guanidines, and aliphatic amines other than those containing adjacent hydroxy and amino groups, such as ethanolamine, in their molecules. Lactose-free caseinogen, purified by Hammarsten's method, gives no reaction, in spite of its high content of serine and threonine units. All samples of gelatin tested gave a slow positive reaction owing, presumably, to the hydroxylysine side-chains. A specimen of hydroxylysine was not available for testing.

INTERFERING SOLUTES—

The usefulness of the test is restricted somewhat because any enediols originally present react with alkaline 1:2-dinitrobenzene without addition of hypochlorite to yield similar violet pigments. Thus these enediols can be distinguished from serine and threonine. Reducing sugars commonly interfere most. Interference from them can be avoided by first titrating with Fehling's reagent and then by applying the test to the sugar-free filtrate. Interference from ascorbic acid and other highly-reactive enediols can be avoided by titrating solutions containing them with iodine in slightly acid solution, until the colour of the iodine just persists. Some native proteins give a slow direct reaction, owing to reducing sugars present, either as part of the protein molecule (albumins, globulins) or as a natural contaminant (crude caseinogen).

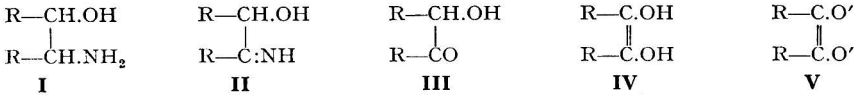
SCOPE OF THE DIRECT 1:2-DINITROBENZENE REACTION—

In solutions more alkaline than pH 8, 1:2-dinitrobenzene develops a violet colour with reducing sugars[1,2,3] or uric acid.[1,2] The colour develops slowly in the cold, rapidly on heating and tends

to become red-brown and fade if the reducing agent and alkali are in excess. The reaction, originally attributed to 1:3-dinitrobenzene, was shown to be caused by the 1:2-isomer, present as a contaminant.[3,4,5] Pure 1:3-dinitrobenzene gives no colour with reducing sugars in alkaline solution, although it forms red pigments with compounds containing reactive methylene groups, such as acetone, creatinine, hydantoins, diketopiperazines and 17-ketosteroids.[6,7,8] Reducing sugars develop a violet colour with aromatic compounds containing nitro groups in the 1:2-position.[9] In 1936, the mechanism of the reaction was elucidated by Kuhn and Weygand,[10] who isolated and identified 2-nitrophenylhydroxyamine as the primary pigment, and observed that ascorbic acid was very effective as a reducing agent. In 1943, the test was independently investigated by Fearon and Kawerau,[11] who concluded that it was selective for any compound functioning or capable of functioning as an enediol. Fearon and Kawerau regret that owing to war conditions they had not access to the paper by Kuhn and Weygand, and consequently failed to give these workers credit for the discovery of the reaction with ascorbic acid and the isolation of the pigments.[12,13] The conclusion that 1:2-dinitrobenzene is a selective reagent for enediols and related compounds has been confirmed.[14,15,16] In general, the test is given by three classes of compounds: α-hydroxyketones, or ketols, –CH(OH)–CO–; α-aminoketones, –CH(NH_2)–CO–, and monosubstituted α-aminoketones, –CH(NHR)–CO–; and hydrazine, monosubstituted hydrazines and hydroxylamine. The test is not given by β- or γ-hydroxyketones, α-hydroxycarboxylic acids, α-ketocarboxylic acids, β-aminoketones, or disubstituted α-aminoketones. Representative reacting ketols include dihydroxyacetone, glucoreductone, ascorbic acid, reducing sugars and steroids carrying an α-hydroxyketone side-chain, such as cortisone. The test also is positive with allantoin and uric acid, both of which can assume a monosubstituted α-aminoketone configuration. It is negative with adenine, guanine, hypoxanthine, hydantoin and creatinine.

MECHANISM OF THE REACTION WITH SERINE AND THREONINE—

It is suggested that the stages in the degradation of an α-hydroxyamine, **I**, by alkaline hypochlorite are: dehydrogenation to the hydroxyimine, **II**, hydrolysis to the ketol, **III**, and rearrangement to the enediol, **IV**, which undergoes ionisation, **V**, in the alkaline solution.



The reaction is promoted by 1:2-dinitrobenzene, which acts as a hydrogen-acceptor. As a result, the enediol is oxidised to the corresponding α-diketone, R–CO–CO–R, and the dinitrobenzene is reduced to 2-nitrophenylhydroxylamine, which ionises to the violet pigment. For the success of the test, it is necessary to work with a fairly low concentration of hypochlorite, so as to avoid its competition with the dinitrobenzene for the oxidisable enediol.

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DEPARTMENT OF BIOCHEMISTRY
TRINITY COLLEGE, DUBLIN

W. R. FEARON
W. A. BOGGUST

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SPECTROGRAPHIC ESTIMATION OF LEAD IN TWIG SAMPLES

AMONG other geochemical analytical techniques, arc spectroscopy of solid samples can yield informative results for a number of elements, especially for non-radioactive metals.[1]

The method now described was devised for the semi-quantitative estimation of lead in twigs from various types of trees in country where sub-outcropping lead-rich veins occur under a cover of soil. The method is sensitive and results can quickly be obtained, as sample preparation is reduced to a minimum.

In determining the elements sodium, potassium, calcium, strontium, manganese, iron and copper in plant leaves, Roach[2] produced their atomic spectra by feeding the material into an air acetylene flame. It was found, however, that by using a Hilger medium spectrograph and burning twigs containing various amounts of lead in a flame of this kind, lead was detected only when

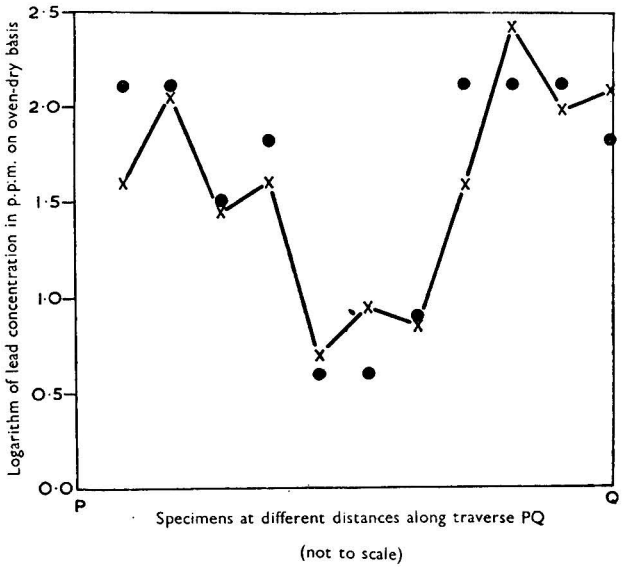

Fig. 1. Comparison of results obtained by the proposed method (dots) and those by Milton and Webb (full line)

present to the extent of several hundredths of 1 per cent. in the oven-dry material. But in the arc the excitation of lead was found to be more intense and the following simple procedure was adopted.

Two pieces of pure carbon rod, 5 mm in diameter, were used to form a vertical direct-current arc; the lower electrode was made the anode and the working gap was 14 mm with a current of 9 amperes. The image of the arc column was focussed on the slit of a Hilger large quartz spectrograph, but only the portion of the arc column 1·5 mm immediately below the cathode was photographed. This cathode-layer portion of the arc corresponded to a distance of 4·5 mm on the slit. The air-dry twig was trimmed to a standard size of about 3 cm long by 3 mm diameter, held in a pair of nickel crucible tongs and introduced into the arc. Care was taken to keep the twig near the bottom electrode (the anode) and to move it into the arc slowly and steadily.

As soon as the specimen reached the arc the shutter was opened and the first 30 seconds of the burn was recorded on a Kodak B10 plate, 2½ by 4 inches, in the region 2750 to 2950 A. Lengths of 2 cm were reduced to white ash in about 15 seconds, the exact time depending on the moisture content, and nearly all the lead had volatilised from the specimen in 30 seconds. By holding the twig and ash in the lower part of the column, radiation from the solid specimen, which would contribute substantially to background on the spectrogram, was not recorded. Twig samples of known lead content were kindly supplied by Dr. J. Webb. They had previously been analysed spectrographically (by Dr. A. Millman) by mixing the ash with an equal weight of pure carbon, loading into an electrode, made the cathode, and recording the cathode layer focussed on the slit of the large Hilger spectrograph.[3] Semi-quantitative estimations were made by visually comparing line intensities with those given by standard mixtures and the sensitivity

limit was found to be 10 p.p.m. of lead in the ash (Pb 2833·07 A), corresponding to about 0·4 p.p.m. in the oven-dry material. In the proposed method the intensities of the line Pb 2833·07 A were estimated against an arbitrary series of fixed intensities and the estimates were compared with the previously determined lead contents. After examining spectrograms from about 30 analysed specimens it was possible to relate the series of fixed intensities to the approximate lead concentrations.

The limit of detection was about 0·5 p.p.m. in the oven-dry material, or half the value taken by Webb and Millman[3] as the "background concentration" for twigs from trees in barren areas, *i.e.*, in areas not overlying mineral veins containing lead. The reproducibility for line intensities corresponding to lead contents up to 50 p.p.m. was found to be ±50 per cent., or less; the error increased somewhat at higher concentrations. In so far as the method is used to detect lead "anomalies" in geochemical prospecting, the reproducibility is adequate, as fairly large concentration differences are being sought. Fig. 1 shows a comparison of the results obtained by Webb and Millman in a traverse over two lead veins and those from the same twigs by the method now described. The whole method from trimming the twigs to the visual estimation of the lead line intensities occupied 50 minutes for the eleven air-dried specimens. The results are in fairly close agreement and the interpretation of both sets of figures is the same, *viz.*, "anomalies" occur at the extremes of the traverse, with barren ground in between.

Thanks are due to Dr. J. S. Webb of the Mining Geology Department, Royal School of Mines, for bringing the problem to the author's notice and for his valuable suggestions during its solution.

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Rothamsted Experimental Station
Harpenden, Herts.

J. R. Butler

July 3rd, 1953

THE DETERMINATION OF TIN IN CANNED FOODS

The method described by Dickinson[1] for the determination of tin in canned foods, although generally applicable, suffers from two disadvantages. First, the colour of the dithiol - tin complex must be measured in Clarke's original method[2] in reflected light with a Lovibond tintometer, so setting a limit to the practical accuracy of the measurement and requiring skilled technique. Secondly, at a concentration greater than one-tenth of that of the tin, preliminary separation of the copper is necessary. Although the copper can be separated by addition of sodium diethyldithiocarbamate and extraction with carbon tetrachloride, as originally described, this step is best avoided as extraction of tin is likely when the amount of the sodium diethyldithiocarbamate is greater than that required to combine with the copper. The element of uncertainty in the method as described in 1945 has been removed by a simple modification and the application of the findings of Williams and Whitehead[3] has permitted the use of a photo-electric instrument.

Interference by copper—

When the modification of Williams and Whitehead[3] is used, in which the intensity of the red dithiol - tin complex is measured in a photo-electric instrument by transmitted light, the interference from copper may be more serious than when the colour intensity is measured visually by reflected light. This is illustrated in Fig. 1, where the effect of added copper on a calibration graph (for which the colour was developed as described below) is shown; the interference may be interpreted as the dual effect of (*i*) turbidity increasing the absorption, particularly when the tin content tends to zero, and (*ii*) reduction in the colour intensity caused by decomposition of the dithiol by copper. The use of sodium diethyldithiocarbamate and extraction with carbon tetrachloride for removal of the copper are strictly reliable only if the quantity of reagent added is equivalent to the amount of copper present; when the reagent is in excess we find that some of the tin also may be extracted. The substitution of diethylammonium diethyldithiocarbamate in chloroform for the sodium salt in carbon tetrachloride allows the efficient separation of the copper from the solution without the loss of tin.

Method

Reagents—

Fusion mixture—A mixture of 3 parts of sodium carbonate and 1 part of potassium cyanide by weight.

Diethylammonium diethyldithiocarbamate—(*a*) Stock solution. Dissolve 3·0 ml of diethylamine in 7·0 ml of chloroform, and then 1·0 ml of carbon disulphide in 9·0 ml of chloroform. Mix

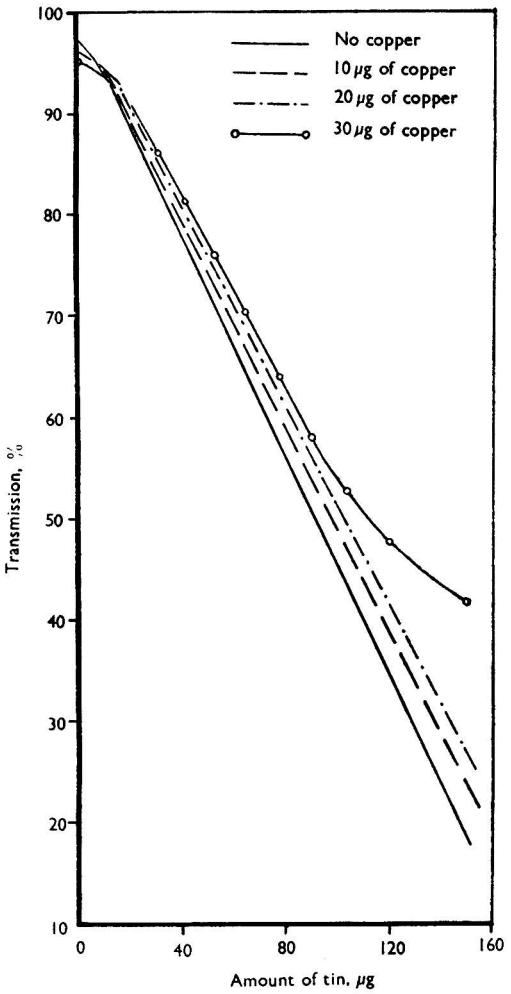

Fig. 1. Effect of added copper on light absorption of tin - dithiol solutions

these two solutions carefully, cool, and store them in a dark bottle in a refrigerator. (*b*) Dilute solution. Dilute the stock solution (1 + 20) with chloroform as required.

Diluted hydrochloric acid (1 + 1).

Teepol solution, 10 *per cent. v/v.*

Dithiol reagent—Dissolve 0·1 g of dithiol (1-methyl-3:4-dimercaptobenzene) in 2·5 ml of 5 *N* sodium hydroxide solution. Add 0·5 ml of thioglycollic acid and make up to 50 ml with water.

This reagent will not keep indefinitely. Ridett[4] says that it will keep several days in a refrigerator and Williams and Whitehead[3] consider that it is stable for 3 weeks, but they do not specify a temperature. Several lots of reagent that we tested all kept for not less than 3 days at 0° C. As the reagent deteriorates results become erratic owing to precipitation and incomplete

colour development. Some typical results illustrating the keeping properties of the reagent are shown in Table I.

TABLE I

DETERIORATION OF THREE LOTS OF DITHIOL REAGENT STORED AT 0° C

Amount of tin, μg	Amount of tin found after storage of reagent for: 0 days, μg	1 day, μg	2 days, μg	3 days, μg	5 days, μg	6 days, μg	7 days, μg	12 days, μg	14 days, μg
0	—	0	0	0	—	—	3	—	23
	0	0	0	8	—	8			
	0	0	0	—	8	—	—	15	
75	—	78	78	80	—	—	80	—	93
	75	80	80	80	—	80			
	75	77	77	—	80	—	—	80	
150	—	155	155	148	—	—	160	—	115
	152	157	158	157	—	130			
	150	155	155	—	155	—	—	150	

PROCEDURE—

Weigh 5 or 10 g of the sample, depending on the expected tin content, into a small porcelain crucible. Dry and char the sample on a hot-plate and heat to ash in a muffle furnace at about 600° C. Add 1 g of fusion mixture and fuse this with the ash by holding the crucible with nickel tongs over a bunsen or meker burner. Cool the crucible, place it in a small beaker with a lip and cover the beaker with a watch glass. Add 10 ml of water to the beaker and by means of a pipette inserted through the lip of the beaker run 10 ml of diluted hydrochloric acid (1 + 1) into the crucible. Boil the contents of the beaker gently for half an hour. Cool and filter. Wash the beaker, flask and filter-paper with water.

If copper is known to be absent or present only in negligible proportions, make the solution up to 50 ml with water in a calibrated flask and continue as described below. Otherwise transfer the solution to a small separating funnel and add 5 ml of the dilute solution of diethylammonium diethyldithiocarbamate in chloroform. Shake and run off the chloroform layer. Extract the aqueous layer with successive 1-ml portions of the reagent until the chloroform layer is colourless. Finally wash the aqueous layer with a few millilitres of chloroform. Make the aqueous layer up to 50 ml with water in a calibrated flask.

To 5 ml of the solution thus prepared add 0·5 ml of diluted hydrochloric acid (1 + 1), 8 drops of Teepol solution and 0·3 ml of dithiol reagent. Make the volume 10 ml with water and place the solution in a bath of boiling water for 2 minutes. Cool and measure the absorption of the clear red solution on the absorptiometer using a 1-cm cell and Ilford No. 604 green filter. If the absorption indicates that the aliquot contained more than 150 μg of tin a smaller amount should be taken.

ACIDITY OF THE FINAL SOLUTION—

The acid concentration is critical when Teepol is used. A certain minimum acidity is necessary in order to promote full colour development, and the amounts suggested in the procedure ensure this necessary minimum concentration of acid. However, in the presence of Teepol there is a maximum acidity above which the solution becomes turbid. Because of this, it is necessary to keep closely to the amount of fusion mixture (1 g) specified. A variation of 10 or even 25 per cent. in this is permissible, but it is advisable to weigh the quantity roughly until such time as the operator can judge by sight to within about 0·25 g.

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THE FRUIT AND VEGETABLE CANNING AND QUICK FREEZING RESEARCH ASSOCIATION
CHIPPING CAMPDEN
GLOUCESTERSHIRE

D. DICKINSON
R. HOLT

July 14th, 1953

THE MEAT CONTENT OF BRINED AND STERILISED SAUSAGES

In the preparation of Vienna sausages (Frankfurter sausages) pasteurised in brine, a lower meat content than is to be expected from the quantities of raw materials used is invariably found when it is calculated by the formula of Stubbs and More,[1] as modified by the Society of Public Analysts and Other Analytical Chemists.[2,3] We have investigated the composition of these sausages in the different stages of normal industrial production. The raw materials consisted of beef, pork and the usual other ingredients, and the same mixture was used for all our experiments. Cellophane casings were filled with this mixture and the sausages were smoked. They were then placed for some minutes in hot water and the cellophane casings were removed. The skinless sausages were packed in 3-kg cans and covered with dilute brine (3 per cent. of sodium chloride). The cans were closed, pasteurised and placed in a room at 10° to 15° C. A part of the sample was prepared as described but without pasteurisation in order to investigate the influence of the pasteurisation on the composition of the final product.

In the laboratory the weights of the sausages and of the brine before closing and after opening the cans were determined. For the chemical analysis sausages from the centre and near the walls of every can were rapidly ground together and moisture content, nitrogen and fat were determined according to Stubbs and More[1] and the S.P.A. Methods.[3] Sodium chloride was determined according to Gerritsma, van de Kamer and Willems.[4] From the analyses the results in Table I, showing the absolute quantities of water absorbed and the protein loss, were calculated.

TABLE I

ABSOLUTE QUANTITIES OF WATER ABSORBED AND THE PROTEIN LOSS

Mode of preparation of sausages	Total weight on closing, g	Absorption of moisture, g	Protein in the brine, g
Smoked, not pasteurised, 3 days in brine ..	2810	464	14
Smoked, pasteurised, 3 days in brine ..	2901	445	16
Smoked, pasteurised, 29 days in brine ..	2840	598	19

The water absorption proves to be important. The protein loss of 19 g in the 2840-g batch, containing 395 g of protein, amounts to a loss of 4·8 per cent. of the total protein. The water absorption and the protein loss have not ended within the first 3 days after pasteurisation.

Table II shows the composition of the sausages in five stages. The lean meat content of the samples is calculated with the factor 100 N/3·5.

TABLE II

COMPOSITION OF SAUSAGES IN DIFFERENT STAGES OF PRODUCTION

	Moisture, %	Nitrogen, %	Lean meat, %
Mixture	49·4	2·08	59·0
Sausages smoked and heated in water ..	44·8	2·27	64·8
Sausages smoked, heated in water, not pasteurised, packed in brine, opened after 3 days	53·1	1·95	55·7
Sausages smoked, heated in water, packed in brine, pasteurised, opened after 3 days	52·6	1·95	55·7
Sausages smoked, heated in water, packed in brine, pasteurised, opened after 29 days	55·7	1·89	54·0

The results in Table II show that the decrease of the lean meat content of sausages pasteurised in brine is caused by two factors: (*a*) during the pasteurisation and during the keeping period after pasteurisation the sausage takes up water from the brine; the final moisture content of the sausage is higher than of the mixture of the raw materials, and (*b*) during the pasteurisation, and to a slight degree during the keeping period after the pasteurisation, nitrogen-containing substances are lost by the sausage and pass into the brine.

It does not seem justifiable to impose specifications for the lean (defatted) meat content of meat products canned in brine, because it is impossible to regulate their moisture content and to prevent the loss of nitrogen into the brine. The Dutch Regulation on Meat and Meat Products has rightly excepted meat products in vinegar and in brine from its requirements (article 4, sub. 6).

Possibly a useful standard would be the obligatory declaration of the total quantity of defatted meat in grams per can.

The authors' thanks are tendered to Zwanenberg's Fabrieken N.V. at Oss, Holland, for the preparation of the samples on an industrial scale and for furnishing all the required information.

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Department of Food Chemistry and Toxicology
University of Utrecht
Catharijnesingel 60
Utrecht
The Netherlands

J. F. Reith
M. J. N. Hofsteede
August 4th, 1953

CONDUCTIMETRIC ANALYSIS AT RADIO-FREQUENCY: SUBMERGED CHOKE METHOD

The rectified radio-frequency method previously described[1] has been further developed. Instead of drawing up samples of the solution into a conductimetric tube fitted with external electrodes, a new type of conductimetric tube that is inserted into the liquid is used.

The new tube is shown in Fig. 1*a*; it consists of two glass tubes, A and B. Around the lower end of the smaller tube, A, a radio-frequency choke, K, is wound, and both are placed inside tube B. The lower ends of the tubes are sealed together. The leads from the choke are attached to metal contact plates, X and X′, on the outside of tube B

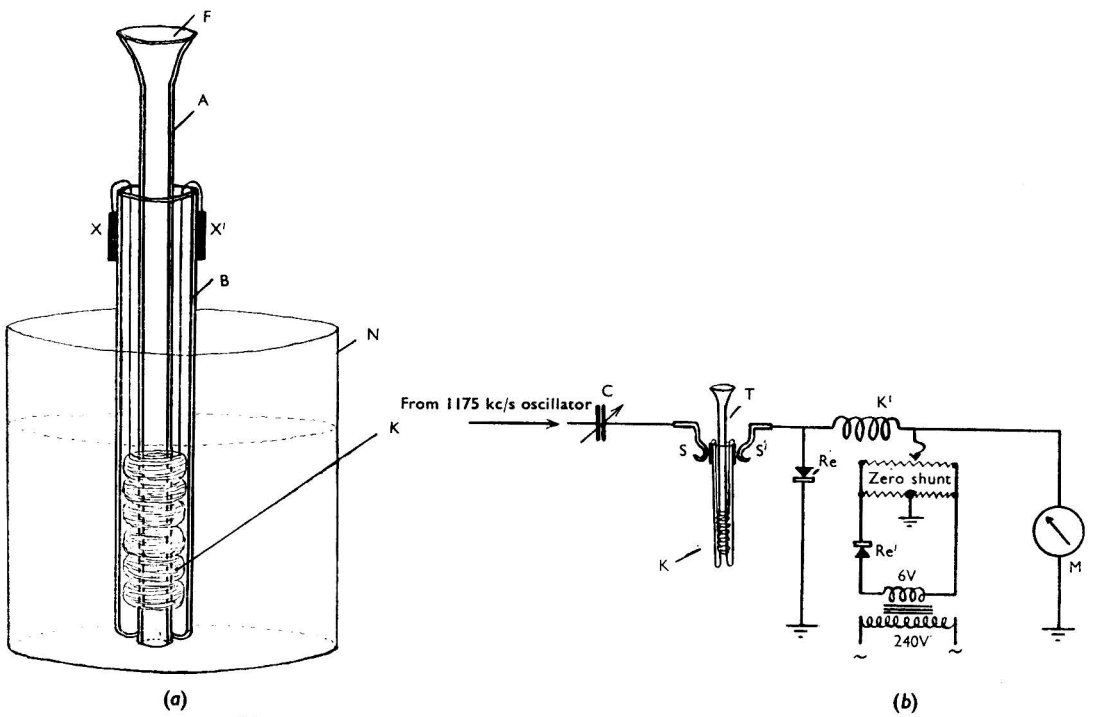

Fig. 1. (*a*) The conductimetric tube; (*b*) circuit diagram

The complete system comprises a 1175 kc/s oscillator,[2] the output from which is regulated by a variable coupling condenser, C, as shown in Fig. 2*b*. The rest of the circuit comprises a metal or other rectifier, Re, a radio-frequency choke, K′, and a zero-shunted micro-ammeter or galvanometer, M.[3] Before immersion, when the choke, K, inside the conductimetric tube, T, is connected

in series with the oscillator and the rectifier (*i.e.*, between the contact springs S and S'), the microammeter is set to show no deflection. When the tube is submerged in the solution, the deflection of the meter is proportional to the conductivity of the solution. The current is controlled by means of the coupling condenser.

A number of titrations and measurements of solution concentration have been carried out by this method. The graphs obtained compare well with those plotted by either rectified radio-frequency or alternating-current methods. Moreover, with the choke type of tube, it is possible to alter the form of the titration curve. When coupling variations are plotted against amounts of reagent the shape of the curve is dependent on the type of coupling condenser used.[4] The submerged choke method is applicable also to the continuous determination and control of solution concentration.[2]

The funnel, F, shown at the top of Fig. 1*a*, is useful when the tube is being washed out.

If only small quantities of solution are available, the container, N, can be a test tube; then, when the conductimetric tube is immersed, the solution that it displaces rises both inside and outside the choke.

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DEPARTMENT OF CHEMISTRY
UNIVERSITY OF SYDNEY
AUSTRALIA

G. G. BLAKE
July 6th, 1953

Apparatus

A MODIFIED LIQUID - LIQUID EXTRACTION APPARATUS

IN a paper on the analysis of nylon and related polymers, Clasper and Haslam describe a liquid - liquid extraction apparatus.[1] When using this apparatus we found that under some circumstances it has the following disadvantage.

The cylindrical main extraction vessel in Clasper and Haslam's apparatus is surrounded by the hot vapours of the extracting liquids rising from the flask attached to the B24 joint to the condenser attached to the B45 joint. Therefore, the temperature inside the main extraction vessel and inside the dip tube and its extension tube can be equal to the boiling temperature of the extracting liquid; the extracting liquid may boil inside these tubes and also in the space below the sintered-glass disc. When this happens the downward movement of the liquid inside the inner tubes will be prevented by the rising bubbles of vapour. Also, drops of extracting liquid travelling up through the sintered glass and through the extracted liquid in the main extraction vessel may boil and carry up some extracted liquid, which may be splashed outside the extraction vessel. These splashes will run down the outside of the vessel and collect in the flask attached to the B24 joint, thereby spoiling the results of the extraction.

When the Clasper and Haslam apparatus was used for the extraction of a solution of nylon in formic acid with light petroleum these difficulties occurred, so a modified apparatus was developed in which extraction proceeded without trouble.

The modified apparatus is shown in Fig. 1. It is a combination of a Soxhlet and the Clasper and Haslam liquid - liquid extraction apparatus. In this apparatus the vapour of the extracting liquid is prevented from travelling straight up through the main extraction vessel by the glass wall at the base of the container and by a liquid seal that closes the by-passing syphon. Instead, the vapour passes up through a side tube and is then condensed as in the original Clasper and Haslam apparatus.

The extracting liquid runs down through the B45 joint to the funnel on the top of the tube and the extraction then proceeds in exactly the same manner as in the original apparatus. In the modified version the extraction takes place at a temperature below the boiling point of the extracting liquid so that no boiling takes place inside the main extraction vessel or in the tubes.

The extracting liquid containing extracted matter overflows the main vessel and collects below it above the glass partition. Here it makes the liquid seal that prevents the entry of vapour into the main extracting vessel through the syphon. Whenever the level of the liquid reaches the higher bend of the syphon it is returned to the flask attached to the B24 joint.

The author is indebted to British Nylon Spinners Limited for permission to publish this note.

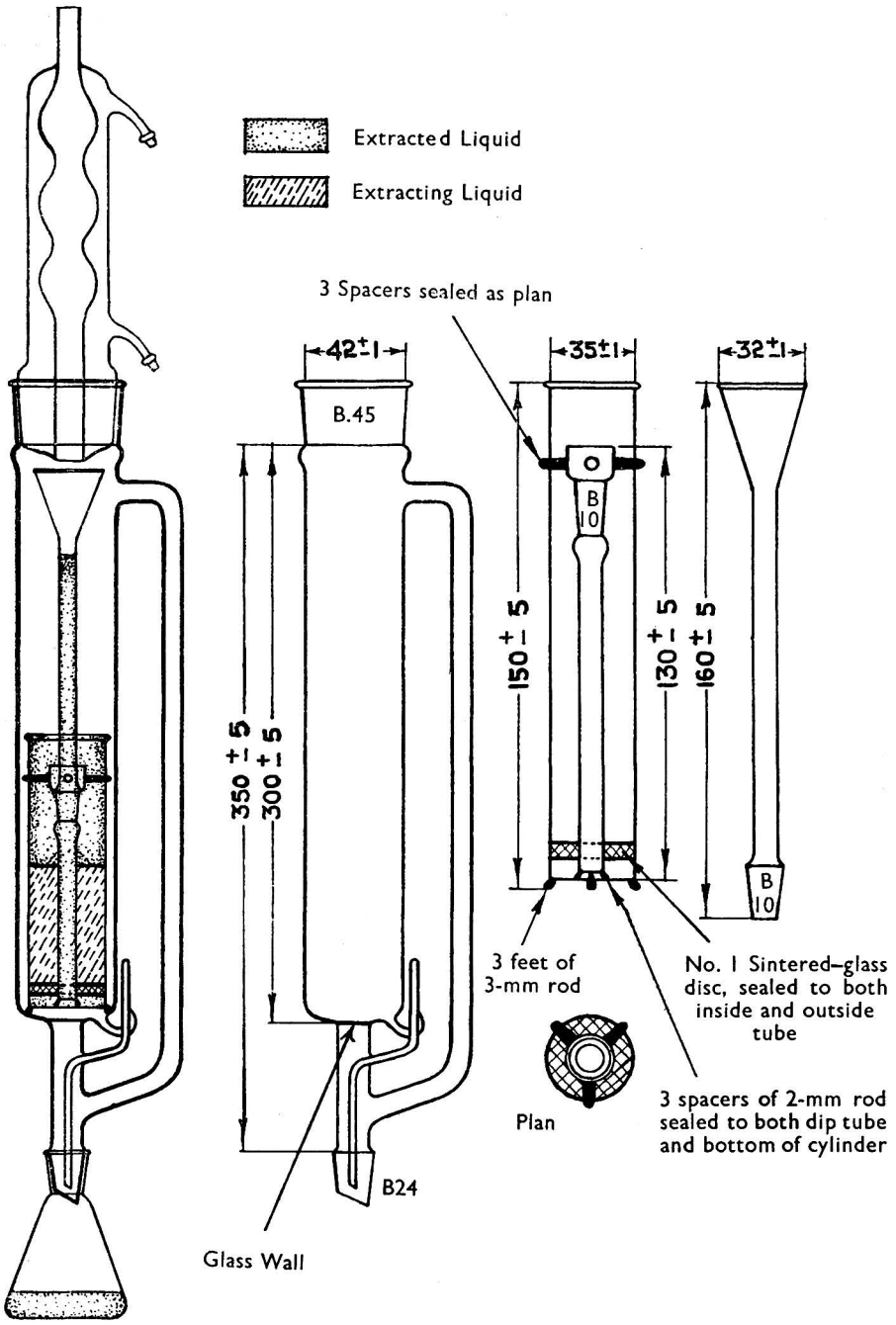

Fig. 1. Modified continuous liquid - liquid extractor. All dimensions are in millimetres

Reference

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Research Department
British Nylon Spinners Ltd.
Pontypool
Monmouthshire

K. W. Mieszkis
August 20th, 1953

Ministry of Food

STATUTORY INSTRUMENT*

1953—No. 1889. The Labelling of Food (Amendment) Order, 1953. Price 2d.

This Order, which came into operation on January 1st, 1954, amends the Labelling of Food Order, 1953 (S.I., 1953, No. 536; Analyst, 1953, 78, 324), and

(a) *permits the use of the description "Atholl Brose" for a product consisting of malt whisky, oatmeal, honey and cream and containing not less than 25 per cent. proof spirit;*

(b) *permits flour, National Flour and National Brown Flour to be sold without a declaration of ingredients in so far as they comply as regards composition with the requirements of the Flour Order, 1953 (S.I., 1953, No. 1282; Analyst, 1953, 78, 566);*

(c) *removes from Table C of the First Schedule to the principal Order specific reference to soft drinks, saccharin tablets and sweetening tablets which are now the subject of Standards Orders and therefore fall within the first item in Table C, which is a Table of pre-packed foods of which the ingredients need not be specified on the label.*

CIRCULAR MF 1/54

Approved Oxidising and Preservative Agents

This circular (price 2d.), dated January 11th, 1954, gives the name of a further product whose use for the cleansing of milk tankers, vessels or appliances has been approved by the Minister of Agriculture and Fisheries and the Minister of Food (see Analyst, 1950, 75, 504, and Analyst, 1953, 78, 326), as follows—

DOMESTOS (AGRICULTURAL GRADE).

LIST OF CURRENT STATUTORY INSTRUMENTS AND STATUTORY RULES AND ORDERS RELATING TO FOOD

This List of Current Statutory Instruments and Statutory Rules and Orders up to and including September 30th, 1953, is a guide to—

(a) *Orders made by or under the authority of the Minister of Food.*

(b) *Orders made by another Minister whose responsibilities in relation thereto have since been transferred to the Minister of Food.*

(c) *Orders made by the Minister of Food jointly with another Minister or Secretary of State; and Orders concerning food, made by the Secretary of State in relation to Scotland.*

(d) *Charges Orders relating to food, made by the Lords Commissioners of Her Majesty's Treasury.*

(e) *Orders and Charges Orders relating to matters which, although not food, are the responsibility of the Minister of Food.*

The list, Sectional List No. 33, includes the prices of the individual Orders and may be obtained from H.M. Stationery Office at cost of postage.

British Standards Institution

AMENDMENT SLIPS†

PRINTED slips bearing amendments to British Standards have been issued by the Institution, as follows—

PD 1725—Amendment No. 2 (October, 1953) to B.S. 1900:1952. Secondary Reference Thermometers (Centigrade scale).

PD 1757—Amendment No. 1 (December, 1953) to B.S. 1797:1952. Tables for use in the Calibration of Volumetric Glassware.

PD 1762—Amendment No. 1 (December, 1953) to B.S. 1992:1953. Butyl Acetylricinoleate.

PD 1763—Amendment No. 1 (December, 1953) to B.S. 1996:1953. Dimethyl Phthalate.

PD 1765—Amendment No. 1 (December, 1953) to B.S. 1792:1952. One-mark Graduated Flasks.

PD 1766—Amendment No. 1 (December, 1953) to B.S. 605:1952. Distillation Receivers (including Crow Receivers).

PD 1767—Amendment No. 1 (December, 1953) to B.S. 615:1953. Kohlrausch Flasks.

PD 1768—Amendment No. 1 (December, 1953) to B.S. 700:1952. Graduated Pipettes and One-mark Cylindrical Pipettes.

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

† Obtainable from the British Standards Institution, Sales Department, 2, Park Street, London, W.1.

Book Reviews

GENERAL BIOCHEMISTRY. By JOSEPH S. FRUTON and SOFIA SIMMONDS. Pp. xii + 940. New York: John Wiley & Sons Inc.; London: Chapman & Hall Ltd. 1953. Price $10.00; 80s.

This is possibly the largest one-volume textbook on biochemistry of the many written in the English language; it can only be reviewed by the method of random sampling, together with a checking by the reviewer of passages on those subjects about which he has, or thinks he has, some specialist knowledge. By both procedures it would appear to live up well to the authors' explicit or implicit claims that they have written a book that is modern in outlook, intelligible at University student level (for it is based on the "course in general biochemistry offered at Yale University"), comprehensive and accurate.

The authors, if one may attempt the clearly impossible task of summarising 900 pages in 50 lines, regard biochemistry as fundamentally the study of enzyme reactions, and this itself simply as an aspect of protein chemistry, physical and dynamic as well as structural and analytic. And so, somewhat unusually, Part I of their book (after a short introductory chapter of 16 pages on "The Scope and History of Biochemistry") is on proteins (160 pages) and is succeeded by Part II (36 pages) on enzymes, Part III (76 pages) on biological oxidation and next Part IV (124 pages) on intermediate metabolism of carbohydrates, including photo-synthesis. Then only do we come to the intermediate metabolism of lipids (Part V, 65 pages) and of nitrogen compounds (Part VI, 91 pages).

Part VII, the last 4 of the 38 chapters in the book, crowds into 69 pages everything else that the authors think it pertinent to write, for this stage of study, about inorganic ions, heat changes in metabolism, hormones, vitamins and growth factors.

There is nothing in this book of patently direct interest to the analyst, but any one who wants to get a clearly written survey of this vast and still growing subject, and wants also to be sure both that nothing of basic importance is omitted from it and that it is thoroughly up-to-date, would be wise to think of spending even the large sum of four pounds on this well-produced and imposing volume.

The authors are to be commended for their scrupulous treatment of "data" as a plural noun, if not always in the strict sense of "things given," though it is a pity that they so insistently use the word "regarding" or "concerning," when all they mean is "about." They are also to be congratulated especially on the width of literature from which their references are taken, British biochemists being given a larger proportion of "credit titles" than are found in some American scientific publications.

I incline to think that reference to the "biological" activity of provitamin D is an unjustifiable extrapolation of what that term is usually taken to mean. There are many physiologists on both sides of the Atlantic who would not accept the authors' apparent conclusion that Borgström's work must be taken as invalidating all, or any, of Frazer's views about the mechanism of fat absorption. On such matters as our knowledge of the vitamin-B_{12} group and of the conversion of carotenoids to vitamin A the book covers quite recent work. Thus there is mention of E. Work's discovery of $\alpha\epsilon$-diaminopimelic acid (1951), which is included in the list of naturally occurring α-amino-acids, and identification of the toxic product of "agene" as methionine sulphoxime. Some of the references are to publications as recent as 1952.

Collective bibliographies, special or general, are absent, all references being given as footnotes, and there is no author index. To be certain of any omission is thus almost impossible, but I have failed to find a reference to Hanes's synthesis of sucrose: his work on phosphorylase conversion of starch in plants to glucose 1-phosphate, however, is noted. It is a little difficult to see any reason for including on page 13, among research publications to be consulted by seekers after further knowledge, the *J. Nutrit.* and *J. Pharmacol. Exptl. Therap.* (both published in U.S.A.), while excluding *Brit. J. Nutrit.* (and especially the accompanying *Proc. Nutrit. Soc.*) and *Brit. J. Pharmacol. Chemotherapy*. Otherwise the list of journals and review publications is well-conceived, that is, comprehensive but judiciously selective.

I have been able to find few faults with this book. This may, I hope, be taken as evidence that there are few faults to be found with it. A. L. BACHARACH

APPLIED INORGANIC ANALYSIS WITH SPECIAL REFERENCE TO THE ANALYSIS OF METALS, MINERALS AND ROCKS. By W. F. HILLEBRAND and G. E. F. LUNDELL. Second Edition. Revised by G. E. F. LUNDELL, H. A. BRIGHT and J. I. HOFFMAN. Pp. xxii + 1034. New York: John Wiley & Sons Inc.; London: Chapman & Hall Ltd. 1953. Price $15.00; 120s.

When the first edition of this book appeared in 1929, discerning analysts in this country realised that a remarkable and authoritative work, destined to become a classic in the literature of analytical chemistry, had been written. The intimate knowledge of the subject and the wide practical experience of the original authors, Dr. Hillebrand and Dr. Lundell, made it inevitable that this should be so. One realised that this was no mere compilation of methods from the literature, but a critical exposition such as could come only from wide knowledge and a first-hand acquaintance with many aspects of a practical subject. In spite of these things, however, the book never became as widely known and used in this country as it deserved to be.

Twenty-four years is a long time to wait for a new edition of a work such as this, and although the revision was begun in the late thirties by Dr. Lundell, the new edition has been long overdue, owing no doubt to the ill-health that compelled him to cease work in 1948 when the revision was two-thirds done. After his death in June, 1950, the new edition was reviewed and completed by Mr. Bright and Dr. Hoffman, and it is fortunate that these two collaborators and colleagues at the National Bureau of Standards have been available and able to complete his work.

The revision is widespread; there is scarcely a page of the old edition that has not been altered, either by the omission of an out-of-date sentence or by the inclusion of a new reference or footnote, or by an extensive addition to the text itself. The chapters on niobium and tantalum, tin, and the platinum metals and gold have been entirely re-written. The chapter on the platinum metals and gold is now the work of Dr. Raleigh Gilchrist, who replaces Dr. Wichers; here the section on the attack of minerals and alloys containing these metals has been greatly extended and a new section of twelve pages gives a procedure for the systematic separation and determination of these metals in the absence of gold. A new chapter on the analytical chemistry of rhenium covers an element not dealt with in the first edition, and should prove a useful addition to the text.

There are many other additions to which attention may well be directed. Additional paragraphs on the accuracy of an analysis remind us of the differences between precision, or reproducibility, and accuracy, or correctness, a distinction too often ignored by many analysts. A new table showing the true weights of substances weighed in air against platinum - iridium and brass weights serves to inform the beginner of the need for taking into account the buoyancy effect of air in work of high accuracy. The section on glassware and porcelain has been expanded, and improved by diagrams showing the comparative resistance of chemical glassware to water, acid and alkaline reagents and buffer solutions, and there is a timely direction of the reader's attention to the surprising amount of contamination by silica that can result from the use of cover-glasses made of ordinary glass. There is a timely warning, too, that the use of electric ovens in ignitions or fusions does not necessarily eliminate contamination, much depending on the previous use to which an oven has been put (pp. 28 and 846), and some figures relating to contamination of sodium carbonate by sulphate and of alumina by boric oxide are given. The section on desiccants has been brought up to date and now includes a table showing the data reported in 1944 by Bower for most of the drying agents in modern use. Under "Common Operations," Scheerer's distillation method for separating arsenic, antimony and tin is described, and the diagram combining the pH values at which metallic hydroxides are precipitated with those obtained by various reagents is a useful new feature.

In the chapter on special operations and techniques information concerning reduction in the Jones reductor has been amplified, but, more important still, the use of the silver reductor has been described. In the reviewer's opinion this is the most elegant and possibly the best method for determining iron, and the authors might well have given a fuller treatment of the method in its application to iron ores; the work of Henry and Gelbach (*Ind. Eng. Chem., Anal. Ed.*, 1944, **16**, 49), in particular, should have been mentioned.

This chapter has been expanded by some thirty pages to include precipitations by tannin, tetraphenylarsonium chloride, *p*-hydroxyphenylarsonic acid, a mention of determinations with dithizone, and a description of electrolytic and photometric methods. As far as the physical methods of analysis are concerned, the authors have been wise enough to confine themselves to brief discussions. Polarography, flame photometry, X-ray analysis, chromatography and mass spectrometry are best dealt with by specialists: the authors recognise this and they have confined themselves to discussions showing only the usefulness and possible applications of these techniques to special problems.

It is good to see included now, *inter alia*, the phosphate method for bismuth, the α-benzoinoxime method for molybdenum, a fuller treatment of the 8-hydroxyquinoline method for aluminium, Pročke and Uzel's test for lithium, the amplification of the chapter on the alkalies, particularly as concerns lithium, rubidium and caesium, the replacement of McBride's by Fowler and Bright's method for standardising potassium permanganate, a fuller discussion of substances that interfere with the precipitation of phosphate as ammonium molybdiphosphate, and the direct precipitation of orthophosphate by magnesia mixture in the presence of a high concentration of citrate ions to prevent interference by calcium, iron and the like. The reviewer can testify that this method gives excellent results with apatites; there is a saving of time and the expensive molybdate reagent, and the values obtained by both methods are in good agreement.

In the new edition, however, there are omissions, deliberate or not, of some importance. For example, there is no mention of ammonium oxalate in the section on the removal of ammonium salts, p. 133; of N-methyldiphenylamine-*p*-sulphonic acid, the best of the diphenylamine indicators, as an oxidation - reduction indicator; of potassium iodate in the standardisation of sodium thiosulphate, in spite of the fact that it is as good as the standards mentioned and much more convenient to use; of Ball and Agruss's work (*J. Amer. Chem. Soc.*, 1930, **52**, 120) on the precipitation of zinc ammonium phosphate; of Lang's induced oxidation method (*Z. anal. Chem.*, 1935, **102**, 8) for total manganese; of Brinkmann and Schmedding's use of $MnSO_4.H_2O$ in manganese determinations (*Z. anal. Chem.*, 1938, **114**, 161); of Michel's modification of the turmeric test for boron (*Mikrochemie*, 1941, **29**, 63); of the Parnas version (*Z. anal. Chem.*, 1938, **114**, 261) of the Kjeldahl method for nitrogen; and of Pugh's method (*J. Chem. Soc.*, 1937, 1824) for chloride or bromide ions by titration with mercurous perchlorate, which, incidentally, is one of the best examples we have of the use of an adsorption indicator, namely, bromophenol blue. Personal experience of these methods shows that they are worthy of consideration by the authors for inclusion in a future revision of their book.

Deliberate omissions, made necessary by considerations of space, are the Part V of the first edition, in which the analysis of soda-lime glass, bauxite and refractories of high alumina content were dealt with, and the tables illustrating the accuracy of determinations for twenty-six elements in a wide range of materials. While accepting the need, one must regret the exclusion of the useful material, particularly the tables, which were so valuable and informative for reference.

In a new edition, and it is to be hoped that the success of the present one will soon call for one, the sequel (see H. W. Fairburn and J. F. Schairer, *Amer. Min.*, 1952, **37**, 744–57) to the co-operative investigation of precision and accuracy in the analysis of silicate rocks (*U.S. Geol. Survey Bulletin*, 1951, No. 980) might well be discussed: the matter is of first importance to all engaged in rock analysis, and the wide discrepancies revealed have disturbed chemists and mineralogists alike. Other work, too recent for inclusion in the present volume, will, no doubt, be dealt with, for example, the investigations at the Chemical Research Laboratory at Teddington on the analysis of uranium and thorium and the fluoride separation of niobium and tantalum on cellulose columns. An extended treatment of the fritting or sintering techniques to include fluxes containing borax would also be welcome.

The text of the present edition is commendably free from typographical errors, as, indeed, in a second edition it should be, the spelling mistakes in the first edition, and there was quite a number of them, having now been corrected. A few, however, have crept in, as is almost inevitable in a work of over a thousand pages. Phenylhydrazine is wrongly spelt on p. 88, "mass" in the footnote to p. 183 should be "brass," the "former" on p. 448 should be the "first-mentioned," "chloride" on p. 734, line 27, should be "iodide," and Mann-Smith on p. 105 and in the index should read Main-Smith. A bad sentence on p. 974 remains, but the jumble of words in lines 16 to 19 on p. 590 of the first edition has now been straightened out to make sense; "lead carbonate precipitated by ammonium carbonate and basic bismuth nitrate, . . ." p. 837, shows the need for and the importance of the humble comma. The former mistake that stannite is SnS_2 has not been repeated; neither has the statement that niobium, "like tantalum, does not give a colour with hydrogen peroxide." Indeed, in the re-written chapter on the earth acids the colorimetric method for niobium, based on the yellow colour that this element gives with hydrogen peroxide in the presence of sulphuric acid, is described in detail. Schoeller's valid criticism (*Analyst*, 1930, **55**, 353) of treating a solution with hydrogen sulphide before determining sulphate is met by a statement that "this procedure would appear to be unreliable because of the possibility of oxidising sulphide ions." The observations that "the separation of mercury from cadmium is based on the insolubility of the sulphide of the former in nitric acid" (p. 256) and this "separation fails if the sulphide [of mercury] was thrown down in a solution containing copper, cadmium, or zinc" (p. 214) have still

to be reconciled, and the description of Dittrich and Freund's salicylate method given on p. 579 is still at variance with the summary of the method given on p. 92 (*cf.* Schoeller, *loc. cit.*). Ferrous ammonium sulphate is, very properly, rejected as a reference substance for the standardisation of potassium permanganate solutions on the basis of its impurity and instability during storage (p. 189), and this being so it is inconsistent to recommend it (p. 400) for standardising titanous sulphate solutions for iron titrations.

The separation of bismuth from copper by the ammonium carbonate method is not as easy as the method given on p. 235 would lead one to suppose, neither is that of chromate from iron by precipitation of the hydrated iron oxide by aqueous ammonia (p. 526). The statement that "iron is quantitatively precipitated by oxalate in ammoniacal solution," p. 619, footnote 23, needs to be reconciled with that on p. 641, footnote 22, where "iron by itself is not precipitated at all by diammonium phosphate in an ammoniacal solution containing ammonium oxalate."

These criticisms are not meant to condemn the book; far from it. The book is so good that one offers criticism only that it may prove helpful and constructive. In the reviewer's opinion, the work is the best of its kind. It is one to be used both in the library *and* at the bench, and it is indispensable to every chemist engaged in "Applied Inorganic Analysis." L. S. THEOBALD

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.

During recent years waste materials of all kinds have been considered for utilisation as a source of chemicals, and from vegetable residues furfural and other furans have become of importance. The parent compound furan, C_4H_4O, is a cyclic, dienic ether stabilised by resonance. It exhibits properties generally associated with vinyl ethers, conjugated dienes and aromatic compounds. It forms derivatives comparable with those of benzene, but in general they are more reactive, substitute more easily and take part in a wider variety of addition reactions. The furan nucleus is more susceptible to cleavage than is that of benzene, and special precautions may have to be taken to retain the identity of the ring; but certain substituted types are notably stable.

Furfural is the most important furan derivative being extensively used as a solvent and in synthetic organic chemistry, particularly for the production of resins, plastics, pharmaceutical products and pesticides.

This is the first comprehensive book devoted solely to the furans and about 700 pages are required to cover their chemistry. A further 100 pages deal with industrial applications of furfural and its derivatives, and there is an Appendix detailing furan resin patents. It should prove of great use to those who use any of the furans or wish to investigate their potentialities.

The analytical chemistry of these compounds is not far advanced, although Angell (*Analyst*, 1947, **72**, 178) has indicated general reactions that may be applied. Many methods for the determination of furfural have been proposed, but none is of general application. They depend either on reactions of the aldehyde group, subject to interference from other carbonyl compounds, or on characteristics of the furan ring, exhibited by other unsaturated compounds. There is no satisfactory method for determining the true furfural content of the commercially pure substance; the figure for this has to be obtained by difference after determination of known impurities. Information is given on methods based on bromine absorption, precipitation, absorption spectrography and colorimetry; analysts will find these useful in particular instances. The classical method of determining pentosans in feeding stuffs or foods, by precipitating with phloroglucinol the furfural obtained from them, is adequate.

Furfural has the ability to dissolve aromatic and other unsaturated compounds selectively in the presence of aliphatic or other saturated substances. This property is used commercially for purifying resinous and oily materials by counter-current extraction or extractive distillation. It might also be useful in the preliminary separation and cleaning of such materials before analysis and for this the comprehensive physical properties listed should be most useful. J. R. NICHOLLS

ORGANIC SYNTHESES. An Annual Publication of Satisfactory Methods for the Preparation of Organic Chemicals. Volume 32. Editor-in-Chief: R. T. ARNOLD. Pp. vi + 119. New York: John Wiley & Sons Inc.; London: Chapman & Hall Ltd. 1952. Price $3.50; 28s.

Perhaps the most noticeable feature of this latest volume is that four of the items—abietic acid, *tert.*-butyl hypochlorite, *cyclo*hexene sulphide and *cyclo*pentadiene—are not permanent under normal conditions of storage. The cyano-group appears in diphenylsuccinonitrile, 2-ethylhexanonitrile, 3-cyano-6-methyl-2-pyridone, 1:1′-dicyanodi*cyclo*hexyl and its two precursors, as well as in cyanogen iodide. Methyl esters present are the acetylenedicarboxylate, *p*-acetylbenzoate and γ-methyl-γ-nitrovalerate, ethyl esters the *cyclo*pent-2-enyl- and ethylidene-malonates, orthocarbonate and *iso*dehydracetate; there is also *p*-chlorophenyl salicylate. Acids are represented by ε-aminohexanoic, thiobenzoic and 10-undecynoic acids, amides by alloxan, *asym.*-dimethylurea and phenylacetamide, imides by β-bromoethylphthalimide, lactones by 4:6-dimethylcoumalin and lactams by 5:5-dimethyl-2-pyrrolidone. Three ketones—2-aminobenzophenone, *iso*amyl β-chlorovinyl ketone and β-tetralone—are described and two alcohols—2:2-dichloroethanol and di-(1-hydroxy*cyclo*hexyl)acetylene. Of the acetals—β-oxo*iso*octaldehyde dimethyl acetal and acraldehyde acetal—the latter has appeared in earlier volumes of the series. Other items included are 2-chloro-1:3-dinitrobenzene, chloro-*tert.*-butylbenzene and naphthalene-1:5-disulphonyl chloride, 1:2-*cyclo*hexanedione dioxime and pyruvaldehyde 1-phenylhydrazone, 2:4-diamino-6-hydroxypyrimidine and flavone and the sodium derivative of nitromalonaldehyde.

As is customary, some of the monographs include intermediate stages and some refer to analgous compounds for which the particular methods can be used. B. A. ELLIS

Publications Received

CHEMICAL METHODS IN INDUSTRIAL HYGIENE. By F. H. GOLDMAN and M. B. JACOBS. Pp. x + 274. New York and London: Interscience Publishers Inc. 1953. Price $3.75; 27s.

L'ANALYSE CHIMIQUE. By CLÉMENT DUVAL. Pp. 128. Paris: Presses Universitaires de France. 1953.

PRACTICAL PHARMACOGNOSY. By T. E. WALLIS, D.Sc., F.R.I.C., Ph.C., F.L.S. Sixth Edition. Pp. x + 238. London: J. & A. Churchill Ltd. 1953. Price 18s.

ORGANISCHE FÄLLUNGSMITTEL IN DER QUANTITATIVEN ANALYSE. By Dr. WILHELM PRODINGER. Third Edition. Pp. xvi + 232. Stuttgart: Ferdinand Enke. 1954. Price (Paper) DM 32; (Cloth boards) DM 34.

BRITISH VETERINARY CODEX 1953. Published by direction of the Council of the Pharmaceutical Society of Great Britain. Pp. xxiv + 737. London: The Pharmaceutical Press. 1953. Price 45s.

SULPHURIC ACID AND THE MANUFACTURE OF PHOSPHATIC FERTILISERS. Pp. 77. Paris: The Organisation for European Economic Co-operation. 1953. Price 450 fr.; 8s. 6d.

EXPERIMENTAL INORGANIC CHEMISTRY. A Guide to Laboratory Practice. By R. E. DODD and P. L. ROBINSON. Pp. xii + 424. Amsterdam and New York: Elsevier Publishing Co.; London: Cleaver-Hume Press Ltd. 1954. Price 42s.

PORTRAITS OF PAST PRESIDENTS

THE custom of supplying Portraits of Past Presidents to members of the Society and subscribers to *The Analyst* has been restored in a modified form. It is no longer possible to supply these photogravure reproductions with all copies of *The Analyst*, as was done before the war, but a sufficient number will be printed to supply gratis copies to all who make application to the Editor, *The Analyst*, 7 and 8, Idol Lane, London, E.C.3.

Two portraits will be available shortly, those of Dr. G. Roche Lynch (President, 1936–37) and Dr. G. W. Monier-Williams (President: 1945–46). A portrait of Dr. E. B. Hughes (President: 1940–42), who is the Society's Bernard Dyer Memorial Lecturer this year, is in preparation.

Orders for any of these three portraits should be sent in before April 30th, 1954.

HER MAJESTY'S COLONIAL SERVICE

A VACANCY exists for a Chemist in the Geological Survey Department, Federation of Malaya. Candidates, under 35, must possess as a minimum qualification an Honours degree in Chemistry from a British University. Duties involve the analysis of minerals, rocks and metallurgical alloys.

Appointment is on probation to the pensionable establishment. The consolidated salary scale (basic salary plus expatriation allowance) is £820–£2044 per annum approximately. Candidates who possess experience in the analysis of rocks, minerals and metallurgical alloys would be allowed to enter the scale at a point higher than £820 per annum, dependent upon length of experience. A variable cost of living allowance, according to family commitments, is also payable.

Quarters, when available, at a rental of between £21 and £84 per annum; free passages for officer and wife and up to three children under the age of 10; free medical attendance for officer and family whilst in Colony; vacation leave—four days for each completed month of resident service.

Apply, in writing, to the Director of Recruitment, Colonial Office, Great Smith Street, London, S.W.1, giving briefly age, qualifications and experience. Mention the reference number CDE 105/60/02.

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. Write Box No. 3855, THE ANALYST, 47, Gresham Street, London, E.C.2.

ASSISTANT, male or female, with experience microbiological assays, required for laboratory in North. Progressive post in pleasant surroundings. Write Box No. 3856, THE ANALYST, 47, Gresham Street, London, E.C.2.

ANALYST required for Materials Research Laboratory. Wide range of work; experience of analysis of ferrous and non-ferrous metals an advantage. A.R.I.C. or equivalent. Salary according to experience. Apply, quoting references, age and salary required to S. Smith & Sons (England) Ltd., Bishops Cleeve, Cheltenham. Reference MAT. 1.

LARGE INDUSTRIAL UNDERTAKING in the West of England requires, for its expanding Research Department, physical and organic chemists with an interest in plant products. Candidates (within age group 23–30) should have good Honours degree and preferably some research experience. Salary according to experience and qualifications. Apply, giving full particulars, to Box No. T.3776, Haddon's, Salisbury Square, Fleet Street, London, E.C.4.

HER MAJESTY'S COLONIAL SERVICE

A VACANCY exists for a Chemist in the Federation of Malaya. Appointment is on probation to the pensionable establishment with consolidated salary according to experience in the scale £1005–£2044 per annum. A variable cost of living allowance, according to family commitment, is also payable.

Income tax at low local rates. Quarters, when available, at a rental of between £21 and £84 per annum. Free passages for officer, wife and up to three children under the age of 10. Leave of four days for each completed month of resident service.

Candidates (preferably 25–27 years) must possess an Honours degree in Chemistry from a British University, together with the Associateship or Fellowship of the R.I.C., plus at least two years' specialised experience in some line (forensic science, toxicology; foods, drugs and water; bacteriology; pharmacy, etc.) relevant to work in Malaya, or research experience indicated by a Ph.D. degree or comparable postgraduate qualification. Duties include the analysis of liquors, toddy, foods, drugs, waters, sewage and petroleum; bacteriological examination of waters and foods; toxicological investigations; blood testing and clinical analyses; examination of exhibits related to poisons, dangerous drugs, bloodstains, counterfeiting, arson, forgery, firearms, bullets, etc.; inspection of petroleum-carrying vessels and of explosives.

Apply, in writing, to the Director of Recruitment, Colonial Office, Great Smith Street, London, S.W.1, giving briefly age, qualifications and experience. Mention the reference number CDE 97/60/01.

QUALIFIED ANALYST (A.R.I.C. or B.Sc.) required for analytical and quality control laboratory near Tadworth, Surrey. Some experience of pharmaceutical and feeding stuffs analysis would be advantage. Write quoting reference A.1. to Box No. 3857, THE ANALYST, 47, Gresham Street, London, E.C.2.

GLAXO LABORATORIES (INDIA) LTD. require a SENIOR ANALYST to take charge of the Analytical Department of their Bombay factory. The post involves the supervision of a considerable number of qualified Indian Chemists and responsibility for analytical and bacteriological control of a wide range of food products, pharmaceuticals and antibiotics. F.R.I.C. Branch "E" desirable, together with previous experience preferably in the control laboratory of an industrial organisation. For a suitably qualified and experienced candidate the commencing salary will be in the rupee equivalent of around £1650 together with generous cost of living and accommodation allowances. A car is provided. Six months paid home leave after each three years' contract. Apply, in writing, to Senior Personnel Officer, Glaxo Laboratories Ltd., Greenford, Middlesex.

ASSISTANT CHEMIST. International Manufacturing Company engaged on a chemical and mechanical process requires an Assistant Chemist who should be capable of progressing up to the position of Works Chemist. Candidates should be up to Inter.B.Sc. or equivalent standard, and have experience of inorganic analysis and quality control methods. The appointment is permanent and pensionable. Apply, with fullest particulars, including age, salary required, previous experience, to the General Manager, Alpha Accessories Ltd., Cae Mawr Factory, Treorchy, Glamorgan, South Wales.

CHEMISTS REQUIRED for control of raw materials and finished products in connection with industrial alcohol and its by-products. Age up to 26 years. Applicants should have degree in Chemistry or equivalent qualification. Salary would depend on qualifications and experience. Apply: Staff Dept., The Distillers Co., Ltd., 21, St. James's Square, London, S.W.1.

QUALIFIED CHEMIST (male) required in General Analytical Laboratory. Age 21–30. Previous experience of food industry desirable, but not essential. Excellent prospects. Pension scheme. Apply to the Chief Chemist, Allied Bakeries Research Laboratories Ltd., Kemp Road, Dagenham, Essex.

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