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Incidental information

No. 13

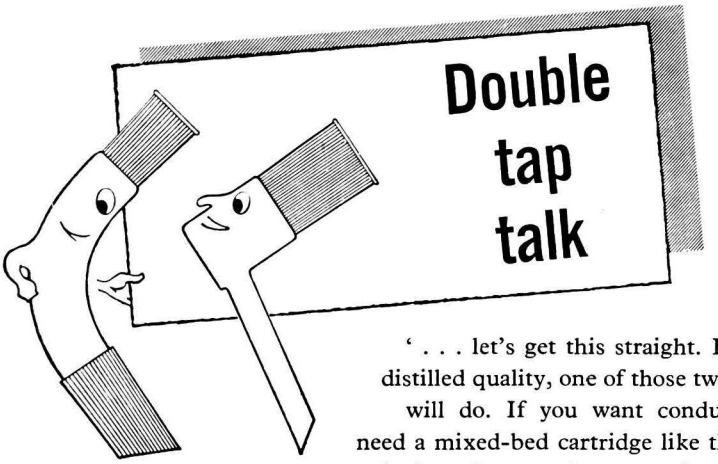
Items of interest from our laboratory notebooks

▶ Most analysts know about 1:10-phenanthroline and many use it for iron determinations. Not so many people seem to know that **4:7-diphenyl-1:10-phenanthroline** is twice as sensitive as 1:10-phenanthroline in the colorimetric determination of iron. There are several papers on the subject but the latest is *Analyst*, 1958, **83**, 80. The reagent is also called **Bathophenanthroline**, and we make it.

▶ Then, again the substitution of methyl groups in the 2:9 positions has the interesting effect of making the reagent insensitive to iron and we then have a selective and sensitive reagent for copper (see *Anal. Chem.*, 1956, **28**, 1158). Hopkin & Williams make **2:9-dimethyl-1:10-phenanthroline** (sometimes called **Neocuproin**).

▶ One does not think of sulphate as a radical one can determine absorptiometrically, but this is now possible for low concentrations. **Barium chloranilate** is the reagent and there are two papers on the subject—*Anal. Chem.*, 1957, **29**, 281 and *Anal. Chem.*, 1958, **30**, 202. Hopkin & Williams make it.

▶ Hopkin & Williams Ltd. were also early off the mark with supplies of the remarkable new colour-producing reagent for fluoride ions, **3-aminomethylalizarin-NN-diacetic acid**, described by Belcher, Leonard and West (*Talanta*, 1959, **2**, 92.) This important reagent is already available from stock.

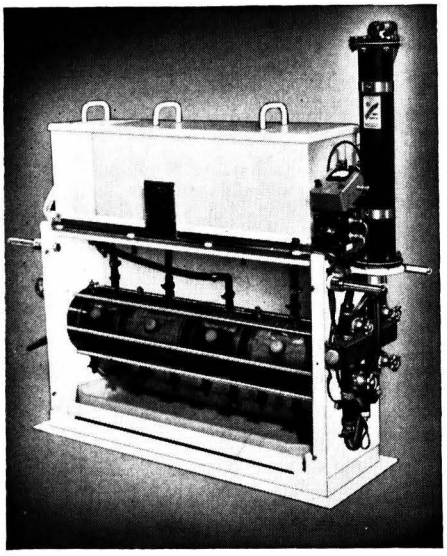


'. . . let's get this straight. If you want water of distilled quality, one of those twin-bed arrangements will do. If you want conductivity quality, you need a mixed-bed cartridge like the "Loughborough" deioniser. But not *both* ways.'

'But there, my dear sir, is where you are wrong! *You can have "distilled" or "conductivity" water—up to 30 gallons an hour of it—from Loughborough's new Stonwata A.300 deioniser.*'

'Now, just a minute! "distilled" or "conductivity" water out of the same tap? That's stretching it a bit far, isn't it?'

'Not a bit. The A.300 is really two units in one. The multi-bed unit produces "distilled" water. You can either use it as it is, or pass it through the mixed-bed cartridge to step it up to "conductivity" level—all at the turn of a tap.'



'Mm! Sounds feasible—but I shudder to think of the regeneration problems.'

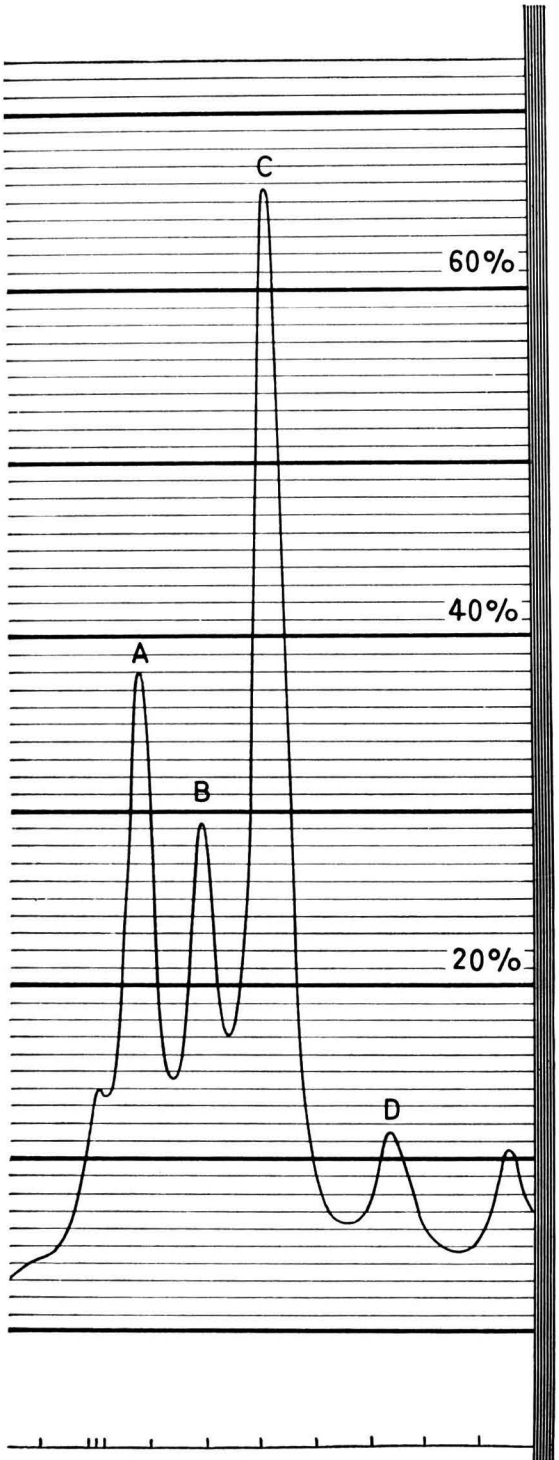
'Not with this one. The clever way the multi-bed unit's been designed makes that very simple—you just fill up the built-in tanks with regenerants, open the valves, and in an hour it is *ready to yield another 300 to 2,000 gallons*, according to your water supply.'

'And the cartridge . . . ?'

'Oh! That goes back to Loughborough, but since it has "distilled" water fed to it, you only need to do this every 1,000 gallons or so.'

'*300-2,000 gallons, eh?* I'm going to find out more about this.'

'Well, you only have to drop a line to . . .



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Quantitative Infrared Intensity Measurements to 0·1% accuracy!

An account has appeared of a procedure* by which the Unicam SP.100 Infrared Spectrometer (standard single prism monochromator) can be adjusted to give routine intensity measurements to an accuracy of 0.1 % transmission. The description shows that this accuracy is maintained to at least 2 per cent transmission levels.

Reliable results to this degree of accuracy are particularly important to the analytical chemist concerned with precise quantitative measurements. The exceptional photometric accuracy of the Unicam SP.100 makes possible this high performance over the whole range of infrared work. With the SP.130 Grating accessory, accurate intensity measurements at high resolution become a routine procedure.

* *Spectrovision No. 6*

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Absorption peaks:

A:	γ- PICOLINE	(792 cm^{-1})
B:	β- PICOLINE	(781 cm^{-1})
C:	2,6- LUTIDINE	(768 cm^{-1})
D:	α- PICOLINE	(746 cm^{-1})

This spectrum, taken in a 0.28 m.m. cell with cyclohexane solvent using single monochromator (rocksalt optics), is typical of those being used regularly for high-accuracy intensity measurements in the analytical laboratories of The Midland Tar Distillers Ltd. (to whom grateful acknowledgement is made).

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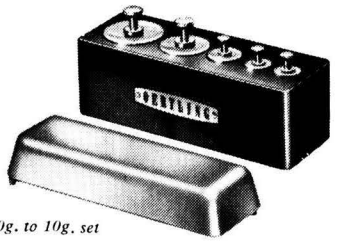

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THE ANALYST

PROCEEDINGS OF THE SOCIETY FOR ANALYTICAL CHEMISTRY

DEATHS

WE record with regret the deaths of

Richard Selwyn Haskew
Samuel Judd Lewis
Thomas Whittaker Lovett
Ernest Pedley
Clarence Arthur Seyler.

Obituary

FREDERICK LEIGH OKELL

FREDERICK LEIGH OKELL was born at Mollington near Chester in 1882. He received his early education at Arnold House School and Kings School, both in the City near his home, and in 1900 he was studying at the Chester School of Science and Art. In the autumn of this same year he became a pupil in the laboratory of William Ffoulkes Lowe, Public Analyst for the City of Chester and most of the counties of North Wales. Three years later he moved to London, where he attended King's College under Professor J. M. Thomson and qualified as Associate of the Institute of Chemistry in 1906. Taking up an appointment as assistant to C. C. Duncan at Worcester County Chemical Laboratory, he acquired valuable experience in applied analytical chemistry. After three years at Worcester he was advised by Sir Herbert Jackson to accept an appointment with the Straits Trading Company in Singapore and he remained with this great firm of tin smelters for nearly twenty-five years, becoming in due course their chief chemist. In 1910 he was elected a Fellow of the Institute of Chemistry. In 1915, when home on leave, rather than rest while his country was at war, he worked at King's College, London, assisting Sir Herbert Jackson on research connected with the glass used in miners' lamps. After his final return to England in 1932 he engaged in private consulting practice.

Soon after establishing his own laboratory in Edgware, Okell, in collaboration with John Lumsden, engaged in an investigation on the iodimetric titration of tin and demonstrated that the procedure, as it was then conducted, was subject to a variable and considerable error due to the dissolved oxygen normally present in the standard iodine solution. It was shown that by displacing air with carbon dioxide the errors were eliminated. This valuable paper was read at the meeting of the Society held on October 2nd, 1935, and in due course published in *The Analyst* (1935, **60**, 803–811).

Later, during the war years, more keen than ever to be fully occupied, he served as assistant to Miss M. H. Pearson, at that time Public Analyst to the County of Middlesex, this work lasting until the war ended. For the majority of men this would have been enough to complete a life's work, but the most distinguished phase of Okell's career was yet to come. Over the years he had acquired a reputation for the number and excellence of his book reviews published in the Society's Journal, and in 1947 he was appointed Assistant Editor of *The Analyst*, working under the late J. H. Lane, who had been appointed Editor in 1945. Lane died in March 1951, and Okell became Editor and ably conducted the duties of this important post for three years. By this time the Council of the Society were concerned to place the appointment in the hands of a younger man, while being equally anxious to have the advantage of Okell's editorial experience and encyclopaedic knowledge of analytical chemistry. Thus it was that in 1954 Mr. J. B. Attrill, who had been Assistant Editor, was appointed Editor, Okell being retained as Advisory Editor, in which position he remained

extremely active, attending on alternate days at the office and always officiating at the monthly meetings of the Publication Committee, until a few weeks before he died on May 9th, 1959, at the age of nearly 77 years. He is survived by his wife May, daughter of Henry Tomkins of Stockton-on-Tees, whom he married in 1914, and their daughter Eleanor. Their son John served in the R.A.F. as a Pilot Officer and was killed in action in August, 1941.

Professional acquaintances knew F. L. Okell not only as a first-class analyst, but also as a genial companion with a fine sense of humour and a wide literary knowledge. Those few who enjoyed his closer friendship also knew of his unimpeachable integrity, his generosity and readiness to help others regardless of the trouble it might entail. *Ave atque vale.*

NOEL L. ALLPORT

A Collaborative Test of Moore and Stein's Resin-chromatographic Method for Determining Amino Acids

BY A. E. BENDER, J. A. PALGRAVE AND B. H. DOELL

(*Research Department, Bovril Ltd.*, 148 *Old Street, London, E.C.*1)

A collaborative assay of a mixture of amino acids by Moore and Stein's resin-chromatographic technique was carried out in ten laboratories. The object was to assess the accuracy of the method as operated in different laboratories.

The results show a high degree of consistency and agreement between the different laboratories. Nine amino acids, glycine, phenylalanine, lysine, valine, serine, threonine, aspartic acid, glutamic acid and tyrosine, were determined with a mean recovery of 100 ± 2 per cent.; the remaining seven, alanine, leucine, *iso*leucine, arginine, histidine, proline and methionine, were determined with an error of ±8 per cent. The cystine in the mixture deteriorated. A second test showed that the determination of this amino acid is still not satisfactory, but that a correction factor can be applied with good results.

The experience of the collaborating laboratories emphasised that the accuracy of the procedure depends upon close attention to the many points of detail and that considerable improvements in recovery of amino acids followed improvements in procedure.

FOR many years, investigation of the chemical and nutritional properties of proteins was hindered by inadequate methods of analysis. The introduction of microbiological and, later, chromatographic techniques was a major advance in this field.

Three reports in particular stimulated us to organise a collaborative test of the chromatographic method. The report of the Rutgers Bureau collaborative test[1] indicated that microbiological methods of determining amino acids were not always reliable. The method is said to be accurate to within ±10 per cent. or better,[2] but in a collaborative test, many of the pure amino acids in a mixture were determined with a much lower accuracy. Moreover, although the absolute deviation of the results in one laboratory was only 0·2 per cent. for twelve amino acids, it was as much as 14·1 per cent. in another laboratory.

The second report was that of Wootton and King,[3] who showed that even relatively simple chemical determinations by different analysts can give startlingly different results. In thirty-six laboratories, a prepared solution was analysed for nine commonly determined constituents. The results ranged from 40 to 140 per cent. of the correct figure for urea, 60 to 180 per cent. for phosphate and 60 to 238 per cent. for creatinine.

The third report, by Beck and Sunderman,[4] showed values ranging between 50 and 950 per cent. of the true value for glucose, between 77 and 170 per cent. for sodium chloride and between 22 and 1040 per cent. for urea.

In investigations of the relationship between the chemical composition of proteins and their nutritive values,[5,6] the biological determination usually has a known variability, but the results for amino acids are of unknown reliability. Tables of the amino acid composition of foods[7,8] show a wide range of values for what are nominally the same proteins. It is not

known whether these differences are caused by the different compositions of the samples analysed or by errors in the method used. An error of even 10 per cent. in the determination of an amino acid can change the apparently limiting amino acid in a protein. For this reason, it is desirable to be able to assess the accuracy of published results for amino acid analyses.

Moore and Stein claimed an accuracy to within ± 3 per cent.[9] for their resin-chromatographic method. Several workers have achieved results in this range by using the method, but there is little information on inter-laboratory variation.

The following workers collaborated with us in the test described in this paper. J. H. Bowes and R. G. H. Elliott, British Leather Manufacturerers' Research Association, Milton Park, Egham, Surrey; D. C. Cusworth, Heather G. Randall and R. G. Westall, University College Hospital Medical School, University Street, London, W.C.1; G. M. Ellinger and E. B. Boyne, Rowett Research Institute, Bucksburn, Aberdeen; A. A. Leach, British Gelatine and Glue Research Association, 2a Dalmeny Avenue, Holloway, London, N.7; F. J. Ley and D. S. Bidmead, Unilever Ltd., Colworth House, Sharnbrook, Bedford; I. L. S. Mitchell and E. Manfield, Glaxo Laboratories Ltd., North Lonsdale Road, Ulverston, Lancs.; M. W. Rees, Department of Biochemistry, The University, Cambridge; A. Robson and B. Skinner, Wool Industries Research Association, Torridon, Headingley, Leeds 6; and E. W. Yemm and A. P. Sims, Department of Botany, The University, Bristol 8.

First test

A solution of amino acids was prepared in our laboratories and samples were despatched in 50-ml polythene containers to the collaborating laboratories. The solution contained 7·22 mg of amino acids (0·91 mg of nitrogen) per ml, as shown in Table I; tryptophan was not included. The solvent was 0·1 *M* citrate buffer, pH 2·5, preserved by thymol. The solution was stored at 0° C except when in transit to the collaborating laboratories.

The amounts of amino acids in the solution approximated to those in milk in order to achieve a ratio similar to that found in natural proteins, *e.g.*, a high concentration of glutamic acid was present and only a small amount of cystine.

Purity of amino acids—

In attempting to assess the accuracy of the method, the purity of the amino acids used is of great importance. At the time we were not aware of any source of amino acids of guaranteed purity, and samples commercially available in this country were used.

All the amino acids except cystine and tyrosine were recrystallised from water and then from 95 per cent. ethanol. Cystine was twice recrystallised by dissolving it in hydrochloric acid, filtering the solution and precipitating the cystine with a saturated solution of sodium acetate. Tyrosine was twice recrystallised by dissolving it in ammonia and precipitating from the filtered solution with hydrochloric acid. All the amino acids were dried over phosphorus pentoxide in a vacuum desiccator for 64 hours and stored in a desiccator until weighed out.

The criteria of purity adopted were (*a*) the colour yield by Yemm and Cocking's method,[10] (*b*) paper chromatography, (*c*) measurement of the carbon dioxide liberated by reaction with ninhydrin[11] and (*d*) examination of the peaks from a chromatographic column.

Colour yield—For each amino acid, the ninhydrin colour yield developed by Yemm and Cocking's method[10] was compared with that of diketohydrindylaminediketohydrindamine. The results in Table II show the colour yields found by the collaborators for their own samples of amino acids. The good agreement for all amino acids, with no consistent differences between laboratories, suggests the absence of significant amounts of non-α-amino impurities. All the results agreed closely with other published figures.[9,10]

Paper chromatography—Approximately 100-μg amounts of each amino acid were spotted on paper and chromatographed in a butanol - acetic acid - water mixture (60 : 15 : 25). After treatment with ninhydrin reagent, only one spot was detected for each amino acid except methionine, which showed two extra spots in the sulphone and sulphoxide positions. This test would not reveal an amino acid impurity present in any amount less than about 2 per cent.

Carbon dioxide determination—The production of carbon dioxide by reaction with ninhydrin[11] was measured. There was no evidence of gross impurity, but the poor agreement between replicate determinations rendered the method of little value.

Examination of column peaks—The mixture was chromatographed on a resin column, and the fractions were de-salted and tested on paper to determine whether or not the peaks were single substances. All peaks were shown to be single substances, except that for methionine, which also contained allo*iso*leucine.

TABLE I

COMPOSITIONS OF AMINO ACID MIXTURES USED

Each laboratory used 1-ml portions of mixture in the first test and 2-ml portions in the second. Figures in parenthesis are weights of hydrochloride

Amino acid	Concentration present in— first test, mg per litre	second test, mg per litre
L-Arginine	256·0 (309·6)	130·0
DL-Histidine	160·0 (197·4)	85·4
L-Lysine	477·0 (714·2)	101·9
L-Tyrosine	312·2	80·7
DL-Phenylalanine	248·0	77·5
L-Cystine	151·2	70·9
DL-Methionine	290·4*	127·4
DL-Serine	327·6	86·1
DL-Threonine	320·5	94·1
DL-Leucine	660·4†	76·3
DL-*iso*Leucine	252·0‡	0·0
DL-Valine	392·9	125·9
L-Glutamic acid	989·8	126·4
DL-Aspartic acid	722·0	149·1
Glycine	566·0	117·6
DL-Alanine	264·2	68·8
DL-Proline	440·0	86·5
DL-Allo*iso*leucine	62·8§	0·0
Cystine solution—		
L-Cystine	—	70·0
Cystine + methionine mixture—		
L-Cystine	—	71·5
DL-Methionine	—	127·5

* Calculated concentration, see below.
† Includes 35·4 mg of leucine present as impurity in *iso*leucine, see below.
‡ *iso*Leucine content of 350·2 mg of sample, see below.
§ Calculated concentration, see below.

METHIONINE, LEUCINE AND *iso*LEUCINE—

Examination of the leucine on a resin column gave a recovery of 97 per cent. in the main peak and a trace of *iso*leucine in a single 1-ml fraction, which amounted to 0·35 per cent. of the total material placed on the column.

Examination of the methionine on a resin column showed the presence of 9 per cent. of methionine sulphone *plus* methionine sulphoxide. Accordingly, the methionine content of the solution was taken to be 91 per cent. of the amount weighed out.

The *iso*leucine was found by resin chromatography to consist of a mixture of 72 per cent. of *iso*leucine, 10·2 per cent. of leucine and 17·8 per cent. of allo*iso*leucine. The amounts of *iso*leucine and leucine weighed were corrected accordingly.

On a 100-cm column when the stepwise-elution technique was used, methionine and allo*iso*leucine were present as a single peak, as shown by paper chromatography. (This agrees with the findings of Piez.[12]) Figures for methionine recovery were therefore based on 91 per cent. of the methionine weighed out *plus* the 62·5 mg of allo*iso*leucine. In one laboratory, a small hump was observed at the end of the methionine peak, which might have been caused by slight separation of the allo*iso*leucine. In another laboratory, a separate small peak was observed after the methionine peak. If this were allo*iso*leucine separating from the methionine peak, then the recovery of methionine in this experiment would be higher than calculated.

CYSTINE AND VALINE—

Samples of the cystine and valine were placed together on one column, and the recoveries were determined by comparison of the colour yields of the same amino acids. For cystine, 144 μg were placed on the column and 148 μg were recovered; for valine, 328 μg were placed on the column and 323 μg were recovered.

TABLE II

COLOUR YIELDS OF AMINO ACIDS

Each collaborator used his own samples of amino acids. In laboratories D (except when shown otherwise), E and F, Moore and Stein's[9] values were used, and in laboratory I, Yemm and Cocking's[10] values were used. Values shown for samples used to prepare test solutions are relative to diketohydrindylidenediketohydrindamine

	Optical density found in—							Optical density found by—		Optical density of samples used to prepare test solutions
Amino acid	laboratory A	laboratory B	laboratory C	laboratory D	laboratory G	laboratory J	laboratory K	Moore and Stein's method	Yemm and Cocking's method	
Glycine ..	0·95	0·98	1·02	1·00	0·93	1·01	1·00	0·95	1·00	0·95
Alanine ..	0·94	0·99	1·03	—	0·96	1·01	1·03	0·97	1·00	1·00
Valine ..	0·95	1·00	1·08	—	0·97	0·98	1·02	0·97	0·99	0·98
Leucine ..	1·00	1·00	1·00	1·00	1·00	1·00	1·00	1·00	1·00	0·96
*iso*Leucine ..	1·00	1·00	1·05	—	0·97	1·00	1·00	1·00	1·00	1·02
Proline ..	—	—	—	—	—	—	—	0·225	—	—
Phenylalanine	0·89	1·00	0·94	1·06	0·91	0·83	0·98	1·00	0·89	0·93
Cystine ..	0·96	1·10	1·18	—	1·10	0·94	—	1·10	1·00	1·16
Methionine ..	1·00	1·00	1·05	1·00	0·98	1·01	1·02	1·02	1·00	0·94
Arginine ..	1·00	1·01	0·98	—	0·99	0·99	1·02	1·01	1·00	0·98
Histidine ..	0·95	1·02	1·06	1·01	0·97	0·97	1·02	1·02	1·00	0·99
Lysine ..	1·08	1·10	1·08	—	1·08	1·11	1·07	1·10	1·08	1·11
Aspartic acid	1·00	1·04	0·95	—	0·96	0·90	1·00	0·94	1·00	0·96
Glutamic acid	1·00	0·99	1·03	—	0·96	1·00	1·01	0·99	1·00	1·03
Serine ..	1·00	0·98	1·00	—	0·96	0·92	1·00	0·95	0·99	0·96
Threonine ..	1·00	0·99	0·95	—	0·96	0·91	1·00	0·94	1·00	0·92
Tyrosine ..	0·89	1·00	0·90	—	0·96	0·89	0·96	1·00	0·89	0·89
Cysteic acid ..	—	1·01	—	0·98	—	—	—	0·99	0·99	—
Methionine sulphone	—	1·00	—	—	—	—	—	1·02	—	—

SECOND TEST

As the cystine appeared to be unstable in the first experiment, it was decided to carry out a second test to determine (*a*) the recovery of cystine from pure solutions, (*b*) the recoveries of cystine and methionine from a solution containing both and (*c*) the recoveries of cystine and methionine from a mixture containing other amino acids.

The amounts used were (*a*) 0·140 mg of cystine per ampoule, (*b*) 0·142 mg of cystine and 0·255 mg of methionine and (*c*) 0·142 mg of cystine and 0·255 mg of methionine *plus* the other amino acids in amounts similar to those used in the first experiment.

The samples were placed in ampoules and freeze-dried to avoid any possibility of deterioration. Both cystine and methionine were the purest samples available from the British Drug Houses Ltd. The purity of the cystine, by nitrogen determination, was 99·8 per cent., and that of the methionine 100·1 per cent. (figures provided by the suppliers). Each amino acid showed a single spot on a paper chromatogram.

Results

First test—

Table III shows the results obtained in each laboratory and the mean recoveries for each amino acid. Table IV shows the figures in terms of deviation from the true values and also the mean deviation for each amino acid, as determined in all the laboratories, as well as the mean deviation for each laboratory. The values for cystine are shown in parenthesis and have been omitted from the calculations for Table IV, as this amino acid appeared to have deteriorated.

The number of determinations made in each laboratory varied between one and five, and the Tables show the mean values. Occasionally, because of poor separation of the peaks or mechanical breakdowns, not all the amino acids were determined in any one experiment. Thus, in Table III, the number of assays, 2/4, means that four runs were made, but individual values were obtained, in some instances, two, three or four times.

Table III

Amounts of amino acids found in first test

Amino acid	Concentration present, mg per ml	Amount of amino acid found in— laboratory A, mg	laboratory B, mg	laboratory C, mg	laboratory C, mg	laboratory D, mg	laboratory E, mg
Number of assays ..	—	2/3	4	3	2/4	2	1
Glycine	0·566	0·537	0·580	0·561	0·561	0·559	0·536
Alanine	0·264	0·311	0·280	0·289	0·280	0·276	0·349
Valine	0·393	0·403	0·420	—	0·376	0·408	0·425
Leucine	0·661	0·578	0·660	0·637	0·635	0·637	0·585
*iso*Leucine	0·252	0·210	0·255	0·247	0·231	0·242	0·240
Proline	0·440	0·607	0·375	0·457	0·450	0·470	0·559
Phenylalanine	0·248	0·287	0·235	0·241	0·243	0·247	0·261
Cystine	0·151	0·254	0·080	—	0·100	0·117	0·102
Methionine *plus* alloisoleucine* ..	0·326	0·294	0·070†	0·329	0·337	0·306	0·218
Arginine	0·256	0·117	0·215	0·266	—	0·270	0·257
Histidine	0·160	0·119	0·135	0·149	—	0·121	0·146
Lysine	0·477	0·466	0·450	0·477	—	0·463	0·503
Aspartic acid	0·722	0·727	0·780	0·729	0·717	0·734	0·780
Glutamic acid	0·990	1·062	1·025	1·034	0·992	0·987	1·021
Serine	0·328	0·277	0·340	0·332	0·320	0·331	0·344
Threonine	0·321	0·320	0·360	0·340	0·333	0·343	0·352
Tyrosine	0·312	0·224	0·295	0·353	0·344	0·312	0·326
Methionine sulphone ..	—	0·028	—	—	—	0·029	—
Elution system		Stepwise	Gradient	Stepwise	Stepwise	Stepwise	Gradient
Resin		Dowex 50X8	Zeo-Karb 225X 4·2	Dowex 50X8	Zeo-Karb 225X4	Dowex 50X12	Dowex 50X4
Cystine determination		As cysteic acid and direct	As cysteic acid. Methionine as sulphone	Direct	Direct	As cysteic acid and direct	Direct
Ninhydrin reagent		Yemm *et al.*[10]	Yemm *et al.*[10]	Moore *et al.*[13]	Moore *et al.*[13]	Moore *et al.*[13]	Moore *et al.*[13]
Column dimensions, cm ..		0·9 × 100	0·9 × 150	0·9 × 100	0·9 × 100	0·9 × 100	0·9 × 150

* See p. 528. † Omitted from calculation of means.

It was emphasised that each laboratory should adopt its usual procedure so that it might be possible to relate any differences in results to methods of procedure. Both stepwise and gradient elution were used; there were eight results on Dowex resin, three on Zeo-Karb and one on Amberlite. There were four variants of the ninhydrin reaction, namely, those of Yemm and Cocking,[10] Moore and Stein[9] and two modifications of the latter.[13,14]

Nine amino acids, namely, glycine, valine, phenylalanine, lysine, aspartic acid, glutamic acid, serine, threonine and tyrosine, were determined to within ±2 per cent. of the true value, and three others, proline, leucine and *iso*leucine, were determined to within ±6 per cent. Of the remainder, the value for alanine, 108 per cent., was due to two poor results, the other eight being fairly good; arginine was also poorly determined in two laboratories. Histidine was the only amino acid that was poorly determined by all collaborators. The recovery of methionine was complicated by the correction factors.

There was a considerable spread in the accuracies attained in the different laboratories. Five laboratories attained an absolute mean deviation for all amino acids (excluding cystine) of less than 5 per cent. and three showed a figure greater than 10 per cent.

TABLE III—*continued*

Amino acid	Amount of amino acid found in— laboratory F, mg	laboratory G, mg	laboratory I, mg	laboratory J, mg	laboratory K, mg	Mean amount found, mg	Recovery, %
Number of assays ..	2/4	5	3	2	2		
Glycine	0·580	0·565	0·582	—	0·540	0·560	99
Alanine	0·283	0·275	0·251	—	0·261	0·285	108
Valine	0·437	0·388	0·398	0·376	0·388	0·402	102
Leucine	0·701	0·622	0·593	0·576	0·626	0·623	94
*iso*Leucine	0·252	0·246	0·236	0·254	0·235	0·242	96
Proline	0·433	0·440	0·405	0·405	0·448	0·458	104
Phenylalanine	0·256	0·257	0·208	0·271	0·231	0·249	100
Cystine	0·131	0·120	0·074	—	0·066	0·116	77
Methionine *plus* alloisoleucine* ..	0·236	0·365	0·278	0·318	0·306	0·299	92
Arginine	0·257	0·261	0·240	—	0·243	0·236	92
Histidine	0·143	0·152	0·144	0·212	0·146	0·148	93
Lysine	0·514	0·476	0·456	0·479	0·484	0·478	100
Aspartic acid	0·759	0·709	0·714	0·633	0·703	0·725	100
Glutamic acid	1·046	1·007	0·980	0·813	0·985	0·996	101
Serine	0·333	0·328	0·308	0·318	0·314	0·321	98
Threonine	0·333	0·332	0·323	0·299	0·317	0·315	98
Tyrosine	0·296	0·311	0·269	0·428	0·309	0·314	101
Methionine sulphone ..	—	—	—	—	—	—	—
Elution system	Stepwise	Stepwise	Stepwise	Stepwise	Gradient		
Resin	Dowex 50X8	Dowex 50X8, Amberlite CG120	Zeo-Karb 225X12	Dowex 50X8	Dowex 50X4		
Cystine determination ..	Direct	Direct	Direct	Direct	Direct		
Ninhydrin reagent ..	Moore *et al.*[13]	Moore *et al.*[13]	Yemm *et al.*[10]	Moore *et al.*[13] (modified)	—		
Column dimensions, cm	0·9 × 98	—	1·0 × 115	0·9 × 100	0·9 × 140		

* See p. 528. † Omitted from calculation of means.

TABLE IV

DEVIATION FROM TRUE VALUES

Amino acid	Deviation found in— laboratory A, %	laboratory B, %	laboratory C on Dowex resin, %	laboratory C on Zeo-Karb resin, %	laboratory D, %	laboratory E, %	laboratory F, %
Glycine	−5·0	+2·0	−1·0	−1·0	−1·0	−5·0	+2·0
Alanine	+17·0	+6·0	+9·5	+6·0	+4·5	+32·0	+7·0
Valine	+2·5	+7·0	—	−4·0	+4·0	+8·0	+11·0
Leucine	−12·5	±0·0	−3·5	−3·5	−4·0	−11·0	+6·0
*iso*Leucine	−16·5	±0·0	−2·0	−8·0	−4·0	−5·0	±0·0
Proline	+37·0	−15·0	+4·0	+2·0	+7·0	+27·0	−1·0
Phenylalanine	+16·0	−5·0	−2·5	−2·0	±0·0	+5·0	+3·0
Methionine *plus* alloisoleucine	−10·0	—	+1·0	+3·0	−6·0	—	−28·0
Arginine	−54·0	−16·0	+4·0	—	+5·5	±0·0	±0·0
Histidine	−25·0	−16·0	−7·0	—	−24·0	−9·0	−10·0
Lysine	−2·0	−6·0	±0·0	—	−3·0	+7·0	+8·0
Aspartic acid	±0·0	+8·0	±0·0	±0·0	+1·0	+8·0	+5·0
Glutamic acid	+7·0	+4·0	+5·0	±0·0	±0·0	+3·0	+6·0
Serine	−14·0	+3·5	+1·0	−2·0	+1·0	+5·0	+1·0
Threonine	±0·0	+12·0	+6·0	+4·0	+7·0	+10·0	+4·0
Tyrosine	−28·0	−5·5	+13·0	±10·0	±0·0	+4·0	−5·0
Mean deviation from true value—							
Absolute	15·4	7·1	4·0	3·5	4·5	10·7	6·1
Algebraic	−5·5	−1·4	+1·8	+0·3	−0·8	+2·9	+0·6

TABLE IV—*continued*

Amino acid	Deviation found in— laboratory G, %	laboratory I, %	laboratory J, %	laboratory K, %	Mean deviation Absolute, %	Algebraic, %
Glycine	±0·0	+3·0	—	−5·0	2·5	−1·1
Alanine	+4·0	−5·0	—	+1·0	9·2	+8·2
Valine	−1·0	+1·0	−4·0	+1·0	4·4	+2·6
Leucine	−6·0	−10·0	−13·0	−5·0	6·8	−5·8
*iso*Leucine	−2·0	−6·0	+1·0	−7·0	4·7	−4·5
Proline	±0·0	−8·0	−8·0	+2·0	11·1	+4·3
Phenylalanine	+4·0	−16·0	+9·0	−7·0	6·3	+0·4
Methionine *plus* alloisoleucine	+12·0	−15·0	+3·0	−6·0	11·7	−7·9
Arginine	+2·0	−6·0	—	−5·0	10·3	−7·7
Histidine	−5·0	−10·0	+33·0	−9·0	14·8	−8·2
Lysine	−1·0	−4·0	−0·0	+1·0	3·2	±0·0
Aspartic acid	−2·0	−1·0	−12·0	−3·0	3·6	+0·4
Glutamic acid	+2·0	−1·0	−18·0	±0·0	4·2	+0·7
Serine	±0·0	−6·0	−3·0	−4·0	3·7	−1·6
Threonine	+4·0	±0·0	−7·0	+1·0	5·0	+3·7
Tyrosine	±0·0	−14·0	+37·0	+1·0	10·7	+1·1
Mean deviation from true value—						
Absolute	2·8	6·6	11·4	3·6		
Algebraic	+0·7	−6·1	+1·4	−2·8		

IMPROVEMENTS IN PROCEDURE—

In laboratory A, whose results showed the greatest mean deviations, both absolute and algebraic, from the true values, the analysis was repeated 2 years later. In the interim, the technique had been modified, and the results of the first and second analyses of the same solution, together with the changes in technique, are shown in Table V.

The solution of amino acids was the same as used originally and had been stored at 0° C for the whole period. The rather low result for tyrosine, the recovery of which was 101 per cent. in the original combined test, may have been due to deterioration. The other amino acids appear to have been remarkably stable, apart from cystine, which gave a low value in the original analysis.

TABLE V

RESULTS OBTAINED IN LABORATORY A IN ORIGINAL TEST AND 2 YEARS LATER

Stepwise elution was used in both series of experiments. Each result is the mean of three determinations

Amino acid	Original recovery, %	Recovery by improved procedure, %
Glycine	95	98
Alanine	117	103
Valine	102·5	103
Leucine	87·5	94
*iso*Leucine	83·5	100
Proline	137	98
Phenylalanine	116	93
Methionine *plus* alloisoleucine	90	—
Methionine	—	98
Alloisoleucine	—	96
Arginine	46	104
Histidine	75	94
Lysine	98	94
Aspartic acid	100	100·6
Glutamic acid	107	101
Serine	86	103
Threonine	100	100·4
Tyrosine	72	90
Resin	Dowex 50	Zeo-Karb 225X8 (particle size 40 μ)
Column height, cm	100	150
Reagent purification	Cellosolve and thiodiglycol redistilled	Cellosolve and thiodiglycol redistilled. Sodium acetate recrystallised. Ninhydrin stored over resin
Blank value (optical density)	0·2 to 0·3	0·07 to 0·09
Colour development	Yemm *et al.*[10]	Jacobs[14]

SECOND TEST——

The second mixture was analysed in two laboratories, and the results are shown in Table VI, which also shows the recoveries found by the same laboratories in the first test.

TABLE VI

RECOVERIES OF CYSTINE AND METHIONINE IN SECOND TEST

Amino acid	Amount of amino acid found, mg	Recovery, %	Recovery in first test, %	Laboratory
Cystine	0·145*	103	—	G
Cystine - methionine mixture—				
Cystine	0·146*	102	—	
Methionine	0·262	103	—	
Mixture as used in test—				
Cystine	0·147	103	80	A
Methionine	0·255	100	109	
Cystine	0·129	91	168	
Methinone	0·234	92	90	

* Corrected to a recovery of 87 per cent. Values for laboratory A not corrected.

The results show that methionine can be determined accurately. The recovery of cystine is only 90 per cent., as was also found by Schram, Moore and Bigwood,[15] who determined it as cysteic acid. This recovery appears to be sufficiently consistent to allow a correction factor to be applied with good results. (More recent figures published by Moore, Spackman and Stein,[16] who used an automatic apparatus, show complete recovery of cystine.)

Discussion of results

Purity of amino acids—

The tests for purity of the amino acids used in the test mixture do not rule out the possibility that small amounts of impurities may have been present. Indeed, all the collaborators reported an unknown peak after that of leucine (possibly norleucine). However, the comparative results of the different laboratories for the same mixture yield useful information.

Accuracy of determinations—

The results show, as would be expected, a range of degrees of accuracy from one laboratory to another. The recoveries of each amino acid, taken as the average for all ten laboratories, are good; nine amino acids were determined within 100 ± 2 per cent. and three within 100 ± 6 per cent. Improvements in technique greatly increased the accuracy. These results are similar to those for 5 to 12 determinations made in one laboratory by Moore and Stein.[17] These workers also found with the earlier procedure, as used in 1951, poor recovery of histidine, as reported here.

Comparison with microbiological assay—

Determinations by procedures of accepted accuracy show notoriously poor agreement when subjected to collaborative examination. In contrast, our results show good agreement. A useful comparison, with one proviso, can be made with the collaborative microbiological assay of an amino acid mixture carried out in eleven laboratories by the Rutgers Bureau.[1] The proviso is that the Rutgers test involved a well developed technique, and the collaborators were generally experienced in the use of the method. In our test, the method had not been in use for many years and several of the laboratories had only recently adopted the procedure and were still in the process of overcoming mechanical difficulties with the fraction collectors. It is perhaps relevant that one collaborator, in laboratory G, who had worked for a brief period with a group trained by Dr. S. Moore, showed the smallest mean absolute deviation from the true values, and laboratory A showed a great improvement in results with experience.

Table VII

Comparison of results found in microbiological and chromatographic collaborative assays

Amino acid	Absolute mean deviation for—		Algebraic mean deviation for—	
	chromatographic assay, %	microbiological assay, %	chromatographic assay, %	microbiological assay, %
Glycine	2·5	3·7	−1·2	+3·7
Alanine	9·2	—	+7·8	—
Valine	4·4	7·1	+3·2	+6·1
Leucine	6·8	3·2	−5·8	−0·1
*iso*Leucine	4·7	8·2	−4·1	+7·7
Proline	11·1	7·3	+4·2	+3·9
Phenylalanine	6·3	3·1	+0·5	+2·4
Methionine	11·7	2·4	−7·9	+2·1
Arginine	10·3	8·0	−7·7	+4·7
Histidine	14·8	8·2	−8·2	+0·5
Lysine	3·2	6·9	±0·0	+1·9
Aspartic acid	3·6	3·8	+0·4	+2·6
Glutamic acid	4·2	7·0	+0·7	+7·0
Serine	3·7	—	−1·6	—
Threonine	5·0	4·8	+3·7	+1·3
Tyrosine	10·7	3·4	+1·1	+2·5

In Table VII, the deviations from the true results are compared with those of the Rutgers collaborative assay. The two sets of results are similar, but our report demonstrates that the resin method is capable of much greater accuracy than the results in Table VII indicate.

Comparison with other collaborative assays—

Collaborative assays, although infrequently carried out, appear to fall into two groups. There is the routine assay, accepted as giving reliable results, and the collaborative assay by a newly developing procedure, research rather than routine. The former group gives much

worse results than does the latter. Collaborative tests by Wootton and King,[3] Beck and Sunderman[4] and Cicvárek and Homolka,[18] consisting of simple determinations, such as those of glucose, sodium chloride and urea, revealed a wide range of errors between the different laboratories. Beck and Sunderman[4] discussed the reasons for these disturbing variations and concluded that they were mainly due to inadequate numbers and poor training of technicians, poor equipment and facilities and lack of understanding between pathologist and staff. It is safe to say that none of these criticisms would normally apply to a research laboratory, and this may explain the higher standards of accuracy in tests of the kind reported here and by the Rutgers Bureau.[1]

Conclusions

As a result of this collaborative test, several general conclusions can be drawn for the guidance of other workers in this field. Among the many factors that must be closely controlled, a number are of outstanding importance.

(*i*) *Separation of peaks*—Many of the poor results were due to incomplete separation of individual peaks. When the peaks are well separated, recoveries are usually good, but when peaks are close, although sufficiently separated to allow a calculation to be made, the recoveries vary. Separation can sometimes be improved by changing pH and temperature.

(*ii*) *Reagent blank values*—The ninhydrin colour reaction can give high blank values owing to impurities in the chemicals used, and impurities can develop even in pure materials during storage. This blank value can be a major source of error and must be kept to a minimum by using freshly recrystallised and redistilled reagents. The ninhydrin can be purified on resin.[14]

(*iii*) *Identification of peaks*—It is necessary, at some stage, to identify the peaks by paper chromatography. There were instances in which peaks had been wrongly identified.

(*iv*) *Resin*—Considerable difficulty has been caused by variations between nominally identical batches of resin, *e.g.*, three batches labelled "8 per cent. cross-linked 200 mesh" contained particle sizes of approximately 20, 40 and 50 μ. The finer resins caused slower running of the column, with asymmetric peaks and low recoveries. This problem has been recently investigated by Hamilton.[19] It is necessary to check each sample of resin for particle size before use. As stated by Moore, Spackman and Stein,[16] the final test of suitability of a resin is the performance of a column prepared from it.

It must be emphasised that our test was carried out on a mixture of amino acids and provides no evidence of the errors introduced by hydrolysis of proteins. The improvements made by Moore, Spackman and Stein[16] had not been introduced when this work was carried out.

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Determination of Calcium in Plant Material by Atomic-absorption Spectrophotometry

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An investigation of interference by phosphorus, aluminium, sodium, potassium and silicon in the analysis of plant material for calcium by atomic-absorption spectrophotometry is described. Interference from these elements has been satisfactorily overcome without precipitation, ion exchange or extraction. Interference by phosphorus, aluminium and silicon is overcome by adding magnesium and sulphuric acid to both sample and standard solutions, and interference by sodium and potassium by adding these elements in excess to both sample and standard solutions. A method for digestion and analysis of plant material is described, and a comparison is made between results by this method and those by other methods.

In the analysis of plant material for calcium by flame-emission methods, many extraneous elements interfere, the most notable being phosphorus and aluminium. Mitchell and Robertson[1] studied the effect of aluminium on calcium emission and found that the addition of strontium to the analytical solutions overcame the interference. Interference by phosphorus on calcium emission has been studied by Leyton,[2] Strasheim and Nell[3] and Hemingway.[4] Methods used to overcome this interference include removal of phosphorus by ion-exchange resins, addition of an excess of phosphorus to the sample solutions and standards and removal of calcium by precipitation as oxalate and then analysis of the precipitate.

Yofè and Finkelstein[5] state that the effect of phosphate on calcium emission from a Beckman oxygen - hydrogen flame can be overcome by the presence of lanthanum or iron in amounts such that the molecular ratio of metal to phosphate is greater than 5 to 1 or 16 to 1, respectively.

This paper describes a study of the application of atomic-absorption spectrophotometry[6,7] to the determination of calcium in plant material. The method should be less subject to interference resulting from the exchange of energy between free atoms in the flame, because, unlike emission analysis, its operation depends on free atoms in the unexcited ground state.

For the analysis of plant material, the procedure should, if possible, not involve precipitation, ion exchange or extraction, since such steps are time-consuming. It should also permit analysis of solutions containing sulphate, since wet digestion with a mixture of nitric, perchloric and sulphuric acids is preferred for dissolution of the inorganic elements in plants.

Description of apparatus

The apparatus used was similar to that described by David[8] and differed only in that a lens was placed between the hollow-cathode tube and the Lundegårdh flame and a second lens between the flame and the spectrograph slit. The first lens had a focal length of 5·8 cm and was so placed as to focus a 1-mm diameter image of the hollow cathode in the centre of the flame. The second lens focused an image of the hollow cathode on the collimator of the spectrograph.

The width of the entrance slit of the spectrograph was 0·1 mm and that of the exit slit on the photomultiplier assembly was 0·2 mm. The latter slit was set on the calcium resonance line at 4226·7 A.

The cathode of the hollow-cathode discharge tube was machined from metallic calcium, and the anode was a strip of zirconium formed into an annular ring. Both were mounted on tungsten wires in a Pyrex-glass tube. The carrier gas in the tube was neon. The current setting of the hollow-cathode discharge tube throughout this work was 50 mA. Neon-filled calcium hollow-cathode tubes were found to operate steadily for only 10 to 30 hours before it became necessary to re-charge them with neon. Since the completion of the work described here, it has been found that a much longer period of steady operation can be achieved by using argon as carrier gas (private communication from Mr. A. Walsh). The close proximity of several weak argon lines to the calcium line at 4226·7 A had negligible effect on sensitivity when argon was used in place of neon.

The air and acetylene pressures applied to the Lundegårdh burner were 36 lb per sq. inch and 40 cm (water gauge), respectively.

Experimental

Height of hollow-cathode beam above burner top—

After the air and acetylene pressures to the burner, and the hollow-cathode tube current, had been set at optimum values, an examination was made of the effect on sensitivity when the position of the passage of the beam from the hollow-cathode tube through the flame was varied.

Table I

Effect of height of hollow-cathode beam above burner top on calcium absorption in presence and absence of phosphorus

Atomic-absorption measurements were made at 4226·7 A

Height of beam above burner top, mm	Absorption of solution containing— 50 p.p.m. of calcium, %	50 p.p.m. of calcium and 100 p.p.m. of phosphorus, %
20	20·5	13·5
15	29·5	17·5
12	38·0	21·0
10	44·5	24·0
8	51·0	26·0
6	57·0	27·0
4	58·0	16·0
3	43·5	3·0

The results in Table I show that a considerable gain in sensitivity can be achieved by adjusting the point at which the beam intersects the flame and that, for a solution containing only calcium chloride, maximum sensitivity occurred when the beam was so close to the base of the flame that its position could not be accurately measured. The decrease in sensitivity at a height of 3 mm was caused by the intersection of the hollow-cathode beam and the cone of unburnt gases within the flame. When phosphate was present, maximum calcium absorption occurred at a point of intersection about 5 mm above the burner top.

Although it is likely that the height of the position of maximum sensitivity would vary from one burner to another and with variation in combustible gas and air pressures, the results in Table I indicate the importance of adjusting the flame position to give maximum sensitivity.

Interference by phosphorus and aluminium—

The height of intersection of the hollow-cathode beam was set at 5 mm above the burner top in all experiments described in this section.

Figs. 1 and 2 show the effects of phosphorus and aluminium on the absorption of solutions containing 25 and 100 p.p.m. of calcium, as chloride; calcium chloride, aluminium chloride and orthophosphoric acid were used in the preparation of these solutions.

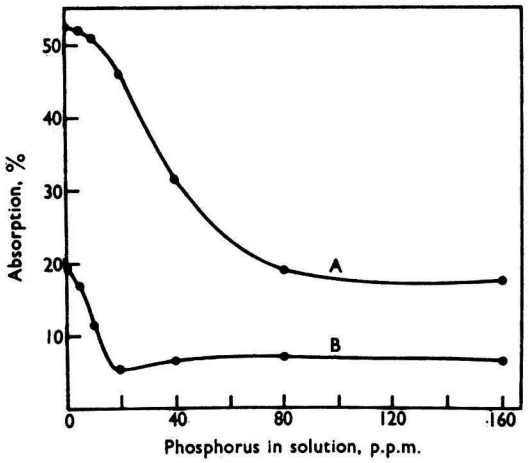

Fig. 1. Effect of phosphate on absorption of the calcium line at 4226·7 A: curve A, solution containing 100 p.p.m. of calcium, as $CaCl_2$; curve B, solution containing 25 p.p.m. of calcium, as $CaCl_2$

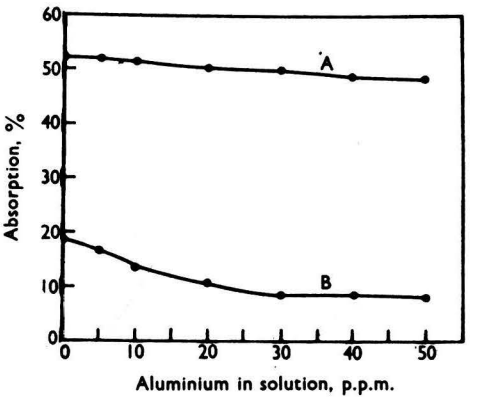

Fig. 2. Effect of aluminium on absorption of calcium line at 4226·7 A: curve A, solution containing 100 p.p.m. of calcium, as $CaCl_2$; curve B, solution containing 25 p.p.m. of calcium, as $CaCl_2$

It can be seen that interference by phosphorus is much greater than that by aluminium and that, for both elements, interference increased as the ratio of element to calcium increased to a point at which greater ratio had no further effect. The curves in Fig. 1 are similar to those obtained in emission by Strasheim and Nell.[3]

The magnitude of interference by phosphorus was so great that it would not have been feasible to overcome it by adding an excess of the element to both sample and standard solutions because of loss of sensitivity. The alternative approach—testing the effect of the presence of other ions in solution on the interference by phosphorus and aluminium on calcium absorption—was therefore attempted. Beryllium, cadmium, nickel, cobalt, magnesium, iron, chromium, molybdenum, manganese, chlorate, bromate, bromide, iodate, iodide, perchlorate, borate, sulphate and ethylenediaminetetra-acetate ions, at concentrations up to 2000 p.p.m., were tested in this manner. Of these ions, only magnesium, iron and beryllium showed a marked effect on calcium absorption. The results for magnesium and iron are shown in Table II and indicated the possible use of these ions to suppress interference by phosphorus and aluminium. Calcium absorption was seriously depressed by beryllium.

Table II

Effects of magnesium and iron on suppression of interference by phosphorus and aluminium

Each solution contained 100 p.p.m. of calcium. Atomic-absorption measurements were made at 4226·7 A

Concentration of phosphorus, p.p.m.	Concentration of aluminium, p.p.m.	Concentration of magnesium or iron, p.p.m.	Calcium absorption in presence of— magnesium, %	iron, %
Nil	Nil	Nil	70·5	70·5
200	40	Nil	28·0	27·5
		500	21·0	39·0
		2000	54·5	36·5

The possibility of adding phosphorus and aluminium to a plant-digest solution, to reduce the proportionate range of variation due to original plant phosphorus and aluminium, and then adding magnesium or iron to control the interference was tested. Neither 2000 p.p.m. of iron nor 5000 p.p.m. of magnesium satisfactorily controlled the large depressive effect caused by the presence of 1000 p.p.m. of phosphorus and 500 p.p.m. of aluminium. The

absorption reading for a solution containing 50 p.p.m. of calcium was 6 per cent. when magnesium was added and only 1 per cent. with iron.

A more detailed study was then made of the effect produced by increasing the ferric chloride and magnesium sulphate concentrations in presence and absence of phosphorus and aluminium, the calcium, phosphorus and aluminium concentrations being within the ranges expected in solutions of plant-material digests. The results are shown in Table III, from

TABLE III

EFFECT OF CONCENTRATIONS OF FERRIC CHLORIDE AND MAGNESIUM SULPHATE IN PRESENCE AND ABSENCE OF PHOSPHORUS AND ALUMINIUM

Each solution contained 200 p.p.m. of calcium. Atomic-absorption measurements were made at 4226·7 A

Concentration of iron added, p.p.m.	Concentration of magnesium added, p.p.m.	Calcium absorption— in absence of phosphorus and aluminium, %	Calcium absorption— in presence of 50 p.p.m. of phosphorus and 10 p.p.m. of aluminium, %	Calcium absorption— in presence of 200 p.p.m. of phosphorus and 40 p.p.m. of aluminium, %
Nil	Nil	85·5	68·0	35·5
250	Nil	79·5	60·0	46·0
500	Nil	72·5	58·0	50·5
750	Nil	70·5	59·5	53·5
1000	Nil	69·0	61·0	56·5
Nil	750	80·5	71·0	36·5
Nil	1500	80·5	78·0	56·0
Nil	2250	81·0	82·0	66·5
Nil	3000	81·0	83·5	75·0

which it can be seen that convergence of calcium-absorption readings in the presence and absence of phosphorus and aluminium occurred, but that coincidence was not attained at the highest levels tested. Since the calcium-absorption readings as the iron concentration was increased appeared to converge to a value considerably lower than that to which readings in the magnesium test apparently converged, the investigation of iron was discontinued.

The results of an experiment to determine the effect of 5000 p.p.m. of magnesium, as sulphate, at different levels of phosphorus and aluminium two or three times greater than those expected in plant-digest solutions are shown in Table IV. Since this did not show

TABLE IV

EFFECT OF 5000 p.p.m. OF MAGNESIUM ON INTERFERENCE BY PHOSPHORUS AND ALUMINIUM AT VARIOUS LEVELS

Magnesium was present as sulphate. Atomic-absorption measurements were made at 4226·7 A

Concentration of calcium, p.p.m.	Calcium absorption in presence of— 400 p.p.m. of phosphorus and 80 p.p.m. of aluminium, %	500 p.p.m. of phosphorus and 100 p.p.m. of aluminium, %	600 p.p.m. of phosphorus and 120 p.p.m. of aluminium, %
Nil	Nil	Nil	Nil
50	36·5	34·0	30·0
200	72·0	69·0	64·0

complete control of interference by phosphorus and aluminium, a similar experiment was made at a magnesium concentration of 10,000 p.p.m. Magnesium chloride was used in this experiment and was found to give practically no control over the interference. This implied that the interference was not controlled by magnesium alone, but by a combined effect of magnesium and sulphate.

Accordingly, it was decided to test the effect of increasing the concentration of magnesium, as chloride, in a solution containing a fixed level (2 per cent. v/v) of sulphuric acid and phosphorus and aluminium concentrations considerably higher than those that would be found in plant-digest solutions. The results are shown in Table V and indicate that control of interference by phosphorus and aluminium was practically complete for the solutions containing 6000 p.p.m. of magnesium and 2 per cent. v/v of sulphuric acid.

TABLE V

EFFECT OF MAGNESIUM CONCENTRATION IN PRESENCE OF 2 per cent. v/v OF SULPHURIC ACID ON INTERFERENCE BY PHOSPHORUS AND ALUMINIUM

Magnesium was present as chloride. Atomic-absorption measurements were made at 4226·7 A

Concentration of phosphorus, p.p.m.	Concentration of aluminium, p.p.m.	Calcium absorption in absence of magnesium, %	Calcium absorption in presence of— 1500 p.p.m. of magnesium, %	3000 p.p.m. of magnesium, %	6000 p.p.m. of magnesium, %	12,000 p.p.m. of magnesium, %
Solution containing 25 p.p.m. of calcium—						
Nil	Nil	17·0	18·0	15·0	13·0	10·0
200	40	2·0	13·5	15·5	14·5	12·0
Solution containing 100 p.p.m. of calcium—						
Nil	Nil	42·5	47·5	45·0	41·0	34·5
200	40	18·5	30·0	41·5	41·5	36·0

The results of a more stringent test on the ability of these concentrations of magnesium and sulphuric acid to control the interference are shown in Table VI, from which it is evident that the variations, except possibly that of the reading for 400 p.p.m. of phosphorus, 10 p.p.m. of aluminium and 100 p.p.m. of calcium, were no greater than would be expected from experimental error. Subsequent work has indicated that the low reading referred to was also due to experimental error.

TABLE VI

EFFECT OF 6000 p.p.m. OF MAGNESIUM AND 2 per cent. v/v OF SULPHURIC ACID ON INTERFERENCE BY PHOSPHORUS AND ALUMINIUM

Atomic-absorption measurements were made at 4226·7 A

Concentration of phosphorus, p.p.m.	Concentration of aluminium, p.p.m.	Absorption of solution containing— 25 p.p.m. of calcium, %	100 p.p.m. of calcium, %
50	Nil	17·5	51·0
	10	17·0	50·5
	20	16·5	50·5
	40	17·0	51·0
	80	17·0	50·0
Nil	10	16·0	48·0
50		16·5	51·0
100		17·0	50·5
200		17·0	49·0
400		16·0	45·0

Variation in magnesium concentration around 6000 p.p.m. at fixed sulphuric acid concentration and variation in sulphuric acid concentration around 2 per cent. v/v at fixed magnesium concentration indicated that the necessary concentrations of magnesium and sulphuric acid were not so critical that small variations caused by magnesium and sulphate in the plant material would affect results.

The results of an experiment to test the control of interference by phosphorus and aluminium at nine levels of calcium showed that control was complete, within the limits of experimental error, at all levels up to 640 p.p.m. of calcium in solution.

Reliable calcium-free sources of sulphuric acid and magnesium are necessary if high concentrations of them are to be added to the plant-digest solutions. The sulphuric acid used was prepared by distilling analytical-reagent grade acid from an all-glass apparatus. Tests showed that the most reliable source of calcium-free magnesium was magnesium metal; three batches of magnesium from different manufacturers were all found to be calcium-free. For addition to solutions to be analysed for calcium, magnesium metal was dissolved in hydrochloric acid that had been distilled from an all-glass apparatus.

A test for variation in sensitivity with height of the point of intersection of the hollow-cathode beam and the flame showed that maximum calcium absorption in the presence of 6000 p.p.m. of magnesium and 2 per cent. v/v of sulphuric acid occurred 6·5 mm above the burner top. The burner height was adjusted to this point in all subsequent work.

Interference by sodium and potassium—

Recovery experiments on four samples of plant material gave results between 15 and 30 per cent. high. These experiments were carried out by adding a known amount of calcium to one of two portions of a sample, analysing both portions and comparing the difference with the amount of calcium added. Atomic-absorption analysis for calcium was carried out in presence of 6000 p.p.m. of magnesium and 2 per cent. v/v of sulphuric acid in both sample and standard solutions.

Of the major inorganic elements in plants, only the alkali metals or silica remained as a possible explanation of the enhancement observed.

Table VII

Effect of low concentrations of sodium and potassium

Each solution contained 6000 p.p.m. of magnesium and 2 per cent. v/v of sulphuric acid. Atomic-absorption measurements were made at 4226·7 A

Concentration of sodium, p.p.m.	Concentration of potassium, p.p.m.	Absorption of solution containing— 25 p.p.m. of calcium, %	100 p.p.m. of calcium, %
Nil	Nil	14·0	41·5
20	Nil	15·0	44·5
40	Nil	15·5	46·0
80	Nil	16·0	47·0
160	Nil	17·0	48·0
Nil	20	15·5	45·5
Nil	40	16·0	46·5
Nil	80	17·5	47·5
Nil	160	18·5	49·5
Nil	Nil	14·0	43·0
20	20	16·0	46·0
40	40	17·0	47·5
80	80	17·5	48·5
160	160	18·5	50·0

Tables VII and VIII show the results of an experiment to determine the effects of sodium and potassium on calcium absorption in presence of 6000 p.p.m. of magnesium and 2 per cent. v/v of sulphuric acid at low and high alkali-metal concentrations, respectively. Although the peak concentrations of sodium and potassium tested were not high enough for maximum calcium absorption to be attained, sufficient information was gained from the results in Table VII to indicate that either sodium or potassium, or both, was the cause of the enhancement observed in the recovery experiments.

If the concentrations of sodium and potassium in plant-digest solutions are assumed to be of the order of 20 and 200 p.p.m., respectively, the results in Table VIII indicate that the enhancement could be overcome by adding 200 p.p.m. of sodium and 1500 p.p.m. of potassium to both sample and standard solutions.

TABLE VIII

EFFECT OF HIGH CONCENTRATIONS OF SODIUM AND POTASSIUM

Each solution contained 6000 p.p.m. of magnesium and 2 per cent. v/v of sulphuric acid. Atomic-absorption measurements were made at 4226·7 A

Concentration of sodium, p.p.m.	Concentration of potassium, p.p.m.	Absorption of solution containing— 25 p.p.m. of calcium, %	100 p.p.m. of calcium, %	200 p.p.m. of calcium, %
Nil	400	17·0	48·0	65·0
50	400	17·0	48·0	65·5
100	400	17·0	48·5	64·5
200	400	17·0	49·0	64·0
400	400	16·5	47·0	64·0
800	400	15·0	46·0	63·0
1600	400	14·5	44·0	62·0
3200	400	14·0	42·5	60·0
6400	400	12·0	38·0	56·0
100	Nil	16·5	47·0	63·5
100	400	17·5	50·0	66·0
100	800	17·5	48·0	64·5
100	1600	17·0	47·0	64·0
100	3200	16·0	46·0	63·0
100	6400	16·0	45·5	63·0
100	12,800	14·0	42·0	60·0

TABLE IX

EFFECT OF SODIUM SILICATE

Calcium was present as chloride. Atomic-absorption measurements were made at 4226·7 A

Concentration of silicon, p.p.m.	Absorption of solution containing— 25 p.p.m. of calcium, %	50 p.p.m. of calcium, %	100 p.p.m. of calcium, %
In absence of magnesium and sulphuric acid—			
Nil	21·5	36·5	56·5
10	18·5	33·5	55·5
20	13·5	31·5	53·5
40	6·0	25·0	48·0
80	3·0	14·5	37·0
160	0·5	—*	—*
320	0·0	—*	—*
In presence of 6000 p.p.m. of magnesium and 2 per cent. v/v of sulphuric acid—			
Nil	13·5	25·0	43·0
10	14·0	26·0	44·0
20	15·0	27·0	45·0
40	15·0	28·0	47·0
80	14·5	27·0	45·5
160	10·0	20·0	35·0
320	5·0	10·0	18·0

* Precipitate was formed.

INTERFERENCE BY SILICATE—

Since silicate is known to interfere with calcium emission from flames, it was decided to examine its possible interference in absorption.

Table IX shows the results for calcium absorption when the concentration of sodium silicate was varied from 0 to 320 p.p.m. of silicon at three calcium levels in presence and absence of 6000 p.p.m. of magnesium and 2 per cent. v/v of sulphuric acid. The calcium in the solutions was present as chloride.

The interesting points in Table IX are the drastic effect of silicate on calcium absorption and the control of this effect below about 40 p.p.m. of silicon by the presence in the solutions of 6000 p.p.m. of magnesium and 2 per cent. v/v of sulphuric acid. The slight enhancement evident at low concentrations of sodium silicate in presence of magnesium and sulphuric acid was subsequently found to be caused by sodium added with the silicate. Enhancement did not occur when measurements were made in presence of an excess amount of added sodium.

Method

Procedure—

To between 0·3 and 0·5 g of dry plant material in a 100-ml Kjeldahl flask add an accurately prepared mixture of 1·25 ml of sulphuric acid and 3·75 ml of perchloric acid, and then add about 15 ml of concentrated nitric acid. Boil gently on a hot-plate until evolution of nitrogen dioxide fumes has ceased and the excess of nitric acid has been removed. (If charring occurs at this stage, add 1 ml of nitric acid, and boil again.) Heat strongly to remove excess of perchloric acid, and shake intermittently to prevent solid matter from adhering to the sides

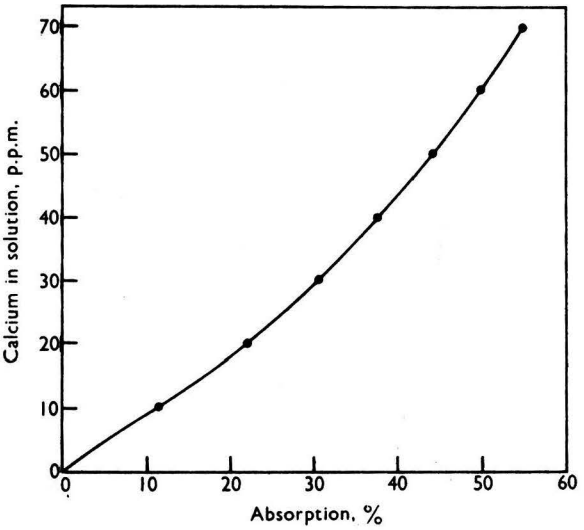

Fig. 3. Atomic-absorption calibration curve for the calcium line at 4226·7 A. The standard solutions contained 6000 p.p.m. of magnesium, as $MgCl_2$, 2 per cent. v/v of sulphuric acid, 200 p.p.m. of sodium, as NaCl, and 1500 p.p.m. of potassium, as KCl

of the flask. When all the perchloric acid has been removed, continue to heat strongly to ensure thorough dehydration of silica. (At this stage, take care not to heat so strongly that fumes of sulphuric acid are evolved.) When cool, place 10 ml of cold water in the flask by pipette, shake thoroughly, and filter the solution through a Whatman No. 42 filter-paper into a 50-ml calibrated flask. Rinse the Kjeldahl flask with another 10-ml portion of cold water and then with two further 5-ml portions. Shake thoroughly after each addition, filter the rinsings, and add the filtrate to the contents of the calibrated flask. Finally, wash the filter-paper with three 5-ml portions of cold water, add the washings to the contents of the calibrated flask, and dilute to the mark with water.

Place a 20-ml aliquot of the solution in a 25-ml calibrated flask, and add 5 ml of a solution containing 3 per cent. of magnesium, 0·1 per cent. of sodium and 0·75 per cent. of potassium, each as chloride. (The solution now contains, apart from the salts contributed by the plant material, 6000 p.p.m. of magnesium, 2 per cent. v/v of sulphuric acid, 200 p.p.m. of sodium and 1500 p.p.m. of potassium.)

Prepare standards to cover the range 0 to 70 p.p.m. of calcium and containing sulphuric acid, magnesium, sodium and potassium in amounts equal to those in the sample solution.

Subject both sample and standard solutions to atomic-absorption spectrophotometric analysis in a manner similar to that described previously.[8] A calibration curve so prepared is shown in Fig. 3.

Note that important points in the digestion of the plant material and dissolution of the digest are the dehydration of silica and the thorough washing of the residue from the digest, as calcium sulphate is only sparingly soluble in water.

Recovery experiments

Two approximately equal portions of each of seven samples of ground air-dried plant material were prepared by coning, quartering and combining opposite quarters of a grab sample from each bulk supply of dry plant matter. After a known amount of calcium had been added to one of each pair, the portions were digested and analysed by the proposed method. A comparison of the amounts of added calcium recovered with the amounts of calcium added is shown in Table X. The weights of dry matter shown were digested and filtered, and the filtrate was diluted to 50 ml. The amounts of calcium shown refer to a 20-ml aliquot of the digest solution.

Table X

Recovery of added calcium from dry plant material

Sample	Approximate weight of dry sample, g	Amount of calcium originally present, μg	Amount of calcium added, μg	Amount of calcium found, μg	Amount of added calcium recovered, μg	Recovery, %
White clover leaf ..	0·18	787	710	1502	715	100·7
Phalaris tops ..	0·75	697	710	1424	727	102·4
Soya bean tops ..	0·19	690	710	1377	687	96·8
Wheat heads.. ..	1·84	477	480	952	475	99·0
Eucalyptus leaf ..	0·51	745	710	1487	742	104·5
Bulked tops	0·14	755	710	1445	690	97·2
Pine needles	1·00	870	720	1537	667	92·6

The results indicate that the proposed method is satisfactorily reliable for the determination of calcium in plant material, particularly as recovery experiments permit assessment of an analytical method independently of other methods.

Comparison with other methods

Table XI shows a comparison of the results of analysis of the digests of five samples of plant materials for calcium by the proposed method, by flame photometry and by titration

Table XI

Determination of calcium by the proposed and other methods

Sample	Amount of calcium found in dry sample by— atomic absorption, %	flame photometry, %	titration, %
Sub-clover tops	0·915	0·900	0·924
Sub-clover tops	0·568	0·567	0·587
White clover leaf	1·053	1·064	1·075
Forest litter	0·770	0·750	0·830
Tobacco leaf	1·320	1·290	1·346

of the oxalate with permanganate. In the flame-photometric method, phosphate was removed by precipitation before analysis and interference by aluminium was suppressed by adding magnesium sulphate to the solution.

Reproducibility

Reproducibility tests were carried out on solutions containing 2·5 and 50 p.p.m. of calcium, as chloride. Seven-millilitre portions of the same solution were placed in 34 Lundegårdh spray bulbs and subjected to atomic-absorption analysis. The absorptions of the two solutions were $6{\cdot}00 \pm 0{\cdot}25$ and $64{\cdot}72 \pm 0{\cdot}84$ per cent., respectively, the variations being standard deviations of single determinations.

Discussion of the method

Since, at least for phosphorus and aluminium, the interferences on calcium in absorption are similar to those observed in emission, it is suggested that most interferences in flame analysis for calcium are of the chemical type in which the calcium is attached to a refractory compound that cannot be easily dissociated at the temperatures attainable in the flame. A comparison of interferences in absorption and emission carried out on the same flame might reveal interferences not in this category, but due to interference in excitation of calcium atoms. If serious interferences of the latter type exist, an atomic-absorption method would have considerable advantages over emission methods.

It is difficult to explain the mechanism by which interference from phosphorus, aluminium and silicon on calcium absorption is controlled by magnesium and sulphuric acid. Since both an anion and a cation are essential, two solid phases may be formed when water is evaporated from a droplet of sample solution, phosphorus, aluminium and silicon entering one solid phase and calcium the other. When combined in different solid phases, phosphorus, aluminium and silicon may not be able to interact with calcium, even in the vapour phase after volatilisation.

Control of interference from phosphate by the presence of iron was apparently less complete in the analysis of solutions for calcium by atomic absorption than in analysis by Yofè and Finkelstein's emission method.[5] An accurate comparison of the method is, however, difficult, as large variations in interference effects occur with variation in flame temperature.[3] No experiment has yet been made to determine the effect of lanthanum on interference by phosphate on calcium absorption.

The lower limit for reasonably accurate determination of calcium by the proposed method is about 1 p.p.m. in solution. The upper limit, which is determined by the slope of the calibration curve, would be about 80 p.p.m. of calcium in solution. If phosphorus, aluminium and silicon were not present in the solutions analysed, the lower limit would be about 0·5 p.p.m. of calcium in solution, since it would not be necessary to add high concentrations of magnesium and sulphuric acid. The presence of 6000 p.p.m. of magnesium and 2 per cent. v/v of sulphuric acid approximately halves the sensitivity. An advantage of atomic absorption is the considerable gain in sensitivity that can be attained, if necessary, either by reflecting the hollow-cathode beam several times through the flame or by using a burner having a long narrow top. Allan[9] describes a burner of length 7·5 cm, which he used to determine magnesium in plant material by atomic absorption. His results indicated that a three-fold gain in sensitivity could be attained when this burner was used in place of a conventional Lundegårdh burner.

Apart from its use in chemical analysis, atomic absorption should find application in the study of conditions within flames. As shown in this paper, the hollow-cathode beam, when focused to a point, provides a means by which the distribution of free atoms in the ground state within the flame can be measured with high precision.

The suppression of interference on calcium absorption by chemically conditioning the solution gives the proposed method considerable advantages over methods involving removal of interfering elements by precipitation, ion exchange or extraction. It makes possible more rapid determinations, removes possible sources of contamination and permits routine analysis by semi-skilled operators.

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Further Developments in the Determination of Oxygen in Beryllium by the Micro Vacuum Fusion Method

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By a development of the technique previously described by Booth and Parker, the micro vacuum fusion method has been successfully applied to the analysis of beryllium containing 0·01 per cent. of oxygen. At this level, the coefficient of variation is 15 to 20 per cent.

Recently we outlined[1] the development of a method for determining oxygen in beryllium by vacuum fusion, which, when applied to the analysis of metal containing several tenths per cent. of oxygen, gave results that were reproducible and in close agreement with those obtained by a chemical method.[2]

Trials in other laboratories indicated that differences in apparatus design and technique appeared to exert a considerable influence on the performance of the method, particularly in the analysis of powdered samples. This behaviour has not been observed in other vacuum fusion analyses; for niobium, thorium, titanium, uranium and zirconium, the results of comparative oxygen determinations made by using apparatus differing both in major design features and in minor details have shown excellent agreement.

Further investigation is necessary to clarify this matter, but the more pressing problem of the extension of the method to the determination of oxygen contents in the range 0·01 to 0·10 per cent. has been solved.

Experimental

In an attempt to resolve the anomalies in the analysis of powdered samples, a series of comparative analyses on a sample of ground French Flake beryllium was carried out in collaboration with another laboratory; the results are shown in Table I.

Table I

Results of comparative analyses of a beryllium-powder sample

Experiment No.	Laboratory	Oxygen content found, %	Mean oxygen content, %	Experimental conditions
1	Woolwich	0·92, 1·02, 0·96, 1·00, 0·99, 1·11, 0·98	1·00	Platinum bath *plus* added tin[1]
		0·99, 1·02, 1·01, 1·00, 1·03, 1·10	1·02	
2	Collaborating laboratory	0·89, 0·97, 1·04, 1·05, 1·10, 1·10	1·02	
3	Woolwich	0·38, 0·43, 0·77, 0·67, 0·37, 0·46, 0·60	0·53	Platinum bath
4	Collaborating laboratory	0·63, 0·74, 0·89, 1·04, 1·06, 1·04	0·90	
5	Woolwich	1·06, 0·72, 0·73, 0·74, 0·83	0·82	Platinum bath*

* Crucible assembly similar to that used by collaborating laboratory.

The results of experiments Nos. 3 and 4 confirmed that the apparatus used in the collaborating laboratory gave better recoveries of oxygen from a platinum bath than did the Woolwich apparatus. The crucible assembly of the latter was then modified in terms of wall-thickness, depth and fit of lid, but the results of analyses in which tin was omitted from the bath remained significantly lower and more erratic than those of analyses in which a tin-platinum bath was used.

Finally, a series of determinations (experiment No. 5) was carried out in which the conditions of test as used in the collaborating laboratory were adhered to as closely as possible, *i.e.*, one of their crucible assemblies was used, the furnace tube was cooled by air and tin was omitted from the bath.

For the extension of the method to oxygen contents in the range 0·01 to 0·10 per cent., the main considerations were (*a*) the sample weight and (*b*) the magnitude and variation of the blank value. Since the achievement of a major reduction in the latter seemed improbable, attention was first turned to the possibility of increasing the sample size.

In a series of exploratory experiments, the weights of beryllium, tin and platinum were systematically varied. Results showed that, for more than 60 mg of tin, the weight used appeared to be uncritical, and 70 mg of tin were used thereafter. For 50 mg of beryllium (the minimum weight considered to be satisfactory), approximately 10 g of platinum were required to obtain consistent results for oxygen. The capacity of the graphite crucible was such that six 50-mg samples of beryllium could be analysed in one series of experiments.

The tentative procedure was tried out on two beryllium samples reputed to contain about 0·1 per cent. of oxygen; the results were as follows—

(*a*) Oxygen found, %	.. ..	0·124,	0·120,	0·105,	0·094,	0·115	
(*b*) Oxygen found, %	.. ..	0·140,	0·137,	0·148,	0·149,	0·150,	0·146

At this level of oxygen content and with a 50-mg sample, the magnitude and variation of the blank value are of minor importance, but before oxygen determinations at the 0·01 to 0·02 per cent. level were attempted the problem was re-investigated.

High and erratic blank values were sometimes experienced from the use of tin foil, and even from analytical-reagent grade tin, whereas the Specpure tin rod more recently used was superior in this respect. The oxygen content of Specpure tin rod, as determined by the normal method (platinum bath at 1900° C), was 80 to 100 p.p.m.

However, if the tin was pre-heated at 1400° C for 1 minute, the apparent oxygen content decreased to between 30 and 40 p.p.m., and this procedure was adopted in subsequent experiments (see p. 548).

This behaviour is explicable in terms of the thermal decomposition of the surface oxide (mainly SnO) to produce oxygen rather than carbon monoxide, since during the initial heating the tin has little or no access to carbon. As a result of discussion with staff of the Tin Research Institute, it was considered to be unlikely that the value of 30 to 40 p.p.m. of oxygen could be significantly reduced. As a precaution, however, the tin subsequently used was heated at 600° C *in vacuo* (less than 10^{-5} mm of mercury) for 3 to 4 hours to achieve homogeneity of residual oxide and its surface was abraded immediately before use.

Seventeen determinations on such material, pre-heated for 1 minute at 1400° C, gave a mean value of 30 ± 6 p.p.m. of oxygen (95 per cent. confidence limits). With approximately equal weights of beryllium and tin, this was considered to be acceptable, and a sample of vacuum-melted beryllium rod having a low and known oxygen content was obtained. Ten samples from one end of this rod and eleven samples from half-way along it were analysed; the results are shown in Table II.

TABLE II

OXYGEN FOUND IN SAMPLES OF VACUUM-MELTED BERYLLIUM ROD

Oxygen content found, %	Mean oxygen content, %	95 per cent. confidence limits,* %
Samples from end of rod—		
0·012, 0·012, 0·014, 0·015, 0·008, 0·012, 0·016, 0·012, 0·013, 0·014	0·013	0·013 ± 0·0016
Samples from half-way along rod—		
0·019, 0·013, 0·010, 0·011, 0·012, 0·011, 0·010, 0·015, 0·013, 0·016, 0·011	0·013	0·013 ± 0·0019

* Uncorrected for blank variance.

Results found for oxygen by the collaborating laboratory by a fast-neutron activation method were 0·010 and 0·011 per cent.

Method

Unless otherwise specified, the operation of the apparatus is as previously described.[3]

Procedure—

Load six approximately 50-mg samples of beryllium, each with 70 ± 5 mg of tin, into the sample "tree." Fill the remaining six spaces with 70-mg pieces of tin. Place 10 g of 14 s.w.g. platinum wire, cut into appropriately sized pieces, in the "tree" to form the platinum bath. Outgas the crucible assembly and platinum bath in the usual way, and then switch off the induction heater. When the temperature of the crucible is less than 600° C, *i.e.*, not visibly red, add 70 mg of tin, and heat the crucible at 1400° ± 50° C for 1 minute and then at 1950° ± 20° C for 2 minutes. Switch off the induction heater.

Ensure that all the evolved gas has been pumped away, add a sample (*plus* tin) to the "cold" crucible, and heat at 1400° ± 50° C for 1 minute and then at 1950° ± 20° C for 2 minutes. Switch off the induction heater, and proceed with the gas analysis.

Repeat the procedure described in the previous paragraph for each sample. Carry out blank determinations in the usual way, and make a check between sample analyses. Should the blank value increase for any reason, add 70 mg of tin, and heat for a few minutes at 1950°C; this treatment should reduce the blank value to the expected level.

Conclusions

The validity of the technique previously described[1] has again been confirmed, and no modifications are required for the analysis of beryllium containing more than 0·1 per cent. of oxygen.

The difference in performance between the Woolwich apparatus and that used in the collaborating laboratory for analysing powdered samples has not been explained, but the experience of other workers in this field may throw further light on the problem.

By increasing the sample weight to about 50 mg and the weight of platinum in the bath to 10 g, and by using vacuum-melted Specpure tin, the method has been successfully applied to the determination of oxygen in beryllium at the 0·01 per cent. level, at which the results have a coefficient of variation of 15 to 20 per cent. and are in satisfactory agreement with those obtained by an entirely different and independent technique, *viz.*, fast-neutron activation.

The confidence limits quoted indicate that the smallest amount of oxygen that can be detected with reasonable certainty is approximately 20 p.p.m.

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Oxidised Nitrogen in Waters and Sewage Effluents Observed by Ultra-violet Spectrophotometry

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Measurement of the ultra-violet absorption at 2100 A affords a rapid method for determining nitrate. For most waters of good organic quality, its sensitivity and accuracy compare favourably with the usual chemical methods, and results are in agreement with such methods. However, for some samples containing nitrate freshly formed by microbiological action, there are discrepancies suggesting the presence of unstable compounds that change to nitrate when kept. The use of a spectrophotometer is therefore suggested as an additional method for investigating nitrification in soils, waters and sewage effluents.

Nitrate has for over half a century been considered in this country as one of the important substances to be determined in waters, because of its possible connection with pollution from sewage or manurial matter. The importance of the determination has been emphasised

in recent years by the recognition of the causation of methaemoglobinaemia in infants given artificial feeds prepared from well waters containing substantial amounts of nitrate.[1] Many investigations have been made of the balance of the various forms of nitrogen before and after microbiological oxidation, and complications have been found.

Although a variety of methods is available for the determination of nitrates, the more accurate procedures are somewhat lengthy. As early as 1913, Thresh[2] showed that methods involving reduction by a zinc - copper couple could give accurate results for nitrate solutions of known concentration, but he made the rather surprising observation about waters that—"For some reason there is often considerable discrepancy, as regards nitrates, in the results obtained by different analysts from the examination of the same water. It has been difficult for me sometimes to believe that all had had samples from the same source, yet the other determinations agreed so closely as to preclude any doubt."

The object of the work described here was to investigate further the possibility of rapid determination of nitrate in waters and effluents by direct observation of the ultra-violet absorption due to nitrate ion.

Ultra-violet absorption

An absorption band due to nitrate ion, having a maximum at 1936 A (log $K_{max.} \sim 4{\cdot}08$) was observed by Scheibe.[3] Hoather[4] referred to the possible use of the ultra-violet absorption of nitrate ion for determining nitrate in waters. The absorption at 2100 A can conveniently be measured, with a reproducibility within about 5 per cent., by using a commercial spectrophotometer. The specific absorption coefficient for 1 p.p.m. of nitrogen in nitrate is as high as 0·55, so that the procedure is extremely sensitive. For nitrite, the corresponding figure is 0·37, but nitrites are usually present only in minute traces. It was shown that there is no significant interference from the other saline constituents present in ordinary natural waters and that for most well waters interference from organic impurity is also negligible. For many river waters and good-quality sewage effluents (laboratory-filtered), reasonable accuracy can be attained by applying a relatively small correction for organic matter in solution, on the empirical basis that the absorption at 2100 A due to organic matter is approximately four times as great as that at 2750 A. The latter wavelength is suitable for assessing organic content because the absorption due to nitrate is extremely low. The optical density at 2750 A (4-cm cell) multiplied by 7 is approximately equal to the "oxygen absorbed" (permanganate value).

Well waters

Agreement between the amounts of nitrate determined by the usual chemical methods and those calculated from the ultra-violet absorption at 2100 A has been satisfactory for hundreds of samples of well waters; a few examples are shown in Table I.

Table I

Nitrate found in well waters

Sample No.	Optical density measured at— 2750 A in 4-cm cell	Optical density measured at— 2100 A in 1-cm cell	Nitrate content— calculated from optical density, p.p.m.	Nitrate content— determined by reduction, p.p.m.
1	0·035	4·3	7·8	7·8
2	0·035	3·7	6·7	6·8
3	0·020	2·3	4·2	3·7
4	0·040	4·4	7·9	8·4
5	0·11	16·5	30	30
6	0·055	0·17	0·2	0·0

For about fifty other samples, however, the nitrate calculated from the ultra-violet absorption was much higher than the nitrate determined chemically. These anomalous results related especially to unusual seasonal conditions, nitrate being added to underground water when percolation starts after autumn rainfall.

These samples did not show any other abnormalities; they had a high standard of organic purity and contained no ammoniacal nitrogen or nitrite. The absorption curve between 2500 and 2100 A has been observed for a few of them and found to be of the usual

form for nitrate. The absorption at 2100 A is independent of pH over a wide range and disappears when the water is reduced with a zinc - copper couple, as in the usual method of determining nitrate by reduction to ammoniacal nitrogen. It was not found to be possible to attribute the unexpectedly high absorption at 2100 A to any impurities other than oxidised nitrogen. Further observations on some similar samples have shown that, if they are kept at room temperature, the nitrate content slowly increases until it is approximately equal to that calculated from the ultra-violet absorption; the latter shows no appreciable change. Examples of this effect are shown in Table II. The nitrate in sample No. 1 was determined by the phenoldisulphonic acid method,[5] the colour being measured at 4100 A with a spectrophotometer. The nitrate in samples Nos. 2 and 3 was determined by reduction,[6] the ammonia being determined by using Nessler's reagent.

TABLE II

VARIATION IN NITRATE CONTENT OF SOME WELL WATERS WITH TIME

Sample No.	Time after collection of sample, days	Nitrate content— calculated from optical density, p.p.m.	Nitrate content— found by chemical methods, p.p.m.
1	2	6·0	4·1
	3	—	4·0
	7	—	4·8
	10	6·4	5·9
2	3	11·3	7·8
	17	11·1	9·8
3	1	39	22
	27	—	35

Samples Nos. 1 and 2 were from different wells, on an outcrop of chalk, used for large public supplies. They contained no nitrite, ammoniacal nitrogen or albuminoid nitrogen, had an "oxygen absorbed" (acid permanganate) figure of only 0·2 p.p.m. and contained little organic carbon. Sample No. 3 was pumped from a large shallow well in gravel; although slightly less pure than the others, it was also free from ammoniacal nitrogen and nitrite.

RIVER WATERS AND SEWAGE EFFLUENTS

A similar discrepancy between nitrate determined chemically and nitrate calculated from ultra-violet absorption (corrected for organic matter, as described above) has been found in some of the few river waters for which comparisons have so far been made. The technique has also been applied in some laboratory experiments on biochemical oxidation (for 14 days) of ammonia in river waters. In some of these experiments, it was found that the spectrophotometric computation of nitrate after oxidation was in agreement with the chemical determination of nitrate (in absence of nitrite), and the balance with the initial nitrogen content was substantially correct. In experiments with certain other samples, the final nitrate content, as determined chemically, was less than half of that expected from the initial ammonia content; however, the ultra-violet absorption at 2100 A approximately indicated the amount of oxidised nitrogen expected (in absence of nitrite).

Similarly, it has been found in a few instances that an apparent high loss of nitrogen in sewage-works filters was accounted for by additional oxidised nitrogen, as indicated by the ultra-violet absorption at 2100 A of the effluent. A series of samples from a sewage works was tested and then kept for a few weeks at room temperature; it was found that the nitrogen apparently missing from the fresh effluent from a group of filters was detected by ultra-violet absorption and that it appeared gradually as nitrate on keeping (the remaining ammoniacal nitrogen was sufficient to produce only a small part of this additional nitrate). Effluent sampled at the same time from a new filter at the same works was of generally better quality and had a nitrate content approximately equal to that calculated from the ultra-violet absorption. The results are summarised in Table III; the samples were composite over a period of 3 hours.

DISCUSSION OF RESULTS

In a large proportion of well waters, and in some river waters and effluents the ultra-violet absorption at 2100 A affords an excellent means of determining nitrate. However,

in some high-quality well waters, river waters and sewage effluents it appears that the content of oxidised nitrogen is not always completely assessed by the usual determinations of nitrite and nitrate. For such samples, ultra-violet absorption indicates the presence of a substantial proportion of the oxidised nitrogen in a form that only appears gradually as nitrate when the sample is kept for a few weeks.

TABLE III

RESULTS FOR SEWAGE AND SEWAGE EFFLUENTS

Time after sampling, days	Ammoniacal nitrogen present, p.p.m.	Albuminoid nitrogen present, p.p.m.	Nitrate content, as nitrogen— calculated from optical density, p.p.m.	Nitrate content, as nitrogen— determined by reduction, p.p.m.
Settled sewage—				
1	33	3·3	—	—
Effluent from new filter—				
1	0·25	0·90	34	32
8	n.d.	n.d.	n.d.	31
Effluent from old filter—				
1	1·3	1·5	35	20
8	n.d.	n.d.	33	26
19	0·1	0·9	n.d.	29
41	n.d.	n.d.	34	33
57	n.d.	n.d.	34	36

Corbet[7] showed that hyponitrite is sometimes produced by bacteria in soil, but it is clear from the ease of oxidation of this substance and from its ultra-violet absorption spectrum that our results are not due to hyponitrite. However, Addison, Gamlen and Thompson[8] described the preparation and some properties of a series of hyponitrites and oxidation products having the formula $Na_2N_2O_x$, in which x is between 2 and 6. As far as information is available, it appears possible that our results may be due to microbiological formation of the last member of this series or some other compound that is comparatively unstable in aqueous solution and gradually changes to nitrate. Some of the complications in determining such substances were described recently[9]; further investigation of our results presents difficulty, partly because samples of interest cannot often be found when desired.

However, observation of the ultra-violet absorption may give additional and essential information in investigations of nitrification in soils, sewage effluents and waters.

The nitrate determinations by the phenoldisulphonic acid method were carried out by Dr. D. A. Brown. Other help has been given by Mrs. I. Meckiffe.

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Determination of Alkylbenzenesulphonates in River Waters and Sewage

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An improved colorimetric method has been developed for the accurate determination of alkylbenzenesulphonates in waters. The procedure is less subject to error from naturally occurring substances than is Longwell and Maniece's method. Agreement with infra-red analysis is good, and the method is of value when infra-red equipment is not available or when a more rapid result is required. The method can also be used satisfactorily on sewage effluent and settled sewage when more accurate detergent analysis of these is needed. The method is simple to perform and combines principles from other reported procedures; it gives good recoveries of known detergent additions and is reproducible.

Alkylbenzenesulphonates are currently the most important anionic synthetic detergents in use, and in recent years much attention has been focused on the determination of traces of these materials in rivers and sewage. Many methods have been proposed, most of which depend on the formation of a coloured salt from the anionic detergent and a dye. The salt is soluble in organic solvents, extractable from an aqueous phase and is determined colorimetrically. Methylene blue is the dye most often used.

Such a method was proposed by Longwell and Maniece,[1] following the setting up by the Minister of Housing and Local Government of a Committee to examine the implications of the increased use of synthetic detergents. This method has become official in this country and is referred to hereafter as such. It was an improvement on an earlier method described by Degens, Evans, Kommer and Windsor[2] in that it overcame interference from inorganic ions. It is recognised, however, that various organic substances present in natural waters and sewage are liable to interfere with the official method and cause high results, the errors being most serious when accurate results are required at extremely low detergent levels, such as in rivers and tap-waters.

Methylene blue was also used in a much more complex method developed in the U.S.A. by Fairing and Short.[3] Moore and Kolbeson[4] recommended substitution of methyl green for methylene blue and extraction of the detergent - dye complex by benzene. This removed some interference, notably from inorganic ions, but other interfering substances remained.

As no colorimetric method is entirely specific, a Sub-Committee of the Association of American Soap and Glycerine Producers' Technical Advisory Committee studied the problem, and its members collaboratively developed a specific quantitative infra-red method[5] for determining traces of alkylbenzenesulphonates in supplies of raw and treated water. This is a most useful referee method and gives positive identification of the detergent. It has, however, three drawbacks: (*i*) it is time-consuming—2 to 3 days elapsed time for an analysis, (*ii*) large samples are required—usually more than 10 litres—and (*iii*) expensive infra-red equipment is necessary. A methylene blue finish can be applied to the method, but disadvantages (*i*) and (*ii*) remain and positive detergent identification is not possible.

It was apparent that the need still existed for a method that would give results of the same order of accuracy as the infra-red method but would avoid its drawbacks, and the work described in this paper was designed to this end. The best features of available colorimetric procedures, notably the official method and that described by Fairing and Short, have been used to provide the basis of the proposed method.

The principal features of these two methods may be summarised as follows. In the official method, interference from inorganic ions is overcome and interference from proteins is reduced by forming the complex with methylene blue in alkaline solution and then washing with an acid solution of methylene blue. In Fairing and Short's method, the detergent is removed from substances causing negative interference, such as proteins, by amine - chloroform extraction from approximately neutral solution. Interference from organic sulphates and other hydrolysable materials is then destroyed by acid hydrolysis. The detergent is

finally isolated from substances causing positive interference by amine - hexane extraction from acid solution and the complex is formed with methylene blue.

We thought that interference from hydrolysable materials should be removed at the start, and acid hydrolysis is the first step in the proposed method. The next step is a modified form of Fairing and Short's amine extractions to isolate the detergent from many interfering substances. Finally, the detergent - methylene blue complex is formed by what is essentially the official procedure. Adsorption of the detergent on carbon was also considered as a preliminary means of purification, but was found to be unsatisfactory.

Experimental

Hydrolysis—

This destroys interference from hydrolysable substances and also removes sulphides; treatment with hydrogen peroxide when sulphides are present, as described in the official method, is therefore unnecessary.

Hydrolysis was complete when the sample, adjusted to 100 ml, was boiled for 1 hour with 50 ml of approximately 1·0 *N* sulphuric acid, the reaction being carried out in a beaker fitted with a cover-glass. There was no indication that alkylbenzenesulphonic acids were lost by steam-distillation, even when acid concentration and time of boiling were doubled and the sample was evaporated to 25 ml. In every experiment, the recovery of alkylbenzenesulphonate so treated was 100 per cent.

Amine extractions—

Fairing and Short claimed that extraction with *n*-hexane separated the detergent - 1-methylheptylamine complex from substances causing positive interference, and this was confirmed. However, these workers had shown that extraction of detergent was not complete when substances causing negative interference, such as proteins, were present, as these materials tended to hold back the detergent and thereby cause low recoveries. This effect had been overcome by carrying out a preliminary extraction of the amine complex with chloroform, a more polar solvent than hexane. By this means, all the detergent was extracted, together with some substances causing positive interference. The latter were then separated by the amine - hexane extraction from aqueous solution.

Fairing and Short's procedure was simplified by carrying out both the chloroform and hexane extractions at pH 7·5, instead of at pH values of 7·5 and 4·8, respectively. Light petroleum (boiling range 40° to 60° C) was used instead of hexane, as it was more readily available in the required grade.

Whereas the amine - chloroform extraction readily afforded quantitative recovery of detergent, the amine - light petroleum extraction initially recovered only 85 to 90 per cent. of the detergent known to be present. This was traced to adsorption of detergent on the glass separating funnels. The problem was solved by washing the separating funnels with methanol containing a trace of heptylamine and adding the washings to the light petroleum extracts; recoveries by this procedure were 100 per cent. Further simplification was achieved by adding the amine dropwise at each extraction instead of preparing dilute solutions daily, as in Fairing and Short's method.

Determination with methylene blue—

To prevent competitive complex formation by the stronger base heptylamine, and hence low results, it is essential to remove all traces of heptylamine before extraction with methylene blue solution. Satisfactory conditions were obtained by boiling the aqueous solution of the amine extract until it was free from solvent and excess of amine, and then boiling for a further 15 minutes with an excess of alkali to decompose the amine - detergent complex.

The determination was then completed by the official method with a few minor modifications. First, it was possible to reduce the reagent blank value by pre-extracting the methylene blue solution with chloroform. It was considered to be preferable to pre-extract only sufficient reagent for 1 day's use, since, after being set aside for several weeks, the extracted dye solution imparted a yellowish tint to the reagent blank solution. This was believed to be caused by decomposition of the chloroform retained in the dye solution. In the absence of chloroform, aqueous methylene blue solutions are stable for long periods.

Secondly, the pH of the alkaline extraction was more closely controlled at 10·5; in the official method, the pH can vary between 10·0 and 10·5. Three extractions of the detergent - dye complex with 15-ml portions of chloroform were preferred to one extraction of 15 ml and then two of 10 ml. It was more convenient and effective to swirl the separating funnel between extractions to dislodge droplets from the sides than to rinse it with 2 ml of chloroform from a burette. For further convenience, only one (neutral) methylene blue solution was prepared. For the acid extraction, 1 ml of 1·0 *N* sulphuric acid was added to the separating funnel containing the neutral dye solution, as required. Sulphuric acid at this concentration is used for the hydrolysis and hence need not be specially prepared.

ADSORPTION ON GLASS—

To prevent losses of detergent by adsorption on the surfaces of the glass apparatus, Fairing and Short took many precautions, which included silicone treatment of all glassware, avoiding the use of concentrated acids in washing, and rinsing with methanol.

Experiments on the recovery of alkylbenzenesulphonate from aqueous solution by extraction with amine - light petroleum mixture showed that 100 per cent. recovery could be attained without silicone treatment of the glass and that the use of concentrated acids in cleaning glassware did not affect the results, as the amine - detergent complex was readily removed by rinsing with methanol - heptylamine mixture. It was further observed that, in alkaline solution, alkylbenzenesulphonate did not adhere to the glass surfaces. To avoid losses by adsorption, hydrolysis and all evaporations are carried out in the same beaker and the final transfer from the beaker is in alkaline solution. The final transfer from the separating funnel, *i.e.*, after extraction with amine - light petroleum mixture, is followed by rinsing with methanol - heptylamine mixture. No other apparatus is used in which losses by adsorption might take place.

It is of the greatest importance to ensure that all glassware is free from adsorbed detergent before a test is started. Various cleaning solutions have been proposed, but the most effective and convenient was found to be methanol or ethanol containing approximately 10 per cent. v/v of concentrated hydrochloric acid. This solution also efficiently removes heptylamine and methylene blue.

METHOD

APPARATUS—

Clean all glassware before use and when changing from extractions with heptylamine to those with methylene blue solution or *vice versa* by rinsing thoroughly with ethanol or methanol to which has been added approximately 10 per cent. v/v of concentrated hydrochloric acid. Finally, rinse the glassware well with water.

REAGENTS—

All materials should be of recognised analytical-reagent grade. Solutions should be prepared in distilled or ion-free water.

Sulphuric acid, approximately 1·0 N.

Sodium hydroxide solution, 10 *per cent. w/v, aqueous.*

Chloroform.

Light petroleum, boiling range 40° to 60° C.

1-*Methylheptylamine.*

Methanol.

Manoxol O.T. solution—Dissolve 1·000 g of Manoxol O.T., sodium dioctylsulphosuccinate, in water, and dilute to 1 litre. Dilute 10 ml of this solution to 1 litre with water to give a solution containing 10 μg of Manoxol O.T. per ml. This is the primary standard.

Alkylbenzenesulphonate solution—Determine the purity of the solid alkylbenzenesulphonate by titration against a cationic reagent; use a recognised procedure, such as that described by Epton.[6] Prepare an aqueous solution containing 10 μg of pure alkylbenzenesulphonate per ml. This is the secondary standard.

Neutral buffer solution—Dissolve 10 g of potassium dihydrogen orthophosphate in 800 ml of water, adjust to pH 7·5 with 10 per cent. w/v sodium hydroxide solution, and dilute to 1 litre.

Alkaline buffer solution—Dissolve 10 g of disodium hydrogen orthophosphate in 800 ml of water, adjust to pH 10·5 with 10 per cent. w/v sodium hydroxide solution, and dilute to 1 litre.

Methylene blue solution—Dissolve 0·2 g of oxidation - reduction grade methylene blue in 1 litre of water. To reduce the blank value, extract sufficient of the reagent for 1 day's use three times with chloroform, and discard the extracts. Do not store the extracted methylene blue solution for more than 1 day, as traces of retained chloroform will slowly decompose and may eventually cause high blank values.

PROCEDURE—

Place a volume of sample containing 25 to 150 μg of anionic detergent in a 400-ml beaker, and adjust the volume to 100 ml by dilution or evaporation as necessary. Use 100 ml of distilled water as blank, and treat exactly as for the sample.

Add 50 ml of 1·0 *N* sulphuric acid, cover with a clock-glass, and boil for 1 hour at a rate such that the volume is about 50 ml at the end of this period; add water, if necessary, to prevent the volume falling below 50 ml. Neutralise the solution to phenolphthalein with 10 per cent. w/v sodium hydroxide solution, added dropwise near the end-point, and transfer to a 250-ml separating funnel with sufficient water to make a total volume of 100 ml. Reserve the beaker for the subsequent collection of chloroform extracts.

Add 10 ml of neutral buffer solution, and extract four times with 25-ml portions of chloroform, to each of which has been added 1 drop of 1-methylheptylamine from a pipette dropper. (For samples that form persistent emulsions, increase the number of extractions to five; most emulsions can be readily broken by rubbing with a paddle-shaped polythene rod.) For each extraction, shake gently and evenly twice a second for 2 minutes. Combine the chloroform extracts in the 400-ml beaker, add 5 ml of water, and evaporate the chloroform and excess of amine on a steam or hot-water bath. Discard the aqueous layer from the separating funnel, which can be used without washing for the extraction with light petroleum.

Wash the sides of the beaker with 10 to 15 ml of water, warm for a few minutes on the bath to ensure complete dissolution, and transfer to the separating funnel with sufficient water to make a total volume of 50 ml. Reserve the beaker for the subsequent collection of light petroleum extracts. Add 10 ml of neutral buffer solution, and extract four times with 25-ml portions of light petroleum, to each of which has been added 1 drop of 1-methylheptylamine. Shake for 2 minutes for each extraction. Run the aqueous layer, as necessary, into a second separating funnel, and combine the light petroleum extracts in the 400-ml beaker.

Discard the aqueous layer after the fourth extraction, place 15 ml of methanol containing 1 drop of 1-methylheptylamine in the separating funnel, and shake for 30 seconds. Run the methanol into the second separating funnel, and again shake for 30 seconds. Run the methanol into the beaker containing the light petroleum extracts. Repeat the rinsing procedure with 25 ml of water, and add this to the methanol - light petroleum mixture.

Evaporate the light petroleum on the steam or hot-water bath, and then boil on a hot-plate until free from excess of amine, as indicated by odour and by the solution becoming colourless to phenolphthalein. Maintain the volume at approximately 50 ml by adding water as necessary. Add 5 ml of 10 per cent. w/v sodium hydroxide solution, and boil gently for 15 minutes. Occasionally rinse the sides of the beaker with water to maintain the volume at approximately 50 ml.

Remove all traces of 1-methylheptylamine from the separating funnel by rinsing with alcohol - hydrochloric acid mixture and then with water, and discard the rinsings. Transfer the sample solution to the separating funnel, neutralise to phenolphthalein with 1·0 *N* sulphuric acid, and add 1 drop of approximately 1·0 *N* sodium hydroxide to adjust the pH to between 8 and 10.

Cool, add 10 ml of alkaline buffer solution, 5 ml of methylene blue solution and 15 ml of chloroform, shake for 1 minute, and allow to settle. Run the chloroform layer into a second separating funnel containing 100 ml of water, 1 ml of 1·0 *N* sulphuric acid and 5 ml of methylene blue solution. Swirl the first separating funnel to dislodge droplets from its sides, and add these drops to the contents of the second separating funnel.

Shake the second separating funnel for 1 minute, and allow the contents to settle. Run the chloroform layer through a funnel containing a glass-wool plug wetted with chloroform into a 50-ml calibrated flask. Swirl the separating funnel as before. Repeat the extraction twice more with 15-ml portions of chloroform, and combine the extracts in the calibrated

flask. Rinse the glass-wool plug with sufficient chloroform to dilute to volume, add the rinsings to the contents of the flask, and mix the solution well.

Measure the optical density of the sample solution against the blank with either a spectrophotometer at 650 mμ or an absorptiometer fitted with an appropriate filter. Use a suitably sized cell. From a calibration graph, determine the amount of detergent, in micrograms, present in the portion of sample analysed (correct for cell size if necessary).

$$\text{Amount of alkylbenzenesulphonate present, as p.p.m of Manoxol O.T.} = \frac{\text{Amount of Manoxol O.T., } \mu g}{\text{Volume of sample, ml}}$$

PREPARATION OF CALIBRATION GRAPHS—

Treat suitable volumes (5, 10, 15 and 20 ml) of alkylbenzenesulphonate solution by the procedure described above, and plot the optical densities so obtained against the number of micrograms of alkylbenzenesulphonate present. The calibration graph should be a straight line passing through the origin.

To convert to Manoxol O.T., prepare separate calibration graphs for the Manoxol O.T. and alkylbenzenesulphonate solutions. Place the appropriate volumes of the standard solutions in separating funnels. Add to the contents of each separating funnel 10 ml of alkaline buffer solution, 5 ml of methylene blue solution and 15 ml of chloroform, shake for 1 minute, and then continue as described above. Calculate a factor from the slopes of the respective graphs to convert micrograms of alkylbenzenesulphonate to micrograms of Manoxol O.T.

RESULTS

INTERFERENCES—

Various natural organic materials have been reported[3] to cause positive interference when samples known to contain no alkylbenzenesulphonate are analysed directly with methylene blue. Since these analyses were made by a methylene blue method other than the official one, a brief check by the latter method was made on aqueous extracts of natural vegetation that had been digested for 5 to 7 days at room temperature. The fact that interference is encountered is shown by the results in Table I. Persistent emulsions were obtained, so that these results are probably too low.

TABLE I

INTERFERENCE FOUND BY OFFICIAL METHOD

Material	Soluble solids present, p.p.m.	Apparent detergent present, as Manoxol O.T., p.p.m.
Wood extract	135	0·25
Bracken extract.. ..	1375	0·59
Leaf extract	775	0·31
Straw extract	1490	0·73

A comparison was then made of the proposed method and the official method (and in one instance, the infra-red method) on a selection of samples likely to cause interference. The results are shown in Table II and indicate significant interference with the official method. The extracts of river-bed and river-bank vegetation apparently contained actual synthetic detergent; the corresponding river water was found to contain 0·06 p.p.m. of detergent by the proposed method and 0·13 p.p.m. by the official method.

When the official method was used on solutions of the detergent in distilled water, further chloroform extracts after the usual three were always colourless. However, in all experiments with samples giving positive interference, further extracts were still blue. This suggests that the methylene blue complexes of the interfering substances are less readily extractable by chloroform from aqueous solution than is the detergent complex. With the proposed method, further extractions at any stage did not increase the final result.

TABLE II

INTERFERENCES FOUND BY VARIOUS METHODS

The samples of peaty water were taken in an area remote from human habitation

Sample	Apparent detergent, as Manoxol O.T., found by— official method, p.p.m.	proposed method, p.p.m.
Urine	2·46	0·02
Aqueous hay extract	0·21	0·01
Aqueous coffee extract	0·68	0·04
Aqueous tea extract	0·16	Nil
Peaty water A	0·13	Nil
Peaty water B*	0·11	Nil
Peaty water C	0·18	Nil
Extract of river-bed vegetation (1800 p.p.m. of soluble solids)	0·77	0·08
Extract of river-bank vegetation (2400 p.p.m. of soluble solids)	0·50	0·23

* No detergent detected by infra-red analysis.

RECOVERY OF DETERGENT—

Calibration graphs plotted from results by the proposed method were identical with those from results by the official method, thereby indicating that no detergent is lost in the various purification steps. Detergent additions were then made to a variety of materials, and the results of recovery experiments are shown in Table III. Although recoveries were slightly high at the lower levels of detergent, the maximum error was equivalent to only 0·03 p.p.m. of detergent. The average recovery is identical to the average detergent addition.

TABLE III

RECOVERY OF ADDED DETERGENT BY THE PROPOSED METHOD

Each result is expressed as Manoxol O.T.

Sample	Detergent originally present, p.p.m.	Detergent added, p.p.m.	Detergent found, p.p.m.	Detergent recovered, p.p.m.
Distilled water	Nil	1·20	1·20	1·20
Peaty water	Nil	1·20	1·17	1·17
Aqueous hay extract	0·03	1·20	1·26	1·23
Urine	0·05	1·20	1·21	1·16
River water A	0·07	1·20	1·24	1·17
River water B	0·03	1·20	1·22	1·19
River water C	0·20	0·12	0·35	0·15
River water C	0·20	0·24	0·47	0·27
River water D	0·20	0·12	0·33	0·13
River water D	0·20	0·24	0·47	0·27

REPRODUCIBILITY—

Two samples of river water having different apparent detergent contents were analysed several times by the proposed method; the results, expressed as Manoxol O.T., were as follows—

Detergent found in river A, p.p.m. .. .. 0·06, 0·06, 0·06, 0·07
Detergent found in river B, p.p.m. .. .. 0·36, 0·35, 0·39, 0·37, 0·35, 0·38

COMPARISON OF METHODS ON RIVER AND TAP-WATER—

The proposed method is considered to be especially applicable to samples of river and tap-water when accurate detergent analysis is required. Accordingly, a detailed comparison was made between the official, infra-red and proposed methods on different samples taken over a period from a single river. The results are shown in Table IV, from which it can be seen that the proposed method shows close agreement with the infra-red (referee) method, but that, owing to positive interference, the official method gives results about 40 per cent. high.

TABLE IV

COMPARISON OF METHODS ON WATER FROM ONE RIVER

Each result is expressed as Manoxol O.T.

Sample No.	Detergent found by— official method, p.p.m.	proposed method, p.p.m.	infra-red method, p.p.m.
1	1·22	1·02	0·91
2	1·47	0·73	1·02
3	1·03	0·78	0·79
4	1·36	1·10	1·07
5	1·17	0·78	0·71
6	1·26	0·91	0·86
7	1·05	0·67	0·68
8	0·97	0·83	0·91
9	1·43	0·91	0·91
10	1·21	0·86	0·90
11	1·34	0·86	0·98
12	0·91	0·68	0·70

Further comparisons were made on samples of water from other rivers and also on one sample of tap-water; the results are shown in Table V. In all determinations, results by the official method are higher.

TABLE V

COMPARISON OF METHODS ON WATER FROM OTHER RIVERS AND TAP-WATER

Each result is expressed as Manoxol O.T.

	Sample No.	Detergent found by— official method, p.p.m.	proposed method, p.p.m.
River A	1	1·84	1·35
	2	1·04	0·56
	3	1·59	1·28
	4	1·39	1·13
	5	1·26	0·89
	6	1·51	1·19
	7	1·75	1·20
	8	1·90	1·65
	9	1·95	1·52
	10	2·24	1·90
	11	1·40	0·98
River B	1	0·23	0·08
	2	0·23	0·07
River C	1	0·13	0·06
River D	1	0·04	Nil
Tap-water ..	1	0·05	Nil

COMPARISON OF METHODS ON SEWAGE EFFLUENT AND SETTLED SEWAGE—

It was considered that the proposed method might give a more reliable result than the official method for alkylbenzenesulphonate not decomposed by sewage works, since many substances present in sewage may cause positive interference when analysed with methylene blue in the usual way.

Results by the proposed, official and infra-red methods were compared for samples of final effluent from sewage that contained approximately 40 per cent. of trade waste, and then by the first two methods only on samples of final effluent from essentially domestic sewage. The samples were taken over a period, and the results are shown in Table VI. The proposed and infra-red methods show close agreement, but results by the official method are higher, to about the same extent, on average, as for river waters. The differences may be expected to vary with the nature of the trade wastes in the sewage.

TABLE VI

COMPARISON OF METHODS ON SEWAGE EFFLUENT AND SETTLED SEWAGE

Each result is expressed as Manoxol O.T. The sewages correspond with the effluents

Sample No.	Detergent found in final effluent by— official method, p.p.m.	proposed method, p.p.m.	infra-red method, p.p.m.	Detergent found in settled sewage by— official method, p.p.m.	proposed method, p.p.m.
Samples from sewage containing waste—					
1	3·9	2·7	2·9	3·7	2·6
2	2·2	1·6	1·9	3·7	2·7
3	2·9	2·6	2·5	4·6	3·6
4	2·5	1·7	1·7	14·0	9·6
5	2·4	1·9	1·8	12·2	9·9
6	3·1	2·0	1·9	14·7	9·7
7	2·9	2·0	2·2	13·9	9·8
8	3·0	1·6	1·9	14·5	10·2
9	4·2	2·2	1·9	9·7	8·9
10	3·6	2·6	2·7	17·0	12·6
11	2·3	2·2	2·3	9·2	7·7
Samples from essentially domestic sewage—					
12	5·3	4·5	—	18·7	14·4
13	4·7	3·1	—	7·3	5·4
14	5·0	4·8	—	12·0	7·8
	5·3	4·9	—		
15	4·1	3·6	—	13·4	9·8
16	5·0	4·3	—	13·2	9·9
17	7·7	5·3	—	12·3	8·4
18	4·2	3·2	—	11·4	9·0
19	7·4	5·7	—	18·9	15·0
20	5·9	5·7	—	8·0	7·2
21	6·2	5·0	—	12·7	9·8
22	7·1	4·9	—	15·3	9·2

Similar comparisons between the official and the proposed methods were made on settled sewage to indicate the extent of interference; these results are also shown in Table VI. Results by the official method are again higher, by 35 per cent. on average.

CONCLUSIONS

An improved colorimetric method based on principles reported in earlier methods has been developed for the more accurate determination of alkylbenzenesulphonates in natural and river waters. Compared with the official method, results are much less subject to interference from other materials present that form extractable complexes with methylene blue.

Agreement with the referee infra-red method is good, but the proposed method is much shorter and more convenient and does not require such specialised equipment. Recovery of known detergent additions from various waters was good, and results are reproducible.

The proposed method can also be used satisfactorily to analyse sewage effluent and settled sewage, for which the official method gives high results. The official method, however, is probably adequate for routine sewage-works control.

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A Routine Cryoscopic Method for Assaying β-Picoline, γ-Picoline and 2:6-Lutidine

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This paper describes a cryoscopic method suitable for the routine assay of β- and γ-picoline and 2:6-lutidine. Errors caused by the presence of moisture or its ingress during the analysis are avoided by applying a correction based on the water content of the sample at the end of the freezing-point determination.

The magnitude of this correction has been determined for each base. The depressions of the freezing-points of these compounds caused by the presence of selected bases in concentrations up to 10 moles per cent. have been measured and found to be linear over the range examined.

Cryoscopic constants and latent heats of fusion have been calculated, and equations connecting freezing-point with purity are presented.

With the greater availability of β-picoline, γ-picoline and 2:6-lutidine in commercially pure form, the need for a precise method of determining purity has become acute. Instrumental methods, such as infra-red spectrometry and gas - liquid chromatography, are, in general, accurate to within ±1 per cent., which is insufficient at the high levels of purity under consideration. Moreover, such methods involve the use of specialised and expensive apparatus. Our aim was to devise a simple, rapid and accurate method, which could be used for controlling the manufacturing process and for assaying deliveries made to customers.

The freezing-points of the pure bases have been reported by Biddiscombe, Coulson, Handley and Herington.[1] Graphs of the freezing-points of some binary mixtures of the three bases have been published by Coulson and Jones[2] and Glowacki and Winans[3]; these sets of graphs are not in good agreement. Biddiscombe and his co-workers also reported the molecular depressions of the freezing-points when *iso*octane was used as solute. We have found no reference to the effect of water on the freezing-points of the bases, although, because of their hygroscopic nature, this is an important factor in a cryoscopic method.

It was decided that a technique based on freezing-points would give the best solution to the problem, provided that information was obtained about the depressions caused by water and various possible basic contaminants.

Experimental

Depression of freezing-points by water—

A series of experiments was made with γ-picoline of about 98 per cent. purity, which contained 0·025 per cent. w/w of water, as determined by the Karl Fischer method. Weighed amounts of water were added to weighed amounts of the picoline, and the freezing-points of the mixtures were plotted against the calculated total water content. The resulting graph indicated a linear relationship up to a water content of 1 per cent. w/w, the maximum examined, but the points were badly scattered about the regression line.

The experiments were repeated with the modification that the water contents of the mixtures were determined by the Karl Fischer method on a portion of each mixture before its freezing-point was determined. The calculated water contents were identical with the water contents by analysis, and the graph of freezing-point against water content again showed considerable scatter about the regression line.

It was inferred that the samples were absorbing water from the atmosphere during the freezing-point determination and that the amount of water so absorbed varied with the length of time taken for the determination.

Samples having known water contents were therefore prepared as before and their freezing-points were measured. Immediately upon completion of the freezing-point determination, the contents of the tube were rapidly melted, with stirring, and brought to a temperature of 15° C. A known volume of the resulting liquid was transferred to the cell of the Karl

Fischer apparatus and its water content (per cent. w/v) was determined. The water content was calculated on a w/w basis by using the density of γ-picoline at 15° C. The results so obtained were plotted against the corresponding freezing-points, and an extremely high degree of correlation was found.

The same technique was applied to one further sample of γ-picoline and two each of β-picoline and 2:6-lutidine. All tests were carried out at a minimum of five different water contents in the range 0·02 to 1·0 per cent. w/w.

During these experiments, it became obvious that a correction for the effect of water could be avoided only with the greatest difficulty and would involve exhaustive drying of the sample and rigorous exclusion of atmospheric moisture. Drying to a water content of about 0·02 per cent. was possible either by heating over fused potassium hydroxide, or by slow distillation by which water was removed as an azeotrope. The exclusion of moisture during the freezing-point determination, *e.g.*, by passing a dry inert gas through the sample, was considered, but was rejected as an unnecessary complication, since the results showed that allowance for the amount of water present could be made with a high degree of precision. The water content of commercial samples of "dry" picolines does not normally exceed 0·2 per cent., and the uncertainty of the correction at this level does not exceed about 0·02° C.

PURIFICATION OF BASES—

The purity of the bases used in the experiments just described ranged from about 96 to about 99 per cent. It was considered that this level of purity was sufficient for determining the effect of water on the freezing-points, but too low to permit accurate assessment of the effect of the individual bases on the freezing-points of each other.

We were able to use, as starting materials, samples of our commercial β- and γ-picoline and 2:6-lutidine, all of which were of at least 98 per cent. purity. Further purification was effected by repeated slow fractional freezing, as described by Biddiscombe, Coulson, Handley and Herington,[1] the process being repeated until no further rise in freezing-point was observed. The freezing-points of the purified bases were similar to those published by these workers for the pure compounds and their infra-red spectra were identical with those of reference standards obtained from the National Chemical Laboratory, Teddington.

Commercial α-picoline of about 98 per cent. purity was further purified by high-efficiency fractional distillation. The final product was analysed by gas - liquid chromatography; no impurities were detected.

Samples of other possible minor impurities, *viz.*, 2-ethylpyridine and 2:4-lutidine, probably of about 95 per cent. purity, were further purified by distillation. Examination of the purified bases by gas - liquid chromatography again showed no impurities.

DEPRESSION OF FREEZING-POINTS BY BASIC IMPURITIES—

Weighed amounts of each impurity in turn were added to weighed amounts of the corresponding main component (see Table I). The water contents of both components of each

TABLE I

MAIN COMPONENTS AND IMPURITIES STUDIED

Main component	Impurities
β-Picoline	α-Picoline γ-Picoline 2:6-Lutidine
γ-Picoline	α-Picoline β-Picoline 2:4-Lutidine 2:6-Lutidine
2:6-Lutidine	α-Picoline β-Picoline γ-Picoline 2-Ethylpyridine

mixture were determined, and the molar concentration of the dry impurity in the total dry mixture was calculated. The freezing-points were corrected to a dry basis by determination of the water content at the end of the test and reference to the graphs described above.

Water contents were always kept as low as possible (below 0·1 per cent.) to minimise the correction. The added impurities covered the range 0 to 10 moles per cent. of the mixture, and the average freezing-point depression produced by 1 mole per cent. of impurity was calculated over this range by the method of least squares.

METHOD

APPARATUS—

The freezing-point apparatus is of the conventional type and consists of an inner test-tube, 150 mm × 25 mm, fitted concentrically by means of a cork inside a wider tube, 150 mm × 40 mm, which acts as an air-jacket. The inner tube is closed by a cork fitted with a suitable thermometer (see below) and a glass stirrer. The stirrer is a glass rod about 3 mm in diameter bent at its lower end into a loop at right angles to the axis of the rod. This loop is of suitable diameter (about 18 mm) to surround the stem of the thermometer and move easily up and down the inner tube. The thermometer is centrally placed in the cork and so positioned that the bottom of its bulb is about 1 cm above the bottom of the inner test-tube.

The cooling liquid is contained in a Dewar jar, internal diameter about 100 mm, to minimise absorption of heat from the atmosphere.

Apparatus for the determination of water content by the Karl Fischer method is also required.

THERMOMETERS—

For γ-picoline, the thermometer used is in accordance with the Standardisation of Tar Products Tests Committee's Schedule T1c (−10° to +20° C subdivided to 0·1° C, calibrated for 100-mm immersion).[4]

For β-picoline, we use a specially made 100-mm immersion thermometer covering the range 0° to −30° C subdivided to 0·1° C (obtained from H. J. Elliott Ltd., E-Mil Works, Treforest, Glamorgan). A satisfactory alternative is an American Society for Testing Materials thermometer No. 33C-52T (−38° to +42° C subdivided to 0·2° C, 51-mm immersion),[5] but the thermometer subdivided to 0·1° C is preferred.

Either the T1c or the 0° to −30° C thermometer may be used for 2:6-lutidine.

PROCEDURE—

Place in the Dewar jar an amount of cooling mixture such that, with the apparatus assembled, the level of liquid in the jar is at least as high as the level of the sample in the inner test-tube. Adjust the temperature of the mixture, immediately before use, to between 6° and 8° C below the expected freezing-point of the sample. (Suitable cooling mixtures can be prepared from solid carbon dioxide and ethanol; alternatively, for temperatures down to about −15° C, crushed ice and sodium chloride can be used and crushed ice and calcium chloride crystals for temperatures down to −25° C.) Place approximately 25 ml of the sample to be tested in the inner test-tube. Fit the stirrer and thermometer, and pre-cool, with stirring, to about 5° C above the expected freezing-point. Rapidly dry the outside of the test-tube, and fit it centrally inside the air-jacket, which is already in place in the cooling bath. Stir gently and continuously, and read the thermometer at 30-second intervals (estimate the temperature to 0·01° C). When the temperature has fallen to the expected freezing-point, introduce a seed crystal as rapidly as possibly, and continue the test. (The seed crystal can be conveniently introduced by raising the stirrer to its highest extent, without removal of the cork from the inner test-tube, and depositing a crystal from a glass rod as low as possible on it. The stirrer is then replaced in the liquid and stirring is continued.) The freezing-point corresponds to the first set of four consecutive readings during which the temperature remains constant. If supercooling occurs, the constant temperature will be observed after the temperature rise. A temperature rise of 1° C is the maximum permissible; if it exceeds this value, repeat the determination on a fresh portion of the sample.

Record the observed freezing-point, F, corrected for any scale error of the thermometer.

Remove the inner test-tube, complete with thermometer and stirrer, without delay, and heat rapidly, with stirring, until the temperature rises to between 14° and 16° C. By pipette, withdraw 20 ml of sample, preferably with use of a filler, and determine its water content (per cent. w/v).

Calculate the corrected freezing-point, F_0, for the dry substance by adding an amount wk, where w is the water content (per cent. w/v) and k is the factor relevant to the base being

tested; the values of k for β-picoline, γ-picoline and 2:6-lutidine are 1·94, 2·55 and 2·72, respectively. (These constants are the means of the figures in Table II converted to a per cent. w/v basis.)

Calculate the purity of the dry sample, P_0, from one of the following expressions—

(*i*) for β-picoline, P_0, mole per cent., $= 100 - \frac{(-18{\cdot}20 - F_0)}{0{\cdot}423}$;

(*ii*) for γ-picoline, P_0, mole per cent., $= 100 - \frac{(3{\cdot}65 - F_0)}{0{\cdot}544}$; and

(*iii*) for 2:6-lutidine, P_0, mole per cent., $= 100 - \frac{(-6{\cdot}10 - F_0)}{0{\cdot}472}$.

The factors in the denominators of these expressions are taken from Table IV (see p. 564).

If many samples are to be tested, it is much more convenient to prepare graphs from these expressions and the above-mentioned values of k.

Results

Table II shows results for the depression of the freezing-points of β- and γ-picoline and 2:6-lutidine by water. The results of each experiment were analysed in the way described below (for 2:6-lutidine)—

Observation No.	1	2	3	4	5
Freezing-point, ° C	−6·70	−7·02	−7·71	−8·10	−8·75
Water content (w), % w/w	0·095	0·197	0·477	0·659	0·895

The equation for the regression line is—

$$\text{Freezing point, } ^\circ\text{C} = -6{\cdot}491 - 2{\cdot}5066\, w,$$

and the standard error of the regression coefficient, k, is 0·061.

Table II

Depression of freezing-points of bases by water

Base	Sample No.	Depression of freezing-point caused by 1% w/w of water in total mixture (k), ° C	Standard error of k	Number of observations
β-Picoline	1	1·851	0·038	7
	2	1·873	0·038	10
γ-Picoline	3	2·413	0·045	8
	4	2·470	0·033	6
2:6-Lutidine	5	2·507	0·061	5
	6	2·532	0·076	7

Table III shows results for the depression of freezing-points of the bases by other bases.

Table III

Depression of freezing-points of bases by other bases

Main component	Freezing-point of main component, ° C	Impurity	Depression of freezing-point (θ) caused by 1 mole per cent. of impurity,* ° C	Standard error of θ	Number of observations
β-Picoline	−18·22	α-Picoline	0·419	0·008	8
		γ-Picoline	0·429	0·006	9
		2:6-Lutidine	0·420	0·010	7
γ-Picoline	+ 3·64	α-Picoline	0·548	0·003	6
		β-Picoline	0·537	0·005	8
		2:4-Lutidine	0·550	0·037	7
		2:6-Lutidine	0·542	0·004	7
2:6-Lutidine	− 6·12	α-Picoline	0·467	0·008	7
		β-Picoline	0·484	0·005	6
		γ-Picoline	0·469	0·010	6
		2-Ethylpyridine	0·467	0·005	7

* Mixture consists of 99 moles per cent. of dry main component and 1 mole per cent. of dry impurity.

The mean values of the freezing-constants in Table III, together with other constants calculated from them, are shown in Table IV.

TABLE IV

FREEZING-CONSTANTS FOR β- AND γ-PICOLINE AND 2:6-LUTIDINE

Main component	Depression of freezing-point caused by 1 mole per cent. of basic impurity, °C	Depression of freezing-point caused by 1 mole of basic impurity per 1000 g of main component, °C	Latent heat of fusion, calories per g
β-Picoline	0·423	3·90	33·2
γ-Picoline	0·544	5·01	30·4
2:6-Lutidine	0·472	5·01	28·3

DISCUSSION OF RESULTS

The figures in Tables II and III for the standard errors of the regression coefficient show the precision of the method and indicate that this is greater for the "base-in-base" experiments than for the "water-in-base." However, most samples of commercially pure bases contain less than 0·2 per cent. of water, and the uncertainty of the water correction at this level is approximately 0·02° C. Samples containing appreciably more water should be dried either by heating under reflux with potassium hydroxide or by slow distillation of the base - water azeotrope.

A "base-in-base" mixture would be expected to form ideal solutions, and the results in Table III show that, for a given main component, there are no significant differences in the effects of the various basic impurities. The grand means have therefore been calculated and are used in the expressions for calculation of purity. The depressions of the freezing-points of β-picoline and 2:6-lutidine differ from those reported by Biddiscombe, Coulson, Handley and Herington,[1] possibly because these workers used *iso*octane as impurity; they agree fairly closely with values deduced from small-scale graphs published by Glowacki and Winans[3] for some binary mixtures of the bases.

"Water-in-base" mixtures appear to form non-ideal solutions, as evidenced by the heat evolved in preparation of the mixtures. The depressions produced by 1 mole per cent. of water, calculated from the results in Table II, differ markedly from the results in Table III.

Our values (Table III) for the freezing-points of the purified bases are slightly lower than those published by Biddiscombe and his co-workers,[1] and the expressions for calculation of purity on p. 563 incorporate these workers' values, *viz.*, −18·20° C for β-picoline, 3·65° C for γ-picoline and −6·10° C for 2:6-lutidine.

The proposed method has been in use in our laboratories for several months, during which time the repeatability of the determination of freezing-point, corrected to the dry basis, has been found to be less than 0·05° C, equivalent to about 0·1 per cent. purity.

I acknowledge the help of Miss S. Rockingham and Mr. L. S. Hickman, by whom most of the practical work was done, and thank the Directors of The Midland Tar Distillers Ltd., Oldbury, for permission to publish this paper.

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A Rapid Volumetric Method for Determining Copper in a Carbonatite Ore

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The sample is digested with a mixture of concentrated nitric, orthophosphoric and perchloric acids. Salts are dissolved by boiling with distilled water, and the permanganic acid formed is reduced by oxalic acid solution. Iron is complexed with sodium fluoride. The pH of the solution is adjusted to between 2·5 and 3 with sodium acetate solution, and the copper is determined iodimetrically.

The proportions of the different minerals constituting the copper-bearing carbonatite at Phalaborwa in the north eastern Transvaal vary sharply in different samples. The copper content varies from a trace to 5 per cent., calcium oxide and iron each vary from 10 to 50 per cent. and phosphorus varies from 1 to 8 per cent., as P_2O_5. For such an ore, the conventional short and long iodide methods give variable results.

Conventional methods

In both short and long iodide methods, the sample is decomposed by digestion with nitric, hydrochloric and sulphuric acids. Heating is continued until fumes of sulphur trioxide are evolved. This produces a heavy precipitate of calcium sulphate from carbonatite samples, and an insoluble residue of magnetite remains in most tests.

Short iodide method—

The sample is decomposed with aqua regia and sulphuric acid until copious fumes of the latter are evolved. When cool, soluble salts are dissolved by boiling with distilled water. The cooled solution is neutralised with ammonia until a permanent precipitate of ferric hydroxide is obtained and then 4 drops of ammonia are added in excess. The precipitate is redissolved in 15 ml of glacial acetic acid and the iron is complexed by adding 2 to 3 g of sodium fluoride.[1] Subsequently, 2 to 3 g of potassium iodide crystals are added to the contents of the beaker, which is swirled for 15 seconds to liberate iodine. The iodine is titrated against standard sodium thiosulphate solution, starch being used as indicator, and the copper content of the sample is calculated.

Results by this method are inaccurate and not reproducible. End-points are transient, possibly owing to (*a*) the masking effect of the insoluble black magnetite on the colour change from blue to white at the end-point and (*b*) the heavy white precipitate of calcium sulphate, which adsorbs copper salts.

Long iodide method—

The sample is decomposed as in the short iodide method and soluble salts are dissolved by boiling with distilled water. A clear solution is obtained by filtering hot and washing with hot distilled water. The volume of the filtrate is adjusted to 300 ml and the solution is boiled. Sufficient 50 per cent. sodium thiosulphate solution to precipitate all the copper present as cuprous sulphide is slowly added. The granular precipitate is separated by filtration, washed with hot distilled water, dried and ignited in a porcelain crucible to cupric oxide, which is then dissolved in diluted nitric acid (1 + 1). The contents of the crucible are quantitatively washed into a 250-ml conical flask. The solution is neutralised with ammonia, a slight excess of ammonia is added and the excess is then removed by boiling. The solution is re-acidified with 15 ml of glacial acetic acid and copper is finally determined iodimetrically as before. No sodium fluoride is needed, as copper is separated from iron, etc.

This method gives low results, owing to adsorption of copper by calcium sulphate. The presence of calcium sulphate not only makes the boiling of the sample and the dissolution of soluble salts difficult and incomplete, but also slows down quantitative filtration and washing considerably. A rapid and accurate method for determining copper in the carbonatite ore was therefore sought.

Strickland states that bornite, chalcocite, chalcopyrite, malachite, azurite, chrysocolla and slags are soluble in hot concentrated orthophosphoric and nitric acids.[2] Perchloric acid also is used in the proposed method to oxidise sulphur.

METHOD

REAGENTS—

Sodium thiosulphate solution—Dissolve 32 g of crystalline sodium thiosulphate and 30 g of sodium carbonate in distilled water. Add 5 ml of chloroform, dilute to 8 litres with distilled water, and mix well. Store in a dark bottle.

Oxalic acid solution, 1 per cent. w/v, aqueous.

Starch solution—Mix 10 g of analytical-reagent grade starch into a paste with distilled water. Pour into 1 litre of boiling distilled water, boil for 1 minute, mix well by stirring, and cool. Store in a glass-stoppered bottle.

Sodium acetate solution, 30 per cent. w/v, aqueous.

STANDARDISATION OF SODIUM THIOSULPHATE SOLUTION—

Place a piece of analytical-reagent grade copper foil in a squat 250-ml beaker, cover with cold diluted nitric acid (1 + 3), and set aside for 5 minutes. Decant the acid, and wash thoroughly with distilled water. Rinse the foil with ethanol and then with diethyl ether, and warm gently until dry. Weigh approximately 20-mg portions of copper into each of three 500-ml conical flasks, and dissolve each portion in 2 to 3 ml of nitric acid. Evaporate to incipient dryness on a hot-plate (low heat) to remove nitrous fumes and excess of acid. Add 15 ml of distilled water to dissolve the copper nitrate, and then neutralise with diluted ammonia solution (1 + 1) until a precipitate of copper hydroxide just forms. Add 15 ml of glacial acetic acid, and cool. Add 2 to 3 g of potassium iodide crystals, and swirl the contents of the flask for 15 seconds to liberate iodine. Titrate against the sodium thiosulphate solution until a pale straw-yellow colour is obtained. Add a few drops of starch solution, and add titrant dropwise until the colour changes sharply from blue to white.

$$1 \text{ ml of sodium thiosulphate solution} \equiv \frac{\text{Weight of copper}}{\text{Titre}}, \text{ g of copper}$$

PROCEDURE—

Weigh 1 to 2 g of sample into a 500-ml conical flask, and add 10 ml each of concentrated nitric and orthophosphoric acids and 2 ml of perchloric acid. Heat on a hot-plate until copious fumes of perchloric acid are evolved, and maintain at this temperature for 20 minutes until all sulphur has been destroyed. Cool, add 120 ml of distilled water, and boil to dissolve all soluble salts. Add 1 per cent. oxalic acid solution dropwise to the boiling solution until the colour changes from pink to blue or until all permanganic acid has been reduced. Add 2 to 3 g of sodium fluoride to the hot solution, and immediately shake the flask to prevent the formation of a "fluoride cake." Add 15 ml of 30 per cent. sodium acetate solution, and thoroughly cool.

Add 2 to 3 g of analytical-reagent grade potassium iodide, and swirl the flask for 15 seconds to liberate iodine. Titrate against standard sodium thiosulphate solution (1 ml ≡ 0·001 g of Cu) until a pale straw-yellow colour is obtained. Add a few drops of starch solution, and add titrant dropwise until the colour changes sharply from blue to white. (The solution should remain white for at least 20 seconds.) Calculate the copper content of the sample.

RESULTS

A comparison of results by the proposed method with those by spectrophotometric determination of copper, the latter being assumed to be correct, for twenty-five ores having copper contents between 0·26 and 5·00 per cent. showed the mean deviation of the volumetric results to be 0·02 per cent. of copper and a standard deviation of less than 0·01 per cent.

A similar comparison with electrolytic determinations of copper in thirty-eight different ores having copper contents between 0·03 and 5·66 per cent. showed the mean deviation of the volumetric results to be 0·02 per cent. of copper and the standard deviation less than 0·01 per cent.

Different amounts of copper were added to a sample containing 10·54 mg of copper, and a series of recovery experiments was carried out by the proposed method; the results were as follows—

Weight of copper added, mg	2·00	11·20	13·00	14·20
Weight of added copper recovered, mg	1·98	11·04	12·82	14·15
Recovery, %	99·0	98·6	98·6	99·6

Conclusions

The proposed method is rapid and highly accurate; this is because (*a*) a clear solution of the sample is readily obtained without filtration and (*b*) decomposition by acid is complete. The sample remains in the same flask throughout, and mechanical loss of copper is therefore limited to a minimum. The presence of up to 50 per cent. of iron can be tolerated, and practically no insoluble magnetite is left in the sample. The method is suitable for the routine analysis of samples containing 0·03 to 5·0 per cent. of copper; forty determinations can be completed in 4 hours.

Interference by manganese, which is oxidised to permanganic acid under the conditions described is prevented by prior reduction with oxalic acid solution. According to Belcher and Wilson,[3] thorium liberates iodine from potassium iodide in acid solution. This is prevented, since thorium is precipitated as fluoride.

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Notes

THE EFFECT OF WATER ON THE DETERMINATION OF TOCOPHEROLS

A satisfactory scheme for detecting and determining tocopherols is based on the procedure devised by Brown[1] and modified by Eggitt and Ward,[2] who used reversed-phase paper chromatography to separate the tocopherols before determining them by Emmerie and Engel's method.[3] Green, Marcinkiewicz and Watt[4] made further improvements by incorporating adsorption paper chromatography into the technique, zinc carbonate being used as adsorbent, and introduced two-dimensional chromatography for separating and identifying the various tocopherols. In any paper-chromatographic method, the tocopherol spots are located on the paper, cut out and eluted with ethanol or other solvents. The concentrations of tocopherols are determined by adding ethanolic solutions of 2:2′-dipyridyl and ferric chloride to the eluates and then measuring the extinctions of the solutions at 520 mμ.

As the paper alone gives a measurable extinction with 2:2′-dipyridyl and ferric chloride, a similar blank sheet of paper must be included with the test papers during chromatography. When the tocopherol spots are cut out and eluted, corresponding pieces of the blank sheet must be treated similarly, and the extinction of the blank solution must be subtracted from that of the tocopherol solution. In early experiments, the value of this blank (the extinction of the solution of 2:2′-dipyridyl and ferric chloride measured in 1-cm cells at 520 mμ with a Unicam SP500 spectrophotometer) was found to vary from 0·060 to as much as 0·120. These variations seriously affected the determination of low concentrations of tocopherols.

During an investigation to find ways to reduce or regularise the blank value, water was added to the dipyridyl - ferric chloride mixture. A significant increase, proportional to the amount of water present, was observed in the extinction of the mixture. The effect of added water is shown in Fig. 1, which summarises the results of several experiments. One reason for the variation in the

blank value became clear. Since, in the second dimension, the papers are developed in an aqueous solvent, it is normal to dry them before the tocopherol spots are cut out. The usual method for drying the papers is to remove the developing solvent by means of a current of nitrogen. This method is extravagant with the gas and is so tedious that little attention may be paid to drying the blank sheet to the same extent as the test sheets. As a result, any residual moisture in the blank sheet will increase the extinction of the blank solution to a value sometimes even greater than that obtained from a faint tocopherol spot on a dry paper.

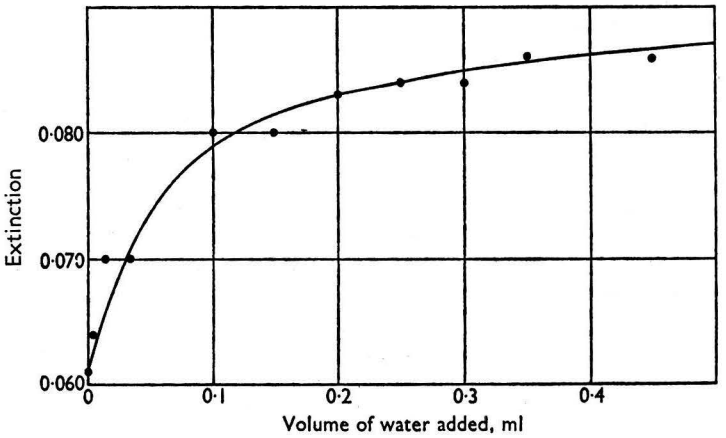

Fig. 1. Effect of adding water to a mixture of ethanolic 2:2′-dipyridyl and ferric chloride. Amounts and strengths of reagents were in accordance with the Analytical Methods Committee's recommendations,[5] and the volume of mixture was made up to 4·6 ml with ethanol throughout. Extinctions were measured in 1-cm cells with a Unicam SP500 spectrophotometer

An improved method for drying the papers was devised. The metal frame supporting the blank and test sheets of Whatman No. 4 filter-paper was spun on a turntable for 6 minutes at 170 r.p.m. in air and in dim light. All papers were dried effectively and equally, and there was no loss of tocopherol. When this procedure was used, the mean extinction of the blank solutions from fifty determinations of tocopherol contents made over 3 months was $0{\cdot}065 \pm 0{\cdot}0046$.

Whatever method is used to dry the papers, it is most important that, for accuracy, the blank sheet of paper should be dried to the same extent as the sheet containing the tocopherols.

I thank Dr. V. H. Booth for his help with this problem.

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Received *April 16th*, 1959

VOLUMETRIC DETERMINATION OF SILVER IN COBALT - SILVER MIXTURE

It appears that no convenient volumetric method is known for determining silver in admixture with cobalt. According to Scott and Furman,[1] a titration cannot be carried out "when highly coloured salts of cobalt, nickel and copper are present over 60 per cent. in the sample," and Treadwell and Hall[2] state that "nickel and cobalt must not be present to any extent because their salts are coloured."

The object of this work was to remove cobalt completely from a mixture of cobalt and silver nitrates. Disodium ethylenediaminetetra-acetate (EDTA) forms an anionic complex with tervalent cobalt, and this forms the basis for the removal of cobalt. When the solution is passed through a column of cation-exchange resin, silver is retained and cobalt is eliminated in the form of its complex.

Method

The ion-exchange column, 12 mm × 80 mm of Amberlite IR-120(H), was prepared in a 50-ml burette having a glass-wool plug at its base and about 2 cm of fine sand above this plug.

Procedure—

In each experiment, the volume of solution taken was such that its total silver content was within the break-through capacity of the column. This solution was evaporated just to dryness to drive off the acid, and the residue was dissolved in a mixture of about 20 ml of water and about 3 ml of 100-volume hydrogen peroxide. To this solution was added an excess of EDTA solution (1 ml of 0·1 *M* EDTA is equivalent to 5·894 mg of cobalt) and the mixture was boiled, with continuous shaking, for a few minutes and then cooled. (During this process, the colour of the solution became an intense violet.) The solution was then passed through the ion-exchange column at a rate of 7 ml per minute. The column was then thoroughly washed with distilled water to remove EDTA and the cobalt complex, and silver was eluted by passing through it 40 ml of hot 16 per cent. nitric acid at a rate of 5 ml per minute. The effluent contained all the silver, which was determined by Volhard's method.

Results

Table I

Amounts of silver found in cobalt - silver mixtures

Cobalt present, %	Calculated amount of silver present, g	Silver found, g	Error, %
34·75	0·04318	0·04316	−0·04
39·65	0·03941	0·03941	0·0
47·31	0·03607	0·03614	+0·19
51·60	0·02698	0·02698	0·0
56·86	0·02428	0·02430	+0·08
61·53	0·02158	0·02158	0·0
71·34	0·01618	0·01615	−0·08
82·04	0·01025	0·01025	0·0
85·36	0·008091	0·008102	+0·13
97·10	0·004316	0·004299	−0·39

In Table I, results by the proposed method are compared with the calculated amounts of silver in various mixtures. It can be seen that the error was not greater than ±0·5 per cent. in any instance, and silver can be determined even when about 3 per cent. by weight is present in the mixture.

Further work on the effective separation and determination of silver in admixture with cobalt, copper and nickel is in progress on similar lines.

I am grateful to Dr. R. Kaushal, Professor of Chemistry, for facilities provided.

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R. L. Shrimal
Received *March 2nd*, 1959

AN INTERFERING ELECTRODE REACTION DURING POLAROGRAPHY OF SULPHUR DIOXIDE SOLUTIONS CONTAINING OXYGEN

THE polarographic reduction products of oxygen, *viz.*, hydrogen peroxide and hydroxyl ions, are known to interfere with many polarographic investigations of oxygen-containing solutions.[1] This Note describes a further example of an interfering electrode reaction observed during the polarography of sulphur dioxide solutions containing oxygen.

During polarography of sulphur dioxide solutions,[2,3] it has apparently been necessary to remove oxygen merely to prevent overlapping or masking of the reduction waves of sulphur dioxide by those of oxygen. It seemed possible that sulphur dioxide present in fruit juices and ciders could be determined in presence of oxygen, since, over the pH range of these samples (3·0 to 4·5), the reduction waves of sulphur dioxide and oxygen are well separated. However, preliminary investigations of solutions containing citrate - sodium hydroxide buffers and sulphur dioxide showed that, although well defined reduction waves were obtained for sulphur dioxide, their heights depended upon the amounts of dissolved oxygen present.

The addition of sulphur dioxide (final concentration 26 p.p.m.) to a buffer solution of pH 3·08, which contained methyl red or gelatin to suppress the oxygen maximum, gave, without de-gassing, a reduction wave for sulphur dioxide only 30 per cent. as high as that obtained when the same amount of sulphur dioxide was added to the previously de-gassed solution. Polarography, without de-gassing, of two buffer solutions (pH 3·00) containing the same concentration of sulphur dioxide (30 p.p.m., as found by iodine titration) but different amounts of oxygen showed that the height of the sulphur dioxide wave for the solution having the higher oxygen content was only half that found for the other solution.

The addition of sulphur dioxide to a buffer solution of pH 4·45, without de-gassing, gave a sulphur dioxide wave only 60 per cent. as high as that obtained when sulphur dioxide was added to the de-gassed buffer solution. That the solutions did, in fact, contain the same amount of sulphur dioxide was shown by Aulenbach and Balmat's method[3]; small amounts of glycerol[3,4] were added to the solutions, which were then made alkaline, de-gassed and re-acidified to a pH between 1 and 2. The heights of the reduction waves for sulphur dioxide were then identical.

It seems probable, therefore, that the low heights of reduction waves of sulphur dioxide observed during polarography of sulphur dioxide solutions containing oxygen are caused by a reaction at the diffusion layer between sulphur dioxide and hydrogen peroxide formed by the preceding reduction of oxygen.

I thank Dr. M. Ingram, Low Temperature Research Station, Cambridge, for helpful discussion.

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Received *May 25th*, 1959

A POSSIBLE SOURCE OF ERROR ARISING IN ANALYSIS FROM THE USE OF POLYTHENE BOTTLES

AFTER using a weakly acid aqueous solution saturated with hydrogen sulphide, contained in a 200-ml polythene wash-bottle, I found that many fillings and rinsings of the bottle with water at intervals over several days were necessary to remove hydrogen sulphide from the bottle. Hydrogen sulphide from the saturated wash liquid had passed into the polythene itself and was released into the space in the bottle when it was set aside. A similar effect was obtained when the bottle was filled with diluted aqueous ammonia, but here the ammonia could be more quickly removed.

Bromine vapour from saturated bromine water rapidly enters the polythene, contact for about 1 minute between solution and polythene being sufficient to leave the latter coloured a light brown. Contact for about 90 minutes leaves the polythene coloured a deep red and, as with hydrogen sulphide, many fillings with water with intervening periods of setting aside the empty bottle are necessary before the bromine is removed from the polythene. This removal is, of course, speeded up by passing a current of air through the bottle.

Difficulties have also been encountered through nitric acid being absorbed in the walls of polythene bottles and subsequently being slowly released (private communication from Mr. W. C. Johnson).

The possibilities in analytical work of error arising from disregard of this behaviour will at once suggest themselves.

The permeability of polythene to certain gases and vapours is well known to the physical chemist, but the analyst may not be so well aware of the phenomenon, and it is the purpose of this Note to direct his attention to what may be an unsuspected source of error in analytical work.

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Received *May 26th*, 1959

Book Review

ORGANIC SYNTHESES. An Annual Publication of Satisfactory Methods for the Preparation of Organic Chemicals. Volume 38. Editor-in-Chief: JOHN C. SHEEHAN. Pp. viii + 120. New York: John Wiley & Sons Inc.; London: Chapman & Hall Ltd. 1958. Price $4·00; 32s.

The thirty-one titled preparations recorded this year are for butenyne and penta-1:4-diene; 4-bromohept-2-ene and 1-nitro-octane; 2-methyldecane-2:5-diol (not previously reported) and 2-bromomethyl-2-hydroxymethylpropanediol; *trans*-stilbene oxide and 5:5-di(hydroxymethyl)-2-phenyl-1:3-dioxan (a monoacetal of pentaerythritol); diphenylacetaldehyde and dicyclohexyl ketone; 2-bromo-*m*-toluic acid, *trans*-2-methyldodec-2-enoic acid, α-methylenelauric acid; undecanedioic acid and its monomethyl ester; diethyl methylenemalonate and β-methylglutaric anhydride; 5-formyl-4-phenanthroic and 6-oxoundecanedioic acids; *p*-chloro-*N*-ethylaniline; benzyltrimethylammonium ethoxide; α-phthalimido-*o*-toluic acid; 3-*o*-chloroanilinopropionitrile and dibromoacetonitrile; methyl *p*-tolyl sulphone; 2-amino-4-anilino-6-chloromethyl-1:3:5-triazine, 2-benzylaminopyridine, 1-methyl*iso*quinoline, tetrahydro-3-hydroxyfuran, tetrahydro-5:5-dimethyl-2-*n*-pentylfuran (not previously reported) and 2-vinylthiophen (for which the refractive index quoted from the literature is obviously misprinted).

Explosions have been reported during the preparation of methoxyacetylene (Vol. 34), diethyl azoformate (Vol. 28) and *o*-toluamide (Coll. Vol. 2), of minor character for the first but very violent for the last; appropriate precautions are indicated.

Perusing the lists of preparations in any of the volumes in this series, one may reasonably pause at times to wonder why some of them have been put forward. For subsequent issues, submitters of methods will be required to resolve in advance any such doubts that might arise.

B. A. ELLIS

IUPAC Reports

THE International Union of Pure and Applied Chemistry intends to publish, from time to time, Reports from its various Sections and Divisions. The reports will be published in pamphlet form, and a loose-leaf binder for them is available from the publishers (price 5s.). The reports may be purchased separately, or a standing order for all reports may be placed with the publishers, Butterworths Scientific Publications, who are the official publishers to IUPAC.

The following reports have recently appeared—

Reports from the Vitamin Assay Subdivision of the Food Division of the Applied Chemistry Section—

The Assay of Vitamin A Oils. (1959. Pp. 7. Price 3s.)

A Report on the Vitamin D Bioassay of Oils and Concentrates. (1959. Pp. 17. Price 6s.)

The Vitamin A Potency of Beta-Carotene. (1959. Pp. 11. Price 6s.)

Report from the Trace Elements in Food Subdivision of the Food Division of the Applied Chemistry Section—

Determination of Copper Content of Foodstuffs: Photometric Method. (1959. Pp. 4. Price 3s.)

Report from the Organic Coatings Division of the Applied Chemistry Section—

A Report on Education and Training in the Paint Industry. (A report, compiled by Dr. L. A. Jordan, C.B.E., from material contributed to a Colloquium held in Paris in July, 1957). (1959. Pp. iv + 30. Price 6s.)

Report from the Commission on Physico-Chemical Symbols and Terminology of the Physico-Chemistry Section—

Manual of Physico-Chemical Symbols and Terminology. Prepared from the publications of the Commission by its President, J. A. Christiansen. (1959. Pp. iv + 27; English and French texts appear on opposite pages. Price 7s. 6d.)

Publications Received

QUALITATIVE ANALYSIS AND ELECTROLYTIC SOLUTIONS. By EDWARD J. KING. Pp. xxiv + 641. New York: Harcourt, Brace and Company Inc. 1959. Price $6.95.

PERFUMES, COSMETICS AND SOAPS, WITH SPECIAL REFERENCE TO SYNTHETICS. Volume I. A DICTIONARY OF RAW MATERIALS TOGETHER WITH AN ACCOUNT OF THE NOMENCLATURE OF SYNTHETICS. By W. A. POUCHER, F.P.S., F.R.P.S. Sixth Edition. Pp. xvi + 463. London: Chapman & Hall Ltd. 1959. Price 75s.

AN INTRODUCTION TO THE STUDY OF CHEMICAL THERMODYNAMICS. By D. H. EVERETT, M.B.E., M.A., D.Sc. Pp. xx + 240. London, New York and Toronto: Longmans, Green & Co. Ltd. 1959. Price 28s.

ANALYTICAL CHEMISTRY OF POLYMERS. Volume XII, Part I. ANALYSIS OF MONOMERS AND POLYMERIC MATERIALS: PLASTICS—RESINS—RUBBERS—FIBERS. Edited by GORDON M. KLINE. Pp. xviii + 666. New York and London: Interscience Publishers Inc. 1959. Price $16.50; 125s.

CONTRIBUTI TEORICI E SPERIMENTALI DI POLAROGRAFIA. Volume IV. Supplemento a "La Ricerca Scientifica." Pp. 361. Padova, Italy: Centro di Polarografia. 1959.

SOVIET RESEARCH IN ANALYTICAL CHEMISTRY OF URANIUM, 1955–1957. Pp. iv + 51. New York: Consultants Bureau Inc. 1959. Price $10.00.

A collection of 10 papers published in English translation by Consultants Bureau from the Russian journals "Journal of Analytical Chemistry" and "Proceedings of the Academy of Sciences of the U.S.S.R. (Doklady)."

BRITISH NATIONAL FORMULARY 1957: SECOND AMENDMENT 1959. Pp. 4. London: The British Medical Association *and* The Pharmaceutical Press. 1959. Price 3d.

It is particularly requested that orders sent to the publishers for this amendment should be accompanied by the remittance and an addressed envelope bearing a 2d. stamp. The booklet measures 4¼ × 6½ inches.

MICROANALISIS ELEMENTAL ORGANICO. By Dr. J. B. NIEDERL and Dr. J. A. SOZZI. Pp. xviii + 285. Buenos Aires: The Authors. 1958. Gratis.

Applications for free copies may be sent to Dr. J. A. Sozzi, Calle Arcos 2073, Buenos Aires (28), Argentina.

ADVANCES IN APPLIED MICROBIOLOGY. Volume 1. Edited by WAYNE W. UMBREIT. Pp. xii + 304. New York and London: Academic Press Inc. 1959. Price $9.50; 76s.

Erratum

JULY (1959) ISSUE, p. 462, 7th line. *For* "chloroform" *read* "water."

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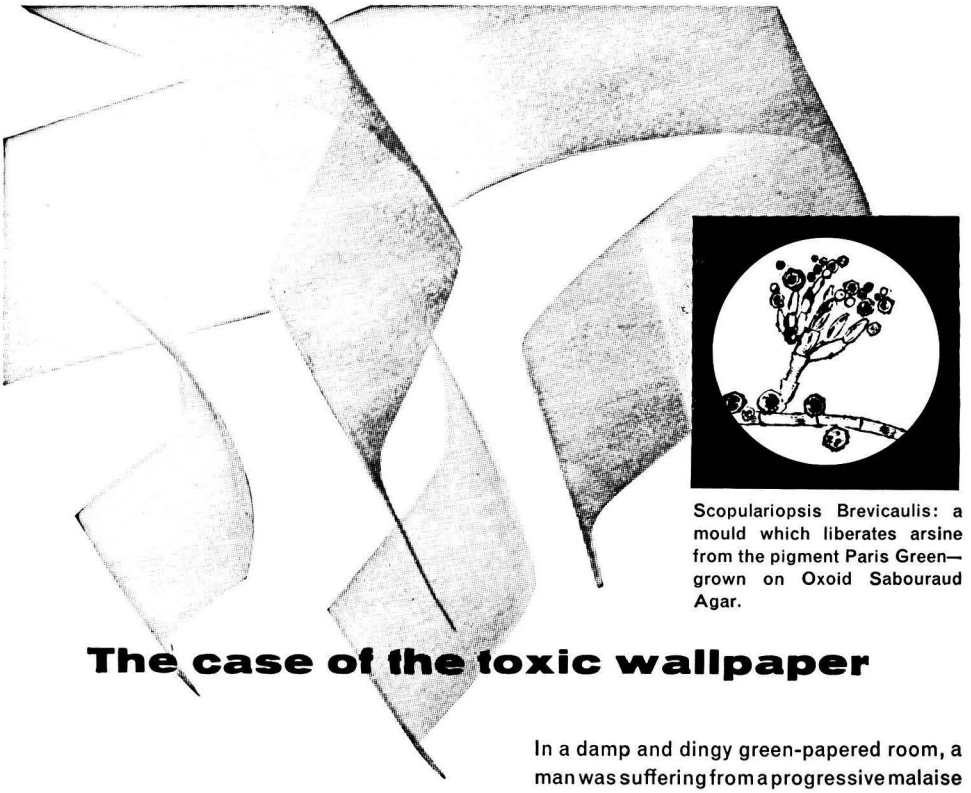

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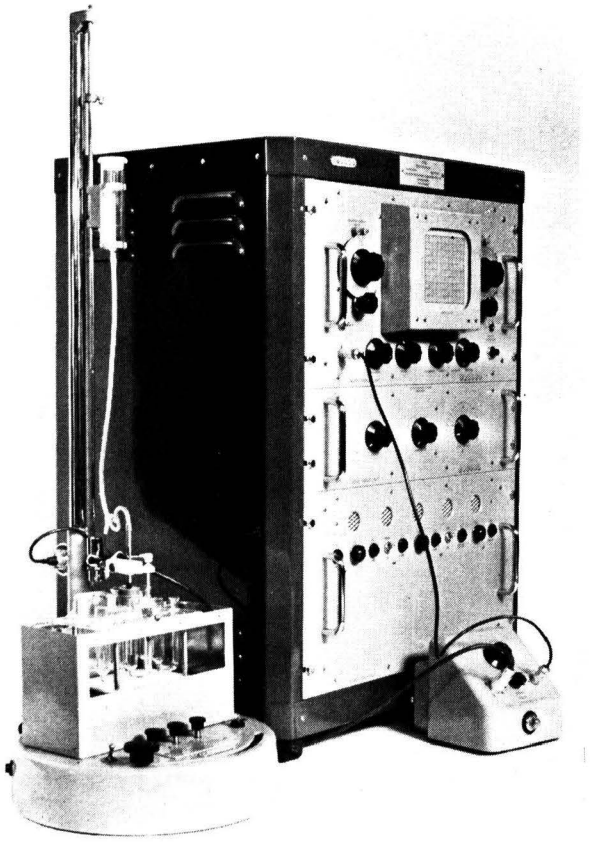

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