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

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

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

Essential Oil Sub-Committee:

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

Metallic Impurities in Foodstuffs Sub-Committee:

Report No. 4. Determination of Zinc.

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

Report No. 1. Determination of Unsaponifiable Matter in Oils and Fats.
Report No. 2. Determination of Unsaponified Fat in Soap. *Out of print.*
Report No. 3. Determination of Free Alkali in Soaps.
Report No. 4. Determination of Free Alkali and Silica in Silicated Soaps.
Report No. 5. Determination of Rosin in Soaps.
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Report No. 1. Assay of Lobelia (*Lobelia Inflata*).
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Report No. 3. Assay of Aconite.
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Report No. 5. Assay of Jaborandi.
Report No. 6. Assay of Ephedra and of Ephidrine in Nasal Sprays.

Fluorine in Foods Sub-Committee:

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

Sub-Committee on Vitamin Estimations:

Report on the Microbiological Assay of Riboflavine and Nicotinic Acid.
The Determination of Carotene in Green-Leaf Material. Part 1. Fresh Grass.
The Determination of Carotene in Green-Leaf Material. Part 2. Green-Leaf Materials other than Grass. (Gratis.)
The Chemical Assay of Aneurine [Thiamine] in Foodstuffs.
The Microbiological Determination of Thiamine.

Tragacanth Sub-Committee:

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

Soapless Detergents Sub-Committee: Examination of Detergent Preparations.

Meat Extract Sub-Committee:

Analysis of Meat Extract.
Determination of Gelatin in Meat Extract and Meat Stocks: Interim Report.

THE ANALYST

PROCEEDINGS OF THE SOCIETY FOR ANALYTICAL CHEMISTRY

DEATH

WE regret to record the death of

Alfred Randolph Campbell.

MICROCHEMISTRY GROUP

AN Ordinary Meeting of the Group was held jointly with the London Section of the Royal Institute of Chemistry at 5.45 p.m. on Friday, May 7th, 1954, at the University, Reading. Dr. A. M. Ward, F.R.I.C., was in the Chair.

Demonstrations were given in the Analytical Chemistry Research Laboratory by I. Smith and J. B. Jepson on "New Methods in Paper Chromatography" and by J. Tinsley on "Shandon Continuous Paper-electrophoresis Apparatus."

The following papers were then presented and discussed in the Zoological Lecture Theatre (see summaries below):—"The Determination of Esterases," by W. N. Aldridge, B.Sc., Ph.D.; "The Determination of Sugars," by G. Harris, Ph.D., A.R.C.S.; "The Measurement of Isotopes of Carbon and Hydrogen," by R. F. Glascock, B.Sc., Ph.D.

During the afternoon, visits were made to Messrs. Huntley and Palmers Ltd., and to the National Institute for Research in Dairying.

THE DETERMINATION OF ESTERASES

DR. W. N. ALDRIDGE said that the two most important methods of determining esterases were those involving manometric techniques and those making use of substrates which, on hydrolysis, produced substances that could easily be determined. In the manometric technique, the acid produced on the hydrolysis of the ester liberated carbon dioxide from a bicarbonate buffer. In the colorimetric techniques, esters containing phenols, phenolphthalein, indoxyl esters, and so on, had been used.

It had long been known that esterases had a low substrate specificity. This had led many workers to assume that they had no specificity and that any ester that might be synthesised would be hydrolysed by *the* esterase. This was quite untrue; nor could it be assumed that the hydrolysis of a particular ester was carried out by one enzyme only. Experiments had been carried out by the author with organo-phosphorus compounds. These had shown that, in rabbit serum, there were two types of esterase that hydrolysed *p*-nitrophenyl and phenyl esters, and that in rat intestinal mucosa there were two esterases that hydrolysed tributyrin. One of these was commonly called lipase. Organo-phosphorus compounds had proved to be extremely useful, and their use would undoubtedly increase the knowledge of this group of enzymes. It was probably true to say that the lack of knowledge of their function was due mainly to the fact that the activities of the different esterases had not yet been separated, nor their properties examined.

THE DETERMINATION OF SUGARS

DR. G. HARRIS said that the need for a precise and versatile method of analysing the carbohydrates in extracts of the raw materials for brewing had been largely met by the use of paper chromatography. Modifications of the quantitative procedures elaborated by N. Nelson (*J. Biol. Chem.*, 1944, **153**, 375) and A. E. Flood, E. L. Hirst

and J. K. N. Jones (*Nature*, 1947, **160**, 86; *J. Chem. Soc.*, 1948, 1679), who used the solvent mixtures ethyl acetate - pyridine - water and ethyl acetate - acetic acid - water, as recommended by M. A. Jermyn and F. A. Isherwood (*Biochem. J.*, 1949, **44**, 402), were described and illustrated by the results of analyses of brewer's wort, barley extracts and beers for their constituent sugars. The separation of the sugars in mixtures of di-, tri-, tetra- and pentasaccharides, as in wort, was best effected with ethyl acetate - pyridine - water, or *n*-propanol - ethyl acetate - water (Albon, N., and Gross, D., *Analyst*, 1952, **77**, 410), but the separation of monosaccharides from one another was best achieved with ethyl acetate - acetic acid - water. Each sugar was determined after location on (Harris, G., and MacWilliam, I. C., *Chem. & Ind.*, 1954, 249) and elution from the paper chromatogram by means of its reduction of the copper ion in Somogyi's reagent (Nelson, *reference above*; Somogyi, M., *J. Biol. Chem.*, 1952, **195**, 19), followed by measurement of the molybdenum-blue colour formed on addition of Nelson's arsenomolybdate reagent and comparison of this colour with that given by a known amount of a standard sugar added to the solution before chromatography. The amounts of each sugar in each of the eluates from the paper chromatogram were found by reference to standard curves showing the relationship between weight of sugar (0 to 200 μg) and colour intensity of the molybdenum blue. Standard curves for glucose, fructose, arabinose, xylose, ribose, sucrose, glucodifructose, maltose, maltotriose, maltotetraose and maltopentaose were shown. Of these sugars, xylose and ribose had been found suitable as added internal standards for wort and beer analyses, and ribose for the analysis of extracts of barley. The standard errors of the determinations of the individual sugars were discussed; these were not greater than ± 3 per cent. for sugars present in amounts of 100 to 300 μg on the chromatograms, but rose to ± 10 per cent. for those present in very small amounts, *e.g.*, 10 to 30 μg.

The defects in the method described above were discussed, the principal objection to it arising from the fact that many polymeric sugars gave little or no reduction of the cupric ion, and must be hydrolysed to monosaccharides before determination. The use of the anthrone - sulphuric acid reagent of R. Dreywood (*Ind. Eng. Chem., Anal. Ed.*, 1946, **18**, 499), as modified by D. L. Morris (*Science*, 1948, **107**, 254), overcame this difficulty, as the hydrolysis and colour development were effected in one step. It suffered from the disadvantage, however, that the colours given by pentoses and pentosans were transient and weak, and the use of these materials as internal standards was inadmissible. A compact apparatus designed by W. D. McFarlane and H. R. Held (*Proc. Eur. Brew. Conv., Nice*, 1953, p. 110) for chromatography was described, and modifications of procedure recommended by Mr. Hall at the Brewing Industry Research Foundation were discussed. Typical results by the method were shown by reference to the analysis of wort, the standard errors of the estimations of the individual sugars being of the same magnitude as for the method previously described.

Some possible future trends in the development of carbohydrate analysis were discussed.

The Measurement of Isotopes of Carbon and Hydrogen

Dr. R. F. Glascock first briefly surveyed the methods available for counting the radioactive isotopes carbon-14 and tritium in solid compounds, and then proceeded to describe in greater detail the more efficient gas-counting methods currently used in the Isotope Section at the National Institute for Research in Dairying. The three isotopes carbon-13, carbon-14 and tritium could be determined in a single 5 or 10-mg sample of organic material (Glascock, *Biochem. J.*, 1952, **52**, 699). By the use of high-vacuum techniques, the sample was burnt quantitatively, the products were trapped in liquid-air chilled traps and separated by subliming away the carbon dioxide at $-78°$ C. This was measured manometrically, so providing, if desired, a measure of the carbon content of the sample. Part was set aside for the mass-spectrometric determination of carbon-13, and measured portions of the remainder were introduced into a gas counter for radioactivity determinations. The gas-counting method used was that of S. C. Brown and W. W. Miller (*Rev. Sci. Instrum.*, 1947, **18**, 496): the counter was filled with carbon dioxide at a pressure of 20 cm of mercury and carbon disulphide at 2 cm, the partial pressure of carbon dioxide being adjusted with inactive carbon dioxide after the addition of the active sample. The counter was operated in the Geiger region.

Although tritium could be measured in a proportional gas counter in the form of hydrogen alone, hydrogen plus methane or as tritiomethane, these gases were subject to several disadvantages. Hydrogen was not condensed at liquid-air temperature and tritium - hydrogen contaminated counters by exchange, either when used alone or in admixture with methane, giving rise to elevated backgrounds—the well-known "memory effect." Methane, although it did not contaminate counters, was also incondensable and was therefore somewhat difficult to manipulate quantitatively. Butane, however, was a good counting gas in the Geiger region and was fully condensed at liquid-air temperature. In the method described by the author (*Nucleonics*, **1951**, **9**, No. 5, 28), *n*-butane was prepared on the 5 to 10-mg scale from water by making it react with dry *n*-butyl magnesium bromide. With freshly prepared reagent the yield was almost quantitative, and it had been shown that no isotopic fractionation occurred during the reaction. Measured portions of the resulting butane were condensed into a gas counter, the pressure was adjusted to 12·5 cm with inactive butane and the counter was operated in the Geiger region.

A slide was shown to illustrate the accuracy and reproducibility of the method. Yields of carbon dioxide from pure compounds were within 1 per cent. of theoretical, and replicate counting and carbon-13 results agreed to within $\pm 1{\cdot}5$ per cent.

PHYSICAL METHODS GROUP

THE Forty-sixth Ordinary Meeting of the Group was held at 6.30 p.m. on Friday, May 28th, 1954, in the Harris Institute, Preston, Lancs. It was a joint meeting with the Liverpool and District Section of the Royal Institute of Chemistry, and was preceded by a visit to the Works of Siemens Lamps Ltd. Mr. A. A. Smales, B.Sc., F.R.I.C., was in the Chair.

The following papers on "Fluorimetry" were presented and discussed:—"A Twin-beam Null-point Fluorimeter," by J. P. Dowdall, B.Sc., A.R.C.S., D.I.C., A.R.I.C., and H. Stretch, A.R.I.C.; "The Quenching of the Fluorescence of Traces of Uranium in Fused Sodium Fluoride by Iron and Plutonium," by G. N. Walton, M.A., B.Sc.

BIOLOGICAL METHODS GROUP

THE Summer Meeting of the Group was held on Wednesday, June 16th, 1954, at the National Institute for Research in Dairying, Shinfield, nr. Reading, Berks.

Dr. S. K. Kon was in the Chair for the morning session, at which the following papers were presented and discussed:—"The Estimation of Depolarising Substances by Use of the Isolated *Semispinalis Cervicis* Muscle of the Chick," by K. J. Child, B.Pharm., A.R.I.C., and Eleanor J. Zaimis, M.D., B.Sc.; "Use of the Frog's *Rectus Abdominis* for Assay of Laudexium and Suxamethonium," by G. B. Chesher, B.Sc., and H. O. J. Collier, B.A., Ph.D. A visit to the laboratory of J. E. Ford and M. E. Gregory followed, to see and discuss the research in progress on the bio-assay of cobalamins.

After lunch a tour was made of the Institute's laboratories. The proceedings terminated at 4.50 p.m. with a vote of thanks moved by Dr. Collier to Professor Kay and his staff for their generous hospitality.

Obituary

SAMUEL ERNEST MELLING

THE sudden death of SAMUEL ERNEST MELLING on April 17th has severed almost the last direct link between the contemporary generation of analytical chemists and those legendary pioneers who founded this Society and who first directed it on its so successful course. Melling was trained in the laboratory of the late A. H. Allen of Sheffield. That must, indeed, have been a remarkable school that could turn out so many men of Melling's calibre, men who attained and maintained eminence and honour in an exacting profession. Melling was proud of being a "Surrey Street" man. He was quietly proud, too, in a natively whimsical way, of the Lancashire town of Wigan where he was born in 1877, and for which he became Public Analyst—his first of many public appointments—nearly forty years ago.

He left Allen's laboratory in 1903 to become partner of the late James Carter Bell, a contemporary and friend of Allen. The appointment for Wigan followed, and thereafter the laboratory at Higher Broughton, with its quaint air of isolation from the strain and urgency of city life, became the centre of one of the busiest practices of analytical chemists in the North of England. Carter Bell died in 1913, and Melling carried on alone until, in 1921, he was joined by the late Edward Ardern. Some of Ardern's outstanding pioneer work on sewage purification had been shared and supplemented by Melling, and the partnership was the natural outcome of the association of two contrasting, but essentially complementary, personalities. With justifiable pride, since it was his life's work and the measure of the success of unremitting effort, Melling watched the practice grow until "Melling and Ardern" became acknowledged as one of the leading firms of consulting chemists in the country. Indeed, he hoped it would be his monument, and it was characteristic that in the last year of his life, when he knew his strength was failing, he undertook an elaborate programme of laboratory reconstruction and re-organisation from which, at the age of 76, a less courageous spirit might well have recoiled.

Melling's professional eminence was recognised by appointment to high offices in many societies, and he regarded as the supreme honour of his career his election to the Presidency of this Society in 1943–45. But an obituary notice cannot be a biography, and it is not for these things that he will be most remembered by his friends. A vivid personality that never failed in any company to be the centre and focus of a group, a master of anecdote, a shrewd but never malicious critic, a histrionic talent that was unsuppressed because it was largely unconscious, and over everything a kindly nature and a disarming and unexpected humility, these made the good companion that Melling so undoubtedly was. To those who knew him well he was more. He was a prodigious worker who spared neither himself nor his associates, a stern critic, a generous opponent, a loyal friend. Above all, he was a deeply religious man, with an unfashionably sincere conviction of the predominant value of spiritual things. Mrs. Melling, a charming and devoted wife, predeceased him by a few months, after several years of failing health. There were no children of the marriage.

It is the modern tragedy that a generation of scientists that is obsolescent at the age of thirty must concentrate all its energy on vocational training and cannot afford the luxury of education. Melling, born in less clamant times, enjoyed a breadth of culture too often denied to his less fortunate successors; his life was the fuller for it. He "ceased upon the midnight with no pain," and we who are left, acquiescent or active in tearing veil after merciful veil from unimaginable horror, might well pause and sigh, not for the passing of an individual, but for ourselves.

J. G. SHERRATT

CRESSACRE GEORGE MOOR

CRESSACRE GEORGE MOOR, who died on February 8th, 1954, shortly before his 86th birthday, had become a prominent figure among analytical chemists at a remarkably early age.

Moor spent two years at Cambridge and two at Oxford studying under Vernon-Harcourt, and became M.A. Cambridge. He was one of the band of workers inspired by A. H. Allen, and some of his first papers were published under their joint names, on the composition of vinegar (*Analyst*, 1893), the detection of exhausted ginger, and changes in butter on storage (*Analyst*, 1894). Before this, in 1892, however, he had read a paper to the British Association

on sewage disposal. He also practised at various times in association with William Chattaway, T. Pearmain, Martin Priest and W. Partridge, at first in London, then both in London and Exeter, and finally again in London. During these early years he was a prolific author, publishing numerous articles, many of which appeared in *The Analyst*, either alone or in conjunction with others, but his name will live chiefly on account of the books of which he was author or joint author. The two best known of these are "Aids to the Analysis of Foods and Drugs," now edited by Dr. J. R. Nicholls, and "Aids to Bacteriology," now edited by Dr. H. W. Scott-Wilson. Other books were "Applied Bacteriology," "The Chemical and Bacteriological Examination of Water" and "Suggested Standards of Purity for Foods and Drugs."

In the early 1890's he became Public Analyst for Exeter, but his allegiance to chemistry was always a divided one, and he resigned the appointment and closed his laboratory in 1901 to go to West Africa prospecting for gold. This venture in mining did not last long, as he was soon back in London to rejoin Chattaway's old laboratory and was appointed Public Analyst for Dorset, Poole and Penzance. During the 1914–18 war he served in the R.A.M.C. as a Captain and, although he remained associated with the practice of Moor and Partridge, he never returned to the laboratory, his natural wanderlust again taking him to Africa to engage in mining, a pursuit in which he never lost interest and on which he had written a book in 1910. He returned to England in the early 1930's and again joined Priest, who was then Public Analyst for Camberwell, in his laboratory in Lewisham, but abandoned this once again to return to mining in Cornwall.

Moor was elected a member of the Society on January 4th, 1893, and served on the Council, 1898–99. He was elected a Fellow of the Institute of Chemistry in 1898.

T. McLachlan

Analytical Methods Committee

REPORT OF THE LEAD PANEL OF THE METALLIC IMPURITIES IN FOODSTUFFS SUB-COMMITTEE

The Determination of Lead in Foodstuffs

The Analytical Methods Committee has received from its Metallic Impurities in Foodstuffs Sub-Committee the following report of its Lead Panel. The Report has been approved by the Analytical Methods Committee and its publication has been authorised by the Council.

Report

The Lead Panel was constituted on February 6th, 1950, with the terms of reference: "to formulate standard and routine methods for the Determination of Lead in Foodstuffs." The purpose of this appointment was in effect to ascertain the precision and accuracy of methods then generally in use, having regard to the possibility of statutory limits of 1 or 2 parts per million, or of even less than 1 p.p.m., being recommended by the Ministry of Food.

At its first meeting the Panel was of the general opinion that the procedure to be adopted should be the development, if possible, of a rapid method, and then of a standard or referee method. For this purpose, samples of fruit syrup and cocoa containing, respectively, about 5 parts and 1 part per million of lead were circulated together with drafts of two methods of analysis, one involving the use of dithizone and the other sulphide for the final assessment. The results proved to be disappointing. Accordingly, a further series of tests with fruit juice and cocoa was undertaken, these two methods again being used, together with a third method that incorporated the colloidal sulphide procedure with the final determination with dithizone as the reagent; in this third method certain precautions were taken, for which the need had been demonstrated by the first test. Again the results were disappointing. The position was then reviewed, and the earlier decision to develop a rapid method before developing a standard method was reversed, largely because it was agreed that the necessity for examining and endeavouring to overcome the many difficulties

that had arisen in the collaborative work to date clearly indicated a standard method as the objective.

The next test was with samples of syrup, salad cream and beer, by a modified dithizone method elaborated by two members of the Committee. In addition, an entrainment method for the beer was outlined. After this test, members of the Panel undertook intensive examination of certain aspects of the method, and then two samples of syrup containing, respectively, 1·5 and 2·5 parts per million of lead were used. The results of this test were still not considered satisfactory. A letter was sent to a number of manufacturers of analytical reagents asking for assistance in getting completely lead-free reagents. Meanwhile collective work on an entrainment method for use with acid extracts from a sample of cocoa was undertaken, but with no better results. A full and detailed report, together with the modified dithizone method finally used, was submitted to the Analytical Methods Committee.

The difficulties (and "snags") that had arisen during the course of this work were as follows—

BLANKS—

The relatively large blanks that were found in the earlier tests presented a major difficulty. It was only as the result of a very considerable amount of work that the possibility of minute traces of lead being universally present was fully appreciated. In a determination in which 5 g of the foodstuff are used, a blank of 10 μg means 2 parts per million, a substantial and significant amount when a prescribed maximum limit of, say, 5 p.p.m. is in force, but one quite unacceptable when the limit is 1 part per million or less. This contamination was found to be due to several causes—

(*a*) Reagents as supplied for ordinary analytical work may contain up to 1 or 2 parts per million of lead. This difficulty has been largely overcome by the enterprise of one or two manufacturers, who can now supply acids and alkalies containing not more than 0·01 part per million of lead, and by careful attention in the method of analysis to the preparation of reagents free from lead by washing them with dithizone solutions. In addition all reagents are stored in Pyrex-glass bottles.

(*b*) The glass of apparatus and bottles may yield lead in the course of usage. The remedy is to use Pyrex glass wherever possible.

(*c*) Atmospheric dust normally contains substantial amounts of lead, often to the extent of several thousand parts per million. Particular attention must therefore be paid to any possibility of dust contamination during any stage of a determination. The ideal laboratory is a separate room, air-conditioned, devoted entirely to this determination.

DESTRUCTION OF ORGANIC MATTER—

Two methods for the destruction of organic matter were considered: oxidation with acids (wet combustion) and incineration to a carbon-free ash (dry combustion). No evidence has been produced that there is any loss of lead by volatilisation if the temperature of incineration does not exceed about 500° C. On the other hand, a loss of lead occurs occasionally with foodstuffs such as fruit juices, which fuse on ignition, as the result of the formation of acid-insoluble lead silicate produced by attack on the silica of the basin. (Dry combustion of such foodstuffs in platinum capsules or basins is not advisable, as traces of platinum may be dissolved on solution of the ash in acid.) The use of magnesium nitrate as an "ashing-aid" was found to overcome this difficulty. The panel was divided in its opinion as to which method for destruction of organic matter was the better, once the initial difficulty of high blanks in the larger amounts of reagents used in the wet combustion method had been overcome.

THE OPTIMUM pH OF THE ACID SOLUTION PREPARED FOR TREATMENT WITH DIETHYLAMMONIUM DIETHYLDITHIOCARBAMATE SOLUTION—

The optimum conditions for the extraction of the whole of the lead, while leaving interfering elements, such as bismuth, unextracted, necessitated a considerable amount of experimental work.

ASSESSMENT OF LEAD DITHIZONATE COLOUR—

The fact that the work for this method was based on the mono-colour technique does not mean that it was considered better than the mixed-colour technique with absorption at defined wavelengths, or the Irving reversion technique. The choice was due to an attempt to make the method available in laboratories where such special apparatus as the Spekker absorptiometer is not available. By the mono-colour technique, an assessment can additionally be made with a tintometer or even by nesslerising. The difficulty that had to be overcome in the mono-colour method was due to the fact that, in the process of washing the excess of dithizone out of the chloroform solution of the lead dithizonate, the use of a solution containing more than a certain percentage of potassium cyanide might result in removal of part of the red solution of lead dithizonate. The procedure laid down in the method does, we believe, avoid this difficulty.

TABLE I

RESULTS ON COCOA

Member	Weight of sample, g	Method of breakdown	Lead found, μg	Lead in blank, μg	Lead in sample, μg	Result, p.p.m.
A	7·0	wet ash	9·0	3·5	5·5	0·79
	5·9	"	9·5	3·5	6·0	1·00
	7·5	dry ash	9·7	3·0	6·7	0·90
	9·0	"	10·3	1·0	9·3	1·03
B	10·0	wet ash	12·7	5·8	6·9	0·69
	10·0	"	14·2	5·8	8·4	0·84
	10·0	dry ash, no $Mg(NO_3)_2$	9·6	2·2	7·4	0·74
	10·0	"	11·4	2·2	9·2	0·92
	9·2	dry ash with $Mg(NO_3)_2$	7·9	2·5	5·4	0·59
C	5·0	wet ash			7·2	1·44
	5·0	dry ash			3·75	0·75
	5·0	"			4·00	0·80
D	5·00	wet ash	7·7	2·5	5·2	1·04
	5·06	"	8·3	2·5	5·8	1·15
	10·02	dry ash	11·5	0·9	10·6	1·06
	10·19	"	9·0	0·9	8·1	0·80
	10·09	"	13·8	1·3	12·5	1·24
	10·00	"	9·4	1·3	8·1	0·81
E	5·0	wet ash			5·1	1·02
	5·0	"			2·8	0·56
	5·0	"			5·4	1·08
	5·0	"			4·6	0·92
	5·0	"			2·3	0·46
F	5·011	wet ash			4·8	0·96
	5·176	"			5·3	1·02
	5·180	"			5·7	1·10
G	10·0	wet ash	13·3	4·1	9·2	0·92
	10·0	"	11·9	2·7	9·2	0·92
	10·0	dry ash	15·3	1·8	13·5	1·35
	10·0	"	12·3	1·8	10·5	1·05

At the suggestion of the Analytical Methods Committee, one final test on cocoa and syrup was undertaken by the Panel. The results of this, in detail, are shown in Tables I and II. The full details of the method are appended. It is hoped that this publication will allow the method to be tried out extensively, and that in a short time the results of outside work will confirm it as a standard or referee method.

One final observation is that the full method is designed to be workable with all kinds of foodstuffs. When the foodstuff does not contain any significant amounts of phosphates or iron (*e.g.*, cereals, sugars or wines), then Section 2A of the method can be omitted.

METHOD FOR LEAD

NOTE—

In the absence of significant amounts of phosphates, bismuth or iron, Section 2A of the method may be omitted. When the full method is used, however, the exact details given must be followed with care.

TABLE II

RESULTS ON SYRUP

Member	Weight of sample, g	Method of breakdown	Lead found, μg	Lead in blank, μg	Lead in sample, μg	Result, p.p.m.
A	6·3	wet ash	7·5	3·5	4·0	0·63
	5·0	"	6·0	3·5	2·5	0·50
	8·2	dry ash	10·5	3·0	7·5	0·92
	8·6	"	4·0	1·0	3·0	0·35
	16·1	"	10·0	1·0	9·0	0·56
B	8·52	wet ash	3·6	2·6	1·0	0·12
	5·42	"	3·2	2·6	0·6	0·11
	8·69	dry ash with $Mg(NO_3)_2$	4·7	2·5	2·2	0·25
C	5·0	wet ash			2·75	0·55
	5·0	"			2·30	0·48
	5·0	"			2·00	0·40
	5·0	"			1·20	0·24
D	5·73	wet ash	5·3	2·5	2·8	0·49
	5·22	"	5·0	2·5	2·5	0·48
	10·06	dry ash	5·2	0·9	4·3	0·43
	10·29	"	5·6	0·9	4·7	0·46
E	6·09	wet ash			1·6	0·26
	7·12	"			1·3	0·18
	15·5	"			5·6	0·35
	13·1	"			4·0	0·31
G	5·2	wet ash	8·7	4·1	4·6	0·89
	5·2	"	8·3	4·1	4·2	0·81
	8·1	"	9·6	3·1	6·5	0·80
	9·8	"	11·3	3·1	8·2	0·84
	10·0	dry ash	9·7	1·8	7·9	0·79

REAGENTS—

All reagents must be substantially "lead-free," either by purchase or by special preparation (see Notes).

Sulphuric acid, concentrated.

Perchloric acid, 60 per cent.

Nitric acid, concentrated.

Hydrochloric acid, 5 N.

Ammonium hydroxide, sp.gr. 0·880.

Ammonium hydroxide, 5 N.

Sodium iodide solution—A 20 per cent. solution in water.

Sodium metabisulphite solution—A 1·25 per cent. solution in water, filtered.

Diethylammonium diethyldithiocarbamate—A 0·5 per cent. solution in chloroform, prepared freshly each day.

Ammonium citrate solution—A 25 per cent. solution in water.

Potassium cyanide solution—A 10 per cent. solution in water. This solution shall be at least 2 days old.

Potassium cyanide wash liquid, 0·5 per cent.—Add 25 ml of 10 per cent. potassium cyanide solution to 475 ml of water and shake this solution with about 10 ml of chloroform and 1 or 2 drops of the dithizone solution. Reject the extract, wash the solution again with chloroform and reject the lower layer. This lower layer must be efficiently collected by swirling the separator, and all the emulsion must be drawn off.

Chloroform—Shake 250 ml of chloroform with 1 ml of 10 per cent. potassium cyanide solution and 25 ml of water containing about 20 drops of 5 *N* ammonium hydroxide, separate and reject the aqueous layer, wash the chloroform with water and filter it.

Dithizone, stock solution—Dissolve dithizone (as supplied) in chloroform, B.P., to give a 0·1 per cent. w/v solution. Filter and store this stock solution in a refrigerator.

Dithizone, working solution—Shake 10 ml of the stock dithizone solution with 9 ml of water and 1 ml of 5 *N* ammonium hydroxide. Separate and reject the lower layer. Draw off the remainder into a centrifuge tube, centrifuge and transfer the clear brown upper layer by teat pipette to a 10-ml burette.

Magnesium nitrate solution—Dissolve 10 g of magnesium nitrate, $Mg(NO_3)_2.6H_2O$, in water and dilute to 100 ml.

Standard lead solution, 1 ml ≡ 0·00001 *g* (10 *μg*) *of lead*—(*a*) Dissolve 1·60 g of lead nitrate in water, add 10 ml of concentrated nitric acid and dilute to 1 litre. (*b*) Dilute exactly 5 ml of solution (*a*) to 500 ml. Prepare dilution (*b*) freshly as required.

1. PRELIMINARY TREATMENT OF THE SAMPLE—

A: *"Wet-ash" digestion*—Digest 5 to 10 g of sample, or 50 ml of a liquid, such as beer, in a Kjeldahl flask with 5 ml of concentrated sulphuric acid, adding nitric acid in small portions in the usual way until the residue remains colourless or pale yellow. Finally add 1 ml of 60 per cent. perchloric acid and heat to fuming point. Cool, dilute with 5 ml of water, heat again to fuming point, add 5 ml of water, then add 10 ml of 5 *N* hydrochloric acid and boil for 5 minutes. If there is any insoluble matter, filter the solution through an acid-washed filter-paper into a conical beaker and wash the filter with hot water.

B: *"Dry-ash" digestion*—Mix in a silica basin 5 to 10 g of sample, or 50 ml of a liquid, such as beer, with 10 ml of magnesium nitrate solution, evaporate the mixture to dryness, char the organic matter over a burner and burn the residue to a white ash in a muffle furnace or over an Argand or similar burner at a temperature not exceeding 500° C.

Moisten the residue with 5 ml of water, add 10 ml of hydrochloric acid and boil the mixture for a few minutes. Filter through an acid-washed filter-paper into a conical beaker and wash the filter with hot water.

2. EXTRACTION AND DETERMINATION OF THE LEAD—

A—To the solution resulting from the preliminary treatment described above add 2 drops of methyl red indicator and then make it just alkaline with ammonium hydroxide, sp.gr. 0·880. Then make the solution just acid with 5 *N* hydrochloric acid and add 10 ml in excess (not more). Warm the solution to 40° to 90° C, add 2 ml of 20 per cent. sodium iodide solution and reduce the liberated iodine with 2 ml of freshly prepared sodium metabisulphite solution. Cool the solution, transfer it to a separating funnel and adjust the volume to 50 to 75 ml. Add 10 ml of diethylammonium diethyldithiocarbamate solution by pipette and shake the funnel vigorously for 30 seconds. Allow the layers to separate and transfer the chloroform layer to a 100-ml flask; then give the aqueous layer two small washes with chloroform without mixing and add these washings to the flask. Repeat the extraction with 10 ml of diethylammonium diethyldithiocarbamate solution and add the second extract to the main extract. Reject the aqueous layer.

To the combined extracts add 2·0 ml of diluted sulphuric acid (1 + 1) and evaporate the chloroform. Add 0·5 ml of 60 per cent. perchloric acid to the residual solution and heat it until fuming occurs and the fuming solution is clear and colourless. Cool the solution, add 10 ml of water and 5 ml of 5 *N* hydrochloric acid, boil it for 1 minute and then cool it again.

B—Add 2 ml of sodium metabisulphite solution, 2 ml of ammonium citrate solution and 2 drops of bromothymol blue indicator. Add 5 *N* ammonium hydroxide to the full blue colour of the indicator. Cool the solution, add 1 ml of 10 per cent. potassium cyanide, transfer it to a 50-ml or 100-ml separating funnel and dilute it to 40 ml. Add by pipette exactly 10 ml of chloroform.

To the solution in the separating funnel add 10 ml of 5 *N* ammonium hydroxide and then dithizone solution, a few drops at a time, with vigorous shaking, until there is a distinct excess. This will be indicated by a change from bright red to purple in the bottom layer and a yellowish shade in the upper layer. Shake the funnel for 30 to 60 seconds and allow the layers to separate.

Run the lower chloroform layer into 50 ml of 0·5 per cent. potassium cyanide wash liquid contained in a second separating funnel, shake this for 30 seconds and allow the layers to separate. The chloroform extract should now be pure red and free from any trace

of blue or green. Dry the outlet of the separator with a small roll of filter-paper, reject a few drops of the chloroform solution to clear the tap of the separator, and filter the rest of the chloroform solution through a dry Whatman No. 41 filter-paper into a dry test tube. Measure the optical density, using a 1-cm cell and Ilford green filter No. 604, or using a spectrophotometer at a wavelength of 520 mμ.

If the amount of lead contained in the sample is such that the optical density of the lead dithizonate is beyond the scale of the instrument used, it is advisable to extract the lead from the chloroform solution by shaking it with 1 per cent. nitric acid solution, diluting this aqueous extract to a known volume and taking an aliquot part for the determination in the way described above.

BLANK OR CONTROL—

Prepare a reagent blank solution under the same conditions as the test, omitting only the sample, and determine the optical density.

STANDARD GRAPH—

Measure 0, 1·0, 2·0, 3·0 and 4·0 ml of standard lead solution into flasks containing 2 ml of diluted sulphuric acid (1 + 1) and dilute each solution to about 15 ml. Add 2 ml of sodium metabisulphite solution, 2 ml of ammonium citrate and 2 drops of bromothymol blue indicator. Add enough 5 *N* ammonium hydroxide to give the full blue colour of the indicator and, after cooling, 1 ml of potassium cyanide solution.

Transfer each solution to a 50-ml or 100-ml separating funnel, dilute it to 40 ml and add exactly 10 ml of chloroform. Add 10 ml of 5 *N* ammonium hydroxide and then dithizone solution, a few drops at a time, with vigorous shaking, until the presence of an excess is indicated. Complete the extraction, with washing, exactly as described for the test solution.

NOTES

"LEAD-FREE" REAGENTS—

Hydrochloric, sulphuric, perchloric and citric acids, ammonium hydroxide and potassium cyanide are now obtainable "lead-free" to an extent that traces of lead contamination do not exceed 0·01 part per million.

In the absence of "lead-free" reagents it is desirable that the reagents be rendered lead-free as follows—

Acids—Sulphuric, nitric and hydrochloric acids can be rendered substantially lead-free by distillation, Pyrex all-glass apparatus being used.

Ammonium hydroxide—Distil 400 ml of ammonium hydroxide, sp.gr. 0·880, from a Pyrex-glass flask fitted with a safety trap into 250 ml of redistilled water kept cold by means of a bath of ice, controlling the pressure of the liberated ammonia gas by adjusting the rate of heating. Subsequently determine the strength of the distillate by titration with *N* hydrochloric acid.

Ammonium citrate solution—Dissolve 125 g of AnalaR ammonium citrate in distilled water up to a volume of 400 to 450 ml, make it faintly alkaline to litmus paper with 5 *N* ammonium hydroxide, and extract it with chloroform and appropriate additions of the stock dithizone solution. Continue extraction until all metals have been removed and the extract is faintly green. Then make the solution acid by adding lead-free 5 *N* hydrochloric acid and extract with further portions of chloroform until the final extract is colourless.

Potassium cyanide, 10 per cent. solution—Dissolve 50 g of AnalaR potassium cyanide in water and dilute to 100 ml. Extract this strong solution with chloroform and 1 or 2 drops of dithizone solution until the extract is no longer coloured red, but has a greenish shade. Use as small an excess of dithizone as possible, because the excess is not readily removed. The excess of dithizone is extracted by shaking the solution with successive portions of chloroform. Dilute the extracted cyanide to 500 ml with water, warm it to remove chloroform and then cool it.

STORAGE OF REAGENTS—

It is advisable to store all "lead-free" reagents in Pyrex-glass bottles. Polythene bottles may be suitable for some reagents, *e.g.*, sodium citrate and potassium cyanide.

MATERIAL FOR APPARATUS—

The use of Pyrex-glass apparatus throughout is advisable.

The Determination of Titanium by High-precision Absorptiometry

By W. T. L. NEAL

The titanium content of titanium-base alloys and pure titanium metal can be determined absorptiometrically with a precision (coefficient of variation) of 0·03 per cent., with a Unicam SP500 spectrophotometer at a wavelength of 4100 A, use being made of the colour of the titanium - hydrogen peroxide compound in solutions with an optical density of 2·5 to 3·0 in 1-cm cells. In this paper an analysis is made of the effect of factors liable to influence the precision and accuracy of the determination, and the techniques required to secure high precision are described in detail.

NORMALLY, absorptiometry is used for the determination of minor or trace elements in a sample, and the accuracy with which this can be achieved is usually better than 1·0 per cent. and can be as good as 0·5 per cent. The procedure is to make a solution of the sample in a suitable solvent, add reagents to produce a colour characteristic for the element to be determined, dilute to a known volume and measure the optical density of the solution at a suitable wavelength relative to a reagent blank or distilled water. This value for the optical density of the solution is compared with a calibration graph produced by measuring the optical density of similarly prepared solutions containing known amounts of the element.

The only essential features making the technique of high-precision absorptiometry more precise than that of conventional absorptiometry are that the optical density of the sample solution is measured, not against a blank solution of zero, or nearly zero, absorption, but against a reference solution containing a known concentration of the element to be determined and coloured in the same way as the sample or test solution; and that the optical densities of these solutions are much higher (in the range 2 to 5) than those used in minor element or trace analysis, which are usually less than 1.[1] It can be shown theoretically (see below) for solutions that obey the Beer - Lambert law, that the greater the concentration of the solutions, the higher will be the precision. How far this statement holds for solutions that do not obey the Beer - Lambert law can only be discovered by experiment. It will be shown that the titanium - hydrogen peroxide solutions do not behave in the way predicted by the theory when their optical density rises above about 1·2 and that there is a certain concentration above which the precision decreases.

The precision of the method is also a function of the spectrophotometer used and of characteristics in the solutions, such as acid concentration and temperature. Extensive preliminary experiments were carried out to assess how closely these variables must be controlled in order to attain the greatest accuracy.

As a result of this work, a method has been developed in which the titanium content of a sample can be determined with a precision (coefficient of variation) of 0·03 per cent. The results can be found for a single sample in $1\frac{1}{2}$ to 2 hours after receipt together with the time required for its solution, which may add $\frac{1}{2}$ to 3 or more hours. Six to eight individual determinations can be carried out per day.

OUTLINE OF METHOD—

The method is based upon the formation of the familiar colour of the titanium - hydrogen peroxide compound in 20 per cent. w/v sulphuric acid and measurement of the relative transmission of the test solution (that is, a solution from the sample being examined), against a reference solution of accurately known concentration by means of a Unicam SP500 spectrophotometer at 4100 A. The concentration of the test solution can then be read from a calibration graph, or by a procedure to be described that avoids the use of a calibration graph.

PRINCIPLE OF METHOD—

For solutions obeying the Beer - Lambert law, the following relationships can be deduced.

Let D_1, D_2, etc. = optical density of solutions 1, 2, etc.
C_1, C_2, etc. = concentration of solutions 1, 2, etc.
I_0 = intensity of incident light.
I_1, I_2, etc. = intensity of light transmitted through solutions 1, 2, etc.
K = a constant that is a function of the solution and of the incident light.
l = path length of light through cell (*i.e.*, cell length).

By definition—

$$D_1 = \log_{10} I_0/I_1 \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad (1a)$$

$$D_2 = \log_{10} I_0/I_2 \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad (1b)$$

and, according to the Beer - Lambert law—

$$D_1 = KlC_1 \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad (2a)$$

$$D_2 = KlC_2 \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad (2b)$$

whence $I_0/I_1 = 10^{KlC_1}$ .. .. (3a)

and $I_0/I_2 = 10^{KlC_2}$ .. .. (3b)

Dividing (3*a*) by (3*b*) we have—

$$I_2/I_1 = 10^{Kl(C_1-C_2)} \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad (4a)$$

$$= 10^{KlC_1(1-C_2/C_1)} \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad (4b)$$

$$= 10^{-D_1(\alpha-1)} \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad (4c)$$

(where $\alpha = C_2/C_1$).

Therefore $$1 - I_2/I_1 = \frac{\Delta I}{I_1} = 1 - 10^{-D_1(\alpha-1)} \quad .. \quad .. \quad .. \quad .. \quad (5a)$$

(where $\Delta I = I_1 - I_2$).

Now, if C_2 is greater than C_1, solution 1 will be used as the reference solution, and the instrument will be adjusted to read 100 per cent. transmission with solution 1 in the light beam; that is $I_1 = 100$.

Thus we may rewrite (5*a*) as—

$$\Delta I = 100[1 - 10^{-D_1(\alpha-1)}] \quad .. \quad .. \quad .. \quad .. \quad .. \quad (5b)$$

where ΔI is expressed as percentage transmission.

In Fig. 1, curve A is the plot of ΔI against corresponding values of D_1 for $\alpha = 1{\cdot}25$. It will be seen that, as D_1 increases, ΔI increases, becoming a maximum at D_1 = infinity.

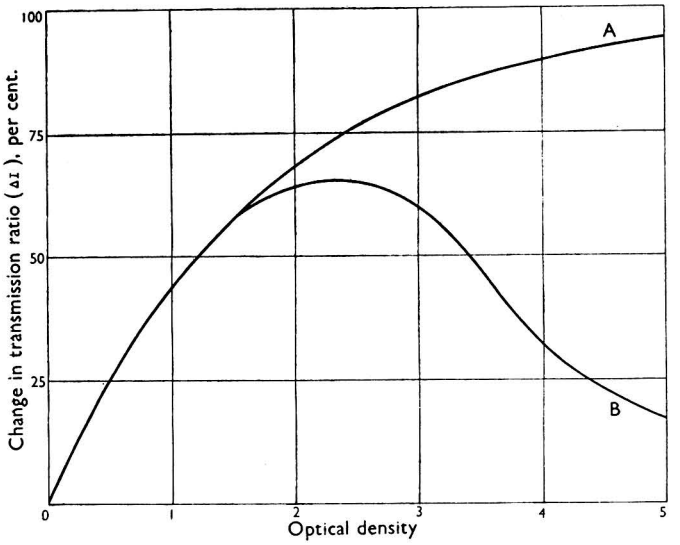

Fig. 1. Theoretical (A) and experimental (B) variation of change in transmission ratio with optical density

Experiments were carried out to compare the behaviour of titanium - hydrogen peroxide solutions with that predicted theoretically. Pairs of solutions were prepared such that while their optical densities varied over the range 0 to 5 (in a 1-cm cell) the ratio of the concentrations of the individual solutions of each pair, α, was constant at 1·25. The transmission ratios of these pairs of solutions were measured, and the results are plotted as curve B, Fig. 1. It will be seen that there is a marked deviation from ideality above an optical density of about 1·2. Theoretical and experimental curves have been plotted for values of α ranging from 1·1 to 1·5, the general form of the curves being the same as that shown in Fig. 1, and in particular the position of the peak of the experimental curve is independent of α.

The theoretical relationships can be analysed mathematically and the position of minimum error can be determined, as has been shown, for example, by Hiskey.[1] Such an approach cannot be made when the solutions show the marked deviation from the Beer - Lambert law indicated in Fig. 1. However, the following semi-empirical approach can be made if it is remembered that the primary requirement is the best possible calibration curve.

The accuracy of the determination of a concentration at any point on a calibration curve is defined exactly as the product of the concentration corresponding to that point and the slope of the curve at that point, where the slope is $d(\Delta I)/dC$, and the error in determining ΔI is constant for all values of ΔI. Now calibration curves are nearly linear over the range $\alpha = 1$ to $\alpha = 1{\cdot}1$ or even 1·2 in the practicable optical density range 0 to 5 or 6, so that the slope, S, of the calibration curve may be considered as equal to $\Delta I/(C_1 - C_2)$, where ΔI is the transmission ratio of the pair of solutions, C_1 and C_2. The over-all accuracy, A, attainable can then be expressed as the product of this slope and the mid-point of the concentration range C_1 to C_2, that is—

$$A = \frac{\Delta I}{(C_1 - C_2)} \times \frac{(C_1 + C_2)}{2}$$

$$= \Delta I(1 + \alpha)/2(1 - \alpha).$$

Hence the accuracy is proportional to ΔI at constant α, and the criterion for the best calibration curve is that it has the greatest ΔI for a given value of α within the range 1·0 to 1·2.

The following limitation applies to this semi-empirical method of assessing the accuracy of a calibration curve. It has been assumed that the most accurate part of a calibration curve on which to work is the steepest part, where α is equal to or not much greater than unity. This is not true for solutions obeying the Beer - Lambert law when D_1 is less than 0·4343 (see Hiskey,[1] Fig. 6), but applies for all higher values of D_1. Hiskey also showed that the minimum error decreases as D_1 increases.

When these conclusions are applied to the experimental curve (Fig. 1), it can be seen that the Beer - Lambert law is obeyed for all optical densities up to about 1·2, so that the minimum possible error on the calibration curves below $D_1 = 0{\cdot}4343$ will still be greater than that for calibration curves at values of D_1 greater than 0·4343. This will in general hold for all systems to which the high-precision absorptiometric method is applied, so that the criterion for maximum accuracy stated above can be safely applied. It will be noted that, as long as solutions obey the Beer - Lambert law, the best calibration curve results from the use of solutions of as high an optical density as possible, with values of α in the range $\alpha = 1{\cdot}0$ to $\alpha = 1{\cdot}2$ (where the slope of the curve is greatest). However, for the titanium - hydrogen peroxide solutions used in the present method, curve B in Fig. 1 shows that the best calibration curves are to be found in the optical density range 2·1 to 2·5, *i.e.*, a concentration range of 15 to 18 mg of titanium per 100 ml of solution.

Other preliminary experiments

In order to assess what influence changes in the many possible variables had on the value determined for the transmission ratio between a pair of solutions, extensive experiments were performed and the results were assessed by normal statistical analysis. These experiments will not be described in detail but the conclusions will be stated and, where it is thought necessary, these will be supported by representative results.

Preparation of cells—

It must be emphasised at the outset that great care is necessary in the preparation of the glass absorption cells. In order to attain reproducible values for the transmission ratio

between a given pair of solutions, it has been found necessary to rinse the emptied cells first with dilute sulphuric acid, twice with either industrial spirit or acetone and then twice with ether. The cells are then inverted on a filter-paper pad to drain and dry, after which the outer optical surfaces should be polished with a clean dry chamois leather. The cells should be filled in such a way that the outer surfaces do not become contaminated with the solution. If such a procedure is adopted, the transmission ratio of a given pair of solutions in the same pair of cells should lie within a range of $\pm 0{\cdot}1$ per cent. transmission after emptying, cleaning and refilling.

VARIABLES THAT ARE A FUNCTION OF THE SOLUTION—

These variables are the acid concentration and temperature of the solution, the amount of reagent added, the stability of the colour of the titanium - hydrogen peroxide compound and other elements present. All but the last of these are inherent in the method, whereas other elements are incidental to the particular specimen being examined. The effect of some elements that are common contaminants of titanium will be described later; here attention will be confined to the inherent variables listed above.

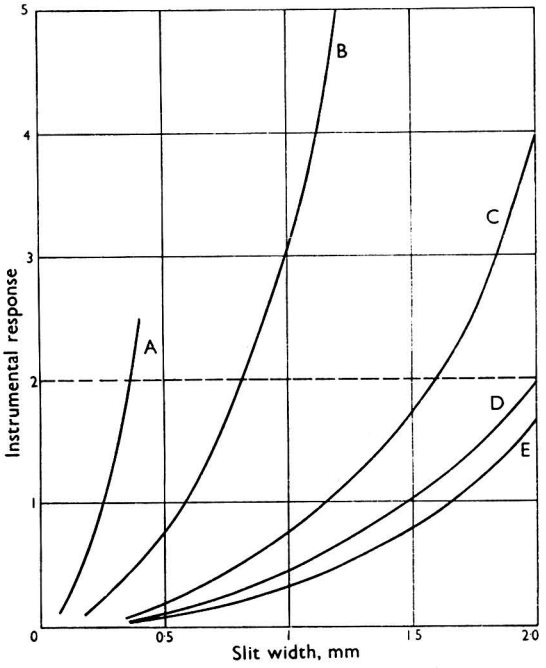

Fig. 2. Variation of instrumental response with slit width of Unicam SP500 spectrophotometer for solutions of various concentrations.

The solutions contained the following amounts of titanium per 100 ml: curve A, 12 mg; curve B, 16 mg; curve C, 20 mg; curve D, 24 mg; and curve E, 32 mg

Amount of reagent—About 3 ml of 20-volume hydrogen peroxide are required to develop the full colour of the titanium - hydrogen peroxide compound in 100 ml of solution containing 15 mg of titanium and 20 g of sulphuric acid, and any excess of hydrogen peroxide does not cause a change in the intensity of the colour.

Stability of colour—The transmission ratio between several pairs of solutions was found to remain constant for at least 14 days.

Variation in acid concentration—This can occur in two different ways. Either the concentration of acid may differ in the individual members of a pair of solutions between which the transmission ratio is to be measured, or it may be the same for the members of a pair while differing from one pair to another. Variations of the first type alter the observed transmission ratio between a pair of solutions. A difference of 2·5 g of sulphuric acid per

litre at a total acid concentration of 200 g of sulphuric acid per litre causes a change in the transmission ratio of 0·1 per cent. (equivalent to 0·02 per cent. of titanium, see below). Variations of the second type do not cause any change in the transmission ratio, even over the range 100 to 300 g of sulphuric acid per litre.

Variations of temperature—These can also occur in two ways. Either the temperature may differ between the individual solutions of a pair, or, while being constant for the members

TABLE I

INFLUENCE OF ACID CONCENTRATION AND TEMPERATURE ON THE TRANSMISSION RATIO BETWEEN A PAIR OF SOLUTIONS WHEN THE ACID CONCENTRATION IS SIMILAR IN BOTH

Reference solution contains 150·00 mg of titanium per litre

Titanium concentration of reference solution, mg per litre	Temperature of reference and test solutions, ° C	Acid concentration of reference and test solutions, g per litre		
		95	190	290
150·04	15·5	99·2	99·3	99·2
	20·5	99·1	99·3	99·3
	24·2	99·1	99·3	99·3
180·05	15·5	41·6	41·7	41·6
	20·5	41·6	42·0	41·7
	24·2	41·7	41·5	41·8

of any pair, may differ from pair to pair. Variations of the first kind are unimportant, as the solutions attain the same temperature within 2 to 3 minutes of the absorption cell being put into the cell holder, *i.e.*, after 2 to 3 minutes a constant value for the transmission ratio is attained. Variations of the second kind correspond to the day-to-day variations of the laboratory temperature and are therefore important. It has been found that temperature variations of this kind do not cause any variation in the transmission ratio of a pair of solutions

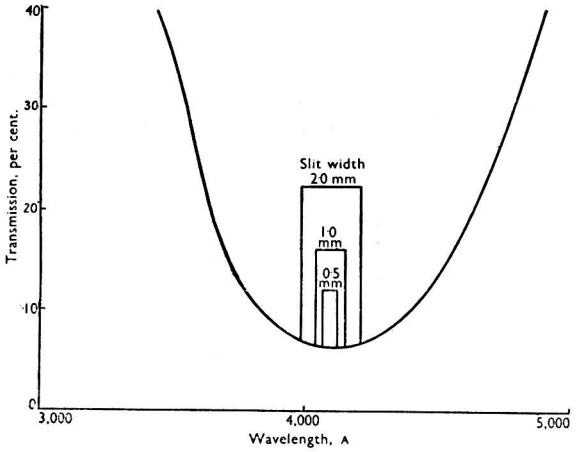

Fig. 3. Absorption curve for titanium - hydrogen peroxide colour, and bandwidths of light corresponding to slit widths of 2·0, 1·0 and 0·5 mm, with a Unicam SP500 spectrophotometer

when the acid concentration of the individual solutions is the same. Table I contains some representative results showing the constancy of the transmission ratios determined under these conditions.

VARIABLES THAT ARE A FUNCTION OF THE SPECTROPHOTOMETER—

When balancing the Unicam SP500 spectrophotometer with a given reference solution in the light beam and the transmission control set to 100 per cent., it is possible to achieve balance at different settings of the slit width and sensitivity control. Only one of these settings may be varied at will, however, the other being a dependent variable. Of these

two variables, only the slit width is calibrated, so that it is necessary to use the slit width as the independent variable. The response of the instrument, which is the deflection of the meter needle for a given change in the setting of the transmission scale, alters as the slit width is changed. The relation between slit width and instrumental response is shown in Fig. 2. (The units on the instrumental response axis are equal to the reciprocal of the change in percentage transmission required to deflect the meter needle one division.) The different curves are produced by placing solutions of different concentration in the light beam, and the horizontal broken line represents the minimum response considered permissible, if the instrument is to be read with certainty to 0·1 per cent. transmission. It corresponds to a deflection of the meter needle of one-fifth of a division for a change of 0·1 per cent. transmission. It has already been shown that the optimum strength of the reference solution is 15 mg of titanium per 100 ml, so that a slit width greater than 0·7 mm should be used (see Fig. 2).

Variation in the slit width also alters the bandwidth of the light passing through the solution and so can alter the value of the transmission ratio between a pair of solutions, by virtue of the fact that the bandwidth extends for different distances on either side of the peak of the absorption curve, as shown in Fig. 3. Such a variation in bandwidth would certainly alter the absolute value of the optical density measured against, say, distilled water, although when, as in this method, two similar solutions are to be compared, the effect may be expected to cancel out, at least in part. Experiments carried out to test this possibility suggest that the effect is not significant. Nevertheless, when dealing with sample solutions that contain other absorbing species, it may be possible to avoid interference by having a small bandwidth for the light from the monochrometer. If this is impossible, then it becomes necessary to apply a correction, as for iron, to be described below. This can only be done if the slit width is kept constant, so that the overlap of the bandwidth on the absorption band of the interfering ions is constant. Hence, it must be arranged that a constant slit width is used. This width should be such that an adequate instrumental response is secured, but no wider. In this work a slit width of 0·8 mm was used. It must be noted that variations in the intensity of the light source, response of the photo-tubes, and so on, such as can occur from instrument to instrument, can alter considerably the positions of the curves shown in Fig. 2, and hence require the use of a different slit width.

CONCLUSIONS—

The necessary conditions for the highest accuracy in the determination of titanium by this method are that (*a*) the concentration of the reference solution should be 150 mg of titanium per litre in 20 per cent. w/v sulphuric acid solution; (*b*) 5 ml of 20-volume hydrogen peroxide solution should be used to colour 100 ml of solution; (*c*) the acid concentrations of the reference and test solutions should not differ by more than 2·5 g of sulphuric acid per litre; (*d*) the slit width of the spectrophotometer should be kept constant and should be as narrow as possible consistent with attaining an adequate response from the instrument; (*e*) the absorption cells should be scrupulously cleaned as described above; (*f*) the transmission ratio of the solutions can be measured at any convenient time after colouring; and (*g*) the transmission ratios of the solutions can be measured at any prevailing laboratory temperature.

METHOD

REAGENTS—

Standard titanium solution—Prepared and standardised as described below.

Sulphuric acid, concentrated—Analytical-reagent grade.

Sulphuric acid, 20 per cent. w/v—Dilute the concentrated acid to a sp.gr. of 1·123 at 20° C.

Hydrogen peroxide, 20-volume.

Ammonium sulphate—Analytical-reagent grade.

APPARATUS—

Balance—A semi-micro balance, weighing to 0·00001 g.

Flasks—Several 100-ml lipped conical flasks, with suitable covers, and 100-ml calibrated flasks, calibrated to 0·01 ml.

Spectrophotometer—Unicam SP500 or Beckmann DU spectrophotometer.

DEFINITIONS—

Standard titanium solution—A stock solution of 150 mg of titanium contained in a known weight of solution the volume of which is 100 ml.

Sample solution—A similar solution prepared from a known weight of sample.

Reference solution—A solution, coloured with hydrogen peroxide, prepared from a 10-ml aliquot of the standard titanium solution.

Test solution—A similar solution prepared from a suitable aliquot of the sample solution.

A set of solutions—This comprises the reference and test solutions, which are coloured and prepared at the same time.

PRELIMINARY REMARKS—

The method is based upon the comparison of a solution of unknown titanium concentration (coloured by the complex formed with hydrogen peroxide) with a similar solution of known titanium concentration, when the transmission ratio of the two solutions is a measure of the difference in concentration. The conditions necessary for an accurate determination of the transmission ratio between these two solutions have already been described. In addition, it is necessary that all aliquots should be taken by weight, as it has been found that aliquots taken volumetrically are not sufficiently reproducible. With volumetric techniques only, the analytical results have a coefficient of variation of 0·07 per cent., but with a weight method for taking the aliquots, the coefficient of variation is reduced to 0·03 per cent. Even if a volumetric method is to be used, the pipette must be recalibrated with solutions containing 5 g of ammonium sulphate and 20 g of sulphuric acid per 100 ml of solution, otherwise the results will be 0·1 to 0·2 per cent. low.

PROCEDURES FOR COMPARING CONCENTRATION—

Two methods are available for comparing the concentrations of the reference and test solutions. One method is to prepare a reference solution of an accurately known concentration, say 15·000 mg of titanium per 100 ml, and to prepare a calibration curve against such a solution. Aliquots of the sample solution are taken such that the concentration of the test solution will lie in the range covered by the calibration curve. The other method is to take aliquots of the standard and test solutions such that both are of approximately the same strength, say to within $\pm 1{\cdot}0$ per cent., and so that the concentration of the reference solution is $15{\cdot}000 \pm 0{\cdot}05$ mg of titanium per 100 ml. Then the difference in the concentration of the reference and test solutions is proportional to the difference in transmission ratio, and the concentration of the test solution can be determined by applying a small correction to the known concentration of the reference solution. In what follows, the former method will be referred to as the calibration-curve method, the latter as the correction method.

Both methods have their sphere of application, the calibration-curve method when a large number of routine determinations have to be undertaken of samples of unknown titanium content, the correction method when determinations of a few titaniferous alloys of approximately known composition have to be made. In the calibration-curve method, it is necessary to check the calibration curve frequently, as any slight change in the transmission control potentiometer will alter the curve to some extent. Again, the points on the calibration curve must be determined in triplicate at least and at small intervals of titanium concentration, if the curve is to be drawn with the required accuracy. Alternatively, fewer points can be determined in triplicate, and an empirical equation to the curve can be deduced, from which other points can be interpolated or a table of transmission ratio against concentration can be made. Such a procedure is lengthy and tedious if only a few determinations are required, the correction method then being preferable, as the slope of the linear correction graph can be determined rapidly; single determinations of a few points and the drawing of the best straight line give an adequate accuracy.

NOTE—If aliquots are to be taken volumetrically, the calibration-curve method must be used, as the aliquots in the correction method must frequently be taken with a burette, and this cannot be done with sufficient accuracy volumetrically.

MEASUREMENT OF TRANSMISSION RATIO—

Two cells are selected and cleaned as already described; one is marked reference and the other is marked test. These cells should be filled with the reference and test solutions, respectively, and the transmission ratio is measured as follows.

The instrument and light source should be turned on 15 minutes beforehand. The wavelength is set to 4100 A and the slit width to the predetermined value, the switch is set to check and the instrument is balanced with the dark-current control. The reference solution is then placed in the light beam, the shutter is opened and the instrument is re-balanced by means of the sensitivity control. The test solution is then placed in the light beam, the switch is turned to "1" and the instrument is balanced by means of the transmission control. The reference solution is then replaced in the light beam and the switch is turned to check. The instrument should still be in balance. If this is not so, the measurement should be repeated. The measurements are repeated after 1-minute intervals until a constant value is attained. This occurs when the temperature of the cells and solutions is the same (the values are constant after 1 to 2 minutes). Except during measurements of the transmission ratio, neither solution should be left in the light beam, or the results may be erratic.

The procedure to be followed when measuring the transmission ratios of a set of solutions is as follows. Both cells are filled with the same reference solution, and the transmission ratio (cell check value) is measured. Initially this must be repeated several times, emptying, cleaning and refilling the cells between each determination, so that a reliable estimate of the value can be made. Subsequently one determination will be sufficient provided that the value agrees with the cell check value found previously to $\pm 0{\cdot}1$ per cent. This cell check should be made before the measurement of the transmission ratios of test solutions is attempted, so as to ensure that the cells are in good order. The reference solution used for the cell check should not be emptied from the reference cell, but should be used as the reference for the determination of the transmission ratios of all the test solutions of the set. Finally, the cell check should again be made, to ensure that the reference solution - reference cell combination has undergone no change. It is advisable to keep the reference cell covered to avoid possible loss by evaporation.

PREPARATION OF THE STANDARD AND SAMPLE SOLUTIONS—

Standard solution—The titanium selected for the preparation of the standard solution should be completely analysed so that the titanium content is accurately known. The accuracy of the method is limited by the accuracy of this value. The standard titanium used in this work was a Van Arkel titanium with the following composition: oxygen, 0·019; hydrogen, 0·022; nitrogen, 0·003; iron, 0·016; manganese, 0·012; aluminium, 0·005; silicon, 0·005; vanadium, 0·002 to 0·003; and lead, 0·002 per cent. Total impurities, 0·087 per cent.; titanium, by difference, 99·91 per cent. Suitable materials for use as standard samples are Van Arkel titanium or arc-melted pure titanium sponge. The sponge itself is not suitable for a standard material, as its composition varies from lump to lump and even within each lump. The selected material should be milled as finely as possible, and an amount weighed out to contain 150·00 mg of titanium. This should be placed in a lipped 100-ml conical flask containing 5·0 g of dry ammonium sulphate. The flask, contents and cover are then weighed to the nearest 0·1 g. About 6 ml $\pm$ 1 ml of concentrated sulphuric acid are added and the flask is heated on a hot-plate to just below the boiling point of the solvent mixture. If the reaction becomes sluggish, or if a white crystalline precipitate appears, more acid (1 ml at a time) should be added. When solution is complete, the flask and contents are cooled and weighed, the difference between the weight of the flask before the addition of the acid and after solution giving the weight of sulphuric acid present. The volume of 20 per cent. w/v sulphuric acid necessary to make the weight of sulphuric acid up to 20 g is calculated, and the contents of the conical flask are transferred to a tared 100-ml calibrated flask with this volume of acid. The transfer is completed with distilled water and the volume made up to the mark at 20° C. The calibrated flask and contents are then weighed and the weight of the solution is calculated; three or four such solutions should be prepared simultaneously to enable the strengths of the individual solutions to be checked (see later).

Sample solutions—These are prepared in the same way as the standard solutions, from 0·1 to 0·2 g of the finely divided sample.

PREPARATION OF THE REFERENCE AND TEST SOLUTIONS—

The required aliquot is taken from the standard or sample solution by means of a pipette or burette and run into a tared 100-ml calibrated flask, whose volume is known to 0·01 ml. This is followed by about 50 ml of 20 per cent. w/v sulphuric acid solution to wash the titanium solution out of the neck of the flask and then by 5·0 ml of 20-volume hydrogen peroxide

solution. The solutions are then made up to the mark with 20 per cent. w/v sulphuric acid solution. It is important when these solutions are made up that they should not differ in temperature by more than 0·2° C, which is equivalent to a change in volume of 0·01 per cent. This is conveniently effected by allowing them to stand in a water-bath or a thermostatically-controlled enclosure at 20° C for 10 minutes. Use of a thermostat is in general more convenient, for, should it be necessary to repeat the preparation of any solution in a given set, this can be done without preparing a fresh reference solution, which would otherwise be necessary.

CHECKING THE STANDARD TITANIUM SOLUTIONS—

Before the standard solutions are used for the preparation of a calibration curve, or for the preparation of reference solutions for making titanium determinations, it is advisable to inter-check them. First a correction curve should be prepared by taking 9·9, 10·0, 10·1 and 10·2-ml aliquots from any one of the reference solutions, weighing them and preparing coloured solutions with hydrogen peroxide as already described. The transmission ratio of each of these solutions against the 10-ml aliquot is measured and the relationship between difference in transmission ratio and difference in concentration is determined by calculation or graphically, assuming a linear relationship. When calculating the concentration of the solutions, the volumes of the calibrated flasks must, of course, be taken into account. Ten-millilitre weighed aliquots are now taken from each of the standard solutions and a set of coloured solutions are prepared, the transmission ratios of which are determined with one of them as the reference solution. The amount of titanium in the standards should then be calculated and must lie within ±0·03 per cent. of the nominal value to be accepted.

PREPARATION OF CALIBRATION CURVE—

Weighed aliquots of 10, 11, 12, 13 and 14 ml are taken from one of the standard solutions and diluted as described. The 10-ml aliquot must be such that its weight is exactly 0·10000 ± 0·00001 of that of the standard solution from which it was taken (this can readily be done with a capillary pipette calibrated in 0·01-ml units and readable to 0·001 ml). With this solution as the reference solution, the transmission ratios of all the solutions, including the 10-ml aliquot, are measured. These measurements should be repeated twice more with a new set of solutions each time. Each individual point is plotted on a graph of adequate size. As it stands, this curve will be relatively inaccurate in the regions midway between the various sets of points. This could be remedied by determining more points, but a simpler way is to deduce from the determined results the values of the transmission ratio for solutions containing exactly 15·0, 16·5, 18·0, etc., mg of titanium per 100 ml and to calculate an equation to the curve. The following is an example of such a calculation—

Original results				
Concentration, C, mg of Ti per 100 ml	Transmission ratio, I, %	$\log_{10} I$	First differences	Second differences
15·0	100·03	2·0000		
			0·2014	
16·5	62·88	1·7986		0·0212
			0·1802	
18·0	41·54	1·6184		0·0206
			0·1596	
19·5	28·76	1·4588		0·0213
			0·1383	
21·0	20·92	1·3205		

In column 4 the differences of the pairs of values of $\log_{10} I$ are given, while in column 5 are given the differences of the figures in column 4. The mean of the figures in column 5 is 0·0210. From the figures in columns 4 and 5, and deducing the appropriate general formulae for the series, it can be shown that the values of $\log_{10} I$ are given by the expression—

$$\log_{10} I = 2 - 0{\cdot}2014n - \frac{0{\cdot}0210n(n-1)}{2}$$

where n is given by $C = 15{\cdot}0 + 1{\cdot}5n$.

From such an equation, intermediate points for the calibration curve can be computed, or a table of values of C calculated for corresponding values of I. The general form of the calibration curve is shown in Fig. 4.

EFFECT OF OTHER IONS—

The alloys examined to date in this laboratory have been virtually pure binary or tertiary alloys, the impurities, *i.e.*, silicon, manganese, vanadium, aluminium, magnesium and calcium, all being present at levels lower than 0·1 per cent. The presence of these elements at this level did not have any effect on the transmission ratios, nor did potassium and ammonium sulphates,

TABLE II

EFFECT OF IRON ON THE TRANSMISSION RATIO OF TITANIUM - HYDROGEN PEROXIDE SOLUTIONS

Concentration of titanium, mg per 100 ml	Transmission ratio of pure titanium solution minus determined transmission ratio in presence of	
	2 mg of iron, %	4 mg of iron, %
15·0	+0·5	+0·9
16·5	+0·3	+0·7
18·0	+0·3	+0·5

when present in amounts up to 1 g per 100 ml of test solution. The effect of iron up to 25 per cent. of the titanium content has been investigated and the results are shown in Table II, in which the amount of iron present is expressed as mg of iron per 100 ml of test solution. It will be seen that the effect of iron varies with the titanium concentration of the test solution. For example, when the titanium concentrations of the test solution is 15 mg per 100 ml, 1 per cent. of iron in the sample is equivalent to 0·007 per cent. of titanium, but when the titanium concentration is 18 mg per 100 ml, 1 per cent. of iron is equivalent to 0·014 per cent. of titanium.

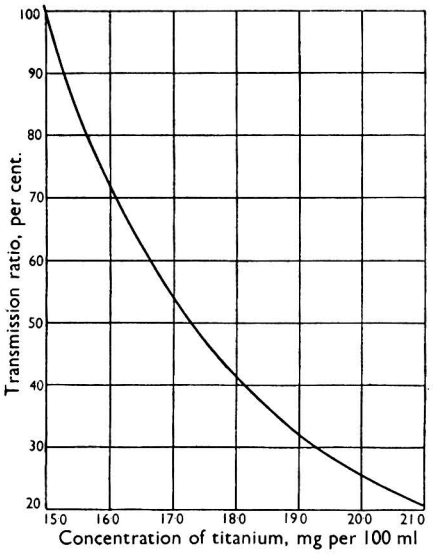

Fig. 4. Calibration curve

TYPICAL RESULTS OF DETERMINATIONS

The following results are typical of many obtained during the analysis of titanium - oxygen and titanium - iron - oxygen alloys. In Table III are shown results determined by taking the aliquots volumetrically, making use of a calibration curve. The following ratios were determined gravimetrically by taking 10-ml aliquots from nine different 100-ml sample solutions—

0·10018, 0·10009, 0·10022, 0·10025, 0·10019, 0·10022, 0·10021, 0·10017, 0·10026; mean 0·10020: coefficient of variation = 0·05 per cent.

In Table IV are shown results found by the correction method with the weight method of taking aliquots. The following are the results of thirteen replicate determinations on a standard Van Arkel titanium—

99·93, 99·95, 99·85, 99·91, 99·91, 99·91, 99·85, 99·91, 99·91, 99·95, 99·91, 99·86, 99·87; mean 99·90: coefficient of variation = 0·03 per cent.

Each figure quoted above and in Tables III and IV is the result of a complete determination, *i.e.*, the titanium content is determined on different portions of the sample; they are not merely replicates in a single sample solution.

TABLE III

TYPICAL RESULTS BY THE CALIBRATION-CURVE METHOD WITH VOLUMETRIC ALIQUOTS

Sample	Amount of titanium found, per cent.			Mean value for titanium, %
1	93·82	93·75		93·79
2	92·88	92·77	92·74	92·80
3	96·54	96·60		96·57
4	95·60	95·54	95·60	95·58
5	94·51	94·49	94·33	94·44
6	92·94	92·95	92·94	92·94
7	92·23	92·31	92·38	92·31
8	94·59	94·40		94·50
9	92·55	92·53		92·54
10	90·70	90·75		90·73

Over-all coefficient of variation: 0·07 per cent.

TABLE IV

TYPICAL RESULTS BY THE CORRECTION METHOD WITH WEIGHT ALIQUOTS

Sample	Amount of titanium found, per cent.		Mean value of titanium, %
1	98·11	98·14	98·13
2	95·86	95·82	95·84
3	99·45	99·45	99·45
4	98·30	98·31	98·31
5	99·70	99·64	99·67
6	95·71	95·68	95·70
7	97·41	97·45	97·43
8	98·41	98·46	98·44
9	94·66	94·68	94·67
10	96·43	96·43	96·43

Over-all coefficient of variation: 0·03 per cent.

DISCUSSION AND CONCLUSIONS

The results by the procedure described have been obtained by the use of normal commercially available apparatus, which has not been modified in any way. The coefficient of variation of these values is 0·03 per cent.; that is, the range within which 95 per cent. of all the values will fall is ±0·06 per cent. This is considerably better than can be achieved by the usual gravimetric procedures, even by the most careful workers. The time taken to complete a determination after the dissolution of the sample is about 90 minutes. This compares favourably with that taken with gravimetric procedures.

The work described above has been carried out as part of the research programme of the National Physical Laboratory, and this paper is published by permission of the Director of the Laboratory.

REFERENCE

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The Determination of Titanium in Uranium - Titanium Alloys by Differential Absorptiometry

By G. W. C. MILNER and P. J. PHENNAH

The differential absorptiometric technique has proved suitable for the determination of from 5 to 75 per cent. of titanium in titanium - uranium binary alloys. The yellow colour produced by titanium with hydrogen peroxide in 2·5 *N* sulphuric acid solutions was used in this work and optical density measurements were made with both the Beckman DU spectrophotometer and the Spekker absorptiometer model H 760. Good agreement was found between the results determined with these instruments, showing that the Spekker absorptiometer has a potential use in the determination of major alloying constituents. Although macro amounts of uranium can be determined differentially, this determination proved impossible in the presence of titanium.

The theoretical principles of differential absorptiometry are summarised.

Hiskey and co-workers[1,2,3] have shown from theoretical considerations that certain absorptiometric determinations can be made with greater precision if the usual water or blank solution is replaced by a standard solution of the same substance at approximately the same concentration. This differential technique has been successfully applied in analysis by various workers using the Beckman DU spectrophotometer, or an equivalent instrument, for optical density measurements. For example, Bastian determined large concentrations of copper[4] and nickel[5] by means of the natural colours of the ions produced by these elements in perchloric acid solutions. Crouthamel and Hubbard[6] determined uranium by a thiocyanate procedure in acetone solution, whilst Neal and Short[7] determined the titanium in pure titanium metal and titanium - titanium dioxide mixtures by means of the titanium - hydrogen peroxide colour.

Many analytical laboratories in this country are equipped with Hilger Spekker absorptiometers and not with the more expensive spectrophotometers. When the differential technique was used for the determination of titanium in titanium - uranium binary alloys, therefore, experiments were made to test the applicability of the new Spekker absorptiometer to differential absorptiometry. Various attempts have been made in the past by different workers to use this type of instrument for the determination of the major constituents of alloy systems. Stross[8] used a system involving the use of neutral filters, these filters being inserted in front of the compensating photocell after the initial balancing of the instrument. Pollak and Nicholas[9] pointed out, however, that this type of technique contravened one of the design principles of this instrument, namely that the light intensity on the indicating photocell should be the same when the instrument is set as when the readings are made. For accurate work, these workers suggested that the neutral filters should be used in front of the indicating photocell only. At a recent meeting of the Physical Methods Group of the Society,[7] however, some doubts were expressed as to the suitability of the Spekker absorptiometer for differential work. In testing this instrument, our method consisted in assaying the alloys for titanium by an established gravimetric method[10] and then by the differential technique, determining the titanium by means of the titanium - hydrogen peroxide colour, making use of both the Beckman spectrophotometer and the Spekker absorptiometer for optical density measurements.

Theoretical principles of differential absorptiometry

Consider first the general case in which the absorptiometer or spectrophotometer is used in the usual way. A solvent blank is used when the instrument is balanced at zero absorption or 100 per cent. transmission. The optical density of the coloured solution is then measured with reference to this setting and the concentration of the solution is determined by reference to a calibration graph, which is prepared by an identical procedure. Suppose that the error in determining a concentration, c_1, by this method is $\pm \chi$ per cent. of the total concentration. This error has no theoretical interpretation, but is defined to include all errors made in the

measurement of the optical density, whether instrumental or operational errors. Moreover, this error will be inherent in all measurements of this kind. The quoted result would be—

$$c_1 \pm \chi \text{ per cent. or } c_1 \pm \frac{\chi c_1}{100} \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad (1)$$

Now consider the differential technique in which the only variation in the method is that a solution of accurately known concentration, c_2, of the substance being determined is used in place of the solvent blank in the balancing of the instrument. Let the value of c_2 be such that $0 < c_2 \leqslant c_1$. The concentration of the substance as measured is the difference, $c_1 - c_2$, to which the same overall error $\pm \chi$ per cent. still applies. The total concentration determined is now the sum of the following two parts: (*a*) a concentration c_2, which is accurately known and therefore has a negligible error, and (*b*) a concentration, $c_1 \pm c_2$, which has an error of $\pm \chi$ per cent. The quoted result would therefore be—

$$c_2 + (c_1 - c_2) \pm \chi \text{ per cent. of } (c_1 - c_2)$$

$$\text{or } c_1 \pm \frac{(c_1 - c_2)\chi}{100}. \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad (2)$$

The error introduced by the differential technique in equation (2) is clearly smaller than that introduced by the usual absorptiometric technique in equation (1). As the value of c_2 is increased, the error is correspondingly reduced to a theoretical limiting value of zero when c_2 equals c_1.

A further deduction can be made from equations (1) and (2). Suppose the value of c_1 is so great that it is impossible to measure the optical density of this solution with reference to the reagent blank. For the Spekker absorptiometer this optical density will be greater than 1·3, as this type of instrument is only calibrated to measure optical density values up to a maximum of 1·3. In these circumstances the usual practice consists in diluting the solution to give a final solution with an optical density that can be measured on the Spekker absorptiometer. This diluting and fractionating method unfortunately introduces the possibility of further errors in the determination of the concentration c_1. With the differential technique for optical density measurements, however, the need for further dilution of the sample solution is either obviated or greatly reduced. In this technique it is only necessary to increase the concentration of the reference standard, c_2, to such a value that the optical density corresponding to the concentration $c_1 - c_2$ is measurable, *i.e.*, the value is less than 1·3 if the Spekker absorptiometer is to be used. In practice it is advisable to choose a value for c_2 so that the optical density reading for the concentration $c_1 - c_2$ is considerably less than the maximum value that can be read on the instrument. Further considerations on the choice of a value for c_2 are given below (see p. 419).

The Hilger Spekker absorptiometer model H 760 is a null-reading type of instrument with a compensating photocell for the determination of the balance point. The method of operation consists in placing the most concentrated solution in the light beam whilst adjusting the instrument to give a zero galvanometer reading at the zero optical density setting by partially closing an iris diaphragm in front of the compensating photocell. When a less concentrated solution is placed in the light beam, the galvanometer is deflected, and it is restored to its zero value by operating the calibrated drum to close the shutter that controls the intensity of the incident light. The difference in optical density between the two solutions is found from the difference in the drum reading. Although the Beckman spectrophotometer is also a null-reading type of instrument, it only uses a single photocell and then the solution of lowest concentration is used in the balancing of the instrument at zero absorption. In spite of this major difference in operation, equations (1) and (2) are applicable to the results recorded with either instrument, as it is simply the optical density difference between the two solutions that is required in the differential technique.

Hiskey based his theoretical treatment of differential absorptiometry on the Beer - Lambert law, which states that—

$$\frac{I_1}{I_0} = -\log_{10} abc_1,$$

where I_0 = intensity of the incident light,
I_1 = intensity of the emergent light from a solution of concentration c_1,
a = absorptivity, and
b = optical path length.

Hiskey developed mathematically the expression—

$$\frac{\Delta c_1}{c_1} = \frac{0{\cdot}4343\ \Delta\left(\frac{I_1}{I_0}\right)}{\frac{I_1}{I_0}\log_{10}\frac{I_1}{I_0}}$$

where Δc_1 is the small error in the concentration, c_1, due to $\Delta\left(\frac{I_1}{I_0}\right)$. Hiskey states that $\Delta\left(\frac{I_1}{I_0}\right)$ is the uncertainty of reading the exact position on the transmission scale of the absorptiometer and, in addition, that the value of $\Delta\left(\frac{I_1}{I_0}\right)$ is constant for any instrument with a linear transmission scale. The error in the concentration is therefore dependent on the magnitude of the error function $\frac{0{\cdot}4343}{\frac{I_1}{I_0}\log_{10}\frac{I_1}{I_0}}$, and reaches a minimum value when the function attains a minimum value. As the error function is in turn dependent on the value of I_1/I_0, it is possible to plot graphically values of $\frac{0{\cdot}4343}{\frac{I_1}{I_0}\log_{10}\frac{I_1}{I_0}}$ as ordinate against values of I_1/I_0 as abscissa, and then the abscissa of the minimum point of this graph corresponds to the value of I_1/I_0 at which the minimum error occurs in the concentration being measured. Measurements of the optical density of solutions containing the titanium - hydrogen peroxide complex with both a Beckman and a Uvispec spectrophotometer, and also with a Spekker absorptiometer, gave results that when plotted produced error-function graphs that were identical for each instrument and corresponded to the curve for $A = 0$ in Fig. 1. The value of I_1/I_0 for the minimum error in the concentration proved to be about 37 per cent. Hiskey extended the above treatment to the differential technique and he developed the expression—

$$\frac{\Delta c_2}{c_2} = \frac{0{\cdot}4343\ \Delta\left(\frac{I_2}{I_1}\right)}{\frac{I_2}{I_1}\left[\log_{10}\frac{I_2}{I_1} + \log_{10}\frac{I_1}{I_0}\right]},$$

where I_2 is the intensity of the emergent light from the unknown concentration, c_2,

I_1 is the intensity of the emergent light from the reference standard concentration, c_1, and—

$$I_2/I_1 = 10^{A - 0{\cdot}4343},$$

A being the optical density of the standard reference solution as measured against a reagent blank.

Similarly, the conditions for the minimum error in the concentration are found by plotting I_2/I_1 against the error function—

$$\frac{0{\cdot}4343}{\frac{I_2}{I_1}\left[\log_{10}\frac{I_2}{I_1} + \log_{10}\frac{I_1}{I_0}\right]}.$$

As I_2/I_1 is a function of A, Hiskey also studied the effect of varying the concentration of the reference standard (and therefore its optical density, A), the resultant graphs being reproduced in curves labelled $A = 0{\cdot}1085$ to $A = 1{\cdot}736$ in Fig. 1. It is clear from Fig. 1 that the minimum value of the error function in a differential technique is always less than that for a normal absorptiometric method and it becomes increasingly less as the concentration of the reference standard is increased. A point is reached, however, when an increase in the concentration of the reference standard produces only a very small decrease in the value of the error function.

The application of Hiskey's theory to the Spekker type of absorptiometer is not immediately evident for several reasons. First, the design of the Spekker absorptiometer is different from that of the Beckman spectrophotometer and the intensity of the emergent light is always adjusted to be constant. Secondly, the Spekker absorptiometer model

H 760 utilises a logarithmic cam arrangement for altering the intensity of the incident light and this results in a linear absorption scale, while the percentage transmission scale is logarithmic and therefore $\Delta\left(\frac{I_1}{I_0}\right)$ can no longer be considered constant. Hiskey's theory must therefore be modified to make it applicable to the Spekker absorptiometer model H 760. No attempt has been made in this paper to analyse mathematically the particular characteristics of the Spekker absorptiometer or to modify existing theory. Instead, the suitability of the

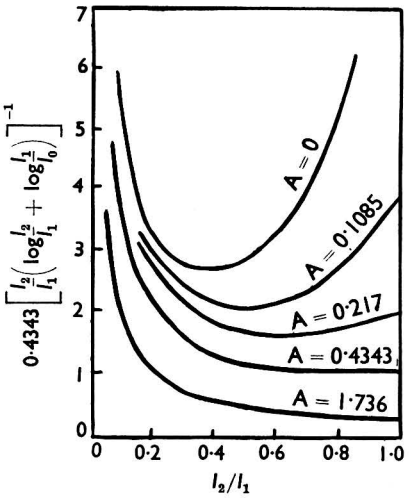

Fig. 1. Curves of the error function for different concentrations of the reference standard

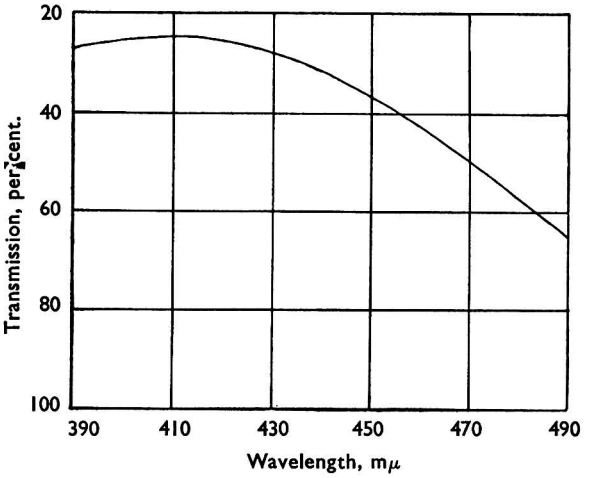

Fig. 2. Transmission curve for titanium - hydrogen peroxide colour

Spekker absorptiometer for differential work has been investigated experimentally by comparing the results found for the titanium content of uranium - titanium binary alloys with those found by the differential method with the Beckman spectrophotometer. Full details of the experimental work and the results found are included in the experimental section.

Experimental

Development of a suitable technique for differential work with the Spekker absorptiometer—

Checking the drum calibration and operation of the drum—The drum calibration of the Spekker absorptiometer used in this work was first checked in accordance with the procedure described by Taylor and Williams.[11] This test was also applied to the Beckman spectrophotometer, and the results are shown in Tables I and II. Repeated tests with the Spekker absorptiometer showed a small error in the scale calibration in the region of zero absorption, and for this reason the part of the drum scale from 0·000 to 0·050 was never used in differential work. The instrument was always initially balanced at an arbitrary zero of 0·050. The operation for adjusting the position of the drum was standardised in this work. The drum was always rotated in the same direction, the chosen direction being from low absorption readings towards readings of higher value.

Technique to overcome cell-length variations—The selection of cells and their use in differential absorptiometry is of considerable importance. A small difference in optical path length may not be apparent when solutions of low optical densities are measured. However, when solutions of relatively high optical densities are being measured, there can be considerable variations in the optical density of a solution if different cells are used. As an example of this effect, the optical density of a titanium solution (18 mg of titanium per 100 ml) containing hydrogen peroxide was measured by two different methods with the Beckman spectrophotometer: (*i*) with the reference standard solution in cell A and the unknown solution under analysis in cell B, when the optical density was found to be 0·167,

and (*ii*) with the reference standard in cell B and the unknown solution in cell A, when the optical density was found to be 0·156.

The large error introduced by the use of badly matched cells can be readily overcome by several methods. The method that was preferred in this work consisted of reversing the order of the cells. In all measurements of the optical density values of solutions, the two chosen cells were arbitrarily marked A and B. Cell A was then filled with the standard reference solution and cell B was filled with the solution of unknown concentration and the optical density difference was measured. Optical density readings were again taken after

TABLE I

RESULTS FOR DRUM CALIBRATION OF THE SPEKKER ABSORPTIOMETER

Solution setting	Reading	Optical density	Individual error
0·000	0·099	0·099	−0·004
0·100	0·202	0·102	−0·001
0·200	0·302	0·102	−0·001
0·300	0·403	0·103	Nil
0·400	0·503	0·103	Nil
0·500	0·601	0·101	−0·002
0·600	0·702	0·102	−0·002
0·700	0·801	0·101	−0·002
0·800	0·903	0·103	Nil
0·900	1·007	0·107	+0·004
1·000	1·105	0·105	+0·002
1·100	1·229	0·129	+0·026
1·200	1·302	0·102	−0·001

TABLE II

RESULTS FOR THE DRUM CALIBRATION OF THE BECKMAN SPECTROPHOTOMETER

Setting	Reading	Optical density	Individual error	Remarks
0·000	0·103	0·103	Nil	To nearest 0·001
0·100	0·203	0·103	Nil	"
0·200	0·303	0·103	Nil	"
0·300	0·403	0·103	Nil	"
0·400	0·503	0·103	Nil	To nearest 0·002
0·500	0·603	0·103	Nil	"
0·600	0·702	0·102	−0·001	"
0·700	0·799	0·099	−0·004	"
0·800	0·905	0·105	+0·002	To nearest 0·005
0·900	1·01	0·11	+0·007	To nearest 0·01
				Range changed here
1·000	1·104	0·104	+0·001	To nearest 0·001
1·100	1·205	0·105	+0·002	"
1·200	1·304	0·104	+0·001	"
1·300	1·406	0·106	+0·003	"
1·400	1·505	0·105	+0·002	To nearest 0·002
1·500	1·606	0·106	+0·003	"

filling cell B with the reference solution and filling cell A with the unknown solution. The mean of these two readings was taken as the true optical density difference. This measuring technique was used in the preparation of all the necessary calibration graphs. In addition, the cells used in the calibration experiments were reserved for all the subsequent work on the determination of unknown concentrations by the differential technique.

Positioning the cells—The positioning of the cells in the Spekker absorptiometer cell carriage can lead to errors unless care is taken to ensure that the cells always occupy the same positions. The technique adopted with this instrument therefore consisted in always placing the cells flush with the side of the carriage nearer the light source. Also, the operation of placing the cell in the light beam was standardised in this work, the cell always being positioned by pushing the carriage towards the rear of the instrument.

THE DETERMINATION OF TITANIUM—

Although titanium forms several coloured complexes suitable for absorptiometric work, investigations were confined to the colour produced by titanium with hydrogen peroxide in 2·5 *N* sulphuric acid solutions. This complex was chosen because of its stability over

reasonable intervals of time, and also because Neal and Short successfully used it for the determination of the titanium content of titanium metal with a Unicam spectrophotometer. The best solvent for uranium - titanium alloys consisted of a mixture of nitric and hydrofluoric acids, but, after the initial dissolution, sulphuric acid was added and the alloy solution was evaporated to fumes of sulphuric acid to remove the nitric and hydrofluoric acids. The alloy solution was then diluted to give the optimum sulphuric acid normality for the production of the coloured titanium complex with hydrogen peroxide. Uranyl ions fortunately do not produce a colour with hydrogen peroxide in acid solutions and give only a small optical density reading with the conditions used for measuring the optical density of the titanium colour. The optimum wavelength for the titanium optical density measurements is in the region of 410 mμ (see Fig. 2), and light of approximately this wavelength can be obtained with the Spekker absorptiometer by means of Ilford No. 601 filters used in conjunction with the mercury-vapour lamp.

When the Spekker absorptiometer is used for differential work, the galvanometer deflection is a major factor in determining the maximum concentration of titanium that can be used in the standard reference solution. In setting the instrument for optical density measurements, the iris diaphragm in front of the compensating photocell is closed, the calibrated drum is next adjusted to zero absorption, or another suitable value, and the light is allowed to pass through the titanium solution on to the indicating photocell. With these conditions the current in the galvanometer circuit is dependent on the intensity of the light falling on the photocell, and the recommended procedure consists in adjusting a resistor in the galvanometer circuit, known as the sensitivity control, until the galvanometer just gives a full-scale deflection. As the concentration of the titanium in the solution is increased, the intensity of the light falling on the indicating photocell is decreased, and eventually a stage is reached when it is impossible to obtain a full-scale deflection of the galvanometer even when the sensitivity control is turned fully clockwise. This state of reduced sensitivity is clearly undesirable in differential absorptiometry, and several methods are available to overcome it. For example, the intensity of the light source could be increased, or alternatively, a more sensitive galvanometer could be used. However, this investigation has been confined entirely to the assessment of the Spekker absorptiometer as supplied by the manufacturers, and the titanium concentrations were chosen to produce a full-scale deflection of the galvanometer.

In addition to the above conditions, it is essential that the Beer - Lambert law is obeyed. A straight-line calibration graph should be produced by plotting the optical density differences between the reference standard and the titanium solution of higher concentration against the increases in concentration over that of the reference standard. Deviations from Beer's law usually occur when the light passing through the solution is not monochromatic, but the use of the mercury-vapour lamp in the Spekker absorptiometer should largely obviate this difficulty. The intensity of this light source is a factor determining the maximum titanium concentration of the reference standard, as it is essential to have sufficient emergent light reaching the photocell to produce a full-scale galvanometer deflection. This kind of limitation does not arise with the Beckman spectrophotometer, as in this instrument a tungsten lamp is used for work in the visible region, and the nature and intensity of the incident beam are controlled by the width of a slit. The higher the concentration of titanium in the reference standard, the greater must be the intensity of the incident beam so as to give a sufficient intensity of the emergent beam. The incident-beam intensity can only be increased with this instrument by further opening of the slit. This operation makes the light less monochromatic, however, and a stage will obviously be reached when Beer's law is not obeyed. An example of the results when Beer's law fails to apply is shown in Fig. 3.

The differential method ensures that the errors from the optical density measurements are minimised as much as possible. Consequently the errors introduced into the determination of the titanium content of alloys will arise mainly from the dilutions needed to adjust the titanium concentration to a value in the region of the optimum reference standard. For the greatest precision it is essential to keep these dilutions to a minimum. When a small volume of the final coloured titanium solution was used, it was considered that suitable dilutions of the sample solution might be troublesome. Moreover, very large final volumes would be wasteful of the reagents used. For these reasons a final volume between these extremes was selected, a volume of 100 ml being considered most suitable for this determination. A good quality grade-A 100-ml calibrated flask was chosen and used for the preparation of all

the titanium solutions. The use of the same flask throughout the experiments avoided the accurate calibration that would be necessary with more than one 100-ml flask.

DETERMINATION OF THE MAXIMUM CONCENTRATION OF THE TITANIUM REFERENCE STANDARD THAT CAN BE USED WITH THE SPEKKER ABSORPTIOMETER—

This maximum concentration was determined with a standard titanium solution containing 1 mg of titanium per ml in 2·5 *N* sulphuric acid. The solution was prepared from Specpure titanium metal sponge. Suitable aliquots of the solution were treated with 4 ml of 100-volume hydrogen peroxide and then each was diluted to 100 ml with 2·5 *N* sulphuric acid. The optical density of each solution was measured in 0·5-cm cells, first at a wavelength of

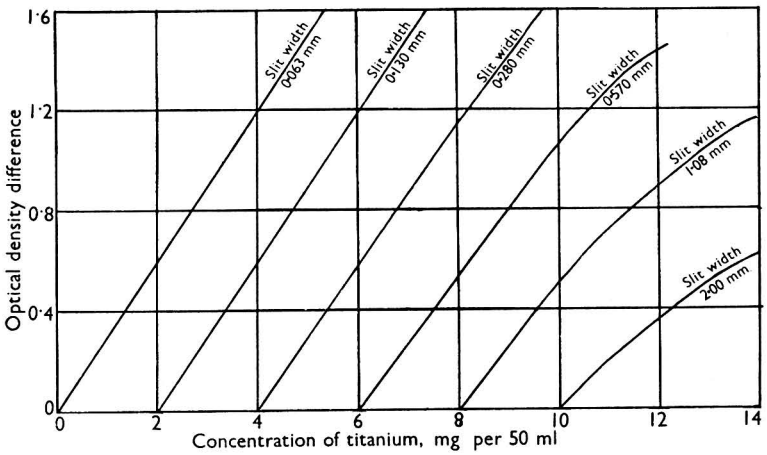

Fig. 3. The effect of increasing the concentration of the reference standard as measured with the Beckmann spectrophotometer at a wavelength of 410 mμ with 1-cm cells. Reference standards containing 0, 2, 4, 6, 8 and 10 mg of titanium per 50 ml were used

410 mμ with the Beckman spectrophotometer, and secondly with Ilford No. 601 filters together with the mercury-vapour lamp and the Spekker absorptiometer. The optical density values for the low titanium concentrations were first measured against a 2·5 *N* sulphuric acid blank. A solution containing 4 mg of titanium per 100 ml was next used in place of the acid blank, and the optical density measurements were repeated. The concentration of the reference standard was then progressively increased, and so were the concentrations of the titanium solutions used for the optical density measurements. This procedure was continued until the Spekker absorptiometer failed to give a full-scale galvanometer deflection, and the experimental results were then plotted on a graph, as shown in Fig. 4. It will be seen from this figure that a linear relationship holds throughout the entire concentration range that was studied with both instruments. From the results of this work, the range from about 12 to 20 mg of titanium per 100 ml of solution was chosen for the determination of titanium in alloys. Before alloys could be analysed, however, it proved necessary to prepare an accurate calibration graph over this range of concentrations. Details of the method used are as follows.

A quantity of Specpure titanium metal sponge was accurately weighed (0·30137 g) and transferred to a platinum dish. A 15-ml portion of nitric acid, sp.gr. 1·42, and then about 7 ml of distilled water were added. After the addition of a few drops of 40 per cent. hydrofluoric acid, the dish and contents were carefully heated on a hot-plate. Further small additions of water and hydrofluoric acid were made periodically until the metal had dissolved completely. The solution was then cooled, 17·5 ml of sulphuric acid, sp.gr. 1·84, were carefully added, and the solution was evaporated to strong fumes of sulphuric acid to remove all nitric and hydrofluoric acids. The solution was cooled, carefully diluted with water and then accurately made up to a volume of 250 ml. This solution contained 1·205 mg of titanium per ml. By means of a 25-ml grade-A burette, graduated in 0·05-ml steps, 12-ml to 16-ml portions of this solution were transferred in turn to the 100-ml calibrated flask.

After the addition of 4 ml of 100-volume hydrogen peroxide, each solution was diluted to the mark with 2·5 *N* sulphuric acid. For the optical density measurements, the solution prepared from the 12-ml aliquot, which contained 14·46 mg of titanium per 100 ml, was used as the reference standard. Separate linear calibration graphs were made for the Spekker absorptiometer and for the Beckman spectrophotometer with optical density differences plotted against titanium concentrations, which were expressed as the number of milligrams of titanium per 100 ml by which the solution differed from the reference standard. These graphs were then used in the following method for determining the titanium content of uranium - titanium alloys containing from 5 to 75 per cent. of titanium with an accuracy of better than ±0·5 per cent.

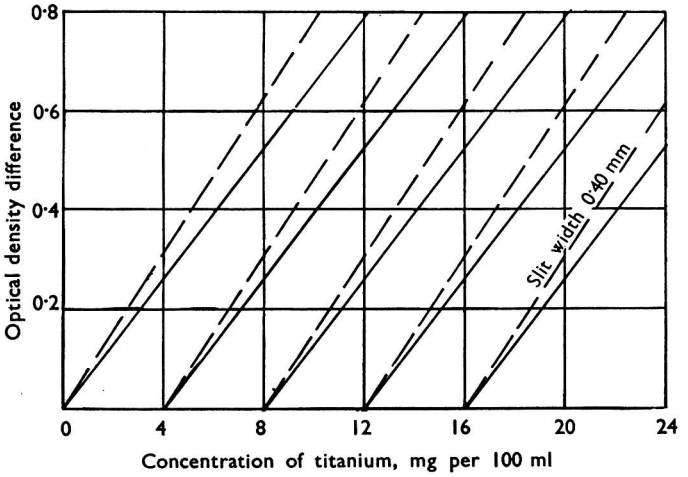

Fig. 4. The effect of increasing the concentration of the reference standard.

Full line: results with the Spekker absorptiometer, Ilford No. 601 filters, mercury-vapour lamp and 0·5-cm cells

Broken line: results with the Beckmann spectrophotometer at a wavelength of 410 mμ, with 0·5-cm cells

Procedure for the analysis of uranium - titanium alloys

Solution of alloy—

Transfer 2 g of sample to a platinum dish and add about 7 ml of water, 15 ml of nitric acid, sp.gr. 1·42, and a few drops of 40 per cent. hydrofluoric acid. Warm the mixture carefully, making further additions of reagent as required until the sample has dissolved (any green residue that remains will dissolve at a later stage during the treatment with sulphuric acid). Cool the solution, add 17·5 ml of sulphuric acid, sp.gr. 1·84, evaporate to fumes of this acid and continue fuming for about 10 minutes. Cool the solution and dilute it accurately with water in a 250-ml calibrated flask.

Preparation of standard titanium solution—

Prepare the following standard solutions of titanium by dissolving the requisite weight of Specpure metal sponge (better than 99·8 per cent. pure) according to the procedure just described: (*a*) 250 ml of solution containing 5·0 mg of titanium per 20 ml and (*b*) a range of standard solutions containing from 12 to 19 mg of titanium per 20 ml.

Preliminary determination of titanium content of the sample—

Transfer a 2-ml aliquot of the alloy solution to a 100-ml calibrated flask then add 2 ml of 100-volume hydrogen peroxide and dilute the mixture accurately to the mark with 2·5 *N* sulphuric acid. Also, transfer a 20-ml aliquot of the standard titanium solution containing 5 mg of titanium per 20 ml to a 100-ml calibrated flask, add 2 ml of 100-volume hydrogen peroxide and dilute it to the mark with 2·5 *N* sulphuric acid. Measure the optical density of each solution with the Spekker absorptiometer and the mercury-vapour lamp, Ilford No. 601 filters and 0·5-cm cells. Use 2·5 *N* sulphuric acid with 2 ml of hydrogen peroxide

per 100 ml as the blank solution. Calculate the weight of titanium in the 2-ml aliquot of sample solution from the ratio of the two optical densities and the weight of titanium in the aliquot of the standard solution.

ACCURATE DIFFERENTIAL DETERMINATION OF THE TITANIUM CONTENT—

Depending on the results of the preliminary determination, take an aliquot of the alloy solution containing from 12 to 19 mg of titanium by means of calibrated grade-A pipettes and transfer it to the 100-ml calibrated flask used in the preparation of the calibration graph. Add 4 ml of 100-volume hydrogen peroxide, dilute the mixture accurately to the mark with 2·5 *N* sulphuric acid and, after mixing, transfer the solution to a clean, dry beaker (solution A).

Take a further aliquot of the alloy solution, transfer it to the same 100-ml calibrated flask, dilute it accurately with 2·5 *N* sulphuric acid and, after mixing, transfer the solution to a clean, dry beaker (solution B).

From the range of standard titanium solutions previously prepared, select an aliquot containing as nearly as possible the same weight of titanium as is present in the aliquot taken from the alloy solution. Transfer this solution to the same 100-ml calibrated flask, add 4 ml of 100-volume hydrogen peroxide and accurately dilute the mixture to the mark with 2·5 *N* sulphuric acid. After mixing, transfer the solution to a clean, dry beaker (solution C).

Adjust the temperature of all solutions to the value used in the preparation of the calibration graph and make the following optical density measurements with either the Spekker absorptiometer and the mercury-vapour lamp, Ilford No. 601 filters and 0·5-cm cells, or the Beckman spectrophotometer at a wavelength of 410 mμ. Use the same cells that were used in the preparation of the calibration graph—

(*a*) Set the scale reading to a value of 0·430 and balance the instrument at this setting with solution B. Measure the optical density due to the uranium in this solution against a 2·5 *N* sulphuric acid blank solution containing 4 ml of 100-volume hydrogen peroxide per 100 ml in the other cell. Interchange the solutions between the cells and repeat the measurements. Calculate the optical density for each measurement and take the arithmetic mean.

(*b*) Set the scale reading to a value of zero or that value found most suitable in the preliminary check (we used 0·05 for our Spekker absorptiometer) with either solution A or solution C in the cell, whichever has the higher optical density. Measure the optical density difference between these two solutions. Interchange the solutions between the cells and repeat the measurements. Calculate the optical density difference for each experiment and take the arithmetic mean.

Calculate the weight of titanium in the alloy sample by the methods shown in the specimen calculations below.

SPECIMEN CALCULATIONS

Two conditions arise in the general application of this method.

(*i*) THE OPTICAL DENSITY OF SOLUTION A IS HIGHER THAN THE OPTICAL DENSITY OF SOLUTION C—

Here the presence of uranium in the alloy solution, A, effectively increases the optical density difference between the two solutions. The uranium contribution must therefore be subtracted.

Example—

Optical density of solution B against sulphuric acid blank = 0·038 (mean of 4 readings).

Optical density difference of solution A against solution C = 0·300 (mean of 4 readings).

True optical density difference = 0·300 − 0·038
= 0·262.

On referring this optical density difference to the calibration graph it was found to correspond to 3·98 mg of titanium per 100 ml. The reference standard contained 12·05 mg of

titanium per 100 ml. Therefore the aliquot of the alloy solution (in solution A) contained (12·05 + 3·98) mg of titanium.

As a 25-ml aliquot of the sample solution (250 ml) was taken for the preparation of solution A, the total weight of titanium in the 2-g sample was therefore—

$$10 \times (12{\cdot}05 + 3{\cdot}98)\text{ mg} = 0{\cdot}1603\text{ g of titanium.}$$

(*ii*) THE OPTICAL DENSITY OF SOLUTION C IS HIGHER THAN THE OPTICAL DENSITY OF SOLUTION A—

Here solution C must be used for the initial adjustment of the Spekker absorptiometer. The presence of uranium effectively reduces the optical density difference between the two solutions. The contribution of the uranium must therefore be added to the observed optical density difference.

Example—

Optical density of solution B against sulphuric acid blank = 0·002 (mean of 4 readings).

Optical density difference of solution C against solution A = 0·105 (mean of 4 readings).

True optical density difference = 0·105 + 0·002 = 0·107.

By reference to the calibration graph, this optical density difference was found to correspond to 1·63 mg of titanium per 100 ml. This reference standard contained 13·37 mg of titanium per 100 ml and, as this concentration proved to be higher than that in solution A, the titanium content of the sample aliquot in solution A was therefore (13·37 — 1·63) mg of titanium.

With this sample it proved necessary to dilute 10 ml to 100 ml with 2·5 *N* sulphuric acid before a 25-ml aliquot was taken for the preparation of solution A. The total weight of titanium in the 2-g sample was therefore—

$$100 \times (13{\cdot}37 - 1{\cdot}63)\text{ mg} = 1{\cdot}174\text{ g of titanium.}$$

DETERMINATIONS OF TITANIUM IN ALLOYS BY THE DIFFERENTIAL METHOD

SYNTHETIC SOLUTIONS—

Synthetic solutions, corresponding to solutions prepared from 2-g portions of alloys covering the range 5 to 75 per cent. of titanium, were prepared from supplies of Specpure titanium metal sponge and Specpure U_3O_8. Both materials were dissolved separately, the titanium being first dissolved in nitric and hydrofluoric acids and then these acids being removed by evaporation to fumes with sulphuric acid. The U_3O_8 was dissolved in nitric acid and the resulting solution was similarly evaporated with sulphuric acid. These two solutions were then mixed and adjusted to a volume of 250 ml and a normality of 2·5 in sulphuric acid. The titanium content of each mixture was then determined according to the above procedure, both the Spekker absorptiometer and the Beckman spectrophotometer being used for optical density measurements. The results of this investigation are shown in Table III.

TABLE III

DETERMINATIONS OF TITANIUM IN SYNTHETIC TITANIUM - URANIUM SOLUTIONS

Nominal composition		Actual composition			Titanium found with	
Titanium, %	Uranium, %	Titanium, g	Uranium, g	Titanium present, %	Beckman spectrophotometer, %	Spekker absorptiometer, %
5	95	0·1039	1·8980	5·19	5·19	5·19
10	90	0·2017	1·7940	10·10	10·08	10·07
25	75	0·4994	1·4990	25·00	24·95	24·96
50	50	0·9990	0·9981	49·97	49·92	49·89
75	25	1·5004	0·5011	74·99	75·18	74·90

ALLOY SAMPLES—

In this work the samples were prepared in the form of thin strips instead of in the form of fine turnings. This technique was adopted to reduce the oxidation of the sample before analysis. The results for titanium by the differential technique described above are shown in columns 2 and 3 of Table IV. There was good agreement between these results and those in column 4, which were determined by a gravimetric procedure described in an earlier report.[10]

TABLE IV

DETERMINATIONS OF TITANIUM IN ALLOY SAMPLES BY THE ABSORPTIOMETRIC AND GRAVIMETRIC PROCEDURE

	Titanium content by analysis		
	Absorptiometric method		
Nominal titanium content of the alloy, %	Beckman spectrophotometer, %	Spekker absorptiometer, %	Gravimetric method, %
10	8·29	8·24	8·45
20	19·30	19·33	19·37
50	46·82	46·88	46·5
60	58·80	58·78	59·0

Thanks are due to Mr. A. A. Smales for suggesting the application of the differential absorptiometric technique to this problem.

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The Determination of Niobium in Stainless Steel

By G. W. C. MILNER and A. A. SMALES

A procedure is described for the determination of the niobium content of stainless steels. The niobium is first separated from many of the other constituents of the steel by precipitation with tannic acid and cinchonine from a slightly acid solution. Silica is used as a carrier for the niobium precipitate, and, after filtration and ignition, the silica is removed as its volatile fluoride. The oxide residue is next fused with potassium bisulphate, and the melt is dissolved by extraction with an aqueous solution of tartaric acid. The niobium concentration is finally determined absorptiometrically, use being made of the yellow colouration produced with potassium thiocyanate. For accurate work it is necessary to incorporate niobium-95 tracer to correct for small losses of niobium occurring in the chemical separation. However, the results for niobium in the absence of the radioactive tracer are generally satisfactory for quality control. The determination of tantalum, by radio-activation, in the separated mixed earth-acid oxides is also briefly discussed.

The methods for the determination of niobium in steels in current use in this country are gravimetric with separation procedures involving either the use of cupferron,[1] or hydrolysis together with the precipitation of niobium as its magnesium salt.[2] The final oxide residues from such procedures contain titanium dioxide and tantalum pentoxide in addition to the niobium pentoxide. The titanium dioxide content is determined absorptiometrically, use being made of the yellow colour produced by this element with hydrogen peroxide, and the necessary correction is applied to the weight of total oxides. The tantalum pentoxide content of the steels is generally assumed to be very small, and the niobium content is then found by applying the factor 0·6990 to the weight of oxides after correcting for titanium dioxide. In these gravimetric methods it is necessary to start the analysis with 5 g of sample, and the chemical separations needed to isolate the niobium from the other steel constituents are rather tedious.

One modern approach to the determination of the niobium content of materials consists in replacing the gravimetric by an absorptiometric technique so that the complete separation of the niobium from other constituents then becomes unnecessary. A method for the determination of niobium in steels by this approach has just recently appeared in the American literature[3]; use is made of the colour produced by niobium with hydroquinone after the preliminary separation of niobium by hydrolysis. We[4] have recently developed an absorptiometric procedure for the determination of niobium in certain types of South African ores. In this method the niobium is first separated from many ore constituents by precipitation with tannic acid and cinchonine from weakly acid solutions, with silica as a carrier. The niobium in the precipitate is finally determined absorptiometrically by means of the niobium - thiocyanate colour, prepared according to Freund and Levitt's[5] recommended conditions; small amounts of niobium lost in the chemical treatment are corrected for by the use of radioactive niobium. In the presentation of this paper at a meeting of the Society, several members asked about the possibility of applying the procedure to the determination of niobium in steels. Subsequent investigations described in this paper have shown that the ore method can be successfully applied to this problem after slight modification.

Experimental

The development of a suitable technique for the complete solution of the sample is the usual initial problem in the analysis of ores and minerals. The recommended method for South African ores and their mineral-dressing fractions, for example, involved attack on the material with hydrofluoric and sulphuric acids in addition to fusions with sodium carbonate and potassium bisulphate. Fortunately, niobium-bearing steels dissolve on treatment with acids, and fusions are therefore unnecessary, so that there is a considerable saving in operational time. After the separation of niobium from many other ore constituents with tannic acid and cinchonine, tungsten and titanium proved to be the only two elements likely to cause serious interference with the absorption measurements in certain circumstances.

For example, when the ratio of niobium pentoxide to tungsten trioxide is 1 to 10 in the final thiocyanate solution, an error of about + 20 per cent. results when 125 μg of niobium pentoxide is determined.[5] This error becomes about + 1·6 per cent. when the niobium pentoxide to tungsten trioxide ratio is 1 to 1. Although small amounts of tungsten are generally present in stainless steels, the ratio of niobium pentoxide to tungsten trioxide in the steels available to us was much greater than 1 and interference from this element was therefore quite small. The colour of the titanium - thiocyanate complex is less sensitive than that of the niobium - thiocyanate complex and whereas a ratio of niobium pentoxide to titanium dioxide of 1 to 10 results in an error of + 5·6 per cent. in the determination of 125 μg of niobium pentoxide, the error is negligible when the ratio has a value of 1 to 1. No difficulties were expected from titanium in steel analysis, therefore, as steels that have been stabilised with niobium generally have a low titanium content.

As in ore analysis, a sample weight of 0·2 g proved suitable for the determination of niobium in steels. In preliminary experiments this weight of steel was dissolved in a platinum dish in 10 ml of diluted sulphuric acid (1 + 1) together with 5 to 10 ml of water. As radioactive niobium was incorporated with each sample so that the efficiency of the niobium separation could be determined, hydrofluoric acid also was used in the initial solution procedure to ensure the exchange between the active and inactive niobium. It proved necessary, however, to remove the hydrofluoric acid before the precipitation with tannic acid, and this was effected by evaporating the sample solution to fumes of sulphuric acid and continuing the heating for about 10 minutes. After the salts were dissolved in water, the tannic acid - cinchonine precipitation procedure was applied and the determination of niobium was completed exactly as for ore samples. Preliminary results for the B.C.S. Steel No. 246 by this procedure were in excellent agreement with the reported figure of 0·82 per cent. for niobium and, moreover, the activity measurements showed that in favourable circumstances as much as 98 per cent. of the niobium in the sample passed into the final tartrate solution. On extending the experiments to different samples, however, it often proved impossible to completely dissolve the salts after heating to fumes with sulphuric acid. This behaviour was attributed to the high chromium content of stainless steels, as chromium sulphate is only soluble in water with difficulty in certain conditions. This difficulty did not occur, however, when the sample was evaporated to fumes of sulphuric acid followed by gentle heating for a short time. Such a technique did not ensure the complete removal of fluoride ions, and so the niobium recoveries were about 90 per cent. This trouble from chromium was not encountered when the sulphuric acid was replaced by 10 ml of perchloric acid, sp.gr. 1·70, the solution then being heated to remove the hydrofluoric acid completely. Details of the procedure incorporating radioactive niobium that was finally adopted are as follows.

Method

Reagents—

Cinchonine solution—Dissolve 5 g of reagent in 100 ml of diluted hydrochloric acid (1 + 1).

Potassium thiocyanate, 3 M—Dissolve 29·0 g of this reagent in water and dilute to a volume of 100 ml.

Stannous chloride, 2 M—Dissolve 4·5 g of stannous chloride and a small pellet of tin in 5 ml of hydrochloric acid, sp.gr. 1·16, and then dilute to 10 ml with water.

Acetone—Use AnalaR quality reagent, which should produce no colouration when 10 ml are mixed with a solution consisting of 10 ml of hydrochloric acid, sp.gr. 1·16, 1 ml of 2 *M* stannous chloride and 5 ml of water.

Niobium tracer—Obtain 1 millicurie of carrier-free radiochemically pure niobium-95 dissolved in a few millilitres of 8 *M* hydrochloric acid from the Radiochemical Centre, Amersham. Add approximately 2 ml of hydrofluoric acid to the solution, transfer it to a polythene bottle with water and dilute it to a volume of about 200 ml. Dilute 25 ml of this stock solution to 250 ml in a second polythene bottle for the working solution and store both solutions behind lead or brick shielding.

Perchloric acid, sp.gr. 1·70.

Hydrofluoric acid, 40 *per cent.*

Sulphur dioxide, saturated solution.

Ammonium chloride.

Sodium hydroxide, 50 per cent. w/v solution.
Tannic acid.
Sulphuric acid, sp.gr. 1·84.
Potassium bisulphate.
Tartaric acid.
Nitric acid, sp.gr. 1·42.

PROCEDURE—

Dissolve 0·20 g of steel in 10 ml of perchloric acid, sp.gr. 1·70, together with about 5 ml of water in a suitable platinum dish (Note 1). Then evaporate to fumes of perchloric acid, cover the dish and continue the heating to oxidise the chromium. Cool the solution, add to it 5 ml of 40 per cent. hydrofluoric acid and a small volume of tracer niobium solution (Note 2), and re-evaporate it to fumes of perchloric acid. Continue heating the solution for several minutes, then cool it, wash the sides of the dish with water and repeat the fuming procedure to remove the hydrofluoric acid. After cooling the residue, dissolve the salts in about 50 ml of water and transfer the solution to a 250-ml squat beaker. To this solution add 10 ml of water saturated with sulphur dioxide and then boil the solution to reduce the chromium and to remove any excess of sulphur dioxide. Add 100 mg of silica in the form of a sodium silicate solution and then 5 g of ammonium chloride, and dilute the resultant solution with water to a volume of about 100 ml. Make the sample solution alkaline to litmus by the addition of a 50 per cent. w/v sodium hydroxide solution and then make it just acid with diluted hydrochloric acid (1 + 1), adding 5 ml of the latter reagent in excess. Dissolve 1 g of tannic acid in the sample solution and then add 5 ml of a cinchonine solution prepared by dissolving 5 g of cinchonine in 100 ml of diluted hydrochloric acid (1 + 1). Add two macerated Whatman accelerators to the solution, heat it to boiling and continue the boiling for 10 minutes. Then allow the mixture to digest while warm for about one hour. Filter through a paper-pulp pad, washing the precipitate with a warm 1 per cent. hydrochloric acid solution containing 1 per cent. of cinchonine. Dry the precipitate and ignite it at about 700° C in the same platinum dish.

Add a few drops of diluted sulphuric acid (1 + 1) to the residue in the platinum dish, followed by about 2 ml of 40 per cent. hydrofluoric acid. Evaporate the mixture to fumes of sulphuric acid to remove silica. Cool the residue and repeat the treatment with a further 2-ml portion of hydrofluoric acid. Then heat the dish strongly to remove the sulphuric acid completely and finally ignite the dish and its contents in a Meker burner flame for a few minutes. Carefully fuse the residue with 2 g of potassium bisulphate to give a clear melt, then let it cool and dissolve the melt in a solution of 1 g of tartaric acid in 20 ml of water. Dilute this solution to a volume of 100 ml with water in a calibrated flask (solution A) and, after thorough mixing, take 10 ml of this solution and dilute it to 50 ml with a 1 per cent. w/v tartaric acid solution (solution B).

Into a 50-ml calibrated flask place 10 ml of hydrochloric acid, sp.gr. 1·16, 1 ml of 2 *M* stannous chloride solution and 5 ml of water. Add 10 ml of acetone and mix the solution thoroughly; then cool it to room temperature by immersing the flask in a water-bath for 15 minutes. With a pipette, place 10 ml of solution B in the 50-ml flask and, after mixing, add 10 ml of 3 *M* potassium thiocyanate solution. Dilute the solution to the mark with water and make optical density measurements after 5 minutes with a 4-cm cell and either a spectrophotometer at a wavelength of 385 mμ or a Spekker absorptiometer with Wratten No. 2 and Chance OV1 filters and a mercury-vapour lamp. Correct the reading for the value found for a blank solution prepared by the above method from a 0·20-g sample of iron. Determine the amount of niobium by referring the corrected sample optical density reading to a calibration graph prepared with a standard niobium solution containing 6 μg of niobium pentoxide per ml in 1 per cent. tartaric acid.

Place 50 ml of the counting-standard solution (Note 2) in a beaker and measure its activity with a γ-scintillation counter. Replace the solution in the beaker by 50 ml of the sample solution A and repeat the count. Correct all counts for the background count of the instrument. Correct the amount of niobium found for experimental losses by multiplying by the factor—

$$\frac{\text{Counts per minute of the counting standard}}{\text{Counts per minute of the sample solution}}.$$

Finally calculate the percentage of niobium in the sample.

NOTE 1—If radioactive niobium is not to be added to the sample, the initial solution procedure can be considerably simplified as follows—

Place 0·20 g of the steel in a 250-ml squat beaker and dissolve the steel in 10 ml of diluted hydrochloric acid (1 + 1) by the dropwise addition of nitric acid, sp.gr. 1·42. Then add 10 ml of perchloric acid, sp.gr. 1·70, and evaporate the solution to fumes of this acid, continuing the evaporation to fully oxidise the chromium. After cooling, dissolve the salts in about 50 ml of water. To this solution add 10 ml of water saturated with sulphur dioxide, and boil it to reduce the chromium and to remove the excess of sulphur dioxide. Complete the procedure as above.

NOTE 2—The volume of tracer solution taken should be such as to result in a 50-ml portion of the final tartrate solution having an activity of a few thousand counts per minute. An equal volume of the tracer solution should also be taken for the counting standard, placed in a 100-ml calibrated flask and diluted to the mark with 1 per cent. tartaric acid solution.

RESULTS

The niobium found when the procedure with radioactive niobium was applied to several typical stainless-steel samples containing from 17 to 22 per cent. of chromium and from 7 to 13 per cent. of nickel are shown in columns 2, 3 and 4 of Table I. The values in column 3 show that by this type of procedure approximately 95 per cent. or more of the niobium in the sample reaches the final tartrate solution. In these circumstances it was considered that the results for niobium in the absence of tracer would be acceptable for many requirements. The initial solution procedure for the steel is considerably simplified when radioactive niobium is not needed, and application of the modified procedure described in Note 1 to the above steels gives the results in column 5 of Table I. With the exception of the British Chemical Standard sample, the steels were also analysed by Bagshawe and Elwell's gravimetric method; these results are reported in column 6 of Table I.

TABLE I

DETERMINATIONS OF NIOBIUM IN STAINLESS STEELS BY ABSORPTIOMETRIC AND GRAVIMETRIC PROCEDURES

Sample number	Results with tracer niobium: Relative γ-activity	Recovery, %	Niobium found, %	Results in absence of tracer niobium in steel, %	Gravimetric results for niobium %	Other elements present: Titanium %	Tungsten %
1 ..	688	96·9	1·04	1·01	1·17	0·18	—
2 ..	673	94·8	1·25	1·17	1·40	0·41	—
3 ..	702	98·9	0·65	0·63	0·69	0·10	—
4 ..	677	95·4	1·03	0·98	1·16	0·05	0·05
5 ..	667	94·0	1·44	1·35	1·52	0·01	0·10
B.C.S. 246	707	99·5	0·80	0·79	0·82*	—	0·22
Counting Standard	710						

* Certified gravimetric figure.

A comparison of the results for niobium found by these methods showed that those by the gravimetric procedure were generally higher than those by the absorptiometric procedures. This lack of agreement was rather disconcerting, and was most serious for samples containing more than 1 per cent. of niobium. It appeared that either the results by the absorptiometric method were erroneous, especially for the steels with the higher niobium contents, or alternatively that the tantalum content of these steels was significant. The behaviour of the absorptiometric method for steels containing about 1 per cent. of niobium or more was tested by taking 0·20-g portions of the steel containing 0·82 per cent. of niobium and adding a standard niobium solution to give samples with increasing amounts of niobium up to a maximum of about 1·5 per cent. The recovery of the added niobium was always complete, which showed that the absorptiometric procedure was perfectly satisfactory for steels with the higher niobium content.

The discrepancies between the absorptiometric and the gravimetric results in Table I indicated the presence of up to 0·1 per cent. of tantalum in some samples. Before attempting to confirm the presence of tantalum in these samples, however, we sought further evidence

in support of the absorptiometric figures in Table I. Bagshawe and Elwell's gravimetric method was applied to 5-g quantities of the steel samples and an accurately weighed quantity (30 mg) of the oxide mixture from each sample was then taken, fused with 2 g of potassium bisulphate, and extracted with 20 ml of a solution containing 5 g of tartaric acid; the resulting solution was diluted to 100 ml with water. A 10-ml portion of this solution was then diluted to 500 ml with a 1 per cent. tartaric acid solution and the niobium - thiocyanate colour was produced from a 10-ml portion of this solution in the normal way. The niobium content of the 30-mg amount of mixed oxides was next determined from the optical density measurements and the niobium percentage was finally calculated from a knowledge of the total weight of mixed oxides found for each sample. The results by this procedure completely substantiated those given in column 4 of Table I.

The tantalum content of the steels was therefore determined by the simplest available method, which was by radioactivation. This method had been used previously for relatively high levels of tantalum by Long[6] and Eichholz,[7] while one of us[8] has described its application to the binary mixture niobium pentoxide - tantalum pentoxide, when, for example, there was no difficulty in determining the tantalum pentoxide content of Specpure niobium pentoxide

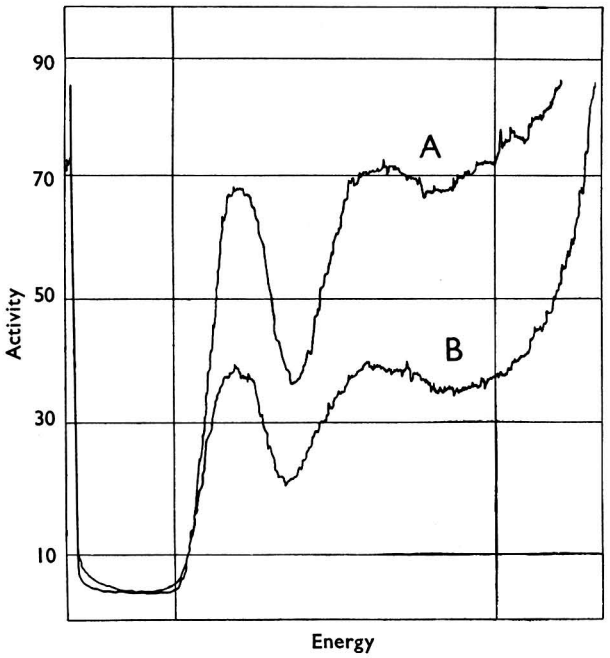

Fig. 1. Gamma spectrum of irradiated mixed oxides from stainless steel No. 1 (curve A) and standard Ta_2O_5 (curve B)

(0·4 per cent.). Beydon and Fisher[9] have also recently discussed this determination. The simplest way of applying the activation method to the steels was to irradiate weighed quantities of about 20 mg of the mixed oxides produced in the gravimetric analysis carried out by Bagshawe and Elwell's procedure. These mixed oxides should contain only niobium and titanium in addition to tantalum, but because small amounts of other elements may be present, γ-counting was done with a sodium iodide crystal spectrometer and a single-channel kick-sorter operating in a narrow channel centred on the 111-day half-life tantalum-182 photopeak at 1·1 MeV. This counting technique makes the direct-activation method for tantalum much more nearly specific than that previously described, but even so it is still possible to have interference from elements giving rise on irradiation to significant levels of radionuclides having γ-energies greater than or near 1·1 MeV, for example, cobalt. The ideal method is, of course, to have a radiochemical separation of the tantalum after irradiation, when the activation method can be made completely specific, but this was unnecessary.

The mixed oxides were irradiated in polythene tubing in the Harwell pile for two hours together with tantalum pentoxide standards. The γ-spectra of an irradiated standard

and sample are shown in Fig. 1. The results are shown in Table II, together with the absorptiometric niobium figures and those for niobium plus tantalum by Bagshawe and Elwell's method.

TABLE II

DETERMINATIONS OF TANTALUM AND NIOBIUM BY DIFFERENT METHODS

Sample number	Tantalum determined by activation, %	Niobium determined by absorptiometry, including tracer, %	Niobium + tantalum determined by gravimetric methods, %
1 ..	0·10	1·04	1·17
2 ..	0·10	1·25	1·40
3 ..	0·06	0·65	0·69
4 ..	0·08	1·03	1·16
5 ..	0·11	1·44	1·52
B.C.S. 246 ..	0·03	0·80	0·82

CONCLUSIONS

It is clear from Table II that the absorptiometric method with a tracer permits an accurate determination of niobium and that the apparent discrepancy between the absorptiometric and gravimetric figures is explained by the tantalum content of the steel samples. If a specific niobium determination is of interest to metallurgical analysts, the thiocyanate absorptiometric method has a decided advantage in speed over the gravimetric method and, as is shown in Table I, even without the use of tracer niobium, results of better than 95 per cent. accuracy are attainable.

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A Rapid Chromatographic Method for the Determination of Vitamin A in Whale-liver Oils

By J. GREEN and D. O. SINGLETON

A rapid method is described for the determination of vitamin A in whole or saponified whale-liver oils. Choice of conditions depends on the presence or absence of anhydrovitamin A. One simple chromatographic treatment permits the drawing of vitamin-A absorption curves in good agreement with the pure vitamin-A curve. The method is not suitable for oils other than whale-liver oils, although its use can lead to considerable elimination of interfering substances.

Vitamin A is accompanied in whale-liver oils by biologically inactive substances, which absorb appreciably in the ultra-violet region and make direct spectrophotometric determination impossible. The chief interfering substance is kitol, present as an ester and isolated first in pure form by Embree and Shantz.[1] Anhydrovitamin A is sometimes present in various amounts, and Gridgeman, Gibson and Savage[2] have shown that non-saponifiable substances are present that interfere with the spectrophotometric analysis of whale-liver oils. Chromatographic methods for the determination of vitamin A in whale-liver oils have been developed by Gridgeman *et al.*,[2] Barua and Morton,[3] and Hjarde.[4] As these methods are lengthy and involve fairly complex conditions of adsorption and elution, there is a need for a rapid and simple procedure.

It has been shown by Green[5] that vitamin A may be quantitatively destroyed on acid-washed floridin earth. It has now been found that, on floridin earth that has been neutralised with ammonia, vitamin A may be quantitatively adsorbed and eluted and, by this means, separated from other interfering substances in whale-liver oils.

Experimental

Conditions for adsorption and elution of vitamin A—

The chromatographic tubes used were 15 cm long and of 1·3 cm internal diameter, fitted with a 6-cm stem of narrow bore. The floridin earth columns measured 3 to 4 cm in height. Traces of acid in the earth must be removed by pre-treatment with ammonium hydroxide. Preliminary experiments showed that washing the earth with a 5 per cent. v/v solution of ammonium hydroxide, sp.gr. 0·880, in water or a 5 per cent. v/v solution of ammonium hydroxide, sp.gr. 0·880, in ethanol neutralised the earth sufficiently. After the alkali treatment, the excess of ammonium hydroxide was removed from the column, either by washing it first with ethanol and then with *n*-hexane or with *n*-hexane alone. Neutralisation of the columns could also be carried out by drawing ammonia vapour through the earth. Vitamin-A esters passed quantitatively through all columns prepared in this manner, but vitamin-A alcohol was totally adsorbed. Anhydrovitamin A was also passed through these columns, but both kitol and kitol esters were retained.

Vitamin-A alcohol could be quantitatively eluted by 80 per cent. of benzene in *n*-hexane. Elution was variable and non-quantitative with *cyclo*hexane and carbon tetrachloride. Kitol esters were easily eluted with benzene, but kitol was eluted only very slowly or not at all.

Anhydrovitamin A—

It was not found possible to separate efficiently anhydrovitamin A from vitamin-A esters on floridin earth. When oils, whole or saponified, were passed through columns of floridin measuring up to 30 cm long, separation was incomplete.

Methods of adsorption and elution had to be carefully chosen to prevent slight formation of anhydrovitamin A from vitamin A. Neutralisation with aqueous ammonium hydroxide caused this reaction to occur more readily than when the column was treated with ethanolic ammonium hydroxide.

The use of ethanol for washing excess of ammonium hydroxide from the column was occasionally satisfactory, but sometimes caused formation of anhydrovitamin A: the latter reaction was greatly increased by washing with aqueous ethanol.

If, after neutralisation with ammonium hydroxide, the column is washed with ether followed by *n*-hexane, all the vitamin A that is subsequently passed through the column is destroyed, with formation of anhydrovitamin A in about 30 per cent. yield. The cause of this remarkable reaction was not elucidated.

QUANTITATIVE STUDIES—

Studies on the quantitative nature of adsorption and elution were carried out with a high-potency vitamin-A oil (potency 270,000 i.u. per g) containing negligible irrelevant absorption. When quantities of the order 500 to 800 i.u. of vitamin A in the whole oil, dissolved in *n*-hexane, were passed through a neutralised column, recoveries of vitamin A were quantitative in the *n*-hexane filtrates. From the saponified oil, by elution with benzene or with 80 per cent. of benzene and 20 per cent. of *n*-hexane, recoveries of eluted vitamin A were 97 to 98 per cent.

TABLE I

ELUTION OF VITAMIN A FROM NEUTRALISED FLORIDIN-EARTH COLUMNS

Eluting solvent	Vitamin A used, i.u.	Vitamin A eluted, i.u.	Recovery in eluate, %
Carbon tetrachloride	700	92	13·2
80 per cent. of benzene and 20 per cent. of *n*-hexane	700	686	97·8
Benzene..	726	707	97·2

Preliminary studies on a crude whale-liver oil showed that, when the non-saponified oil was used, neutralisation of the adsorbent with ammonia gas produced eluates that, on spectroscopic examination, gave better vitamin-A curves than eluates produced by other methods. When the saponified oil was used, neutralisation with ethanolic ammonium hydroxide was the method of choice.

METHOD

REAGENTS—

Floridin earth—As supplied by British Drug Houses, Ltd., "prepared for Emmerie's test." Sieve the earth to remove particles passing a 160-mesh sieve. The material should conform to the specification given by Green[6] and may be prepared by this method.

n-*Hexane*—Laboratory-reagent grade.

Ethanol—Pure and absolute.

Benzene—Analytical-reagent grade.

APPARATUS—

Chromatographic tubes, 15 cm long and of 1·3 cm internal diameter, fitted with a 6-cm stem of narrow bore are required.

PROCEDURE WITHOUT SAPONIFICATION—

Pour sufficient floridin earth into a chromatographic tube on top of a small glass-wool plug until the height of the column is 3 cm. Insert the stem of the tube through a cork into the neck of a 100-ml filter flask containing a small quantity of ammonium hydroxide, sp.gr. 0·880. Apply suction to the top of the tube so that air and ammonia vapour are drawn through the adsorbent at such a rate that gentle turbulence occurs. The temperature of the adsorbent rises considerably during the neutralisation. After 2 to 3 minutes, cease suction and remove the adsorbent to a small beaker. Place a small filter-paper circle on the glass-wool plug and return the floridin earth to the tube. Place a second filter-paper circle on top of the column and gently press it down.

Pour 25 ml of *n*-hexane in 5-ml portions through the column and discard the filtrate. Change the receiver, then pour into the column 5 ml of a solution of the whale-liver oil in

n-hexane. This solution should contain 500 to 800 i.u. of vitamin A, although smaller quantities may be used. Elute the column directly with 30 ml of *n*-hexane and make up the eluate to 50 ml with *n*-hexane. Evaporate a portion of this solution to dryness, taking the usual precautions to prevent loss of vitamin A. Dissolve the residue in *cyclo*hexane and determine the vitamin-A content with a photo-electric spectrophotometer. Any small solvent error can be found by a blank determination.

PROCEDURE WITH SAPONIFICATION—

Saponify 0·5 to 1·0 g of the whale-liver oil and dissolve the non-saponifiable fraction in *n*-hexane. Take sufficient floridin earth to make a column 3 cm in length and make it into a slurry with 10 ml of a 5 per cent. solution of ammonium hydroxide, sp.gr. 0·880, in ethanol. Pour the slurry on to a filter-paper circle mounted on a glass-wool plug in the chromatographic tube. Place a second filter-paper on the surface of the column and press

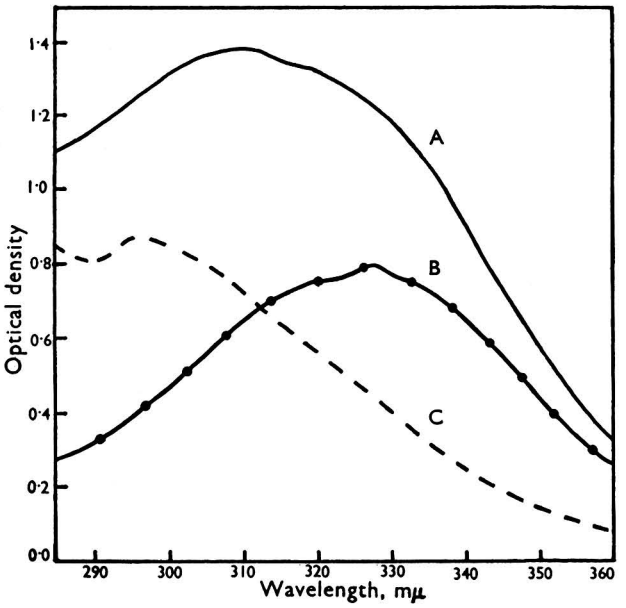

Fig. 1. The extent of removal of irrelevant absorption from a crude whale oil, without saponification. Curve A, absorption of crude oil; curve B, absorption of oil after removal of irrelevant absorption by chromatography; curve C, irrelevant absorption

it down. Wash the column with five 5-ml portions of *n*-hexane and discard the filtrate. Change the receiver and pass 5 ml of the vitamin-A solution (containing 500 to 700 i.u.) through the column. Wash the column with five 5-ml portions of *n*-hexane. The filtrate contains only anhydrovitamin A. Change the receiver, and elute with six 5-ml portions of 80 per cent. benzene and 20 per cent. *n*-hexane. Collect the eluate and make up to 50 ml. Evaporate a portion to dryness for spectrophotometric determination of vitamin A, as described above. A blank determination will give the small solvent correction.

RESULTS AND DISCUSSION

WHALE-LIVER OIL—

Whale-liver oils containing appreciable amounts of anhydrovitamin A must be assayed by the procedure involving saponification.

Table II shows the results found for these crude whale-liver oils, for which the determined vitamin-A curves are compared with the curves given for pure *trans*-vitamin-A acetate and free alcohol given by Cama, Collins and Morton.[7] Differences are shown, as is usual, at a number of points, when the extinction at the maximum is taken as 1·000. The oils D

and E give, without saponification, curves that have peaks at 327·5 mμ and are in close agreement with the curve for vitamin-A acetate between 300 and 350 mμ. Whale-liver oil L, however, contains anhydrovitamin A and therefore cannot be assayed without saponification. The results for the same three whale-liver oils, after saponification, are shown. The curves are very similar to the curve for vitamin-A acetate, maxima being in every curve at 326·5 mμ. In the example of oil L, anhydrovitamin A has been separated from vitamin A by chromatography after saponification. Fig. 1 shows the extent of removal of irrelevant absorption from a crude whale-liver oil, without saponification.

Table II

Determination of vitamin A in three crude whale-liver oils

		Oil D		Oil E		Oil L	
	Wave-length, mμ	E ($E_{max.}$ = 1·000)	Difference	E ($E_{max.}$ = 1·000)	Difference	E ($E_{max.}$ = 1·000)	Difference
Unsaponified oils	300·0	0·570	+0·021	0·574	+0·019	0·579	+0·024
	312·5	0·855	−0·002	0·865	+0·008	0·857	0·000
	327·5	1·000	0·000	1·000	0·000	1·000	0·000
	337·5	0·862	+0·007	0·867	+0·010	0·877	+0·020
	345·0	0·697	+0·002	0·696	+0·001	0·725	+0·030
	360·0	0·316	+0·017	0·317	+0·018	0·372	+0·073
Saponified oils	300·0	0·573	+0·020	0·565	+0·012	0·581	+0·008
	312·5	0·869	+0·012	0·872	+0·005	0·871	+0·004
	326·5	1·000	0·000	1·000	0·000	1·000	0·000
	336·5	0·875	+0·018	0·874	+0·017	0·874	+0·017
	345·0	0·667	0·000	0·671	+0·004	0·678	+0·011
	360·0	0·286	+0·006	0·295	+0·009	0·300	+0·020

Assays and reproducibility—

The reproducibility of the assay was determined by a series of six determinations on whale-liver oil L after saponification. Table III shows that the reproducibility is satisfactory.

Table III

Precision of assays of whale-liver oil L

Number of assays	6
Mean vitamin-A potency	41,300 i.u. per g
Range of determined potencies	39,800 to 42,900 i.u. per g
Standard error of mean	515

In general, no other type of oil gives results as good as those found with whale-liver oils. The method, however, removes some interfering material from other oils containing large quantities of compounds responsible for irrelevant absorption. Trials with oils containing vitamin A_2 and vitamin A_1 showed no separation of the two substances on floridin earth, even when long columns were used.

Separation of vitamin-A alcohol and ester on floridin is clearly possible in the absence of other interfering substances. In the examination of a whale-liver oil, however, the alcohol and ester proportion can only be determined by two assays, one on the whole oil and one on the saponified oil, as benzene elutes kitol esters from floridin. Hence, if the amount of free alcohol is small, its determination is masked by losses that may occur during saponification.

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Walton Oaks Experimental Station
Vitamins Ltd.
Tadworth, Surrey

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An Examination of Scottish Heather Honey

By T. J. MITCHELL, E. M. DONALD and J. R. M. KELSO

Ling honey from the nectar of *Calluna vulgaris* is unique among European honeys in colour, taste, viscosity and the property of thixotropy. The present work sought to find an explanation of this difference by the determination of nitrogen, mineral matter (percentage of ash) and colloid content of samples of honey drawn from widely scattered districts in Scotland and Northumberland. Forty-two samples were examined, of which 30 were predominantly ling honey.

Correlation was found between the pH and the ash content of the honeys. There was some evidence of relationship between the colloid content and the total nitrogen and the thixotropy. The relation between these properties was not exact, probably because of the variation in floral source of the honeys.

Badollet and Paine[1,2] have devised methods that use basic dyes for determining colloids, and Paine *et al.*[3,4] have shown that the colloids in American honeys contain a high fraction of nitrogenous compounds; it is now accepted that the nitrogenous compounds in honey are proteins, amino-acids and melanoidins. The formation and determination of amino-acids and melanoidins has been investigated by Ambler,[5,6] Ambler and Snider,[7] and Lothrop and Gertler.[8] The physical and chemical properties of honey have been adequately summarised by Root.[9]

Pryce-Jones,[10,11,12,13] and Lothrop and Paine[3] are responsible for most of the work done on the proteins of honey. De Boer and Kniphorst[14] working on Dutch honey were the first to use the term thixotropy in connection with honeys. Cameron, Mitchell and Westwood[15] in 1951 investigated twenty samples of heather honey, and their results are incorporated in Table I.

Cleaning of samples—

The honey as received contained wax, dust and particles of dead bees as impurities. Each sample was heated to 60° C, then stirred briskly and strained through ten layers of fine muslin gauze.

Determination of colloid—

About 20 g of honey were weighed into a tared beaker and dissolved in 50 ml of distilled water. The solution was heated with 25 ml of 10 per cent. trichloroacetic acid, with stirring, and the precipitate was allowed to settle for 48 hours. The precipitate was filtered on a dry tared filter-paper, washed with three 10-ml portions of 2·5 per cent. aqueous trichloroacetic acid and then with three portions of distilled water. The paper was dried to a constant weight in a vacuum oven at 40° C.

Total nitrogen in honey—

Investigations by Taylor,[16] Hitchcock and Belden,[17] Weissman and Schoenheimer,[18] Miller and Houghton,[19] Marcali and Rieman,[20] and Dahl and Oehler[21] on the micro and semi-micro determination of nitrogen had demonstrated the reliability and comparative speed of the method when homogeneous materials were examined. Mitchell *et al.*[15] modified Hitchcock and Belden's method, making use of copper sulphate, selenium and potassium sulphate. They found that normal honey samples were not sufficiently homogeneous to give satisfactory replication by Pregl's micro method of analysis for nitrogen with 5 to 35-mg portions of honey.

Determination of nitrogen—About 150 mg of honey were weighed into a small glass tube and placed in a 25-ml Kjeldahl digestion flask with 4 mg of copper sulphate, 8 mg of potassium sulphate, 0·8 mg of selenium powder and 5 ml of concentrated sulphuric acid. The digestion required 3 to 3½ hours. Neutralisation was effected in the distillation apparatus[17] with a solution containing 500 g of sodium hydroxide and 80 g of sodium thiosulphate in 500 ml of distilled water, and the mixture was made alkaline by adding a further 15 ml

TABLE I

CHARACTERISTIC VALUES FOR VARIOUS HONEYS

Number	Source	Kind	Water content, %	Ash on dry material, %	Colloid on dry material, %	Nitrogen on dry material, %	pH	Free acid, ml of 0·1 *N* NaOH per 100 g of honey	Thixotropy ratio	Colour	Series
1	Thurso, Caithness (Comb) ..	Clover (+ Ling)	23·7	0·04	0·16	0·08	3·59	37·7	1	Light cream	B
2	Rafford, Forres	Ling	17·6	0·24	0·48	0·13	3·69	61·0	2	Dark amber	"
3	Thurso, Caithness	Clover (+ Ling)	19·0	0·16	0·31	0·11	3·77	33·6	3	Cream	"
4	Bushy Creek, Kyle, Ross-shire	Bell	17·8	0·22	0·26	0·18	3·86	55·4	3	Brown	"
5	Dornie, Kyle, Ross-shire ..	Bell	19·6	0·36	0·51	0·24	3·89	64·6	1·5	Brown	"
6	Kyle, Ross-shire	Bell	20·7	0·13	0·85	—	4·02	—	—	Amber	A
7	New Galloway, Castle Douglas	Ling (+ Bell)	19·0	0·33	0·31	0·16	4·03	45·2	2	Light brown	B
8	Glenalmond, Perthshire ..	Mixture	22·0	0·23	2·74	0·17	4·18	—	2	Cream	A
9	Scaniport, Inverness-shire ..	Mixture	20·7	0·28	1·68	—	4·20	—	5	Cream	"
10	Dinnet Moor, Aberdeenshire	Ling (+ Bell)	20·0	0·33	0·79	0·21	4·28	26·6	3	Light brown	B
11	Dornie, Kyle, Ross-shire ..	Mixture	19·0	0·20	1·26	—	4·32	—	2	Cream	A
12	Balnain, Inverness-shire ..	Mixture	19·4	0·23	1·28	—	4·33	—	9	Cream	"
13	Carr Bridge, Inverness-shire	Bell	17·7	0·11	1·25	—	4·34	—	1	Cream	"
14	Perth College	Mixture	21·1	0·23	1·46	0·18	4·36	—	15	Amber	"
15	Beauly, Inverness-shire ..	Ling	23·3	0·23	1·71	0·18	4·39	—	3	Cream	"
16	Dalnaglar, Glenshee ..	Ling (+ Bell)	17·4	0·42	1·36	0·21	4·44	30·7	2·5	Dark amber	B
17	Muir of Ord, Ross-shire ..	Ling	20·7	0·20	3·34	0·19	4·49	—	3	Light cream	A
18	Tongue, Sutherlandshire ..	Ling	19·0	0·18	1·57	—	4·53	—	2	Light cream	"
19	Calside, Paisley	Ling	18·8	0·41	0·80	0·14	4·53	22·0	3	Dark amber	B
20	Hundleshope, Peebleshire ..	Ling	21·2	0·56	1·95	0·28	4·54	24·4	4	Amber	"
21	Carnwath, Lanarkshire ..	Ling	21·6	0·54	1·94	0·29	4·57	23·1	21	Light amber	"
22	Bankfoot, Perthshire ..	Ling (90%)	21·5	0·22	1·61	—	4·59	—	7	Amber	A
23	Rogart, Sutherlandshire ..	Ling	20·7	0·22	1·79	—	4·60	—	2	Cream	"

TABLE I—*continued*

Number	Source	Kind	Water content, %	Ash on dry material, %	Colloid on dry material, %	Nitrogen on dry material, %	pH	Free acid, ml of 0·1 *N* NaOH per 100 g of honey	Thixotropy ratio	Colour	Series
24	Skelmorlie, Ayrshire ..	Ling	21·4	0·53	2·79	0·38	4·66	29·4	>100	Amber	B
25	Assynt, Evanton, Ross-shire	Ling	18·0	0·52	2·00	0·27	4·70	22·8	7	Amber	"
26	Lammermoors, Selkirkshire	Ling	23·2	0·31	2·81	—	4·71	—	20	Dark cream	A
27	Forres	Ling	24·3	0·69	2·21	0·38	4·72	24·9	56	Dark amber	"
28	Assynt, Ross-shire	Ling	23·6	0·32	2·23	—	4·72	—	18	Cream	B
29	Drumelzier Moor, Peebles-shire	Ling (+ Bell)	20·0	0·51	1·58	0·30	4·73	21·8	19	Dark amber	"
30	Halkirk, Caithness	Ling	26·7	0·30	2·37	—	4·77	—	10	Amber	A
31	Port William, Newton Stewart	Ling	23·1	0·68	2·58	0·37	4·82	18·7	35	Amber	B
32	Crieff, Perthshire	Ling (75%)	22·8	0·26	1·76	0·22	4·83	—	4	Light amber	A
33	Raedykes, Fetteresso, Stone-haven	Ling	19·8	0·41	1·88	0·29	4·84	17·7	66	Light amber	B
34	Stow, Midlothian	Ling	22·3	0·26	2·54	—	4·86	—	12	Light amber	A
35	Longframlington, Northumberland	Ling	22·8	0·29	3·02	—	4·87	—	15	Amber	A
36	Dores, Inverness-shire ..	Ling	20·6	0·69	1·81	0·27	4·93	19·0	9	Dark amber	B
37	Dornock Farm, Crieff, Perth-shire	Ling	23·2	0·30	2·72	0·29	4·98	20·4	>100	Dark amber	"
38	Perth College, 10 years old..	Ling	20·3	0·28	3·02	—	5·02	—	3	Very dark amber	A
39	Galashiels, Roxburghshire..	Ling	19·0	0·29	2·52	0·21	5·11	—	>100	Dark amber	"
40	Blackhills, Auldearn, Nairn	Ling	20·0	0·60	2·63	0·35	5·14	16·0	1	Amber	B
41	Lammermuir Hills, East Lothian	Ling	24·5	0·68	2·70	0·36	5·46	20·7	80	Light amber	"
42	Dallas, Morayshire	Ling	22·1	0·63	1·93	0·30	5·60	12·9	5	Light amber	"

of the reagent. The distillate was collected in 0·2 *N* hydrochloric acid and the excess of acid was titrated with 0·2 *N* sodium hydroxide, with methyl red as indicator.

MINERAL MATTER IN HONEY—

Twelve different elements, in addition to carbon, hydrogen and oxygen, are commonly found in honey. These elements are silicon (as silica), iron, copper, manganese, chlorine, calcium, potassium, sodium, phosphorus, sulphur, aluminium and magnesium. Calcium and iron are mainly present as phosphates in a form that can be readily assimilated by the human system.

Work done in 1932 by H. A. Schuette and K. Remy[22] showed that a darkly coloured honey has a high mineral content and also that iron, copper and manganese predominate in the mineral matter of dark honey. The mineral matter has been shown to consist mainly of alkaline materials.

Ling honey, being dark coloured, can therefore be expected to have a reasonably high mineral content.

The simplest method of determining the amount of mineral matter in a honey is to ignite a weighed sample of the honey, and from the weight of ash to calculate the mineral content of the honey. By this method some of the chlorine and sulphur will be lost by volatilisation; this is prevented by sulphation. An arbitrary 10 per cent. is deducted from the result.

An average value of ash residue for dark honeys is 0·26 per cent., the range of values determined for a variety of honeys being 0·10 to 0·61 per cent.

Determination of ash—Approximately 2 g of honey were weighed accurately into a small platinum crucible and were treated with 10 drops of concentrated sulphuric acid and then the mixture was heated gently over a flame. When the mass had become completely charred, the crucible was heated in a muffle furnace at 800° C for 30 minutes. The determination was made in duplicate.

ACIDITY OF HONEY—

The acids in honey have a great influence on the taste of honey from different floral sources. Malic and citric acids are the main ones found in honey, together with traces of acetic, succinic and formic acids. These acids, besides imparting a tart taste to the honey, have a pronounced influence on the other flavouring substances present.

If sufficient alkali is added to honey to neutralise the acids exactly, the flavour of the honey is altered and becomes decidedly insipid.[9]

In honey with a high mineral content, *e.g.*, buckwheat or heather honey, a salty taste may be detected. This may be due to the high mineral content causing a decrease in the acidity and thus altering the flavour.

The degree of acidity of honey can be determined by a comparative method such as the evaluation of pH. Three factors control the acidity of the honey, *viz.*—

(*a*) the nature of the individual acids present;
(*b*) the total amount of acids present; and
(*c*) the influence of other substances such as the minerals in the honey.

As all heather honey probably contains the same organic acids, the first factor will not greatly affect the degree of acidity.

Generally, honey with a high mineral content has a high pH value, *e.g.*, honeydew and ling honey, even though the amount of acid present is high. The action of the minerals in honey in reducing the acidity is a buffer effect and is not only important in its influence on the flavour of honey, but also affects such factors as colour formation and yeast growth. Substances responsible for the acidity of honeys amount to about 0·1 per cent.[9] Generally, pH values for honey lie in the range 3·6 to 4·2.

Determination of pH—Approximately 10 g of honey were weighed out accurately into a 100-ml beaker and the honey was made into a solution with about 25 ml of distilled water. The honey solution was transferred to a 100-ml calibrated flask and made up to the mark with distilled water. The pH of this solution was then determined immediately by the glass electrode method.

Free acid in honey—The standard A.O.A.C. procedure was followed in the determination of the free acid in honey.

Results—The pH of honey was found to lie between 3·59 for a honey that was mainly clover and 5·60 for a honey that was claimed to be pure ling. The free acid varied between 64·6 ml of 0·1 *N* sodium hydroxide per 100 g of honey for the bell heather sample and 12·9 ml per 100 g of honey for the sample of pH 5·60; phenolphthalein was used as indicator. The pH and free-acid values were obviously closely related, for honeys of the same floral type and ling honeys had low free-acid values and a high pH.

Previous work

In 1932, Chataway[23] determined the moisture content of a honey by measuring its refractive index and its viscosity and by comparing these results with the values for its moisture content found by the standard A.O.A.C. procedure.

From this work, which established the relationship between refractive index and moisture content and between viscosity and moisture content, Chataway drew up tables enabling the refractive index and viscosity of a honey to be converted into the corresponding value for moisture content.

The change of refractive index of a honey with increase in temperature was found to be −0·00023 per degree over the range 15° to 25° C.

In 1933, Chataway[24] investigated the determination of the moisture content of a honey by a densimetric method; although the results were good for highly mobile liquids, it was observed that the hydrometer was inaccurate when used for highly viscous honeys. In order to overcome this defect, once the hydrometer had been placed in the honey, a layer of water was poured over the honey. This layer of water dissolved the honey adhering to the stem of the hydrometer and allowed the hydrometer to reach its true equilibrium point. A correction was made to allow for the effect of the layer of water above the honey, and the results were reproducible.

It was found that the density of the honey, like the refractive index and the viscosity, was related to its moisture content.

In 1939, Oppen and Schuette[25] examined 29 honeys from different floral sources and showed that the relative viscosities were correlated with the moisture content. Viscometric and A.O.A.C. drying methods gave results that differed by only 0·2 per cent. on an average.

Choice of method

As heather honey is thixotropic, a method entailing measurement of viscosity would not give results that could readily be interpreted. The honey would need to be in the true liquid or sol form to give comparative results and a few of the honeys would require to be dried by the standard A.O.A.C. procedure for use as absolute values and as a comparison with the values from the work done by Chataway.

The densimetric method would entail the construction and calibration of a large sensitive hydrometer. The refractometric method with a Zeiss - Abbé sugar refractometer at 20° C was chosen for speed and accuracy.

Thixotropy in honey

Scott-Blair[26] in 1935 noted that heather honey usually contained a higher percentage of moisture than ordinary honey. As the honey did not show any tendency to ferment, it must hold its moisture in a different way from the other honeys. Heather honey showed a variable viscosity in the unstirred state and the viscosity fell sharply on stirring. He also showed that the honey did not have a definite yield value, *i.e.*, the viscosity did not become infinite for small stresses.

Pryce-Jones[11] in 1936 demonstrated that removal of the colloids entirely destroyed the thixotropy of heather honey.

In 1943, Munro[27] carried out work on the viscosity and thixotropy of a variety of American honeys, mainly sage, aster, buckwheat, clover and goldenrod, and British heather honey. He concluded that there was a direct relationship between the colloid content and the thixotropy of heather honey and he showed that fermentation had no significant influence on the viscosity and thixotropy of a honey. Munro's apparatus was based on the falling ball apparatus used by Chataway.[23] The tube was 16 mm in diameter and the steel ball had a diameter of 0·62 cm. The time for the steel ball to fall through a distance of 14 cm in the honey, which had not been stirred for at least 24 hours, was measured by a stop-watch and

compared with the time required for the same operation directly after the honey had been stirred. This ratio was called the thixotropy ratio.

As the viscosity of a fluid possessing structural viscosity depends on the rate of shear, and this rate of shear so found will be different in the two observations, the results are not a quantitative measure of the ratios of viscosities after stirring and after the honey has been left at rest.

Apparatus—

The viscometer consisted of a round glass tube, 1 inch in diameter and 20 cm in length. This size of tube was found to be necessary in order to use a steel ball that did not take an excessive time to fall and also to allow for wall effect on the fall of the ball. The ball was $\frac{1}{4}$ inch in diameter, thus giving a d to D ratio of 0·25 as compared with 0·39 in the method used by Chataway and Munro. The outside of the tube was marked with a file to give the required distance of 14 cm for the fall of the ball. A distance of 2 cm was allowed in which the ball could attain an even falling velocity.

In order to see the ball in some of the more cloudy honeys, a light was set up at a distance of 1 foot from the viscometer. To avoid any likelihood of heating the honey, this light was used only while timing the fall of the ball.

Method—

Honey was poured into the tube to within 1 cm of the top and allowed to set for 24 hours. A steel ball was then introduced just below the surface of the honey and timed over the 14 cm with a stop-watch. As soon as the ball reached the bottom, the honey was stirred vigorously and a second ball send down and timed. The ratio of these readings was the thixotropic ratio. The experiment was repeated with a larger ball ($\frac{5}{16}$ inch in diameter) and the ratio found from these readings did not differ appreciably from the previous values.

Results—

The values found for the thixotropic ratio varied from 1 for the comb honey (mainly clover) to more than 100 for the pure ling samples.

Honey samples 20, 25 and 40 (Table I) showed much increased thixotropy after 16 hours in an oven at 40° C. They were examined for moisture, but no appreciable difference was found between their original and final moisture contents. The change of thixotropy could thus have been due not to loss of moisture, but rather to some change in the chemical or physical constitution of the honey, *e.g.*, formation of melanoidins or coagulation of the colloids in the honey.

Discussion of results

It will be seen that by arranging the samples in order of pH value the ling honeys are clearly segregated. There is also a tendency for the ash residue to increase as the pH rises, and there is almost the same rise in colloid content as in pH. There was little or no correlation between the colloid content and the colour, and no relation could be traced between the colloid content and the district where the honey was gathered.

The bell heather and the clover samples had markedly low protein, ash and colloid contents compared with the ling honeys.

The average water content was 20·3 per cent. and the average pH value 4·52 per cent. The range of water content was from 17·4 to 26·7 per cent. On a dry basis the ash content ranged from 0·04 to 0·69 per cent., the total nitrogen from 0·08 to 0·38 per cent. and the colloid precipitated by trichloroacetic acid from 0·16 to 3·34 per cent. The results for colloid content are appreciably higher than those reported by J. Pryce-Jones.[10,11,12,13] It was noted that the colour tended to increase with increasing acidity (see Table I).

The bell heather and clover honey samples did not exhibit thixotropy and had a different taste and a lower colloid and nitrogen content than ling honey. It appears that the degree of thixotropy shown by ling honeys may be due to their higher content of colloidal substances, including proteins.

CORRELATION OF ASH AND pH—

The samples containing a greater percentage of mineral matter have a higher pH. The correlation between the results is only general, as the samples of honey besides containing honey from the sources named, may contain some from other floral sources that will have an effect on the properties and analysis of the honey.

CORRELATION OF THIXOTROPY, COLLOID CONTENT AND TOTAL NITROGEN—

Some evidence of correlation was found between the colloid content and the thixotropy, and between the colloid content and the total nitrogen. The results are shown in Table II, arranged in order of increasing colloid content.

TABLE II

CORRELATION BETWEEN COLLOID CONTENT AND THIXOTROPY AND TOTAL NITROGEN

Number	Colloid content	Nitrogen %	Thixotropic ratio
1	0·16	0·08	1
4	0·26	0·18	3
3	0·31	0·11	3
7	0·31	0·16	2
2	0·48	0·13	2
5	0·51	0·18	3
10	0·79	0·21	3
19	0·80	0·14	3
6	0·85	—	1
13	1·25	—	1
11	1·26	—	2
12	1·28	—	9
16	1·36	0·21	2·5
14	1·46	0·18	15
18	1·57	—	2
29	1·58	0·30	19
22	1·61	—	7
9	1·68	—	5
15	1·71	0·18	3
32	1·76	0·22	4
23	1·79	—	2
36	1·81	0·27	9
33	1·88	0·29	66
42	1·93	0·30	5
21	1·94	0·29	21
20	1·95	0·28	4
25	2·00	0·27	7
27	2·21	0·38	56
28	2·23	—	18
30	2·37	—	10
39	2·52	0·21	>100
34	2·54	—	12
31	2·58	0·37	35
40	2·63	0·35	1
41	2·70	0·36	80
37	2·72	0·29	>100
8	2·74	0·17	2
24	2·79	0·38	>100
26	2·81	—	20
35	3·02	—	15
38	3·02	—	3
17	3·34	0·19	3

As with the ash and pH, no exact relation was found between the thixotropy, colloid content and total nitrogen.

It must be observed that some of the samples described in Table I as "ling honey" may be from mixed floral sources. A true ling honey should remain gelatinous and be

distinctly brown in colour, and from this point of view it is difficult to find a clear cut relationship between colour and the colloid or protein content in the present series of samples.

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Technical Chemistry Department
Royal Technical College
Glasgow

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The Aspecific Detection of Preservatives in Foods by a Simple Fermentation Test with Special Reference to Cured Meat Products

By D. A. A. MOSSEL

The stability of non-sterile canned solid foods that are not allowed to contain preservatives is occasionally increased by adding small amounts of highly toxic antimicrobial agents, *e.g.*, derivatives of bromoacetic acid and phenylmercury compounds. Chemical methods for detecting each of the various preservatives are cumbersome and time-consuming. It was therefore thought worth while to try to adapt the Kluyver fermentation test as an aspecific reaction for antimicrobial compounds.

For testing the solid food (especially cured meat products) a portion is extracted with 0·5 per cent. tartaric acid solution (pH 3·0 ± 0·2) and a similar portion is extracted with 0·1 per cent. aqueous sodium hydroxide (pH 8·0 ± 0·2). The extracts are pasteurised at pH 3, enriched by adding 0·5 per cent. of Difco yeast extract and 2·5 per cent. of dextrose, and the pH is adjusted to 4·0. The extracts are then inoculated with sufficient bakers' yeast to give 10^4 cells per ml of solution and they are placed in Einhorn fermentation tubes. After incubation for 24 to 30 hours at 24° ± 2° C, the volume of gas formed is measured.

Brominated acetic acid derivatives can be detected when present in meat products at concentrations corresponding to 5 mg of bromine per kg. The method also permits the detection of 0·1 per cent. of hexamethylenetetramine, 0·5 per cent. of boric acid and 0·1 per cent. of benzoic acid. Sodium chloride, potassium nitrate and sodium nitrite do not interfere at the maximum levels found in meat products, *i.e.*, 5, 0·2 and 0·02 per cent., respectively.

It has been demonstrated that some food manufacturers occasionally increase the stability of foods that should not contain preservatives, such as canned non-sterile cured meat products, by adding small amounts of very active antimicrobial compounds. In many instances highly toxic agents are used for this purpose, such as derivatives of monobromoacetic acid[1] or phenylmercury compounds.[2]

Chemical methods for the detection of the various compounds mentioned are available, but as a rule are cumbersome and often time-consuming. It was therefore of value to see whether the presence of such preservatives could be demonstrated by applying the microbiological method that has been used with success in detecting preservatives in beverages[3] and in milk.[4]

Preliminary research

Direct inoculation—

The first method studied as a means of revealing the presence of added antimicrobial agents in canned cured meats was a modification of Lignières' direct test on milk.[5] Blank and preserved samples of about 2 to 5 g were inoculated by grinding them asceptically in a mortar with typical meat spoilage bacteria, such as *Pseudomonas* and *Bacillus* strains, so that the samples contained about 10^3 live cells per gram. They were incubated at 24° ± 1° C. Spoilage was indicated by organoleptic changes and, in severe instances, by gas formation.

It appeared that the concentrations of preservatives detectable in this way were much higher than those encountered in practice. The test was therefore worthless.

Diffusion tests—

Next, a modification of the cup test, suggested by Jacquet and Steeg,[6] was tried. The samples under investigation were placed in a hole of about 15 mm diameter cut into the centre of nutrient agar plates lightly seeded with the meat spoilage bacteria referred to above. Here again the sensitivity appeared to be insufficient.

This type of test works for milk but not for canned meats, for the following reason. Effective preservation of milk should inhibit relatively high numbers of organisms (10^3 to 10^5 per ml), particularly under the conditions that prevail in the test. Preservatives in canned foods, on the contrary, have only to inhibit up to about 100 organisms per gram under conditions that are in themselves already slightly inhibitive: the bacteria and spores to be inhibited are more or less heat-damaged, and some of them are retarded by the decreased partial pressure of oxygen. It may happen that concentrations of a given preservative effective in preventing spoilage in canned foods do not have any influence in the type of test in which fresh inocula are used outside cans.

As these types of test were insufficiently sensitive for the purpose, it was decided to work with a concentrated extract of the foods under investigation and to use *Saccharomyces cerevisiae* as an inoculum, since in previous investigations this organism had shown a very high sensitivity, as little as 2×10^{-5} per cent. of ethyl monobromoacetate, for example, being detectable by its use.[7]

EXTRACTION - FERMENTATION TECHNIQUES—

The preservatives so far found in meat products are soluble in water. Hence the extraction can be effected with aqueous solutions. Some of the preservatives are only stable at low pH (*e.g.*, derivatives of bromoacetic acid), whereas others can only be extracted at fairly high pH (*e.g.*, boric and benzoic acid). It was therefore necessary to carry out parallel extractions with acidulated water, pH about 3, and alkaline water, pH about 8.

Saccharomyces cerevisiae in the form of bakers' yeast-cakes was chosen as the test strain, because it is generally available in a constant type, which makes the method also applicable in laboratories not containing special microbiological departments.

The method had to be applied to extracts prepared from non-sterile material under non-aseptic conditions. In order to prevent potential interference with the development of the test strain by inhibitory contaminants, the extracts had to be sterilised before inoculation. This was always done by heating the extracts for 1 minute at 80° C at a pH of 3. The method was invariably effective, as at pH 3 thermal death times are very short.[8]

Finally, the sterilised extracts had to be supplied with a source of carbon and micronutrients.[9,10] For this purpose 2·5 per cent. of dextrose, applied as a sterile 25 per cent. w/v solution, and 0·5 per cent. of Difco yeast extract, added as a sterile 12·5 per cent. w/v solution, were used.

The fermentation test was carried out at pH 4·0, where the activity of the acidic preservatives such as benzoic acid, which require a relatively low pH for antimicrobial action,[11] is not impaired[12,13,14] and where the growth of bakers' yeast is not inhibited.[15]

METHOD

EXTRACTION—

Extract 10 g of the sample in a blender for 10 minutes with 100 ml of 0·5 per cent. tartaric acid solution. Extract another 10-g sample in the same way with 100 ml of 0·1 per cent. sodium hydroxide solution. Filter both extracts and adjust their pH to $3{\cdot}0 \pm 0{\cdot}2$ with a few drops of 30 per cent. w/w alkali or acid, as the case may be.

STERILISATION OF THE EXTRACTS—

Transfer the extracts to plugged sterile 250-ml Erlenmeyer flasks, heat them, with vigorous shaking, in a water-bath at about 85° C until the temperature of the extracts rises to $80° \pm 1°$ C, and keep them at that temperature for 1 minute. Check the temperature in a control flask filled with 100 ml of tap water and subjected to the same heat treatment.

ENRICHMENT AND ADJUSTMENT OF THE pH OF THE EXTRACTS—

Add aseptically to both extracts (*a*) 10 ml of a sterile 25 per cent. w/v solution of pure dextrose and (*b*) 4 ml of a sterile 12·5 per cent. w/v solution of Difco dehydrated yeast extract.

Adjust aseptically the pH of the extracts to $4{\cdot}0 \pm 0{\cdot}2$ by adding a few drops of a sterile 30 per cent. w/w potassium hydroxide solution. Determine the number of drops required by a trial electrometric titration.

INOCULUM—

From a commercial bakers' yeast-cake that has been stored in a refrigerator for not more than 3 days, prepare under aseptic conditions a 10 per cent. w/v dispersion in sterile 0·85 per cent. sodium chloride solution. Dilute this dispersion aseptically three successive times 1 + 9 with sterile 0·85 per cent. sodium chloride solution to give an inoculum containing about 10^6 live cells per ml.

INOCULATION AND INCUBATION—

Inoculate both extracts with 1 per cent. v/v of the yeast suspension containing 10^6 cells per ml and transfer them to sterile Einhorn (Smith) fermentation tubes. Incubate them at 24° ± 2° C and measure the volume of gas formed after 24 to 30 hours.

RESULTS

SENSITIVITY OF THE METHOD—

The levels of detection in ground cured ham were determined for a few preservatives, *viz.*, ethyl monobromoacetate, hexamethylenetetramine, boric acid and benzoic acid. For this purpose levels of detection may be defined as AD_{50}, *i.e.*, those that cause a reduction in gas formation of at least 50 per cent.

The results are shown in Table I. All levels refer to the method of extraction giving the highest result.

TABLE I

LEVELS OF DETECTION (AD_{50}) OF SOME PRESERVATIVES IN GROUND HAM

Preservative	Extraction medium	Level, % of ham
Ethyl monobromoacetate	acid	1×10^{-3}
Hexamethylenetetramine	acid	1×10^{-1}
Boric acid	alkaline	5×10^{-1}
Benzoic acid	alkaline	1×10^{-1}

The levels of detection of the classical preservatives are of the same order as, or a lower order than, the effective levels in proteinaceous foods.[16] For brominated preservatives the concentrations used in meat products so far are not exactly known, but they appear to be of the order of 10 to 25 mg of bromine per kg,[17] which corresponds to 0·002 to 0·005 per cent. of ethyl monobromoacetate. The test developed is therefore suitable in this respect.

RELIABILITY OF THE METHOD—

The potential weak point of the proposed method was the inhibition of the yeast strain by salts passing from the cured meats into the extracts. If the final concentrations of these compounds in the extracts were high enough to inhibit *Saccharomyces cerevisiae* at pH 4, false positive results could be expected.

Examinations of about 25 Dutch and imported canned hams, carried out in the author's Institute, have so far revealed that the maximum concentrations of the various salts were 5 per cent. of sodium chloride, 0·2 per cent. of potassium nitrate and 0·02 per cent. of sodium nitrite. These findings are in conformity with Jensen's specification[18] for curing practice in the U.S.A., where ham contains 3·5 per cent. of sodium chloride, 0·15 per cent. of sodium nitrate and 0·008 per cent. of sodium nitrite.

In order to study the inhibitive action of these levels of the salts in ham on the yeast fermentation under the conditions of the test, 0·5 per cent. of sodium chloride, 0·02 per cent. of potassium nitrate and 0·002 per cent. of sodium nitrite were dissolved in a solution containing 2·5 per cent. of dextrose and 0·5 per cent. of yeast extract at pH 4. It appeared that this solution was fermented by about 10^4 cells per ml of *Saccharomyces cerevisiae* in the same way as a blank solution of dextrose and yeast extract of the same pH.

Hence false positive results owing to the presence of curing salts in the extracts are not to be feared.

Discussion of results

The method that has been developed permits the aspecific detection of the presence in cured meat products of effective levels of preservatives. The salts normally used in preparing these commodities do not interfere with the test.

It should be noted that this test is useless for detecting the current antibiotics, such as penicillin, subtilin, chloramphenicol, aureomycin and terramycin, as they show only very weak inhibitory properties ($AD_{50} > 50$ mg per litre) towards yeasts.[19] If these antibiotics are to be detected, there is no need for the method described in this paper, as excellent methods have already been developed for the examination of animal feeding stuffs enriched with antibiotics.[20,21]

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Central Institute for Nutrition Research, T.N.O.
Utrecht, The Netherlands

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Design and Operating Technique of a Vacuum Drying Oven

Part II. Solids in Cane Molasses

By S. D. GARDINER and F. J. FARMILOE

With a new carefully designed vacuum oven, a drying method at 69° to 70° C was devised for cane molasses. Values for true solids were determined to within ±0·1 per cent. An ash correction was derived to permit solids determined by refractometry with the use of sucrose tables to replace the lengthy and difficult determination of solids by drying. Equations were derived relating true solids to refractometer solids, invert sugar and sulphate ash, and true solids to refractometer solids, invert sugar and sulphate ash minus a correction for sodium and potassium sulphates. This correction involves determinations with a flame photometer and gives more accurate results.

The determination of water in cane molasses by drying in a vacuum oven will give results that increase continually with time of drying.[1] The present work was initiated to obtain a true result. Four main problems existed: first, the design of a suitable vacuum oven; secondly, the prevention of formation of some decomposition products; thirdly, correction for loss caused by formation of others; and fourthly, the retention of water had also to be prevented as far as possible.

The design of a suitable vacuum oven has already been described.[2] Loss of weight owing to formation of decomposition products, even at low temperatures, undoubtedly occurs. That due to amino-acid - glucose condensation has been largely eliminated by using air currents to obviate catalysis by water vapour,[3] and any residual error from this cause, and decomposition of the labile fructose, has been overcome by graphical correction. Decomposition has been found by experiment to be a straight-line function, additive to the graph of true percentage loss of weight plotted against time. The last and most difficult problem is to find whether the percentage loss figures, after correction for decomposition, really indicate that all the water, combined or otherwise, has been expelled. If the drying conditions in the early stages encourage crust formation, the losses will be low, falsely indicating the water content of the molasses. To make sure that this is not so, the following factors have been incorporated in the vacuum oven drying method described in this paper—

(*i*) A surface extender with good thermal conductivity should be used.
(*ii*) The extender should present maximum surface area and interparticulate voids.
(*iii*) A thin layer of extender should be used.
(*iv*) The ratio of extender to solids should be as high as possible.
(*v*) In order to ensure an efficient distribution of solids on the extender, sufficient water should be added, avoiding an excess.
(*vi*) A sufficient bleed of dry air should be maintained.
(*vii*) The air bleed should be intimately directed over the extender.
(*viii*) It is important that heating should be started at a low temperature and increased steadily to the operational temperature. During this time, most of the total water (original and added) is removed.

Method

Apparatus—

Oven—The special vacuum drying oven, thermostatically controlled to ±0·5° C and designed specifically for analytical work,[2] including drying train of alumina and barium oxide, with liquid-detergent film device as air-flow meter.

Dishes—Six aluminium dishes, 1·0 cm deep by 5·0 cm diameter, with tight-fitting lids.

Mixing rods—Six glass mixing rods with flattened bent ends, 4·0 cm by 0·3 cm.

Desiccator—A barium oxide desiccator with a copper cooling block of large thermal capacity.

Balance—An aperiodic balance with fully automatic weight-loading device, sensitive to 0·1 mg and taking a maximum load of 200 g.

Other glassware—A thin 200-ml beaker with a thin clock-glass cover and rod, a 60-ml stoppered vessel and a 0 to 5-ml graduated pipette.

SURFACE EXTENDER—

B.D.H. aluminium, "medium powder," grease free, gristed to pass B.S. sieve 200.

OPERATING TECHNIQUE—

Trial and error tests indicated that the best value for the ratio of total water to weight of solids was 4·8. Similarly, the volume of diluted molasses required was that corresponding to 0·40 g of solids. Two simple equations were derived satisfying these conditions—

$$\text{Water to be added} = (\text{weight of molasses taken}) \left[\left(\frac{\text{per cent. of solids}}{17{\cdot}2} \right) - 1 \right] \quad (1)$$

$$\text{Volume of diluted molasses} = \frac{37{\cdot}4\ (\text{weight of diluted molasses})}{(\text{per cent. of solids})\ (\text{weight of molasses})} \quad (2)$$

In equations (1) and (2) the uncorrected values of refractometer solids should be used.

Approximately 25 g of molasses are placed in the tared 200-ml beaker and weighed with the glass cover and stirring rod. As calculated from equation (1), about 85 ml of water are added, the whole is then well stirred, covered and reweighed. The diluted molasses is immediately transferred to the 60-ml stoppered vessel, nearly filling it. The suspended matter in the diluted molasses must not be allowed to settle, otherwise the portion transferred will not be representative. The large weight of molasses taken is necessary to minimise sampling errors. The calculated volume of diluted molasses, which from equation (2) approximately equals 2·2 ml, is transferred by means of the graduated 0 to 5-ml pipette (running out from 0 to 2·2 ml), into the centre of the dry aluminium powder. Some difficulty may be experienced in wetting the surface extender. The stoppered vessel must be well shaken before the aliquot increments are transferred to the dishes. Tests are carried out in duplicate. The weight of the sample added is found by difference to within $\pm 0{\cdot}1$ mg.

After many tests the straightforward pipette method, as outlined above, was chosen instead of a weighing-burette method. Speed, simplicity and reliability were the deciding factors. Errors due to evaporation of the sample, or absorption of water from the atmosphere by the dry extender, were hardly detectable.

The dishes contain $8 \pm 0{\cdot}1$ g of aluminium powder. The lids are placed underneath the dishes. They are dried in the recess in the vacuum oven, together with the glass rods, for approximately 16 hours (overnight) at 69° to 70° C, with an air bleed of 100 ml per minute. The lids are placed over the dishes in the oven recess before they are transferred to the copper cooling block in the desiccator. Cooling times in the desiccator are 10 and 15 minutes for 4 and 6 dishes, respectively. After adding the diluted molasses, as described above, and very careful mixing with the glass rods, which are subsequently left inside, the dishes are dried as before, except that this time heating is started at 50° C, and the temperature is slowly raised to 70° C and kept there for the remainder of the drying period. The gradual rise in temperature facilitates the removal of water from the particulate surfaces of the extender and prevents crust or skin formation. The extra water added probably assists this action. The air bleed is continuously pre-dried, although this is unwarranted except in later stages of drying.[4] Pre-heating does not occur and is unnecessary.

To plot the graph of percentage loss of weight against time, weights are recorded at the following times: after 45 minutes, the temperature having risen to 70° C; after 5 hours; at 17 to 20 hours; at 32 to 35 hours; and at 47 to 50 hours. It is essential that these time intervals are observed, for the losses of weight are very small and can only be measured successfully with the sensitive aperiodic balance recommended.

All the results by vacuum oven drying were corrected for decomposition in the following manner. The later straight section of the graph of percentage loss of weight against time

was extrapolated back to cut the 45 minutes ordinate at the corrected percentage loss figure. Fig. 1 shows that the graph of percentage loss of weight against time, curve III, is the graphical sum of two curves, *viz.*, the true drying curve, I, with the later section parallel to the time axis, and a straight line for decomposition, II, starting at 45 minutes. If decomposition were to start after several hours of heating, the drying curve would show an inflection; it does not, as is proved by experiment. Decomposition was therefore considered to start when

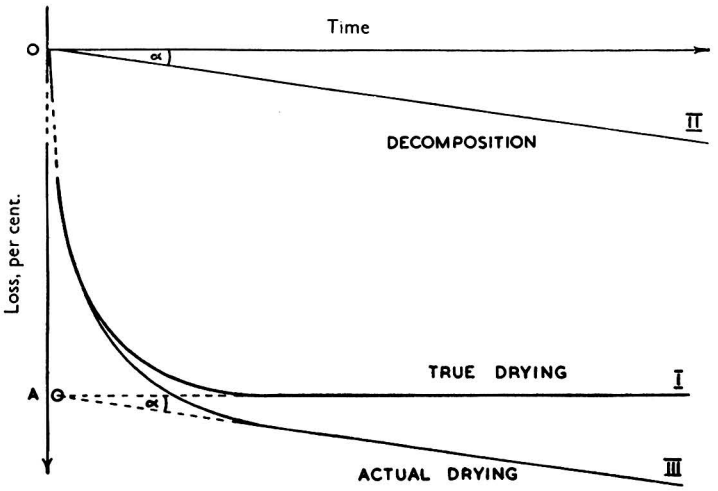

Fig. 1. Diagram showing how the actual drying curve is derived from the addition of the decomposition and true drying curves

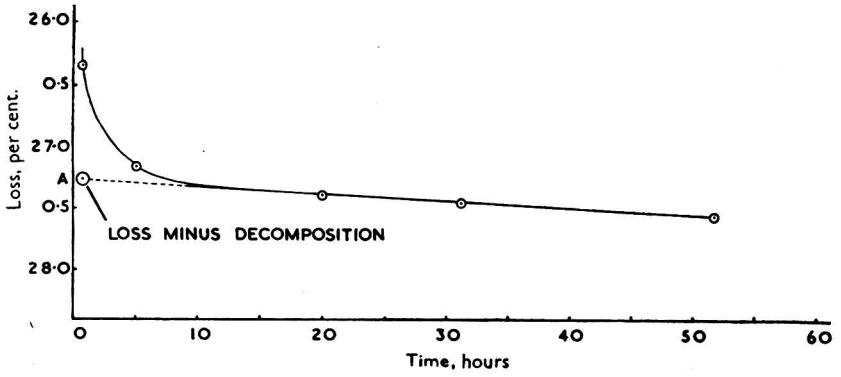

Fig. 2. A typical drying curve for cane molasses at a temperature of 69° to 70° C

the temperature first reached 70° C. Fig. 2 shows a typical graph of percentage loss of weight against time for cane molasses. Point A is the percentage loss figure, corrected for decomposition (Figs. 1 and 2). Duplicate tests agreed to within $\pm 0{\cdot}05$ per cent. loss, and two completely separate dilution and drying operations to within $\pm 0{\cdot}10$ per cent. loss.

If only approximate results are required, the percentage loss after 5 to 10 hours can be taken as correct to within 0·2 per cent. For carrying out the test rapidly, the dishes and powder and rods can first be dried at 105° C for 1 hour.

Determination of true solids in cane molasses—

Solids in cane molasses were determined by the drying method already described and were termed "true solids." Sixteen samples were tested, all being free from undissolved crystals, and the results are shown in Table I.

TABLE I

DETERMINATION OF AN ASH CORRECTION FACTOR FROM SULPHATE ASH CONTENT

Number	Solids by vacuum oven drying		Solids by refractive index		Sulphate ash, %	Invert sugar, %	Ash correction factor
	Test, %	Mean, %	Test, %	Average, %			
1	74·63	74·78	77·00	77·12	11·50	20·02	0·0032
	74·92		77·10				
			77·25				
2	77·92	77·84	79·85	80·02	10·29	24·76	0·0033
	77·75		79·85				
			80·40				
			79·60				
			80·07				
			80·33				
3	77·98	78·02	80·73	80·70	9·97	24·12	0·0041
	78·05		80·67				
4	72·79	72·73	74·70	74·70	8·49	19·70	0·0038
	72·66		74·40				
			74·95				
5	72·69	72·73	74·95	75·03	9·03	18·69	0·0040
	72·76		75·43				
			74·95				
			74·80				
			75·03				
6	72·94		75·83	75·81	10·33	20·88	0·0043
			75·75				
			75·95				
			75·91				
			75·63				
7	72·70		75·80	75·85	10·00	19·97	0·0048
			75·90				
8	73·27		76·17	76·14	10·35	19·12	0·0043
			76·10				
9	73·13		75·93	76·02	10·10	21·16	0·0044
			75·83				
			76·20				
			76·10				
10	72·25	72·00	75·26	75·24	9·31	21·54	0·0054
	71·74		75·27				
			75·23				
			75·20				
11	73·49		76·20	76·26	9·63	19·74	0·0044
			76·22				
			76·30				
			76·30				
12	72·94		75·95	76·07	9·65	20·06	0·0050
			76·15				
			76·10				
13	72·88		75·20	75·25	9·55	21·11	0·0040
			75·30				
14	72·89		76·00	76·11	9·43	19·53	0·0052
			76·07				
			76·10				
			76·27				
15	73·10		75·55	75·55	9·87	21·23	0·0039
			75·55				
16	73·10		75·83	75·92	9·94	20·76	0·0044
			76·00				
					Average value	..	0·0043

ASH CORRECTIONS BASED ON SULPHATE ASH CONTENT—

Further determinations were made of apparent solids by direct determination of refractive index, sucrose tables were used and these figures were corrected for invert sugar. Invert sugar was determined by Lane and Eynon's method, with pre-defecation by potassium oxalate solution and alumina cream. Sulphate ash determinations, termed "gravimetric ash,"[5] were also made, so that a possible further correction could be derived to enable refractometer

results, with corrections, to replace the lengthy and more difficult determination of solids by drying.

The invert sugar correction for refractometer apparent solids is given by $+0{\cdot}00025 \times$ true solids $\times$ invert sugar.[6] A further correction was devised so that the solids could be adjusted for ash content, and this was found to be $-0{\cdot}0043 \times$ true solids $\times$ sulphate ash. Combining these two equations—

$$\text{True solids} = \frac{\text{refractometer solids (sucrose tables)}}{1 - 0{\cdot}00025 \text{ invert sugar} + 0{\cdot}0043 \text{ sulphate ash}}$$

The results are shown in Table I.

MODIFIED ASH CORRECTIONS BASED ON CONTENT OF ALKALI SALTS (POTASSIUM AND SODIUM SULPHATES)—

It was realised that the composition of the sulphate ash might cause variations of the correction factor, and in order to decide this, the amounts of potassium and sodium sulphates in the sulphate ash were determined with a flame photometer.

The results are shown in Table II.

TABLE II

DETERMINATION OF THE MODIFIED ASH CORRECTION FACTOR TO ALLOW FOR THE SODIUM AND POTASSIUM CONTENT

Number	Sulphate ash, %	Sodium sulphate, %	Potassium sulphate, %	Sulphate ash — sodium and potassium sulphates	Modified ash correction factor
1	11·50	0·4	7·6	3·5	0·010
2	10·29	0·6	5·7	4·0	0·009
3	9·97	0·3	5·2	4·5	0·009
4	8·49	0·7	3·9	3·9	0·008
5	9·03	0·8	4·1	4·1	0·009
6	10·33	0·3	5·6	4·4	0·010
7	10·00	0·3	5·3	4·4	0·011
8	10·35	0·3	5·8	4·3	0·010
9	10·10	0·3	5·8	4·0	0·011
10	9·31	0·3	4·4	4·6	0·011
11	9·63	0·3	4·8	4·5	0·009
12	9·65	0·2	4·5	5·0	0·010
13	9·55	0·5	5·3	3·8	0·010
14	9·43	0·3	4·1	5·0	0·010
15	9·87	1·1	4·9	3·9	0·010
16	9·94	0·3	4·6	5·0	0·009
				Average value	0·010

It was previously known[7,8] that for samples such as golden syrup, in which the alkali sulphates predominated, the ash correction for refractometer solids was at a minimum or was zero, and this has been further confirmed with the new drying oven.

A modified ash correction has been devised from Table II, *viz.*, [$0{\cdot}010 \times$ true solids $\times$ (sulphate ash — potassium and sodium sulphates)]. This correction gives more accurate results, but necessitates determinations with a flame photometer.

Combining the new correction with that for invert sugar—

$$\text{True solids} = \frac{\text{refractometer solids (sucrose tables)}}{[1 - 0{\cdot}00025 \text{ invert sugar} + 0{\cdot}010 \text{ (sulphate ash} - \text{potassium and sodium sulphates)}]}$$

Table III shows the difference between solids by refractometer corrected for invert sugar and sulphate ash, the average ash correction factor of 0·0043 being used, and solids by vacuum oven drying.

TABLE III

COMPARISON OF VACUUM OVEN DRYING SOLIDS WITH CORRECTED REFRACTOMETER SOLIDS

Average ash correction factor of 0·0043 used

Sample	Solids by vacuum drying, % (*a*)	Refractometer solids: From sucrose tables, % (*b*)	Refractometer solids: Corrected for invert, % (*c*)	Refractometer solids: Corrected for invert and ash constituents, % (*d*)	Difference, % (*a*−*d*)
1	74·8	77·1	77·5	73·8	1·0
2	77·8	80·0	80·5	77·1	0·7
3	78·0	80·7	81·2	77·9	0·1
4	72·7	74·7	75·1	72·4	0·3
5	72·7	75·0	75·3	72·5	0·2
6	72·9	75·8	76·2	73·0	−0·1
7	72·7	75·9	76·3	73·2	−0·5
8	73·3	76·1	76·5	73·2	0·1
9	73·1	76·0	76·4	73·2	−0·1
10	72·0	75·2	75·6	72·7	−0·7
11	73·5	76·3	76·7	73·7	−0·2
12	72·9	76·1	76·5	73·5	−0·6
13	72·9	75·3	75·7	72·7	0·2
14	72·9	76·1	76·5	73·5	−0·6
15	73·1	75·6	76·0	72·9	0·2
16	73·1	75·9	76·3	73·2	−0·1

Table IV shows the difference between solids by refractometer corrected for invert sugar and ash constituents, the average modified ash correction factor of 0·010 being used, and solids by vacuum oven drying.

TABLE IV

COMPARISON OF VACUUM OVEN DRYING SOLIDS WITH CORRECTED REFRACTOMETER SOLIDS

Average modified ash correction factor of 0·010 used

Sample	Solids by vacuum drying, % (*a*)	Refractometer solids: From sucrose tables, % (*b*)	Refractometer solids: Corrected for invert, % (*c*)	Refractometer solids: Corrected for invert and ash constituents, % (*d*)	Difference, % (*a*−*d*)
1	74·8	77·1	77·5	74·9	−0·1
2	77·8	80·0	80·5	77·4	0·4
3	78·0	80·7	81·2	77·7	0·3
4	72·7	74·7	75·1	72·3	0·4
5	72·7	75·0	75·3	72·3	0·4
6	72·9	75·8	76·2	73·0	−0·1
7	72·7	75·9	76·3	73·1	−0·4
8	73·3	76·1	76·5	73·3	0·0
9	73·1	76·0	76·4	73·5	−0·4
10	72·0	75·2	75·6	72·3	−0·3
11	73·5	76·3	76·7	73·4	0·1
12	72·9	76·1	76·5	72·9	0·0
13	72·9	75·3	75·7	72·9	0·0
14	72·9	76·1	76·5	72·9	0·0
15	73·1	75·6	76·0	73·1	0·0
16	73·1	75·9	76·3	72·6	0·5

DISCUSSION OF RESULTS

A temperature of 69° to 70° C should ensure that dextrose monohydrate will lose its water of hydration, and that the bend in the graph of percentage loss of weight against time will be as pronounced as possible. Slight decomposition is inevitable when labile

fructose is present. It was not assumed that because dextrose hydrate will lose practically all its water of hydration at 60° C under ideal conditions[7] that it will do so when present in molasses. Golden syrup, which contains 24 per cent. of dextrose, will lose nearly all its water, combined or otherwise, when dried at 69° to 70° C by the method described in this paper. Cane molasses contains only about 10 per cent. of dextrose and gives a better shaped drying curve with less decomposition. There is no reason to assume that water of hydration will be retained. Raffinose pentahydrate is not present in cane molasses.[9]

According to Brieghel-Müller and Kirt,[10,11] the best working temperature for drying sucrose and impure sucrose solutions was 70° C. The authors easily dried a sucrose solution[12] with 23·04 ± 0·01 per cent. of solids. The result by drying was accurate to within ±0·01 per cent., remaining constant on heating from 17 to 49 hours. The aluminium powder, after wetting and vacuum drying, showed no retention of water. Extraction with light petroleum in a Soxhlet apparatus proved that the powder was free from grease. Under a $\frac{2}{3}$-inch objective, the particles looked like granulated zinc, very irregular and rough with pointed ends and slightly knurled surfaces, suggesting that there would be much "edge-effect" evaporation. When the fine grist particles are separated from the medium powder, the coarser particles must be completely rejected. It is inadvisable to attempt size reduction by mechanical or other means for fear of losing the shape characteristics.

White quartz sand was compared with aluminium powder at various pH values. Four solutions of pH 3·2, 4·0, 4·9 and 6·2 were prepared from potassium hydroxide and aconitic acid[13] (the principal organic acid present in cane molasses). These solutions were simultaneously dried on aluminium powder and quartz sand. The results showed that there was no difference in the degree of inertness of aluminium for pH values of 3·2 to 6·2. The pH values of diluted molasses tested were from 5·2 to 5·6.

Conclusion

Analysts without the use of a flame photometer for sodium and potassium determinations can, with the simpler formula, be assured that their results will approximate to the correct value for drying solids. At a late stage of the investigation it seemed likely that the important constituents of the ash affecting refractometer readings were calcium and magnesium sulphates, which might more simply be estimated by a hardness test and expressed as calcium sulphate. This possibility is under investigation.

The view has been expressed by some sugar technologists that water content is of little importance. Nevertheless, in refineries, particularly where a solids balance is calculated as well as a sugar balance, the necessity for a correct water or solids determination in molasses is undisputed, and the refractometer remains as the most satisfactory instrument for the purpose.

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NOTE—Reference 2 is to Part I of this series.

TATE AND LYLE RESEARCH LABORATORY
WESTERHAM ROAD
KESTON, KENT

January 27th, 1954

Notes

DETERMINATION OF BORATE IN SOLUTIONS CONTAINING COBALT OR CHROMIUM

It has been reported earlier that the determination of borate in the presence of metal ions is unsatisfactory.[1,2] The conventional method of separating the metal as hydroxide before the borate determination is time-consuming and gives low values for the borate. We have been able to develop methods in which the effects of the metal ions are nullified by complex formation. An iodide complex for copper[1] and a thiosulphate complex for silver[2] have been found satisfactory. It has now been observed that cobalt as a cobalticyanide complex and chromium as a soluble chromate do not interfere in the determination of borate.

Cobaltous ions can be easily converted into potassium cobaltocyanide, which is oxidised to the cobalticyanide. This complex is stable in presence of dilute acid and alkali and it does not interfere with the standard procedure for borate determination. When the original cobalt concentration is high, the bright yellow colour of the cobalticyanide complex interferes with the correct determination of the end-point (neutralisation of the mineral acid), and most of the cobalt must be removed by precipitation as silver cobalticyanide. The precipitate can be easily coagulated and removed by filtration.

Solutions containing chromic and borate ions are treated with sodium peroxide, when a soluble chromate is formed. This chromate tends to oxidise the ordinary dye indicators and so interferes with correct recognition of the end-point. This can be avoided by precipitating the chromate as barium chromate. The precipitate is soluble in strong mineral acids, but in weak acid solution the solubility product is less than $2{\cdot}3 \times 10^{-10}$. Therefore, from an acidified solution containing barium ions, the chromate is completely precipitated before the neutralisation of the mineral acid is complete. The borate can then be conveniently titrated. An excess of barium ions does not interfere in the determination. The method has distinct advantages over that in which chromium is precipitated as hydroxide by the addition of iodide and iodate.[3]

Method

Reagents—

Potassium cyanide solution, 14 per cent. w/v.

Barium chloride solution—Prepare a 5 per cent. w/v solution from $BaCl_2.2H_2O$.

Hydrochloric acid, 2 N approximately—Dilute 1 part of the concentrated acid with 5 parts of water.

Silver nitrate solution, 0·1 N.

Nitric acid, dilute—Dilute 1 part of the concentrated acid with 10 parts of water.

Details of the preparation of standard alkali and glycerol solutions are given in earlier work.[2]

Procedure for quantities of cobalt below 0·06 g—

To the solution containing cobalt and boric acid contained in a 350-ml conical flask, add potassium cyanide solution from a burette until the brown precipitate first formed just dissolves to give a yellow solution. Avoid an excess of potassium cyanide, as this on acidification will give hydrogen cyanide. If this hydrogen cyanide is not removed by boiling, the final value found for the boric acid will be too high. Add hydrogen peroxide, boil to complete the oxidation and then add 5 to 10 ml of dilute nitric acid. Dilute to a total volume of 150 ml and heat to gentle boiling. Carefully neutralise the excess of nitric acid and complete the titration of the boric acid in the usual way.

With a mixture of borax and cobalt solutions, the procedure is the same except that the precipitate that is formed when the two solutions are mixed is dissolved in nitric acid.

Procedure in presence of an excess of cobalt—

Proceed as before to the oxidation with hydrogen peroxide, when an intense yellow colour is observed. Make the solution distinctly acid with dilute nitric acid and then add silver nitrate solution dropwise until the supernatent liquid is only light yellow in colour. Avoid an excess of silver nitrate, as this would create a new difficulty. Warm to coagulate the precipitate, filter it off and wash it with warm water. Use the filtrate for the determination of boric acid as described above.

TABLE I

TEST DETERMINATIONS OF BORIC ACID IN SOLUTIONS CONTAINING COBALT

Cobalt taken, g	Boric acid taken, g	Boric acid found, g	Error, %
0·003	0·1000	0·1002	+0·2
0·006	0·1920	0·1929	+0·5
0·009	0·0900	0·0900	nil
0·012	0·1246	0·1237	−0·7
0·015	0·0794	0·0799	+0·6
0·030	0·1893	0·1877	−0·8
0·060	0·2000	0·2004	+0·2
0·090	0·0902	0·0900	−0·2
0·120	0·1570	0·1568	−0·1
0·150	0·1935	0·1929	−0·3

That the determination of boric acid after separation of the cobalt as hydroxide is unsatis factory has also been confirmed here. Even with the utmost care and repeated washing of the precipitate, the values found for boric acid were nearly 3 per cent. low (results not reported). In Table I results of similar determinations of boric acid, after the cobalt was masked as potassium cobalticyanide, are given. The last five experiments were carried out by the second procedure, which involves the elimination of cobalt as silver cobalticyanide. The only precaution necessary here is to add a volume of silver nitrate solution sufficient to remove only the greater part of the cobalt complex, but not all. When the above procedure is followed, results are obtained within an accuracy of $\pm 1{\cdot}0$ per cent.

PROCEDURE FOR A MIXTURE OF BORIC ACID (OR BORAX) AND CHROMIC CHLORIDE—

To the solution of chromic chloride contained in a 350-ml conical flask, add an accurately weighed quantity of boric acid and then 0·5 to 1·0 g of sodium peroxide. Dilute the solution with about 20 to 25 ml of water and boil it under a reflux condenser, when it becomes clear. Cool the solution, add 20 to 30 ml of dilute hydrochloric acid and then 10 to 20 ml of barium chloride solution. If necessary, add more hydrochloric acid so that no barium chromate is precipitated at this stage. Dilute the solution to a total volume of about 150 ml and heat it carefully to boiling.

TABLE II

TEST DETERMINATIONS OF BORIC ACID IN A MIXTURE WITH CHROMIC CHLORIDE AFTER PRECIPITATING THE LATTER AS BARIUM CHROMATE

Chromium taken, g	Boric acid taken, g	Boric acid found, g	Error, %
0·0051	0·1048	0·1045	−0·3
0·0101	0·1200	0·1202	+0·2
0·0255	0·0960	0·0955	−0·5
0·0356	0·1562	0·1560	−0·1
0·0510	0·0800	0·0798	−0·2
0·0510	0·1440	0·1447	+0·5

Cool the solution and neutralise most of the free mineral acid with 2 *N* sodium hydroxide solution until the barium chromate first formed no longer dissolves. Then add 2 to 3 drops of methyl red and neutralise the rest of the mineral acid slowly with the standard alkali. The rate of formation of the barium chromate precipitate increases towards the end of the neutralisation. Titrate the boric acid in the usual way in presence of glycerol, with phenolphthalein as indicator.

When a mixture of sodium pyroborate and chromic chloride is taken, the procedure is the same. The precipitate of chromium borate, even if formed, does not affect the oxidation to chromate. The results of determinations are shown in Table II.

PROCEDURE FOR MIXTURE OF CHROMIC AND BORIC ACIDS—

To the solution of chromic and boric acids contained in a 350-ml conical flask, add 10 to 20 ml of barium chloride solution and heat to gentle boiling. Cool and dilute the solution to a total volume of about 150 ml with distilled water free from carbon dioxide. Titrate the chromic

TABLE III

TEST CO-DETERMINATIONS OF BORIC ACID AND CHROMIC ACID IN MIXTURES

Chromic acid taken, ml of 0·1 *N* solution	Chromic acid found, ml of 0·1 *N* solution	Error, %	Boric acid taken, g	Boric acid found, g	Error, %
4·60	4·63	+0·6	0·0665	0·0666	+0·2
9·20	9·15	−0·5	0·1254	0·1247	−0·6
13·80	13·78	−0·2	0·0910	0·0913	+0·3
18·40	18·41	+0·1	0·1264	0·1256	−0·6

acid with standard alkali, using methyl red as indicator. The indicator should be added only after the precipitation of barium chromate has started. After the neutralisation of the chromic acid, the boric acid is titrated in the usual way. Typical results are shown in Table III.

DISCUSSION OF RESULTS

The results of the determination of boric acid in a mixture with chromic chloride are given in Table II. The results were similar when sodium pyroborate was used, and so they are not shown. The determination of the end-point was easy, and the results were accurate, being within ±0·6 per cent. of actual values. The test solution in all experiments contained an excess of barium chloride, and results indicate that an excess of barium ions does not interfere with the determination of boric acid. This has also been confirmed by titrating solutions of boric acid in the presence of a large excess of barium chloride solution (results not given).

The results of the determinations of either acid are accurate to within ±0·6 per cent. The proposed method can be used with advantage for the determination of boric acid from a mixture with chromic acid.

The author wishes to thank Dr. M. H. Khundkar for his keen interest during the progress of the work.

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DEPARTMENT OF CHEMISTRY
UNIVERSITY OF DACCA
EAST PAKISTAN

S. Z. HAIDER
October 26th, 1953

THE DETERMINATION OF LOW BROMINE ABSORPTION VALUES

THERE are several reasons why organic compounds, such as solvents, can contain small amounts of unsaturated impurities. The solvents concerned may have been separated initially from much larger quantities of unsaturated compounds, as is so with benzene, toluene, xylene and so on, when the final removal of olefinic contamination is accomplished by washing with sulphuric acid. Alternatively, the unsaturated compounds may have been produced by the thermal decomposition of heat-sensitive compounds, such as polyhydric alcohols. Judgment of the quality of these materials often depends on the degree of unsaturation.

The usual methods for the determination of the bromine number, such as the Francis[1] or McIlhiney[2] methods, are designed to measure bromine numbers in the 1 to 200 range and they use bromine solutions that are approximately 0·5 *N*. The sample size is usually a few grams and it rarely exceeds 10 g. Bromine numbers below 1·0 are often reported as "less than 1·0" and a maximum precision of ±0·1 of the bromine number is attainable.

The method of bromine number determination that we have developed applies to samples possessing a bromine number of less than 1·0. Very dilute bromine solutions are used and the sample size is adjusted to give readily measurable titration volumes for minute differences in bromine number. With bromine solutions of this dilution, substitution does not seem to occur and the application of any correction for substitution, such as that applied by McIlhiney,[2] is unnecessary. The method consists, therefore, of the addition of an excess of a standard bromine solution to the sample, and then the unused bromine solution is determined in the normal manner through the liberation of iodine from potassium iodide. Acetic acid has been found to be the most

satisfactory solvent for the bromine as no unsaturated compounds could be detected in this solvent, and its miscibility with water is an added advantage.

Method

Reagents—

Bromine in acetic acid—Dissolve 0·75 ml of bromine in 2·5 litres of glacial acetic acid. Adjust the concentration of this solution so that 20 ml of it are equivalent to 20 to 25 ml of 0·01 *N* sodium thiosulphate solution. Store it in the reservoir of an all-glass automatic burette and protect it from light.

Sodium thiosulphate, 0·01 N *solution.*

Potassium iodide solution—A 10 per cent. w/v solution of potassium iodide in water. It is essential that a fresh solution be prepared each day.

Starch indicator solution—A 1 per cent. solution of starch in water.

Procedure—

With a pipette or by weighing, place a suitable quantity of the sample in a dry 500-ml glass-stoppered iodine flask. Reference to Table I will permit the selection of a suitable sample size.

Table I

Selection of sample size

Bromine number range of sample	Sample weight, g
0·10 to 1·00	1·0
0·01 to 0·10	10
0·001 to 0·01	50
0·0001 to 0·001	100

Cool the flask in iced water for 10 minutes. Add 10 ml of bromine solution to it from the automatic burette. Place the stopper in the flask and swirl it gently, and then replace it in the ice-bath for 3 minutes. Add 10 ml of potassium iodide solution and 100 ml of distilled water and thoroughly mix the contents. Titrate the liberated iodine with 0·01 *N* sodium thiosulphate solution, adding starch indicator towards the end of the titration. Make a blank determination omitting the sample. Calculate the bromine number from the difference between the two titration values.

Accuracy of the method—

A check on the accuracy of the method was made by determining the bromine numbers of prepared solutions of allyl alcohol and crotonaldehyde. Samples of these two compounds were purified by distillation and aqueous solutions of a range of bromine number were prepared by weighing. Table II shows the excellent agreement that exists between the values for the bromine

Table II

Comparison of determined values of bromine number with known values

Crotonaldehyde solutions bromine number		Allyl alcohol solutions bromine number	
Prepared values	Determined values	Prepared values	Determined values
0·0050	0·0049	0·0004	0·0004
0·0080	0·0082	0·0011	0·0011
0·0100	0·0099	0·0042	0·0041
0·0398	0·0400	0·0529	0·0513
0·0797	0·0800	0·0965	0·0966

number as determined experimentally and the known values of the prepared solutions. The method, therefore, provides a sensitive measure of unsaturated compounds that are present in minute quantities; the titration difference of 0·1 ml corresponds to a bromine number measurement of 0·00008 when 100-g samples are used.

Applications of the method

Trace quantities of unsaturated compounds are often deleterious when present in certain products, as the properties of odour and taste are often affected by the presence of such impurities.

For instance, glycols that are suitable for use as tobacco humectant show satisfactorily low values for bromine number, whereas odourous glycols give values that are significantly higher. High quality aromatic solvents should contain only minute quantities of olefinic hydrocarbons, and the evaluation of the residual unsaturation in these solvents is usually made by the application of various "acid wash" tests. A direct relationship has been established between the "acid wash" colour, as evaluated by a Spekker absorptiometer, and the determined bromine number. Methanol, ethanol and *iso*propanol have been shown to contain trace unsaturation, but no measurable unsaturation has been detected in the commonly used halogenated solvents, such as chloroform and carbon tetrachloride.

The method may also be used for the determination of unsaturation in substances of which only micro quantities are available. Then it is preferable to dissolve a weighed quantity of the sample in a relatively large volume of a suitable solvent before testing it.

The authors wish to express their thanks to the Management of Petrochemicals Limited for permission to publish this work and to Dr. E. T. Borrows for the encouragement that has been given to them to carry out the experimental work.

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Analytical Development Laboratory
Petrochemicals Limited
Partington Industrial Estate
Urmston, Manchester

V. W. Reid
J. D. Beddard
January 13th, 1954

A CHROMATOGRAPHIC PROCEDURE FOR THE DETERMINATION OF "PYRETHRINS" IN PYRETHRUM EXTRACTS

Qualitative chromatographic methods for separating "pyrethrins" I and II have been described.[1,2,3] Further work has given a quantitative method of separation that is suitable for the assay of pyrethrum extracts in *n*-hexane; pyrethrum extracts in mineral oil can be assayed after removal of the solvent. Dyes are used to standardise the adsorbent and to follow the progress of the elution.

Experimental

Reagents—

Alumina adsorbent—Chromatographic-analysis grade alumina, which is standardised as described below.

Dye solution—Dissolve separately Sudan yellow and Sudan red (chromatographic-standardisation grade) in *n*-hexane to give saturated solutions, and then mix equal volumes of these solutions.

n-*Hexane solvent, free from aromatic hydrocarbons*—Technical grade *n*-hexane is sufficiently and conveniently purified by successive percolation through two long columns of activated charcoal (100 cm × 2 cm approximately).

n-*Hexane - diethyl ether solvents*—*n*-Hexane (as above) containing 10 per cent. and 20 per cent. v/v of analytical-reagent grade diethyl ether.

Apparatus—

A glass column 15 cm long and 8 to 9 mm internal diameter, with a B 14 socket to take a B 14 250-ml tap funnel at the upper end and a B 19 cone to take a B 19 tap unit at the lower end.

Method

Procedure for preparation of column—

Plug the lower end of the column with glass-wool, fit the tap, which is lubricated with a mixture of gum arabic and glycerol (approximately equal porportions heated together), and charge the column with 5 g of alumina. Wash the column through with 50 ml of ether, and then close the tap and fill the column with *n*-hexane; after placing a stopper in the open end, invert the column and allow the alumina to run down it, then return it to its normal position and briskly rotate the column by rolling it between the palms of the hands. Open the tap, and apply slight air pressure to the top of the column until the *n*-hexane has run down to the level of the top of the alumina.

PROCEDURE FOR STANDARDISATION OF THE ALUMINA—

Pour 1 ml of the mixed dye solution into the column and begin running in the 10 per cent. ether - *n*-hexane reagent from the tap funnel. Start collecting the eluate immediately the Sudan yellow reaches the plug of glass-wool, and note the volume when the pink band of the Sudan red just reaches the same point. The latter dye should arrive there when between 70 and 95 ml of eluate have been collected; otherwise, the batch of alumina must be treated until it has the requisite activity, either by exposing it to a humid atmosphere if the volume of eluate is greater than 95 ml, or by heating it at 100° to 110° C if the volume is below 70 ml.

PROCEDURE FOR THE SEPARATION AND DETERMINATION OF THE "PYRETHRINS"—

Prepare a fresh column, as described above, containing the standardised alumina. Mix 4 ml of pyrethrum solution in *n*-hexane (containing 10 to 30 mg of total "pyrethrins") with 1 ml of the mixed dye solution in a small beaker and transfer the solution quantitatively to the top of the column. Wash out the beaker several times with a little *n*-hexane and add the washings to the column after suitable intervals. Start elution with the 10 per cent. ether - *n*-hexane reagent; as soon as the Sudan yellow reaches the plug of glass-wool, collect the eluate, containing the "pyrethrin-I" fraction, in a 100-ml calibrated flask. When the pink Sudan red band reaches the plug, change the receiver for a 200-ml calibrated flask and continue the elution with the 20 per cent. ether - *n*-hexane reagent until 200 ml of eluate, containing the "pyrethrin-II" fraction, have been collected. The whole process should be completed on the same day; air pressure is applied to the column during the later stages of elution, if necessary, to achieve this.

After making the eluates up to volume, take from each fraction a one-hundredth aliquot, remove the solvent in a current of clean dry nitrogen or carbon dioxide, and dissolve each residue in 20 ml of aldehyde-free 95 per cent. ethanol. Determine the ultra-violet absorption of a 1-cm thickness of these ethanolic solutions by a spectrophotometer, at a wavelength of 224 mμ for the "pyrethrin-I" fraction and at 229 mμ for the "pyrethrin-II" fraction. Then—

$E_{1\,cm} \times 17{\cdot}85$ = mg of "pyrethrin I" originally put on column.

$E_{1\,cm} \times 20{\cdot}39$ = mg of "pyrethrin II" originally put on column.

These conversion factors are empirical. They are based on parallel analyses of a pyrethrum extract by the current Association of Official Agricultural Chemists' mercury reduction method[4] and the present chromatographic method, as the extinction coefficients of the four "pyrethrin" analogues are not known with certainty.[3]

For pyrethrum extracts in mineral oil, first remove the solvent by distillation at a low pressure (less than 0·001 mm of mercury) and at a temperature not exceeding 40° C,[5] and then dissolve the residue in *n*-hexane to give a concentration within the limits specified above.

Crude pyrethrum extracts contain plant pigments that separate in the eluates but which do not seriously interfere with the use of the marker dyes; with a little experience the progress of the critical Sudan red band can be readily followed.

RESULTS

Table I shows some of the results of the chromatographic method in comparison with the A.O.A.C. mercury reduction method.

TABLE I

COMPARISON OF RESULTS OF "PYRETHRIN" DETERMINATIONS

	Chromatographic method				A.O.A.C. mercury reduction method			
Sample	Number of determinations	"Pyrethrin I," % w/v	"Pyrethrin II," % w/v	Total "Pyrethrins," % w/v	Number of determinations	"Pyrethrin I," % w/v	"Pyrethrin II," % w/v	Total "Pyrethrins," % w/v
A	2	0·30 (0·00)*	0·24 (0·01)	0·54	3	0·28 (0·02)	0·23 (0·01)	0·51
B	5	0·19 (0·02)	0·30 (0·03)	0·49	1	0·19	0·31	0·50
C	9	0·27 (0·02)	0·24 (0·03)	0·51	1	0·27	0·24	0·51
D	3	0·38 (0·03)	0·17 (0·01)	0·55	3	0·34 (0·02)	0·16 (0·01)	0·50
E	1	0·34	0·42	0·76	2	0·01 (0·01)	0·42 (0·00)	0·76
F	9	0·22 (0·02)	0·17 (0·03)	0·39	1	0·22	0·18	0·40

* The figures in parentheses indicate total ranges, *e.g.*, the result 0·19 (0·02) summarises a series of readings extending from 0·18 to 0·20, with a mean of 0·19.

The chromatographic method shows a repeatability scarcely inferior to that of the mercury reduction method; it has the advantage of being considerably more rapid and, by suitable staggering, a number of determinations can be made simultaneously.

Alternative methods of determination[6,7,8,9] (not requiring a spectrophotometer) of the chromatographically-separated "pyrethrins" have been investigated, but they have proved unsatisfactory, either because of the difficulty of applying them with the small quantities involved, or because of the inherent colour of the solutions.

Thanks are due to Dr. A. J. Feuell for advice and encouragement, to Miss M. W. Jarvis for carrying out some of the analyses, and to the Director of the Colonial Products Laboratory for permission to publish this note.

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AMENDMENT SLIPS*

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Book Reviews

THE CHEMISTRY OF SYNTHETIC DYES. Volume II. By K. VENKATARAMAN. Pp. xvi + 705–1442. New York: Academic Press Inc. 1952. Price $15.00; 120s.

This volume firstly continues the description of dyes on a chemical basis (see *Analyst*, 1953, **78**, 131). The anthraquinone group, with its several facets—mordant, acid, vat, and so on—not unexpectedly accounts for nearly a third of the descriptive matter now presented. Other fairly substantial classes are the indigoids, the sulphur dyes, the azine and triphenylmethane groups and the cyanines, but the phthalocyanines and the sulphurised and solubilised vat dyes are not overlooked. As before, there are digressions, which here are mainly concerned with naturally occurring colouring matters; this aspect, which alone would serve for a book on its own, accounts for most of the brief chapter on the naphthaquinones. The chapter on miscellaneous dyes includes some information on colour formers in photography, fluorescent dyes and certain pigments for lacquers such as co-precipitated acid and basic dyes and *cyclo*hexylamine and allied salts of water-soluble dyes.

Chapters are devoted to a consideration of the constitution of dyes in relation to light-fastness, to the degradation of cellulose under the influence of certain dyes and to substantivity. The final chapter deals with the identification, analysis and evaluation of dyes, so far as any general methods can be given; the problem is perhaps well summed up in the statement "an important requirement for success in dyestuff analysis is the possession of a complete range of authentic samples with which a direct comparison can be made."

The indexes to the combined volumes are very welcome, but it is only fair to warn that too much must not be expected from that of dye names, extensive though it is. Thus, of some 200 foreign dyes used to-day in this country in at least moderately large quantities, the names of only 20 will be found; some of the others are quite new, some are new formulations based on existing dyes, some are old friends under new names and for some the information is not as yet generally available. Moreover, it must be remembered that the new edition of the "Colour Index" will list 25,000 names—a book in itself.

The author would have carried out a useful task had he merely correlated the information set out in the numerous B.I.O.S. and F.I.A.T. reports. In fact, he has done much more, *e.g.*, by drawing on both patent and chemical literature. A work of this magnitude is inevitably expensive; this is a pity, as it must of necessity limit the appeal of an interesting, important and colourful branch of chemistry.

B. A. ELLIS

ORGANIC SYNTHESES. Volume 33. Editor-in-Chief: CHARLES C. PRICE. Pp. vi + 115. New York: John Wiley & Sons Inc.; London: Chapman & Hall Ltd. 1953. Price $3.50; 28s.

In contrast to the previous volume, this latest addition to the series includes a much higher proportion of heterocyclic compounds, *viz.*, 2:4-diamino-6-phenyl-*s*-triazine, 2:2-dimethylpyrrolidine, furfurylidene diacetate, 3-methylbenzofuran, nicotinonitrile, pyridine-N-oxide, 2-pyrollidinopropranol and thiophen-3-carboxylic acid. Acids are represented by atrolactic, fluorene-9-carboxylic, α-phenylcinnamic and αββ-triphenylpropionic acids; allied products are itaconyl chloride, *o*-phenylene carbonate, di*tert.*-butyl malonate, diethyl γ-oxopimelate and *cyclo*butane-1:1-dicarboxylate. In the aldehyde and ketone family are "butylchloral," *o*-nitrocinnamaldehyde, 6-nitroveratraldehyde and 3-acetamidobutanone, 1:4-naphthaquinone, stearone and α-tetralone; the analyst, as such, might be interested in *p*-dimethylaminobenzaldehyde and also in *m*-nitrobenzazide. Other items included are *cis*-stilbene, *m*-nitrodiphenyl and *m*-nitrostyrene, pent-4-yn-1-ol, cresol, benzhydryl 2-chloroethyl ether, alloxantin dihydrate, naphthalene-1:5-dithiol and dimethylketen.

As is customary, some of the methods described include preliminary stages that for others, are given as separate monographs. A warning is issued that the sodium derivative of nitromalonaldehyde, described in Volume 32, is potentially explosive.

B. A. ELLIS

THE JOURNAL OF ANALYTICAL CHEMISTRY OF THE U.S.S.R. IN ENGLISH TRANSLATION. Volume VII, No. 1, January–February, 1952. Pp. 80. New York: Consultants Bureau, 152, West 42nd Street, New York, 18, N.Y. 1953. Annual subscription $80.00; Single issue $15.00. Reprints of single papers $7.50.

The appearance of this number of "The Journal of Analytical Chemistry of the U.S.S.R.," the first to be translated into English in full, will be welcomed by English-speaking analysts.

It contains eight papers originally published in February, 1952, as the first of the bi-monthly issues of the Russian journal. The contents are—

"The Solubility of Precipitates in the Presence of Common Ions and Other Ions," by A. K. Babko; "Physico-Chemical Analysis of Systems of Importance in Analytical Chemistry. XX. The Solubility of Precipitates in Complex (Real) Analytical Systems," by I. V. Tananaev, I. B. Mizetskaya and A. D. Vinogradova; "The Complex Study of Precipitates," by A. V. Nikolaev and M. P. Elentukh; "Complex Compounds and their Application in Analytical Chemistry," by D. I. Ryabchikov; "Thiourea Complexes of Low Solubility and their Application in Analysis," by K. B. Yatsimirsky and A. A. Astasheva; "The Application of Mathematical Methods for Evaluating the Results of Chemical Analysis," by E. G. Gracheva; "Microcrystalloscopic Reactions for Nitrates and Nitrites," by N. M. Korenman and A. A. Belyakov; "New Azo-Indicators of the Methyl Orange Series, and the Relation between the Structure and pH Changes of Azo-Indicators," by V. I. Kuznetsov and G. N. Kosheleva.

Obituary notice of Mikhail Maksimovich Fainberg.

Resolutions of the Conference on Classical Methods of Chemical Analysis in the Geochem Institute of the Academy of Sciences, U.S.S.R., held in Moscow, November 14th to 17th, 1951.

Review of "Colorimetric Analysis," by A. K. Babko and A. T. Pilipenko.

The English translation is published in the form of a photolithographic reproduction of typescript sheets, clipped together in a strong paper cover. The work has been well carried out by the Consultants Bureau of New York, a commercial firm specialising in the translation of foreign scientific literature.

Photostatic reproduction is expressly prohibited by the copyright owners, but copies of this and other translations of Russian journals can be inspected at the Chemical Society's library, to which members of our Society have access. F. L. OKELL

BRITISH VETERINARY CODEX 1953. Pp. xxiv + 737. London: The Pharmaceutical Press. 1953. Price 45s.

As a result of a suggestion made by the Pharmaceutical Society of Great Britain to the Royal College of Veterinary Surgeons and the British Veterinary Association, this work, similar to the British Pharmaceutical Codex but devoted to medicinal substances and preparations used in veterinary practice, has been produced. A British Veterinary Codex Committee was appointed by the Council of the Pharmaceutical Society, and the present volume is the outcome of three and a half years' work of this Committee and its six Sub-Committees. The main function of the book is to define standards for the quality of medicinal substances and preparations used for veterinary purposes, but, in fact, being modelled on the British Pharmaceutical Codex, it contains a vast amount of ancillary information, much of it of great value to analysts and all others associated with veterinary work.

There are three main divisions of the book: Part I, of 424 pages, consists of monographs on drugs, chemicals and related substances, Part II contains the monographs on antisera, vaccines and related products, and Part III comprises the Formulary and covers 118 pages. All titles are in English, the old Latin names, when such exist, being relegated to the synonyms. There follow the Appendixes, modelled on the lines of the corresponding section of the British Pharmacopoeia (where it is entitled "Appendices") and these include reagents used in assays and tests, quantitative tests for arsenic and lead, limit tests and a most useful list of alternative names, including trade names, for substances defined in the B. Vet. C. There is also a therapeutic and pharmacological index, and the work concludes with a closely printed general index of 41 pages.

The 431 monographs on drugs and chemicals embrace all substances ordinarily used in the treatment of animal diseases, and, for drugs already described in the British Pharmacopoeia or the British Pharmaceutical Codex, the same standards usually apply and modified grades are only recognised when there is evidence of a substantial veterinary use for material of lower grade. From the analyst's point of view, special interest attaches to the 66 monographs on substances not already defined in the B.P. or B.P.C. Doubtless the Analytical Standards Sub-Committee (Chairman: Dr. D. C. Garratt) were mainly concerned with drafting the standards and tests for these, and it is noticeable that an assay is included whenever possible; for many organic drugs, however, it has been necessary to fall back upon a determination of the nitrogen content, as, obviously, in the short time available, specific methods could not be developed. There is a monograph for technical grade benzene hexachloride, and it is interesting to note that a polarographic assay for the γ-isomer content is described. The monograph on Cetomacrogol 1000 (polyethylene glycol 1000 monocetyl ether, a non-ionic emulsifying agent) affords an example of the care expended on the analytical aspects of the British Veterinary Codex.

It is to be noted that the monographs are not only concerned with standards but also include extremely useful sections on action and uses and on toxicity, while, because of the great variety of doses, separate instructions are given, when appropriate, for horses (and foals), cattle (and calves), sheep (and lambs), pigs, dogs, cats, turkeys and fowls. The therapeutic notes have been most carefully and comprehensively written and sometimes, as, for example, in the monographs on benzylpenicillin, streptomycin hydrochloride, sulphanilamide and phenothiazine, extend to more than a page of closely-printed type.

In veterinary practice, antisera and vaccines are probably more widely used than in the human field, especially for prophylactic purposes, because the protection of animals, which are exposed to much greater risk of infection, by the hygienic methods adopted among humans would be uneconomic. Thus protection by immunisation is important in veterinary work, and this fact is reflected in the large number of preparations included in Part II of the Codex, there being 65 monographs, variously concerned with antisera, vaccines, toxoids and tuberculins, which follow the same pattern as those of Part I.

The Formulary of Part III is, again, modelled on B.P.C. lines, and there are among the 300 items numerous examples of assays whenever such can be applied. Especially interesting among these are the individual assays of the active constituents of intramammary injection of penicillin with dihydrostreptomycin, the latter constituent being determined microbiologically either by direct plate assay with a strain of *Bacterium coli* susceptible to dihydrostreptomycin but not to penicillin or by initial inactivation of the penicillin with penicillinase and then the use of *Bacillus subtilis*. For the assay of the penicillin, a special peptone - yeast extract medium (which inhibits the effect of dihydrostreptomycin) is used in conjunction with a defined strain of *Staphylococcus aureus* as test organism.

Doubtless, as experience is gained in the daily use of this great work of reference, errors and shortcomings will emerge, giving opportunities for investigators to publish papers; and, in due course, with the advancement of veterinary science, a new Codex will make its appearance. But, for the present, all concerned, however remotely, with this vast and difficult subject will be grateful to those who put so much of their effort and knowledge into the production of the first British Veterinary Codex. N. L. ALLPORT

SPOT TESTS. Volume I. INORGANIC APPLICATIONS. By F. FEIGL, Eng., D.Sc. Translated by R. E. OESPER, Ph.D. Fourth Edition. Pp. xii + 518. Amsterdam and New York: Elsevier Publishing Co.; London: Cleaver-Hume Press, Ltd. 1954. Price 45s.

This excellent work has now been divided into two volumes. The present one, Inorganic Applications, follows the general arrangement of the relevant parts of the third English edition (reviewed in *The Analyst*, **1949**, **74**, 427). Within this arrangement, however, there has been substantial extension. The first chapter, "Development, Present State and Prospects of Inorganic Spot Test Analysis," has been revised in co-operation with Professor P. W. West, whose work in the development of the principles and practice of spot tests is widely known. Professor West has also written, with considerable expansion, the second chapter on "Spot Test Techniques."

Most of the volume deals with spot tests for cations and anions and shows evidence of careful revision. The former treatment has been adhered to, whereby details are given of tests that the author and his co-workers have investigated, while other promising tests are given as notes. About seventy-five new tests have been included, and others have been expanded from notes to detailed descriptions.

The chapters on "Tests for Free Elements" and "Systematic Analysis of Mixtures by Spot Reactions" have small additions and the chapter on "Applications," while omitting the organic field, is larger than before.

It is perhaps too early to expect the author's considered views on the use of the newer complexing agents in rendering spot tests more selective; we may hope to see more mention of this in the next edition. The formulae shown for some complexes appear to lack sufficient evidence. References are now collected at the end of each chapter; this seems a pity, as a glance to the foot of a page at a name or a date can often be adequate to an analyst who is familiar with the field of his enquiry.

The text and diagrams have been re-set to save space; nevertheless, the volume contains over a hundred pages more than was devoted in the previous edition to inorganic work. Professor Oesper's translation maintains the high quality of previous editions. The new flexible back makes for easy handling; it remains to be seen whether it will survive as well as boards the constant handling that the book deserves to have from every analytical chemist. DAVID W. WILSON

Publications Received

ENERGY TRANSFER IN HOT GASES. National Bureau of Standards Circular 523. Pp. iv + 126. Washington: U.S. Government Printing Office. 1954. Price $1.50.

VAPOUR PRESSURE OF ORGANIC COMPOUNDS. By T. EARL JORDAN. Pp. x + 266; 153 Plates. New York and London: Interscience Publishers Inc. 1954. Price $14.50; 108s.

THE PROPERTIES OF TIN. Pp. 55. Greenford, Middlesex: Tin Research Institute. 1954. Price 2s. 6d.

MÉTHODES ET RÉACTIONS DE L'ANALYSE ORGANIQUE. Volume III. RÉACTIONS COLORÉES ET FLUORESCENCES. By M. PESEZ and P. POIRIER. Pp. vi + 299. Paris: Masson et Cie. 1954.

PRACTICAL METHODS IN BIOCHEMISTRY. By F. C. KOCH and M. E. HANKE. Sixth Edition. Pp. x + 537. London: Baillière, Tindall & Cox Ltd. 1953. Price 38s. 6d.

OUTLINE OF CHEMISTRY. A SUMMARY FOR FIRST YEAR STUDENTS. By E. J. MANSON. Translated and revised by H. W. TURNER, B.Sc. Pp. viii + 150. London and Glasgow: Blackie & Son Ltd. 1954. Price 5s.

INTERMEDIATE PRACTICAL CHEMISTRY COURSE. By L. M. TURTON, Ph.D. Pp. vi + 138. London and Glasgow: Blackie & Son Ltd. 1954. Price 5s.

INDUSTRIAL AND MANUFACTURING CHEMISTRY. Part II. INORGANIC. Volumes I and II. By G. MARTIN, D.Sc., Ph.D., F.R.I.C. Sixth Edition by W. FRANCIS, M.Sc., Ph.D., F.R.I.C., F. Inst. F. Pp., Vol. I, xxiv + 600; Vol. II, xxii + 491. London: The Technical Press Ltd. 1954. Price Vol. I, 70s.; Vol. II, 70s.

NAME REACTIONS IN ORGANIC CHEMISTRY. By A. R. SURREY. Pp. viii + 192. New York: Academic Press Inc.; London: Academic Books Ltd. 1954. Price $4.00.; 32s.

PRACTICAL PHYSICAL CHEMISTRY. By A. FINDLAY. Eighth Edition, revised and edited by J. A. KITCHENER. Pp. xiv + 364. London and New York: Longmans, Green and Co. Ltd. 1954. Price 18s.

THE VITAMINS: CHEMISTRY, PHYSIOLOGY, PATHOLOGY. Volume I. Edited by W. H. SEBRELL, jun., and R. S. HARRIS. Pp. xiv + 676. New York: Academic Press Inc.; London: Academic Books Ltd. 1954. Price $16.50; 132s.

CLAYTON'S THE THEORY OF EMULSIONS AND THEIR TECHNICAL TREATMENT. Fifth Edition by C. G. SUMNER, M.Sc., Ph.D., F.R.I.C. Pp. viii + 669. London: J. & A. Churchill Ltd. 1954. Price 72s.

Errata

MARCH (1953) ISSUE, p. 181, 4th line from foot of page. *For* "Serum sodium = 48·88 ($x-y$) mg per ml," *read* "Serum sodium = 48·88 ($x-y$) mg per 100 ml."

MAY (1954) ISSUE, p. 273, 2nd line below Table I. *For* "Irving and Rossotti's[6]" *read* "Irving and Rossotti's.[1]"
IBID., p. 277, 2nd line below Table VI. *For* "Belcher" *read* "Belcher, Nutten and Stephen."
IBID., p. 277, 2nd line below Fig. 3. *After* "Belcher" *add* "*et al.*[6]"

MAY (1954) ISSUE, p. 291, 3rd line from foot of page. *For* "m.p. 225° C" *read* "m.p. 255° C."

PORTRAITS OF PAST PRESIDENTS

A FURTHER photogravure portrait in the series of Portraits of Past Presidents, that of Mr. S. E. Melling (President: 1943–44), of whom an Obituary Notice appears on p. 396, is in preparation. Members of the Society and subscribers to *The Analyst* who wish to receive a gratis copy of this portrait should apply to the Editor, *The Analyst*, 7–8, Idol Lane, London, E.C.3, before September 30th, 1954.

REPORT OF THE ANALYTICAL METHODS COMMITTEE

THE Report of the Thiamine (Microbiological) Panel of the Sub-Committee on Vitamin Estimations, "The Microbiological Determination of Thiamine," reprinted from *The Analyst*, March, 1954, **79**, 118–121, is now available from the Secretary, the Society for Analytical Chemistry, 7–8, Idol Lane, London, E.C.3; price to members 1s. 6d. and to non-members 2s. 6d. Reports of the Analytical Methods Committee are only obtainable from the Secretary (not through Trade Agents) and remittances must accompany orders.

A VACANCY exists for an Assistant of either sex in the Analytical Department of a food factory in S.W. England. Applicants, who should hold a university degree or equivalent, are invited to write, stating age, qualifications and salary required, to the Chief Chemist and Bacteriologist, Aplin and Barrett, Ltd., Newton Road, Yeovil, Somerset.

ALBRIGHT & WILSON LTD., Chemical Manufacturers, invite applications for the post of Research Analyst in the Laboratory of their factory at Barry (Glam.). The duties involve the investigation into and the development of new methods for the analysis of organo-silicon compounds and intermediates associated with the manufacture of silicones. Candidates, aged under 30, must have an Honours Degree in Chemistry or A.R.I.C. Experience of classical and modern analytical techniques, together with the ability to control a small analytical research section, is essential. Commencing salary according to age, experience and qualifications. There is a non-contributory pension scheme. Apply to the Appointments Officer, Albright & Wilson Ltd., Oldbury, Birmingham.

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ALBRIGHT & WILSON LTD., Chemical Manufacturers have vacancies for Laboratory Assistants for shift duties at their factory at Barry, Glam. The work will involve plant control analysis and pilot plant production. Candidates should be aged 22 to 26, have completed National Service and have Inter B.Sc., or equivalent. In addition to basic salary, which depends on age, qualifications and experience, a shift allowance is payable. The Company possesses a non-contributory pension scheme. Apply to the Appointments Officer, Albright & Wilson Ltd., Oldbury, Birmingham.

CHEMIST required to assist in Laboratory of large engineering company in Luton. Some knowledge of oil and grease analysis essential. Applications in writing, giving full details of age, education and experience, should be addressed to Box No. 3865, THE ANALYST, 47, Gresham Street, London, E.C.2.

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Apply in writing to the Director of Recruitment, Colonial Office, Great Smith Street, London, S.W.1, giving briefly age, qualifications and experience. Mention the reference number BCD 97/14/01.

CHEMISTS of Inter. B.Sc. standard are required for important analytical work of varying character. Salary dependent on age, qualifications and experience. Reply in writing with full particulars to the Personnel Manager, The Fairey Aviation Co., Ltd., Hayes, Middlesex.

A VACANCY occurs for a Lady Chemist and Bacteriologist with B.Sc. or equivalent qualification on the staff of the laboratories of Vine Products Ltd., of Villiers Road, Kingston-on-Thames, Surrey. Some experience in the analysis of foods and beverages is essential. The salary offered is £750, but would vary according to qualifications and experience. A superannuation scheme is in operation. Please communicate with the Works Manager by letter or 'phone (Kingston 5641).

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