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Summaries of Papers in This Issue

An Evaluation of the Methods for Determining Residual Chlorine in Water

Part I. Free Chlorine

An evaluation has been made of nine colorimetric and three titrimetric methods available for determining free chlorine in water. The criteria used in comparing the methods were stability of the coloured products, reproducibility, specificity, sensitivity, limit of detection, linearity of the calibration graphs, accuracy, effect of temperature, stability of the reagent and simplicity and convenience.

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N. J. NICOLSON

The Water Research Association, Ferry Lane, Medmenham, Marlow, Bucks.

Analyst, 1965, **90**, 187–198.

The Use of Electrolytic Hygrometers for the Determination of Water and Hydrogen

Factors relevant to the installation and operation of moisture meters as in-line monitors are discussed. Results on the general performance of these instruments are given, with special reference to cell efficiency and flow-rate and pressure of the sample gas.

A calibration system is described for the range 10 to 1000 v.p.m. of moisture. Calibration over this range of moisture concentration is achieved by catalytic oxidation over a platinum surface at 500° C of known amounts of hydrogen in the presence of an excess of oxygen. The known amounts of hydrogen are obtained from standard gas mixtures or by electrolysis of dilute sulphuric acid.

Two modifications to the standard instrument are discussed. The first involves a method of rapid regeneration of a saturated cell and the second permits the moisture contents of gases to be determined at pressures up to a maximum of 200 p.s.i.g. without a pressure-reducing valve.

The use of the instrument for determining hydrogen in metals and gas mixtures is described.

J. A. J. WALKER and P. CAMPION

United Kingdom Atomic Energy Authority, Reactor Materials Laboratory, Wigshaw Lane, Culcheth, Warrington, Lancs.

Analyst, 1965, **90**, 199–209.

The Effect of Nitrilotriacetic Acid Impurity on the Standardisation of Solutions of Ethylenediaminetetra-acetic Acid

Commercial and some analytical-reagent grades of ethylenediaminetetra-acetic acid have been found to contain significant amounts of nitrilotriacetic acid, and it is shown that this impurity can give rise to different factors when solutions of ethylenediaminetetra-acetic acid are standardised against different metals with the accepted indicators. Potentiometric investigations also show that ethylenediaminetetra-acetic acid and nitrilotriacetic acid give separate end-point steps. Either step may be detected by indicators, depending on the titration conditions and the metal used.

The presence of nitrilotriacetic acid also has a deleterious effect on the end-point sharpness.

R. N. P. FARROW and A. G. HILL

The British Drug Houses Ltd., B.D.H. Laboratory Chemicals Division, Poole, Dorset.

Analyst, 1965, **90**, 210–215.

The Determination of Small Amounts of *N*-Isopropyl-*N'*-phenyl-*p*-phenylenediamine

N-Isopropyl-*N'*-phenyl-*p*-phenylenediamine can be determined by visual titration in chloroform with toluene-*p*-sulphonic acid. The method is simple, rapid and accurate. Details are given for applying this technique to the analysis of raw and vulcanised rubbers containing the diamine.

J. R. DAVIES

The Natural Rubber Producers' Research Association, 48–56 Tewin Road, Welwyn Garden City, Herts.

Analyst, 1965, **90**, 216–219.

The Mass-spectrometric Determination of Certain Trace Impurities in Gases

The determination of impurities in gases with an MS2G mass spectrometer is, with few exceptions, restricted to a lower limit in the region of 50 to 100 v.p.m. In certain instances, by modifying the normal procedure and concentrating the impurity before analysis, determinations can be made at a much lower level, the increase in sensitivity being up to 4000 times the normal.

Methods have been developed that have been used to determine, with a coefficient of variation of better than 4 per cent., 1 v.p.m. of helium in nitrogen, 1 v.p.m. of hydrogen in argon, 3 v.p.m. of carbon dioxide in hydrogen and 20 v.p.m. of hydrogen in helium.

R. T. PARKINSON and L. TOFT

Master General of Ordnance Inspectorates, Chemical Inspectorate, Royal Arsenal, Woolwich.

Analyst, 1965, **90**, 220–227.

The Determination of Carbon in Sodium

A method has been developed for determining free carbon in sodium at the part per million level. Sodium is removed by distillation at 600° C for 8 hours and the carbon in the residue determined by combustion in an excess of oxygen at 1200° C. The carbon dioxide formed is measured manometrically. Additions of carbon to sodium for the levels 3, 6 and 12 p.p.m. have been recovered satisfactorily with a standard deviation of 0·5 p.p.m. of carbon. The possibility of loss of carbon because of interaction with sodium oxide has been investigated.

J. A. J. WALKER and E. D. FRANCE

United Kingdom Atomic Energy Authority, Reactor Materials Laboratory, Wigshaw Lane, Culcheth, Warrington, Lancs.

Analyst, 1965, **90**, 228–233.

Colorimetric Determination of Copper in Plants

A rapid and accurate method is described for determining copper by the colorimetric measurement of the complex formed between copper and bis-cyclohexanone oxalyldihydrazone. To apply the method to the analysis of plant material, dry ashing with subsequent digestion of the ash with aqua regia was employed; this procedure was found to be equal in accuracy and superior in speed and convenience to a more commonly used wet-ashing procedure.

The method is sensitive to 0·4 p.p.m. of copper in solution, and its analytical precision, expressed as a coefficient of variation, is 0·8 per cent. When applied to plant analysis, its precision is lower, and when averaged for three plant species, the error of a single determination on a given sample on any day is 2·0 per cent.

K. R. MIDDLETON

Rubber Research Institute of Malaya, P.O. Box 150, Kuala Lumpur, Malaysia.

Analyst, 1965, **90**, 234–240.

A Modified Method for Determining Traces of Nitrilotriacetic Acid in Ethylenediaminetetra-acetic Acid

Short Paper

R. N. P. FARROW and A. G. HILL

The British Drug Houses Ltd., B.D.H. Laboratory Chemicals Division, Poole, Dorset.

Analyst, 1965, **90**, 241–242.

Interference of Ammonium Ions in the Determination of Reducing Sugars by the Colorimetric Method of Somogyi and Nelson

H. FALANGHE *Short Paper*

Department of Chemistry, Instituto Zimotécnico, Escola Superior de Agricultura "Luiz de Queiroz," University of São Paulo, Piracicaba, São Paulo, Brazil.

Analyst, 1965, **90**, 242–243.

A Method for Determining *N*-Hydroxyurethane for Use in Metabolic Studies

S. S. MIRVISH *Short Paper*

Department of Experimental Biology, The Weizmann Institute of Science, Rehovoth, Israel.

Analyst, 1965, **90**, 244–246.

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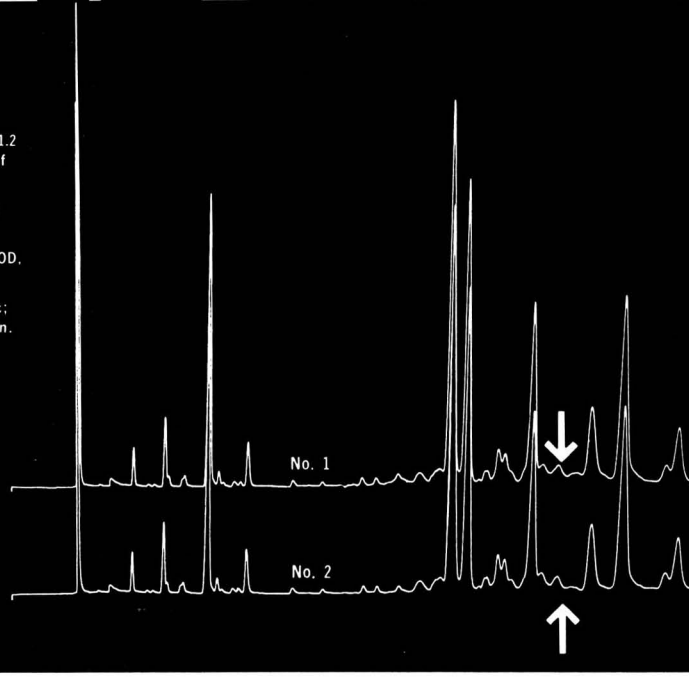

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An Evaluation of the Methods for Determining Residual Chlorine in Water

Part I. Free Chlorine

BY N. J. NICOLSON

(*The Water Research Association, Ferry Lane, Medmenham, Marlow, Bucks.*)

An evaluation has been made of nine colorimetric and three titrimetric methods available for determining free chlorine in water. The criteria used in comparing the methods were stability of the coloured products, reproducibility, specificity, sensitivity, limit of detection, linearity of the calibration graphs, accuracy, effect of temperature, stability of the reagent and simplicity and convenience.

The barbituric acid method was the best laboratory colorimetric method for use in the absence of combined chlorine, *e.g.*, monochloramine. In the presence of combined chlorine, however, the *NN*-diethyl-*p*-phenylenediamine method was more satisfactory, provided that the colour was measured immediately. The 3,3′-dimethylnaphthidine method seemed most applicable to field work in the absence of combined chlorine. The titrimetric procedures were less reproducible than the spectrophotometric procedures, the *NN*-diethyl-*p*-phenylenediamine - ferrous ammonium sulphate titration being better than the other two, but requiring some temperature control. The main disadvantage of the other methods was a lack of reproducibility or specificity.

ALL domestic water supplies in this country are disinfected by chlorination, and it is the practice to maintain a small concentration of residual chlorine in the water that reaches the consumer. In the absence of ammonia or organic compounds, the chlorine is present as hypochlorous acid or hypochlorite, and is referred to as free chlorine. When ammonia or organic compounds are present, substances may be formed that contain nitrogen - chlorine bonds, *e.g.*, monochloramine (NH_2Cl) and dichloramine ($NHCl_2$), and such forms of chlorine are referred to as combined chlorine. These two chlorine fractions are of interest because of their different bactericidal activity and stability.

Numerous methods are available for determining both these fractions, but some of these are known to give conflicting results. A few of the methods had been compared,[1,2,3,4] but no previous attempt had been made to compare extensively all of the available methods. The object of the present investigation was to compare the methods available for determining free chlorine in water, and most of the comparisons were made with solutions that contained no chlorine in the combined form. The determination of free and combined chlorine will be considered in detail in the next paper of this series.

The criteria used in comparing the methods were: reproducibility, specificity, sensitivity, limit of detection, recovery of chlorine, effect of temperature, stability of the reagent and simplicity and convenience. For colorimetric procedures, stability of the colour and adherence to Beer's law were also considered.

From an assessment of the above criteria it should be possible to select the method most suited to a particular purpose, *e.g.*, laboratory, field or plant-control work.

METHODS AVAILABLE—

Aromatic amines—Chlorine in water reacts with certain aromatic amines, forming coloured oxidation products. The determination of chlorine usually involves either the measurement of the colour produced or titration with a reducing agent until the colour is just discharged. Aromatic amines that have been used include: *o*-tolidine in acidic solution,[5,6,7,8] *o*-tolidine in neutral solution,[9,10] *NNN′N′*-tetramethylbenzidine,[11] *p*-amino-*NN*-dimethylaniline,[12] *NN*-diethyl-*p*-phenylenediamine,[13] tetrakis-(*p*-dimethylaminophenyl)ethylene[14] and 3,3′-dimethylnaphthidine.[15]

Methods based on the Zincke - König reaction—An alkali metal cyanide is allowed to react with chlorine, producing cyanogen chloride that in turn is allowed to react with a solution of an amine in pyridine, producing an intense colour. Amines that have been used are benzidine,[16,17] sulphanilic acid[18] and barbituric acid.[19]

Amperometric titration—Chlorine is titrated with sodium arsenite[20] or phenylarsenoxide[1] solution to an amperometric end-point.

Iodimetric titration—Potassium iodide is allowed to react with chlorine, and the liberated iodine titrated with thiosulphate solution with starch indicator. This is recommended[6] for concentrations of chlorine greater than 1 p.p.m.

Indophenol reaction—Sodium phenate and aniline react with chlorine, producing an indophenol dye.[21]

Methyl orange—Methyl orange is bleached by chlorine, and this reaction has been used for determining chlorine photometrically[22,23,24] and titrimetrically.[25,26,27]

α-Naphthaflavone—α-Naphthaflavone reacts with chlorine,[28] producing coloured products, the intensity and hue of which vary with the concentration of chlorine.

Ultraviolet absorption—Free and combined chlorine show specific absorption peaks in the ultraviolet region,[29,30,31] but measurement of these is a relatively insensitive method for determination.

Experimental

Apparatus—

Glassware—All glassware used for solutions of chlorine or chlorine complexes was treated overnight with water containing 10 p.p.m. of chlorine and rinsed before use, to ensure the absence of a chlorine demand.

Spectrophotometer—All colorimetric measurements were made with a Hilger and Watts Uvispek H700 spectrophotometer.

Amperometric titrator—A commercial titrator (Code No. A-554011B), manufactured by Wallace and Tiernan Limited, London, was used throughout this study.

Reagents—

Chemicals—AnalaR grade chemicals were used whenever possible; otherwise the purest grade available was used. Commercial 3,3′-dimethylnaphthidine, however, was found to be impure and was purified. The crude amine was dissolved in ethanol, precipitated as the sulphate with dilute sulphuric acid, filtered off and washed with ethanol; the washed precipitate was heated gently with dilute aqueous sodium hydroxide solution, filtered off and recrystallised from ethanol.

Dilution water—All dilution water used had to be chlorine-demand free and chlorine free. It was conveniently prepared by passing distilled water down a mixed-bed ion-exchange column, then chlorinating it to give a residual chlorine content of 2 to 3 p.p.m. After it had been stored in a stoppered carboy for a minimum of 16 hours, 5-litre batches of the water were dechlorinated by subjecting them to ultraviolet irradiation for about half an hour with a Hanovia fluorescence lamp (model 16) without the filter, the water being stirred during the treatment. No ammoniacal nitrogen could be detected with the Nessler method, indicating a nitrogen concentration of $<0{\cdot}003$ p.p.m.

Standard solution of chlorine—A 10 p.p.m. solution of chlorine in water was chosen for the working standard. This solution was relatively stable, gave reasonably high titres on standardisation against 0·0025 N sodium thiosulphate, and allowed aliquots for the final dilution in the range 0·1 to 0·5 p.p.m. to be measured accurately.

Chlorine gas was washed with water and then passed into a flask of dilution water, giving a pale green solution containing about 4000 p.p.m. of chlorine, as determined iodimetrically; 5 ml of this solution were diluted with about 2 litres of dilution water, giving a solution having a chlorine concentration in the range 9 to 11 p.p.m.

The standard solution of chlorine was prepared daily and standardised iodimetrically against $\sim$0·0025 N sodium thiosulphate, which in turn was standardised against 0·01 N potassium iodate.[6] The relative standard deviations obtained in eight replicate standardisations were 0·12 and 0·08 per cent., respectively.

The standard solution containing 10 p.p.m. of chlorine was stored in an aspirator connected to a gravity-feed burette, and covered to minimise photo-decomposition. At the end of the day it was re-standardised to determine the extent of the decomposition. The

average decomposition experienced, calculated from 30 daily results, was 0·01 p.p.m. (*i.e.*, about 0·1 per cent.). This represents the decomposition after a period of about 6 hours. Any slight error resulting from the use of standard solutions of chlorine of different ages was minimised by the randomisation of the order of testing the methods.

Preparation of monochloramine—To about 200 ml of dilution water in a 2·5-litre amber-glass bottle were added 2 ml of 0·075 per cent. ammonium chloride solution. To 1800 ml of dilution water in a beaker, 0·25 ml of water containing about 4000 p.p.m. of chlorine was added and the pH adjusted to 10 with sodium carbonate. The solutions were mixed in the amber-glass bottle and stored for 1 to 2 hours before use.

Preparation of dichloramine and trichloramine—Dichloramine and trichloramine were prepared according to the methods of Chapin.[32,33] The solutions, after being stored to ensure completion of reaction, were diluted to give solutions containing 50 p.p.m. of total chlorine, as determined iodimetrically; 1 ml of the diluted solutions was further diluted to 100 ml when a solution containing 0·5 p.p.m. of dichloramine or trichloramine was required.

Preliminary study

A preliminary study was made of all the methods reported in the literature. The factors examined at this stage were sensitivity, linearity of calibration graphs, formation and stability of coloured products and general reproducibility. Any method that obviously had serious disadvantages was excluded from further study, and the more promising ones were examined in greater detail.

No attempt was made to modify the procedures given, and the instructions recommended in the original methods were, in most instances, closely followed. If they were insufficient or ambiguous, however, the instructions were modified after some preliminary work.

The preliminary study enabled familiarity with the methods to be developed, this being very important in the subsequent measurement of their precision. The wavelength of maximum absorption, $\lambda_{max.}$, was also determined for the colorimetric methods, and was used in later work when it differed from the wavelength recommended.

As a result of this preliminary study, the following modifications were introduced. In the *NNN'N'*-tetramethylbenzidine method[11] the strength of the reagent was decreased from 1 to 0·1 per cent. w/v, and the volume used in the test was increased from 0·1 to 1·0 ml. In the barbituric acid method, the optical densities were measured at 582 mμ rather than at 578 mμ, the recommended wavelength.[19] A 0·2 per cent. w/v solution of tetrakis-(*p*-dimethylaminophenyl)ethylene in acetone was prepared, and 1 ml was used in the test after 1 ml of glacial acetic acid had been added to the sample. This procedure was found to give greater sensitivity and colour stability than the original method. The optical densities were measured at 740 mμ rather than at 720 mμ.[14]

Table I

Methods excluded from further study

Method	Reason for exclusion
p-Amino-*NN*-dimethylaniline	Reagent unstable in solution.
Potassium cyanide - benzidine - pyridine ..	When used under the specified conditions, precipitation occurred. Colours produced were very variable. A relatively insensitive method.
Methyl orange	Gave non-linear calibration graphs. Order and rate of mixing the reagent and sample affected the extent of bleaching obtained.[23]
Indophenol	Direct measurement in aqueous solution was insensitive. Extraction method with chloroform could not be confirmed.
α-Naphthaflavone	When used under the specified conditions, precipitation occurred immediately. The method depends on change in hue as well as intensity, and is unsuitable for instrumental use.

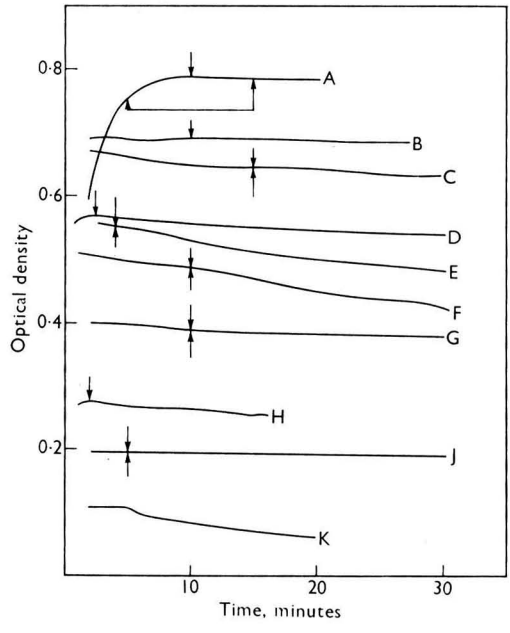
The methods excluded from further study are listed in Table I, with the reasons for their exclusion.

Final evaluation

The twelve remaining methods were evaluated in greater detail.

Stability of coloured products—

The relative stabilities of the coloured products produced by the various reagents are illustrated in Fig. 1, together with the time of development recommended in the literature and the time of development used throughout the evaluation. Table II lists the relative decompositions of the colours produced. The results show that the recommended time of



A Barbituric acid
B Tetrakis-(*p*-dimethylaminophenol)ethylene
C *o*-Tolidine (I.W.E.)[6]
D *NN*-Diethyl-*p*-phenylenediamine
E *o*-Tolidine (A.P.H.A.)[7]
F Modified Milton (sulphanilic acid)
G *NNN'N'*-Tetramethybenzidine
H Neutral *o*-tolidine,[10]
J 3,3'-Dimethylnaphthidine
K Neutral *o*-tolidine[9]

Fig. 1. Curves showing stabilities of coloured products
↑ Time of development recommended in the literature
↓ Time of development used throughout the evaluation

development for free chlorine is usually quite arbitrary. In most instances, the colour fades with time. For the *NN*-diethyl-*p*-phenylenediamine colorimetric method, no time of development was specified. The colour produced was found to be stable for a period of 2 to 3 minutes, and this period of development was used. For the barbituric acid method, a time of colour development of 10 minutes was used, as the colour showed reasonable stability only after 8 to 12 minutes rather than after the 5 to 15 minutes recommended.[19]

Reproducibility—

In the comparison of the reproducibility of the methods, the technique described below was adopted. Predetermined volumes of the standard solution of chlorine were taken and diluted, and the chlorine present was determined by the method under consideration. Two concentrations of chlorine were used, 0·1 and 0·5 p.p.m., that were in the range most likely to be encountered in practice.

The reproducibility of each method was determined statistically at the two levels of concentration from the analyses of a series of duplicate samples and blank solutions, carried out over a period of ten days. This experimental design has been described by Morrison

and Wilson.[34] The order of testing, both within individual methods and between methods, was randomised to avoid systematic error.

Statistical analyses of the results (analysis of variance) permitted any significant differences between the methods to be established, at a probability level of 1 per cent. The results are summarised in Table III.

TABLE II

MEAN RATE OF DECOMPOSITION OF COLOUR

Method	Time of development, minutes	Mean rate of decomposition, per cent. per minute
Barbituric acid	8 to 12	Nil
Tetrakis-(*p*-dimethylaminophenyl)ethylene	5 to 10	Nil
o-Tolidine (I.W.E.)[6]	15	0·1
NN-Diethyl-*p*-phenylenediamine	2 to 3	Nil
o-Tolidine (A.P.H.A.)	5	0·5
Sulphanilic acid	5 to 15	0·5
NNN'N'-Tetramethylbenzidine	5 to 15	0·3
Neutral *o*-Tolidine[10]	2 to 5	0·5
3,3'-Dimethylnaphthidine	2 to 10	0·06
Neutral *o*-tolidine[9]	2 to 10	4·2

TABLE III

REPRODUCIBILITY OF METHODS

	Method	Relative standard deviation, per cent. Concentration of chlorine, p.p.m.		Methods that were significantly worse than the given method. Concentration of chlorine, p.p.m.	
		0·1	0·5	0·1	0·5
1	Barbituric acid	3·48	1·85	3, 6, 7, 10, 11, 12	3, 6, 9, 10, 11
2	Tetrakis-(*p*-dimethylaminophenyl)ethylene	4·59	1·85	6, 12	3, 6, 9, 10, 11
3	*o*-Tolidine (I.W.E.)[6]	7·9	3·66	12	—
4	*NN*-Diethyl-*p*-phenylenediamine	4·04	1·95	3, 6, 10, 11, 12	3, 9, 10, 11
5	*o*-Tolidine (A.P.H.A.)	4·14	3·01	3, 6, 10, 12	—
6	Sulphanilic acid	9·43	3·40	—	—
7	*NNN'N'*-Tetramethylbenzidine	6·76	3·13	12	—
8	3,3'-Dimethylnaphthidine	5·52	1·82	12	3, 6, 9, 10, 11
9	Neutral *o*-Tolidine[10]	5·23	3·73	12	—
10	Amperometric titration	7·28	3·94	12	—
11	*NN*-Diethyl-*p*-phenylenediamine (ferrous ammonium sulphate titration)	7·20	4·53	12	—
12	Neutral *o*-tolidine[9] (ferrous ammonium sulphate titration)	16·4	2·76	—	—

INTERFERENCES—

Two types of interference were possible: firstly, from those substances that gave a positive response with the reagent alone (*e.g.*, other oxidising agents), and secondly, from those substances that, although they had no effect on the reagent alone, interfered with the determination of free chlorine in some other way.

Oxidising agents—The apparent concentration of chlorine, in parts per million, found in the presence of certain oxidising agents is given in Table IV.

The methods that involved the use of aromatic amines were seriously affected by many oxidants other than chlorine. Manganese (Mn^{3+} and MnO_4^-) and bromine (Br_2) interfered with all such methods. Similarly, only a few of the amines were free from interference from iodine. Little or no interference was observed from iron^{III}, nitrite and chlorite.

The initial tests were carried out in distilled water, but subsequently it was shown that iron^{III} dissolved in a surface water gave a significant yellow colour with the *o*-tolidine reagent alone; this effect had not been observed in distilled water. Further tests showed that humic and fulvic acids isolated from the River Thames turned *o*-tolidine yellow in distilled water containing iron^{III}.

In the *o*-tolidine test, the addition of arsenite[8] to the sample before the addition of the reagent permitted a correction to be applied for the interference of other oxidants present.

TABLE IV

RESPONSE OF REAGENTS ALONE TO OXIDISING AGENTS, EXPRESSED AS THE APPARENT CONCENTRATION OF CHLORINE

Interfering substance	Apparent concentration of chlorine, p.p.m., found by method—											
	1	2	3	4	5	6	7	8	9	10	11	12
Iron, Fe^{3+}, 0·5 p.p.m.	Nil	—	0·01	Nil	Nil	Nil	—	—	0·05	Nil	Nil	—
Manganese, Mn^{3+}, ~0·5 p.p.m. as chlorine	0·01	0·60	1·12	0·97	1·10	0·03	1·14	0·13	—	0·02	1·22	1·89
Manganese, MnO_4^-, 0·5 p.p.m. as chlorine	Nil	0·50	0·47	0·38	0·52	0·01	0·37	0·51	0·41	0·35	0·45	0·40
Nitrite, NO_2^-, 0·15 p.p.m. as nitrogen	Nil	—	0·02	Nil	0·02	—	—	—	—	Nil	Nil	—
Chlorite, ClO_2^-, 0·5 p.p.m. as chlorine	Nil	—	0·03	0·01	0·02	—	—	—	—	0·03	Nil	—
Bromine, Br_2, 0·5 p.p.m. as chlorine	0·04	0·40	0·42	0·39	0·49	0·49	0·35	0·47	0·33	0·59	0·42	0·40
Iodine, I_2, 0·5 p.p.m. as chlorine	Nil	0·50	0·03	0·56	0·03	0·01	0·02	0·51	0·49	0·65	0·53	0·54
Fulvic acid, 18° Hazen + 0·5 p.p.m. of Fe^{3+}	Nil	0·89	0·30	Nil	0·02	—	0·64	0·22	—	—	Nil	Nil
Humic acid, 15° Hazen + 0·5 p.p.m. of Fe^{3+}	Nil	0·96	0·35	Nil	0·02	—	0·80	0·23	—	—	Nil	Nil
Monochloramine, 0·5 p.p.m.	0·17	0·01	0·46	0·04	0·55	0·20	0·55	0·52	0·02	Nil	Nil	Nil
Dichloramine, 0·5 p.p.m.	0·03	Nil	0·27	0·03	0·44	0·05	0·23	0·08	0·03	Nil	Nil	Nil
Trichloramine, 0·5 p.p.m.	0·39	0·20	0·19	0·09	0·40	0·31	0·18	0·11	0·18	0·23	0·11	0·23

1 Barbituric acid.
2 Tetrakis-(*p*-dimethylaminophenyl)ethylene.
3 *o*-Tolidine, (I.W.E.).[6]
4 *NN*-Diethyl-*p*-phenylenediamine.
5 *o*-Tolidine (A.P.H.A.).
6 Sulphanilic acid.
7 *NNN'N'*-Tetramethylbenzidine.
8 3,3'-Dimethylnaphidine.
9 Neutral *o*-tolidine.
10 Amperometric titration.
11 *NN*-Diethyl-*p*-phenylenediamine (ferrous ammonium sulphate titration).
12 Neutral *o*-tolidine (ferrous ammonium sulphate titration).

TABLE V

EFFECT OF INTERFERING SUBSTANCES ON THE RECOVERY OF CHLORINE, EXPRESSED AS THE CONCENTRATION OF CHLORINE FOUND

Interfering substances		Concentration of chlorine, p.p.m., found by method—											
		1	2	3	4	5	6	7	8	9	10	11	12
Alkalinity	630 p.p.m. as $CaCO_3$	0·51	precipitate formed	blue colour	0·50	0·50	0·49	—	0·49	green colour	0·45	0·49	0·50
	315 p.p.m. as $CaCO_3$	—	0·49	0·50	—	—	—	green precipitate	—	0·49	—	—	—
	158 p.p.m. as $CaCO_3$	—	—	—	—	—	—	0·50	—	—	—	—	—
Fulvic acid, 100° Hazen		0·50	0·35	0·26	0·47	0·05	0·13	0·20	0·10	0·03	0·05	0·45	Nil
Carbon dioxide, saturated solution		—	—	0·50	—	0·50	—	—	—	—	—	—	—
Clay suspension, 70 p.p.m. of Speswhite Kaolin		0·50	0·49	0·49	0·50	0·49	—	0·48	0·51	—	0·38	0·51	0·48

1 Barbituric acid.
2 Tetrakis-(*p*-dimethylaminophenyl)ethylene.
3 *o*-Tolidine (I.W.E.).[6]
4 *NN*-Diethyl-*p*-phenylenediamine.
5 *o*-Tolidine (A.P.H.A.).
6 Sulphanilic acid.
7 *NNN'N'*-Tetramethylbenzidine.
8 3,3'-Dimethylnaphthidine.
9 Neutral *o*-tolidine.
10 Amperometric titration.
11 *NN*-Diethyl-*p*-phenylenediamine (ferrous ammonium sulphate titration).
12 Neutral *o*-tolidine (ferrous ammonium sulphate titration).

Similarly, it was found that this *o*-tolidine - arsenite "blank" could be used to correct for the interfering effect of iron^{III} in the presence of humic or fulvic acids. Further tests with solutions containing humic or fulvic acids and 0·5 p.p.m. of iron^{III} showed that all those amine reagents used in acidic solution gave a similar, but not identical, interference. That is, the interference was not due to an inherent yellow complex formed between the iron^{III} and the fulvic or humic acid, but appeared to be caused by oxidation taking place in acidic solution. However, 0·5 p.p.m. of iron^{III} is unlikely to be encountered in practice in a treated water. The interference produced by 0·1 p.p.m. of iron^{III} and fulvic or humic acid was much lower.

The barbituric acid method was virtually free from serious interference, although bromine responded partially to the reagents. The amperometric titration method was affected by manganese (MnO_4^-), bromine and iodine.

The effect of combined chlorine upon the determination of free chlorine is considered briefly in the following paragraph.

Effect of combined chlorine—All the reagents responded, to a greater or lesser extent, to all forms of chlorine. It was the rate of the reaction between the reagent and the chlorine compound that determined the extent of the response obtained in the time specified in the method. The pH of the final solution was the most important single factor in determining the response that was obtained. Hence, the rate of the reaction is dependent on the pH.

Excluding those methods that claim to differentiate the various chlorine fractions, no method responded to the total chlorine present in combined form. The greatest response, from 80 to 100 per cent., depending on the nature of the combined chlorine, was obtained with the *o*-tolidine (A.P.H.A.) method.

Five methods were reported that claimed to differentiate free chlorine from total chlorine: *NN*-diethyl-*p*-phenylenediamine (colorimetric),[13] *NN*-diethyl-*p*-phenylenediamine (ferrous ammonium sulphate titration),[13] neutral *o*-tolidine,[9] neutral *o*-tolidine[10] and amperometric titration.[1] The three titrimetric methods did not respond to solutions of monochloramine and dichloramine, provided that the titrations were carried out immediately after the sample and reagent were mixed. Some breakthrough of combined chlorine was observed with the *NN*-diethyl-*p*-phenylenediamine and neutral *o*-tolidine colorimetric methods during the period that elapsed before measurement of the colour. This suggests that the colour should be measured immediately to minimise this breakthrough. Results to date also suggest that it is necessary to use freshly prepared reagents and samples cooled to 15° C or below, to obtain the lowest apparent concentration of free chlorine. Old reagents were found to give higher and less consistent results.

Other interferences—The reagents were added to solutions of 0·5 p.p.m. of chlorine containing sodium hydrogen carbonate, fulvic acid, carbon dioxide and clay suspension, respectively. The amount of chlorine found, in parts per million, is given in Table V.

Sodium hydrogen carbonate interfered only when the acid in the reagent was insufficient to overcome the alkalinity. Since some hard waters have alkalinities in excess of 250 p.p.m. as calcium carbonate, this is an important factor. One method only, the *NNN'N'*-tetramethylbenzidine method, incorporated insufficient acid to overcome an alkalinity of 315 p.p.m. as calcium carbonate. There was a tendency for clay suspensions to give slightly low results, especially with acidic reagents. This can probably be attributed to adsorption of the coloured complex on to the surface of the clay particles.[35]

The claim of Goldenberg[36] that carbon dioxide caused low recoveries of chlorine with the *o*-tolidine methods could not be substantiated, even when a saturated solution of carbon dioxide in water was used.

The apparent interference of fulvic acid was probably due to its being oxidised by chlorine, and cannot be regarded as a true interference. Subsequently, it was shown that a chlorinated surface water consumed further chlorine when the pH was decreased. Lower concentrations of residual chlorine were obtained with methods involving the use of acidic reagents compared with the results obtained with methods operating close to the pH of the water (~8). Acidic reagents may, therefore, give low results with samples containing organic matter.

SENSITIVITY AND LIMIT OF DETECTION—

The limit of detection, as defined by Roos,[37] is a function of the reproducibility of the blank value. If the blank value were perfectly reproducible, the limit of detection would be determined by the smallest discrimination obtainable with the apparatus being used. Sandell[38]

defined sensitivity in terms of the number of micrograms of element, converted to the coloured product, that in a column of solution having a cross-section of 1 sq. cm has an optical density of 0·001.

The calculated figures for the limit of detection and the sensitivity are given in Table VI, and apply only if the reagent obeys Beer's law down to zero concentration.

TABLE VI

SENSITIVITY AND LIMIT OF DETECTION

Method	Limit of detection, p.p.m.	Sensitivity, μg per sq. cm	Optical density per cm per p.p.m. of chlorine
Barbituric acid	0·00088	0·00047	2·11
Tetrakis-(*p*-dimethylaminophenyl)ethyelene	0·0027	0·0015	0·656
o-Tolidine (I.W.E.)[6]	0·00054	0·0012	0·835
NN-Diethyl-*p*-phenylenediamine	0·0040	0·0036	0·276
o-Tolidine (A.P.H.A.)	0·00063	0·0014	0·719
Sulphanilic acid	0·0011	0·00081	1·24
NNN'N'-Tetramethylbenzidine	0·00055	0·00094	1·06
3,3'-Dimethylnaphthidine	0·0020	0·0019	0·525
Neutral *o*-tolidine[10]	0·0031	0·0031	0·320
Amperometric titration	0·012*	—	—
NN-Diethyl-*p*-phenylenediamine (ferrous ammonium sulphate titration)	0·011	—	—
Neutral *o*-tolidine[9] (ferrous ammonium sulphate titration)	0·043*	—	—

* Calculated from the standard deviation obtained for each sample, as blank values of zero were obtained.

Some reagents showed a slight departure from Beer's law at concentrations below 0·1 p.p.m. The calibration graphs became slightly concave and cut the chlorine axis at a finite concentration. Because of this, the calculated limits of detection for these reagents are falsely low by this finite concentration. Departures from Beer's law that were observed are given in Table VII.

The reason for the departure from Beer's law at low concentrations is not known. It may be due to impurities in the organic amines causing a slight chlorine demand, or to a second reaction that becomes of increasing importance at low concentrations.

Despite the large differences in sensitivity exhibited by the reagents, it does not appear to be a dominating factor in determining chlorine in the range 0·1 to 0·5 p.p.m. This is confirmed by the barbituric acid and *NN*-diethyl-*p*-phenylenediamine methods, which differed in sensitivity by a factor of about eight, yet were not significantly different in reproducibility. Sensitivity may become increasingly important as the concentration approaches zero.

TABLE VII

DEPARTURES FROM BEER'S LAW

Method	Approximate concentration, in p.p.m., at which the calibration graph becomes curved	Approximate concentration, in p.p.m., at which the calibration graph cuts the chlorine axis
Barbituric acid	>1·0	Nil
Tetrakis-(*p*-dimethylaminophenyl)ethylene	0·15	0·01
o-Tolidine (I.W.E.)[6]	0·12	0·004
NN-Diethyl-*p*-phenylenediamine	>1·0	Nil
o-Tolidine (A.P.H.A.)	0·08	0·004
Sulphanilic acid	>1·0	Nil
NNN'N'-Tetramethylbenzidine	0·08	Nil
3,3'-Dimethylnaphthidine	0·15	0·012
Neutral *o*-tolidine	>1·0	0·02

RECOVERY OF CHLORINE—

Titrimetric methods—The mean recoveries of chlorine obtained with the three titrimetric methods (based on the volume of reducing agent required to reach the respective end-points) are given in Table VIII. The reasons for the low recoveries are uncertain. As the titrations

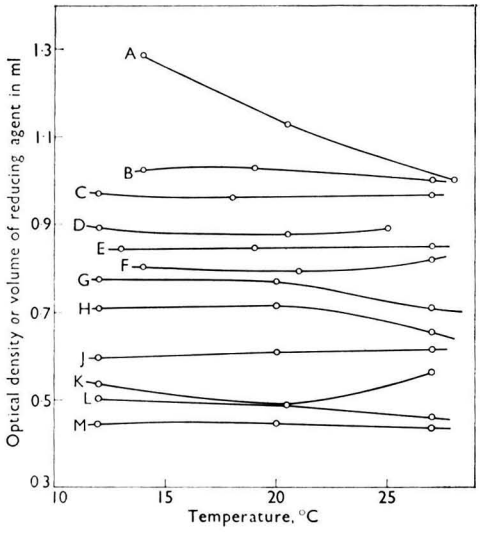
involved swirling or agitation of the solution, it is possible that chlorine was lost to the atmosphere. The commercial amperometric titrator used in this study is fitted with a high-speed stirrer, and it was shown that when solutions of chlorine were agitated with this for 90 seconds (the average duration of a titration), 5 to 7 per cent. of the chlorine was lost.

TABLE VIII

MEAN RECOVERIES OF CHLORINE

Method	Mean recovery of chlorine, per cent. Concentration of chlorine, p.p.m. 0·1	0·5
Amperometric titration	85·7	86·5
NN-Diethyl-*p*-phenylenediamine (ferrous ammonium sulphate titration)	90·8	96·5
Neutral *o*-tolidine (ferrous ammonium sulphate titration)	76·6	98·0

There is also some doubt whether the reaction between chlorine and the phenylarsenoxide used in the amperometric titration proceeds to completion in a reasonably short time. Solutions of chlorine that had been titrated to the amperometric end-point with phenylarsenoxide solution always gave a slightly positive reaction for chlorine when analysed subsequently by several of the colorimetric methods. If the same solutions were irradiated with an ultraviolet lamp after the end-point was reached, no chlorine could be detected.



A Sulphanilic acid
B 3,3′-Dimethylnaphthidine
C Barbituric acid
D *NNN′N′*-Tetramethylbenzidine
E Tetrakis-(*p*-dimethylaminophenol)ethylene
F Neutral *o*-tolidine
G *o*-Tolidine (I.W.E.)[6]
H *o*-Tolidine (A.P.H.A.)[7]
J *NN*-Diethyl-*p*-phenylenediamine
K *NN*-Diethyl-*p*-phenylenediamine (F.A.S.)
L Neutral *o*-tolidine (F.A.S.)
M Amperometric titration

Fig. 2. Curves showing effect of temperature

The methods involving the use of ferrous ammonium sulphate as the reducing agent are dependent upon the formation of coloured oxidation products. It was shown in Fig. 1 that the coloured products formed with both methods fade on standing. Since titrations take a finite time, apparent loss of chlorine would result.

Colorimetric methods—In the colorimetric methods, provided that the procedure used for the analysis of a sample is the same as that used in the preparation of the calibration graph, the recovery should be close to 100 per cent. A chlorine demand in the dilution water, however, would result in apparently low recoveries of chlorine. In the present experimental work, considerable care was taken to remove any chlorine demand from the water. The fact

TABLE IX

PERFOMANCE OF THE COLORIMETRIC METHODS

Criterion	Method 1	2	3	4	5	6	7	8	9
Production of stable colours	C	B	C	C	C	C	C	B	C
Reproducibility	A	A	B	A	A	C	B	A	C
Specificity for chlorine	B	C	C	C	C	C	C	C	C
Sensitivity	A	A	A	B	A	A	A	A	A
Low limit of detection	A	B	B	A	B	A	B	B	B
Linear calibration graph	A	C	C	A	C	A	C	C	C
Independence of temperature	A	A	C	B	C	C	B	B	B
Stability of reagent	C	C	A	C	A	A	A	A	A
Simplicity and convenience	C	C	A	C	A	C	A	A	C

A Criterion satisfied.
B Criterion partially satisfied.
C Criterion not satisfied.

1 Barbituric acid.
2 Tetrakis-(*p*-dimethylaminophenyl)ethylene.
3 *o*-Tolidine (I.W.E.).[6]
4 *NN*-Diethyl-*p*-phenylenediamine.
5 *o*-Tolidine (A.P.H.A.).
6 Sulphanilic acid.
7 *NNN'N'*-Tetramethylbenzidine
8 3,3'-Dimethylnaphthidine.
9 Neutral *o*-tolidine.

that three of the calibration curves passed through the origin strongly suggests that the procedure adopted was successful. Additional evidence was provided by the coincidence of calibration graphs for the barbituric acid method prepared with standard solutions of chlorine or cyanide.[4]

EFFECT OF TEMPERATURE—

The effect of the temperature at which the analysis was carried out is illustrated in Fig. 2. In general, temperatures in the range 12° to 20° C had little or no effect, whereas high temperatures gave high or low results. The results showed the sulphanilic acid reagent to be the most dependent on temperature with a temperature coefficient of −1·8 per cent. per °C. The *NN*-diethyl-*p*-phenylenediamine (ferrous ammonium sulphate titration) method gave high results at both high and low temperatures, and both of the *o*-tolidine methods and the *NNN'N'*-tetramethylbenzidine method gave high results at high temperatures. The barbituric acid and tetrakis-(*p*-dimethylaminophenyl)ethylene reagents were virtually independent of temperature.

STABILITY OF REAGENT—

It is convenient in routine analysis to prepare enough reagent to last several weeks. A few of the reagent solutions used in this evaluation tended to decompose on storage, giving blank values that increased in colour with age of the reagent. There was no evidence, however, over a period of one or two weeks that the results were adversely affected. Reagents that were stable for several weeks were mainly of the *o*-tolidine type, *e.g.*, *o*-tolidine, *NNN'N'*-tetramethylbenzidine and 3,3'-dimethylnaphthidine. The least stable reagent solutions were those of *NN*-diethyl-*p*-phenylenediamine, tetrakis-(*p*-dimethylaminophenyl)ethylene and barbituric acid.

Palin[13] showed that, with *NN*-diethyl-*p*-phenylenediamine oxalate reagent, by the time the optical density of the blank solution had quadrupled, low recoveries were obtained with a solution containing 3 p.p.m. of chlorine. Throughout the present studies, *NN*-diethyl-*p*-phenylenediamine sulphate reagent was used. This reagent kept for periods of up to four weeks, after which time significantly high blank values and low recoveries of chlorine were observed.

Conclusions

Colorimetric methods—

The ideal colorimetric method for determining free chlorine should satisfy the criteria previously listed. The performance of the methods in relation to these criteria has been presented factually in the preceding section of this paper. It is, however, a matter of opinion whether the performances were satisfactory. The author's opinion on these points is presented in Table IX.

Of the nine colorimetric methods evaluated, the barbituric acid method appeared the most suitable laboratory method for determining free chlorine in the absence of combined chlorine. It was reproducible, sensitive, relatively specific and independent of temperature. It was, however, less convenient to use than some other methods; the final colour was dependent on time, and the reagent decomposed when stored. The method has the disadvantage of responding only partially to combined chlorine, restricting the use of the method in practice.

Although the *NN*-diethyl-*p*-phenylenediamine method was less specific than the barbituric acid method it was otherwise just as accurate. These two methods are particularly suitable at low concentrations of chlorine, as their calibration graphs pass through the origin. The reagent tended to decompose in solution, and after four weeks gave high blank values and noticeably lower results. The *NN*-diethyl-*p*-phenylenediamine method has one significant advantage over the barbituric acid method: it can be used to differentiate free chlorine from combined chlorine, provided that the colour is measured immediately.

For chlorine concentrations of 0·1 p.p.m. and above, three further methods were equally suitable for determining free chlorine in the absence of organic matter: *o*-tolidine (A.P.H.A.), tetrakis-(*p*-dimethylaminophenyl)ethylene and 3,3′-dimethylnaphthidine. In the presence of organic matter, low chlorine results may be obtained. The *o*-tolidine (A.P.H.A.) method determines total chlorine, whereas the remaining two methods determine free chlorine *plus* a part of the combined chlorine, depending on its nature or on the age of the reagent.

Of the five methods so far discussed, the 3,3′-dimethylnaphthidine method seemed the most applicable to field work. The method is simple to use, is reproducible, involves the use of only one reagent solution (which is stable for several weeks) and gives a colour with chlorine that is almost independent of the time of development within the period 2 to 30 minutes. One precaution that was found necessary was the purification of the commercially available reagent.

The *o*-tolidine (A.P.H.A.) method was shown to be more reproducible than the *o*-tolidine (I.W.E.)[6] method at a concentration of chlorine of 0·1 p.p.m. Otherwise, the performances were similar. One difference between the methods is that in the I.W.E. method the reagent is added to the sample and the pH is gradually lowered to 2·1, whereas in the A.P.H.A. method the sample is added to the reagent, so that the pH remains close to 1·3.

The remaining colorimetric methods, *i.e.*, the sulphanilic acid, *NNN′N′*-tetramethylbenzidine and neutral *o*-tolidine[10] methods, were found to be unsuitable, mainly because of a lack of reproducibility.

Titrimetric methods—

The three titrimetric methods evaluated were found to be inferior to the better colorimetric methods, when used in conjunction with an accurate spectrophotometer. The precision of the two types of procedure may be more comparable if a filter photometer is used. The *NN*-diethyl-*p*-phenylenediamine (ferrous ammonium sulphate) method was better than the other two, but adequate temperature control is essential to achieve optimum performance.

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The Use of Electrolytic Hygrometers for the Determination of Water and Hydrogen

J. A. J. WALKER AND P. CAMPION

(*United Kingdom Atomic Energy Authority, Reactor Materials Laboratory, Wigshaw Lane, Culcheth, Warrington, Lancs.*)

Factors relevant to the installation and operation of moisture meters as in-line monitors are discussed. Results on the general performance of these instruments are given, with special reference to cell efficiency and flow-rate and pressure of the sample gas.

A calibration system is described for the range 10 to 1000 v.p.m. of moisture. Calibration over this range of moisture concentration is achieved by catalytic oxidation over a platinum surface at 500° C of known amounts of hydrogen in the presence of an excess of oxygen. The known amounts of hydrogen are obtained from standard gas mixtures or by electrolysis of dilute sulphuric acid.

Two modifications to the standard instrument are discussed. The first involves a method of rapid regeneration of a saturated cell and the second permits the moisture contents of gases to be determined at pressures up to a maximum of 200 p.s.i.g. without a pressure-reducing valve.

The use of the instrument for determining hydrogen in metals and gas mixtures is described.

FOR the past 40 years the National Bureau of Standards in America has used an electrochemical method,[1,2] based on the change in electrical resistance of a hygroscopic film, for determining water vapour. The sensitivity of this method is of the order of 10 v.p.m. and has a range maximum of 2 per cent. by volume of water vapour. The method suffers from the disadvantage that the conducting film must be renewed at intervals of not greater duration than half a day.

In 1959 Keidel published a paper[3] on the determination of water by direct amperometric measurement. The basis of this method has been used in commercially available moisture monitors of the type manufactured by Beckman and by Consolidated Electrodynamics Corporation. All the work reported in this paper has been done with Consolidated Electrodynamics Corporation instruments.

The principle of operation is based on the electrolysis of water that has been quantitatively and continuously removed from the sample-gas stream by a film of phosporus pentoxide. The electrolysis current is directly related by Faraday's law to the mass flow-rate of water into the instrument and is used to determine accurately the concentration of water in the gas stream. A microammeter is used to achieve a direct reading of moisture content for a given flow-rate.

The essential unit is the electrolytic cell. The sample of gas is passed over the phosphorus pentoxide film into the cell. Water is removed from the gas, which passes out of the cell and into a flow controller. The electrolysis current is measured on a microammeter and may be attenuated. For a sample gas flow-rate of 100 ml per minute, the range of the instrument is 1 to 1000 v.p.m. of water.

The inlet and outlet ports are of the compression-coupling type and are situated at the rear of the unit. There is provision for feeding a signal to a potentiometric recorder.

INSTALLATION OF MOISTURE MONITORS

Detailed installation instructions are contained in the manufacturer's handbook and will not be described. Considerations regarding the type and state of pipework, connections and valves are important, however, and will be discussed.

PIPEWORK—

Several different types of tube, both metallic and non-metallic, were investigated to ascertain the suitability of any type or types for inclusion in a system in which moisture is to be monitored.

Each tube was prepared as given below—

A 25-cm length was cut from each of five tube materials, and the internal surfaces of the three metal tubes were degreased by sucking acetone and then air through the bore with a water-pump. Water was then passed through each of the five tubes to saturate the internal surface. Surplus water was removed by sucking air through each tube for at least 30 minutes.

Dry argon (containing <1 v.p.m. of water vapour) was passed (flow-rate, 100 ml per minute) through each test length of tube until the moisture content of the exit gas was <10 v.p.m. of water vapour or had reached a constant value. The results are shown in Fig. 1.

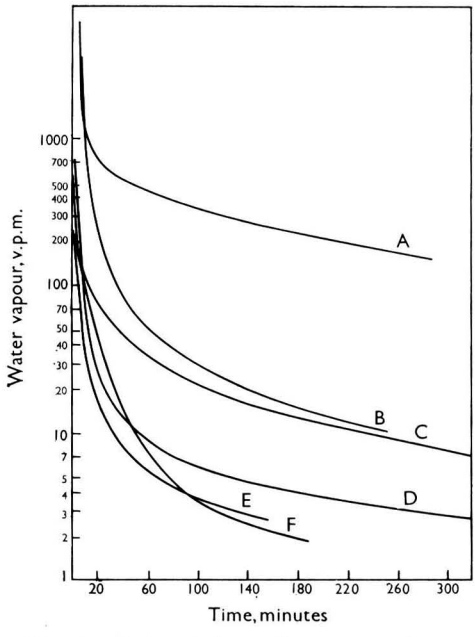


Fig. 1. Moisture-desorption curves for various materials. Curve A, nylon; curve B, copper; curve C, polythene; curve D, Teflon (polytetrafluoroethylene); curve E, nickel; curve F, stainless steel

It was found that stainless steel and nickel were comparable in respect to the amount of moisture adsorbed on the internal surfaces. Teflon tube was slightly worse in this respect, whereas polythene, copper and nylon tubes contained even more adsorbed moisture. The value of the moisture content of the argon at the exit of the nylon tube levelled off at about 150 v.p.m., and this figure represents an approximate measure of the permeability of the tube to atmospheric moisture. The choice of tube in respect to the amount of adsorbed water is between stainless steel and nickel. Of these two materials, stainless steel is probably the more efficient if other factors, such as corrosion resistance, are considered.

A further test was carried out on an internal-surface cleaning technique for the stainless-steel tube. This technique consists of the stages given below—

(1) Passing acetone and then air through the tube to remove grease.

(2) Heating the tube to about 700° C while helium flows through it.

(3) Passing aqueous solutions of nitric acid (10 per cent. v/v) and hydrofluoric acid (5 per cent. v/v) through the tube until the effluent is only slightly coloured.

(4) Passing water, acetone and air through the tube.

Three pieces of stainless-steel tube of equal length were tested for adsorbed moisture after the internal surfaces had been saturated with water vapour. One piece of tube was cleaned by the above technique, the second washed with solvent and the third was tested as received. Dry argon (water vapour content, 0·5 v.p.m.) was passed through each tube in turn, and

the moisture content of the exit gas monitored. The results are shown in Fig. 2. It is evident that the chemically cleaned tube contains less adsorbed moisture than the other two tubes, and this indicates the efficacy of the cleaning operation.

CONNECTIONS—

There are three well established types of connectors that are of use in the installation of moisture monitors. These are—

(*a*) Demountable O-ring joints.

(*b*) Modification of (*a*) to exclude brazing.

(*c*) Compression couplings.

The prime consideration in the choice of joint used concerns possible contamination of the stream of gas by moisture from the joint itself. Obviously, it is necessary to use a joint that is leak-proof, both under high vacuum and under pressure. Even extremely small leaks cannot be tolerated, since, under vacuum or pressure, moisture will diffuse into the stream of gas, resulting in an erroneous moisture reading.

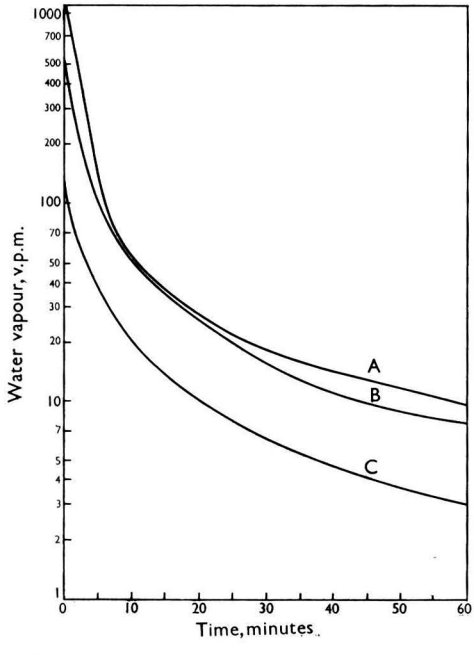
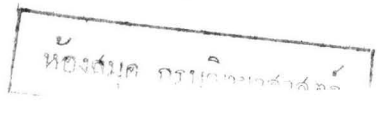


Fig. 2. Variation of moisture-desorption curves for stainless-steel tube, length 125 cm, bore 0·030 inch, after different cleaning techniques. Curve A, as received; curve B, solvent washed; curve C, chemically cleaned

In addition, brazing of pipework to couplings involves the use of a flux that is a ready source of water. Most of the flux may be removed from the external surfaces of the braze during the cooling of the joint in water. Flux that has flowed down between the pipe and the coupling may be removed by drilling into the coupling.

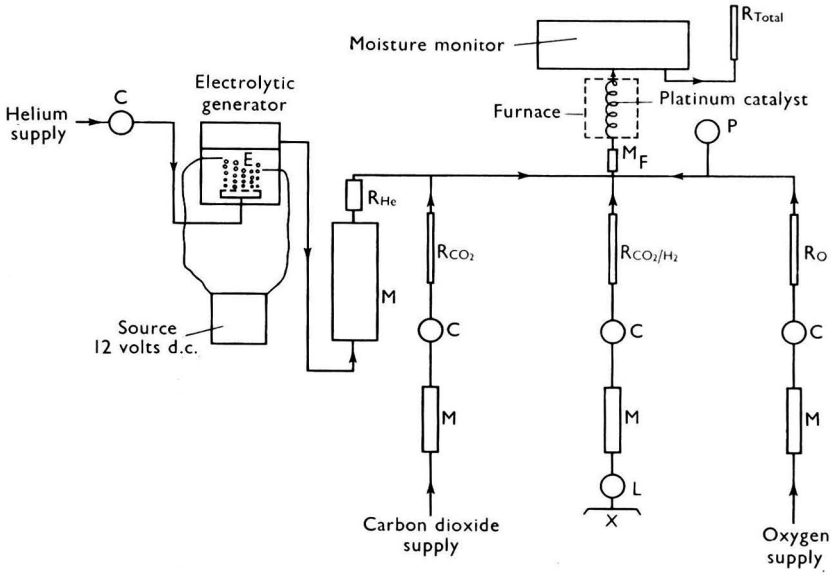
A modification of the O-ring coupling involves the male part and consists of removing the spigot around which the O-ring is normally positioned. The rest of the male is drilled to give a sliding fit with the pipe and the O-ring is positioned around the end of the pipe, which takes the place of the removed spigot. This modification and the removal of flux, by cutting back into the female coupling with a drill, produce a joint that is leak-tight and is not a source from which moisture may "weep" into the stream of gas.



Compression couplings are to be recommended for joining pieces of pipe. They allow a gas-tight seal to be made in less time than is required to make an O-ring joint, and there is no moisture contamination from flux.

VALVES—

Needle valves of the Hone type are highly satisfactory for installation in systems in which the moisture content of the gas is to be determined. They are made of stainless steel with a Teflon seat (which may be renewed if necessary), and are compact. They are suitable for systems with a working gas-pressure of 1000 p.s.i.g., and may be heated to 200° C. Some



C = Millaflow flow controllers
E = Electrolyte, 2 per cent. v/v aqueous solution of sulphuric acid
L = Loosco pressure regulator
M = Molecular sieve, 5A, driers
X = Standard gas mixture, *x* v.p.m. of hydrogen in carbon dioxide

M_F = Final molecular-sieve drier
P = Pressure gauge, 0 to 15 p.s.i.g.
R_{He} = Helium rotameter
R_{CO_2} = Carbon dioxide rotameter
R_{CO_2/H_2} = Carbon dioxide - hydrogen rotameter
R_O = Oxygen rotameter
R_{Total} = Total gas-flow rotameter

Fig. 3. Apparatus for the calibration of moisture monitors

gauge heads for bottled gases and pressure reducers are not satisfactory, because of moisture contamination or hold-up from the rubber diaphragm used in the reduction valves. Those pressure reducers having a stainless-steel diaphragm are much to be preferred and are commercially available.

TEMPERATURE OF PIPEWORK, JOINTS AND VALVES—

The time delay in registering a change in moisture concentration on a moisture monitor depends on (*a*) the rate of flow of the sample gas through the cell, and (*b*) the rate at which equilibrium is attained between adsorbed moisture on the internal tube-surfaces and moisture in the sample gas. The cleaning technique already described results in a faster rate of equilibration. Increasing the temperature of the pipework decreases the adsorption of moisture, and it is therefore advisable to heat all pipework, joints and valves to a maximum of 200° C, wherever practicable.

Methods of calibration

Two methods for calibrating moisture monitors have been used. In both methods, hydrogen was oxidised to water over a heated platinum catalyst in the presence of an excess of oxygen, and the calibration was carried out at atmospheric pressure. A minimum of experimental results are included here, since the apparatus was very similar to that used for the determination of hydrogen in metals, described fully in a later section.

In the first method, mixtures of hydrogen and carbon dioxide were prepared, and the exact hydrogen concentration determined by helium-ionisation gas chromatography.[5] The hydrogen concentration (and hence the moisture concentration) was varied between 10 and 1000 v.p.m. by quantitatively adding hydrogen-free carbon dioxide to the appropriate standard gas mixture. The apparatus is outlined in Fig. 3.

The second method involved the use of the electrolysis of dilute sulphuric acid as the source of hydrogen. The apparatus is shown schematically in Fig. 3. At 25° C and at a flow-rate of 100 ml per minute of sweep gas passing through the generator, a current of 13·2 μA produces a concentration of 1 v.p.m. of hydrogen. Therefore, by adjusting the current passing through the generator, calibration over the range of the moisture monitor was achieved (see Fig. 4).

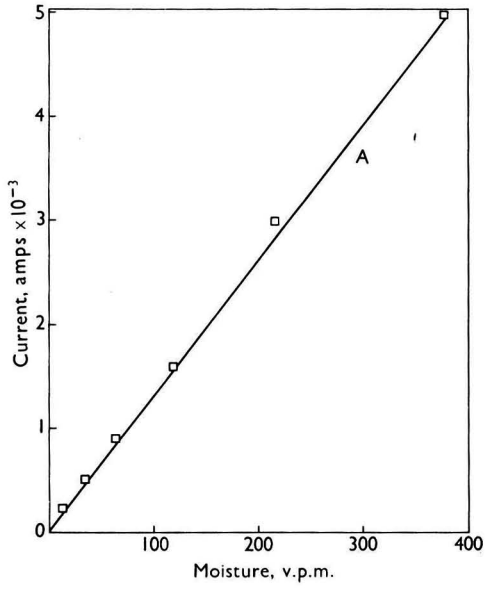


Fig. 4. Graph showing efficiency of the cell for removing moisture from gas by a film of phosphorus pentoxide. Curve A, theoretical calibration curve; points □, actual meter readings. Helium flow-rate, 100 ml per minute

Frequent calibration of electrolytic moisture monitors is unnecessary, since the instruments are based on a quantitative, as distinct from an empirical, principle. Generally, electrical faults in the cell or electronic circuit may be detected with a test meter, and leakage from O-rings and compression seals, by a pressure test. A high or off-scale reading is not necessarily indicative of a faulty instrument. For example, during the commissioning of a rig for studying carbon dioxide - graphite reactions, suspected malfunction of a moisture monitor was shown to be caused by desorption of copious amounts of moisture from newly fabricated pipework. Therefore, if unexpected results are obtained, a rapid calibration determines the accuracy of the moisture monitor.

Little electronic trouble has been experienced, but deterioration of the O-ring seals on the cell nozzles has occured. The cause of this deterioration is unknown, but one possibility is the reaction between phosphoric acid from a "flooded" cell and the neoprene O-ring.

General performance of moisture monitors

Cell efficiency—

An important part of a moisture monitor is the electrolytic cell, and some factors relating to its performance have been discussed previously.[4]

Two factors regarding cell efficiency have been tested, namely—

(*a*) That moisture is completely removed from the sample gas by the phosphorous pentoxide film.

(*b*) That the hydrogen generated in the cell is equivalent to the reading of the moisture-monitor meter.

Complete removal of water from the sample gas was tested for by introducing a known concentration of water into helium and passing it through a moisture monitor. The apparatus was of the same type as that used for calibrating a moisture monitor by electrolytic generation of hydrogen.

The meter reading was compared with the theoretical concentration of moisture as calculated from the generator current. The results are shown in Fig. 4 and agree satisfactorily with the theoretical curve.

A check of the hydrogen concentration in the effluent gas from the moisture monitor was made by helium-ionisation gas chromatography. The sample gas used in this experiment was carbon dioxide from a bulk supply, and the hydrogen concentration was determined for different flow-rates. The results are shown in Table I and demonstrate the ability of the moisture monitor to determine the moisture content at flow-rates other than 100 ml per minute.

Table I

Electrolysis-cell efficiency

Flow-rate of carbon dioxide, ml per minute	Moisture monitor reading (corrected to a flow-rate of carbon dioxide of 100 ml per minute), v.p.m.	Hydrogen concentration in effluent gas, as v.p.m. of moisture
100	129	128, 130
81	130	133, 133
65	131	130, 135
46	141	133, 137
23·5	149	141, 154

Variation in the flow-rate of the sample gas—

Several tests were made to ascertain the effect of change in the flow-rate of the sample gas on the indicated moisture content. It was found that a linear relationship existed between the flow-rate and the indicated moisture content. Graphs of moisture-monitor readings *versus* flow-rates for several gases have invariably shown a positive intercept on the ordinate at zero flow. In the tests that were conducted, the intercept varied from 2 to 6 v.p.m. of moisture For a carbon dioxide ring-main in these laboratories, which is monitored continuously, this intercept occurs at about 5 v.p.m. of water.

Baumann[6] has represented the total cell current as—

$$I_T = I_{water} + I_r + I_R$$

where I_T is the total current measured,
I_{water} is the electrolysis current from the moisture contained in the sample gas,
I_r is the background current (residual current) of the cell and
I_R is the current caused by the recombination of hydrogen and oxygen.

Thus, from this equation, it would appear that the 5 v.p.m. ordinate intercept is caused by residual current across the cell. However, results have shown that this explanation is an oversimplification. Firstly, when a molecular-sieve drier was placed before the moisture monitor sampling the carbon dioxide ring-main, the ultimate meter reading decreased to 1 to 2 v.p.m. of moisture. Secondly, as the flow-rate of carbon dioxide is progressively decreased, the moisture and effluent hydrogen concentrations increase as shown in Table I. This means that at low flow-rates of the sample gas, the concentration of moisture is increased slightly, relative to higher flow-rates. The most likely explanation is that a small, constant

amount of moisture is desorbed from surfaces in contact with the sample gas and the effect is most noticeable at low flow-rates. Therefore, the original I_{water} term in Baumann's equation can be represented by—

$$I_{water} = I_S + I_D$$

where I_S is the current due to the moisture inherent in the sample gas and
I_D is the current due to the moisture desorbed from sample-line surfaces.

VARIATION OF PRESSURE OF THE SAMPLE GAS—

It is important that the effect of fluctuations of sample-gas pressure on the meter reading of a moisture monitor is known. A series of experiments was therefore planned to determine the extent of this effect. Argon was used as the test gas and the moisture in the argon was monitored for three different flow-rates. Within each flow-rate the pressure of the sample gas was increased from 20 to 100 p.s.i.g. in 10 p.s.i.g. steps. The results are shown in Table II.

TABLE II

VARIATION OF PRESSURE OF THE SAMPLE GAS

Flow-rate of argon through moisture monitor, ml per minute at N.T.P.	Approximate equilibration time, *i.e.*, time for meter reading to return to original reading	Initial decrease in indicated moisture content of gas, v.p.m.
9	2 to 3 hours	6 to 12
103	20 minutes	3 to 6
200	<5 minutes	3 to 5

It was observed that, for a given flow-rate, an initial decrease in apparent moisture content occurred and this decrease was constant for each pressure step. The approximate equilibration time decreased markedly for an increase in argon flow-rate. At low flow-rates (*e.g.*, 10 ml per minute) a gas-pressure change in a system that is monitored for moisture will cause a lower moisture reading to be registered for some hours after the pressure change has taken place. For a system in which changes in pressure occur by nature of the experiment or operation of the system, a high flow-rate through the moisture monitor (*e.g.*, 200 ml per minute) is necessary, largely to obviate a spurious reading of moisture content.

The initial decrease may be explained as follows—

Consider a surface with condensed moisture that is in equilibrium with moisture in the gas phase. Increasing the gas pressure to twice its initial value will increase the partial pressure of the moisture in the gas to twice its initial value. The system, moisture (in gas phase) - condensed moisture, is not in equilibrium because of the partial-pressure increase, and hence, moisture will condense on the surface. The gas (pressure = 2 × initial pressure) is therefore slightly denuded of moisture and, since the flow-rate is constant, an apparent decrease in moisture content is registered. When equilibrium is re-established the moisture content returns to the value registered before the pressure change.

MODIFICATIONS TO INSTRUMENTS

Two practical modifications have been made. The first involves the regeneration of a saturated cell. At extremely high concentrations of water, the electrical resistance of the phosphorus pentoxide film is reduced to such a value that the potential drop across the cell approaches 2 volts or less. When the voltage is <2 volts, a limiting-current resistor in the circuit protects the electronic components from the overload current. Thus, an adequate source of current is required to electrolyse the water in the cell. A cheap, robust d.c. generator is that of a 1-amp battery charger. The output of the charger is connected across the cell contacts after the cell has been removed from its housing. Regeneration is rapid (a few minutes only are required) and may be completed by re-inserting the partially dry cell in the moisture monitor and then switching on the current. This method of quick regeneration has been used successfully and is especially useful when large surface areas of new pipework are being de-gassed for the first time.

The second modification makes possible the use of the electrolytic cell in a gaseous system at a working pressure of 200 p.s.i.g. or thereabouts. This modification was required since it was suspected that "hold-up" of moisture was occurring in the low-pressure sampling pipework. In essence, the cell containment is modified so that the normal type of cell may

still be used. These modifications include its fabrication from stainless steel, so that the containment will withstand pressures very much higher than the working gas pressure of the rig. Hence, if a leak develops in the cell (unlikely) or in the O-ring seals at the inlet or outlet nozzles, gas may leak into the containment vessel, but not into the atmosphere. There is an added advantage that the cell will only malfunction during the time required for the gas pressure in the containment to build up to the gas pressure in the rig. Thereafter, leakage will virtually cease and erroneous moisture readings will only arise from back diffusion of adsorbed moisture on the inside surface of the contaminant vessel and the outside surface of the cell.

Continuous monitoring of hydrogen in gases

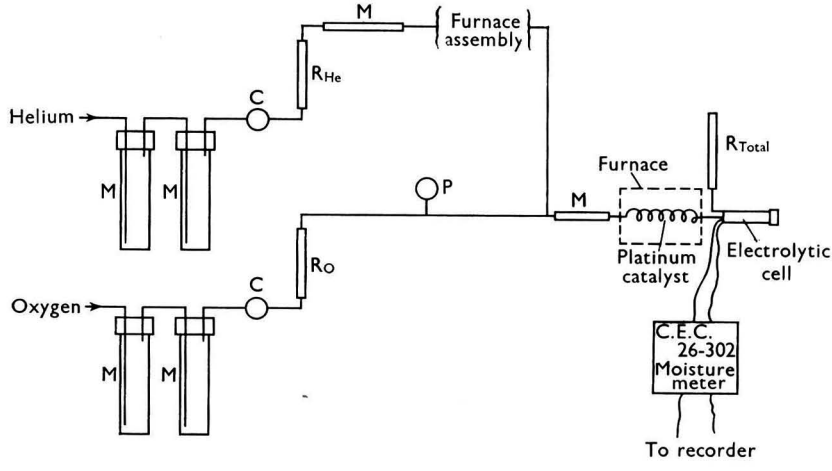
Research into the magnitude of the carbon dioxide - graphite reaction has shown that minor constituents of the carbon dioxide coolant can markedly affect the rate of reaction. One of these constituents is hydrogen, and a continuous record of hydrogen concentration can be obtained with the calibration unit previously described (see Fig. 3). Methane and carbon monoxide do not interfere during the catalytic oxidation of hydrogen at 500° C, but oxidation of higher hydrocarbons can occur. The amounts of oxidation found in three alkanes are shown below—

Gas..	Ethane	Propane	Butane
Oxidation, per cent.	7	15	28

A gas mixture containing one or more of these alkanes will create a positive error in the reading of the instrument. This may be corrected from a knowledge of the alkane content of the gas mixture. Further experiments to increase the efficiency and specificity of the instrument are in hand.

The determination of hydrogen in radioactive zircaloy specimens

A zirconium pressure tube, in contact with high-temperature high-pressure coolant water, corrodes, resulting in hydrogen pick-up by the tube. Hydrogen has a deleterious effect on the impact properties of zirconium alloys, and therefore the extent of hydride contamination represents a potential limit to the life of a pressure vessel.



C = Millaflow flow controllers
M = Molecular-sieve driers
P = Pressure gauge
R_{He} = Helium rotameter
R_O = Oxygen rotameter
R_{Total} = Total gas-flow rotameter

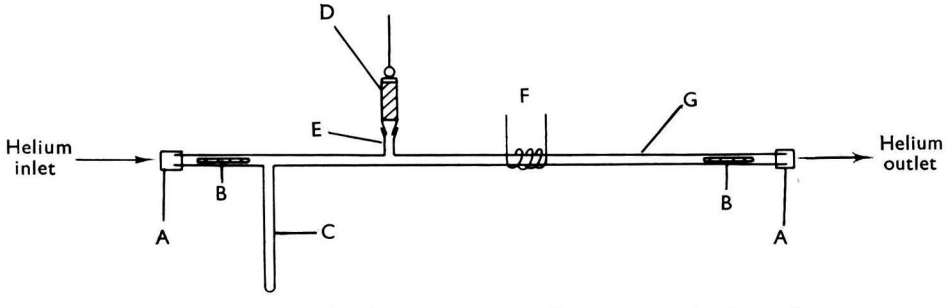
Fig. 5. Schematic diagram of apparatus for the determination of hydrogen in zircaloy-2

An experiment was initiated to provide results on the effect of irradiation of zircaloys containing 100 to 500 p.p.m. of hydrogen, and it was necessary to analyse each specimen at the point of fracture.

Many methods[7,8] are available for determining hydrogen in metals, but in the present work the choice was restricted considerably by the specific activity of the irradiated zircaloys, namely 0·5 curies per gram. Probably the two most widely used methods are vacuum extraction and vacuum fusion, but both entail several manual operations that are impractical with a remote-handling technique. Another serious objection to a vacuum technique was the possibility of leaks occurring within the radioactive-handling cell, and consequently, it was decided to investigate carrier-gas techniques that could be operated at atmospheric pressure. At elevated temperatures, zirconium was found to react extremely vigorously with oxygen when determinations with reactive carrier-gas were attempted, and the possibility of forming radioactive aerosols could not be discounted. Therefore, an inert carrier-gas technique was adopted. Helium was preferred to argon, since it was available in sufficient purity (containing <1 v.p.m. of hydrogen and methane) to eliminate any purification except drying. Briefly, the method entails heating the specimen at 1400° C under flowing helium, oxidising the released hydrogen to water over a platinum catalyst in the presence of an excess of oxygen and measuring the amount of water produced with a Consolidated Electrodynamics Corporation moisture monitor, type 26–302. A curve of moisture level *versus* time is plotted, the area under the curve integrated and the volume of hydrogen released by the specimen calculated.

Experimental

The apparatus is shown diagrammatically in Fig. 5. All pipework, with the exception of the catalyst, was constructed of 0·030-inch i.d. stainless steel or 0·050-inch i.d. chemically clean copper, and, wherever possible, compression couplings were used to minimise the number of brazed joints. The large-capacity driers were fabricated from stainless steel, filled with Union Carbide (Linde) molecular sieve, type 5A, and maintained at a pressure of 50 p.s.i.g. The desired helium and oxygen flow-rates were maintained by Millaflow flow controllers, designed to regulate inlet-gas pressures of 20 to 100 p.s.i.g. Upstream from the flow controllers, the apparatus was at atmospheric pressure. A pressure gauge was included on the oxygen inlet-line to indicate faulty conditions, *e.g.*, blocking of the electrolytic cell, and facilitate pressure checks if a leakage was suspected. Two further driers, located immediately in front of the furnace assembly and the catalyst, removed any moisture desorbed from tube surfaces into the helium stream. Flow-rates were measured on 5 to 50-ml rotameters, calibrated *in situ* by means of a rising-soap-bubble meter. The critical flow-rate was that of helium (20 ml per minute), and the method is independent of the oxygen flow-rate. A flow-rate of 3 ml of oxygen per minute was found to be sufficient for complete oxidation of the hydrogen released from specimens containing 500 p.p.m. of hydrogen.



A = De-mountable Edwards ¼-inch couplings
B = Mild steel enclosed in glass
C = "Spent" sample arm
D = Lead-weighted B10 cone
E = Specimen-introduction socket
F = Radyne 1-kW R.F. unit
G = Silica tube, 9-mm outer diameter throughout

Fig. 6. Furnace assembly for the determination of hydrogen in zircaloy-2

Specimen heating was by means of a Radyne 1-kW radio-frequency unit, the work coil of which coupled directly on to the specimen. The use of a susceptor, *e.g.*, graphite, was avoided for two reasons. Firstly, the positioning of a specimen on a susceptor often involves

accurate specimen manipulation, and secondly, de-gassing of susceptor materials can be a lengthy procedure. The furnace assembly is shown in Fig. 6, and was fabricated from 9-mm o.d. silica tubing. A six-turn work coil of $\frac{1}{8}$-inch o.d. annealed copper tube wound closely round the furnace tube, easily attained the operating temperature of 1400° C. Temperature control inside the radioactive-handling cell was achieved by determining the relation between the applied voltage and the mass of the specimen, for an operating temperature of 1400° C, before introduction of the active specimens. Specimens, introduced through a dry B10 socket that was sealed during the analysis by a lead-loaded cone, were moved magnetically into the radio-frequency coil and, after analysis, into the "spent" sample pocket.

Construction of the catalyst was as given below—

A 4-foot length of $\frac{3}{16}$-inch nominal bore stainless-steel was chemically cleaned and three 4-foot lengths of platinum ribbon (0·1 × 0·01 inch) were inserted at the ends, together with two silver-wool plugs to retain any fine dust particles. The electrolytic cell of the moisture monitor was mounted directly on to the end of the catalyst so that "hold-up" of the produced moisture was reduced to negligible proportions. The catalyst was wound in a spiral form and enclosed in a furnace at 500° C.

The leads from the electrolytic cell were connected to a Consolidated Electrodynamics Corporation moisture monitor, type 26–302, and the meter output fed to a 0- to 10-mV Honeywell recorder.

Results and discussion

The efficiency of the catalyst at various temperatures was ascertained by analysing the helium - oxygen effluent gas for hydrogen. A helium-ionisation gas chromatograph was used and 0·05- to 1·00-ml "plugs" of hydrogen were injected into the helium gas-stream before the catalyst. Below 350° C, hydrogen was present in the effluent gas, the concentration depending upon the amount of hydrogen injected. Above 400° C, the effluent-hydrogen levels did not exceed 0·5 v.p.m.

The manufacturers claim an accuracy of ±5 per cent. for the 26–302 moisture monitor. This was shown to be a conservative estimate by introducing known volumes (0·06 to 0·60 ml) of hydrogen into the helium carrier-gas and determining the volume of hydrogen recovered. The results are listed below—

Volume of hydrogen injected, ml ..	0·190, 0·427, 0·598, 0·060, 0·118, 0·237, 0·296, 0·356
Volume of hydrogen determined, ml ..	0·192, 0·430, 0·593, 0·060, 0·116, 0·240, 0·292, 0·349

The method for calculating hydrogen concentration from the graphs of moisture content *versus* time was as given below—

In Fig. 7 the ordinate and abscissa values at 1 cm are 2000 v.p.m. of water and 0·835 minutes, respectively.

Thus, the volume of helium, in ml, corresponding to 1 cm of abscissa = $20 \times 0{\cdot}835 = 16{\cdot}7$, and the volume of hydrogen, in ml per sq. cm = $\frac{2 \times 10^3 \times 16{\cdot}7}{10^6} = 3{\cdot}33 \times 10^{-2}$. The area under the curves was measured with a planimeter having an accuracy of ±0·1 sq. cm.

Table III

Hydrogen found in zircaloy specimens

	Concentration of hydrogen found, p.p.m.			
Helium carrier-gas technique	285, 269 286, 273 275, 278 283	433, 447 431, 424 446, 427 420, 435	180, 178 174, 170 173, 180 185, 187 183, 188 184	114, 121 119, 115 122
Vacuum-extraction technique ..	272 278	430 427 423	178 176 184	120 117

The performance of the furnace assembly was tested by analysing zircaloy specimens that had been annealed at 600° C to ensure complete homogeneity, by the helium carrier-gas and the well established vacuum-extraction techniques. Comparative results are listed in Table III.

The optimum sample weight was found to be 0·1 ± 0·02 g. The purging times required for complete removal of oxygen from the furnace assembly depended upon the time taken to insert the specimens. The time required for placing the specimen into the furnace was approximately 1 minute. A purge time with helium of 5 minutes was shown to be satisfactory for the removal of possible air contamination.

The rapid heating rate achieved by the radio-frequency unit ensures that the bulk of the hydrogen is expelled rapidly (Fig. 7). The moisture monitor had an upper limit of 20,000 v.p.m. of water and specimens containing >500 p.p.m. of hydrogen tended to read

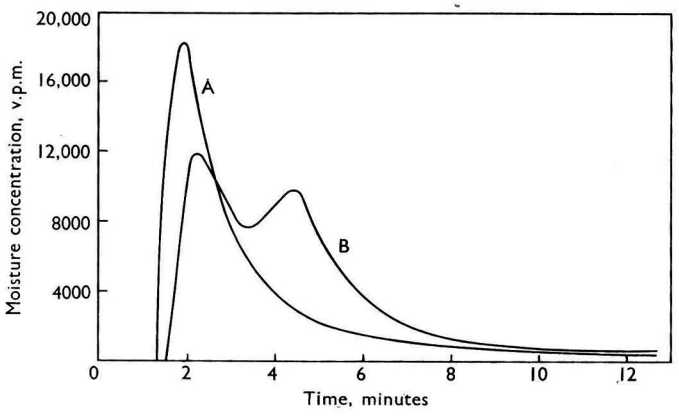


Fig. 7. Graphs of moisture concentration against time, illustrating single-peak and double-peak heating techniques. Curve A, specimen containing 524 p.p.m. of hydrogen; curve B, specimen containing 592 p.p.m. of hydrogen. Curves not to scale

off-scale. This was overcome by decreasing the initial temperature of the specimen to 1250° C for 2 minutes and then increasing it to the operating temperature of 1400° C. The total analysis time with this double-peak procedure was not significantly different from that of the single-peak procedure.

After insertion of the specimen, no further manual operations are necessary. This means that the operator need have none of the acquired skill necessary for operating conventional vacuum systems.

The calculation of results involves integration with a planimeter, and this is a time-consuming procedure. Experiments are now under way to fit an automatic integrator to the existing apparatus. Initial results for the helium carrier-gas determination of hydrogen in steels, platinum, nickel, etc., appear promising, and further experiments with these materials are in progress.

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The Effect of Nitrilotriacetic Acid Impurity on the Standardisation of Solutions of Ethylenediaminetetra-acetic Acid

By R. N. P. FARROW AND A. G. HILL

(*The British Drug Houses Ltd., B.D.H. Laboratory Chemicals Division, Poole, Dorset*)

Commercial and some analytical-reagent grades of ethylenediaminetetra-acetic acid have been found to contain significant amounts of nitrilotriacetic acid, and it is shown that this impurity can give rise to different factors when solutions of ethylenediaminetetra-acetic acid are standardised against different metals with the accepted indicators. Potentiometric investigations also show that ethylenediamintetra-acetic acid and nitrilotriacetic acid give separate end-point steps. Either step may be detected by indicators, depending on the conditions of titration and the metal used.

The presence of nitrilotriacetic acid also has a deleterious effect on the sharpness of the end-point.

It has often been stated that solutions of ethylenediaminetetra-acetic acid (EDTA), which is usually used in the form of a sodium salt, may give different titre values when they are standardised under different conditions or against different metals.[1,2] This has been attributed to the presence of polyvalent cationic impurities present in the EDTA.[1,2] It was noticed in these laboratories, however, that one sample of EDTA gave titre values differing by about 1 per cent. when it was titrated against equimolar amounts of high-purity zinc and cadmium under the same conditions. Spectrographic examination of the EDTA showed only traces of polyvalent cations and thus it seemed probable that the discrepancy was caused by organic impurities.

Nitrilotriacetic acid (NTA) is known to occur in EDTA[3] and it was found in the sample of EDTA being investigated. It was then decided to investigate the effect of NTA on the standardisation of EDTA in more detail.

Determination of NTA in commercially available samples of EDTA

NTA can be determined in the presence of EDTA by polarography in the presence of an excess of cadmium.[3] Samples of EDTA from several commercial sources were examined by this method, and the results are given in Table I. It can be seen that quite heavy contamination occurs in almost all samples.

Effect of NTA on the standardisation of EDTA

Reagents—

Zinc, cadmium, lead and bismuth—B.D.H. specially pure grade (assay not less than 99·999 per cent.). Suitable portions were washed successively with 5 N hydrochloric acid, 5 N nitric acid, water, industrial methylated spirit, acetone and diethyl ether and allowed to dry at room temperature.

Manganese metal—Electrolytic grade (assay not less than 99·9 per cent.) was treated in the same way as the other metals, except that the wash with nitric acid was omitted.

Magnesium metal—Spectrographically standardised. This was obtained from Johnson, Matthey & Co. Ltd. and contained not more than 0·01 per cent. of total metallic impurities. The metal was washed before use in the same way as manganese.

Calcium carbonate—AnalaR grade that had been dried at 250° C before use.

EDTA—The alcohol-precipitation method of purifying EDTA[4] removes only a small portion of the NTA. Precipitation with hydrochloric acid is rather more efficient, but even then several precipitations are required to remove the NTA completely. The EDTA used was subjected first to six precipitations with hydrochloric acid and then to a single precipitation of the disodium salt with alcohol. This treatment reduced the NTA content to less than 0·002 per cent. The purified EDTA was mixed with NTA in the correct proportions to give NTA contents of up to 1 per cent. Approximately decimolar solutions were prepared

by dissolving 37·22 g of EDTA in sufficient water to produce 1 litre of solution. The solutions were stored in polythene bottles.

Buffer solution, pH 10—Ammonium chloride, 67·5 g, and 570 ml of ammonia solution, sp.gr. 0·88, were dissolved in sufficient water to produce 1 litre of solution.

The water used was of AnalaR quality, and all other reagents were of the highest purity available.

TABLE I

NTA CONTENTS OF COMMERCIALLY AVAILABLE EDTA

Source*	Grade	Form of EDTA	NTA found, per cent.
A	Laboratory reagent	Disodium salt (6 batches)	0·50, 0·38, 0·37 0·07, 0·11, 0·05
	Pure	Dilithium salt	0·01
B	Laboratory reagent	Disodium salt	0·03
C	Pure	Disodium salt	0·42
	Pure	Acid	0·19
	Technical	Acid	0·80
D	—	Disodium salt	0·52
	—	Acid	0·58
E	—	Disodium salt	3·1
	—	Acid	1·9
F	Technical	Disodium salt	1·1
	Pharmaceutical	Disodium salt	1·3
	—	Acid	0·42
G	Laboratory reagent	Disodium salt	0·65
H	Laboratory reagent	Disodium salt	<0·01
I	—	Disodium salt	<0·01
	—	Acid	0·18
J	—	Disodium salt	0·07
	—	Acid	0·12
K	Technical	Acid	0·54

* Sources are located in Great Britain (A, B, C, D, E, F), Germany (G), France (H, I, J) and America (K).

METHODS USED FOR STANDARDISING EDTA—

Standard solutions were prepared from the primary standards mentioned above. Zinc, cadmium and lead were dissolved in dilute nitric acid, and bismuth was dissolved in nitric acid, sp. gr. 1·42. Most of the oxides of nitrogen were removed by heating on a steam-bath overnight, and any traces remaining were removed by adding ascorbic acid before titration. Calcium carbonate, magnesium and manganese were dissolved in dilute hydrochloric acid. These solutions were diluted to a suitable volume and weighed. Aliquots, sufficient to require a titre of about 45 ml, were weighed out and titrated under the conditions stated below. A conventional grade-A 50-ml burette was used. The calibration error for this burette was constant over the range 40 to 50 ml and was ignored. Temperature corrections were applied when necessary. All titrations were performed in duplicate and the factors obtained agreed to within 0·0005, except in a few instances at high concentrations of NTA, under which conditions the deterioration of the end-point adversely affected the reproducibility. The factor, f, was then calculated from the relation—

$$= \frac{\text{theoretical titre}}{\text{titre found}}$$

All factors were then re-calculated relative to zinc on the arbitrary basis that the factor determined against zinc at pH 10 is 1·0000 at each level of NTA.

The titration conditions used were as described below—

Zinc, cadmium and manganese (at pH 10)—The standard solution was diluted to about 150 ml with water and 0·1 g of ascorbic acid was added. After 1 to 2 minutes, 10 ml of buffer solution (pH 10) and one Alfloc total-hardness indicator tablet (Solochrome black T) were added. The solution was titrated with 0·1 M EDTA until the last traces of red disappeared and the solution was blue.[5]

Magnesium—The method was the same as that for zinc, cadmium and manganese, except that the temperature of the solution was maintained at 40° to 50° C.[5]

Lead—The standard solution was diluted to about 100 ml and 0·1 g of ascorbic acid was added. After 1 to 2 minutes, 5 g of hexamethylenetetramine and about 0·1 g of methyl

thymol blue complexone dispersion (1 per cent. in solid potassium nitrate) were added. The solution was titrated with 0·1 M EDTA until the last trace of blue disappeared and the solution was colourless.[6]

Calcium—The method used was the same as that for lead, except that the hexamethylenetetramine was replaced by 5 ml of diethylamine.[6]

Bismuth—The standard solution was diluted to about 150 ml and 0·5 g of ascorbic acid was added. After 1 to 2 minutes, 3 to 5 drops of a 0·1 per cent. aqueous solution of catechol violet were added and dilute ammonia solution was introduced until the solution was blue. The solution was titrated with 0·1 M EDTA until the last trace of red disappeared and the solution was yellow.[7]

Zinc (at pH 6)—The standard solution was diluted to about 75 ml and 0·1 g of ascorbic acid was added. After 1 to 2 minutes 3 to 5 drops of a 0·5 per cent. aqueous solution of xylenol orange were added and hexamethylenetetramine was introduced until the solution turned red. The solution was titrated with 0·1 M EDTA until the last trace of red disappeared and the solution was yellow.[8]

Results

The results obtained are shown in Fig. 1. It can be seen that increasing amounts of NTA impurity in the EDTA result in an increasing divergence between the factors produced by different metals. In most instances, the relationship between the factor and the NTA content is approximately rectilinear.

The factor for solutions standardised against calcium was 0·4 per cent. higher than that for zinc when no NTA was present. Since this throws suspicion on the quality of the primary standard, a further batch of calcium carbonate was purified by the method of Gjems.[9] This gave the same result. The factor obtained by titration against magnesium was lower than that for zinc.

Potentiometric investigation

The divergence of the factors obtained by titrating zinc and cadmium standards was unexpected, since the stability constants of the EDTA and the NTA complexes are similar and the titrations were carried out under identical conditions. In view of this, it was thought worthwhile to examine the titration of these two metals by potentiometric techniques.

The theory and use of mercury as a pM indicator electrode in complexometric titrations has been described by Reilley, Schmid and Lamson.[10,11] In this work the total volume of the solution was approximately 100 ml at the end-point. The pH was buffered by dissolving 0·4 g of ammonium nitrate in the solution and adding 5 N ammonia until the pH was 9 to 10. Fifty microlitres of a 0·05 M mercury - EDTA complex were added and the solution was titrated with 0·1 M EDTA. A mercury indicator electrode was used and the potential was measured against a saturated calomel electrode with a Cambridge pH meter.

Table II

Factors calculated from different potentiometric end-point steps

NTA present, per cent.	End-point step used	Factor
0	Potentiometric	0·9996
	Visual	1·0004
0·5	First zinc and first cadmium	1·0003
	Second zinc and second cadmium	0·9997
	First zinc and second cadmium	0·9912
	Second zinc and first cadmium	1·0090
	Visual	1·0090
1·0	First zinc and first cadmium	0·9998
	Second zinc and second cadmium	0·9998
	First zinc and second cadmium	0·9809
	Second zinc and first cadmium	1·0191
	Visual	1·0170

When pure EDTA was used a good single step was obtained, but if NTA was added to the EDTA, two end-point steps were found. Fig. 2 shows the shapes of the potentiometric curves obtained with zinc; those for cadmium were similar. It is presumed that the two successive end-points are due to EDTA and NTA, respectively.

The fact that two end-points are obtained allows the ratio of factors for EDTA standardised against cadmium and zinc to be calculated in four ways, depending on which end-point is used for each metal. The calculations show that the factor obtained from the first end-point break for cadmium and the second break for zinc are in reasonable agreement with those obtained by visual titration with Solochrome black T as indicator (see Table II). This indicates that Solochrome black T detects the EDTA end-point step in the titration of cadmium, but the NTA end-point in the titration of zinc.

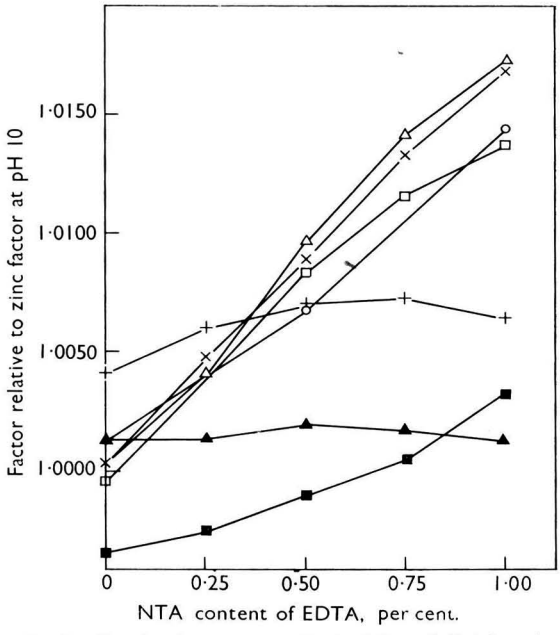

Fig. 1. Graphs showing the effect of the nitrilotriacetic acid content of EDTA on the factor for 0·1 M EDTA standardised against various standard solutions—

△ = bismuth
× = cadmium
○ = zinc (at pH 6)
□ = lead
+ = calcium
● = magnesium
▲ = manganese

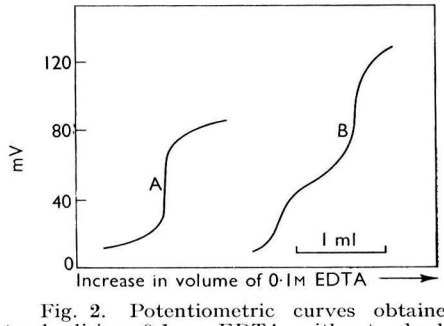

Fig. 2. Potentiometric curves obtained on standardising 0·1 M EDTA with standard zinc solution at pH 10. Curve A, pure EDTA; curve B, EDTA *plus* 1 per cent. of nitrilotriacetic acid

Effect of NTA on the sharpness of end-points

During this work it was noticed that increasing amounts of NTA in the EDTA had an adverse effect on the quality of the indicator colour change at the end-point, with the result

that the disappearance of the colour of the metal - indicator complex tended to be prolonged. This affected the reproducibility of duplicate titrations when significant amounts of NTA were present.

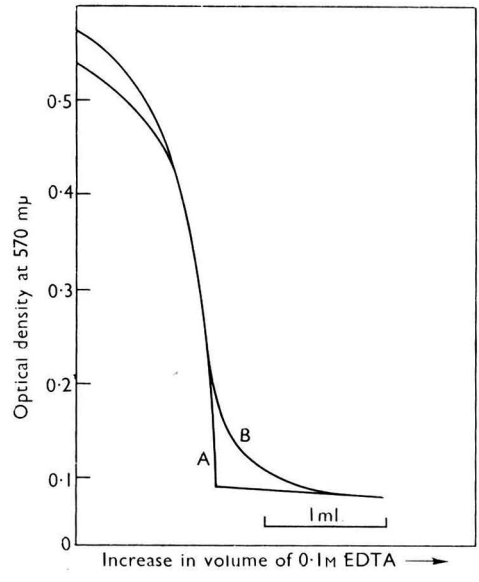

Fig. 3. Curves of the optical density at 570mμ of a 2 per cent. solution of cadmium chloride treated with 0·1 M EDTA. Curve A, pure EDTA; curve B, EDTA *plus* 1 per cent. of nitrilotriacetic acid.

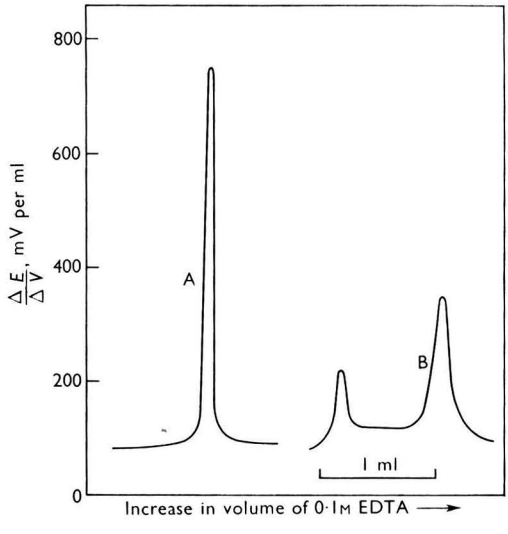

Fig. 4. Differential potentiometric curves obtained on standardising 0·1 M EDTA with standard zinc solution at pH 10. Curve A, pure EDTA; curve B, EDTA *plus* 1 per cent. of nitrilotriacetic acid

In order to confirm this effect, the end-point was examined spectrophotometrically. A 200-ml stock solution containing 4 g of hydrated cadmium chloride, 40 ml of buffer solution (pH 10) and 6 ml of a 0·1 per cent. solution of Solochrome black T in industrial methylated

spirit was prepared, and 50-ml aliquots were titrated with pure EDTA and with EDTA containing 1 per cent. of NTA. The titration was carried out in a 5 × 5 × 5-cm cell, and the change in optical density at 570 mμ was followed with a Beckman DU spectrophotometer. The resulting titration curves are shown in Fig. 3, which show clearly how the disappearance of the red colour is prolonged when the titrant contains NTA.

The potentiometric titration curves (see Fig. 2) also show that the sharpness of the end-point is lowered by the presence of NTA. This is most clearly shown in the differential form of the curves (see Fig. 4).

Discussion

This work shows that the presence of NTA in EDTA used for complexometric titrations has two practical effects—

(*i*) Standardisation factors obtained from different metals or from the same metal under different titration conditions may not agree.

(*ii*) There is often a deterioration in the quality of the end-points.

Since commercial EDTA almost invariably contains NTA, these effects must be borne in mind when solutions are standardised.

The factors obtained by titration of different primary standards show a linear relationship to the amount of NTA impurity present. Exceptions to this are lead, which shows a slight falling off above 0·5 per cent. of NTA, calcium and magnesium. It can be seen from Fig. 1 that, if those for calcium and magnesium are ignored, the curves obtained can be divided roughly into two groups: (*a*) bismuth, cadmium, zinc (at pH 6) and lead, and (*b*) manganese and zinc (at pH 10). Potentiometric titrations indicate that this difference is caused by the fact that with group (*a*) metals the EDTA end-point is detected by the indicator, whereas with group (*b*) the indicator reacts to the NTA end-point step. This effect is possibly caused by differences in the stabilities of the metal - indicator complexes, but the relevant stability constants are not available to confirm this.

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The Determination of Small Amounts of *N*-Isopropyl-*N'*-phenyl-*p*-phenylenediamine

By J. R. DAVIES

(*The Natural Rubber Producers' Research Association, 48–56 Tewin Road, Welwyn Garden City, Herts.*)

N-Isopropyl-*N'*-phenyl-*p*-phenylenediamine can be determined by visual titration in chloroform with toluene-*p*-sulphonic acid. The method is simple, rapid and accurate. Details are given for applying this technique to the analysis of raw and vulcanised rubbers containing the diamine.

N-Isopropyl-*N'*-phenyl-*p*-phenylenediamine is used extensively as an antioxidant and antiozonant in rubber compounds, and, as part of a programme of research undertaken by the Association, a method was required for determining the diamine at the 0- to 20-mg level contained in (*i*) chloroform solutions and (*ii*) raw- and vulcanised-rubber samples.

Previous methods used for amine-type antioxidants have included colorimetric, elution and paper-chromatographic techniques, which are often long and tedious.

The colorimetric methods used for naphthylamine derivatives,[1] in which benzoyl peroxide or 3-methylbenzothiazolin-2-one hydrazone hydrochloride are used as reagents, cannot be applied successfully to *N*-isopropyl-*N'*-phenyl-*p*-phenylenediamine. Hilton[2] determined *p*-phenylenediamine derivatives by oxidising an extract of rubber with cupric acetate to form the highly coloured Wurster salt, and concluded that this method was unsuitable owing to interference from oxidation products formed during the ageing of rubber.

However, Lorenz and Parks[3] have described a potentiometric titration of 20- to 100-mg amounts of *N*-isopropyl-*N'*-phenyl-*p*-phenylenediamine with 0·1 N perchloric acid in glacial acetic acid, and several authors[4,5,6] have determined weak organic bases having dissociation constants down to 10^{-11}, by titration with toluene-*p*-sulphonic acid in aprotic solvent systems.

Since *N*-isopropyl-*N'*-phenyl-*p*-phenylenediamine is a weak base having a dissociation constant of about 10^{-8}, it was thought that its determination would be possible with a non-aqueous acid - base titration technique, and since the sensitivity of the titration of weak organic bases is increased by using solvents of low dielectric constant, it was decided to investigate the titration of *N*-isopropyl-*N'*-phenyl-*p*-phenylenediamine in chloroform, with 0·005 N toluene-*p*-sulphonic acid as titrant.

Experimental

In order to establish the feasibility of using a visual titration technique and gain an insight into the mechanism of the neutralisation process, potentiometric titration of the diamine was performed with 0·005 N toluene-*p*-sulphonic acid. A Cambridge pH meter covering the pH range 0 to 14 was used in conjunction with a simple electrode system incorporating a glass electrode and reference calomel electrode. The shape of the titration curve obtained by plotting a graph of pH *versus* volume of titrant showed a well defined end-point occurring at an apparent pH of 4·0, corresponding to the neutralisation of only one amine-nitrogen of the molecule. Presumably the amino group flanked on both sides by the aromatic phenyl groups is not basic enough under these conditions to give a relatively sharp potentiometric break.

Methyl orange in chloroform (indicator pH range in water, 2·9 to 4·6) gave a sharp colour change from green to red at exactly the potentiometric equivalence point. As this is well defined, and the indicator transition coincides with the end-point, a simple visual titrimetric procedure is proposed in this paper.

Method

Reagents—

Chloroform—Use analytical-reagent grade material.

Methyl orange indicator solution—Extract 1·0 g of methyl orange with 100 ml of chloroform, and filter the extract.

Toluene-p-*sulphonic acid*, 0·005 N—Dissolve 0·4755 g of toluene-*p*-sulphonic acid monohydrate (molecular weight, 190·22) in 5 ml of absolute alcohol in a 500-ml calibrated flask and dilute the solution to the mark with chloroform. Keep the solution in an automatic microburette to prevent evaporation of the solvent, and standardise it before use with pure *sym*-diphenylguanidine (m.p. 148° to 149° C). Carry out all titrations at room temperature, 20° ± 2° C.

PROCEDURE—

To an accurately weighed amount (0 to 20 mg) of *N*-isopropyl-*N*′-phenyl-*p*-phenylenediamine in a 250-ml conical flask, add 25 ml of chloroform and 4 or 5 drops of methyl orange indicator solution. Titrate the mixture with the standard toluene-*p*-sulphonic acid to a red-violet end-point.

CALCULATION—

1 ml of 0·005 N toluene-*p*-sulphonic acid ≡ 1·13 mg of *N*-isopropyl-*N*′-phenyl-*p*-phenylenediamine.

There is no solvent blank correction.

RESULTS

The proposed method was applied to samples of *N*-isopropyl-*N*′-phenyl-*p*-phenylenediamine (m.p. 77·5° C), dissolved in chloroform. The results are given in Table I. If the figures obtained are examined by regression analysis, it can be shown that the results are unbiased, and have a standard error of ±0·05 mg, which cannot be demonstrated to be proportional to the amount present.

TABLE I

DETERMINATION OF *N*-ISOPROPYL-*N*′-PHENYL-*p*-PHENYLENEDIAMINE

Weight taken, mg	Volume of 0·005 N toluene-*p*-sulphonic acid added, ml	Weight found, mg
1·50	1·35	1·52
2·72	2·38	2·69
5·14	4·55	5·14
8·10	7·27	8·22
12·50	11·10	12·53
14·10	12·43	14·07
17·93	15·90	17·98
20·80	18·50	20·90

The method was then applied to the analysis of rubber samples containing added *N*-isopropyl-*N*′-phenyl-*p*-phenylenediamine and was investigated as described below.

RAW NATURAL RUBBER—

The *N*-isopropyl-*N*′-phenyl-*p*-phenylenediamine was incorporated into the rubber, either by mixing it in a conventional rubber-mill or by swelling the rubber in ethyl acetate containing a known concentration of diamine, the excess of solvent being removed by evaporation.

The rubber was cut into small pieces weighing not more than 100 mg each, and a 1-g sample was accurately weighed into a 250-ml extraction flask. To prevent the pieces of rubber sticking together and slowing the rate of extraction, small glass beads were added to the flask. The sample was extracted for 12 hours by heating it under reflux with 50 ml of boiling acetone. Extraction with acetone was preferred to chloroform, which completely dissolved the rubber and interfered with the subsequent titration in which toluene-*p*-sulphonic acid was used. The rubber was removed by filtration, the acetone distilled off and the residue dissolved in 10 ml of chloroform. The chloroform solution was titrated with 0·005 N toluene-*p*-sulphonic acid to the methyl orange end-point.

A blank correction is necessary for natural rubber, the determination being made by extracting with 50 ml of acetone a 1-g control sample of raw natural rubber containing no added antioxidant, the procedure being identical to that for the test sample.

Typical results are given in Table II for the determination of the diamine added to raw natural rubber by milling.

TABLE II

DETERMINATION OF *N*-ISOPROPYL-*N'*-PHENYL-*p*-PHENYLENEDIAMINE ADDED TO RAW NATURAL RUBBER BY MILLING

Weight of rubber sample taken, g	Volume of 0·005 N toluene-*p*-sulphonic acid added, ml	Amount of diamine found, per cent. w/w	Amount of diamine added, per cent. w/w
1·012	0·20	—	—
1·013	8·40	0·92	1·00
1·013	16·50	1·82	2·00

The results suggest that some loss of the diamine occurs in the milling process.

Typical results are given below for the determination of *N*-isopropyl-*N'*-phenyl-*p*-phenylenediamine added by swelling—

Calculated amount of diamine added, mg (approximately) ..	8	9·5	10	10
Amount of diamine found, mg	8·20	9·61	9·90	9·95

In the experiments in which 10 mg of diamine were added, the samples contained equimolar amounts of zinc dibutyldithiocarbamate, and the effect of this compound on the titration of the diamine was investigated. The results given below were obtained with 12·5 mg of the diamine, and show that only slight interference is observed from a twofold excess of zinc dibutyldithiocarbamate—

Weight of dithiocarbamate taken, mg	0	12·5	25·0
Weight of diamine found, mg	12·54	12·54	12·65

VULCANISED RUBBER—

N-Isopropyl-*N'*-phenyl-*p*-phenylenediamine was incorporated into the rubber by milling before vulcanisation at 140° C for 30 minutes.

The original composition (parts by weight) of the mix is given below—

Natural rubber R.S.S.	100
Zinc oxide	5
Stearic acid	2
N-Cyclohexyl-2-benzthiazoylsulphonamide	0·6
Sulphur	2·5
N-Isopropyl-*N'*-phenyl-*p*-phenylenediamine	1 or 2

The vulcanised rubber was cut into small pieces, each weighing not more than 100 mg and a 1-g sample accurately weighed into a 250-ml extraction flask. The experimental procedure was then identical to that described for raw natural rubber. As for raw natural rubber a blank correction is necessary, the value being determined by extracting a 1-g sample

TABLE III

DETERMINATION OF *N*-ISOPROPYL-*N'*-PHENYL-*p*-PHENYLENEDIAMINE IN VULCANISED RUBBER

Weight of rubber sample, g	Volume of 0·005 N toluene-*p*-sulphonic acid added, ml	Amount of diamine added, per cent. w/w	Amount of diamine found, per cent. w/w
1·000	1·01	—	—
1·000	8·36	0·91	0·83
1·000	16·40	1·81	1·74
~1	—	0·91	0·83, 0·84, 0·83
~1	—	1·81	1·75, 1·70, 1·74

of vulcanised rubber of identical composition to that of the test sample, except that no antioxidant is added. Typical results shown in Table III, show that some loss of *N*-isopropyl-*N'*-phenyl-*p*-phenylenediamine occurs during the milling and vulcanisation processes.

Conclusion

Although the extraction procedure is lengthy, the titrimetric analysis can be used to determine *N*-isopropyl-*N'*-phenyl-*p*-phenylenediamine in (*i*) chloroform solutions and (*ii*) raw and vulcanised rubbers.

This work forms part of a programme of research undertaken by the Natural Rubber Producers' Research Association.

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The Mass-spectrometric Determination of Certain Trace Impurities in Gases

By R. T. PARKINSON and L. TOFT

(*Master General of Ordnance Inspectorates, Chemical Inspectorate, Royal Arsenal, Woolwich*)

The determination of impurities in gases with an MS2G mass spectrometer is, with few exceptions, restricted to a lower limit in the region of 50 to 100 v.p.m. In certain instances, by modifying the normal procedure and concentrating the impurity before analysis, determinations can be made at a much lower level, the increase in sensitivity being up to 4000 times the normal.

Methods have been developed that have been used to determine, with a coefficient of variation of better than 4 per cent., 1 v.p.m. of helium in nitrogen, 1 v.p.m. of hydrogen in argon, 3 v.p.m. of carbon dioxide in hydrogen and 20 v.p.m. of hydrogen in helium.

In recent years the demand for special high-purity gases has increased considerably and, to ensure that such gases comply with the relevant specifications, analytical methods of high sensitivity and selectivity are required. If the undesirable impurities include the inert gases, then the only methods available are those based on differences in physical properties.

MS2G mass spectrometers (Associated Electrical Industries Ltd.) are in use in this Inspectorate for the analysis of gases, and, although this is a technique that has the great advantage of high specificity, in general the lower limit for the determination of an impurity in a gas is restricted to the 50 to 100 v.p.m. region, the actual limit depending on the relative ionisation properties of the impurity and the base gas. As it was required to determine helium in nitrogen at levels of less than 10 v.p.m., hydrogen in argon at 5 v.p.m., hydrogen in helium at 20 v.p.m. and carbon dioxide in hydrogen at levels of 100 v.p.m. and below, it was apparent that the ion beams produced from the impurities either would not be detected by the instrument or would be too weak for accurate measurement. As only marginal improvements in sensitivity can be obtained by modifying the instrument's detector system, consideration was given to means of improving the sensitivity by (*i*) increasing the sample pressure in the analyser tube of the mass spectrometer and (*ii*) concentrating the impurity before analysis. This paper is concerned with the investigation of these two techniques with particular reference to the analysis of certain high-purity gases.

Experimental

The use of abnormally high analyser-tube pressures—

The normal sample volume used for gas analysis with the MS2G mass spectrometer is approximately 1 c.c., which produces an analyser-tube pressure of about 10^{-7} torr.

An appreciable gain in sensitivity was obtained by increasing the sample pressure in the analyser tube. With samples of helium, argon and nitrogen, analyser-tube pressures in the region of 5×10^{-6} torr could be tolerated. At still higher tube pressures, the ionisation efficiency decreased and the impurity-ion beams were reduced in intensity. The optimum pressure varied between instruments and had to be determined before a specific analysis was made.

At these pressures, the major ion beams were far too intense for measurement, and the amount of base gas present was derived from a measurement of a less abundant ion beam. For helium, which produces no minor ion beams of sufficient intensity, the amount present was calculated from the relation between the mass-4 ion beam and the pressure in the sample reservoir (see under "Procedure I," p. 225).

Although this technique resulted in an appreciable increase in sensitivity (see Table I), it was still not sufficient for the analyses required for argon, nitrogen and hydrogen. It did make possible, however, the determination of hydrogen in helium at the 20 v.p.m. level (see under "Procedure I"). Eighteen replicate experiments on a standard mixture consisting of helium containing 20 v.p.m. of hydrogen gave a mean result of 19·7 v.p.m. of hydrogen, with a standard deviation of 0·7 v.p.m.; the results ranged from 18·9 to 22·0 v.p.m.

At high tube pressures, the ionisation characteristics of gases can vary from those obtained at normal pressures, and it is preferable to calibrate the mass spectrometer against standard gas blends. An indication of the effect of a base gas on the ionisation of a trace impurity can be obtained by using the double inlet system of the instrument. The impurity

TABLE I

IMPROVEMENTS RESULTING FROM HIGH ANALYSER-TUBE PRESSURES

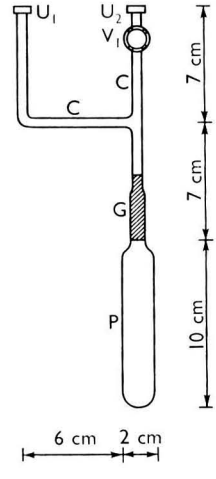
Impurity	Base gas	Sensitivity Impurity-ion beam, inches* per v.p.m.— at normal pressure	at optimum high pressure	Improvement factor
Hydrogen	Argon	0·0003	0·01	33
Hydrogen	Helium	0·0003	0·05	167
Helium	Nitrogen	0·0001	0·01	100

* The amplifier of the MS2G mass spectrometer has ten sensitivity ranges, and all ion-beam measurements are finally converted to range-seven readings for calculating results. The conventional method of measuring ion beams is to denote a full-scale deflection of ten divisions on the meter, when range seven is used, as ten "inches," and the measuring range of the instrument is from 0 to 3000 inches. In practice, it is considered that 0·05 inch is the smallest measurement that can be made.

is introduced into one side of the inlet system in a calculated amount, so as to give the required ion-beam reading at a particular impurity level. The base gas, also in a calculated amount, is treated likewise on the other side. Both sides of the inlet system are then opened to the analyser tube together, and any effect on the previous beam readings noted.

THE USE OF IMPURITY-CONCENTRATION TECHNIQUES—

(*i*) *Helium in nitrogen and hydrogen in argon*—For these determinations, the impurity was concentrated by passing the sample gas into a simple apparatus (see Fig. 1) consisting of a glass tube fitted with a valve and a side arm, the tube being cooled in liquid nitrogen.

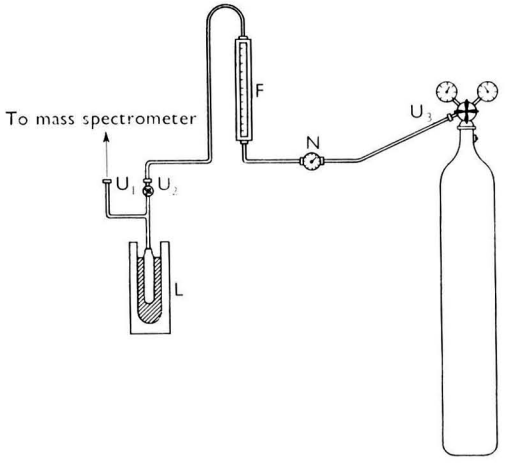


U_1, U_2 = Metrovac unions
V_1 = $\frac{1}{8}$-inch Metrovac valve
P = Pyrex glass
C = $\frac{1}{4}$-inch o.d. copper tubing
G = Glass-to-metal seal

Fig. 1. Diagram of liquefier

At flow-rates that are stated in the detailed methods, the base gases were liquefied and the impurities remained in the gaseous phase. The gas mixture above the surface of the liquefied gas was then analysed mass spectrometrically at a normal sample pressure. This procedure gave low and inconsistent results, and analysis of successive fractions of the gas

mixture showed a variable, but continuous, decrease in its impurity content. It was, therefore, necessary to remove and measure all the impurity contained in the liquefier. This was accomplished by removing from the liquefier three successive high tube-pressure doses of gas. In this operation the gas mixture was expanded into the evacuated sample reservoir of the mass spectrometer, the resultant pressure drop causing the liquefied gas to boil, thus effectively removing a large proportion of the impurity with each dose. The impurity-ion beam was measured for each dose, the impurity content of the original gas being proportional to the sum of these ion beams. For helium in nitrogen, no helium was detected in the fourth and subsequent doses. For hydrogen in argon, the sum of the hydrogen beams from the three doses remained essentially constant, although three successive doses did not remove all the hydrogen from the liquefier, and the coefficient of variation was better than 3 per cent. The hydrogen measured in fourth and subsequent doses was little above background level.



U_1, U_2 = $\frac{1}{8}$-inch Metrovac unions
U_3 = Connector (as supplied by British Oxygen Co.)
L = Liquefier
N = Edwards needle valve
F = Flow-meter

Fig. 2. Diagram of liquefaction apparatus

With this technique it is essential to measure the volume of sample passed into the liquefier; this can be determined by using a measuring device based on mercury displacement (see under "Procedure II*b*," p. 226). A less accurate method is to calculate the sample volume by measuring the volume of liquefied gas and converting this to its equivalent volume in the gaseous state. Still another method is to allow the liquefied gas to boil off through a gas meter after the impurity has been determined. The use of a flow-meter or gas meter to measure the volume of sample passing into the liquefier is not possible, because the pressure in the liquefier does not remain constant, but gradually increases as more of the sample is liquefied. A flow-meter may be used, however, if the sample is compared with a standard (see under "Procedure II*a*," p. 226). In this procedure the standard is passed through a flow-meter into a liquefier for a definite time at a constant flow-rate, the impurity then being determined as outlined above. The sample is treated identically, and the impurity content derived from the ratio of the impurity-ion beam of the sample to that of the standard (see under "Procedure II*a*"). In this instance, the flow-meter is used only to ensure that the same amount of gas is passed into the liquefier for both standard and sample, and it is not used to measure the actual amounts. If a standard gas is not available, then the impurity content is calculated from the impurity-ion beam and from the relation between a high-pressure dose and the total sample volume (see under "Procedure II*b*").

When argon is liquefied in order to concentrate the hydrogen impurity (see under "Procedure III *a*," p. 226), with the apparatus shown in Fig. 2, the gas is passed into the liquefier at a rate of 2 litres per minute as indicated by the flow-meter. At this flow-rate, liquefaction and partial solidification of the argon takes place. At flow-rates of less than

about 1 litre per minute, however, liquefaction does not always occur, although more than 10 litres of gas may have been passed into the liquefier. Under these conditions the argon in the liquefier is in the vapour state, whose density approaches that of liquid argon as more and more gas is passed into the liquefier.

This process of concentrating the impurity before a high analyser-tube pressure is used for the analysis, results in a large increase in sensitivity compared with normal mass-spectrometric analysis. When this technique is applied to the determination of helium in nitrogen, the increase in sensitivity is about 1300 times, with a detection limit, based on twice the standard deviation, of 0·05 v.p.m. With hydrogen in argon, the increase is about 4000 times and the detection limit is 0·06 v.p.m.

Analysis of samples of nitrogen of known helium content, in which the concentration technique (see under "Procedure II*b*") was used, *i.e.*, not run in conjunction with a standard gas, but with due allowance made for helium recovery (double-inlet check, previously mentioned), gave the results shown in Table II.

TABLE II

RESULTS FOR HELIUM IN NITROGEN (BATCHWISE METHOD)

Helium content, v.p.m.	Sample volume, ml	Helium found, v.p.m.
0·9	2160	0·9
	4320	0·9
8·7	432	7·9
	2160	8·0
	4320	8·4
150	432	152, 147, 142, 151, 149, 141, 142*

* Mean of these results = 146 v.p.m.; standard deviation 4·6 v.p.m.

The precision of the concentration technique, when a sample was run in conjunction with a standard (see under "Procedure II*a*"), was determined by using two standard gas blends, one containing 0·9 and the other 8·7 v.p.m. of helium in nitrogen. The 8·7 v.p.m. standard was then used as the reference sample and the 0·9 v.p.m. standard treated as an unknown sample.

Six replicate experiments gave a mean result of 0·91 v.p.m. and a standard deviation of 0·03 v.p.m., the range of results being from 0·87 to 0·94 v.p.m.

A similar procedure was used for determining trace amounts of hydrogen in argon. A standard blend containing 5·0 v.p.m. of hydrogen was used as a reference sample and a 0·9 v.p.m. standard was treated as an unknown. In this series, ten replicate experiments gave a mean result of 0·84 v.p.m. and a standard deviation of 0·03 v.p.m., the results ranging from 0·78 to 0·88 v.p.m.

(*ii*) *Carbon dioxide in hydrogen*—The hydrogen was first analysed mass spectrometrically with one normal dose of gas. A known amount of the sample (approximately 150 ml), contained in a glass flask, was then cooled in liquid nitrogen to condense the carbon dioxide. (It was found that a cooling-time of at least 15 minutes was necessary to ensure complete condensation.) After cooling, the non-condensable gases were removed by evacuation, the flask warmed up to normal temperature, the separated carbon dioxide introduced into the mass spectrometer and the mass-44 ion beam measured. The carbon dioxide content of the original gas was calculated from this measurement, and from the ion-beam measurements of the normal analysis, the ratio of one normal dose of gas to the total sample volume being taken into account (see under "Procedure IV," p. 227).

The normal method for determining carbon dioxide in hydrogen was found to have a positive bias of from 30 to 60 v.p.m. at levels below about 1000 v.p.m. This is probably owing to the desorption of carbon dioxide from the internal surfaces of the mass spectrometer when a normal operating pressure of hydrogen is introduced into the instrument. The condensation technique does not suffer from this defect, since the gas taken into the instrument is practically pure carbon dioxide, giving rise to comparatively large ion beams, and contribution of carbon dioxide by desorption is relatively insignificant.

The increase in sensitivity that can be gained by concentrating the carbon dioxide is mainly limited by the size of sampling flask and cooling trap that can be conveniently handled. At the 50 v.p.m. carbon dioxide level, the mass-44 ion beam produced from a 150-ml gas sample is of ample intensity for mass-spectrometric measurement, and the detection limit with this sample volume is 0·5 v.p.m. of carbon dioxide, compared with about 50 v.p.m. for the normal method, an appreciable gain in sensitivity. If necessary, this can be improved by using a larger sample volume.

In order to compare the accuracy of the "condensation" method with the normal method, several standard gas bands were analysed, covering the range 3 to 3000 v.p.m. of carbon dioxide in hydrogen. The results (see Table III) show the normal method to be inaccurate below about 1000 v.p.m., whereas the "condensation" method shows a satisfactory recovery of carbon dioxide over the entire range.

TABLE III

RECOVERY OF CARBON DIOXIDE

Actual carbon dioxide content, v.p.m.	Normal method		Condensation method	
	Carbon dioxide found, v.p.m.	Recovery, per cent.	Carbon dioxide found, v.p.m.	Recovery, per cent.
3320	3300	99	3320	100
2620	2560	98	2590	99
2470	2530	102	2470	100
1030	1090	106	1020	99
515	585	113	502	97
120	177	148	117	98
96	133	140	91	95
70	103	147	67	96
48	79	165	45	95
24	70	290	23	95
3·0	36	—	3·1	103

The precision of the "condensation" method was determined at five different levels of carbon dioxide content. These results (see Table IV) show the precision of the method to be satisfactory.

TABLE IV

PRECISION OF THE "CONDENSATION" METHOD

Carbon dioxide content, v.p.m.	Number of results	Standard deviation, v.p.m.
3350	6	42
1130	6	12
95	14	1·2
24	5	0·3
3·0	4	0·08

THE PREPARATION OF STANDARD GAS BLENDS—

(*i*) *Hydrogen in argon and helium in nitrogen*—As a gas flow is required when a dynamic system is used for concentrating the impurity, and a comparatively large volume required for determining the precision, each standard was prepared by evacuating a gas cylinder (nominal capacity of 200 cu. ft of gas at S.T.P.), by adding the required amount of impurity and then filling the cylinder to a known pressure with pure base gas.

(*ii*) *Hydrogen in helium*—This standard was prepared as described above except that a gas cylinder of nominal capacity of 40 cu. ft of gas at S.T.P. was used.

(*iii*) *Carbon dioxide in hydrogen*—For this determination, in which a static system is used for concentrating the impurity, only a small volume of gas is required and the standards were prepared in a 3-litre glass flask. This flask was attached to a manifold that was fitted with a mercury manometer and the necessary valves for evacuation and intake of gas. The required amounts of carbon dioxide and hydrogen were measured by reference to the manometer. Blends below 500 v.p.m. of carbon dioxide were prepared by diluting a higher-level blend with hydrogen.

METHODS

MASS SPECTROMETER OPERATION CONDITIONS—

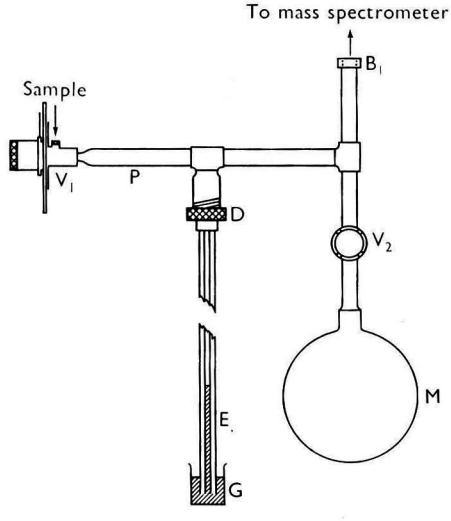
Electron-accelerating voltage:	70 volts.
Ion-accelerating voltage:	1975 volts.
Ion repeller:	Beams tuned to second maximum.
Ion gauge:	Range, 10^{-6}.

The above conditions are for normal operation. When the high-tube-pressure technique is used, the ion gauge is switched to the 10^{-5} range. It is possible that the optimum operating pressure for this technique, on a particular instrument, may be in excess of 10^{-5} torr. In this instance, as the filament and high-tension supplies are automatically out of circuit when the ion gauge is on the 10^{-3} or 10^{-4} range, the instrument would have to be modified to allow operation at the higher pressure.

At high pressures, only one maximum is produced when the beam height is plotted against the ion-repeller voltage. Beams are tuned to this maximum.

PROCEDURE 1: FOR HYDROGEN IN HELIUM—

Connect a manometer to the gas-inlet system of the mass spectrometer so that the pressure in the sample reservoir can be measured. (The apparatus shown in Fig. 3 for the determination of carbon dioxide in hydrogen can be utilised for this purpose).



V_1 = Edwards needle valve
V_2 = Polythene diaphragm valve
B_1 = Metrovac union
P = Pipework
D = Woodward coupling
E = Manometer
M = Sampling flask
G = Mercury reservoir

Fig. 3. Diagram of apparatus for determining carbon dioxide

Determine the optimum operating pressure, P_1, for the instrument, by increasing the pressure of sample in the reservoir in stages, by using helium containing about 100 v.p.m. of hydrogen. Plot the mass-2 ion beam against the reservoir pressure.

Evacuate the apparatus, then introduce the gas to be analysed into the sample reservoir so that a mass-4 ion beam is produced that approaches the maximum intensity measurable. Note this beam reading, A, and the pressure, P_2, in the sample reservoir.

Increase the pressure of sample in the reservoir up to the predetermined optimum, P_1, and note the mass-2 ion-beam reading, B.

Calculate the hydrogen content of the sample, which is given by the equation—

$$\text{Hydrogen content, v.p.m.} = \frac{B \cdot S_b \cdot P_2 \times 10^6}{A \cdot S_a \cdot P_1}$$

where A = mass-4 ion-beam measurement,
B = mass-2 ion-beam measurement,
P_1 = optimum operating pressure,
P_2 = pressure of sample in reservoir that produces the mass-4 ion beam, A,
S_a = relative sensitivity of helium and
S_b = relative sensitivity of hydrogen.

PROCEDURE II: FOR HELIUM IN NITROGEN—

(*a*) *Dynamic method*—Determine the optimum operating pressure for the instrument by increasing the analyser-tube pressure in stages, by using a sample of nitrogen containing a trace amount of helium and plotting the mass-29 ion beam against the mass-4 ion beam. When the optimum pressure has been determined, proceed in the manner described below—

Assemble the apparatus as shown in Fig. 2 and connect it to the mass spectrometer at the sample-inlet valve. Evacuate the system in the normal manner. Close the sample-inlet valve on the mass spectrometer, cool the liquefier tube in liquid nitrogen and pass the sample into the liquefier at an indicated flow-rate of 200 ml per minute for 20 minutes. (The flow-rate will require periodic adjustment owing to pressure changes in the cooling limb.) After 20 minutes close the valve on the liquefier and turn off the gas flow.

Admit one normal dose of gas from the liquefier into the sample reservoir of the mass spectrometer. Locate the mass-29 ion beam then admit more gas from the liquefier, increasing the reservoir pressure gradually until the mass-29 beam reaches the predetermined level. Close the inlet valve.

Allow about 2 minutes for conditions to stabilise, then measure the mass-4 helium ion beam.

Pump away the dose.

Measure the helium-ion beam on two further high-pressure doses and total the three mass-4 measurements.

Disconnect the apparatus from the mass spectrometer; remove the liquid-nitrogen container and allow the condensed nitrogen to boil away, making sure that the liquefier is open to atmosphere.

Repeat the entire procedure with a standard gas. Calculate the helium content of the sample from the ratio of the helium-ion beams, on sample and standard.

(*b*) *Batchwise method*—Assemble the apparatus as shown in Fig. 2, but replace the flow-meter, F, by a measuring device capable of delivering a known amount of sample into the liquefier.

Pass 4 litres of sample into the liquefier, and then close the liquefier valve.

Analyse the gas mixture in the liquefier as described under "Dynamic method."

Combine the three helium-ion beam measurements, and calculate the helium content of the sample, which is given by—

$$\frac{A \cdot S_{He} \cdot V_2 \times 10^6}{B \cdot C \cdot S_N \cdot V_1} \text{ v.p.m.}$$

where A = total value of mass-4 measurements,
B = mass-29 measurement,
C = mass-28 - mass-29 ratio,
S_{He} = relative sensitivity of helium,
S_N = relative sensitivity of nitrogen,
V_1 = total sample volume and
V_2 = volume of nitrogen equivalent to the mass-29 measured at the optimum working pressure, *i.e.*, volume of nitrogen contained in one high-pressure dose.

PROCEDURE III: FOR HYDROGEN IN ARGON—

The methods are similar to those described for the determination of helium in nitrogen, the only difference being—

(*a*) *Dynamic method*—(*i*) Use an indicated flow-rate of 2 litres per minute for 10 minutes for the sample flow-rate and (*ii*), measure the mass-2 and mass-36 ion beams.

(*b*) *Batchwise method*—(*i*) Use a sample volume of 10 litres and (*ii*) measure the mass-2 and mass-36 ion beams.

PROCEDURE IV: FOR CARBON DIOXIDE IN HYDROGEN—

Assemble the apparatus shown in Fig. 3 and connect it to the sample-inlet system of the mass spectrometer.

Evacuate the apparatus, then fill flask M to approximately atmospheric pressure with the gas sample. Note the pressure on the manometer.

Close the valve on the flask, fill a Dewar vessel with liquid nitrogen so that flask M is immersed, and cool the flask for at least 15 minutes.

After the flask has been cooled, admit one normal dose of gas (by means of the doser in the gas-inlet system) into the mass spectrometer. Measure the mass-2 ion beam, A, and all other ion beams present.

Pump away the dose and also the non-condensable gases in flask M.

Close the valve on flask M, remove the Dewar vessel and allow the flask to warm up to normal temperature.

When the flask has attained normal temperature, open the valve on the flask and allow the gas to expand into the sample reservoir.

Measure the mass-44 ion beam, B, and calculate the carbon dioxide content of the sample, which is given by—

$$\frac{B \,.\, S_b \,.\, V \times 760 \times 10^6 \,(V_r + V_p + V_f)}{(A \,.\, S_a + \dfrac{B \,.\, S_b \,.\, V \times 760 \,(V_r + V_p + V_f)}{V_f \,.\, P_s \,.\, V_r} + Z)\, V_f \,.\, P_s \,.\, V_r}$$

where A = mass-2 ion-beam measurement,
B = mass-44 ion-beam measurement,
S_a = relative sensitivity of hydrogen,
S_b = relative sensitivity of carbon dioxide,
V = volume, at a pressure of 760 mm of mercury, of one dose of gas metered by the mass-spectrometer doser,
V_f = volume of sampling flask M,
V_r = volume of mass-spectrometer reservoir,
V_p = volume of pipework in apparatus,
P_s = pressure of sample in flask M, in mm of mercury, and
Z = the sum of the products of all other ion beams (except masses 2 and 44) and their relative sensitivities.

Apart from the ion-beam measurements, the sampling pressure and the relative sensitivities, the other terms are constant. The expression can therefore be simplified to—

$$\frac{B \,.\, S_b \,.\, Q \times 10^6}{(A \,.\, S_a + \dfrac{B \,.\, S_b \,.\, Q}{P_s} + Z)\, P_s} \text{ v.p.m.,} \qquad \text{where } Q = \frac{V \times 760 \,(V_r + V_p + V_f)}{V_f \,.\, V_r}$$

When the purity of the sample exceeds 99 per cent., then minor ion beams are an insignificant fraction of the total and the expression can be further simplified to—

$$\frac{B \,.\, S_b \,.\, Q \times 10^6}{A \,.\, S_a \,.\, P_s} \text{ v.p.m.}$$

DISCUSSION

The methods developed have greatly increased the sensitivity of certain mass-spectrometric determinations.

It is considered that—

(*i*) If so required, the sensitivities of the methods described could be further increased by using a larger sample volume for analysis.

(*ii*) The techniques could be equally well applied to the determination of hydrogen, helium and neon in argon, nitrogen or other similarly condensable gases.

(*iii*) The carbon dioxide determination could be extended to gases other than hydrogen.

(*iv*) The simple liquefaction apparatus could possibly have a use in fields other than mass spectrometry.

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The Determination of Carbon in Sodium

By J. A. J. WALKER and E. D. FRANCE

(*United Kingdom Atomic Energy Authority, Reactor Materials Laboratory, Wigshaw Lane, Culcheth, Warrington, Lancs.*)

A method has been developed for determining free carbon in sodium at the parts per million level. Sodium is removed by distillation at 600° C for 8 hour and the carbon in the residue determined by combustion in an excess of oxygen at 1200° C. The carbon dioxide formed is measured manometrically. Additions of carbon to sodium for the levels 3, 6 and 12 p.p.m. have been recovered satisfactorily with a standard deviation of 0·5 p.p.m. of carbon. The possibility of loss of carbon because of interaction with sodium oxide has been investigated.

When sodium is used as a reactor coolant, its carbon content is of importance from the point of view of the carburisation or decarburisation of steels with which it is in contact. To help in establishing the mechanism and extent of this carburisation - decarburisation, a method for determining carbon in sodium was developed.

Carbon may exist in sodium as free carbon, carbon in solution, carbide and carbonate. The method to be discussed deals with the determination of free carbon, soluble carbon and any carbide not decomposed either by heating to 600° C, or by hydrochloric acid vapour.

Two methods for determining carbon in sodium or sodium - potassium alloys have been described.[1,2,3] The method developed by Pepkowitz and Porter[1] involves the conversion of the sodium to sodium sulphate and wet oxidation of the carbon to carbon dioxide. The carbon dioxide is measured by a volumetric differential freeze-out technique.

In another method, due to Stoffer and Phillips,[2] 100-mg samples are oxidised at 950° C in a stream of purified oxygen. The carbon present is converted to carbon dioxide, which is absorbed by Ascarite contained in tared U-tubes.

Both methods claim to be satisfactory in the 0·005 to 0·010 per cent. w/w range, which was well above the level thought to be of interest in the circulating sodium-rig experiments at Culcheth. Exploratory work at Culcheth on the Pepkowitz and Downer method,[4] in which a conductimetric finish is used, showed that blank values for the method were high when compared with the low carbon contents to be determined. The apparatus blank value of 0·030 mg of carbon found when the Stoffer and Phillips combustion technique was used, is equivalent to 300 p.p.m. of carbon for an approximate 100-mg sample weight. It was thought that development of this method for larger sample weights would be time consuming, because of manipulative difficulties.

It was therefore decided to use a distillation technique to remove the sodium and to determine the carbon in the residue by a low-pressure volumetric procedure after combustion in an excess of oxygen.

Experimental

Apparatus—

The equipment required for determining carbon in sodium may be conveniently divided into two parts for the purpose of description.

The sampling of sodium from a rig is carried out in an evacuable glove box to eliminate atmospheric contamination. The glove box is attached to a sodium loop and contains the silica crucibles that hold the sodium during distillation, a sample holder and a distillation pot (Fig. 1), both made of stainless steel.

The remainder of the apparatus consists of a complete low-pressure micro carbon apparatus (Fig. 2), as used in the determination of carbon in metals.[5] Cylinder oxygen is passed through a purifier of platinised asbestos (at 900° C) with subsequent removal of carbon dioxide by soda asbestos. The purified oxygen is fed into an alumina combustion tube maintained at 1200° C by a platinum-wound furnace. The latter is controlled by a variable auto-transformer of the Variac type, coupled in series with a temperature controller. At the exit of the combustion-tube and heated to approximately 600° C is a roll of copper gauze. This will convert any carbon monoxide produced to carbon dioxide and act as an efficient scrubber for chlorine. The chlorine may arise from the thermal breakdown of (*a*) hydrogen chloride

that is incompletely removed after the acidification stage and (*b*) the sodium chloride formed during the acidification stage (see p. 231).

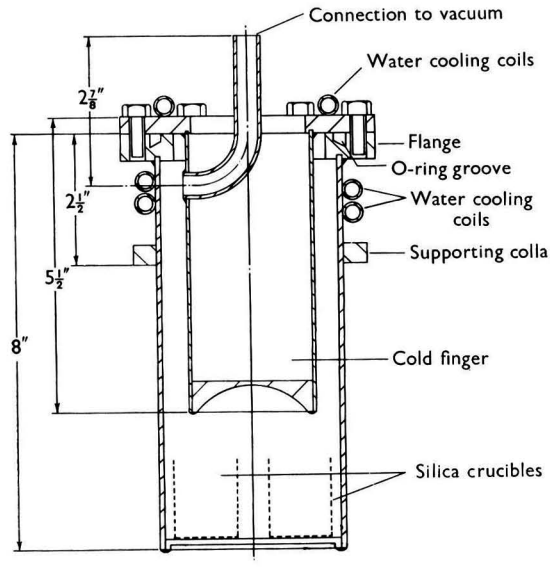
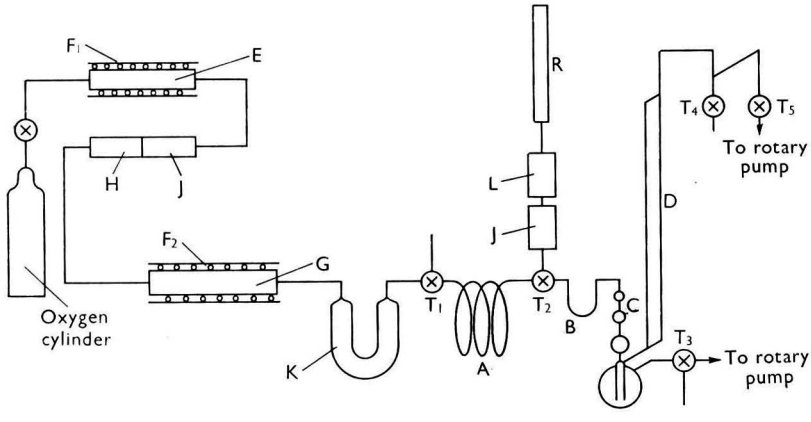


Fig. 1. Diagram of distillation pot



A = Multiple loop
B, C = Calibrated U-tube and bulbs
D = Mercury manometer
E = Platinised asbestos
F_1 = Nichrome-wound furnace at 900° C
F_2 = Platinum-wound furnace at 1200° C
G = Combustion tube
H = Anhydrone drier
J = Soda-asbestos scrubbers
K = Drier containing phosphorus pentoxide and glass wool
L = Phosphorus pentoxide drier
R = Rotameter
T_1, T_2, T_3 = Three-way taps
T_4, T_5 = Taps

Fig. 2. Schematic diagram of apparatus for the micro-determination of carbon

Attached to the end of the combustion tube is a moisture scrubber of phosphorus pentoxide mixed with finely chopped glass wool. After passage through the scrubber, the evolved carbon dioxide is condensed out with a multiple glass loop, which is immersed in a liquid-oxygen cold trap during the combustion operation.

Outline of the method

The determination of carbon in sodium may be divided into three stages, namely—

(*a*) Preparation of silica crucibles.
(*b*) Sampling of liquid sodium and its removal by distillation.
(*c*) Combustion of the residue in the crucible and measurement of the carbon dioxide formed.

The crucibles are made from pure silica tube (19-mm o.d.), closed at one end, and are of 6- to 7-ml capacity. They are not directly handled throughout the whole of this operation or subsequently. The crucibles are placed in a muffle furnace and heated at 1000° C for a minimum period of 18 hours, or until required.

Both the crucible holder and distillation pot are pickled in 3 N hydrochloric acid and thoroughly washed in water before use. The crucible holder (containing four silica crucibles) and distillation pot are then loaded into the evacuable sampling box, which is attached to the sampling port of a circulating sodium loop. In addition, a 10-ml nickel sampling-bucket and forceps are placed in the sampling box.

The box is evacuated to $<1\ \mu$ pressure and filled with purified argon. Sodium is transferred in the nickel bucket from the loop to three of the silica crucibles, which are completely filled (7 g of sodium). The fourth silica crucible is used for a blank determination. The crucible holder and its four crucibles are placed in the distillation pot, and the latter is sealed, removed from the sampling box and connected to the distillation rig.

Air trapped in the connecting lines between the distillation unit and the pot is removed by evacuation to $<1\ \mu$ pressure by using a mercury-diffusion pump backed by a rotary pump. The valve on the top of the pot is opened gradually and the pot evacuated. The temperature of the pot is then maintained at 600° C for 8 hours by means of side and base heaters, after which time all the sodium will have distilled from the crucibles on to the cold finger. The pot is allowed to cool while being evacuated continuously. It is then isolated from the pumping system and removed from the distillation unit.

The pot is brought to atmospheric pressure by gradual admittance of air, and the crucible holder is removed. Neutralisation of the sodium oxide present in the carbon residue in the silica crucibles is carried out by placing the crucible holder and crucibles in a vessel containing hot hydrochloric acid. The crucibles remain in contact with the vapour for 1½ hours and are then dried in an oven at 110° C for a further hour.

Carbon in the crucible residue is determined by combustion in a stream of oxygen. The silica crucible is pushed into the combustion tube at 1200° C and swept by a stream of purified oxygen. The carbon dioxide formed from the combustion of the residue is swept by the oxygen stream into the multi-loop trap (cooled in liquid oxygen), where it condenses. It is transferred by sublimation to a calibrated volume, connected to a mercury manometer and the pressure increase noted.

The carbon content of the original sample may then be calculated, since the net weight of carbon found and the original weight of sample (found by reference to the capacity of the crucible) are known.

Preparation of standards—

The problem of dispensing microgram amounts of carbon into a silica crucible is difficult. An obvious method would be to dissolve a known weight of carbonaceous material in a solvent and transfer a measured amount to a crucible. The solvent could be removed by volatilisation, leaving a fixed weight of carbon. This technique was tried out on four substances, namely, sucrose and melamine in aqueous solution, benzene hexachloride in benzene and colloidal graphite in white spirit. Measured amounts of these substances were added to sodium, the sodium removed by distillation and the residual carbon determined.

Both sucrose and melamine gave consistently low recoveries, and a possible explanation might be the loss of carbon by gaseous evolution of a carbon compound. For benzene hexachloride, no reaction with sodium occurred, and the additions of colloidal graphite gave recoveries of poor reproducibility.

It was found that a dry mix of graphite and ammonium chloride was a satisfactory means of dispensing carbon in known amounts. The mix was prepared by adding 0·1 g of Reactor Grade "A" graphite (<300 mesh) to 100 g of AnalaR ammonium chloride, thoroughly grinding the mixture in a mortar and passing it through a 60-mesh sieve. The <60 mesh

fraction was re-ground and passed through a 100-mesh sieve. After the carbon content of the mix had been determined, the <100-mesh fraction was used for adding measured amounts of carbon to sodium.

The carbon content of the graphite - ammonium chloride mix was determined by weighing suitable amounts of the mix into prepared silica crucibles and removing the ammonium chloride by sublimation. The sublimation was carried out in a distillation pot at 400° C for 2 hours *in vacuo*; mechanical entrainment of the graphite in the ammonium chloride vapour was prevented by fitting the crucible with a loose silica lid. The residual graphite was determined by the combustion method previously described.

Standard additions of graphite to sodium were made by the same procedure, except that sodium was added to each crucible after the ammonium chloride separation and the carbon was determined after distillation of the sodium.

Results

Treatment of crucibles—

It was thought that the carbon blank value of the silica crucibles might be improved by an additional treatment to the preliminary heating at 1000° C. Consequently, several silica crucibles were immersed in a 20 per cent. hydrofluoric acid solution, thoroughly washed with water and compared with untreated crucibles. The results of this comparision show that there is no significant difference in the average carbon blank value, that of the untreated crucibles being 4·8 μg of carbon (standard deviation, $S = 2{\cdot}3$ μg) and that of the treated crucibles 3·3 μg of carbon ($S = 2{\cdot}1$ μg).

Acidification of distilled residue—

The poor reproducibility of the carbon results on a series of samples from the same sodium loop indicated that contamination of the distillation residue by atmospheric carbon dioxide might be occurring. Contamination could occur during the short transfer time for the crucible from distillation pot to combustion tube. Neutralisation of the sodium oxide in the residue with hydrochloric acid vapour would obviate this error, and a comparison of untreated and treated residues gave average carbon contents of 28 μg ($S = 8{\cdot}0$ μg) and 9·6 μg ($S = 3{\cdot}0$ μg), respectively.

It was apparent from these results that acid treatment of the distillation residue was necessary, and the acidification stage was incorporated in the analytical procedure.

Homogeneity of graphite - ammonium chloride mix—

A nominal graphite content of 100 μg per 0·1 g of graphite - ammonium chloride mixture was produced, and a series of carbon determinations made on the residues after sublimation of the ammonium chloride. The results on a series of 0·1-g samples of mixture indicated a satisfactory degree of homogeneity for the graphite - ammonium chloride mix, the average net weight of carbon found (after a crucible blank value deduction of 3 μg) being 82 μg ($S = 4$ μg).

Dispersion of graphite in sodium—

A satisfactory standard could only be obtained if it could be shown that dispersal of the graphite in the sodium was achieved. An experiment was therefore performed in which a piece of graphite (0·5-inch cube) was heated in 35 g of sodium at 250° C for a period of 48 hours, after which time the graphite was removed and the sodium cooled, re-melted and poured into silica crucibles.

The carbon residue in each crucible was determined by the normal procedure after removal of the sodium by distillation. Four such determinations were made, and the background carbon content of the sodium determined. The results obtained substantiated the assumption that graphite had been distributed through the sodium, the weights of carbon found in the four determinations being 118, 158, 189 and 165 μg per 7 g of sodium, and the background level of carbon in the sodium being 16 μg (average of four results).

Carbon standards—

The method was tested for accuracy at the three levels 3, 6 and 12 p.p.m. of carbon in sodium. The results are shown in Table I.

Discussion

The method described gives satisfactory reproducibility and accuracy for determining elemental carbon in sodium, as assessed by additions of the graphite - ammonium chloride mixture. It has been shown that the graphite will be suspended in the sodium during distillation and therefore is a reasonable representation of conditions under which carbon may be present in it.

Table I

Recoveries of added carbon at levels of 3, 6 and 12 p.p.m.

Level, p.p.m.	Weight of added graphite, μg	Background carbon in sodium, μg	Net recovery of carbon, μg
3	20	30 (7 results) $S = 9{\cdot}6$ μg	22 (10 results) $S = 3{\cdot}5$ μg
6	41	10 (7 results) $S = 3{\cdot}1$ μg	44 (10 results) $S = 4{\cdot}0$ μg
12	82	6 (6 results) $S = 2{\cdot}0$ μg	85 (10 results) $S = 3{\cdot}1$ μg

The method assumes that adequate facilities exist for the sampling of sodium into the silica crucibles. It is suitable for sodium and sodium - potassium mixtures, and, as both are highly reactive metals, sampling in an inert atmosphere is necessary to obviate contamination.

It has been suggested[6] that sodium oxide may react with carbon during the distillation to give either carbon monoxide or sodium carbonate, both of which would be subsequently lost. This would lead to a low carbon result, so the possibility of losses through such reactions has been investigated.

Loss of carbon as carbon monoxide—

At 527° C the equilibrium vapour pressure of carbon monoxide for the reaction—

$$Na_2O + C \rightleftarrows CO + 2Na$$

is $9{\cdot}08 \times 10^{-6}$ mm of mercury. This suggests that carbon monoxide formation and hence loss of carbon from the system, is unlikely, as was shown by the experiments described below—

Sodium (containing 500 μg of oxygen) and 1000 μg of lamp-black carbon (previously de-gassed for 36 hours at 1000° C) were heated at 550° C for 1 hour. Any evolved gas was collected and analysed for carbon monoxide and methane by means of a helium-ionisation chromatograph.[7] It was found that the carbon monoxide and methane produced were each equivalent to <1 p.p.m. of carbon, if a 7-g sample of sodium was assumed.

The sodium and lamp-black were heated for a further 4 hours at 550° C to simulate distillation conditions, and the evolved gases collected and analysed. Again, the carbon monoxide and methane found were equivalent to <1 p.p.m. of carbon for a 7-g sample. It was therefore concluded that loss of carbon as carbon monoxide and methane was not significant.

Conversion of carbon to carbonate—

If carbonate were formed during the distillation, it would react with the hydrochloric acid vapour at the acidification stage, producing carbon dioxide and leading to loss of elemental carbon. The likelihood of such a conversion was investigated by the experiment described below—

Lamp-black carbon (1000 μg) that had previously been de-gassed at 1000° C for 36 hours, and 6 g of sodium that had an oxygen content of 500 μg, were distilled for 5 hours at 550° C. The cooled crucible was removed in a carbon dioxide-free atmosphere and the carbonate in the distillation residue determined by an electrical conductivity method. It was found that the conversion of carbon to carbonate was <0·1 per cent. w/w. This result suggests that errors in the analysis of sodium for carbon are not caused by loss of carbon as carbonate.

Decomposition of carbides—

The most likely carbides to be found in the distillation residue are those of iron, chromium and calcium. These carbides decompose in the presence of hot hydrochloric acid vapour and do not contribute to the total carbon found by combustion.

Conclusion

A method has been described for determining free carbon in sodium. The accuracy and reproducibility of the method have been verified and found to be satisfactory for the range 3 to 12 p.p.m. of carbon.

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Colorimetric Determination of Copper in Plants

By K. R. MIDDLETON

(*Rubber Research Institute of Malaya, P.O. Box* 150, *Kuala Lumpur, Malaysia*)

A rapid and accurate method is described for determining copper by the colorimetric measurement of the complex formed between copper and biscyclohexanone oxalyldihydrazone. To apply the method to the analysis of plant material, dry ashing with subsequent digestion of the ash with aqua regia was employed; this procedure was found to be equal in accuracy and superior in speed and convenience to a more commonly used wet-ashing procedure.

The method is sensitive to 0·4 p.p.m. of copper in solution, and its analytical precision, expressed as a coefficient of variation, is 0·8 per cent. When applied to plant analysis, its precision is lower, and when averaged for three plant species, the error of a single determination on a given sample on any day is 2·0 per cent.

Copper is significant in plant nutrition, generally because of its function in enzymatic oxidation and reduction.[1] In this investigation, it is important because of its beneficial effect[2] on the nodulation of legumes, used in Malayan rubber plantations as cover crops, and also because of its postulated rôle in the biosynthesis of rubber hydrocarbon in the tree *Hevea brasiliensis*.[3]

A study of these problems demands a rapid, sensitive and specific method for determining copper; for this purpose the reagent biscyclohexanone oxalyldihydrazone seemed suitable, particularly because of its insensitivity to cations other than copper in plant ash.[4,5] Procedures for using the reagent have been recently proposed,[4,5] but preliminary tests showed that modifications were required to improve accuracy and precision.

To determine traces of elements in plants, digestion with concentrated acids (wet ashing) is usually preferred to dry ashing,[6] but dry ashing is economical in time, apparatus and reagents. Copper determinations involving both ashing methods were therefore compared for leaf samples of three different materials: *Hevea brasiliensis* and two legumes, *Pueraria phaseoloides* and *Calopogonium mucunoides*. This investigation has confirmed previous work[7] that indicated that wet and dry ashing could be made equally effective; the latter is therefore recommended for determining copper by the proposed procedure.

Method

Reagents—

All reagents should be of analytical-reagent grade.

Neutral red indicator solution, 0·003 per cent. w/v, aqueous.

Alkaline citrate reagent—Prepare a 2 N solution of sodium citrate in 2 N sodium hydroxide.

Ammonium acetate reagent—Prepare a 0·4 N solution of ammonium hydroxide in 1·6 N ammonium acetate. Adjust the pH of this solution to 9·1 by adding ammonia solution or acetic acid as required.

Biscyclohexanone oxalyldihydrazone reagent, 0·5 per cent. w/v, in 50 per cent. v/v ethanol.

Aqua regia—To 50 volumes of hydrochloric acid, sp.gr. 1·18, add 25 volumes of nitric acid, sp.gr. 1·42, and 25 volumes of water.

Procedure—

Ashing and digestion—Weigh a dry, finely ground, sub-sample of plant material, containing from 0·02 to 0·06 mg of copper, into a 60-ml silica dish (when contamination is suspected, the dish should be cleaned by thoroughly digesting it with hot aqua regia). After the material has been carefully charred on a hot plate, heat the dish in a muffle furnace at a temperature of 550° to 600° C until a uniformly white ash is obtained (2 hours' heating usually suffices). Remove the dish from the furnace, add 5 ml of aqua regia to its cooled contents and evaporate the solution to dryness on a boiling-water bath. Then add 10 ml of 2·5 N nitric acid and allow the mixture to digest on the bath for 30 minutes. Filter the warm digest into a 50-ml calibrated flask, wash the dish, the residue and the filter several times with small amounts of hot water, taking care not to exceed a final volume of 30 ml.

Determination of copper—Add 1 ml of neutral red indicator solution to the calibrated flask, neutralise the contents with the alkaline citrate reagent and add 5 ml of ammonium acetate reagent. Allow the mixture to cool, add 1 ml of biscyclohexanone oxalyldihydrazone reagent, swirl the solution thoroughly and dilute it to 50 ml with water. After 30 minutes, measure the optical density of the solution at 600 mμ in a 4-cm cell, by using a photo-electric absorptiometer and setting the instrument against a reference cell, filled with the solution from a blank determination. Although this technique automatically applies a blank correction, the actual blank value should be measured against water in the reference cell, as a precaution against contaminated reagents or apparatus.

Calibration—A test of the above procedure on standard solutions of copper sulphate showed that the concentration of copper, in parts per million, in solution was linearly related to the optical density, E, of the biscyclohexanone oxalyldihydrazone - copper complex, as shown below—

Copper content = 0·007 ($\pm$0·004) + 1·203 ($\pm$0·005) E; r = 0·9999 (14 degrees of freedom)

The highly significant nature of this least-square regression shows that the Beer - Lambert law is obeyed, and since an examination of residual error, as described by Middleton and Westgarth,[8] also showed that it did not differ significantly from a straight line passing through the origin, a simplified equation, copper content = 1·212 E (600 mμ), may be used for the concentration range 0·4 to 1·2 p.p.m. in solution.

Results

Variation in the colour intensity of the biscyclohexanone oxalyldihydrazone - copper complex with pH was studied by adding increasing amounts of concentrated sodium hydroxide solution to acidified solutions of copper sulphate in the presence of ammonium citrate. Colours were developed in 50-ml volumes by the procedure described above, and the buffering capacity of the same amount of ammonium citrate in the presence of an excess of sodium hydroxide was also measured. The results of both investigations are summarised in Fig. 1.

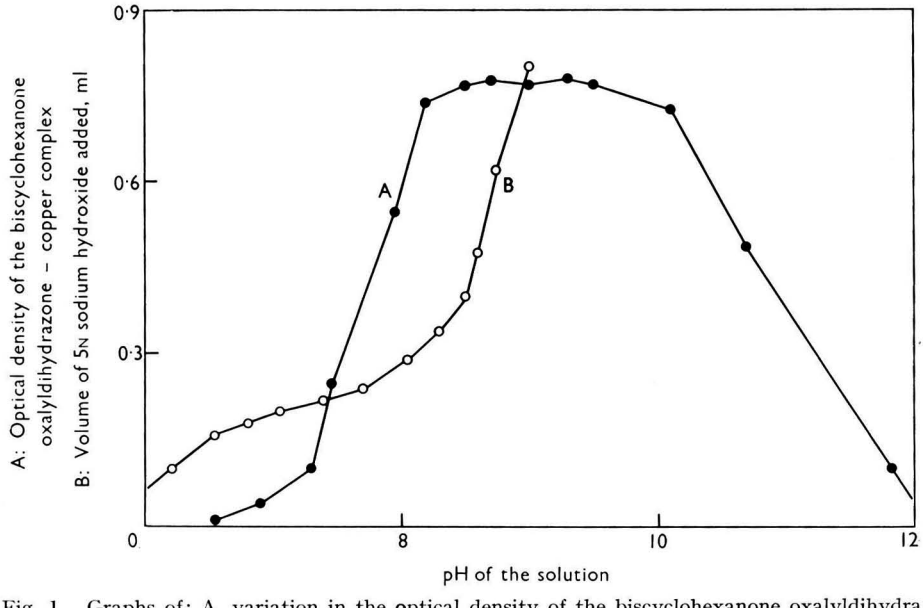

Fig. 1. Graphs of: A, variation in the optical density of the biscyclohexanone oxalyldihydrazone - copper complex with pH; B, the buffering capacity of ammonium citrate

The effect of variation in the concentration of ammonium ions on the speed of development and subsequent stability of the coloured biscyclohexanone oxalyldihydrazone - copper complex, at a constant pH and a constant concentration of citrate ions in the solution, is shown in Fig. 2.

A comparison of wet ashing, as recommended by Gorsuch,[6] with dry ashing, as proposed in this paper, was carried out on six different plant samples. The mean values of results

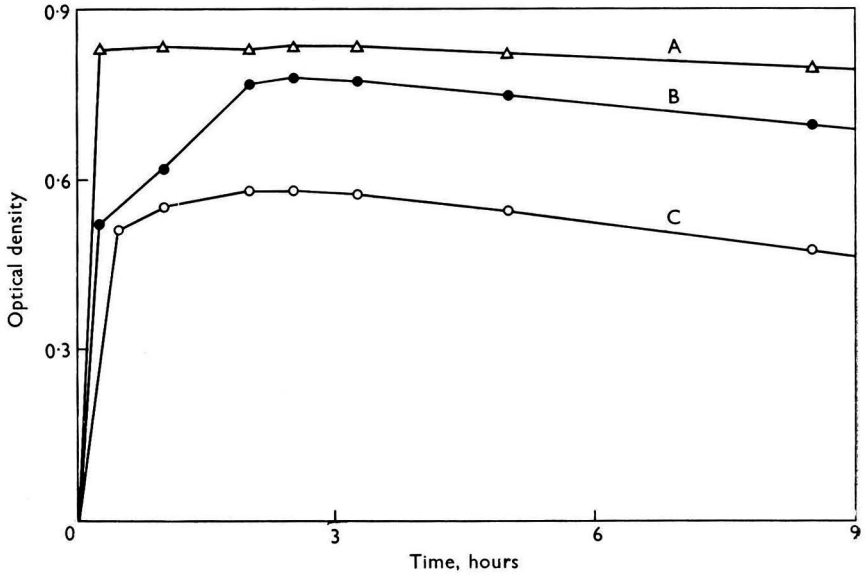

Fig. 2. Stability and speed of development of the biscyclohexanone oxalyldihydrazone - copper complex, as shown by variation of optical density with time: curve A, 0·5 N sodium citrate in 0·08 N ammonium acetate, pH 9·3; curve B, 0·5 N sodium citrate, without ammonium acetate, pH 9·2; curve C, 0·5 N sodium citrate in 2·4 N ammonium acetate, pH 9·4

for the determinations of copper are given in Table I, together with a summary of a statistical examination of individual results.

TABLE I

COPPER IN *Hevea brasiliensis*, *Pueraria phaseoloides* AND *Calopogonium Mucunoides*: COMPARISON OF DRY WITH WET ASHING

Values within the Table are means of duplicate determinations. The minimum significant difference is the minimum difference between corresponding means, significant at the 5 per cent. level

		Copper content, p.p.m. of oven-dry material, of—					
		Hevea brasiliensis		*Pueraria phaseoloides*		*Calopogonium mucunoides*	
Sample	Day	Wet ashing	Dry ashing	Wet ashing	Dry ashing	Wet ashing	Dry ashing
A	1	11·2	11·4	16·1	16·0	12·4	12·7
	2	11·2	11·5	16·0	16·2	12·5	12·4
B	1	16·4	16·3	23·7	23·2	20·9	20·3
	2	15·6	15·9	23·0	23·8	20·4	20·8
Minimum significant difference		0·4		0·4		0·4	
Mean values* ..		13·6	13·8	19·7	19·8	16·6	16·6
Minimum significant difference		0·4		0·4		0·4	

* In the comparison of the mean values, the error term used contained significant interactions and was therefore larger than the error between duplicates used for other comparison in the Table.

The recovery of varying amounts of copper from prepared solutions, containing salts of potassium, magnesium, calcium, manganese, iron, aluminium, boron, zinc and molybdenum (in the proportions in which they occur in leaves), was investigated in a 4 × 5 factorial

experiment, the determination being made in duplicate on two separate days. A summary of a statistical examination of individual results is given in Table II. The concentrations of the elements listed above are in multiples, from the lowest amounts normally found in *Hevea brasiliensis* (level 1) to the highest amounts expected for *Pueraria phaseoloides* and *Calopogonium mucunoides* (level 4); they therefore cover the range of possible interference for these three plants.

TABLE II

PERCENTAGE RECOVERY OF COPPER FROM PREPARED SOLUTIONS CONTAINING SALTS OF POTASSIUM, MAGNESIUM, CALCIUM, MANGANESE, IRON, ALUMINIUM, BORON, ZINC AND MOLYBDENUM

The elements are in the proportions in which they occur in plant leaves. Results within the Table are means of four determinations and the minimum significant difference is the minimum difference between corresponding means, significant at the 5 per cent. level

	Percentage recovery of copper						
	Concentration of the listed elements						
Copper added, p.p.m.	level 0	level 1	level 2	level 3	level 4	Means	Minimum significant difference
0·3	98·5	98·0	102·0	97·0	98·5	98·8	
0·6	100·8	100·3	101·3	99·6	100·1	100·4	0·5
0·9	100·3	100·2	100·5	99·5	99·0	99·9	
1·2	99·7	99·3	99·7	98·1	98·2	99·0	
Minimum significant difference.. ..	..	..	..	1·1			
Mean values* ..	99·8	99·5	100·9	98·5	99·0		
Minimum significant difference.. ..	..	..	..	1·8			

* In the comparison of mean values for concentration levels, the error term used contained a significant day × level interaction, and was therefore larger than the error term used for other comparisons in the Table.

The degree of bias in results obtained by the proposed method was studied by analysing increasing weights of plant material as suggested by Youden[10]; the results are summarised in Fig. 3.

DISCUSSION

For the coloured complex formed between copper and biscyclohexanone oxalyldihydrazone, the pH and concentration of ammonium ions needed for maximum colour intensity, speed of development and subsequent stability, have been discussed by other workers,[4,5,9] but not precisely defined. In the procedure described above, maximum colour intensity is attained between pH 8·5 and 9·5 (see Fig. 1), and although sodium borate has been proposed[5] as a means of controlling pH, it may be easily shown by experiment that ammonium salts have a more potent buffering effect; ammonium citrate is particularly effective (see Fig. 1) over the specified pH range.

The presence of citrate ions as chelating agents is essential to prevent turbidities forming in the alkaline solution, and citrate ions are most effective when added before or during neutralisation. For this reason, prior addition of ammonium citrate has been suggested,[5] but if this is done, the buffering effect of ammonium ions obscures the end-point and makes adjustment of pH within the specified range difficult. A more satisfactory procedure involves the introduction of citrate ions during neutralisation of the acid-digested ash and, after the end-point has been reached, the addition of an ammonium acetate buffer solution to ensure that a final pH within the range 8·5 to 9·5 is attained. For a 5-g sample of the materials used in this investigation, a minimum final citrate concentration of 0·3 N is needed; however, the final concentration may be regulated to suit different conditions, by varying the amount of nitric acid used to digest the ash, and hence the amount of citrate ions introduced during neutralisation.

Fig. 2 shows that in the presence of a small amount of ammonium ions (curve A) the colour complex had formed completely in 15 minutes and remained stable for 3 hours, but in the

absence of ammonium ions (curve B) and in the presence of an excessive amount of ammonium ions (curve C), colour development was slow and incomplete, and in both instances the stability of the complex was appreciably less. Fig. 2 summarises a more detailed investigation that showed that the optimum range for the final ammonium ion concentration was from 0·08 to 0·40 N; this study was made with the concentration of citrate ions constant, but varying the amount of citrate separately did not have a detectable effect on colour development and stability.

COMPARISON OF DRY WITH WET ASHING—

For the accurate determination of copper in plant material, organic matter must be completely destroyed and the element totally released in an ionic form. For this purpose, wet ashing is generally preferred to dry ashing, on the grounds that copper is immobilised during the dry-ashing process. In a detailed investigation, Gorsuch[6] concluded that wet

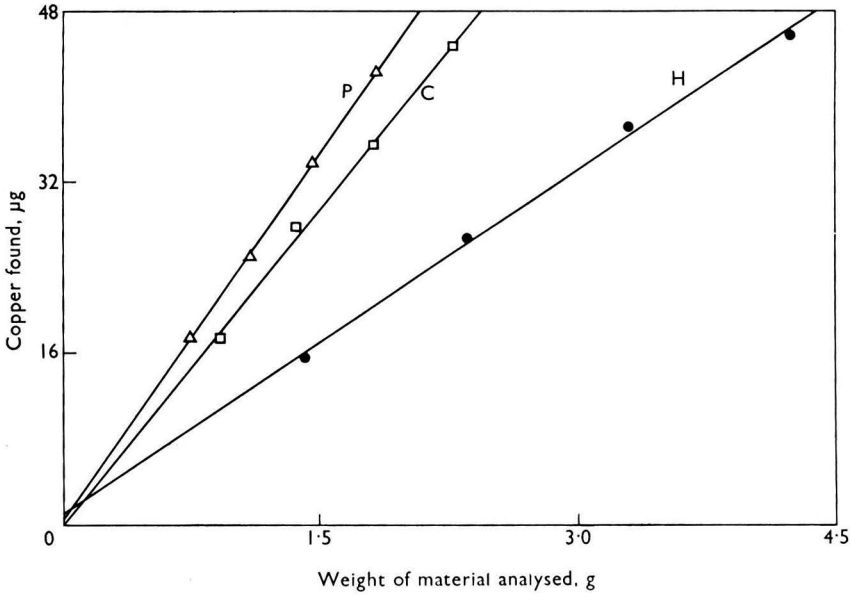

Fig. 3. Regression for relation between amount of copper found and amount of material used, for three different plants. Results shown are means of two determinations on oven-dry material

Curve	Material	Relation between copper found, µg, (y) and weight of sample taken, g, (x)	Correlation coefficient, r	Degrees of freedom
P	*Pueraria phaseoloides*	$y = 0{\cdot}27\ (\pm 0{\cdot}65) + 22{\cdot}88\ (\pm 0{\cdot}48)\ x$	0·999	6
C	*Calopogonium mucunoides*	$y = 0{\cdot}07\ (\pm 0{\cdot}91) + 19{\cdot}63\ (\pm 0{\cdot}44)\ x$	0·998	6
H	*Hevea brasiliensis*	$y = 0{\cdot}97\ (\pm 0{\cdot}84) + 10{\cdot}74\ (\pm 0{\cdot}28)\ x$	0·998	6

ashing was more generally satisfactory, but he did not preclude the possibility of dry ashing being satisfactory under certain conditions; and it has been shown for certain plant materials[7] that subsequent treatment with aqua regia prevented losses often associated with dry ashing.

A comparison of mean values in Table I also shows that, provided the dry ash is digested with aqua regia, wet and dry ashing give similar results for copper determined by the proposed procedure. This conclusion was confirmed by a statistical examination of the regression of copper determined by dry ashing on copper determined by wet ashing, in which the highly significant least-square regression obtained was shown not to differ significantly from a straight line of unit slope drawn through the origin.

ACCURACY OF THE METHOD—

A statistically designed procedure for detecting bias in an analytical method has already been fully discussed in relation to the determination of iron and aluminium in plants[11]; a similar procedure has been followed in this investigation.

Fig. 3 summarises the results obtained by analysing varying weights of three different plant materials. The standard errors of parameters, obtained by regressing the amount of copper found on the weight of material taken for analysis, indicate that the regression lines do not differ significantly from straight lines passing through the origin. This was confirmed by a statistical examination of the residual error in the area of the regression line, by using a procedure already described,[8] and is a demonstration that there is no constant bias. If there were constant bias, the lines would make significant intercepts on the ordinate axis; but the absence of such intercepts does not preclude the possibility of variable bias, proportional to the amount of material analysed.

However, the results summarised in Table II show that, for concentrations of copper above 0·3 p.p.m. in solution, elements from the leaf ash of plants do not interfere seriously in the determination of copper, since recoveries of 98 per cent. or more were obtained. This is a demonstration that neither constant nor variable bias occurred to an important extent in analyses of prepared solutions corresponding in composition to the ash of the three plant materials. When considered in conjunction with the test summarised in Fig. 3, this finding indicates the absence, for practical purposes, of both constant and variable bias in determinations of copper by the proposed method.

An examination of Table II shows that significantly low recoveries were frequently obtained when the concentration of copper in solution was less than 0·6 p.p.m.; this points to a limit in the sensitivity of the reagent, and a more detailed investigation of this point (not recorded in this paper) indicated that 0·4 p.p.m. was the practical limit.

PRECISION OF THE METHOD—

The overall precision of an applied analytical method may be defined as the error associated with a single determination on a given sample on any day.[11] This error covers analytical, weighing, sub-sampling, ashing and day components, and of these, the weighing component is normally extremely small and may be ignored.

In the proposed method, although overall day means were similar, means for individual samples and materials (Table I) often differed significantly from day to day. A statistical examination of the individual results summarised in Table II also provided an estimate of day error, in this instance associated with the analytical part of the procedure only. This estimate, though significant, was relatively small, suggesting that the day error shown in Table I contained an additional component associated with the sub-sampling and ashing operations. For determinations made on plant material (Table I), analytical sub-sampling and ashing components were unavoidably confounded in the replicate error, but for determinations made on prepared solutions (Table II) the replicate error was an estimate of analytical variability only.

From statistical analyses of individual results, the various error components discussed above have been computed as coefficients of variation, *i.e.*, $\frac{100 \times \text{standard error}}{\text{mean values obtained}}$, and they are as given below—

Analytical error, per cent.	0·8
Analytical error *plus* sub-sampling error, per cent.—	
For *Hevea brasiliensis*	1·6
For *Pueraria phaseoloides*	1·2
For *Calopogonium mucunoides*	1·0
Error of a single determination on a given sample on any day, per cent.—	
For *Hevea brasiliensis*	2·7
For *Pueraria phaseoloides*	1·8
For *Calopogonium mucunoides*	1·7

The error terms from which these estimates were computed were not found to be significantly different, and were therefore pooled to give 2·0 per cent. as an average estimate, for the three materials, of the error of a single determination on a given sample on any day.

It should be noted that in all instances the analytical error is, as expected, smaller than the combined errors, and is somewhat lower than that normally anticipated for a colorimetric method.[12]

Conclusions

A rapid colorimetric method for determining copper has been described and, by an appropriate statistical study, has been shown to give accurate results when applied to analysis of plants. Determinations, made after a modified procedure had been used for bringing copper into solution, were found to give results similar to those obtained by using a more usual wet-ashing procedure.

Both analytical and overall precision were satisfactory; a relatively small day variation in results was associated both with the sub-sampling and ashing of plant material, and also with the analytical part of the method.

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SHORT PAPERS

A Modified Method for Determining Traces of Nitrilotriacetic Acid in Ethylenediaminetetra-acetic Acid

By R. N. P. FARROW and A. G. HILL

(*The British Drug Houses Ltd., B.D.H. Laboratory Chemicals Division, Poole, Dorset*)

Commercial ethylenediaminetetra-acetic acid (EDTA) usually contains traces of nitrilotriacetic acid (NTA), which has an adverse effect on the use of EDTA in complexometric titrations. NTA causes variations between factors obtained by standardising the EDTA against different metals and also has an adverse effect on the quality of the indicator colour change.[1]

Daniel and LeBlanc have published an elegant polarographic procedure for determining NTA.[2,3] This method is based on the addition of an excess of cadmium ion at pH 7 to 10 with subsequent polarography. The polarographic wave for the cadmium - NTA complex precedes that for the cadmium - EDTA complex and is easily measured.

This method has now been simplified and adapted to make it more suitable for occasional use. It has been found unnecessary to use potassium nitrate as supporting electrolyte. The method of adding the correct amount of cadmium can be simplified by titrating an aliquot; this obviates the need for a pH meter and is much quicker. The standard-addition method of calculating results is more suitable for occasional use than a calibration curve. Since the addition of NTA may require more cadmium than is present in the solution, it is safer to add the NTA in the form of the cadmium complex.

By using a K1000 cathode-ray polarograph (Southern Analytical Ltd.) together with an increased sample weight, the sensitivity of the method can be increased by a factor of at least 10, and NTA contents down to 20 p.p.m. can be determined.

Method

Reagents—

Cadmium chloride solution—Dissolve 50 g of hydrated cadmium chloride in water and dilute the solution to 1 litre.

Ammonia - ammonium chloride buffer solution, pH 10—Dissolve 67·5 g of ammonium chloride and 570 ml of ammonia solution, sp.gr. 0·88, in sufficient water to produce 1 litre.

Standard NTA solution—Dissolve 1 g of NTA in water with the addition of sufficient 5 N sodium hydroxide to give a clear solution and dilute it to 100 ml in a calibrated flask. Titrate a 50-ml aliquot with cadmium chloride in the way described under "Procedure" and add the required amount of cadmium chloride solution to the remaining 50 ml in the calibrated flask. Adjust the pH of the solution to about 8 and dilute the solution to 100 ml.

1 ml of solution ≡ 5 mg of NTA.

Preparation of the sample—

Technical-grade samples often contain ethylenediamine-*NN'*-diacetic acid and another unidentified impurity, both of which interfere with the determination. These impurities must be removed by acid hydrolysis by using the method of LeBlanc.[3]

When extremely pure samples are examined, a larger sample weight must be used. Up to 50 g can be taken, but sufficient sodium hydroxide must be added to form the tetrasodium salt of EDTA. In these instances, slightly less than the theoretical amount of solid cadmium chloride should be added during the preparation of the sample solution.

Procedure—

Dissolve 1 g of EDTA in water (with the addition of 5 N sodium hydroxide for the acid) and dilute the solution to 100 ml. Remove a 50-ml aliquot with a dry pipette and dilute this with about 100 ml of water. Wash the pipette out into the original calibrated flask. Treat the aliquot with 10 ml of ammonia - ammonium chloride buffer solution and a little Solochrome black T. Titrate the solution with cadmium chloride until the indicator turns red, and note the volume used. Add the same volume of cadmium chloride solution *plus* 0·1 ml in excess to the contents of the calibrated flask. Adjust the pH of the solution to about 10 with 5 N sodium hydroxide. The

solution should now be slightly hazy, indicating that an excess of cadmium is present. Dilute the solution to 100 ml and transfer 2·5 ml to the dry cell of a K1000 cathode-ray polarograph. Deoxygenate the solution in the usual way, and begin polarography with a start potential in the region of −0·65 volts. Add a suitable amount of standard NTA solution by means of a micro-syringe, and determine the NTA content by the standard-addition technique.[4]

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Interference of Ammonium Ions in the Determination of Reducing Sugars by the Colorimetric Method of Somogyi and Nelson

BY H. FALANGHE

(*Department of Chemistry, Instituto Zimotécnico, Escola Superior de Agricultura "Luiz de Queiroz," University of São Paulo, Piracicaba, São Paulo, Brazil*)

THE method of Somogyi[1] and Nelson,[2] based on the reduction of cupric ions by glucose and originally developed for determining glucose in blood, is now widely used for determining reducing sugars.

In the course of a research project in this Department in which reducing sugars were being determined by the method of Somogyi and Nelson, it was observed that the results suffered from the interference of ammonium salts present in the solutions being analysed. This occurred whenever the ratio of the concentration of ammonium ions to that of the sugars was 1 to 6 or greater, unless a careful exclusion of air was made during the reaction. The cuprous oxide precipitated during the determination of glucose is readily re-dissolved when ammonium ions are present,

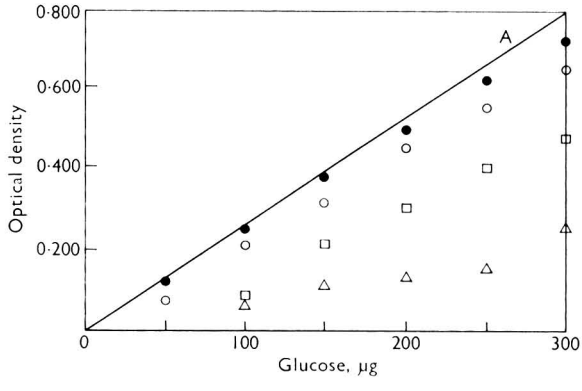

Fig. 1. Effect of added ammonium ions (as ammonium tartrate) on the optical density at 520 mμ of glucose solutions. Curve A, standard curve for glucose; and—

Points	Addition of ammonium ions per 2 ml, μg
●	50
○	500
□	1000
△	1500

and consequently re-oxidation of the dissolved precipitate is readily effected. The overall effect is that the amount of molybdenum blue formed decreases as the concentration of ammonium ions in the solution increases, as seen in Fig. 1, which shows that the optical density corresponding to

the amount of molybdenum blue formed decreases with the increase of ammonium ion concentration in the glucose solutions. Table I shows the results obtained when the method was applied to solutions containing different ratios of ammonium ion concentration to sugar concentration. Further, it can be shown that ammonium ions present in the ratio of one part of ammonium ions to six parts of sugar in the ammonium salt - sugar solutions affect the result, at least at the upper limit of the method (300 μg of glucose). It can also be seen that when the upper limit of the method is approached, the difference between the amount of sugar present and that found are greater. In spite of this, the percentage error is generally greater when the glucose concentraton is 50 μg per 2 ml, *i.e.*, approaching the lower limit of the method.

TABLE I

RECOVERY OF GLUCOSE IN THE PRESENCE OF AMMONIUM SALTS BY THE METHOD OF SOMOGYI AND NELSON

Glucose present, μg per 2 ml	Glucose found,* μg per 2 ml, in the presence of—											
	50 μg of ammonium ions per 2 ml			500 μg of ammonium ions per 2 ml			1000 μg of ammonium ions per 2 ml			1500 μg of ammonium ions per 2 ml		
	(*a*)	(*b*)	(*c*)	(*a*)	(*b*)	(*c*)	(*a*)	(*b*)	(*c*)	(*a*)	(*b*)	(*c*)
50	40	46	42	26	24	30	0	0	0	0	0	0
100	86	94	92	62	80	60	20	30	62	16	20	26
150	134	144	146	118	118	124	72	78	108	36	40	36
200	178	186	190	160	168	180	120	114	134	50	50	42
250	232	228	230	206	204	210	132	150	196	76	54	48
300	266	270	272	248	242	252	186	178	242	86	96	92

(*a*) Ammonium was present as ammonium sulphate.
(*b*) Ammonium was present as ammonium tartrate.
(*c*) Ammonium was present as ammonium acetate.
* Each result is the mean of two determinations.

It was observed in this Department that ammonium sulphate, ammonium acetate and ammonium tartrate are partially removed from the sugar solutions by deproteinisation with zinc sulphate and sodium hydroxide,[3] and that this treatment decreases the interference of ammonium ions. However, a detailed study of the removal of these salts and of the eventual removal of other ammonium compounds by deproteinisation was not made. A simpler solution to the problem of the interference was accomplished by passing the reducing sugar - ammonium salt solutions through a column (60 mm × 12-mm internal diameter) of Folin Decalso (Decalso F) before the sugars were determined. The effectiveness of this preliminary treatment on the removal of the interfering ammonium ions is demonstrated by the results given in Table II.

TABLE II

OPTICAL DENSITIES OF GLUCOSE SOLUTIONS CONTAINING AMMONIUM SALTS*

Amount of ammonium ions added to 200 μg of glucose per 2 ml	Salt present	Optical density† at 520 mμ
Nil	—	0·535
1500	Ammonium sulphate	0·540
1500	Ammonium acetate	0·530
1500	Ammonium tartrate	0·535

* Determined by the method of Somogyi and Nelson after preliminary treatment of the solution in a column of Folin Decalso.
† Results are the mean of four determinations.

I thank Dr. P. A. Bobbio for his helpful discussions and Miss T. D. Nogueira for her technical assistance.

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A Method for Determining *N*-Hydroxyurethane for Use in Metabolic Studies

By S. S. MIRVISH

(*Department of Experimental Biology, The Weizmann Institute of Science, Rehovoth, Israel*)

Several of the carcinogenic aromatic amines have been shown to be oxidised *in vivo* to the corresponding hydroxylamines, and these may well be the biologically active agents.[1] It was suggested that the carcinogen urethane (NH_2.CO.OEt) exerts its effects after a similar conversion to *N*-hydroxyurethane (HONH.CO.OEt), which is itself carcinogenic,[2,3] and such a conversion was subsequently reported in the rat.[4] This paper describes a method for determining *N*-hydroxyurethane for use in studying the metabolism of both it and urethane. Similar procedures may also be applied to related *N*-hydroxy compounds, *e.g.*, the anti-cancer agent hydroxyurea (private communication from Dr. F. S. Philips, Sloan-Kettering Institute, New York).

Experimental

Determination of *N*-hydroxyurethane in pure solution—

Aqueous solutions of *N*-hydroxyurethane show ultraviolet-absorption maxima at 208 mμ (molar extinction coefficient, $\epsilon = 220$) in 0·1 N hydrochloric acid and at 217 mμ ($\epsilon = 3800$) in 0·05 N sodium hydroxide. The relatively strong absorption in alkaline solution may be used as a method of analysis, but the absorption is rather unstable, decreasing about 1 per cent. per minute, and many substances would interfere by absorbing at similar low wavelengths.

Methods for analysing the related hydroxamates (*R*.CO.NH.OH) were then examined. The common method, which makes use of the purple complex with ferric ions, was not applicable to *N*-hydroxyurethane, owing to the weak colour of the complex. Thus, with a ferric perchlorate reagent,[5] *N*-hydroxyurethane showed maximal absorption at 555 mμ ($\epsilon = 23$), compared with the absorption maxima at 500 to 530 mμ ($\epsilon = 700$ to 1300) reported for hydroxamates.[5]

The sensitive method of Bergmann and Segal[6] involves direct oxidation of hydroxamates with iodine to give nitrite, and subsequent diazo coupling. Prior hydrolysis to hydroxylamine is not necessary, though free hydroxylamine is also determined. When this method, as modified by Seifter, Gallop, Michaels and Meilman,[5] was applied directly to *N*-hydroxyurethane, the results were about 40 per cent. of those obtained for equivalent amounts of hydroxylamine, showing that *N*-hydroxyurethane is less easily oxidised by iodine than are hydroxamates. However, when *N*-hydroxyurethane was first heated with 7 N hydrochloric acid for 90 minutes at 100° C to hydrolyse it to hydroxylamine, and the procedure of Seifter *et al.* then modified by adding an excess of sodium acetate to neutralise the hydrochloric acid, the results rose to 97·3 per cent. of the values obtained for equivalent hydroxylamine control samples. The standard procedure gave a linear standard curve for amounts between 0·1 and 0·5 μmoles (*i.e.*, 10·5 and 52·5 μg) of *N*-hydroxyurethane in the initial solution of 1·0 ml.

A check that the hydrochloric acid treatment did in fact hydrolyse the *N*-hydroxyurethane to hydroxylamine was provided by the use of thin-layer chromatography, which showed the disappearance of the *N*-hydroxyurethane spot ($R_F = 0{\cdot}50$) after hydrolysis, and the appearance of a spot corresponding to hydroxylamine at the base-line. The chromatography was carried out on kieselguhr with a benzene - ethyl acetate (1 + 1) mixture as the solvent, and the spots were treated with iodine solution. This resulted in the formation of a brown colour, which faded rapidly from the hydroxylamine and hydrolysate spots.*

The *N*-hydroxyurethane was heated in the routine method with 7 N hydrochloric acid for 90 minutes, since the results were found to be maximal after heating for 60 to 180 minutes. After similar treatment of 0·5 μmoles of hydroxylamine for 90 minutes, the recovery was 93·0 per cent. as compared with that of unheated control samples. After a heating period of 180 minutes, 83 per cent. of the hydroxylamine was recovered. Thus the hydroxylamine liberated from the *N*-hydroxyurethane was slowly destroyed by the hot hydrochloric acid, but not quickly enough to affect the usefulness of the method. Under the conditions used (pH = 3·4) the results were maximal after oxidation of hydroxylamine with iodine for 2 to 10 minutes, as under the conditions of Bergmann and Segal[6] (pH = 4·3).

* The chromatographic system and the means of detection were kindly recommended by Dr. A. Bendich, Sloan-Kettering Institute, New York.

Pure *N*-hydroxyurethane was kept for months in air at 0° C with little or no change in the refractive index or the ultraviolet absorption in alkaline solution, demonstrating the stability of the pure compound. A 0·01 M solution of *N*-hydroxyrethane was unaffected by incubation for 18 hours at 37° C, as shown by the iodine-oxidation and ultraviolet-absorption methods.

ANALYSIS OF *N*-HYDROXYURETHANE - HYDROXYLAMINE MIXTURES—

The differential determination of *N*-hydroxyurethane and hydroxylamine is necessary, for example, to detect hydrolysis of the former compound. In the procedure of Yashphe, Halpern and Grossowicz,[7] mixtures of hydroxamates and hydroxylamine are analysed by making use of the fact that, at a pH of 2·3 and at temperatures between 10° and 15° C, hydroxamates are almost unattacked by iodine whereas hydroxylamine is rapidly oxidised. This is in contrast to the conditions of Bergmann and Segal (pH = 4·3), under which both are attacked. After treatment of *N*-hydroxyurethane with iodine at pH 2·2 and 0° C for 10 minutes (without prior hydrolysis with hydrochloric acid), the final colour was found to be only 2 to 4 per cent. of that given by hydroxylamine control samples. Colour development was more rapid at room temperature than at 10° to 15° C as recommended. Six analyses on each of three mixtures of *N*-hydroxyurethane and hydroxylamine (in ratios of 3 + 1, 1 + 1 and 1 + 3), gave overall recoveries of 96·1 per cent. for *N*-hydroxyurethane and 100·3 per cent. for hydroxylamine.

TABLE I

RECOVERY OF *N*-HYDROXYURETHANE AND HYDROXYLAMINE FROM VARIOUS SOLUTIONS

Compound determined	Solutions from which the compound was recovered	Standard used*	Procedure used	Number of analyses	Recovery of compound (mean ± standard deviation), per cent.
N-Hydroxyurethane	pure solution	hydroxylamine	(*i*)	14	97·3 ± 3·4
Hydroxylamine	pure solution	hydroxylamine (not heated with hydrochloric acid)	(*i*)	20	93·0 ± 4·0
N-Hydroxyurethane	*N*-Hydroxyurethane - hydroxylamine mixtures	*N*-hydroxyurethane	(*ii*)	18	96·1 ± 9·4
Hydroxylamine		hydroxylamine	(*ii*)	18	100·3 ± 6·5
N-Hydroxyurethane	horse serum	*N*-hydroxyurethane (in pure solution)	(*iii*)	16	99·0 ± 6·1
N-Hydroxyurethane	mouse blood	*N*-hydroxyurethane (in pure solution)	(*iii*)	15	93·4 ± 3·8
N-Hydroxyurethane	liver homogenate	*N*-hydroxyurethane (in pure solution)	(*iv*)	15	95·3 ± 5·5

* Standards were treated in the same manner as the sample solutions.

DETERMINATION OF *N*-HYDROXYURETHANE IN SERUM AND BLOOD—

When the procedure for determining *N*-hydroxyurethane was applied directly to serum or blood containing this compound, the recovery was only 60 to 80 per cent.; but after precipitation of the proteins with trichloroacetic acid, the recovery rose to 99·0 per cent. for horse serum and 93·4 per cent. for fresh mouse blood. The trichloroacetic acid treatment was therefore included in the standard procedure for blood, which then showed a linear standard curve for amounts of between 0·4 and 2 μmoles (42 and 210 μg) of *N*-hydroxyurethane in the initial solution (containing 0·5 ml of blood). The recovery was unaffected by incubation of the *N*-hydroxyurethane with blood for 3 hours at 37° C before addition of the trichloroacetic acid. The sensitivity is adequate for measuring levels of *N*-hydroxyurethane in blood in carcinogenicity studies, as these have generally used doses of 0·5 to 1 mg per g of body weight,[2,3] *i.e.*, 250 to 500 μg of *N*-hydroxyurethane per 0·5 ml of blood (assuming even distribution of the *N*-hydroxyurethane).

DETERMINATION OF *N*-HYDROXYURETHANE IN LIVER HOMOGENATE—

The recovery of *N*-hydroxyurethane from fresh mouse-liver homogenate was only 65 to 75 per cent. after precipitation with trichloroacetic acid, as for blood. The recovery was similarly low when *N*-hydroxyurethane was added to aliquots of the supernatant liquid obtained after the trichloroacetic acid treatment, showing that the losses were caused by a reaction with the supernatant liquid and not with the precipitate. Both *N*-hydroxyurethane and hydroxylamine (0·5 μmole of each) showed 30- to 70-per cent. losses on being heated with hydrochloric acid in the presence of

glucose (50 μmoles), suggesting that the reacting components of the supernatant liquid were glucose and, more particularly, glycogen (the latter is not precipitated by trichloroacetic acid and could react after being hydrolysed to glucose by the hydrochloric acid), and that the hydroxylamine liberated by the hydrochloric acid hydrolysis, rather than the *N*-hydroxyurethane itself, was reacting. Treatment with aqueous trichloroacetic acid and subsequent addition of alcohol containing additional trichloroacetic acid, on the basis of model experiments that showed almost complete precipitation of glycogen, increased the recovery of *N*-hydroxyurethane to 95·3 per cent.

The procedure for *N*-hydroxyurethane - hydroxylamine mixtures was used to show that the trichloroacetic acid - alcohol treatment produces 20 to 30 per cent. hydrolysis of *N*-hydroxyurethane to hydroxylamine in the absence of liver, but this fact did not affect the recovery of *N*-hydroxyurethane from liver homogenates, as the hydroxylamine was apparently also determined. The principal results from the above determinations are given in Table I.

METHODS

Preparation of *N*-hydroxyurethane—

This compound was synthesised as described previously[8] and distilled under vacuum[9] to give an oil with the correct elemental analysis, $n_D^{20} = 1{\cdot}4490$ and density = 1·2200 g per ml at 25° C.

Procedures—

(*i*) *Determination of* N-*hydroxyurethane in pure solution*—Solutions of *N*-hydroxyurethane (0·1 to 0·5 μmoles) in 1 ml of water were heated with 2 ml of hydrochloric acid, sp.gr. 1·18, at 100° C for 90 minutes, and then diluted with water to 10 ml. To 2-ml aliquots were added 3 ml of 2 M sodium acetate, and the method was then continued as described by Seifter *et al.*[5] except that the colour was developed for 30 instead of 10 minutes.

(*ii*) *Analysis of* N-*hydroxyurethane - hydroxylamine mixtures*—The method of Yashphe, Halpern and Grossowicz[7] was used, except that the iodine oxidation was carried out at 0° C for 10 minutes, and the final colour was developed at room temperature for 30 minutes, without addition of hydrochloric acid.

(*iii*) *Recovery of* N-*hydroxyurethane from serum and blood*—One millilitre of 0·002 M *N*-hydroxyurethane was mixed with 0·5 ml of horse serum or heparin-treated mouse blood, and 0·5 ml of water. Three millilitres of 0·3 M trichloracetic acid were added. After centrifugation of the mixture, a 1-ml aliquot of supernatant fluid was analysed.

(*iv*) *Recovery of* N-*hydroxyurethane from liver homogenate*—To 0·2 ml of mouse-liver homogenate (prepared from 1 g of liver and 3 ml of 0·02 M phosphate buffer, pH 7·4) were added 0·2 ml of 0·01 M *N*-hydroxyurethane, 0·5 ml of water, and after mixing, 0·5 ml of M trichloracetic acid. After mixing again, 7 ml of ethanol containing 0·25 M trichloracetic acid were added, and the material was spun in a centrifuge. One millilitre of water was added to a 2-ml aliquot of supernatant fluid and, after evaporation at 95° C for 1 hour to remove the ethanol, the residual solution was analysed.

I thank Professor I. Berenblum for helpful advice, and Miss Ruth Plager for technical assistance. The investigation was supported by grant CA 05263 of the National Cancer Institute, United States Public Health Service.

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Book Reviews

HANDBOOK OF INDUSTRIAL INFRARED ANALYSIS. By ROBERT G. WHITE. Pp. xiv + 440. New York: Plenum Press. 1964. Price $19.50.

The realisation that infrared spectrometry is now a commonplace important tool in many industrial laboratories has recently encouraged several authors to produce text books based on experiences gained in their own field. This book has been written for the analytical chemist as an entirely practical manual with virtually no theoretical treatment. It has, therefore, "become almost a recipe book" for the man on the bench.

In common with similar volumes, the contents are divided into chapters dealing with: History; Theory and Terminology; Instrumentation; Technique; Qualitative Analysis; Quantitative Analysis; Applications; and Literature. There is no doubt that the subject matter is very well covered and will afford all workers a wealth of practical detail. It is particularly suited to the industrial analyst making first excursions into the field of infrared spectroscopy. He will find comprehensive information concerning the latest instruments and accessories; the manipulative instructions are particularly pertinent and embrace very recent advances. In the chapter on qualitative analysis, the diagnostic schemes commonly associated with such authors as Cross and Bellamy are fused with methods for storing and retrieving data to give a system that should materially assist the beginner in establishing his own diagnostic framework. The chapter on applications can only be regarded as a reference source to other detailed work, but the coverage is so vast that little more could be expected. In many sections the book gives the unpleasant impression of being an extended card-index system. Indeed, it is claimed that some 1600 references have been selected from a total of 4000, but perhaps inclusion in the script has over-emphasised the catalogue nature of the text. The value of this type of book is frequently enhanced by material included in ancillary chapters or in an appendix. In addition to several author-references pages and a general index, tables of reciprocals and optical density - transmittance conversions are very useful.

Annoyance may be felt with the apparent transparent quality of the paper, with some inferior freehand drawings and with photographs of actual spectra having smudged grey backgrounds.

It is difficult to assess the degree of levity permissible in a book of this kind. Does the author have to comment on the distress caused to housewives by spectroscopists purloining their hairclips or raiding their boudoirs for cleansing tissue? Most analysts regard frustration as an occupational hazard, but hardly expect invective and prayer to be offered as suggested solutions to their problems. These comments in no way attempt to decry the essentially practical value of the contents: it is a pity that its price precludes purchase by less affluent workers. W. L. SHEPPARD

MICRO-ANALYSIS IN MEDICAL BIOCHEMISTRY. By I. D. P. WOOTTON, Ph.D., M.A., M.B., B.Chir., F.R.I.C., M.C.Path. Fourth Edition. Pp. x + 254. London: J. & A. Churchill Ltd. 1964. Price 30s.

The earlier editions of this invaluable practical manual were written by the late Professor E. J. King alone or in collaboration with the present author, and have been widely used in clinical-chemistry laboratories throughout the world; indeed, there have been translations of the previous editions into Spanish, Italian, Serbo-Croat and Arabic.

The book contains up-to-date accounts of methods for almost every qualitative or quantitative chemical analysis that the laboratory may be called upon to perform, and the "dipper" and "dropper" tests are well represented. The introduction to volumetric analysis and the account of radioactive-isotope tests, which appeared in earlier editions, have been wisely dropped Any worker who would need to acquire experience in the techniques described must start with some knowledge of the fundamentals of volumetric analysis, and the application of radioactive isotopes to the analysis and the investigation of disease is now so extensive that it quite properly needs a separate manual. In addition to the routine methods described in previous editions, there are now new sections on the preparation and preservation of specimens, the assessment of acid - base balance in the body, a very brief account of high-temperature flame photometry for determining calcium and magnesium and a screening test and a simple chromatographic method for the detection and identification, respectively, of barbiturates. These last are particularly important because of the ever-increasing incidence of poisoning. Other new methods that have been introduced include the use of chromium sesquioxide as a faecal marker for balance studies, an account

of the quantitative analysis of renal calculi and the use of cellulose acetate for the electrophoresis of plasma proteins. The increased importance of the measurements of enzymes in the body fluids for the diagnosis of disease has resulted in a separate chapter. That iso-enzymes get no more than passing mention perhaps reflects the current attitude at the Post-graduate Medical School on their significance in the present state of knowledge as a diagnostic aid. The short chapter on steroids is sensible and limited to the determination of urinary 17-oxosteroids and 17-oxogenic steroids, plasma 11-hydroxycorticosteroids and urinary pregnanetriol. Too many practical manuals of clinical chemistry include complicated procedures that are the province of the highly specialised laboratory rather than the general clinical-chemistry department. Professor Wootton has played an important part in introducing quality control, and the new section dealing with this will bring home to workers the need for regular internal checks of accuracy of the methods used.

The Chemical Pathology Department at the Post-graduate Medical School trains numerous pathologists and clinical chemists from overseas. It is appropriate, therefore, that the methods used in that department and described in this book should as far as possible not only be above reproach, but also make use of readily available apparatus and equipment. Nevertheless, it has been necessary to include in the present edition an account of automatic analysis, which has become indispensable in allowing laboratories to meet the ever-increasing numbers of requests. Although the user of the AutoAnalyzer is best trained by attending one of the practical courses arranged by the manufacturers of the apparatus, Professor Wootton is to be congratulated on his concise account in only 22 pages, which should enable a worker in an isolated laboratory to set up and use the apparatus. For the worker who has already used the AutoAnalyzer, various minor, hitherto unpublished, modifications have also been included. However, many laboratories possess neither the financial resources nor the demands to purchase this equipment, and it is comforting that corresponding manual procedures have been fully described. Although this latest edition contains fewer references to the original methods than one would prefer, it will be an essential purchase for all concerned with the analysis of clinical specimens, and at the remarkably low price of 30s., rightly deserves to be a best-seller. C. H. GRAY

INTRODUCTION TO ELECTROANALYSIS. By L. L. LEVESON. Pp. viii + 120. London: Butterworth & Co. (Publishers) Ltd. 1964. Price 15s.

The author's intention to provide a concise introduction to electro-analysis, for students at undergraduate level, is successfully fulfilled in this limp-covered first edition.

In the first chapter, electrochemical analysis is classified into methods according to the electrical property measured, and each method is examined in succeeding chapters under the headings Potentiometry, Voltammetry, Amperometric Titration, Coulometry and Conductometry A bibliography, arranged according to the subject matter of each chapter, a list of reviews, journals and abstracts, and a comprehensive index, complete the book.

The subject matter presupposes a basic knowledge of electrochemistry, but the theory of each aspect of electro-analysis is clearly and adequately explained, in general terms, to cover the practical applications of each method. The book would form a sound basis for a course on electro-analysis, and should stimulate further reading.

Although some of the diagrams lack detail, the text is well presented and the book is value for money. I. R. SCHOLES

Erratum

MARCH (1965) ISSUE, p. 141, Fig. 3, 2nd compound, 4th row of numbers. *For* "7505" *read* "5750".

Notice to Authors

THE Society publishes papers on all aspects of the theory and practice of analytical chemistry, fundamental and applied, inorganic and organic, including chemical, physical and biological methods. Such papers may describe original work or may present in review form a critical evaluation of the existing state of knowledge on a particular facet of analytical chemistry. Papers may be submitted for publication by members of the Society or by non-members.

Papers and all correspondence relating thereto should be sent to the Editor of *The Analyst*, 14 Belgrave Square, London, S.W.1.

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Manuscript—Papers should be typewritten in double spacing one one side *only* of the paper. Three copies (top and two carbon copies) should be sent to the Editor, and a further copy retained by the author.

Title and synopsis—The title should be brief but descriptive, and must pin-point the original features of the work. All papers must be accompanied by a short synopsis of about 100 to 250 words; this should give the principle of the method, draw attention to its novel features and indicate its scope and sensitivity. Contributions to the Short Papers section do not require synopses.

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NOTES ON THE WRITING OF PAPERS FOR *The Analyst*

Manuscripts should be in accordance with the style and usages shown in recent copies of *The Analyst*.* Conciseness of expression should be aimed at: clarity is increased by adopting a logical order of presentation, with suitable paragraph or section headings.

Descriptions of new methods should be supported by experimental results showing accuracy, precision and selectivity.

The recommended order of presentation is as indicated below—

(*a*) Synopsis.

(*b*) Statement of object of investigation and, if necessary, historical introduction and account of preliminary experimental work; these need be no longer than is necessary for the understanding of the new material.

(*c*) Description of method. When working details are given, they should, if possible, be given in the imperative mood. Well known procedures must not be described in detail.

(*d*) Presentation of results.

(*e*) Statistical analysis of results. Any statistical evaluation of results should be in accordance with accepted practice.

(*f*) Discussion of scope and validity.

(*g*) Summary and conclusions.

Tables, diagrams, etc.—The number of tables should be kept to a minimum. Column headings should be brief. Tables consisting of only two columns may often be arranged horizontally. No lines should be ruled in tables in the manuscript. Tables must be supplied with titles and be so set out as to be understandable without reference to the text.

* Rules for nomenclature in "Handbook for Chemical Society Authors 1961" (price 21s. from the Chemical Society, Burlington House, London, W.1) are followed. The Shorter Oxford English Dictionary is followed for spelling, but some words are given that Dictionary's secondary alternative spelling.

Tables or graphs may be used, but not both for the same set of results, unless important additional information is given by so doing.

In general, graphs should have a reasonable number of co-ordinate lines, and not only the two main axes. The information given by a straight-line calibration graph can usually be conveyed adequately as an equation in the text.

Diagrams and graphs should be drawn in Indian ink on Bristol board, stout paper or tracing cloth, not larger than foolscap size and with at least 1-inch margins all round. The use of squared paper should be avoided. All lettering should be inserted lightly in black lead pencil at the appropriate place in the diagram, and will be replaced by type in block-making. All lines in Indian ink should be firmly drawn and sufficiently thick to stand reduction.

Drawings should be specially prepared for submission to *The Analyst*, as they cannot normally be returned and may be modified or cut in the course of block-making.

Three sets of illustrations should be provided, two sets of which may be photographic or dyeline copies of the originals, or pencil sketches, for transmission to the referee; there is no need to prepare Indian-ink duplicates.

Photographs—Photographs should only be submitted if they convey essential information that cannot be shown in any other way. They should be submitted as glossy prints made to give the maximum detail. Colour photographs can only be accepted when a black-and-white photograph fails to show some vital feature.

Abbreviations—Normality and molarity are generally expressed as decimal fractions (*e.g.*, 0·02 N, 0·375 M). Abbreviational full stops are omitted after the common contractions of metric units (*e.g.*, ml, g, μg, mm) and after °C, °F, μ, Å and other units represented by symbols; litre and metre, when without prefixes, are printed in full.

Abbreviations other than those of recognised units should be avoided in the text; symbols and formulae are not used instead of the names of elements and compounds in the text, but may be used in addition to names when they are necessary to avoid ambiguity, *e.g.*, to specify crystalline composition, as in $CuSO_4.5H_2O$, to show structure or in equations.

Percentage concentrations of solutions should be stated as "per cent. w/w" (alternatively "g per 100 g"), as "per cent. w/v" (alternatively "g per 100 ml") or as "per cent v/v." Concentrations of solutions of the common acids, however, are often conveniently given as dilutions of the concentrated acids, such as "diluted hydrochloric acid (1 + 4)," which signifies 1 volume of the concentrated acid mixed with 4 volumes of water. This avoids the ambiguity of 1 : 4, which might be equivalent to *either* 1 + 4 *or* 1 + 3.

References—References should be numbered serially in the text by means of superscript figures, *e.g.*, Mackenzie and Mitchell[1] or Furman,[2] and collected in numerical order under "REFERENCES" at the end of the paper. They should be listed, with the authors' initials, in the following form (double-spaced typing)—

1. Mackenzie, R. C., and Mitchell, B. D., *Analyst*, **1962**, **87**, 420.
2. Furman, N. H., *Editor*, "Standard Methods of Chemical Analysis," Sixth Edition, D. Van Nostrand Co. Inc., New York and London, **1962**, Volume **1**, p. **863**.

For books, the edition (if not the first), the publisher and the place and date of publication should be given, followed by the volume or page number, or both if required.

The entry of "personal communications" in the reference list is not justified; full acknowledgment of such unpublished sources should be made in the text or in the acknowledgments at the end of the paper.

Authors must, in their own interest, check their lists of references against the original papers; second-hand references are a frequent source of error. The number of references must be kept to a minimum.

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FOUNDED 1874. INCORPORATED 1907.

THE objects of the Society are to encourage, assist and extend the knowledge and study of analytical chemistry and of all questions relating to the analysis, nature and composition of natural and manufactured materials by promoting lectures, demonstrations, discussions and conferences and by publishing journals, reports and books.

The Society includes members of the following classes:—(*a*) Ordinary Members who are persons of not less than 21 years of age and who are or have been engaged in analytical, consulting or professional chemistry; (*b*) Junior Members who are persons between the ages of 18 and 27 years and who are or have been engaged in analytical, consulting or professional chemistry or *bona fide* full-time or part-time students of chemistry. Each candidate for election must be proposed by three Ordinary Members of the Society. If the Council in their discretion think fit, such sponsorship may be dispensed with in the case of a candidate not residing in the United Kingdom. Every application is placed before the Council and the Council have the power in their absolute discretion to elect candidates or to suspend or reject any application. Subject to the approval of Council, any Junior Member above the age of 21 may become an Ordinary Member if he so wishes. A member ceases to be a Junior Member on the 31st day of December in the year in which he attains the age of 27 years. Junior Members may attend all meetings, but are not entitled to vote.

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Scientific Meetings of the Society are usually held in October, November, December, February, April and May, in London, but from time to time meetings are arranged in other parts of the country. Notices of all meetings are sent to members by post.

All members of the Society have the privilege of using the Library of The Chemical Society. Full details about this facility can be obtained from the Librarian, The Chemical Society, Burlington House, Piccadilly, London, W.1.

The Analyst, the Journal of the Society, which contains original and review papers, information about analytical methods and reviews of books, and has a world-wide distribution, and *Proceedings of the Society for Analytical Chemistry*, in which are reported the day-to-day activities of the Society, are issued monthly to all Ordinary and Junior Members. In addition, all Ordinary Members receive *Analytical Abstracts*, providing a reliable index to the analytical literature of the world.

Forms of application for membership of the Society may be obtained from the Secretary, The Society for Analytical Chemistry, 14 Belgrave Square, London, S.W.1.

LOCAL SECTIONS AND SUBJECT GROUPS

THE North of England, Scottish, Western and Midlands Sections were formed to promote the aims and interests of the Society among the members in those areas.

Specialised Groups within the Society are concerned with the study of various branches of analytical chemistry of specialised or topical interest. Groups dealing with such topics as Microchemical Methods, Biological Methods, Thin-Layer Chromatography, Atomic-Absorption Spectroscopy and a Special Techniques Group, covering very new developments and specialised physical methods, are at present active—and further Groups are formed from time to time as the need arises.

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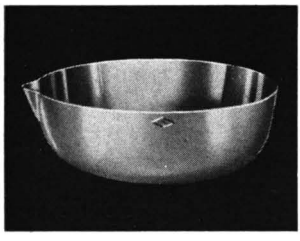

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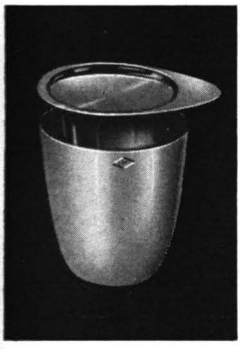

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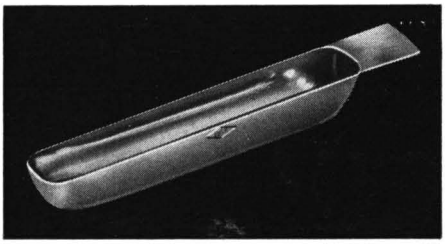

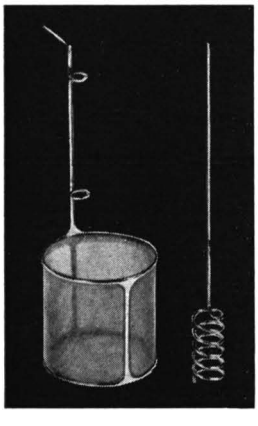

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