THE ANALYST

Rapid Automated Determination of Biphenyl in Citrus Fruit Rind*

By F. A. GUNTHER and D. E. OTT

(Department of Entomology, University of California Citrus Research Center and Agricultural Experiment Station, Riverside, California)

A totally automated analytical procedure for determining the fungistat biphenyl in citrus fruit rind has been developed. Small pieces of hand-peeled rind are automatically homogenised in water and steam-distilled to liberate the biphenyl, which is trapped in cyclohexane solution; this solution is exhaustively extracted to remove interfering steam volatiles, and the biphenyl remaining is read at 246 mμ in a continuous flow recording spectrophotometer. Time required from the introduction of rind samples to read out of biphenyl present is 9 minutes, with about 15 minutes for the first sample. The useful range is from 1 to about 150 p.p.m. on a whole-fruit basis with a reproducibility of about 3 per cent. The method has been applied most extensively to Valencia oranges.

THE fungistat biphenyl has been used commercially since about 1935[1] to protect citrus fruits from the normally extensive post-harvest decay caused principally by the so-called blue-green moulds, *Penicillium digitatum* and *P. notatum*. Despite the objectionable odour of biphenyl it is still used in immense tonnages, wherever citrus is grown, as a mould inhibitor on the multi-billion dollar citrus crops in storage and transit around the world. A combination of fortuitous properties makes the chemical biphenyl unique for this purpose: it is non-toxic, non-carcinogenic,[2] inexpensive, its vapour tension - temperature relationships[1] are ideal for the purpose, it can be easily handled and is a sublimable solid, and its vapour is highly fungistatic to the omnipresent blue and green moulds in their rind locales. With any of the usual methods of application to citrus fruits it does not penetrate through the ntact rind but resides in the wax "layers" and oil sacs of the rind until the fruits are adequately aired, allowing it to escape slowly by vaporisation.

Because of its strong and persistent odour, however, much effort during the past 20 years has been expended to find a less offensive but otherwise satisfactory substitute for biphenyl. Thousands[1] (Souci, S. W., private communication; Eckert, J. W., private communication) of candidate chemicals have been screened in many laboratories for this purpose, but an equally efficient and acceptable substitute citrus fruit fungistat or fungicide has not yet been found.

Because of its unusually low mammalian toxicity[2] the United States' legal tolerance for biphenyl on and in citrus fruits and products is 110 p.p.m. on a whole-fruit basis; this is the highest specific tolerance value yet assigned to any pesticide by the U.S. Food and Drug Administration, except for some organobromine fumigants calculated as inorganic bromide. To stimulate efforts to find a less offensive substitute, however, some other countries have accorded lesser tolerance values to biphenyl in this usage. For example, Great Britain and France allow a still realistic maximum of 70 p.p.m., whereas in 1965 The Netherlands' government legislated[3] the completely unrealistic and fungistatically ineffective (Eckert, J. W., private communication) maximum of 30 p.p.m.

Absolute enforcement of any pesticide tolerance restriction requires the extensive use, with confidence, by both the producing agency and the ultimate consuming agency—whether

* University of California Citrus Research Center and Agricultural Experiment Station Paper No. 1698. This material was presented in part at the September, 1965, Technicon Symposium "Automation in Analytical Chemistry," New York.

it be wholesale buyer, importer or regulatory official—of an adequate residue analytical method. Adequacy in this instance relates to satisfactory reproducibility in many different laboratories, to a minimum detectability that will comfortably bracket the lowest amount permissible, to simplicity of operation because of the world-wide shortage[3] of trained residue analysts and to rapidity because of the thousands of samples that should be involved. It should also relate to the ability of the operation and required apparatus to be standardised so as to circumvent the unfortunate errors[4] almost always introduced when a residue analytical method produced by one laboratory is adapted to the always slightly different equipment, supplies, conditions and personnel training in another laboratory.

Because of the importance of biphenyl over three decades, there are dozens of residue analytical methods for this fungistat; Rajzman[1] has reviewed in detail the most acceptable ones, and has pointed out the advantages and disadvantages of the many analytical approaches involved. Because it was readily adaptable, one[5] of these methods has now been totally automated, from fruit or fruit rind to p.p.m. of biphenyl present, to provide a method that is fast, reliable, operationally simple, and unique in that it is a standardisable biphenyl-residue screening method.[3] This method represents the near-ultimate in pesticide residue analysis. It is the first example of complete automation, from homogenisation in water of the fruit or fruit rind to recorder read out 9 minutes later of the amount of biphenyl present in that sample relative to fortified control samples, within the range of 1 to about 150 p.p.m. on a whole-fruit basis.

Method

Apparatus—

AutoAnalyzer modules (Technicon Controls Inc., Ardsley, New York) are connected as shown in Fig. 1, and are listed as follows.

Solidprep sampler.

Proportioning pumps, two.

Digestor, with accessories for distillation use.

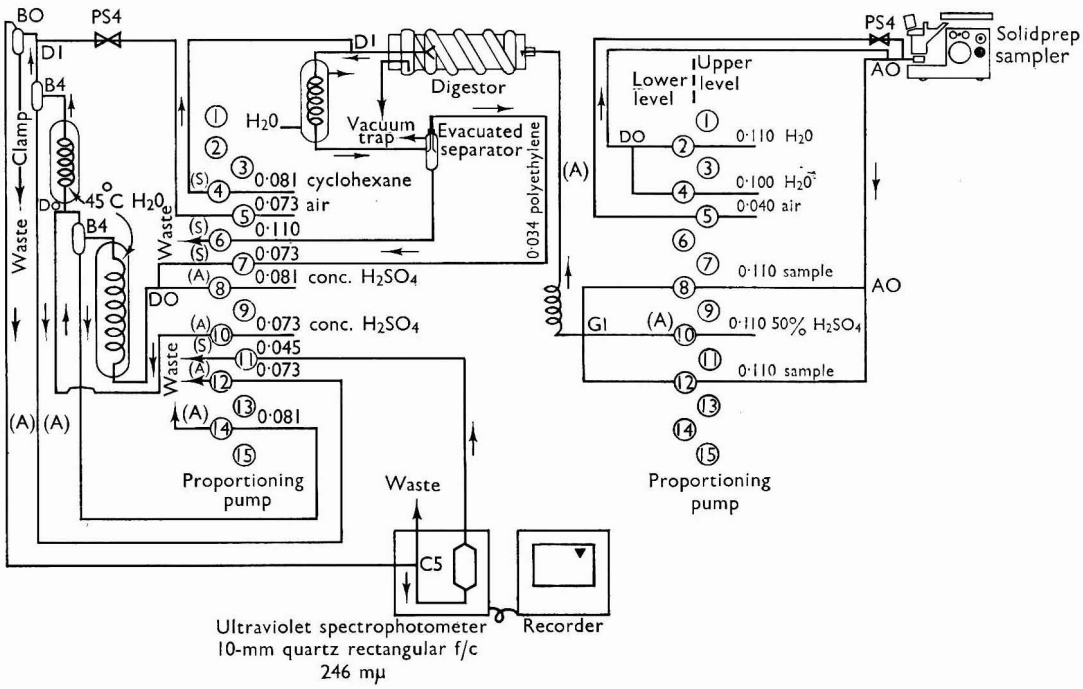

Fig. 1. Automated system for biphenyl residues in citrus fruit rind. The Solidprep sampler is set to pump 45 ml of water as homogenising fluid, and the rate of sampling is 7 per hour. Tube sizes are in inches

(A = Acidflex tubing; S = Solvaflex tubing)

Evacuated separator (see Fig. 2)—For simplicity in drawing this figure, the waste digestant liquid at the end of the helix is shown as being aspirated into the vacuum source; in practice, however, the most convenient disposal is to pass to waste through an all-glass or plastic water aspirator.

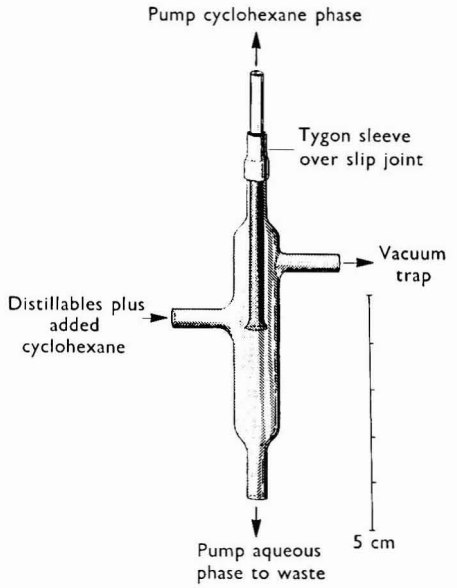

Fig. 2. Details of evacuated separator essential to the biphenyl-residue automated system

Ultraviolet spectrophotometer—The spectrophotometer is dual beam, with a 10-mm light path rectangular quartz flow-cell in the sample side, and nothing in the reference side.

Strip-chart recorder, 10 mV.

Glassware and tubing (see Fig. 1).

ASSEMBLY OF APPARATUS—

The detailed flow diagram for the apparatus is schematically presented in the standard manner in Fig. 1; a few minor changes may be needed in a particular system for smooth and reliable operation. Thus, the flow-rates controlled by some of the tubings in the two pumps may need to be varied from those shown in the figure in order to achieve optimum flow characteristics. In particular, the diameter of the pump tubing at position 11 on the left-hand pump may need to be increased for maximum flow, or decreased to keep air bubbles out of the spectrophotometer cell. Also, the rate of flow of material pumped from the bottom of the evacuated separator may have to be varied to achieve good separation within the separator.

Acidflex sleeving over joints and transmission tubing is used throughout the system where there is possibility of contact with concentrated sulphuric acid, except where indicated otherwise. All joints (glass to glass, flexible-tubing to glass, or flexible-tubing to flexible-tubing) associated with the sampling system from the homogeniser vessel to the pipette which introduces sample and digestant into the digestor helix must be butt joints (no connecting nipples) to avoid blockage by particles of orange rind. For the same reason the tubing from the mixing coil in this same stream to this introduction pipette should be Acidflex sleeving rather than transmission tubing. The introduction pipette should be long enough to introduce the sample directly into the heated zone; accumulation of rind and other particles occurs if introduced into an unheated zone. Biphenyl-exhausted particles will accumulate in the cool downstream end of the helix, however, but here there is no risk of their subsequent dislodgement and contamination of another sample; the helix must be cleaned after about 1 week when in constant use to remove these gross accumulations.

PROCEDURE—

This system utilises either analytical-reagent grade or spectrograde cyclohexane* for extraction of the distillate, and analytical-reagent grade sulphuric acid for the cyclohexane wash series. The Solidprep sampler is set to pump 45 ml of water as homogenising fluid, and is operated with a standard factory set programmer; the homogeniser motor is set for medium speed during the sampling cycle. The vacuum pump connected to the evacuated separator (Fig. 2) is set for about 2 inches of mercury, the requirement for the separator, which in turn supplies the vacuum to collect the vapours in the collection funnel. This funnel is inserted into the exit end of the digestor helix which rotates at 20 r.p.m. and is heated by setting the heater controls 1 and 2 at 3·0 and 5·0 amps, respectively.

The ultraviolet spectrophotometer is set at the pre-determined absorption maximum for biphenyl in cyclohexane solution (246 mμ under the present conditions, although Gunther *et al.*[5] specify 248 mμ). A 0·062-inch horizontal aperture slit is used in the air path of the reference side of the dual-beam spectrophotometer. To keep the measuring cell clean, the following procedure when shutting down the system is suggested: as rapidly and nearly simultaneously as possible, the transmission tubing that is connected to the cell and to the pump tube which pulls the continuous stream of cyclohexane through the cell, is disconnected at the nipple joint from that pump tube, while a small pinch clamp is tightened over the three tubings which are connected collectively to the spectrophotometer (see Fig. 1); finally, a similar pinch clamp on the waste line connected to the bottom of the BO glass fitting is loosened to by-pass the contents in that fitting to waste.

When starting the system up, and after equilibrium conditions are reached, a continuous stream of cyclohexane pours into the BO fitting, and the foregoing procedure is performed in reverse. The chart drive of the recorder is turned on, and when the base-line becomes stable after about 15 minutes the Solidprep sampler is started with an empty cup in the first position followed by samples of previously chopped fruit rind† weighed into sample cups in every third successive cup, with two intervening empty cups between each sample for "wash" purposes; in this mode of operation the rate of sampling is 7 per hour. For routine screening analyses one empty "wash" cup will probably suffice, thus decreasing the time required per analysis by one-third. With series of samples containing both very high and very low levels of biphenyl, however, two cups for washing are necessary (see diffusion on Fig. 4).

DISCUSSION OF THE METHOD

A simplified flow diagram of the basic steps in this biphenyl analytical system is shown in Fig. 3. The rind previously chopped into approximately $\frac{1}{4}$-inch or smaller cubes is auto-

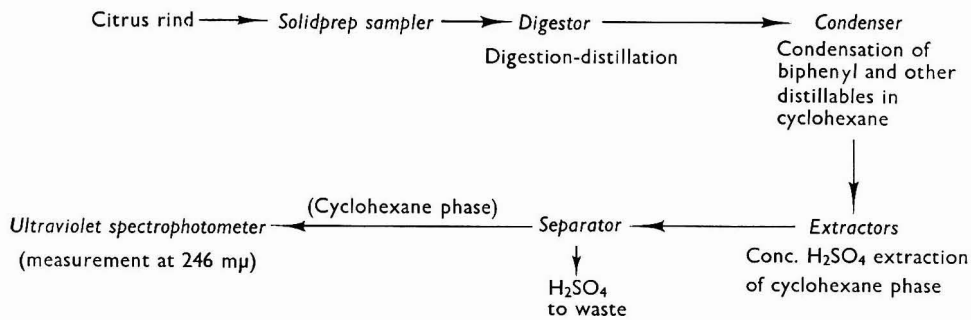

Fig. 3. Simplified flow diagram of the basic steps in the automated system for biphenyl residues in citrus fruit rind

matically homogenised in water, and then steam-distilled in the presence of sulphuric acid to liberate the biphenyl *plus* citrus oils (mostly terpenoids) and waxes. These steam volatiles are trapped in cyclohexane, the oils and waxes are quantitatively extracted into concentrated sulphuric acid, and the biphenyl left in the cyclohexane is determined at 246 mμ. In the original manual method[5] some *p*-cymene escaped the room-temperature acid washing or was

* The manual method[5] requires the use of spectrograde cyclohexane.

† $\frac{1}{4}$-inch or smaller cubes prepared by a Hobart or similar food chopper.

formed[6] from other terpenoids; to minimise this highly variable interference it was oxidised with permanganate, then washed out with more sulphuric acid. The present continuous acid extraction step at 45° C either removes *p*-cymene without the necessity for an oxidation step, or else the small-sized starting sample (2 g of rind against 150 g of whole fruit for the manual method) effectively reduces this particular background contribution to nil in this measuring system.

One of the major obstacles in the development of this automated procedure was the problem of continuously separating an aqueous phase from an immiscible solvent phase, while at the same time having these two phases under reduced pressure. This was overcome with a special glass evacuated separator. As it is new as well as essential for the biphenyl system it has been shown in detail in Fig. 2. This device does not guarantee an absolutely non-aqueous stream out of the top. After the slip joint has been optimally set under equilibrium and fixed conditions for vacuum pump and digestor temperature, the occasional small amounts of aqueous phase and occasional air bubbles are entrained in the emergent cyclohexane solution; these are either sufficiently constant, or in such small amounts that the net reproducibility of results is not affected.

Typical chart recordings are produced in Fig. 4, and were obtained from 1 or 2-g starting samples of chopped Valencia orange rind. These recordings are from the system operated for 7 consecutive, noise-free hours according to the flow diagram in Fig. 2. Thirty-four samples and 8 controls and fortified controls were run that day with no special (single) operator attention. The average time interval was therefore 9 minutes per test, exclusive of about 30 minutes for warm-up and 15 minutes for shut-down. Note the absence of significant background (Fig. 4) from even 2 g of control rind, and also the close agreement between

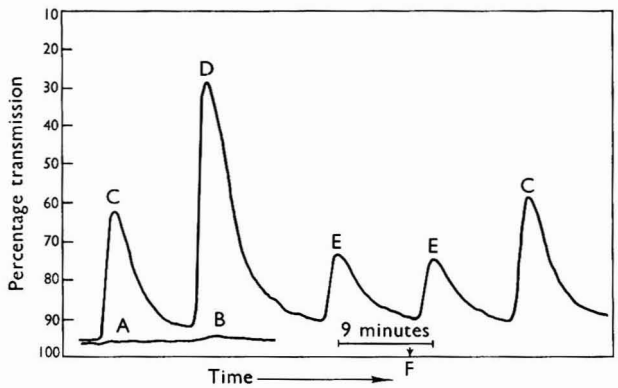

Fig. 4. Chart recordings obtained from the biphenyl-residue automated sytem: A, 1 g of Valencia orange rind control; B, 2 g of rind; C, 1 g of rind fortified with biphenyl at 350 p.p.m.; D, 2 g of rind fortified with biphenyl at 350 p.p.m.; E, 1 g of rind from the biphenyl-treated carton of fruit; F, sampling cycle origin of last peak

replicates by comparison of ordinate intercepts of peak maxima alone. For greater precision, peak height (or area) in absorbance units should be used: this is the difference in absorbance units between the peak maximum and a base-line drawn in the usual manner beneath the peak.

Absorbance unit - peak height measurements were obtained in this way for a series of controls (rind from untreated samples) fortified in the range 35 to 700 p.p.m. to establish a fortified-control standard curve. To 1 or 2-g portions of chopped control rind, weighed into every third sampler cup, were added appropriate volumes (up to 2 ml) of a standard solution of 350 μg per ml of biphenyl in 95 per cent. ethanol, or a standard volume of solutions of varying strengths. There were no measurable differences between the 1 or 2-g samples of control rind run alone through the system and the 1 or 2-g samples of control rind each containing 1 ml of 95 per cent. ethanol. The resultant standard curve had a slope of 195 p.p.m. of biphenyl per 0·1 absorbance unit. Reproducibility results were as follows. The lowest point, 35 p.p.m. in the rind (a value which is roughly equivalent to 7 pp.m. based on the

U.S. Food and Drug Administration practice of weight of whole fruit,* and well below The Netherlands' tolerance of 30 p.p.m. mentioned earlier), was replicated six times yielding a mean value of 0·017 ± 0·001 absorbance unit; a next higher value at 70 p.p.m. was replicated four times, with a mean value of 0·037 ± 0·004 absorbance unit; the mean value at a 175 p.p.m. level, from three replicates, was 0·082 ± 0·005 absorbance unit; other absorbance values averaged 0·200 at 350 p.p.m. and 0·350 at 700 p.p.m. These values were not corrected for a background of 0·003 absorbance unit per gram of control rind.

For greater minimum detectability, 2-g samples of rind can be used routinely, or a recorder range expander can be added with 1-g samples: the 2*X* position is feasible but noise and base-line shifting usually exclude the 4*X* and 10*X* positions. Two-gram samples of grapefruit and lemon rind are necessary for adequate sensitivity and precision when AutoAnalyzing these citrus fruits.

To examine samples representative of commercial practice, half of a field box of tree-ripened Valencia oranges was stored for 6 days at 25° to 30° C in a standard vented citrus fruit shipping carton. Standard, commercial, biphenyl-treated liners were placed in the bottom and top of the carton, and for extreme dosage one was placed in the middle with oranges above and below it. The other half box of oranges was kept in a separate room as a control. Fruits were sampled from the top of the "treated" carton for AutoAnalysis, producing the two peaks labelled E in Fig. 4. From the fortified-control standard curve, 175 ± 8 p.p.m. of biphenyl were in and on the rind of this treated fruit, equivalent to 33 ± 2 p.p.m. on a whole-fruit basis.

The primary problem that has occurred with the present system is that occasionally there is excessive signal noise to the recorder associated with the physical composition of the stream flowing through the measuring cell. However, if the cell tubing connections are all clamped off when this noise appears, and the cyclohexane stream is momentarily by-passed (according to directions in the shut-down procedure) to leave in the light path a full and static-condition cell, the noise immediately stops, yet there are no visible air bubbles or other contaminants. This problem is more annoying than serious, however, as peaks recorded during these periods quantitate comparably to smooth peaks from equivalent samples if a curve drawn midway through the noise is used for measurement purposes. The noise rarely lasts longer than 30 minutes at a time; if it starts in the middle of a peak that peak will probably be lost for quantitative purposes because there is a marked base-line shift at the start of the noisy period.

At present it is not possible to relate directly and simply the slope of the fortified control standard curve to that of a primary standard curve with biphenyl alone. Without a "keeper," (*e.g.*, orange oil), biphenyl alone shows large and variable losses during the Solidprep homogenisation cycle. The reason for lower responses from the few exploratory samples of fortified grapefruit and lemon rind, compared (Table I) with fortified Valencia orange rind, may be due to lower amounts of oils and waxes as "keepers" in the first two varieties. Thus, standardisation must always be in terms of fortified controls of the same variety as the unknowns. Both automated and manual[5] methods are compared in Table I.

Background values from untreated (control) samples are collated in Table II in comparison with corresponding samples run by the manual[5] method. It is clear from these results that variable backgrounds from grapefruits and lemons were not a cause of the lower recoveries shown in Table I.

Despite these minor disadvantages, the advantages of speed, convenience, precision and reproducibility, coupled with more than adequate sensitivity for monitoring purposes, result in an automated system that should merit further evaluation for eventual adoption by control laboratories around the world as a screening method for biphenyl residues in citrus fruit rind. With this system, speed of analysis is reduced to minutes-per-sample rather than the hours-by-sample required by the several manual methods at present being used both in the United States and in Europe. With cups in the Solidprep sampler arranged as recommended, the time lapse between successive samples is 9 minutes. This was the loading used in the present investigation to achieve the maximum accuracy from assured adequate purging of the system between samples containing, at random, from none to more than 175 p.p.m. of biphenyl each on a whole-fruit basis. In screening operations routine samples

* Mature Valencia oranges contain 18·7 ± 6·3 per cent. rind based upon 297 measurements, Navel oranges contain 22·1 ± 7·3 per cent. rind (567 measurements), lemons contain 30·0 ± 8·5 per cent. rind (632 measurements), and grapefruit contain 23·0 ± 3·2 per cent. rind (47 measurements).

would contain from about 30 to about 120 p.p.m., and this meticulous purging might not be necessary; in this situation every other cup could contain a sample with consequent reduction of average time per analysis to 6 minutes.

In its present form this method should also work with whole fruit that has been made into a purée and commercial fruit products, but these applications should be checked with fortified samples for reproducibility, sensitivity and efficiency before routine application. Applications to grapefruits and lemons should incorporate enough fortified controls to establish a reliable recovery value as related to Valencia oranges at 100 per cent.

Table I

Illustrative comparative recoveries and reproducibilities of automated and manual biphenyl methods on commercially treated fruits in the approximate range of 30 to 150 p.p.m. on a whole-fruit basis

Variety	Average recovery, per cent.		Reproducibility, per cent.	
	Automated* (2-g sample)†	Manual (150-g sample)	Automated (2-g sample)†	Manual (150-g sample)
Grapefruit	38 (44 p.p.m.)	100‡	±2	±3‡
Lemon	52 (58 p.p.m.)	99	±2	±1
Orange, Valencia	100	98	±3	±1
Orange, Navel	§	100	§	±1

* From standard curves prepared from fortified controls, compared to Valencia oranges at assumed 100 per cent. recovery.

† Rind only.

‡ Unpublished results developed in 1959 by Gunther and co-workers in routine application to commercial shipments in Hamburg, W. Germany.

§ Navel oranges were not in season at the time of this study; the manual results illustrate recoveries for possible comparisons by others in routine applications of the present method.

Table II

Illustrative background values from control samples with automated and manual methods on a whole-fruit basis

Variety	Background			
	Absorbance units		Equivalent, p.p.m.	
	Automated (2-g samples)*	Manual (150-g samples)†	Automated (2-g samples)*	Manual (150-g samples)†
Grapefruit	0·004 ± 0·001	0·005 ± 0·003‡	1·9 ± 0·3	1·5 ± 0·6‡
Lemon	0·004 ± 0·001	0·008 ± 0·002	2·6 ± 0·5	1·8 ± 0·4
Orange, Valencia	0·003 ± 0·001	0·010 ± 0·004	1·1 ± 0·2	3·1 ± 0·9
Orange, Navel	§	0·004 ± 0·001	§	1·0 ± 0·2

* Rind only.

† Whole fruit.

‡ Unpublished results developed in 1959 by Gunther and co-workers in routine application to commercial shipments in Hamburg, W. Germany.

§ Navel oranges were not in season at the time of this study; the manual results illustrate recoveries for possible comparisons by others in routine applications of the present method.

References

1. Rajzman, A., *in* Gunther, F. A., *Editor*, "Residue Reviews," Springer-Verlag, Heidelberg and New York, Volume 8, 1965, p. 1.
2. van Stratum, P. G. C., "The Toxicity of the Citrus Fungistat Diphenyl," Central Institute for Nutrition and Food Research T.N.O., *Report No.* R1838, The Netherlands, November, 1964.
3. Gunther, F. A., and Ott, D. E., "Automation in Analytical Chemistry," 1965, Technicon Symposium, New York, N.Y., 1965.
4. National Academy of Sciences—National Research Council, Pesticide Residues Committee, Report on "no residues" and "zero tolerance," Washington, D.C., June 1965.
5. Gunther, F. A., Blinn, R. C., and Barkley, J. H., *Analyst*, 1963, **88**, 36.
6. Gunther, F. A., *Adv. Pest Control Res.*, 1962, **5**, 191.

Received *February 14th*, 1966

Determination of Carbon in Steel by a Dynamic Infrared System

By G. WHITE and P. H. SCHOLES

(*British Iron and Steel Research Association, Metallurgy Division, Hoyle Street, Sheffield* 3)

A simple, automatic apparatus has been developed for the rapid determination of carbon in steel. It is based upon the continuous measurement of carbon dioxide evolved during the high temperature combustion of steel in oxygen by using a specially designed infrared gas analyser and integration system. When this is used in conjunction with a conventional resistance-tube furnace, the speed of the determination varies from 40 to 55 seconds for mild and low alloy steels, and slightly longer for highly alloyed materials.

Considerable progress has been made during recent years in the development of combustion apparatus for determining carbon in steel. There are now several analysers available commercially in Europe and North America.[1] These new instruments are entirely automatic. The operator has only to place a weighed sample into a refractory container and insert this into a combustion furnace. From this stage the determination proceeds automatically, the operator being required only to read a meter and perform a simple conversion to the percentage of carbon. Sometimes this is unnecessary as the percentage of carbon is shown directly.

The commercial analysers can be classified into three groups according to the technique used to measure the carbon dioxide evolved during the high temperature combustion of steel samples in oxygen. These measuring systems are (*a*) electrochemical, mainly those of German origin, (*b*) thermal conductivity (used in instruments made in North America), and (*c*) infrared absorption (used in a British and a French instrument). Current developments in the techniques available for the rapid sampling of molten steel permit the use of these analysers for process control purposes, and another rather arbitrary grouping is possible in terms of instrument time. For the electrochemical instruments this is normally $2\frac{1}{2}$ to 4 minutes, being rather longer for the French infrared analyser, and 60 to 90 seconds for the thermal-conductivity instruments and the British infrared analyser. With instruments of the latter group it is, therefore, possible to determine the carbon content of, for example, large open-hearth furnaces at 5-minute intervals.

With the exception of the Canadian Thermocarb,[2] the commercial process analysers incorporate a static measurement system in which the combustion gas is first collected before measurement. One of the disadvantages of this approach is that conditions must be carefully pre-arranged to ensure that combustion is completed before measurement of carbon dioxide takes place. For maximum speed it seemed preferable to use a dynamic approach, in which the carbon dioxide content of the combustion gas is monitored continuously by using a fast-response detector with electronic integration. Previous work by the authors had shown that remarkably fast combustion of samples, in the form of turnings and solid pins, could be obtained with an inexpensive resistance-tube furnace. In the system used, combustion takes place in the presence of excess oxygen, and the combustion gas is pumped from the furnace at a fixed rate.

Measurement of the evolved carbon dioxide by infrared absorption is an attractive alternative to thermal-conductivity measurement, and is free from many of the difficulties arising from the instrumental instability of the latter technique. The principle is not new, it was first proposed by Lay[3] in 1955. Le Controle de Chauffe of Paris published a brochure in 1962 describing a prototype carbon analyser based upon infrared measurements, and in the following year, Tipler[4] described an analyser designed principally for determining low carbon contents in steel and silicon - iron. The latter instrument has now been modified by Hilger-I.R.D. Limited for more general application.[5] Both instruments incorporate static measurement systems of the type mentioned earlier. In early 1963, an infrared analyser was kindly made available to the authors by Hilger-I.R.D. Limited for initial experiments on the dynamic measurement of the evolved carbon dioxide.

In this paper, the development of a dynamic carbon-in-steel analyser, the Dynacarb, is described. The primary consideration in design is that the apparatus should be inexpensive, have a rapid throughput time and be capable of application to all types of steel.

Experimental

Preliminary experiments were carried out with a standard single range, direct-reading infrared analyser supplied by Hilger-I.R.D. Limited. Combustion patterns for various types of steel were recorded with a moving-chart potentiometer, and it was found that, with furnace temperatures in excess of 1300° C and high oxygen flow-rates, combustion of most samples was complete in less than 1 minute. The recorded pattern of carbon dioxide concentration *versus* time was extremely symmetrical, but from attempts to relate carbon content to integrated peak area it was clear that the response of the analyser was not adequate to detect all of the carbon dioxide present in the gas. Experiments designed to slow down the rate of carbon dioxide evolution and its subsequent introduction to the analyser were not successful, and the obvious requirement was an analyser with a much more rapid response-time.

A second infrared analyser was obtained that had a response-time of about 200 milliseconds, and was suitable for measuring concentrations of carbon dioxide up to 12 per cent. by volume. This instrument, which incorporated a high speed recorder suitable for obtaining combustion patterns, had previously been used as a prototype for experimental work in determining carbon dioxide in respiratory gases.

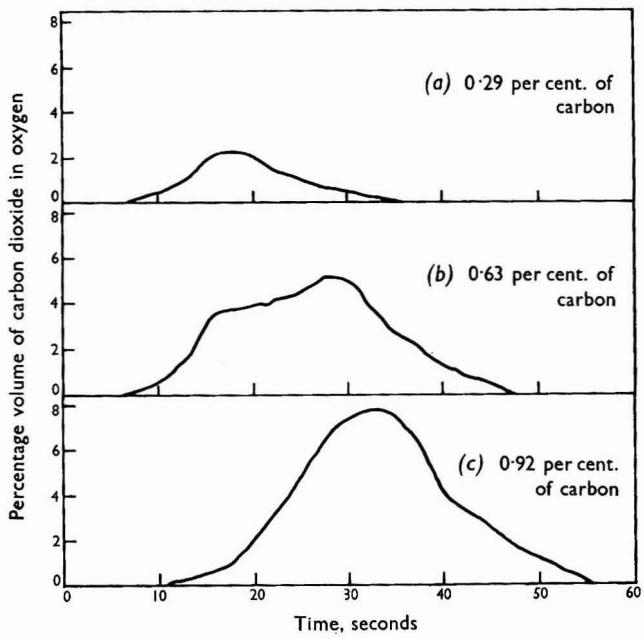

Fig. 1. Typical combustion patterns. Temperature 1350° C, flow-rate 900 ml per minute

In general, the recorded patterns showed a reasonable degree of symmetry, but, in a few instances, irregular combustion conditions caused minor rapid fluctuations in the carbon dioxide content of the evolved gases. The maximum carbon dioxide concentration at any one instant did not exceed 8 per cent. by volume. Typical combustion patterns are presented in Fig. 1. Measurement of the area under the peak gave reproducible values, provided that the oxygen flow-rate through the analyser was maintained at a constant level.

Mixing tubes of various capacities were inserted into the flow system at the entrance side of the analyser, in order to decrease the proportion of carbon dioxide in the combustion gas. The values obtained were similar to those obtained in the absence of expansion tubes, confirming that instrument response was sufficient to detect all of the carbon dioxide passing through the analytical cell.

These experiments showed that dynamic infrared measurement of carbon dioxide was feasible, and an apparatus based upon these principles was designed in conjunction with Hilger-I.R.D. Limited.

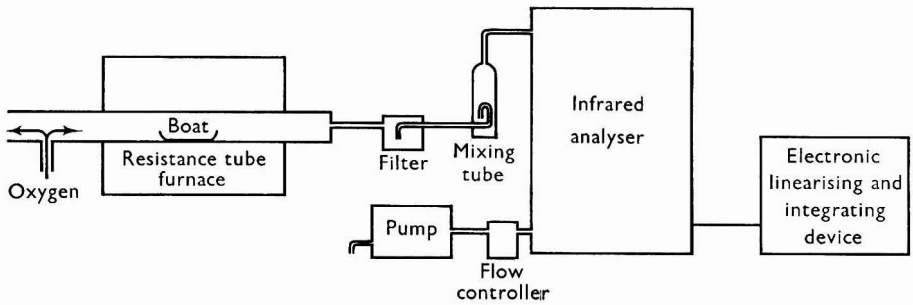

Fig. 2. Block diagram of prototype apparatus

APPARATUS—

A block diagram of the Dynacarb apparatus is shown in Fig. 2.

Gas analyser—The gas analyser is a modified, general-purpose instrument incorporating a specially designed analytical cell for the measurement of high velocity gas streams. It is suitable for use with carbon dioxide concentrations up to 10 per cent. by volume in oxygen, and has a response-time better than 200 milliseconds.

As the electrical output of infrared analysers is logarithmic in function, it was necessary to incorporate a linearising circuit in order to produce a signal that could be integrated and displayed on a meter. This was accomplished by dividing the curve relating meter-reading to voltage-output into a number of segments.[6] Each segment has an associated printed circuit card with a silicon diode and an adjustable potentiometer. Bias is applied to each diode to ensure that it will pass only current higher than the voltage of selected ordinates of the meter reading - voltage relationships; the voltage of each segment is then adjusted by its potentiometer to give an over-all linear relationship. During combustion of a sample, the linearised signal is stored by a suitable condenser, and is continuously displayed on a meter throughout the determination.

Oxygen flow—A pump maintains a constant flow of gas through the apparatus. Fluctuations in flow-rate, caused by irregular combustion, are avoided by the supply of a large excess of oxygen during ignition of the sample. Excess oxygen escapes from the mouth of the tube, and provides an adequate seal against the atmosphere without the need for any form of closure. Large fluctuations in the percentage of carbon dioxide evolved are smoothed by passing the gas through a small pre-mixing vessel attached to the analyser.

The effect of changes in flow-rate was studied by injecting identical volumes of carbon dioxide into the flexible tubing of the apparatus by means of a Hamilton gas-tight syringe, and recording the meter deflections at various flow-rates. In Fig. 3, it can be seen that there is a plateau region between 900 and 1000 ml of oxygen per minute. The reasons for this are

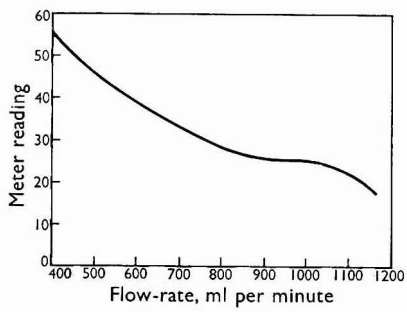

Fig. 3. Effect of the flow-rate, 5·0 ml of carbon dioxide injected each time

obscure, but Bartley (in a private communication) has suggested that it may be caused by a change in the nature of the gas flow through the analytical cell. It seems probable that, at flow-rates below 900 ml per minute, the flow is laminar, but above this value a certain amount of turbulence is introduced and, while the over-all flow-rate is unaffected, there is some slowing down in the passage of carbon dioxide through the cell.

A flow-rate of 950 ml per minute was, therefore, used in subsequent work; this was achieved by means of a rotary pump and adjustable flow regulator.

Formation of iron oxides—

When combustion of samples takes place at high temperatures with fast oxygen flow-rates carry-over of iron oxide becomes troublesome and it is essential to filter the gas stream efficiently before passing it through the analyser. Filters made from cotton-wool and glass-wool become blocked and cause fluctuations in the flow-rate. The problem was overcome by using a calico filter-cloth in a small glass container of the type used with American high frequency furnaces. This proved to be most effective and may easily be cleaned by tapping the container or gently brushing the filter-cloth.

Choice of flux and blank determination—

The pick-up of carbon from extraneous sources arises from (*a*) the oxygen supply, (*b*) the combustion boat and (*c*) the fluxing materials. After purification of the oxygen supply and pre-ignition of the combustion boat, the contribution from (*a*) and (*b*) is negligible at the carbon levels investigated.[7] Pre-ignition of the boats is best performed in one tube of a twin-tube furnace. After cooling in air for a few minutes, the boat is loaded with the sample and flux and then ignited in the second tube. Under these conditions the blank value can be attributed solely to the flux.

Two fluxing materials, tin powder and lead foil, have proved satisfactory. The addition of lead foil to carbon and low alloy steel gives a fairly smooth combustion and a minimum carry-over of iron oxide. With careful handling the blank is of the order of 30 p.p.m. of carbon. For more complex steels and solid-pin samples, lead is not suitable and additions of tin powder must be made. Combustion is not as smooth and carry-over of iron oxide is greater, but the blank values are lower, and are in the range of from 10 to 20 p.p.m. of carbon.

Calibration of the analyser—

The infrared analyser is initially set up with a gas mixture nominally containing 8·6 per cent. of carbon dioxide in oxygen. With the prototype, it is not possible to adjust the integrator meter to obtain a direct reading of the percentage of carbon for a specified sample. Scale calibration may be achieved by burning a number of standard steels in oxygen, and constructing a graph relating meter divisions to carbon content. It is preferable, however, that calibration should be independent of standardised steels and, therefore, it is better to inject varying volumes of high purity carbon dioxide (volume corrected to S.T.P.) into the system with a Hamilton gas-tight syringe. The relationship between integrated meter reading and carbon dioxide is linear and remains constant over long periods, provided that the infrared analyser is free from electronic drift. This possibility must be checked several times during the course of each working day by using the setting-up gas mixture. A simple slide-rule conversion after each test is all that is necessary to obtain the percentage of carbon.

The instrumental precision of the analyser was assessed by injecting a series of identical volumes of carbon dioxide into the system. The coefficient of variation of measurements, obtained for repetitive injections, is about 1 per cent.: this value is, however, limited by the precision of the injection syringe used in these tests.

Analytical performance with resistance and high frequency combustion furnaces

For reasons of economy and simplicity, the Dynacarb apparatus is primarily intended to be used with a resistance-tube combustion furnace. Tests have also been made with a high frequency furnace and a comparison of analytical speeds is given in Table I.

TABLE I

COMPARISON OF ANALYSIS TIMES WITH RESISTANCE AND HIGH FREQUENCY COMBUSTION FURNACES

Millings and drillings		Instrument time, seconds: Resistance heating	Instrument time, seconds: High frequency heating
Mild and medium-carbon steel		40 to 50	35 to 45
Low alloy and high-carbon steel		45 to 55	40 to 50
Stainless steel		45 to 50	30 to 40
High-carbon alloy steel		65 to 75	50 to 60
Pin samples—			
5-mm diameter	Mild steel	55 to 70	35 to 45
	High-carbon steel	60 to 80	40 to 50
3-mm diameter	Carbon and low alloy steel	50 to 65	35 to 45
	High-carbon steel	65 to 80	45 to 55

RESISTANCE HEATING—

There was no difficulty in obtaining the complete combustion of samples of even the most complex alloy steel in the form of millings and drillings, with a conventional tube furnace. The best results were obtained by using 26-mm i.d. aluminous-porcelain combustion tubes that were maintained at a temperature of 1350° to 1400° C by silicon carbide heating rods. Instrument time, from the time of inserting the loaded refractory boat into the tube up to the time taken for reading the integrated signal from the meter, varied from 40 to 55 seconds for carbon and low alloy steel, to 65 to 75 seconds for alloy steels containing about 1 per cent. of carbon. Samples of stainless steels ignited quite readily with analysis times rarely exceeding 55 seconds, and a limited number of tests indicated that nickel-base alloys could be analysed within about 60 seconds.

For maximum speed in steelworks process control, suction samples are taken in preference to a small cast sample which requires milling or drilling before analysis. One method is to insert the tip of an evacuated tube into a spoon sample of de-oxidised molten steel. The high temperature leads to fusion at the tip, and the sudden suction that is produced causes the metal to enter and fill the tube, so producing a solid rod of 3 to 4-mm diameter. A suitable alternative procedure is to aspirate the molten steel into a glass tube by suction from a rubber bulb. A piece, weighing approximately 1 g, is then cut from the cooled rod or pin and used for analysis.

A number of pin samples have been analysed with the Dynacarb apparatus. Preliminary tests on samples of low alloy steels from Samuel Fox & Company indicated that complete combustion was obtained in a resistance furnace, provided that the sample was covered with tin powder. Analysis time, for 3 and 5-mm diameter samples, was related to the carbon content of the sample, and varied from 60 to 80 seconds. Modern tube furnaces are capable of continuous operation at temperatures well in excess of 1400° C; it was possible to reduce the minimum analysis time to about 50 seconds by increasing the furnace temperature to 1450° C. During subsequent trials of the instrument at the English Steel Corporation Ltd., suction samples were taken alongside the conventional samples that were intended for direct-reading spectrographic analysis. Excellent comparisons with spectrographic results were obtained on steels of different steel-making compositions.

HIGH FREQUENCY HEATING—

Comparative tests were made with a Radyne 5-Mc generator, fitted with a quartz-glass combustion chamber containing a movable refractory pedestal to support the combustion crucibles.

The speed of combustion, by high frequency heating, is dependent upon the method of passing oxygen through the combustion chamber. When oxygen entered the bottom of the chamber below the refractory crucible, combustion times were found to be of the same order as those obtained with resistance heating. When the flow was reversed and passed through a jet directly above the crucible, extremely rapid ignition was achieved and the

total analysis time was approximately 30 seconds. With such rapid evolution of gas, however, the localised concentration of carbon dioxide may exceed 10 per cent. by volume, thereby overloading the infrared analyser, particularly when high-carbon steels are being analysed. It was, therefore, necessary to design a special oxygen-delivery jet that would ensure adequate mixing and dilution of the carbon dioxide in the combustion chamber. This was made from 4-mm glass tubing with a tapered jet of 2 to 3-mm diameter. Several 1-mm holes, bored into the wall of the tube above the orifice, created sufficient turbulence in the chamber to maintain the carbon dioxide level at below 10 per cent. As described earlier, the combustion gases were pumped through the analyser, and excess of the oxygen was allowed to escape into the atmosphere through one arm of a T-piece situated in front of the combustion chamber.

With high frequency heating, all types of steel in the form of millings or drillings could be analysed within 50 seconds. Solid-pin samples required a further 5 seconds for complete combustion.

Results

Many standard steels have been analysed; the results of the analyses are presented in Table II, and are based upon instrument calibration by injection of high purity carbon dioxide. Good agreement was obtained with certified values, and analytical precision varied from about 1 per cent. coefficient of variation at the 1 per cent. level of carbon content, to 3 or 4 per cent. at the 0·05 per cent. carbon level. There was no significant difference in precision for samples analysed with both the resistance and high frequency combustion furnaces.

Table II

Results obtained with resistance and high frequency combustion furnaces

B.G.S. No.	Type	Certificate value, percentage of carbon	Resistance heating		High frequency heating	
			Percentage of carbon	Standard deviation	Percentage of carbon	Standard deviation
260/1	High purity iron	0·014	0·016	0·002	—	—
265/1	Carbon steel	0·043	0·043	0·001	—	—
295	Carbon steel	0·265	—	—	0·26	0·004
238/1	Carbon steel	0·21	0·21	0·004	—	—
293	Carbon steel	0·63	0·64	0·005	0·63	0·004
159/2	Carbon steel	0·54	0·54	0·009	0·54	0·003
215/1	Carbon steel	0·925	0·93	0·006	0·92	0·015
224/1	Chrome - vanadium steel	0·50	0·50	0·008	0·51	0·012
290	13 per cent. manganese steel	1·17	1·17	0·016	1·15	0·010
241/1	High speed steel	0·85	0·85	0·011	0·84	0·009
220/1	High speed steel	0·93	0·94	0·008	—	—
235/1	Stainless steel	0·042	0·042	0·002	—	—
235/2	Stainless steel	0·072	0·071	0·001	0·074	0·002
310	Nimonic 90	0·098	0·098	0·001	0·097	0·003
261	Stainless steel	0·083	0·086	0·003	—	—
247/3	White cast iron	3·00	2·97	0·028	—	—
203/1	Ferrochrome alloy	0·045	0·042	0·001	—	—
203/2	Ferrochrome alloy	0·027	0·026	0·002	—	—
Results obtained in trials at E.S.C. Ltd.—						
239/2	Carbon steel	0·295	0·294	0·008	—	—
238/1	Carbon steel	0·21	0·207	0·010	—	—
293	Carbon steel	0·63	0·64	0·008	—	—

As a further confirmatory test of performance under steelworks conditions, 15 samples of stainless steel, 4 nickel-base alloys and 10 samples of high-speed tool-steel were analysed at the English Steel Corporation, and the results were compared with those obtained by using the Wöstoff Carmhograph conductimetric analyser for low carbon contents,[7] and the British Standard gravimetric method[8] for the high-carbon tool-steels. The results in Table III are in favourable agreement with these alternative procedures. During these tests, 3 standard steels were each analysed by 3 operators at frequent intervals over a period of 2 weeks, in order to provide a more realistic assessment of precision, which would include variation between different operators (see Table II).

TABLE III

RESULTS OBTAINED DURING STEEL WORKS ROUTINE OPERATION

Cast No.	Wösthoff Carmhograph, percentage of carbon	Dynacarb, percentage of carbon
Stainless-steel samples—		
5157	0·017	0·019
2509	0·035	0·037
4085	0·068	0·069
2507	0·049	0·055
2603	0·057	0·059
2719	0·095	0·098
2497	0·078	0·080
4080	0·027	0·037
4090	0·041	0·044
4091	0·052	0·053
4092	0·056	0·053
4093	0·27	0·25
FW 14	0·037	0·036
FW 2515	0·059	0·065
FK 07	0·051	0·060
Nickel-base alloys—		
Nimonic S207	0·042	0·045
Nimonic S85	0·012	0·008
Nimonic H8707	0·029	0·031
Nimonic Y1041	0·28	0·28
High speed tool-steel samples—	Standard method, percentage of carbon	
RA 44	0·74	0·75
RA 45	0·74	0·72
RA 46	0·73	0·72
RA 47	0·75	0·74
RA 53	0·78	0·80
RA 54	0·83	0·84
RA 55	0·83	0·84
RA 56	0·81	0·83
RA 57	0·82	0·81
RA 58	0·83	0·84

The full-scale meter deflection of the Dynacarb is nominally equivalent to 1·2 per cent. of carbon on the basis of a 1-g sample weight, and a "×4" scale expansion is provided giving full-scale deflection, equivalent to 0·3 per cent. of carbon. For samples containing more than about 1 per cent. of carbon, a smaller sample weight must be used. The lower level of detection is of the order of 0·005 per cent. of carbon and, at this percentage, the 95 per cent. confidence limits for the mean of two results is $\pm 0{\cdot}0015$ per cent. of carbon.

CONCLUSIONS

The application of infrared absorption to the determination of carbon in steel by a dynamic system has been shown to be feasible. Precise and accurate results may be rapidly obtained on a wide range of steel-making compositions, by using samples in the form of millings, drillings and solid pins. The proposed apparatus may be used either with a high frequency or resistance combustion furnace, but as the advantages of the former are marginal, a simple resistance-tube furnace is recommended.

The chief advantages of a dynamic system are simplicity and economy. Capital costs are lower than with alternative instruments that incorporate high frequency heating and automated gas collection. The system is continuously flushed with oxygen, and there is no possibility of the carry-over of carbon dioxide from one sample to the next. Combustion processes can be followed, and completion of the analysis is immediately obvious. A further advantage is the use of an open tube, excess of oxygen providing a seal against the atmosphere during combustion.

We acknowledge with gratitude the full co-operation of Mr. W. Bartley, Managing Director, and his colleagues of Hilger-I.R.D. Limited. In addition, thanks are given to Mr. L. Kidman for his permission to make trials at English Steel Corporation, to Mr. R. Staham, Samuel Fox and Company, who supplied pin samples, and to Mr. P. Barker, who performed some of the experimental work.

References

1. Scholes, P. H., "Proceedings of the Sixteenth Chemists' Conference," British Iron and Steel Research Association, London, **1963**, p. 26.
2. Hines, W. G., Addinall, R. L., and Orten, J. P., *J. Metals*, **1964**, 165.
3. Lay, J. O., *Metallurgia*, **1955**, **51**, 109.
4. Tipler, G. A., *Analyst*, **1963**, **88**, 272.
5. Waldock, P., *Hilger J.*, **1965**, **9**, 18.
6. Rippon, K. P., and Smith, F., *Brit. Instn Radio Engrs J.*, **1962**, **24**, 127.
7. Scholes, P. H., *Rep. B.I.S.R.A.*, MG/D/244/62.
8. British Standard 1121 : Part 11 : 1948.

Received *January 24th*, **1966**.

The Determination of Aluminium in Iron and Steel

By J. A. CORBETT

(*Physical Metallurgy Section, Commonwealth Scientific and Industrial Research Organisation, Australia*)

AND B. D. GUERIN

(*Metallurgy Department, University of Melbourne, Australia*)

Various colorimetric reagents have been examined for their sensitivity in a standard method for determining aluminium in ferrous metals. Interfering elements are removed by a mercury-cathode separation followed by cupferron - chloroform extraction. In the method adopted, aluminium is determined by measuring the optical density of its complex with Alizarin red S - calcium reagent. The method has been tested with a wide range of steels.

THIS project was undertaken at the request of the Committee on Sampling and Analysis of Ferrous Metals, Standards Association of Australia, as part of its programme of developing standard methods for the analysis of steels. After a survey of published methods the authors concluded that none was completely satisfactory as a standard method applicable to all types of steels. As a result, the investigation described here was undertaken.

The determination of aluminium at low levels is particularly susceptible to errors arising from the introduction of extraneous aluminium, and a high degree of analytical skill is required to prevent contamination by minute traces of this ubiquitous element. In addition to this avoidable random introduction of aluminium, there is an unavoidable pick-up of aluminium from impurities in reagents and from dissolution of aluminium from glassware. This need not be a serious problem because, in the hands of an experienced analyst, the blank from these sources can be quite reproducible. The method of analysis should be designed to keep the magnitude of this aluminium pick-up within reasonable limits. As so many analytical reagents can be a source of minute traces of aluminium, a method should in general require only small additions of reagents, restricting, in particular, the use of alkaline reagents and avoiding their contact with glass. The storage of all reagents in polythene containers reduces the risk of contamination. It should be emphasised, however, that it is the reproducibility of the blank rather than its magnitude that imposes the lower limit to the range of aluminium that can be determined, and, in fact, the blank may be greater than the net amount of aluminium in the sample aliquot at very low levels of aluminium. It is unlikely, however, that satisfactory reproducibility will be attained with very high aluminium pick-up.

PRELIMINARY SEPARATIONS

There are no known colorimetric reagents that are specific for aluminium. On the contrary, with all reagents there is only a rather restricted list of elements that do not interfere with the colour-forming reaction, and an efficient separation of most of the elements occurring in steels will be necessary. Several methods[1,2] have been described, in which interfering elements form complexes instead of separating. Such methods involve the use of large amounts of reagents and are applicable only to certain types of steels. For a standard method to deal with all classes of steels and irons, an efficient separation of most of the elements present will be necessary.

Blair, Power, Griffiths and Wood[3] have discussed previously published separations of aluminium from iron which they have classified under the headings: precipitation; solvent extraction; chromatography; and mercury-cathode electrolysis. They selected the last as the most suitable technique for their method for determining trace amounts of aluminium. When used with a d.c. supply of sufficient power output, electrolysis in a perchloric acid medium over a mercury cathode provides a rapid and elegant method of removing many alloying elements, including manganese, nickel, chromium and molybdenum, as well as the iron. In view of the requirement to keep reagent additions to a minimum this electrolytic technique is the obvious choice for a preliminary separation.

MERCURY-CATHODE ELECTROLYSIS—

The design of a mercury-cathode apparatus for rapid electrolysis should allow the use of high currents and reasonably low electrolyte temperatures, both factors increase the hydrogen over-voltage on mercury, and contribute to current efficiency. Metals amalgamating with the mercury tend to lower the hydrogen over-voltage so that the use of contaminated mercury may result in imperfect separations. The use of a magnet beneath the cell to attract deposited ferro-magnetic metals below the surface of the mercury can thus improve cell efficiency.

In this investigation we used a water-cooled Melaven cell operating at 10 amps and a magnetic mercury-cathode cell (similar to the design of Center, Overbeck and Chase[4]), operating at 12 to 15 amps. Both designs have proved satisfactory for this work, as no doubt would other types of cells designed to operate at these current levels.

Electrolysis of 0·5-g samples of plain carbon steels were completed in less than 15 minutes, but molybdenum-bearing stainless steels may require up to 1 hour for completion.

Blair[3] and his colleagues showed that traces of chloride interfere with the electrolysis, and that repeated fuming with perchloric acid is needed to remove such traces when the composition of the steel, in particular its chromium content, necessitates the use of hydrochloric acid in the attack of the sample. We have found that if the solution is boiled to allow perchloric oxidation of chromium to its higher valency state, double fuming at this high temperature will remove the chloride.

During electrolysis there is a tendency for elements to be deposited in the order of their electrode potentials, although some simultaneous deposition does occur. With stainless steels it is found that complete deposition of nickel and then iron will occur before that of chromium. Molybdenum, if present, will be the last of these alloying elements to be completely deposited. Spot tests should be used to test for the completeness of deposition of iron, chromium and molybdenum (see Notes). The spot test for chromium,[5] in which one drop of solution is used, will detect the presence of 50 μg of chromium in the electrolyte.

It has not been found necessary to include a spot test for manganese. With steels containing up to 2 per cent. of manganese, less than 0·2 mg of manganese remains in the electrolyte when deposition of iron is complete. With 12 per cent. manganese steels the presence of manganese in the electrolyte becomes obvious because of the anodic oxidation to permanganate or oxides of manganese. When electrolysis is continued until the electrolyte becomes colourless, up to 1·3 mg of manganese remains in the electrolyte. However, even this amount of manganese is below interference level with the principal colorimetric reagents for aluminium.

FINAL SEPARATION

Of the elements that have been mentioned in the literature as steel constituents, those that would be present in solution after electrolysis are titanium, vanadium, zirconium, beryllium and phosphorus. There could also be traces of iron, manganese, chromium or molybdenum, at levels below the limits of detection of the spot tests. Various methods are available to isolate aluminium from these elements.

Sodium hydroxide was used by Scholes and Smith,[6] Hill[2] and Studlar and Eichler[7] to precipitate, as hydroxides, some of the elements listed above. The remainder were complexed with hydrogen peroxide to prevent interference with the colorimetric reagent used. The probability of aluminium pick-up from alkaline reagents has been mentioned. A further danger with this technique is the possibility of loss of aluminium as aluminium phosphate when a high phosphorus alloy is encountered.

Claassen, Bastings and Visser[1] used a series of solvent extraction separations with 8-hydroxyquinoline and chloroform, with complexing reagents and different values of pH. A knowledge of the sample composition is necessary, and with some steels a further separation with cupferron is required.

A cupferron - solvent extraction seemed preferable to the above techniques and has been widely used for this type of separation. It was decided to investigate its application to the present method.

CUPFERRON PRECIPITATION - CHLOROFORM EXTRACTION—

Blair, Power, Griffiths and Wood[3] have been concerned to remove residual traces of iron which cause severe interference with the Eriochrome cyanine R reaction with aluminium. They made a small addition of cupferron with chloroform extraction of cupferrates to remove

traces of iron, titanium and vanadium. As an additional precaution they added hydrogen peroxide to ensure that any excess of titanium or vanadium was in the non-interfering oxidised state. There is a possibility of error in this technique. If titanium is present in greater than trace amounts it will react with all of the added cupferron and will be extracted, leaving iron present in the aqueous layer. Those workers' restriction on the amount of cupferron apparently arose from their desire to keep the total aluminium pick-up as low as possible. We preferred to ensure the complete removal of iron, titanium, zirconium, molybdenum and vanadium at this stage by repeating the addition of cupferron and chloroform extraction until the chloroform extract was colourless. Hydrogen peroxide, which interferes with some colorimetric reactions of aluminium, can now be omitted, so the choice of colorimetric reagent is widened.

The acidity of the aqueous phase during chloroform extraction of cupferrates should be controlled. Slight losses of aluminium by extraction occur if the pH is above 0·4, and the extraction of iron is retarded in solutions that are too strongly acid.[8] Our experiments have indicated that a satisfactory separation can be effected in molar acid solution, so an appropriate addition of acid is made before the separation.

The repeated cupferron - chloroform extraction necessary with some alloy steels may result in a slightly increased, but reproducible, pick-up of aluminium. Provided the blank determination for such steels is given identical treatment no error is introduced by this technique.

It should be noted that any traces of manganese and chromium in the electrolyte after mercury-cathode electrolysis will not be removed by the cupferron - chloroform extraction.

The only other elements likely to be present in irons and steels, and which are not removed by the two separations described or by a preliminary filtration of the insoluble matter, are the aluminium, and also magnesium, beryllium and phosphorus.

Assuming that the sample weight is limited to 0·5 g, and the aliquot taken for colour development is not greater than one-fifth of the electrolyte, the levels at which elements could be present in the aliquots are as follows—

Beryllium	200 μg for 0·2 per cent. beryllium alloy
Chromium	10 μg assuming the spot test is effective
Magnesium	200μg for 0·2 per cent. alloy
Manganese	300 μg for 12 per cent. manganese steel
Phosphorus	1000 μg for 1 per cent. phosphorus alloy

Colorimetric reagents

A Unicam spectrophotometer SP600 was used for all colorimetric work described.

After studies of the published colorimetric reagents used for aluminium, five reagents were considered to merit detailed investigation. These were—

Alizarin red S - calcium reagent.
Arsenazo.
Eriochrome cyanine R (Solochrome cyanine RS).
8-Hydroxyquinoline.
Stilbazo.

The investigation of each of these reagents has included: (*a*) the determination of the optimum wavelength for photometric measurement, from a study of graphs of optical density against wavelength for the reagent and the aluminium complex; (*b*) conformity with Beer's law; (*c*) calculation of sensitivity, which has been expressed: (*i*) as the extinction coefficient ϵ with respect to 1 gram atom of aluminium per litre of solution (as advocated by International Union of Pure and Applied Chemistry), and (*ii*) as the concentration range of aluminium corresponding to the optical density range 0 to 1·0 in the cell size recommended for the particular reagent; (*d*) interference studies, for the most part confined to those elements which could be present following the two major separations. Elements given as not interfering have been tested at least to the levels listed above.

The results of the above work are shown in Table I.

TABLE I

RESULTS OF INVESTIGATIONS ON COLORIMETRIC REAGENTS

	Alizarin red S - calcium reagent	Arsenazo	Eriochrome cyanine R (Solochrome cyanine RS)	8-Hydroxyquinoline	Stilbazo
Optimum wavelength for colour measurement	490 mμ	580 mμ	532 mμ	392 mμ	520 mμ
pH for colour development	4·4 to 4·65	6·1 to 6·8	Approximately 6·1	Extraction at pH 4·9 to 5·0	Approximately 6·8
Conformity to Beer's law. (Range tested shown in brackets.)	Conforms (0 to 80 μg of aluminium per 100 ml)	Conforms (0 to 100 μg of aluminium per 100 ml)	Slight but consistent deviations detected (0 to 60 μg of aluminium per 100 ml)	Conforms (0 to 300 μg of aluminium per 100 ml)	Deviations suggesting the the existence of more than one compound (0 to 100 μg of aluminium per 100 ml)
ϵ at optimum wavelength	$1{\cdot}8 \times 10^4$	$1{\cdot}2 \times 10^4$	$6{\cdot}75 \times 10^4$	$6{\cdot}7 \times 10^3$	$3{\cdot}8 \times 10^4$
Concentration range and cell size	0 to 80 μg of aluminium per 100 ml in 2-cm cells	0 to 100 μg of aluminium per 100 ml in 2-cm cells	0 to 60 μg of aluminium per 100 ml in 0·5-cm cells	0 to 100 μg of aluminium per 100 ml in 4-cm cells	0 to 70 μg of aluminium per 100 ml in 1-cm cells
Interferences: Effect on optical density at optimum wavelength—					
(*a*) Beryllium	Slight increase	Drastic increase	Drastic increase	None	Slight increase
(*b*) Other elements listed on page 492 at levels specified	40 μg Be $\equiv$ 1 μg Al None	0·7 μg Be $\equiv$ 1 μg Al None. Higher levels of chromium cause increase	2 μg Be $\equiv$ 1 μg Al None. Higher levels of chromium cause decrease	None	40 μg Be $\equiv$ 1 μg Al None
Notes on reagent	The reaction of sodium alizarin sulphonate with aluminium in the presence of calcium ions has been used in analysis of rocks, slags and coal ash.[10,11,12]	The orange coloured aqueous solution forms a cherry-red complex with aluminium.[13] (The reagent used was from Tokyo Kasei Kogyo Co. Ltd., Japan.)	Acidified (nitrated) solutions of Merck's Eriochrome cyanine R and Gurr's Solochrome cyanine RS were found to give identical reactions. This reagent has been widely used for aluminium determinations in steels.[3,6,7]	Aluminium hydroxyquinolinate is extracted from the aqueous phase with chloroform yielding a pale yellow solution. This reaction has been used in steel[14] and cast-iron[15] analyses.	The reaction with aluminium to form a reddish brown complex has been used in steel analyses.[16,17]

PRACTICABILITY—

In addition to the investigations outlined above we have introduced the concept of the "practicability" of a colorimetric reagent and have considered the five reagents in this respect. Under this term we have included rapidity of development of the coloured complex and its stability, the stability of the reagent solution and the effect on the optical density of slight changes in conditions such as pH and buffer concentration. All of these factors play a part when the reproducibility of a method is determined experimentally. Another factor is the optical density of the reagent. If the reagent absorbs appreciably at the optimum wavelength it is not practicable to use a large excess of reagent and there is a tendency for the calibration graph to deviate from linearity towards the upper end, unless the equilibrium constant for the complex formation is high. Absorption by the reagent reduces the slope of the calibration graph, and also, in the type of instrument used here, limits the cell size that can be used without unduly opening the slit.

CHOICE OF COLORIMETRIC REAGENT—

Each of the 5 colorimetric reagents investigated in detail has sufficient sensitivity for determining aluminium in steels and irons. We have found that the lower limit of detection of aluminium is determined by the reproducibility of aluminium pick-up in the blank and sample rather than by the sensitivity of the colorimetric reagents tested. It would seem, then, that the choice of reagent for this standard method should depend primarily on the reproducibility of the colour-forming reaction rather than its sensitivity. One criterion for a standard method is that it should give satisfactory results in the hands of a competent analyst. On this basis all of the above reagents can be considered as satisfactory as it is possible to obtain accurate reproducible results for aluminium with each reagent, provided the relevant conditions are sufficiently closely controlled. The factors affecting reproducibility are different for each reagent, and with some of the reagents are difficult or irksome to control in practice. It was therefore considered that reproducibility tests, made under the ideal conditions for each reagent, would not provide a realistic basis for comparison. The factors affecting reproducibility, that we have discussed in the section on "Practicability," provide results for a more realistic appraisal of the reproducibility which could be expected under laboratory conditions, and it was consideration of these factors that guided our final choice of reagent for the standard method. Close control of pH and buffer concentration should present no difficulty provided that the analyst takes the elementary precaution of preparing a sufficient volume of buffer solution to deal with all samples, blanks and standards in the batch of analyses. Close control over time of standing is, however, irksome, when measurements are being made against a reference solution whose density is also time-dependent. Strict adherence to Beer's law is desirable as it obviates the need for close plotting of the calibration graph with each batch of analyses.

With Eriochrome cyanine R close control over time of standing is essential because the optical densities of both the aluminium complex and reference solutions decrease on standing. There are slight deviations from Beer's law.

Stilbazo has similar defects, the deviations from Beer's law being slightly more severe.

The lower sensitivity of the 8-hydroxyquinoline complex is compensated by the low absorption of the reference solution which permits the use of larger cells and smaller dilutions for absorption measurements. The colour development involving a chloroform extraction is not as simple as with the other reagents, and the coloured solution is light sensitive and subject to the disadvantage of a volatile liquid.

Aqueous solutions of arsenazo are stable, and the optical density of the aluminium complex remains constant in the interval from 1 to 5 hours after colour development. The reagent itself has a low absorption at the wavelength used. It suffers from severe interference from beryllium. This is not regarded as a serious defect; however, this element is found in a few special stainless alloys and experimental batches of steels.

Aqueous solutions of Alizarin red S are stable for about 2 weeks and the optical density of the calcium - aluminium complex remains constant in the interval from 1 to 4 hours after colour development. Absorption by the excess reagent is not severe.

We are of the opinion that arsenazo and Alizarin red S - calcium reagent are the two most suitable reagents for use in a standard method for determining aluminium. The latter

has the slight advantage that interference from beryllium is almost negligible. In deciding to recommend the Alizarin red S - calcium reagent to the Australian Committee on Analysis of Ferrous Metals we were further influenced by the wealth of experience in its use by analytical chemists in the analysis of non-metallic materials.

APPLICATION OF ALIZARIN RED S - CALCIUM REAGENT TO THE METHOD—

The aluminium, before colour development, is in dilute perchloric acid solution. The pH for colour development may be in the range 4·4 to 4·65, but it must be kept uniform to within ±0·02 units. The acid solution can be brought directly to the required pH by adding a high concentration of buffer solution, but this addition was found to cause a marked decrease in the optical density of the aluminium complex. As with the other colorimetric reagents for aluminium the buffer concentration must be kept reasonably low for efficient colour development. With the resulting low buffer capacity it is necessary that before colour development the solution is neutralised with sodium hydroxide followed by a small measured excess of acid to re-dissolve the aluminium. The addition of 10 ml of a buffer of M sodium acetate - M acetic acid (pH 4·75) brings the final pH within the required limits.

As sodium hydroxide is a likely source of trace aluminium, the amount required to neutralise the solution has been kept to a minimum by evaporating the perchloric acid solution almost to dryness before neutralisation.

The addition of 7 mg of Alizarin red S provides sufficient excess of reagent to ensure linear calibration over the range 0 to 80 μg of aluminium. Ideally, 0·5-g portions of high purity iron should be used in the blank and standard. As "pure" irons, commercially available, contain traces of aluminium, this addition is not recommended.

The time required to complete the determination of aluminium in a single steel sample is approximately 8 hours.

METHOD

The method is applicable to all steels and irons, and has a range from 0·002 to 10 per cent. of aluminium.

APPARATUS—

Mercury-cathode cell—The cell should be designed to operate at a current of at least 10 amps and should be water-cooled.

Spectrophotometer—Any instrument suitable for measuring the optical density of a solution at 490 mμ may be used.

REAGENTS—

All reagents should be of the highest purity obtainable and distilled water should be used throughout. Certain types of analytical-grade reagents are unsuitable because of the presence of either aluminium or other impurities. All solutions should be stored in polyethylene or polypropylene containers.

Cupferron, 2 per cent. w/v—Dissolve 2 g of cupferron in 50 ml of water and dilute the solution to 100 ml. Filter if necessary. This solution should be colourless and must be prepared each day.

Sodium hydroxide, 2 M—Dissolve 80 g of sodium hydroxide pellets in 700 ml of water in a polyethylene container, cool the solution and dilute it to 1 litre.

Hydrochloric acid, 0·2 M—Dilute 18 ml of hydrochloric acid (sp.gr. 1·18) to 1 litre.

Phenolphthalein indicator—Dissolve 0·1 g of phenolphthalein in 50 ml of ethanol and dilute the solution to 100 ml with water.

Calcium chloride—Dissolve 14 g of calcium carbonate in 50 ml of hydrochloric acid (50 per cent. v/v). Boil the solution for 2 minutes. Cool and dilute to 1 litre.

Buffer solution—Dissolve 140 g of hydrated sodium acetate ($CH_3COONa.3H_2O$) in water. Add 60 ml of glacial acetic acid and dilute to 1 litre.

Alizarin red S solution, 0·14 per cent. w/v—Dissolve 140 mg of Alizarin red S in 75 ml of water and dilute the solution to 100 ml.

Chromium spot-test, solution A—Dissolve 10 g of sodium hydroxide pellets in 50 ml of water, cool the solution and add to it 50 ml of hydrogen peroxide (6 per cent.).

Chromium spot-test, solution B—Dissolve 0·5 g of diphenylcarbazide in 50 ml of glycerol. (This solution is satisfactory for several days.) To 5 ml of the glycerol solution add 5 ml of

sulphuric acid (25 per cent. v/v) and 5 ml of glacial acetic acid. (This solution is satisfactory for 1 to 2 hours.)

Iron and molybdenum spot-test solution C—Dissolve 5 g of ~~tin(II)~~ sodium thiocyanate ($NaSCN.H_2O$) in 50 ml of water and dilute the solution to 100 ml.

Iron and molybdenum spot-test solution D—Dissolve 32 g of stannous chloride ($SnCl_2.2H_2O$) in 40 ml of hydrochloric acid (sp.gr. 1·18) and dilute the solution to 100 ml with water.

PROCEDURE—

Transfer 0·5 g of sample to a 100-ml beaker, add to it 10 ml of nitric acid (50 per cent. v/v) and allow it to digest until the solvent action ceases (Note 1). Add 5 ml of perchloric acid (60 per cent.) and evaporate to fumes of perchloric acid. Allow the mixture to fume for 1 minute with the cover removed (Note 2). Cool the residue, add to it 10 ml of water, heat to dissolve soluble salts and filter the mixture through a small filter-paper. Wash the filter-paper with hot water and reserve the filtrate (A) (Note 3). Transfer the paper to a platinum crucible, char, then ignite it at a temperature not exceeding 1000° C. Cool, and moisten the residue with 5 or 6 drops of dilute sulphuric acid (20 per cent. v/v), add to the residue 2 ml of hydrofluoric acid and evaporate to dryness. Heat the residue to 800° C for several minutes, then fuse it with 0·5 g of sodium hydrogen sulphate. Cool the mixture, add 10 ml of water and dissolve the solid by heating (Note 4). If the total aluminium is required add this extract to filtrate (A). If separate results are required for acid-soluble and acid-insoluble aluminium, treat the extract as described in Note 5. Transfer the solution to the mercury-cathode cell with a minimum amount of water. The volume of electrolyte should not exceed 70 ml. Electrolyse at 10 to 15 amps, washing down the cover and inside of the cell with water after 30 minutes. Continue the electrolysis until deposition is complete, *i.e.*, until spot tests indicate that iron or, if present, chromium and molybdenum have been removed from the electrolyte (Note 6). Remove the electrolyte and filter it immediately into a 100-ml standard flask (Note 7) with the minimum volume of water for washing, and make the solution up to the mark. Transfer by pipette a 20-ml aliquot (Note 7) into a 200-ml separating funnel. Add to the solution 2 ml of hydrochloric acid (50 per cent. v/v) and mix. Introduce 1 ml of cupferron solution (2 per cent. w/v), shake the mixture, and allow it to stand for 5 minutes. Add 15 ml of chloroform, shake the solutions for 30 seconds, allow the two phases to separate and run the chloroform layer into a beaker. Extract the aqueous layer with a further 10 ml of chloroform, then run off the chloroform layer. Add 1 ml of cupferron (2 per cent. w/v) to the aqueous portion, mix, and allow the solutions to stand for 5 minutes. Add 10 ml of chloroform and shake the solutions for 30 seconds, allow the layers to separate, note whether the chloroform layer is coloured (Note 8) and run off the chloroform layer. Run the aqueous layer into a 100-ml beaker.

Evaporate the aqueous portion to about 5 ml, add 1 ml of nitric acid (50 per cent. v/v) and evaporate to fumes of perchloric acid. Continue the evaporation until no free liquid is visible although fumes of perchloric acid are still being emitted. If drops of perchloric acid remain on the beaker wall, carefully wash down with water and repeat the evaporation. Cool, add 10 ml of water and warm to dissolve salts. Cool, add two drops of phenolphthalein solution and add sodium hydroxide (2 M) from a polythene wash-bottle until the colour just changes to pink. No more than 2 to 4 drops of sodium hydroxide should be required. Titrate with 0·2 M hydrochloric acid until the solution becomes colourless and add 1·0 ml in excess.

Transfer the solution to a 100-ml calibrated flask and dilute to 50 ml. Add, in order, with a burette and shaking the solution after each addition, 2 ml of calcium chloride solution 10 ml of buffer solution and 5 ml of Alizarin red S solution (0·14 per cent. w/v); dilute the solution to the mark with water. Allow the solution to stand for 1 hour, transfer it to a 2-cm cell and measure the optical density at 490 mμ against the reference solution. (See under Calibration.)

REAGENT BLANK—

Each sample must be accompanied by a reagent blank solution. The treatment of the blank must be identical with that of the sample throughout the method. Measure the optical density at 490 mμ against the reference solution. (See under Calibration.)

CALIBRATION—

Aluminium solution—Dissolve 1·757 g of aluminium potassium sulphate ($Al_2(SO_4)_3K_2SO_4.24H_2O$), in distilled water, add 1 ml of sulphuric acid (sp.gr. 1·84) and dilute to 1 litre.

Dilute 100 ml of the above solution to 1 litre with water. 1 ml ≡ 10 μg of aluminium.

Calibration procedure—To 50 ml of water in a 100-ml calibrated flask add 1·0 ml of 0·2 M hydrochloric acid, and proceed with colour development as described under "Procedure." Allow the solution to stand for 1 hour. This is the reference solution.

Each sample must be accompanied by a standard. This is prepared by measuring with a burette a 35-ml portion (Note 9) of the diluted aluminium standard solution into a 100-ml beaker, and proceeding through all steps of the method. The treatment of the standard must be identical with that of the sample. Measure the optical density at 490 mμ against the reference solution and deduct the reagent blank to give the optical density due to 70 μg of aluminium in the aliquot.

CALCULATIONS—

Deduct the reagent-blank optical density from the test-solution optical density and convert to weight of aluminium in the aliquot taken, by reference to the calibration standard. Hence calculate the percentage of aluminium in the sample.

NOTES—

1. The addition of hydrochloric acid is necessary to effect dissolution of some alloy steels. An addition of 5 ml of hydrochloric acid (sp.gr. 1·18) is suitable.

2. If hydrochloric acid has been used special care must be taken to remove it. Evaporate to fumes of perchloric acid, and with the cover on the beaker boil the solution vigorously for 30 seconds to oxidise the chromium. Remove the cover and allow the mixture to fume freely for another 30 seconds. Cool, wash down the sides of the beaker with water and repeat this evaporation to fumes, boiling and fuming, to remove the last traces of chloride.

3. If tungsten is present remove the filtrate and wash the paper with 10 ml of ammonia solution (50 per cent. v/v), then with water.

4. The fused residue from some complex alloys may not completely dissolve in water. If it does not, transfer the extract to a 100-ml beaker and boil it to dissolve the residue as far as possible. Add the solution and the insoluble residue to the main filtrate A.

5. For the determination of acid-insoluble aluminium, add to the extract, filtered if necessary, 1 ml of perchloric acid (60 per cent.), 2 ml of hydrochloric acid (50 per cent. v/v) and continue with the cupferron extraction as described under "Procedure."

6. In each of the following spot tests, 1 drop of the electrolyte is placed in a small porcelain crucible or in the depression of a porcelain spot plate.

Iron—Add 1 drop of nitric acid (50 per cent. v/v) and 1 drop of the sodium thiocyanate solution *C*. A red colouration indicates the presence of iron.

Chromium—Add 1 drop of solution *A*, mix and then add two drops of solution *B*, and mix. The resulting solution should be acid. An immediate purple colouration indicates the presence of chromium.

Molybdenum—Add 1 drop of solution *C* and 2 drops of solution *D*. A pink to red coloration indicates the presence of molybdenum.

7. The aliquot must not contain more than 70 μg of aluminium. When necessary the sample weight, the dilution, or the aliquot can be adjusted to conform to this limit. If the aliquot is less than 20 ml it should be diluted to 20 ml with water. The aliquot should be approximately 0·5 molar with respect to perchloric acid. If necessary, make an appropriate addition of perchloric acid (60 per cent.).

8. If this chloroform extract is coloured, repeat the addition of 1 ml of cupferron (2 per cent. w/v) and the extraction with chloroform, until the chloroform extract is colourless.

9. The aliquot for colour development should contain 70 μg of aluminium. With standards accompanying steels containing more than 0·07 per cent. of aluminium a proportionately larger initial amount of aluminium will be necessary.

RESULTS

Tests were made on a group of N.B.S. and B.C.S. steels with aluminium contents ranging from 0·002 to 6·98 per cent. The results are shown in Table II.

These results show that aluminium can be determined with satisfactory accuracy. Statistical tests at low levels of aluminium indicated that the lower limit of detection of the method should be not greater than 0·001 per cent. of aluminium. At this lower limit a pick-up of 6 μg of aluminium in the blank-determination aliquot was sufficiently reproducible to enable an additional 1 μg of aluminium in the sample aliquot to be detected.

TABLE II

RESULTS OF TESTS ON N.B.S. AND B.C.S. STEELS, ALUMINIUM CONTENT 0·02 TO 6·98 PER CENT.

Sample	Certificate value, percentage of aluminium	Laboratory 1			Laboratory 2		
		Mean result	No. of determinations	Standard deviation	Mean result	No. of determinations	Standard deviation
N.B.S. 106 (low alloy steel)	1·06	1·060	5	0·009	1·054	5	0·011
N.B.S. 55e (ingot iron)	0·002	0·0032	5	0·0009	0·0013	5	0·0005
N.B.S. 170a (open hearth steel)	0·046	0·0483	3	0·0006	—	—	—
B.C.S. 241/1 (high speed steel)	*	0·0229	5	0·0019	0·0246	5	0·0013
B.C.S. 271 (mild steel)	0·008	0·0083	5	0·0002	0·0080	5	0·0005
B.C.S. 233† (magnet alloy)	6·98	7·05	5	0·041	7·07	5	0·059
B.C.S. 246 (18/12 stainless with niobium, molybdenum)	‡	0·0027	3	0·0002	0·0026	2	—

* No certificate value is given for B.C.S. 241/1. Scholes and Smith[6] found 0·024 per cent. of aluminium.
† Certificate and experimental values for B.C.S. 233 are for soluble aluminium.
‡ No certificate value is given for B.C.S. 246.

Further reproducibility tests are now being carried out by a panel of fellow members of the Ferrous Analysis Committee in order to determine the 95 per cent. confidence limits for incorporation in the proposed standard.

The authors wish to acknowledge helpful discussions at meetings of the Committee on Sampling and Analysis of Ferrous Metals, Standard Association of Australia.

REFERENCES

1. Claassen, A., Bastings, L., and Visser, J., *Analytica Chim. Acta*, 1954, **10**, 373.
2. Hill, U., *Analyt. Chem.*, 1959, **31**, 429.
3. Blair, D., Power, K., Griffiths, D. L., and Wood, J. H., *Talanta*, 1960, **7**, 80.
4. Center, E. J., Overbeck, R. C., and Chase, D. L., *Analyt. Chem.*, 1951, **23**, 1134.
5. Evans, B. S., and Higgs, D. G., *Analyst*, 1945, **70**, 75.
6. Scholes, P. H., and Smith, V. D., *Iron Steel Inst.*, 1962, **200**, 729.
7. Studlar, K., and Eichler, V., *Chemist Analyst*, 1962, **51**, 68.
8. Short, H. G., *Analyst*, 1950, **75**, 420.
9. Parker, C. A., and Goddard, A. P., *Analytica Chim. Acta*, 1950, **4**, 517.
10. Shapiro, L., and Brannock, W. W., *Bull. U.S. Geol. Surv.*, 1956, No. 1036–C.
11. Archer, K., Flint, D., and Jordan, J., *Fuel, Lond.*, 1958, **37**, 421.
12. British Standard 1016 : Part 14, 1963.
13. Kuznetsov, V. I., and Golubstova, R. B., *Zav. Lab.*, 1955, **21**, 1422.
14. Sandell, E. B., "Colorimetric Determination of Traces of Metals," Third Edition, Interscience Publishers Inc., New York, 1959, 243.
15. Rooney, R. C., *B.C.I.R.A. J. Res. and Dev.*, 1958, **7**, 436.
16. Jean, M., *Analytica Chim. Acta*, 1954, **10**, 526.
17. Wetlesen, C. U., *Ibid.*, 1962, **26**, 191.

Received *May 26th*, 1965

A Chemiluminescence Method for Determining Ozone

By D. BERSIS and E. VASSILIOU

(*Nuclear Research Center "Democritus," Aghia Paraskevi Attikis, Athens, Greece*)

A method for determining ozone is described which is characterised by the direct recording and automatic determination of ozone within a wide range of concentrations. The development of this method is based on the use of a chemiluminescent solution that is stable, and shows a linear relationship between the light emitted and the ozone concentration. A combination of rhodamine B with gallic acid in ethanol is satisfactory in operation and does not itself emit light. The electronic instrumentation used is relatively simple. Other methods of ozone analysis based on this principle meet with much difficulty, owing to the direct oxidation of the chemiluminescent compound. The present method, by contrast, involves the use of gallic acid as an ozone acceptor, and rhodamine B, which remains unchanged during the measurement, as a photon emitter. Observations made with an oscillograph of the light emitted by single bubbles of ozonised air passing through the chemiluminescent solution give valuable information about the response-time of the system.

THE increasing interest in the applications of ozone, and their importance in the fields of radiation chemistry, upper atmosphere technology, industrial organic chemistry, etc., gives rise to a constantly growing number of projects dealing with basic research in ozone chemistry. Therefore, the development of good and rapid methods of ozone analysis is required.

The methods for the determination of ozone that have been developed so far are mainly chemical,[1,2] electrochemical[3,4,5,6] and optical.[7,8] Each of these methods has advantages and disadvantages, so that the method that is to be used must be selected according to the individual requirements and conditions. In general, however, the direct and continuous indication or, better, automatic recording of the results is desirable. Methods with procedures of this kind can be found in the literature,[5,6,9] but most of them are relatively slow in response, or require complicated instrumentation. Some methods are also hindered by the presence of gases, such as nitrogen dioxide and sulphur dioxide, which interfere more or less strongly.

Within the range of optical methods, a field at present being developed, is one in which the light emitted by chemiluminescence reactions is used. The use of modern techniques of photon-counting combined with chemiluminescent systems of high efficiency can give rise, mainly from the point of view of sensitivity and response-time, to an ideal method of analysis. Nevertheless, a method based on these principles has not been developed to the extent expected, at least for ozone, owing to the fact that the chemiluminescent compound formed is constantly being destroyed during analysis, thus complicating the results. Re-cycling of the solution containing the chemiluminescent compound causes further complications.

Experimental

Air supply—

A small rotary compressor, fluid metering type 8, Weldon Tool Co., was used, to which a manometer and a flow-meter were attached to control the pressure and the flow-rate.

The air stream was freed from carbon dioxide and dried by means of two columns containing granulated potassium hydroxide and silica gel, respectively.

Ozone production—

Ozonised air was produced by the following systems according to requirement—

(*a*) A conventional Siemens ozoniser (Pyrex glass) with a wall thickness of 1 mm, gap distance of 3 mm and total volume of 11 ml. The two electrodes were filled with a sodium chloride solution (10 per cent. w/v).

(*b*) A 5-fold ozoniser, *i.e.*, five ozonisers, each like the one described above, connected in such a manner as to split the air stream into five equal streams as it enters. The five streams meet again at the common outlet of the ozonisers. By means of a suitable H.T. commutator switch, it was possible to energise any number of the individual ozonisers, as required by the experiment.

The high voltage was supplied by a H.T. transformer (50 c/s) and controlled by means of a Variac connected to a suitable stabiliser, and was continuously monitored.

To exercise additional control over the ozone concentration in the ozonised air stream, a suitable trap, K, (Fig. 1 (*b*)), containing granules of dry potassium hydroxide was used. The volume of ozone decomposed could be regulated by two taps, T_1 and T_2, connected to suitable micrometric screws for fine adjustment.

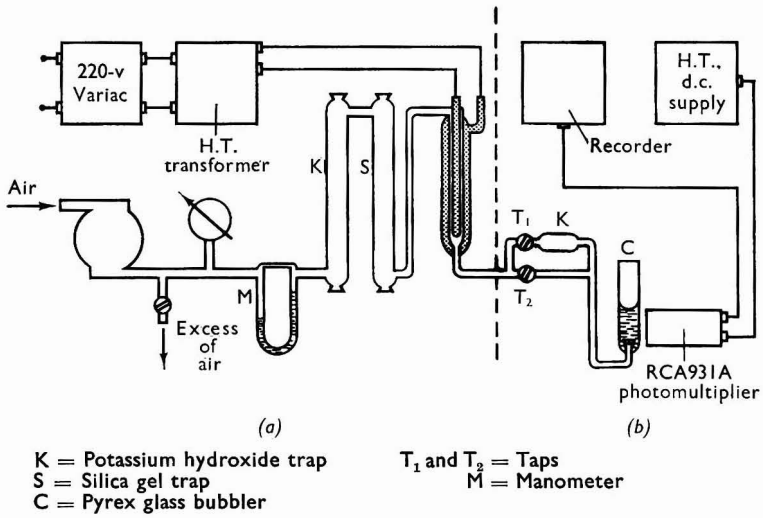

Fig. 1. Apparatus for the automatic determination of ozone: (*a*), calibration unit; (*b*), analysis unit

CALIBRATION OF THE OZONISER—

The calibration of the ozoniser, with respect to ozone production, was carried out as described by Ehmert.[6]

According to this method, a suitable reaction vessel containing a neutral 2 per cent. potassium iodide solution and a certain amount of dilute sodium thiosulphate solution is attached to the apparatus. The ozonised air bubbling through this vessel reacts with the potassium iodide, so liberating free iodine, which in turn reacts with the sodium thiosulphate. The residual sodium thiosulphate is then measured and compared with that of a blank. For this measurement, 4 platinum electrodes are used. An electric potential of about 0·18 volt is applied between two of them. At this voltage no electrolysis takes place as long as sodium thiosulphate is present, because of polarisation. The second pair of electrodes is connected to a suitable current source, so that iodine is liberated by electrolysis, and this reacts immediately with the sodium thiosulphate present. When the whole of the sodium thiosulphate has been consumed, the free iodine causes depolarisation of the first pair of electrodes, and a current flows which is linear with time. By using Faraday's constant, the amount of iodine can be calculated from the values of current and time.

PHOTOMETRIC ASSEMBLY—

A Pyrex glass bubbler, C, (Fig. 1 (*b*)), of approximately 20 mm i.d., containing 10 ml of chemiluminescent solution, was used as a photometric cell. The porous diaphragm was of the G2 type. A second bubbler was connected in series with the first to ensure that no ozone escaped observation.

An RCA 931 A photomultiplier, connected to a Varian G 11 A pen recorder through a pre-amplifier, or to an oscilloscope, and to a stabilised d.c. high tension supply, (Fig. 1 (*b*)), was also used.

SOLUTION—

The chemiluminescent solution was prepared by dissolving 2·5 g of gallic acid and 0·03 g of rhodamine B in 1 litre of ethanol (96 per cent. v/v).

METHOD AND RESULTS

PROCEDURE—

The gas stream to be analysed with respect to ozone concentration is passed through trap K, (Fig. 1 (*b*)), if required (*i.e.*, if the ozone concentration is too high), and is then bubbled through the reaction cell, C. The light emitted energises the photomultiplier, which in turn gives a signal to the recorder. This signal, as will be seen later, is proportional to the ozone concentration when the stream flow remains constant. The recorded area is used to determine the absolute amount of ozone passed through the reaction cell.

CALIBRATION OF THE APPARATUS—

To calibrate the ozone-analysis apparatus, use was made of the calibration unit shown in Fig. 1 (*a*).

It was found, by using Ehmert's method,[6] that the production of ozone was 0·17 per cent. v/v under the following conditions—

gas, air; high tension, 7 kV; stream flow, 64 ml per minute; pressure, 17 inches of water; temperature, 20° C.

The ozonised air stream was led into the photometric cell, and the d.c. high tension supply connected to the photomultiplier was regulated so that the recorder showed an indication in support of the direct reading, *e.g.*, of 17.

LINEARITY AND LIMITS—

To examine the linearity of the method, use was made of the calibration unit (Fig. 1 (*a*)), in which the single ozoniser was replaced by the 5-fold one.

The five single ozonisers, as already indicated, were connected in such a manner that equal amounts of gas could pass through each. Equivalent amounts of ozone, therefore, were produced in each ozoniser under the same conditions. In practice, small differences occurring in the production of ozone by each ozoniser were corrected by fine adjustment of the high tension acting on each ozoniser.

The concentration of ozone produced by each ozoniser was 0·07 per cent. v/v under the conditions described above. The percentage concentration of ozone was calculated with respect to the total stream flow, which was 64 ml per minute, and must not be confused with the flow through each ozoniser, which was approximately 13 ml per minute.

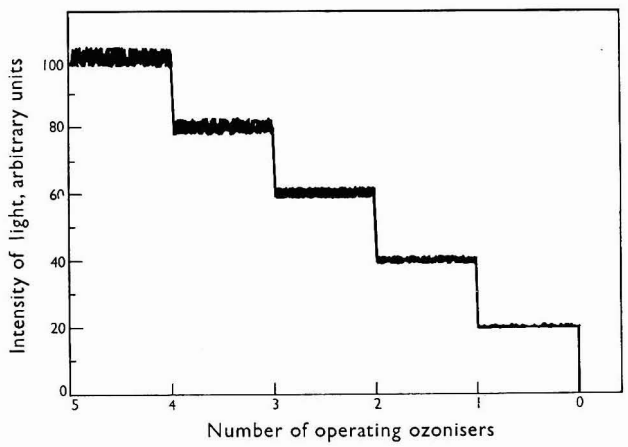

Fig. 2. Operation of the five-fold ozoniser

The tap T_1, (Fig. 1 (*b*)), was entirely closed; T_2 was opened and the d.c. high tension adjusted so that the recorder gave a reading of 100 when all five ozonisers were operating. By switching off one, two, three and four ozonisers the recorder gave readings of 80, 60, 40

and 20, respectively, (Fig. 2). It can, therefore, be concluded that in the region between 0·07 and 0·35 per cent. v/v, the light emitted by the chemiluminescence reaction is linearly related to the ozone concentration.

To determine whether this function is also linear in lower concentrations of ozone, the following operations were carried out—

With all five ozonisers switched on, the ozone decomposition trap, K, (Fig. 1 (*b*)), was adjusted by means of taps T_1 and T_2, so that the recorder gave a reading of 20. This represented an ozone concentration of 0·07 per cent. v/v. On increasing the photomultiplier sensitivity by means of the d.c. high tension supply, a reading of 100 was given (the ozone concentration remaining at 0·07 per cent. v/v). Switching off afresh one, two, three and then four ozonisers, the recorder gave readings of 80, 60, 40 and 20, respectively. The function, therefore, is also linear within the region 0·07 to 0·014 per cent. v/v.

Adopting the same technique, an ozone concentration of 0·0003 per cent. v/v was reached, the function remaining linear.

STABILITY OF THE CHEMILUMINESCENT SOLUTION—

Experiments with the continuous bubbling of ozonised air through 10 ml of chemiluminescent solution showed that the readings are stable for at least 20 hours when the stream flow is 64 ml per minute, and the ozone concentration 0·01 per cent. v/v, allowance being made for the evaporation of alcohol.

INFLUENCE OF TEMPERATURE—

Change of temperature by $\pm 10°$ C does not influence the results of analysis. This lack of effect with temperature change applies to the chemiluminescent solution only, and not to the ozonisers that were used in developing the method. Temperature change in the ozonisers largely affects the rate of ozone production.

INFLUENCE OF OTHER GASES—

As may be appreciated, nitrogen, oxygen and similar gases do not interfere at all.

Nitrogen dioxide and sulphur dioxide were each mixed with air and the mixtures were passed separately through the chemiluminescent solution to test whether they would (*a*) react with simultaneous emission of light, (*b*) destroy the solution.

It was found that in neither instance was light emitted; nor was the chemiluminescent solution destroyed, for, after passing ozone through it again, the reading remained unchanged, *i.e.*, the reading was the same before and after nitrogen dioxide and sulphur dioxide had been passed through the solution.

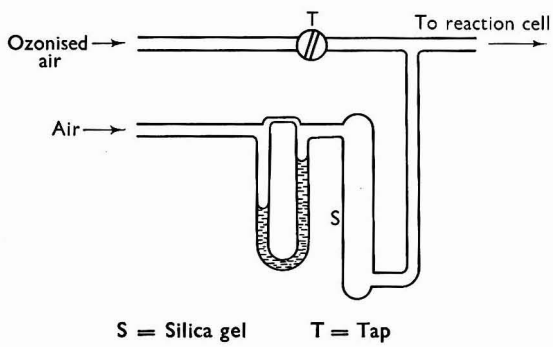

Fig. 3. Apparatus for increasing the stream flow without change in the absolute volume of ozone per time-unit

INFLUENCE OF THE STREAM FLOW—

The apparatus outlined in Fig. 3 was used to study the effect of the gas-stream flow on the emitted light. The flow was increased at the outlet of the ozoniser in order to avoid[10] change in the rate of ozone production.

It was found that increasing the flow up to 200 ml per minute does not influence the reading of the recorder when the rate of ozone production remains constant and within the limits already mentioned.

RESPONSE-TIME OF THE CHEMILUMINESCENT SOLUTION—

To determine the response-time of the solution, oscillographic observations of the light emitted by single bubbles were made (Fig. 4 (*a*)). Similar experiments were also conducted by using a second solution which contained no gallic acid (Fig. 4 (*b*)), and to make a better comparison of the results obtained, the heights of the pulses given by this second solution were arbitrarily equalised with those given by the first solution by adjusting the sensitivity of the oscilloscope.

It can be seen that the time of fall, considerable (approximately $\frac{1}{2}$ second) for rhodamine B, (Fig. 4 (*b*)), becomes almost zero for the mixture with gallic acid, (Fig. 4 (*a*)).

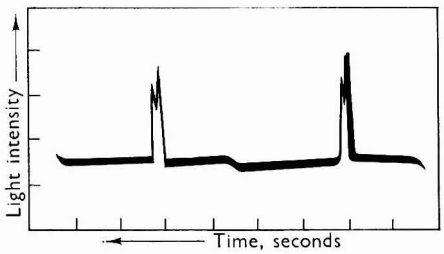

Fig. 4 (*a*). Light emitted by single bubbles of ozonised air through a mixture of gallic acid and rhodamine B in ethanol

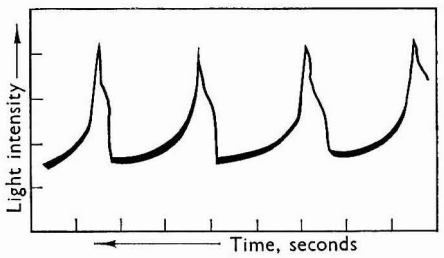

Fig. 4 (*b*). Light emitted by single bubbles of ozonised air through rhodamine B in ethanol

REPRODUCIBILITY OF RESULTS—

It was found from a large number of recordings conducted at several ozone concentration levels under constant conditions, that the chemiluminescent solution (within its stability limits, as already stated) showed a fluctuation smaller than ± 1 per cent.

DISCUSSION

Although some attempts have been made to use the light emitted during chemiluminescence reactions as a means of ozone analysis, all have encountered serious difficulties, mainly arising from (*a*) the continuous decrease of the concentration of the chemiluminescent compound that results in non-linear response of emitted light as a function of ozone concentration, and (*b*) the low level of the intensity of light emitted during the chemiluminescence reactions used.

It must, therefore, be concluded that an analytical method based on the conversion of chemical energy into light, with measurement of the latter, can be successful only if the concentration of the chemiluminescent compound remains unchanged during analysis, *i.e.*, if the chemiluminescent compound does not take a direct part in the chemical reactions occurring in the reaction vessel.

Because such a change occurs, solutions like those used by Biswas and Dahr,[11] and Briner,[12] although emitting light under the influence of ozone, are nevertheless not suitable for ozone analysis. They are mainly useful for continuous recording of the results.

To avoid this difficulty, Bernanose and Réné[13] used chromatographic paper impregnated with solutions of luminol or rhodamine B. Even so, however, although regular luminescence is observed, this method cannot be used for continuous recording as the concentration of the light-emitting compound diminishes rapidly. Bernanose's method also encounters another difficulty. Chromatographic paper-discs were used, containing only a small amount of chemiluminescent compound (of the order of 1 μg) so that the intensity of the light emitted should have been accordingly low. This may have been the reason why a Lallemand 18-step photomultiplier, which has a sensitivity about 10 times higher than that of the IP 21 RCA, was used.

In the present work it was realised that only the protection of the chemiluminescent substance by another compound could lead to the solution of the problem. The latter compound should react with ozone more easily than does the former and its concentration should

be relatively high. Further, during the reaction with ozone either the compound itself or its reaction products, should be able to transfer to the chemiluminescent compound an amount of energy such that the latter would be only temporarily excited, and then return to its ground state by emitting a photon.

A combination of rhodamine B, as a chemiluminescent compound with gallic acid in ethanol, was found to possess the desirable properties.

Use was made of gallic acid for the following 8 reasons—

It contains three hydroxyl groups and therefore† has a good quantum yield.

It has no induction time because of the presence‡ of a carboxyl group.

It protects rhodamine B from direct oxidation.

The oxidation products of gallic acid are not coloured and, therefore, no screening effect takes place.

Its solution in ethanol is not affected at all by the atmospheric oxygen.

It is not characterised by self-emission of light.

The excited molecules or reaction products of gallic acid seem to be at such energy levels (under the influence of ozone) that the energy transfer to rhodamine B, which is finally responsible for the emission of light, becomes possible with a satisfactory quantum yield.

The reaction of gallic acid with ozone, the energy transfer to rhodamine B and the subsequent emission of light have, in total, a much faster response than does the reaction of rhodamine B alone with ozone (*cf.* Fig. 4 (*a*) with Fig. 4 (*b*)).

Rhodamine B was used because—

(*i*) It appears to be a good acceptor of the energy provided by the reaction of gallic acid with ozone.

(*ii*) It is stable to oxygen.

(*iii*) Unlike luminol, it does not self-emit (although the self-luminescence of luminol can be inhibited[16] by using α-naphthol or 3-indazolinone-4-carboxylic acid, other complications arise).

(*iv*) The light that it emits is suited to the S4 surface of the photomultiplier that was used.

(*v*) It is not oxidised directly by ozone in the presence of gallic acid.

The authors of the present work have produced unpublished evidence that the reactions taking place may be summarised as follows—

$$\text{gallic acid} + \text{ozone} \longrightarrow \text{A}^* + \text{oxygen}$$

$$\text{rhodamine B} + \text{A}^* \longrightarrow \text{rhodamine B}^* + \text{B}$$

$$\text{rhodamine B}^* \longrightarrow h\nu + \text{rhodamine B}$$

where A* is an excited intermediate, or excited intermediates, resulting from the reaction between gallic acid and ozone and B is the final product, or products, of the oxidation.

Taking into account (*a*) the sequence of the reactions, (*b*) the fact that rhodamine B remains unchanged during the process, and (*c*) that the concentration of gallic acid is much higher than that of rhodamine B (50 : 1), the advantages of this system can be readily appreciated when it is compared with other methods in which direct oxidation of a chemiluminescent compound is used.

The following conclusions can be drawn—

As regards the use of the 5-fold ozoniser, it can be said that it is the most dependable source of ozone in that concentrations are kept constant and related accurately by certain ratios (*e.g.*, 1 to 2, 1 to 3, 1 to 4 and 1 to 5).

If, instead of the taps T_1 and T_2 of the potassium trap (Fig. 1 (*b*)), a capillary tube is used in one or the other branch of the apparatus, a fixed proportion of the ozone would be destroyed. Partial decomposition of the ozone could also be achieved thermally. This method, however, requires closer control.

† It has been observed that in reactions of polyphenols with ozone the light sum increases rapidly with increasing numbers of hydroxyl groups.

‡ In the presence of carboxyl groups, the emission of light begins simultaneously with the reaction. The delay otherwise observed is called the induction time.

A G2 porous diaphragm was used in the construction of the bubbler, which acted as the reaction cell, C (Fig. 1 (*b*)), as this grade of diaphragm gives fine bubbles without the need to increase considerably the pressure of 17 inches of water of the ozonised air.

It must be pointed out that the production of ozone in the ozoniser falls as the pressure increases and, therefore, all experiments must be carried out at the same pressure. For the single ozoniser that was used in the present work, it was found that by changing the pressure from 5 to 35 inches of water (the stream flow being constant and equal to 64 ml per minute), the ozone concentration changed from 0·10 to 0·08 per cent. v/v.

It has already been mentioned that the time of linear response of 10 ml of the chemiluminescent solution is 20 hours for a stream flow of 64 ml per minute, and an ozone concentration of 0·01 per cent. v/v. This time can be extended, either by increasing the dimensions of the reaction cell, or by suitable adjustment of the potassium hydroxide trap, K (Fig. 1 (*b*)). If by the latter, the time of linear response can easily become 700 hours with the conditions described above.

In the instance of the solution containing gallic acid (Fig. 2), the occurrence of light fluctuation, which is almost entirely absent from similar curves taken by using rhodamine B alone in ethanol, is an additional measure of the fast response of this solution.

The incidental noise originates from the statistical behaviour of the bubbles passing through the chemiluminescent solution.

The intensity of light emitted is a measure of the ozone concentration only if the stream flow is constant. Generally, however, it is a measure of the absolute amount of ozone passing through the reaction cell per unit of time.

The fact that by this method concentrations of ozone down to 0·0003 per cent. v/v only were recorded, does not exclude at all the possibility of measuring much lower concentrations. For example, the modern low noise photomultipliers make it possible to count photons readily, one by one.

A rough calculation of the quantum yield of the system used in the present work, shows that an emitted photon corresponds to about 10^5 molecules of ozone, *i.e.*, about 10^{-11} μg of ozone.

These extreme figures, which lie beyond the limits even of radioactivation analysis, show the future possibilities of chemiluminescence as a tool in analytical chemistry.

This work was done under the auspices of the Greek Atomic Energy Commission.

References

1. Morozov, N. M., *Zh. Analit. Khim.*, 1960, **15**, 367.
2. Zehender, F., and Stumm, W., *Mitt. Geb. Lebensmittelunters. u. Hyg.*, 1953, **44**, 206.
3. Wartburg, A. F., Brewer, A. W., and Lodge, J. P., jun., *Air & Wat. Pollut.*, 1964, **8** (1), 21.
4. Mast, G. M., and Saunders, H. E., *I.S.A. Trans.*, 1962, **1**, 325.
5. Hersch, P., and Deuringer, R., *Analyt. Chem.*, 1963, **35**, 897.
6. Ehmert, A., *in* "Ozone Chemistry and Technology," Advances in Chemistry Series 21, American Chemical Society, Washington, D.C., 1959, p. 128.
7. Osherovich, A. L., and Rodionov, S. F., *Atm. Ozone* (Moskow: Mosk. Univ.), Sb., 1961, 72.
8. Alway, C. D., and Slomp, G., jun., *in* "Ozone Chemistry and Technology," Advances in Chemistry Series 21, American Chemical Society, Washington, D.C., 1959, p. 108.
9. Regener, V. H., *Ibid.*, 1959, **21**, 121.
10. Bersis, D., and Katakis, D., *J. Chem. Phys.*, 1964, **40** (7), 1997.
11. Biswas, J., and Dhar, N., *Z. anorg. allg. Chem.*, 1928, **173**, 125.
——, ——, *Ibid.*, 1931, **199**, 400.
12. Briner, E., *Helv. Chim. Acta*, 1940, **23**, 320.
13. Bernanose, A. J., and Réné, M. G., *in* "Ozone Chemistry and Technology," Advances in Chemistry Series 21, American Chemical Society, Washington, D.C., 1959, p. 7.
14. Bersis, D. S., *Z. phys. Chem.*, 1959, **22**, 328.
15. Uemura, K., *Science and Crime Detection*, 1954, **7**, 6.

Received *July 5th*, 1965

The Determination of Tantalum by the Solvent Extraction of a Tantalum - Pyrogallol Complex

By BETSY BIRABEN SCOTT

(*Facultad de Química y Farmacia, Universidad Nacional de La Plata, Argentina*)

A colorimetric procedure for determining up to 1·2 mg of tantalum in the presence of up to 20 mg of niobium, or up to 180 mg of tungsten, has been developed. The colourless tantalum - pyrogallol complex is extracted into ethyl acetate at pH between 4·5 to 6·0 by means of tetrahexyl or tetrabutyl ammonium iodide and back-extracted with acidified ammonium oxalate (pH 2·0). The yellow complex obtained is measured spectrophotometrically at 400 mμ.

Tantalum can be determined colorimetrically when admixed with niobium and tungsten by selectively extracting a colourless tantalum - pyrogallol complex, in the presence of tetrabutyl or tetrahexyl ammonium iodide, into ethyl acetate and back-extracting with acidified ammonium oxalate. The molar extinction coefficient of the yellow complex is 2135 at 400 mμ. Vanadium, chromium and molybdenum interfere less than in the original procedure of Hunt and Wells. Titanium remains as a serious interference.

Nudelman[1] has studied the effect of quaternary ammonium compounds on the extraction of metal - pyrogallol complexes into ethyl acetate. He observed that a colourless tantalum complex was extractable in the presence of tetrahexyl or tetraheptyl ammonium iodide. This observation has been developed into a method for the determination of tantalum in the presence of excess niobium and other ions. Usually corrections have to be applied to pyrogallol absorptiometric methods for determining tantalum when niobium or tungsten are present,[2,3] but in the new procedure 1 mg of tantalum can be determined in the presence of 20 mg of niobium and 180 mg of tungsten. Titanium interferes with the determination and must be removed.

Experimental

The effect of pH and quaternary ammonium iodide on extraction—

Aliquots of a tantalum solution containing 0·9 mg of metal were extracted at different pH's with additions of tetrahexyl ammonium iodide (THAI), or the tetrabutyl salt (TBAI) into ethyl acetate and then back-extracted with ammonium oxalate solution acidified to pH 2. The measured absorbances at 400 mμ are given in Tables I and II.

Table I

Effect of pH on extraction of tantalum (0·9 mg) in the presence of THAI and TBAI

pH	Absorbance	
	THAI	TBAI
4·0	0·490	0·500
4·2	0·515	—
4·3	0·522	0·525
4·5	0·525	—
4·9	0·520	0·527
5·0	0·535	—
5·6	0·525	—
6·4	0·522	0·532
6·8	0·500	0·518

TABLE II

EFFECT OF THAI AND TBAI ON THE EXTRACTION OF TANTALUM (0·9 mg) AT pH 4·5 TO 5·0

THAI, mg	Absorbance	TBAI, mg	Absorbance
5	0·200	20	0·480
8	0·528	40	0·526
15	0·531	80	0·532
30	0·520	100	0·531
45	0·500	120	0·520

A certain amount of quaternary salt is required for extraction into the organic phase, but an excess prevents back-extraction into the aqueous phase.

INTERFERENCE BY NIOBIUM—

Amounts of niobium up to 4 mg are without effect; between 4 and 8 mg a larger amount of tetrahexyl ammonium iodide must be taken to ensure quantitative extraction of tantalum; above 9 mg too much tetrahexyl ammonium iodide (required for extraction into the organic phase) prevents quantitative stripping. Tetrahexyl ammonium iodide can be used successfully with 9 mg of niobium but only over a restricted range of pH. Tetrabutyl ammonium iodide allows up to 20 mg of niobium to be present, and extraction is possible over a wider pH range than with tetrahexyl ammonium iodide (see Tables III and IV).

TABLE III

EFFECT OF THAI AND TBAI ON THE INTERFERENCE OF NIOBIUM IN THE DETERMINATION OF TANTALUM (0·9 mg) AT pH 4·5 TO 5·0

Niobium, mg	THAI	Absorbance	TBAI	Absorbance
—	15	0·531	80	0·530
0·45	15	0·530	80	0·528
0·90	15	0·531	80	0·530
3·6	15	0·527	80	0·530
3·6	30	0·526	—	—
6·3	30	0·513	—	—
9·0	8	0·258	—	—
9·0	15	0·470	—	—
9·0	40	0·492	—	—
9·0	50	0·450	—	—
18·0	—	—	80	0·535
27·0	—	—	80	0·470
36·0	—	—	120	0·460
36·0	—	—	200	0·442

TABLE IV

EFFECT OF pH ON THE INTERFERENCE OF 9 mg OF NIOBIUM IN THE DETERMINATION OF TANTALUM (0·9 mg)

	Absorbance in the presence of—	
pH	THAI, 40 mg	TBAI, 80 mg
4·5	—	0·531
4·7	0·490	—
4·9	0·491	0·530
5·3	0·458	—
5·5	—	0·532
5·8	0·340	—
5·9	—	0·528
6·2	—	0·529

INTERFERENCE BY TUNGSTEN—

Although the tungsten - pyrogallol complex is insoluble in ethyl acetate in the presence of tetrahexyl ammonium iodide, it can interfere because extractable coloured products are formed. Spectral studies on the reaction between tungsten, pyrogallol and quaternary ammonium ions show that the existence of a definite stoicheiometric compound is dubious, and suggest the oxidative nature of the process as the colour intensity increases, not only

with acidity (Table V) and ammonium salt concentration, but also with time. Thus when niobium is present, the restricted pH allows only 11 mg of tungsten to be present without

TABLE V

EFFECT OF pH ON THE INTERFERENCE BY 18·4 mg OF TUNGSTEN IN THE DETERMINATION OF TANTALUM (0·9 mg)

	Absorbance in the presence of—	
pH	THAI, 15 mg	TBAI, 60 mg
4·5	—	0·580
4·7	—	0·558
5·0	0·590	—
5·2	0·562	—
6·1	0·555	0·547
6·5	0·530	—
6·6	—	0·542

interference. In the absence of niobium, up to 180 mg of tungsten do not cause interference if the pH is maintained at 6, and a minimum amount of tetrahexyl ammonium iodide is used.

TABLE VI

EFFECT OF TUNGSTEN IN THE DETERMINATION OF TANTALUM (0·9 mg) AT A pH OF 4·5 TO 5·0

	Absorbance in the presence of—	
Weight of tungsten, mg	THAI, 15 mg	TBAI, 80 mg
5·5	0·531	0·532
9·2	0·529	0·551
11·0	0·530	0·570
12·9	0·538	—
18·4	0·590	—

TABLE VII

EFFECT OF TUNGSTEN ON THE DETERMINATION OF TANTALUM (0·9 mg) AT A pH OF 6

Weight of tungsten, mg	Absorbance in the presence of 15 mg of THAI
18	0·528
92	0·527
184	0·532
202	0·548

Tetrabutyl ammonium iodide is unsuitable for use with tungsten because of the extractability of the tungsten - pyrogallol complex.

OTHER INTERFERENCES—

The interferences studied only include those which usually accompany tantalum after a hydrolytic concentration starting from more complex materials.

Molybdenum, vanadium and chromium interfere to a lesser extent than in Hunt and Wells' technique (see Table VIII).

Concentrations of fluoride and phosphate as high as 5×10^{-2} M cause insignificant errors in the procedure. Boric acid interferes by retaining tantalum in the aqueous phase; sulphate does not interfere.

TABLE VIII

INTERFERENCE OF CATIONS IN THE DETERMINATION OF TANTALUM

	Tantalum oxide equivalent, mg	
1 mg of metal oxide	Hunt and Wells	Proposed method
Molybdenum trioxide	0·68	0·015
Vanadium pentoxide	0·44	0·12
Chromic oxide	—	0·06

COLOUR PRODUCTION AND STABILITY—

Colour measurements were made after 30 minutes in order to obtain perfect separation of phases; the colour was stable for at least 1 day. The absorbance is linear up to 1·2 mg of tantalum.

PROCEDURE—

Test solutions—Tantalum (10^{-3} M) and niobium (10^{-2} M) solutions were prepared by dissolving the appropriate amount of spectrographically pure metal in a platinum dish with 5 ml of 48 per cent. hydrofluoric acid and a few drops of concentrated nitric acid. After concentration on a steam-bath to about 2 ml, 5 ml of concentrated sulphuric acid were added, evaporating to sulphur trioxide fumes. The diluent was 4 per cent. ammonium oxalate solution. Tungsten (5×10^{-2} M and 1 M), molybdenum (1×10^{-2} M), vanadium and chromium (5×10^{-2} M) solutions were prepared by dissolving the corresponding salt in distilled water.

A titanium (1×10^{-2} M) solution was prepared in the same way as tantalum except that titanium dioxide was used as the starting material.

The molar solutions of phosphate and fluoride were prepared by dissolving the respective ammonium salt in distilled water.

PREPARATION OF CALIBRATION GRAPH—

The standard curve was prepared by taking aliquots of the 0·2 mg per ml tantalum solution and proceeding as indicated in Method (*a*) or (*d*). The curve was plotted over the range 0 to 1·2 mg of pure metal.

REAGENTS—

Ammonium oxalate solution, 4 per cent. w/v, aqueous.

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

Sulphuric acid solution, 5 per cent. w/v, aqueous.

Pyrogallol reagent (A)—Dissolve 10 g of pyrogallol in 100 ml of analytical-reagent grade ethyl acetate (this solution keeps well for several weeks).

Pyrogallol reagent (B)—Dissolve 10 g of pyrogallol and 0·1 g of tetrahexyl ammonium iodide in 100 ml of analytical-reagent grade ethyl acetate.

Pyrogallol reagent (C)—Dissolve 10 g of pyrogallol and 0·3 g of tetrahexyl ammonium iodide in 100 ml of ethyl acetate.

Tetrabutyl ammonium iodide solution—Dissolve 1 g in 100 ml of 4 per cent. ammonium oxalate solution.

Acid ammonium oxalate—Acidify 4 per cent. w/v ammonium oxalate solution with concentrated sulphuric acid until a pH of 2 is attained.

METHOD—

Prepare the sample solution by dissolving the metal with hydrofluoric acid and a few drops of nitric acid (see Test solutions), or by fusing the oxides with potassium bisulphate. After preparing the solution and evaporating, dilute with ammonium oxalate solution. Use an amount of sample solution containing not more than 1 mg of tantalum.

(*a*) *Samples containing niobium up to 4 mg and tungsten up to 11 mg*—Introduce an aliquot of sample solution into a 100-ml separating funnel and dilute to 20 ml with ammonium oxalate solution after adjusting the pH to between 4·5 and 5·0 with sodium sulphite solution (if necessary correct with sulphuric acid solution). Extract with a 10-ml portion of pyrogallol reagent (B) and shake the mixture vigorously for at least 2 minutes. Re-extract the clear aqueous phase with 5 ml of the same reagent. Combine the organic layers and wash twice with 2-ml portions of 4 per cent. ammonium oxalate, waiting each time until the aqueous solution is clear. Add 20 ml of acid ammonium oxalate solution to the organic layer and shake them together vigorously for 2 minutes. Wait for 30 minutes and read the absorbance at 400 mμ in a 1-cm cell against a blank similarly prepared.

(*b*) *Samples containing niobium between 4 and 8 mg and tungsten up to 11 mg*—All conditions are the same except that it is necessary to extract the first time with 10 ml of pyrogallol reagent (C). The second extraction is performed with 5 ml of reagent (B).

(*c*) *Samples containing tungsten up to 180 mg and niobium up to 1 mg*—This is essentially the same procedure as in (A), but the extraction is performed at a pH of 6·0 to 6·5.

(*d*) *Samples containing niobium up to* 20 *mg and tungsten up to* 6 *mg*—Add the sample and 5 ml of tetrabutyl ammonium iodide solution to a 100-ml separating funnel. Add ammonium oxalate solution to a total volume of about 20 ml. Correct the pH as above to between 4·5 and 6·5. Extract initially with 10 ml of pyrogallol reagent (A), and then with 5 ml of the same reagent after adding 3 ml of tetrabutyl ammonium iodide solution. Treat the collected extracts in the same manner as when reagent (C) is used.

Results and discussion

By using the appropriate procedure the figures obtained for the determination of 0·9 mg of tantalum in the presence of excess niobium and tungsten are collected in Table IX.

Table IX

Determination of tantalum (0·9 mg) in the presence of niobium and tungsten

Niobium, mg	Tungsten, mg	THAI, mg	TBAI, mg	pH	Absorbance
4	11	15	—	4·6	0·528
7	11	35	—	4·7	0·527
0·9	180	15	—	6·2	0·530
18	5·5	—	80	5·8	0·531

The extraction of the tantalum complex into ethyl acetate is quantitative. Absorbances for the same concentration, with and without extraction, show an insignificant difference (less than 2 per cent.). An unstable yellow colour appears on acidifying the residual aqueous phase with sulphuric acid, which resembles the yellow[4] complex $TaO(C_2O_4)Py^-$, but tests show that it is caused by reaction between pyrogallol and bisulphite. The residual aqueous solution remains colourless after acidification when extraction is carried out at a lower pH of between 4·0 and 4·5.

In agreement with Lucachina[5] it was found that the oxalate ion must be present in the final aqueous phase; the yellow complex was not formed when the organic phase was extracted with sulphuric acid of the same pH as the oxalate extractant.

Other quaternary ammonium salts such as tetraheptyl ammonium iodide, tetrahexyl and tetrabutyl ammonium bromides give similar results.

I wish to thank R. A. Wells, former Director of the National Chemical Laboratory, Teddington, for providing the facilities to carry out the work. I am also indebted to A. Woolf for his reading of the manuscript and to J. A. Catoggio and G. Smith for their helpful suggestions.

References

1. Nudelman, O., Thesis, 1964 (unpublished paper), Facultad de Química y Farmacia, La Plata, Argentina.
2. Catoggio, J. A., and Rogers, L. B., *Talanta*, 1962, **9**, 387.
3. Hunt, E. C., and Wells, R. A., *Analyst*, 1954, **79**, 345.
4. Babko, A. K., and Lucachina, V. V., *Ukr. Khim. Zh.*, 1962, **28**, 371; *Chem. Abstr.*, 1962, **57**, 9445e.
5. Lucachina, V. V., *Ibid.*, 1963, **29**, 689; *Chem. Abstr.*, 1963, **59**, 14574g.

Received *May 24th*, 1965

Flame-photometric Determination of Sodium and Potassium in Manganese Ores

By B. G. RUSSELL

(The National Institute for Metallurgy, Yale Road, Milner Park, Johannesburg)

Two procedures are described; in one, the sample is dissolved in hydrochloric acid, interfering elements are precipitated with 8-hydroxyquinoline in ammoniacal solution, and the precipitate then extracted into chloroform. Sodium and potassium are determined in the aqueous phase by means of a filter flame photometer.

The second procedure is more suitable for routine use and involves the dissolution of the sample in hydrochloric acid, followed by the addition of sulphuric acid and aluminium nitrate to suppress interferences, and the direct evaluation of the sodium and potassium contents of the solution by means of either a prism or a filter flame photometer. Comparative results obtained by this alternative procedure on instruments of these two types are given.

Growing importance is being attached to the reliable determination of sodium and potassium contents of South African manganese ores, and this problem is aggravated by the widely discrepant results currently reported by suppliers and customers on the same sample.

Table I

Results of the determination of sodium and potassium in South African manganese ores

	Analysis by suppliers		Analysis by customers	
Sample No.	Sodium oxide, per cent.	Potassium oxide, per cent.	Sodium oxide, per cent.	Potassium oxide, per cent.
1	0·56	0·89	0·41	1·08
2	0·48	0·76	0·36	0·61
3	0·65	1·14	0·46	0·46
4	0·44	0·51	0·12	0·61
5	0·51	0·62	0·14	0·93

The classical gravimetric procedures of J. Lawrence-Smith[1] and Berzelius[2] for determining sodium and potassium were found to be too time-consuming and inaccurate for present purposes.

Grimaldi[3] has successfully applied a "standard-addition" method to the determination of sodium and potassium in siliceous rocks, but we were unsuccessful in applying this procedure to the analysis of manganese ores, owing to the non-linear relationship between emission and alkali content of the sample.

The choice of a flame-photometric procedure for determining sodium and potassium is largely dictated by the type of instrument available. Instruments involving the use of a prism are invariably more expensive than those in which filters are used. If a simple filter instrument, typified by the "EEL" flame photometer (obtainable from Evans Electroselenium Ltd., Harlow, Essex), can be used, it has economic advantages.

The "EEL" flame photometer has been described in detail by Collins and Polkinhorne,[4] and was used in the investigational work described in this paper. To obtain further information on the performance of this instrument many of the solutions examined on it were also examined on a Beckman Model DU spectrophotometer that had been fitted with a flame attachment.

The mutual interferences of alkali metals,[5] calcium,[6,7,8] and some anions[4] have already been investigated. Farrow and Hill[9] have also studied the effect of cations in the determination of alkali metals, and reported interferences by the chief constituents of manganese ores, *i.e.*, iron and manganese.

The relatively cool air - coal gas or propane - butane flame of the "EEL" instrument excites fewer elements than the higher temperature flames of other instruments. Cationic interferences are therefore less with an "EEL" instrument; however, a filter instrument is not sufficiently selective for accurate determination of sodium and potassium, because emissions due to iron and manganese are known to pass through the filters.[9]

Bond and Stace[5] found that the use of narrow wavelength-band filters largely eliminates emission from calcium and strontium and, although the use of such filters might be an advantage in eliminating interference from iron and manganese emissions, these filters were not readily available.

Method I—Suppression of interference by solvent extraction

Experimental

Preparation of a solution of the sample—

Hydrochloric acid was used to dissolve the samples because sodium and potassium are present in manganese ores as cryptomelane and ephesite, both of which are readily soluble in this acid. This was later verified by the good agreement of the sodium and potassium values obtained in repeat tests in which the samples were decomposed with hydrofluoric acid.

Separation of interfering substances—

Manganese is the major interfering element in the analysis of manganese ores. It is not readily absorbed on a cation-exchange resin unless it is present in solution as permanganate. This, however, presents difficulties that arise from the marked tendency for manganese in solution to precipitate as manganese dioxide during oxidation.

Few reagents, other than 8-hydroxyquinoline, precipitate manganese quantitatively. Many other metals are also quantitatively precipitated by 8-hydroxyquinoline in alkaline solution, but alkalis tend to co-precipitate, and errors are introduced if the precipitate is removed by filtration.

If, however, the precipitate is extracted into chloroform, no such loss of alkalis occurs. If calcium and magnesium are present, it is essential to use a large excess of 8-hydroxyquinoline, but it is claimed that the presence of butyl Cellosolve increases the solubility of the magnesium complex in chloroform,[10] and so reduces the amount of chloroform required for the extraction. Butyl Cellosolve also has the advantage of reducing the amount of chloroform necessary for the extraction of other metal 8-hydroxyquinolinates; it also aids separation of the organic and aqueous phases. If only a small amount of calcium is present, the calcium precipitate may be removed by filtration after the chloroform extraction.

The precipitation of manganese is quantitative only if the pH of the solution is between 7 and 9·5, and a reducing agent, *e.g.*, hydroxylamine,[11] is present.

When an acetic acid solution of 8-hydroxyquinoline was used, the extraction of manganese was incomplete, and subsequent removal of the large amount of ammonium acetate formed was difficult and time-consuming. When, however, an alcoholic solution of the reagent was used, these problems were not encountered.

Preparation of standards—

To minimise error, acidities were adjusted so that each solution contained 3 per cent. v/v of perchloric acid before it was sprayed into the flame. Standard solutions containing known amounts of both sodium and potassium oxides, in the ratio of 1 to 4, were used for calibration purposes, as this was approximately the ratio of sodium to potassium found in the samples investigated.

It has been shown[5] that wide variations in this ratio are permissible because the mutual interference of sodium and potassium is negligible if the amount of either does not exceed that of the other by a factor of more than 10.

Method

Reagents—

All reagents should be of the highest purity obtainable.

Ammonia solution—Pass ammonia gas into water in a plastic bottle until a saturated solution of ammonia is obtained. This reagent, as supplied, usually has an unacceptably high sodium content.

8-*Hydroxyquinoline* (8 *per cent. w/v*)—Dissolve 100 g of the reagent in 70 ml of 96 per cent. ethanol, then add 900 ml of water. Filter the solution through a Büchner funnel, and wash the precipitate with water. Continue to draw air through the precipitate for about 30 minutes, then transfer it to a dark bottle and store it, preferably in a refrigerator.

Dissolve 8 g of the purified reagent in 100 ml of ethanol; prepare the reagent solution daily.

Standard sodium solution—Dissolve 1·8860 g of sodium chloride (dried at 105° C) in water, add 5 ml of perchloric acid (sp.gr. 1·58), and dilute the solution to 1 litre.

Dilute this solution 10 times for use.

1 ml of solution ≡ 0·1 mg of sodium oxide

Standard potassium solution—Dissolve 1·5830 g of potassium chloride (dried at 105° C) in water. Add to this solution 5 ml of perchloric acid (sp.gr. 1·58) and dilute the solution to 1 litre. Dilute this solution 10 times for use.

1 ml of solution ≡ 0·1 mg of potassium oxide

Keep the volumes of all reagent solutions to a minimum and ensure accurate compensation for the blank by standardising the amount of each reagent added at each stage of the procedure.

APPARATUS—

Use quartz apparatus wherever possible, and make extractions in Pyrex separating funnels. Calibrated flasks made of soda-glass were used, but these did not introduce any detectable error.

Use a filter (*e.g.*, "EEL") flame photometer with an air - coal gas flame.

PROCEDURE—

Determine a blank on the reagents with each batch of samples.

Transfer the sample (see Note 1) to a 150-ml quartz beaker, and add 15 ml of hydrochloric acid (sp.gr. 1·16). Evaporate the solution to dryness on a hot-plate, then cool it slightly. Add about 2 drops of hydrochloric acid (sp.gr. 1·16) and 20 ml of water. Boil the solution gently to dissolve soluble salts; allow to cool. Transfer the entire contents of the beaker to a calibrated flask and dilute the solution to the mark.

Filter the solution through a dry filter-paper into a dry quartz beaker, and transfer a 20-ml aliquot, representing not more than 0·29 g of sample, to a 250-ml quartz beaker.

Add 2 g of hydroxylammonium chloride, dilute the solution with water to about 150 ml, then add 10 ml of the 8-hydroxyquinoline solution. Adjust the pH of the solution to between 7 and 9, testing with indicator paper; heat it to about 60° C (do not boil), then cool.

Transfer the entire contents of the beaker to a 150-ml Pyrex separating funnel, and add to the solution 2 ml of butyl Cellosolve and 15 ml of chloroform. Shake the funnel for about 30 seconds, then draw off and discard the chloroform phase. Repeat the extraction twice, with 5-ml portions of chloroform.

Transfer the aqueous solution to a 150-ml quartz beaker; if necessary, filter the solution through a Whatman No. 40 filter-paper; wash the filter-paper sparingly with water.

Add 3 ml of nitric acid (sp.gr. 1·42), evaporate the solution to dryness, then cool. Add 2 ml of perchloric acid (sp.gr. 1·54), again evaporate the solution to dryness, volatilise the excess of perchloric acid and then cool. Add 1·5 ml of perchloric acid (sp.gr. 1·54), warm gently to dissolve soluble salts, then transfer the clear solution to a 50-ml calibrated flask and dilute with water to the mark.

Determine the sodium and potassium emissions of the solution at 589 and 766·5 mμ, respectively, and calculate the sodium and potassium oxide contents of the sample from calibration graphs prepared by using the appropriate standard sodium and potassium solutions.

NOTE 1—

When analysing batches of samples containing the same approximate ratios of sodium and potassium, it is advantageous to prepare calibration standards containing both sodium and potassium in similar ratios to the samples. When the ratio of the one element to the other exceeds 10 to 1, this technique becomes necessary to compensate for mutual interference of the two elements.

PRECISION OF THE METHOD—

This is shown in Table II. The samples contained about 45 per cent. of manganese, and 13 per cent. of iron, 3·5 per cent. of silica, 1·1 per cent. of barium oxide and 4 per cent. of alumina.

TABLE II

DETERMINATION OF SODIUM AND POTASSIUM BY PROPOSED METHOD

	Sodium oxide		Potassium oxide	
Sample number	2	4	2	4
Determinations	11	7	14	7
Mean value	0·193 per cent.	0·207 per cent.	0·843 per cent.	0·682 per cent.
Standard deviation	0·016	0·014	0·022	0·030
Coefficient of variation ..	8·3 per cent.	6·8 per cent.	2·7 per cent.	4·4 per cent.

COMPARISON OF RESULTS—

The standard deviation was higher for the determination of sodium than it was for the potassium determination. A probable explanation lies in the proportionately higher blank in the sodium determination: the mean of 5 determinations was 0·072 per cent. of sodium oxide; the corresponding blank in the potassium determination was a mean of 0·036 per cent. of potassium oxide for 6 determinations.

RECOVERIES OF ADDED SODIUM AND POTASSIUM—

To assess the extent of any loss of alkalis in the proposed procedure, additions equivalent to 2·0 mg of sodium oxide and 5·0 mg of potassium oxide were made to a series of 0·5-g samples of purified manganese dioxide.

Recoveries of sodium oxide varied between 98·1 and 100·6 per cent. and the blank values between 22 and 65 μg; the standard deviation was 0·035 μg, and the coefficient of variation was 1·7 per cent.

Recoveries of potassium oxide varied between 92·0 and 102·0 per cent. and the blank values were between 8 and 49 μg; the standard deviation was 0·167 μg, and the coefficient of variation was 3·5 per cent.

These recoveries show that there is no appreciable loss of sodium or potassium in the proposed procedure and emphasise the necessity to make frequent blank determinations, especially with each batch of samples.

CONCLUSION

The method is satisfactory for determining sodium and potassium in manganese ores. The procedure is, however, time-consuming and not suitable for application on a routine basis.

As very few elements interfere, the method is ideally suited for establishing the sodium and potassium content of samples of manganese ores of variable composition. The investigation of a batch of 7 samples and a blank can be completed in about 10 working hours.

METHOD II—SUPPRESSION OF INTERFERENCES BY THE ADDITION OF ALUMINIUM

EXPERIMENTAL

LIMITATIONS OF A FILTER FLAME PHOTOMETER—

The main limitation of a filter instrument is due to the transmission of iron and manganese emissions through the sodium filter, and iron emission through the potassium filter. Evans Electroselenium Ltd. reported that the presence of aluminium suppresses interference due to calcium,[6,8] and it was decided to investigate the suppression effect of aluminium on iron and manganese emissions.

According to Collins and Polkinhorne,[4] hydrochloric acid and chlorides seriously suppress sodium and potassium emissions when the chloride concentration exceeds 0·012 N. Therefore, chlorides introduced during the dissolution of the sample should be removed by evaporating the sample solution with a measured excess of sulphuric acid.

TABLE III

FLAME PHOTOMETER READINGS SHOWING THE EFFECT OF SULPHURIC ACID ON EMISSION OF SODIUM AND POTASSIUM

Sulphuric acid, normality	Sodium oxide, 30 p.p.m.		Potassium oxide, 30 p.p.m.	
	"EEL"	Beckman	"EEL"	Beckman
Nil	66·5	60·5	58·0	63·0
0·05	—	61·5	—	63·0
0·1	—	62·0	—	—
0·4	—	62·0	—	66·0
0·5	65·0	—	61·0	—
0·7	—	62·0	59·0	64·5
1·0	63·5	62·0	59·0	62·5
1·5	—	—	58·0	—
2·0	61·0	60·0	56·0	57·5
3·0	58·5	57·0	52·5	53·0

Both instruments were adjusted to give 100 divisions deflection equivalent to sodium oxide and potassium oxide, each at the 50 p.p.m. level.

Results in Table III show, in general, that sulphuric acid does not have an erratic or pronounced effect on the emissions of sodium and potassium, provided that the strength of this acid is about 0·7 N. It was decided, therefore, to standardise the amount of sulphuric acid present at 0·8 N, and avoid an excessive loss of this acid when solutions were evaporated to remove chlorides. The next aim was to establish the effect of added aluminium, and the tests made are summarised in Table IV; the strength of sulphuric acid in these and all subsequent tests was maintained at 0·8 N.

TABLE IV

FLAME PHOTOMETER READINGS SHOWING THE EFFECT OF ALUMINIUM (AS NITRATE AND SULPHATE) ON EMISSIONS OF SODIUM AND POTASSIUM

Aluminium added, p.p.m.	30 p.p.m. of sodium oxide with aluminium added as—				30 p.p.m. of potassium oxide with aluminium added as—			
	Nitrate		Sulphate		Nitrate		Sulphate	
	"EEL"	Beckman	"EEL"	Beckman	"EEL"	Beckman	"EEL"	Beckman
0	66·5	66·5	66·5	66·5	58·0	63·0	58·0	63·0
25	—	—	67·0	—	—	—	—	—
50	—	66·5	—	65·0	—	—	—	62·0
75	—	—	—	—	—	—	59·0	—
100	67·0	66·5	67·0	66·5	58·5	—	59·0	64·0
150	—	—	—	—	—	—	59·5	—
200	—	—	—	67·0	—	—	—	66·0
300	67·5	69·0	67·0	—	58·5	65·0	60·0	—
500	—	—	67·0	68·0	—	—	59·5	68·0
1000	66·5	68·0	66·0	66·0	58·0	65·0	59·0	66·0
1500	—	—	62·0	64·0	—	—	58·5	65·0
2000	66·5	66·0	59·0	62·0	58·0	60·0	56·5	63·5
3000	66·5	—	—	—	58·0	—	—	—
4500	—	63·0	—	—	54·5	55·0	—	—
5000	66·5	63·0	—	—	53·0	55·0	—	—
6000	66·5	63·0	—	—	—	55·0	—	—

Instrumental settings as for Table III.

The results contained in Table IV show that the sodium emission is constant when the concentration of aluminium (as nitrate) exceeds 1000 p.p.m. and that the potassium emission is reasonably steady for concentrations of aluminium (as nitrate) up to 3000 p.p.m.

If the aluminium is added as sulphate, the emissions of both sodium and potassium vary. The sulphate concentration must therefore be kept constant, and this requirement precludes the use of aluminium sulphate in place of aluminium nitrate to suppress interferences arising from the presence of iron and manganese.

TABLE V

FLAME PHOTOMETER READINGS SHOWING THE EFFECT OF IRON AND MANGANESE ON EMISSIONS OF SODIUM AND POTASSIUM

Iron or manganese added, p.p.m.	30 p.p.m. of sodium oxide, with added—				30 p.p.m. of potassium oxide, with added—			
	Iron		Manganese		Iron		Manganese	
	"EEL"	Beckman	"EEL"	Beckman	"EEL"	Beckman	"EEL"	Beckman
0	66·5	66·5	66·5	66·5	61·5	63·0	61·5	63·0
1000	75·5	—	—	70·5	63·0	—	—	63·0
2000	83·0	65·0	74·5	71·0	66·0	61·0	61·5	63·5
4000	>100	—	—	71·5	83·0	—	—	66·0
5000	—	65·5	79·0	—	—	62·5	61·5	—
8000	—	—	—	73·0	—	—	—	66·5
10,000	—	68·0	92·0	75·5	—	64·0	70·0	67·0

Concentration and instrumental settings as for Table VI.

Table V shows that the potassium emission is unaffected by up to 5000 p.p.m. of manganese, although the presence of iron at the 1000 p.p.m. level causes high readings to be obtained. Interference due to the presence of iron is satisfactorily suppressed in the presence of between 2000 and 5000 p.p.m. of aluminium (as nitrate), as shown in Table VI.

TABLE VI

FLAME PHOTOMETER READINGS SHOWING THE EFFECT OF ALUMINIUM NITRATE ON THE COMBINED INTERFERENCE OF IRON AND MANGANESE ON THE EMISSIONS OF SODIUM AND POTASSIUM

Aluminium added (as nitrate), p.p.m.	Sodium oxide, 30 p.p.m.		Potassium oxide, 30 p.p.m.	
	"EEL"	Beckman	"EEL"	Beckman
0	—	79·5	75·0	72·0
150	—	81·0	—	76·5
300	—	78·0	71·0	74·0
500	—	76·5	—	72·5
600	—	76·0	69·5	66·5
1000	92·0	75·0	65·5	66·5
2000	88·0	—	61·5	—
3000	86·0	75·0	61·5	66·5
5000	84·0	75·0	61·5	66·5
10,000	84·0	82·5	<60·0	58·0
12,000	84·0	—	—	—

The solutions contained 1400 p.p.m. of iron and 4880 p.p.m. of manganese. Instrumental settings were as for Table IV.

Interference effect on the sodium emission by both iron and manganese (Table V) is probably a result of the light from the respective emission lines at 586·8 mμ and 586·0 mμ passing through the sodium filter. The presence of aluminium (as nitrate) in concentrations

TABLE VII

DETERMINATION OF SODIUM AND POTASSIUM IN THE PRESENCE OF MANGANESE AND IRON

Manganese added, p.p.m.	Sodium oxide, p.p.m.*		Potassium oxide, p.p.m.*	
	"EEL"	Beckman	"EEL"	Beckman
1000	33·0	30·0	30·4	30·8
2000	33·6	30·3	30·4	30·2
3000	35·0	36·5	30·7	30·7
4000	36·8	30·0	30·2	30·6
Iron added, p.p.m.				
1000	36·0	30·0	30·6	30·4
2000	37·6	30·1	29·9	30·0
3000	39·9	30·4	30·4	30·8
4000	41·8	31·4	30·5	30·6

* 30 p.p.m. added: aluminium, 5000 p.p.m.

above about 3000 p.p.m. partly suppresses this interference (Table VI), but, as the results in Table VII show, errors are incurred if the amounts of iron and manganese are variable, therefore, iron and manganese equivalent to the amounts of these elements present in the sample must be added to the standards used for calibrating the instrument. This is not necessary for potassium determinations as the interference of iron and manganese is completely suppressed by the addition of between 2000 and 5000 p.p.m. of aluminium (as nitrate).

COMPARISON OF A FILTER INSTRUMENT WITH A PRISM INSTRUMENT—

Experiments conducted on the "EEL" instrument (Table V) were repeated on the Beckman instrument and, for convenience, the two series of results are placed side by side in Table V. A comparison of these results shows that interference arising from the presence of manganese and iron is less pronounced with a prism instrument than it is with a filter instrument. At this stage, it was thought that valuable information could be obtained by repeating the experiments, the results of which are shown in Tables III, IV and VI; on this occasion a prism instrument was used, and, for convenience, the two series of results are placed side by side in these tables.

The monochromator of a prism instrument enables a narrow band-pass to be obtained that largely eliminates emission bands at 586·8 and 586·0 mμ.

Conclusions reached from these additional experiments with a prism instrument make it possible for both sodium and potassium to be determined in the presence of manganese and iron without compensating for these elements in the standards used to prepare the calibration graph.

COMPARISON OF THE TWO METHODS WITH OTHER METHODS—

Each of the 5 manganese ore samples examined (Table VIII) contained about 3·5 per cent. of silica, 1·1 per cent. of barium oxide and 4·7 per cent. of alumina. The manganese contents ranged from 40 to 45 per cent. and the iron contents from 12 to 17 per cent.

Results obtained by procedures given in the references and by the recommended methods referred to in this paper are shown in Table VIII. The samples were South African manganese ores.

METHOD

REAGENTS—

All reagents should be of the highest purity obtainable.

Aluminium nitrate solution—Dissolve 713 g of hydrated aluminium nitrate ($Al(NO_3)_3.9H_2O$) in water, and dilute the solution to 1 litre. This solution contains 50,000 p.p.m. of aluminium.

Standard sodium and potassium solutions—See Method I.

APPARATUS—

Use an "EEL" flame photometer with an air - coal gas flame, and a Beckman Model DU with a flame attachment and an oxy - hydrogen flame.

PROCEDURE—

Determine a blank on the reagents with each batch of samples.

Transfer the sample (see Note 1 under Method I) to a 250-ml quartz beaker, add 20 ml of hydrochloric acid (sp.gr. 1·16) and boil the solution for about 15 minutes. Add 10 ml of 8 N sulphuric acid and evaporate the solution until fumes of sulphuric acid begin to appear (avoiding any significant loss of this acid), then cool the solution. Dilute the solution with water to about 30 ml, then filter it quantitatively through a Whatman No. 40 filter-paper into a 100-ml calibrated flask containing 10 ml of the aluminium nitrate solution (see Note 2). Dilute the solution with water to the mark.

Determine the sodium and potassium emissions of the solution at 589 and 766·5 mμ, respectively, on either of the two types of flame photometer, and calculate the sodium and potassium oxide contents of the sample from appropriate calibration graphs prepared by using the standard sodium and potassium solutions.

If a filter instrument is used for the determination of sodium oxide, add, as nearly as possible, the same amount of iron and manganese as that present in the sample solution, to the standard sodium solution before preparing the calibration graph.

NOTE 2—

After taking into account the aluminium content of the sample, this addition should provide a final 100-ml solution containing between 3000 and 5000 p.p.m. of aluminium, if a prism flame photometer is used; otherwise, the range should be between 3000 and 6000 p.p.m. of aluminium.

PRECISION OF THE METHOD—

This is shown in Table IX. The samples were similar in composition to those analysed in Table II.

TABLE VIII

COMPARISON OF METHODS

Sample number	Method given in Ref. 1.	Method given in Ref. 3	Method I, "EEL"	Method II "EEL"	Method II Beckman
	Sodium oxide, per cent.				
1	0·50	0·28	0·22	0·22	0·22
2	0·48	0·39	0·19	0·17	0·17
3	0·47	0·42	0·20	0·20	0·20
4	0·41	0·50	0·21	0·21	0·21
5	0·51	0·41	0·23	0·20	0·19
	Potassium oxide, per cent.				
1	1·12	1·18	1·01	1·01	1·00
2	0·96	0·93	0·84	0·79	0·80
3	1·02	1·14	1·11	0·95	0·94
4	0·74	0·68	0·68	0·66	0·62
5	1·58	1·22	0·98	0·92	0·91

TABLE IX

DETERMINATION OF SODIUM AND POTASSIUM BY PROPOSED METHOD II

	Sodium oxide "EEL"	Sodium oxide Beckman	Potassium oxide "EEL"	Potassium oxide Beckman
Determinations	12	12	12	12
Mean value, per cent.	0·212	0·213	0·670	0·632
Standard deviation	0·007	0·006	0·007	0·001
Coefficient of variation, per cent. ..	3·3	2·6	1·1	1·7

Table X shows the results obtained by another laboratory on six international standard samples with a Zeiss PMQ II spectrophotometer with flame attachment.

TABLE X

DETERMINATION OF SODIUM AND POTASSIUM IN INTERNATIONAL STANDARDS BY METHOD II

Sample	Sodium oxide, per cent. Value by Method II	Sodium oxide, per cent. Accepted mean value	Potassium oxide, per cent. Value by Method II	Potassium oxide, per cent. Accepted mean value
T–1[12]	4·46	4·39	1·28	1·23
SY–1[13]	3·40	3·24	2·64	2·75
G–1[14]	3·37	3·39	5·60	5·52
N.B.S. No. 91[15] ..	8·48	8·48	3·22	3·25
STD–GH[16]	3·82	3·75	4·62	4·70
STD–GR[16]	3·90	3·80	4·48	4·50

CONCLUSIONS

The direct determination of both sodium and potassium by Method II is reliable if a prism instrument is used.

With a filter instrument, iron and manganese interfere in the determination of sodium only, but this interference can be overcome by adding iron and manganese to the calibration

solutions. For the determination of potassium only, a filter instrument can be used with advantages in speed and simplicity and without any serious loss of precision.

Results obtained by Method II (and Method I) are more reliable than those obtained by alternative procedures examined.[1,3]

I thank Dr. R. E. Robinson, Director of the National Institute for Metallurgy, for valuable advice and for permission to publish this paper, Mr. T. W. Steele for his helpful suggestions throughout this project, and Mr. J. Ferguson of the Geology Department, Witwatersrand University, for the results quoted in Table X.

References

1. Lawrence-Smith, J., *Amer. J. Sci.*, 1871, **50**, 269.
2. Berzelius, J. J., *Annln Phys.*, 1824, **1**, 169.
3. Grimaldi, F. B., *Prof. Pap. U.S. Geol. Surv.*, 1965, No. 400B, 225.
4. Collins, G. C., and Polkinhorne, H., *Analyst*, 1952, **77**, 430.
5. Bond, R. D., and Stace, H. C. T., *Ibid.*, 1958, **83**, 679.
6. Leaflet Reference No. 1704.16 "EEL" 1162, Evans Electroselenium Ltd., Harlow, Essex.
7. Bond, R. D., and Hutton, J. T., *Analyst*, 1958, **83**, 684.
8. Leaflet Reference No. 1704.8, Evans Electroselenium Ltd., Harlow, Essex.
9. Farrow, R. N. P., and Hill, A. G., *Talanta*, 1961, **8**, 116.
10. Umland, F., and Hoffman, W., *Analytica chim. Acta*, 1954, **11**, 120.
11. Welcher, F. J., *Editor*, "Organic Analytical Reagents," Vol. I, D. van Nostrand Co. Inc., New York, 1947, p. 264.
12. Tanganyika Geological Survey, Standard Geochemical Sample T–1, Supplement No. 1 (1961).
13. Webber, G. R., *Geochim. cosmochim. Acta*, 1965, **29**, 229.
14. Ingamells, C. O., and Suhr, N. H., *Ibid.*, 1963, **27**, 897.
15. Natn. Bur. of Stand., Washington, D.C., Standard Sample No. 91, "Opal Glass."
16. Roubault, H., and Govindaraju, H. de la R., *Sciences Terre*, 1963, **9**, No. 4, 339.

Received *September 9th*, 1963
Amended, *April 25th*, 1966

SHORT PAPERS

Determination of Cyclamate in Soft Drinks by Gas Chromatography

By M. L. RICHARDSON and P. E. LUTON

(*John & E. Sturge Ltd., Lifford Chemical Works, Kings Norton, Birmingham* 30)

A GAS-CHROMATOGRAPHIC procedure has been published by Rees for the determination of sodium cyclamate in soft drinks,[1] but we have had difficulty in applying this method to soft drinks obtained from several sources.

According to Beck[2] if the acid content of a solution is greater than that corresponding to pH 0·65, cyclamic acid is formed which, unlike sodium cyclamate, is soluble in the solvents involved in the procedure and hence is discarded by the solvent extraction steps.

We have confirmed Beck's observations, and have also shown that as the organic acid content of these samples varies (often considerably), this must be taken into account, otherwise erroneously low cyclamate values are obtained.

In the procedure described by Rees, the cyclohexene produced by the nitrite reaction is determined as a basis for calculating the sodium cyclamate content of the sample. Other products, such as monochlorocyclohexane, cyclohexanone and cyclohexanol (see Fig. 1) are also formed, and this stresses the importance of rigid standardisation in preparing solutions for gas chromatographic evaluation, to ensure that the amount of cyclohexene produced is reproducible. In our modified method, the test solution is acidified with sulphuric acid in preference to hydrochloric acid before it is reacted with sodium nitrite and this precludes the formation of monochlorocyclohexane.

Feigl[3] also reports that a reaction product is cyclohexanol.

The proposed procedure has been applied to three random samples of soft drinks and sodium cyclamate values of 0·30 (0·31), 0·49 (0·50) and 0·21 (0·21) per cent. were obtained; values in parenthesis were obtained when the same samples were analysed by an alternative method (see *Note*).

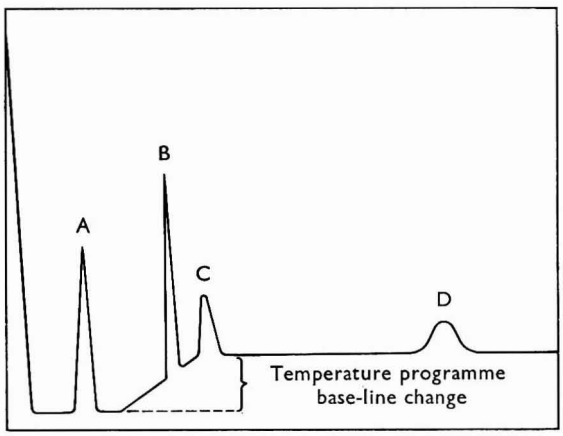

Fig. 1. Gas chromatography showing peaks of: A, cyclohexene; B, monochlorocyclohexane; C, cyclohexanone; D, cyclohexanol

Method

Apparatus—

Pye 104 Series model 14 gas chromatograph with flame ionisation detector.
Column, 5 *ft*—10 per cent. polyethylene glycol 400 on 100 to 120-mesh Celite.
Carrier gas—45 ml per minute of argon.
Hydrogen—45 ml per minute.

Air—500 ml per minute.
Attenuation—1 × 10^3.
Initial period—3 minutes at 50° C.
Temperature programme—16° C per minute to 50° to 120° C.
Final period—20 minutes.
Chart speed—30 inches per hour (Speedomax W recorder).

REAGENTS—

Sulphuric acid, 10 N.
Sodium cyclamate solution, 0·20 *per cent. w/v, aqueous.*
Zinc acetate solution—Add 21·9 g of zinc acetate dihydrate and 3 ml of glacial acetic acid to water and dilute the solution to 100 ml.
Potassium ferrocyanide solution, 10·6 *per cent. w/v, aqueous.*
Light petroleum, boiling range 30° *to* 40° C—Analysis by the conditions above should give no peaks corresponding to benzene or cyclohexene. If peaks are obtained, re-distil the light petroleum and collect the fraction distilled at 30° to 35° C.
Light petroleum solution—Add enough benzene (about 2 drops) to 50 ml of the light petroleum, so that on analysis of a 1-μl sample with the conditions given above, a benzene peak is obtained, 4 to 5 inches high.
Sodium nitrite, 0·5 M.

PROCEDURE—

Transfer by pipette, 2 ml of sodium cyclamate solution (0·20 per cent.), 18 ml of water, 2 ml of sulphuric acid (10 N), 1 ml of light petroleum solution and 1 ml of sodium nitrite solution into a 25-ml calibrated flask. Stopper the flask, shake the contents for a few seconds and release the stopper carefully. Continue this process for a further 3 minutes until there is no sign of effervescence on releasing the stopper. Add water if required, so that the solvent enters the neck of the flask. By means of a 1-μl syringe, transfer a 1-μl sample of the light petroleum solution to the top of the column and record a chromatogram.

Repeat the above process with 4, 6, 8 and 10 ml of cyclamate solution and 16, 14, 12 and 10 ml of water, respectively. Plot a graph of the area of the cyclohexene peak relative to that of the benzene peak against the concentration of sodium cyclamate.

ANALYSIS OF COMMINUTED ORANGE DRINKS—

Transfer by pipette, an aliquot of comminuted orange drink containing less than 50 mg of sodium cyclamate (preferably about 25 mg) into a 100-ml beaker, add water to produce a volume of about 40 ml, adjust the pH to 0·95 to 1·05 with sulphuric acid and transfer the solution to a 50-ml calibrated flask. Add 1 ml of zinc acetate solution and 1 ml of potassium ferrocyanide solution, shake the mixture well and dilute to the mark with water. Filter the solution through a Whatman No. 90 filter-paper and extract a portion of the filtrate with three 50-ml aliquots of chloroform, and then twice with 25-ml portions of light petroleum. Transfer by pipette, 20 ml of the final aqueous solution, 1 ml of light petroleum solution and 1 ml of sodium nitrite solution to a 25-ml calibrated flask and then complete the determination as described under "Procedure" from "Continue this process" to "record a chromatogram." Calculate the area of the cyclohexene peak, relative to that of the benzene peak and, by means of the calibration curve, determine the concentration of sodium cyclamate in the original comminuted orange drink.

Note—We, however, consider this procedure to be lengthy and it involves the use of expensive instrumentation; in view of this we recommend the much simpler direct procedure described in the following paper (*Analyst*, 1966, **91**, 522).

We thank the Directors of John & E. Sturge Limited for permission to publish this paper.

REFERENCES

1. Rees, D. I., *Analyst*, 1965, **90**, 568.
2. Beck, K. M., *Fd Technol.*, 1957, **11**, 156.
3. Feigl, F., "Spot Tests in Organic Analysis," Sixth Edition, Elsevier Publishing Co., Amsterdam, 1960, p. 542.

Received *December 3rd*, 1965

The Determination of Cyclamate in Soft Drinks by Titration with Sodium Nitrite

By M. L. RICHARDSON and P. E. LUTON

(*John & E. Sturge Ltd., Lifford Chemical Works, Kings Norton, Birmingham* 30)

The present recommended method is based on the reaction—

$$-NHSO_3^- + NO_2^- \longrightarrow SO_4^{2-} + N_2,$$

the end-point of which is determined electrometrically.

The British Pharmacopoeia 1963[1] prescribes this reaction for the assay of a number of drugs containing the –NH– grouping, such as benzocaine, dapsone, procaine hydrochloride and suramin. The National Formulary XII[2] also uses a similar procedure, but in its First Supplement deletes the use of the electrometric end-point leaving starch - iodide paper as the method of end-point detection. This deletion was carried out so that the analyst is directed to perform the procedure in only one manner, that is, no alternative procedures may be stated in the National Formulary.[2]

The starch - iodide external indicator procedure has also been recommended for the cyclamate content of comminuted drinks,[3] but owing to other constituents present has proved suitable only as a rough check.

The proposed method is, however, much simpler and quicker than that earlier described by Rees[4] and modified by ourselves.[5]

Recoveries

Various volumes of sodium cyclamate solution (5·00 ml of sodium cyclamate solution ≡ 4·76 ml of 0·1 M sodium nitrite solution) were added to 100 ml of a comminuted drink containing the usual amounts of benzoic acid and saccharin and the solution then acidified with 5 ml of 5 N hydrochloric acid. Recoveries were as follows.

Volumes of cyclamate solution added, ml	Volume of 0·1 N sodium nitrite added, ml	Recovery, per cent.
0	0	—
5	4·72	99·1
15	14·45	102·0
25	23·20	98·3

Reproducibility

Drink	Percentage w/v of sodium cyclamate	Number of determinations	Coefficient of variation, per cent.
A	0·21	4	4·5
B	0·38	3	<1·0
C	0·58	3	1·5
D	0·29	3	<1·0

Experimental

Apparatus—

Titration vessel—Fit a 150-ml beaker with two similar platinum-wire electrodes of 1 mm diameter and 5 cm long, wound in a helix of 0·6 cm diameter and 1 cm long. The electrodes should be about 2·5 cm apart. Clean the electrodes, when necessary, by immersing them for about 30 seconds in boiling concentrated nitric acid containing 0·5 per cent. iron(III) chloride, and then rinse thoroughly in distilled water.[1]

Polarising potential—The circuit is as described in the British Pharmacopoeia[1]; the polarising potential should be adjusted to give the maximum galvanometer response as indicated in Fig. 1, which also shows the effect of too high and too low polarising potentials. We found that a polarising voltage of 40 mV was suitable, but stress that the voltage depends on the size, shape and geometry of the electrodes.

Reagents—

Hydrochloric acid, 5 N.

Sodium nitrite, 0·1 M—Standardise against sulphanilic acid.[1]

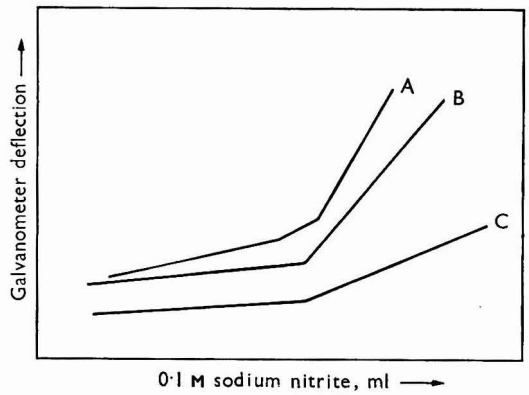

Fig. 1. Selection of polarising potential: curve A, voltage too high (by 10 mV); curve B, voltage ideal; curve C, voltage too low (by 10 mV)

PROCEDURE—

Transfer 100 ml of comminuted drink to a 150-ml beaker, add 5 ml of 5 N hydrochloric acid, and mix with a magnetic stirrer. Place the electrodes in position and titrate with 0·1 M sodium nitrite solution in suitable increments (in the region of the end-point these should not be greater than about 0·1 ml of titrant), recording the galvanometer readings for each addition. Plot a titration curve and deduce the end-point by extrapolation. Calculate the sodium cyclamate present.

Note—The burette jet must be kept below the level of the solution being titrated.

We thank the Directors of John & E. Sturge Ltd. for permission to publish this paper.

REFERENCES

1. British Pharmacopoeia 1963, p. 1024.
2. National Formulary XII, 1965, p. 68, 362.
3. "Sucaryl Sweetener in Soft Drinks: Technical Information," Abbott Laboratories Ltd.
4. Rees, D. I., *Analyst*, 1965, **90**, 568.
5. Richardson, M. L., and Luton, P. E., *Ibid.*, 1966, **91**, 520.

Received *December 3rd*, 1965

The Determination of Ethanolamine and Serine in Phospholipids

BY A. J. DE KONING*

(*Fishing Industry Research Institute, University of Cape Town, Rondebosch, Cape Town, South Africa*)

THE quantitative analysis of phospholipids is frequently based on the determination of the products they yield on hydrolysis, *e.g.*, choline from lecithins and sphingomyelins, and ethanolamine together with serine from cephalins. Several methods for the determination of ethanolamine and serine are described in the literature. For instance, periodate oxidation has been described by Dittmer, Feminella and Hanahan,[1] and paper-chromatographic determinations have been studied by Levine and Chargaff[2] and also by Magee, Baker and Thompson.[3] The paper-chromatographic procedures proved unreliable in our laboratory.

This paper describes the determination of ethanolamine (100 to 1000 μg) and serine (100 to 500 μg) by their separation on a cation-exchange resin, and subsequent colorimetric determination with ninhydrin. It is essentially Moore and Stein's procedure[4] applied to a phospholipid hydrolysate and its accuracy is better than 10 per cent.

* Present address: University of Basutoland, Bechuanaland and Swaziland, P.O. Roma, via Maseru, Basutoland, Southern Africa.

Method

Reagents—

Ethanolamine—British Drug Houses Ltd. laboratory reagent (assay not less than 99 per cent.). Re-distil *in vacuo* (b.p. 64° C at 4 mm pressure), n_D^{20} 1·4538, (value quoted in the literature,[5] 1·4539).

Serine—DL-Serine, obtainable from the California Foundation for Biochemical Research, served as standard compound. Found: carbon, 34·35 per cent.; hydrogen, 6·77 per cent.; $C_3H_7O_3N$ requires carbon, 34·29 per cent.; hydrogen, 6·72 per cent.

Stock solution of ethanolamine and serine—Prepare a solution containing 2·22 mg of ethanolamine and 0·71 mg of serine per ml in N hydrochloric acid.

Citrate buffer solutions of pH 3·25, 5·0 and 5·28—These solutions were prepared in 5-litre volumes, adjusted if necessary to the required pH with hydrochloric acid or sodium hydroxide, and stored at 0° C.

(*a*) pH 3·25. Dissolve 105 g of citric acid, 41 g of sodium hydroxide and 52 ml of concentrated hydrochloric acid in 5 litres of water;

(*b*) pH 5·0. Dissolve 525 g of citric acid and 200 g of sodium hydroxide in 5 litres of water;

(*c*) pH 5·28. Dissolve 122 g of citric acid, 72 g of sodium hydroxide and 34 ml of concentrated hydrochloric acid in 5 litres of water.

Before use, boil the buffer solution for 10 seconds and add 5 ml of Brij solution (prepared by dissolving 35 g of Brij 35, obtainable from the Atlas Powder Company, in 100 ml of water) per litre. To the buffer of pH 5·28 add 1 g of the disodium salt of EDTA per litre.

Cation-exchange resin—The ion-exchange resin used was Amberlite C.G.120, type 2 (Fisher Scientific Company). A long column (120 × 0·9 cm) was used for serine and a short column (30 × 0·9 cm) for ethanolamine determinations. The columns were jacketed and operated at 50° C. Before use, the columns were washed with 0·2 N sodium hydroxide and then with the appropriate buffer solution (about 25 ml for the short column and 100 ml for the long column).

Ninhydrin—Dissolve 2·0 g of ninhydrin (Merck's AnalaR reagent) in 50 ml of Cellosolve and mix with 50 ml of citrate buffer solution of pH 5·0 containing 80 mg of tin(II) chloride (anhydrous Merck's AnalaR reagent). Store the reagent under nitrogen in a dark bottle and use within 6 hours of preparation.

Procedure—

About 600 mg of a phospholipid was hydrolysed with 25 ml of 2 N hydrochloric acid in a sealed ampoule at 120° C for 24 hours. The ampoule was opened and the solution filtered. The residual tar was thoroughly leached with hot water and poured into the same filter. After evaporation of the filtrate on a steam-bath, the material was dissolved in 25·0 ml of water in a calibrated flask. Ethanolamine and serine were determined in 1-ml and 3-ml aliquots, respectively.

Determination of ethanolamine—

The hydrolysate containing 100 to 1000 μg of ethanolamine was placed on to the short column and eluted at 50° C with a citrate buffer of pH 5·28. Fractions of 2 ml were collected with an automatic fraction-collector. Tubes 30 to 80 were treated with 4 ml of the ninhydrin solution and the colour was developed at 100° C for 20 minutes. After appropriate dilution with an isopropanol - water (3 + 7) mixture, the optical density of the colour was read in an Evelyn colorimeter at 565 mμ. Under the conditions described, ethanolamine was eluted in tubes 50 to 60; the extra tubes served to obtain a blank value for the ninhydrin colour. The optical densities were plotted against tube number, and ethanolamine was determined quantitatively by comparing the peak area with that of a known amount of ethanolamine, or more conveniently, by means of the optical density factor (see below). Also, a known amount of ethanolamine was usually added to the phospholipid and the percentage recovery determined.

Determination of serine—

The hydrolysate containing 100 to 500 μg of serine was eluted on the long column with the citrate buffer of pH 3·25 at 50° C. Fractions of 1 ml were collected, and tubes 90 to 140 were treated with 2 ml of the ninhydrin reagent and analysed as described for ethanolamine. A serine percentage recovery test was also included.

TREATMENT OF A STANDARD MIXTURE—

Five ml of the stock solution, *i.e.*, a mixture of 11·10 mg of ethanolamine and 3·55 mg of serine was "hydrolysed" with 25 ml of 2 N hydrochloric acid at 120° C for 24 hours. The reaction mixture was evaporated to dryness and dissolved in 25·0 ml of water in a calibrated flask as described above. An aliquot of 1 ml was taken for the preparation of a standard ethanolamine chromatogram, and of 2 ml for a serine chromatogram. The optical density factors of ethanolamine and serine were found by the following expression—

$$\frac{\Sigma \text{ (optical densities} - \text{blanks)}}{\text{mg of substance}}$$

i.e., the optical density per mg of substance. The amounts of ethanolamine and serine in a hydrolysate may be readily calculated by means of these factors.

DISCUSSION AND RESULTS

The quantitative determination of ethanolamine and serine in phospholipid hydrolysates is complicated by the fact that the phospholipids often contain small amounts of polypeptide impurities.[6] Either rigorous purification of the phosphatide before hydrolysis is necessary, or ethanolamine and serine must be completely separated from other ninhydrin-reacting substances in the hydrolysate. Accordingly, serine was eluted with the buffer of pH 3·25 on the long column which separates it adequately from its nearest homologues, threonine and glutamic acid. This treatment also separated serine completely from phosphoryl ethanolamine and 2-aminoethyl phosphonic acid, compounds which are ninhydrin-reactive and present in hydrolysates of certain phospholipids.[7,8,9] It should be emphasised here that this separation is not achieved with the short column as described by Dittmer *et al.*[1]

Ethanolamine was treated as a basic amino-acid and was eluted from a short column with the buffer of pH 5·28. The position of ethanolamine was between histidine and arginine when a mixture of basic amino-acids was separated on this column. Ammonia emerged just after ethanolamine and formed a double peak with it. Large quantities of ammonia were formed when the

TABLE I

RECOVERY OF ETHANOLAMINE AND SERINE ADDED TO PHOSPHOLIPIDS

Sample	Percentage present		Percentage added		Total percentage determined		Percentage recovery	
	Ethanolamine	Serine	Ethanolamine	Serine	Ethanolamine	Serine	Ethanolamine	Serine
Hake flesh	1·60	0·34	1·53	0·32	3·07	0·69	98	105
Hake liver	1·83	0·39	—	0·10	—	0·47	—	96
Rock lobster (hepatopancreas)	1·93	0·36	0·50	1·05	2·42	1·42	100	101
Rock lobster (roe) ..	1·74	0·10	0·66	—	2·58	—	108	—

phospholipid mixture was hydrolysed with 6 N hydrochloric acid at 120° C (*cf.* Dittmer *et al.*[1]). Under our hydrolytic conditions, *viz.*, 2 N hydrochloric acid at 120° C, ammonia formation was negligible. Table I shows the results obtained in the analysis of several marine phospholipids. Percentage recovery tests of ethanolamine and serine indicate that the accuracy of the present method is within 10 per cent.

REFERENCES

1. Dittmer, J. C., Feminella, J. L., and Hanahan, D. J., *J. Biol. Chem.*, 1958, **233**, 862.
2. Levine, C., and Chargaff, E., *Ibid.*, 1951, **192**, 465.
3. Magee, W. L., Baker, R. W. R., and Thompson, R. H. S., *Biochim. Biophys. Acta*, 1960, **40**, 118.
4. Moore, S., and Stein, W. H., *J. Biol. Chem.*, 1954, **211**, 893.
5. Hodgman, C. D., *Editor*, "Handbook of Chemistry and Physics," Fortieth Edition, The Chemical Rubber Publishing Co., Cleveland, Ohio, 1958, p. 976.
6. de Koning, A. J., *Biochim. Biophys. Acta*, 1964, **84**, 467.
7. Crone, H. D., and Bridges, R. G., *Biochem. J.*, 1963, **89**, 111.
8. Rouser, G., Kritchevsky, G., Heller, D., and Lieber, E., *J. Amer. Oil Chem. Soc.*, 1963, **40**, 425.
9. de Konig, A. J., *Nature*, 1966, **210**, 113.

Received *August 24th*, 1966

The Paper Chromatography of Some Purines, Pyrimidines and Imidazoles

By M. N. KHATTAK, N. T. BARKER and J. H. GREEN

(*Department of Nuclear and Radiation Chemistry, University of New South Wales, Kensington, Sydney, Australia*)

Quantitative determination and separation of various hydroxypurines, amino-hydroxypyrimidines and two imidazoles have been attempted by using paper-chromatographic techniques. Few other methods are applicable for their separation. During experiments on the radiation decomposition of nucleic acid constituents the opportunity arose to study the chromatographic behaviour of a number of rather rare compounds. About 24 solvents were tried, and of these the 7 solvent systems which were found more efficient were used. R_F values, absorption maxima and the percentage recoveries of the compounds were found.

Experimental

Compounds—

The pyrimidines and the three purines (2-hydroxy, 8-hydroxy and 2,8-dihydroxy) were kindly supplied by Professor Adrian Albert, Australian National University, Canberra. Purine,6-hydroxypurine (hypoxanthine), 2,6-dihydroxypurine (xanthine) and 2,6,8-trihydroxypurine (uric acid) were commercial products (obtained from British Drug Houses Ltd. and L. Light Laboratories Ltd.). 4-Amino-5-imidazole carboxamide was obtained from Calbiochem (U.S.A.) and 4-amino-5-imidazole carboxamidine was prepared by heating adenine in glacial acetic acid in a sealed tube at a high temperature.[1] All the solvents used for developing were analytical-reagent grade. Propanol and butanol were re-distilled in an all-glass apparatus under an atmosphere of nitrogen.

Developing solvent systems—

The systems used in the investigations, each being freshly prepared, were as given below—

(*i*) butanol - water (86 + 14),[2]
(*ii*) propanol - water (77 + 23),[3]
(*iii*) butanol - propionic acid - water (43 + 27 + 30),[4]
(*iv*) t-butanol - ethyl methyl ketone - formic acid - water (40 + 30 + 15 + 15),[5]
(*v*) t-butanol - ethyl methyl ketone - ammonia (15 N) - water (40 + 30 + 10 + 20),[5]
(*vi*) isopropanol - hydrochloric acid (10 N) - water (65 + 18·4 + 16·6),[6]
(*vii*) ammonium bicarbonate (16 per cent.).

Apparatus—

Strips of Whatman No. 1 chromatographic paper (10 × 50 cm) were cut to allow up to 40 cm for movement of the solvent. A Shandon 20-inch rectangular chromotank 2130 was used throughout the work. An ultraviolet lamp (Philips No. 92123) enclosed in a light-tight box which had a circular hole covered by a Chance glass filter (maximum transmission at 250 mμ) was used to detect the ultraviolet absorbing or fluorescent spots in the chromatograms. Micro-pipettes (Misco type) were used for spotting the solutions on the paper.

Procedure—

Solutions of the pure compounds at concentrations of 10^{-2} to 10^{-4} M were applied at 3-cm intervals on the paper strips. Optimum amounts were up to 50 μg in the spots, each of which had a maximum diameter of 5 mm. All the chromatograms were developed by uni-dimensional descending technique at room temperature (20° to 25° C), and after development, the chromatograms were dried by hanging in a fume-hood for 1 hour. Some were further dried in a large oven at 50° C. The separated spots were located in the ultraviolet light and marked. Uric acid spots were not visible in ultraviolet light; however, by spraying the paper with Folin - Ciocalteu's reagent, their positions were found. The marked areas were cut and eluted in a known volume of 0·1 N hydrochloric acid or 0·1 N ammonia solution. The eluates were filtered, and the concentrations were measured in a Beckman DU spectrophotometer at the appropriate $\lambda_{max.}$ in 1-cm silica cells.

Results and discussion

The absorption maxima and percentage recoveries are given in Table I. R_F values (the mean of 5 experiments reproducible to $\pm 0{\cdot}01$) are shown in Table II, with absorption or fluorescence in ultraviolet light.

There is a gradual lowering of R_F values in the purines. It is to some extent related to the solubilities of these compounds. Purine itself is highly soluble, but when a group (especially hydroxyl) enters at the 2-position, the resulting product is less soluble. When all the three positions (2, 6 and 8) are occupied, the compound is completely insoluble. The fact that the R_F values of these substances are the lowest could be due to their low solubilities. The variations in the R_F values of the pyrimidines and imidazoles could be explained similarly in terms of the structures and solubilities of these compounds.

The results of the chromatographic studies reported in this paper have been used in the identification and measurement of the products formed in the γ-radiolysis of de-aerated aqueous solutions of various nucleic acid constituents.[8,9]

Table I

Absorption maxima and percentage recoveries of compounds chromatographed

Compounds	$\lambda_{max.}$,* mμ	Recovery,* per cent.
Group A—		
Purine	260	98
2-Hydroxypurine	264	95
6-Hydroxypurine (Hypoxanthine)	248	95
8-Hydroxypurine	280	96
2,6-Dihydroxypurine (Xanthine)	268	80
2,8-Dihydroxypurine	310	85
6,8-Dihydroxypurine	257	86
2,6,8-Trihydroxypurine (Uric acid)	290	75
Group B—		
4,5-Diamino-2-hydroxypyrimidine	305	80
4,5-Diamino-6-hydroxypyrimidine	257	85
4,5-Diamino-2,6-dihydroxypyrimidine	260	60
2,4,5-Triaminopyrimidine	268	65
4,5,6-Triaminopyrimidine	265	65
Group C—		
4-Amino-5-imidazole carboxamidine	287	70
4-Amino-5-imidazole carboxamide	269	85

* Average of three experiments.

Table II

R_F values × 100

Compounds	Solvent systems (*i*)	(*ii*)	(*iii*)	(*iv*)	(*v*)	(*vi*)	(*vii*)	Absorption or Fluorescence
Group A—								
Purine	60	75	75	60	66	60	42	Absorption
2-Hydroxypurine	16	37	48	37	40	41	80	Absorption
6-Hydroxypurine (Hypoxanthine)	25	54	59	50	41	41	60	Absorption
8-Hydroxypurine	33	63	50	54	51	46	56	Absorption
2,6-Dihydroxypurine (Xanthine)	22	60	22	32	37	43	45	Absorption
2,8-Dihydroxypurine	14	27	26	30	33	40	60	Fluorescence
6,8-Dihydroxypurine	30	33	31	26	35	38	40	Absorption
2,6,8-Trihydroxypurine (Uric acid)	5	17	12	14	23	22	40	—
Group B—								
4,5-Diamino-2-hydroxypyrimidine	7	31	45	27	7	6	4	Fluorescence
3,4-Diamino-6-hydroxypyrimidine	6	20	60	32	40	30	60	Fluorescence
4,5-Diamino-2,6-dihydroxypyrimidine	0	5	30	6	5	6	—	Fluorescence
2,4,5-Triaminopyrimidine	10	33	58	23	36	30	55	Fluorescence
4,5,6-Triaminopyrimidine	11	35	—	37	53	38	64	Fluorescence
Group C—								
4-Amino-5-imidazole carboxamidine	16	22	55	42	35	41	62	Absorption
4-Amino-5-imidazole carboxamide	23	34	66	57	58	58	70	Absorption

REFERENCES

1. Cavallieri, L. F., Tinker, J. F., and Brown, G. B., *J. Amer. Chem. Soc.*, 1949, **71**, 3976.
2. Smith, J., and Markham, R., *Biochem. J.*, 1949, **45**, 294.
3. Korn, E. D., Charalampons, F. C., and Buchanan, J. M., *J. Amer. Chem. Soc.*, 1953, **75**, 3610.
4. Goodman, M., *U.S. Atomic Energy Commission, Report No.* UCRL–1961, September, 1952, 67.
5. Fink, K., Cline, R. E., and Fink, R. H., *Analyt. Chem.*, 1963, **35**, 3, 389.
6. Ekert, B., and Monier, R., *Nature*, 1960, **188**, 309.
7. Hems, G., *Archs. Biochem. Biophys.*, 1959, **82**, 437.
8. Khattak, M. N., and Green, J. H., *Aust. J. Chem.*, 1965, **18**, 1847.
9. ——, ——, *Int. J. Radiat. Biol.*, in the press.

Received *May 28th*, 1965

The Determination of Total Sulphur in Soil and Plant Material

BY I. A. CHAUDHRY AND A. H. CORNFIELD

(*Chemistry Department, Imperial College of Science and Technology, London, S.W.7*)

WHEN many samples of soil or plant material need to be analysed for their total sulphur content, bomb methods and apparatus with combustion trains are hardly suitable. Simple digestion and combustion procedures, in which many different reagents are used, have also been described. Among these reagents have been oxidising salts, with or without acid, peroxides and sodium bicarbonate.[1,2,3,4] The authors tried most of these reagents and found that the highest values for total sulphur in a number of soils were obtained by using the magnesium nitrate - nitric acid method.[4] Potassium chlorate[3] usually gave slightly lower total sulphur values. Sodium peroxide, hydrogen peroxide, potassium perchlorate and sodium bicarbonate gave very low values.

In the original magnesium nitrate - nitric acid method[4] a relatively low combustion temperature (350° C) is used and a long combustion period (overnight) is required, presumably to ensure complete oxidation. In a preliminary test on 3 soils it was found that when a 3-hour combustion period at either 350° or 550° C was used, total sulphur values were lower than with combustion overnight at 350° C. The magnesium nitrate was replaced by potassium nitrate, which, used like the magnesium nitrate with nitric acid, was then tested. It was found that the latter method (potassium nitrate - nitric acid) gave the highest values for total sulphur and that a 3-hour combustion period gave as high values as overnight combustion.

The results obtained for 10 soils that varied in texture from sands to clays by using (*i*) the proposed method (potassium nitrate - nitric acid and 3-hour combustion at 550° C), (*ii*) the magnesium nitrate - nitric acid method and combustion overnight at 350° C[4] and (*iii*) the potassium chlorate method (3-hour combustion at 550° C)[3] are shown in Table I. A turbidimetric method,

TABLE I

COMPARISON OF RESULTS OF TOTAL SULPHUR DETERMINATIONS FOR 10 SAMPLES OF AIR-DRIED SOIL

Soil texture	Total sulphur found, per cent.,* by— Potassium nitrate - nitric acid method	Magnesium nitrate - nitric acid method	Potassium chlorate method
Sandy	0·096	0·092	0·087
Sandy	0·037	0·034	0·031
Sandy loam	0·061	0·057	0·054
Loam	0·032	0·028	0·029
Loam	0·105	0·101	0·094
Loam	0·098	0·096	0·089
Silt loam	0·033	0·029	0·025
Silt loam	0·046	0·043	0·038
Clay loam	0·019	0·015	0·014
Clay	0·074	0·068	0·068
Mean	0·0601	0·0563	0·0529
Range of coefficient of variation	0 to 5·6 per cent.	1·5 to 10 per cent.	3·0 to 10 per cent.
Mean coefficient of variation	2·19 per cent.	3·79 per cent.	5·85 per cent.

* Each result is the mean of duplicates.

based on those described by Butters and Chenery[4] and Massoumi and Cornfield,[5] was used to determine sulphate in the digests. With every sample of soil the potassium nitrate - nitric acid method gave higher values for total sulphur than the magnesium nitrate - nitric acid method. In addition, the range of the coefficients of variation, and its mean, were lower for the former method. The potassium chlorate method usually gave the lowest total sulphur values.

TABLE II

RECOVERY OF SULPHUR FROM SOIL SAMPLES

Recovery of 500 μg of sulphur added as methionine to soil samples*

Soil No.	Oxidant	Original sulphur in soil, per cent.	Methionine sulphur recovered, μg	Methionine sulphur recovered, per cent.	Coefficient of variation, per cent.
1	Potassium nitrate - nitric acid..	0·0445	490	98	1·44
	Magnesium nitrate - nitric acid	0·0441	467·5	93·5	2·26
	Potassium chlorate	0·0439	460	92	3·07
2	Potassium nitrate - nitric acid..	0·109	502·5	100·5	0·70
	Magnesium nitrate - nitric acid	0·104	455	91	1·55
	Potassium chlorate	0·108	457·5	91·5	2·31
3	Potassium nitrate - nitric acid..	0·062	495	99	1·43
	Magnesium nitrate - nitric acid	0·059	472·5	94·5	0·78
	Potassium chlorate	0·058	445	89	1·59
4	Potassium nitrate - nitric acid..	0·083	485	97	1·45
	Magnesium nitrate - nitric acid	0·082	475	95	1·48
	Potassium chlorate	0·079	460	92	3·07

* Each result is the mean of duplicates.

Table II shows recoveries of known added amounts of sulphur (in the form of methionine) from four of the soils. For potassium nitrate - nitric acid, recoveries ranged from 96 to 101 per cent. (mean 98·6 per cent.); for magnesium nitrate - nitric acid, 90 to 96 per cent. (mean, 93·5 per cent.); and for potassium chlorate, 88 to 94 per cent. (mean, 91 per cent.). It is seen, therefore, that potassium nitrate - nitric acid is a better reagent than the other two for determining total sulphur in soils.

The potassium nitrate - nitric acid method was also tested as a procedure for determining total sulphur in plant materials. Table III shows that there was complete recovery of methionine sulphur added to 4 plant materials.

TABLE III

RECOVERY OF SULPHUR FROM PLANT MATERIALS

Recovery of 500 μg of sulphur added as methionine to plant material with the potassium nitrate - nitric acid method*

Plant material	Original sulphur found, per cent.	Methionine sulphur recovered, μg	Methionine sulphur recovered, per cent.	Coefficient of variation, per cent.
Dwarf peas	0·275	495	99	1·42
Oat straw	0·14	492·5	98·5	0·71
Young grass	0·355	500	100	0·0
Hay	0·17	497·5	99·5	0·71

* Each result is the mean of duplicates.

Details of the potassium nitrate - nitric acid method for digesting soils and plant materials are described below.

METHOD

REAGENTS—

Fuming nitric acid.

Nitric acid, 25 per cent. v/v—Prepare from analytical-reagent grade nitric acid.

Digesting solution—Dissolve 100 g of AnalaR potassium nitrate in 600 ml of distilled water. Add 350 ml of concentrated nitric acid and dilute to 1 litre.

PROCEDURE FOR SOILS—

Weigh 1 g of the soil (air-dried and ground to pass a 0·5-mm sieve) into a 50-ml tall beaker. Add 10 ml of the digesting solution and evaporate to dryness on a steam-bath. Place the beaker in an electric furnace, heat to 550° C and maintain at this temperature for 3 hours. After cooling, add 5 ml of 25 per cent. nitric acid v/v, and again digest the contents for 1 hour on a steam-bath. Extract the soluble salts with distilled water and filter the solution through a Whatman No. 42 filter-paper. Dilute to a known volume and take a suitable aliquot of the filtrate for the turbidimetric determination of sulphate.

PROCEDURE FOR PLANTS—

Weigh accurately 150 to 200 mg of finely ground plant material into a 50-ml tall beaker. Add 3 ml of fuming nitric acid, cover the beaker with a watch-glass and set aside for 15 minutes. Wash the watch-glass and add the washings to the beaker. Evaporate the contents to dryness on a steam-bath. Then add 10 ml of the digesting solution and proceed as described under soils.

An appropriate blank is always run with each batch of determinations. It was found that blank values with potassium nitrate were as low as those when magnesium nitrate (prepared from Specpure magnesium) was used.

REFERENCES

1. Bardsley, C. E., and Lancaster, J. D., *Proc. Soil Sci. Soc. Amer.*, 1960, **24**, 265.
2. Aidinyan, R. Kh., *Pochvovedenie*, 1957, 49.
3. Saalbach, E., Kessen, G., and Judel, G. K., *Landw. Forsch.*, 1962, **15**, 6.
4. Butters, B., and Chenery, E. M., *Analyst*, 1959, **84**, 239.
5. Massoumi, A., and Cornfield, A. H., *Ibid.*, 1963, **88**, 321.

Received *January 4th*, 1966

A Simple Colorimetric Finish for the Johnson-Nishita Micro-distillation of Sulphur

BY G. A. DEAN

(*Commonwealth Scientific and Industrial Research Organization, Division of Soils, W.A. Regional Laboratory, Nedlands, Western Australia*)

THE usual methylene-blue finish for the routine micro determination of sulphur in soils and plants by the Johnson - Nishita distillation method is somewhat tedious and often too sensitive[1] unless a very small sample is taken, which results in sampling difficulties. No suitable reagent giving a sensitive colour on direct absorption of hydrogen sulphide could be found, and a procedure based on a colloidal metal sulphide[2] was eventually developed. The method is suitable for determining amounts of sulphur up to 200 μg, and Johnson - Nishita units designed for the methylene-blue finish may be used without modification.

PRINCIPLE—

The hydrogen sulphide generated by the Johnson - Nishita digestion is absorbed in a measured volume of N sodium hydroxide. An intermediate wash is unnecessary as traces of reducing agent do not interfere. This sodium hydroxide is mixed with a measured volume of bismuth reagent to give colloidal bismuth sulphide in acetate buffer containing 1 per cent. gelatin. No special precautions or further operations are necessary. The clear yellow-brown colour is measured in 1-cm cells at 400 mμ, or at a longer wavelength if a lower sensitivity is required.

Some advantages of using bismuth are given by Boltz.[2] The first use of colloidal bismuth sulphide in acetate buffer was described by Field and Oldach.[3]

HYDROGEN SULPHIDE ABSORBENT—

A solution of N sodium hydroxide was found to be an extremely efficient absorber for hydrogen sulphide, allowing high nitrogen flow-rates and hence short distillation times.[4] For example, when 1000 μg of sulphur, as sulphate, were digested for 10 minutes in a standard Johnson - Nishita distillation unit[5] with a nitrogen flow-rate of 500 ml per minute, a second N sodium hydroxide absorber collected only 0·4 μg of sulphur, and less than 4 μg were distilled from the unit in the next 30 minutes.

Air oxidation of the alkaline sulphide is prevented by the use of nitrogen. Air oxidation in aerated N sodium hydroxide at 20° C was found to be independent of sulphide concentration between 0 and at least 3 p.p.m., and was about 0·07 p.p.m. per hour, which, in the present method, is equivalent to a decrease in optical density of 0·001 in 5 minutes. Hence no special precautions are required during the transfer.

When the final bismuth - acetate buffer solution was used as the absorbent instead of sodium hydroxide, recovery of 100 μg of sulphur was 78 per cent.

COLOUR DEVELOPMENT—

Full optical density was reached immediately after mixing, and in diffused daylight was stable for at least 3 days. The optical density and absorption maximum were independent of temperature of mixing (sodium hydroxide at 20° C, bismuth reagent between 0° and 50° C), age and rate of addition. Colloid protection was excellent; the solutions were clear and sparkling and were unaffected by boiling. Gum arabic gave results similar to gelatin, but some samples caused slow oxidation of the bismuth sulphide and hence fading of the colour by about 1 per cent. per hour.

SENSITIVITY

Absorption maximum occurred between 360 and 365 mμ, but traces of iodide from the Johnson - Nishita reducing agent caused slight interference (no intermediate wash was used; the reducing agent was as recommended by Gustafsson[3]). At 400 mμ, sensitivity to sulphide was decreased by one-fifth but interference from iodide was negligible. At 400 mμ an optical density of 0·01 in a 1-cm cell was equivalent to 1·8 μg of sulphur in 30 ml, or about one-sixth the sensitivity of methylene blue.[1] In agreement with previous observations,[3] Beer's law was obeyed up to an optical density of at least 1·4 (250 μg of sulphur in 30 ml). At 480 mμ the sensitivity was about one-half of that at 400 mμ.

PRECISION

Hydrogen sulphide in N sodium hydroxide was added directly in 20-ml aliquots to 10-ml aliquots of bismuth reagent; the average optical density and standard deviation were—

Solution A	0·298 $\pm$ 0·0012 (0·39 per cent., 9 determinations)
Solution B	0·933 $\pm$ 0·0021 (0·22 per cent., 5 determinations)

The over-all mechanical precision (excluding that of the spectrophotometer, a Unicam SP600), calculated from the observed precision of the component operations, was $\pm$0·15 per cent.

For 8 calibration curves, each determined on a different day with standards of 50 and 100 μg distilled in the usual manner, Beer's law was obeyed to a standard deviation of 0·55 per cent., the same as the calculated mechanical precision, and the standard deviation of the slope from the mean was 3 per cent., or about the same as for methylene blue. The main source of error is therefore the distillation rather than the finish.

If the order of addition was reversed, the optical density was somewhat higher but less precise, and was critically dependent on the rate and manner of addition. For example, with solution B above, the average optical density and standard deviation for four determinations were 1·040 $\pm$0·014 (1·4 per cent.). When tubes were re-used after rinsing with water, no significant interference from adsorbed traces of bismuth could be detected. Reversing the order of addition simplifies the procedure but the results are too dependent on technique to justify its use without previous tests.

METHOD

REAGENTS—

The amounts given are sufficient for 100 analyses.

Bismuth reagent—This is 1500 p.p.m. of bismuth and 1 per cent. gelatin in 4 N acetic acid. Heat 2 g of analytical-reagent grade bismuth subnitrate or 3·4 g of analytical-reagent grade bismuth nitrate pentahydrate in 230 ml of glacial acetic acid until dissolved, filter if necessary through a Whatman No. 50 filter-paper, cool, add 30 g of gelatin dissolved by warming in about 500 ml of water, and dilute to 1000 ml. The reagent is stable indefinitely.

Sodium hydroxide, N—Dissolve 80 g of sodium hydroxide in 2 litres of water.

PROCEDURE—

Provide the outlet of the Johnson - Nishita distillation unit with a suitable receiver, such as a 6 $\times$ 1-inch test-tube, with the outlet tube reaching to the bottom. Transfer 20 ml of N sodium

hydroxide by pipette* into the dry receiver and distil hydrogen sulphide in the usual manner with a nitrogen flow-rate of between 500 and 600 ml per minute for 15 minutes. During this time place 10 ml of bismuth reagent by pipette into any suitable dry vessel such as a 6 × 1-inch test-tube. Detach the receiver and pour the solution into the bismuth reagent, allow a few seconds' drainage, and mix it thoroughly. Measure the optical density at 400 mμ against a reagent blank solution. After use, steam the receiver vigorously for 5 to 10 seconds and drain for 1 minute; the combined action of the steam and residual sodium hydroxide keeps the receiver perfectly clean from run to run indefinitely.

Alternatively, provided tests have shown that a reversed order of addition gives acceptable results (see above), with a pipette run 10 ml of bismuth reagent directly into the receiver by using a rigidly standardised technique, mix thoroughly and measure the optical density as above. After use, rinse thoroughly with water and allow to drain; steaming is unnecessary.

Treat standards containing 0 to 200 μg of sulphur in a similar way. Beer's law is obeyed.

Results

Ground pine-needle samples of 100 mg, ashed according to the method of Steinbergs *et al.*,[6] were analysed by a Johnson - Nishita distillation unit with various finishes. The mean percentage of sulphur found and standard deviations were—

By methylene blue	.. ..	0·0885 ± 0·0024 (9)
By bismuth sulphide	gelatin, 400 mμ	0·0882 ± 0·0012 (6)
	gum arabic, 363 mμ ..	0·0877 ± 0·0024 (16)

The figures in parenthesis are the number of determinations.

Discussion

The present finish has been used in this laboratory for many hundreds of plant analyses. Compared with the methylene-blue finish it is quicker, more convenient and gives identical results. With a bank of 12 units a distillation occupied 35 minutes from start to start, excluding the time required for colour measurement. If duplicate receivers are used the time would be decreased.

The procedure given is suitable for up to 200 μg of sulphur, or more if the wavelength is increased, and up to 1470 μg can be tolerated before the theoretical capacity of the bismuth reagent is exceeded. Even at this concentration no flocculation occurs on standing overnight, and the sulphur is readily determined by transferring an appropriate aliquot to 30 ml of reagent blank solution. However, such large amounts of sulphur may not be distilled completely.[5]

It is important to keep the nitrogen flow-rate above 500 ml per minute; at 300 ml per minute recovery of sulphur from standard plant samples was 82 per cent.

References

1. Gustafsson, L., *Talanta*, 1960, **4**, 227.
2. Boltz, D. F., *Editor*, "Colorimetric Determination of Nonmetals," Interscience Publishers Inc., New York and London, 1958, p. 270.
3. Field, E., and Oldach, C. S., *Ind. Engng Chem., Analyt. Edn*, 1946, **18**, 665.
4. Gustafsson, L., *Talanta*, 1960, **4**, 236.
5. Johnson, C. M., and Nishita, H., *Analyt. Chem.*, 1952, **24**, 736.
6. Steinbergs, A., Iismaa, O., Freney, J. R., and Barrow, N. J., *Analytica Chim. Acta*, 1962, **27**,158.

Received *February 14th*, 1966

* The use of an autopipette is indispensable in routine work. In this work Daffert type autopipettes were used.

The Oxidation of Hydroxylamine in Sodium Hydroxide in the Presence of Copper(II)

By J. H. ANDERSON*

(*Long Ashton Research Station, University of Bristol, Somerset*)

The oxidation of hydroxylamine to nitrous oxide at pH 8 by copper(II) salts in air produces traces of nitrite.[1] No nitrite is formed, however, from the spontaneous decomposition of hydrogen hyponitrite, $HN_2O_2^-$, to nitrous oxide.[1] It was suggested[1] that copper(II) oxidised hydroxylamine to a transient intermediate, which contained a single nitrogen atom and which was represented by

* Present address: Fisons Pharmaceuticals Ltd., Holmes Chapel, Cheshire.

the hypothetical compound, nitroxyl, (NOH); this might decompose to nitrous oxide with great speed and only small amounts might be further oxidised to nitrite.

Partington[2] states that copper(II) oxidises solutions of hydroxylamine in sodium hydroxide to nitrous oxide. In the present work a nitrogenous gas, but no nitrite, is produced in the absence of air; in the presence of air appreciable amounts of nitrite are produced, suggesting that the oxidation involves two steps. Hyponitrite does not decompose under these conditions.

Methods

Reagents used were as previously described.[1] Changes in the volume of gases, resulting from the oxidation of hydroxylamine in air or under nitrogen, were measured by performing the reactions at 30° C in double side-arm Warburg flasks in the previously described manner.[1] The flasks contained 200 μmoles of sodium hydroxide, and copper sulphate and additions as shown in Table I. Reactions were started by adding 5 μmoles of hydroxylammonium chloride from the side-arm, bringing the total volume to 2·5 ml. The hydrogen cyanide vapour used for inhibiting certain reactions was derived from 0·2 ml of a suspension of 10 per cent. calcium hydroxide in 0·7 M calcium cyanide[3] held on filter-paper in the centre well. Nitric oxide was determined by oxidation with a solution of alkaline permanganate placed in a side-arm.[4] The gas produced in the absence of air was calculated as nitrous oxide. After the stated period of reaction, samples were spun in a centrifuge to remove the precipitates of copper hydroxide and were then analysed as previously described.[5,6]

Gas analysis was performed with the model MS.2H mass spectrometer (Associated Electrical Industries). The reactions (volume 2·5 ml) were performed under reduced pressure in Thunberg tubes.[1] These were cooled to −60° C to reduce the vapour pressure of water, and were evacuated with a rotary vacuum pump until a pressure of 0·4 mm of mercury was attained. After temperature equilibration at 30° C the reaction was initiated by adding hydroxylammonium chloride from the side-arm. When the oxidation was complete, the tube was cooled to −60° C and the gas was allowed to expand into the chamber of the mass spectrometer.

Results

Oxidation of hydroxylamine in the absence of air—

Warburg experiments performed under nitrogen showed that a suspension of 100 μmoles of copper(II) hydroxide in the presence of 1 millimole of sodium hydroxide oxidised 10 μmoles of hydroxylammonium chloride to 5·3 μmoles of nitrous oxide (or nitrogen). The reaction was complete in 20 minutes, and yellow copper(I) hydroxide was formed. The gas probably accounted for the hydroxylamine nitrogen added, because no nitric oxide was formed and no hydroxylamine, nitrite or hyponitrite was found in the residual reaction mixture. When the gas (produced under reduced pressure) was analysed in the mass spectrometer, masses 44 and 30 were detected, showing the presence of nitrous oxide and of the nitrosonium ion, NO^+, which is formed by the destruction of nitrous oxide under these conditions. Negligible amounts of nitrogen were found other than that of residual air. These were determined by measuring the mass-32 peak (oxygen).

Experiments performed under reduced pressure with 5 μmoles of hydroxylamine in the presence of 1 millimole of sodium hydroxide showed that the recovery of hydroxylamine was 90 per cent. after 1 hour. When small amounts of copper(II) were added (12·5 and 125 mμmoles) the recoveries of hydroxylamine (86 and 81 per cent., respectively) were slightly decreased. No nitrite was formed. Possibly the small disappearance of hydroxylamine was caused by a metal-ion impurity (in the sodium hydroxide) which was reduced by the oxidation of hydroxylamine to nitrous oxide, and oxidised by the reduction of hydroxylamine to ammonia. The impurity might be replaceable by copper. Nitrite (2·5 μmoles), which was added to the reactions in the presence and absence of copper, was quantitatively recovered after 1 hour and did not decrease the recoveries of hydroxylamine. These findings suggest that the lack of production of nitrite from hydroxylamine and an excess of copper(II) in the absence of air does not arise from the reduction of nitrite by copper(I) or hydroxylamine.

As nitrite is formed from hydroxylamine and copper in air, the reaction would seem to involve two steps: (*a*) the oxidation of hydroxylamine by copper(II) to an intermediate which spontaneously decomposes to gas; (*b*) the oxidation of the intermediate to nitrite by oxygen.

Stability of hyponitrite—

Sodium hyponitrite (7 μmoles) in the presence of 1 millimole of sodium hydroxide did not form nitrous oxide or nitrite when 30 μmoles of copper(II) were added in the absence of air, or

when 2·5 μmoles of copper(II) were added in the presence of air. These results show that hyponitrite is not an intermediate in the conversion of hydroxylamine to nitrous oxide or nitrite.

OXIDATION OF HYDROXYLAMINE IN THE PRESENCE OF AIR—

The observed decrease in gas volume (calculated as oxygen) associated with the oxidation of hydroxylamine in air (Table I) was not equal to the theoretical uptake calculated as oxygen,

TABLE I

THE PRODUCTS OF THE COPPER-CATALYSED OXIDATION OF HYDROXYLAMINE

Periods of reaction: group A, 16 hours; group B, 6 hours.

Group	Amount of copper sulphate added, mμmoles	Additions	Time required for attaining stationary readings	Decrease in gas volume, calculated as μmoles of oxygen	Hydroxylamine disappearance, μmoles	Nitrite formation, μmoles	Nitrate formation, μmoles	
							Found	Calculated
A	0	—	6 hours	6·2	5·0	2·0	2·4	2·8
	25	—	10 minutes	1·9	5·0	2·3	0·0	0·0
	0	Cyanide	—	4·9	2·9	1·3	—	2·4
	25	Cyanide	16 hours	6·9	5·0	1·9	2·4	3·3
B	0	—	—	3·6	3·2	1·3	—	1·5
	1·25	—	60 minutes	5·2	5·0	3·5	0·8	1·1
	2·5	—	20 minutes	4·2	5·0	3·1	0·3	0·7
	12·5	—	10 minutes	1·9	5·0	2·2	0·0	0·0

in accordance with equation (1), from the nitrite production. In certain experiments, however, nitrate was produced in amounts which were almost equal to those calculated by multiplying the difference between the observed and theoretical decreases in gas volume by the factor 2/3 derived from equation (2). The hydroxylamine that was not accounted for as nitrite and nitrate was probably converted to nitrous oxide, which was not determined. The production of this gas from hydroxylamine, according to equation (3), does not involve a change in the volume of gases. The contraction in volume that resulted from the greater solubility of 1 μmole of nitrous oxide compared with that of 1 μmole of oxygen was calculated to be approximately 0·1 μmole of oxygen. This was small and was not taken into account when calculating the nitrate formation.

$$NH_2OH + O_2 = NO_2^- + H_2O + H^+ \quad .. \quad .. \quad .. \quad (1)$$

$$NH_2OH + 1\tfrac{1}{2}O_2 = NO_3^- + H_2O + H^+ \quad .. \quad .. \quad .. \quad (2)$$

$$2NH_2OH + O_2 = N_2O + 3H_2O \quad .. \quad .. \quad .. \quad .. \quad (3)$$

Table I shows that 5 μmoles of hydroxylamine present with 0·2 millimole of sodium hydroxide disappeared completely in 6 hours forming 2·0 μmoles of nitrite and 2·4 μmoles of nitrate (group A). When a small amount of copper(II) (25 mμmoles) was added, the oxidation of hydroxylamine to 2·3 μmoles of nitrite was complete in 10 minutes and no nitrate was formed (group A). Cyanide inhibited the oxidation of hydroxylamine in the presence, or absence, of copper(II), and nitrite (and nitrate) were formed (group A). These results suggest that the metal-ion impurity is reduced by the oxidation of hydroxylamine and oxidised by oxygen. In these respects the impurity is probably replaceable by copper. Cyanide probably binds the metal ions. Increasing the amount of copper added from 1·25 to 12·5 mμmoles increased the rate of oxidation of hydroxylamine but decreased the formation of nitrite *plus* nitrate from 4·3 to 2·2 μmoles (group B). These findings suggest that the formation of nitrous oxide was increased.

DISCUSSION

Latimer[7] states that alkaline conditions favour the decomposition of hydroxylamine to nitrous oxide and ammonia. The decomposition studied in the present work is oxidative, but it is always possible that small amounts of ammonia were formed in the presence and absence of air by the reduction of hydroxylamine with a reduced metal-ion impurity or copper(I).

The analysis performed with the mass spectrometer showed that the gas produced by the quantitative oxidation of hydroxylamine with an excess of copper(II) in the absence of air contains nitrous oxide. Possibly the hypothetical compound, nitroxyl, is the intermediate from which the nitrous oxide is spontaneously formed, as indicated by equation (4).

$$2\,(NOH) = N_2O + H_2O \quad .. \quad .. \quad .. \quad .. \quad (4)$$

As nitrite is produced from hydroxylamine and copper(II) in air, the oxidation would seem to involve two steps. The first step would be the oxidation of hydroxylamine to nitroxyl by copper(II) according to equation (5). As solutions of hyponitrite in sodium hydroxide are stable, it seems unlikely that hyponitrite is an intermediate of the conversion of nitroxyl to nitrous oxide. The second step is apparently the oxidation of nitroxyl to nitrite. Probably the reaction requires molecular oxygen as indicated by equation (6) and does not involve copper(II) as oxidant.

$$NH_2OH + 2Cu(II) = (NOH) + 2Cu(I) + 2H^+ \quad (5)$$

$$(NOH) + \tfrac{1}{2}O_2 = NO_2^- + H^+ \quad (6)$$

The oxidation of copper(I) by oxygen may produce peroxide. This would oxidise nitrite to nitrate. The finding that increasing the copper concentration decreased the yield of nitrite *plus* nitrate (group B, Table I) may be compared with previous results[1] in which increasing the copper concentration from 1 to 1000 μM at pH 8 increased the rate of oxidation of 1 mM hydroxylamine to nitrous oxide, but only about 5 per cent. of the hydroxylamine was converted to nitrite. Possibly a high concentration of hydroxyl ions is required for the oxidation of nitroxyl to nitrite.

I thank Dr. K. W. Dunning for helpful discussions of the typescript, and Dr. R. Clampitt for performing the analysis with the mass spectrometer.

References

1. Anderson, J. H., *Analyst*, 1964, **89**, 357.
2. Partington, J. R., "General and Inorganic Chemistry," Second Edition, Macmillan and Co. Ltd., London, 1951, p. 554.
3. Robbie, W. A., *Meth. Med. Res.*, 1948, **1**, 307.
4. Anderson, J. H., *Biochem. J.*, 1965, **94**, 236.
5. ——, *Ibid.*, 1964, **91**, 8.
6. ——, *Analyst*, 1963, **88**, 494.
7. Latimer, W. M., "The Oxidation States of the Elements and their Potentials," Second Edition, Prentice Hall Inc., New Jersey, 1952, p. 97.

Received *August 31st*, 1965

Limits of Sensitivity of Detection of Aluminium in Amorphous and Crystalline Aluminium Oxide by X-ray Diffractometry

By C. J. TOUSSAINT and G. VOS

(*Euratom, Chemistry Department, Analytical and Mineral Chemistry Section, Ispra, Italy*)

It is frequently necessary to know the limit of detection that can be obtained in quantitative determinations by X-ray diffraction, but few results have been published. Recent work on the limit of sensitivity of silicon in lithium fluoride and tungsten[1] and of structural constituents in metallographic samples[2] are examples.

During research on the determination of aluminium in sintered aluminium powders, we made a study of the minimum detectable amount of aluminium in an aluminium oxide matrix with two types of diffractometer, one with a curved crystal monochromator, the other with a filter to eliminate the K_β radiation. Assuming that the diffraction line is free from interference, the limit of detection of a crystalline phase in a mixture of powders generally depends on: the absolute intensity of the particular diffraction maximum from the crystalline phase; the mass absorption coefficient of the matrix; and an instrumental factor (type of diffraction technique and equipment, experimental conditions).

Experimental

Two different types of diffractometers were used. One was the CGR* diffractometer, equipped with a curved quartz monochromator in front of the reflection specimen, proportional counter and de-mountable X-ray tube with copper anode. The applied voltage and beam current were 45 kV and 15 mA, respectively. The other was the Philips diffractometer with sealed-off copper tube, proportional counter, nickel filter (to eliminate copper K_β radiation) and pulse-height analyser.

* Compagnie Générale de Radiologie: Paris, France.

Further instrumental parameters were slit divergence and scatter 1°, receiving slit width 0·2 mm. Excitation conditions were 40 kV and 20 mA, and 50 kV and 20 mA (fine focus or normal focus tube).

The (111) reflection of aluminium has been chosen as analytical line. The minimum composition detectable may be defined as that concentration which yields an intensity equal to three times the standard deviations of the background intensity. Thus—

$$\text{Limit of detection} = \frac{3.C\sqrt{B.T}}{P.T}$$

where P = intensity of the peak in counts per second.

B = intensity of the background in counts per second.

T = counting time in seconds.

C = percentage weight of aluminium.

The limits of detection were determined with a counting time of 100 seconds. The intensity of the peak was obtained from a sample containing 0·5 per cent. of aluminium, while the background was measured with pure aluminium oxide samples.

Results and discussion

The limits of detection are given in Table I. As well crystallised aluminium gives strong reflections and the mass absorption coefficient of the matrix is only 28 cm² per g (copper K_α), aluminium can be determined to about 0·0080 per cent. in crystalline aluminium oxide. In unfavourable circumstances, a matrix of amorphous aluminium oxide in which the broad halo of

Table I

Limits of detection of aluminium in crystalline and amorphous aluminium oxide found by different X-ray diffractometer techniques

Apparatus	Limits of detection: Matrix amorphous, aluminium oxide	Limits of detection: Matrix crystalline, aluminium oxide
Diffractometer with monochromator, tube load 675 watts	0·0550 per cent.	0·020 per cent.
Diffractometer with filter—		
(*a*) Fine focus tube, tube load 800 watts	0·0480	Not determined
(*b*) Normal focus tube, tube load 1000 watts ..	0·0270	0·0100
Diffractometer without filter—		
(*a*) Fine focus tube	0·0350	Not determined
(*b*) Normal focus tube	0·0220	0·0080

diffraction emanating from the amorphous regions occurs at about the same wavelength as the aluminium (111) reflex, therefore giving rise to high background counting rates, limits of 0·03 per cent. can be attained. In Fig. 1 are shown diffractometer traces of an amorphous aluminium oxide sample containing 0·5 per cent. of aluminium under different experimental conditions.

The most unfavourable results were obtained with the diffractometer equipped with the crystal monochromator. As the two diffractometers used were of a different type it is impossible to give a quantitative explanation. It is interesting to note that Parrish and Kohler,[3] with the same type of apparatus and for the usual type of measurements, obtained results of the P/B ratios that were about the same, when they compared a quartz crystal monochromator placed behind a transmission specimen with the pulse-height discrimination method. In certain circumstances, however, such as when the analytical line lies in the low-angle region, the crystal monochromator should give better results.

The influence of the total power applied to the X-ray tube has considerable influence on the sensitivity. On examination of Table I, we conclude that this plays an important rôle. The poor results obtained with the CGR diffractometer arise from this, as the maximum power that could be applied was only 675 volts.

Eliminating the filter gave only a slight increase in the sensitivity.

In conclusion, we believe that with modern X-ray diffractometer apparatus, limits of detection between 0·1 and 0·01 per cent. under similar conditions can be obtained.

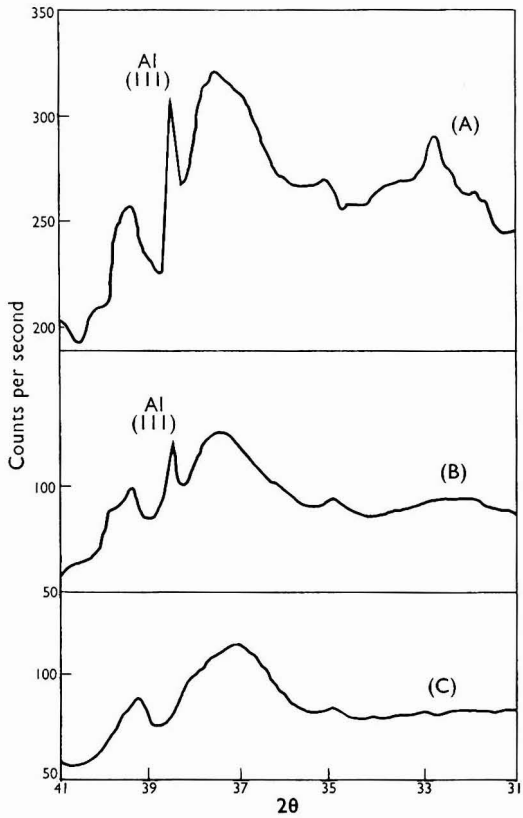

Fig. 1. X-ray diffractometer traces of an amorphous aluminium oxide sample containing 0·5 per cent. of aluminium. Instrumental parameters: copper radiation, fine focus tube, 40 kV and 20 mA, scanning speed $\frac{1}{2}°$ per minute

References

1. Parrish, W., and Taylor, J., Int. Symp. X-Ray Microsc., No. 2, Stockholm, Elsevier, Amsterdam, 1960, 458.
2. Kudielka, H., Möller, H., *Arch. EisenhüttWes.*, 1963, **34**, 181.
3. Parrish, W., Kohler, T. R., *Rev. Scient. Instrum.*, 1956, **27**, 795.

Received *August 13th*, 1965

Analytical Methods Committee

REPORT PREPARED BY THE MEAT PRODUCTS SUB-COMMITTEE

Nitrogen Factor for Kidney

THE Analytical Methods Committee has received the following Report from its Meat Products Sub-Committee. The Report has been approved by the Analytical Methods Committee and its publication has been authorised by the Council.

REPORT

The Meat Products Sub-Committee of the Analytical Methods Committee responsible for the preparation of this Report was constituted as follows: Dr. S. M. Herschdoerfer (Chairman), Mr. S. Back, Mr. P. J. Cooper (appointed 1965), Mr. P. O. Dennis, Mr. J. R. Fraser (resigned 1965), Mr. H. C. Hornsey, Dr. A. J. Kidney, Mr. T. McLachlan, Dr. R. A. Lawrie, Dr. A. McM. Taylor and Mr. E. F. Williams, with Mr. P. W. Shallis as Secretary.

In the course of its investigations on the nitrogen factors of different types of meat the Sub-Committee has already reported on pork,[1] beef,[2] chicken,[3] liver,[4] veal[5] and turkey.[6] Two reports were also issued on the nitrogen content of rusk filler.[7,8]

The Sub-Committee has now completed its determinations of the nitrogen content of kidneys and its findings are summarised in Fig. 1.

In a similar way as for the various types of meat, kidneys have a variable nitrogen content. On the other hand, it was interesting to note that there was very little difference between the mean values for the kidneys of the three species examined by the Sub-Committee. The over-all weighted mean was 2·69 per cent.

RECOMMENDATION

The Sub-Committee recommends an average factor of 2·7 for use in the analysis of kidney products.

REFERENCES

1. Analytical Methods Committee, *Analyst*, 1961, **86**, 557.
2. ——, *Ibid.*, 1963, **88**, 422.
3. ——, *Ibid.*, 1963, **88**, 583.
4. ——, *Ibid.*, 1964, **89**, 630.
5. ——, *Ibid.*, 1965, **90**, 256.
6. ——, *Ibid.*, 1965, **90**, 581.
7. ——, *Ibid.*, 1961, **86**, 560.
8. ——, *Ibid.*, 1965, **90**, 579.

	No. of samples	Range	Mean
OX KIDNEYS (fresh)	14	2·48–2·93	2·71
OX KIDNEYS (frozen)	25	2·26–3·07	2·69
LAMB KIDNEYS (fresh): (2 kidneys from same carcase minced)	12	2·46–2·98	2·67
PIG KIDNEYS (fresh): (8 to 12 kidneys minced)	12	2·41–2·90	2·67

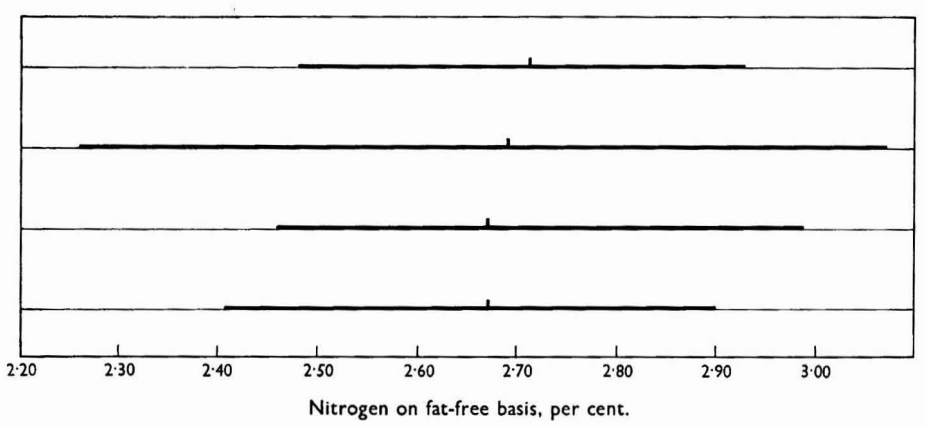

Fig. 1. Nitrogen contents of kidneys from different species. Horizontal lines represent the range of nitrogen contents, short vertical lines indicate the average values

Analytical Methods Committee

REPORT PREPARED BY THE FISH PRODUCTS SUB-COMMITTEE

Nitrogen Factor for Cod Flesh

THE Analytical Methods Committee has received the following Report from its Fish Products Sub-Committee. The Report has been approved by the Analytical Methods Committee and its publication has been authorised by the Council.

REPORT

The Fish Products Sub-Committee was appointed by the Analytical Methods Committee in 1963 and its constitution is: Dr. S. M. Herschdoerfer (Chairman), Dr. J. H. Bushill (deputy—Mr. W. C. A. Wise), Dr. C. L. Cutting, Mr. J. R. Fraser (resigned June, 1965), Mr. B. J. Hasberry (deputy—Mr. D. J. Ward), Dr. W. T. Little, Dr. J. A. Lovern, Mr. T. McLachlan and Mr. P. J. Cooper (appointed August, 1965), with Mr. P. W. Shallis as Secretary. The Sub-Committee's terms of reference are: "(*a*) To establish the essential characteristics of fish and differences in these characteristics caused by decomposition or other changes; (*b*) To recommend methods for determining the amount of fish present in food products."

Cod is the fish used most frequently as an ingredient in manufactured products—fish fingers, fish cakes, fish pastes, etc.—and the Sub-Committee examined the available methods for determining the fish content, expressed as cod, of such products. It was considered at the time that a method based on the nitrogen content of the fish was the most promising one, and accordingly a search of the relevant literature was carried out and arrangements were also made for analyses to be carried out in a number of laboratories.

In a paper on "Seasonal Variations of Fat, Water Solubles, Protein and Water in Cod Fillets," Dambergs[1] reported the results obtained in monthly analyses of samples of muscles of medium-size Nova Scotia inshore cod (*Gadus morrhua* L.). He observed that the protein content of the muscles reached a maximum in October to November and then gradually diminished to a minimum in May. This cyclic variation was observed in all parts of the fillets, *i.e.*, the head, middle and tail sections.

Similar observations had been made on North Sea cod by Ironside and Love,[2] who concluded that the variations in protein were influenced by the spawning effort and by starvation during the winter months.

The Sub-Committee arranged to examine head, middle and tail sections of fillets as well as whole fish, to compare results obtained on freshly caught fish and on fish purchased in markets and to investigate as far as possible fish from different fishing grounds.

The sampling instructions issued to all participating laboratories were that the fish should be filletted, the skin removed from the fillets and then the whole, or the appropriate portions, of the fillets should be minced and the minced flesh thoroughly mixed before the sample was removed for analysis. Details of the determination of moisture by vacuum drying at 70° C and of the determination of nitrogen by the Kjeldahl method with mercuric oxide as catalyst were also issued to all participating laboratories.

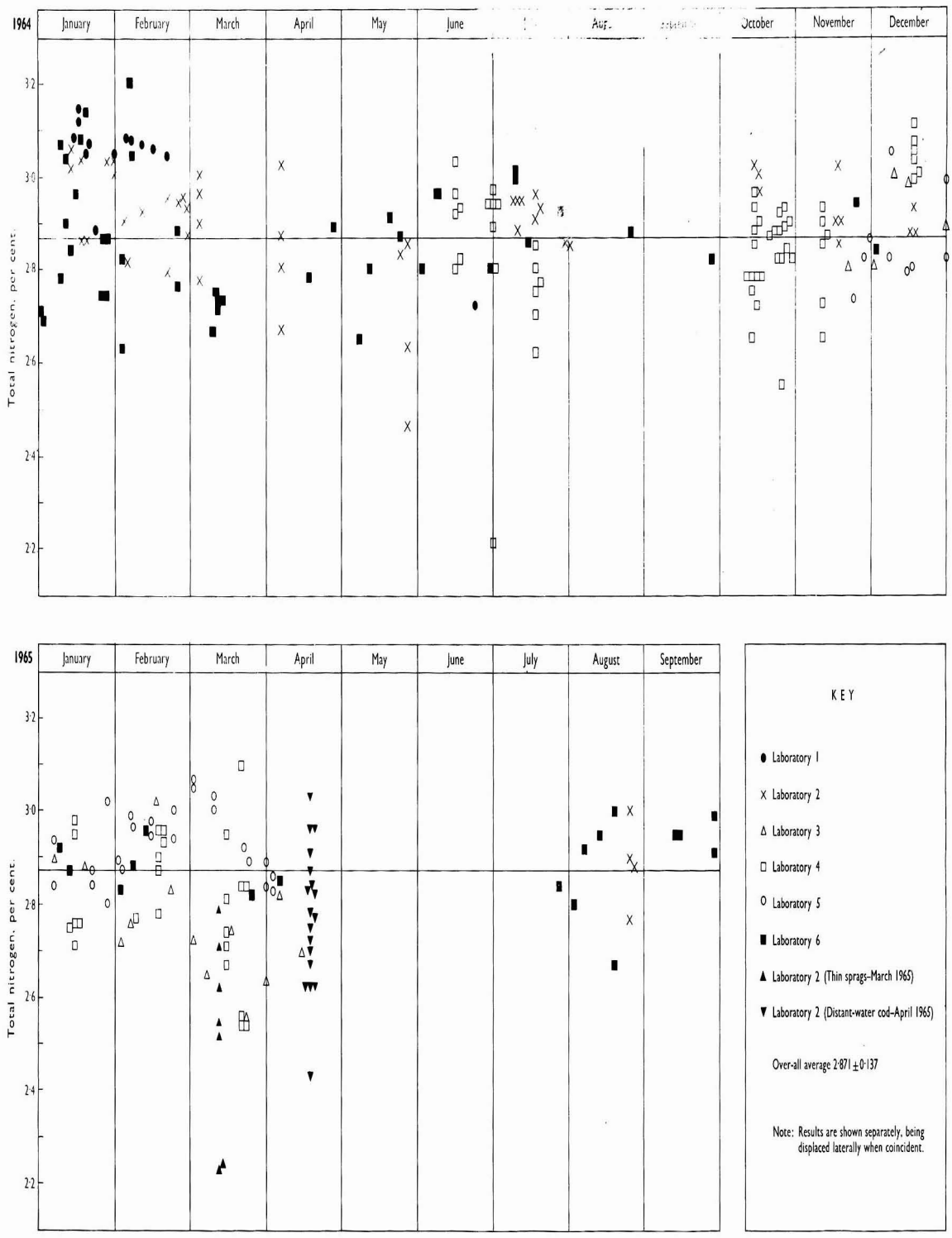

Fig. 2. Nitrogen content of cod, January, 1964, to September, 1965

The results obtained in the period January, 1964, to September, 1965, are summarised in Figs. 1 and 2.

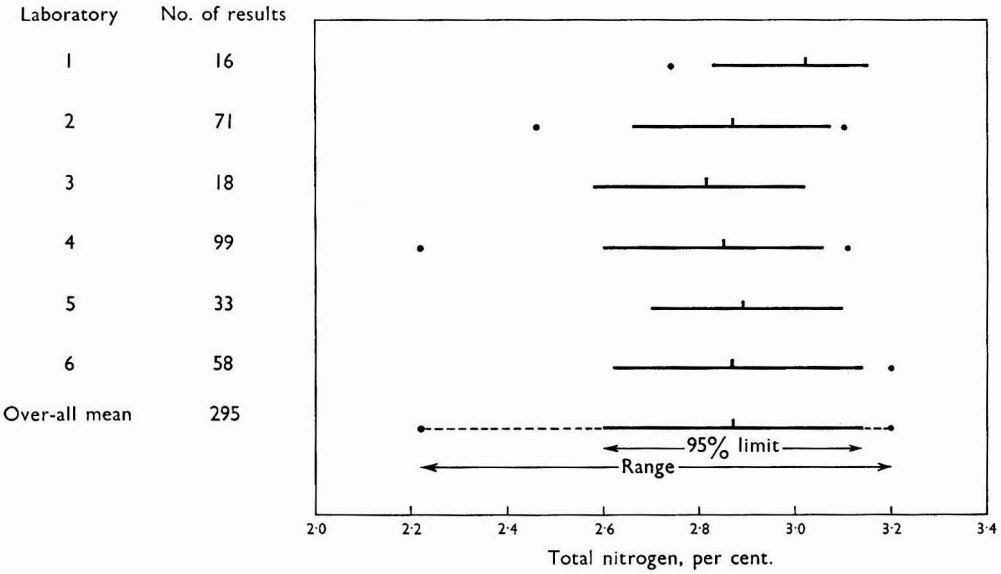

Fig. 1. Means, normal limits (95 per cent.) and ranges of results from different laboratories, January, 1964, to September, 1965

These results were submitted to a statistical analysis to determine the average percentages of nitrogen, their normal limits of variation and to establish how much of this variation was due to the causes listed below—

A. Differences between seasons of the year.
B. Differences between parts of the fish analysed.
C. Differences between fish of different sizes.
D. Differences between fresh, stored and frozen fish.
E. Differences between fishing grounds.

The over-all mean value for the nitrogen content of cod flesh on 295 samples was 2·871 per cent., with a standard deviation of $\pm 0{\cdot}137$.

Discussion of results

(*a*) A seasonal variation in the nitrogen content was confirmed in the samples examined, although it was noted that these periods were somewhat different from those reported by Dambergs.

(*b*) As expected, no difference was found between the left and right sides of fish. The head portion had a higher and the tail portion a lower nitrogen content than the middle portion.

(*c*) The size of the fish does not appear to have any significant influence on the nitrogen content of the flesh.

(*d*) Fresh fish examined within a few hours of capture and also pre-rigor, had a higher than average nitrogen content, whereas fish kept on ice for 10 to 12 days had a lower than average nitrogen content.

(*e*) The apparent influence of the fishing grounds was indicated by the higher nitrogen levels observed in cod from Iceland and the Faroes and the lower nitrogen content of cod of distant-water origin. Lower figures can also be due to the loss of nitrogen on prolonged storage in ice.

Recommendation

After due consideration of all the relevant data the Sub-Committee recommends that an average nitrogen factor of 2·85* should be used in the analysis of cod products.

Acknowledgment

The Sub-Committee thanks those listed below for their help and communications—

Birds Eye Foods Ltd.
British Food Manufacturing Industries Research Association.
J. Lyons & Co. Ltd.
Ministry of Technology, Torry Research Station.
Ross Foods Ltd.
Unilever Ltd.

References

1. Dambergs, N., *J. Fish. Res. Bd Canada*, 1964, **21**, 703.
2. Ironside, J. I. M., and Love, R. M., *J. Sci. Fd Agric.*, 1958, **9**, 597.

* The Sub-Committee has followed the usual practice of the Meat Products Sub-Committee in recommending an average nitrogen factor expressed to the nearest 0·05 per cent.

Book Reviews

Stable Radicals. By Anatoli Leonidovich Buchachenko. Pp. viii + 180. New York: Consultants Bureau. 1965. Price $15.00.

The appearance of this book is timely, as it gives a fairly comprehensive and up-to-date account of the properties of stable, electrically neutral radicals. The space allotted to different aspects of the subject varies considerably; but this is mainly attributable to gaps in the present state of knowledge rather than to a lack of balanced treatment on the part of the Russian authors. As with most monographs of this type, a revised edition will sooner or later be required, dependent upon the rate at which the subject is extended. Nevertheless, it will be of great value to chemists concerned with such matters as kinetics of reactions, polymerisation, oxidation - reduction processes and the like.

The first chapter is devoted to the general methods used in the detection and investigation of the behaviour of stable radicals, among which electron paramagnetic resonance predominates. This method is described in considerable detail and this chapter could be read with profit even by those spectroscopists to whom the technique is unfamiliar. In the ensuing chapters reference is frequently made to the various derivations discussed in this basic section, and for this reason, if for no other, it is a pity that no index is provided.

Chapters 2 to 4 deal successively with radicals formed with tervalent carbon, monovalent oxygen and bivalent and quadrivalent nitrogen. Chapter 4 contains something of importance to analysts, particularly with respect to the well known diphenylpicrylhydrazyl (DPPH) radical. Magnetic and electrical properties of stable radicals are treated briefly in Chapter 5, while the last chapter deals with the use of stable radicals in the investigation of chemical reactivity, particularly with respect to oxidation - reduction processes. As in many other connections, chromatography is often enlisted as a valuable purification technique in the isolation and separation of stable radicals.

Finally, the book is well produced and the molecular formulae and spectra are clearly printed, while plentiful literature references are given at the end of each chapter. A word of praise is due to the translators who have produced a most readable, yet clearly expressed text. There are a few trivial and irritating misprints, particularly in the latter part of the book, suggestive of rather casual proof-reading.

F. G. Angeli.

CELL K. By RODERICK P. KERNAN, D.Sc. Pp. viii + 152. London: Butterworth & Co. (Publishers) Ltd. 1965. Price 27s. 6d.

This little monograph warrants review in these columns only because one of its seven chapters describes the assay of potassium in tissues and body fluids. In fact, it provides a fascinating account of some effects of the evolution of living organisms from primitive creatures that existed millions of years ago when the oceans contained about equal amounts of sodium and potassium. As sea-water now contains about thirty times more sodium than potassium, the latter element must have been absorbed preferentially by living matter. Ingenious mechanisms have evolved whereby most living cells maintain higher concentrations of potassium than the fluids by which they are bathed. With other ions, cellular potassium plays a major part in the electrical properties of resting and active cells; those of nerves and muscles have been well studied on account of the changes in electrical potential and polarisation which take place during nerve conduction and muscle contraction. Cell potassium also plays a major part in many metabolic reactions of the body.

This book provides an interesting account which can be read as an academic exercise, and perhaps for pleasure, by all those analysts whose knowledge of physical chemistry and electrochemistry has remained up to date. C. H. GRAY

KIRK-OTHMER ENCYCLOPEDIA OF CHEMICAL TECHNOLOGY. Second Edition. Volume 7. Dialysis to Electron-spin Resonance. Edited by HERMAN F. MARK, JOHN J. MCKETTA, jun., and DONALD F. OTHMER. Executive Editor: ANTHONY STANDEN. Pp. xvi + 903. New York, London and Sydney: Interscience Publishers, a division of John Wiley & Sons Inc. 1965. Price £16 18s.; price per volume for subscribers to the complete set of 18 volumes £13.

It has naturally been the policy of the reviews of this series of volumes (see *Analyst*, 1963, 88, 899, *et seq.*) to draw special attention to sections of the contents having special reference to chemical analysis and associated subjects. As a rule these have been part of an individual monograph, and as such they have been subsidiary to the main subject matter of the monograph. This of course does not detract from their importance; chemical analysis has been described as "the hand-maiden of chemistry." From time to time, however, as successive volumes appear there have been monographs devoted exclusively to specific branches of chemical analysis. From this point of view the recently published Volume 7 is of special importance, in that it deals with Electroanalytical methods which, together with the following monograph on Electrochemistry, comprise 115 pages.

The former monograph starts with a short but succinct theoretical chapter that includes the latest conceptions of the subject, such as chronopotentiometry (the variation of potential with time), a specific name for which is polarography. Then follows one of the main sections which is on potentiometry, both direct and depending on zero faradic currents, *i.e.*, precipitation and oxidation - reduction titrations. The section on apparatus for this work is not developed in detail. This is understandable as "do-it-yourself" apparatus is now seldom used for this type of work; certainly not in this country and probably to an even smaller extent in the United States. It is more usual to purchase ready made equipment from a reliable manufacturer. The treatment of the subject is sufficient to indicate the principles of such apparatus without descriptions of particular instruments in any detail; nevertheless, some notes on the relative merits of the various makes would have been of value to the newcomer in this field.

The applications of polarography to both qualitative and quantitative analysis are fully discussed. Here again the instrumental side, both manual and recording, is dealt with generally rather than in particular. Of special interest in the latter connection are the recent developments in the use of operational amplifiers of the type used in modern analogue-computer circuits; and the use of 3-electrode circuits (also known as potentiostats) to remedy the loss of potential in overcoming the internal resistance of a cell. Another little known technique is amperometric titration, in which a rotating platinum electrode is used.

The next main heading is electrolysis methods, more commonly known as electro-deposition, although strictly this is a particular instance in which insoluble products are formed. In the monograph, this is described under electro-gravimetry, and a distinction is made between it and coulometry, *i.e.*, the measurement of the quantity of electricity which accomplishes the depletion of a sample from a solution by electrolysis. There is a brief reference to the numerous devices that have been described for the integration of current - time curves in controlled-potential

coulometry. These are of considerable potential importance especially for process control, and they can be chemical, electromechanical or electronic in nature.

The final section of this monograph deals with conductance methods (more commonly known as conductimetry), and the treatment follows the same lines. The very useful type of conductivity cell having electrodes whose distance apart can be varied is not mentioned. There are 99 literature references.

The 57-page article on Electrochemistry is purely theoretical, and is best studied in conjunction with the first part of the monograph on Electroanalytical methods. The alphabet has made them neighbours, but unfortunately in the reverse order of the natural sequence. On reading these 2 important articles, one is impressed by the necessity for standardisation of the nomenclature of the subject. This is apparent even from the comments made above in this review.

Other monographs based on the ramifications of electrical techniques are Electrodialysis and Electrodecantation (electrogravitational separation). The latter depends upon the stratification phenomena that occur when colloidal dispersions are subjected to an electrical field between vertical membranes, permeable to the electrical current and impermeable to the colloid. Apparently laboratory equipment of this nature can be used for chemical preparatory work, although the principal practical application has been to the concentration of rubber latex. Under Electrodialysis, applications to the removal of salt from water, de-ashing sugar solutions, de-acidification of citrus juices, de-contamination of milk, sulphite pulp and waste recovery are all considered; the last, however, is as yet of academic interest only.

The monograph on Electrolytic Machining Methods provides another example of ambiguity of nomenclature. Many would feel that it is more correctly described as electrochemical machining, and indeed it is so listed in the present Encyclopedia, but with an appropriate cross-reference. It is useful to have a record of recent advances in this field, because, although at present the applications are not yet widely used in industry, they appear to have a future especially where stress-free metal objects are involved. The short section on Electrical Testing provides an introduction to the subject which otherwise is of limited interest to analysts and, indeed, to chemists. However, electron-spin resonance spectroscopy is an important and sensitive technique for detecting unpaired electrons, and as such has many applications in the analytical field. A 29-page monograph deals with it clearly and adequately.

Previous reviews of this series have commented on the strange situation that self-contained monographs arranged in a purely alphabetical order inevitably produces. In the present volume, for example, the author of the section on Dialysis differs from the author of Electrodialysis; and, moreover, neither monograph makes reference to the other. A further article deals with Diffusion Separation Methods at some length; this is related, perhaps somewhat remotely, to Electrodecantation. One comprehensive section on separation methods might have brought all these into their correct relative perspective. However, as Diffusion Separation Methods follows Diffusion (a short, 12-page section), there is some argument for combining these two.

A somewhat unexpected monograph in a chemical encyclopaedia is Dimensional Analysis, a purely mathematical tool, enabling a set of variables to be reduced to a set of dimensionless products of the variables. Of a similar fringe character is Economic Evaluation, a short but excellent monograph which tells the chemist in simple terms just about as much as he needs to know on the financial evaluation of a proposed course of action. Drilling Fluids (20 pages) is another off-track subject. Chemically, it is of some importance in view of the wide variety of chemical substances used in the preparation, maintenance and utilisation of drilling fluids. Examples of their functions are to increase density or viscosity, form seals, reduce filtrate loss, and act as stabilisers, emulsifiers, inhibitors, bactericides, lubricants and flocculants. As may be expected a wide variety of chemical substances is involved, although strangely, there is no reference to the latest addition to the list, namely dextran, which has given promising results in preliminary trials in the United States.

Food chemists will turn with special interest to the monograph on Eggs. Although this does not deal with analytical methods, it contains some valuable data on the compositions of various types of processed eggs. Similarly, the monograph on Dry Cleaning lists the operations and chemicals involved. It also makes some attempt to evaluate dry cleaning performance, a difficult matter when water-soluble soil removal is measured in terms of the removal of sodium chloride, glucose and a water-soluble food dye from rayon. The chemistry of clothes marking, and even "coinop" (self service) dry cleaners are included.

In all there are 32 monographs in this volume. Most of those not mentioned above are relatively short, with the exception of Dyes and Dye Intermediates, their applications and evaluation,

followed by Natural Dyes and Reactive Dyes as separate monographs. These 4 dyestuffs monographs total 168 pages. It is interesting to learn from the monograph on Natural Dyes that logwood is still of great importance, nearly one million pounds' weight having been consumed in 1964 in the United States alone. Further detailed comments on individual monographs is not possible, but mention should be made of those on Diamines and Diarylamines (31 pages), Diatomite, Diphenyl, Distillation, Diuretics, Driers (including metallic soaps), Drying and Drying Agents, Drying Oil and Elastomers.

This volume once again demonstrates the wide scope, up-to-date treatment and high standard of presentation by both authors and publishers, which have characterised the 6 previous issues. Despite the claims of an international approach to the subject matter made in the Preface to Volume 1, the list of authors appears to be wholly American, and the bibliographies reflect this trend. It is only fair to add that this does not appear to detract materially from the value of the subject matter; however, it does mean that statistics and view-points are not as widely spread as one might expect in a work of this kind. JULIUS GRANT

ANALYTISCHE CHEMIE IN DER INDUSTRIELLEN PRAXIS. No. 2. By Prof. WOLFGANG LEITHE. Pp. xvi + 412. Frankfurt: Akademische Verlagsgesellschaft. 1964. Price DM 60.

Books on analytical chemistry are almost always written purely for specialists or students of the subject, and I find it interesting that I have now read the third book in 2 years (one each in French, English and German) written to inform the non-specialist about present-day analytical chemistry. This seems to indicate a revival of general interest in a subject that concerns all chemists to some extent.

Dr. Leithe has wide experience both as a professor and as head of a large industrial laboratory, and few people are in a better position to appreciate the gap between academic teaching and industrial practice. He writes for the newcomer to industry and starts by indicating the range of industrial analysis. Before going on to describe, very briefly, almost all current techniques from gravimetric analysis to differential thermo-analysis, he has about 30 pages on laboratories, their siting and fittings, sampling and samplers, laboratory personnel, costing and the recording of results. To the experienced analyst this is the most interesting section of the book. It is virtually impossible to describe all analytical techniques in 270 pages, but this is a very good attempt. I would, however, comment on the variable value of the diagrams. The photograph of the piston burette on p. 35 and the sketch of the optics of the Abbé refractometer on p. 172 tell one nothing unless one is already familiar with the apparatus; on the other hand, the diagram of the infrared spectrophotometer on p. 164 is clarity itself.

The next section (pp. 301 to 342) on the analytical needs of certain industries is, in my opinion, too short to be really informative.

The book concludes with a section on the analysis of gaseous and liquid effluents (pp. 343 to 386), and a brief sketch of automatic methods of analysis (pp. 387 to 402). These should give the student an adequate outline of the subjects, but too much space is given to sulphur dioxide. There is a table giving the reagents used in a well known German series of gas-detector tubes for toxic gases.

Perhaps I ought to say that unless the reader has a much better knowledge of German than the average modern student, he will need to have at hand a *good* German dictionary.

H. N. WILSON

ANALYTICAL CHEMISTRY OF BORON. By A. A. NEMODRUK and Z. K. KARALOVA. Pp. xii + 236. Jerusalem: Israel Program for Scientific Translations. Distributed in Great Britain and the Commonwealth, South Africa, Eire and Europe by the Oldbourne Press, London. 1965. Price 103s. 6d.

Very few analysts have never been asked to determine boron, and when parts, or fractional parts, per million of boron are involved this invariably presents a problem.

If the material is a solid, the problem arises of how best to obtain the sample in solution, without loss of boron, in a form suitable for the direct evaluation of boron, or its subsequent isolation as methyl borate, and a recommended procedure for one class of material often fails if it is applied to another.

Difficulties associated in the *initial* stages of this determination have led to the development of separations based on pyrolysis, chromatography, the use of ion exchangers, the mercury-cathode cell, etc., but methods used in the *final* stages of this determination may be described as "variations on the same theme."

The first two chapters of this book describe the fundamental physical and chemical properties of boron and its compounds, and subsequent chapters deal with methods of detection, separation, and determination of the element, including the more important analytical procedures for determining boron in various naturally occurring and industrial materials.

Based on information contained in over 1200 Soviet and foreign references up to early 1963, and the authors' personal experience, published methods are discussed, and comment is made on their respective merits, limitations, sensitivity, accuracy and reproducibility, and methods that have proved more reliable in practice receive special attention.

The only adverse comment on this book is that it does not deal with some of the more recent important developments that show a marked tendency towards the use of procedures for the *direct* determination of boron at very low levels in certain selected materials. This, however, is understandable in view of the inevitable delays associated with double publications.

The book, which was first published in Moscow in 1964, is a translation of one of a series of about 50 monographs to be published by The Institute of Geochemistry and Analytical Chemistry, U.S.S.R. Already about 10 of this series have been either translated and published in the English language, or are in the course of being translated for publication.

Published translations often contain irritating, if insignificant, misprints and inconsistencies, but this cannot be said of this book, and all concerned, especially the translator and proof-reader are to be congratulated for presenting such a clearly written English version.

A publication such as this, that deals in considerable detail with the subject up to the time of its initial publication, is an asset to any analytical laboratory, because it covers, in about the right amount of detail, a wide variety of materials ranging, alphabetically, from animal tissues to zirconium alloys. W. T. ELWELL

CHEMISTRY OF ORGANIC SULFUR COMPOUNDS IN PETROLEUM AND PETROLEUM PRODUCTS. Proceedings of the 3rd Scientific Session, held in Ufa, June 3–8, 1957. General Editor: Prof. R. D. OBOLENTSEV. Pp. xii + 364. Jerusalem: Israel Program for Scientific Translations. Distributed in Great Britain and the Commonwealth, South Africa, Eire and Europe by Oldbourne Press, London. 1965. Price 90s.

In June 1957, a Congress on the Chemistry of Organic Sulphur Compounds was held at Ufa; the proceedings of the Congress were published in Russia in 1959, edited by Professor R. D. Obolentsev. This work has now been translated by the Israel Programme for Scientific Translations, the translation being the text of this book.

The reason for the Congress was the increased availability of high sulphur-content crude oils (*e.g.*, Tuimazy deposits), which prompted Soviet scientists to work out a centrally co-ordinated research programme in this field in order to attempt to solve 2 problems: to develop basically new methods of primary processing of sulphur-containing petroleums; and to develop existing processing procedures by inclusion of new methods of petroleum distillation causing de-sulphurisation, such that sulphur could be used in other ways.

This book gives an account of the proceedings of the fundamental studies carried out up to that time. Divided into 6 parts, the proceedings range over the following aspects of sulphur chemistry: synthesis, characterisation, and analysis of sulphur-containing organic compounds; the isolation and composition of organic sulphur products in petroleum; thermo-catalytic transformations of organic sulphur compounds; corrosive and tar-forming properties of sulphur crudes; the utilisation of organic sulphur compounds; and toxicology of certain sulphur compounds.

To the analyst at first glance there does not appear to be much of interest, but the first two sections are full of analytical procedures, including extensive use of chromatography for separating the vast range of compounds encountered. Details of an oscillographic polarograph for analysis of sulphur compounds is also given. Naturally only Russian research was given at the conference but reference is made to work in Western Europe and America.

The book is relatively free from errors (although in some places benzene is spelt benzine), and the standard of translation is high. The major criticism is that the length of time since the original Russian publication (7 years), causes the book to be only of reference value, because most of the information is now already known to those working in this field. G. NICKLESS

Errata

JUNE (1966) ISSUE, p. 343, 4th line of synopsis. *For* "of the indicator" *read* "of the phenol."

JULY (1966) ISSUE, p. 431, 10th line under Fig. 4. *For* "for tetramethyl lead and 86 to 89 per cent. for tetramethyl lead" *read* "for tetraethyl lead and 86 to 89 per cent for tetramethyl lead."