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Summaries of Papers in This Issue

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Information on retention times, flow rates and the backflushing technique is given, together with diagrams of the valves used. For the detection of components the micro ionisation cross-section detector is used. Detector response is discussed for different components, and the results of the determination of response factors relative to nitrogen are given. The output of the detector is shown to be linear, except for hydrogen, and the repeatability of the results obtained with this apparatus is described. It is shown that a comprehensive analysis of town gas can be performed in approximately one hour, considerably faster than by conventional methods of gas analysis.

G. BLAKEMORE and G. E. HILLMAN

Central Laboratory, West Midlands Gas Board, Nechells, Birmingham 7.

Analyst, 1965, **90**, 703–714.

Manual and Semi-automatic Methods for the Determination of the Lead Content of Urine

Two methods for the determination of the lead content of urine are described. The first stage in each method is the destruction of organic matter by digestion with nitric acid; this is followed by dry ashing at 500° C and the preparation of a solution of the ash. In the first method the lead content of the ash solution is determined manually by a single-extraction, mixed-colour technique with dithizone as the colorimetric reagent. In the second method the lead content is determined automatically under similar, but not identical, conditions. A Technicon AutoAnalyzer, with an extraction coil and an optical cell that are specially designed for the purpose, is used for the automatic lead determination. The conditions of analysis are designed to minimise interference from bismuth, and it is shown that the concentrations of bismuth likely to occur in urine do not cause any significant error in the determination of lead. Concentrations of calcium, magnesium and phosphate considerably in excess of those normally present in urine can also be tolerated. Both methods are suitable for routine use, being relatively quick and easy to perform.

E. V. BROWETT and R. MOSS

The Associated Octel Co. Ltd., Ellesmere Port, Cheshire.

Analyst, 1965, **90**, 715–726.

The Determination of Oxygen in Sodium

A method has been developed for the determination of oxygen in sodium in the range 0 to 100 p.p.m. Sodium is removed by distillation and the oxygen is determined by titration with sulphuric acid. The coefficient of variation for the range 0 to 100 p.p.m. of oxygen is 10. The effect of titratable impurities associated with sodium oxide in the distillation residue has been examined. The possible loss of oxygen as carbon monoxide from the reaction between sodium oxide and carbon has been investigated and found to be negligible.

J. A. J. WALKER, E. D. FRANCE and W. T. EDWARDS

United Kingdom Atomic Energy Authority, Reactor Materials Laboratory, Culcheth, Warrington, Lancashire.

Analyst, 1965, **90**, 727–731.

The Rapid Dissolution of Plutonium Dioxide by a Sodium Peroxide Sinter, Followed by Determination of the Plutonium Content by Differential Spectrophotometry

A method is described for the dissolution of plutonium dioxide and the determination of the plutonium content by differential spectrophotometry. The plutonium dioxide is brought into solution by mixing with excess of sodium peroxide and heating at 400° C for 10 minutes, followed by the addition of a similar amount of sodium hydroxide and re-heating for a further 10 minutes. The cold sinter is then extracted with water and transferred to hydrochloric acid solution containing hydroxylammonium chloride. The solution is heated to decompose the peroxide and reduce the plutonium to the tervalent state. The plutonium concentration is then determined by differential spectrophotometry in 4-cm cells at 565 mμ, comparing with standards prepared from plutonium metal. Mean recoveries on 100-mg amounts of plutonium dioxide that had been ignited at 850° C were 99·8 per cent. with a coefficient of variation of 0·3 per cent. Similar results were obtained on plutonium dioxide that had been ignited to 1550° C, by slightly extending the sintering time.

G. W. C. MILNER, D. CROSSLEY, I. G. JONES and G. PHILLIPS

Analytical Chemistry Branch, Atomic Energy Research Establishment, Harwell, Nr. Didcot, Berkshire.

Analyst, 1965, **90**, 732–735.

An Automated Micro Determination of Blood-Glucose with the AutoAnalyzer

A glucose oxidase technique is described for the determination of blood-glucose with the AutoAnalyzer. A recorder deflection of 90 to 100 transmission lines is possible for a glucose concentration of 12·5 mg per 100 ml. Only 0·025 ml of blood is required for the determination of the glucose in the range 0 to 500 mg per 100 ml, and a sufficient volume of the diluted sample remains for a repeat analysis if required. Sixty solutions can be analysed in one hour. The cost of the reagents is one penny for every three samples analysed.

D. E. FAULKNER

Biological Control Laboratories, Wellcome Foundation, Acacia Hall, Dartford, Kent.

Analyst, 1965, **90**, 736–744.

Iodimetric and Iodatometric Determination of Thiocarbonate Sulphur

Oxidimetric methods for determining the strength of aqueous potassium thiocarbonate solution by use of iodine, potassium iodate, and iodic acid are discussed. The iodimetric method is based on the interaction of potassium thiocarbonate with a known excess of iodine in the presence of 0·5 N to N hydrochloric acid, and a back-titration of the unreacted iodine with a standard solution of sodium thiosulphate, with starch or carbon tetrachloride as indicator. The amount of sulphur undergoing oxidation corresponds to one of the sulphur atoms of tri-thiocarbonate, while the other two sulphur atoms form carbon disulphide. Satisfactory results have been recorded by taking an aliquot volume of potassium thiocarbonate solution, so that the oxidisable sulphur is only 2 to 8 mg. Greater amounts of sulphur affect the efficiency of the end-point owing to the trapping of iodine by a coherent film of sulphur. Alternatively, titration of potassium thiocarbonate with potassium iodate under similar conditions gives a similar reaction which also results in the oxidation of only one-third of the total sulphur. However, iodic acid is found to oxidise all three sulphur atoms of thiocarbonate, so affording a differentiation of thiocarbonate sulphur from sulphide sulphur and establishing the molar relationship: $HIO_3 \equiv K_2CS_3 \equiv 3H_2S$.

K. N. JOHRI and KIRPAL SINGH

Department of Chemistry, University of Delhi, Delhi 7, India.

Analyst, 1965, **90**, 745–749.

Methods for Micro and Semi-Micro Determination of Thiamine in Pharmaceutical Preparations

Methods are described for the semi-micro titrimetric determination, and micro-colorimetric determination of thiamine in pure solutions and in mixtures of pharmaceutical preparations.

They depend on precipitation of thiamine with a special iodobismuthic acid reagent, and determination of the iodine content of the complex by direct titration with *N*-bromosuccinimide, or the bismuth content of the complex with EDTA, or dissolution of the complex in potassium iodide - acetone mixture and measurement of the red colour of the solution at 345 mμ, or in a photoelectric colorimeter with a violet filter.

The methods have been applied to different forms of thiamine-containing preparations, and the results are compared with the official gravimetric silicotungstic acid method and the fluorimetric method.

The average mean error for the semi-micro method is $\pm 0{\cdot}5$ per cent. for amounts of thiamine from 1 to 10 mg, and for the micro method the error is about $\pm 1{\cdot}7$ per cent. for amounts of thiamine ranging from 5 to 150 μg.

F. SAID, M. M. AMER, K. N. GIRGIS and Z. A. GEORGE

Analytical Chemistry Department, Faculty of Pharmacy, Cairo University, Cairo, U.A.R.

Analyst, 1965, **90**, 750–755.

The Spectrophotometric Determination of Manganese after Oxidation with Sodium Perxenate

Short Paper

R. W. BANE

Argonne National Laboratory, Argonne, Illinois, U.S.A.

Analyst, 1965, **90**, 756–758.

Anthrone as a Reagent for Determining Carbohydrate in Rats' Milk and Related Materials

Short Paper

ADELE MITTWOCH

Human Nutrition Research Unit, National Institute for Medical Research, The Ridgeway, London, N.W.7.

Analyst, 1965, **90**, 759–762.

Notice to Authors

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Intending authors should consult the current Notice to Authors, last published in full in *The Analyst*, **1965**, **90**, 249, reprints of which can be obtained on application to The Editor, *The Analyst*, 14, Belgrave Square, London, S.W.1. All papers submitted will be expected to conform to the recommendations there laid down, and any that do not may be returned for amendment.

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Comprehensive Fuel-gas Analysis by Gas Chromatography

BY G. BLAKEMORE AND G. E. HILLMAN

(Central Laboratory, West Midlands Gas Board, Nechells, Birmingham 7)

An apparatus is described, suitable for the comprehensive routine analysis of town gas of widely varying composition. The apparatus incorporates 5 columns, which are all used at the same temperature. The columns, together with the components eluted, are (*i*) a molecular sieve for the elution of permanent gases excepting hydrogen, (*ii*) silica gel for elution of carbon dioxide and C_2 hydrocarbons, (*iii*) deactivated alumina for the elution of C_2 to C_5 hydrocarbons, (*iv*) deactivated alumina for the elution of C_6 hydrocarbons (if required), benzene and toluene and (*v*) a molecular sieve for the elution of hydrogen only.

Information on retention times, flow rates and the backflushing technique is given, together with diagrams of the valves used. For the detection of components the micro ionisation cross-section detector is used. Detector response is discussed for different components, and the results of the determination of response factors relative to nitrogen are given. The output of the detector is shown to be linear, except for hydrogen, and the repeatability of the results obtained with this apparatus is described. It is shown that a comprehensive analysis of town gas can be performed in approximately one hour, considerably faster than by conventional methods of gas analysis.

THE almost complete revolution in gas-making processes during the past 7 years has had a profound effect on the requirements for gas analysis. With conventional plant making coal gas, and carburetted water-gas from gas oil, the Bone and Wheeler or the Gooderham soap-film apparatus was considered suitable. Hydrocarbons of lower molecular weight were mainly confined to C_1 and C_2 saturated and unsaturated compounds, and were readily determined by the chemical absorption and combustion methods of such apparatus. With the advent of light-petroleum distillate for gas making, the limitations of this type of apparatus immediately became apparent, since much larger concentrations of C_3 to C_6 hydrocarbons are present in some of the gases produced, *e.g.*, carburetted water-gas. Determination of these hydrocarbons was uncertain, results were variable, and, accordingly, other methods of analysis were investigated. Methods based on infrared spectroscopy and mass spectrometry were satisfactory, but the high cost of the apparatus precluded their use for most laboratories.

The technique of gas chromatography was developing rapidly at about this time and some workers turned to this for the solution to the problems of hydrocarbon analysis.[1,2] Methods of gas analysis by chromatography are now well established, but so far there has been no method described whereby an analysis of town gas may be carried out for the constituents present in concentrations of 0·01 per cent. v/v and above, without recourse to the use of several columns, different column temperatures and one or more detectors.

In the West Midlands Gas Board area, gas may be produced by as many as eight different methods of manufacture. Although the physical characteristics of the gas will remain essentially constant, the composition may vary widely and consequently, a versatile apparatus is required for analyses. A Janák-type apparatus[3] has been in use for some time now for routine works analyses. This apparatus gives sufficient information with regard to the major constituents present, but is not sensitive enough to permit the determination of components such as acetylene, butadiene, benzene, cyclopentadiene, the knowledge of the concentrations of which is essential in the evaluation of plant performance, particularly with the modern processes used. Experiments with the improved detector for the Janák apparatus described by Brown and Satsmadjis[4] were not entirely satisfactory and other detectors were therefore investigated. Katharometers and thermistors were rejected after investigation because of the need for rigorous control of temperature and carrier-gas flow, and also for calibration over a wide range of composition, thus making the use of standard

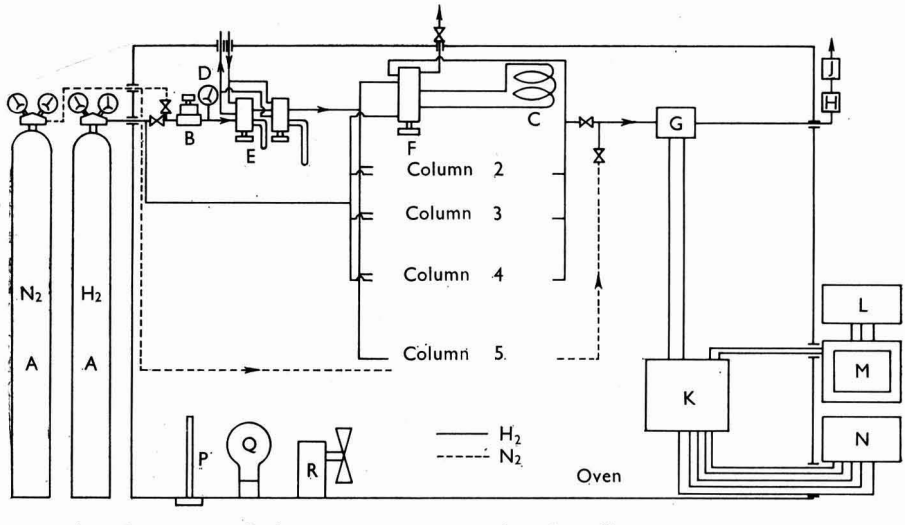
gases mandatory. The ionisation cross-section detector[5,6] appeared to be the most promising, and an apparatus has been described incorporating this detector.[7] In 1963 Lovelock, Shoemake and Zlatkis[8] published details of the micro ionisation cross-section detector, which they claimed was more sensitive than the previous detector by a factor of 10, and this paper describes the results achieved with this detector. We thought that it would be beneficial if the apparatus could be operated isothermally, and, because this was not possible with partition columns, the use of adsorption columns as described by Scott[2,9] was investigated.

Method

Apparatus—

The apparatus consists of the units shown in Fig. 1. All electronic units are designed for installation in standard 19-inch racks. Since the apparatus is intended for continuous use it is desirable that the analysis time be as short as possible. Accordingly, a duplicate oven has been constructed with its own d.c. amplifier. The stabilised power-supply unit has consequently been designed to supply two amplifiers.

Oven—The oven (18 × 12 × 12 inches) is made of sheet aluminium with Widney Dorlec angle brackets and ends, and is insulated internally with ½-inch expanded-polystyrene sheet. The thermostat unit is a Sunvic controller type TS3, and the oven is heated by a 40-watt bulb with a small fan for air circulation.



A = Carrier-gas cylinders
B = Carrier-gas control valve
C = Columns
D = Pressure gauge
E = Sample-injection valves
F = Backflush valves
G = Detector
H = Molecular-sieve filter
J = Soap-film meter
K = Amplifier
L = Integrator
M = Recorder
N = Stabilised power-supply unit
P = Thermostat
Q = 40-watt bulb
R = Fan motor

Fig. 1. Diagram of the apparatus

Carrier-gas flow regulators—

The carrier-gas flow is controlled by Negretti and Zambra flow regulators type R182, the inlet column pressure being measured by a standard 0 to 30 p.s.i. gauge. Sample injection is by two push-type 6-port gas-sample injection valves with O-ring seals. These have been constructed at this laboratory and are based on a design by Pratt and Purnell.[10] A diagram of this valve is shown in Fig. 2 (*a*). Sample loops of volume 0·462 ml and 3·62 ml are connected to these valves and the appropriate loop may be selected as required. The backflush valves are of a similar type, with suitably modified ports, and are shown in Fig. 2 (*b*).

Columns—These are made from ¼-inch o.d. annealed copper tubing of various lengths and have packings as described below. All flow lines are of ⅛-inch o.d. annealed copper

tubing, and connections are made with "Enots" compression fittings (obtainable from Benton & Stone Ltd., Aston Brook Street, Birmingham, 6). The micro ionisation cross-section detector[8] used is shown in Fig. 3 and is constructed of brass instead of stainless steel. The radioactive source used is the standard-tritium target type TRT2 obtainable from the Radiochemical Centre, Amersham. This is safe once inside the detector, but should be handled with forceps if removed for cleaning. Before using these targets the Ministry of Housing and Local Government and the Factory Inspectorate should be notified, and registration under the Radioactive Substances Act (1960) obtained. Tritium targets are not classified as dangerous but the Act requires those using radioactive substances to be registered.

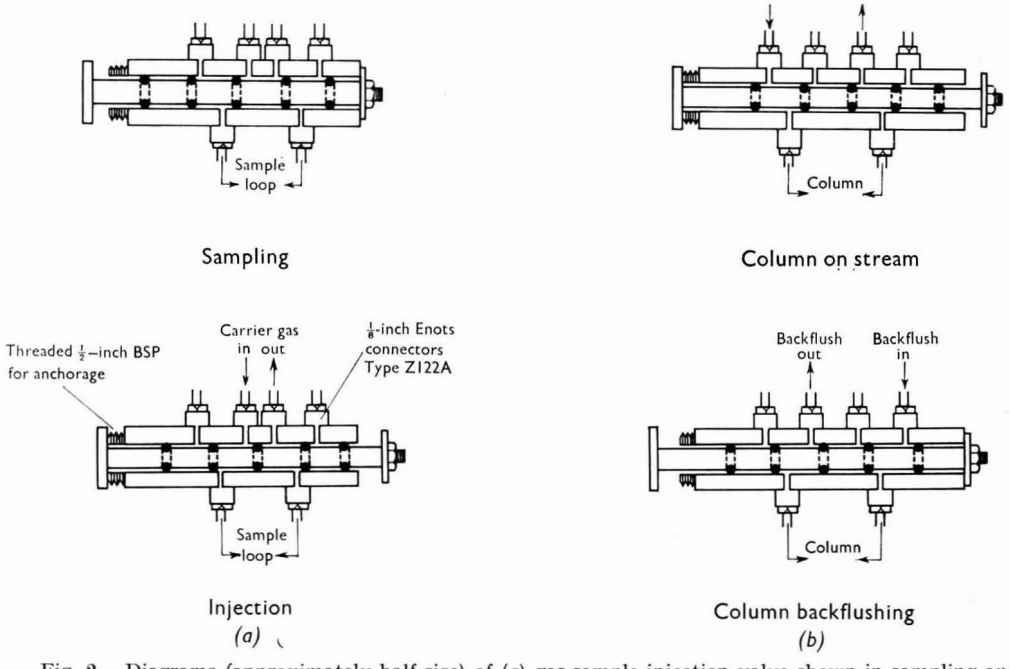


Fig. 2. Diagrams (approximately half size) of (*a*) gas-sample injection valve shown in sampling and injection positions and (*b*) backflush valve shown in column-on-stream and backflushing positions

To prevent stray signals from entering the detector, it is contained within an earthed brass cylinder 3 inches in length. With a 10^9-ohm resistor in the amplifier it is possible to measure currents of the order of 3×10^{-12} amp, and it is most important that stray signals be prevented from entering the input circuit of the amplifier. Initially, interference was caused by the

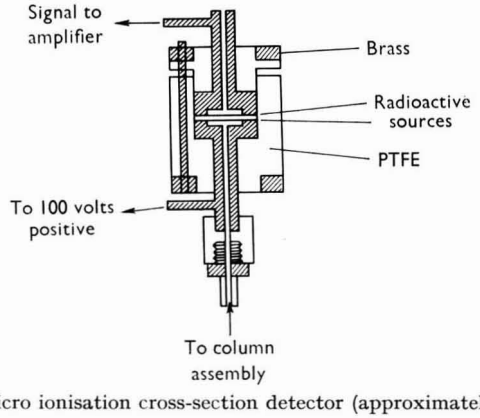


Fig. 3. Micro ionisation cross-section detector (approximately half size)

operation of the thermostat, and this was effectively prevented by decoupling all mains leads and thermostat points with 0·02-μF 300-volt a.c. working condensers.

A molecular-sieve filter is connected to the outlet of the detector to prevent ingress of moisture and consequent rapid deterioration of the columns. The carrier-gas flow, if required, may be measured at the outlet of the filter with a soap-film meter.

D.C. amplifier—This is shown in Fig. 5, and is housed in the oven in its own carefully screened compartment. It was designed originally by Thomson, of the Dyson Perrins Laboratory, Oxford, and has been suitably modified to enable the backing-off voltage and the polarising voltage for the detector to be obtained from the power supply. The construction of the amplifier is not critical, but the input circuit is at high impedance, so that all leads should be screened where necessary and kept as short as possible. It is most essential that a constant-voltage heater supply be used.

Recorder—The Kent Multilec Mark III recorder has six sensitivity settings (1 to 50 mV), and has been modified to read zero at 10 per cent. of full-scale deflection. The different sensitivities can, of course, be incorporated in the d.c. amplifier if a recorder of only 1 mV f.s.d. is used.

Stabilised power-supply unit—The stabilised H.T. supply[11] and L.T. supply[12] will give 20 mA at 200 volts (±0·1 per cent.) and 1 amp at 12 volts (±1·0 per cent.), respectively. The construction of this unit is not critical provided conventional practice is followed. The OC36 transistor must be mounted on an insulated heat sink, which should be sited clear of any hot-running components. Component details and values are given in the Appendix, p. 713, and the circuit diagram is shown in Fig. 4.

Integrator—This is designed by I. Stansfield, of the Central Laboratory, South Western Gas Board, Bristol, and is at present the subject of a patent application; it is therefore not possible to describe it. The integrator has a linear count rate from 10 per cent. to 100 per cent. of the full-scale deflection of the recorder and a maximum count rate of 3000 per minute.

COLUMNS—

The columns used, together with the components eluted, are cited below.

Column 1—This consists of 6 feet of Linde 13X molecular-sieve type (40–60 mesh) and is used for the elution of oxygen and argon, nitrogen, methane, and carbon monoxide. (It is most important that type 13X molecular sieve is used as isobutane may be eluted and interfere with any of the permanent gases if type 5A is used.) Argon and oxygen are eluted as a composite peak. In any particular fuel gas the argon to nitrogen ratio is approximately constant and thus the argon can be easily calculated, although this may lead to errors of up to 0·05 per cent. Should the percentage of the two gases be required individually, then oxygen may be removed with chromous chloride solution, enabling argon to be determined alone.

Column 2—This consists of 10 feet of Peter Spence type H alumina, (100–120 mesh), deactivated with 20 per cent. w/w of sodium hydroxide. It permits the elution of ethane, ethylene, propane, propene, acetylene, isobutane, butane, *trans*-but-2-ene, isobutene/but-1-ene, *cis*-but-2-ene, isopentane, 1,3-butadiene and pentane, in that order. A typical chromatogram from this column is shown in Fig. 6.

Column 3—This consists of 6 feet of silica gel (40–60 mesh) and is used for the elution of ethane, carbon dioxide and ethylene.

Column 4—This consists of 12 inches of Peter Spence type H alumina, (100–120 mesh), deactivated with 40 per cent. w/w sodium hydroxide and is used for the elution of benzene and toluene.

Column 5—This consists of 6 feet of Linde molecular-sieve type 13X (40–60 mesh) and is used for elution of hydrogen only.

Column Technique—To ensure maximum sensitivity hydrogen is used as the carrier gas for elution of all components except hydrogen, for which nitrogen is used.

The carrier-gas flow is 30 ml per minute through all columns, measured at the outlet of the detector, and the column temperature is 32° C ± 0·2° C. Peaks on columns 2, 3 and 4 are symmetrical, but it must be emphasised that the alumina-deactivating technique of Scott[9] should be strictly followed, otherwise some asymetry will occur. Peaks on column 1

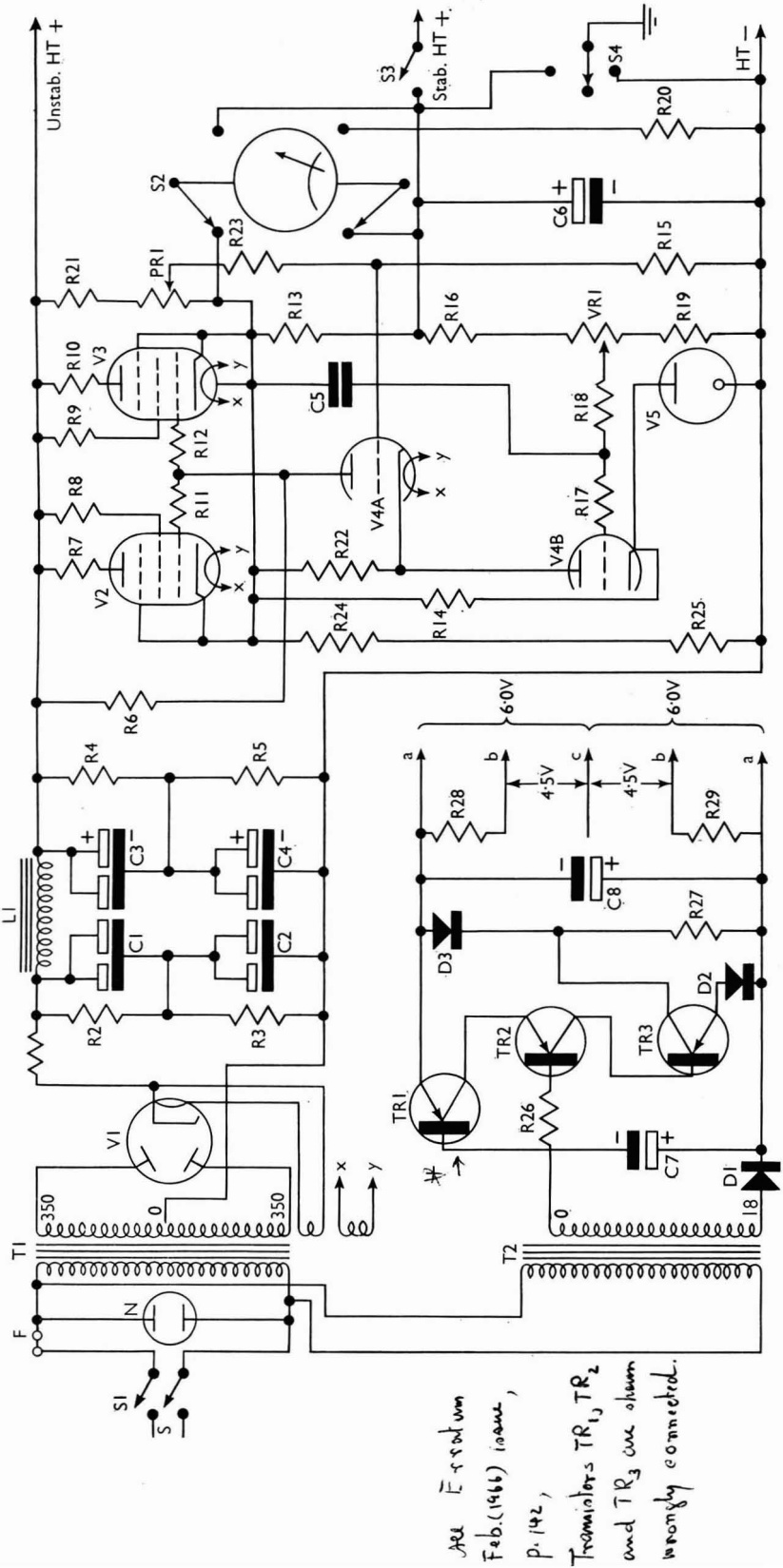


Fig. 4. Circuit for stabilised power-supply unit for d.c. amplifiers (For list of components, see Appendix, p. 713)

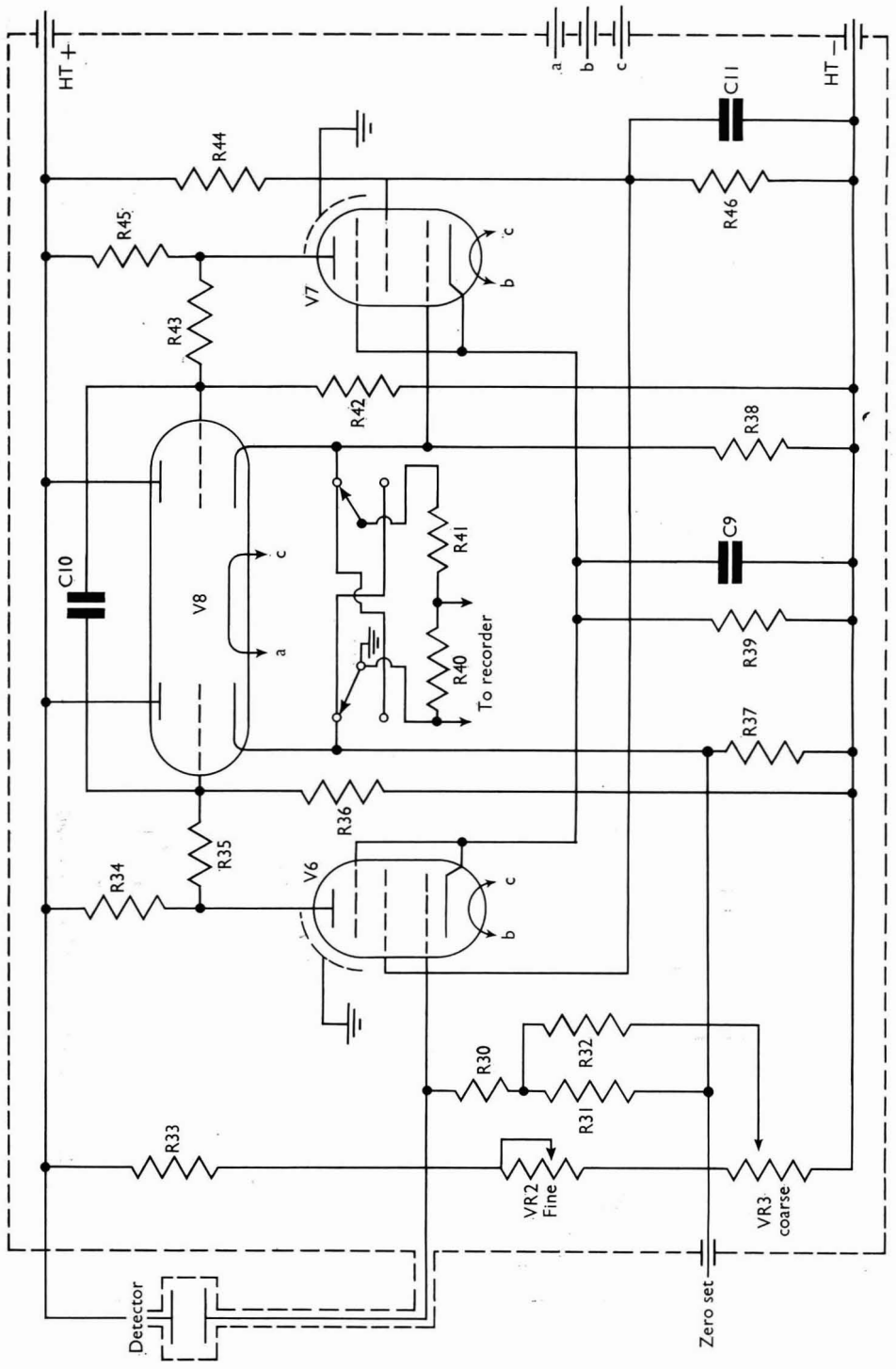


Fig. 5. Circuit for d.c. amplifier (For list of components, see Appendix, p. 713)

TABLE I

RETENTION TIMES OF COMPONENTS

Column 1		Column 2		Column 3	
Component	Time, minutes	Component	Time, minutes	Component	Time, minutes
Oxygen and argon	3·5	Propane	2·3	Ethane	4·5
Nitrogen	6·0	Propene ..	3·3	Carbon dioxide ..	6·0
Methane	8·5	Acetylene ..	3·9	Ethylene ..	7·5
Carbon monoxide	12·0	Isobutane ..	4·7		
		Butane ..	5·3		
		Isobutene ..	8·5		
		Pentane ..	14·6		

Column 4		Column 5	
Component	Time, minutes	Component	Time, minutes
Benzene	8	Hydrogen ..	3
Toluene	24		

are to some extent asymetric, but as an integrator is used, this is not inconvenient. All columns are backflushed with carrier gas (30 ml per minute) when not in use, thus removing heavier components that would interfere with subsequent analyses.

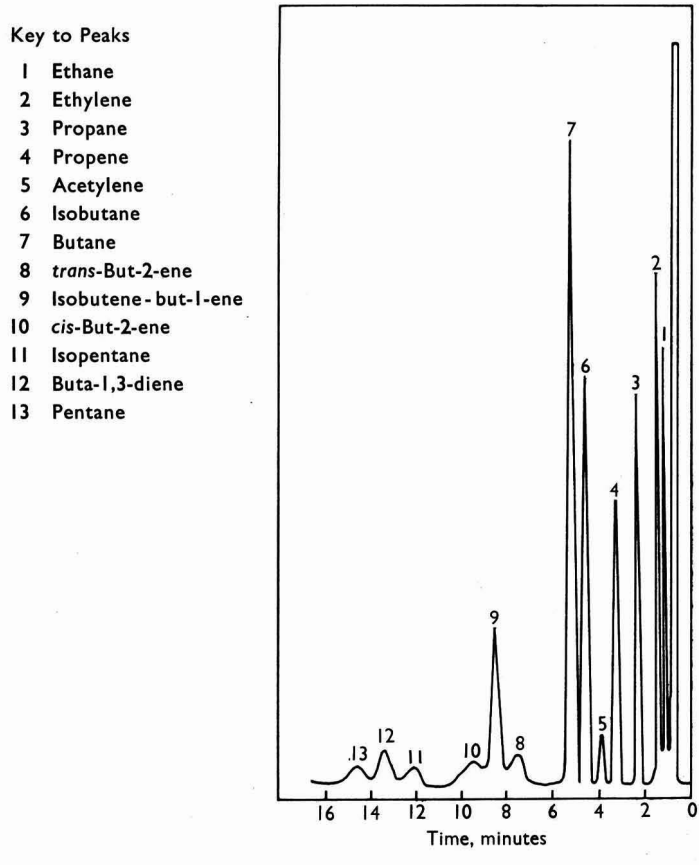


Fig. 6. Chromatogram of elution of C_1 to C_5 hydrocarbons on de-activated alumina

PROCEDURE—

Switch the apparatus on and allow it to warm up, half-an-hour usually being sufficient to bring the apparatus to the required temperature of 32° C. Select the appropriate column by pushing in the requisite backflush valve, and adjust the carrier-gas flow by means of the controller to 30 ml per minute. Adjust the sensitivity of the recorder, which must be found by experiment. Adjust the base-line of the chromatogram to 10 per cent. of f.s.d. by means of the coarse and fine controls on the amplifier. The sample loops used on this particular apparatus are 0·462 ml and 3·62 ml and all gases are sampled at atmospheric pressure. The size of the loop used depends on the type of sample being analysed. In general, the 3·62-ml loop is used for all gases except refinery gases and hydrogen. The volumes stated above have been measured carefully and include the dead space in the sample-injection valve. To inject the sample, push the sample valve in, leave for 30 seconds, and then return the plunger to its original position. Note the integrator count after each peak has emerged. When all the components have been eluted, pull the plunger of the backflush valve out, thereby reversing the carrier-gas flow on that particular column. Select the next column by pushing its backflush valve in, and repeat the procedure as above.

Determine the concentration of each component by means of the equation—

$$\text{Concentration, per cent.} = \frac{AD}{BF} \text{ v/v}$$

where A = the integrator count for the component, F = the response factor relative to nitrogen, and B = integrator count for 1 per cent. v/v of nitrogen (determined previously with a standard gas) and D is the recorder sensitivity factor.

Determine the hydrogen concentration by comparison with a standard of known hydrogen concentration, injecting the sample of standard gas by the procedure described above.

The time taken to determine permanent gases, C_2 to C_5 hydrocarbons and benzene is approximately 60 minutes. This time can be reduced to 45 minutes if two recorders and two amplifiers are used. The power-supply unit has been designed to supply two amplifiers with this time reduction in mind.

If the apparatus is not in continuous use, pass carrier gas at about 10 ml per minute through all columns with the backflush valves in the backflush position, thereby maintaining carrier gas in the columns.

RESULTS

The accuracy of the results obtained is necessarily dependent on (*a*) the linearity and response of the detector; (*b*) the errors due to sample injection, temperature and pressure variations of the columns, and variations in carrier-gas flow, and (*c*) the errors associated with the electronic parts of the apparatus, *i.e.*, the amplifier, the integrator, etc.

The errors listed in (*c*) have been minimised by careful attention to design.

Of the errors under (*b*), those derived from sample injection are very small because of the excellent design of the valve, the sample repeatability being better than $\pm 0{\cdot}5$ per cent. Temperature variations are negligible because of the thermostatic control, as are the variations of carrier-gas flow with this type of regulator. It may be noted that no correction is made for variation in pressure. Careful consideration was given to this point, but it was found to be unnecessary for routine estimations. Undoubtedly errors could be reduced at the expense of the time involved in applying the appropriate correction to each component. The standard deviations given below were determined without measuring the pressure, and the errors obtained were considered to be satisfactory for routine analysis.

Linearity—Fig. 7 shows graphs of the linearity of the detector response to nitrogen and butane. It can be seen that over the range of sample size used, the detector response is linear. Linearity for all the components determined by this apparatus has been measured and, with the exception of hydrogen, has been found to be excellent. If small samples of hydrogen are used, *e.g.*, below 0·7 ml, linearity is good. The non-linearity with large samples probably arises from the fact that nitrogen is used as a carrier gas, and with an electrode separation of 2 mm, the detector is being used at the limit consistent with linearity.

TABLE II

DETECTOR PROPERTIES

Ionisation efficiency	3×10^{-8}
Noise level	3×10^{-13} amp
Background current	6×10^{-9} amp
Minimum detectable concentration at a carrier-gas flow rate of 30 ml per minute (butane as test gas)	$1{\cdot}0 \times 10^{-5}$ ml
Carrier gas	Hydrogen
Detector volume	0·08 ml
Detector time constant	0·2 seconds

Detector sensitivity—The properties of the detector are given in Table II. These measurements were determined with a polarising voltage of 100 volts and a carrier-gas flow of 30 ml per minute, the detector and the column being held at 32° C ± 0·2° C. The detector is not operated at its maximum sensitivity because to do this would require unrealistic carrier-gas flow rates, which would lead to excessive retention times. It is therefore desirable to sacrifice sensitivity for convenient operation.

Variation of carrier-gas flow rate in the range 10 to 70 ml per minute had little or no effect on the standing current of the detector, *i.e.*, the base-line of the recorder, but large variations did alter the base-line somewhat. Polarising voltages of 90 to 300 volts had no effect on either the standing current or the output of the detector, showing that all charges produced were being collected.

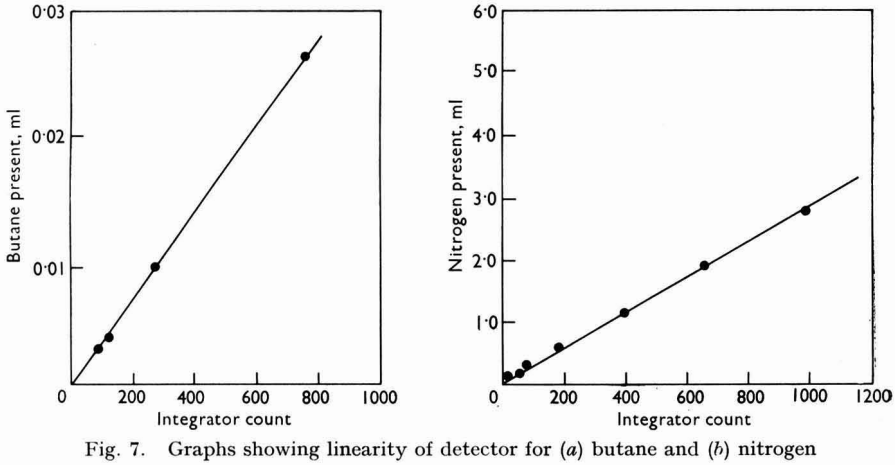


Fig. 7. Graphs showing linearity of detector for (*a*) butane and (*b*) nitrogen

Detector response—Lovelock *et al.*[5,8] state that the response of the detector is predictable from knowledge of the ionisation cross-section and the molecular weight of the substance involved; we have not found this. Boer,[5] when using the macro ionisation detector, found some deviation for compounds of molecular weight below 60.

TABLE III

RESPONSE FACTORS RELATIVE TO NITROGEN

Component	Observed factor	Calculated factor
Oxygen	1·31	1·62
Nitrogen	1·00	1·00
Carbon monoxide	1·09	1·42
Carbon dioxide	1·87	2·46
Methane	1·08	1·29
Ethane	2·15	2·58
Propane	3·98	3·88
Butane	5·55	5·18
Acetylene	1·45	1·69
Ethylene	1·73	2·13
Propene	3·78	3·43
Isobutene	2·90	4·74
Benzene	5·08	5·92

Table III shows the response factors relative to nitrogen compared with those calculated from the ionisation cross-section of the component and carrier gas.

It can be seen that agreement is not good and the difference is far greater than would be caused by experimental error in the measurements. The calculated values in Table III are obtained from the values quoted by Lovelock *et al.*[8] for the ionisation cross-section for tritium radiation. It appears, therefore, that for compounds of lower molecular weight, the relationship is approximate only and more information is required about the ionisation cross-section for tritium radiation, before the relationship can be established with any certainty. Nevertheless, this does not detract from the fact that detector response is constant. These factors have been determined a number of times during the past 6 months, and, although change has occurred in response, it is random and within the limits of experimental error, and it may be inferred that the detector does not age rapidly. It is possible that over a period of years the response of the detector will diminish because of the decrease in the emission of β-radiation from the tritium source (the half-life of tritium is 12·5 years), and to this end response factors should be checked occasionally so that the sources may be replaced when necessary.

Repeatability of the apparatus—

Table IV shows the repeatability of the apparatus with standard gases that had been analysed by mass spectrometry. In Table IV, σ is the standard deviation obtained from the equation

$$\sigma = \sqrt{\frac{\Sigma x^2 - \frac{(\Sigma x)^2}{n}}{n-1}}$$

where x = integrator count and n = number of determinations, and is the 66 per cent. confidence limit. The t factor, which reflects the number of degrees of freedom, is 2·2 and $2{\cdot}2\sigma$ is the 95 per cent. confidence limit.

Table IV

The repeatability of the apparatus

Component	Mean number of integrator counts	Concentration of component in standard gas, per cent. v/v	$2{\cdot}2\sigma$	$2{\cdot}2\sigma$ as per cent. of mean	$2{\cdot}2\sigma$ as per cent. v/v
Oxygen	14·7	0·06	1·8*	12	±0·012
Nitrogen	437	13·3	5·3	1·2	±0·16
Methane	458	12·7	5·1	1·1	±0·14
Carbon monoxide	783	21·3	8·8	1·1	±0·23
Carbon dioxide	1856	5·7	42	2·3	±0·13
Hydrogen	470	47·1	6·3	1·3	±0·61
Ethane	162	2·24	8	4·9	±0·10
Ethylene	226	3·83	12	5·3	±0·20
Propane	198	0·30	5·5	2·8	±0·008
Propene	184	0·26	7·0	3·8	±0·010
Acetylene	42	0·18	5·7*	13·5	±0·024
Isobutane	362	0·41	10·5	2·9	±0·012
Butane	653	0·73	19·0	2·9	±0·021
Isobutene	246	0·53	18·3	7·4	±0·040

* $2{\cdot}2\sigma$ is high because of the small volume of the component present.

For the determination of the standard deviation, at least 16 samples were analysed for each of the components stated. The column headed "$2{\cdot}2\sigma$ as per cent. v/v" shows the repeatability as a percentage of composition. It can be seen that the repeatability is generally better than 5 per cent. of the amount present, and sometimes better than 2 per cent. Repeatability is emphasised because standard gas mixtures are extremely difficult to prepare with accuracy and no facilities were available for making such mixtures. It is probable that the error involved on this apparatus is of the same order as the repeatability and should not be greater.

Conclusions—

The apparatus is suitable for the comprehensive analysis of town and other fuel gases of a widely varying nature in a reasonable time. It has been in use almost continuously

for 12 months, analysing approximately 25 samples per week, and it is expected that during a normal working day 6 gases could be analysed with ease. Routine maintenance is negligible and, during the time of operation, only the molecular-sieve column has been changed, owing to the apparatus being left open to the atmosphere during a holiday period and consequent deactivation of the molecular sieve. Carbon dioxide is tenaciously retained on both molecular sieve and alumina, and it was thought that this might affect the column performance, but there has been no evidence of this to date, so it would appear that the backflushing technique is adequate. Day-to-day column variations are slight and are within experimental error as shown in Table IV. Air, or the standard hydrogen and nitrogen mixture, is used for checking the detector response to nitrogen. A nitrogen and hydrogen mixture can be easily prepared and accurately analysed on conventional gas-analysis apparatus. The total of all the constituents determined should approximate to 100 per cent. In practice, totals of between 98 and 102 per cent., often between 99 and 101 per cent., are obtained. Errors are largest when there is a large amount of one component in the gas sample. In all cases the results are normalised, that is, corrected to a total of 100 per cent.

The apparatus is eminently suitable for routine applications, and the cost of the components is not excessive, being approximately £500, inclusive of the recorder. Rigidly controlled conditions are not required, and all components are determined at slightly above ambient temperature. The apparatus therefore possesses certain features that are desirable, but not always obtainable in a commercial chromatograph except at great expense.

We thank the Chairman of the West Midlands Gas Board for permission to publish this paper, and are grateful to Data Publications Limited for permission to reproduce the circuit diagrams of the stabilised power-supply unit.

We also thank Mr. T. A. Dick, Area Chief Chemist, West Midlands Gas Board, for his help and encouragement, and all our colleagues who helped in the constructional and analytical work, especially Mr. T. E. Maltby, without whose help the construction of this apparatus would not have been possible.

Appendix

List of components used in the electronic units

All resistors are ½ watt 20 per cent. tolerance and all capacitors are 500 volt d.c. working unless otherwise stated.

Stabilised power-supply unit—

R_1	=	47-ohm 3-watt resistor
R_2, R_3, R_4, R_5	=	100,000-ohm 1-watt resistors
R_6	=	1-megohm 5 per cent. high-stability resistor
R_7, R_{10}	=	10-ohm resistors
R_8, R_9	=	68-ohm resistors
R_{11}, R_{12}	=	1000-ohm resistors
R_{13}	=	0·5-ohm 1-watt resistor
R_{14}	=	47,000-ohm 2-watt resistor
R_{15}	=	150,000-ohm 5 per cent. high-stability resistor
R_{16}	=	56,000-ohm 1-watt 5 per cent. resistor
R_{17}	=	1000-ohm resistor
R_{18}	=	100,000-ohm resistor
R_{19}	=	22,000-ohm 1-watt resistor
R_{20}	=	500,000-ohm 1-watt 5 per cent. resistor
R_{21}, R_{22}	=	220,000-ohm 5 per cent. high-stability resistors
R_{23}	=	100,000-ohm 5 per cent. high-stability resistor
R_{24}	=	22,000-ohm resistor
R_{25}	=	47,000-ohm 1-watt resistor
R_{26}	=	10,000-ohm resistor
R_{27}	=	3300-ohm 1-watt resistor
R_{28}, R_{29}	=	12-ohm 2-watt resistors
VR_1	=	25,000-ohm 1-watt wire-wound resistor
PR_1	=	100,000-ohm carbon pre-set resistor
C_1, C_2, C_3, C_4	=	32-μF capacitors
C_5	=	0·05-μF capacitor
C_6	=	8-μF capacitor
C_7	=	2000-μF 15-volt working capacitor
C_8	=	3000-μF 15-volt working capacitor
V_1	=	GZ34 valve
V_2, V_3	=	EL81 valves

V_4 = ECC81 valve
V_5 = 85A2 valve
TR_1 = OC36 transistor
TR_2 = OC72 transistor
TR_3 = OC71 transistor
D_1 = TM64 or BYZ 13
D_2, D_3 = 6·3V Zenerdiodes, OAZ290
T_1 = Mains transformer 0–350V primary, 350–0–350V secondary at 180 mA, 5V at 3 amp and 6·3V at 4 amp
T_2 = Heater transformer 0–230V primary, 0–18V secondary at 3 amp
L_1 = Smoothing choke 10H 180 mA d.c.
S_1, S_2 = Double-pole double-throw switches
S_3 = Single-pole double-throw switch
S_4 = Single-pole 3-way switch
N = Neon bulb
F = 3-amp cartridge fuse

D.C. amplifier—

R_{30} = 1000-megohm resistor.
R_{31} = 1-megohm 5 per cent. high-stability resistor
R_{32} = 3·9-megohm 5 per cent. high-stability resistor
R_{33} = 100,000-ohm 5 per cent. high-stability resistor
R_{34} = 220,000-ohm 5 per cent. high-stability resistor
R_{35} = 820,000-ohm 5 per cent. high-stability resistor
R_{36} = 1-megohm 5 per cent. high-stability resistor
R_{37}, R_{38} = 20,000-ohm 5 per cent. 2-watt resistor
R_{39} = 100,000-ohm 5 per cent. high-stability resistor
R_{40} = 10-ohm 1 per cent. resistor
R_{41} = 1800-ohm 1 per cent. resistor
R_{42} = 1-megohm 5 per cent. high-stability resistor
R_{43} = 820,000-ohm 5 per cent. high-stability resistor
R_{44} = 150,000-ohm 5 per cent. high-stability resistor
R_{45} = 220,000-ohm 5 per cent. high-stability resistor
R_{46} = 800,000-ohm 5 per cent. high-stability resistor
VR_2 = 1000-ohm wire-wound resistor
VR_3 = 100,000-ohm wire-wound resistor
C_9, C_{10}, C_{11} = 0·1-μF capacitors
V_6 = ME1400 valve
V_7 = ME1400 or EF37A valve
V_8 = 12AT7 (ECC81) valve

References

1. Boreham, G. R., and Marhof, F. A., Gas Council Research Communication GC54, 1958.
2. Scott, C. G., *J. Inst. Petrol.*, 1959, **45**, 118.
3. Blakemore, G., *Analyst*, 1962, **87**, 737.
4. Brown, G. M., and Satsmadjis, J., *Coke Gas*, 1961, 48.
5. Boer, H., *in* Desty, D. H., *Editor*, "Vapour Phase Chromatography," Butterworths, London, 1957, p. 177.
6. Lovelock, J. E., *Anal. Chem.*, 1961, **33**, 162.
7. Gardner, W. F., and Farrow, N. W., "The Development of a Gas Chromatograph for the Routine Analysis of Town Gas." Paper presented to the the London and Southern Junior Gas Association, 26th April, 1963.
8. Lovelock, J. E., Shoemake, G. R., and Zlatkis, A., *Anal. Chem.*, 1963, **35**, 460.
9. Scott, C. G., *in* van Swaay, M., *Editor*, "Gas Chromatography, 1962," Butterworths, London, 1962, p. 36.
10. Pratt, G. L., and Purnell, J. H., *Anal. Chem.*, 1960, **32**, 1213.
11. Guy, H., *Radio Constr.*, October, 1961, 206.
12. Bridge, N. W., *Radio Constr.*, June, 1962, 818.

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Manual and Semi-Automatic Methods for the Determination of the Lead Content of Urine

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Two methods for the determination of the lead content of urine are described. The first stage in each method is the destruction of organic matter by digestion with nitric acid; this is followed by dry ashing at 500° C and the preparation of a solution of the ash. In the first method the lead content of the ash solution is determined manually by a single-extraction, mixed-colour technique with dithizone as the colorimetric reagent. In the second method the lead content is determined automatically under similar, but not identical, conditions. A Technicon AutoAnalyzer, with an extraction coil and an optical cell that are specially designed for the purpose, is used for the automatic determination of lead. The conditions of analysis are designed to minimise interference from bismuth, and it is shown that the concentrations of bismuth likely to occur in urine do not cause any significant error in the determination of lead. Concentrations of calcium, magnesium and phosphate considerably in excess of those normally present in urine can also be tolerated. Both methods are suitable for routine use, being relatively quick and easy to perform.

THERE is a need for a rapid and accurate method for the determination of lead in urine in connection with the medical control of personnel engaged in industries in which metallic lead or lead compounds are processed. Published methods for the determination of lead in urine or other biological materials, such as those of Bambach and Burkey[1] and Irving and Butler,[2] involve a lead - bismuth separation prior to the colorimetric determination of lead as dithizonate. The "United States Public Health Service" method for determining lead in air and in biological materials, recently described and evaluated with particular reference to the analysis of blood samples by Kennan *et al.*,[3] involves a similarly complicated procedure. Such methods are too time-consuming to be ideal for routine application. In other methods, such as that of Cholak, Hubbard and Burkey,[4] the lead is separated by co-precipitation. This technique considerably shortens the time required to perform the analysis, but it is our experience that such techniques are unreliable as the lead is not always quantitatively removed from the urine.

Nelson and Hamm[5] point out that the state of chemical combination of lead excreted in the urine is not known, but give some evidence indicating that lead is bound as a chelate, having stability intermediate between that of lead citrate and the lead - EDTA chelate. It is considered, therefore, that the first stages in the determination of lead in urine are to destroy all the organic matter present, then to dissolve the ash to ensure that the lead present is in the inorganic state for the determination.

Thousands of determinations of lead in urine have been performed in our laboratories by a method essentially that of Bambach and Burkey,[1] but we have no evidence of bismuth ever being present in significant amounts in the urine-ash solutions. There is therefore a *prima facie* case for a simple and rapid colorimetric technique based on a single extraction procedure and use of dithizone for the determination of lead in urine-ash solutions.

However, when large numbers of urine samples are collected for lead determination, it is almost certain that some of the samples will be provided by individuals who are taking stomach powders or tablets containing bismuth compounds, without this being known to the medical examiner. It was first necessary to determine the bismuth content of such urine samples, in order to assess the potential interference due to bismuth in lead determinations based on a single extraction with dithizone.

Thirty-eight samples of urine were collected from 9 individuals known to be taking "anti-acid" stomach powders or tablets incorporating bismuth compounds. The bismuth content of each of these urine samples was determined. The urine was ashed and a solution of the ash prepared as described in Method 1 below. The bismuth content of this solution was then determined colorimetrically by means of dithizone, according to the method

described by Sandell,[6] which involves a lead - bismuth separation at pH 3·4. The highest bismuth content found was 30 μg per litre, and the mean of the 38 values obtained was 11 μg per litre.

Alternative methods for the determination of lead in urine were then developed in which these concentrations of bismuth can be tolerated, one method involving manual analysis of the ash solution and the other involving automatic analysis by means of the Technicon AutoAnalyzer. In both methods, the lead determination is preceded by digestion with nitric acid and ashing at 500° C in order to destroy the organic matter present in the urine. No attempt was made to develop an automatic procedure for this preliminary step in the analysis.

METHOD 1—MANUAL LEAD DETERMINATION

GENERAL PRECAUTIONS TO BE OBSERVED—

Pyrex glassware must be used throughout and all new items of glassware must first be treated with chromic acid. They must then be washed with nitric acid and distilled water immediately before use, on each occasion that they are used. All other precautions necessary to prevent casual contamination must be taken.

All distilled water must be essentially free from heavy metals. This may be achieved by distilling the water in an all-Pyrex apparatus, or by passing "ordinary" distilled water through a mixed-bed de-ioniser before use.

REAGENTS—

Nitric acid, concentrated—Redistil AnalaR nitric acid in an all-Pyrex still; discard the first 10 per cent. of distillate and leave 10 per cent. undistilled.

Nitric acid, diluted (1 + 19)—Dilute 50 ml of redistilled nitric acid to 1 litre with distilled water.

Nitric acid, dilute (1 + 99)—Dilute 200 ml of diluted nitric acid (1 + 19) to 1 litre with distilled water.

Ammonia solution, sp.gr. 0·880—AnalaR grade.

Dithizone—As supplied by Eastman Kodak.

Hydroxylammonium chloride, 1 per cent. w/v solution—Dissolve 1 g of AnalaR hydroxylamonium chloride in 100 ml of distilled water and make just alkaline to *o*-cresol red with ammonia.

Weak dithizone solution, 5 mg per litre, in chloroform—Wash 1 litre of AnalaR chloroform with 50 ml of 1 per cent. hydroxylammonium chloride solution, then dissolve 5 mg of dithizone in 1 litre of "washed" chloroform. Store this solution in a refrigerator.

Strong dithizone solution, 30 mg per litre, in chloroform—Dissolve 30 mg of dithizone in 1 litre of "washed" chloroform. Store this solution in a refrigerator.

Thymol blue indicator, 0·2 per cent. solution—Triturate 2 g of thymol blue with 43·0 ml of 0·1 N sodium hydroxide solution and dilute to 1 litre with distilled water.

Citrate - cyanide reagent—Weigh 350 g of AnalaR citric acid monohydrate into a 2-litre beaker, and add about 200 ml of distilled water and 2 ml of thymol blue indicator solution. Slowly add ammonia solution, with cooling, until the neutral colour (green) is obtained. Between 150 and 200 ml will probably be required. Dilute to about 1400 ml with distilled water, add 20 g of AnalaR hydroxylammonium chloride and re-adjust the pH to neutrality to thymol blue by adding a few drops of ammonia solution. Transfer the solution to a separating funnel and remove any lead present by shaking with successive 5-ml portions of strong dithizone solution until the latter retains its green colour. Retain the last portion of dithizone solution in the separating funnel and add 50 g of AnalaR potassium cyanide dissolved in about 200 ml of distilled water. Shake the funnel, discard the dithizone extract and wash the aqueous solution with AnalaR chloroform to remove the excess of dithizone. Add 20 ml of thymol blue indicator solution, and dilute to 2 litres with distilled water. Store this solution in a refrigerator.

Standard buffer, pH 9·0—Take 50 ml of a solution containing 12·369 g of boric acid and 14·911 g of potassium chloride per litre, add to the solution 21·40 ml of 0·2 M sodium hydroxide and dilute to 200 ml with distilled water. Add thymol blue indicator solution at the rate of 3 ml to each litre of buffer solution.

Stock standard solution containing 1 *mg of lead per ml*—Dissolve 0·7993 g of AnalaR lead nitrate, previously powdered and dried at 105° C in dilute nitric acid (1 + 99) and make the solution up to 500 ml.

Standard solution containing 1 *μg of lead per ml*—Prepare from the stock standard solution of lead by appropriate two-stage dilution with dilute nitric acid (1 + 99).

APPARATUS—

Muffle furnace—This should have a large heating chamber, and be capable of operating at 500° C.

Spectrophotometer—Unicam SP600 or equivalent instrument.

Crystallising dishes—These should be made of Pyrex glass, measuring 94 mm × 50 mm, and have a lip.

Clock glasses—These should be made of Pyrex glass and be 10 cm in diameter.

Separating funnels—These should be made of Pyrex glass, have a capacity of 150 ml and be calibrated at 50 ml.

PREPARATION OF THE SOLUTION FOR ANALYSIS—

If a clear, freshly-voided sample of urine is available, measure 50 ml into a crystallising dish and add 5 ml of redistilled nitric acid.

If the urine sample is aged or if it contains suspended matter, decant it from the sample bottle into a measuring cylinder and note its volume. Wash the sample bottle with an amount of concentrated nitric acid equal to 10 per cent. of the sample volume, add the acid washing to the urine and then transfer the acidified urine back to the sample bottle. Measure an aliquot equivalent to 50 ml of urine into the crystallising dish.

Place the dish containing the acidified urine on a hot-plate, cover it with a clock glass and boil the acidified sample to dryness. Care must be taken to avoid loss due to excessive frothing. Transfer the dish containing the residue, still covered with the clock glass, to a muffle furnace and maintain at 500° C for 5 minutes. This is normally sufficient to obtain a clean white ash, but if any charred organic matter remains, this may be removed by spotting it with nitric acid, re-heating on the hot-plate and again ashing at 500° C for 5 minutes.

Allow the dish to cool, add about 30 ml of diluted nitric acid (1 + 19) and heat to boiling on the hot-plate to dissolve the ash. Transfer the acid solution to a 150-ml Pyrex separating funnel, wash the dish with distilled water, add the washing to the funnel, and make the solution up to 50 ml with distilled water.

PROCEDURE FOR THE MANUAL DETERMINATION OF LEAD CONTENT—

Measure 70 ml of the standard buffer solution of pH 9·0 into a separating funnel, for use as a reference in the control of the pH values of the sample solutions.

Measure 20 ml of citrate - cyanide reagent from a burette into a separating funnel containing 50 ml of urine-ash solution. Immediately add ammonia solution or nitric acid, dropwise from a burette, until the colour matches that of the reference of pH 9·0, when viewed in diffuse daylight. By this means the pH value of the solution may be controlled to within ±0·1.

Transfer 10 ml of the weak dithizone solution by pipette into the separating funnel. Shake the funnel for 60 seconds, lift the stopper momentarily to release any pressure that may have developed and allow to stand until the chloroform layer is absolutely clear.

Dry the stem of the funnel and then fill a 2-cm optical cell with the dithizone extract. Measure the optical density at 510 mμ against chloroform.

Prepare a calibration curve by measuring known volumes of standard lead solution (1 μg per ml) up to 10 ml, into separating funnels, making the volume of each up to 50 ml and then following the procedure detailed above. Determine the lead content of the samples by reference to this calibration curve.

Valid results are obtained by this procedure when the amount of lead present in the sample solution does not exceed 10 μg of lead (corresponding to 200 μg of lead per litre of urine). If the result obtained indicates that more than 10 μg of lead is present, the analysis must be repeated on a smaller volume of urine.

METHOD 2—LEAD DETERMINATION BY MEANS OF AN AUTOANALYZER

GENERAL PRECAUTIONS TO BE OBSERVED—

The precautions described for the manual method must be observed.

REAGENTS—

The following reagents, described under Method 1, are required for Method 2.
Nitric acid, concentrated.
Nitric acid, diluted (1 + 19).
Nitric acid, dilute (1 + 99).
Ammonia solution, sp.gr. 0·880.
Dithizone.
Hydroxylammonium chloride, 1 per cent. w/v solution.
Weak dithizone solution.
Strong dithizone solution.

The following reagents are required specifically for Method 2.

Ammonium citrate solution, 50 per cent. w/v—Weigh 865 g of citric acid monohydrate, AnalaR, into a 2-litre beaker, add about 200 ml of distilled water and place the beaker in a cooling bath of ice and water. Add the minimum volume of *o*-cresol red indicator solution to give a definite colour and then add concentrated ammonia solution slowly, with stirring, until the red colour of the alkaline form of the indicator is just developed. Dilute the solution to 2 litres with distilled water and then transfer it to a separating funnel. Remove any lead in this solution by shaking with successive 20-ml portions of strong dithizone solution until the initial green colour of the dithizone solution remains unchanged. Wash the citrate solution with chloroform to remove dissolved dithizone and set it aside until the aqueous phase is completely clear, and then discard the chloroform phase. Filter the ammonium citrate solution through a small plug of lead-free glass wool.

Wash solution—Measure 400 ml of ammonium citrate solution and 400 ml of diluted nitric acid (1 + 19) into a 2-litre volumetric flask and dilute to the mark with distilled water. This solution is used for dissolving urine ash and also as a wash solution in the subsequent automatic analysis.

Sulphite - cyanide - ammonia buffer solution—Dissolve 10 g of anhydrous sodium sulphite and 5 g of potassium cyanide in about 100 ml of distilled water. Transfer the solution to a separating funnel and remove any lead present by shaking with successive 20-ml amounts of strong dithizone solution until the initial green colour of the dithizone remains unchanged. Wash the solution with chloroform to remove dissolved dithizone, allow to stand until the aqueous phase is completely clear and then discard the chloroform phase. Filter the sulphite - cyanide solution through a small plug of lead-free glass wool, add 30 ml of concentrated ammonia solution, and dilute to 2 litres with distilled water.

Stock standard solution containing 1 mg of lead per ml—Dissolve 0·7993 g of AnalaR lead nitrate, previously powdered and dried at 105° C, in dilute nitric acid (1 + 99) and make the solution up to 500 ml.

Standard solution containing 1 μg of lead per ml—Transfer 10 ml of the stock standard solution of lead and 10 ml of concentrated nitric acid to a 1-litre volumetric flask by pipette. Dilute to volume with distilled water. Transfer 25 ml of this solution to a 250-ml volumetric flask and add 45 ml of diluted nitric acid (1 + 19) and 50 ml of ammonium citrate solution by pipette. Make up to volume with distilled water.

This solution contains 1 μg of lead per ml and the same concentrations of nitric acid and ammonium citrate as the wash solution and the sample solutions prepared according to the procedure given below.

Working standard lead solution—Prepare a series of working standard solutions by dilution of the standard lead solution (1 μg per ml) with wash solution, to cover the range 0·05 to 0·4 μg of lead per ml. These solutions correspond to the concentrations obtained in the ash solutions from urine samples containing 25 to 200 μg of lead per litre.

PREPARATION OF THE SOLUTION FOR ANALYSIS—

The procedure in Method 1 is adopted, up to and including heating the dish containing the urine ash in a muffle furnace at 500° C for 5 minutes.

Allow the dish to cool. Add 10 ml of wash solution and heat on a hot-plate until the solution just boils. Immediately remove the dish from the hot-plate, allow it to stand for about 5 minutes and then transfer the solution to a 25-ml Pyrex volumetric flask. Repeat this procedure with a second 10-ml portion of wash solution, transferring the solution and any undissolved solids to the flask. Wash the dish with about 4 ml of wash solution and transfer the washings quantitatively to the flask. Allow the combined solution and washings to cool to room temperature and make up to volume with wash solution.

APPARATUS—

The apparatus consists essentially of standard AutoAnalyzer modules as supplied by Technicon Instruments Ltd., but much of the necessary glassware was specifically designed for this application. The manifold assembly and flow diagram is shown in Fig. 1 and the important points in the analytical system are indicated below.

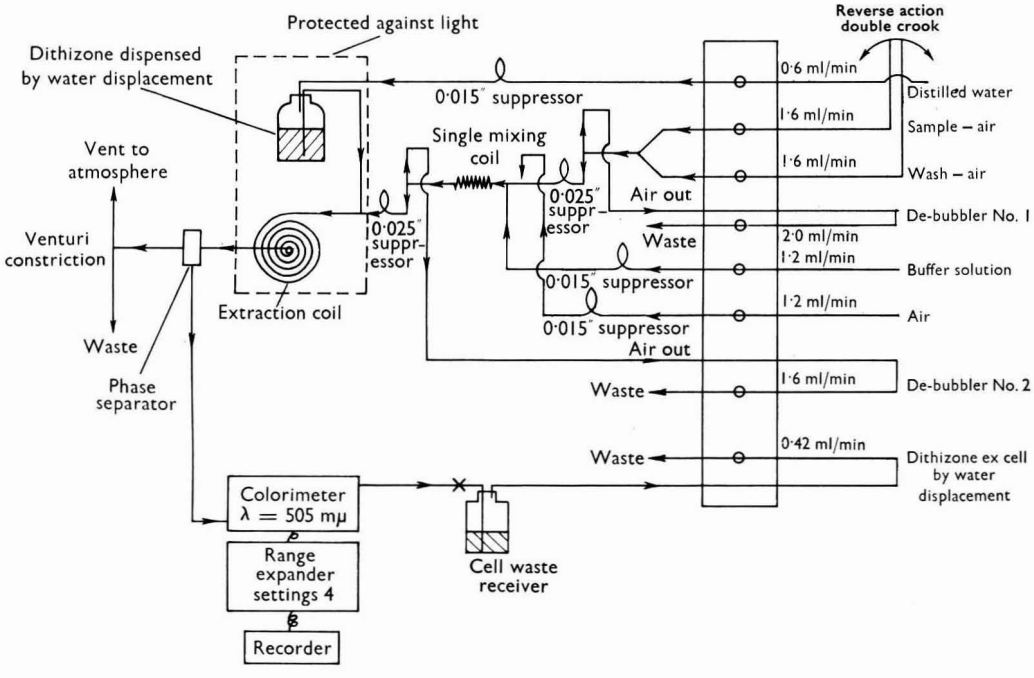

Fig. 1. Manifold assembly and flow diagram of apparatus

Sampling—A standard reverse-action double-crook sampler is used in conjunction with a constant-head device. This is similar to that described by Scholes and Thulbourne,[7] except that the tubing carried by the arm of the crook enters the wash solution in the constant-head reservoir through a narrow guide tube as shown in Fig. 2. This minimises the risk of casual contamination of the wash solution.

Control of pH of the aqueous phase—The change from liquid to air in one arm of the crook and from air to liquid in the other arm cannot be synchronised both at the beginning and the end of each sampling period. Early trials showed that this caused irregularities in the bubble pattern of the sample - wash stream which corresponded to the movements of the sample crook. Injection of buffer solution into such an unevenly segmented stream leads to cyclic variations in the pH value of the stream after the mixing coil, which cannot be tolerated in the extraction of lead with dithizone in the later stage of the system. Therefore it is necessary to remove the bubbles from the sample - wash stream and re-segment it with air, prior to the injection of the buffer solution.

The air bubbles are removed from the buffered aqueous phase immediately before the injection of the dithizone solution.

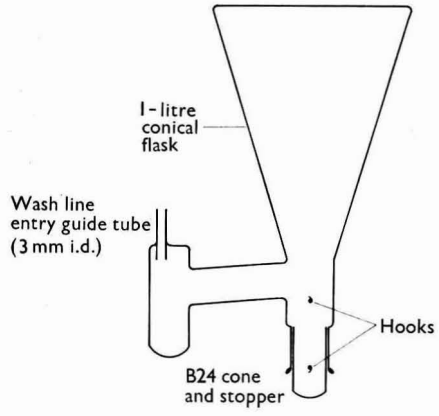

Fig. 2. Constant-head device

Extraction and separation—The dithizone solution is injected into the system by displacement with water and serves to segment the aqueous phase flowing through the extraction coil. Various designs of extraction system were tried. Vertical extraction coils packed with small glass beads were found to be unsuitable as they totally destroyed the regular flow pattern, which must be maintained in order to obtain precise results. Standard mixing coils in the horizontal position tended to induce surging flow and were therefore not satisfactory. The extraction coil finally developed for this duty comprises a horizontal "pancake" spiral made from 10 feet of 2·7 mm internal diameter Pyrex-glass tubing having 10 complete turns.

It is necessary to protect the dithizone solution reservoir and the extraction coil from light.

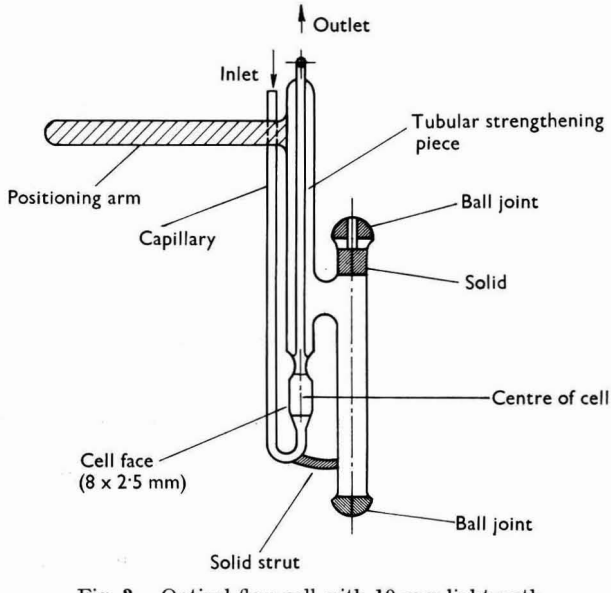

Fig. 3. Optical flow cell with 10-mm light path

Phase separation and optical-density measurement—After separation of the two phases, the amount of lead present is determined by measuring the optical density of the chloroform phase. Two types of optical cell are currently available with the Technicon colorimeter, one a rectangular type from which the liquid overflows, and one a flow-through cuvette through which the liquid can be pumped. The volume of the rectangular cell is large compared with the pumping rates employed, resulting in totally inadequate sensitivity for this application. The necessary sensitivity could be achieved by means of a 15-mm flow-through cuvette,

but stable performance could not be obtained. The instability was attributed to the formation of a film on the end-windows of the cuvette from the non-aqueous phase being pumped through.

A new type of optical cell was therefore designed. It was made to fit in the rectangular flow-cell carriage, which is a standard item of AutoAnalyzer equipment. The general arrangement is shown in Fig. 3.

The cell is rectangular, having two optical faces approximately 8 mm × 2·5 mm and a light path of 10 mm. The capillary tubing leading to and from the cell is joined to the rectangular portion in such a way as to avoid any abrupt change in cross-section, which would cause turbulence or "streaming" in the flow of liquid through the cell at the flow rates employed. The liquid is pumped upwards through the cell, at right angles to the light path.

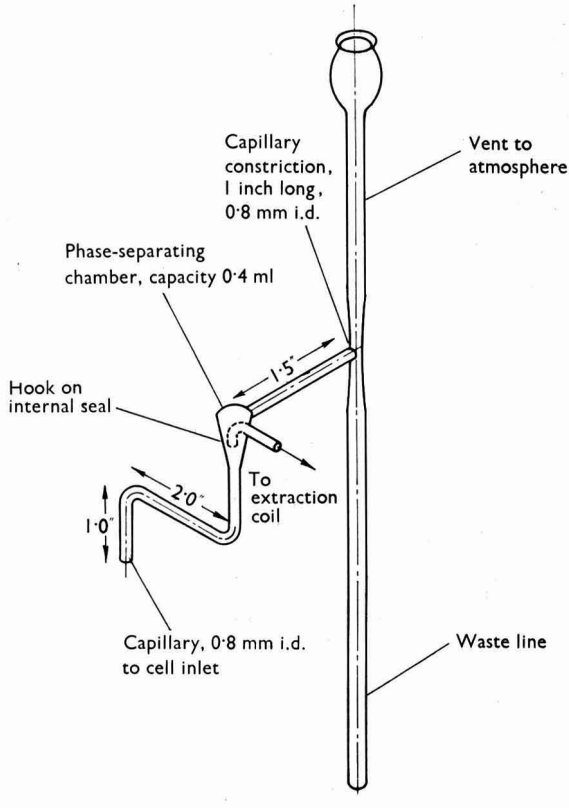

Fig. 4. Phase separator

A phase separator was developed for use with this optical cell, and this is shown in Fig. 4. The volume of the separating chamber is about 0·4 ml. Most of the chloroform phase is pumped from the bottom of this chamber to the cell inlet. The aqueous phase and a little of the chloroform phase overflow from the top of the separating chamber through a horizontal capillary outlet that joins a capillary section in a vertical waste line. A continuous uniform rate of flow to waste is achieved by means of this capillary restriction in the waste line, thus minimising any fluctuations in rate of flow through the separating chamber.

The colorimeter is fitted with 505-mμ light filters, and a range expander is required to amplify the signal from the photocells to the recorder.

PROCEDURE FOR THE AUTOMATIC DETERMINATION OF LEAD—

Assemble the apparatus as shown in the flow diagram (Fig. 1). Switch on the machine with the crook of the sampler in the wash solution, the cell-waste-receiver inlet tap closed and the pump tubing from this receiver disconnected. Pump all reagents through the system

to waste via the phase-separator overflow line. When a steady flow pattern is achieved, connect the cell-waste-receiver pump line, open the tap and pump the chloroform phase through the cell. Switch on the recorder-chart drive and position the cell to give maximum deflection on the chart before adjusting the position of the base-line with the range expander on the setting "S4."

Transfer the working standard solutions and sample solutions to the sample cups and place them on the sample tray, alternating them with cups containing wash solution. Set the sampler to operate at a rate of 40 per hour and switch it on as soon as the base-line on the recorder chart is steady. This gives an effective rate of 20 samples per hour.

After aspiration of the last sample, allow wash solution to be aspirated until the recorder pen has returned to the base-line. The time interval from aspiration of the sample to recording the corresponding peak on the chart is about 10 minutes.

Draw a calibration curve of peak height against lead concentration of the working standard solutions. Determine the lead content of the samples by reference to this calibration curve. The calibration curve is not rectilinear.

If the height of the peak corresponding to a particular sample indicates a lead content greater than 200 μg per litre, the analysis must be repeated on an appropriate dilution of the urine-ash solution with wash solution to bring the lead concentration within the range of the working standards.

The reagent compositions and pumping rates are designed to bring the pH value of the aqueous phase to about 8·90 for the dithizone-extraction stage. Valid results are obtained if the pH value lies between 8·7 and 9·0. The aqueous effluent from the phase separator must be tested periodically, to ensure that the extraction is taking place within this range of pH values.

Results

Method 1—A large volume of urine was acidified and ashed under the conditions described. The ash was dissolved in diluted nitric acid (1 + 19) and the volume of the ash solution made up to the initial volume of the urine. The lead content was determined by the analysis of a 50-ml portion of this solution according to Method 1. Known amounts of standard lead solution (1 μg per ml) were added to other 50-ml portions of the urine-ash solution, and these solutions were then analysed by means of Method 1, with the following results—

Lead added, μg per litre of urine	80	80	100	100	120	120
Lead found after subtraction of blank value, μg per litre of urine	78	77	98	99	120	118

The blank value on urine alone was 16 μg of lead per litre.

Method 2—A large volume of urine of low lead content was acidified and ashed. The ash was dissolved in equal, measured volumes of diluted nitric acid (1 + 19) and distilled water, and the same volume of ammonium citrate solution was added. This solution of the ash was then diluted to a volume equal to two-fifths of the initial volume of the urine, and 20-ml portions of this solution, with and without the additions of known amounts of working standard lead solution, were diluted to 25 ml with distilled water. These solutions were then analysed on three separate occasions by Method 2. The results are shown in Table I.

Table I

Recovery of added lead by Method 2

Lead added, μg per litre of urine	Lead found after subtraction of blank, μg per litre of urine		
	(1)	(2)	(3)
28	27	26	26
31	29	29	30
44	45	47	42
50	52	52	50
63	66	65	62
94	95	93	94
125	124	125	125

The blank value on urine alone was 10 μg of lead per litre.

INTERFERENCE BY BISMUTH—

Early experimental work with the AutoAnalyzer showed that the flow system described above can be used for the determination of trace amounts of lead in the presence of citrate and cyanide over a wide range of pH values, provided that the conditions of extraction remain constant for both sample solutions and standards. The peak height corresponding to a given concentration of lead increases as the pH of extraction increases from 8·5 to 9·5, but it was found that bismuth was also readily extracted at pH 9·5.

The ammonia content of the buffer solution was varied to give lower pH values for extraction. Nitric acid - ammonium citrate solutions containing known amounts of bismuth, and also nitric acid - ammonium citrate extracts of urine ash containing known amounts of bismuth, were analysed by means of the AutoAnalyzer. The "apparent lead content," shown in Table II, was found by reference to calibration curves prepared by the analysis of similar solutions containing known amounts of lead, determined under identical conditions in each case.

TABLE II

EXTRACTION OF BISMUTH UNDER CONDITIONS DESIGNED FOR THE AUTOMATIC DETERMINATION OF LEAD AT VARIOUS pH VALUES

Base-stock	Bismuth added, μg per litre of urine	Apparent lead content, μg per litre of urine		
		pH 9·15	pH 8·90	pH 8·60
Nitric acid - ammonium citrate	Nil	Nil	Nil	Nil
	20	9	6	5
	50	21	16	12
Urine-ash solution	Nil	16	16	16
	20	21	20	19
	50	29	27	24

It can be seen from Table II that the extraction of bismuth is retarded at lower pH values and is further retarded by the presence of salts derived from urine ash. The optimum pH for extraction of lead with minimum interference from bismuth was established as being in the range 8·7 to 9·0.

A bulk-ash solution was prepared from urine of low lead content under the conditions described in Method 2. Standard bismuth nitrate solution was added to give the equivalent of 50 μg of bismuth per litre of urine (several times greater than the amounts found in the urine of individuals taking medicaments containing bismuth). Different amounts of standard lead solution were added to portions of this solution and these solutions, containing both added bismuth and added lead, were analysed by Method 2. The results are shown in Table III.

TABLE III

RECOVERY OF ADDED LEAD BY THE AUTOMATIC METHOD FROM URINE-ASH SOLUTIONS CONTAINING 50 μg OF BISMUTH PER LITRE

Lead added, μg per litre of urine	Lead found after subtraction of blank, μg per litre of urine	Error, μg per litre of urine
50	60	+10
60	68	+ 8
70	75	+ 5
80	85	+ 5
90	94	+ 4
100	106	+ 6
120	125	+ 5
140	143	+ 3
160	161	+ 1
180	181	+ 1
200	199	− 1

The blank value on urine alone was 16 μg of lead per litre.

A further bulk sample of urine of low lead content was ashed and the ash dissolved under the conditions described in Method 1. Standard bismuth nitrate solution was added to the urine-ash solution to give the equivalent of 50 μg of bismuth per litre of urine, and different amounts of standard lead solution were added to 50-ml portions. These 50-ml portions were then analysed by Method 1. The results are shown in Table IV.

TABLE IV

RECOVERY OF ADDED LEAD BY THE MANUAL METHOD FROM URINE-ASH SOLUTIONS CONTAINING 50 μg OF BISMUTH PER LITRE

Lead added, μg per litre of urine	Lead found after subtraction of blank, μg per litre of urine	Error, μg per litre of urine
60	64	+4
70	76	+6
80	82	+2
90	96	+6
100	112	+2
120	120	nil
140	138	−2
160	164	+4
180	182	+2
200	194	−6

The blank value on urine alone was 8 μg of lead per litre.

Thus the extraction of bismuth decreases with increasing amounts of lead in the system, by both the manual and automatic methods. The interference in either method due to amounts of bismuth that may be present in urine samples is slight at low lead concentrations, and is negligible at those concentrations that indicate significant absorption of lead by the individual excreting the urine.

INTERFERENCE BY CALCIUM, MAGNESIUM AND PHOSPHATE—

The possibility of interference occurring in the application of the single-extraction procedure to urine samples having abnormally high concentrations of alkaline-earth phosphates was investigated. The normal concentrations of calcium, magnesium and phosphate in urine are given by Wootton[8] as—

Calcium, 14 mg per 100 ml, expressed as Ca
Magnesium, 12 mg per 100 ml, expressed as Mg
Phosphate, 306 mg per 100 ml, expressed as PO_4^{3-}

Three large samples of urine were obtained and the lead content of each was adjusted to about 100 μg per litre by the addition of standard lead solution. Six portions, each of 50 ml, were taken from each sample. To four of the portions of urine A were added calcium (as calcium chloride), magnesium (as magnesium chloride) and phosphate (as ammonium hydrogen phosphate) to give an increase in the concentration of these ions equal to the normal concentrations quoted by Wootton.[8] Twice these amounts were added to each of 4 portions of urine B and three times these amounts to each of 4 portions of urine C. The lead contents of the samples with and without added alkaline-earth phosphates were then determined by Method 1. The results are shown in Table V.

TABLE V

RECOVERY OF LEAD BY METHOD 1 IN THE PRESENCE OF ADDED CALCIUM, MAGNESIUM AND PHOSPHATE

Urine with added lead	Calcium added, mg per 100 ml	Magnesium added, mg per 100 ml	Phosphate added, mg per 100 ml	Lead found, μg per litre
A	nil	nil	nil	104, 104
	14	12	306	101, 100, 102, 103
B	nil	nil	nil	102, 102
	28	24	612	98, 98, 100, 102
C	nil	nil	nil	108, 104
	42	36	918	102, 101, 104, 101

The ash solutions prepared from urine C with added calcium, magnesium and phosphate became slightly opalescent when the pH value was adjusted to 9·0 after the addition of the citrate - cyanide reagent. Precipitation occurred in the solutions prepared from both urine B and urine C containing added calcium, magnesium and phosphate when they were shaken with dithizone solution after pH adjustment. This did not cause any significant loss of lead nor did it cause any manipulative difficulty.

Another large sample of urine was taken and its lead content adjusted to about 100 μg per litre. Ten portions of 50 ml each were then taken. To 4 portions were added twice the normal concentrations of calcium, magnesium and phosphate, and to 4 other portions were added 3 times the normal concentrations of these ions. All 10 portions were then analysed for lead content by Method 2. The ash prepared from the samples with added alkaline-earth phosphate dissolved slower than is usual in the ammonium citrate - nitric acid wash solution, and in two cases some of the ash remained undissolved. No precipitation occurred at any stage in the AutoAnalyzer system. The results are shown in Table VI.

TABLE VI

RECOVERY OF LEAD BY METHOD 2 IN THE PRESENCE OF ADDED CALCIUM, MAGNESIUM AND PHOSPHATE

Calcium added, mg per 100 ml	Magnesium added, mg per 100 ml	Phosphate added, mg per 100 ml	Lead found, μg per litre
nil	nil	nil	104, 98
28	24	612	97, 97, 92,* 103
42	36	918	95, 95, 99, 92*

* The ash did not completely dissolve in the wash solution.

These results indicate that the interference due to the presence of alkaline-earth phosphates is negligible when up to 4 times the normal concentrations are present.

COMPARISON OF THE MANUAL AND AUTOMATIC METHODS—

A number of routine urine samples were divided into 2 parts after acidification with nitric acid. One portion was analysed by Method 1 and the other portion by Method 2.

The results, which indicate very satisfactory agreement between the two methods, are summarised in Table VII.

TABLE VII

COMPARISON OF RESULTS OBTAINED BY THE MANUAL AND THE SEMI-AUTOMATIC METHODS OF DETERMINING LEAD IN URINE

Concentration range, μg of lead per litre	No. of samples	Mean deviation, μg of lead per litre	Standard deviation, μg of lead per litre
10–60	24	<1	$\pm 2{\cdot}7$
60–150	30	<1	$\pm 3{\cdot}5$

CONCLUSIONS

The methods described were developed specifically for the routine determination of the lead content of urine. It is shown that the concentrations of bismuth that are likely to occur in urine do not cause undue error in either the manual or the automatic procedure for lead determination. Thus either method could be readily adapted to the determination of trace amounts of lead in other substances where the ratio of lead to bismuth is similar to that in urine, or where bismuth is known to be absent. If bismuth is known to be absent then the automatic method for lead determination may be modified so that the extraction with dithizone takes place in the range pH 9·3 to 9·7. This enhances the sensitivity of the lead determination.

It is also shown that valid results for lead content are obtained in the presence of concentrations of calcium, magnesium and phosphate several times greater than those normally present in urine. Much higher concentrations of alkaline-earth phosphates could not, however, be tolerated.

We acknowledge the assistance given by Mr. F. Wainwright in making the optical cell and other glassware required for the automatic method, and also the help given by Mr. E. H. Lowe in obtaining some of the results quoted on the effects of bismuth and of alkaline-earth phosphates, and for performing some of the trials in which the results by the manual and automatic methods were compared.

We thank The Associated Octel Co. Ltd. for permission to publish this paper.

References

1. Bambach, K., and Burkey, R. E., *Ind. Engng. Chem., Analyt. Edn.*, 1942, **14**, 904.
2. Irving, H. M., and Butler, E. J., *Analyst*, 1953, **78**, 571.
3. Keenan, R. G., Byers, D. H., Saltzman, E., and Hyslop, F. L., *Amer. Ind. Hyg. Ass. J.*, 1963, **24**, 481.
4. Cholak, J., Hubbard, D. M., and Burkey, R. E., *J. Ind. Hyg. Toxicol.*, 1948, **30**, 59.
5. Nelson, K. W., and Hamm, R. E., *A.M.A. Archs. Ind. Hlth*, 1958, **17**, 38.
6. Sandell, E. B., *Editor*, "Colorimetric Determination of Traces of Metals," Third Edition, Interscience Publishers Inc., New York and London, 1959, p. 347.
7. Scholes, P. H., and Thurlbourne, C., *Analyst*, 1964, **89**, 466.
8. Wootton, I. D. P., "Micro-analysis in Medical Biochemistry," Fourth Edition, J. and A. Churchill Ltd., London, 1964, p. 4.

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The Determination of Oxygen in Sodium

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A method has been developed for the determination of oxygen in sodium in the range 0 to 100 p.p.m. Sodium is removed by distillation and the oxygen is determined by titration with sulphuric acid. The coefficient of variation for the range 0 to 100 p.p.m. of oxygen is 10. The effect of titratable impurities associated with sodium oxide in the distillation residue has been examined. The possible loss of oxygen as carbon monoxide from the reaction between sodium oxide and carbon has been investigated and found to be negligible.

The use of sodium as a coolant in fast-reactor systems necessitates careful control of its oxygen content, since the corrosion of fast-reactor materials may be oxygen dependent.

A method was required that could be used to determine the oxygen content of sodium over the range 0 to 100 p.p.m. The method chosen had to be suitable for eventual routine application to a number of dynamic sodium test-loops and for an analysis frequency of 30 determinations per day.

Three principle techniques are in current use. In the method of Pepkowitz and Judd[1] sodium is separated from sodium oxide as an amalgam. The oxide is then determined by acid titration. In a modification of this technique Steinmetz and Minushkin[2] converted the oxide to the water equivalent and determined this with Karl Fischer reagent. In another procedure, by White, Ross and Rowan,[3] based on the Wurtz synthesis, the sodium is removed by reaction with butyl bromide. The residual sodium oxide is then determined by acid titration. In a third method developed by Humphreys,[4] the sodium is removed by vacuum distillation. The residue of sodium oxide is then dissolved in water and determined by acid titration.

The removal of sodium with either mercury or butyl bromide was not considered practical from a routine manipulative point of view. A survey[5] of the literature relating to the two techniques suggested that the reproducibility and accuracy were in doubt. Preliminary work on these techniques substantiated this. Initial experience[6] with the distillation method indicated that this would be the most suitable for routine analysis, provided that certain objections to the technique could be resolved.[7] The distillation residue may include material other than sodium oxide, such as calcium and magnesium oxides, sodium hydride and sodium carbonate. The presence of one or more of these components would lead to a positive titration error. Also a loss of oxide during the distillation may occur owing to the reaction—

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

This reaction is unlikely, however, on thermodynamic grounds. Both these objections have been investigated and are discussed later.

Experimental

Apparatus—

The sampling equipment is in two parts. The stainless-steel distillation pot,[8] which is illustrated on p. 229 of *The Analyst*, April, 1965, consists of a flange with a cold finger and an extraction valve, and a pot to which the flange is bolted. A neoprene O-ring separates the flange and pot, and copper coils surround upper and lower sections to enable the O-ring to be continuously cooled during the distillation.

The second part of the sampling equipment is a stainless-steel crucible holder which holds four nickel crucibles, each of capacity approximately 7 ml. Good thermal contact between the base of the distillation pot and each crucible is necessary to ensure complete distillation. Temperature measurements are made with a thermocouple in external contact with the base of the distillation pot. Internal temperature measurements of the base of the pot have been taken and were lower than the externally measured temperature by 50° to 70° C.

The distillation rig consists of a vacuum line with two attachment points. The vacuum is maintained by an oil-diffusion pump backed by a single-stage rotary pump, and a pressure

of less than 1 micron of mercury is maintained. The distillation pot is surrounded by a heater unit consisting of a base and a side heater, each fitted with a heater control.

Outline of the method

All the work contained in this report was carried out using a dynamic sodium test-loop.[9] This rig incorporates an evacuable glove-box adjacent to the sampling well. The equipment in the glove-box was manipulated by means of neoprene gloves. Contamination caused by the permeation of oxygen and moisture through the gloves is negligible. To demonstrate this, sodium, in nickel crucibles, was exposed to the glove-box atmosphere for three times the normal sampling period; no measurable increase in the oxygen content of the sample could be detected.

Before sampling, four crucibles are placed in the well of sodium and left immersed in the flowing sodium for at least 24 hours. This ensures that any oxide on the surface of the crucible is reduced by the sodium. Alternatively, the crucibles may be cleaned by "hydrogen firing." With this process, the four crucibles and holder are heated to 500° C for 2 hours in a distillation pot containing hydrogen at a pressure of approximately 5 p.s.i. The hydrogen is removed by evacuation, and the pot and its contents are allowed to cool to room temperature.

To sample, the distillation pot with the valve open, is placed in the test-loop glove-box. The whole assembly is then evacuated to a pressure of less than 1 micron of mercury and maintained at this level for a minimum of 12 hours to ensure complete out-gassing of the equipment. After this period, the glove-box is brought to atmospheric pressure with purified argon containing less than 1 v.p.m. of oxygen and less than 5 v.p.m. of water. The port between the glove-box and the sampling well is opened, and the crucibles are removed from the sodium and placed in the crucible holder. The holder is then lowered into the pot, and the two sections of the distillation pot are bolted together. The pot valve and the sampling-well port are closed. The pot is removed from the sampling box and connected to the distillation rig.

If hydrogen-fired crucibles are used, they can be filled by dipping them into the molten sodium. Samples of the sodium in the solid form can be melted in a stainless-steel crucible and poured into hydrogen-fired crucibles. This must be done in an oxygen-free-argon atmosphere, and care must be taken to out-gas the heater and the stainless-steel crucible before use, by heating them for a prolonged period under vacuum at a higher temperature than that required to melt the sodium.

The connecting link on the distillation rig between the pot and the vacuum line is evacuated to a pressure of approximately 1 micron of mercury. The valve on the pot is then opened gradually (after temporarily by-passing the diffusion pump) and the pot is evacuated to a pressure of less than 1 micron of mercury. The cold finger is filled with water, and the cooling coils (including a detachable coil dipping into the cold finger) are connected to a water supply.

The temperature of the pot is raised to 350° C and maintained at this level for 2 hours. The temperature is then increased to 550° C and distillation continued for a further 4 hours. After this period, all the sodium has distilled on to the cold finger. The pot is allowed to cool to room temperature still under continuous vacuum. It is then isolated from the pumping system and removed from the distillation unit.

The pot is brought to atmospheric pressure by opening the valve slowly and the holder containing the crucibles is removed. The sodium oxide residue in the crucibles is dissolved in a dilute aqueous ethanolic (97 + 3) solution of bromocresol purple (0·05 per cent. w/w solution, neutralised before use) and titrated with 0·01 N sulphuric acid. The concentration of oxygen is calculated from this titration, the weight of sodium sample being calculated from the volume of the crucible used.

Preparation of standards—

A technique of adding microgram amounts of oxygen to sodium with an acceptable degree of accuracy was developed after investigating various methods. The obvious choice was the addition of mercuric oxide, whose high weight-to-oxygen ratio makes it easier to weigh a suitable amount directly into the crucible. Also it is easily reduced by sodium to give sodium oxide *plus* volatile mercury. Some degree of success was obtained with this reagent, but, presumably due to its low thermal stability, the addition of molten sodium to crucibles containing known amounts of mercuric oxide led to loss of oxygen, and recoveries were variable.

The high thermal stability of zinc oxide, together with its ease of reduction with sodium to sodium oxide and volatile zinc,[10] suggested it as an alternative standard, but because of the low weight-to-oxygen ratio, some means of dilution before addition was required. Attempts to add zinc oxide were made by placing a solution of zinc nitrate in the nickel sampling crucibles and evaporating and heating to 450° C under vacuum. However, the crucibles were badly corroded by the oxides of nitrogen produced during the thermal decomposition of zinc nitrate. Dilution of zinc oxide with a solid diluent that could be removed by sublimation was next investigated. An ammonium chloride - zinc oxide mixture was prepared, but during sublimation of the ammonium chloride the zinc was also removed, presumably as zinc chloride. A mixture of AnalaR zinc oxide and naphthalene was found satisfactory, and was prepared by grinding approximately 0·7 g of zinc oxide with 50 g of pure naphthalene. The mixture was separated on a 60-mesh sieve. The fraction passing through this sieve was re-ground and separated through a 100-mesh sieve. The fraction passing through the 100-mesh sieve was used for measured additions of zinc oxide by weighing the mixture into hydrogen-fired crucibles and subliming the naphthalene at approximately 200° C, after which the crucibles were heated for 2 hours in an oven at 105° C. For the nominal 5 p.p.m. oxygen-recovery work, small nickel trays approximately 1 cm × 1 cm × 0·25 cm were constructed from nickel foil (0·012 cm thick). The naphthalene - zinc oxide mixture was weighed into each tray and the naphthalene removed by vacuum sublimation at 100° C. Each tray, containing a known amount of zinc oxide, was added to a nickel crucible. The ratio of the zinc oxide - naphthalene mixture was standardised by heating 0·5-g portions in a small tared crucible at 200° C to constant weight. The original mixture was used for recoveries in the range 50 to 100 p.p.m. For recoveries below 50 p.p.m. this mixture was diluted approximately 4-fold.

The standard additions were made in the above manner and molten sodium, from a dynamic rig with a lower oxygen level, was added by means of a stainless-steel scoop. The crucibles so prepared were then treated as detailed in the method. After titration, the resulting solutions were examined for residual zinc. It was found that a maximum of 5 per cent. of the added zinc remained behind after distillation, presumably as sodium zincate. This indicates that sodium oxide was the main product. Full conversion of zinc oxide to sodium oxide is not essential as the compounds ZnO, Na_2O and Na_2ZnO_2 all titrate with the same oxygen equivalence—

$$Na_2O + H_2SO_4 \longrightarrow Na_2SO_4 + H_2O$$
$$ZnO + H_2SO_4 \longrightarrow ZnSO_4 + H_2O$$
$$Na_2ZnO_2 + 2H_2SO_4 \longrightarrow Na_2SO_4 + 2H_2O + ZnSO_4$$

Results

Sampling technique—

In the method described, the sodium may be sampled from the loop sampling-well by either filling hydrogen-fired crucibles with sodium by using a nickel scoop, or by immersing the crucibles in the well. It was found that the results from both these sampling techniques showed reasonable agreement. The standard deviation calculated on 16 results (8 from each sampling method with the same sodium source) was 1 p.p.m. at the 2 p.p.m. level.

Traces of metallic impurities—

A spectrographic analysis of the distillation residue from loop sodium for trace metal impurities was carried out and the results are shown in Table I.

Table I

Traces of metallic impurities in the distillation residue

Metal	Concentration, p.p.m.
Ba, Be, Co, Mg, Mn, Mo, Sr, Ti, V ..	≤0·1
Al, Bi, Cr, Pb	≤0·2
Si, Zr	≤0·3
Ca, Ni, Sn, Nb	≤0·5
W, Zn	<0·7
Ag	2

FLAME-PHOTOMETRIC DETERMINATION OF SODIUM OXIDE—

Interference in the titration caused by traces of metallic impurities may be obviated by determining the sodium in the titration solution flame photometrically. Good agreement has been found between the methods of determining sodium oxide in the residues, and the results are shown in Table II.

TABLE II

COMPARISON OF THE METHODS OF DETERMINING THE OXYGEN IN THE RESIDUE

	Sodium loop	
Method of determining the oxygen	Sample A, oxygen found, p.p.m.	Sample B, oxygen found, p.p.m.
Directly, by titration	23	8
	25	9
Indirectly, by flame-photometry ..	25	10
	26	10

TYPICAL ANALYSES—

The results of routine analyses on sodium test-loops at Culcheth by the method described are shown in Table III. Each set of four results is associated with one distillation pot.

TABLE III

TYPICAL ANALYSES OF SODIUM TEST-LOOPS

Nominal level of oxygen, p.p.m.	Oxygen found, p.p.m.	
5	4, 4, 4, 8	5, 5, 5, 5
	4, 2, 6, 3	3, 3, 5, 5
10	11, 9, 13, 11	10, 10, 10, 9
	8, 10, 7, 12	9, 9, 8, 8
	8, 11, 12, 11	
20	23, 22, 25, 23	22, 22, 26, 21
	22, 24, 24, 25	23, 23, 22, 28
30	32, 32, 34, 37	26, 24, 28, 28
	28, 31, 30, 26	26, 26, 25, 29
40	36, 37, 35, 40	45, 44, 46, 48
	44, 37, 48, 41	
50	50, 47, 44, 48	53, 55, 54, 51

OXYGEN STANDARDS—

Oxygen recovery experiments, adding oxygen by the diluted zinc oxide technique, were carried out at the 5, 10, 25, 50 and 100-p.p.m. levels of oxygen in sodium. The results are shown in Table IV.

TABLE IV

RECOVERY OF OXYGEN ADDED TO 7 g OF SODIUM

Oxygen added, p.p.m.	Background oxygen in sodium, p.p.m.	Oxygen recovered, p.p.m.
5	1	5·0 (6 results) S = 1·0
10	5	10·0 (8 results) S = 1·0
25	3	23·0 (9 results) S = 2·0
53	8	56·0 (4 results) S = 4·0
105	5	96·0 (4 results) S = 11·0

DISCUSSION

In the determination of oxygen by acid titration of the residue remaining after distillation, it is assumed that only sodium oxide is left. Magnesium and calcium oxides are the most likely impurities in the residue. Magnesium and calcium reduce sodium oxide and their oxides have a high thermal stability. The results shown in Table I indicate that the alkaline-earth metal contribution to the titre is insignificant. However, a check on the sodium content of the distillation residue may be made with a flame photometer.

The presence of sodium carbonate in the residue after distillation would interfere with both titration and flame-photometric determinations. It would have to be determined separately and allowed for in the titration. In the development of the present method, the sodium carbonate concentration in the residue after distillation of sodium from a test-loop was found conductimetrically to be less than 3 p.p.m. Since 8 p.p.m. of residual sodium carbonate give a titration equivalent to 1 p.p.m. of oxygen, the concentration of sodium carbonate found makes a negligible contribution to the final titre.

The possibility of sodium hydride being present in the residue after distillation is discounted, owing to its high hydrogen-dissociation pressure at the distillation temperature. The concentration of hydrogen in loop sodium was shown, by an extraction method,[11] to be less than 0·25 p.p.m. under normal operating conditions.

It is reported that sodium hydroxide is unstable in the presence of sodium at the test-loop temperature (<400° C) and will eventually decompose to sodium oxide and sodium hydride.[12] Therefore, if sodium hydroxide was present in the distillation residue, it would titrate as if it were sodium oxide and not affect the measured oxygen content of the sample.

At the distillation temperature the equilibrium vapour pressure of carbon monoxide in the reaction—

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

is approximately 9×10^{-6} torr. The low partial pressure of carbon monoxide indicates that loss of oxygen should not be significant. However, the application of a vacuum to this system (as in the vacuum distillation stage) will tend to shift the equilibrium to the right, resulting in loss of oxygen. Possible loss by this means was investigated. Sodium, containing 500 μg of oxygen (as mercuric oxide) and 1000 μg of lamp-black (previously degassed for 3 hours at 1000° C), was distilled at 550° C for 1 hour. All the gas evolved during distillation was collected and examined for carbon monoxide with a helium ionisation chromatograph.[13] The carbon monoxide found was equivalent to approximately 1 p.p.m. of oxygen on a 6-g sample of sodium.

The system was heated for a further period of 4 hours at 550° C to simulate distillation conditions and the gas evolved collected and carbon monoxide determined. The amount of carbon monoxide found was equivalent to approximately 1 p.p.m. of oxygen on a 6-g sample of sodium. Workers at D.E.R.E. using ^{14}C-labelled carbon have confirmed that insignificant amounts of ^{14}C were evolved in the gas phase, which was pumped off and collected for counting. It was concluded that loss of oxygen caused by reaction between sodium oxide and carbon was not significant.

Conclusion

A method has been devised for the determination of oxygen in sodium in the range 0 to 100 p.p.m. Recoveries of oxygen added to sodium were satisfactory, and in this range the coefficient of variation was of the order of 10. The method has been used on a routine basis for the past 5 years.

We wish to record our gratitude to the loop-operation staff of the Liquid Metals Section, R.M.L. Culcheth, for their help.

References

1. Pepkowitz, L. P., and Judd, W. C., *Anal. Chem.*, 1950, **22**, 1283.
2. Steinmetz, H., and Minushkin, B., U.S.A.E.C. Research and Development Report, NDA 2154–6, 1961.
3. White, J. C., Ross, W. J., and Rowan, R., Oak Ridge National Laboratory Report ORNL 1286, April, 1952.
4. Humphreys, J. R., Nuclear Sci. and Eng. AICLE Preprint 67, 1955.
5. Soenen, M., *Centre d'Etude de L'Energie Nucleaire* Report NIR 1436, 1963.
6. Thacker, R., U.K. Atomic Energy Authority Industrial Group Report TN/C.609, 1957.
7. Epstein, L. F., G.E.C. R. and D. Report GEST–2012, 1963.
8. Walker, J. A. J., and France, E. D., *Analyst*, 1965, **90**, 229.
9. Tyzack, C., *The Reactor*, 1962, **1**, No. 2, 16, U.K.A.E.A., Risley, Warrington.
10. Mellor, J. W., "Comprehensive Treatise on Inorganic and Theoretical Chemistry," Longmans, London, 1961, Volume 2, Supplement II, Part 1, p. 474.
11. Walker, J. A. J., and Seed, H., *Analyst*, 1965, **90**, 18.
12. Mellor, J. W., *op. cit.*, p. 475.
13. Berry, R., *in* van Swaay, M., *Editor*, "Gas Chromatography 1962," Butterworths, London, 1962, p. 321.

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The Rapid Dissolution of Plutonium Dioxide by a Sodium Peroxide Sinter, Followed by Determination of the Plutonium Content by Differential Spectrophotometry

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A method is described for the dissolution of plutonium dioxide and the determination of the plutonium content by differential spectrophotometry. The plutonium dioxide is brought into solution by mixing with excess of sodium peroxide and heating at 400° C for 10 minutes, followed by the addition of a similar amount of sodium hydroxide and re-heating for a further 10 minutes. The cold sinter is then extracted with water and transferred to hydrochloric acid solution containing hydroxylammonium chloride. The solution is heated to decompose the peroxide and reduce the plutonium to the tervalent state. The plutonium concentration is then determined by differential spectrophotometry in 4-cm cells at 565 mμ, comparing with standards prepared from plutonium metal. Mean recoveries on 100-mg amounts of plutonium dioxide that had been ignited at 850° C were 99·8 per cent. with a coefficient of variation of 0·3 per cent. Similar results were obtained on plutonium dioxide that had been ignited to 1550° C, by slightly extending the sintering time.

The difficulty of dissolving plutonium dioxide, particularly after ignition at high temperatures (850° C or greater) is widely acknowledged. The present methods include attack with nitric acid containing hydrofluoric acid,[1] or halogen acids,[2] or hydrochloric and perchloric acids at high pressure.[3] Fusions with bisulphate or bifluoride have also been proposed,[4] but these are time-consuming and in some respects hazardous if not carried out with great care. At A.E.R.E., the spectrophotometric determination of plutonium, by measurement of the Pu^{III} peak at 565 mμ[5] in a reduced dilute hydrochloric acid solution, is in routine use. Hitherto, this measurement was applied to plutonium dioxide after dissolution in nitric acid with added hydrofluoric acid. This approach necessitated evaporation to remove all the hydrofluoric acid and to decrease the nitric acid concentration to a low level before addition of the reducing agent in dilute hydrochloric acid. The dissolution process, therefore, as well as being slow, was also incompatible with the solution conditions required for the subsequent plutonium determination. During investigations into the suitability of fusion techniques as means of dissolving various plutonium-containing ceramics and cermets, it was noted that sintering with sodium peroxide at 500° C was successful in attacking a mixture of plutonium and chromium oxides. The cold sinter, on extraction with water and nitric acid, gave a completely clear solution. This was successfully repeated with plutonium dioxide alone. A more detailed investigation was therefore carried out with a view to developing a rapid technique for the dissolution of plutonium dioxide as a preliminary to the determination of plutonium by differential spectrophotometry.

Experimental

Apparatus—

Platinum crucibles—These should be of 30-ml capacity with suitable lids.
Muffle furnace—A Gallenkamp "Hotspot" or similar equipment is suitable.
Spectrophotometer—A Unicam SP500 or a similar instrument is used.

Reagents—

Sodium peroxide, AnalaR.
Hydrochloric acid—Concentrated AnalaR reagent.
Hydrochloric acid - hydroxylammonium chloride solution—Molar hydrochloric acid containing 5 per cent. w/v hydroxylammonium chloride.

RADIOCHEMICAL SAFETY—

The hazards involved in handling plutonium compounds necessitated that the whole of the work reported in this paper be carried out in glove-boxes.

Previous workers[6] have shown that, for the best results by the sodium peroxide sinter technique, the sample must be finely divided and intimately mixed with excess of sodium peroxide. The temperature should be kept below 500° C to minimise attack on crucibles. In this application platinum vessels were used, as contamination by iron or nickel would interfere with the spectrophotometric determination of the plutonium. The first experiments were carried out with 100-mg amounts of plutonium dioxide (ignited at 850° C) of smaller mesh size than 100 B.S.S. and mixed with 1 g of sodium peroxide. The crucible and its contents were heated at 400° C for 1 hour in a muffle furnace. The contents of the crucible were extracted with dilute perchloric acid, and the solution was evaporated to destroy excess of peroxide and finally made up to 50 ml with the hydrochloric acid - hydroxylammonium chloride solution. The plutonium content was determined by differential spectrophotometry.[5] Some good results were obtained but in general the recoveries were approximately 2 per cent. low, and it could be seen that small amounts of plutonium dioxide remained unattacked by the sintering process. Increasing the quantity of sodium peroxide used to 2 g did not improve the recoveries.

It had been noted that, during the sintering procedure, the contents of the crucible gradually liquefied, presumably due to the formation of sodium hydroxide. It was considered that a melt containing more sodium hydroxide might give a consistency enabling the contents of the crucible to be mixed by swirling, and that this might result in a more complete attack on the plutonium dioxide. The technique was therefore modified to include the addition of sodium hydroxide. A 100-mg sample of plutonium dioxide was mixed with 0·5 g of sodium peroxide and heated in a muffle furnace for 10 minutes at 490° C. The crucible was then removed from the furnace, 0·5 g of sodium hydroxide in pellet form was introduced and the ignition was repeated for a further 10 minutes. The results were better, in that no residues were observed, but the recoveries were still approximately 1 per cent. low. It was concluded at this stage that, although the modified sintering procedure was satisfactory, the extraction technique was not, some of the plutonium not being in the correct valency state for spectrophotometric determination as Pu^{III}.[5,7]

The sinter could be extracted either with water or with dilute hydrochloric acid. Extraction with water might lead to low recoveries due to hydrolysis of plutonium species, which would then evade reduction to Pu^{III}. Acid extraction of an alkali melt might be too vigorous, and hydrochloric acid in the presence of nascent oxygen might lead to attack on the crucible. There was also the need to keep the acid content low in order to favour subsequent reduction of the plutonium to the tervalent state. In one series of 10 experiments, each melt was extracted with 3·0 ml of water and then washed into 5 M hydrochloric acid containing the requisite amount of hydroxylammonium chloride. A yellow - green precipitate was obtained which re-dissolved with difficulty on warming. The mean recovery for plutonium was 99·3 per cent. with a coefficient of variation of 0·3 per cent. The use of a slightly different technique, which involved evaporating the solution with 5 M hydrochloric acid before the addition of hydroxylamine, did not improve the plutonium recovery. At this stage it was noted that the platinum crucible was stained a brown colour after extraction with water. This stain could be removed by washing with 5 M hydrochloric acid. A series of 11 experiments was then carried out in which each melt was extracted with 5 M hydrochloric, evaporated, the plutonium reduced and a spectrophotometric measurement made. The reaction on extraction was extremely vigorous and the recoveries were invariably high, averaging 100·6 per cent. with a coefficient of variation of 1 per cent. It was found in separate experiments that the brown stain on the platinum crucibles contained plutonium, but extraction with 5 M hydrochloric acid removed sufficient platinum to interfere with the spectrophotometric measurements, giving slightly high results. To avoid the contamination by platinum a series of sinters was carried out at 400° C. The results showed that the platinum crucibles were not attacked at this lower temperature, since there was no sign of staining, and that plutonium dioxide dissolved completely.

A further examination of the aqueous extraction of the cold sodium peroxide sinter showed that two reactions were possible, depending upon the volume of water used. If the sinter was extracted with about 3·0 ml of water and then transferred to a dilute acid solution, a green precipitate was obtained. This precipitate (presumably Pu^{VI} peroxide) dissolved

with difficulty on warming, with the evolution of oxygen. The resultant solution had a typical Pu^{III} absorption spectrum. Addition of hydroxylammonium chloride to this solution and then spectrophotometric measurement of Pu^{III} gave low recoveries. If, on the other hand, the sinter was extracted with about 1·5 ml of water and warmed gently for a few minutes before transferring to a dilute acid solution (to neutralise the excess alkali), the resulting solution had a typical Pu^{VI} absorption spectrum. The inclusion of hydroxylammonium chloride in the dilute acid solution in this instance gave a Pu^{III} solution without the formation of any precipitate. It seemed desirable to extract the sinter in a way that avoids the formation of a precipitate not readily decomposed. The extraction technique was therefore modified and carried out with about 1·5 ml of water. The resulting solution or slurry was transferred dropwise with a polyethylene Pasteur pipette to a beaker containing 3 ml of concentrated hydrochloric acid *plus* 5 ml of 5 per cent. hydroxylammonium chloride solution. The crucible was then washed with 1 ml of concentrated hydrochloric acid to remove the final traces of the sinter. A series of determinations carried out with this technique gave a mean recovery of 99·8 per cent. with a coefficient of variation of 0·3 per cent.

PROCEDURE—

Weigh 100 mg of plutonium dioxide and add this to 0·5 g of sodium peroxide in a 20-ml platinum crucible. Mix well with a small nickel spatula, but avoid spreading plutonium dioxide on to the crucible walls. Heat the crucible in a muffle furnace at 400° to 420° C for 10 minutes, and then remove it and add to its contents 0·5 g of caustic soda pellets. Replace the crucible in the furnace and heat it for a further 10 minutes. Increase both heating periods to 15 minutes for plutonium dioxide samples ignited at 1000° C and above. Remove the crucible, swirl the contents gently and allow it to cool. (The hot melt is black with the denser material at the bottom of the crucible.) Add 1 ml of water to the sinter and cover the crucible with a platinum lid. Allow the dissolution reaction to proceed for 5 to 10 minutes. Warm the crucible gently on a hot-plate and inspect frequently. Remove from the hot-plate if the reaction becomes too vigorous. When the reaction has almost ceased, add a further 0·5 ml of water to the mixture and warm it gently until all the solid material disperses. (A black solution of suspension is obtained.) Transfer the extract dropwise by means of a Pasteur pipette to a 50-ml beaker containing 3 ml of concentrated hydrochloric acid *plus* 5 ml of hydrochloric acid - hydroxylammonium chloride solution. Stir during the addition, and rinse the crucible with two 2-ml portions of water and one 1-ml portion of concentrated hydrochloric acid, adding the rinsings to the solution in the beaker. Finally, rinse the crucible with two 2-ml portions of water. Warm the solution on a hot-plate for about 15 minutes. (The solution becomes clear after 5 minutes, but heating is continued for a further 10 minutes to ensure the complete reduction of the plutonium and the decomposition of the peroxide.) Cool the solution and dilute to 50 ml with hydrochloric acid - hydroxylammonium chloride solution. Carry out differential spectrophotometric measurements on this solution at 565 mμ in 4-cm cells.

RESULTS

The procedure was tested on two samples of plutonium dioxide, one having been ignited at 850° C and the other at 1550° C. Both samples were ground to a fine powder in an agate mortar, but the exact mesh-size was not determined. The results, which are shown in Table I, are expressed as percentage recoveries, assuming $PuO_{2\cdot00}$ stoicheiometry. This assumption might introduce a small error in determinations with the oxide ignited at 850° C, but this is negligible relative to the precision obtained. The plutonium determinations were carried out by differential spectrophotometry against standards prepared from plutonium metal.

TABLE I

RESULTS OF THE DETERMINATION OF PLUTONIUM IN TWO SAMPLES OF PLUTONIUM DIOXIDE

Temperature of sample ignition, °C	Recovery, per cent.	Mean recovery, per cent.	Coefficient of variation
850°	99·30, 100·24, 99·62, 99·66, 99·55, 100·10, 100·00, 99·70, 99·80, 100·15	99·81	0·3 %
1550°	99·85, 100·05, 99·70, 99·51, 100·00, 99·95, 99·71, 100·05	99·85	0·2 %

Discussion

The dissolution of plutonium dioxide by the sinter technique is extremely rapid in comparison to the alternative methods available. The method is specifically aimed at a spectrophotometric measurement of the Pu^{III} colour. Sufficient work has been carried out, however, to show that a considerable influence can be made on the valency state of plutonium during the leaching of the sinter. Solutions of Pu^{VI} can be obtained by keeping the volume of the extract low before acidifying, and visual evidence in the preliminary work seemed to indicate that Pu^{IV} solutions are obtained on extraction with nitric acid. This aspect of the technique would be very useful in the adaptation of the dissolution procedure to other analytical methods. We have also found the peroxide sinter technique to be suitable for the dissolution of mixed plutonium and uranium carbides, mixed plutonium and uranium oxides, and plutonium cermet materials.

We thank Mr. G. Weldrick for carrying out some of the spectrophotometric measurements.

References

1. Katz, J. J., and Seaborg, G. T., "The Chemistry of the Actinide Elements," Methuen & Co. Ltd., London, 1957.
2. Cleveland, J. M., *J. Inorg. Nucl. Chem.*, 1964, **26**, 1470.
3. Metz, C. F., and Waterbury, G. R., *in* Kolthoff, I. M., and Elving, P. J., *Editors*, "Treatise on Analytical Chemistry," Volume 9, Interscience Publishers, New York, 1962.
4. Feldman, C., *Anal. Chem.*, 1960, **32**, 1727.
5. Phillips, G., *Analyst*, 1958, **83**, 75.
6. Rafter, T. A., *Ibid.*, 1950, **75**, 485.
7. Phillips, G., U.K. Atomic Energy Authority AERE Report M678.

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An Automated Micro Determination of Blood-Glucose with the AutoAnalyzer

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A glucose oxidase technique is described for the determination of blood-glucose with the AutoAnalyzer. A recorder deflection of 90 to 100 transmission lines is possible for a glucose concentration of 12·5 mg per 100 ml. Only 0·025 ml of blood is required for the determination of the glucose in the range 0 to 500 mg per 100 ml, and a sufficient volume of the diluted sample remains for a repeat analysis if required. Sixty solutions can be analysed in one hour. The cost of the reagents is one penny for every three samples analysed.

Several automated methods based on the glucose oxidase - peroxidase - dye reaction for the determination of true glucose have been reported.[1,2,3,4] With these methods the cost of reagents for the analysis of one sample varies from 9d. to 1¼d. Although the sensitivity of these methods is adequate for the estimation of blood-glucose in a relatively large volume of blood, the methods are not sufficiently sensitive for the determination of blood-glucose concentrations in the small samples obtainable from small laboratory animals.

The blood diluent, a modification of that used by Wincey and Marks,[2] contains 0·9 per cent. w/v of sodium chloride, making the solution isotonic with the blood samples and improving their stability. The concentration of formaldehyde in the blood diluent is 0·05 per cent. w/v and this does not interfere with the method.

To overcome interference from sodium fluoride, magnesium sulphate is used on the donor side of the dialyzer.[3]

Method

Apparatus—

AutoAnalyzer (*Technicon Instruments Ltd.*)—This consists of a sampler unit, proportioning pump, dialyzer with Cuprophan membranes, modified heating bath at 20° C, a colorimeter fitted with a 15-mm tubular flow-cell and 640-mμ filters, and a single-pen recorder with a built-in "X2" range expansion. The range expansion has been built into the recorder to allow the sample peaks to be read with greater accuracy.[5]

The modifications to the heating bath consist of a reduction of the volume of the delay coil to 12 ml and the fitting of a cooling coil. The cooling coil, 7 inches in diameter and of similar shape to the glass delay coil, is made from an 8-foot length of ⅛-inch-bore copper tubing. It is placed between the delay coil and the wall of the heating bath. The inlet and outlet to the coil pass through the spare holes in the top of the heating bath.

The temperature of the heating bath is maintained at 20° C ± 0·1° C by an adjustable thermo-regulator and the cooling coil. Cold tap-water from a simple constant-head device is passed through the copper cooling coil at 15 to 45 litres per hour, thus ensuring that the temperature of the analytical stream is not influenced by ambient temperature fluctuations.

Blood pipettes—Pipettes are made from Pyrex-glass capillary tubing (Scientific Supplies Co. Ltd.), of internal diameter 0·45 to 0·55 mm for the 0·025-ml pipette and 0·85 to 1·00 mm for both 0·05 and 0·1-ml pipettes. These pipettes are calibrated with an Agla micrometer syringe, and a graduation mark is made with a glass-cutting knife.

Reagents—

Blood diluent—Dissolve 1000 units of heparin in 0·9 per cent. w/v sodium chloride. Add 0·125 ml of filtered 40 per cent. w/v formaldehyde and make up to 100 ml with 0·9 per cent. w/v sodium chloride. This solution should be prepared each week.

Sodium acetate buffer solution—Dissolve 27·2 g of analytical-reagent grade sodium acetate trihydrate in 900 ml of distilled water. Add glacial acetic acid until the pH value is 5·0 ± 0·1 and make up to 1 litre.

o-*Tolidine stock solution*—Prepare 1 per cent. w/v stock solution of *o*-tolidine (BDH laboratory reagent) in absolute ethanol. This solution is stable for many months at room temperature.

Peroxidase solution—Dissolve 20 mg of peroxidase, Rz factor = 1, [Hughes and Hughes (Enzymes) Ltd.,] in 100 ml of acetate buffer solution. Store this solution at 4° C.

Fermcozyme 653A—A liquid preparation of glucose oxidase (750 units per ml) [Hughes and Hughes (Enzymes) Ltd.] Store this preparation at 4° C.

Diluent—Dissolve 40 g of analytical-reagent grade magnesium sulphate heptahydrate in distilled water and make up to 1 litre.

Recipient—Dissolve either 16·1 g of analytical-reagent grade anhydrous sodium sulphate (or 36·5 g of analytical-reagent grade sodium sulphate decahydrate) and 1 ml of Triton X-100 (Rohm and Hass) in distilled water and make up to 1 litre.

Solution of enzymes—Add 7 ml of Fermcozyme 653A and 10 ml of peroxidase stock solution to 133 ml of sodium acetate buffer solution. This solution should be prepared just before use.

Dye solution—Swirl 3·0 ml of *o*-tolidine stock solution into 147 ml of sodium acetate buffer solution. This solution should be prepared just before use.

Standard glucose solutions—Prepare a "stock" standard solution containing 50 mg of analytical-reagent grade D-glucose per 100 ml of saturated benzoic acid. The solution must be allowed to stand at room temperature for 24 hours to allow the equilibrium between α and β-glucose to be attained. This is necessary because the method described is specific for β-glucose.[6] Prepare standard glucose solutions by diluting the "stock" solution with saturated aqueous benzoic acid solution to give solutions containing 2·5, 5·0, 7·5, 10 and 12·5 mg per 100 ml.

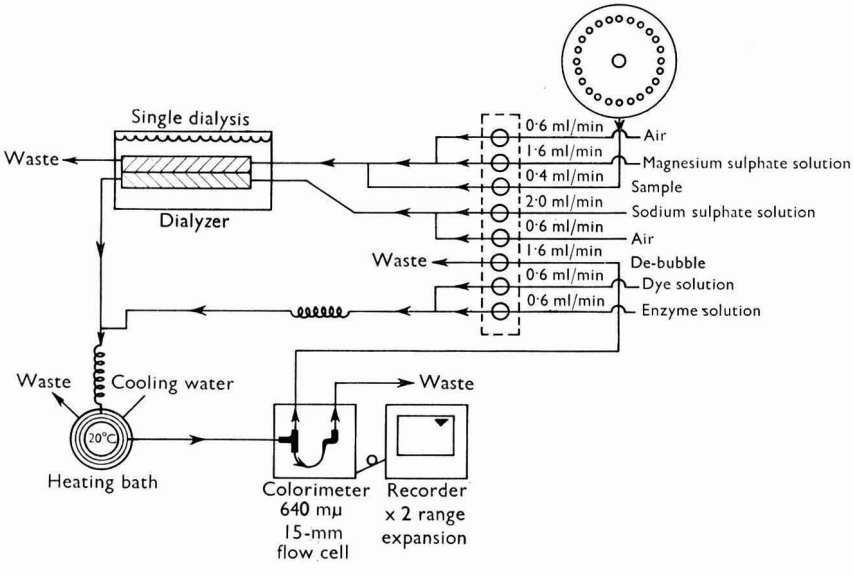

Fig. 1. Flow-diagram for the automated micro determination of blood-glucose

PROCEDURE—

The analyses are performed with single dialysis and the manifold shown in the flow diagram (Fig. 1). The sampler module is set to run at 60 samples per hour. Mixing coils are omitted before dialysis in order to reduce sample contamination; reproducibility is not affected. Samples of blood are diluted in the sample cups, the volume of blood taken and the dilution made depending on the anticipated concentration of blood glucose in the sample. Blood may be diluted as follows—

0·1 ml of blood *plus* 0·9 ml of blood diluent.
0·05 ml of blood *plus* 0·95 ml of blood diluent.
0·025 ml of blood *plus* 0·975 ml of blood diluent.

These dilutions adequately cover blood-glucose concentrations of 0 to 125 mg per 100 ml, of 0 to 250 mg per 100 ml, and of 0 to 500 mg per 100 ml, respectively. A calibration curve is drawn daily from the results obtained with standard glucose solutions of 2·5, 5·0, 7·5, 10·0 and 12·5 mg per 100 ml, which are duplicated at each level.

The concentration of glucose, in mg per 100 ml of blood, was found from the recorder peak by means of the AutoAnalyzer chart-reader, calibrated for the appropriate range.

Results

Factors influencing the kinetics of the enzyme system—

(*i*) *pH*—The optimum pH for the enzyme system is 5·0. Variations of ±0·1 do not appreciably affect the sensitivity (see Fig. 2).

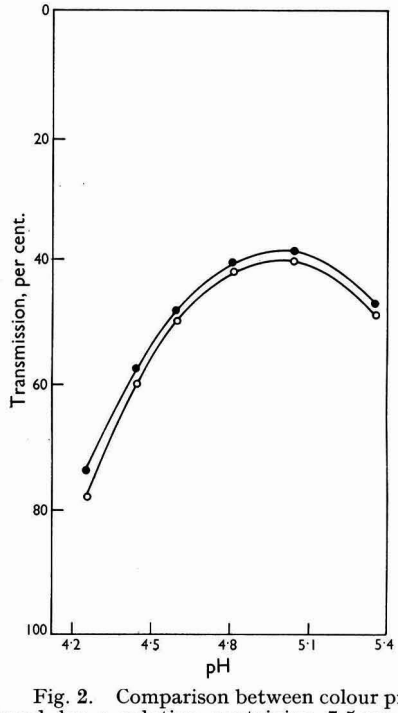

Fig. 2. Comparison between colour produced by a solution containing 7·5 mg of glucose per 100 ml with 6 months' old, ● and fresh, ○, fermcozyme 653A preparations at various pH values

Optimum reproducibility is only attained if the chromogen is presented to the flow cell at maximum colour development. Since incubation time cannot conveniently be altered, it is essential that maximum colour should be developed at the same time with all batches of Fermcozyme 653A. When the enzyme is stored at 4° C there is no loss of activity during 6 months (Fig. 2). It is therefore both convenient and economical to obtain sufficient enzyme for this period. Only two checks for optimum pH are then required each year.

The optimum pH can be determined most conveniently using the AutoAnalyzer. A series of sodium acetate buffer solutions is prepared with a pH range of 4·0 to 5·3. Enzyme and dye solutions are prepared in these buffer solutions. A standard glucose solution is aspirated at each buffer pH and its colour intensity recorded. By plotting colour intensity against the buffer pH, the optimum pH for the colour reaction may be obtained (Fig. 2). The continual aspiration for 15 minutes of a solution containing 12·5 mg of glucose per 100 ml should give limits of not more than ±1·0 of a transmission line (Fig. 3).

(*ii*) *Concentration of peroxidase*—Concentrations of peroxidase greater than 0·33 mg per 100 ml of enzyme solution are not rate limiting (Fig. 4*b*). The concentration chosen allows for adequate substrate saturation.

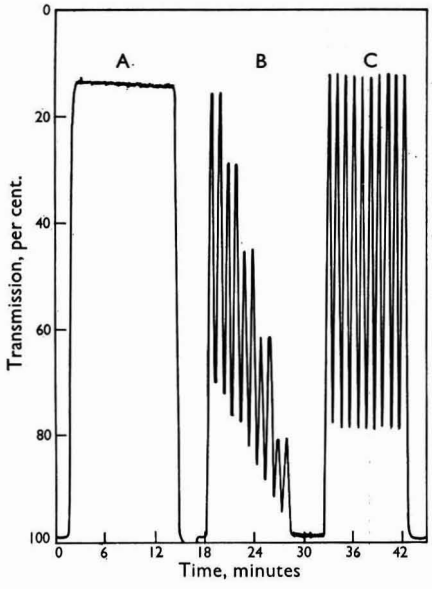

Fig. 3. Recordings showing: (A), the possible limits of reproducibility by means of continual aspiration of a solution containing 12·5 mg of glucose per 100 ml; (B) the reproducibility of duplicates for standard solutions containing 12·5, 10·0, 7·5, 5·0 and 2·5 mg of glucose per 100 ml (C) the error obtained for replicates of a manually diluted blood sample

(*iii*) *Concentration of glucose oxidase* (*Fermcozyme* 653*A*)—The solution of enzymes contains 3500 units of glucose oxidase per 100 ml. If this concentration is doubled the optical density and thus the sensitivity is increased by 10 per cent., but the reagent cost is almost doubled. Because of the saving in cost, the 10 per cent. loss in possible sensitivity with 3500 units of enzyme per 100 ml is considered to be justified (Fig. 4*a*).

(*iv*) *Concentration of dye*—The concentration of *o*-tolidine in the dye solution is 20 mg per 100 ml. The rate-limiting concentration is 1 mg per 100 ml (Fig. 4*c*).

CONTAMINATION BETWEEN SUCCESSIVE SAMPLES—

The maximum sampling rate is governed by the contamination between successive samples (carry-over), and the reproducibility desired. "Carry-over" is the main factor limiting the sampling rate for any particular determination.

Five standard solutions, containing 2·5, 5·0, 7·5, 10·0 and 12·5 mg of glucose per 100 ml, were used to study the "carry-over" effect. Samples from each of these standard solutions were analysed for glucose content after being arranged in an order such that each concentration both preceded and followed the other four concentrations twice. The analyses were then repeated using the same design, but this time identical samples were submitted in pairs, and for the subsequent calculations only the second of each pair was considered. This was based on the hypothesis that the second of a pair would provide a more accurate determination. Finally, a set of eight identical samples was analysed consecutively at three of the standard concentrations. The three designs were repeated on four occasions over a period of 5 weeks.

During the periods between experiments about 200 blood samples were processed in the manifold each day for a 5-day working week. The dialyser membrane was changed after the first two experiments in order to consider changes in membrane "hold-up" volumes and any consequent changes in "carry-over."

Means and coefficients of variation were calculated for each set of 8 determinations; the results are shown in Table I.

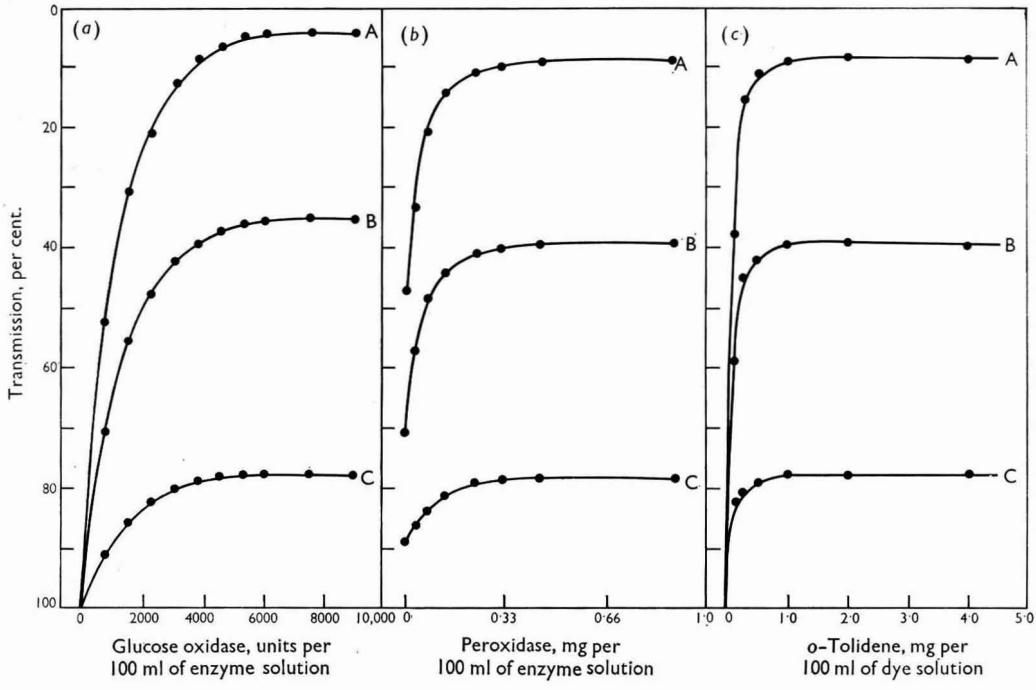

Fig. 4. The influence of (*a*) glucose oxidase concentration, (*b*) peroxidase concentration and (*c*) dye concentration on the amount of colour produced by solutions containing 12·5 mg (curves A), 7·5 mg (curves B) and 2·5 mg (curves C) of glucose per 100 ml

TABLE I

COMPARISON OF MEAN GLUCOSE CONCENTRATIONS AND COEFFICIENTS OF VARIATION FOR VARIOUS CONCENTRATIONS OF GLUCOSE IN AN INVESTIGATION OF THE "CARRY-OVER" EFFECT

Date	Method of sampling‡	Mean* glucose concentrations determined for known (standard) glucose concentrations, mg per 100 ml					Coefficients of variation† for standard glucose concentrations, mg per 100 ml				
		25	50	75	100	125	25	50	75	100	125
September 23rd, 1963	A	26	50·5	75	100	124	3·0	0·8	0·7	0·5	0·6
	B	26	51	76	100·5	125·5	2·2	0·7	0·7	0·5	0·7
	C	25·5	—	75	—	124·5	0·8	—	0·7	—	0·3
October 8th, 1963	A	26	50	75	100	124·5	1·2	0·6	0·7	0·4	0·5
	B	25·5	50	75·5	101	125	0·7	0·5	0·4	0·5	0·6
	C	25·5	—	75·5	—	125	1·1	—	0·6	—	0·3
October 22nd, 1963	A	26	50·5	75·5	100·5	126·5	1·7	0·7	1·5	0·9	0·9
	B	25	50	75·5	100	125·5	1·7	1·3	1·4	1·0	1·1
	C	24·5	—	74·5	—	124	1·0	—	0·9	—	1·1
October 28th, 1963	A	26	50	74	99·5	124	2·9	2·0	1·2	0·8	0·6
	B	24·5	50	75	101	125·5	1·1	0·5	1·0	0·6	0·4
	C	25	—	76	—	126	0·5	—	0·6	—	0·3

* Each value is the mean of eight determinations.

† Coefficient of variation, *i.e.*, the standard deviation expressed as a percentage of the mean reading.

‡ A—Five standard glucose solutions were arranged in an order such that each concentration preceded and followed the other four concentrations twice.

B—The second of duplicate pairs of five standard glucose solutions were arranged in the same order as in A.

C—Three standard glucose solutions were arranged so that each concentration was aspirated eight times consecutively.

If any appreciable "carry-over" occurred, greater variation would be obtained with the single determinations than with the second of a pair. Also, if there was a change in sensitivity with time during each separate experiment, then the paired readings would produce greater variation than the consecutive readings, since their determinations were taken over a longer period than the consecutive readings. The coefficients of variation obtained were compared using Student's t test. The mean coefficients for single readings were not significantly different ($P > 0{\cdot}2$) from those for the duplicate readings at any of the five concentrations, suggesting that there was no significant "carry-over."

The duplicate coefficients were not significantly different ($0{\cdot}5 > P > 0{\cdot}1$) from the consecutive ones, suggesting that there was no significant change in sensitivity with time. The only significant difference obtained ($0{\cdot}05 > P > 0{\cdot}01$) was for the 2·5 mg of glucose per 100 ml solution; for which the mean coefficient for single readings was significantly greater than that for consecutive readings. Continual use of the manifold gave no indication of an increase in the "carry-over."

Glucose recoveries—

Glucose recoveries were determined on the blood from rabbits, guinea-pigs and rats, which had been fasted for 18, 16 and 15 hours, respectively. The recoveries were investigated using glucose solutions equivalent to 25, 50, 75 and 100 mg per 100 ml for an over-all 1 in 20 dilution of blood and glucose. Four recoveries, one at each glucose concentration, were performed on the blood from each of 6 animals, thus giving 24 recoveries for each species. Duplicates were run for each recovery. The results are recorded in Table II.

TABLE II

GLUCOSE RECOVERIES AND COEFFICIENTS OF VARIATION WITH RABBIT, GUINEA-PIG AND RAT BLOOD

Species	Added glucose, mg per 100 ml	Mean recovery, per cent.	Range of recovery, per cent.	Coefficient of variation	Fasting blood-glucose range, mg per 100 ml
Rabbit	25	101	95 to 104	3·0	68 to 96
	50	100	98 to 100	0·8	
	75	99	97 to 100	1·1	
	100	98	97 to 98	0·5	
Guinea pig ..	25	99	96 to 100	1·6	73 to 99
	50	98	96 to 100	1·5	
	75	100	99 to 100	0·7	
	100	99	98 to 100	0·6	
Rat	25	98	96 to 104	3·4	48 to 76
	50	97	96 to 100	1·7	
	75	99	96 to 101	1·8	
	100	98	96 to 100	1·5	

Each recovery is the mean of results from six animals. Samples were analysed in duplicate.

Precision of analysis—

Since it is necessary for samples to be manually diluted before automated analysis is performed, the possible introduction of errors was considered.

Ten operators in turn performed 10 individual analyses from one heparinised blood pool. The results showed that the method was very precise (see Table III). There was no significant difference ($0{\cdot}50 > P > 0{\cdot}20$) between the coefficient of variation for each operator and that for an undiluted glucose solution (8·5 mg per 100 ml), showing that dilution of the blood samples did not significantly affect the precision of the method. The progressive reduction of the blood-glucose concentration is attributed to glycolysis in the heparinised blood sample because of the absence of preservative. The extent of glycolysis is enhanced by the cumulative time of the operators preparing their respective dilutions.

TABLE III

COEFFICIENT OF VARIATION AND MEAN VALUE OF 10 DETERMINATIONS OF BLOOD-GLUCOSE CONCENTRATION FOR A BLOOD SAMPLE DILUTED 10-FOLD BY 10 OPERATORS

Operator	1	2	3	4	5	6	7	8	9	10	Glucose solution*
Glucose, mg per 100 ml	86·7	86·7	86·4	86·6	84·4	84·8	84·8	83·1	83·3	82·4	85·9
Coefficient of variation	0·7	0·5	0·7	0·8	0·6	0·6	0·7	0·6	0·8	0·8	0·6

* Determinations were also made on a glucose solution of known concentration ≡ 85 mg per 100 ml.

SUBSTANCES INTERFERING WITH THE METHOD—

Fourteen substances were examined at three or four concentrations each in conjunction with four concentrations of glucose to see if they interfered with the method. The concentrations of the substances examined for interference were chosen to embrace their possible physiological range, including concentrations found after medication (*e.g.*, ascorbic acid) and their possible use in some analytical procedures (*e.g.*, fluoride). The following substances in the maximum concentrations examined did not interfere with the method—oxidised glutathione (30 mg per 100 ml), potassium oxalate (300 mg per 100 ml), sodium fluoride (200 mg per 100 ml), lactic acid (30 mg per 100 ml), glycogen (12 mg per 100 ml), fructose (6 mg per 100 ml), maltose (10 mg per 100 ml), xylose (50 mg per 100 ml) and heparin (8000 units per 100 ml).

Uric acid, ergothioneine, ascorbic acid, reduced glutathione and formaldehyde were found to interfere with the method. The results are shown in Table IV.

TABLE IV

GLUCOSE RECOVERIES IN THE PRESENCE OF INTERFERING SUBSTANCES

Glucose concentration, mg per 100 ml	Glucose recovered,* %, in presence of— Uric acid, mg per 100 ml 2·5	5·0	10	20	Ergothioneine, mg per 100 ml 1·25	2·5	5·0	Ascorbic acid, mg per 100 ml 4	10	20
25	96	84	74	62	94	92	86	103	88	26
50	97	90	82	69	97	95	88	100	91	69
75	97	94	87	74	97	95	91	101	101	76
100	99	100	88	80	98	96	95	107	98	80

Glucose concentration, mg per 100 ml	Glucose recovered,* %, in presence of— Glutathione (reduced), mg per 100 ml 30	60	120	Formaldehyde, per cent. w/v 0·05	0·1	0·2	0·4
25	98	96	93	100	100	97	94
50	96	91	86	100	98	96	90
75	95	88	81	100	98	96	90
100	97	89	79	100	98	95	90

* Each value is the mean of two observations.

Although uric acid, ergothioneine, ascorbic acid and glutathione interfere with the method at the concentrations shown, glucose recoveries using an over-all 1 in 20 dilution of blood from either rabbit, guinea-pig or rat have shown that the physiological concentrations of these substances do not seriously interfere. A concentration of 0·05 per cent. w/v formaldehyde as used in the blood diluent does not cause interference.

PROPERTIES OF BLOOD DILUENT—

The results in Table V show the comparison of the keeping properties at 4° C of glucose in blood kept in the blood diluent of Wincey and Marks,[2] and those of blood kept in the modified diluent. The same sample of blood was used in both instances. Blood kept in the former diluent showed a gradual increase in glucose concentration over a period of 5 days. This is probably due to the release of free glucose from a glucogenic substance present in the blood cells.

The results also show a small initial difference, which in part can be attributed to the formaldehyde concentration in the diluent of Wincey and Marks.[2] The results are shown in Table V. Since this diluent also caused haemolysis, the release of ergothioneine and glutathione could also be a contributory factor.

Marks and Lloyd[4] have shown that ergothioneine and glutathione inhibit colour development and have used an isotonic diluent to prevent haemolysis.

TABLE V

COMPARISON OF BLOOD-GLUCOSE CONCENTRATION OF SAMPLES STORED IN TWO DIFFERENT BLOOD DILUENTS

Diluent	Initial concentration* of blood-glucose, mg per 100 ml	Concentration of blood-glucose, mg per 100 ml, after—				
		1 day	2 days	3 days	4 days	5 days
A	86	92	94	97	99	97
B	88	88	87	88	88	88

* Mean of 4 results.

Diluent A consisted of 1000 units of heparin and 0·25 ml of 40 per cent. w/v formaldehyde (≡ 0·1 per cent.) made up to 100 ml with distilled water (Wincey and Marks[2]).

Diluent B consisted of 1000 units of heparin and 0·125 ml of 40 per cent. w/v formaldehyde (≡ 0·05 per cent.) made up to 100 ml with 0·9 per cent. w/v sodium chloride.

DISCUSSION

Previous automated determinations of blood-glucose have required more blood for an estimation than can be conveniently obtained from small laboratory animals. It is possible, with the proposed method, to determine a blood-glucose concentration in as little as 0·025 ml of blood. A second analysis may also be made on the remaining diluted sample, should this be necessary. The time between sample aspiration and presentation (the lag time) is approximately 7 minutes, and this rapidity enables close monitoring of blood-glucose levels.

It is inevitable, owing to the high cost of enzymes, that any enzymic method is likely to be more expensive than conventional copper and ferricyanide methods. However, the proposed method is less expensive than previously reported enzymic methods.

The physiological ranges of uric acid in the serum of the rat, rabbit and guinea-pig are 1·8 to 3·0, 1·0 to 4·3 and 1·3 to 5·6 mg per 100 ml, respectively.[7] From Table IV it can be seen that the percentage interference of uric acid in the determination of glucose increases both with increasing concentrations of uric acid and with decreasing concentrations of glucose. The results suggest that, for normal concentrations of uric acid, an under-estimate of the blood-glucose concentration in the normoglycaemic range of 6 per cent. or less would occur. This is in good agreement with the results obtained by Hill and Kessler,[1] who showed that normal physiological concentrations of uric acid interfered with their method, resulting in under-estimation by 4 per cent. of the glucose concentration. Marks and Lloyd[4] also showed under-estimations of 6 per cent. in the presence of normal plasma concentrations of uric acid.

However, glucose recoveries from rabbit, guinea-pig and rat blood show that the under-estimation by the proposed method is no more than 3 per cent. for a 1 in 20 dilution of blood (see Table II). It is probable that this method is unsuitable for the measurement of blood-glucose of gouty patients where the uric acid concentration may be as high as 5 to 15 mg per 100 ml of blood.[8,9] Intracellular glutathione and ergothioneine do not interfere, as haemolysis does not occur in the modified diluent proposed for this method.

The interference caused by the presence of ascorbic acid in glucose solutions has also been noted by previous workers.[10,11,12] Normal blood levels of ascorbic acid are less than 1 mg per 100 ml[7] and do not cause serious interference.

CONCLUSIONS

A method is described for the determination of true blood-glucose in small laboratory animals. The method has been in continual use for 3 years and has proved to be rapid, precise, sensitive and economical.

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References

1. Hill, J. B., and Kessler, G., *J. Lab. Clin. Med.*, 1961, **57**, 970.
2. Wincey, C., and Marks, V., *J. Clin. Path.*, 1961, **14**, 558.
3. Discombe, G., *Ibid.*, 1963, **16**, 170.
4. Marks, V., and Lloyd, K., *Proc. Assoc. Clin. Biochem.*, 1963, **2**, No. 7, 176.
5. Taylor, I. E., and Marsh, M. M., *Ann. N.Y. Acad. Sci.*, 1960, **87**, 775.
6. Middleton, J. E., and Griffiths, W. J., *Brit. Med. J.*, 1957, **2**, 1525.
7. Spector, W. S., *Editor*, "Handbook of Biological Data," W. B. Saunders Co., Philadelphia and London, 1956, p. 53.
8. Talbott, J. H., and Coombs, F. S., *J. Amer. Med. Ass.*, 1938, **110**, 1977.
9. Jacobson, B. M., *Ann. Intern. Med.*, 1938, **11**, 1277.
10. Cawley, L. P., Spear, F. E., and Kendall, R., *Amer. J. Clin. Path.*, 1959, **32**, 195.
11. Salomon, L. L., and Johnson, J. E., *Anal. Chem.*, 1959, **31**, 453.
12. Sunderman, F. W., jun., and Sunderman, F. W., *Amer. J. Clin. Path.*, 1961, **36**, 75.

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Iodimetric and Iodatometric Determination of Thiocarbonate Sulphur

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Oxidimetric methods for determining the strength of aqueous potassium thiocarbonate solution by use of iodine, potassium iodate, and iodic acid are discussed. The iodimetric method is based on the interaction of potassium thiocarbonate with a known excess of iodine in the presence of 0·5 N to N hydrochloric acid and a back-titration of the unreacted iodine with a standard solution of sodium thiosulphate, with starch or carbon tetrachloride as indicator. The amount of sulphur undergoing oxidation corresponds to one of the sulphur atoms of tri-thiocarbonate, while the other two sulphur atoms form carbon disulphide. Satisfactory results have been recorded by taking an aliquot volume of potassium thiocarbonate solution, so that the oxidisable sulphur is only 2 to 8 mg. Greater amounts of sulphur affect the efficiency of the end-point owing to the trapping of iodine by a coherent film of sulphur. Alternatively, titration of potassium thiocarbonate with potassium iodate under similar conditions gives a similar reaction which also results in the oxidation of only one-third of the total sulphur. However, iodic acid is found to oxidise all three sulphur atoms of thiocarbonate, so affording a differentiation of thiocarbonate sulphur from sulphide sulphur and establishing the molar relationship: $HIO_3 \equiv K_2CS_3 \equiv 3H_2S$.

The efficiency of potassium thiocarbonate reagent as a sulphide and thiocarbonate precipitant in qualitative analysis previously reported,[1,2,3,4,5] has justified investigations into its applications to quantitative analysis. This, however, has necessitated standardisation of samples of aqueous potassium thiocarbonate. In a recent communication,[6] a titrimetric (indirect) method and a gravimetric method for standardising concentrated aqueous solutions of potassium thiocarbonate were reported, in which thallous nitrate was used for precipitating the thiocarbonate radical. The total sulphur in potassium thiocarbonate was determined gravimetrically by weighing the dry, reddish-brown thallous thiocarbonate. Subsequent verification of the thallium(I) contents of the products was obtained after their reaction with hydrochloric acid and titration against standard potassium iodate solutions under the conditions described by Andrews.[7] For routine assaying of concentrated aqueous potassium thiocarbonate solutions, the use of standard thallous nitrate solution in a known excess for precipitating the thiocarbonate radical and the back-titration of the excess thallium(I) with standard potassium iodate solution had been suggested. The results obtained were in good agreement with those already known.

Iodimetric and gravimetric methods[8,9,10] for determining sulphur in soluble sulphides have been described in the literature. In the present communication, results of the direct methods of iodimetric and iodatometric estimation of sulphur in 0·01 to 0·04 M solutions of potassium thiocarbonate are discussed.

THE IODIMETRIC METHOD

Reagents—

Iodine solution, 0·1 N—This solution should be prepared from resublimed iodine (May and Baker Ltd.).

Sodium thiosulphate, 0·1 N—Prepare this from analytical-reagent grade (Merck, G.R.) material and standardise against iodine.

Hydrochloric acid, N—Prepare from analytical-reagent grade material (Merck, G.R.).

Starch solution, 1 *per cent., aqueous.*

Carbon tetrachloride—Use analytical-reagent grade material (AnalaR).

SAMPLE—

Potassium thiocarbonate, 2 M—An aqueous solution was prepared by the direct method,[6] and after standardisation was used as a stock solution. This was diluted to between 0·01 and 0·04 M, as these dilute solutions need to be freshly prepared.

PROCEDURE—

Transfer 10 ml of 0·1 N iodine solution to a 250-ml conical flask and add N hydrochloric acid to bring the volume to 50 ml. Add from a pipette an aliquot of the sample such that the amount of sulphur separated is within the 2 to 8-mg range. Shake the reactants and titrate the excess iodine against 0·1 N sodium thiosulphate solution, using carbon tetrachloride or starch solution as indicator. Calculate the potassium thiocarbonate content of the sample solution from the relation—

$$1 \text{ ml N } I_2 \equiv 0{\cdot}0932 \text{ g } K_2CS_3 \equiv 0{\cdot}01603 \text{ g S}$$

The last figure is the amount of oxidisable sulphur (*i.e.*, one-third of the total sulphur) in 0·0932 g of potassium thiocarbonate.

DISCUSSION

It is evident from the results shown in Table I that the reaction of potassium thiocarbonate with acidified iodine is such that only one sulphur atom undergoes oxidation. The amount of sulphur undergoing oxidation corresponds to one-third of the total sulphur present in the compound. Iodimetric oxidation of potassium thiocarbonate in the presence of 0·5 N to N hydrochloric acid is found to occur by the following series of reactions, which shows the removal of 2 sulphur atoms as carbon disulphide—

$$K_2CS_3 + 2HCl \longrightarrow 2KCl + H_2CS_3$$

$$H_2CS_3 + I_2 \longrightarrow 2HI + CS_2 + S$$

$$K_2CS_3 + 2HCl + I_2 \longrightarrow 2KCl + 2HI + CS_2 + S$$

$$\text{Thus, } K_2CS_3 \equiv H_2S \equiv I_2$$

The evolution of carbon disulphide serves to differentiate thiocarbonate from sulphide; with potassium thiocarbonate solutions of high concentration carbon disulphide is easily noticed on shaking the reacting solutions. However, the results of the titrimetric evaluation of potassium thiocarbonate are accurate with 0·01 to 0·04 M solutions, only if an aliquot of sample solution is taken so that 2 to 8 mg of sulphur are precipitated during the complete reaction with acidified iodine. Potassium thiocarbonate solution must be added to the acidified iodine solution and not vice versa. This obviates losses caused by the escape of hydrogen sulphide generated by oxidation and also eliminates any side-reactions.

The results obtained with potassium thiocarbonate solutions of concentrations greater than 0·04 M were not satisfactory, owing either to the adsorption of iodine by sulphur produced in the reaction, or to the uncontrolled generation and oxidation of hydrogen sulphide. Carbon tetrachloride is preferable to starch as indicator, 2 to 5 ml serving efficiently for the extraction of the last traces of iodine and giving a clearly defined end-point. The limiting sensitivity of the starch end-point in the iodimetric titration of ionic sulphur compounds is aggravated by the enclosure of iodine by a coherent film[11] of sulphur separated during the reaction. The amount of iodine necessary to produce a permanent blue colour with starch under favourable conditions is of the order of 0·001 to 0·002 milli-equivalents.[12] In comparison, 0·0004 milli-equivalents of iodine are found to be sufficient for extraction into a carbon tetrachloride layer and to impart a pink colour to it. When more than 5 mg of sulphur is separated, starch is the preferred indicator. The optimum concentration of hydrochloric acid for satisfactory results is 0·5 N to N. Solutions of potassium thiocarbonate more dilute than 0·01 M change into sulphides, polysulphides and thiosulphate on standing, and therefore must be freshly prepared for use.

THE IODATOMETRIC METHOD

Potassium iodate serves as an excellent primary standard[13,14,15,16] in oxidimetric titrations. Iodic acid has many analytical applications[17] recorded in the literature, but there is no reference to its reaction with thiocarbonates. Potassium iodate solution in the presence of 0·5 N to N hydrochloric acid has been used to determine sulphur in potassium thiocarbonate

solution. In the present study, iodic acid is found to have valuable application in differentiating between the sulphur derived from thiocarbonates and that derived from sulphides and in the determination of the total sulphur in potassium thiocarbonate with appreciable accuracy.

REAGENTS—

Potassium iodate, 0·025 N—Prepare from AnalaR grade material, by dissolving in redistilled water.

Iodic acid, 0·025 N—Prepare from E. Merck, G.R. grade material, by dissolving in redistilled water.

Starch solution, 1 *per cent., aqueous.*

SAMPLE—

Potassium thiocarbonate, 2 M—An aqueous solution prepared as described in the iodimetric method.

PROCEDURE—

Transfer 10 ml of 0·025 N potassium iodate solution to a 250-ml conical flask and acidify with 10 to 15 ml of N hydrochloric acid. Introduce dropwise, freshly-prepared 0·02 M potassium thiocarbonate solution from a burette with constant shaking, until a permanent colour of iodine is seen in the solution. Add a few drops of starch solution and continue the addition of potassium thiocarbonate until only a yellow tinge remains. The blue colour of the starch - iodine complex is discharged by a slight excess of potassium thiocarbonate, thus marking the end point.

DISCUSSION

The generation of free iodine during the first step of the reaction of potassium thiocarbonate with potassium iodate acidified with hydrochloric acid, and its final reduction to iodide supports the sequence of reactions—

$$(i) \quad 2KIO_3 + 5K_2CS_3 + 12HCl \longrightarrow I_2 + 12KCl + 5S + 5CS_2 + 6H_2O$$

$$(ii) \quad I_2 + 2HCl + K_2CS_3 \longrightarrow 2HI + 2KCl + S + CS_2$$

$$i.e., \quad 2KIO_3 + 14HCl + 6K_2CS_3 \longrightarrow 2HI + 14KCl + 6S + 6CS_2 + 6H_2O$$

$$\text{Thus, one-third } KIO_3 \equiv K_2CS_3$$

The results, which are shown in Table II, are in good agreement with the known amounts present of potassium thiocarbonate solutions of concentrations that would give 2 to 8 mg of precipitated sulphur. The results show that only one sulphur atom of thiocarbonate is oxidised, *i.e.*, one-third of the total sulphur. Quantitatively the reaction of potassium thiocarbonate with potassium iodate is similar to that of soluble sulphides,[18] except that in

TABLE I

RESULTS OF THE DETERMINATION OF SULPHUR IN POTASSIUM THIOCARBONATE WITH IODINE IN THE PRESENCE OF 0·5 N TO N HYDROCHLORIC ACID

Strength of potassium thiocarbonate solution, M	Volume of potassium thiocarbonate solution taken, ml	Titre of 0·1 N iodine, ml	Total sulphur present, mg	Sulphur found,* mg
0·01	5	1·01	4·80	1·62
	10	2·00	9·60	3·20
	20	4·01	19·20	6·42
	25	5·02	24·00	8·03
	30	6·05	28·80	9·68
0·02	5	2·01	9·60	3·22
	10	4·00	19·20	6·40
0·04	2	1·60	7·68	2·56
	4	3·21	15·36	5·14
	6	4·82	23·04	7·71
0·1	1	2·03	9·60	3·25
	2	4·05	19·20	6·48

* Oxidisable sulphur is one-third of the total sulphur.

TABLE II

RESULTS OF THE DETERMINATION OF SULPHUR IN POTASSIUM THIOCARBONATE WITH POTASSIUM IODATE IN THE PRESENCE OF 0·5 N TO N HYDROCHLORIC ACID

Volume of 0·025 N potassium iodate taken, ml	Titre of 0·025 M potassium thiocarbonate, ml	Total sulphur present, mg	Sulphur found,* mg
5	3·14	6	2·01
10	6·25	12	4·00
15	9·35	18	5·98
20	12·45	24	7·97
25	18·70	30	9·95

* Oxidisable sulphur is one-third of the total sulphur.

the former, carbon disulphide is evolved. With higher concentrations of hydrochloric acid (4 to 6 N), the sequence of reactions is different in that iodine monochloride is formed instead of free iodine in the first step. This is further reduced by potassium thiocarbonate to iodide, as a second step. The results obtained if 4 to 6 N hydrochloric acid is used, however, are not reproducible although they always indicate that more than one sulphur atom of the thiocarbonate radical has undergone oxidation. Obviously this is caused by the speedy or uncontrolled transformation to hydrogen sulphide and carbon disulphide of thiocarbonate acid produced by the action of 4 to 6 N hydrochloric acid on potassium thiocarbonate. The difference in the results is caused by the varying amounts of hydrogen sulphide produced from potassium thiocarbonate at low pH values.

Potassium thiocarbonate solutions of concentrations less than 0·01 M are not suitable for titration, as such weak solutions readily undergo transformation and results are not satisfactory.

THE METHOD WITH IODIC ACID

PROCEDURE—

Transfer 10 ml of 0·025 N iodic acid solution to a 250-ml conical flask. Add dropwise to the solution 0·02 M potassium thiocarbonate, freshly prepared by dilution of its standard stock solution, until a permanent faint yellow colour of iodine remains. Add a few drops of starch indicator solution and continue the addition of 0·02 M potassium thiocarbonate until the blue colour is discharged at the end-point. At least two concordant readings should be obtained.

DISCUSSION

The reaction of potassium thiocarbonate with iodic acid is such that all 3 sulphur atoms undergo oxidation—

$$HIO_3 + K_2CS_3 \longrightarrow KI + KHCO_3 + 3S$$

$$\text{Thus, } HIO_3 \equiv K_2CS_3 \equiv 3H_2S$$

Thus the equivalent weights of iodic acid and potassium thiocarbonate are one-sixth of their molecular weights.

Iodatometric oxidation of all 3 sulphur atoms in potassium thiocarbonate by iodic acid is possible because of two factors. First, the reaction between potassium thiocarbonate and iodic acid is direct, unlike that between hydrochloric acid and potassium iodate. With hydrochloric acid, thiocarbonic acid is first liberated from potassium thiocarbonate as an intermediate step before its interaction with iodate-iodine is possible. Secondly, iodic acid is relatively much weaker than hydrochloric acid, as evident from the values[19] $pK_a = 0{\cdot}77$; $K_{inst} = 1{\cdot}67 \times 10^{-1}$, for iodic acid. The reaction between iodic acid and potassium thiocarbonate is gradual, so permitting all 3 sulphur atoms to undergo oxidation. There is a change of 6 electrons for each mole of iodic acid undergoing reduction to iodide and for each mole of potassium thiocarbonate undergoing oxidation to sulphur.

As the precipitation of elemental sulphur is 3-fold in the oxidation with iodic acid, starch is preferable to carbon tetrachloride as an indicator, because the latter extracts not only iodine but also some sulphur, thereby obscuring the end-point. In the presence of elemental sulphur the discharge of the blue colour of the starch - iodide complex is easily noticed. The difficulty caused by the last traces of iodine being enclosed by a coherent

TABLE III

RESULTS OF THE DETERMINATION OF TOTAL SULPHUR IN POTASSIUM THIOCARBONATE WITH IODIC ACID

Volume of 0·025 N iodic acid taken, ml	Titre of 0·02 M potassium thiocarbonate, ml	Sulphur present, mg	Sulphur found, mg	Sulphur present *minus* sulphur found, mg
5	1·05	2	2·01	+0·01
10	2·08	4	4·00	0·00
15	3·12	6	5·99	−0·01
20	4·15	8	7·97	−0·03
25	5·10	10	9·91	−0·09

film of sulphur is obviated to a great extent by titrating the minimum amount of iodic acid with potassium thiocarbonate so that not much sulphur is produced and titre values are kept within permissible limits of error.

The fact that all 3 sulphur atoms of thiocarbonate are oxidised by iodic acid and no carbon disulphide is evolved, is supported by the negative result of a colorimetric test.[20] In this test a drop of the solution should produce a stable pink colour if acetone and elemental sulphur are present together with free carbon disulphide.

CONCLUSIONS—

Results are quantitative with 0·01 to 0·04 M potassium thiocarbonate. Suitable dilution of more concentrated solutions is necessary to obtain accurate results. With iodic acid under optimum conditions all the 3 sulphur atoms of thiocarbonate are oxidised, thus affording a good method of determining and differentiating thiocarbonate from other sulphur compounds.

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REFERENCES

1. Johri, K. N., *J. Sci. Ind. Res. India*, 1959, **18B**, 430.
2. ——, *J. Indian Chem. Soc.*, 1961, **38**, 935.
3. ——, *Analyst*, 1961, **86**, 487.
4. ——, *Indian J. Appl. Chem.*, 1963, **26**, 114.
5. ——, "Chemical Analysis without Hydrogen Sulphide using Potassium Trithiocarbonate," Asia Publishing House, Bombay 1, 1963.
6. ——, and Singh, K., *Indian J. Chem.*, 1965, **3**, 158.
7. Andrews, L. W., *J. Amer. Chem. Soc.*, 1903, **25**, 756.
8. Jacobs, M. B., "The Analytical Chemistry of Industrial Poisons, Hazards and Solvents," Second Edition, Interscience Publishers, New York, 1949, p. 323.
9. Scott, W. W., "Standard Methods of Chemical Analysis," Fifth Edition, D. Van Nostrand, New York, 1939, Volume 1, pp. 911–915, and 923.
10. Duval, C., "Inorganic Thermogravimetric Analysis," Elsevier Publishing Company, Amsterdam, 1953, pp. 134–135.
11. Brunck, O., *Z. anal. Chem.*, 1906, **45**, 541.
12. Levin, L., and Swann, W. B., *Talanta*, 1958, **1**, 276.
13. Jamieson, G. S., "Volumetric Iodate Methods," Chemical Catalog Co., New York, 1926.
14. Kolthoff, I. M., and Stenger, V. A., "Volumetric Analysis," Second Edition, Interscience Publishers, New York, 1947, Volume 2, p. 88 *et seq.*
15. Stenger, V. A., *Anal. Chem.*, 1951, **23**, 1540.
16. Tuthill, S. M., Sprague, R. S., and Stoeker, W. C., *Ibid.* 1964, **36**, 1994.
17. Smith, G. F., "The Analytical Applications of Periodic Acid, Iodic Acid, and their Salts," Fifth Edition, G. Frederick Smith Chemical Co., Columbus, Ohio, 1950.
18. Bethge, P. O., *Anal. Chim. Acta*, 1954, **10**, 310.
19. Albert, A., and Serjeant, E. P., "Ionization Constants of Acids and Bases," Methuen and Co. Ltd., London, 1962.
20. Urbanski, T., *Talanta*, 1962, **9**, 799.

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Methods for Micro- and Semi-micro Determination of Thiamine in Pharmaceutical Preparations

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Methods are described for the semi-micro titrimetric determination, and micro-colorimetric determination of thiamine in pure solutions and in mixtures of pharmaceutical preparations.

They depend on precipitation of thiamine with a special iodobismuthic acid reagent, and determination of the iodine content of the complex by direct titration with *N*-bromosuccinimide, or the bismuth content of the complex with EDTA, or dissolution of the complex in potassium iodide - acetone mixture and measurement of the red colour of the solution at 345 mμ, or in a photoelectric colorimeter with a violet filter.

The methods have been applied to different forms of thiamine-containing preparations, and the results are compared with the official gravimetric silicotungstic acid method and the fluorimetric method.

The average mean error for the semi-micro method is $\pm 0{\cdot}5$ per cent. for amounts of thiamine from 1 to 10 mg, and for the micro method the error is about $\pm 1{\cdot}7$ per cent. for amounts of thiamine ranging from 5 to 150 μg.

NUMEROUS analytical procedures, including biological, microbiological fermentative, physical and chemical methods, have been employed for the assay of thiamine.[1] Most important of these methods in pharmacy are the chemical methods, which include colorimetric,[2] fluorimetric,[3,4] gravimetric[5,6,7] and volumetric procedures of the complexometric and non-aqueous types.[8]

The fluorimetric assay of thiamine in pharmaceutical preparations is hampered by the presence of interfering substances such as excipients, flavouring and colouring matter, as well as other active organic and inorganic ingredients included in the finished product, which quench or diminish the fluorescence of thiochrome.

The Adamson and Handisyde[2] modification of Bertrand's gravimetric procedure,[5,6] in which silicotungstic acid is used, offers several advantages, the most important being greater accuracy.[7] However, the method is not directly applicable to products containing such ingredients as syrups, betaine, choline or nicotinamide. Interference due to complex formation necessitates preliminary chromatographic separation before fluorimetric or gravimetric procedures, which in this instance lengthens an already long procedure.

Budesinsky and Vanikova[8] used an excess of Dragendorff's reagent for the quantitative precipitation of thiamine, and then determined the excess of reagent by titration with EDTA.

On repeating Budesinsky's work, we found that Dragendorff's reagent precipitated thiamine quantitatively from pure solutions only. In the presence of relatively high concentrations of sodium or potassium acetate, citrates, tartrates, orthophosphates and chlorides, precipitation was delayed or completely prevented. This difficulty was finally eliminated by modifying the precipitating reagent. When the concentration of bismuth was increased and that of potassium iodide decreased, the interfering action of the above salts was consequently eliminated, and the precipitation of thiamine became complete and more or less specific.

The new reagent did not produce any precipitate with any of the other B-complex factors, such as riboflavine, pyridoxine, nicotinic acid, nicotinamide, calcium pantothenate and cyanocobalamin, nor with ascorbic acid. Affected thiamines, such as thiochrome and hydrovitamin, as well as its hydrolytic products, the pyrimidine and thiazole moieties, are not precipitated by the reagent.

Choline and betaine are the only two components similarly precipitated. However, this difficulty could be avoided by washing the precipitate repeatedly with alcohol until the alcoholic washings were free from any red colour (the colour of the soluble choline or betaine complex). Excluding most alkaloids, no other substances encountered in this work were found to interfere by precipitation.

Thiamine was then determined in the insoluble bismuth iodide complex iodometrically by determining the iodine content of the complex, or complexometrically by determining the bismuth content with standard EDTA solution.

Thiamine was also determined by dissolving the complex formed in acetone - potassium iodide mixture to give a red solution; the colour intensity was read either in a spectrophotometer at 345 mμ or in a photo-electric colorimeter with the appropriate filter.

METHOD

REAGENTS—

Stock bismuth solution—Dissolve 73 g of bismuth oxynitrate in 250 ml of hydrochloric acid, sp.gr. 1·18, dilute the solution to a litre with water, and shake it well. This solution is approximately 0·25 M and keeps indefinitely.

Concentrated potassium iodide solution, 40 per cent. w/v, aqueous—Dissolve 40 g of potassium iodide in 1 per cent. aqueous ascorbic acid solution, and dilute to 100 ml with water.

Iodobismuthic acid solution—To 15 ml of the concentrated potassium iodide solution, add, in a fine stream and with continuous mixing, 20 ml of stock bismuth solution, and then dilute the mixture with 65 ml of water while stirring. This reagent must be freshly prepared.

Dilute potassium iodide solution, 4 per cent. w/v, aqueous.

Standard potassium iodide solution, 0·005 M.

Standard N-bromosuccinimide, 0·005 M—Dissolve 0·89 g of pure *N*-bromosuccinimide in about 800 ml of water and dilute the solution to 1 litre with water. Standardise the reagent as follows: introduce 20 ml of 0·005 M potassium iodide in a 250-ml glass-stoppered conical flask, add 40 ml of hydrochloric acid, sp.gr. 1·18, cool the mixture in ice to 5° C, and titrate it with *N*-bromosuccinimide solution, using chloroform to indicate the end-point; calculate the exact molarity.

Standard zinc solution, 0·01 M—Dissolve 0·8138 g of analytical-reagent grade zinc oxide (previously dried by heating to 900° to 1000° C for 20 minutes and cooling in a desiccator over calcium chloride) in the minimum volume of hydrochloric acid, sp.gr. 1·18, with the aid of heat. Cool the solution to room temperature and dilute it to 1 litre with doubly distilled water.

Eriochrome black T solid indicator—Dilute one part of Eriochrome black T with 400 parts of analytical-reagent grade sodium chloride.

Ammonia buffer solution, pH 10—Dissolve 67·5 g of pure ammonium chloride in about 100 ml of doubly distilled water, add 570 ml of ammonia solution, sp.gr. 0·88, and sufficient doubly distilled water to bring the volume to 1 litre.

Standard EDTA solution, 0·01 M—Dissolve 3·729 g of analytical-reagent grade ethylenediaminetetra-acetic acid, disodium salt dihydrate, in a few millilitres of water, and dilute the solution to 1 litre. Standardise the prepared reagent against the standard zinc solution as follows: place 20 ml of 0·01 M zinc solution in a 250-ml conical flask, add 10 ml of ammonia buffer solution and a speck of Eriochrome black T. Titrate the solution with the prepared EDTA solution until the wine-red colour of the solution changes sharply into clear blue.

PROCEDURE

SEMI-MICRO (TITRIMETRIC) METHOD—

Transfer 5 ml of the sample solution (containing 1 to 20 mg of thiamine hydrochloride) to a 25-ml centrifuge tube. Add 5 ml of iodobismuthic acid solution, mix well and allow to stand for 5 minutes. Add 2 ml of alcohol and spin the mixture in a centrifuge for 10 minutes at about 3500 r.p.m. Decant the supernatant liquid and wash the precipitate three times each with 5 ml of alcohol, taking care to break the precipitate and wash the sides of the tube, and spin in the centrifuge for 5 minutes each time; then decant off the clear supernatant liquid. After the last washing and decantation drain the tube well. The determination of thiamine in the contents of the tube may be carried out by either of the following methods.

(*a*) *By titration of the iodine content of the complex*—Transfer the precipitate to a 500-ml glass-stoppered conical flask with the aid of 20 ml of water and 40 ml of hydrochloric acid and shake it until the precipitate dissolves completely. Cool the solution to about 5° C, and titrate with 0·005 M *N*-bromosuccinimide.

During the titration, iodine is produced in the solution and gradually increases in concentration until, on further addition of *N*-bromosuccinimide solution, the brown colour

of iodine starts to fade. When the colour of the solution is pale yellow, add 5 ml of chloroform and continue the titration with frequent vigorous shaking until the violet colour disappears from the chloroform layer.

Each millilitre of 0·005 M *N*-bromosuccinimide is equivalent to 0·2108 mg of thiamine hydrochloride or 0·2046 mg of thiamine mononitrate.

The results obtained by this procedure are shown in Table I.

(*b*) *By titration of the bismuth content of the complex*—Transfer the contents of the centrifuge tube to a 250-ml conical flask by using about 2 ml of nitric acid and 20 ml of water. Add a sufficient volume of 5 per cent. sodium thiosulphate solution to remove the liberated iodine. Add a known excess (about 20 ml) of the standard EDTA solution and neutralise the solution to litmus with ammonia solution. Add 10 ml of ammonia buffer solution, a speck of Eriochrome black T and titrate the excess of EDTA with standard zinc solution to the first wine-red colour.

Each millilitre of 0·01 M EDTA is equivalent to 1·6864 mg of thiamine hydrochloride or 1·6368 mg of thiamine mononitrate.

The results obtained by applying this method are shown in Table I.

TABLE I

COMPARISON OF RESULTS FROM THE TWO TITRIMETRIC METHODS

Iodimetric method			Complexometric method		
Thiamine			Thiamine		
present, mg	found, mg	Recovery, per cent.	present, mg	found, mg	Recovery, per cent.
1	0·994	99·4	5	4·88	97·6
1	1·005	100·5	5	4·88	97·6
2	2·010	100·5	5	4·88	97·6
2	1·989	99·5	5	5·06	101·2
2	1·989	99·5	10	9·86	98·6
2	2·010	100·5	10	9·95	99·5
2	2·010	100·5	10	9·78	97·8
4	3·935	98·5	10	9·95	99·5
4	3·956	98·9	20	19·90	99·5
10	9·901	99·0	20	19·90	99·5
10	10·048	100·5	20	20·08	100·4
Average		99·5	Average		99·0
Standard deviation =		0·6	Standard deviation =		1

MICRO (PHOTOMETRIC) METHOD—

Transfer 1 ml of the sample solution (containing from 5 to 150 μg but preferably not less than 30 μg) to a 10-ml centrifuge tube and precipitate, wash and decant as previously described in the semi-micro procedure.

To the residue in the centrifuge tube, add 0·1 ml of concentrated potassium iodide solution and 8 ml of acetone and shake until the precipitate has dissolved. Measure the optical density of the red colour in a spectrophotometer at 345 mμ within 5 minutes. The colour starts to fade after 5 minutes, especially in daylight.

The results obtained by this method are shown in Table II.

TABLE II

RESULTS OF THE PHOTOMETRIC DETERMINATION OF THIAMINE

Thiamine used, μg	Optical density	Thiamine found, μg	Error, per cent.	Thiamine used, μg	Optical density	Thiamine found, μg	Error, per cent.
5	0·086	4·8	−4	60	1·050	60·9	+1·5
10	0·170	9·8	−2	70	1·205	69·9	−0·14
20	0·350	20·3	+1·5	80	1·410	81·5	+1·9
30	0·515	29·8	−0·7	90	1·540	89·0	−1·1
40	0·680	39·3	−1·7	100	1·680	97·0	−3
50	0·900	52·0	+4	150	2·600	150·0	0

DETERMINATION OF THIAMINE IN PHARMACEUTICAL PREPARATIONS—

Pharmaceutical preparations containing thiamine, either solely or in mixtures with other vitamins or other ingredients of a different nature, were assayed by both the iodimetric method and by the micro method. For the sake of comparison the thiamine content of these preparations was also determined by the gravimetric silicotungstic acid method[9] and the fluorimetric method.[4] The following procedures were adopted for the different types of preparation.

(*a*) *Tablets*—A weighed quantity of the powdered tablets was treated as directed by the British Pharmacopoeia, 1958, for "Aneurine tablets." The final volume of solution was adjusted to result in a suitable concentration of thiamine; *i.e.*, 1 to 20 mg per 5 ml for the iodimetric method and 30 to 50 μg for the micro method.

(*b*) *Ampoules and syrups*—The sample was simply diluted with water to give a solution with a suitable concentration of thiamine.

(*c*) *Capsules*—Ten capsules were sliced longitudinally and introduced into a beaker containing about 25 ml of water. The mixture was well stirred and, as soon as the capsules were washed of their contents, the clear liquid was decanted into a 250-ml calibrated flask. The residue in the beaker was washed with 3 successive 10-ml portions of water, these added to the flask and the volume made up to 250 ml with water.

After thorough mixing, 5 ml of the solution were withdrawn, 10 ml of 10 per cent. sodium tungstate solution were added and the solution mixed. Ten ml of 2 per cent. v/v sulphuric acid were added, the solution was mixed and allowed to stand for a few minutes. Precipitated gelatin was filtered off, the filter being washed several times with water. The filtrate and washings were diluted to a suitable concentration for either the semi-micro or micro procedures.

It should be noted that the more rapidly this treatment is done, the less gelatin will be dissolved in the vitamin extract.

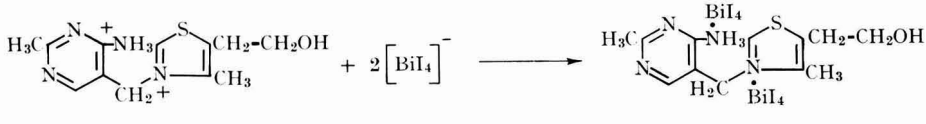
CONTROL EXPERIMENTS—

To ascertain the accuracy of the methods in their application to pharmaceutical preparations, control experiments were carried out by adding a known quantity of thiamine to a sample whose thiamine content had already been determined, and the total thiamine content then determined. The percentage recoveries of the added thiamine had almost the same accuracy as those previously found with pure thiamine solution.

The results obtained by applying the methods to 8 different pharmaceutical preparations are shown in Table III.

DISCUSSION

It is suggested that the reaction between thiamine and iodobismuthic acid proceeds in the following manner—



This means that every molecule of thiamine - bismuth iodide complex contains two atoms of bismuth and 8 atoms of iodine. This was found to be so on determining the bismuth content with EDTA and the iodine content with *N*-bromosuccinimide. The factors used in calculating the thiamine content, as well as the iodine and bismuth contents, were based on the assumption that the above formula is correct and worked to give stoicheiometric results.

The use of *N*-bromosuccinimide in the determination of iodides was suggested by the reported oxidative activity of that compound.[10,11] Using it as redox reagent, similar to an iodate, we obtained good results.[9,12] The results in Table I show the accuracy of the method, which has an error of $\pm 0{\cdot}5$ per cent. and a standard deviation not more than 0·6.

With the pharmaceutical preparations listed in Table III, the method gave reliable determinations of the vitamin in both simple and complex preparations. The gravimetric method, however, showed marked deviations, especially in the analysis of complex mixtures as in samples Nos. 3 to 6, where an error of about −30 to −23 per cent. is noticed.

TABLE III

A COMPARISON OF THE RESULTS FROM THE VARIOUS METHODS FOR THE DETERMINATION OF THIAMINE IN PHARMACEUTICAL PREPARATIONS

			N-bromosuccinimide (semi-micro) method			Silicotungstic method		Colorimetric (micro) method			Fluorimetric method	
Sample No.	Pharmaceutical preparation	Stated thiamine content	Thiamine found,* mg	Proportion of stated amount, %	Recovery† of added thiamine, %	Thiamine found,* mg	Proportion of stated amount, %	Thiamine found,* mg	Proportion of stated amount, %	Recovery‡ of added thiamine, %	Thiamine found,* mg	Proportion of stated amount, %
1	Alphavit B_1 ampoules	100 mg/ml	97·21	97·2	99·9	95·39	95·4	98·18	98·2	99·0	98·00	98·0
2	Alphavit B_1 tablets	100 mg/tablet	89·85	89·9	99·7	88·23	88·2	96·10	96·1	99·0	100·0	100·0
3	Vitacid B-complex vial	100 mg/ml	104·80	104·8	99·9	71·47	71·5	104·0	104·0	99·5	103·2	103·2
4	Beco tablets	20 mg/tablet	18·75	93·8	99·3	12·58	62·9	18·59	93·0	101·07	18·67	93·4
5	Phosphoplex-C syrup	80 mg/100 ml	80·96	101·2	100·2	70·24	87·8	79·44	99·3	100·0	79·20	99·0
6	Phosphoplex with iron	80 mg/100 ml	95·07	118·8	99·8	79·07	98·8	94·74	118·4	99·2	87·44	109·3
7	C-Bex syrup	0·2 mg/ml	0·27	135·0	101·3	§	§	0·27	135·0	98·5	0·27	135·0
8	Vitotal capsules	2·5 mg/capsule	§	§	§	§	§	3·1	124·0	100·0	3·25	130·0

* Each result represents the average of at least two determinations.
† 1 to 5 mg added in each control experiment.
‡ 20 μg added in each control experiment.
§ In these instances the thiamine content was too low to be detected by the method in use.

The determination of iodine in the complex offers a more sensitive method than the determination of bismuth as each molecule of complex contains 8 atoms of iodine compared with 2 atoms of bismuth. However, the determination of bismuth in the precipitate[13] with EDTA gave conforming results with slightly higher error than those of the iodimetric method.

The bismuth iodide - thiamine complex is insoluble in alcohol, ether, chloroform and light petroleum. It is sparingly soluble in potassium iodine solution, giving a yellow colour, and in acetone, giving a red colour. It is readily soluble in acetone - potassium iodide mixture, giving an intense red colour. This latter property was utilised in the colorimetric determination of the complex. It was found that a ratio of one part of potassium iodide solution (40 per cent.) to 80 parts of acetone is the best for producing the maximum intensity of the red colour which is stable for 5 minutes. The maximum absorption of the colour is at 345 mμ, and the optical density is proportional to the concentration of thiamine. The average $E^{1\%}_{1cm}$ was calculated as 1400 for thiamine hydrochloride and 1358 for thiamine mononitrate.

When this method was applied to pure thiamine solutions, a mean error of about $\pm 1{\cdot}7$ per cent. was found. With pharmaceutical preparations a similar accuracy was attained, and results by the proposed method conform with those obtained by use of the U.S.P. fluorimetric method for the determination of thiamine.[4] However, the latter method is open to interferences from the decomposition products of thiamine itself, particularly to thiochrome, as well as the presence of any fluorescent material in the mixture.

References

1. Sebrell, W. H., jun., and Harris, R. S., "The Vitamins," Academic Press Inc., New York, Volume III, 1954.
2. Association of Vitamin Chemists, "Methods of Vitamin Assay," Second Edition, Interscience Publishers Inc., New York, 1951.
3. Garratt, D. C., "Quantitative Analysis of Drugs," Chapman and Hall, London, 1955.
4. United States Pharmacopoeia, Sixteenth Edition.
5. Bertrand, G., *Bull. Soc. Chim. France*, 1899, **21**, 434.
6. ——, *Compt. Rend.*, 1899, **128**, 742.
7. Vannatta, E. E., and Harris, L. E., *J. Amer. Pharm. Ass., Sci. Edn.*, 1959, **48**, 30.
8. Budéšinský, B., and Vanikova, E., *Českosl. Farm.*, 1956, **5**, 579; *J. Pharm. Pharmacol.*, 1958, **10**, 577.
9. British Pharmacopoeia 1958.
10. Barakat, M. Z., and Abdulla, A., *Analyst*, 1960, **85**, 285.
11. Barakat, M. Z., Abdel Wahab, M. F., and Sadr, M. M., *Anal. Chem.*, 1955, **27**, 536.
12. Said, F., Amer, M. M., and George, Z. A., *Bull. Faculty of Pharmacy, Cairo University*, 1964, **2**, 59.
13. Wilson, C. L., and Wilson, D. W., *Editors*, "Comprehensive Analytical Chemistry," Volume IB, Elsevier Publishing Co., Amsterdam, 1960, p. 355.

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SHORT PAPERS

The Spectrophotometric Determination of Manganese after Oxidation with Sodium Perxenate

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The spectrophotometric determination of manganese by the distinctive colour of the permanganate ion has been used for many years. The two oxidising agents commonly employed are periodate and persulphate.[1] The periodate method, first proposed in 1917 by Willard and Greathouse,[2] was used in 1942 at the Metallurgical Laboratory of the University of Chicago for the determination of manganese in uranium metal and its compounds, after prior separation of the uranium.[3] Later in 1942, I demonstrated the feasibility of a direct determination of manganese in the presence of uranium by using periodate. Procedures involving this reagent have been described for the determination of manganese in uranium[4] and uranium - manganese alloys.[5]

Sodium perxenate, first prepared in 1963,[6] is a very powerful oxidising reagent with an oxidation potential estimated to be 3·0 volts in acid solution.[7] It is capable of oxidising Mn^{II} to permanganate almost instantaneously in dilute acid solution at room temperature and without a catalyst.[7] The method for qualitative or quantitative application is rapid and simple. Its use in the spectrophotometric determination of manganese results in a considerable saving of time and effort, since it eliminates the necessity of heating, maintaining at near-boiling temperature and cooling to room temperature before spectrophotometric measurement.

Method

Apparatus—

A Beckman DU spectrophotometer was used to measure the optical density of the permanganate colour at 525 mμ, with the slit width set at 0·03 mm.

Sodium perxenate reagent—

Although the chemistry of perxenates has been extensively described,[7] some properties of sodium perxenate that may be of particular interest to the analytical chemist will be reviewed. Sodium perxenate was first discovered as a hydrolysis product of xenon hexafluoride in concentrated sodium hydroxide solution.[6] It can be precipitated in purer form and with better yield by passing ozone into a solution of xenon trioxide in M sodium hydroxide. The trioxide can be prepared by the hydrolysis of xenon hexafluoride in the presence of magnesium oxide.[7] The sodium compound is a white crystalline solid that corresponds to the composition $Na_4XeO_6.xH_2O$. It may contain from 0 to 8 molecules of water of crystallisation depending on the manner in which it is dried. The reagent I used was dried in a vacuum over magnesium perchlorate and the value of x was approximately 0·5. Sodium perxenate is a very stable compound and can be stored indefinitely at room temperature. It dissolves in water giving a clear, light yellow, alkaline solution that slowly decomposes evolving oxygen and forming Xe^{VI}. A saturated solution of this reagent is approximately 0·025 M.

Octavalent xenon is unstable in acid solution, rapidly evolving oxygen and forming Xe^{VI}. Both Xe^{VIII} and Xe^{VI} will oxidise iodide to iodine. Therefore, with two titrations, the sodium perxenate can be assayed for total chemically bound xenon and the mean valency determined. Since only octavalent xenon is effective in the instantaneous oxidation of Mn^{II} to permanganate, it is important to know the oxidising power of the salt. For the first titration, the salt is dissolved in 90 ml of a solution containing 4 g of potassium iodide and 2 g of sodium bicarbonate (to furnish carbon dioxide to expel oxygen from the flask).[8] The solution is acidified by the addition of 6 ml of diluted sulphuric acid (1 + 2), and the liberated iodine is titrated with thiosulphate to a starch end-point. From this titration the total oxidising power of the reagent may be determined. For the second titration, another portion of the salt is dissolved in water and acidified. Excess of iodide is added and the tri-iodide formed is titrated. The titre determined from the second titration is six times the xenon molarity and is a measure of the chemically bound xenon. The ratio of the titre obtained by the first titration to the titre obtained by the second titration

multiplied by 6 gives the effective valency of the xenon, from which the percentage of xenon present as Xe^{VIII} can be determined. Although hydrochloric acid is commonly used in iodide titrations, it cannot be used in this instance, since perxenate oxidises chloride to chlorine in acid solution.[6] The titre for the second titration would be too great because the chlorine can oxidise the iodide to iodine. Either perchloric or sulphuric acid can be used.

An analysis of sodium perxenate dried in a vacuum over magnesium perchlorate gave the following results: Na_2O, 38·0 per cent.; XeO_4, 58·3 per cent. and H_2O, 2·9 per cent. The salt had 7·94 equivalents of oxidising power per g-atom of xenon. An aqueous solution of the reagent has a pH of 11·6 and decomposes to the extent of 10 per cent. in 24 hours.

Preparation of the reagent solution—Dissolve 200 mg of sodium perxenate in distilled water and dilute to 100 ml. The reagent solution should be prepared freshly each day.

PROCEDURE—

Qualitative—Add a few milligrams of solid sodium perxenate to several millilitres of a dilute sulphuric, perchloric or nitric acid solution of the sample in a test-tube, and shake it vigorously. The purple colour of permanganate will appear immediately if manganese is present. A spot plate can be used to make the test with only a few drops of solution. The acidity must be at least 0·1 N to prevent the formation of hydrated manganese dioxide. The presence of ions that are more easily oxidised than Mn^{II}, such as chloride, iodide, ferrous or chromous, will interfere. Addition of a large excess of sodium perxenate gives a positive test if manganese is present, provided that the interfering ions are not present in unduly large amounts.

Quantitative—Dissolve the uranium metal, oxide or alloy whose manganese content is to be determined, in nitric acid. Evaporate to remove the excess acid. Dilute to a convenient volume, and transfer a 10-ml aliquot to a 50-ml calibrated flask. The acidity should be in the range 0·1 to 2 N. Slowly add 10 ml of the sodium perxenate solution from a pipette, shaking the flask as vigorously as possible during the addition. Dilute to volume, and measure the optical density at 525 mμ against a reference prepared by diluting a 10-ml aliquot of the sample solution to 50 ml. The amount of manganese is calculated from a c/a ratio or calibration curve.

At 525 mμ, uranium has a very low optical density. A solution containing 2 g of uranium per 50 ml, when measured against water as a reference in 5-cm cells, had an optical density of 0·006 (equivalent to 98·8 per cent. transmission).

One-centimetre cells were used in the range 50 to 500 μg of manganese per 50 ml. Five-centimetre cells were used for smaller concentrations of manganese down to 5 μg per 50 ml.

DISCUSSION

A comparison of the results obtained with sodium perxenate and potassium periodate in controls both with and without uranium is shown in Table I.

TABLE I

EXPERIMENTAL RESULTS

(*i*) *In the presence of uranium*—

	Oxidising agent							
	Sodium perxenate						Potassium periodate	
Uranium, g per 50 ml ..	1	1	1	1	1	2	1	1
Manganese present, μg ..	50	100	250	250	500	5·0	100	250
Manganese found, μg ..	50	100	245	245	503	5·2	100	242

(*ii*) *In the absence of uranium*—

	Oxidising agent					
	Sodium perxenate			Potassium periodate		
Manganese present, μg ..	5·0	250	500	150	250	400
Manganese found, μg ..	5·0	248	500	155	250	395

Sodium perxenate will rapidly oxidise Mn^{II} to permanganate in dilute acid solutions at room temperatures. It can be used conveniently in both qualitative and quantitative applications. The dissolution of the salt in water and the oxidation of Mn^{II} are represented by the following equations—

$$Na_4XeO_6 + H_2O \longrightarrow 4Na^+ + OH^- + HXeO_6^{3-}$$

$$5HXeO_6^{3-} + 2Mn^{2+} + 9H^+ \longrightarrow 2MnO_4^- + 5XeO_3 + 7H_2O$$

In acid solution, sodium perxenate rapidly decomposes according to the equation—

$$HXeO_6^{3-} + 3H^+ \longrightarrow XeO_3 + \tfrac{1}{2}O_2 + 2H_2O$$

The efficiency of the oxidation by perxenate depends to a large extent upon the way the reagent is added to the manganese solution and upon the pH. Although the addition of solid sodium perxenate to the acid test solution will oxidise the manganese, it has been found to be much more efficient to add the reagent as an aqueous solution with shaking or rapid stirring of the test solution.

Addition of sodium perxenate to a neutral Mn^{II} solution will precipitate brown hydrated manganese dioxide. In very dilute acid solution, of pH about 2, the permanganate colour develops completely, but a brown precipitate forms after several hours. The optimum condition seems to be the development of the colour in about 0·1 N acid solution, and then the addition of sulphuric acid to give an approximately 2 N acid solution in which the colour is stable. Very high acid concentrations and the presence of other substances rapidly oxidised by perxenate inhibit full development of the colour.

The colour is stable for at least 3 hours. However, it is recommended that the optical density be measured as soon as possible after development.

Although chlorides interfere with the development of the permanganate colour in acid solution, tests indicated that, at low acidities, small concentrations of chloride are without effect. For example, complete colour development occurred when 1 drop of concentrated hydrochloric acid was present in 25 ml of a solution containing 250 μg of manganese, and also in another solution when 2 ml of perchloric acid was present. However, in a similar manganese solution containing 2 ml of perchloric acid and 1 drop of hydrochloric acid, no colour developed on addition of sodium perxenate.

For the determination of manganese in dilute acid solutions containing no other metal ions or interfering anions, and for the determination of manganese in relatively pure uranium or uranium - manganese alloys, perxenate is superior to periodate or persulphate as an oxidising reagent. However, the suitability of sodium perxenate as an oxidising agent should be tested for each application. Much remains to be done in investigating the effects of other metal ions, masking or complexing agents and in determining the optimum conditions for its use.

There is no doubt that the reagent will find many applications in analytical chemistry. In a preliminary test of its use in the determination of manganese in steel, an NBS cast iron containing 0·603 per cent. of manganese was analysed with sodium perxenate; 0·59 per cent. of manganese was found. However, a very large excess of sodium perxenate was required. The oxidation of chromous chromium to dichromate has been demonstrated and is under investigation. The oxidations of iodate to periodate, and $cobalt^{II}$ to $cobalt^{III}$ have been observed.[7] In neutral or basic solution, sodium perxenate will oxidise milligram amounts of $neptunium^{IV}$ and $plutonium^{IV}$ hydroxides to the sexavalent state. In acid solution, neptunium and americium are only partially oxidised to the sexavalent state; a large excess of the reagent, however, would probably give complete oxidation.[9]

I thank J. G. Malm and E. H. Appelman for preparing and providing the sodium perxenate. I also thank N. Egan for the determination of water in the sodium perxenate.

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References

1. Sandell, E. B., "Colorimetric Determination of Traces of Metals," Third Edition, Interscience Publishers Ltd., London, 1959, p. 608.
2. Willard, H. H., and Greathouse, L. H., *J. Amer. Chem. Soc.*, 1917, **39**, 2366.
3. Johnston, W. H., United States Atomic Energy Commission Report, CC–342, 1942, p. 25.
4. Rodden, C. J., "Analysis of Essential Nuclear Reactor Materials, U.S. Government Printing Office, Washington, D.C., 1964, p. 828.
5. Gibson, M., U.K. Atomic Energy Authority, A.E.R.E. Report C/R–350, 1949.
6. Malm, J. G., Holt, B. D., and Bane, R. W., *in* Hyman, H. H., *Editor*, "Noble Gas Compounds," University of Chicago Press, Chicago, Illinois, 1963, p. 167.
7. Appelman, E. H., and Malm, J. G., *J. Amer. Chem. Soc.*, 1964, **86**, 2141.
8. Willard, H. H., and Furman, N. H., "Elementary Quantitative Analysis," Second Edition, D. Van Nostrand Co., New York, 1935, p. 212.
9. Cohen, D., Argonne National Laboratory, Argonne, Illinois, private communication, 1964.

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Anthrone as a Reagent for Determining Carbohydrate in Rats' Milk and Related Materials

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A METHOD was required for the determination of total carbohydrate in rats' milk and the partially digested curd from the stomachs of suckling rats. Chromatographic investigation had drawn attention to two factors that were to govern the choice of method. First it was found that the amount of total carbohydrate recovered on the chromatograms was less than that expected. The figures for carbohydrate calculated "by difference," and also those reported in the literature were appreciably higher. This fact, together with other evidence obtained later, suggested that some of the carbohydrate in rats' milk is linked to nitrogenous compounds and is retained by the protein precipitants used as a preliminary to chromatography. Further, only about half the carbohydrate revealed on the chromatograms was present as free lactose. Most of the remainder was an unidentified sugar or sugar complex; some experimental evidence was obtained suggesting that it corresponded to a constituent of rats' milk isolated by Roberts, Pettinati and Bucek,[1] and lending support to their belief that it was an isomer of lactose. In addition, small amounts of glucose and galactose were found in the stomach contents.

A method was sought that did not involve de-proteinisation and in which the various carbohydrate components elicit an additive response. Colorimetric determination with a reagent containing anthrone is suitable in that it satisfies both requirements. According to Morris,[2] a compound sugar gives the same depth of colour as an equivalent amount of a mixture of its hydrolysis products. He also states that extraneous material can be tolerated to a limited extent. Fagen, Sibbach and Hussong[3] describe a method for the determination of lactose in dairy products, and it was hoped that this would be applicable to rats' milk and the stomach clots of suckling rats. Standardisation in this method is scant, however, and unreliable results were obtained. A survey of the literature pointed to the need to establish optimum conditions for the analysis of the materials under investigation.

DEVELOPMENT OF METHOD—

Early methods were handicapped by the lack of control resulting from the heat of mixing sugar solution and anthrone reagent. This was eliminated by Black,[4] who dissolved carbohydrate (carboxymethylcellulose) and anthrone in acid of the same strength. Chou and Tobias[5] followed this principle in the determination of individual sugars in ice cream after elution from a chromatogram; they used 27·5 N sulphuric acid as solvent. Chromatographic isolation was not feasible for rat-milk samples, as they are partially insoluble and an estimation of total carbohydrate was required. It was found that the freeze-dried and defatted preparations (non-fatty solids) could be dissolved entirely in 27·5 N sulphuric acid. Exploratory tests with lactose, glucose and galactose showed that the absorption peak is at 620 mμ, and maximum colour is attained after heating for 5 minutes at 100° C. The graph of optical density against weight of sample was linear over the range 0 to 125 μg of lactose, but the greatest accuracy was achieved within the range 75 to 125 μg.

The colour intensities of lactose, glucose and galactose were compared. It was found that, within the limits of experimental error, the relative figures agreed with those of Morris.[2]* Thus, an equimolecular mixture of the monosaccharides gave the same optical density as an equivalent amount of the parent substance.

The question of possible interference by protein was considered. Morris[2] had obtained the same result for lactose determined in diluted whole milk both before and after de-proteinisation. However, the protein - carbohydrate ratio is higher in curd than in milk, reaching a value of 10 to 1. Seifter *et al.*[6] obtained a positive response with protein, which was attributed to its tryptophan content, but the error when the protein - carbohydrate ratio was 20 to 1 amounted to only 1·5 per cent. Shetlar[7] found that, although tryptophan itself depressed the intensity of the carbohydrate - anthrone colour at 620 mμ, the tryptophan in serum protein had no effect, so that compensating

* It must be recorded, however, that the relative optical densities obtained by Chou and Tobias[5] differed from those of Morris. Thus, the relative values for lactose and glucose and for sucrose and fructose were different. The standards in the method of ice-cream analysis of Chou and Tobias were determined after elution from paper chromatograms, and it is possible that the amount recovered from paper varies for individual sugars.

reactions must occur in the natural product. In view of these authors' findings, serious interference was not expected, but the chemical system is complex, and the matter was put to the test. It was found that the response of lactose in the presence of 10 times its weight of casein was similar to that of pure lactose. Prolonged storage of concentrated lactose solutions in 27·5 N sulphuric acid at room temperature caused darkening and gave low results. The loss was greater in the presence of protein and led to serious inaccuracy. Storage of dilute solutions at below 5° C had no adverse effect.

Method

Reagents—

Sulphuric acid, 27·5 N—Add 1 litre of AnalaR sulphuric acid (sp.gr. 1·84) to 438 ml of distilled water. Check the normality by titration with standard sodium hydroxide solution. Store this reagent in a glass-stoppered bottle.

Note—In some early experiments the final colour obtained was brown instead of green. The cause of this was traced to the contamination of sulphuric acid that had been stored in a bottle with a bakelite cap. Helbert and Brown[9] reported that the cap liners used by some manufacturers of acid give a trace of colour with the anthrone test for carbohydrate, which affects the blank. They transferred their concentrated acid to glass-stoppered bottles as a routine and the practice has been adopted in the Unit's laboratory.

Anthrone reagent—Dissolve 0·16 g of anthrone in 100 ml of 27·5 N sulphuric acid. Allow the reagent to mature for 1 hour before use, and discard when 24 hours old.

Procedure—

Weigh 15 to 25 mg of non-fatty solids into a 5-ml beaker; weigh as rapidly as possible, and keep the beaker covered with foil because of the extremely hygroscopic nature of the material. Dissolve the sample in 27·5 N sulphuric acid, dispersing the lumps with the aid of a short, pointed glass rod. Transfer to a 50-ml calibrated flask and dilute to the mark with the same acid. The resulting solution contains from 22 to 90 μg of carbohydrate per ml. Prepare, similarly, a solution of lactose in 27·5 N sulphuric acid containing 50 μg per ml. As soon as each solution is prepared store it at below 5° C until it is required.

Measure aliquots containing 75 to 125 μg of carbohydrate into Pyrex tubes (17 × 180 mm), dilute to 4 ml with 27·5 N sulphuric acid, and add 8 ml of anthrone reagent. Place the tubes in a boiling-water bath for 5 minutes, transfer to a cold-water bath for 3 minutes, and allow to temper for 30 minutes. Measure the optical density at 620 mμ in a spectrophotometer against pure 27·5 N sulphuric acid.

Whatman glass-fibre paper should be used for wiping the outside of pipettes and for removing drops of acid from the outside of glassware when transferring the acid solution. Although some glass fibre is liable to be introduced there is no danger of contamination by cellulose, which would affect the result.

Results

There was an unaccountable day-to-day variation in the depth of colour developed. The $E^{1\%}_{1cm}$ for pure lactose monohydrate ranged from 386·4 to 422·2 with a mean of 416·4. The relative optical densities of lactose, glucose and galactose were not affected by this variation. The presence or otherwise of casein had no effect on the depth of colour obtained. The changes in the extinction coefficient are therefore considered immaterial, provided that standards are included in each set of determinations.

Typical results obtained on standards are shown in Table I.

In the preparation of standards, AnalaR lactose monohydrate and AnalaR glucose were used, and were dried over phosphorous pentoxide before being weighed. Galactose was recrystallised twice and also dried over phosphorous pentoxide. The preparation of purified casein used (Genatosan Ltd.) had a negligible carbohydrate content.

The optical density of a blank solution of 27·5 N sulphuric acid was 0·33.

The optical densities, expressed as a percentage of the optical density of pure lactose solution, are shown in Table II.

The results obtained on rat's milk are given below—

	Albino strain	Hooded strain
Total carbohydrate (as lactose) of whole milk	2·44 per cent.	2·36 per cent.

The determinations were carried out on bulked samples of milk from 8 rats. Results on duplicate aliquots of the same solutions agreed within 2 per cent. of the mean value (*i.e.*, the discrepancies

in the figures above were less than 0·05 per cent.). The amounts of samples available were insufficient for complete determinations to be carried out in duplicate. The figures fall within the

TABLE I

THE OPTICAL DENSITIES OF CARBOHYDRATE SOLUTIONS, WITH AND WITHOUT THE ADDITION OF CASEIN, *AFTER REACTION WITH ANTHRONE REAGENT*

Carbohydrate	Optical density, at 620 mμ, of a solution containing, in 12 ml—		
	100 μg of carbohydrate	100 μg of carbohydrate *plus* 500 μg of casein	100 μg of carbohydrate *plus* 1000 μg of casein
Lactose	0·347	0·352	0·349
Glucose	0·451	0·449	0·448
Galactose	0·261	0·258	0·253

TABLE II

COMPARISON OF THE OPTICAL DENSITIES OF CARBOHYDRATE SOLUTIONS AFTER REACTION WITH ANTHRONE REAGENT

Carbohydrate	Optical density at 620 mμ, expressed as a percentage (Lactose = 100), of a solution containing, in 12 ml—		
	100 μg of carbohydrate	100 μg of carbohydrate *plus* 500 μg of casein	100 μg of carbohydrate *plus* 1000 μg of casein
Lactose	100	101·6	100·7
Glucose	133·1	132·5	132·1
Galactose	72·6	71·7	70·1
Half sum of glucose *plus* galactose	102·9	102·1	101·1

range of those obtained by Glass[8] for the carbohydrate content (total solids less the sum of the fat *plus* protein *plus* ash) of the milk of 15 black-strain rats. The range was 2·2 to 3·1 per cent., and the mean 2·6 per cent.

The carbohydrate content determined by the method described was compared with the carbohydrate content calculated "by difference," by analysing two preparations of the non-fatty solids of stomach contents. The results of the analysis are shown in Table III.

TABLE III

RESULTS OF ANALYSIS OF TWO SAMPLES OF NON-FATTY SOLIDS FROM RATS' STOMACH CONTENTS

	Sample 1	Sample 2
Moisture, per cent.	1·69	1·62
Protein (N × 6·38), per cent.	73·21	71·15
Ash, per cent.	16·15	18·18
Carbohydrate "by difference," per cent.	8·95	9·05
Total	100·00	100·00
Carbohydrate determined by the method described, per cent. as lactose	9·59	8·67

The non-fatty solids of samples 1 and 2 constituted 18·79 per cent. and 17·94 per cent., respectively, of the stomach contents. The discrepancies between carbohydrate determined by the method described and carbohydrate calculated "by difference" therefore correspond to only 0·12 per cent. and 0·07 per cent., respectively, of the total wet weights of the samples.

Finally, carbohydrate determinations were carried out on the same two preparations of non-fatty solids from the stomach contents with added lactose, and the results are shown in Table IV.

TABLE IV

RESULTS OF RECOVERY EXPERIMENTS WITH ADDED LACTOSE

	Sample 1	Sample 2
Weight of sample, mg	23·56	23·90
Calculated content of carbohydrate, mg	2·259	2·072
Weight of added lactose, mg	1·5	2·0
Total carbohydrate "expected," mg	3·759	4·072
Total carbohydrate found, mg	3·615	4·210
(Carbohydrate found/carbohydrate "expected") as a percentage	96·2	103·4
Recovery of added carbohydrate, per cent.*	90·6*	106·9*

* These figures are obviously distorted and have no real meaning.

Conclusions

The method described gives the same intensity of colour with lactose as with an equivalent amount of its hydrolysis products. The optical density is unaffected by the presence of casein if the weights of carbohydrate and casein are in the ratio of 1 to 10.

Results obtained with rats' milk agreed with those reported elsewhere, and results of experiments on the non-fatty solids of stomach contents suggest that the accuracy is within ± 4 per cent. of the theoretical.

The method may have wider applications, such as in the analysis of certain dairy products.

References

1. Roberts, H. R., Pettinati, J. D., and Bucek, W., *J. Dairy Sci.*, 1954, **37**, 538.
2. Morris, D. L., *Science*, 1948, **107**, 254.
3. Fagen, H. S., Sibbach, R. V., and Hussong, R. V., *J. Dairy Sci.*, 1954, **37**, 10.
4. Black, H. C., *Anal. Chem.*, 1951, **23**, 1792.
5. Chou, T. C., and Tobias, J., *J. Dairy Sci.*, 1960, **43**, 1031.
6. Seifter, S., Dayton, S., Novic, B., and Muntwyler, E., *Arch. Biochem.*, 1950, **25**, 191.
7. Shetlar, M. R., *Anal. Chem.*, 1952, **24**, 1844.
8. Glass, R. L., Ph.D. Thesis, University of Minnesota, July 1959.
9. Helbert, J. R. H., and Brown, K. D. B., *Anal. Chem.*, 1955, **27**, 1791.

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Book Reviews

THIN-LAYER CHROMATOGRAPHY: A LABORATORY HANDBOOK. Edited by EGON STAHL. P. xvi + 553. Berlin, Heidelberg and New York: Springer-Verlag; New York and London: Academic Press Inc., Publishers. 1965. Price DM 68.

Chromatography in its various forms is an acknowledged technique in the laboratories of the chemist and biologist wherever separation methods are needed, and no form has proved to be so simple, quick and versatile for non-volatile compounds as thin-layer, which was a very early form of the chromatographic technique. However, the name of Stahl has become acknowledged worldwide for his development of the thin-layer technique as we know it to-day. Consequently, a handbook edited by him must inevitably be regarded as a classic in the field. There is so much useful information collected in this book that it is difficult to discuss adequately all that has been included—sufficient to say that Stahl and his co-authors have attempted, and with success, to include all that was known at the time of writing. One can find discussions of the theory and practice of chromatography as typified by thin-layer chromatography, for the general and special problems of separation, but also, and the reviewer regards this as important, the preparation of the material before chromatography. As an example of this, where natural products are involved, the extraction of the species from all its associated biological material is often described, as well as the concentration of the constituents to a point where chromatography will be successful. This is truly a handbook that the laboratory worker in many fields will find invaluable.

After an historical introduction, there is a very full discussion and description of the equipment used for preparing plates, together with the method of doing so, the types of separation chambers employed, spraying equipment and hoods, standard conditions for reproducibility in thin-layer chromatography, and finally the basic equipment required. This chapter by Stahl is well written, and much of the information is of general interest, but to the beginner in thin-layer chromatography it might appear that only one form of equipment is satisfactory, *i.e.*, the most expensive. It might be pointed out here, that much good work has been done with improvised apparatus, and this is what many British laboratories have had to start with. If R_F values have to be used then complete standardisation of conditions is necessary for reproducibility, but often for ordinary work such stringent observance is not essential, though "sloppy" working will lead to poor chromatography. A further chapter by Stahl on "special techniques" describes modified forms of thin-layer chromatographic procedure. From the practical point of view, the sections on "Coating Materials," "Documentation," "Quantitative Estimation" and "Isotope Techniques," by D. Waldi, H. Glanshirt and K. Mongold are all equally informative and thorough in their treatment, and provide the reader with good guidance. One valuable point not mentioned is the use of infrared spectroscopy for identifying the structure of unknown compounds, and there is little mention of preparative thin-layer chromatography and the problem of preparing the larger and thicker layers. Undoubtedly these will be included in the next edition.

The section on "Theoretical Aspects of Thin-Layer Chromatography," by M. Brenner *et al.*, provides some 60 pages on the theory of chromatography and needs to be studied very carefully if advantage is to be taken of the concepts in experimental work. The ease of making a chromatogram tends to make one lazy about its theoretical aspects, but this section is a sharp reminder that there is now a considerable background of theoretical literature. For the non-mathematician and non-physicist this section may, however, be too difficult to appreciate fully. It is praiseworthy that Stahl has thought fit to include it in this handbook.

More than two thirds of the book is devoted to the special sections on aliphatic lipids; terpene derivatives; vitamins; steroids; organic bases; pharmaceutical products; the use of thin-layer chromatography in clinical diagnosis and pharmacology; synthetic organic materials; dyestuffs; additives in food and household articles, etc.; hydrophilic constituents of plants; amino-acids and derivatives; nucleic acids and nucleotides; and sugars and derivatives. Those interested in these fields will find a wealth of information useful to the beginner and stimulating to the experienced. For those outside these fields there is much good reading, and from here one may get inspiration for one's own particular problems. The inorganic aspects of thin-layer chromatography have been discussed by H. Seiler, whose earlier work with inorganic species is well known. Clearly this is a comparatively undeveloped field, restricted to cations and common anions in the main, and to the concepts of classical qualitative analysis. Little mention is made here of exploring by thin-layer chromatography the chemistry of sulphur or phosphorus, though recent work has shown the possibilities of this approach.

Finally, there is a section "Spray Reagents for Thin-Layer Chromatography," with full practical details; terminology of thin-layer chromatography in English, German and French; a list of commercial suppliers—fairly international; and a table for conversion of R_F into R_M values.

No doubt in a book of this comprehensive nature, experts in each field will find errors and omissions that a reviewer may overlook, but surely no one in the analytical field can afford to ignore this book. It should be a book on "hand" in libraries and many laboratories.

F. H. POLLARD

AUTOMATIC METHODS IN VOLUMETRIC ANALYSIS. By D. C. M. SQUIRRELL, B.Sc., F.R.I.C. Pp. x + 201. London: Hilger & Watts Ltd. 1964. Price 42s.

Currently all books dealing with automation in analysis are timely, for there is now an overwhelming interest in such developments. As the author of this book points out, automation for its own sake is not sufficient, unless it shows an advantage either in saving time where this is necessary (to provide information regularly), or in superiority over, or equality with, manual methods from the point of view of reproducibility or accuracy. The methods described are based upon the author's experience of situations in which automation can be satisfactorily applied to volumetric analysis. These may be fully automatic or semi-automatic depending upon the nature of the analysis.

Apart from the introductory discursive chapter on "Automation—when and why?," there are six chapters dealing with titration to set end-points; "Full scale recording potentiometric methods"; "Full scale titrations in non-aqueous media"; "Other instrumental titration methods"; "Continuous and on-stream process analysis"; and "Ancillary automatic equipment." Each chapter deals essentially with details of experimentation rather than theoretical principles, and the selection of the determinations described are primarily the personal choice of the author from the requirements of his own industry. This represents a prejudice that does not necessarily detract from the value of the book, for it is comparatively easy to apply the ideas expounded to other fields of work, with the working out of details to suit the new problems.

The rapidly developing technique of non-aqueous analysis receives careful attention, and well illustrates the bias of the methods described. In this chapter the following are discussed—Titration of bases: amines, indirect determination of acetic anhydride, amine-amide mixtures, amine oxides and phosphines, amine end-groups in nylon, acrylamide, mixtures containing alkali metals, determination of metals as bases; Titration of acids: carboxyl groups in polymers and copolymers, carboxyl end-groups in nylon 6, determination of traces of formic acid, phenols, etc.; Functional group analysis. Inorganic applications are limited to chloride and sulphate estimations. In many places quite detailed instructions are given, but this, though frequent, is not general.

If the choice of limitations of subject matter is overlooked, then one can recommend this book as a useful contribution to the field of automation, and worthy of study both by student

and experienced analyst alike. The growing tendency to do the analysis directly on the plant must also make this book of interest to the plant manager and others responsible for more routine testing and control.

F. H. POLLARD

OPEN TUBULAR COLUMNS IN GAS CHROMATOGRAPHY. By L. S. ETTRE. Pp. xx + 164. New York: Plenum Press. 1965. Price $4.95.

There are many books on gas chromatography in general, but this is the first one, as far as the reviewer is aware, that concentrates on the heart of chromatography where the process of separation takes place, namely the column. The name "Open tubular columns" refers to what have formerly been known as "Capillary Columns," and it is a pity that the author has chosen this title which will mean little to those not aware of this change in terminology.

The book is written for the "practical gas chromatographer" so that he may better understand the process he is using—the reviewer would like to think that many of those now content to squirt a sample into an expensive piece of equipment will take the trouble to read this book and get a better appreciation of what they are doing. The text is well arranged and lucidly written; there are few wasted words. As indicated already, it deals specifically with "capillary columns," which in one part are compared with "packed columns," but the author is definitely a "capillary column" man and one has to remember this when taking his assessment of the situation. This is not to say that he is unfair to packed columns, but rather that the beginner could be led astray.

One part of the book is devoted to the preparation of the column; this is undoubtedly valuable and useful to all who wish to use this type of column. To round off the book, there is a brief discussion on the rest of the chromatographic system, with special reference to the requirements of the ancillary equipment where great detector sensitivity is needed; a specialised bibliography; and supplements on calculations of the "air peak time" by using both Peterson and Hirschs' and Gold's methods.

A useful book, well arranged and written, but will probably only be appreciated by a limited number of gas chromatographers.

F. H. POLLARD

GUIDE TO GAS CHROMATOGRAPHY LITERATURE. By AUSTIN V. SIGNEUR. Pp. vi + 351. New York: Plenum Press. 1964. Price $12.50.

Of all forms of chromatography, none has rocketted into use more than gas chromatography, and the problem of a research worker is to find all the references pertinent to his own particular field. Abstracts and reviews can help, but these are often scattered in different volumes, so that a comprehensive book of references is very welcome.

This is such a volume and represents a mammoth task of listing approaching 8000 references, in alphabetical order of the author first named in the paper, and with something like 2000 individual authors. From this can be judged the army of workers in this field known and unknown. The thoroughness of the references might be judged in that even 150 "anon" contributions are listed.

The value of such a book lies in the ease with which one can refer to the subject required, but this is where this book fails, for the subject index is not sufficiently detailed. One admits the difficulty here, but it is nevertheless a pity that such a valuable book does not have the facility to have such a good subject cross reference. It might also have been better to separate reviews and books from research papers.

In spite of the criticism made, it is a good book to have around in the library, and on the desk of anyone interested in gas - liquid chromatography and gas - solid chromatography.

F. H. POLLARD

AN INTRODUCTION TO MICROBIOLOGY. By W. B. HUGO, B.Pharm., Ph.D., F.P.S. Pp. x + 146. London: William Heinemann Medical Books Limited. 1965. Price 20s.

This book is apparently the first of an intended series of "Pharmaceutical Monographs" written by pharmacists for students of pharmacy. What a pity this is not mentioned on the cover or title page! One has to discover it either by reading the general preface or by stumbling across it in the text in such phrases as "toxins . . . and immunological products will be dealt with in Volume 4," or "sterilisation is described in a separate monograph in this series." But this is not the fault of the author, nor does it detract from the quality and content of the present volume.

Apart from the last chapter, the subject is treated from the biochemical and structural aspects of the cell. In spite of this apparently academic approach, however, the author, in an unusually

refreshing manner, has skilfully and successfully included so many points of practical interest that even the tyro must learn from it something of the basic principles of bacterial growth and metabolism. The last of the four chapters of the book is handled in a somewhat different way and deals with microbial taxonomy, with particular emphasis on those organisms most likely to be of interest to the pharmacist.

If one wished to be precise one could criticise the definition on page 51 of "thermal death rate," the statement on page 66 "It is clear that there is a limit to the amount of substrate that a given amount of enzyme can deal with . . ."—without referring to time factor, and the definition on page 71 of "fermentation"—nowadays so many fermentations involve synthetic processes. But these are comparatively small points.

The book is well produced in a semi-hard binding suitable for laboratory use, and it contains some good electron micrograph reproductions. There is a also a short list of journals and review volumes recommended for reading.

G. SYKES

CHEMISTRY AND BIOLOGY LABORATORIES: DESIGN - CONSTRUCTION - EQUIPMENT. By WERNER SCHRAMM. Translation Editor, J. M. LEYTHAM. Translator, Mrs. M. JANSEN. Pp. x + 225. Oxford, London, Edinburgh, New York, Paris and Frankfurt: Pergamon Press, 1965. Price 105s.

This English edition is, in the main, a translation of the second German edition although, in order to avoid confusion, a lot of statistical information about constructional materials has purposely been omitted.

Werner Schramm's book presents considered German views on the fundamental principles involved in the planning, design and construction of chemical and biological laboratories. It seeks to provide a background of information that will enable scientists, consultants, architects and builders to avoid constructional errors.

Information is provided on the general planning and dimensions of laboratories; on building land and construction; on the various services such as gas, water and electricity; on protection against fire, excessive noise and other hazards and on laboratory furniture, in particular on the equipment needed in laboratories involving different functions. The equipment required for animal houses is not neglected and laboratories for biological, bacteriological and radiochemical work are all dealt with in considerable detail.

Finally, there are several examples of laboratories used for particular chemical and biological purposes, and information is provided about some of the basic rules that must be adhered to in the construction of these laboratories.

The book is well produced. The diagrams are particularly informative, but some of the photographs are far from satisfactory.

The translation appears to have been carried out very efficiently and the treatise is very readable. The bibliography would have been improved by the inclusion of the English equivalents of the references that are made. Moreover, in using the translation one is struck by the absence of a subject index; the table of contents at the beginning of the book is hardly a satisfactory substitute.

This book can be recommended. Chemists who use it as a source of ideas and subsequently visit some of the more important laboratories making use of these ideas will give all the better advice when called upon to play their part in the design and construction of new laboratories.

J. HASLAM

SOME ELECTRICAL AND OPTICAL ASPECTS OF MOLECULAR BEHAVIOUR. By MANSEL DAVIES. Pp. x + 190. Oxford, London, Edinburgh, New York, Paris and Frankfurt: Pergamon Press. 1965. Price 15s.

Physical methods that give information regarding the internal structures of inorganic or organic molecules are of the greatest interest and importance. In these days of complex mathematical concepts regarding mass, interacting force fields, etc., it is refreshing to be reminded that a direct application of the laws of electrostatics can give the most rewarding information regarding the behaviour of molecules exposed to static or oscillating electric fields.

This book has considerable didactic value; the student should easily cope with the mathematical presentation of the Mosotti - Clausius - Debye theory of dipole moments. The more sophisticated "cavity field" theory of Onsager follows naturally for liquid media.

The theoretical text is well supported with good general descriptions of the techniques employed for the determination of permittivity, dipole moment, conductivity, dielectric dispersion, etc.

The Kerr effect is describing following a detailed discussion of molecular polarisability; the stereochemical phenomenon is associated with the Drude-Kuhn theory, and a consideration of scattered radiation leads to a description of the Raman effect.

There are not many works wholly devoted to studies of the interaction of electromagnetic radiation, including light, with molecular matter. Anyone who wishes to do some really fundamental thinking on what Sir William Bragg aptly called the "Nature of Things" could do no better than study the many examples and problems given in this comprehensive work. It provides a solid foundation for much future research and a lucid description of what has been achieved so far.

D. T. LEWIS

VACUUM MICROBALANCE TECHNIQUES. Volume 4. Edited by PAUL M. WATERS. Pp. xxii + 288. New York: Plenum Press. 1965. Price $13.50.

This book consists of 18 papers read at the Vacuum Microbalance Conference, Pittsburgh, May 7–8th, 1964, in which microbalances of all the main types in use are described. These include an automated quartz spiral balance, pivoted and suspended beam balances, and a quartz crystal oscillator balance.

In a number of the papers considerable constructional and operational detail is given, which would have been made clearer in places by the use of line diagrams, rather than uninformative photographs. Two papers describe the construction of pivoted beam balances that are fully bakeable, and therefore suitable for use in ultra high vacuum systems.

For users of vacuum microbalances in analytical applications, the papers of most interest may be those describing the application of commercial instruments, such as the Cahn electrobalance, in studies of adsorption, thermal degradation of polymers, and reactions of solids with gases. Most of the papers emphasise problems of spurious mass change, and the methods by which the difficulties were overcome. In particular, much attention is given to the spurious effects of thermo-molecular flow, including an effect at high pressures which Thomas and Williams considered to be due to thermo-molecular flow within the pores of their sample.

It is inevitable in such a narrow field that the annual publication of volumes of this type results in much repetition of similar material from year to year, with relatively slight changes of emphasis and detail. These volumes are excessively expensive in the opinion of this reviewer, and with an almost 50 per cent. increase in price since Volume 3, Volume 4 of the series cannot be recommended as good value.

D. W. BASSETT

SOLUBILITIES OF INORGANIC AND ORGANIC COMPOUNDS. Volume 2. TERNARY AND MULTICOMPONENT SYSTEMS. Part 2. Edited by PROF. H. STEPHEN, O.B.E., D.Sc., F.R.I.C., and DR. T. STEPHEN, M.Sc., Ph.D. Pp. vi + 947–2053. Oxford, London, Edinburgh, New York, Paris and Frankfurt: Pergamon Press. 1964. Price £12 10s.

This is the most recently published volume of the series, and presents useful data for those interested in these systems, some of which may be of interest to chromatographers who use ternary and multicomponent eluting solvents.

F. H. POLLARD

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