THE ANALYST

Society for Analytical Chemistry Gold Medal

COUNCIL, on the recommendation of its Honours Committee, has decided to award the first Society for Analytical Chemistry Gold Medal to

HERBERT NEWTON WILSON

formerly Analytical Group Manager, Imperial Chemical Industries Ltd. (Billingham Division and, later, Agricultural Division).

H. N. Wilson has spent the major part of his career in the Research Department's analytical laboratories at I.C.I., Billingham, where he helped to build up and later took complete charge of, an analytical department well equipped and of outstanding quality, covering an extremely wide field. Sections included were sampling, standards, raw materials and finished products (from argon to cement, and numerous organic chemicals as the Division's research and manufacturing programme expanded). The Billingham laboratories have always been early users of new techniques, and were among the first British industrial laboratories to establish a microchemical section, to use spectrophotometry, polarography, gas chromatography and X-ray fluorescence analysis. The extent and quality of the training available and the personal influence of "H.N." is seen in the list of men, now in senior positions elsewhere, who were under him in all or part of the formative stages of their careers, including—

J. Borrowdale, Chief Chemist, Richard Thomas & Baldwins Ltd., Scunthorpe.
W. T. Elwell, Chief Analyst, Imperial Metals Industries Ltd., Birmingham.
M. Gibson, Technical Manager, J. C. Gregory & Son Ltd., Stoke-on-Trent.
H. Hollis, Director of Chemical Inspection, Ministry of Defence, Woolwich.
J. R. Hudson, Research Manager, Brewing Industry Research Foundation, Caterham.
J. Norris, Director, J. H. Marks & Co. Ltd., Cleckheaton.
G. E. Penketh, Chief Analyst, I.C.I. Ltd. (Heavy Organic Chemicals Division).
P. A. Raine, Chief Chemist, Crown Cork Co. Ltd.
A. Robertson, Personnel Director, I.C.I. Ltd. (Mond Division).
A. A. Smales, Head of Analytical Chemistry Branch, A.E.R.E., Harwell.

Wilson's published work covers a wide range of analytical problems dating back to the 1930's when papers on such diverse topics as selenium in sulphur and the determination of OH groups by the pyridine - acetic anhydride method were published; through the 1940's on amines, trace elements in phenol, uranium in urine, arsenic in glass, nitromethane in air; to a very productive period in the '50's and '60's including some outstanding work on the determination of silicate and phosphate. He was also joint editor of both editions of "Chemical Analysis—The Working Tools." His latest book, just published, "An Approach to Chemical Analysis—Its Development and Practice" can be recommended to all analytical chemists and their "customers" for its wide scope and penetrating observations.

H. N. Wilson was the first Royal Institute of Chemistry examiner for the Fellowship in General Analytical Chemistry from 1941 to 1949, and served on the Council of that body in 1945–46 and 1949–52. Within the Society for Analytical Chemistry, of which he has been a member for over 30 years, he served on Council in 1947–48 and is perhaps best known for his chairmanship of the joint S.A.C. - A.B.C.M. Committee on Trade Effluent Analysis and for the resultant monograph of approved methods. For some years before 1962 he was also a member of the Fertiliser Manufacturers' Association analytical committee and served on the scientific sub-committee of the Fertilisers and Feeding Stuffs Act, 1926, Standing Advisory Committee of the Ministry of Agriculture, Fisheries and Food.

For his contributions to chemical analysis the Society is proud to award its first Society for Analytical Chemistry Gold Medal to HERBERT NEWTON WILSON.

An Automatic Apparatus for the Determination of Titanium

BY C. L. DENTON AND J. WHITEHEAD
(*British Titan Products Company Ltd., Billingham, Co. Durham*)

A method is described for the determination of titanium in solution. The titanium is reduced with cadmium in a column-type reductor and titrated with ferric alum solution to a potentiometric end-point. The determination is carried out automatically once the sample solution has been placed in the instrument, and the result is obtained in approximately 7 minutes. Results are given demonstrating the excellent precision obtainable with the instrument.

IN the titanium pigment industry it is necessary to analyse a large number of samples, mostly liquid, for titanium content. The method most frequently used for this determination is to reduce the titanium to the tervalent state and oxidise with a standard oxidising agent.[1,2] Agents which have been used for reducing titanium include the following: aluminium, fusible alloys, cadmium, iron, zinc, bismuth, lead, slightly amalgamated zinc and liquid amalgams of zinc, bismuth, lead, tin or cadmium.

Solid reducing agents are generally used in a column or Jones-type reductor, and liquid reducing agents in the separating-funnel type of reductor of which the Nakazono is a typical developed form.[3] Solid reducing agents may also be added directly to the solution, a typical example of this being aluminium. After reduction is complete, excess aluminium dissolves in the solution and does not interfere in the subsequent titration.[4] Cathodic reduction has also been used, particularly in the coulometric generation of tervalent titanium.[5]

Reduced titanium solutions can be titrated with a variety of standard titrimetric reagents. Among the commonest of these are solutions of potassium dichromate, permanganate or bromate, ceric sulphate and ferric solutions. The end-point may be determined using indicators such as methylene blue, diphenylamine, indigo carmine or sodium or potassium thiocyanate. The titration may also be completed potentiometrically.

Solutions containing titanous salts are unstable and easily oxidised by the atmosphere, but this may be avoided by the use of an inert atmosphere or by adding the titanous solution to an excess of oxidising agent. Alternatively, the solution may be run into an excess of ferric sulphate solution and the ferrous equivalent titrated with standard oxidising agent.

Even in the most rapid of the above methods, the manipulative time is still considerable, and it is evident that an automatic instrument would achieve a large saving in man-hours. It was envisaged that such an instrument would carry out automatically a determination of titanium in a solution which had been manually inserted into the instrument. At the end of the determination the result would be displayed in digital form and the instrument would be ready for another determination.

The above methods were critically examined in order to select the most suitable for further consideration. Initially, attempts were made to use liquid amalgams since they are rapid and efficient in action. The titanium solution was agitated with the amalgam in a glass cylinder, by means of a mechanically operated perforated glass piston, until all the titanium was reduced to the tervalent form. The reduced solution was then run off into a titration vessel via a side arm, situated at the amalgam level. The reduction appeared to be complete, but slight oxidation took place during transference to the titration vessel despite the presence of an inert atmosphere of carbon dioxide and the presence of potassium thiocyanate which stabilises tervalent titanium. In view of this and mechanical difficulties, no further work was done on this method.

Electrolytic reduction is potentially very attractive, but it was shown that the current densities required to reduce the quantity of titanium in the average sample in a reasonable time were too high.

The column-type of reductor was next investigated. Experience has shown that cadmium metal granules (1 to 2 mm) are superior to amalgamated zinc as reducing agent in a column-type reductor. A column-type reductor, packed with cadmium, was therefore fitted with a solenoid-operated outlet valve and platinum level probes in the top of the column. The cadmium was supported on a sintered-glass disc protected by a plug of glass-wool.

With this system, the complete reduction and washing of a sample solution took about 15 to 20 minutes, but this time progressively increased as the granules decreased in size and packed into the base of the column.

This was clearly unsuitable for an automatic method and consequently the upward flow of liquid was investigated.

A simple apparatus was assembled to investigate this technique. The sample was pumped from a beaker into the bottom of the column using a mechanically operated nylon syringe and two glass non-return valves. Level probes were used to control the reduction and washing processes, and the solution was finally titrated manually. Consistent reduction efficiencies and constant reduction times were obtained over many determinations, indicating that the upward flow was fluidising the column and preventing it from packing. This reduction procedure was accordingly adopted and incorporated into the final apparatus.

Ferric alum solution was chosen as titrant in view of the fact that many of the sample solutions contained ferrous iron which would react with such oxidising agents as potassium dichromate and ceric sulphate.

The nature of the samples, which varied appreciably in colour, precluded the use of photometric methods for end-point detection, and a potentiometric method was therefore selected for use. The change in potential at the end-point was enhanced by the presence of potassium thiocyanate, and this was added in all titrations. It has the further advantage of stabilising the tervalent titanium to atmospheric oxidation and the colour change at the end-point provides a visual check on the accuracy of the instrument.

Several methods of adding titrant were examined. The standard burette with location of meniscus by light source and photoelectric cell was rejected because it is too complex. Preliminary work with an early form of peristaltic pump indicated that it was not sufficiently accurate and it would be difficult to incorporate in an automatic system. Frequent calibration would also be necessary.

A glass syringe pipette, operated by a precision calibrated leadscrew driven by a synchronous motor, was next investigated. A cam on the top of the leadscrew operates a mechanical counter, which is adjusted so that the full traverse of the syringe, *i.e.*, 50 ml, is equivalent to 1000 revolutions. This method, which was proved to be completely satisfactory for dispensing accurate volumes of liquid, was incorporated into the automatic titrator. Very careful lining up of the syringe and leadscrew was found to be necessary to prevent breakage of the syringe. This was avoided by using a shorter piston made from Teflon with an O-ring seal fitted to the end. These have proved to be extremely satisfactory in use, and breakages have been almost eliminated.

General description of the instrument

End-point detector—

The end-point detector is essentially the same as that described by Brown and Weir,[6] which is incorporated in the Model 34 Titromatic Analyser made by Electronic Instruments Ltd. It has, however, been modified to give a direct reading in pH units. The modified circuit is given in Fig. 1, and a list of components can be found in Appendix I. The modifications are as follows:

A 100–0–100 microammeter with series resistor R_{30} (5600 ohms) is connected across the cathodes of V_4. R_{18} is omitted, and the value of R_{19} is changed to 22,000 ohms. R_{40A} and R_{40B} form part of a twin-ganged potentiometer, connected so that as one increases the other decreases. This is the set buffer control. R_{41} is a potentiometer to give the required pH scale length, and R_{20} is a 25,000-ohm helical potentiometer that gives a direct indication of the pH of the solution.

The manual-check auto switch is converted to a two-position rotary switch giving manual and auto positions only. The neon lamps, V_{12} and V_{13} (normally in the Brown - Weir unit) that are used to indicate "Titrate on" and "Titrate off," are omitted, and have been replaced by the meter connected across the cathodes of V_4.

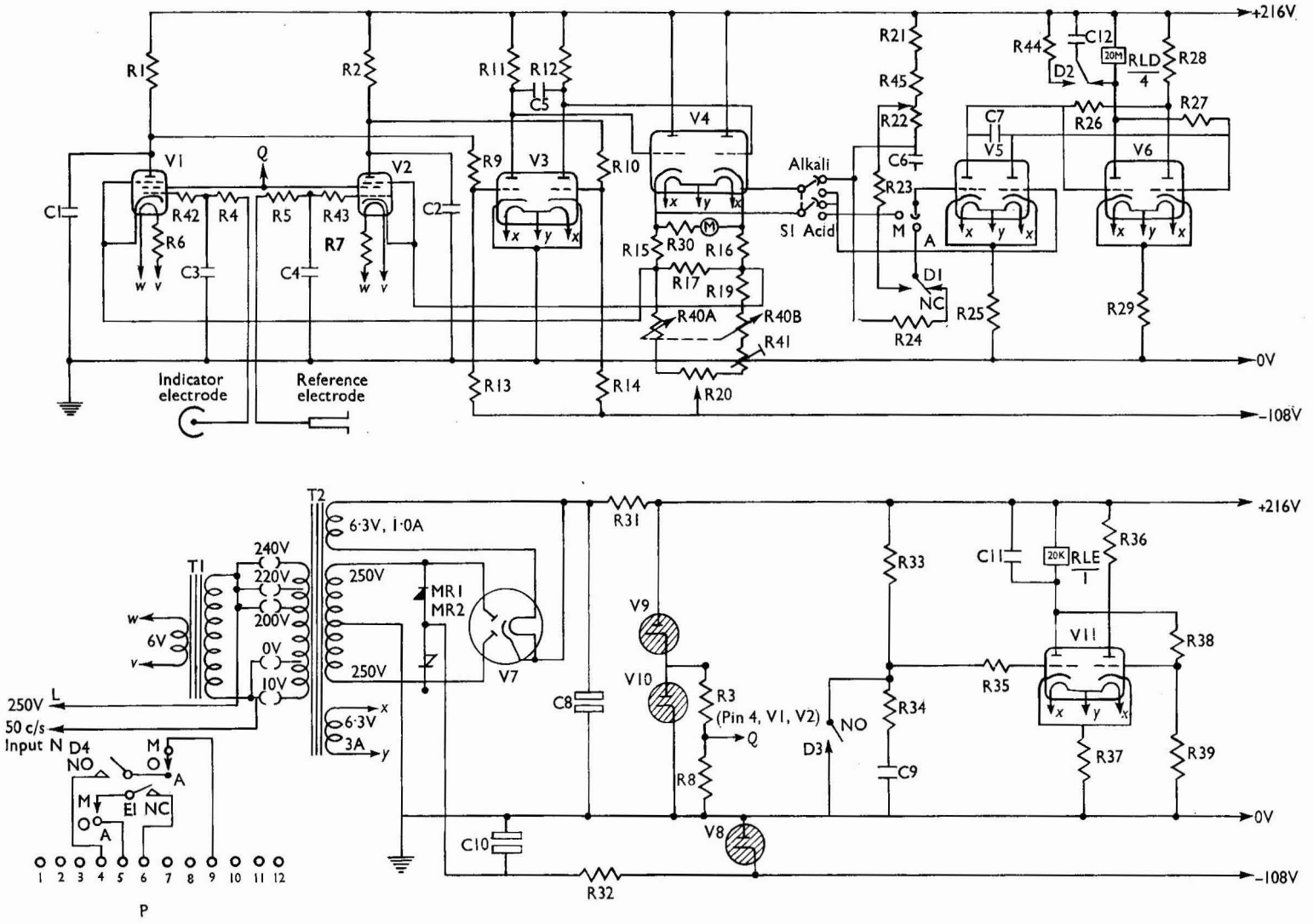
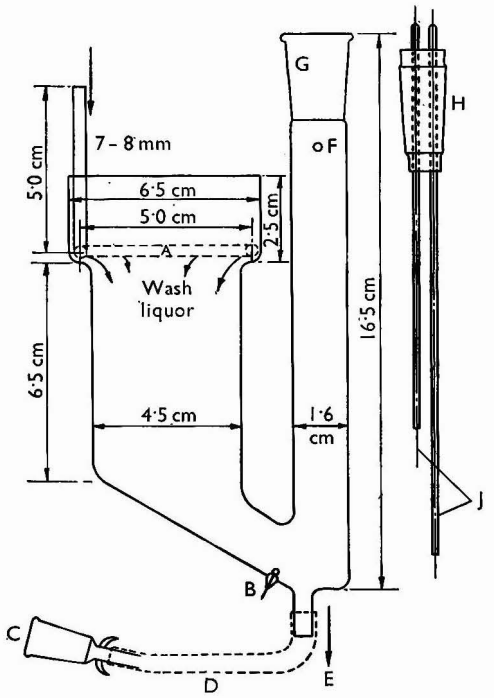


Fig. 1. End-point detector circuit diagram

The value of R_{22}, the anticipation control, is changed to 25,000 ohm, with R_{45}, 180,000 ohm, connected from the top end of R_{22} to R_{21}.

The E1 contact is now made normally closed to operate the sequence unit.

The end-point detector is capable of being used for pH or potentiometric titrations.



A = 5-cm diameter ring with several holes to run wash liquor into beaker
B = Platinum probe fused through glass
C = B10 socket
D = PVC tubing
E = Outlet to glass filter
F = Air hole
G = QVF B14 socket
H = PTFE cone to fit B14 socket
J = 1 × 25-s.w.g. platinum wire fused through end of glass tubing and soldered to tinned-copper wire

Fig. 2. Sample beaker and probes

SAMPLE BEAKER—

This is a modified 250-ml squat-type beaker, and a diagram of it is shown in Fig. 2.

The top of the beaker is widened to take the wash liquor manifold. This consists of a 7 to 8-mm diameter tube, with holes at 1-cm intervals through which wash liquor is passed to rinse the sides of the beaker. The tube is recessed to prevent solution spraying straight into the beaker and not washing the sides. The beaker has a sloping base so that a minimum volume of solution is retained in the beaker when a change is made to the next sequence in the operations. The volume of wash solution is determined by three probes which are placed in a side arm to avoid accidental wetting by the wash solution. As a further precaution against spurious contact, the connecting wires are encased for most of their length in glass-tubing.

SAMPLE TRANSFER PUMP—

A 20-ml nylon syringe is used for this purpose. The syringe is driven by a 250-volt, a.c., 50 c/s geared induction motor, running at 4·75 r.p.m., connected to the syringe by means of a crankshaft. The arrangement can be seen in Fig. 3.

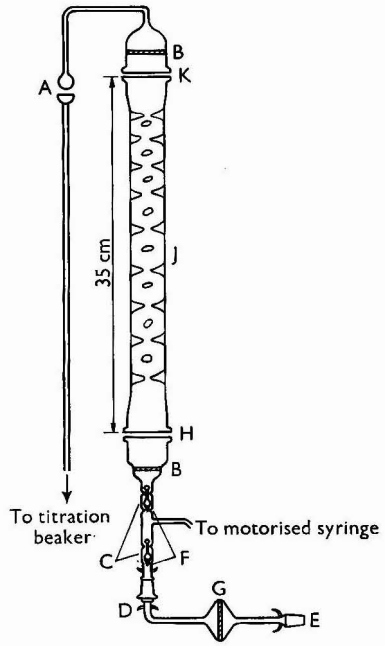
REDUCTOR—

A diagram of the apparatus is shown in Fig. 4.

The solution is pumped from the sample beaker to the reductor through a non-return valve system, consisting of hollow, ground-glass valves weighted with mercury, fitted to the base of the reductor column so that the solution can only pass from the beaker to the column. Since small particles of solid material interfere with the operation of the glass valves, all

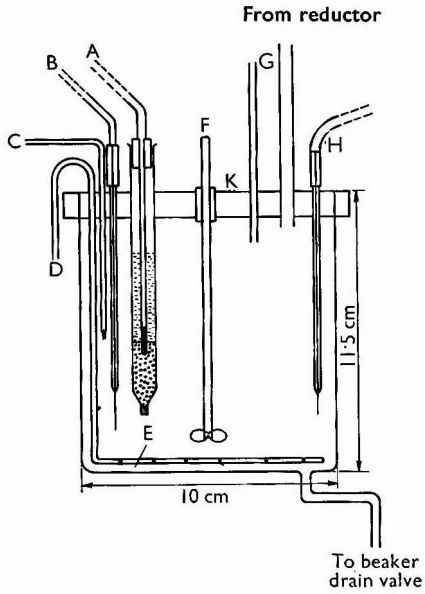
solutions used must be free from suspended matter, and a No. 1 porosity sintered-glass filter is fitted in the sample line as an additional precaution.

The glass reductor column is 20 mm in diameter and 35 cm long. The column has a series of indentations to improve distribution, and is fitted with a 75-mm No. 0 porosity sintered-glass filter at the base to support the cadmium reducing agent. A similar filter is fitted at the upper end to prevent cadmium passing into the titration beaker. Two clip-on joints are fitted to the column to facilitate re-packing. The space between the top joint and the sintered-glass filter is also packed with glass-wool as an additional precaution. To the upper end is attached an outlet tube leading into the titration beaker.



A = QVF ball joint, m.s. 12/2 BS
B = 75 × 0 sintered disc
C = Glass valves weighted with mercury
D = B10 cone and socket
E = B10 cone
F = Constrictions ground to form valve seat
G = 7 × 1 sintered disc
H = 1-inch clip-on joint
J = 20-mm bore with indentations
K = 1-inch clip-on joint

Fig. 4. Reductor column and valve assembly



A = Calomel electrode
B = Platinum earth electrode
C = Titrant delivery
D = Carbon dioxide inlet
E = Carbon dioxide ring
F = Stirrer
G = Thiocyanate delivery
H = Platinum indicating electrode
K = Perspex beaker top

Fig. 5. Titration beaker and electrodes

TITRATION BEAKER AND ELECTRODE SYSTEM—

This is shown in Fig. 5. It is made from a 1-litre beaker, the top of which is cut off to the dimensions shown, and a drain outlet fitted. The lid of the beaker is machined from solid Perspex, and is drilled to take the calomel reference and two platinum electrodes, which are held in position by clips attached to the centre bridge-piece. The lid also contains holes through which pass the stirrer, the sample solution, the inert purge gas, the potassium thiocyanate inlet and the titrant. The inert gas and drain outlet are both controlled by standard solenoid valves that are obtainable from Baird and Tatlock Ltd., Chadwell Heath, Essex. All the valves used in the instrument are of the energise - to - open type. The stirrer

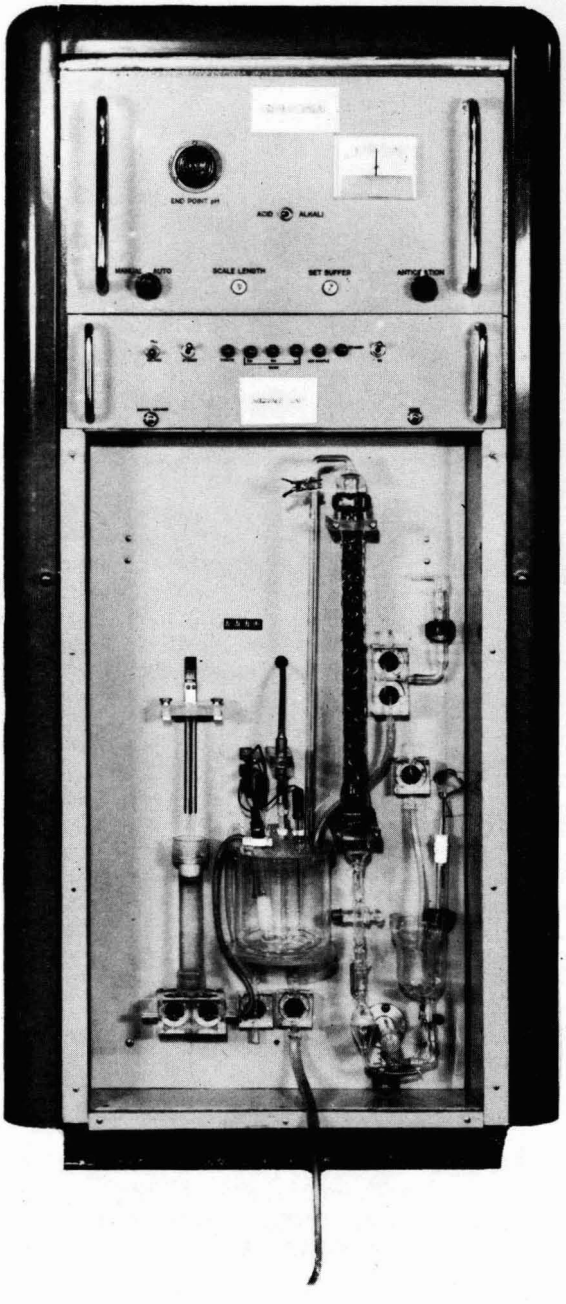


Fig. 3. General view of titrator

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has a shaft of diameter 5 to 6 mm, and is fitted with four blades. The bearing is machined from PTFE, and the drive is by a flexible shaft from a 456 r.p.m., 250-volt a.c., 50 c/s geared induction motor.

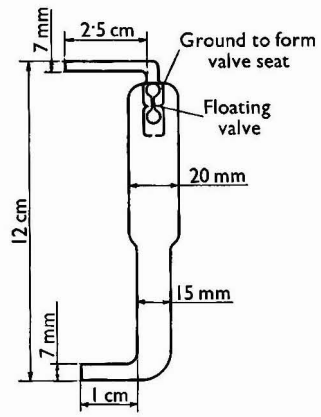

Fig. 6. Potassium thiocyanate vessel

Fig. 6 shows the vessel from which 10 ml of potassium thiocyanate solution are transferred to the beaker. Solenoid valves are used to control the filling and emptying of the vessel, the seal being effected by a floating glass valve which is simpler and more reliable than a system of level electrodes.

The inert-gas supply is also controlled by a solenoid valve.

A standard calomel electrode with a porous plug base is used. The platinum indicating and earth electrodes consist of 25 s.w.g.-platinum wire fused into a glass tube so that a length of 1 cm is exposed.

TITRANT SYRINGE—

This consists of a glass syringe of 20-mm i.d. barrel, and a piston made of PTFE with a rubber sealing ring. The piston is attached to a leadscrew driven by a 250-volt a.c., 50 c/s, 19-r.p.m. reversible geared induction motor. The leadscrew also drives a mechanical counter which indicates the amount of titrant added. The early form of syringe consisted of a glass piston and barrel, and considerable care had to be taken in the assembly of the drive mechanism to prevent fracture of the syringe. Replacement of the piston by one machined from Teflon, fitted with a rubber O-ring seal eliminated this trouble, and at the same time increased the accuracy of the delivery.

The filling and emptying of the syringe is controlled by a parallel pair of solenoid valves mounted in a Perspex block. When the syringe is full, a micro-switch, Sw_6, is operated which automatically closes the fill valve. The empty valve is opened when the drive motor starts to operate.

If, in the preliminary experiments, a speed of delivery was selected such that overshooting of the fill position or the end-point did not occur, the rate of filling of the syringe was very slow and the duration of determination was lengthened appreciably. Accordingly, a two-speed gearbox was designed and fitted to the drive, so that a fast rate could be used for most of the filling and emptying, and a slow rate when the end-point or the fill positions were being approached. A diagram of the gearbox system is shown in Fig. 7. The change-over is operated by a micro-switch, Sw_7, when the syringe is being filled, and by an anticipation control when the end-point is approached.

CONTROLS—

The titrator consists of three basic units, all of which are readily detachable and replaceable. The end-point indicator, set-buffer control, scale-length control, manual - auto switch and anticipation control are contained in the end-point unit which is housed in the upper part of the instrument. A switch is also incorporated to allow for change in the direction of the titration.

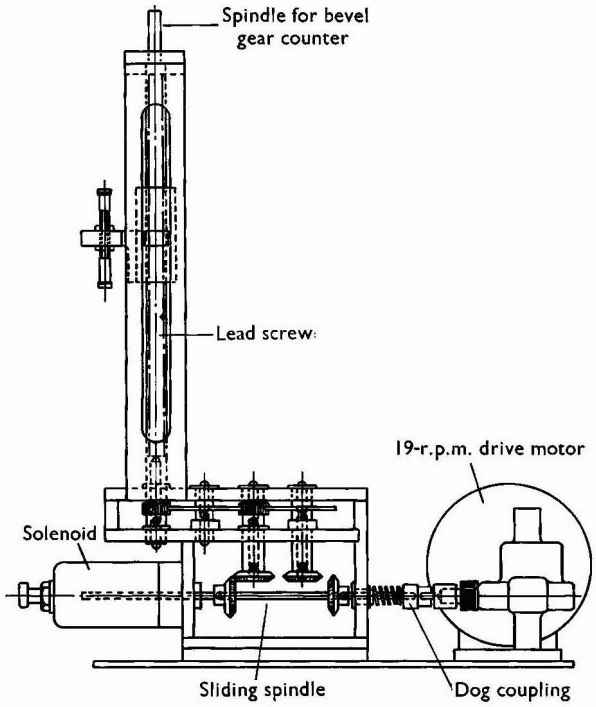

Fig. 7. Two-speed gearbox

The centre unit, which controls the sequence of operations, contains the mains on - off switch, the start button, the manual advance control and indicating lights. A fill-deliver control is also installed for use in priming the syringe when a new one is being fitted. The manual advance control enables wash stages to be by-passed if required.

The lower section houses the titration assembly.

Sequence of operations

The instrument has been designed so that many of the operations take place simultaneously, resulting in a considerably reduced cycle time; this is summarised in Table I. Before starting a determination, the ferric alum, potassium thiocyanate and wash solution reservoirs should all be filled, and the ferric alum syringe should be primed by manual operation of the fill-deliver control. The instrument should be switched on for approximately 1 hour before use to allow the end-point system to become stabilised. The "stand-by" light, L_1, should be illuminated to indicate that the Uniselector is in the first or "stand-by" position. Reference should be made to the sequence unit circuit shown in Fig. 8, for details of the circuit, and to the appendix for component details.

The sample solution is transferred to the sample beaker and the start button pressed. This closes Sw_{1a} and Sw_{1b} which energises relay RLS. Closure of S1a maintains relay RLS in an energised state when the "start" button is released. Energising relay RLS moves the Uniselector to position 2, indicated by the lighting of L_2.

Uniselector position 2—

Contact S1b is closed, thereby opening the potassium thiocyanate delivery valve, and potassium thiocyanate is added to the beaker. S1c is closed and the circuit through RLB to earth is completed via electrode probes.

The wash syringe motor is started when S2 is closed and S3a opens causing the beaker drain valve to close.

Relay RMF is then energised by change over of S3b. Closure of MF1 energises the "fill titrant" valve, causing it to open.

TABLE I

SUMMARY OF OPERATIONS CARRIED OUT AT EACH UNISELECTOR POSITION

Uni-selector position	Potassium thiocyanate deliver	Inert-gas valve	Beaker drain valve	Ferric alum feed valve	Titration syringe	Wash solution valve	Wash syringe motor
1	Closed	Closed	Open	Closed	Stopped	Closed	—
2	Open. Solution added to titration beaker	Open	Closed	Open Closed	Syringe fills with ferric alum at fast rate until the last 50 revs., which are added at the slow rate. When the zero end stop is reached, the ferric alum feed valve is closed	Closed	Pumps sample solution from beaker, through the reduction column to the titration beaker. When the liquid level falls to the lower level probe, the Uni-selector moves to position 3
3	Open	Open	Closed	Closed	—	Open. Solution added to top level probe, then valve closes (first wash)	Solution pumped as above, and when the liquid level falls to the lower probe the Uniselector moves to position 4
4	Open	Open	Closed	Closed	—	Open. Solution added as above (second wash)	As above. When the lower probe is reached, the Uniselector moves to position 5
5	Open	Open	Closed	Closed	—	Open. Solution added as above (third wash)	As above. When the lower probe is reached, the Uniselector moves to position 6
6	Closed. Vessel fills	Open	Closed	Closed	Titration starts at slow rate for first 50 revs., then at fast rate until the anticipation is reached. At the end of the titration, *i.e.*, when the end-point potential is maintained for 20 seconds, the Uniselector moves to position 1	Closed	Stopped

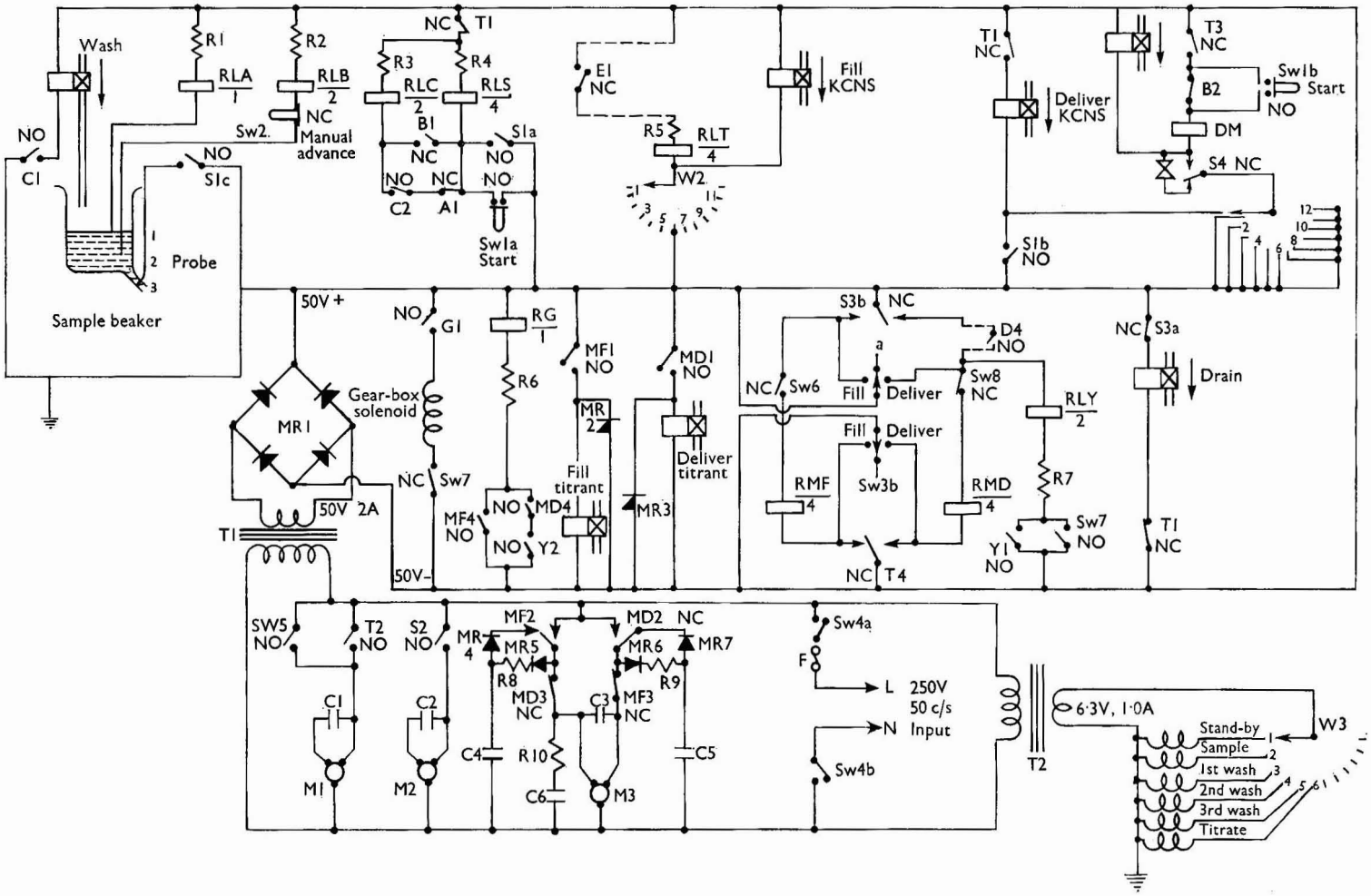

Fig. 8. Sequence unit circuit diagram

The titrant motor is started when MF2 changes over and MF3 opens. The phase difference ensures that the titrant motor starts rotating in the correct direction and titrant is pumped to the syringe.

The rate of filling the syringe is increased by changing the gear drive. This is accomplished when MF4 is closed and relay RLG is energised. GI is closed which energises the gearbox solenoid and the gear is changed to the fast rate.

Immediately before the syringe is full, the rate of filling is decreased to avoid overshooting the end-point. This is achieved by the opening of Sw_7 when the leadscrew reaches a pre-set position. The gearbox solenoid is de-energised and the gear is changed back to the slow rate.

When the titrant syringe is full, Sw_6 is opened; this de-energises RMF. Contact MF2 reverts to the normal position and the titrant motor stops, the braking components ensuring that the motor stops instantaneously.

The above operations are all carried out on position 2 of the Uniselector. The sample is gradually pumped from the sample beaker to the reductor column and the beaker is washed out with three lots of wash solution. The detail of how this is done is as follows:

When the liquid level falls below probe 2, relay RLB is de-energised and closing of B2 energises the Uniselector coil. B1 closes which energises relay RLC. This relay remains energised because C2 is closed. C1 closes, thus causing wash solution to pass into the beaker.

UNISELECTOR POSITIONS 3 TO 5—

When the liquid makes contact with probe 2, relay RLB is energised which opens B2. This advances the Uniselector to stage 3, lamp 2 is switched off and lamp 3 switched on. Note that B1 is opened, but owing to C2 being closed relay RLC remains energised.

When the liquid level makes contact with probe 1, relay RLA is energised which opens A1. This de-energises relay RLC which opens C1 and the flow of wash solution stops.

This wash procedure is repeated until three washes have been completed and the uniselector is on position 5.

UNISELECTOR POSITION 6—

When the Uniselector is moved to position 6, relay RLT is energised. T1 is opened which (*a*), closes the potassium thiocyanate deliver valve, (*b*), keeps the drain valve closed and (*c*), de-energises relays RLS and RLC. This stops the wash syringe motor. T2 is closed, so starting the stirrer motor. T3 opens to maintain the Uniselector in position 6. T4 changes over and relay RMD is energised which (*a*), opens the titrant delivery valve and (*b*), starts the titrant motor in the delivery direction. Note that D4 is always closed at the beginning of a titration. Titrant is then delivered to the beaker at the slow rate.

During the titration the potassium thiocyanate vessel is filled, since the fill valve is directly in contact with position 6 on the Uniselector.

When the relay RLY is energised owing to Sw_7 being closed by the lead screw, the contact Y1 maintaining the circuit through RLY when the Sw_7 circuit is broken, Y2 closes, energising relay RLG and so causing the gear to change so that titrant is delivered at a fast rate.

At the anticipation setting, D4 opens, relay RLY is de-energised and Y1 and Y2 open. Relay RLG and hence the gearbox solenoid are de-energised and the titrant addition reverts to the slow rate. Relay RMD is de-energised and the titration stops.

The titration is controlled by the signal from the end-point detector. Closure of D4 causes addition of titrant to occur. Titrant is added in short bursts until the potential difference between the electrodes is the same as the end-point potential and has remained unchanged for a pre-set period. E1 opens and de-energises relay RLT so that T3 closes and the Uniselector operates on its motoring contacts and returns to position 1 indicated by lamp L_1.

In this position T1 closes and the beaker drain valve opens. Since position 1 of bank 1 on the Uniselector is not earthed, the carbon dioxide valve closes.

METHOD

CALIBRATION AND STANDARDISATION—

To standardise the end-point detector a high-impedance potential source of 0 to 500 millivolts is required. Disconnect the electrodes from the end-point detector, connect the millivolt supply and proceed as follows:

Set the end-point pH control to 400 and the potential supply to 100 millivolts. Adjust the set-buffer control until the meter reads zero. Change the potential supply to 300 millivolts and rotate the end-point pH control until the meter reads zero. The end-point pH control should read 800. If the reading is not 800, adjust the scale length control and repeat the previous operations until the correct scale length is obtained.

Disconnect the potential source and connect the electrodes to the end-point detector. Transfer to the titration vessel a titanium solution which has been titrated to the end-point. Set the end-point pH control to 250 and adjust the set-buffer control until the meter reads zero. The instrument is now set approximately to the required end-point.

Set the "anticipation" control to a value which will cause the first anticipation to occur about 5 ml from the end-point. This value can only be found by trial and error.

Weigh accurately to 0·1 mg a catch weight of approximately 0·9 g of titanium dioxide (99·9 per cent. purity) which has been previously dried in an air-oven at 105° C. Transfer to a 250-ml beaker, and dissolve by heating with 40 ml of concentrated sulphuric acid and 30 g of ammonium sulphate. Cool and dilute to 500 ml in a calibrated flask with distilled water. Pipette a 50-ml aliquot into the sample beaker, set the manual - auto switch to the manual position and press the "start" button. When the titanium solution has been reduced and transferred to the titration beaker, continue the titration manually and construct a curve of titrant *versus* potential difference. Select the setting for the end-point by inspection of the graph and use this value for all subsequent titrations. Set the manual - auto switch to auto and repeat the titration using a 50-ml aliquot each time.

The calibration should be carried out in triplicate, and the volume of ferric alum used in each case noted in revolutions. The scatter between replicate titrations should be within ±0·1 per cent. The weight of titanium dioxide per revolution can then be calculated.

N.B.—This procedure eliminates the need to standardise the ferric alum before use on the titrator.

REAGENTS—

Cadmium granules—1 to 2 mm in size.

Potassium thiocyanate solution, 20 per cent. w/v.

Wash solution—Prepare 10 per cent. w/v ammonium sulphate in sulphuric acid (1 + 50).

Ferric alum solution, approximately 0·0625 N—Dissolve 1200 g of ferric alum, A.R., in 400 ml of concentrated sulphuric acid and 7 litres of water. Dilute to 36 litres and mix well. Oxidise any ferrous iron by adding 0·1 N potassium permanganate solution until one more drop will just colour 50 ml of the solution. Then add 20 ml in excess.

PROCEDURE—

Any substance which is reduced by cadmium metal and re-oxidised by ferric alum will interfere in the determination. Interfering agents include chromium, vanadium, niobium, tungsten, uranium, platinum, molybdenum, arsenic and antimony, as well as nitric acid and certain organic compounds. Those which occur in titaniferous materials are chromium, vanadium, niobium and molybdenum. Chromium, vanadium and molybdenum reduce to a definite valence and may be allowed for in the determination if their concentration is known.

To determine the titanium content of a solution, a suitable aliquot is transferred to the sample beaker, the manual - auto control is set to auto and the start button pressed. At the end of the determination the weight of titanium dioxide present in the solution is calculated from the reading on the counter and the conversion factor for the ferric alum titrant. Note that there is no need to rinse the titration beaker between determinations because the residual solution is at the end-point condition and will not interfere in the titration of the next sample.

RESULTS

The accuracy of the instrument was assessed by making 30 determinations on the same sample. The following results were obtained—

TiO_2, per cent. w/v	15·35	15·36	15·37	15·38	15·39
Number of results	1	9	4	3	5

TiO_2, per cent. w/v	15·40	15·41	15·42	15·44
Number of results	3	3	1	1

Average determination—15·38 per cent.
Standard deviation—0·023 per cent.
Coefficient of variation—0·145 per cent.

Performance

A complete determination takes 7 minutes, during which time the operator is available to carry out other work such as the preparation of the next sample. The instrument has resulted in a substantial saving of time and has also increased the accuracy of the determination by the use of a potentiometric instead of a colorimetric end-point. It is natural that such an instrument requires careful maintenance, and a scheme of routine preventative maintenance has been drawn up which has greatly contributed to reducing the down-time of the instrument. It has been found that particular attention should be paid to the electrodes and to ensuring that the solutions are free from insoluble particles. The cadmium granules should be sifted to remove any fine particles before use.

The instrument has been in use in the laboratories of this Company for longer than a year and has given satisfactory service during this period. The total cost of the instrument, including labour, materials and commissioning, is approximately £550.

We thank Mr. R. Hutton and Mr. D. L. Suttill for technical assistance, and the Directors of British Titan Products Company Ltd., for permission to publish this paper.

Appendix I

Components list for end-point detector (Fig. 1)

Unless otherwise stated, the resistors have a tolerance of ±2 per cent. and are made of high stability carbon.

R_1, R_2, R_{26} R_{27},	= 1·8-megohm, 0·25-watt resistors
R_3	= 27,000-ohm, 0·25-watt resistor
R_4, R_5, R_9, R_{10}, R_{23}, R_{39}	= 1-megohm, 0·25-watt resistors
R_6, R_7	= 10-ohm, 0·25-watt resistors
R_8, R_{19}, R_{28}, R_{36}	= 22,000-ohm, 0·25-watt resistors
R_{11}, R_{12}, R_{13}, R_{14}, R_{38}, R_{43}	= 2·2-megohm, 0·25-watt, resistors
R_{15}, R_{16}	= 39,000-ohm, 0·25-watt resistors
R_{17}	= 1800-ohm, 0·25-watt resistor
R_{21}	= 2-megohm, 0·25-watt resistor
R_{24}	= 10-megohm, 0·25-watt resistor
R_{25}	= 560,000-ohm, 0·25-watt resistor
R_{29}, R_{37}	= 33,000-ohm, 0·25-watt resistors
R_{30}	= 5600-ohm, 0·25-watt resistor
R_{33}	= 33-megohm, 2-watt resistor
R_{34}, R_{44}	= 1000-ohm, 0·25-watt resistors
R_{35}	= 470,000-ohm, 0·25-watt resistor
R_{42}	= 22-megohm, 0·25-watt resistor
R_{45}	= 180,000-ohm, 0·25-watt resistor
R_{20}	= 25,000-ohm, 4-watt, 10-turn helically-wound potentiometer, with a resistance tolerance of 5 per cent. and linearity tolerance of ±0·5 per cent.
R_{22}	= 25,000-ohm, 1-watt, wire-wound potentiometer with a tolerance of ±20 per cent.
R_{31}	= 3300-ohm, 5-watt, wire-wound resistor with a tolerance of 5 per cent.
R_{32}	= 22,000-ohm, 5-watt, wire-wound resistor with a tolerance of 5 per cent.
R_{40A}, R_{40B}	= 100,000-ohm, 0·5-watt, twin-gang potentiometer with a tolerance of ±20 per cent. R_{40A} increases as R_{40B} decreases.
R_{41}	= 100,000-ohm, 1-watt, wire-wound potentiometer with a tolerance of ±20 per cent.
C_1, C_2	= 0·005-μF tubular paper capacitors, 500-volt, d.c., working
C_3, C_4	= 0·001-μF tubular paper capacitors, 500-volt, d.c., working
C_5	= 0·25-μF tubular paper capacitors, 500-volt, d.c., working
C_6	= 1-μF tubular paper capacitor, 500-volt, d.c., working
C_7	= 0·05-μF tubular paper capacitor, 500-volt, d.c., working
C_8	= 32-μF electrolytic capacitor, 450-volt, d.c., working
C_9	= 2-μF paper capacitor, 600-volt, d.c., working
C_{10}	= 8-μF electrolytic capacitor, 500-volt, d.c., working
C_{11}	= 0·02-μF tubular paper capacitor, 500-volt, d.c., working
C_{12}	= 20-μF metallised paper capacitor, 150-volt, d.c., working
MR_1, MR_2	= 250-volt, r.m.s., silicon metal rectifiers
S_1	= Round, double-pole, double-throw toggle switch
S_2	= 3-pole, 2-way wafer switch
V_1	= ME 1400 valve
V_2	= EF 37A valve
V_3	= ECC 83 valve
V_4, V_5, V_6, V_{11}	= 6060 valves

V_7	= EZ 80 valve
V_8, V_9, V_{10}	= 108 C1 valves
T_1	= Constant voltage transformer, input: 240 volts, 50 c/s; output: 6·0 volts
T_2	= Main transformer, input: 200–250 volts; output: 250–0–250 volts, 60 mA; 6·3 volts, 3 amps and 6·3 volts, 1 amp
RLD/4	= 20,000-ohm coil, 4-pole change-over, Post Office type 3000 plug-in relay
RLE/1	= 20,000-ohm coil, 2-pole change-over, Post Office type 3000 plug-in relay
M	= Centre-zero microammeter, 2·5 inch diameter, 100–0–100 μA full-scale deflection
P	= Painton Multicon 12-pole socket

Appendix II

Components list for the sequence unit (Fig. 8)

R_1, R_2, R_3, R_4, R_5, R_6, R_7	= 650-ohm, 1-watt, high-stability carbon resistors
R_8, R_9	= 600-ohm, 5-watt, wire-wound resistors
R_{10}	= 100-ohm, ¼-watt, high-stability carbon resistor
C_1, C_2, C_3	= 1-μF, 500-volt d.c. working paper capacitors
C_4, C_5	= 64-μF, each composing 2 32-μF, 450-volt d.c. working, electrolytic capacitors
C_6	= 0·1-μF, 500-volt d.c. working, tubular paper capacitor
MR_1	= REC 23a metal bridge rectifier, 54 volts r.m.s., 1 amp input, 50 volts d.c. output
MR_2, MR_3, MR_4, MR_5 MR_6, MR_7	= REC 51A silicon rectifier, 250 volts r.m.s., 500 mA
RLA, RLB, RLC RLG, RLS, RLT, RLY	= Miniature plug-in relays, 24-volt d.c., 650-ohm coil, 4-pole change-over contacts rated at 3 amps a.c.
RMD, RMF	= Miniature flag-in relays, 48-volt d.c., 1850-ohm coil, 4-pole change-over contacts rated at 250 volt, 3 amp a.c.
Sw_1	= Push to make, release to break, double-pole double-throw switch
Sw_2	= Push to break, release to make, double-pole double-throw switch
Sw_3	= Centre-off, double-pole double-throw toggle switch
Sw_4, Sw_5	= Round double-pole double-throw toggle switches
Sw_6, Sw_7	= Micro-switches
Sw_8	= Micro-switch
F	= ¾-inch 2-amp fuse
T_1	= Transformer: primary, 250 volts; secondary, 50 volts, 2 amps
T_2	= Standard filament transformer: primary, 250 volts; secondary, 6·3 volts
DM and W_1, W_2, W_3	= 3-bank, 12-position, plug-in type Uniselector unit, 250-ohm coil
Lamps	= 6·3 volts, 1-watt, panel lamps

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The Polarographic Determination of Lead after Cation-exchange Separation

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From M hydrofluoric acid solution, lead, cobalt, copper(II), manganese(II), nickel and a small part of chromium(III) are strongly adsorbed on a column of strongly acidic cation-exchange resin in the hydrogen form, while other elements present in steel are either not adsorbed or only weakly adsorbed, and are removed from the column on washing it with M hydrofluoric acid.

On elution with 2 M hydrochloric acid, the lead is removed from the column and determined by d.c. polarography. This method is applied to the determination of lead (>0·01 per cent.) in steels.

THE direct polarographic determination of lead in the presence of aluminium, chromium(III), cobalt, copper, iron(II), manganese, nickel, tin(IV) and zinc is straightforward, and alloys containing these elements have been satisfactorily analysed for lead.[1,2,3] However, the polarographic determination of lead is more difficult in the presence of titanium(IV) and molybdenum(VI), elements often present in high-alloy steels, because these species often produce reduction waves that interfere with the lead wave.

Hamza and Headridge,[4] using M ammonium fluoride adjusted to pH 7 as the base electrolyte, obtained a reversible reduction wave for lead, $E_{\frac{1}{2}} = -0{\cdot}453$ volt against a S.C.E., with which there is no interference from molybdenum(VI), titanium(IV) and vanadium(IV). However, if that base electrolyte was used for the direct determination of lead in steel, there would be interference from iron(III), which produces an irreversible reduction wave, $E_{\frac{1}{2}} = -0{\cdot}77$ volt against a S.C.E., that interferes with the lead wave when the molar ratio of iron(III) to lead exceeds 4 to 1. Although attempts were made to remove interference from iron(III) by reducing it quantitatively to iron(II), these were unsuccessful because iron(II) is a powerful reducing agent in M ammonium fluoride.

It was, therefore, decided to examine the possibility of separating lead from iron(III), molybdenum(VI), titanium(IV) and vanadium(V) using a cation-exchange resin.

Headridge and Dixon[5] have reported that aluminium, iron(III) and vanadium(V) are scarcely adsorbed on the cation-exchange resin, ZeoKarb 225, in the hydrogen form, from M hydrofluoric acid. On the other hand, cobalt, copper (II), nickel and manganese(II) are strongly adsorbed. The behaviour of chromium(III) was unusual. From boiled solutions, the chromium(III) was obviously present in two complexes not in rapid equilibrium. The complex present in major amount was not adsorbed by the cation-exchange resin from M hydrofluoric acid, but the minor complex, possibly $Cr(H_2O)_4F_2^+$, was strongly adsorbed.

Nikitin[6] has reported that lead is adsorbed by a cation-exchange resin from M hydrofluoric acid. This is to be expected since lead, like copper(II), complexes only weakly with fluoride,[7] and copper(II) is strongly adsorbed by ZeoKarb 225 from M hydrofluoric acid. arsenic(III) and (V), antimony(III) and (V), tin(IV), titanium(IV), zirconium, niobium(V), tantalum, molybdenum(VI) and tungsten(VI) are either not adsorbed or only weakly adsorbed by a cation-exchange resin from M hydrofluoric acid.[6,8,9]

A simple method is therefore available for separating lead from elements that interfere with its polarographic determination. The alloy is dissolved in a mixture of hydrofluoric and nitric acids, the excess of nitric acid is removed by evaporation, and a M hydrofluoric acid solution of the metallic ions is passed down a column of ZeoKarb 225 in the hydrogen form. Lead, cobalt, copper(II), manganese(II), nickel and a small fraction of the chromium(III) are adsorbed. On washing with 16 column volumes of M hydrofluoric acid, arsenic(V), antimony(V), aluminium, iron(III), tin(IV), titanium(IV), zirconium, vanadium(V), niobium(V), tantalum, molybdenum(VI), tungsten(VI) and most of the chromium(III) are removed from the column.

Hydrochloric acid (2 M) was considered to be a suitable eluant for lead. Most, or all, of the copper(II), cobalt, chromium(III), manganese(II) and nickel accompany the lead, but cobalt, manganese and nickel do not interfere with the polarographic determination of lead in a base electrolyte of M hydrochloric acid. Copper in amounts considerably in excess of the lead causes difficulties with the d.c. polarographic determination of lead in M hydrochloric

acid, but not with a differential cathode-ray or pulse polarographic determination. Chromium(III) interferes with the polarographic determination of lead in M hydrochloric acid, and, if more than a trace of chromium(III) is present, a base electrolyte of 0·5 M acetic acid - 0·5 M sodium acetate - 0·5 M sodium chloride may be used. There is no interference from chromium(III) in this base electrolyte.

A polarographic method based on this scheme is now described for the determination of lead in steels.

EXPERIMENTAL

APPARATUS—

Polarograph—A Sargent model XV polarograph was used.

Polarographic cell—This was a Meites-type H-cell with a saturated calomel electrode in the electrode compartment and an agar-saturated potassium chloride bridge. The volume of solution used in the solution compartment was 40 ml. The cell was immersed in a water tank thermostatically controlled at 25·0° C. Oxygen-free nitrogen was used to free the solution from dissolved oxygen.

Polythene column—This was constructed as follows. The bottom of part of a polythene specimen tube, of length 2·0 cm and internal diameter 1·3 cm, was drilled with eight holes of diameter 0·05 cm, and half-filled with polythene drillings. A polythene disc, of diameter 1·35 cm, was also drilled with eight holes of diameter 0·05 cm, and was forced into the specimen tube until it came into contact with the drillings. A piece of polythene tubing, 34 cm long with an internal diameter of 1·0 cm and external diameter of 1·3 cm, was then inserted into, and welded to, the specimen tube. A short piece of flexible plastic tubing was pushed over the lower end of the specimen tube and fitted with a screw clamp.

An aqueous slurry of ZeoKarb 225 (SRC14), of mesh size 52 to 100, was added to the column to produce a resin bed 3·8 cm high and 3·0 ml in volume. The top 30 cm of the column acted as a reservoir. The resin was washed with 5 M hydrochloric acid to convert it entirely to the hydrogen form, and then by water until free from chloride ions. It was then ready for use.

REAGENTS—

Hydrochloric, hydrofluoric and nitric acids—These were of analytical-reagent grade.

High-purity iron—This was Specpure iron obtained from Johnson, Matthey and Company Limited.

Standard lead solution—Prepare an exactly 10^{-3} M solution from the appropriate weight of analytical-reagent grade lead nitrate crystals, the lead content of which has been previously determined by a complexometric titration with standard EDTA solution. The lead solutions used in obtaining the calibration graph are prepared from this standard lead solution by diluting with 2 M hydrochloric acid and water.

METHOD—

Dissolve 1 g of steel in 15 ml of 40 per cent. w/w hydrofluoric acid plus 1 ml of nitric acid, sp.gr. 1·42. Evaporate the solution just to dryness and dissolve the residue in 5 ml of 40 per cent. w/w hydrofluoric acid. Re-evaporate just to dryness. Dissolve the residue in 25 ml of 10 M hydrofluoric acid and dilute the solution to 250 ml in a graduated flask. Immediately transfer the solution to a dry polythene bottle.

Add a suitable aliquot of the M hydrofluoric acid solution to the column of ZeoKarb 225 resin at a flow-rate of approximately 2 ml minute^{-1}, such that the quantity of lead, copper, cobalt, manganese, nickel and chromium(III) does not exceed 0·8 millimoles (Notes 1 and 2). Then pass 50 ml of M hydrofluoric acid through the column at a flow-rate of approximately 2 ml minute^{-1}, followed by 10 ml of water.

NOTE 1. Only 2 per cent. of the total chromium(III) is retained by the cation-exchange resin. Make allowance for this when calculating the quantity of adsorbable cations.

NOTE 2. The column of cation-exchange resin has a total capacity of 3·2 millimoles for doubly charged ions. By restricting the total quantity of strongly adsorbed ions to 0·8 millimoles, only the top 25 per cent. of the column will be occupied by these ions. This is considered to be an adequate safety factor to ensure that no lead is removed when 50 ml of M hydrofluoric acid are subsequently passed through the column.

Elute all of the lead from the column with 50 ml of 2 M hydrochloric acid at a flow-rate of 2 ml minute^{-1}, collecting the effluent in a 100-ml graduated flask. Dilute the solution to the mark with water.

Place 40 ml of this solution in the solution compartment of the polarographic cell and record a polarogram over the potential range of 0 to −1·0 volt against a S.C.E. When more than a trace of chromium(III) is present in the alloy, transfer 50 ml of the M hydrochloric acid solution by pipette into a 100-ml graduated flask, make up to the mark with 2 M sodium acetate solution, and record a polarogram with this solution. Measure the lead diffusion current at −0·55 volt against a S.C.E. and determine the concentration of lead in the solution from a suitable calibration graph. Hence calculate the amount of lead in the alloy. The authors used a lead calibration graph prepared from six solutions in the concentration range of 10^{-5} to 10^{-4} M. The standard deviation of the error in diffusion current was 0·010 μA corresponding to a relative standard deviation of 1·4 per cent. at a lead concentration of 10^{-4} M. The error in diffusion current is expressed by i_d (measured) - i_d (calculated), where the values of i_d (calculated) are points exactly on the straight-line calibration graph of diffusion current *versus* concentration.

Results

Analysis of synthetic solutions—

Four synthetic solutions of iron(III) plus lead in M hydrofluoric acid were prepared and carried through the cation-exchange and polarographic procedures. Each synthetic solution contained 1 g of Specpure iron. The volume of each solution passed through the column was such that the lead concentrations of the solutions being polarographed were in the range of 4×10^{-5} to 10^{-4} M. The recoveries of lead are shown in Table I.

Table I

The recoveries of lead after a cation-exchange separation from iron(III)

Lead taken, mg	1·00	4·11	8·19	20·3
Lead found, mg	1·00	4·08	8·22	20·3

Analysis of steels—

Two steels were analysed by the recommended method using the calibration graph mentioned previously. The results are shown in Table II.

Table II

Results for the determination of lead in steels

Alloy	Lead content, per cent.	Lead found polarographically, per cent.
Mild steel, BCS 329	0·050	0·050, 0·050
Lead steel, BCS 212/1 ..	0·22	0·21, 0·22

Discussion

A cation-exchange separation of lead prior to its polarographic determination is not actually necessary with the two steels analysed above, but the results, in conjunction with those for the synthetic solutions, are proof of the reliability of the separation scheme.

Although the above results are satisfactory, the metallurgist is primarily interested in amounts of lead less than 100 p.p.m. Because the d.c. polarograph is incapable of producing precise results for lead determinations at concentrations below 100 p.p.m. in the alloy by the above method, we were unable to examine the full potentialities of the method. However, we see no reason why the lower limit of determination should not be lowered to 1 p.p.m. by using a more sensitive polarograph such as a differential cathode-ray or pulse polarograph. Parts per million of trace metals in alloys have already been determined using a square-wave polarograph.[1]

The method should be particularly suitable for the determination of lead in alloy steels containing titanium, vanadium, niobium, tantalum, molybdenum and tungsten, all of which are soluble in a mixture of hydrofluoric and nitric acids. The method could also be applied

to the determination of lead in niobium- and tungsten-base alloys, etc. With all alloys precautions must, of course, be taken to ensure that the column is not overloaded with cobalt, copper, nickel and manganese, which are adsorbed with the lead.

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The Determination of Sodium in Aluminium Alloys by Flame Spectrophotometry with Fuel-rich Flames to Reduce Interference

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The determination of trace quantities of sodium in aluminium alloys by flame spectrophotometry offers a rapid and accurate control procedure. When conventional flames with a balanced fuel - air mixture are used, molecular oxide band spectra of iron and manganese are strongly excited, and interfere with the measurement of the sodium emission. The interference is much less in fuel-rich flames while the sensitivity to sodium is slightly increased. The use of fuel-rich flames therefore provides a more versatile and accurate method than those hitherto used. As in most methods for the determination of sodium it is advantageous to add lithium as an internal standard to compensate for minor variations in conditions. The results are of general application to the analysis of other materials which may contain iron and manganese, and possibly nickel and chromium.

The deleterious effects of small amounts of sodium in aluminium - magnesium alloys were first described by Ransley,[1] who showed that as little as 10 p.p.m. of sodium could cause embrittlement leading to cracking on hot-rolling of the ingots. With large ingots even smaller sodium contents have adverse effects, and it is therefore necessary to be able to carry out accurate analysis before fabrication of the ingot.

Spectrographic determination of sodium is used in routine control but requires chemically-analysed metal standards for calibration. Careful attention to the sampling procedure is also necessary because of the tendency for sodium to segregate. Flame spectrophotometry offers a highly sensitive means of determining sodium,[2] and has been used to establish the calibration values of spectrographic standards. Moreover, samples can also be taken by drilling the solid ingot, thus avoiding segregation difficulties. Matelli[3] described a method using a Beckman DU spectrophotometer with flame attachment capable of determining down to 10 p.p.m. of sodium in aluminium alloys. By using the more sensitive Unicam SP900 flame spectrophotometer, Hine and Bates[4] extended the range to 1 p.p.m. without using methanol to intensify the emission. However, in applying the latter method to the analysis of a variety of aluminium alloys it has been found that errors can result from interference unless the metal used for calibration is identical in composition (other than sodium content) with the sample being analysed. In particular, differences in iron and manganese contents between the sample and calibration metal give rise to serious errors. In this paper it is shown that the extent of interference is governed by the type of flame used and that with cool, fuel-rich flames the interference is slight.

Experimental

Apparatus—

Flame spectrophotometer—Unicam SP900 with acetylene - air flame.

All other apparatus should be made of quartz, translucent silica or polythene, as appropriate.

Reagents—

Sodium-free alloy for calibration—Place a clean piece (10 to 50 g) of alloy, of the same type as that to be analysed, in a graphite crucible in a vacuum distillation apparatus capable of maintaining a vacuum of at least 10^{-5} mm of mercury. A suitable apparatus is described

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on p. 24 of "Analysis of Aluminium and its Alloys."[2] Heat to 900° C for 1½ hours. Cool, remove the specimen, pickle the surface in dilute hydrochloric acid, rinse and dry. Repeat the vacuum treatment. Reduce by milling or drilling with scrupulously clean tools. Take 1-g portions of the metal for calibration.

Pure water—Pass water (preferably distilled) through a mixed-bed resin, *e.g.*, Amberlite MB3, using all-plastic apparatus.

Hydrochloric acid, 6 N—Prepare from the purest obtainable reagent; if necessary, distil in quartz.

Sodium chloride stock solution—Dry pure sodium chloride at 105° C; dissolve 2·5418 g in water and make up to 1000 ml in a volumetric flask. Transfer immediately to a polythene bottle. 1 ml = 1 mg of sodium.

Sodium chloride working solutions—Prepare dilutions of the stock solution such that 1 ml = 10 μg of sodium and 1 ml = 1 μg of sodium, respectively.

Lithium chloride stock solution—Dissolve 5·32 g of lithium carbonate in a minimum of dilute hydrochloric acid in a platinum dish. Heat gently to expel carbon dioxide, cool and make up to 1000 ml in a volumetric flask. Transfer immediately to a polythene bottle. 1 ml = 1 mg of lithium.

Lithium chloride working solutions—Prepare dilutions of the stock solution such that 1 ml = 100 μg of lithium and 1 ml = 10 μg of lithium, respectively.

Other metals used were of Johnson and Matthey "Specpure" grade.

PROCEDURE (FOR RANGE 0 TO 10 P.P.M. OF SODIUM)—

Weigh 1 g of sample into a 250-ml silica beaker; at the same time weigh six 1-g samples of sodium-free alloy for calibration into other beakers. Wash beakers and contents by decantation three times with de-ionised water, carefully draining off the water each time. Add 30 ml of 6N hydrochloric acid, cover with a silica watch-glass and warm to start the reaction, finally heating to complete solution. Evaporate to incipient crystallisation of salts and allow to cool. Re-dissolve the salts in 25 ml of water and transfer (filtering if necessary) into 100-ml quartz volumetric flasks. Add to each 5 ml of lithium solution (1 ml = 10 μg of lithium) and to five of the calibration solutions add 2, 4, 6, 8 and 10 ml of standard sodium solution (1 ml = 1 μg of sodium) retaining the remaining one as a blank. Adjust to volume with de-ionised water, mix and transfer to clean polythene bottles.

Set up the flame photometer using suitable slit and gain control settings and adjust the flame as indicated below. Aspirate the highest standard and arrange to give 90 to 95 per cent. galvanometer deflection when set on the sodium line at 589 mμ. Aspirate all the standards and samples, clearing between each by passing de-ionised water, and record the sodium emission readings. Re-set the instrument to the wavelength of the lithium peak at 670·7 mμ to give a galvanometer deflection of 40 to 60 per cent. and again aspirate standards and samples, recording the lithium emissions. Subtract the blank sodium reading from the readings for the standards and samples. Plot the sodium-to-lithium emission ratios against sodium content and read the contents of the samples from the graph.

For sodium contents above 10 p.p.m. use the stronger sodium and lithium solutions for calibration, with appropriately lower gain settings on the instrument.

NOTE—In the case of alloys containing more than 1 per cent. of silicon, ignite the filtered silicon residue in a platinum crucible, treat with hydrofluoric and nitric acids and evaporate to dryness. Dissolve the residue in a little hydrochloric acid and add to the main solution.

FLAME BACKGROUND—

An acetylene - air flame was used in these experiments. The air was set at 23 p.s.i. throughout, as lower pressures are insufficient to give good atomisation of the solution. Variation of flame conditions was obtained by adjusting the gas pressures. For the examination of traces of sodium it is obviously necessary to keep contamination and over-all background down to a minimum. The background at 589 mμ was therefore measured at a series of acetylene pressures using a solution of sodium-free N8* alloy (1 g per 100 ml). The instrument settings were arranged to give full deflection of the galvanometer with a solution containing 0·1 μg of sodium per ml using pure water as the reference solution.

* The alloy designations used throughout are those of B.S. 1470.

The results are shown in Fig. 1, from which it is seen that minimum background is obtained at positions A or B. At position B the flame is fuel-rich and just verging on luminosity. At position A it is air-rich and unstable. Position C represents the balanced flame usually used and recommended by the manufacturers; it is achieved by starting with a gas pressure of about 10 cm and turning down the gas until the blue burner cones just verge on instability. Surprisingly, this conventional flame gave high background, and the gas-rich flame type B appeared to be preferable. However, this experiment alone was not conclusive since background is composed of several factors, including sodium impurity in the reagents, effects of reagents on the flame and, possibly, effects of alloying elements in the metal. Conceivably, the higher emission with flame C could be reflecting a greater sensitivity to the sodium impurity unavoidably present.

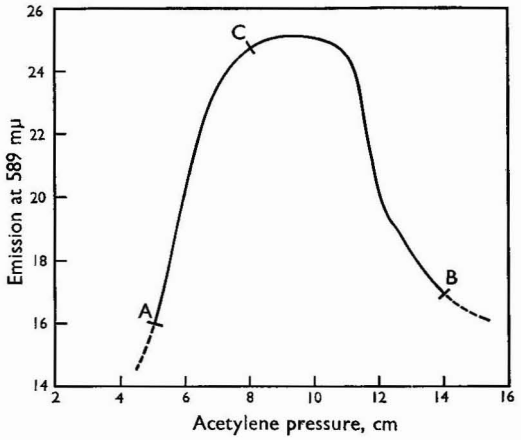

Fig. 1. Effect of flame condition on background emission at 589 mμ. A: Air-rich and unstable flame; B: Fuel-rich flame and C: Balanced flame

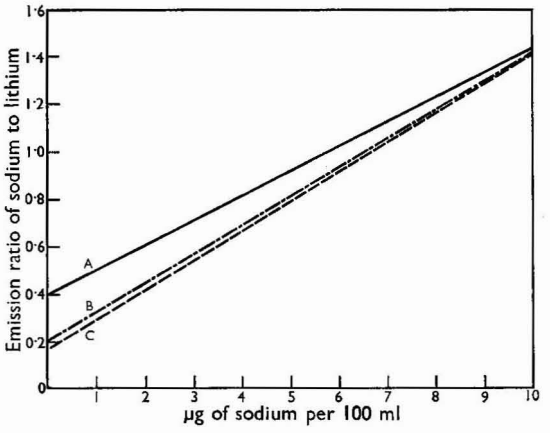

Fig. 2. Calibration graphs for different flame conditions. Acetylene pressure: (A) 8 cm; (B) 14 cm; (C) 4 cm

Calibration curves were therefore constructed for the three types of flame, again using N8 alloy as the calibration metal. The results are shown in Fig. 2. This figure demonstrates again the higher background with the conventional balanced flame, but also shows that the sensitivity to sodium is actually better in the other types of flame.

To determine the reason for the higher background intercept with the conventional balanced flame, other experiments were carried out. No effect was found by adding magnesium up to the equivalent of 10 per cent. to calibration solutions, hence the magnesium content of N8 alloy was not the cause. This alloy also contains about 0·8 per cent. of manganese, 0·3 per cent. of iron and 0·2 per cent. of silicon (the silicon is removed in processing so this could not be the cause of the background). Similar behaviour in different flame types was found with commercially pure aluminium (0·4 per cent. of iron, 0·3 per cent. of silicon) but not with super-purity metal (99·99 per cent. of aluminium). Thus attention was given to the effects of iron and manganese on background emission.

Effect of Iron and Manganese

Both iron and manganese are shown by Dean[5] to exhibit pronounced molecular oxide bands in the region of sodium emission. The exact situation at 589 mμ is difficult to assess because of sodium impurity in the solutions used, but it seems possible that excitation of oxides could vary considerably in different flame types and hence give rise to the anomalous effects observed. Solutions were prepared containing 1 mg and 5 mg of iron per 100 ml, and also 5 and 10 mg of manganese per 100 ml. The spectra recorded showed much higher emission from iron and manganese in balanced and air-rich flames than in the fuel-rich flame. However, the presence of sodium impurity in these solutions complicates the interpretation, and a more convincing experiment was carried out. Duplicate samples of aluminium were dissolved in hydrochloric acid and 10 mg of iron added to the solution. Iron was extracted from one solution with di-isopropyl ether and the spectra of the solutions then recorded in

three types of flame. The results are shown in Fig. 3. In each case the broken line shows the emission from sodium only (after iron extraction) while the full line shows the spectrum with superimposed emission from molecular iron oxide. These results show conclusively that iron oxide band spectra interfere seriously in the determination of sodium when conventional balanced or air-rich flames are used for excitation, but that interference is slight in a fuel-rich flame.

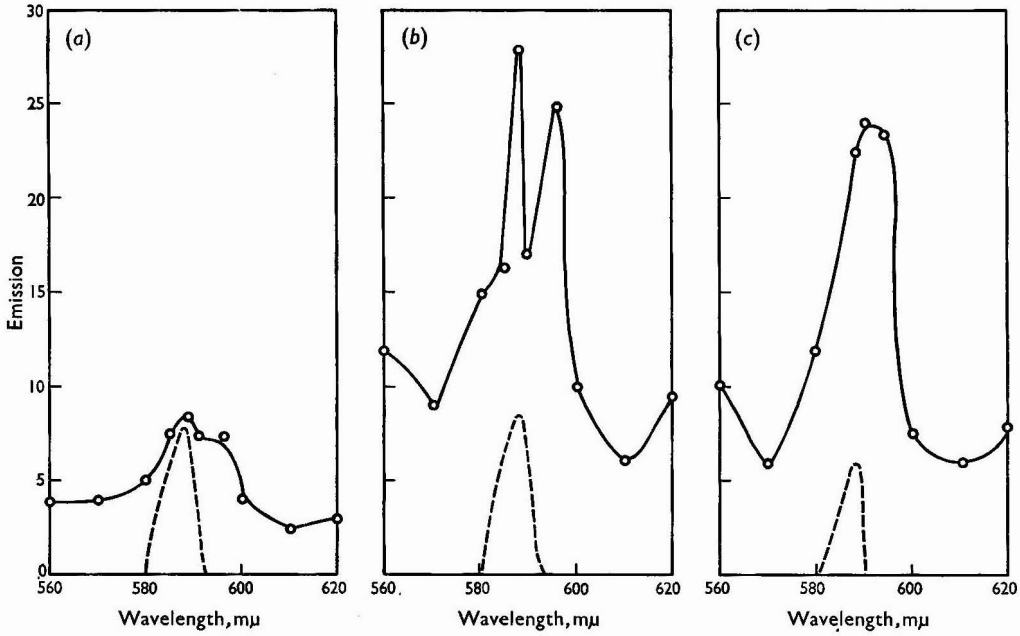

Fig. 3. Spectra of solutions before and after extraction of iron (10 mg per 100 ml). Broken lines, iron-free spectrum; continous line, iron with sodium impurity. (*a*) air pressure, 23 p.s.i.; acetylene pressure, 14 cm; (*b*) air pressure, 23 p.s.i., acetylene pressure, 8 cm; (*c*) air pressure, 23 p.s.i., acetylene pressure, 4 cm

The effect of increasing iron contents on the emission from 5 μg of sodium per 100 ml, in the presence of aluminium, is shown in Fig. 4 for the three flame types. Similar results were obtained for manganese additions except that the magnitude was lower with manganese. Again it is obvious that the fuel-rich flame is least sensitive to interference and gives an almost horizontal line, whereas the most air-rich flame has the steepest slope.

Magnitude of errors

The magnitude of errors caused by iron or manganese interference depends on the relative contents of these elements in the calibration metal and the sample. The errors will be positive if the sample contains more of these elements than the calibration metal and negative if it contains less. The error is always at a minimum with a fuel-rich flame. Some examples are given in Table I. In this table the figure given as the established value is the best estimate of sodium content obtained by a radioactivation method. The calibration metal was commercially pure aluminium containing about 0·25 per cent. of iron.

Table I

Results obtained on aluminium alloys in fuel-rich and air-rich flames

Composition, per cent.		Established sodium,	I Fuel-rich flame sodium found,	II Air-rich flame sodium found,	
iron	manganese	p.p.m.	p.p.m.	p.p.m.	Remarks
1·2	0·04	2	3·2	8·5	Serious iron effects in II
0·22	0·3	7	6·3	10	Manganese effect in II
0·3	0·8	1·1	1·5	4·0	Manganese effect in II
0·16	0·17	3·2	3·8	5·0	—
0·01	0·01	<0·5	0	−2·2	Negative errors in II due to lower iron content of sample

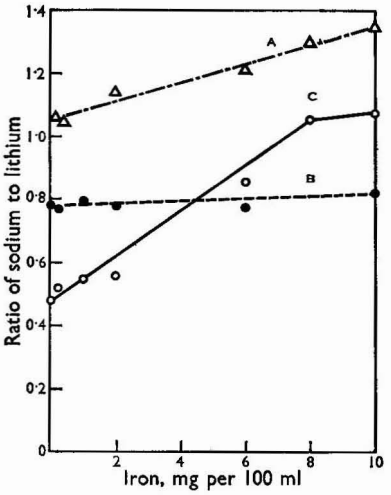

Fig. 4. Effect of increasing iron on emission of 5 μg of sodium per 100 ml. Acetylene pressure: Graph A (△), 8 cm; graph B (●), 14 cm; graph C (○), 4 cm

Flame reproducibility and precision using fuel-rich flame

The fuel-rich flame shown to be preferable from the point of view of interferences is less familiar than the conventional balanced flame, and possibly less convenient to work with. However, after some experience it is possible to reproduce the flame and obtain consistently good results. The general stability of the instrument under these conditions is excellent, as shown by the following experiment. A solution of an aluminium - magnesium alloy containing 3·5 p.p.m. of sodium was prepared, the instrument set up and a calibration carried out; 6 readings were taken on the sample solution and the sodium content determined. The flame was then turned out, re-lit, re-adjusted and the procedure repeated to a total of 6 times. The "within-run" standard deviation found was equivalent to ±0·15 p.p.m. of sodium and the "between-run" value was also ±0·15 p.p.m.

The over-all precision of the method, established by processing separate weighed samples of several alloys was found to be about ±0·5 p.p.m. for a content of 3 p.p.m. of sodium.

Definition of flame types

The figures for fuel pressure given in the text, and illustrations as representing the flame types used, only provide a general indication of the value. The exact pressure to use depends upon the age and condition of the burner jet and other variables. Final adjustment of the flame is therefore carried out visually according to the following instructions. (For the Unicam SP900 instrument, with air pressure at 23 p.s.i., the approximate acetylene pressures are shown in brackets.)

Balanced flame—This is the conventional flame in which there is sufficient air to give steady and complete combustion of the fuel. It is obtained by starting with a slightly fuel-rich flame and then turning down the gas pressure until the inner blue burner cones just become unstable, then increasing the pressure slightly until the cones regain stability (8 to 10 cm of acetylene pressure).

Air-rich flame—The fuel pressure is turned down to the point where the flame will only just remain alight. The flame is weak, short and barely visible (4 to 5 cm of acetylene pressure).

Fuel-rich flame—The fuel pressure is turned up until the flame becomes luminous and then turned back until the luminosity just disappears (11 to 14 cm of acetylene pressure).

Discussion and conclusions

The interference caused by excitation of molecular oxide bands of iron and manganese was the most important aspect of a larger investigation into the determination of trace amounts of sodium in aluminium alloys. Other work showed that the acidity of the solution

affected the emission from sodium and that there were complex interactions between various factors. However, by close standardisation of the procedure, and the use of lithium as an internal standard to compensate for small variations in conditions, accurate results could be obtained.

The errors caused by iron or manganese, or both, when a conventional flame is used, are of sufficient magnitude that the calibration metal must be of almost identical composition with the sample to obtain correct results. In practice this would mean subjecting a portion of each sample to vacuum distillation to serve as its own calibration metal. On the other hand, although some interference still occurs in fuel-rich flames, it is so small that calibration metal of similar alloy type to the sample can be used without incurring appreciable error. In fact, several groups of alloys, such as the aluminium - magnesium series, are sufficiently similar in iron and manganese contents to allow common standards of sodium-free calibration metal to be used.

Of other common alloying elements, it has been found that copper, zinc and magnesium have no appreciable effect, but a few less common elements such as nickel and chromium (which are known to give oxide bands) may have similar effects to iron and manganese. However, the use of calibration metal of the same alloy type in conjunction with a fuel-rich flame would be likely to give accurate results in these cases also.

It is not clear whether the lower emission from iron and manganese oxides in fuel-rich flames is a result of the lower temperature of such flames, or whether the reducing character of the flame suppresses the formation of oxides. Fassel[6,7] and his co-workers have carried out extensive investigations into the flame spectra of rare-earth elements and of vanadium, niobium, etc. They have shown that, whereas in conventional (stoicheiometric) flames monoxide band spectra of these elements predominate, in fuel-rich flames distinctive line spectra appear which allow quantitative determination of the elements. Fassel considers that though the fuel-rich flames are cooler, the main reason for the predominance of line spectra is that the conditions for monoxide formation are unfavourable (reducing species present) and lead to a high atom population in the flame. A similar explanation fits all of the observations made in the present work. Analogous effects were also found in the determination of lithium in aluminium alloys, and the wider investigation of unconventional fuel-rich flames may result in improvements in flame spectrophotometry procedures for many elements. Although the work was concerned with the analysis of aluminium alloys, the results are of general application, and could be of significance in the determination of sodium in biological samples, plant materials and minerals. For all analyses where iron or manganese, or both, may be present, the use of a fuel-rich flame, just verging on luminosity will give the greatest freedom from interference coupled with high sensitivity.

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The Determination of Water in Lubricating Oils by a Near-infrared Spectrophotometric Method

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The water content of clear mineral oils can be quickly and accurately determined by dissolving the wet oil in ethyl acetate and measuring the near-infrared absorbance of the solution, relative to a similar reference solution previously dried by molecular sieves. In discoloured or cloudy, wet oils the water can be removed by azeotropic distillation with ethyl acetate, and the water content of the distillate determined spectroscopically. With the latter procedure, errors that arise at low water concentrations may be due to the absorption of atmospheric moisture by the solvent.

Many workers have shown that water can be simply, rapidly and accurately determined in such liquids as glycerol, ethylene and propylene glycols, methanol, isopropanol, butanol and fuming nitric acid, by near-infrared spectrophotometry.[1,2,3,4,5] In general, the method has been considered to be applicable to the determination of water in alcohols, aldehydes, amines, esters and ketones.[6] Because spectrophotometric measurements can be rapidly made over a single sharp absorption peak with a high degree of accuracy, it would be useful if the technique could be extended to hydrocarbons, particularly lubricating oils. Undiluted oils are difficult to manipulate into absorption cells; measurements would be greatly simplified by the use of a solvent, not particularly hygroscopic and yet easily dried, for the oil and a reasonable amount of water. Ethyl acetate is a solvent which fulfils these conditions. Water in water - oil emulsions could also be determined using this procedure. Neither typical lubricating-oil additives nor lubricating oils interfere with the near-infrared water absorption band at 5290 cm^{-1}.

Difficulties arise in the spectroscopic determination of water in used oils. These contain finely divided particles that are suspended in the oil and scatter much of the incident radiation, so introducing intolerably large errors. No simple method of removing the suspended material is available, and so this difficulty is overcome by the rapid azeotropic distillation of the water from the oil with ethyl acetate. The water content of the ester distillate is then determined spectroscopically. A similar procedure has been used to determine the water content of powders.[7]

Method and results

Reagents—

Ethyl acetate—Dry ethyl acetate (Hopkin and Williams AnalaR grade) was further dried for at least 48 hours over molecular sieves (with approximately 25 g per 2½ litres).

Molecular sieves, Linde Air Products 4A grade (supplied by B.D.H.)—After using the sieves, regenerate them in a stream of argon at 250° to 300° C for 10 hours.

Calibration—Beer's law measurements—

Near-infrared spectrophotometric measurements were made on a Unicam SP700 instrument, with 10-mm stoppered cells.

Standard solutions of water in ethyl acetate (or in ethyl acetate and oil) were prepared by the weight-fraction method, from solvents (or solutions) previously dried over molecular sieves. The absorbance of these wet solutions relative to the dry solvent (or solution) was measured. The results obtained are shown in Fig. 1.

Removal of water from ethyl acetate by molecular sieves—

To 57 g of ethyl acetate containing 0·424 per cent. of water, w/w were added 5·9 g of molecular sieves. Samples of the solution were withdrawn at intervals; their absorbance was measured and then the samples were returned to the bulk of the solution.

Results—

Time, in minutes	10	20	35	55	85	125	230	360	480
Percentage of water (w/w) in solution	0·290	0·210	0·155	0·105	0·070	0·040	0·020	0·015	0·007

The above results were obtained when the samples were withdrawn at the times indicated. Identical results for the same water content were obtained for solutions of 40 g of ethyl acetate and 17 g of oil, regardless of whether the oil was of the straight or detergent type. In each instance the time required to remove half the water from the solutions was 20 minutes.

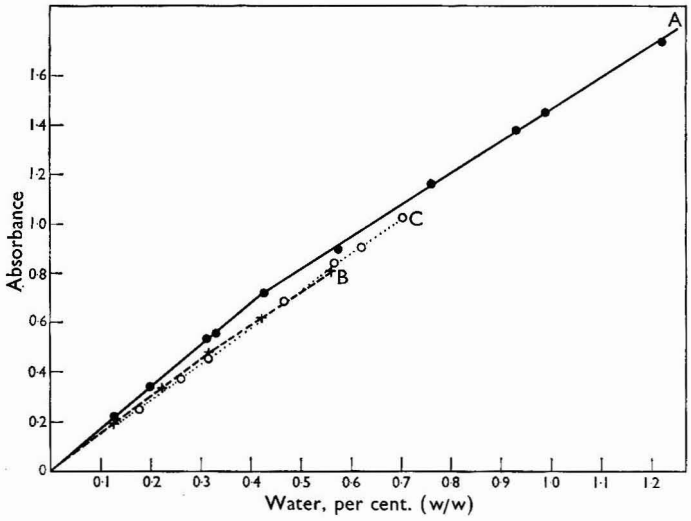

Fig. 1. Beer's Law relationships for water in lubricating oil samples. Graph A, in ethyl acetate; graph B, in ethyl acetate and straight oil, 2 to 1, v/v; graph C, in ethyl acetate and detergent oil, 2 to 1, v/v

UPTAKE OF ATMOSPHERIC MOISTURE BY ETHYL ACETATE—

Ten-ml samples of ethyl acetate were measured into eight similar specimen-tubes, 7·5 cm in length and 1·7 cm in diameter. These tubes were placed in a tank saturated with water vapour, at 24° C. One tube was immediately withdrawn to determine the small "handling correction," and the other tubes were withdrawn at intervals.

Results—

Time, in minutes	30	63	90	110	195	255	305
Percentage of water (w/w) dissolved (corrected)	0·034	0·081	0·102	0·124	0·215	0·278	0·374

The above results were obtained when the samples were withdrawn at the times indicated.

AZEOTROPIC DISTILLATION APPARATUS—

Attach a round-bottomed, side-arm flask of 500 ml capacity to a lagged column 50 cm long and 3 cm in diameter, which has been loosely packed with glass rings. Connect this, via a distillation head and a 35-cm long condenser, to a calibrated receiver protected by a drying tube. Support the flask-heating mantle by means of a laboratory jack so that it can be lowered for fast cooling. Before use, dry the apparatus by distilling at least 80 ml of dry ethyl acetate.

REMOVAL OF WATER BY AZEOTROPIC DISTILLATION—

The apparatus described above was used to distil 345 ml of ethyl acetate containing 1·360 per cent. of water, w/w. The near-infrared absorbance of 5-ml samples withdrawn at intervals from the distillation flask was determined.

Volume of ethyl acetate distilled, in ml ..	50	100	150	200	250
Percentage of water (w/w) remaining ..	0·565	0·337	0·150	0·072	0·018
Percentage of water removed	58·5	75·2	89·0	94·7	98·7

DETERMINATION OF WATER IN OIL SAMPLES BY USING AZEOTROPIC DISTILLATION—

Wash a 50-ml wet-oil sample into the distillation flask with 100 ml of ethyl acetate. Add two dry anti-bumping granules and distil the mixture until 80 ml of distillate have been collected. Measure the absorbance of the distillate in the region 5450 to 5150 cm^{-1}, and determine the percentage of water in the distillate by reference to the calibration graph (Fig. 1).

It was found that the results for twelve 50-ml samples, containing between 0·0440 and 0·2550 g of water, determined by this method showed an average error of 0·04 per cent. The maximum error was +2·6 per cent.

DIRECT DETERMINATION OF WATER IN NEW OR CLEAR OILS—

Dissolve 33 ml of wet oil in ethyl acetate and make the volume up to 100 ml. Measure the near-infrared absorbance of this solution relative to a similar solution that has been dried over molecular sieves. Find the water content of the solution from the ethyl acetate and oil calibration graph.

DISCUSSION

Meeker, Critchfield and Bishop[6] have shown that dry reference solutions for near-infrared spectrophotometry can be quickly obtained by using molecular sieves, type 4A. Their results by this method, for the water determination in ethyl acetate and a number of other organic liquids, were in close agreement with those obtained by the Karl Fischer method. This investigation has shown that this drying technique is as equally effective for ethyl acetate - oil mixtures as for ethyl acetate alone.

Water can be accurately determined in new or clear oil samples by measuring the absorbance of such systems as water - ethyl acetate - oil or water - diethyl ether - acetone - oil, in the region 5450 to 5100 cm^{-1}. The former system is the simpler of the two for practical use. It is not particularly hygroscopic, absorbing approximately 0·001 per cent. of its own weight of atmospheric moisture per minute, under the experimental conditions used. The ethyl acetate can be readily recovered, and in the proportion of two parts of ethyl acetate to one part of oil (by volume), approximately 0·5 per cent. of water (*i.e.*, 1·5 per cent. of water with respect to the oil), readily dissolves. A disadvantage of this system is that ethyl acetate precipitates some oil additives, but this factor does not lead to errors in the determination of the water content of the oil. The system, diethyl ether - acetone - oil (5·5 to 2·5 to 2·0, by volume) is less convenient; solvent recovery is more difficult, and cell-window cleaning before measurement is troublesome due to solvent creepage. This system does, however, take up rather more water (2·5 per cent. with respect to the oil).

The experimental procedure indicated has the advantage that a dry-oil solution, for reference purposes, can be readily obtained from a wet-oil sample. The wet oil is dissolved in ethyl acetate, and the solution is divided into two parts, one of which is dried with molecular sieves for about 24 hours. This forms the reference solution from which calibration solutions containing, say, 0·1, 0·2, 0·3 and 0·4 per cent. of water may be prepared. A calibration graph is then constructed and the absorbance of the retained wet-oil solution determined. Such a procedure would be tedious if it had to be carried out for every oil sample, but where the same type of oil is dealt with many times only a few minutes are required for each determination, as the same reference solution and calibration graph can be used in each case. Calibration graphs are very similar for straight or detergent oils (Fig. 1), and if some accuracy can be sacrificed, an "average" calibration graph can be used. However, if the maximum degree of accuracy is required, solutions should be made up by weight rather than volume, and the base-line accuracy of the spectrophotometer periodically checked against solutions of known water content. Under such conditions the limit of accuracy previously suggested[6] for the near-infrared determination of water at 5290 cm^{-1}, *i.e.*, $\pm$0·02 per cent. absolute, in the range 0·02 to 1·00 per cent. of water, can probably be improved to $\pm$0·004 per cent. absolute above the 0·075 per cent. water level.

The absorbance of ethyl acetate solutions of many used and all cloudy oils cannot be measured spectroscopically as the solutions scatter much light. In these instances the water in the oil may be removed by azeotropic distillation with ethyl acetate and the amount of water in the distillate determined spectrophotometrically. The efficiency of water-removal by this type of distillation was illustrated by distilling 250 ml of ethyl acetate from 345 ml

of the ester containing 1·360 per cent. of water. Examination of the distillation residue at this stage showed that 98·8 per cent. of the water had been removed. So, for an ethyl acetate and oil solution containing only up to about 0·5 per cent. water, all the water will have been removed if the volume is reduced to one-fifth by distillation. This is the procedure that was adopted.

To determine the accuracy of this method, known amounts of water were added to mixtures of ethyl acetate and oil which had previously been dried over molecular sieves. The ester was distilled and the water content of the distillate determined. For twelve such samples containing 0·1 to 0·6 per cent. of water with respect to the oil, the average error was 0·04 per cent., with a maximum error of +2·6 per cent. The accuracy of the method is highest for oil samples containing more than 0·3 per cent. of water. At lower water concentration levels the accuracy decreases, possibly due to the now important effect of atmospheric moisture during the transference of solutions. For new or clear oil samples good agreement was obtained between the direct spectrophotometric and azeotropic distillation methods.

It is interesting that Beer's law, for solutions of water in ethyl acetate, is only obeyed up to a concentration of 0·42 per cent. w/w of water. Deviations that occur above this value are possibly due to the association of water molecules. A similar deviation was observed for solutions of water in the esters, ethyl propionate and methyl acetate.

I thank Mr. J. Duncalf for help with the preliminary experimental work.

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The Determination of Residual Anionic Surface-active Reagents in Mineral Flotation Liquors

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A method is described for the determination of residual amounts of anionic surface-active reagents, used as flotation promoters, in mineral flotation liquors. The method is equally effective for the long carbon chain carboxylates and the anionic non-soapy surface-active agents. The reagent used is the cationic copper(II) triethylenetetramine complex, which reacts in alkaline solution with anionic surface-active agents to give an adduct that can be extracted into an isobutanol - cyclohexane mixture. The copper associated with the surface-active anion in the extract is determined photometrically as the coloured complex using diethylammonium diethyldithiocarbamate. Long chain carboxylates with carbon numbers from C_{14} to C_{22}, as well as anionic non-soapy surface-active reagents, can be determined in the range 0·2 to 10 p.p.m. The effect of a number of likely interferences has been investigated.

Anionic surface-active agents, particularly the carboxylate soaps, are extensively used in the mining industry as promoters for the separation of minerals by flotation. A simple and rapid method of determining residual amounts of these reagents is required for plant control, and also for investigating their adsorption on to mineral surfaces.

The usual method of analysis for traces of anionic surface-active agents is that of Jones,[1] modified by Longwell and Maniece,[2] in which a cationic dye, methylene blue, is added and the coloured adduct extracted into chloroform solution for photometric measurement. Many similar dye-adduct extraction systems have been reported[3] but these generally fail when applied to the carboxylic acid soaps. The quaternary dye pinacyanol has been suggested as a reagent for the determination of laurates in weakly alkaline solution using bromobenzene as extractant for the adduct,[4] but the reagent is too unstable for routine use. Tomlinson and Sebba[5] determined traces of soaps in neutral solution with the cationic dye crystal violet. The adduct formed was removed by a process described as "ion flotation" and the optical density of the residual dye solution measured. The reaction is non-stoicheiometric, requires precise control of conditions and is very susceptible to interference from dissolved inorganic ions.

Milligram amounts of long chain fatty acids in aqueous solution were determined photometrically by Ayers[6] and by Iwayama[7] by extracting the coloured cobalt or copper(II) soaps into chloroform. Metal cations are more attractive as reagents than organic dyes that are difficult to obtain in an adequate state of purity, may not be completely stable and frequently tend to adsorb on to the surfaces of vessels used in the analysis. On the other hand, the extinction coefficients of the cobalt or copper(II) soaps in organic solution, as in the methods of Ayers and of Iwayama, are too low to be used for the present purpose. However, it has been found that the sensitivity can be considerably increased by adding a sensitive colorimetric reagent for the metal concerned to the metal-soap extract. This procedure has the advantage that slight differences in the extinction coefficients of the metal soaps of the various fatty acids used do not affect the optical density of the extract. A suitable reagent for copper(II) soaps is dimethylammonium diethyldithiocarbamate. The extinction coefficient of the copper(II) complex is about 11,000, and both the reagent and the complex are soluble in organic solvents.

Attempts to extract microgram amounts of fatty acids from neutral or near neutral aqueous solution into organic solvents have not been successful. At this concentration level no extraction occurs when cupric nitrate is added, as in the method of Ayers, but some extraction does take place when an acetate buffered triethanolamine solution is used as described by Iwayama. Here, however, the reagent blank is very high. A better extraction occurs when alkaline solutions of complex copper ammines are used in place of the simpler copper(II) salts. Thus, for example, traces of soaps extract into chloroform with copper(II)

ions in the presence of alkali hydroxide and an excess of ammonia. Recoveries using this method tend to be low and erratic, but can be improved by replacing the cuprammonium ion by the complex triethylenetetramine copper(II) cation. The necessary alkaline pH can conveniently be achieved by incorporating the required base in the copper reagent solution.

Experimental

Preparation of the reagent—

The reagent is prepared with a slight excess of triethylenetetramine in addition to that necessary to complex the copper, thus avoiding precipitation of copper as the hydroxide on adding alkali. There is a tendency for copper(I) oxide to precipitate slowly from the reagent when either sodium or potassium hydroxide is used, but a completely stable reagent can be prepared by using monoethanolamine as the base. This forms a complex with copper(II) ions which does not react with soaps to give extractable products, and is much less stable[8] ($\log K = 16{\cdot}48$) than that of triethylenetetramine[9] ($\log K = 20{\cdot}6$). The separation of the organic phase is also much cleaner when monoethanolamine is used as the base.

Choice of extractant—

Of a wide range of organic liquids tried, only chloroform and the immiscible aliphatic alcohols give anything approaching complete extraction of the soap adducts. Chloroform gives a lower extraction than the alcohols and this is obtained only at very high pH values. The alcohols, however, tend to give cloudy extracts containing aqueous entrained phase; also the reagent blanks are high and increase as the ionic strength of the aqueous phase is increased. The entrainment effect is least with isobutanol, although particularly high reagent blanks are obtained. The addition to the isobutanol of either benzene or cyclohexane, which do not themselves extract the soap adducts, not only reduces the reagent blanks but also prevents entrainment of the aqueous phase without affecting the recovery. Because it has a much lower toxicity, the use of cyclohexane is preferred to that of benzene.

Reagents—

Copper - triethylenetetramine reagent—Dissolve 25 g of copper(II) nitrate trihydrate in 125 ml of water and stir slowly into a solution containing 16·25 g of triethylenetetramine in 125 ml of water. Add 250 ml of monoethanolamine in 250 ml of water and dilute with water to 1 litre.

Isobutanol - cyclohexane extractant—Mix 200 ml of isobutanol with 800 ml of cyclohexane.

Diethylammonium diethyldithiocarbamate solution—Dissolve 2 g of diethylammonium diethyldithiocarbamate in 100 ml of isobutanol. Prepare freshly every 2 days.

Standard soap solution—Dissolve 0·250 g of the required pure fatty acid in 200 ml of methylated spirit, add 1 ml of 0·88 sp.gr. ammonia solution and dilute with water to 1 litre. Standard solutions of non-soapy surface-active agents may be prepared in water or alcohol - water mixture.

Procedure—

Perform the extractions in 75-ml boiling tubes fitted with size B24 interchangeable ground-glass stoppers. Centrifuge samples which are cloudy, or which contain particulate matter, until clear. Transfer a 25-ml sample by pipette to an extraction tube and add 5 ml of the copper - triethylenetetramine reagent, followed by exactly 10 ml of the isobutanol - cyclohexane extractant. Stopper the tubes and invert rapidly 100 times. After the phases have separated, transfer the organic layer to a dry test-tube by means of a dropping pipette fitted with a rubber bulb. Mix the extract with two drops of the diethylammonium diethyldithiocarbamate solution and allow to stand in a dark place for 15 minutes. Measure the optical density relative to the extractant in 2-cm cells at a wavelength of 435 mμ. A blank determination must be made.

For calibration purposes, take aliquots of a standard solution in a series of extraction tubes and dilute each to 25 ml with water to give a range of concentrations from zero to 10 p.p.m. Complete the determinations as described above.

Discussion

The presence of traces of surface-active material in reagents or on glassware must be avoided. One batch of triethylenetetramine, which is only readily available in technical quality, gave a 0·3 p.p.m. intercept on the calibration graph. This can be overcome by adding an equivalent amount of sodium lauryl sulphate to the reagent, but at the cost of a slightly higher blank. Distilled water from an all-glass still has been found satisfactory for the determination. Glassware should be treated with chromic acid, rinsed with water, alcohol and chloroform and allowed to drain dry.

Composition of the extractant—

The addition of cyclohexane to the isobutanol extractant has two effects. These are the reduction of the reagent blank and the prevention of entrainment of aqueous copper solution when soaps are present. With too much cyclohexane the recovery is reduced and cloudy extracts are obtained. These effects are shown in Fig. 1. The best extractant contains 80 per cent. by volume of cyclohexane, and it can be seen that the recovery is not critically dependent upon its exact composition. Identical results are obtained when the cyclohexane is replaced by benzene, but hexane almost completely suppresses extraction.

Efficiency of extraction—

No further improvement in extraction occurs after shaking the tubes 40 times, but vigorous agitation sometimes produces a fairly stable emulsion when more than 5 p.p.m. of a soap is present. By rapidly inverting the tubes 100 times, in place of shaking, maximum extraction is obtained and the phases usually separate completely within 10 minutes.

The volume of the organic phase after extraction is 8·5 ml, that of the aqueous phase having increased by 1·5 ml. Successive extractions of the aqueous phase with more of the same extractant result in an increasing isobutanol concentration in the aqueous phase, thus giving increased blanks and entrainment of aqueous copper solution. By performing second and subsequent extractions with 8·5 ml of (5 + 80) isobutanol - cyclohexane mixture, the original extraction conditions are maintained. Under these conditions no further recovery can be detected in a second extraction.

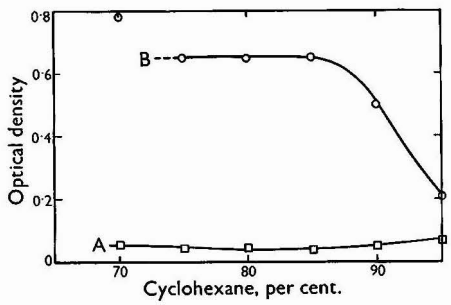

Fig. 1. Effect of addition of cyclohexane to the isobutanol extractant: curve A, blank; curve B, sample corrected for blank

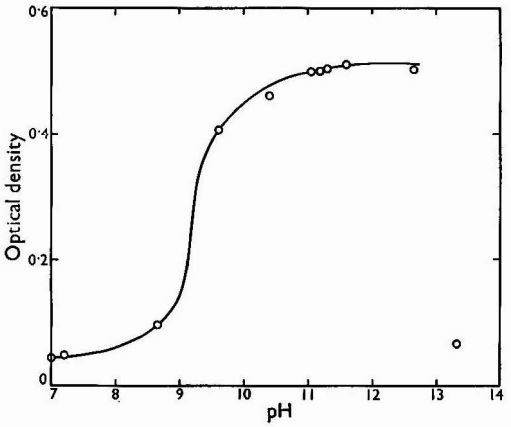

Fig. 2. Effect of pH on the determination of 4·74 p.p.m. of oleic acid

Effect of pH—

From Fig. 2 it can be seen that a large increase in extraction occurs as the pH is raised above a value of 9, and that it reaches a maximum between pH 11 and pH 12·6. When the pH is raised above 13 there is a noticeable lightening in the blue colour of the aqueous phase, and the extraction falls to a low value. With the recommended reagent, the aqueous phase after extraction has a pH of 11·6.

DEVELOPMENT OF COPPER(II) DITHIOCARBAMATE COLOUR—

Janssen[10] has determined the stability constant for the copper(II) diethyldithiocarbamate system and gives log $K = 28{\cdot}8$. This is sufficiently high for the coloured complex to be expected to form readily in the presence of triethylenetetramine. Whereas, in practice, the addition of a considerable excess of triethylenetetramine has no effect on colour development, the reaction is slow under the conditions used. This can be seen from Fig. 3, which also shows that the dithiocarbamate complex is not stable to light. After addition of the reagent the extract should be allowed to stand in the dark for 15 minutes.

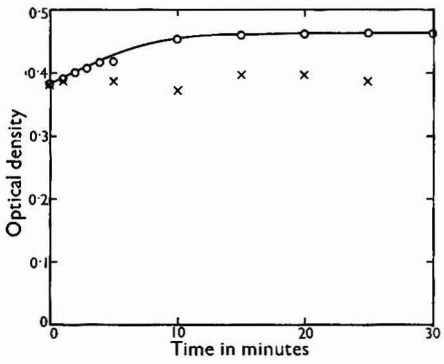

Fig. 3. Development of copper(II) dithiocarbamate colour: O, protected from light; X, exposed to light

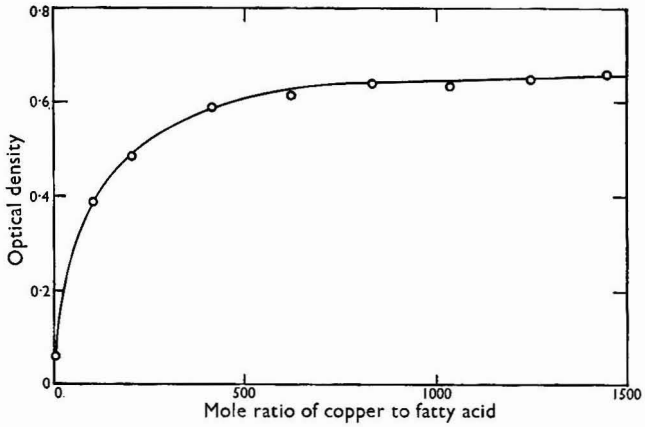

Fig. 4. Effect of reagent concentration on the extraction

REAGENT CONCENTRATION—

The relationship between the optical density of the extract and the mole ratio of copper(II) triethylenetetramine complex to oleic acid is shown in Fig. 4. The very large excess of copper reagent required for the reaction to approach completion suggests that the adduct formed between the fatty acid anion and the complexed copper cation must be highly dissociated. For a high recovery, the mole ratio of copper to fatty acid must be at least of the order of 1000.

COMPOSITION OF THE EXTRACTED SPECIES—

Chaberek and Martell[11] state that triethylenetetramine forms with divalent copper a square planar tetraco-ordinated (1 + 1) ion, and Schwarzenbach[9] has determined the stability constant log $K = 20{\cdot}6$. It is very likely that it is this stable chelate which reacts with fatty acid anions to give a neutral adduct which can be extracted into organic solvents. Because of the high dissociation of the adduct, the Job method of continuous variation[12] and the mole ratio method[13] are not suitable for determining its composition. The Harvey and Manning slope-ratio method[14] is usually effective in such cases, but cannot be applied here as very stable emulsions are formed when the necessary very large excess of surface-active reactant is present.

However, on making the assumption that extraction is sensibly complete, a comparison of the measured molar extinction coefficients for copper(II) diethyldithiocarbamate in the equilibrated extractant, and for oleic acid taken through the full procedure provides an approximate figure for the combining ratio of oleic acid with the copper complex.

The measured molar extinction coefficient for copper(II) diethyldithiocarbamate, prepared from copper(II) oleate in (5 + 80) isobutanol - cyclohexane mixture, is 10,700. For oleic acid taken through the procedure the value found is 5280, after correction for the reduction in volume of the extractant. The ratio between these figures is 2·03, which is close to the expected ratio of 2 molecules of oleic acid for each molecule of copper, supporting the view that the extracted compound is probably a neutral ion-association system.

APPLICATION TO OTHER SURFACE-ACTIVE ANIONS—

A calibration for oleic acid is given in Fig. 5 and shows a close adherence to the Beer - Lambert law. Similar calibrations are obtained with other fatty acids from myristic ($C_{13}H_{27}COOH$) to behenic ($C_{21}H_{43}COOH$). Lauric acid ($C_{11}H_{23}COOH$) gives low recoveries and no extraction occurs with capric acid ($C_9H_{19}COOH$). Very low and erratic recoveries are obtained with the almost insoluble montanic acid ($C_{27}H_{55}COOH$). The method is also effective for non-soapy anionic surface-active agents of the alkyl sulphate, alkyl phosphate and alkyl - aryl sulphonate types.

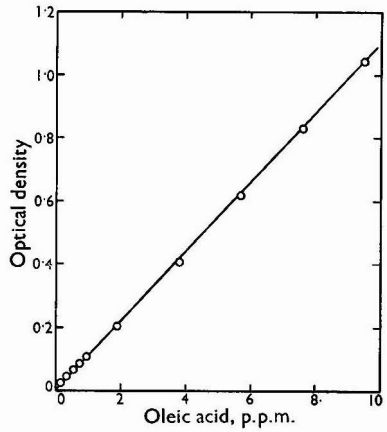

Fig. 5. Calibration for oleic acid

When plotted on a molar basis, the calibrations for various anionic surface-active agents all lie fairly close to one another. Comparative optical densities for a number of these materials are given in Table I. The optical density figures quoted are those given by a 10^{-5} molar solution of the surface-active agent, under the conditions of the determination.

TABLE I

RELATIVE MOLAR SENSITIVITY OF THE METHOD FOR A NUMBER OF ANIONIC SURFACE-ACTIVE AGENTS

Surface-active agent	Optical density for a 10^{-5} molar solution
Lauric acid	0·016
*Myristic acid	0·305
*Palmitic acid	0·316
*Stearic acid	0·314
*Oleic acid	0·312
Behenic acid	0·287
*Sodium lauryl sulphate	0·333
Sodium cetyl sulphate	0·278
Sodium dodecylbenzene sulphonate	0·266
Sodium di-2-ethylhexyl sulphosuccinate	0·328
Di-n-nonyl phosphoric acid	0·334

* Purity of materials checked by analysis, other substances are of reagent chemical grade.

REPRODUCIBILITY OF THE METHOD—

Sixteen determinations were made of the oleic acid concentration in a 4·74 p.p.m. solution. The mean was 4·76 p.p.m. and the standard deviation 0·05.

INTERFERENCES—

Flotation systems with soaps are frequently adversely affected by the presence of dissolved cations that precipitate insoluble metal soaps. The soap remaining in solution is determined in this procedure after spinning the sample liquor in a centrifuge to remove all insoluble material. If it is required to determine the total amount of soap present, both in soluble and insoluble form, the effect of some of the precipitating cations can be overcome by the addition

of EDTA. This liberates carboxylate ions from many soaps, while forming a copper(II) complex of lower stability [15](log $K = 18{\cdot}86$) than that of triethylenetetramine. However, a new calibration must be made with EDTA added as the extraction is slightly reduced in its presence.

The addition of 1 ml of a 6·25 per cent. solution of disodium ethylenediamine tetra-acetate dihydrate before the copper - triethylenetetramine reagent, is sufficient to complex the dissolved cations present in most hard waters in Great Britain.

The effect on the determination of oleic acid, of the presence of a number of common cations, and also of some anions which might be present in flotation liquors, is shown in Table II.

TABLE II

EFFECT OF VARIOUS IONS ON THE DETERMINATION OF 4·74 PARTS PER MILLION OF OLEIC ACID

Ion	Concentration present, p.p.m.	Added as:	No EDTA present: Oleic acid found, p.p.m.	No EDTA present: Error, per cent.	EDTA added: Oleic acid found, p.p.m.	EDTA added: Error, per cent.
K^+	240	KNO_3	4·60	−2·9	—	—
NH_4^+	240	NH_4NO_3	3·84	−19·0	—	—
	48		4·60	−2·9		
Mg^{2+}	120	$MgSO_4$	3·35	−29·3	4·58	−3·3
Ca^{2+}	240	$CaCl_2$	2·02	−57·4	4·83	+2·0
Al^{3+}	240	$Al(NO_3)_3$	2·58	−45·5	2·92	−38·4
	120		3·38	−28·7	3·87	−18·4
Mn^{2+}	240	$MnSO_4$	0·59*	−87·5	3·72*	−21·6
Fe^{3+}	240	$Fe(NO_3)_3$	3·02*	−36·3	3·84*	−18·9
	120		3·63*	−23·4	3·97*	−16·2
Co^{2+}	240	$Co(NO_3)_2$	2·67*	−43·8	4·58	−3·3
	120		4·05	−14·5	4·78	+0·9
Ni^{2+}	240	$Ni(NO_3)_2$	3·35	−29·3	4·63	−2·2
	120		3·98	−16·0	4·95	+4·4
Zn^{2+}	240	$ZnSO_4$	3·71	−21·7	4·76	+0·4
F^-	240	NaF	4·86	+2·5	—	—
Cl^-	240	NaCl	4·74	0	—	—
SO_4^{2-}	240	Na_2SO_4	4·54	−4·3	—	—
PO_4^{3-}	240	Na_2HPO_4	4·49	−5·3	—	—
$B_4O_7^{2-}$	240	$Na_2B_4O_7$	4·41	−7·0	—	—
CO_3^{2-}	240	Na_2CO_3	4·60	−2·9	—	—
CN^-	100	KCN	4·60	−2·9	—	—
CH_3COO^-	240	CH_3COONa	4·60	−2·9	—	—
CrO_4^{2-}	240	K_2CrO_4	4·57	−3·5	—	—
Silicate	240	Water glass	4·61	−2·7	—	—

* Some precipitation of metal compounds occurred in these cases.

Non-soapy surface-active agents, which do not generally form insoluble metal salts, are less affected by cationic interference. Thus for a 5 p.p.m. solution of sodium lauryl sulphate in the presence of 240 p.p.m. of calcium as calcium chloride, the concentration found was 4·86 p.p.m., an error of −2·8 per cent., whereas the error caused for oleic acid is over 50 per cent., although this difference was not apparent in the presence of EDTA.

Traces of long chain alkyl amines do not interfere seriously with the determination of fatty acids, but the surface-active quaternary amines completely inactivate an equivalent amount of soap.

APPLICATION TO FLOTATION SYSTEMS—

Much of the preceding part of this paper has dealt with ideal systems. In practice flotation liquors may contain considerable amounts of residual solids and slimes. Although these generally remain in the aqueous phase after the extraction, the adsorbed surface-active agent tends to be stripped by the reagent and is determined. Most filter materials adsorb anionic surface-active agents to a considerable extent, even from pure solutions. Nylon filter-cloth is free from this defect but has a relatively high porosity. Particulate matter can readily be removed, however, by centrifuging the sample before analysis.

A number of anionic surface-active agents have been successfully recovered from laboratory test flotations. Continuous analysis records of anionic surface-active agents are being obtained by the use of the Technicon AutoAnalyzer. The application of the method to this system, and the results obtained in flotation experiments will be the subject of a future publication.

Conclusion

The determination of anionic surface-active agents, including the fatty-acid soaps, in mineral flotation liquors can be done by extraction of the adduct with the copper - triethylenetetramine complex, followed by photometric determination of the coloured copper - diethyldithiocarbamate complex in the organic phase. The method should also find application in fields other than in the mineral flotation industry.

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Polarographic Determination of Arsenic in Steel

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A method for the polarographic determination of arsenic in steel is described. It consists of the reduction of arsenic(V) to arsenic(III) in 9·5 N hydrochloric acid solution by means of potassium iodide in the presence of ascorbic acid, and of the extraction of arsenic(III) with chloroform. Arsenic is then re-extracted from chloroform with 0·2 M ascorbic acid and determined polarographically with the same ascorbic acid solution as supporting electrolyte. The method was checked on NBS steel standards and synthetic mixtures. Arsenic contents greater than 0·01 per cent. can thus be determined easily without concentrating in the re-extraction step.

MANY results are recorded in the literature on the polarographic behaviour of arsenic in different supporting electrolytes. Arsenic must be in the three-valence state, since arsenic(V) does not give any analytically useful polarographic wave. Bayerle,[1] Kacirkova[2] and Lingane[3] have shown that arsenic(III) is reduced at the dropping-mercury electrode in the presence of mineral acids as supporting electrolytes, usually producing two waves with half-wave potentials ranging from −0·3 to −1 volt against the S.C.E., depending upon the acid concentration. According to Kryukova,[4] arsenic(III) is reduced with hydrochloric acid solution giving four waves which are dependent on the hydrochloric acid concentration. Kryukova has also shown that the reduction of arsenic(III) with tartaric and lactic acid solution gives two well-defined waves that can be used for the polarographic determination of arsenic. However, in alkaline solutions arsenic(III) is oxidised to arsenic(V), giving an anodic wave, the half-wave potential of which is −0·26 volt against the S.C.E.[5] Šušić[6] was the first to establish that arsenic(III) can be polarographically determined at different pH values with ascorbic acid as the supporting electrolyte.

EXPERIMENTAL

All attempts to determine arsenic in steel by a direct polarographic method were unsuccessful. Although ascorbic acid reduces ferric ions to ferrous ions and some other ions to their lower valence state, it cannot eliminate the influence of some interfering ions, such as molybdenum and vanadium, for steel. In addition, high concentrations of iron in the solution increase the residual current so that small amounts of arsenic cannot be determined accurately. Therefore, we decided to reduce arsenic(V) to arsenic(III) with the potassium iodide, and then to extract arsenic(III) with chloroform. The iodimetric method for the determination of arsenic, based on the reduction of arsenic(V) to arsenic(III) with potassium iodide in concentrated hydrochloric acid solution, is well known. Milayev and Varasnina[7] have also described a method for the separation of arsenic from some other elements by reducing arsenic(V) to arsenic(III) with potassium iodide, and extracting arsenic(III) with carbon tetrachloride from concentrated hydrochloric acid solution. Our choice of chloroform as the extractant instead of carbon tetrachloride was based on the polarographic behaviour of chloroform. In fact, chloroform is reduced at the dropping-mercury electrode at a more negative potential than arsenic, and therefore does not interfere with the polarographic wave of arsenic. This is important as, in the re-extraction step, small amounts of organic solvent pass over into the aqueous phase and may interfere with the determination of arsenic if they are reduced at a potential close to the reduction potential of arsenic, as occurs with carbon tetrachloride. The re-extraction of arsenic(III) from the organic into the aqueous phase occurs rapidly and quantitatively, so that the extracted arsenic can be re-extracted easily with the ascorbic acid solution that was used as the supporting electrolyte for the polarographic determination of arsenic.

In the reduction of arsenic(V) to arsenic(III) and extraction of arsenic(III) in the presence of ferric ions, elementary iodine is liberated as the reaction product of ferric ions and arsenic(V) with potassium iodide. Most of the free iodine is liberated from the reaction of potassium

iodide and ferric ions if the ferric ion concentration is higher than that of arsenic. Therefore the reduction and the extraction of arsenic from steel samples was carried out in the presence of ascorbic acid. The rôle of ascorbic acid is to suppress the liberation and extraction of elementary iodine. The amount of ascorbic acid used is sufficient to reduce about ten times the amount of ferric ions present to ferrous ions. The reduction of arsenic(v) is ensured by the presence of 0·01 M potassium iodide, which is about 20 to 50 times the equivalent concentration of arsenic(v), and is almost unconsumed in the reactions.

REAGENTS—

All materials used should be of analytical-reagent grade. The ascorbic acid solution should be freshly prepared, and the chloroform should not contain more than 0·2 per cent. of alcohol.

Solid ascorbic acid and 0·2 M *ascorbic acid solution.*
Hydrochloric acid, 9·5 M.
Nitric acid, sp.gr. 1·4.
Sulphuric acid, sp.gr. 1·84.
Potassium iodide, 0·1 M.
Chloroform.

PROCEDURE—

Weigh 0·2 to 0·5 g of steel sample, depending upon the arsenic content, and dissolve it in 10 to 20 ml of a mixture of nitric acid, sulphuric acid and water (4 to 1 to 4). Evaporate to dryness and dissolve the residue in 9·5 M hydrochloric acid. Transfer the solution to a 25-ml volumetric flask and fill it up to the mark with 9·5 M hydrochloric acid solution. Place 2 to 5 ml of this solution in a separating funnel, add 0·2 to 0·5 g of solid ascorbic acid and 0·1 to 0·3 ml of 0·1 M potassium iodide solution and extract twice with an equal volume of chloroform by vigorously shaking the funnel for 10 minutes. Then re-extract the arsenic from the organic phase into 2 to 5 ml of 0·2 M ascorbic acid solution in the polarographic vessel, stirring the two phases by bubbling nitrogen through the solutions for a few minutes; without separating the layers determine the arsenic polarographically in the aqueous phase. The half-wave potential of arsenic is between 0·8 and 0·9 volt against the S.C.E. For a small arsenic content in steel, the arsenic should be concentrated in the re-extraction step of the procedure.

RESULTS

Following the above procedure we have carried out the determination of arsenic in synthetic mixtures prepared from standard solutions of iron, arsenic and some other elements. The ratio of iron to arsenic was approximately the same as that usually met in steel samples. The composition of synthetic mixtures and the amount of arsenic determined are given in Table I. The results obtained show that the agreement between taken and found amounts of arsenic is satisfactory and is within the limits of the accuracy of the polarographic method.

TABLE I (*a*)

DETERMINATION OF ARSENIC IN SYNTHETIC MIXTURES

Arsenic determined × 10^4 M

Determination No:	1	2	3	4	5	6	7		Taken arsenic,
Mixture								Average	× 10^4 M
A	1·60	1·61	1·65	1·58	—	—	—	1·62	1·60
B	0·808	0·782	0·845	0·808	0·750	0·831	0·831	0·808	0·800
C	0·377	0·420	0·421	0·377	0·426	0·392	0·382	0·399	0·400

TABLE I (*b*)

COMPOSITION OF MIXTURES

	Iron, g per ml	Chromium, g per ml × 10^4	Manganese, g per ml × 10^4	Molybdenum, g per ml × 10^4	Vanadium, g per ml × 10^4	Arsenic, × 10^4 M
A	0·01117	1·37	2·75	3·84	2·50	1·60
B	0·02234	3·42	2·75	3·84	2·50	0·800
C	0·02234	3·42	2·75	3·84	2·50	0·400

Each determination was performed with 2 ml of the solution of the corresponding mixture and no concentration was made in the re-extraction step.

The recommended method was further checked by determining arsenic in four standard NBS steel samples, which contained from 0·1 to 0·018 per cent. of arsenic. The results obtained are given in Table II and are found to be in good agreement with the certificate values for arsenic content in steel standards. The polarographic waves of arsenic obtained in the determination of arsenic in NBS steel sample No. 464–D are shown in Fig. 1. There are four curves; two of them represent the arsenic content in the sample after re-extraction, and the other two the content of arsenic in the same solution after the addition of a standard.

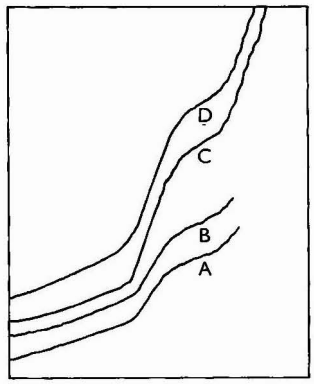

Fig. 1. Polarographic determination of arsenic in 0·4000 g of NBS steel, No. 464-D. Curves A and B, arsenic content of sample; curves C and D, arsenic found after addition of standard. Polarographic conditions: Radiometer PO 3h polarograph, V = 2 ml (from 25 ml), V_s = 0·05 ml, $C_s = 1{\cdot}5 \times 10^{-4}$ g per ml, sensitivity = 1 × 100

TABLE II

DETERMINATION OF ARSENIC IN NBS STEEL

NBS number:	461–A (0·028 per cent. of arsenic), g taken		462–B (0·046 per cent. of arsenic), g taken		463–C (0·10 per cent. of arsenic), g taken		464–D (0·018 per cent. of arsenic), g taken	
	0·3075	0·3000	0·2878	0·2144	0·2020	0·2181	0·4000	0·4111
Determination No.	Arsenic found, per cent.				Arsenic found, per cent.			
1	0·0294	0·0297	0·0461	0·0446	0·108	0·098	0·0229	0·0193
2	0·0316	0·0289	0·0436	0·0456	0·101	0·104	0·0182	0·0172
3	0·0266	0·0279	0·0469	0·0442	0·095	0·099	0·0182	0·0182
4	0·0287	0·0296	0·0438	0·0458	0·094	0·098	0·0181	0·0162
5	0·0273	0·0317	0·0460	0·0471	0·096	0·102	0·0176	—
6	0·0291	0·0269	0·0456	—	—	—	—	—
Average	0·0288	0·0291	0·0453	0·0443	0·099	0·100	0·0190	0·0177

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A Simple Chromatographic Method for Determining the Basic Amino-acids in Protein Hydrolysates

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A new micro method utilising charcoal chromatography has been developed for the determination of the individual basic amino-acids in protein hydrolysates. It is based on the fact that both the aromatic amino-acids and basic amino-acid picrates are strongly adsorbed by active-charcoal columns, and that the latter can be freed from the aromatic amino-acids by treatment of the adsorbent with aqueous ethyl acetate solution. The separation of the individual basic amino-acids depends on differential decomposition of their picrates on the columns by various eluents. The method can be used for the determination of histidine in the presence of other imidazole derivatives.

The earlier chemical methods that were commonly used in the determination of the basic amino-acids in protein hydrolysates suffered from the disadvantages of being tedious and non-specific. The problem was not entirely solved by the use of the time-consuming microbiological and enzymic assays. In recent years, many ion-exchange chromatographic and electrophoretic methods have been introduced for the analysis of the basic amino-acids. Although the new procedures have overcome most of the difficulties inherent in the older methods, they require highly specific conditions and involve the use of special equipments and materials. The present investigation was therefore undertaken with the object of developing a simple analytical micro procedure for the determination of histidine, lysine and arginine in protein hydrolysates.

Charcoal adsorption has been used by several workers for the quantitative removal of the aromatic amino-acids from protein hydrolysates, prior to the separation of the other amino-acids.[1,2,3,4] The quantitative separation of the basic amino-acids from protein hydrolysates can be efficiently accomplished with the method described by Robson and Selim.[1] These authors have shown that the basic amino-acid picrates are strongly retained by active charcoal. Later, charcoal chromatography was used by Selim *et al.*[5,6] for the quantitative removal of the aromatic and basic amino-acids in a single operation. Therefore, by treating the protein hydrolysate with picric acid and applying the mixture to a column of charcoal, it is possible to wash out all of the aliphatic amino-acids with dilute acetic acid, while the aromatic amino-acids and basic amino-acid picrates remain strongly adsorbed on the charcoal.

In the present study, further work on charcoal chromatography has revealed that treatment of the charcoal column, containing the aromatic amino-acids and the basic amino-acid picrates, with aqueous ethyl acetate results in the complete elution of the aromatic amino-acids, whereas the picrates of the basic amino-acids remain firmly retained by the adsorbent. It was also found that when the charcoal column containing the adsorbed picrates is treated with a dilute aqueous pyridine solution, the histidine dipicrate is readily decomposed and all of the histidine appears in the eluate, while the picric acid remains in the column. Both lysine and arginine picrates are unaffected by the pyridine treatment and remain strongly retained by charcoal. It has been shown that aqueous ammonia readily decomposes lysine picrate on charcoal, but has no effect on the adsorbed arginine picrate. Accordingly, further treatment of the column with dilute aqueous ammonia leads to elution of lysine, whereas the picric acid and arginine picrate remain firmly held by the adsorbent. Arginine can then be recovered quantitatively from the column by passing a dilute hydrochloric acid solution through the column. This decomposes the arginine picrate, and arginine passes out with the eluent leaving the picric acid strongly adsorbed on the charcoal.

All these results furnish the basis of the present method. By the new technique the basic amino-acid can be recovered from the charcoal column and collected in three separate fractions. The first, or pyridine fraction, consists of a pure solution of histidine and is entirely free from tyrosine and other basic amino-acids. Histidine can therefore be

readily determined in this fraction colorimetrically, by the Pauly reaction. The second, or ammonia fraction, contains no amino-acids except lysine, which can therefore be determined in this fraction, after removal of ammonia, by the colorimetric ninhydrin method. The third, or hydrochloric acid fraction, contains all the arginine and as the latter is not contaminated by any other amino-acid, it can be readily determined in the acid eluate, after being completely freed from ammonia, by applying the colorimetric ninhydrin method.

The applicability and the adequacy of the method have been demonstrated by analysing micro amounts of various proteins, not exceeding 2 to 3 mg, and the results are reproducible and comparable with those obtained by using other methods. The high recoveries achieved by analysing both pure standard solution of the individual basic amino-acid, as well as protein hydrolysates to which known amounts of the basic amino acids were added, reveal the validity and accuracy of the method. Also, the specificity of the present technique for the determination of the basic amino-acids in protein hydrolysates has been adequately examined. This procedure has the advantage that it can also be used for determining histidine in the presence of other imidazole derivatives. It is simple, rapid and economical in reagents and does not involve the need for special equipment.

Experimental

Reagents—

All reagents used were of the highest purity obtainable commercially. Pyridine was re-distilled before use. The nitrogen content of proteins was determined by the standard micro-Kjeldahl procedure. Duplicate determinations were made on each sample and the values presented are the average of these determinations.

Charcoal—The charcoal used in all experiments was a B.D.H. Ltd. active de-colourising charcoal. It was purified by the procedure previously reported.[1] The washed charcoal was further treated before use with 5 per cent. acetic acid.

Egg albumin—E. Merck Darmstadt (nitrogen, 15·01 per cent. of moisture-free and ash-free protein; moisture, 12·01 per cent.; ash, 4·3 per cent.).

Blood albumin (bovine)—B.D.H. Ltd. (nitrogen, 15·15 per cent. of moisture-free and ash-free protein; moisture, 10·95 per cent.; ash, 8·8 per cent.).

Gelatin—Coignet's gold label leaf gelatin (nitrogen, 17·09 per cent. of moisture-free and ash-free protein; moisture, 14·3 per cent.; ash, 4·05 per cent.).

Edestin—B.D.H. Ltd. (nitrogen, 14·68 per cent. of moisture-free and ash-free protein; moisture, 11·2 per cent.; ash, 6·1 per cent.).

Apparatus—

Micro chromatographic, Pyrex tubes were used for the charcoal adsorption (7 mm in internal diameter and 15 cm in effective length). Small funnels are incorporated at the end of each tube.

The colorimetric determinations were carried out with a Unicam spectrophotometer, model SP500, with 1-cm quartz cuvettes.

Procedure—

Hydrolysis of proteins—Weigh 2 to 3 mg of the protein into a 2-inch length of glass tubing (4 mm in internal diameter) sealed at one end. Add to the protein, by pipette, 0·1 ml of 6 N hydrochloric acid, and seal off the tube. Place the tube in an oven at 110° C for 24 hours; then open the seal and remove the acid by evaporating the solution to dryness on the steam-bath. Dissolve the dried residue in 1 ml of 0·2 M acetate buffer at pH 5·6.

Adsorption of the aromatic amino-acids and basic amino-acid picrates on active charcoal—Transfer the buffered hydrolysate quantitatively to a small flask and make up the volume of the solution to approximately 20 ml with water. To this solution add 1 ml of a standard aqueous picric acid solution (containing 4 mg of picric acid), and shake the mixture thoroughly for 1 minute. Pass the resulting solution through a micro-chromatographic tube packed with 150 mg of active charcoal (already washed in the column with 10 ml of 5 per cent. acetic acid) at about 2 ml per minute. Then treat the column with 20 ml of aqueous 5 per cent. acetic acid to remove any retained free amino-acids other than the aromatic ones.

Elution of the aromatic amino-acids—Apply 40 ml of a freshly prepared aqueous solution of 5 per cent. ethyl acetate to the column at 2 ml per minute. We found that the first 20 ml

of the ethyl acetate eluate contained practically all of the phenylalanine and tyrosine, and that the last portion of the eluate gave, when tested, a completely negative ninhydrin reaction.

Elution of histidine and its determination—Treat the charcoal column, still containing the basic amino-acid picrates, with 25 ml of a 2 per cent. aqueous solution of pyridine at 2 ml per minute. The first 10 to 15 ml of the eluate, we found, contained all of the histidine as indicated by the Pauly colour reaction. Evaporate the resulting eluate *in vacuo*, and make up the volume of the concentrate to 10 ml with water. This histidine fraction was shown to be entirely free from tyrosine by the 1-nitroso-2-naphthol colour reaction and by paper chromatography.

Introduce 4-ml aliquots from the histidine fraction into 25-ml measuring flasks, and neutralise the pyridine present by adding 0·5 ml of N hydrochloric acid. Add to these solutions 5·5 ml of water, and determine the histidine content of the final mixtures colorimetrically by following exactly the Macpherson modification of the Pauly reaction.[7] Read the optical density of the resulting red coloured solution at 530 mμ in the spectrophotometer against water. Calculate the histidine concentration by using a standard solution of the amino-acid, prepared at the same time by the following method. Introduce 3 ml of an aqueous solution containing 0·03 mg of histidine into the flask and treat the solution with 4 ml of a 2 per cent. pyridine solution. Add, successively, 0·5 ml of N hydrochloric acid and 2·5 ml of water. The colour was then developed in the same manner as with the unknown, and its absorbance measured in the spectrophotometer.

Elution of lysine and its determination—Apply 25 ml of an aqueous solution of 0·1 N ammonia to the adsorbent at 2 ml per minute. We found that the last fraction of the percolate, when tested after the complete removal of ammonia, gave an entirely negative ninhydrin reaction. Treat the colourless lysine fraction with sufficient dilute alcoholic potassium hydroxide solution until the fraction is distinctly alkaline to phenolphthalein. Remove the ammonia by evaporating the lysine solution to dryness *in vacuo*. Repeat the evaporation procedure once again, after the addition of water and ethanol. The air bubbled into the distillation flask was already freed from any ammonia by its passage through a trap containing dilute sulphuric acid. Add a little water to the ammonia-free residue, and neutralise the resulting solution with N hydrochloric acid and make the solution up to 5 ml. This lysine solution was shown, by paper chromatography, to be free from other amino-acids. Determine the lysine in 1-ml aliquots of solution by the modified ninhydrin colour reaction, according to Rosen.[8] Measure the absorbance of the final coloured solution at 570 mμ in the spectrophotometer against a reagent blank that has been treated in the same manner. Calculate the lysine concentrations from the optical densities with the aid of a calibration curve prepared from a graphical plot of optical densities against μmoles of leucine (0·025 to 0·175). The μmoles of leucine obtained from the curve were converted into μmoles of lysine by using a conversion factor of 103 which corresponds to the percentage yield of lysine, based on leucine as 100 per cent., in the same way as demonstrated by Rosen.[8]

Elution of arginine and its determination—Treat the charcoal column, still containing the arginine, with 25 ml of N hydrochloric acid. Make the colourless eluate distinctly alkaline to phenolphthalein by adding a dilute alcoholic solution of potassium hydroxide, and then aerate the solution at room temperature for 1 hour. Free the air that is used from any possible contamination by ammonia with a dilute sulphuric acid trap. Then make the arginine solution neutral to litmus with N hydrochloric acid and evaporate the solution to dryness *in vacuo*. Dissolve the resulting residue in a little water and make the solution up to 5 ml. We found on examining of this solution by paper chromatography, that it was completely free from any other amino-acids. Determine the arginine, with 1-ml aliquots, by the same colorimetric ninhydrin method described above for lysine. The μmoles of leucine obtained from the calibration curve were converted into μmoles of arginine by using a conversion factor of 100.

RESULTS

The applicability of the method was determined by analysing four different proteins, *viz.*, egg albumin, serum albumin, gelatin and edestin. The analyses were run in triplicate and the results were found to be reproducible. In all experiments duplicate samples of the individual basic amino-acid fractions, obtained from the column, were analysed. In Table I are shown the values of the basic amino-acids of the above proteins, as determined by the

TABLE I

BASIC AMINO-ACID CONTENTS OF PROTEINS EXPRESSED ON 16 GRAMS OF PROTEIN NITROGEN

Protein	Amino-acid	Present procedure: Amino-acid, g	Present procedure: Mean, g	Other methods	
Egg albumin	histidine	2·5, 2·6, 2·58	2·6	2·5, Miller *et al.*[12]	2·6, Long[13]
	lysine	6·45, 6·52, 6·6	6·5	6·5, Long[13]	6·5, Block and Weiss[14]
	arginine	6·3, 6·22, 6·2	6·2	6·2, Long[13]	6·3, Miller *et al.*[12]
Serum albumin	histidine	3·3, 3·37, 3·4	3·4	3·4, Block and Weiss[14]	3·6, Keller and Block[15]
	lysine	8·7, 8·66, 8·7	8·7	8·4, Block and Weiss[14]	8·6, Keller and Block[15]
	arginine	5·96, 5·9, 6·0	6·0	5·4, Keller and Block[15]	5·9, Block and Weiss[14]
Gelatin	histidine	0·85, 0·8, 0·82	0·8	0·8, Block and Weiss[14]	0·8, Long[13]
	lysine	5·45, 5·48, 5·5	5·5*	4·5, Long[13]	4·8, Block and Weiss[14]
	arginine	8·3, 8·2, 8·2	8·2	7·8, Block and Weiss[14]	8·1, Eastoe[16]
Edestin	histidine	2·3, 2·25, 2·23	2·3	2·5, Block and Weiss[14]	2·5, Kimmel and Smith[17]
	lysine	2·75, 2·8, 2·78	2·8	2·5, Kimmel and Smith[17]	2·8, Block and Weiss[14]
	arginine	14·8, 15·01, 14·9	14·9	14·9, Kimmel and Smith[17]	15·4, Yemm and Folkes[18]

* This value includes hydroxylysine.

present method and compared with previously reported figures. As indicated in Table I, the results obtained are in good agreement with those results cited by other workers. The accuracy of the new procedure was demonstrated by running recovery experiments simultaneously with the unknowns. These were performed by the addition of known amounts of histidine, lysine and arginine, in two concentration ranges, to each protein hydrolysate before application of the latter to the charcoal column. Analyses were then made for the recovery of the added amino-acids. The results of these experiments are given in Table II, and they show that high recoveries, ranging from 97 to 99 per cent. were achieved by the present technique. The time required for the analyses, run in duplicate, of the three basic amino-acids in protein hydrolysates is 3 hours, and 4 to 5 hours for analysing 6 samples simultaneously.

TABLE II

RECOVERY OF THE INDIVIDUAL BASIC AMINO-ACIDS ADDED TO ALIQUOTS OF PROTEIN HYDROLYSATES EQUIVALENT TO 2 TO 3 mg OF PROTEIN

Protein	Amino-acid	Amino-acid added, μmole	Amino-acid recovered, μmole	Recovery, percentage
Egg albumin	histidine	0·15	0·149	99
		0·32	0·311	97
	lysine	0·2	0·196	98
		0·34	0·337	99
	arginine	0·27	0·265	98
		0·55	0·535	97
Blood albumin	histidine	0·25	0·245	98
		0·32	0·318	99
	lysine	0·2	0·198	99
		0·34	0·330	97
	arginine	0·17	0·168	99
		0·57	0·566	99
Gelatin	histidine	0·1	0·098	98
		0·25	0·243	97
	lysine	0·2	0·194	97
		0·68	0·670	97
	arginine	0·57	0·564	99
		0·85	0·83	98
Edestin	histidine	0·15	0·148	99
		0·25	0·242	97
	lysine	0·25	0·244	98
		0·34	0·336	99
	arginine	0·75	0·737	98
		1·20	1·18	98

CAPACITY OF CHARCOAL—

The use of micro columns packed with 150 mg of active charcoal was found to be quite sufficient to hold, as picrates, all the amounts of basic amino-acids that are present in aliquots of protein hydrolysates which are equivalent to 3 mg of proteins. Such columns have enough room to retain the excess free picric acid and to permit the separation of the basic amino-acids in the manner previously described. Robson and Selim[1] have shown that while amounts of picric acid in slight excess of those equivalent to the arginine and histidine present, in the molar ratio of 1 to 1 for the former and 2 to 1 for the latter, are enough for the complete removal of these amino-acids by charcoal, picric acid in amounts four to five times that required for the formation of lysine picrate should be present to secure the complete retention of lysine by charcoal. On this basis, it was found that treatment of the protein hydrolysate (2 to 3 mg of protein) with 4 mg of picric acid is quite sufficient for the conversion of all of the basic amino-acids present into their picrates, and for their quantitative adsorption on to charcoal.

RECOVERY—

In order to test the validity and specificity of the method for the determination of the basic amino-acids in protein hydrolysates, aliquots of pure standard solutions of histidine, lysine monohydrochloride and arginine monohydrochloride were each treated with 4 mg of picric acid and applied separately to micro columns of charcoal (150 mg). Each time, the adsorbent was treated with 20 ml of 5 per cent. acetic acid followed by 40 ml of 5 per cent. ethyl acetate. Histidine, lysine and arginine were further eluted from their columns in exactly the same way as previously described. The percentage of the basic amino-acids recovered was determined as before. These experiments were then repeated, but with two known amino-acid mixtures. Mixture (1) consisted of the three basic amino-acids, while mixture (2) was prepared from the three basic amino-acids, phenylalanine and tyrosine and a number of the aliphatic amino-acids. The latter included glycine, alanine and glutamic acid, all of which are weakly adsorbed by charcoal, as well as isoleucine, leucine and methionine which are relatively strongly retained by charcoal. In each experiment, the pyridine, ammonia and hydrochloric acid fractions were collected from the columns and analysed for their basic amino-acid contents. The results of these analyses, run in duplicate, are given in Table III, and they indicate that the recoveries of the basic amino-acids were high and average from 97 to 99 per cent. From the figures given in Table III, it would appear that the presence of the aliphatic and aromatic amino-acids does not interfere with the determination of the basic amino-acids by the present technique.

TABLE III

RECOVERY OF THE INDIVIDUAL BASIC AMINO-ACIDS FROM PURE SOLUTIONS AND AMINO-ACID MIXTURES

Material analysed, volume 25 ml	Amino-acid added, μmole	Amino-acid recovered, average value, μmole	Recovery, percentage
Pure histidine solution	0·75	0·740	99
Pure lysine solution	0·75	0·728	97
Pure arginine solution	0·75	0·742	99
Mixture (1)—			
Histidine	0·4	0·395	99
Lysine	0·4	0·390	98
Arginine	0·4	0·391	98
Mixture (2)—			
Histidine	0·5	0·486	97
Lysine	0·5	0·493	98
Arginine	0·5	0·485	97
Tyrosine	1·0	—	—
Phenylalanine	1·0	—	—
Glutamic acid	4·0	—	—
Glycine	2·0	—	—
Alanine	3·0	—	—
Leucine	3·0	—	—
Isoleucine	2·0	—	—
Methionine	0·5	—	—

SPECIFICITY—

The reliability and specificity of the method were also demonstrated by examining the pyridine, ammonia and hydrochloric acid fractions obtained in the above experiments, as well as in those carried out on egg-albumin hydrolysate, for the presence of any contaminating amino-acids. Each time the histidine fraction was found to be completely free from tyrosine, as indicated by the 1-nitroso-2-naphthol colour reaction, according to the method of Cerriotti and Spandrio.[9] The behaviour of the above fractions, on a two-dimensional chromatogram, with water-saturated 2,4,6-collidine and water-saturated phenol - ammonia was also examined. The results showed that the final histidine, lysine and arginine solutions were not contaminated with any other amino-acids. However, δ-hydroxylysine picrate will be adsorbed by charcoal together with the lysine picrate, and therefore, the presence of hydroxylysine in protein hydrolysates will interfere with the quantiative assay of lysine. This, however, does not present a difficulty as the limited distribution of hydroxylysine in proteins greatly minimises the incidence of this interference.

DISCUSSION

Charcoal chromatography, which has proved its value as a convenient procedure for the quantitative removal of the aromatic and basic amino-acids from protein hydrolysates,[5,6,10,11] was selected as an effective tool for the micro determination of the individual basic amino-acids in the present investigation.

In agreement with the work of Schramm and Primosigh,[2] it has been found that the acetic acid treatment of charcoal, before and after the percolation of the hydrolysate, is necessary for facilitating the complete removal of the adsorbed amino-acids. No attempts were made in this work to prevent, or minimise, the catalytic action of active charcoal on amino-acids by poisoning the adsorbent with hydrocyanic acid solution, or hydrogen sulphide-saturated water. However, under the experimental conditions used, it was found that the absence of such poisoning of charcoal does not affect the results to any appreciable extent. It appears that if the whole process of charcoal chromatography is carried out rapidly at high rates of liquid flow, with the active charcoal purified and washed, as previously described, there will be hardly any risk of the catalytic effect of charcoal playing an important rôle. The irreversible adsorption of amino-acids will also be avoided. The use of micro and very short columns of purified and acetic acid pre-treated charcoal has greatly helped to meet the above requirements. In addition, if any traces of aliphatic amino-acids escape removal from the column when the latter is washed with 5 per cent. acetic acid, these will be eluted, together with the aromatic amino-acids, by treatment with ethyl acetate.

The present chromatographic method has the advantage that many of the imidazole derivatives do not interfere with the histidine determinations, and therefore the method will be of value in the determination of histidine in the presence of these compounds. From the results of preliminary experiments it was found that the imidazole derivatives that fail to form dipicrates are not strongly retained by charcoal. So imidazole, imadazole acetic acid and imidazole propionic acids, in the presence of picric acid, are readily eluted from charcoal columns by 5 per cent. acetic acid or an aqueous solution of ethyl acetate. Histamine, however, is readily adsorbed as the dipicrate on charcoal and is therefore not washed out from the column by acetic acid or ethyl acetate. Unlike histidine, it is unaffected by the pyridine treatment and accordingly its presence with histidine does not interfere with the determination of the latter. The adsorbed histamine can be readily eluted from the column with 0·1 N ammonia.

CONCLUSION

This method is simple, rapid, accurate and specific for the determination of the individual basic amino-acids in protein hydrolysates. It gives results that are reproducible and comparable with those previously reported. It has also the advantage that it can be used for the determination of histidine in the presence of other imidazole derivatives.

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The Micro Determination of Cyanide: Its Application to the Analysis of Whole Blood

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An introduction to the existing methods for the analysis of cyanide is given and some of the limitations to the methods are pointed out.

A modification of the Epstein method is described, in which Cavett blood-alcohol flasks are used. This method can be applied to small samples of 2 ml volume containing 0·2 μg of cyanide. By strict control of the conditions it is shown that a high degree of accuracy can be achieved. Interference by heavy metal ions is avoided by using 2 mg of the disodium salt of EDTA per ml of blood. It is therefore suggested that this anti-coagulant should be used when the blood is collected. Experiments on the partition of cyanide in whole blood showed that 5 minutes' exposure resulted in more than 70 per cent. of cyanide being bound to haemoglobin. This value remained unchanged in the presence of a transport inhibitor.

THE necessity to determine small amounts of cyanide formed by *Ps. aeruginosa* in small samples of whole blood or tissue led to the development of the method that is described.

Techniques suitable for determining low concentrations of cyanide are based on the König[1] reaction for the synthesis of pyridine dyes. The method developed by Aldridge[2] involves the coupling of cyanogen bromide with a pyridine - benzidine mixture. The carcinogenic nature of the benzidine has led to its substitution by other amines, notably *p*-phenylenediamine (Bark and Higson[3]). Even so, relatively large samples are necessary to carry out this technique. The original Aldridge method has been applied by Tompsett[4] to biological material. But although it is suitable for determining low concentrations of cyanide, the samples to be analysed must contain at least 5 μg of hydrogen cyanide.

The Epstein[5] method is more sensitive and therefore appeared to be more suitable for the determination of small samples of low cyanide concentration. In this method cyanogen is converted to cyanogen chloride, which is then converted to glutaconaldehyde by chloramine T. The excess of chloramine T is reduced by 3-methyl-1-phenyl-2-pyrazolin-5-one. The presence of pyridine in the reagent prevents the reduction of the cyanogen chloride that is formed. A small amount of 4,4′-bis-(3-methyl-1-phenyl-2-pyrazolin-5-one) in the reagent causes the formation of a blue dyestuff suitable for quantitative determination. The exact nature of the reaction is unknown.

The principle of this reaction has been applied by some workers to the determination of cyanide in small volumes of blood. Feldstein and Klendshoj[6] used the modification described by Boxer and Rickards[7] and adapted it for micro diffusion analysis by using Conway No. 1 units. In the absence of a special oscillating table it was found that recovery never exceeded 70 per cent., even at a dilution of 1 in 4.

Feldstein and Klendshoj[6] also found that the apparent recovery of the cyanide, added to whole blood, decreased on standing for a few hours, but reached the expected levels after 24 hours' storage. An enzyme system, within the red cell, capable of removing cyanide was suggested.

The method described below offers a simple modification of a commercially available apparatus and avoids the use of an oscillating table. The factors leading to incomplete cyanide recovery from whole blood were also investigated and allowance was made for these.

Method

Apparatus—

For the micro diffusion work a modification of the Cavett flask was used,* as shown in Fig. 1. On the floor of the flask a small cup was mounted with Evostik. This was prepared from a polystyrene specimen tube (external measurements 3·8 cm × 1·5 cm). The tube was

* Supplied by Quickfit & Quartz Ltd., Staffs., England.

sawn off to permit the insertion of a wall, 0·5 cm high. In this wall a slot of 1·5-mm width was sawn. The capacity of such a cup is approximately 0·5 ml and, because it is "non-wettable," fluid does not readily leave it. The flask must be washed with detergent and water only and dried with warm air. The Evostik attachment has withstood frequent use, but the more permanent Araldite is also suitable.

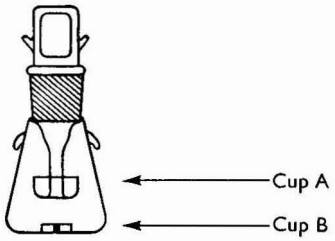

Fig. 1. Modification of the Cavett flask used for micro diffusion work (quarter scale)

REAGENTS—

Pyrazolone reagent—This must be prepared freshly on the day of use, as otherwise the colour yield may be variable. Dissolve 240 mg of 3-methyl-1-phenyl-2-pyrazolin-5-one in 100 ml of water at 75° C; this requires about 1½ hours. Dissolve 20 mg of 4,4′-bis-(3-methyl-1-phenyl-2-pyrazolin-5-one)* in 20 ml of pyridine by shaking them together. Just before use, mix these two solutions.

Sodium hydroxide, 0·1 N.

Sodium dihydrogen phosphate, M.

Sulphuric acid, 15 *per cent. v/v.*

Chloramine T solution, 0·1 *per cent., aqueous*—Keep solution refrigerated. Just before use, add 1 volume of this solution to 3 volumes of M sodium dihydrogen phosphate, and cool the resulting solution to about 5° C.

Whole blood—Out-dated, citrated blood-bank material.

Standard cyanide solution—Dissolve 500 mg of potassium cyanide in 1000 ml of 0·1 N sodium hydroxide. Suitable dilutions of this stock solution with de-ionised water are prepared before use.

PROCEDURE—

Moisten the ground-glass neck of the flask (see Fig. 1) with distilled water. Introduce 1·2 ml of 0·1 N sodium hydroxide into cup A. Tilt the flask about 25° by resting it on a suitable support. Introduce 4 ml of water on to the floor of the flask, then add to the water either 2 ml of aqueous standard solution containing 200 mg of the disodium salt of EDTA per 100 ml, or whole blood containing 2 mg of the disodium salt of EDTA per ml of blood. Fill cup B with 0·5 ml of 15 per cent. sulphuric acid and replace the stopper, righting the flask. Attach the springs, and again tilt the flask to about the same angle as before. Tap the floor of the flask slightly to allow the acid to flow out. After about 30 seconds rotate the flask twice on a flat surface, and allow diffusion to proceed for 2 hours. At the end of this period withdraw a 1-ml sample from cup A, cool it to approximately 5° C and add 0·2 ml of buffered chloramine T solution. Mix the solutions thoroughly. After 2 minutes, add 3 ml of pyrazolone reagent rapidly. Allow the colour to develop for 45 minutes, then add 2 ml of n-butanol to extract the coloured product. Determine the absorbancy of the supernatant liquid, obtained after spinning the solution in a centrifuge, at 615 mμ and 1-cm light path. It is found that Beer's law is obeyed for concentrations from 10 to 100 μg per 100 ml. The colour is stable for at least another 15 minutes.

* Supplied by Koch-Light Laboratories, Colnbrook, England.

Results

Two types of substances may be reasonably expected to interfere. The presence of sulphydryl groups could lower the apparent values. Free sulphydryl groups are known to react with cyanide, and the *in vitro* formation of thiocyanate from proteins has already been demonstrated by Pascheles.[8] Catsimpoolas and Wood[9] carefully investigated the reaction between cyanide and albumin and showed that thiocyanate was only formed at pH 8 or higher.

To investigate possible small changes in apparent cyanide levels, artificial cyanide solutions in plasma were prepared in the presence or absence of 5×10^{-5} M sodium salt of *p*-chloromercuribenzoate. This concentration of the mercuric salt had been shown previously not to complex with the cyanide, but was sufficient to block the free sulphydryl groups in plasma. Even after standing for 24 hours both sets gave the same values. It was therefore concluded that the presence of a blocking agent was not necessary.

Table I

THE RECOVERY OF ADDED CYANIDE FROM WHOLE BLOOD IN THE PRESENCE OF THE DISODIUM SALT OF EDTA

2 mg of the disodium salt of EDTA per ml of whole blood

With EDTA			Without EDTA			Percentage increase with EDTA
Cyanide, μg per cent.	$E^{1\,cm}_{615\,m\mu}$	Average	Cyanide, μg per cent.	$E^{1\,cm}_{615\,m\mu}$	Average	
10	0·105		10	0·085		
10	0·100	0·103	10	0·088	0·088	14
10	0·105		10	0·090		
20	0·210		20	0·100		
20	0·213	0·212	20	0·195	0·193	9
20	0·213		20	0·193		
30	0·340		30	0·300		
30	0·342	0·341	30	0·304	0·302	11
30	0·340		30	0·302		

Heavy metal ions are the second group of interfering materials and would also produce a negative error as they readily form cyanide complexes that are not easily dissociated. Whole blood contains small concentrations of ionic iron and copper. The disodium salt of EDTA was added in sufficient concentration to complex these and also to act as an anticoagulant. Table I shows the results of such an experiment, which clearly demonstrate the advantage of using EDTA. The apparently greater difference at 10 μg per cent. may well be with significance, as the absorbancy values are rather low.

Table II

REPRODUCIBILITY

20 determinations in 3 batches

Whole blood, 20 μg per cent. of hydrogen cyanide, 2 mg per ml of the disodium salt of EDTA

No.	$E^{1\,cm}_{615\,m\mu}$	No.	$E^{1\,cm}_{615\,m\mu}$
1	0·215	11	0·215
2	0·216	12	0·214
3	0·218	13	0·217
4	0·215	14	0·318
5	0·214	15	0·215
6	0·215	16	0·215
7	0·217	17	0·214
8	0·213	18	0·216
9	0·213	19	0·214
10	0·214	20	0·214

Mean = 0·2152 = 20 μg per cent.
$2\sigma = \pm 0{\cdot}0014 = \pm 0{\cdot}26$ μg per cent.
Standard error of mean = ±0·031 μg per cent.

As the addition of EDTA had been established to be advantageous, all further experiments were carried out in its presence. Table II shows the reproducibility attainable with

TABLE III

RATE OF COLOUR DEVELOPMENT

Time, minutes	$E^{1\,cm}_{615\,m\mu}$	Percentage of maximum
10	0·509	84·5
20	0·564	93·7
30	0·600	99·9
40	0·602	100
50	0·602	100

one cyanide concentration only. For 95 per cent. confidence limits at 20 μg per cent., the value was $\pm 0{\cdot}26$ μg per cent. or 1·3 per cent. within the optimal absorbancy range.

Various modifications of the Epstein method utilise differing times for maximal colour development. This was therefore investigated with the method described above. As shown in Table III, 45 minutes or more gave maximal values.

TABLE IV

THE EFFECT OF WHOLE BLOOD DILUTION ON CYANIDE RECOVERY

Whole blood, ml	Water, ml	Cyanide added, μg per cent. of hydrogen cyanide*	$E^{1\,cm}_{615m\mu}$	Percentage of aqueous standard
1	5	50	0·246	100·0
2	4	40	0·243	99·0
3	3	30	0·210	85·3
4	2	20	0·151	61·1
5	1	10	0·137	55·7

* Mean of two individual determinations.

As the rate of gas liberation is affected by the viscosity of the medium, the effect of using various dilutions of whole blood was determined. Table IV demonstrates that gas liberation is incomplete if dilutions of less than 1 in 3 are used.

The geometry of the diffusion chamber significantly affects the time required for absorption efficiency to approach 100 per cent. This has been shown in detail by Conway[10] for the chambers developed by him.

TABLE V

THE EFFECT OF DIFFUSION TIME ON ABSORPTION EFFICIENCY

Potassium cyanide in 0·1 N sodium hydroxide
1 ml $\equiv$ 0·2 μg of hydrogen cyanide

Time, hours	$E^{1\,cm}_{615\,m\mu}$	Average	Percentage absorbed
0·5	0·177		
0·5	0·173	0·176	82·8
0·5	0·178		
1·0	0·196		
1·0	0·196	0·196	92·0
1·0	0·195		
2·0	0·210		
2·0	0·212	0·211	99·2
2·0	0·210		
3·0	0·213		
3·0	0·215	0·213	100
3·0	0·211		

This factor was therefore investigated for the proposed modification, and Table V shows that after 2 hours' diffusion 99 per cent. of the 3-hour value is given. The 2-hour diffusion time was adopted for convenience.

Because of earlier statements that the cyanide disappears from whole blood and that plasma could also be used, it seemed important to obtain information on the partition of added cyanide within the blood elements.

Table VI shows that after 5 minutes' exposure, over 70 per cent. of the cyanide was bound to the haemoglobin. This value should probably be somewhat higher as the stroma fraction was not washed, and was therefore slightly contaminated with haemoglobin. It

TABLE VI

PARTITION OF CYANIDE ADDED TO WHOLE BLOOD

Time of exposure = 5 minutes; packed cell volume = 34 per cent.

Material	$E^{1\,cm}_{615\,m\mu}$	Percentage recovery of cyanide added
Whole blood	0·323	100
Whole blood	0·326	100
Aqueous standard	0·324	100
Cells	0·236	73·5
Cells	0·232	
Plasma—undiluted	0·062	12·9
Stroma (4 ml blood) ..	0·086	13·6
		Recovery = 98·5 per cent.

* The contents of the cells were adjusted with saline to the original volume of blood, plasma and stroma absorbancy were corrected to appropriate volumes.

remained to be seen whether the cyanide reached the cell contents by a pure-diffusion process, or whether active transport was involved. For this purpose ouabain, an inhibitor to active cation transport, was added in high concentration. It can be seen from Table VII that the results were similar to those obtained without the use of an inhibitor, and it is reasonable to assume that cyanide freely diffuses and presumably forms cyanmethaemoglobin.

TABLE VII

THE EFFECT OF OUABAIN 10^{-3} M ON THE CYANIDE PARTITION IN WHOLE BLOOD

Time of exposure = 5 minutes; packed cell volume = 34 per cent.

Material*	$E^{1\,cm}_{615\,m\mu}$	Percentage recovery of cyanide added
Whole blood	0·348	100
Whole blood	0·348	100
Cells	0·265	76·4
Cells	0·266	
Plasma—undiluted	0·096	9·4
Plasma—undiluted	0·097	
Stroma (4 ml blood) ..	0·012	14·6
		Recovery = 100·4 per cent.

* The contents of the cells were adjusted to the original volume of blood, plasma and stroma absorbancy were corrected to appropriate volumes.

It becomes clear from these experiments that plasma must never be used for analysis and, provided whole blood is used, the proposed modification can be adopted with accuracy for cyanide levels above 10 μg per cent. Levels of about 5 μg per 100 ml can be determined with similar accuracy if 4 ml of sample are available. The stopper, after the first absorption, is inserted into a second flask containing a fresh 2-ml sample of the same specimen to which 4 ml of water are added, and the hydrogen cyanide is liberated by the addition of 0·5 ml of 15 per cent. v/v sulphuric acid.

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Determination of Particulate Matter in Intravenous Fluids

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Methods for the examination of intravenous solutions for suspended particles have been investigated. A visual inspection method has been devised, which enables samples to be graded by comparison with reference materials. For quantitative work, techniques for particle-size analysis with a Coulter Counter have been developed. A survey of all the solutions commercially available in Australia has been made, and limits for the acceptance of such solutions have been proposed.

Attention has recently been drawn to the hazards associated with particulate matter present in intravenous fluids,[1,2] and there is clearly a need for control of this type of contamination. No such control can be effected unless the particles can be sized and counted with reasonable accuracy. In addition, it may be necessary to distinguish different types of particles, particularly to recognise bast and other fibres,[1,2] and to establish limits for them. As a first step, however, it appears that a considerable improvement would result if limits for total particulate matter were established, without discrimination of type.

Many methods of examination of solutions are possible, of which the following have been considered in this work—

(*a*) Visual examination in diffuse light. This enables larger particles to be detected, the lower size limit being about 50 microns. This method is used by many manufacturers for inspection of finished products, but leads to considerable operator fatigue, and is not quantitative.

(*b*) Visual examination by light scattering (Tyndall effect). Garvan and Gunner[1] used apparatus of this type, the light beam being introduced through the bottom of the bottle and the particles viewed from the horizontal position. As, however, the interest is mainly in particles that are large compared with a wavelength of visible light, this method gives insufficient discrimination of particle types. Particles over about 1 micron in diameter give most of their scatter at small angles to the direct emergent beam, and relatively little scatter in the 90° direction. Colloidal particles, *i.e.*, those small compared with a wavelength of visible light, scatter equally about the normal. Thus large particles are less readily visible, and are seen against a diffuse background if any colloidal matter is present. Small angle viewing eliminates the colloidal background almost completely, and is much more satisfactory for detection of particles over about a micron in diameter. An apparatus for this purpose is described below.

(*c*) Filtration through a membrane filter, followed by microscopic examination of the retained particles. This method is of value for the examination of many products, but in our experience is not ideally suited to solutions having relatively low counts, such as the best of the commercial intravenous solutions. Apart from the tedious nature of the counting process, there is a danger that particles having a similar refractive index to the filter may be missed, especially as they are viewed against the granular background of the filter.

(*d*) Resistance counters (Coulter, Ljungberg, Sansar). This type of instrument has been found to be most suitable for particle-size analysis, and has been used for the survey which occupies the remainder of this paper. Only electrolytes have been examined so far, but methods of handling non-electrolytes by dilution with a suitable electrolyte are being developed. One previous publication[3] has discussed the use of a resistance counter for examination of normal saline solution, a single count being made of particles greater than 1·3 microns.

The visual examination methods, (*a*) and (*b*), are carried out on unopened bottles, while the last two, (*c*) and (*d*), are destructive, *i.e.*, they have to be carried out by opening the containers; both types of test are necessary so that all bottles can be checked semi-quantitatively and more accurate counts taken on representative samples.

Experimental

(*a*) Light scattering apparatus—

This apparatus produces a sharply defined ribbon-shaped beam of light, projected horizontally into the bottle. The optical scheme is shown in Fig. 1. The light source, A, is an 8-volt, 50-watt iodine - quartz projector lamp. An image of the filament is focused by the condenser, B, on to an aperture, D, (the aperture is made equal in size to the image). A stop, C, immediately in front of the condenser defines the shape of the final beam; a vertical slit has been found to be the most satisfactory.

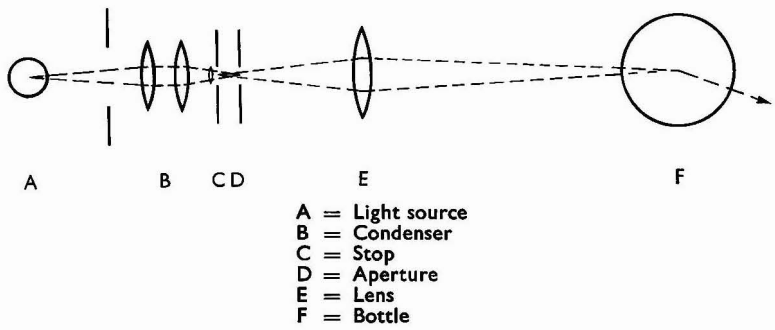

Fig. 1. Light-scattering apparatus for the visual detection of small particles

The lens, E, of approximately 8 to 10-cm focal length, projects an image of the slit, C, into the centre of the bottle. The bottle itself acts as a cylindrical lens and helps to keep the beam fairly uniform in cross-section.

The apparatus fits into a box about 14 inches long and 8 inches wide, divided into compartments by baffles that support the optical elements. An extra baffle in front of the lamp carries a heat filter, and a final baffle is situated just in front of the sample; the whole interior is painted matt black. The final beam is a ribbon about 1·5 cm high and 1 to 2 mm wide. The end of the box is open, and the sample is viewed at an angle of 20° to 30° from the direct transmitted beam. With a little practice it is possible to form some judgment of the sizes of particles present. Particles over about 5 microns diameter and of moderately high refractive index are seen as twinkling spots of light, often appearing to change colour as the angle of viewing changes. Small particles, over about 1 micron, can be seen as rather faint but definite spots of light.

(*b*) Resistance counter—

A Coulter Counter model A (Medical) was used, with a 100-micron aperture. The threshold and threshold zero potentiometers were replaced by 10-turn "Helipots" fitted with "Duodials" for easier and more accurate setting.

The counter was checked at intervals by placing a 10,000-ohm load resistor across the electrodes and injecting pulses obtained by clipping and differentiating 50-c.p.s. a.c. mains. A switch was fitted to over-ride the mercury contacts so that the counter could be re-set, started and stopped. Counts of mains pulses were made for 1 minute; the count was invariably within the accuracy of reaction time when the counting period was controlled by manual switching while observing a stop-watch.

To avoid contamination from airborne particles, solution administration sets were used for sampling, and the sample container was handled in the down-draft from a Vokes absolute filter. Instead of the beaker normally used to hold samples in the counter, a tube of 2·5-cm diameter was used as the sample container. This tube had a side arm into which the tube of the administration set could be inserted. A drain tube on this sample container led to a T-piece, one arm of which led to the inner electrode vessel, so that the two electrode compartments could be flushed through in series to avoid waste of sample. In use, the outer tube was filled and flushed through to the inner tube several times. The inner compartment needs only to have the same electrolyte concentration as the outer, and does not need to have the same particulate concentration, as the flow is inwards only. After electrolyte balance

had been reached, the outer vessel was drained and two or three changes of solution made in the outer sample container. It was necessary to ensure that there was no continuous column of liquid between the sample container and the tubing of the administration set (see Fig. 2).

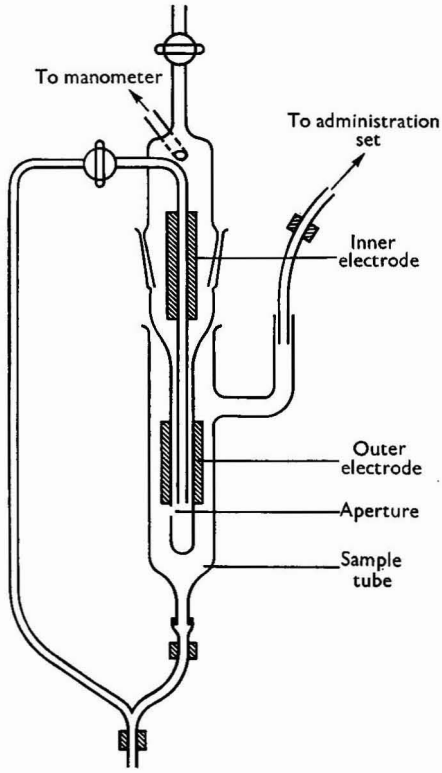

Fig. 2. Sample vessel for Coulter Counter

The counter was calibrated with a Dow polyvinyltoluene latex of 3·49-micron particle diameter. The calibration method given in the instructions supplied with the instrument is adequate when the electrolyte remains constant, but separate calibration for each electrolyte would be inconvenient.

From theory[4] the resistance across the aperture is—

$$r = \frac{4\rho l}{\pi D^2} \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad (1)$$

For a particle of high specific resistivity and diameter less than about 10 per cent. of the aperture diameter, the resistance change due to passage of a particle is—

$$\Delta r = \frac{4\rho d^3}{1{\cdot}5\ \pi D^4} \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad (2)$$

It therefore follows that—

$$\frac{\Delta r}{r} = \frac{d^3}{1{\cdot}5\ D^2 l} \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad (3)$$

where d is particle diameter, D the aperture diameter, l the effective aperture length and ρ the specific resistivity of the solution. For a given aperture—

$$\frac{\Delta r}{r} = ad^3 \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad (4)$$

where a is a constant for the aperture.

The voltage pulse ΔE due to passage of a particle of effective diameter d is—

$$\Delta E = \frac{E_0 \Delta r}{ry\,(R + r + c)} \sqrt{\frac{x^2y^2}{1 + x^2y^2}} \quad .. \quad .. \quad .. \quad .. \quad (5)$$

or

$$\Delta E = \frac{E_0 a d^3}{y\,(R + r + c)} \sqrt{\frac{x^2y^2}{1 + x^2y^2}} \quad .. \quad .. \quad .. \quad .. \quad .. \quad (5a)$$

where R is the aperture current resistor (from 50,000 ohms to 6·4 megohms), x the shunt impedance of the input circuit[4] (100,000 ohms), E_0 the H.T. voltage (300 volts),

$$y = (1/R + 1/r + 1/G) \quad .. \quad .. \quad .. \quad .. \quad .. \quad (6)$$

G is the input resistor of the first amplifier stage (1 megohm), and c is a 15,000-ohms resistor in series with R and r.

We can then put

$$d^3 = K't'y\,(R + r + c)\sqrt{\frac{1 + x^2y^2}{x^2y^2}}$$

$$= K't'\,(R + r + c)\,\sqrt{x^{-2} + y^2} \quad .. \quad .. \quad .. \quad .. \quad (7)$$

or if r is less than about 30,000 ohms,

$$d^3 = K't'\,(1/R + 1/r)\,(R + r + c) \quad .. \quad .. \quad .. \quad .. \quad .. \quad (8)$$

where K' is a constant which does not depend on r, and t' is the threshold reading on a 0 to 100 scale.

The factors given by Coulter to correct readings at each aperture current setting to those of aperture current 1, involve cancellation of the r term in equation (5) so that the calibration constant,[4] as given by Coulter, is not independent of r.

In use, it was found convenient to measure the voltage between the electrodes for an aperture current setting of 7 or 8 (mean of both polarities), and calculate r from the relationship—

$$r = \frac{E\,(R + c)}{E_0 - E}$$

and then to calculate the threshold setting for a series of particle diameters by substituting for r and suitable aperture current (R) values in equation (7) or (8). Counts at 2·0, 2·5, 3·5, 5·0, 7·5, 10·0, 15·0 and 20·0-micron particle diameter were determined for each solution, means of several readings being taken for each setting. The container was gently shaken and allowed to stand until air bubbles had dispersed. When opening a new container, successive counts at a single size setting were taken until consistent counts were obtained to eliminate errors due to contamination by sample tubes or particles liberated by piercing the closure.

It is possible that other models of resistance counter could be used for this type of work; it is essential, however, that the counter used should be capable of operating over a wide range of resistance, and some form of aperture current control and facilities for calibration with varying aperture currents is essential.

Results

Samples, representing all the solutions commercially available in Australia, were obtained from seven manufacturers and examined; four were of Australian manufacture, the others being of U.K. or German origin.

Table I

Counts for selected particle diameters

Manufacturer	Number of samples	2-μ particle diameter			3·5-μ particle diameter			5-μ particle diameter			10-μ particle diameter		
		mean	best	worst	mean	best	worst	mean	best	worst	mean	best	worst
A1	29	11760	2262	38182	3401	376	14434	1286	126	5148	152	(14)	424
A2	16	2255	378	6896	586	120	1468	186	38	346	28	(10)	84
B	11	2212	520	8880	592	84	2498	199	32	912	26	(6)	78
C	22	185	64	844	50	20	172	(18)	(8)	38	(8)	—	—
D	25	1275	418	3362	296	44	816	97	36	778	(17)	(6)	32
E	9	4863	1260	22292	1092	228	4582	363	76	1416	35	(6)	124
F	7	245	208	562	73	20	222	27	(10)	88	(10)	—	(12)
G	18	241	22	814	48	(6)	102	(18)	—	40	(9)	—	14

For each batch of solution, a set of 6 to 10 containers was examined visually with the low-angle light-scattering apparatus described above. When all samples appeared to be similar, one or two bottles were taken at random for testing, but when appreciable differences were noticed, the best and worst bottles were taken.

Table I shows the counts for selected particle diameters, the figures in the body of the table being the number of particles per ml of solution greater than the stated size. Solutions of different composition showed no systematic trends as far as could be judged from the small number of batches of each which have been examined, so the results on each manufacturer's products have been characterised by taking the mean of all samples, supplemented by the best and worst counts from the manufacturer concerned. The results in Table I appear to come from a skew distribution. Examination of the detailed results confirm this suggestion; comparisons of the mid-range (mean of highest and lowest counts) with the median and mean for the counts for the 3·5-μ particle diameter are shown in Table II. Table III shows the

TABLE II

COMPARISON OF MID-RANGE VALUES FOR THE COUNTS OF THE 3·5-μ PARTICLE DIAMETER

Manufacturer	Mid-range	Median	Mean
A1	7384	2420	3401
A2	794	510	586
B	1291	470	592
C	96	37	50
D	430	252	296
E	2405	356	1092
F	121	35	73
G	54	51	48

The presence of a tail on the high side of the distribution is most marked in the A1 and E series.

results for the best and worst bottles from a given sample in cases where visual examination showed differences. Since the number of bottles for each batch was small, the differences do not fully indicate the range within a batch.

TABLE III

VARIATIONS WITHIN A BATCH SHOWN BY COMPARISON BETWEEN BEST AND WORST BOTTLES

Manufacturer	2-μ particle diameter		3·5-μ particle diameter		5-μ particle diameter		10-μ particle diameter	
	best	worst	best	worst	best	worst	best	worst
A	2826	5294	562	902	192	234	22	32
B	1326	1780	310	576	98	202	(6)	22
D	958	2404	126	208	36	44	(10)	(10)
	418	698	94	178	39	86	—	(20)
	1740	2880	418	598	104	202	(8)	(20)
	614	1072	198	210	68	80	(6)	(16)
E	1454	4092	356	1316	162	332	(10)	—
	1266	1986	230	348	86	102	24	32
G	130	308	44	92	(14)	28	(10)	(14)

Two sets of samples were obtained from manufacturer A, the second being of solutions made early in 1965. The second set gave, on the whole, lower counts than the first, and the differences justified listing the two sets separately. No significant trends were apparent within the range of samples from other manufacturers, but qualitative comparisons of earlier samples showed that some manufacturer's products had improved appreciably over a period of 2 or 3 years before these samples were taken. On the other hand, products made by manufacturer C in 1961 were very similar to current production.

Very low counts suffer from large errors because there are a few spurious pulses, including one or two due to switching. Counts less than 20 have been placed in parenthesis to show that they are of doubtful accuracy. An investigation of these spurious pulses is being made; if they are randomly distributed in time, increasing the volume counted will not by itself increase the accuracy. Errors only become appreciable with the larger particles in the better quality solutions.

The results shown in Tables I and II indicate a wide variation between samples from any one manufacturer (the standard deviation being comparable to the mean values). It is, however, clear that there is a considerable difference in over-all quality of solutions from different manufacturers in respect of particulate contamination.

Sources of particles

The particles may arise from many sources—inadequate filtration, particles detached from the filter, particles in the bottles as received, contamination by the wash-water and particles derived from the closures. Garvan and Gunner[1,2] consider that rubber bungs are the main source. Some manufacturers are using rubber bungs coated with a flexible lacquer. In one manufacturer's products, lacquered bungs were used in 6 batches scattered through the production dates, and in the absence of other systematic trends could be used as a comparison of the two types.

Table IV

Comparison of lacquered and normal bungs

Bungs	Number of samples	2-μ particle diameter			3·5-μ particle diameter			5-μ particle diameter			10-μ particle diameter		
		mean	best	worst	mean	best	worst	mean	best	worst	mean	best	worst
Normal	23	13850	4622	38182	4120	1596	14434	1580	422	5148	183	42	424
Lacquered	6	4939	2262	7824	1049	376	1856	315	126	708	52	14	120

The mean and extreme results are shown in Table IV. The means show about a 3-fold improvement in the counts for bottles with lacquered bungs, although there was appreciable overlap between the individual results.

Samples of these two types of bungs were supplied by the manufacturer concerned. One bung of each type was rinsed several times with water previously filtered through a 0·45-μ Millipore filter, then autoclaved in a flask containing 100 ml of 0·9 per cent. sodium chloride solution. A batch of solution treated similarly, without a bung, was used as a control. The counts are shown in Table V. It is clear that the lacquered bungs can be

Table V

Counts on solutions autoclaved in contact with rubber bungs

Bungs	2-μ particle diameter	3·5-μ particle diameter	5-μ particle diameter
Control (no bung) ..	1312	346	126
Lacquered bung	1992	426	150
Unlacquered bung ..	75354	9252	1500
Lacquered bung: control	680	80	24
Unlacquered bung: control	74042	8906	1374

made almost particle-free by simple rinsing. The comparison does not, of course, show that all lacquered bungs are better than unlacquered; there are considerable differences in composition of the rubber compound, especially in the types of filler used, finish of mould surfaces, etc., and a great deal will also depend on the properties of the lacquer used. It must be strongly adherent and flexible enough not to crack where the bung is deformed or abraded.

Solutions in plastic containers showed the lowest counts (manufacturers C, F and G). Two manufacturers used heat-sealed polythene bottles and one, PVC bags with rubber closures. None of the foregoing, of course, necessarily shows that rubber-closed glass containers cannot be made with low counts, only that the products in plastic containers are, on the whole, more free from contamination than those in glass. There is an overlap between counts for the worst plastic containers and the best glass bottles.

Particle size distribution

Most of the samples examined give a reasonable approximation to a straight line when log count is plotted against log size. Very low counts, which are almost certainly too high, deviate from this. A few results are shown in Fig. 3.

Conclusions

It is clear that there is a wide range in the degree of particulate contamination of solutions at present on sale. Samples from each manufacturer show a considerable spread, but there are significant differences between the means for various manufacturers.

A particle size distribution made with a resistance-type counter appears to be the most convenient and accurate means of quantitative analysis of particulate matter. The choice of limits for an acceptable solution must be an arbitrary one, and will need to be adjusted as manufacturing practice improves and medical evidence on the harmful effects accumulates. At present, it is suggested that a solution is acceptable if the counts per ml are less than 1000 particles at 2·0 μ, 250 particles at 3·5 μ, 100 particles at 5 μ and 25 particles at 10 μ.

A 5-fold reduction in these limits may be a reasonable ultimate objective. Improvements in the technique for examination of solutions are needed to cover two deficiencies—

(*a*) low counts are not accurate, and some modification of the sampling and counting system will be necessary to increase accuracy. This will become more important as the solution quality improves;

(*b*) to count particles in dextrose solutions and other non-electrolytes it will be necessary to add an electrolyte in such a way that no uncontrolled contamination is introduced. Techniques for doing this are now being studied.

Further investigation is needed to provide supplementary tests for specific particle types, *e.g.*, fibres, but the quantitative assessment of over-all contamination is an essential first step to better control of solution quality.

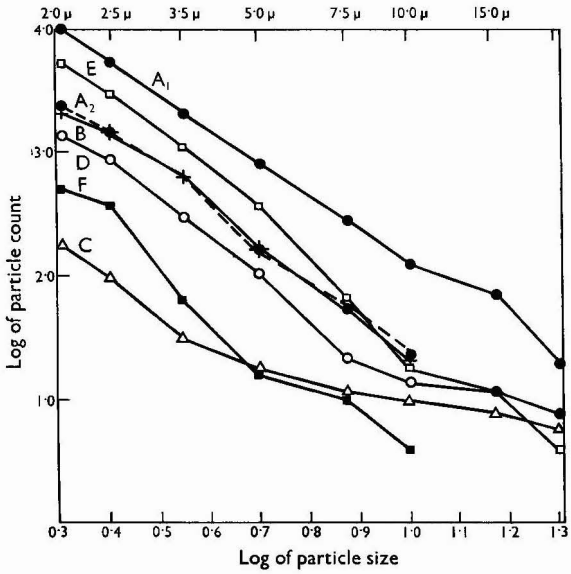

Fig. 3. Particle-size distribution for samples of: curve A_1, 0·9 per cent. sodium chloride solution; curve A_2, potassium-free maintance fluid; curve B, 4·3 per cent. dextrose in 0·18 per cent. sodium chloride solution; curve C, Darrow's solution; curve D, tissue repair solution, No. 1; curve E, 5·0 per cent. dextrose in 0·9 per cent. sodium chloride solution; curve F, 0·9 per cent. sodium chloride solution

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SHORT PAPERS

The Amperometric Titration of Submillinormal Concentrations of Iodine with Mercury(I) Perchlorate

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Iodine can be determined by mercury(I) titration to a visual or a potentiometric end-point.[1] The present work concerns the amperometric mercury(I) titration of submillinormal concentrations of iodine by methods similar to those used for iron(III)[2] and copper(II).[3]

Experimental

Reagents—

Use analytical-grade reagents and distilled or de-mineralised water throughout.

Mercury(I) perchlorate, 0·01 to 0·02 N in N perchloric acid—Prepare as described by Berka *et al.*,[1] but suitably dilute with N perchloric acid. Store over metallic mercury. Standardise by titration of iodine as indicated below.

Potassium iodate, 0·01000 N (= 0·001667 M).

Sulphuric acid, approximately 0·1 N.

Potassium iodide, approximately N in boiled-out water.

Apparatus—

Use conventional apparatus for amperometric titration at a rotating platinum electrode.[3] Since titrations are carried out at zero potential with respect to the saturated calomel electrode,

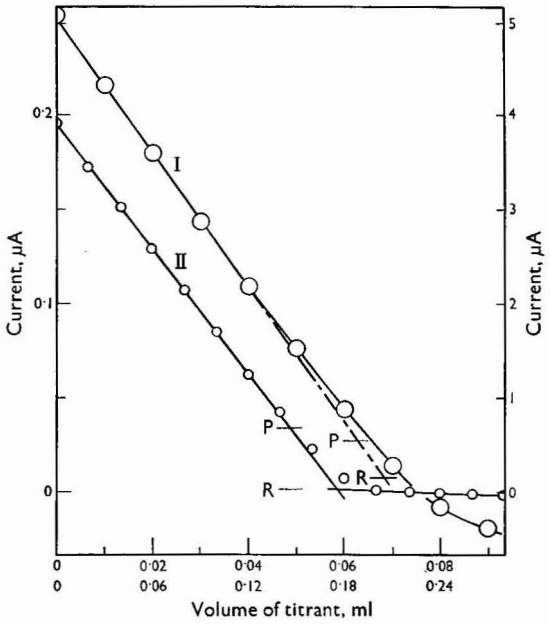

Fig. 1. Pre-addition titration of iodine in sulphuric acid and 0·02N potassium iodide. P and R are the pre-addition and residual current lines, respectively. Iodine, sulphuric acid and approximate titrant normalities are, respectively: curve I, $2·6 \times 10^{-6}$, 0·1, and 0·002 (left ordinate and upper abscissa); curve II, 5×10^{-5}, 5·0, and 0·02 (right ordinate and lower abscissa)

a polarising device is not needed. At the end of each day clean the electrode by the method described previously.[3] At the beginning of each day, pre-condition the platinum electrode by running a preliminary titration of iodine until the titrant is present in slight excess.

PROCEDURE—

(*A*) Place 50 ml of 0·1 N sulphuric acid in the titration cell. Insert the platinum electrode and salt bridge and de-oxygenate with a stream of nitrogen. Add 1 ml of N potassium iodide, stop the gas stream and inject sufficient 0·01 N potassium iodate to produce iodine, equal to about 20 per cent. of that contained in the sample. After 1 minute, note the current reading, P, then at once inject the sample solution. Read the current after a further minute, then titrate with approximately 0·02 N mercury(I) perchlorate until the current has fallen to, or near, zero. Allow an interval of 1 minute between a titrant addition and the reading of the current. Find the end-point graphically as the intersection of the linear portion of the titration curve and the line: current = P.

(*B*) Proceed as in (*A*) up to the stopping of the gas stream. Note the residual current, R, then at once inject the sample solution and titrate as in (*A*). Find the end-point graphically by producing the linear portion of the titration curve to cut the line: current = R.

RESULTS AND DISCUSSION

In all experiments, iodine was produced by the addition of a controlled excess of potassium iodide and an aliquot of standard potassium iodate solution to 50 ml of an acid medium through which nitrogen had been bubbled for 25 to 35 minutes. The results given in Table I are expressed as the apparent normality of the titrant, and were obtained at room temperature in the range 21° to 24° C. End-points were located by procedures (*A*) and (*B*) and, when possible, by a conventional method involving the extrapolation of the arms of an L-shaped titration curve.[4] Currents in the post-equivalence region were anodic and tended to increase with time.[2,3] All observations in this region were therefore completed within a total time of about 4 minutes. Not recommended because of this current instability, the L-curve method failed completely when the iodine concentration was less than about 10^{-5} N (Fig. 1, curve I).

TABLE I

TITRATION OF IODINE IN ACIDIFIED 0·02 N POTASSIUM IODIDE WITH APPROXIMATELY 0·02 N MERCURY(I) PERCHLORATE

Iodine* concentration, μ N	Acid medium	Apparent mercury(I) millinormality		
		Procedure *A*	L-curve	Procedure *B*
50	0·3 N potassium thiocyanate - 0·02 N perchloric acid	20·8	20·3	20·5
50	0·3 N potassium thiocyanate - 0·02 N perchloric acid†	20·0	19·5	19·9
50	0·02 N perchloric acid†	19·9	19·2	19·6
50	0·1 N perchloric acid	21·0	20·4	20·8
50	0·1 N sulphuric acid	21·0‡	21·0‡	21·1‡
50	0·5 N sulphuric acid	20·8	20·9	21·0
50	1·0 N sulphuric acid	20·5	20·4	20·4
50	3·0 N sulphuric acid	19·8	19·7	19·7
50	4·0 N sulphuric acid	18·9	19·1	19·1
50	5·0 N sulphuric acid	18·2, 17·2, 16·0	17·9, 17·3, 15·5	18·0, 17·4, 15·6
26	0·1 N sulphuric acid	20·3	20·6	20·8
8	0·1 N sulphuric acid	20·8	failed	21·9
2·6	0·1 N sulphuric acid	2·10§	failed	2·20§
0·8	0·1 N sulphuric acid	2·05§	failed	2·03§

* Introduced as potassium iodate.
† 0·1 N potassium iodide.
‡ Means of 7 runs; standard deviations ±0·27, ±0·42 and ±0·36 for procedure (*A*), L-curve, and procedure (*B*), respectively.
§ Titrant diluted 10-fold.

The 0·3 N potassium thiocyanate - 0·02 N perchloric acid - 0·02 N potassium iodide medium used previously[3] was satisfactory, and the omission of potassium thiocyanate had no significant effect. The concentration of potassium iodide is not critical, but should be kept quite low to avoid positive errors in the determination of iodine. These errors may be due to slight oxidation of iodide by residual traces of dissolved oxygen.

Titration is also possible in a sulphuric acid - potassium iodide medium. The acidity is not critical, and the residual current at a properly cleaned and pre-conditioned platinum electrode is small (typically less than 0·05 μA at an electrode of sensitivity 146 μA per millimole of silver(I) per litre).[3] In 0·1 N sulphuric acid - 0·02 N potassium iodide, titrations of 5×10^{-5} N iodine by procedures (*A*) and (*B*) are precise to within 1·5 per cent. and 2 per cent., respectively.

Normal titration curves of 5×10^{-5} N iodine were obtained at an acidity as great as 5 N (Fig. 1, curve II), but the results differed by nearly 20 per cent. from those obtained in 0·1 N acid. These results in 5 N sulphuric acid were reproducible to within about 5 per cent., so that this medium is fairly satisfactory if it is also used in the standardisation of the titrant.

This work was carried out with the partial support of the United States Atomic Energy Commission (Contract AT(30-1)-1977), and was completed at the Imperial College of Science and Technology, London. The facilities afforded by the College authorities, in particular by Professors R. M. Barrer and T. S. West, are gratefully acknowledged.

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Replacement of Benzidine by Copper Ethylacetoacetate and Tetra Base as Spot-test Reagent for Hydrogen Cyanide and Cyanogen

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RECENTLY the Society for Analytical Chemistry issued a warning[1] against the use of benzidine, which has been recognised as a carcinogenic compound. The Ministry of Labour is therefore proposing a general prohibition of its manufacture, handling, storage and use. As the authorities in other countries are expected to follow suit, chemical analysis and especially spot-test analysis will be greatly affected. About sixty spot-test procedures are known in which benzidine is used as a reagent. The appeal made by the Society for Analytical Chemistry to replace benzidine by alternative non-toxic reagents is therefore of the utmost importance.

The use of benzidine as a reagent in spot-test analysis depends almost entirely on its ability to form a blue quinoidic oxidation product from the base,[2] as a result of direct or induced redox reactions mainly carried out in an acetic acid medium. The following applications come into this category—

INORGANIC SPOT-TEST ANALYSIS[3]—

Detection of water-soluble oxidants.
Tests for metal ions that give water-insoluble higher oxides.
Tests for acids and salts that reduce manganese dioxide.
Tests for the metallic and non-metallic elements that form heteropolyacids with molybdenum trioxide.
Tests for normal and complex cyanides by the generation of volatile hydrogen cyanide.
Tests for mercuric cyanide through the pyrolytic release of cyanogen.
Tests for elemental sulphur, selenium and tellurium by dry heating with mercuric cyanide.[4]

ORGANIC SPOT-TEST ANALYSIS[5]—

Preliminary tests of ignition residues containing manganese, lead, phosphorus pentoxide and arsenic pentoxide.
Detection of pyridine and its derivatives via glutaconicaldehyde, and of the aldehydes that form coloured Schiff bases with benzidine.
Tests for compounds that reduce manganese dioxide, *e.g.*, ascorbic acid.
Detection of the pyrolytic release of hydrogen cyanide and/or cyanogen from certain nitrogen-containing compounds.
Detection of the functional groups that release hydrogen cyanide by the action of nascent hydrogen.
Detection of the functional groups that react pyrolytically with mercuric cyanide to produce volatile hydrogen cyanide or sulphur dicyanide.

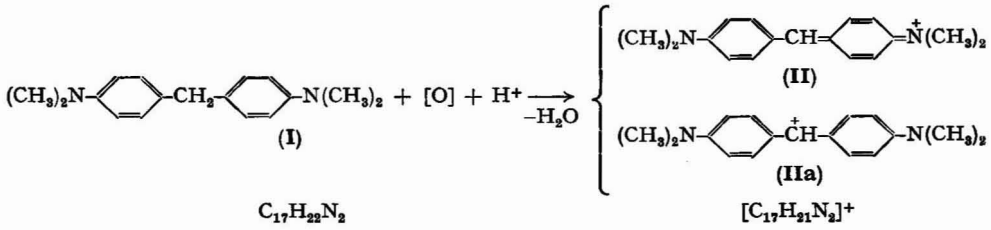
In all of these tests benzidine can be replaced by *o*-tolidine, with the exception of those for hydrogen cyanide, cyanogen and the dicyanides of sulphur, selenium and tellurium. Tetra base (4,4′-tetramethyldiaminodiphenylmethane), already recommended by Trillat[6] for detecting traces of lead dioxide, has also been shown to be a suitable reagent for detecting the presence of higher metal oxides as well as the formation of phosphomolybdates.

There are certain difficulties to be overcome in replacing benzidine in the well known test for hydrogen cyanide gas described by Sieverts and Hermsdorf.[7] This test is based on the appearance of a blue colour ("benzidine blue") when hydrogen cyanide or cyanogen gas comes into contact with filter-paper moistened with an aqueous solution of copper acetate and benzidine acetate. The replacement of benzidine by *o*-tolidine, dianisidine, diphenylbenzidine or by tetra base does not produce a satisfactory result.

We have examined the behaviour of mixtures of copper salts with free tetra base in non-aqueous solutions, and found that mixtures of tetra base with copper oleate or copper naphthenate, show a distinct blue colour when exposed to hydrogen cyanide or cyanogen gas. The deep colour of these copper salts is, however, a disadvantage in detecting the blue colour produced in the cyanide test.

The behaviour of mixtures of tetra base and inner-complex copper salts, dissolved in chloroform, was surprising. It has been stated that copper hydroxyquinolinate, copper salicyldoxime, copper cupferron, copper neocupferron, copper picolinate and copper acetylacetone do not give this reaction with tetra base. In contrast to these salts, however, the inner-complex copper ethylacetoacetate was found to give perfect results. Obviously the chelation of copper in this salt is not as stable as in the other inner-complex salts mentioned. This non-toxic reagent can therefore be recommended for use in the detection of hydrogen cyanide and cyanogen.

The colour-test described below is based on the formation of an oxidation product of tetra base (**I**); it contains the quinodic or carbonium cation[8] (**II**) or (**IIa**).



This redox reaction occurs in the presence of higher metal oxides and also with chlorine, bromine or iodine vapours. As a contrast, the weak oxidants copper ethylacetoacetate ($CuAc_2$) and cyanogen have no effect. Obviously the equilibria of the redox reactions

$$2CuAc_2 + C_{17}H_{22}N_2 \rightleftharpoons 2CuAc + [C_{17}H_{21}N_2]Ac + HAc \quad .. \quad .. \quad (1)$$

$$(CN)_2 + C_{17}H_{22}N_2 \rightleftharpoons [C_{17}H_{21}N_2]CN + HCN \quad .. \quad .. \quad .. \quad (2)$$

lies so far to the left that no colour appears. However, when the condition of (1) or (2) is realised, in the presence of copper ions or cyanide ions, the formation of the coloured oxidation product is suitable for detecting hydrogen cyanide and cyanogen. The net reactions are—

$$2CuAc_2 + C_{17}H_{22}N_2 + 2HCN \rightarrow 2CuCN + [C_{17}H_{21}N_2]Ac + 3HAc \quad .. \quad (3)$$

$$2CuAc_2 + 2C_{17}H_{22}N_2 + (CN)_2 \rightarrow 2CuCN + 2[C_{17}H_{21}N_2]Ac + 2HAc \quad .. \quad (4)$$

It must be emphasised that only salts with the cations (**II**) and (**IIa**) are blue. The necessary acid is formed as in equations (3) and (4). When exposed to ammonia vapours the colourless carbinol base is formed. With the addition of acetic acid the blue colour reappears.

Method

Reagent—

Dissolve about 5 mg each of copper ethylacetoacetate* and tetra base in 1 to 2 ml of chloroform Although this slightly green solution is stable for some days when stored in closed bottles, it is recommended that a freshly prepared solution should be used.

* The pure salt is manufactured by, and available from, Lobachemie, Vienna IXX, Austria.

PROCEDURE—

Liberate the hydrogen cyanide or cyanogen, by wet destruction or pyrolysis, in a micro test-tube covered with filter-paper moistened with a drop of reagent. Previous evaporation of the solvent is not necessary. The appearance of a blue circular fleck on the almost colourless reagent paper indicates a positive result. The blue colour disappears when exposed to ammonia vapour.

The limit of identification is 1 microgram of hydrogen cyanide.

DISCUSSION

The advantages of this test are that the reagent, whose components are stable, is easily prepared and that the blue fleck formed on the reagent paper is stable for some days. This is not so when the aqueous, unstable, benzidine-containing reagent of Sieverts and Hermsdorf is used; the latter forms black copper sulphide in the presence of the hydrogen sulphide which is evolved simultaneously with the hydrogen cyanide. In contrast to this, the new reagent is effective even in the presence of some hydrogen sulphide with the hydrogen cyanide.

The colour test is characteristic for volatile hydrogen cyanide and cyanogen and also sulphur, selenium and tellurium dicyanides. Cyanogen can be identified in the presence of hydrogen cyanide by a colour test described elsewhere[9] that does not involve the use of benzidine.

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A Projection Method for Inspection of Ampoules

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A MODIFIED 35-mm slide projector has been used to project an enlarged image of the contents of ampoules, vials, etc., for detection of particulate matter. The key point is that the ampoule is immersed in a rectangular glass tank (about 2 inches from front to back) filled with a liquid having a similar refractive index to the contents; water is suitable for most inspections.

An ampoule in air gives a very poor image because of reflections that result in most of the image being dark; poor results are also obtained if the immersion liquid matches the refractive index of the glass. The use of water as immersion fluid, however, gives a clear image with the glass walls apparently seen in section, and any particles of adequate size can be detected even if not sharply in focus. A magnification of about 25 is recommended, enabling particles over about 40 microns to be detected and sizes to be estimated readily; at this magnification, the whole contents of ampoules up to about 10 ml can be seen at once.

There are so many types of projector that it is difficult to give much detail on the modifications needed. A projector of fairly open design is easier to adapt; some have a fair amount of open space between the slide carrier and projection lens, so removal of the slide carrier would provide enough space for a suitable tank. With other designs part of the casing can be cut away, or the lens mounting can be fitted to a bracket to bring it 1 to 2 inches farther away from the condenser to allow space for the tank. A projector with a 100-mm lens gives the required magnification at a distance of about 6 feet; a 150 to 200-watt lamp in a projector with an f/3·5-lens gives enough illumination for use in a moderately darkened room, but a lamp of higher wattage allows the lens to be stopped down to about f/5·6, or less, and this increases the depth of focus so that all particles are in reasonably sharp focus.

In use, the ampoule is shaken, preferably with a swirling motion so that the particles move in a spiral, and quickly placed in a position in the tank that brings the side walls sharply into focus. Air bubbles quickly clear, apparently falling because the image is inverted; they can readily be distinguished from large and heavy particles, while smaller particles usually remain suspended for an appreciable time. The swirling motion of the contents brings all particles into focus twice in each rotation, even if the depth of focus is not very great.

Particles present in the tank would appear at first to be a nuisance, but they are normally well out of focus and can be readily detected by their movement relative to the ampoule. Passing a stream of water, filtered if necessary, through the tank keeps the number of these particles to a negligible level. Continuous replacement is necessary because fresh particles are introduced with each ampoule.

The projection method places the examination on a reasonably quantitative basis and makes it possible to propose a standard for acceptance of injectable solutions. It is suggested that a batch of ampoules should be considered satisfactory if, in any 10 ampoules taken at random, (*a*) total counts do not exceed 10 particles over 50 microns and (*b*) there are no particles over 1 mm diameter. There is much less operator fatigue than in visual inspection, so the method should be of value for inspection on a production line, as well as for examination of check samples.

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The Determination of Water in Beryllium Oxide

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The water content of various beryllium oxide powders is of interest for correlation with sintering studies. By thermal analysis and infrared studies,[1] it has been shown that water chemisorbed on beryllium oxide is firmly held, and is removed by heating in vacuum only at 800° C. Therefore methods dependent upon the complete removal of all chemisorbed water (*e.g.*, weight loss on heating to 800° C, or heating to 800° C in a stream of dry gas and determination of water by absorption) although possible, are complicated by factors such as the simultaneous loss of sulphate and surface carbonate present. The Karl Fischer method[2] was found to be unsatisfactory because it is impossible to obtain a reproducible end-point for titration, even by using a null-point conductivity technique with the oxide stirred in methanol. This is due either to a slow reaction between the Karl Fischer reagent and the chemisorbed hydroxyl groups, or to the reaction of the reagent with beryllium oxide, similar to that which occurs with calcium oxide, magnesium oxide, zinc oxide, aluminium oxide, mercuric oxide and cuprous oxide.[3]

We have investigated three methods for determining the water content of beryllium oxide: (*i*) infrared spectrophotometry of the oxide as a mull; (*ii*) exchange with deuterium oxide; (*iii*) reaction with 2,2-dimethoxypropane. All these methods have an infrared finish. The recommended procedures are given here, together with essential details of the experimental work.

Experimental

The infrared spectrophotometry was carried out with a model 21, Perkin Elmer, infrared spectrophotometer. Conventional equipment was used for the sample-mulling techniques which were carried out in a glove-box flushed with dry nitrogen. The method was established by examining mulls prepared under different conditions and containing various proportions of beryllium oxide (from different sources), mixed with potassium cyanide and hexachlorobutadiene. Standard samples for calibration were prepared from mixtures containing known weights of beryllium hydroxide.

In the experiments based on exchange, precautions were taken to "condition" all the glassware that came into contact with the deuterium oxide. All operations involving the contact of deuterium oxide with the atmosphere were performed in a glove-box flushed with dry nitrogen.

Methods

Infrared spectrophotometry of the oxide as a mull—

A mixture of approximately equal weights of sample and potassium cyanide as an internal standard (dried in vacuum at 80° C for several hours) is accurately weighed out. This is thoroughly

mixed by grinding in a large agate mortar; a sample of this mixture is then placed in a small agate mortar and ground vigorously for 2 minutes. (This and the previous operation are carried out in a glove-box flushed with dry nitrogen.) Two or three drops of hexachlorobutadiene are added and the grinding continued for a further 2 minutes. It may be necessary to add a further drop or two of hexachlorobutadiene during this period to obtain a satisfactory mull. Bradley and Potts[4] indicate that care is necessary in the preparation of mulls for quantitative work and we confirm this. The mull is examined between rock salt plates with a Perkin Elmer, model 21 spectrophotometer under the following conditions: resolution 980; gain 4·5; speed 0·5 μ minute^{-1}; response 1; suppression 0. The 5000 cm^{-1} to 1600 cm^{-1} region is scanned twice in order to reduce errors in absorbance measurements. A 5-fold ordinate scale expansion is used if necessary. The absorbance at 3300 cm^{-1} and 2050 cm^{-1} is measured by the base-line density method.[5] It was found impossible, with the above procedure, to obtain mulls of potassium cyanide alone that did not show a weak absorption band at 3300 cm^{-1}. To correct for this, a curve is drawn up in which absorbance at 2050 cm^{-1} (A_{2050}) is plotted against the absorbance at 3300 cm^{-1} (A_{3300}) for a series of potassium cyanide mulls prepared by using the above procedure. The correction required to A_{3300} corresponding to a measured A_{2050} in a beryllium oxide - potassium cyanide mixture is determined from the curve and subtracted from the measurement to give A'_{3300}. The ratio A'_{3300}/A_{2050} is corrected for the amount of sample and internal standard in the mixture to give the ratio Z—

$$Z = \frac{A'_{3300}}{A_{2050}} \times \frac{\text{Weight of internal standard}}{\text{Weight of sample}}$$

From the ratio Z, the hydroxyl content of the sample is determined from the standard calibration curve, constructed by preparing mixtures of one given sample of beryllium oxide with beryllium hydroxide (hydrated); the hydroxyl content being determined by beryllium assay. Within the range 0·5 to 5 per cent. of hydroxyl content, Lambert - Beer's law is observed.

Exchange with deuterium oxide—

A known weight of the oxide (about 1 g) is added to a known weight of deuterium oxide (about 10 g, 99·781 per cent. w/w) in a glass-stoppered centrifuge tube. All apparatus coming into contact with deuterium oxide is first washed with deuterium oxide, baked at 120° C for several hours and cooled in a desiccator before use. All operations involving contact of deuterium oxide with the atmosphere are performed in a glove-box flushed with dry nitrogen. The tube is shaken for 5 minutes, spun in a centrifuge, and a sample of the liquid phase is analysed for deuterium oxide by Gaunt's method.[6] Hence the weight of water originally present in the sample is readily calculated.

Reaction with 2,2-dimethoxypropane—

The procedure used is similar to that given by Bishop and Critchfield.[7] A 5-ml portion of 0·1 N methanesulphonic acid, 2 ml of 2,2-dimethoxypropane and 20 ml of carbon tetrachloride are transferred by pipette into a dried stoppered conical flask and a known weight of oxide (about 1 g) is added. The flask is shaken periodically for 8 hours and allowed to stand for a further 16 hours. With beryllium oxide the supernatant liquor is quite clear at this stage, and a sample is withdrawn and its infrared spectrum recorded in the region 1600 to 1900 cm^{-1} by the use of 0·1-mm calcium fluoride cells and standard instrumental conditions. A blank determination is performed simultaneously. The water in the sample is determined from the acetone absorbance at 1710 cm^{-1} from a calibration curve.

Results and discussion

Some results are shown in Table I.

Table I

Percentage of water in three beryllium oxide samples by three methods

Sample number	Method (*i*), infrared spectrophotometry	Method (*ii*), deuterium oxide exchange	Method (*iii*), reaction with 2,2-dimethoxypropane
1	0·9	1·02	1·1
2	1·2	1·13	1·1
3	0·3	0·36	0·3

Three different samples of beryllium oxide of commercial origin were examined. All three methods gave satisfactory results. The time taken for a determination by means of method (*i*) is approximately 30 minutes. An advantage of this method is that a surface reaction or exchange is not involved.

Method (*ii*) is the most accurate of the three methods, and also the most sensitive to small amounts of water.

Method (*iii*) is the most time-consuming, but it is possible to process a number of samples without a great deal of attention. The slow reaction of 2,2-dimethoxypropane is no doubt due to steric hindrance involved in reaction with chemisorbed hydroxyl groups; an initial rapid reaction being due to reaction with the physically absorbed water.

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The Determination of Quinizarin in Hydrocarbon Oil

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The analytical method used for the detection, determination and identification of one of the prescribed markers, quinizarin (1,4-dihydroxyanthraquinone), in "marked" gas oils and marker concentrates has been described by Harrison and Heaysman.[1] The references in this paper to the use of recrystallised quinizarin as standard and, later, to the use of commercial-quality quinizarin for marking gas oils, with typical average optical densities of about 0·243 for 1 cm of 0·001 per cent. w/v solutions of such quinizarin in gas oil, have now been shown to be misleading, in that analysts have been basing calculations on the use of commercial quinizarin as standard. As the marking regulations refer to a concentration of 1,4-dihydroxyanthraquinone, they can be interpreted as referring only to pure quinizarin. Consequently, all results should be based on the use of pure recrystallised quinizarin as standard.

While investigating the effect of using quinizarin samples of different degrees of purity, we have shown that the repeatability of the method is influenced by lighting conditions, particularly when the alkali is present, any illumination leading to a fading in colour of the blue-violet quinizarin - sodium hydroxide complex with a reduced quinizarin recovery. The stated repeatability of 1 per cent. claimed by Harrison and Heaysman[1] cannot therefore be achieved unless the lighting conditions are controlled.

Consequently the analytical method has been re-examined and a stricter procedure, described later, has been developed. By using this new procedure and introducing varying times for the sodium hydroxide extraction, the effects of extraction time and of light conditions on the analysis of mixtures of 10 ml of cyclohexane (containing 10 p.p.m. of quinizarin) and 40 ml of gas oil have been examined. Table I gives typical recovery values under three general types of lighting conditions, *viz.*, total darkness, ordinary laboratory conditions (fluorescent strip-lighting), which varied slightly according to the scattered daylight prevailing, and direct sunlight, varying with haze and occasional cloud interruption.

Results

Table I

Recovery of quinizarin from DERV

Light conditions	Percentage of recovery after extraction times of—					
	10 minutes	20 minutes	25 minutes	30 minutes	40 minutes	60 minutes
Total darkness	96·4	96·6	—	93·3	88·0	63·6
	96·8	96·6	—	91·9	84·7	82·6
Ordinary conditions* ..	95·8	93·8	—	81·5	—	—
	95·1	95·2	—	84·0	—	—
Direct sunlight	85·8	88·6	74·8	66·9	62·6	38·0
	93·1	87·6	75·1	69·4	59·9	42·6

* The levels in illumination in ordinary conditions and in direct sunlight may vary by 1 to 100.

As indicated by these results, any exposure to direct sunlight is undesirable, and even in total darkness losses result if contact with the the alkali is unduly prolonged. Under ordinary daylight conditions, with 10 minutes in the alkaline stage of each extraction, the average recovery of 95·5 per cent. compares fairly well with 96·6 per cent. under the ideal conditions of total darkness, and 10 minutes should suffice for adequate layer-separation.

We therefore recommend that the following precautions should be observed—

The duration of the extractions with sodium hydroxide should be minimised. (10 minutes should suffice to ensure an adequate separation of the oil and alkaline layers.)

Bright illumination conditions, especially direct sunlight, should be avoided.

The alkaline extract should be transferred to a second separating funnel containing hydrochloric acid. (6 ml of 1 to 1 acid should suffice for three alkali extractions.)

Adequate time should be allowed for the separation of the final cyclohexane extract from the aqueous parent layer. Short separation periods sometimes result in the presence of a spray of fine aqueous droplets in the cyclohexane extract, giving rise to optical-density readings which are up to 25 per cent. too high.

It is essential that the peak maximum be located for each sample of quinizarin examined. It has been noted that the indicated wavelength of the maximum of the absorption peak at 520 mμ can vary from 519 to 521 mμ.

Procedure

Shake 50 ml of the gas oil, suspected to contain quinizarin, with 5 ml each of sodium hydroxide solution (5 per cent. w/v) and butanol in a 100-ml separating funnel for 45 seconds. When the two layers have separated (this may take up to 10 minutes) run the aqueous phase into a second separating funnel containing 6 ml of hydrochloric acid (1 to 1), and wash it through with about 2 ml of water. Extract the oil with a further 5 ml of sodium hydroxide solution by shaking the funnel for 45 seconds. Allow the layers to separate and run the aqueous layer into the second separating funnel. Wash the extract through with water as before. Repeat this operation with a further 5 ml of sodium hydroxide solution. If the third extract is not colourless, extract with further 5-ml portions of sodium hydroxide, if necessary introducing some hydrochloric acid into the second separating funnel. Add 10 ml of the spectroscopically pure cyclohexane to the acidified extract and shake the funnel for 30 seconds. After separation of the two layers, run the aqueous layer to waste. Use a dry pipette to transfer the amber liquid to a 1-cm cell of a suitable spectrophotometer. With pure cyclohexane as blank, determine the position of the absorption peak around 519 to 521 mμ and read the optical density. This is the most pronounced peak and it is also the farthest from any possible interference from components of the gas oil.

A standard for comparison purposes can be prepared as follows—10 ml of a standard quinizarin solution in cyclohexane (10 p.p.m.) are added to 40 ml of quinizarin-free gas-oil. The revised procedure, previously described, is then applied to this mixture and the optical density at the absorption maximum is determined and compared with the optical density of the original standard solution in cyclohexane. Recovery, R, should be at least 96 per cent. The results of the quinizarin analyses of a batch of samples may then be adjusted by a factor of $100/R$.

The repeatability of the revised method is found to be less than 1 per cent. on solutions of quinizarin in unmarked gas oil. However, recent experience in analyses of marked gas oils has provided a mean difference between 13 pairs of duplicate samples of 2·5 per cent. and a maximum difference of 5·8 per cent.

Where identification of quinizarin is required, the absorption spectrum from 420 to 540 mμ is plotted with a suitable automatic-recording spectrophotometer.

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The Effect of Particle Size on Back-scattered X-ray Correction Methods in On-stream X-ray Fluorescence Analysis

BY K. G. CARR-BRION

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THE measurement of back-scattered fluorescent X-rays from a block containing a suitable element, placed on the side of the slurry flow remote from the X-ray source has been described.[1] It was used as a means of correcting for variations in the fluorescent X-ray intensity from the slurry, caused by changes in the composition and content of the solid component of the slurry. If this method is used for on-stream analysis without considering the effect of changes in particle size, further errors could occur. It can be predicted quite easily from basic X-ray absorption theory[2] that this back-scattered fluorescent X-ray intensity should increase as the particle size in a slurry of constant composition increases. However, it has also been shown[3] that the fluorescent X-ray intensity from particles in an aqueous slurry will decrease as the particle size in a slurry of constant composition increases.

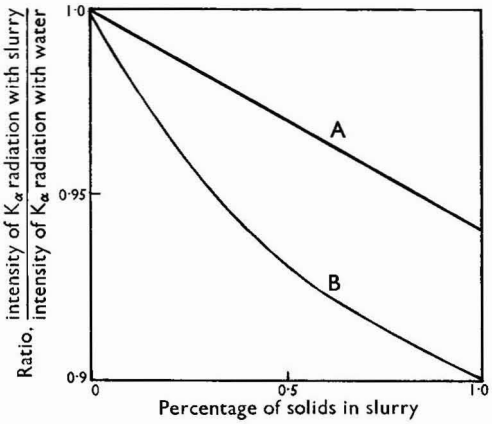

Fig. 1. Effect of change in particle size on back-scattered cadmium K_α intensity: curve A, particles from 100 to 150 mesh; curve B, particles less than 300 mesh

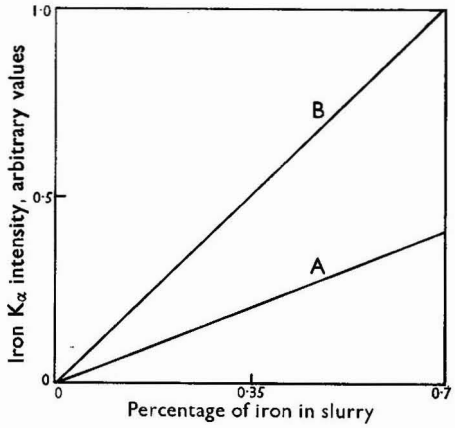

Fig. 2. Effect of change in particle size on iron K_α intensity from the slurry: curve A, particles from 100 to 150 mesh; curve B, particles less than 300 mesh

It was decided to test these predictions experimentally, with a Phillips PW 1540 X-ray spectrograph adapted to take an inverted slurry presenter. It was found that the fluorescent intensity from the reference block—in this case made of cadmium—increased with increasing particle size in the haematite - water slurry (see Fig. 1), while the fluorescent iron K_α intensity from the particles in the slurry decreased (see Fig. 2). Similar effects would be expected with all elements capable of being determined on-stream by X-ray fluorescence analysis.

A decrease in the size of the X-ray absorbing particles will therefore cause a marked increase in the apparent solid content (see Fig. 1). This solid content is used, either directly or indirectly, to obtain the concentration of the element being determined in the solid component, since the fluorescent X-ray intensity is a function of the concentration of the element in the slurry as a whole. Even if separate means of correcting for real changes in solid content are incorporated, the use of back-scattered radiation will in no way compensate for particle-size effects. The measured value of the X-ray mass-absorption coefficient will accentuate these effects to a degree dependent on the system being examined. Thus if the method is used to correct for variations in the composition and content of the solid component of the slurry, a correspondingly greater control of particle-size variations is necessary. It should be mentioned that this effect has also been observed in heterogeneous solid samples when back-scattered or transmitted methods of correcting for matrix effects are used,[4] but that under these circumstances, sufficiently close control of particle size makes it possible to obtain accurate quantitative results.

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Interference from Silica in Phosphate Analysis

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In a recent publication,[1] automatic methods for determining orthophosphates and acid-hydrolysable phosphates on the AutoAnalyzer were described. These automatic methods were based on generally accepted methods[2] with some slight modifications. It was shown, however, that when samples were analysed by both the manual and the automatic methods, statistically identical results were obtained.

By using these methods in surveys of polluted and unpolluted lakes, fjords and rivers in Norway, it was found that unexpectedly high values of acid-hydrolysable phosphates were observed, particularly in unpolluted waters. The method was therefore believed to be subjected to interference from a substances normally present in natural waters. Although the manual method is claimed to be free from interference,[2] it was thought that the high values were caused by the interference of silica; this element is determined by a basically identical procedure, except for the lower acidity required for its determination.

The main difference between the orthophosphate and acid-hydrolysable phosphate methods is that the sulphuric acid is added to the sample before the molybdate in the acid-hydrolysable phosphate method, while in the orthophosphate method a mixture of the two reagents is added to the sample, the final reagent concentrations being the same in both methods. When heating of the sample was omitted in the acid-hydrolysable phosphate method, the same degree of interference was obtained. This indicated that the interference could be due solely to the different ways of adding reagents in the two methods.

When the molybdate concentration of the reagent in the acid-hydrolysable phosphate method was reduced from 10 to 5 per cent., the interference from silica was only 3 μg of phosphate phosphorus per litre per mg of silicate per litre. With further reduction below 5 per cent. in molybdate concentration, the sensitivity of the method was considerably decreased. Thus, although the molybdate concentration could be reduced to 5 per cent. without any reduction in sensitivity occurring, there was still under these conditions a significant interference from silica.

The sulphuric acid solution used for hydrolysis was then made 7 N, and the molybdate solution was made 2 N with respect to sulphuric acid and 5 per cent. with respect to molybdate, the final concentration of sulphuric acid being as before. This modified procedure resulted in no interference from silicic acid up to 10 mg of silicate per litre.

Therefore, in order to eliminate the interference from silica in the method for determining acid-hydrolysable phosphates described previously,[1] the following modifications in the reagents described therein (page 31) should be made—

(*a*) substitute 7 N sulphuric acid for 9 N sulphuric acid used for hydrolysis;

(*b*) substitute a 5 per cent. ammonium molybdate in 2 N sulphuric acid solution for the solution of 10 per cent. ammonium molybdate.

The molybdate concentration could be reduced from 10 to 5 per cent. in the orthophosphate method without any reduction in sensitivity, so making this procedure a little cheaper.

The previous and the modified procedures were used to analyse 80 sea-water samples and 15 fresh-water samples for acid-hydrolysable phosphates. They were also analysed for silica. The difference in values obtained from the two phosphate methods should be due to the interference of silica in the original procedure. These values and the silica values have been subjected to correlation analysis. The correlation coefficients of the results (see Table I) for the interference values and the silica values are highly significant (P = less than 0·1 per cent.), thus indicating that silica is the interfering substance in the previous method.

TABLE I

CORRELATION BETWEEN INTERFERENCE VALUES AND SILICA VALUES IN THE ACID-HYDROLYSABLE PHOSPHATE METHOD

		Sea-water	Fresh water
Number of samples		80	15
		Mean values	
Previous method	μg of phosphorus per litre	50·95	55·0
Modified method	μg of phosphorus per litre	42·30	20·5
Difference	μg of phosphorus per litre	8·65	34·5
SiO_2	mg of silicate per litre	0·98	3·9
SiO_2 as P	μg of phosphorus per litre	8·82	35·1
Correlation coefficient		0·8993	0·8500

1 mg of silicate per litre = 9 μg of phosphorus per litre.

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The Collection of Fractions Separated by Gas-Liquid Chromatography

BY M. D. D. HOWLETT AND D. WELTI

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MANY methods have been reported for trapping gas - liquid chromatography fractions for further infrared spectroscopic examination. Some methods are too crude to trap efficiently fractions containing less than 1 mg of component, others are too specific in their applications. We would like to draw attention to a method of trapping that combines two techniques not generally used together.

The two techniques are (*a*), the trapping of the fraction in a tube containing normal gas - liquid chromatography column packing, and (*b*), Swoboda's total-trapping technique.

The use of a stationary phase on the support reduces the vapour pressure of the sample component more than Shearer's method[2] of using support material on its own. The sample components can also be stored more easily when the stationary phase is present.

The combination of these techniques is suitable for trapping the more volatile components, liquids, or components soluble in common infrared solvents. For less volatile compounds or solids,

and particularly those which are insoluble in the common infrared solvents, we used a third method which is a variation on Leggon's method[3] for trapping fractions in potassium bromide powder. This was suggested by the success of Philpotts and his colleagues[4] in handling 1 to 100 microgram samples from liquid - solid chromatographs.

Gas - liquid chromatography column packing, total-trapping technique

A 9-cm long, 3-mm i.d. tube packed with 7 per cent. Apiezon "L" on 60 to 80 mesh celite or firebrick, which has been previously stripped, is fitted on to the exit port of the detector by-pass system. Any sensitivity of the detector to the increase in back-pressure caused by fitting the tube is eliminated by building a suitable restriction into the by-pass. The tube is cooled with crushed solid carbon dioxide, and a calcium chloride drying-tube is fitted to the other end. After collecting the fraction, the tube is sealed with caps and stored at a low temperature until required. It is then inserted into a small copper tube, 7·5 cm long, 1 cm i.d., electrically heated to 220° C. One end of the collecting tube is connected to a miniature conventional cold-trap (Fig. 1), and the other end is connected to the argon supply via a rotameter and needle valve.

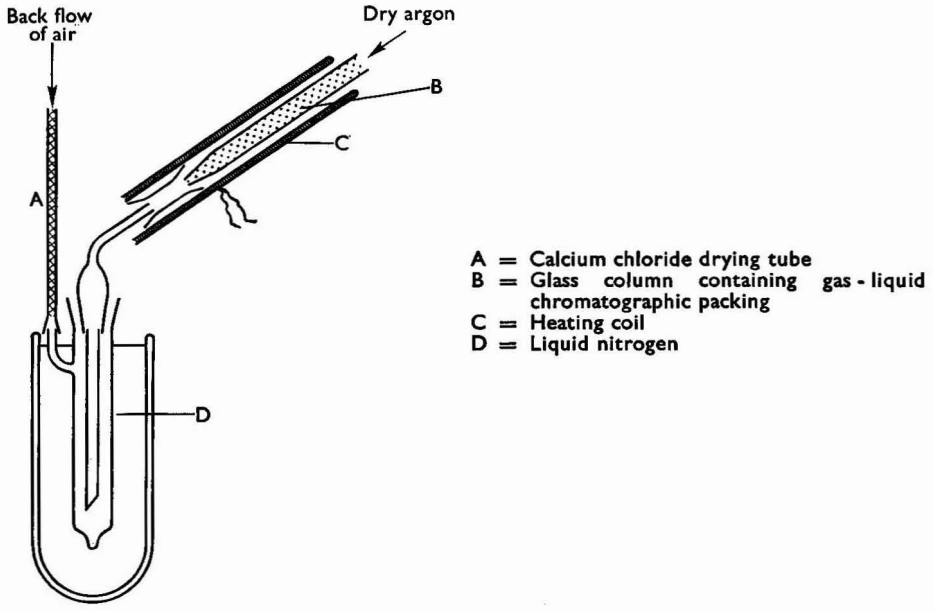

Fig. 1. Apparatus for the column packing, total-trapping technique

The miniature cold-trap consists of a test-tube, 10 cm long, 1·2 cm i.d., made from a standard Quickfit B10 socket with a tapered bottom, and fitted with a side arm placed 0·5 cm from the neck. The side arm is fitted with a B5 cone on which a drying tube is placed. A centre tube is prepared from a Quickfit MF 15/0 joint, 3·5 mm i.d. with a stem 6·5 cm long. The other end is fitted with a B5 cone that is attached to the short column via two glass sockets as shown in Fig. 1.

When the collecting tube has been heated for 60 seconds the trap is immersed in liquid nitrogen, and argon (60 ml per minute) is passed through the system for up to 5 minutes.

The condensing argon traps the sample completely,[1] and as the vapour pressure of liquid argon at −196° C is about 200 mm of mercury it causes a back-flow of 140 ml per minute of air, which is dried in a calcium chloride drying-tube fitted to the outlet of the trap. In 5 minutes, 0·9 ml of condensed gas is collected. No side effects with oxygen sensitive compounds have been observed. When any doubt has arisen, the air inlet has been placed directly above the liquid nitrogen.

The collecting tube is removed from the trap and a bubble flow-meter is attached in its place. The Dewar flask is slowly lowered down the trap so that the argon boils at a rate of less than 5 ml per minute. When all the argon has been removed, the trap is sealed at both ends with Quickfit caps. Liquids with a high vapour pressure are cold-distilled into the tip of the trap by immersing

the tip in liquid nitrogen.[5] Higher-boiling components are centrifuged into the tip without loss. The fractions can be examined as liquids or solutions by normal infrared methods with either microcells or a beam condenser.

The times for eluting various components from the collecting tubes, which were measured by attaching a flame-ionisation detector to the tube in place of the cold trap, were: ethyl acetate, 50 seconds; isobutyl acetate, 120 seconds; octanol, 150 seconds; eugenol, 300 seconds. High-boiling polar materials "tail" badly on elution from the collection tubes.

Examination of both the "total-trapping" step and the whole sequence of operations gave recoveries of greater than 90 per cent. for 2 mg of ethyl acetate (as determined by the absorbance of the infrared carbonyl stretching band measured in 50 μlitres of carbon tetrachloride). By inference, the efficiency of the collecting tube must be close to 100 per cent. Similarly, 0·1 mg of ethyl caproate gave recoveries of greater than 85 per cent. Spectra of 0·05-mg samples can be obtained that show all the dominant bands without beam condensation or scale expansion. Beam condensation tends to evaporate volatile samples, but scale expansion will increase the sensitivity to below 0·01 mg.

The storage efficiency of the collecting tubes was also determined by infrared methods. The percentage recoveries of ethyl acetate and isobutyl acetate were—

	Percentage recovery after—						
	0 hours at	5 hours at			24 hours at		
	17° C	−78° C	−10° C	17° C	−78°	−10° C	17° C
Ethyl acetate.. ..	100	100	75	52	82	48	40
Isobutyl acetate ..	100	100	95	98	102	98	91

No appreciable amounts of water were detected after 24 hours' storage.

A single component can be processed in 45 minutes, but six components can be processed in parallel and their spectra determined in less than 3 hours.

This method has been used successfully in practice for 18 months on complex mixtures.

THE TRAPPING OF LESS VOLATILE COMPOUNDS IN POTASSIUM BROMIDE POWDER—

The fractions are collected directly into a short tube, 10 cm long and 2·5 mm i.d., fitted with B5 cones at each end, and containing a plug (approximately 50 mg) of dry, powdered potassium bromide. A constriction in the tube wedges the powder close to the chromatograph outlet. The tube is cooled with solid carbon dioxide, and a drying tube is attached to the outlet. The tube can be capped at both ends and stored in a refrigerator.

The disc is prepared in a normal disc holder (23 mm in diameter, slot-size, 1 mm × 5 mm). The potassium bromide plug from the tube is placed on top of a small amount of dry powder at the bottom of the slot. The slot is "topped up" with more dry powder. The disc holder is placed in a die loaded with a pressure of 200 lb per sq. inch for 90 seconds. Where the components are sufficiently involatile the die is also evacuated. The disc can be examined spectroscopically with or without a beam condenser.

This method can be used routinely for samples sizes down to 0·05 to 0·1 mg. Good spectra were obtained even with 0·025 mg of the marginally volatile liquid 2,4-xylenol, using a beam condenser. With a good technique, sample sizes of less than 0·01 mg could be examined. The compressibility of liquids during pressing will reduce the efficiency of the process. For instance, 0·1 mg of 2,4-xylenol gave a recovery efficiency of 37 per cent.; 0·05 mg, 52 per cent.; but 0·025 mg, 96 per cent.

The trapping efficiency has been examined with a flame-ionisation detector attached to the outlet of the trap. No losses occur if the trap is cooled in solid carbon dioxide. At room temperature, 70 per cent. of 2,4-xylenol was lost before the peak was complete. Interference from water is negligible, provided that the potassium bromide powder is continually protected from water vapour.

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Received *April 7th*, 1965

Book Reviews

VITAMIN ASSAY; TESTED METHODS. By ROLF STROHECKER AND HEINZ M. HENNING. Translated by D. D. Libman. Weinheim: Verlag Chemie, GmbH. Pp. 360. 1965. Price DM 48; $12.00.

The German edition of this book was published in 1963 and was reviewed by me in *The Analyst*, 1964, **89**, 375. The translation has been carried out by D. D. Libman, and this has now made available, to a much wider reading sphere, an excellent compilation of methods of vitamin assay.

In the previous review, I mentioned the comprehensive nature of the work and its scope. For those interested in all phases of vitamin assay this is an ideal volume of reference, and I am quite sure that, with the appearance of this English edition, it will find its place in many laboratories in which this work is carried out.

For those dealing purely with chemical methods, there are plenty of methods to suit the taste: good choice is given of ultraviolet absorption, photometric, polarographic and chromatographic techniques, all of which are made available to the reader.

Those more interested in microbiological methods will also find this a useful reference book, and although the detail of the technique might appear to be a little on the scanty side it is well referenced, and the reader may readily fill in the gaps by going to the original work on the subject.

I can say no more in tribute to this volume except that since I reviewed it in 1964 it has found regular use and is probably the most widely used of all vitamin books in my laboratory.

R. F. MILTON

DOUBLE LAYER AND ELECTRODE KINETICS. By PAUL DELAHAY. Pp. xii + 321. New York, London and Sydney: Interscience Publishers, a division of John Wiley & Sons Inc. 1965. Price 110s.

An extended review and critical appraisal of this field has been needed for some time. None is better qualified than Professor Delahay to write on this topic, and he does so with his usual competence. This, of course, means that the result is strong meat. At times progression is leisurely and assimilation is effortless; at other times one is led into fascinating but concentrated detail so that connecting links get submerged and the over-all picture dislimns. A good deal of concentration is needed in certain places, but is well rewarded.

The book is divided into two almost equal parts. The first deals with the double layer and can be read independently of the second, but to make full use of the second part on kinetics an intimate knowledge of double layer phenomena is necessary. The valuable and extensive documentation extends through 1964, and is used in part to extend the coverage of the text to more specialised studies.

After an excellent brief historical introduction, Part I on the double layer follows and is divided into 5 chapters covering first, the thermodynamics of the ideal polarised electrode and second, the structure of the diffuse double layer without specific adsorption. Then follow 2 chapters dealing with adsorption: third, the structure of the double layer with specific adsorption and fourth, adsorption at an ideal polarised electrode. The final chapter in this part deals briefly with electrode systems other than the mercury - aqueous solution interface. It is in respect to solid electrodes that the subject is least well developed, indeed, is still in the qualitative stage, as Delahay emphasises in a very good summary of the problems involved. Part II, on electrode kinetics, is again divided into 5 chapters dealing with: first, the kinetics of simple electrode processes without specific adsorption; second, with processes involving more than one step; third, with the correlation between electrode kinetics and double layer structure in the absence of specific adsorption; fourth, with kinetics of electrode processes with chemisorption of reactants or products, and last with potential-dependent adsorption of reactants and products. Good indexes are provided.

This is no impersonal review. Results are critically compared and interpreted with judgment and the author does not hesitate to express his own views. An outstanding feature of the book is the extensive collection of diagrams reproduced from the original literature which facilitate the comparison of theory with experiment. This monograph will undoubtedly assume the rôle of the authoritative source on this subject for both analytical and electrochemical research and development for some time to come.

E. BISHOP

STEROID-SPEKTRENATLAS: ATLAS OF STEROID SPECTRA. By Dr.-Ing. WALTER NEUDERT and Dr.-Ing. HORST RÖPKA. Translated by Dr. JOHN B. LEANE. Pp. viii + 471 + scale. Berlin, Heidelberg and New York: Springer-Verlag. 1965. Price DM 144.

This is an important work. The only other atlas of steroid spectra is that by Dobriner and colleagues (Volume 1, 1953; Volume 2, 1958) and this deals exclusively with the infrared spectra of 656 steroids. In this new work, information is presented on the infrared, ultraviolet and nuclear magnetic resonance spectra of 900 steroids. In addition, optical rotation and dipole-moment data are given. The compounds are numbered making cross reference easy.

Because, in the Dobriner atlas, emphasis lay on the identification of a particular steroid, the spectra there were recorded in general for the ranges 1800 to 1600 and 1500 to 700 cm^{-1}. Six hundred of the spectra refer to solutions, the remaining 56 are for potassium bromide dispersions. In the Neudert - Röpke atlas, infrared spectra are given for all the 900 compounds, which, apart from the three liquids, are all for potassium bromide dispersions. In addition, 64 of the compounds are examined in nujol suspension and three in chloroform solution. The range covered is from 2·5 to 15·0 μ. (A transparent scale is provided for reading the corresponding cm^{-1} values.) The size of the graphs is somewhat small (147 × 60 mm), but they are printed clearly. Ultraviolet spectra are given uniformly for methanol solutions, and all the 900 compounds are referred to 41 actual spectra. Nuclear magnetic resonance spectra are provided for 95 compounds. Full practical details are given for the procedures used to obtain all the absorption spectra.

The last third of the book is concerned with a discussion of absorption spectroscopy leading up to the assignment of bands to the particular groups in the compounds studied. Reference to the previous graphs is easy. There is a valuable section on dipole moments and one on punched-card systems which includes, of course, the coding system adopted in the atlas.

The selection of compounds in this work is directed, in large measure, by the steroid research work of the Schering A.G. However, the main types of steroid compounds are included, though there are some omissions, *e.g.*, equilenin. Literature sources for the compounds themselves are unfortunately, not given. Although it might have been a formidable task it would have been worth the effort.

It is impossible in so short a space to do justice to a work of this nature. There is a wealth of information here which is inherently valuable, both to the general chemist and to the steroid specialist. The work is especially valuable for the amount of material that has been collected into one volume, and bearing this in mind the price is reasonable. J. S. WHITEHURST

HANDBOOK OF ULTRAVIOLET METHODS. By ROBERT G. WHITE. Pp. viii + 365. New York: Plenum Press. 1965. Price $17.50.

This book is an extended card index catalogue of 1600 analytical methods for pharmaceuticals, fine chemicals, foods, soaps, cosmetics, etc., classified in alphabetical order of principal author, with a chemical-compound subject index. Sometimes the abstract gives adequate information for direct analysis without reference to the original literature. The abstracts were drawn from about 260, mainly English, journals during the period 1940 to 1964. There is every indication that adequate coverage of the pertinent techniques has been achieved.

The contents will be particularly useful to the newer, small laboratory and should prove a blessing to the inexperienced analyst confronted with inadequate facilities for literature search. Ideally, frequent new editions will be required to keep the contents up-to-date, but the volume can prove a solid foundation for building a comprehensive card index system. I hope this book proves the forerunner of many similar publications in techniques other than ultraviolet spectroscopy. W. L. SHEPPARD

LABORATORY METHODS IN INFRARED SPECTROSCOPY. Edited by R. G. J. MILLER. Pp. 164. London: Heyden & Son Ltd. Price 45s.; $6.50.

The analyst using infrared spectroscopy is faced with the separate problems of first obtaining a satisfactory spectrum and then getting his information from it. The second of these has been well documented for some time, but systematic information on the practical side has appeared comparatively recently, and then usually only as part of a complete treatment. For those who wish to buy a separate book on laboratory methods, this is by far the best value for money.

The first few chapters cover the general ground of using a spectrometer, handling samples, recording the spectrum and coping with optical materials. The others give detailed information on obtaining spectra at extreme temperatures, dealing with polymeric materials of various kinds, using aqueous solutions and dispersions, and studying crystals with polarised radiation. The

identification of chromatography fractions is probably the most important application of infrared spectroscopy, so the excellent account presented here is most welcome. The tracking down of spurious bands is treated with humour, and the short notes on this topic lead off with a practical enthusiasm that lasts the book through.

The editor has done a good job in choosing the material, and any disagreement on emphasis is largely a matter of personal preference. The chapter on spectrometers might well have included a comparison of the performances of commercial instruments. The description of grinding and polishing optical materials seems too long; cells are usually made commercially nowadays. It is a pity that the original plan to cover quantitative analysis was changed although, to be fair, most of the principles and problems are discussed under other headings and this is, alas, a dying art.

The separate chapters are written by different specialists who have avoided the pitfall of repetition with some success. Indeed, where two authors do include the same topic, something of further interest often arises. For instance, there is healthy disagreement between chapters about the importance of the attenuated total reflection method for aqueous solutions, and some very important limitations of the bromide disc technique are mentioned under chromatography but not under sample handling. The alcohol inhibitor had not been removed from the sample of chloroform that was used to record the spectrum given among the solvents, in spite of good advice from the tracker down of spurious bands, who should find further scope for his activities in the spectra of cyclohexane and tetrahydrofuran.

This book is well arranged in cross-referenced chapters with sections and sub-sections but would still benefit from an index. The diagrams are excellent and the text is clear except for the presentation of a few equations and a number of trivial misprints. A. R. PHILPOTTS

TRANSITIONAL ELEMENTS. By EDWIN M. LARSEN. Pp. xii + 183. New York and Amsterdam: W. A. Benjamin Inc. 1965. Price (cloth) $4.95; (paper) $2.45.

The avowed intent of this text is to introduce the chemistry of the transitional elements to university chemists. As a result of the development in the analytical chemistry of the transitional elements in recent years, it is more than ever desirable that the practising analyst should have a good working knowledge of the specialist chemistry of these metals. Such a knowledge is neatly summarised in this little book. The five chapters are headed: The Properties of the Transitional Elements; The Metals and their Compounds; The Structures of the Metals and their Compounds; Solution Chemistry; and Co-ordination Chemistry. In addition, there are useful appendices entitled: Writing Net Equations; Writing Equilibrium Constants; Conventions for Standard Electrode Potentials; Nomenclature of Complex Compounds; Tables of Data and Reference Texts. The reviewer would, however, query the quaint choice of certain reference texts in the last appendix.

The book is lucidly written and contains many of the quite excellent figures and diagrams that one has come to associate with Benjamin books.

As a means of obtaining an abbreviated, but broad, introduction to the transitional elements, this book makes a valuable contribution, and also serves as an excellent introduction to further reading in this field. EDWARD ABEL

PHOTOSYNTHETIC BACTERIA. By E. N. KONDRAT'EVA. Pp. vi + 243. Jerusalem: Israel Program for Scientific Translations. Distributed in Great Britain and the Commonwealth, South Africa, Eire and Europe by the Oldbourne Press, London. 1965. Price 72s.

The photosynthetic bacteria form an ill-defined group, and probably represent the simplest of living organisms. Some are responsible for an appreciable fraction of the sulphur deposits in nature—by oxidation of the hydrogen sulphide previously produced by sulphate-reducing bacteria. They have not, however, so far achieved the economic importance of the latter group. Their interest lies largely in the fact that they afford an opportunity for studying photosynthesis uncomplicated by respiration or by liberation of free oxygen. It was the study of this process that first led to the view that the oxygen released by higher plants is derived from water and not from carbon dioxide, as was previously thought.

The present volume contains no new work, but gives a well translated and admirably arranged summary of the literature to date. Some 1200 references are given, of which 234 are in the Russian language. Biochemical aspects receive special attention, notably the physiology of the group, the mechanism of photosynthesis, and the analysis and composition of the carotenoids and the photosynthetic pigments— bacteriochlorophyll in the purple, bacterioviridine in the green sulphur bacteria.

The publication of this book should do much to stimulate research on this difficult and interesting group of bacteria. L. D. GALLOWAY