THE ANALYST

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New Horizons

THE last decade has seen a wealth of new weapons added to the analyst's armoury, permitting the attainment of an accuracy and speed hitherto undreamt of. In the main these tools have been physical instruments, *i.e.*, the polarograph, the mass spectrometer, the quantometer, flame photometers, wide-range spectrographs, radioactivity-measuring devices and, most popular of all, absorptiometers allowing the use of photometric methods. Looking at all this progress it seems as though the analyst of the future will be a physicist rather than a chemist. Gone forever are the days of the analyst who was as much an artist as a chemist. Indeed in those days, unless an analyst was an artist, he could have had little success with his primitive but difficult separations. What pleasure it was to watch a true craftsman analyse a rock specimen, mainly by gravimetric methods, taking four days for fourteen elements. With what loving care did he dehydrate his silica, separate his iron and aluminium and at length estimate, however inaccurately, his alkalis.

The analyst must, however, look forward without regrets. Analytical chemistry has the proud role of "handmaiden of all the sciences," and without the analyst very little progress has ever been made. Any method, therefore, that will give an accurate answer rapidly must be used, even if it would appear to be a trifle soulless to the old-time craftsman now occupied collecting the results coming out printed from the quantometer.

Of all the new tools, two alone can claim to be really chemical in nature; ion-exchange resins and the chelating reagents, such as ethylenediaminetetra-acetic acid. Both these methods are playing a very important and growing part in recent advances in analytical chemistry. Both have no doubt new fields to conquer, but it is the future of ion-exchange resins that will be considered here briefly.

In practically every field of analysis the cation and anion-exchange resins have found abundant use, as can be seen from the recent bibliography published in *The Analyst* (April, **1953**, **78**, 220–252). Now, however, come striking advances that open up entirely new horizons. These are the use of chelating resins and resins showing very selective properties. Skogseid (Thesis, Oslo, 1948) was one of the first to suggest the use of a chelating resin; he caused picryl chloride to react with nitrated and reduced polystyrene and followed this by nitration. The resin so formed acted as a cation-exchange resin and had a greatly increased potassium affinity. Other workers prepared resins with diketone groups, and others with active groups of the chlorophyll and haemin series. Yet again other workers prepared resins containing chelating groups of the amino-acid type; such resins were found to have a high affinity for copper, and with the bivalent transition metals the order of affinity corresponded to the Irving and Williams order of stability observed with soluble complexes, *i.e.*, $Mn < Co < Ni < Cu$.

First reactions to these new resins by workers in this field were cautious, since the resins were difficult to obtain or to make. In addition, it appeared that they were not very specific and reports on their behaviour were conflicting. Now, however, come more promising developments. N. Grubhofer and L. Scheith (*Naturwiss.*, **1953**, **40**, 508) have made an entirely new type of resin by boiling the finely grained Amberlite XE-64 with thionyl chloride - pyridine, so converting the carboxylic groups to acid chloride groups. The pale yellow product contained 20 per cent. of chlorine and was found to be very reactive towards phenols, amino acids, proteins and so on. The ester produced with the secondary O-H of quinine contained 25 per cent. of quinine and was found to be an active anion-exchange resin with optically active basic centres; with this it was possible to separate racemic mixtures of acids. Mandelic acid, for instance, was split in chloroform solution into its optically active components.

Grubhofer and Scheith have also examined polyaminostyrene resins. These compounds after diazotisation were coupled with proteins in weakly alkaline solution and the excess of diazo groups was saturated with 2-naphthol. The protein in the resin was found to retain its enzymic activity if diastase, pepsin, ribonuclease or carboxypeptidase was used as coupled constituent. Starch run through a diastase resin column no longer reacts with iodine; the carboxypeptidase column converts chloroacetyl-DL-alanine to L-alanine and chloroacetyl-D-alanine.

At about the same time J. P. Cornaz and H. Deuel (*Experientia*, 1954, **10**, 137) reported that the hydroxamic acids prepared from acrylic and pectic acids have a strong selectivity for ferric ions. Still more recently, Miles *et al.* prepared an unusual resin with highly selective properties. They prepared the resin by the mercuration of an alcohol-soluble phenol-formaldehyde resin with mercuric acetate in ethanol solution, the resulting polymer being precipitated as a yellow powder containing about 35 per cent. of mercury. This new resin has the property of selectively removing mercaptans from aqueous solutions, the mercaptans afterwards being readily and easily eluted by a solution of 2-mercaptoethanol or hydrogen sulphide. Glutathione, cysteine and coenzyme A are all retained by this resin. It is obvious that there will be many uses for such unusual properties in the examination of biological fluids, quite apart from uses such as the analysis of petroleum fractions.

It would therefore appear that the newer types of resins, chelating and specifically selecting, have come to stay, and we may confidently expect further exciting discoveries in this new and most interesting field. G. H. O.

PROCEEDINGS OF THE SOCIETY FOR ANALYTICAL CHEMISTRY

MIDLANDS SECTION

AN Ordinary Meeting of the Section was held at 6 p.m. on Wednesday, May 25th, 1955, in the Chemistry Department, The University, Edgbaston, Birmingham, 15. The Chairman of the Section, Mr. J. R. Leech, J.P., presided.

The following papers on the use of a new reagent for the determination of sulphate were presented and discussed: "4-Amino-4'-chlorodiphenyl as a Reagent for the Determination of Sulphate," by A. J. Nutten, B.Sc., Ph.D., F.R.I.C.; "The Determination of Sulphur in Coals after Combustion in the Calorimetric Bomb," by H. C. Wilkinson, M.Sc., A.M.Inst.F., A.R.I.C.; "A Semi-micro Method for the Determination of Sulphur in Rubber," by B. B. Bauminger, Ph.D., A.R.I.C.; "The Determination of Sub-micro Quantities of Sulphate," by A. S. Jones.

Before the meeting there was a tour round the Organic and Analytical Research Sections of the Chemistry Department of Birmingham University.

BIOLOGICAL METHODS GROUP

AN Ordinary Meeting of the Group was held in the Lecture Hall of the Royal Society for Tropical Medicine and Hygiene, Manson House, 26 Portland Place, London, W.1, at 2.30 p.m. on Friday, May 13th, 1955. Dr. L. J. Harris, F.R.I.C., Chairman of the Group, was in the Chair.

The subject of the meeting was "Biological and Microbiological Methods of Estimating Vitamin B_{12}" and the following papers were presented and discussed: "Introductory Address," by F. A. Robinson, M.A., LL.B., F.R.I.C.; "The Microbiological Estimation of Vitamin B_{12} in Serum," by R. H. Girdwood, M.D., Ph.D., F.R.C.P., Ed., M.R.C.P.; "The Estimation of Vitamin B_{12} in Animal Feeding Stuffs with *Lactobacillus leichmannii* and *Ochromonas malhamensis* as Test Organisms," by D. H. Shrimpton, B.A., Ph.D.; "The Estimation of Vitamin B_{12} in Milk," by Margaret E. Gregory, Ph.D., A.R.I.C.; "Biological Methods of Estimating Vitamin B_{12}," by Marie E. Coates, Ph.D., F.P.S.; "A Critical Analysis of the Method of Vitamin-B_{12} Assay with *Euglena gracilis* as Test Organism," by W. R. Pitney, M.D., M.R.A.C.P.

In the absence of Mr. F. A. Robinson, his paper was read by Mr. E. H. Fitzgerald.

Portable High-frequency Titrimeter

By J. P. DOWDALL, D. V. SINKINSON and H. STRETCH

(*Presented at the meeting of the Society on Wednesday, April 6th, 1955*)

A stable easily constructed battery-operated titrimeter with a tuned-anode - tuned-grid oscillator operating at 15 to 20 Mc/s is described. Examples of its use are given; these include titrations of ionisable chlorine and fluorine separately and in mixed solution, acid - base titrations and the titration of organic bases in non-aqueous solution.

In a search for an improved method of fluoride titration, attention was directed to a paper by Jensen and Parrack[1] describing an instrument in which the titration vessel is located within the field of the anode coil of a high-frequency oscillator, and the end-point of the titration is determined by observing the change in anode-current reading. The apparatus of Jensen and Parrack, however, suffers from certain disadvantages, notably an undesirable heating effect on the solution being titrated.

Anderson, Bettis and Revenson[2] make use of a grid-dip oscillator, whilst Blaedel and Malmstadt[3,4] describe titrimeters in which the end-point is determined by measurement of frequency change during the titration. In recent work, Fujiwari and Hayashi[5] described an apparatus in which reception of a constant-power modulated high-frequency signal is modified during the course of a titration, owing to the presence of the solution in the field of the grid coil of the detector.

All the methods cited above are relatively complex and require for their operation considerable power, which is normally derived from the mains. The titrimeter that is the subject of this paper is simply constructed, portable and powered by batteries, the power consumption being very small.

A high-frequency method is of special value in the titration of highly coloured solutions, or in other cases where visual indicators are unsatisfactory. It is a valuable alternative to conductrimetric procedures, as it has certain advantages over the latter in that no electrodes are present in the titration solution. It is also easier to set up.

Theoretical considerations

The basis of these titrations appears to be complex, but, as pointed out by Forman and Crisp,[6] a simple solution may be obtained by considering the types of polarisation effects involved. In an aqueous solution of a strong electrolyte subjected to a high-frequency electric field, the two types of polarisation chiefly responsible for the power loss are the molecular (dipolar) polarisation of the solvent molecules and what may be termed the "ionic polarisation" of the electrolyte.

In the latter case, the maximum absorption of power occurs at a frequency, f, equal to $\frac{2\pi}{\tau}$, where τ is the "relaxation time" of Debye and Falkenhagen.[7] This relaxation time is given by the relation—

$$\tau = \frac{8{\cdot}85 \times 10^{-11} D_0}{\Lambda_\infty \gamma},$$

where D_0 is the dielectric constant of water, Λ_∞ the equivalent conductance of the electrolyte at infinite dilution and γ the molar concentration of the solution.

For a given temperature, D_0 is constant, and for a simple electrolyte of any given type (uni-univalent, uni-bivalent and so on), γ is also roughly constant, so that—

$$\tau \simeq \frac{\text{constant}}{\gamma}$$

and

$$f \simeq K_e \gamma,$$

where K_e is a constant for each simple electrolyte.

Hence the frequency at which maximum loss occurs increases with the concentration of the solution; for example, with a 0·02 *N* solution of sodium chloride, this frequency is of the order of 35 Mc/s, and with a 0·2 *N* solution is 250 Mc/s. Forman and Crisp show that this simple relation is followed by a number of electrolytes; their results indicate that, for a given concentration, the maximum rate of change of dielectric constant occurs at approximately the frequency at which the maximum absorption of power takes place.

In a tuned-anode - tuned-grid oscillator the valve is normally self-biased to near cut-off by the voltage developed across the grid coil, together with the grid capacitor and resistor. When high-frequency energy is absorbed by the titration solution, the anode current increases in order to compensate for the lost energy, and the grid bias is automatically re-adjusted. If the energy loss is too large, however, insufficient voltage is developed across the grid coil, nd the valve is momentarily biased on the vertical slope of the anode-current - grid-voltage curve. As a result, the anode current increases sharply, and the valve ceases to generate high-frequency energy.

The loss of energy is proportional to the concentration of the solution. The instrument described below can be used for electrolyte concentrations up to approximately 0·03 *N*.

Description of apparatus

A tuned-anode - tuned-grid oscillator is used to generate high-frequency energy. The circuit is shown in Fig. 1, and the general appearance of the instrument is shown in Fig. 2. The titration solution is placed in a suitable cell located within the anode coil. A battery triode (3A4) of high mutual conductance and low power consumption is used as the oscillator

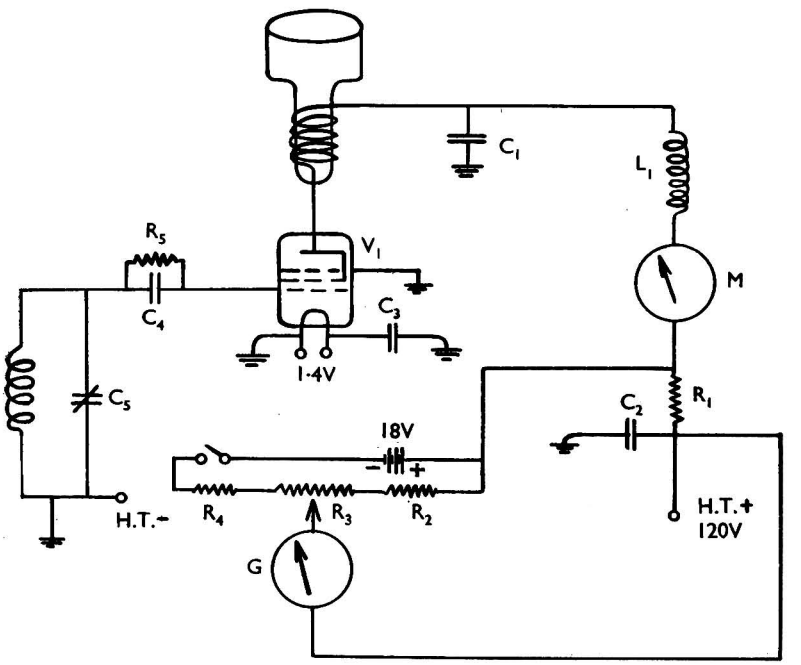


Fig. 1. Circuit diagram of the high-frequency titrimeter

C_1 = 100-$\mu\mu$F condenser
C_2 = 0·005-μF condenser
C_3 = 0·1-μF condenser
C_4 = 100-$\mu\mu$F condenser
C_5 = 160-$\mu\mu$F variable condenser
L_1 = radio-frequency choke
M = milliammeter (0 to 25 mA)
G = galvanometer (full-scale deflection, 40 μA)
R_1 = 1000-ohm, 5-watt resistance
R_2 = 10,000-ohm resistance
R_3 = 15,000-ohm resistance
R_4 = 10,000-ohm resistance
R_5 = 40,000-ohm resistance

valve. If a large power valve is used, an undesirable heating effect occurs in the solution. The anode coil is tuned by its own self capacity, which results in a high dynamic resistance, so permitting oscillation to be maintained over the greatest possible range of solution concentration.

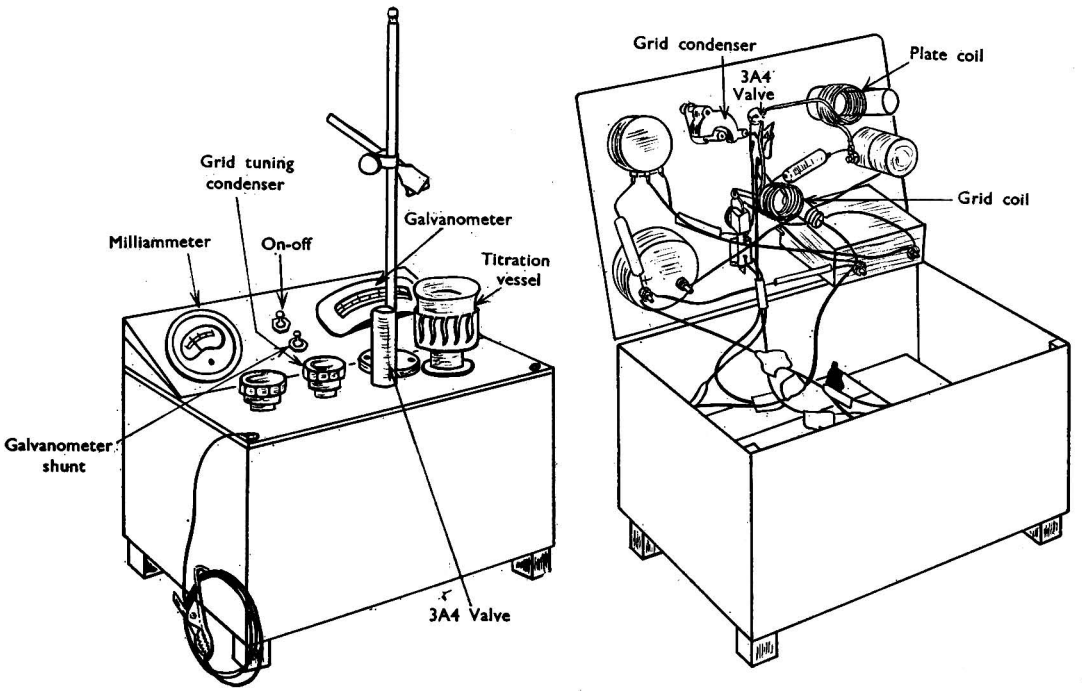


Fig. 2. General view of the high-frequency titrimeter

The coil, of 14 S.W.G. enamelled copper wire, consists of 5 turns, $\frac{1}{8}$-inch spacing between turns, wound to fit closely around the titration vessel described below. The grid coil consists of 4 to 5 turns of 16 S.W.G. enamelled copper wire, wound to a diameter of $\frac{3}{4}$ inch with $\frac{1}{8}$-inch spacing between turns. Both coils are self-supporting. The specifications are relatively uncritical, merely affecting the range of oscillation frequencies.

A milliameter (0 to 25 mA) is included in the anode circuit to allow preliminary adjustment of the current, and a sensitive galvanometer with an arrangement for backing-off the greater part of the current is switched in when readings are being taken during the course of a titration.

The instrument, including batteries, is contained in a copper box, the oscillator components and titration vessel being attached to the lid. The screening of the titration vessel is extended above and below the lid, so that the liquid level is always in the screened portion of the tube. Thorough screening of the entire instrument and a good earth connection are required for the avoidance of hand capacity effects, and the self-contained power supply assists materially in this direction. Mechanical rigidity of the titration vessel is also essential. The vessel consists of a 6-inch by 1-inch Pyrex-glass tube flared at the upper end for a distance of $1\frac{1}{2}$ inches to a diameter of 2 inches. A small motor-driven stirrer is used to mix the solution during titration. A trial titration with water in both burette and titration cell established that no change in anode current resulted from a change in liquid level.

The sensitivity of the instrument is related to the slope of the anode-current - grid-voltage curve. If the anode current is set at the beginning of a titration to a low value, the current shift per millilitre addition of titrant will be small. If the initial current is high, the current shift will be increased. In this later condition, re-setting of the galvanometer in the course of a titration may be necessary and, if the sensitivity has been set too high, the valve may cease oscillating during the titration.

The pin numbering of the 3A4 valve is as follows: 1, F—; 2, A; 3, G2; 4, G1; 5, F (C.T.) and G3; 6, A; 7, F+.

The valve is of the alternative series - parallel filament connection type, and the filaments were used in parallel with a 1·4-volt battery.

Method of use

The solution under test is put by pipette in the titration cell and diluted so as to bring the liquid level into the flared-out portion of the cell. The stirrer is started, and the anode current is adjusted to about 9 to 10 mA by means of the grid-tuning condenser. The backing-out current is switched on, and the galvanometer needle is adjusted to a position near the zero end of the scale by means of the control potentiometer.

Additions of the titrant are made and the galvanometer readings recorded. It may be necessary to re-adjust the galvanometer needle with the backing-out potentiometer during the course of a titration. As the end-point is approached, a marked change of galvanometer deflection per unit volume of titrant added occurs, and in many cases a reversal in direction of galvanometer reading will take place.

Application to ionisable fluorine determination

Most existing methods for the determination of fluoride ion may be grouped into three classes, *viz.*—

(*a*) isolation of hydrofluoric acid by steam-distillation, followed by titration with standard alkali solution,

(*b*) gravimetric determination as lead chlorofluoride and

(*c*) titration with certain metallic salt solutions in which the formation of a lake with an added dyestuff is inhibited in the presence of fluoride ion, *e.g.*, thorium nitrate as titrant together with Solochrome blue as the indicator.

All these methods have been examined. Method (*a*) was insufficiently specific. Method (*b*) was critical as to concentration of many common ions. In method (*c*) the end-point depended on the visual matching of the colour of finely divided suspensions. In practice, different operators had dissimilar views as to the end-point.

In view of these findings, a high-frequency method was examined.

Cerous nitrate was initially used as titrant; however, the normal salt is strongly acidic, and the resulting curve rose continuously and showed a change of slope at the equivalence point. An attempt was made to obtain sharper end-points with this reagent by the addition of weak bases, but was only partly successful. Cerous acetate was tried, and sharp end-points were obtained. The reagent, however, underwent slow oxidation, the solution becoming cloudy on standing. Cerous acetate was finally replaced by lanthanum acetate, which gave equally satisfactory titration curves, and the solution was quite stable on storage.

With lanthanum acetate as titrant for sodium fluoride solutions in the concentration range 0·025 to 0·013 *N* in approximately 0·013 *N* acetic acid solution, results are obtained within ±1 per cent. of the fluoride ion present in the solution.

A typical set of readings is as follows—

Volume of lanthanum acetate solution, ml	8·0	9·0	10·0	11·0	12·0	13·0	14·0	15·0
Galvanometer reading	200	274	343	400	428	325	229	100

The lower range of sensitivity may be extended to a titration of solutions of 0·006 *N* fluoride ion by the use of a more sensitive galvanometer having a full-scale deflection of 6 mA, and this represents the lower usable limit of the present tuned-anode - tuned-grid titrimeter.

The degree of accuracy attained in the analysis of an organo-fluorine compound after decomposition of fluoride ion is shown by the following results—

Weight of fluorine present in compound, g	0·0532	0·0567	0·0464	0·0620	0·0458	0·0636
Total volume of titrant, ml	37·71	41·40	33·00	44·34	32·88	45·90
Weight of fluorine found, g	0·0529	0·0580	0·0463	0·0621	0·0461	0·0643

1·0 ml of lanthanum acetate solution = 0·001402 g of fluorine.

A one-third aliquot was taken for titration.

CONTROL TESTS—

Direct blank tests on reagents are not normally possible with the method, since it is difficult to titrate traces of ionisable fluorine in the presence of fairly large quantities of other ions. A more effective method is to add known amounts of fluoride to the reagents, and titrate in the normal manner.

EFFECT OF ACIDS AND BASES ON THE TITRATION—

Bases that displace lanthanum from its salts and acids that give insoluble lanthanum salts must be removed from solution. Weak bases, such as aniline, do not affect the end-point, and acids appear to have an effect proportional to their pH, a weak acid being tolerable to a greater degree than a strong one.

Acetic acid—Lanthanum acetate requires the addition of a small amount of acetic acid to assist solution and to prevent formation of basic salts. Acetic acid in concentration less than 0·1 *N* has no harmful effects in the titration of 0·025 to 0·013 *N* fluoride solutions. With 0·0013 *N* fluoride, acetic acid concentrations above 0·013 *N* cause high end-points. The acetic acid concentration was therefore standardised at 0·013 *N*. In this manner,

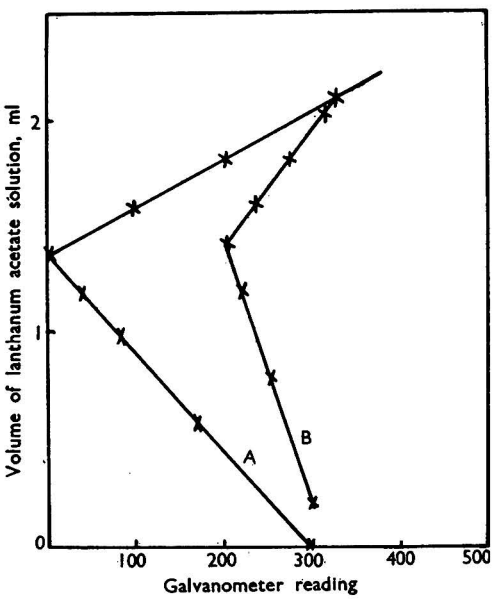


Fig 3. Titration of fluoride in the presence of ammonium chloride: curve A, about 0·1 g of sodium fluoride; curve B, about 0·1 g of sodium fluoride and 0·2 g of ammonium chloride

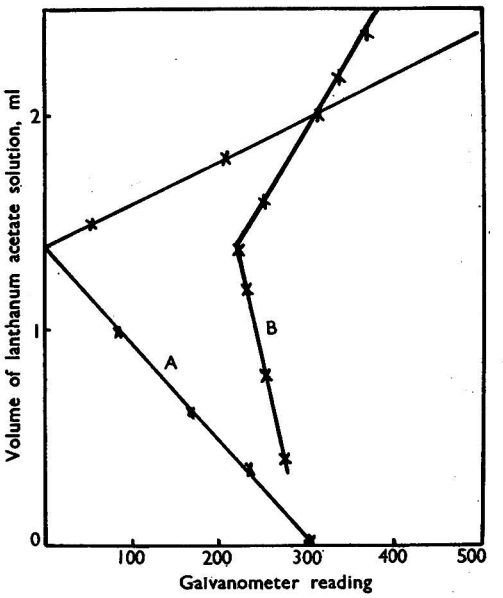


Fig. 4. Titration of fluoride in the presence of sodium chloride: A, about 0·1 g of sodium fluoride; B, about 0·1 g of sodium fluoride and 0·2 g of sodium chloride

relatively high changes in pH during the titration are avoided, and the effect of traces of alkali and carbon dioxide is minimised.

Nitric acid—Up to 0·02 *N* nitric acid has no harmful effect on titrations of 0·013 *N* fluoride solutions. Above this concentration of nitric acid the resulting damping of the tuned circuit makes it difficult to maintain oscillation.

Hydrochloric acid—This was not specifically tried, but solutions containing sodium chloride and nitric acid gave correct values for the fluoride present.

EFFECT OF CERTAIN SALTS—

The effect of all salts, apart from those that react with cerium or lanthanum acetate, is to damp the tuned circuit, thus effectively limiting the titration range. Titrations of 0·013 *N* fluoride solutions are not significantly affected by sodium, ammonium, nitrate, chloride, acetate or silicate ions present in similar concentration to the fluoride. The effect

of ammonium and sodium chlorides are shown in Figs. 3 and 4. The end-point obtained in the presence of phosphate ions is unreliable.

Method for determination of ionisable fluorine

Reagents—

Sodium fluoride—Dissolve 4·20 g of freshly dried purified sodium fluoride in 1 litre of water, and determine the fluoride in a suitable aliquot by the lead chlorofluoride method.

Lanthanum acetate—Dissolve 8·0 g of lanthanum acetate in a little warm water containing 0·5 to 1·0 ml of glacial acetic acid and dilute to 1 litre.

Procedure—

Switch on the instrument 10 to 15 minutes before use.

Introduce the solution to be titrated into the titration vessel, followed by the necessary amount of 0·1 *N* acetic acid to produce a 0·012 *N* acetic acid solution, the liquid level being in the flared-out portion of the titration vessel.

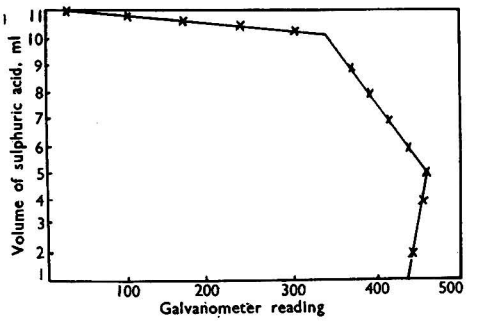


Fig. 5. Titration of 5 ml of 0·1 *N* sodium carbonate with 0·05 *N* sulphuric acid

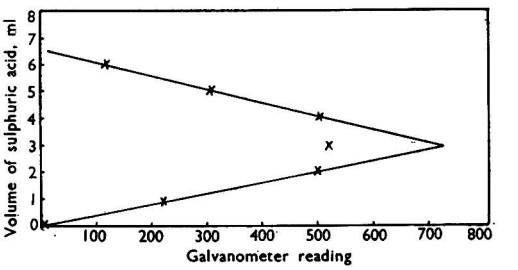


Fig. 6. Titration of 3 ml of 0·02 *N* sodium hydroxide with 0·02 *N* sulphuric acid

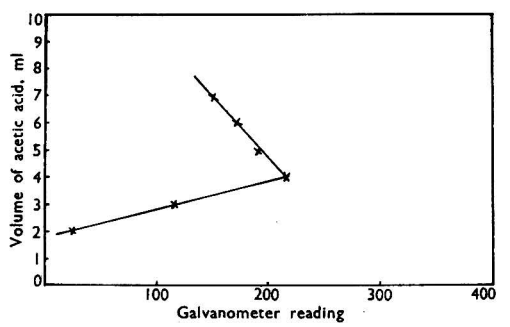


Fig. 7. Titration of 0·1 *N* sodium carbonate with 0·25 *N* acetic acid

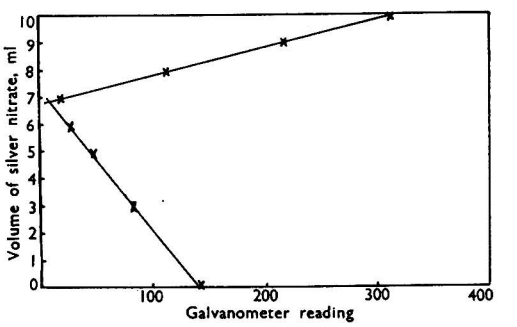


Fig. 8. Titration of 0·1 *N* sodium chloride with 0·1 *N* silver nitrate

Start the stirrer and rotate the grid-tuning control to bring the valve into a state of oscillation (indicated by minimum value of anode current), then de-tune slightly to a current reading of 9 to 10 mA. Switch on the backing-out current and adjust the potentiometer to bring the galvanometer needle to a reading near the lower end of the scale. A sensitive galvanometer must be protected by a shunt until this adjustment is made.

Make equal additions of titrant, and record the galvanometer readings. Near the end-point the deflection per millilitre decreases and, when the end-point is passed, reverses direction. Record five readings past the end-point. If necessary, re-set the galvanometer by means of the backing-out control during the course of the titration. Empty the titration vessel by means of a suction tube.

Titration of fluoride and chloride in the same solution—

At approximately 0·013 *N* concentration of each ion in solution, fluoride, which is preferably titrated first, with lanthanum acetate, may be determined to ±1 per cent. of the

quantity present. This is followed by titration of the chloride with silver nitrate. This titration is more precise than the fluoride titration; equilibrium is rapidly attained and reversal of the galvanometer needle can be followed visually with the addition of single drops of titrant at the end-point.

Acid - base and silver nitrate - halide titrations

Figs. 5, 6, 7 and 8 show representative titrations. In some cases the end-point is so sharp that it is unnecessary to plot the results.

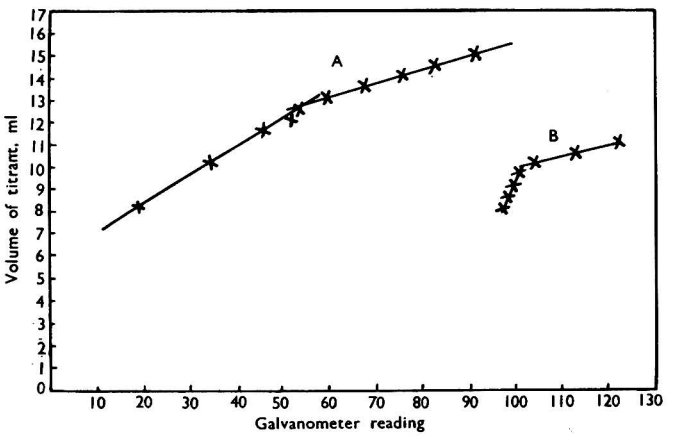
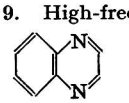
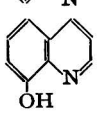


Fig. 9. High-frequency titration of organic bases

A, 

Theoretical equivalent weight 130·4
Found 130

B, 

Theoretical equivalent weight 145·4
Found 145

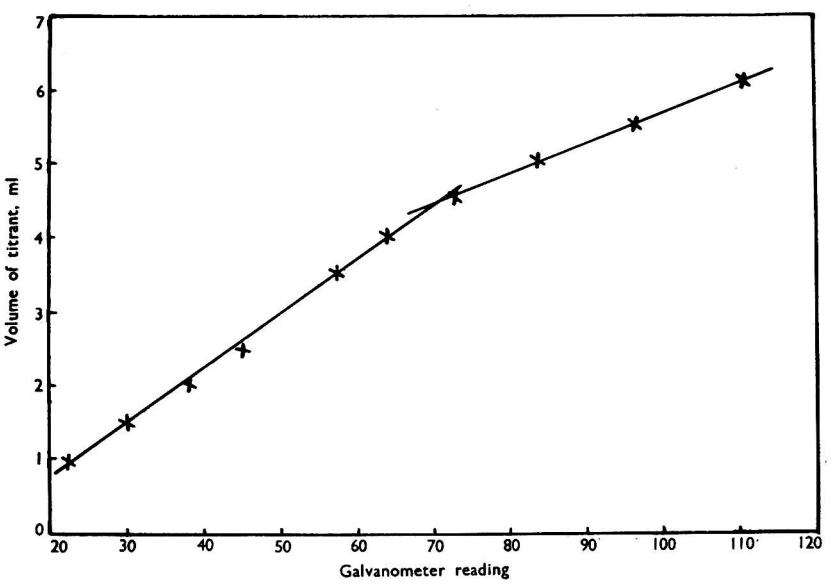
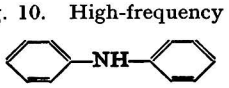


Fig. 10. High-frequency titration of organic bases



Theoretical equivalent weight 169·2
Found 170

Titration of organic bases

At the request of Mr. E. S. Lane, of A.E.R.E., Harwell, experiments were made on the titration of organic bases in non-aqueous media.

The method adopted was similar to that of Wagner and Kauffman.[8] The base, dissolved in the requisite amount of glacial acetic acid in the titration cell, was titrated with perchloric acid in glacial acetic acid. The perchloric acid was standardised against potassium hydrogen phthalate as described by Seaman and Allen.[9] Satisfactory results were obtained, as shown in Figs. 9, 10 and 11. This aspect is being further investigated by Mr. Lane.

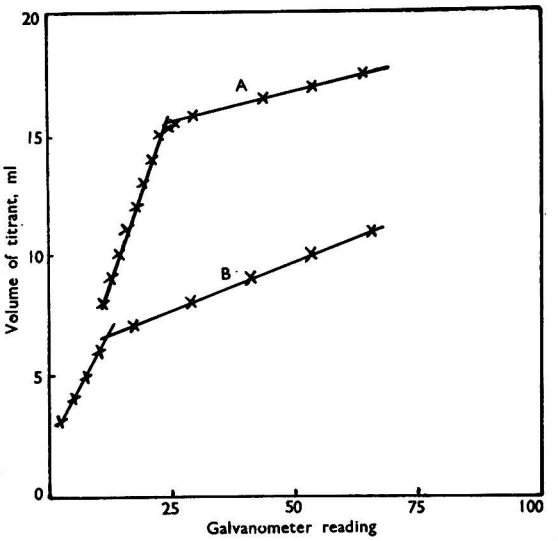
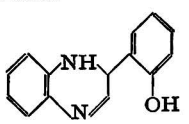


Fig. 11. High-frequency titration of an organic acid and an organic base. A, 8-hydroxyquinoline in neutralised dimethylformamide titrated with 0·1 *N* sodium methoxide in benzene-methanol

B,



Theoretical equivalent weight	210
Found	210

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Ministry of Supply
Chemical Defence Experimental Establishment
Porton, Wilts.

November 17th, 1954

Fractionation of Crude Fumagillin by Distribution Methods

By R. R. GOODALL and J. K. LANDQUIST

(*Presented at the meeting of the Physical Methods Group on Tuesday, January 18th,* 1955)

An intractable product, related to gliotoxin, occurring with fumagillin among the metabolites of a strain of *Aspergillus fumigatus* had high amoebicidal activity. A counter-current fractionation in the system benzene - light petroleum and ethanol - water demonstrated the presence of three fractions, only one of which had amoebicidal activity and this was identified as fumagillin.

In the development of an improved method for the bulk purification of fumagillin, advantage was taken of its acidic properties. Separation from neutral or less acidic impurities was achieved by distribution between a solvent and an aqueous buffer solution of pH 9, with recovery of the fumagillin from the aqueous phase.

During a study of the metabolic products of the mould *Aspergillus fumigatus* (strain A61), our colleague Dr. C. T. Calam isolated a crude extract with amoebicidal properties. This was subsequently separated by a chromatographic method into the known antibiotic fumagillin (Eble and Hanson[1]) and an intractable product that was called "thiogillin" and contained as its major constituent a substance related to gliotoxin. A fraction of this material, "thiogillin B," which had been purified as far as possible by chromatography, had high amoebicidal activity (active *in vitro* at 1 in 27×10^6, fumagillin being active at 3 to 9 times this dilution). We undertook further purification of this fraction by counter-current distribution in order to ascertain whether the biological activity was due to contamination with fumagillin or to the presence of an unknown amoebicidal substance. The results obtained encouraged us to apply distribution methods to the extraction of fumagillin from the crude fermentation product.

Counter-current fractionation of "thiogillin b"—

"Thiogillin B" is not sufficiently soluble in water for an aqueous phase to be used in distribution studies, but the mixed-solvent system detailed in Table I enabled appropriate distribution ratios to be obtained between the two liquid phases.

Table I

Distribution of "thiogillin b"

Mixture	Light petroleum, boiling range 60° to 80° C	Benzene	Ethanol	Water	Distribution ratio*
1	1 volume	1 volume	1 volume	1 volume	0·44
2	0·4 volume	1·6 volumes	1 volume	1 volume	0·89

* $\frac{\text{Concentration in upper layer}}{\text{Concentration in lower layer}}$.

The second mixture was selected for counter-current studies, since the observed distribution ratio is nearer to unity. Twenty-millilitre portions of the light petroleum - benzene phase (which separates as the upper layer after equilibration by shaking) were placed in each of a series of 22 glass-stoppered tubes. Ethanol - water phase (20 ml) was introduced into tube 1 and then 0·643 g of "thiogillin B." After solution and equilibration, the lower layer was transferred to tube 2 by means of a hypodermic syringe and 20 ml of fresh lower layer were introduced into tube 1. Equilibration and transfer was continued throughout the series in the well known manner.

After distribution was completed, 0·5-ml aliquots were withdrawn from both layers in each alternate tube (1, 3, 5, etc.) and evaporated to dryness. The residue was dissolved in a measured volume of methanol, and the ultra-violet absorption was determined.

The distribution by weight, determined by evaporating the remaining contents of each tube to dryness, showed that the material had separated into three parts with peak levels

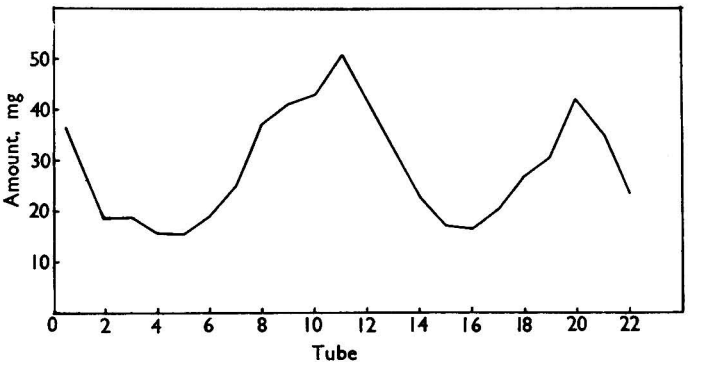

Fig. 1. Weight distribution of "thiogillin B" after counter-current distribution; 640 mg used and 630 mg recovered. Solvents—top layer: light petroleum, 20 per cent., and benzene, 80 per cent.; bottom layer: ethanol, 50 per cent., and water, 50 per cent.

at tubes 1, 11 and 20 (Fig. 1). The material in tubes 1 to 3 showed the absorption spectrum of fumagillin (Fig. 2*b*; compare also with the spectrum of the sample before distribution (Fig. 2*a*) and with the spectrum of crystalline fumagillin (Fig. 3)), that in tubes 6 to 14 had

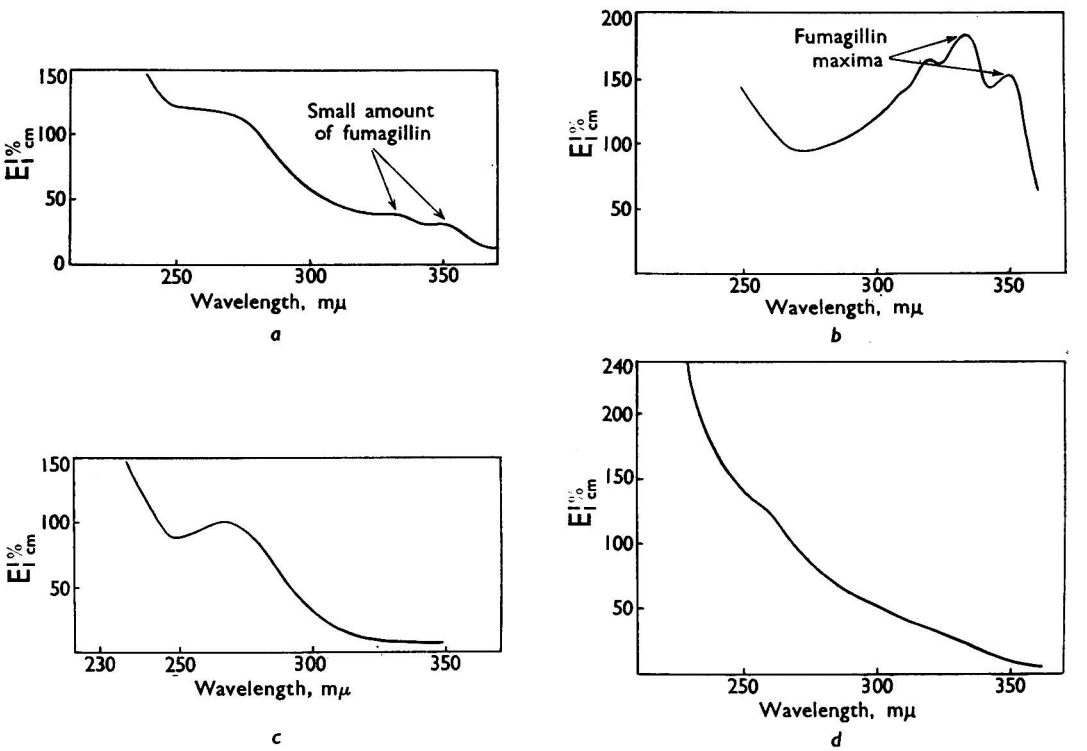

Fig. 2. Ultra-violet absorption spectra of fractions from "thiogillin B"—

(*a*) "Thiogillin B" before fractionation.
(*b*) Sample from stage 1 of 22-stage counter-current distribution
(*c*) Sample from stage 11 of 22-stage counter-current distribution
(*d*) Sample from stage 21 of 22-stage counter-current distribution

gliotoxin-like absorption (Fig. 2*c*) and the substance in stages 18 to 22 showed no characteristic ultra-violet absorption, apart from high end-absorption below 240 mμ (Fig. 2*d*). Nitrogen and sulphur were absent from fractions 1 to 4, but were present in all the other fractions. Fractions 1 to 4 consisted of amorphous white solids, and the other fractions were brown gums or glasses.

Fractions 11 to 13 were combined and submitted to a second 22-stage distribution in the same solvent mixture to eliminate any remaining trace of fumagillin. The middle fractions (9, 10 and 11) from this experiment were combined to give 33 mg of material that had no *in vitro* activity against *Entamoeba histolytica*. Fraction 20 of the original series was also inactive *in vitro*, and it was therefore demonstrated that the activity of the original "thiogillin B" was due entirely to the presence of residual fumagillin.

PURIFICATION OF FUMAGILLIN—

Fumagillin is an acid sparingly soluble in water but soluble in aqueous methanol, in which it displays a pK intermediate between those of benzoic and acetic acids. Although in the experiment described above fumagillin was separated from a mixture by counter-current extraction, it seemed likely that the crude fumagillin obtained from the fermentation broth by solvent extraction might be more readily purified on the large scale by utilising its acidic properties as a basis for separation from neutral or less acidic impurities. In a preliminary study, the distributions of crystalline fumagillin between water-immiscible solvents and dilute aqueous buffer solutions were determined (Table II), fumagillin concentrations being determined by ultra-violet absorption spectrophotometry.

TABLE II

DISTRIBUTION OF CRYSTALLINE FUMAGILLIN BETWEEN SOLVENTS AND AQUEOUS BUFFER SOLUTIONS

Solvent	Distribution ratio (solvent/aqueous concentration)	pH
Benzene	1·9	7·8
	0·61	9·0
Toluene	0·04	9·0
Ethylene dichloride	0·82	9·0
Chloroform	4·0	9·0
Butanol	18·0	9·0
	12·0	10·0
3:5:5-Trimethylhexan-1-ol	0·87	9·0
*cyclo*Hexanol	17·0	9·0
Ethyl acetate	0·48	9·0
Butyl acetate	75·0	7·0
	32·0	8·0
	0·68	9·0
	0·13	10·0

NOTES—

1. Preparations of the buffer solutions (pH 7 and 8 are 0·05 *M* phosphate, pH 9 and 10 are 0·05 *M* borax) are given by Britton.[2]
2. The concentration of fumagillin before equilibration was always in the range of 2 to 20 mg per cent.

The distribution of fumagillin from crude material was then examined. A sample of crude solids obtained by extraction of the broth at pH 7 with chloroform was dissolved in benzene and extracted with an equal volume of dilute aqueous borax buffer[2] (pH 9). The extraction was repeated. Here it may be observed that fumagillin and impurities are decomposed in aqueous solutions above pH 10, so that the operating pH must never be in excess of 9. The combined buffer extracts were brought to pH 5 with phosphoric acid and the fumagillin was extracted into chloroform. In this way the fumagillin concentration was raised from approximately 7 per cent. in the crude solids to approximately 23 per cent. The notable improvement was apparent at the next stage, at which the product from the buffer was readily crystallised from a minimum of acetone. The colourless needles obtained after washing with chilled acetone and drying had an ultra-violet absorption spectrum corresponding to 94 per cent. purity ($E^{1\%}_{1\,cm}$ at 334 mμ = 1464 and $E^{1\%}_{1\,cm}$ at 350 mμ = 1335) and a m.p. of 197° to 199° C when melted quickly. At the benzene - buffer distribution stages, the distribution ratio was adversely affected when the concentration in benzene exceeded 1·5 per cent. or

that of the borax buffer exceeded 0·05 *M* before extraction. Trials with crude thiogillin in benzene (15 per cent. w/v) in conjunction with a 2 *M* glycine buffer (pH 9) were unpromising.

For technical use butyl acetate is the most attractive solvent, and it was established that it could be used at all stages of the purification* (except for the final crystallisation). Amongst the other solvents noted in Table II, butanol and *cyclo*hexanol are not suitable because of unfavourable distribution at pH 9 and 3:5:5-trimethylhexan-1-ol is not volatile enough.

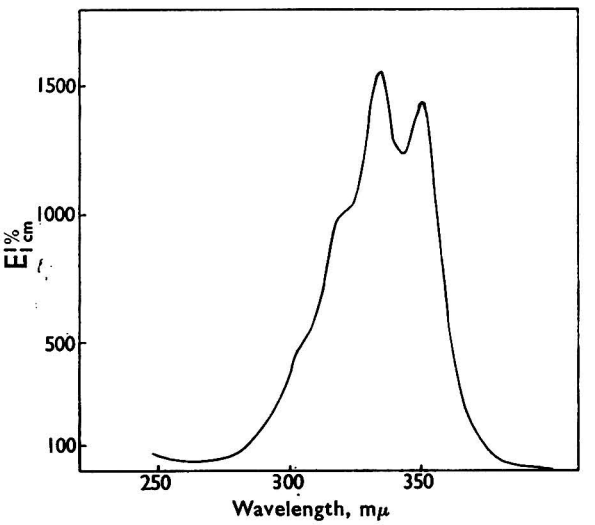

Fig. 3. Ultra-violet absorption spectrum of crystalline fumagillin

Data: 0·89 mg per 100 ml solution in distilled analytical-reagent grade methanol; slit width for wavelengths up to 265 mμ, 1 mm; slit width for wavelengths from 265 to 360 mμ, 0·3 mm.

λ	$E^{1\%}_{1\,cm}$
235 mμ (min.)	89
305 mμ	inflexion
320 mμ	inflexion
334 mμ (max.)	1555
350 mμ (max.)	1435

The over-all recovery was 60 per cent. from a broth containing approximately 2·5 g of fumagillin per 100 litres. This method of purification represents a considerable advance in convenience and yield over the methods formerly employed in these laboratories and over those described by Asheshov, Strelitz and Hall[3,4] and Eble and Hanson.[1]

* Patent protection pending.

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January 22nd, 1955

Solvent Extraction in the Analysis of the Precious Metals

By W. A. E. McBRYDE

(*Presented at the meeting of the Physical Methods Group on Tuesday, January 18th, 1955*)

Present-day applications of solvent extraction for the analytical separation of gold and the six platinum metals are summarised. The types of compounds whose solvent extraction is discussed include halogen complexes, oxides, complexes with stannous chloride and organic complexes. The choice of method is usually governed by the environment of the metal being separated and by the subsequent operations in the over-all analysis. Another case of solvent extraction discussed is that of the metals themselves from reducing fluxes into collecting buttons of lead or other metals as, for instance, in the fire assay.

THE separation of the platinum metals, whether on the industrial scale or in the laboratory, still leaves room for improvement, especially in the case of small quantities. It seems appropriate as new techniques like solvent extraction, chromatography and so forth are developed and improved that the help they afford with old problems should be reviewed and evaluated. Whilst solvent extraction is not a new technique by any means, its use has become more widespread and new knowledge of the chemistry involved has contributed to its more intelligent application. Furthermore, the extraordinary successes that have resulted from the application of partition chromatography to inorganic separations seem to justify some reference to this technique in this paper inasmuch as solvent extraction plays a part in it. Finally, a few words will be added to suggest directions of investigation that may prove fruitful in improving another old extraction technique, peculiar to the precious metals, namely, the fire assay.

The information presented in this paper reveals many gaps in our knowledge and invites a few interesting comparisons. In few cases reported so far have quantitative data been provided to support the choice of a particular solvent for a given separation. Also, it would be of value to be able to compare a number of organic reagents whose compounds, say, with palladium have been recommended for the extraction and separation of this element from the remaining precious metals.

It will be convenient to discuss the several examples in groups according to the kinds of substances involved in the extraction.

HALOGEN COMPLEXES

It has long been known that chloroauric acid can be extracted from hydrochloric acid solutions into a number of ethers and esters.[1,2] The extraction bears some resemblance to that of iron^{III} in that the distribution of gold depends on the concentration of acid in the aqueous phase, and that for very small concentrations of acid the distribution with ethers favours the aqueous phase. Gold may thus be extracted from a hydrochloric acid solution, and subsequently, if the ethereal solution is shaken with water, the gold is returned to an aqueous solution. The same behaviour is displayed by hydrobromic acid solutions of gold.[3,4] No mechanism for the extraction has been proposed, but by analogy with iron^{III} one might guess that $HAuCl_4$ or $HAuBr_4$ is the extracted species. The literature on the extraction of ferric chloride reveals that the mechanism is probably much more complicated than this, and a few experiments performed by the writer in connection with the extraction of gold bromide revealed anomalies that confirm the complexity of the extracted species.

From the standpoint of a practical separation, the removal of gold from various platinum and other metals was confirmed in the case of extraction from hydrobromic acid into di*iso*-propyl ether.[3] Indeed, for the separation of bromo complexes this ether is preferable to ethyl acetate, although the latter had been recommended by Yoe and Overholser[5] for separating the chloro complexes of gold and palladium. One point in connection with the extraction of bromo complexes requires some clarification. In the paper just mentioned, osmium was

shown to be partly extracted into ether. The osmium solution in these experiments had been prepared by dissolution of osmium tetroxide in alkali; the constituents present when this solution is treated with hydrobromic acid cannot be known with certainty. It has since been observed that no significant amount of osmium is extracted from a solution prepared from ammonium bromo-osmate. It may also be noted that under the conditions stated only minute amounts of iridium were extracted into di*iso*propyl ether; Bock, Kusche and Bock[4] imply that some iridium was extracted into diethyl ether.

Although palladium may be extracted from hydriodic acid solutions into *iso*butyl methyl ketone, and a separation from platinum and rhodium possibly effected thereby, it is doubtful whether the separation possesses the practical advantages found in other methods and therefore it has not been investigated in detail.[6] Kitihara reported that palladium and four other platinum metals were not extracted from hydriodic acid into diethyl ether.[7] The same author has investigated the extraction of osmium fluoride and the platinum-metal chlorides from hydrofluoric acid solutions into ether, and reports that no extraction occurred.[8] The extraction of gold^{III} from 8 *M* nitric acid solutions into ether has been noted,[9] but the writer is not aware of the incorporation of this work into any practical separation.

Reference has already been made to the inclusion of partition chromatography within the scope of this review. Broadly speaking, we are concerned here with the partition of salts of the precious metals between a stationary aqueous phase, held in the interstices of a column of cellulose, and a moving phase containing one or more organic solvents. It has been found that these metals are carried along to different extents by the solvent and, given the proper conditions, they can be spread out and separated in the form of bands along a strip of paper. Thus, Burstall, Davies, Linstead and Wells[10] have shown the separation of gold and the six platinum metals by means of a solvent containing ethyl (or *iso*propyl) methyl ketone and hydrochloric acid. Under the conditions chosen iridium became tervalent and moved along with the rhodium, so that a second operation was required for the separation of these two. Lederer,[11] by means of a solvent containing butanol, hydrochloric acid and nitric acid, kept iridium quadrivalent, in which condition it travelled with platinum in the solvent, while the separation of the other metals was about the same as observed by Burstall *et al.* The findings of these authors are supported by the work of Shibata and Uemera,[12] who found the same order of development for gold, platinum, palladium and rhodium when the solvent was ether or mixed butanol and pentanol with hydrochloric acid. These authors, and also Anderson and Lederer,[13] have separated gold from platinum and palladium, ether being used as the moving solvent. Kember and Wells[14] similarly separated gold from platinum metals and selected base metals on paper strips or cellulose columns with the aid of an eluting solvent consisting of ethyl acetate and nitric acid.

Burstall and Wells[15] have extended the use of organic solvent - acid mixtures to the removal of gold, as aurocyanide, from an anion-exchange resin. Several organic solvents were investigated, with best results from a solution of acetone and hydrochloric acid, and practically complete elution by a solution of methanol and hydrochloric acid.

Complexes with stannous chloride

Solutions of five of the platinum metals in hydrochloric acid yield coloured products when treated with stannous chloride. These reactions are frequently used for spot tests and for colorimetric determinations of certain of the metals. It had been thought, until recently, that these coloured products contained the platinum metals in some lower valency state, *e.g.*, chloroplatinous acid, or perhaps that they were colloidal dispersions of the metals similar to the many-coloured varieties of gold produced by stannous chloride. New and important light has been shed upon the matter by the work of Ayres and Meyer.[16,17] Concerning the interaction of chloroplatinates and stannous chloride, it now appears necessary to assume the existence of a complex species containing tin and platinum in solution, and possibly formulated as $(PtSn_4Cl_4)Cl_4$. Similar experimental information is wanting for the other platinum metals, but the behaviour of palladium, rhodium and iridium prompts the guess that similar reactions occur with these metals also. The coloured complexes are extracted, altogether or in part, by oxygen-containing solvents such as esters and ethers.

The behaviour of palladium is unusual in two respects. Wölbling[18] has recorded that, if palladium solutions are first made ammoniacal and then acidified to about *M* before the addition of stannous chloride, the palladium colour is not extracted. The work of Ayres and Meyer[16] does not throw much light on this statement, but does demonstrate that the

product formed from palladium solutions varies a good deal according to the concentration of hydrochloric acid and stannous chloride. A second anomaly described by these authors concerns the interference of palladium on the colorimetric determination of platinum after extraction into pentyl acetate. They found that "interference increased up to a concentration of 5 p.p.m. [of palladium], after which it remained constant up to concentrations of 50 p.p.m. or greater." This unaccountable observation seems only to bear out the greater complexity of the palladium system compared to that of platinum.

This extraction procedure is remarkably effective for separating small amounts of platinum from moderate amounts of base metals and possesses the advantage that the separated platinum is already available for an absorptiometric determination. It seems to offer no help with the problem of separating the individual members of the platinum group.

Oxides

The distribution of osmium tetroxide between carbon tetrachloride and water has been thoroughly studied.[19] Whilst no direct analytical application of this information has been made, Sauerbrunn and Sandell[20] have utilised this distribution ratio to establish the dissociation constants for osmic acid.

Organic compounds

Many organic reagents in inorganic analysis owe their usefulness to the formation of chelate compounds with inorganic ions. The chelate species may be ionic, like Ru(*o*-phenanthroline)$_3^{\cdots}$, or molecular, like Pd(dimethylglyoxime)$_2$. In the latter event appreciable solubility in organic solvents may be expected, with the possible application of solvent extraction. It is also well known that some selectivity of reaction can be achieved by regulating the pH of the aqueous solution containing these reagents.

Although many chelate compounds of the precious metals have been prepared and characterised, it is also true that many of the organic reagents that have been recommended for analysis of the platinum metals either do not yield chelate compounds or yield products of uncertain or undetermined composition. One difficulty in the way of easy formation of chelate compounds is the fact that the common habitat for these metals is in stable complex ions such as $AuCl_4'$, $PtCl_6''$, $RuCl_5OH'''$. Ryan[21] has recently discussed the nature of several compounds investigated for use in analyses of the platinum metals. He has shown that these appear to be predominantly of the form $(RH)_2MX_6$ (R = amine, M = platinum metal, X = halogen) or $R_2M'X_2$ (R = monodentate amine, M' = Pd or Pt). Whether this observation may be taken as representative of the majority of organic reagents for the analysis of platinum metals remains to be established by more critical examination of the composition of many compounds. The consequences of Ryan's generalisation upon the prospects for solvent extraction will be apparent. The first class of compounds involving hexachloro (hexabromo and so on) complexes will presumably be essentially ionic in character and these will exist as substances only in the solid state. Reagents forming such compounds would not be expected to enhance the solvent extraction of the platinum metals. The second category apparently consists of non-ionic substances related to $Pd(NH_3)_2Cl_2$, and solubility in organic solvents may be expected. Among recorded applications of solvent extraction of organic derivatives of the platinum metals, palladium and bivalent platinum compounds are well represented, while the other platinum metals and quadrivalent platinum appear rarely or not at all.

From the examples that follow it will be seen that several procedures are available for the extraction of co-ordination compounds of palladium, and that, provided platinum remains quadrivalent, an analytical separation of palladium from the other platinum metals is possible. Further, platinum may be removed by solvent extraction after the removal of, or together with, palladium under conditions that favour its reduction to the bivalent state. No other separation among these elements has been developed to a point that would permit its analytical application.

The only recorded instance of solvent extraction of an organic derivative of gold is that of the compound formed with *p*-diethylaminobenzylidenerhodanine, which may be extracted into ether or chloroform.[22] However, there appears to be no particular purpose in making such an extraction.

Small amounts of palladium may be separated from silver, gold and the other platinum metals by extraction of its dimethylglyoxime complex, $Pd(C_4H_7O_2N_2)_2$, into chloroform.[23]

A small amount of nitric acid in the aqueous phase prevents the reduction of gold to metal or the formation of the platinous complex with this reagent. Although Ayres and Tuffly[24] were unable to separate 10 to 20 mg of palladium by this procedure, Fraser, McBryde and Beamish[25] state that it is applicable to amounts as small as 2 μg and as great as 1 mg. These authors have also separated palladium from large amounts of lead and from other base metals, interference from iron^{III} being avoided by the addition of ethylenediaminetetra-acetic acid.

Ryan[26,27] has developed a similar extraction procedure for palladium, in which the complex formed by *p*-nitrosodiphenylamine, $Pd(C_{12}H_{10}N_2O)_2Cl_2$, is extracted into ethyl acetate, ether or chloroform. In the cold the other platinum metals (except Pt^{II}) do not react with this substance and so remain in the aqueous phase. When the aqueous solution is warmed, platinum, iridium and rhodium produce coloured products, the first being extractable into the same solvents. It is believed that the effect of heating is to cause reduction of platinum to the bivalent state, in which it does react with this reagent in the same way as palladium^{II}. In comparing these two procedures it is interesting to record that dimethylglyoxime prevents the reaction of palladium with *p*-nitrosodiphenylamine. Recently, Yoe and Kirkland[28] used *p*-nitrosodimethylaniline in much the same way to separate 200 μg to 1 mg of palladium from smaller amounts of platinum. The extraction in this case was limited to pure chloroform.

1-Nitroso-2-naphthol is said to be a more sensitive reagent than dimethylglyoxime for the precipitation of palladium[29] and it is specific for palladium among the platinum metals. The compound $Pd(C_{10}H_6NO_2)_2$ can be extracted into toluene to give an orange solution, whilst the derivative of 2-nitroso-1-naphthol yields a violet solution in toluene. These extracts have been recommended for absorptiometric determination of the metal.[30]

Dithizone forms characteristically coloured products with gold, palladium and platinum^{II} which are extracted into chloroform. Young[23] has utilised these reactions to provide a volumetric determination of these metals as they occur in assay beads. The titrant is an empirically standardised solution of dithizone in chloroform. Under the recommended conditions the other platinum metals do not interfere, although in alkaline or ammoniacal solutions all the platinum metals form dithizonates that are soluble in chloroform or carbon tetrachloride.[31]

Another volumetric procedure in which solvent extraction plays a part, although different from the preceding one, is that proposed by Pollard for the determination of palladium[29] or platinum.[32,33] Here a standardised solution of sodium diethyldithiocarbamate is added to decolorise the compounds formed by palladium or platinum in hydrochloric acid and stannous chloride. The product of the decolorising reaction is a voluminous precipitate that can be removed by extraction into a supernatant layer of benzene. The end-point of the titration occurs when the aqueous phase has been decolorised, and from the volume of the reagent solution used the amount of metal is calculated. A recent development of this procedure proposed by Yoe and Kirkland[28] is the simultaneous separation of palladium and platinum from the other platinum metals by extraction into chloroform of their addition products with sodium diethyldithiocarbamate. Potassium iodide in a moderately concentrated acidic solution serves to reduce the platinum to the bivalent state.

In the examples that follow, the organic complexes have not been as well characterised and there is less evidence of selectivity. There is obvious need for further investigation in some cases.

A number of derivatives of thiourea form complexes with various of the platinum metals under appropriate conditions, and some of these can be extracted into immiscible solvents. Thus, Ayres and Tuffly[24] undertook to separate 10 to 25 mg of palladium by extraction of its phenylthiourea complex with pentyl acetate. A separation from gold was achieved, but the reagent suffers from lack of specificity, especially as regards platinum. Thiosemicarbazide has been recommended as an absorptiometric reagent for platinum,[34] and the blue-coloured product can be extracted into acetic esters or pentanol. The same reagent and two diphenyl derivatives form coloured products with ruthenium that can be extracted into ether,[35] and Sandell[22] has recommended the use of the phenyl derivative for the colorimetric determination of ruthenium, stating that the red or red-violet product can be extracted into butanol. Oxine (8-hydroxyquinoline) is reported[22] to form compounds with palladium and tervalent ruthenium which are soluble in chloroform, but no data are furnished for reactions of oxine with the other precious metals.

The ammine-type compounds formed by chlororhodites and chloro-iridites with pyridine, *e.g.*, $Rhpy_3Cl_3$, can be extracted into chloroform and have served for the separation of these two elements.[36]

METALS

The fire assay is an analytical operation peculiar to the precious metals. Although not ordinarily so considered, it may be regarded as a sort of solvent extraction in part. The solvent here is molten lead, formed in fine droplets by the reduction of litharge during the fusion of a suitable charge that includes an ore containing the precious metals. The latter are collected in the lead which settles out from the vitreous melt to form a separate phase. This lead-base alloy, on cooling, is known as a button. The choice of lead for this purpose is due partly to its great density, but more especially to the ease with which it can be separated from the precious metals by the operation of cupellation.

It has been shown that cupellation is accompanied by losses of several of the platinum metals in various ways,[37] and there is every likelihood that reliable assays for platinum metals may require analysis of the button itself. This would require the determination of perhaps a milligram of precious metal in the presence of several grams of lead, and this is not an easy matter. The task has been rendered easier by the application of ion-exchange resins, for it has been shown that the commonest platinum metals can be separated by a column of resin from relatively large amounts of lead and other base metals.[38,39] If the cupellation step is to be eliminated for the assay for the platinum metals, then the assayer is no longer restricted to lead as the extracting metal during fusion of the ore and flux. There are reasons, based upon geochemical distribution and upon the alloying properties of these elements, for guessing that iron or nickel or both might be better collectors in the assay fusion. A very extensive and practical field of investigation is thus indicated.

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A Colorimetric Method for the Determination of Copper in Alloyed Steels with 2:2′-Diquinolyl

BY W. T. ELWELL

A colorimetric procedure is recommended for the determination of copper in many metals and alloys. It has been successfully applied to a variety of materials, including plain carbon and highly alloyed steels, ferro-alloys (tungsten, molybdenum, vanadium and titanium), pure cobalt and pure nickel, containing copper over the range 0·001 to 1 per cent. The procedure is not, however, recommended as a precise method when the copper content exceeds about 0·25 per cent.

An oxidised solution of the sample is reduced with sulphurous acid, adjusted to a pH value of 5 to 6, then extracted with a solution of 2:2′-diquinolyl in amyl alcohol in the presence of citric acid. The violet colour, which is proportional to the amount of copper present, does not fade and is measured in any convenient way.

THE determination of copper in steels and ferro-alloys almost invariably depends upon a preliminary isolation of copper as sulphide or thiocyanate. These procedures are reliable but slow, and limitations are imposed when the weight of sample is restricted and the copper content is below about 0·15 per cent.

Work has been published recently[1,2] on the use of 2:2′-diquinolyl as a sensitive reagent containing a "copper specific" group. This reagent, which is about eight times more sensitive than sodium diethyldithiocarbamate, was first mentioned in this capacity by Breckenridge, Lewis and Quick.[3] Two papers have appeared recently, one by Cheng and Bray[4] for the determination of copper in soil and plant materials, the other by Hoste, Eeckhout and Gillis[5] for the determination of copper in water, animal tissues and blood plasma. Hoste *et al.* extended their procedure to the examination of two "synthetic" steel solutions, and our experimental work on actual steel samples was well advanced when their publication was noted; but, to the best of our knowledge, there is no report in the literature on the application of 2:2′-diquinolyl to actual samples of highly alloyed steels, and our attempts to apply Hoste's conditions to such samples, containing upwards of 5 per cent. of chromium, were not successful.

The experimental work reported here is an independent approach to the problem and incorporates many features which are considered necessary for the successful application of the 2:2′-diquinolyl procedure to the analysis of metallurgical samples.

EXPERIMENTAL

The 2:2′-diquinolyl procedure is usually applied to "simple" solutions, adjusted to a pH value of between 4 and 7, after reduction of the copper, in cold solution, with hydroxylamine hydrochloride. The cuprous compound is extracted with amyl alcohol, used as solvent for the reagent, and the violet colour is measured photometrically.

In view of the specific nature of 2:2′-diquinolyl it seemed reasonable to expect that, by some simple modification, the procedure could be extended to solutions containing relatively large amounts of metallic ions. Preliminary qualitative experiments indicated that, provided

a suitable complexing reagent, *e.g.*, citric acid, was present to prevent precipitation of hydroxides, the procedure could be applied directly to steel solutions. On the basis of these experiments a tentative procedure was applied to 50-mg samples of pure iron containing added copper ranging from 0·02 to 0·10 mg: the equivalent of 0·04 to 0·20 per cent. of copper. The results obtained were very promising and enabled an extinction factor to be derived for application to other samples rich in iron.

The tentative procedure was applied to four plain carbon steels that had previously been examined for copper by a preliminary sulphide precipitation and also to four British Chemical Standard Steels (issued by The Bureau of Analysed Samples Ltd.); the results are shown in Table I.

TABLE I

DETERMINATION OF COPPER IN STEELS

Sample	Copper			Remarks*
	By tentative procedure, %	Previously determined value, %	Certificate value,* %	
Plain carbon steel 61A	0·104	0·10		
Plain carbon steel 61B	0·086	0·09		
Plain carbon steel 62	0·073	0·075		
Plain carbon steel 63	0·076	0·08		
B.C.S. No. 224 (Cr, 1·5 per cent.; V, 0·25 per cent.)	0·07		0·07	
B.C.S. No. 235, Stainless 18/8 (Ti, 0·6 per cent.; W, 0·7 per cent.)	0·75		0·98	
B.C.S. No. 246, Stainless 18/12 (Mo, 2·9 per cent.; Nb, 0·8 per cent.)	0·05		0·13	Average of 3 results
B.C.S. No. 261, Stainless 18/12 (Nb, 0·7 per cent.)	0·03		0·06	Single result only

* Many of the British Chemical Standards are not intended to be used as copper standards. Where a certified value has been included, without reference in the Remarks column, the copper content is an approved value.

In view of the favourable results for the plain carbon steel, the results for the alloy steels were disappointing. No marked improvement was observed when there was a threefold increase in the amount of hydroxylamine hydrochloride used, or when hydrazine was used as an alternative reducing agent at room temperature. It was therefore suspected that either chromium or nickel or both was causing serious interference.

One important feature of the procedure had not been overlooked, *viz.*, the efficiency of extraction of the cuprous compound by the organic solvent. Our earlier work was based on a single extraction, since we had shown on "simple" solutions, and on solutions containing only iron as a major constituent, that this gave, within practical limits, complete extraction. Hoste *et al.* indicate that the efficiency of a single extraction, under conditions comparable with those of our tentative procedure, is about 99 per cent.[5] It was appreciated that to increase the number of extractions would have the disadvantage of decreasing the depth of colour to be measured. This is not a serious problem; a larger absorption cell could be used, but it is desirable from many aspects to complete the determination after a single extraction. It was shown that, even after prolonged shaking, recoveries of copper from solutions of alloy steels were invariably low. This could not be associated with the stability of the colour, since we had shown qualitatively that the colours of some solutions remained constant after standing for 48 hours. In order to establish to what extent further extractions were necessary, B.C.S. No. 246 was re-examined. A sample of a solution of B.C.S. No. 246, to which copper was added to simulate a steel containing 0·24 per cent. of copper, was also examined. Solutions of these steels were extracted three times with the organic solvent. The amount of copper recovered from each extraction is shown in Table II.

Similar experiments were made with a solution of B.C.S. No. 235, but it was only after a total of six extractions that complete recovery (0·98 per cent. of copper) was achieved.

A result of 0·09 per cent. of copper on B.C.S. No. 261 was obtained after three extractions;

this is much higher than the provisional value of 0·06 per cent. supplied with the sample. This high result was later confirmed (see Table IV).

In order to establish the extent to which chromium and nickel interfered, 0·5 g of sample No. 61A was dissolved in hydrochloric acid, oxidised with nitric acid (to ensure complete solution of copper) and the solution was evaporated to dryness to remove excess of nitric acid.

TABLE II

EXTRACTION OF COPPER FROM STEEL

	Copper found in	
	B.C.S. 246, %	B.C.S. 246 + added copper, %
First extract	0·090	0·168
Second extract	0·024	0·062
Third extract	0·007	0·010
Total	0·121	0·240

The residue was dissolved in hydrochloric acid and diluted to 100 ml with water. Various amounts of standard nickel and chromium solutions were added to 10·0-ml aliquots of the steel solution; copper was then determined by the tentative procedure (single extraction); the results were as follows, the chromium and nickel being given as equivalent percentages of the weight of sample 61A taken (calculations are based on a 50-mg sample)—

Chromium added, per cent. ..	—	5	10	15	20	—	—	—	—
Nickel added, per cent. ..	—	—	—	—	—	5	7·5	10	15
Copper found, per cent. ..	0·108	0·074	0·048	0·012	0·014	0·111	0·118	0·126	0·135

These results show conclusively that recovery of copper from solutions of alloy steel decreases with increase in chromium content. The high results obtained in the presence of nickel are caused by copper present in the metallic nickel (not high-purity quality) used in the preparation of the standard solution. The chromium standard was prepared from analytical-reagent grade potassium dichromate (reduced with sulphur dioxide and adjusted to pH 6·0 in the presence of a slight excess of citric acid).

This unexpected interference by chromium led us to make a number of qualitative tests, which indicated that, provided the chromium was in the chromous condition, quantitative recoveries were possible. In one series of qualitative tests, copper was extracted from a chromous solution, the chromous solution was then allowed to oxidise, an equivalent amount of copper was added to the solution and the extraction was repeated; this gave only a faint positive reaction for copper. The inconvenience of keeping all the chromium in the chromous state caused us to seek some alternative procedure.

The standard oxidation potentials are stated to be[6]—

$$Cu^{\cdot} \rightleftharpoons Cu^{\cdot\cdot} + e' \qquad E_0 = -0{\cdot}167$$
$$Fe^{\cdot\cdot} \rightleftharpoons Fe^{\cdot\cdot\cdot} + e' \qquad E_0 = -0{\cdot}771$$
$$Cr^{\cdot\cdot} \rightleftharpoons Cr^{\cdot\cdot\cdot} + e' \qquad E_0 = +0{\cdot}41.$$

So one would not expect the interference to be caused by $Cr^{\cdot\cdot\cdot}$ acting as an oxidising agent.

At the time this experimental work was proceeding we were also investigating the determination of small amounts of copper in chromium salts containing very small amounts of iron. The results obtained were variable and consistently low, but in one experiment, in which the chromium was in the tervalent condition, ferrous iron was added to simulate the composition of a steel sample, and there was qualitative evidence that the copper recovery had been considerably improved. It appeared that when a large excess of chromium was present it was necessary to have a large excess of ferrous iron present, more than would be normally produced when a cold ferric solution was treated with hydroxylamine hydrochloride. This led us to seek the best and simplest means of ensuring the presence of sufficient ferrous iron to prevent re-oxidation of copper after reduction. Hydroxylamine and hydrazine had proved inefficient reducing agents at room temperature, and increased amounts of these reagents, and also sulphurous acid, were separately applied at higher temperatures.

When sample B.C.S. No. 246 was re-examined by reduction of the boiling solution, about 85 per cent. recovery of copper was obtained with hydroxylamine, but when hydrazine

and sulphurous acid were used separately, complete recovery was achieved by a single extraction in each case. Sulphurous acid is cheap and is readily available in a very pure condition; it was therefore decided to investigate further the use of this reagent.

We confirmed that, using a specified volume of sulphurous acid and boiling for 5 minutes to ensure complete reduction of iron, the amount of sulphur dioxide remaining had no harmful effect on the final colour; the excess is probably desirable in that it ensures reducing conditions during the extraction and subsequent measurement of the colour. With a standard copper solution, it was found necessary to add a ferrous salt in order to obtain a constant extinction factor. Other modifications introduced at this stage aimed at making the method applicable to alloy steels containing either tungsten or molybdenum or both; a simplified method of pH control to avoid the use of a pH meter was also incorporated.

The revised procedure was first applied to a low-alloy steel (B.C.S. No. 252), and the effect of various time factors on the stability of the colour was investigated, the results being shown below. All tests were made in duplicate.

The results obtained after the solutions had been shaken and allowed to stand for various periods before the organic layers were separated and examined were as follows, B.C.S. No. 252 having a certified value of 0·11 per cent. of copper—

Standing period, minutes	5	10	30
Test A: copper found, per cent.	0·123	0·122	0·121
Test B: copper found, per cent.	0·122	0·123	0·122

The results obtained for B.C.S. No. 252 after the solutions had been shaken and allowed to stand for 5 minutes before removal of the organic layers are as follows; the coloured solutions were retained in covered cells and subsequently examined at regular intervals as indicated—

Standing period, minutes..	Nil	10	30	60	120
Test A: copper found, per cent.	0·122	0·120	0·118	0·116	0·113
Test B: copper found, per cent.	0·122	0·121	0·118	0·117	0·114

The revised procedure was next applied to samples No. 61A and B.C.S. No. 246 and a sample of No. 61A to which had been added the equivalent of 20 per cent. of chromium. (Calculations are based on a 50-mg sample.) The following results were obtained—

Sample No.	61A	61A + Chromium	B.C.S. No. 246
Copper found, per cent.	0·110	0·114	0·121

On the basis of these good results the revised (recommended) procedure was applied to a variety of metals (Table III) and ferrous alloys (Table IV), the majority being British Chemical Standards.

TABLE III

RESULTS FOR PURE METALS OF CERTIFIED (SPECTROGRAPHIC) 0·001 PER CENT. COPPER CONTENT

Copper added, per cent.	Nil	0·001	0·005	0·010	0·020	0·050
Copper found in*—						
High-purity iron,† per cent.	0·001	0·001	0·005	0·010	0·018	0·049
High-purity cobalt, per cent.	0·002	0·001	0·005	0·011	0·023	0·047
High-purity nickel, per cent.	0·002	0·001	0·004	0·010	0·022	0·053

* After deduction of percentage of copper in pure metal. † Not a British Chemical Standard.

METHOD

Optical densities are measured at 20° C at a wavelength of 546 mμ. Recommended filters for use with the Spekker photo-electric absorptiometer (mercury-vapour lamp) are Calorex H503 and Ilford No. 604.

REAGENTS—

All reagents must be of the highest purity obtainable. The use of distilled water is essential; it must be free from copper.

Sulphurous acid—Prepared by saturating water with sulphur dioxide.

2:2′-*Diquinolyl solution*—Dissolve 50 mg of the reagent in 100 ml of amyl alcohol. (Redistilled amyl alcohol, as supplied for milk testing, is suitable.)

PROCEDURE—

Transfer 0·5 g of the steel millings to a suitable beaker and dissolve the sample in about 5 ml of concentrated hydrochloric acid, sp.gr. 1·18 (Note 1), cool, oxidise with a slight excess of concentrated nitric acid, sp.gr. 1·42, and evaporate to dryness; do not bake. Add a few drops of concentrated hydrochloric acid, sp.gr. 1·18, and evaporate as before. Cool, extract the residue with warm water and add a few drops of 20 per cent. v/v hydrochloric acid, if

TABLE IV

RESULTS FOR VARIOUS STEELS

Sample	Lab. Ref. No.	B.C.S. No.	Copper: New procedure, %	Copper: Certificate value,* %	Remarks*
	61A		0·11	0·10	Previous laboratory results
	61B		0·091	0·09	
	62		0·074	0·075	
	63		0·078	0·08	
Plain carbon steel		154	0·031	0·03	
		161	0·041	0·02	Single result only
		163	0·055	0·05	
		213	0·13	0·12	
		218	0·16	0·16	
High-purity iron		260	0·006	0·003	Single result only
High-purity iron		149	0·001	⩽0·002	
Cast steel		230	0·085	0·085	
Low-alloy steel		251	0·082	0·09	
Low-alloy steel		252	0·12	0·11	
Low-alloy steel		254	0·12	0·11	
Low-alloy steel		258	0·18	0·185	
Chrome - vanadium steel ..		224	0·072	0·07	
13 per cent. of chromium steel		211	0·085	0·08	
18/8 steel		209	0·055	0·035	Single result only
18/8 - Ti steel	674		0·15	0·15	Previous laboratory result
18/8 - Ti - W steel		235	0·96	0·98	
18/12 - Nb steel		261	0·096	0·06	Single result only
18/12 - Nb - 3Mo steel ..		246	0·12	0·13	Average of 3 results
16W, 3Cr, 4Co, 0·5 Mo steel ..		167	0·05	0·05	Single result only
17Ni, 1Cr, 0·3 Mo steel ..		225	0·10	0·09	Average of 3 results
Ferromolybdenum		200	0·082	0·07	
Ferrotungsten		202/2	0·021	0·02	Single result only
Ferrotitanium		243	0·082	0·085	Average of 2 results
Ferrotitanium		243/2	1·16	1·21	
Ferromanganese		208	0·025	0·025	Average of 3 results
Ferrovanadium		61†	0·31	0·29	U.S. National Standard

* Many of the British Chemical Standards are not intended to be used as copper standards. Where a certified value has been included, without reference in the Remarks column, the copper content is an approved value.

† N.B.S. No.

necessary, to obtain complete solution of soluble salts (Note 2). Dilute with water to about 25-ml and add 25 ml of sulphurous acid. Boil for 5 minutes, cool, add 15 ml of 50 per cent. citric acid solution and transfer to a 100-ml calibrated flask (Note 3). Dilute with water to the calibration mark (20° C) and mix well.

Transfer a 10·0-ml aliquot to a 100-ml beaker and carefully add 10 per cent. w/w ammonia solution until the pH value of the solution is between 5 and 6 (Note 4). Add 2 ml of 5 per cent. sodium acetate solution and transfer to a 50-ml glass-stoppered, graduated separating funnel provided with a short 1-inch tapered stem. Adjust the volume with water to 17·5 ml, mix thoroughly, then add 10·0 ml of the 2:2′-diquinolyl reagent. Shake vigorously for about 2 minutes, then allow the two layers to separate for 5 minutes. Run off the lower aqueous layer, then dry the inside of the stem with filter-paper. Transfer the coloured organic layer,

via a dry filter-paper, into a 1-cm cell, and measure the optical density; use the blank solution in the compensating cell. Then—

$E_{1cm} \times 0{\cdot}109$ = copper, mg (Note 5)
i.e., $E_{1cm} \times 0{\cdot}218$ = copper, per cent. (with 0·5 g per 100 ml used).

NOTES—

1. This weight of sample is recommended in order to minimise sampling error (see also Note 3) and should be used when the copper content does not exceed about 0·2 per cent. When the copper content is high (about 1 per cent.), take the same weight of sample, but adjust the final volume to 1 litre before withdrawing the 10·0-ml aliquot. The method is not, however, primarily intended for samples containing so much copper.

Steels which do not dissolve readily in the hydrochloric acid may be dissolved in the nitric acid or a mixture of the two acids.

In the examination of ferro-alloys, *e.g.*, ferrotungsten or ferromolybdenum, transfer the prepared sample to a small platinum dish provided with a lid, and add 5 to 10 ml of 50 per cent. v/v sulphuric acid and 5 ml of 40 per cent. w/w hydrofluoric acid. Dissolve the sample in concentrated nitric acid, sp.gr. 1·42, added dropwise.

In the examination of ferromanganese, ferrovanadium or ferrotitanium, transfer the prepared sample to a beaker, add 5 to 10 ml of 50 per cent. v/v sulphuric acid and dissolve in concentrated nitric acid, sp.gr. 1·42, added dropwise.

In both cases evaporate, using a radiant heater, to fumes of sulphur trioxide, cool, extract with warm water and a few drops of 20 per cent. v/v hydrochloric acid, then proceed as described in the procedure. These instructions are intended to serve as a general guide to the method of obtaining a satisfactory solution of the material. Each ferro-alloy must be considered individually.

Determine a blank on all reagents; proceed exactly as described in the procedure.

2. When the sample contains either tungsten or molybdenum or both, a precipitate will be present at this stage and should be ignored.

If necessary add iron at this stage (or after transferring the 10·0-ml aliquot) to ensure the presence of at least 40 mg of iron (200 mg of $FeSO_4.7H_2O$) in the final 17·5 ml. Additional iron is not necessary in the examination of alloy steels of the type shown in Table IV.

3. In the examination of samples high in tungsten or molybdenum, add 15 per cent. w/w ammonia solution until the precipitated tungstic or molybdic acid has just dissolved, then acidify with 20 per cent. v/v hydrochloric acid, preferably to a pH value of between 5 and 6.

When the copper content of any sample is below about 0·02 per cent., do not dilute to 100 ml and take an aliquot, but use the entire sample. When the copper content is above this value, it is permissible to weigh out a proportionately smaller sample and use the entire sample (see also Note 1). In these cases proceed as described for a 10·0-ml aliquot and keep the volume as low as possible before adding the 2:2′-diquinolyl reagent.

4. Some solutions show characteristic colour changes during neutralisation. A sufficiently accurate adjustment of the solution can be made with the aid of a piece of 0·5-mm platinum wire, as follows.

Add the ammonia solution until a small drop of the test solution is just alkaline to blue litmus paper, then add just sufficient 10 per cent. citric acid solution to render it acidic to the same test paper.

Alternatively the solution can be adjusted with the aid of two indicators spotted out in small drops on to a white tile. Add the ammonia solution until the test solution is alkaline to methyl orange but acidic to methyl red. At this stage add 1 more drop of the ammonia solution.

In either case test the solution against a narrow range pH paper; a pH value of between 5 and 6 should be indicated.

There is, of course, no objection to the use of a pH meter.

5. This factor was obtained with high-purity iron to which had been added various amounts of a standard copper solution; it must be determined for every individual instrument and filter. The optical densities were measured on a Spekker absorptiometer as indicated.

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Determination of Copper in Plant Materials by Means of Zinc Dibenzyldithiocarbamate

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A method is described for the rapid absorptiometric determination of copper in plant materials over the range 0 to 15·0 p.p.m. The organic matter is destroyed by digestion with sulphuric, nitric and perchloric acids. The copper, after dilution of the acid solution, is extracted with a solution of zinc dibenzyldithiocarbamate in carbon tetrachloride, and the optical density measured at a wavelength of 440 mμ. The method is shown to be free from interference by other commonly occurring metals in plant materials and to give good recovery of added copper.

The importance of copper in herbage is such that any new reagent for its determination is worth investigation. Sodium diethyldithiocarbamate has probably been the most widely used reagent for the determination of copper.

Ekman and Lundell[1] used a wet-ashing technique, and, after removal of silica, dissolved the ash in ammonium citrate, and determined the copper by means of sodium diethyldithiocarbamate. Iron was found to interfere even in the presence of large amounts of the citrate ion and, if interference was detected, the iron was removed by shaking the sodium diethyldithiocarbamate complex in carbon tetrachloride with an equal volume of dilute ammonia (Nydahl[2]).

Piper[3] digested the plant material with a mixture of nitric, perchloric and sulphuric acids, extracted the copper by means of dithizone from an acid solution and, after evaporating off the solvent and digesting the residue, finally determined the copper as the diethyldithiocarbamate complex. This procedure may give greater purity, but losses of copper or greater blanks may occur, owing to the longer technique involved.

Martens and Githens[4] have shown that small amounts of copper can be separated directly in acid solutions from relatively large amounts of most other metals by means of zinc dibenzyldithiocarbamate. No precipitations or filtrations are required, and the addition of the citrate ion appears unnecessary. The reagent solution and the colour of the extracted copper dibenzyldithiocarbamate are stable, and the acid solution remaining after extraction may be used to determine other metals, such as cobalt, molybdenum and so on.

The use of zinc dibenzyldithiocarbamate as a reagent for determining copper in plant materials was accordingly investigated. Recently, Abbott and Polhill[5] have shown that the dibenzylammonium and potassium salts of dibenzyldithiocarbamic acid are equally effective.

Experimental

Effect of interfering elements—

Solutions containing standard amounts of copper, from 10 to 40 μg, were extracted from 50 ml of 5 per cent. v/v sulphuric acid, with and without the addition of potentially

* Present address: Laporte Chemicals Ltd., Luton, Beds.

interfering metals, with 20 ml of a 0·01 per cent. solution of zinc dibenzyldithiocarbamate in carbon tetrachloride. After the extract had been shaken for 1 minute it was filtered through a Whatman No. 1 filter-paper and the optical density measured in ¾-inch tubes on the Unicam SP350 spectrophotometer.

The results shown in Table I indicate that there is little interference over the range 10 to 40 μg of copper from 1000 μg of iron, 100 μg of cobalt and 1000 μg of manganese.

TABLE I

RECOVERY OF COPPER IN THE PRESENCE OF OTHER IONS

Metal ion added	Amount of metal ion added, μg	Copper, μg	Copper recovered, μg
Iron	1000	10·0	10·0
Iron	1000	20·0	20·0
Iron	1000	30·0	30·0
Iron	1000	40·0	39·9
Iron	1000		
Cobalt	100	10·0	10·1
Manganese	1000		
Iron	1000		
Cobalt	100	20·0	20·3
Manganese	1000		
Iron	1000		
Cobalt	100	40·0	40·0
Manganese	1000		
Molybdenum	100	20·0	20·0
Molybdenum	500	20·0	20·4
Molybdenum	1000	20·0	20·9

RECOVERY OF COPPER AFTER DIGESTION—

Amounts of copper from 10 to 40 μg were digested with a mixture of 3 ml of concentrated sulphuric acid, 15 ml of concentrated nitric acid and 2 ml of perchloric acid, and finally, after treatment with hydrogen peroxide, the solution was diluted and extracted with 20 ml of 0·1 per cent. solution of zinc dibenzyldithiocarbamate in carbon tetrachloride.

Results showed that there was no loss of copper during the digestion, when compared with standards extracted directly from 3 per cent. v/v sulphuric acid.

METHOD

REAGENTS—

Sulphuric acid, concentrated—Micro-analytical purity.
Nitric acid, concentrated—Distilled.
Perchloric acid, 60 per cent. w/w—AnalaR.
Hydrogen peroxide, 100 volume—AnalaR.
Carbon tetrachloride.
Zinc dibenzyldithiocarbamate—A 0·01 per cent. w/v solution in carbon tetrachloride of the solid supplied by Hopkin and Williams Ltd.

PROCEDURE—

Digestion of sample—Weigh 2 g of plant material into a dry 100-ml Kjeldahl digestion flask. Add 3 ml of sulphuric acid, 2 ml of perchloric acid and 15 ml of nitric acid. Digest over a small burner for about ½ hour until the solution is colourless. Cool, add 2 ml of hydrogen peroxide and heat to fuming. When cool, add 10 ml of water and again heat to fuming. Set aside to cool.

Extraction of copper—Add 20 ml of water, transfer to a 100-ml separating funnel and dilute to about 50 ml. Add 20 ml of 0·01 per cent. zinc dibenzyldithiocarbamate in carbon tetrachloride and shake for 1 minute. Filter the yellow-coloured carbon tetrachloride layer through a Whatman No. 1 filter-paper and measure the optical density of the extract in ¾-inch tubes at a wavelength of 440 mμ. Carry out a blank determination on all reagents. Read off the amount of copper present from a standard curve prepared as described below.

Preparation of standard curve—Weigh 0·3928 g of copper sulphate, $CuSO_4.5H_2O$, and

dissolve it in about 100 ml of water. Add 5 ml of concentrated sulphuric acid and dilute to 1000 ml; 1 ml of solution contains 100 μg of copper.

Dilute 10 ml of this concentrated solution to 1 litre with glass-distilled water; 1 ml of this solution contains 1·0 μg of copper.

Measure 0, 10, 20, 30 and 40-ml portions of the dilute copper solution into a 100-ml separating funnel, add 2·5 ml of concentrated sulphuric acid and dilute to 50 ml. Extract with 20 ml of the reagent as described above and measure the optical density in $\frac{3}{4}$-inch tubes at a wavelength of 440 mμ.

Results

A number of samples have been examined for their copper content, with and without the addition of copper. Results for the recovery of added copper are shown in Table II.

Table II

Copper in herbage, p.p.m.	Copper added, p.p.m.	Copper found, p.p.m.	Copper recovered, p.p.m.
10·0	5·0	15·0	5·0
11·6	5·0	16·6	5·0
10·2	5·0	15·2	5·0
2·0	5·0	7·0	5·0
3·8	5·0	8·7	4·9
2·2	5·0	7·3	5·1
3·8	5·0	9·0	5·2
8·2	5·0	13·2	5·0
10·2	5·0	15·2	5·0
7·9	5·0	13·2	5·3
7·2	5·0	12·3	5·1
7·8	5·0	12·7	4·9

Conclusions

The use of zinc dibenzyldithiocarbamate as a reagent for determining copper in plant materials has been shown to give good recovery of added copper and to be free from interference from other elements commonly present in herbage. The digestion mixture and procedure chosen leads to no loss of copper.

Grateful thanks are given to the Ministry of Agriculture and Fisheries for permission to publish this paper.

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"Oxygen Absorbed" from Acid Permanganate in the Presence of Chloride

By R. F. ROBERTS

When chlorides are present in samples examined by the "oxygen absorbed" test, the results are frequently high. This is shown not to be due to loss of chlorine from the sample solution, as was formerly thought, but probably to reaction between the chlorine and some organic substances. By substituting phosphoric acid for the sulphuric acid used in the original method the effect of chloride can be made negligible. In the absence of chloride the modified method gives results similar to those obtained by the original method.

The well known "oxygen absorbed" from acid permanganate test has been used for many years in the assessment of the quality of sewage effluents, and has also been applied to industrial effluents.

The procedure given in the Ministry of Health Methods[1] consists essentially in adding the requisite amount of sample to a known amount of standard potassium permanganate in dilute sulphuric acid. The mixture is made in a stoppered bottle and kept at 26·7° C for 4 hours, then cooled quickly, potassium iodide is added and the liberated iodine titrated with standard thiosulphate solution.

It is known that chlorides interfere with the test, but the literature contains little further information on the subject. Thresh, Beale and Suckling[2] attribute the interference to loss of the chlorine liberated from chloride and permanganate in the presence of sulphuric acid. They advise enclosing in the stoppered bottle a sealed glass ampoule containing potassium iodide solution. After the 4-hour incubation the ampoule is fractured by a sharp shake of the closed bottle and the loss of chlorine is then avoided.

Experimental

In preliminary experiments tests were carried out on solutions of sodium chloride in distilled water at different concentrations between 0 and 10 per cent., no precautions being taken to prevent loss of chlorine. In every case the final titration was equivalent to the initial amount of permanganate taken. In an attempt to encourage loss of chlorine two of the samples were shaken with the stoppers removed after incubation, but there was no evidence of loss of chlorine.

The test was then applied to samples of river water and solutions of organic substances. Different amounts of sodium chloride were added to portions of stock solutions containing organic matter to form solutions ranging up to 15 per cent. of sodium chloride. The "oxygen absorbed" test was then carried out on each. Generally, high figures were obtained when sodium chloride was present. Duplicate tests by the potassium iodide ampoule technique showed it had no effect on the values obtained. The results are given in Table I.

The results obtained in Table I indicate that the errors are not caused by loss of chlorine, but are probably due to reaction between the chlorine and certain organic substances. In the presence of urea an appreciable error is obtained when the solution contains 0·3 per cent. of sodium chloride and above. In solutions containing less than 0·3 per cent. of sodium chloride the error is not serious.

Means of preventing this interference of chloride were sought and finally it was found that the interference could be reduced to a negligible amount by substituting diluted phosphoric acid (1 + 3) for the diluted sulphuric acid (1 + 3) used in the method.

The test as described in the Ministry of Health Methods[1] was carried out on various substances in the presence and absence of sodium chloride, and the results were compared with those obtained by substituting phosphoric acid for sulphuric acid. The results are shown in Table II.

TABLE I

EFFECT OF CHLORIDE ON THE "OXYGEN ABSORBED" WITH AND WITHOUT THE "POTASSIUM IODIDE AMPOULE" PRECAUTION

Experiment number		"Oxygen absorbed" without potassium iodide ampoule, p.p.m.	"Oxygen absorbed" with potassium iodide ampoule, p.p.m.
1	River water alone	7	—
2	River water + NaCl to form 1 per cent. solution	9	—
3	River water + NaCl to form 2 per cent. solution	11	—
4	River water + NaCl to form 3 per cent. solution	13	12
5	River water + NaCl to form 5 per cent. solution	13	12
6	River water + NaCl to form 10 per cent. solution	—	12
7	River water + NaCl to form 15 per cent. solution	12	11
8	Distilled water + urea	1	—
9	Distilled water + urea + NaCl to form 0·1 per cent. solution	1	—
10	Distilled water + urea + NaCl to form 0·3 per cent. solution	3	—
11	Distilled water + urea + NaCl to form 0·5 per cent. solution	7	—
12	Distilled water + urea + NaCl to form 1·0 per cent. solution	11	—
13	Distilled water + urea + NaCl to form 2·0 per cent. solution	17	—
14	Distilled water + urea + NaCl to form 3·0 per cent. solution	18	—
15	Distilled water + urea + NaCl to form 5·0 per cent. solution	16	17
16	Distilled water + sugar	1	—
17	Distilled water + sugar + NaCl to form 5·0 per cent. solution	6	—
18	River water alone	8	—
19	River water + NaCl to form 5·0 per cent. solution	14	14
20	River water + urea	8	—
21	River water + urea + NaCl to form 5·0 per cent. solution	31	31

NOTES ON TABLE I—

(*a*) Results are given to the nearest 1 p.p.m.

(*b*) Experiments 1 to 7 were carried out on one sample of river water; numbers 18 to 21 were carried out on a sample taken on another day.

(*c*) Experiments 4, 5 and 7 were repeated with double the amount of permanganate: similar results were obtained.

(*d*) Solutions of urea and sugar were each approximately 20 p.p.m.

(*e*) Experiments 9 to 14 were repeated with 1000 p.p.m. of urea present; the results were then about doubled.

(*f*) The presence of chloride had no effect on the figures obtained on solutions of approximately 20 p.p.m. of ethanol, tartaric acid, tannic acid, salicylic acid and succinamide.

TABLE II

"OXYGEN ABSORBED" TEST ON VARIOUS SUBSTANCES WITH AND WITHOUT SODIUM CHLORIDE, (*a*) SULPHURIC ACID AND (*b*) PHOSPHORIC ACID BEING USED

Experiment number		"Oxygen absorbed," sulphuric acid being used		"Oxygen absorbed," phosphoric acid being used	
		Sodium chloride absent, p.p.m.	5 per cent. of sodium chloride present, p.p.m.	Sodium chloride absent, p.p.m.	5 per cent. of sodium chloride present, p.p.m.
1	Distilled water + 10 p.p.m. of urea	1	11	1	2
2	Distilled water + 20 p.p.m. of urea	1	18	1	3
3	Distilled water + 40 p.p.m. of urea	1	>35*	1	2
4	Distilled water + 100 p.p.m. of urea	1	>35*	1	3
5	Distilled water + 1000 p.p.m. of urea.. ..	1	>35*	1	3
6	Distilled water + 20 p.p.m. of dried blood ..	1	5	1	2
7	Distilled water + 100 p.p.m. of dried blood ..	8	18	8	11
8	Distilled water + 100 p.p.m. of glue	6	26	5	6
9	Distilled water + 100 p.p.m. of meat extract ..	6	16	6	8
10	Distilled water + 100 p.p.m. of vegetable extract	9	21	8	9
11	Distilled water + 20 p.p.m. of sugar	1	6	1	1
12	Distilled water + 20 p.p.m. of aminoacetic acid	1	16	1	3
13	Distilled water + 100 p.p.m. of aminoacetic acid	1	>35*	1	3
14	Distilled water + urine	13	>35*	12	14

NOTES ON TABLE II—

(*a*) Results are given to the nearest 1 p.p.m.
(*b*) Experiments 2, 3 and 5 were repeated with a solution containing 10 per cent. of sodium chloride. Similar results to those given with 5 per cent. of sodium chloride were obtained.

* The Ministry of Health Methods recommends that not more than 50 per cent. of the permanganate used in the test should be consumed. In the above experiments 50 per cent. of the permanganate represented 35 p.p.m. of "oxygen absorbed."

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IMPERIAL CHEMICAL INDUSTRIES LIMITED
SALT DIVISION
WINSFORD

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New Reagents for the Colorimetric Determination of Atropa Alkaloids

By F. M. FREEMAN

Details of an absorptiometric method for the determination of atropine and related alkaloids are given. The method is based on the nitration of the phenyl grouping and the subsequent production of a relatively stable colour with dimethylformamide and tetraethylammonium hydroxide.

Of the methods described for the determination of small quantities of hyoscyamine and related alkaloids, the Vitali Morin[1] is the one that has probably been most commonly employed.

The reaction has been applied to belladonna and its preparations and related products by Allport and Wilson.[2] It has been further investigated by various authors, including James and Roberts[3] and Ashley,[4] who have determined the conditions governing the reaction. In particular, Ashley has pointed out the need for strict control of the water content of the solvents used in this method.

Although the Vitali Morin reaction as modified by Ashley[4] was found to be capable of good results, it was felt that further improvements could be made if consideration were given to the broader principles involved in this reaction.

The Vitali Morin is a specific example of the well known colour reaction between acetone and aromatic nitro compounds in the presence of sodium hydroxide. In this reaction dinitro compounds generally give a purplish-red colour, whereas trinitro compounds give a blood-red colour. The addition of base alone is sometimes sufficient to produce a colour with certain trinitro compounds, for example, 1:3:5-trinitrobenzene. Carr[5] has described the compounds capable of reacting with aromatic nitro compounds in the presence of base as those possessing methyl, methylene or, more rarely, methine groups adjacent to electronegative groups. Examination of solvents reported to be capable of producing colour in the Vitali Morin reaction,[3] in the light of this theory, showed that they did in fact possess these characteristics. These solvents were acetone, ethyl methyl ketone, malonic ester and pyridine. Other solvents that I have investigated on the basis of Carr's deductions were methyl cyanide, dimethylformamide and diethylaminoethanol.

As the presence of a base is essential to the reaction, consideration has also been given to this aspect of the subject. With acetone or pyridine as the solvent, when ethanolic solutions of potassium hydroxide are employed, the colour stability is poor, being critically dependent on the water content of the reaction mixture.

Sodium methoxide in benzene - methanol was found to be superior to ethanolic potassium hydroxide in some respects. The most promising results, however, were obtained by the use of tetraethylammonium hydroxide, which has been reported to give a red colour with nitrobenzene in acetone.[5] Of the solvents described, preliminary tests indicated that dimethylformamide was the solvent of choice when used in conjunction with tetraethylammonium hydroxide. The following experiments were carried out to determine the conditions governing this reaction.

Experimental

In the experiments described below aliquots of an ethanolic solution of hyoscyamine hydrochloride containing 0·050 mg per 1 ml were evaporated to dryness and nitrated by adding 0·2 ml of fuming nitric acid, which was subsequently removed by evaporation. The residue was dissolved in successive small portions of dimethylformamide and transferred to a 10-ml stoppered flask for coloration.

Variation in base concentration—

The effect of varying the concentration of tetraethylammonium hydroxide is shown in Fig. 1. Various amounts of a 1·25 per cent. solution of tetraethylammonium hydroxide in dimethylformamide were added to a solution of the nitrated alkaloid in the same solvent. The optimum amount for the maximum colour development in a reaction volume of 10 ml

lay between the limits of 0·7 ml of a 1·25 per cent. solution, below which little colour was developed, and 0·5 ml of a 25 per cent. solution, above which turbidity developed. A volume of 0·3 ml of 25 per cent. aqueous solution of tetraethylammonium hydroxide was chosen as being satisfactory for maximum colour development in a reactive volume of 10 ml. It is probable that Fig. 1 corresponds to the neutralisation curve of the reaction mixture.

Dilution by dimethylformamide of the colour produced on addition of 0·7 ml of a 1·25 per cent. solution of the hydroxide was non-linear and may be due to the presence of acidic impurities in the dimethylformamide, as the reaction was capable of being reversed by the addition of acid. A further quantity of tetraethylammonium hydroxide, however, restored the full colour.

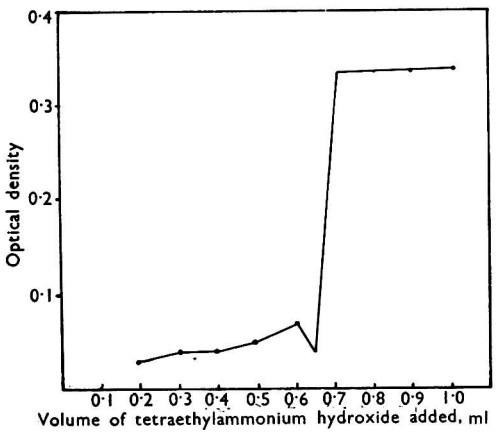

Fig. 1. Colour development of nitrated alkaloid in dimethylformamide with a 1·25 per cent. solution of tetraethylammonium hydroxide

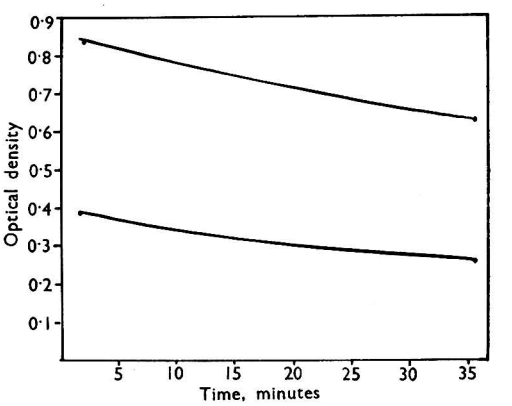

Fig. 2. Stability of the colour in two hyoscyamine solutions

STABILITY OF COLOUR—

Fig. 2 shows the rate at which the colour faded in two hyoscyamine solutions of different concentrations. The nitration was carried out as previously described, the colour being developed by the addition of 0·3 ml of 25 per cent. aqueous tetraethylammonium hydroxide. The slow rate of fading permits measurements to be taken without undue haste. Exposure to light was found to have little effect on the rate of fading.

EFFECT OF THE ADDITION OF WATER ON COLOUR DEVELOPMENT—

The effect of the addition of water to the reaction mixture before the addition of tetraethylammonium hydroxide was investigated, and it was found that a satisfactory intensity of colour could be produced in the presence of up to 20 per cent. of water, the intensity falling as the percentage of water increased. Thus the water content of the dimethylformamide, which is usually less than 0·1 per cent., has no effect on the colour developed.

EFFECT OF WATER CONTENT ON COLOUR STABILITY—

It was found that reaction mixtures containing up to 10 per cent. of water showed little change in the rate of fading when compared with mixtures containing less than 0·1 per cent. of water.

ABSORPTION CURVES—

The absorption curve of the colour produced by the methods previously described had a maximum at 540 mμ.

Method

Apparatus—

Unicam SP500 spectrophotometer or similar instrument capable of measurement in the visible region; 1-cm and 4-cm cells.

Reagents—

Dimethylformamide—Laboratory-reagent grade (as supplied by The British Drug Houses Ltd.).

Tetraethylammonium hydroxide—A 25 per cent. aqueous solution; laboratory-reagent grade (as supplied by The British Drug Houses Ltd.).

Hyoscyamine hydrochloride.

Nitric acid, fuming—Analytical-reagent grade.

Procedure—

The recommended method, which can be applied to pure solutions containing hyoscyamine and related alkaloids or to their galenicals after partial purification by the method of Allport and Wilson,[2] is as follows.

Evaporate an aliquot containing approximately 0·05 to 0·15 mg of alkaloid to dryness on a steam-bath and nitrate by the addition of 0·2 to 0·3 ml of fuming nitric acid. Remove the nitric acid by evaporation and transfer the residue by means of small portions of dimethylformamide to a 10-ml calibrated flask. Add 0·3 ml of 25 per cent. aqueous tetraethylammonium hydroxide and dilute to the mark with dimethylformamide.

Set the flask aside for 5 minutes, and then measure the colour produced at 540 mμ in 1-cm cells, with dimethylformamide as a blank. The sensitivity of the reaction can be increased by the use of 4-cm cells; however, this involves an increase in the reaction volume to 15 or 20 ml and necessitates a corresponding increase in the amount of tetramethylammonium hydroxide solution added. The calibration graph is linear.

Other applications

This reaction has been successfully applied to the determination of phenylacetic acid, benzylpenicillin and dibenzylethylenediaminedipenicillin, and also in part to the determination of chloramphenicol, details of which will be described elsewhere.

The author acknowledges the encouragement of Dr. F. J. Smith of the College of Technology, Liverpool.

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The Absorptiometric Determination of Mercury in Urine

By A. C. ROLFE, F. R. RUSSELL and N. T. WILKINSON

A method has been developed for the absorptiometric determination of mercury in urine. The method is rapid and gives complete recovery of mercury in samples of urine that have been standing for several days, which is not the case with other methods that have been tried.

The method depends on the oxidation of the urine, contained in a bottle that will withstand slight pressure, with nitric acid and potassium permanganate at about 85° C. From the resulting solution mercury is extracted with dithizone and any interfering substances are removed from the mercury dithizonate by application of a "re-extraction" procedure. The colour of the mercury dithizonate is measured on a photo-electric absorptiometer.

Mercury can be determined over a range of 0 to 100 μg with an accuracy of ±1 μg below 50 μg and ±3 μg for amounts between 50 and 100 μg.

For a considerable time we have had to determine mercury in the urine of various persons who have been under medical observation.

During the course of our work we have examined the methods of Strafford and Wyatt,[1] Haddock and Williams,[2] Hubbard,[3] Cholak and Hubbard,[4] Kozelka[5] and Milton and Hoskins.[6] The results of our experimental work showed that the methods generally gave low recoveries of mercury when it was added to fresh urine in the form of a solution of mercuric sulphate. If the urine was allowed to stand for some time after the addition of the mercuric sulphate before applying the test, the results obtained by each method were even lower.

Experimental

Our first experiments were based on the method of Strafford and Wyatt.[1] This method is very lengthy, but we did not carry out the procedure in its entirety, since after decomposition of the urine in the special apparatus and subsequent co-precipitation of the mercury as sulphide together with added copper it is necessary to separate the mercury from a nitric acid solution of the two sulphides by electrolysis before proceeding to the colorimetric determination with *p*-dimethylaminobenzylidenerhodanine.

With the introduction of diphenylthiocarbazone (dithizone) as a reagent for the determination of mercury, we considered that it might be unnecessary to separate the mercury by electrolysis, and we carried out determinations on the solution of the mixed sulphides by a titration - extraction procedure.

The results are given in Table I.

Table I

Determination of Mercury in 100-ml samples of fresh urine

Experiment number	Mercury added as mercuric sulphate, μg	Mercury found, μg	Recovery, %
1	50	35	70
2	100	53	53
3	25	22	88
4	75	37	49
5	135	99	73
6	195	105	54
7	250	153	61

The mercury in experiments 1 and 2 was determined immediately; in experiments 3 to 7 the urine was allowed to stand for 5 days after the addition of mercury.

We became aware that the dithizone extraction - titration of mercury was liable to slight error, since various titres of the dithizone solution could be obtained if the standardisation of the solution was carried out with different amounts of mercury. This error did not, however, account for the low recovery of mercury in the experiments given in Table I.

We then decided to examine the various stages of the method of Strafford and Wyatt[1] to determine whether loss of mercury occurred at any stage. At the same time we adopted an absorptiometric finish to the determination, a solution of dithizone in toluene being used, which we had shown to give results of a high order of accuracy. The tests were carried out in the absence of urine, but at each stage full recovery of the added mercury was obtained. From this we deduced that the low recovery in the method of Strafford and Wyatt is associated with the destruction of the organic matter.

The method of Haddock and Williams[2] was then tried.

To 50 ml of the urine contained in a 250-ml beaker, 5 ml of sulphuric acid, sp.gr. 1·84, and 1 g of potassium permanganate were added. The solution was stirred well and heated on a steam-bath for 10 minutes. Then 0·5 g of oxalic acid was added, the solution was again stirred and allowed to remain on the steam-bath until all the precipitated manganese dioxide was destroyed. The solution was then cooled, diluted to 100 ml and transferred to a 250-ml separating funnel; 5 ml of dithizone solution (0·01 per cent. of purified dithizone in carbon tetrachloride) were added and the mixture was shaken vigorously. The dithizone extract was run into a 50-ml separating funnel and the extraction of the solution was repeated with two further 5-ml portions of dithizone solution. The combined dithizone extracts were washed with 4 ml of 0·1 *N* hydrochloric acid and the lower (dithizone) layer was run into a 50-ml separating funnel. The 0·1 *N* hydrochloric acid washing was shaken with 2 ml of dithizone solution and this dithizone extract added to the other combined extracts.

To the combined dithizone extracts 4 ml of diluted hydrochloric acid (1 + 1) were added and the solution was shaken vigorously. The dithizone layer was separated and two further extractions with 4-ml portions of diluted hydrochloric acid (1 + 1) were carried out. The acid extracts were combined and placed in a 250-ml separating funnel and washed once with 3 ml of carbon tetrachloride. The carbon tetrachloride washing was rejected.

The hydrochloric acid solution was diluted with 90 ml of water and titrated with a dilute solution of dithizone in carbon tetrachloride. This dilute solution of dithizone was prepared from the 0·01 per cent. solution so that 1 ml was equivalent to approximately 0·001 mg of mercury, the precise strength being determined by titrating a standard mercury solution.

A blank test was carried out on the reagents used.

The results are shown in Tables II and III.

TABLE II

DETERMINATIONS CARRIED OUT IMMEDIATELY AFTER THE ADDITION OF MERCURIC SULPHATE SOLUTION TO FRESH URINE

Mercury added, μg	Mercury found, μg	Recovery, %
5·0	5·0	100
10·0	9·5	95

TABLE III

DETERMINATIONS CARRIED OUT 5 DAYS AFTER THE ADDITION OF MERCURIC SULPHATE SOLUTION TO FRESH URINE

Mercury added, μg	Mercury found, μg	Recovery, %
5·0	4·1	82
10·0	7·1	71

It was considered that on allowing the mercury to stand in contact with the urine for any length of time a complex organo-mercuric compound was formed, and it was not decomposed with sulphuric acid - potassium permanganate solution. We thought, however, that, although this mercury did not react with dithizone, it was likely that sufficient ions might be present to obtain precipitation of the mercury as sulphide, since mercuric sulphide has a solubility product of 4×10^{-53}.

Quantities of mercury as mercuric sulphate were added to fresh urine and either immediately or after standing for 5 days the organic matter was destroyed by the procedure recommended by Haddock and Williams. To the finally prepared solution, diluted to 300 ml,

1 ml of 0·04 *M* copper sulphate solution was added, and then hydrogen sulphide was passed through the solution for 15 minutes. The mixed precipitate of copper and mercury sulphides was filtered off, and finally brought into solution as described by Strafford and Wyatt.[1]

Mercury was determined in the solution by the absorptiometric method described later, commencing at "Measure a suitable aliquot of the solution. . . " on p. 530. The results obtained when the test was carried out immediately after addition of the mercury to the urine were satisfactory, but when carried out 5 days after the addition the results were low, being very similar to those shown in Table III.

Hubbard[3] in his investigations on the determination of mercury in urine decomposed the urine with potassium permanganate in the presence of sulphuric acid. The mercury was added as a solution of mercuric nitrate, and the author claims satisfactory results. The reagent used for the colorimetric determination was di-2-naphthylthiocarbazone, which is claimed to be more sensitive than diphenylthiocarbazone, which we have used. We did not think that any advantage would be gained by using the new reagent, since our previous experiments had shown that complete recovery of mercury from aqueous solutions of mercuric salts was obtainable with dithizone and that urine treated with sulphuric acid and potassium permanganate did not yield all its mercury when the mercury was added as mercuric sulphate and the urine allowed to stand for 5 days; therefore, no tests were done on this method.

Cholak and Hubbard[4] in a paper on the micro-determination of mercury in biological materials follow the procedure described by Hubbard[3] for the decomposition of urine, *i.e.*, hot digestion with sulphuric acid and potassium permanganate. For the same reason as mentioned above no tests were carried out on the method.

The digestion method of Kozelka[5] was then tried. In this method it is recommended first to reduce the volume of the urine by boiling after acidification and to reject the distillate, and then to proceed with the destruction of the organic matter. We found, however, that the distillate contained mercury, so we decided to carry out the digestion procedure for the destruction of organic matter directly on 50 ml of urine without preliminary boiling.

In the final solution the mercury was determined by the absorptiometric method described later, commencing at "Measure a suitable aliquot of the solution. . . " on p. 530.

The results are given in Tables IV and V.

TABLE IV

DETERMINATIONS CARRIED OUT IMMEDIATELY AFTER THE ADDITION OF MERCURIC SULPHATE SOLUTION TO FRESH URINE

Mercury added, μg	Mercury found, μg	Recovery, %
50	35·0	70·0
100	87·5	87·5

TABLE V

DETERMINATIONS CARRIED OUT 5 DAYS AFTER THE ADDITION OF MERCURIC SULPHATE SOLUTION TO FRESH URINE

Mercury added, μg	Mercury found, μg	Recovery, %
50	42·5	85·0
100	82·5	82·5

The digestion method of Milton and Hoskins[6] was then tried. In this method it is recommended that 1 litre of urine should be digested with sulphuric acid and potassium permanganate; mercury was then separated as the sulphide. We have shown that preliminary separation of the mercury as sulphide is unnecessary, and therefore we carried out the digestion on a much smaller volume of urine, and on the products of the digestion we carried out a direct extraction and absorptiometric determination of the mercury, the procedure being as follows.

A 100-ml portion of urine was placed in a 250-ml round-bottomed flask and 10 ml of sulphuric acid, sp.gr. 1·84, and 2·5 g of potassium permanganate were added. The flask was fitted with a reflux condenser (ground-glass joint) and the solution was boiled for 2 hours. The solution was cooled and the excess of potassium permanganate was destroyed by the

gradual addition of oxalic acid crystals. Then 6 per cent. w/v potassium permanganate solution was added until a slight excess was present, indicated by the solution being slightly pink. This slight excess of permanganate was removed by addition of 5 ml of 10 per cent. w/v hydroxylamine hydrochloride solution.

The solution was filtered and the filter was washed with water; the filtrate and washings were diluted to 250 ml in a calibrated flask. Mercury was determined on a 100-ml aliquot by the procedure described later, commencing at "Measure a suitable aliquot. . . " on p. 530.

The results obtained by this procedure are given in Tables VI and VII.

TABLE VI

DETERMINATIONS CARRIED OUT IMMEDIATELY AFTER THE ADDITION OF MERCURIC SULPHATE SOLUTION TO FRESH URINE

Mercury added, μg	Mercury found, μg	Recovery, %
Nil	Nil	—
10	10·5	105·0
50	40·5	81·0
100	81·5	81·5

TABLE VII

DETERMINATIONS CARRIED OUT 5 DAYS AFTER THE ADDITION OF MERCURIC SULPHATE SOLUTION TO FRESH URINE

Mercury added, μg	Mercury found, μg	Recovery, %
Nil	Nil	—
10	8·3	83·0
50	35·5	71·0
100	72·5	72·5

It had been shown that no mercury is lost when a precipitate of mercury and copper sulphides is dissolved in a mixture of nitric and sulphuric acids in the presence of a small amount of filter-paper pulp and the filter-paper is eventually destroyed by the acid.

This is not so, however, if a digestion of urine, to which has been added a solution of mercuric sulphate, is carried out with nitric and sulphuric acids. A 20-ml portion of urine was placed in a Kjeldahl flask, and 20 μg of mercury (as mercuric sulphate), 8 ml of diluted sulphuric acid (1 + 1) and 5 ml of concentrated nitric acid were added. The solution was heated to fuming and the organic matter was oxidised by further small additions of nitric acid. The solution was diluted and the mercury determined by the absorptiometric method described later, commencing at "Measure a suitable aliquot of the solution. . . " on p. 530. Of the 20 μg of mercury added only 2·5 μg were found.

The above results show that, of the methods tried for destruction of organic matter, that with potassium permanganate in the presence of sulphuric acid would find favour in its simplicity, but this method does not give complete recovery of mercury added to urine that has been allowed to stand for any length of time. We decided, therefore, to try an oxidation with nitric acid, as follows.

A 50-ml portion of the urine was placed in a 400-ml beaker and a known amount of mercury was added as a solution of mercuric sulphate. Then 25 ml of nitric acid were added and the solution was heated at 75° C for 30 minutes. A 6 per cent. w/v solution of potassium permanganate was added to the hot solution until a slight excess was present. The solution was cooled and the excess of permanganate destroyed by adding a 10 per cent. w/v solution of purified hydroxylamine hydrochloride. The solution was neutralised with ammonia, and then diluted sulphuric acid (1 + 1) added to bring the solution to pH 0·2. The mercury in the solution was determined by the absorptiometric dithizone method described later, commencing at "Measure a suitable aliquot of the solution. . . " on p. 530.

It was found that with a comparatively large concentration of nitric acid re-extraction of the mercury dithizonate is essential, otherwise high results are obtained. The results are given in Tables VIII and IX.

TABLE VIII

DETERMINATIONS CARRIED OUT IMMEDIATELY AFTER THE ADDITION OF MERCURIC SULPHATE TO FRESH URINE

	Mercury added, μg	Mercury found, μg	Recovery, %
Without re-extraction	50	60	120
	100	130	130
With re-extraction	50	45	90
	100	94	94

TABLE IX

DETERMINATIONS CARRIED OUT 5 DAYS AFTER THE ADDITION OF MERCURIC SULPHATE TO FRESH URINE

	Mercury added, μg	Mercury found, μg	Recovery, %
Without re-extraction	50	50	100
	100	120	120
With re-extraction	50	42	84
	100	90	90

In view of the promising results shown in Tables VIII and IX we considered that by treating the urine with a mixture of strong oxidising agents complete destruction of the organic matter would probably be obtained. It was also decided to carry out the oxidation in a closed vessel to prevent any loss of mercury by volatilisation. Experiments showed that digestion of the urine with a mixture of nitric acid and potassium permanganate gave complete recovery of the mercury from urine when determined 5 days after the addition of the mercuric sulphate solution. The results obtained are given in Tables X and XI and the method is described on pp. 528 to 530.

TABLE X

DETERMINATIONS CARRIED OUT IMMEDIATELY AFTER THE ADDITION OF MERCURIC SULPHATE TO 50 ml OF FRESH URINE

Mercury added, μg	Mercury found, μg	Recovery, %
Nil	0·75	—
20	19·75*	98·7
50	49·5*	99·0
100	102·5*	102·5

* Corrected for blank.

TABLE XI

DETERMINATIONS CARRIED OUT 5 DAYS AFTER THE ADDITION OF MERCURIC SULPHATE TO 50 ml OF FRESH URINE

Mercury added, μg	Mercury found, μg	Recovery, %
Nil	0·75	—
20	18·8*	94·0
50	49·3*	98·6
100	97·5*	97·5
20	19·2*	96·0
50	49·4*	98·8
100	98·0*	98·0

* Corrected for blank.

A sample of urine was obtained from a person exposed to a mercury hazard. The mercury was immediately determined in the fresh urine by the method described on pp. 528 to 530. Mercuric sulphate solution was then added to the urine and the mixture set aside for 5 days. Total mercury was determined in the sample after standing for 5 days with and without added mercuric sulphate solution. The results are given in Table XII.

TABLE XII

DETERMINATIONS OF MERCURY IN URINE

	Mercury added, μg	Total mercury found, μg	Mercury found after correction for original content, μg	Recovery, %
Determined immediately on fresh urine ..	Nil	89·5	—	—
Determined after standing for 5 days ..	Nil	90·0	—	—
Determined 5 days after the addition of mercuric sulphate	50	143·5	53·5	107·0
Determined 5 days after the addition of mercuric sulphate	100	193·5	103·5	103·5

EFFECT OF COPPER—

The effect of copper was investigated. Known amounts of mercury (as mercuric sulphate) and copper (as copper sulphate) were taken. To each solution 7 ml of diluted sulphuric acid (1 + 1) were added and the solutions were diluted to 100 ml. The solutions were transferred to 250-ml pear-shaped separating funnels, 5 ml of hydroxylamine hydrochloride solution were added to each and the mercury was extracted by the method described below, commencing at " . . . add 3 ml of dithizone extraction solution. . . " on p. 530.

The results are shown in Table XIII.

TABLE XIII

EFFECT OF COPPER ON DETERMINATION OF MERCURY

Mercury added, μg	Copper added, μg	Optical density
2	Nil	0·123
2	1000	0·129
10	Nil	0·443
10	1000	0·450
20	Nil	0·840
20	1000	0·835

METHOD

SAMPLING—

Use 16-oz. W.M. glass-stoppered bottles (previously rinsed with warm diluted nitric acid (1 + 1), then scrubbed with a test tube brush and sodium carbonate). Wash the bottles well with tap water and finally rinse with distilled water.

REAGENTS—

Nitric acid, sp.gr. 1·42.

Sulphuric acid, diluted (1 + 1)—Carefully add 1 volume of sulphuric acid, sp.gr. 1·84, to 1 volume of distilled water, stirring during the addition.

Hydrochloric acid, 5 N.

Ammonia, sp.gr. 0·88.

Potassium permanganate, 6 *per cent. solution.*

Phenol red indicator.

Ammoniacal hydroxylamine solution—Dissolve 25 g of hydroxylamine hydrochloride in approximately 60 ml of water, add 0·2 ml of phenol red indicator and make alkaline with ammonia, sp.gr. 0·88, to the full red colour of the indicator. Cool, and extract with a 0·01 per cent. w/v solution of dithizone in chloroform, using 5-ml portions until the last extract remains green; then wash the solution free from excess of dithizone by repeated extraction with 10-ml portions of chloroform. Warm the solution until the excess of chloroform is removed, cool, filter and dilute to 250 ml.

Purification of dithizone (*diphenylthiocarbazone*)—Dissolve 1 g of the commercial product in 75 ml of pure chloroform, filter, and shake the filtered solution, contained in a 250-ml separating funnel, with four successive 100-ml portions of 1 per cent. v/v ammonia. Combine the orange coloured aqueous solutions, filter through a filter-paper into a beaker and precipitate

the dithizone from the filtrate by rendering it slightly acid with 5 *N* hydrochloric acid. After it has settled, separate the precipitate by filtration on a clean sintered-glass crucible, using suction, and wash it free from acid with distilled water. Dry the well drained precipitate over sulphuric acid *in vacuo* for 3 to 4 days, protecting the material from light. The purified solid so prepared, when stored in the dark, is stable for at least 6 months.

Dithizone extraction solution—Dissolve 0·02 g of purified dithizone in 250 ml of redistilled toluene.

Toluene, sulphur-free and redistilled.

Sodium hydroxide—A 0·2 *N* solution containing 20 ml of ammoniacal hydroxylamine solution per litre.

Mercuric sulphate solutions—(*a*) *Stock solution*—Weigh accurately 0·4 to 0·5 g of pure dry mercury into a 100-ml beaker, add 10 ml of water, cover the beaker with a watch-glass

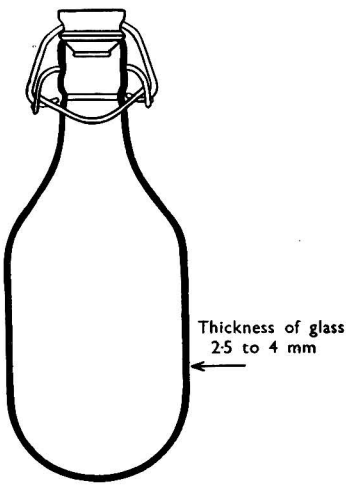

Fig. 1. Glass pressure bottle (a screw stopper may be used as alternative to the swing stopper)

and add gradually 10 ml of nitric acid, sp.gr. 1·42; warm until the mercury has completely dissolved. Add 25 ml of diluted sulphuric acid (1 + 1) and evaporate until fumes of sulphur trioxide are evolved. Cool, dilute cautiously with 50 ml of water, boil for 1 minute and cool. Transfer to a 500-ml calibrated flask, dilute to the mark with water and mix well.

(*b*) *Dilute solution*—Measure from a 25-ml burette such a volume of solution (*a*) as will contain 0·0100 g of mercury into a 1-litre calibrated flask, add 5 ml of diluted sulphuric acid (1 + 1) and dilute to the mark with water.

Then 1 ml ≡ 10 μg of mercury.

PROCEDURE—

Transfer 50 ml of the urine to a 350-ml glass pressure bottle (Fig. 1), add 20 ml of nitric acid and 20 ml of potassium permanganate solution; gently mix the solutions and place the stopper on the bottle. Place the bottle in a suitable beaker (usually a 600-ml beaker) and add water to the beaker until its level is well above the level of the solution in the pressure bottle; place the beaker on a boiling-water bath and heat for 2 hours. Remove from the bath and cool thoroughly. Shake the bottle and invert it so that the solution will come into contact with the vapour in the bottle. Carefully remove the stopper, covering it with a towel during the removal. Rinse the stopper with distilled water, adding the rinsings to the contents of the bottle.

If at this stage excess of permanganate is not present, add potassium permanganate solution 1 ml at a time until an excess is present when the solution has been standing for 2 minutes after the addition. Remove excess of permanganate by adding ammoniacal hydroxylamine solution. Add a few drops of phenol red indicator and ammonia until the solution attains the full red colour of the indicator, keeping the solution cool during the addition of ammonia. Add 18 ml of diluted sulphuric acid (1 + 1) and 10 ml of ammoniacal

hydroxylamine solution, transfer the solution to a 250-ml calibrated flask, cool, dilute to the mark and mix. Set aside for 3 hours or preferably overnight.

Measure a suitable aliquot of the solution (usually 100 ml) containing not more than 100 μg of mercury and place it in a 250-ml pear-shaped separating funnel; add 3 ml of the dithizone extraction solution and 7 ml of toluene and shake vigorously for 30 seconds. Allow the organic solvent to separate and observe the colour of the dithizone; excess of dithizone is indicated by its green colour, or brownish-green colour if much mercury is present. If excess of dithizone is not present, indicated by the toluene layer being a golden-brown colour, continue the addition of the dithizone extraction solution 0·5 ml at a time with shaking as described above until excess of dithizone is indicated. Allow to separate, then run off and discard the lower (aqueous) layer, wash the toluene layer with 10 ml of distilled water without mixing, and run off and discard the washings.

Add 10 ml of 5 *N* hydrochloric acid and shake vigorously for 30 seconds. Allow to separate, run the lower layer into a second separating funnel and wash the toluene - dithizone layer twice without shaking, using 50 ml of distilled water for each washing; add the washings to the hydrochloric acid extract contained in the second separating funnel. Reject the dithizone - toluene layer.

To the hydrochloric acid extract and washings add 5 ml of ammoniacal hydroxylamine solution, a volume of dithizone extraction solution equal to that used in the initial extraction, and 7 ml of toluene; shake vigorously for 30 seconds. Allow the two liquids to separate and run off and discard the lower layer.

Wash the toluene - dithizone layer once with 10 ml of water without shaking, run off and discard the washings. Remove excess of dithizone by adding 10 ml of 0·2 *N* sodium hydroxide, shake for 20 seconds, then allow the layers to separate. Run off and discard the lower aqueous layer. Repeat the extraction with the 0·2 *N* sodium hydroxide reagent twice more, rejecting the aqueous layer each time. If mercury is present, the toluene layer will be a golden-brown colour. Run off the last aqueous layer as much as possible, dry the stem of the separating funnel with a strip of filter-paper and then filter the toluene - mercury dithizonate solution through a dry Whatman No. 41 filter-paper (9 cm diameter) containing about 0·5 g of anhydrous sodium sulphate; collect the filtrate in a dry 25-ml calibrated flask. Rinse the separating funnel a few times with small volumes of toluene, pass the rinsings through the filter and collect them in the flask. Dilute the filtrate and washings with toluene to the mark and mix.

Measure the optical density with a Spekker photo-electric absorptiometer, using either a 4-cm or 1-cm cell (according to the depth of colour of the solution) and Calorex H503 heat filters and Ilford No. 602 blue filters in conjunction with a tungsten-filament lamp. Use toluene in the comparison cell. A blank determination should be carried out on all reagents used in the method.

Establish calibration curves for the Spekker absorptiometer with 1-cm and 4-cm cells as follows. Measure accurately known volumes of the dilute mercuric sulphate solution, representing 1, 2, 4, 10, 20, 30, 50, 70 and 100 μg of mercury, into each of nine 250-ml pear-shaped separating funnels. Add 7 ml of diluted sulphuric acid (1 + 1) to each solution and dilute to 100 ml. Add 5 ml of ammoniacal hydroxylamine solution and extract the mercury from each with dithizone exactly as described above. Measure the optical density of each solution, using the 4-cm cell to cover the range 0 to 30 μg of mercury and the 1-cm cell to cover the range 0 to 100 μg of mercury. Carry out a blank determination on the reagents used. Plot the curves with amount of mercury, in μg, as abscissae and optical densities as ordinates.

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Microbiological Assay on Large Plates

Part III. High Throughput, Low Precision Assays*

By K. A. LEES and J. P. R. TOOTILL

Methods are presented for the simultaneous assay of from about 10 to 50 samples by large-plate microbiological assay. The emphasis throughout is on the highest practicable throughput and these methods are recommended for such preliminary or routine work as calls for numbers of assays rather than precision of individual results.

In Part I[1] of this series methods suitable for use in routine laboratory assays (standard error about ± 5 per cent.) were presented. Part II[2] dealt with the more complex methods required for precision assays (standard error about ± 1 per cent.) suitable for the evaluation of biological standards and the like. This final Part considers the problem of high throughput assays, when only moderate precision (standard error about ± 10 per cent.) is required, but when the number of samples to be simultaneously assayed is usually more than 10.

In investigational work on fermentation problems a large number of initial experiments (requiring a correspondingly large number of assays) often have to be carried out to provide the leads for later, more exacting, work. No final conclusions are demanded from such preliminary work, for any leads given will automatically be checked in succeeding confirmatory experiments. Such large-scale preliminary work is often undertaken with the expectation of a substantial increase in yield of a microbiologically active material; the primary need is then for a microbiological assay capable of handling simultaneously a large number of samples, the precision and accuracy of the determinations being of secondary importance. Indeed no potency determination at all may be required, but simply a "limit test" to show whether or not the unknown sample exceeds a certain potency.

A series of designs that fulfil these requirements and discard all superfluous checks for validity are given below.

Type I: Completely random arrangement

The microbiological plate assay of certain crude fermentation products, particularly those that stimulate growth of the test organism, are inherently inaccurate. Other growth stimulating substances are, in general, present, to a small extent at least. Vitamin-B_{12} assay is particularly subject to this type of interference; indeed, no plate method completely specific for this vitamin has yet been published.

Our experience based on estimating the magnitude of the effect of interfering substances by means of bioautographs and recovery assays shows that the precision obtainable from four to six replicate zones per sample at one dilution level, placed at random on a large plate (*i.e.*, with no special statistical precautions to eliminate errors due to temporal and positional effects) will result in standard errors of about 10 per cent.—of the same order of magnitude as the inherent inaccuracies in assaying crude preparations of vitamins B_{12}—provided an organism not unduly sensitive to growth-promoting impurities is used.

The absence of any special statistical precautions to eliminate temporal and positional effects should not be taken to imply that the high standard of plate preparation (involving such operations as uniform seeding, controlling agar thickness and so on) can be relaxed. In fact, the onus is on the microbiologist to ensure that these effects do not become so large as to exceed the inherent inaccuracies in this type of assay.

Accordingly, the arrangement we use for the vitamin-B_{12} assay of fermentation liquors is the simplest possible, namely, a completely random arrangement of four to six zones per sample on a large microbiological assay plate, and an appropriate number of levels of standard with similar replications.

The plating out of the assay solutions should be so performed as to preserve the advantages of randomisation; that is to say, all of one solution should not be plated out before all of the next and so on. We find it most convenient to preserve the usual practice of plating out

* For particulars of Parts I and II of this series, see reference list, p. 535.

in row and column order, the randomisation ensuring that any particular solution has the same chance of being plated out at any particular time and in any particular position as at any other time or position.

Type II: Youden and balanced lattice squares

With antibiotics the inherent inaccuracies are of less magnitude than those discussed above, although here also no great precision is required in preliminary work. In these circumstances a replication of about four to six zones per sample is all that is needed, although the removal of error due to positional and temporal effects is now worth while.

The general conduct of the assay is as already outlined. Single dilution levels of the unknowns are used, the mean zone sizes from which are compared with those from a standard at one level or more, but the arrangement is not completely random, designs being employed to eliminate temporal and positional effects.

To obtain high throughput the number of samples (including the standard dilutions) may exceed either the horizontal or the vertical dimension of the design, or both. This involves the comparisons between the samples being to some extent confounded; for simplicity of computation and equality of precision in assaying the various samples this partial confounding should be balanced, to ensure that the loss of information is spread evenly over all samples.

Designs of this type fall into two classes: those in which the confounding is confined to one dimension only, namely, Youden squares, and those in which the confounding is equal in both dimensions, namely, balanced lattice squares.

(*a*) Youden squares—

In these designs the number of samples exceeds the vertical but not the horizontal dimensions of the design; hence the design employed is in fact a rectangle. Each row of the design contains a complete set of all solutions used in the assay, thus ensuring that positional and temporal effects operative between the rows are eliminated. In the columns a balanced arrangement is employed whereby each solution in the assay occurs equally frequently with every other. Thus positional and temporal effects can be eliminated from the results by utilising the comparisons between solutions only where they occur together in the same column or columns.

An example of such a design is given in Table I. The numbers in the odd horizontal rows represent the actual positions in which the eleven solutions involved in the assay have been plated out; those in the even rows show the zone sizes obtained from them.

Table I

11 × 5 Youden square for eight unknowns

A	B	C	D	E	F	G	H	I	J	K
7	6	1	9	11	8	2	5	10	3	4
23·2	23·6	23·8	23·3	24·4	21·2	24·3	25·8	23·3	23·9	24·3
11	10	5	2	4	1	6	9	3	7	8
24·4	23·6	25·7	24·3	24·5	24·1	24·0	23·7	23·8	23·5	21·5
2	1	7	4	6	3	8	11	5	9	10
24·5	24·2	23·8	24·3	24·0	23·8	21·8	24·7	25·7	23·2	23·2
4	3	9	6	8	5	10	2	7	11	1
24·4	24·0	23·4	24·0	21·6	25·7	23·4	24·5	23·5	24·2	24·1
1	11	6	3	5	2	7	10	4	8	9
24·4	24·8	23·5	24·2	25·7	24·0	23·5	23·6	24·4	21·6	23·4

As is usual in the procedures described in this series of papers, the plating out of solutions is carried out in row and column order, beginning in the top left-hand corner; a template containing the numbers referring to the solutions (as described above) is placed under the assay plate.

The computation of these assays cannot readily be done directly from the design and use is made of a form on which the zone sizes are entered in positions convenient for computation rather than as they occur on the design. Such a form is reproduced in Fig. 1.

The operator reads the developed plate vertically, beginning with column A, and enters the zone size for each solution in the appropriate blank space in column A on the form, opposite

MICROBIOLOGICAL ASSAY LABORATORY, SEFTON PARK. **DESIGN K**

Assay solution number. ↓ This Design must be read columnwise.

Date 1/9/54. | Square No. 9 | Plated by B.S. | Computed by D.E.M.
Type Penicillin. | Diluted by B.S. | Read by D.E.M. | Checked by E.H.

	A	B	C	D	E	F	G	H	I	J	K	V	rV	T	rV-T	Potency		Sample	Dilution
1	24.4	24.2	23.8			24.1					24.1	120.6	603.0	596.6	+ 6.4	6.10	1	1	1/100
2	24.5			24.3		24.0	24.3	24.5				121.6	608.0	599.1	+ 8.9	6.35	2	2	"
3		24.0		24.2		23.8			23.8	23.9		119.7	598.5	596.2	+ 2.3	5.70	3	3	"
4	24.4			24.3	24.5				24.4		24.3	121.9	609.5	598.4	+ 11.1	6.60	4	4	"
5			25.7		25.7	25.7		25.8	25.7			128.6	643.0	602.2	+ 40.8	$S_1 = 10$	5	≡ 1000	u./ml.
6		23.6	23.5	24.0	24.0		24.0					119.1	595.5	597.7	- 2.2	5.30	6	5	1/100
7	23.2		23.8				23.5		23.5	23.5		117.5	587.5	595.2	- 7.7	$S_2 = 5$	7	≡ 500	u./ml.
8					21.6	21.2	21.8			21.6	21.5	107.7	538.5	588.9	- 50.4	$S_3 = 2.5$	8	≡ 250	u./ml.
9			23.4	23.3				23.7		23.2	23.4	117.0	585.0	595.5	- 10.5	4.70	9	6	1/100
10		23.6					23.4	23.6	23.3		23.2	117.1	585.5	596.7	- 11.2	4.63	10	7	"
11	24.4	24.8			24.4			24.7		24.2		122.5	612.5	600.0	+ 12.5	6.70	11	8	"
	120.9	120.2	120.2	120.1	120.2	118.8	117.0	122.3	120.7	116.4	116.5	1313.3	6566.5	6566.5	0.0				

Fig. 1. Proforma for 11 × 5 Youden square computation

the assay solution number concerned. Care must be taken with these entries; although the numbers in each of the columns on the form and the design are the same, they do not occur in the same order.

Computation from the entries on the form proceeds as follows—

1. Sum columns A, B, C, D, etc., entering the results at the foot.

2. Sum the rows 1, 2, 3, 4, etc., entering the totals in column V. The sum of column V should check with the sum of the sums of columns A, B, C, etc.

3. Multiply each entry in column V by r (where r is the number of replications employed; $r = 5$ in the present example), and enter the results in column rV. The sum of column rV should be r times that of column V.

4. For each assay solution sum all the totals of the columns containing that assay solution result, *e.g.*, for solution 1 in Fig. 1 sum column totals A, B, C, F and K. Enter these totals in column T. The sum of column T should equal that of rV.

5. Subtract T from rV for each assay solution and enter result with appropriate sign in column rV — T. The algebraic sum of these entries should be zero.

6. Where an assay is being performed plot the values rV — T of the standards on semi-logarithmic paper against the appropriate dose-level, due allowance being made for sign.

7. Read off the rV — T values for all other solutions from this standard curve, thus obtaining the potency of the sample dilutions.

A list of designs of this type is given by Fisher and Yates[3] (Table 21). A full analysis, if required, may be carried out as shown there (page 19). We have found the 13 × 4, 11 × 5 and 16 × 6 designs to be most practicable. (The 16 × 6 design can, if necessary, be adapted to accommodate eight solutions at two dose levels.) The standard error of the assay involving 5 replicates is about ±7 per cent.

Several randomisations of the design or designs chosen should be prepared and mounted on templates. In this manner plating out is expedited and repeat or successive assays can be performed with different randomisations.

(*b*) BALANCED LATTICE SQUARES—

This type of design is particularly suitable when a large number of solutions are involved and no assay is required, but simply a test to show whether the potency of any unknown solution exceeds that of the standard. These designs consist of a number of replicate squares, each one containing all the solutions involved, but arranged in a different manner. Each solution appears equally frequently with every other in either the rows or the columns of the designs.

Such designs are possible whenever the number of solutions involved is the square of a prime or the square of a power of a prime.

In practice we have found the seven by seven and the nine by nine arrangements, catering for forty-nine and eighty-one solutions with a replication of 4 and 5, respectively, to be the most practicable.

The loss of information due to the confounding can be quite appreciable. For example, in the seven by seven designs only three-quarters of the information is available from comparisons within the squares, the remaining one-quarter being lost in the comparisons between the rows and columns. If the plate has been well prepared, the positional effects are likely to be small and, further, since each square is plated out individually before proceeding to the next, the temporal effects within each square may also be small, as the total time involved in plating the forty-nine solutions is not large. These considerations show that an appreciable amount of information may be contained in the contrasts between the rows and between the columns.

In certain circumstances only limited amounts of material are available, so that only a few assay zones can be obtained from each sample, *e.g.*, in mass selection programmes of various types. There is then no question of being able to increase the replication per sample in order to achieve additional precision. The only remedy is to make the fullest use of the information

contained in the zones available. In such circumstances the need to carry out the large number of simultaneous comparisons involved in the selection programme is best met by lattice square designs, and the need to make full use of the information available requires recovery of inter-column and inter-row information.

Computation from these designs is, however, tedious, and their use can only be justified when the need for high throughput is coupled with difficulties in preparing more than a small number of replicates of each sample and so outweighs the cost of computation.

An example of a balanced lattice square design suitable for twenty-five solutions is shown in Table II. The computation of these designs is given by Cochran and Cox.[4] The designs should be randomised before use.

TABLE II

5 × 5 BALANCED LATTICE SQUARES FOR TWENTY-FIVE SOLUTIONS

A	B	C	D	E
F	G	H	I	J
K	L	M	N	O
P	Q	R	S	T
U	V	W	X	Y

Square I

A	G	M	S	Y
V	C	I	O	P
R	X	E	F	L
N	T	U	B	H
J	K	Q	W	D

Square II

A	H	O	Q	X
L	S	U	C	J
W	E	G	N	P
I	K	R	Y	B
T	V	D	F	M

Square III

The presentation of this paper and its predecessors in the series[1,2] would not have been possible without the technical help of several of our colleagues, and in particular the highly skilled assistance of Mr. D. E. Miller and Mr. F. Wood. The material of the three papers was originally presented to the Biological Methods Group on Thursday, December 11th, 1952.

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2. ——, ——, *Ibid.*, 1955, **80**, 110.
3. Fisher, R. A., and Yates, F., "Statistical Tables," Fourth Edition, Oliver & Boyd Ltd., Edinburgh, 1953.
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NOTE—Reference 1 is to Part I; reference 2 is to Part II.

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The Analysis of Binary Molybdenum-base Alloys

By G. H. BUSH and D. G. HIGGS

This paper describes methods of analysis evolved for the determination of 15 elements in binary molybdenum-base alloys. The elements considered are aluminium, boron, chromium, cobalt, copper, iron, manganese, nickel, niobium, silicon, tantalum, titanium, tungsten, vanadium and zirconium.

The results of analyses carried out on synthetic mixtures of the various elements with molybdenum are shown to indicate the reproducibility obtainable.

Investigations into the production of binary alloys of molybdenum with a large variety of metals by the vacuum - arc process called for fairly close analytical control both of the raw materials and the final alloy. The methods to be described have been used during the past 2 years with a wide variety of alloys and have proved satisfactory both from the point of view of accuracy and ease of manipulation.

The production of molybdenum-base alloys with a view to a systematic investigation of their commercial uses has only been undertaken seriously in recent years, so that reference in the published literature to methods for the analysis of molybdenum-base alloys are extremely few, and indeed the only methods seen are those described by Maurer in a private communication.[1] Although evolved independently, methods described in this paper for the determination of chromium, cobalt, manganese and silicon are similar to those used by Maurer and differ only in detail, whilst other methods considered differ more materially or are not described by this author. We are, however, indebted to Maurer for a suggestion put forward for the purification of niobium and tantalum hydroxides by acid - sulphite treatment, which we have embodied in the paper.

In the methods to be described, chromium, copper, manganese, silicon and vanadium are directly determined in the presence of major amounts of molybdenum, whilst aluminium, cobalt, iron, nickel, niobium, tantalum, titanium and zirconium are determined after a double precipitation from alkaline solution. Even a double precipitation in alkaline solution fails to remove the last traces of molybdenum from titanium, which is finally determined as the cupferron complex. Residual molybdenum is separated as the sulphide from zirconium, after one precipitation of the latter in alkaline solution by the method to be described under boron.

It is well known that molybdenum can be precipitated completely as sulphide by saturating an alkaline molybdate solution with hydrogen sulphide and decomposing the thiomolybdate formed, by the addition of diluted sulphuric acid in slight excess, and this principle is used in the determination of boron. The separation is open to the criticism that the minor constituent is determined in the filtrate after removal of the major constituent by precipitation, but we have shown that molybdenum sulphide does not occlude boron when the precipitation is carried out as described. Tungsten is separated from molybdenum by removing the latter as sulphide by precipitation from a slightly acid solution containing tartaric acid. The tungsten is determined absorptiometrically as tungsten thiocyanate, the effect of residual traces of molybdenum being suppressed by the use of titanous chloride.

In the course of experimental work it was found that the best solvents for molybdenum alloys were—

(*a*) hydrochloric acid, sp.gr. 1·18, with the addition of sufficient nitric acid, sp.gr. 1·42, to maintain vigorous dissolving action, and

(*b*) diluted sulphuric acid (1 + 3) with sufficient nitric acid, sp.gr. 1·42, to effect solution.

One or other of these solvents is effective in opening up all the alloys considered. The use of diluted nitric acid alone is to be avoided, since it usually results in the deposition of molybdic oxide with consequent retardation of further chemical action.

The results shown in Tables I to XV have been obtained on synthetic mixtures of the various elements with molybdenum and indicate the order of recovery obtained by the

methods of analysis described. Pure chemical reagents and distilled water should be used throughout.

Aluminium

The determination of aluminium by means of 8-hydroxyquinoline is preferred to either the ammonium hydroxide or ammonium phosphate precipitation, since it has been found that unless great care is taken these methods lead to high results.

Unless care is exercised during the double precipitation of aluminium hydroxide, it is likely that some molybdate would still be retained; should this be so, it is probable that the molybdenum will be precipitated as its oxinate along with the aluminium.

PROCEDURE—

Weigh accurately 1 to 5 g of sample, depending on the amount of aluminium present, and place it in a 400-ml squat beaker. Add 50 ml of hydrochloric acid, sp.gr. 1·18, and heat with sufficient nitric acid, sp.gr. 1·42, to effect solution.

When dissolved, add 100 ml of water and make alkaline with ammonium hydroxide, sp.gr. 0·880, to the colour change of phenol red (pH 8·4). Boil for a few minutes and filter through a Whatman No. 41 filter-paper, washing well with warm water containing a few drops of ammonium hydroxide (see Note 1).

Dissolve the precipitate into the original beaker with hot diluted hydrochloric acid (1 + 1), dilute to 100 ml and re-precipitate the aluminium, boil, filter and wash as before.

Dissolve the precipitate in hot diluted hydrochloric acid (1 + 1) into the original beaker, dilute to 100 ml and add ammonium hydroxide, sp.gr. 0·880, until most of the acid is neutralised and the precipitation of aluminium hydroxide just fails to take place. Add 10 g of ammonium acetate, heat to 60° to 70° C and precipitate the aluminium by the addition of 50 to 60 ml of a 2 per cent. w/v solution of 8-hydroxyquinoline in 2 *N* acetic acid. Digest until the precipitate forms large flocks, filter the hot mixture through a weighed sintered-glass crucible of porosity No. 4, wash well with hot water and dry at 110° C to constant weight.

NOTE—1. Small amounts of aluminium are not immediately precipitated in a filterable form, and it is recommended that, when the amount present is small, the solution and precipitate should be allowed to stand for several hours, preferably overnight, before filtration.

RESULTS—

Typical results are shown in Table I.

TABLE I

RECOVERY OF ADDED ALUMINIUM FROM ADMIXTURE WITH MOLYBDENUM

Molybdenum taken, g	5·0	5·0	2·0	2·0	1·0	1·0
Aluminium added, g	0·00248	0·00496	0·00496	0·00993	0·00993	0·01985
Aluminium found, g	0·00245	0·00496	0·00495	0·01044	0·01007	0·01925
Aluminium added, %	0·050	0·099	0·25	0·50	0·99	1·98
Aluminium found, %	0·049	0·099	0·25	0·52	1·01	1·92

Boron

This determination is described in a published paper.[2] The molybdenum is separated as the sulphide and the boron subsequently titrated with sodium hydroxide by a potentiometric method.

Chromium

The Vignal method has not been found suitable for the determination of chromium in molybdenum alloys, since a white turbidity develops during the oxidation with permanganate, and subsequently a brownish-red coloration persists even after boiling with manganous sulphate. The ammonium persulphate - silver nitrate oxidation method has been found most suitable.

PROCEDURE—

To 2 g of alloy in a 600-ml conical flask add 50 ml of water, 15 ml of sulphuric acid, sp.gr. 1·84, and heat with sufficient nitric acid, sp.gr. 1·42, to effect complete solution. Remove the nitrous fumes by boiling, and dilute with 200 ml of hot water. Add 5 ml of

4 per cent. w/v manganous sulphate solution, a few fragments of porous boiling-plate together with 0·5 g of silver nitrate and 1·5 to 2·0 g of ammonium persulphate. Boil the solution for 10 minutes. (The manganese present will not be oxidised to permanganate until all the chromium is oxidised; thus it serves as a useful indicator of complete oxidation.)

Destroy the permanganate by the addition of 0·2 to 0·3 g of sodium chloride and, if the pink colour persists, add diluted hydrochloric acid (1 + 3) dropwise to the boiling solution until the pink colour is discharged; continue boiling to expel the chlorine generated. Cool thoroughly, reduce the chromium with a measured excess of 0·1 *N* ferrous ammonium sulphate and titrate the excess with 0·1 *N* potassium permanganate, using 2 drops of 1 per cent. w/v disulphine blue as internal indicator, until 1 drop of oxidant changes the colour of the indicator from green to golden brown.

RESULTS—

Typical results are shown in Table II.

TABLE II

TITRATION OF ADDED CHROMIUM IN PRESENCE OF MOLYBDENUM

Molybdenum taken, g	.. ..	2·0	2·0	2·0	2·0	2·0	2·0
Chromium added, g	.. ..	0·0017	0·0035	0·0087	0·0130	0·0217	0·1046
Chromium found, g	.. ..	0·0017	0·0035	0·0088	0·0131	0·0219	0·1052
Chromium added, %	.. ..	0·085	0·18	0·44	0·65	1·08	5·23
Chromium found, %	.. ..	0·085	0·18	0·44	0·66	1·09	5·26

Cobalt

Direct electro-deposition of cobalt from ammoniacal solution does not take place completely in presence of molybdenum, even when the latter is present in relatively small amount, hence complete separation is essential for quantitative determinations. Apart from this prerequisite no difficulty has been encountered, and the following procedure is quite satisfactory.

PROCEDURE—

To an appropriate weight of sample contained in a 600-ml squat beaker add 40 ml of hydrochloric acid, sp.gr. 1·18, and heat with sufficient nitric acid, sp.gr. 1·42, to effect solution.

When dissolved, cool, and add 20 per cent. w/v sodium hydroxide until the solution is alkaline, then add 5 ml in excess. Add a small quantity of solid sodium peroxide, dilute to 300 ml and boil gently for about 10 minutes.

Allow the precipitate to settle and decant the clear liquid through a paper pulp, add a little pulp to the remaining precipitate, mix, transfer to the filter and wash with hot water.

Dissolve the precipitate into the original beaker with hot diluted sulphuric acid (1 + 3) containing a small quantity of 20-volume hydrogen peroxide, wash well and re-precipitate the cobalt in a volume of about 300 ml with 20 per cent. sodium hydroxide and sodium peroxide as described previously. After the precipitate has been washed with hot water it should be free from molybdenum.

Dissolve the precipitate in 20 ml of hot diluted sulphuric acid (1 + 3) containing a few millilitres of 20-volume hydrogen peroxide and boil to decompose the excess of peroxide.

Cool, add 25 ml of 20 per cent. w/v ammonium sulphate and make alkaline with ammonium hydroxide, sp.gr. 0·880, adding 15 ml in excess. Add about 0·5 g of sodium sulphite to ensure removal of any peroxide and electrolyse the solution in a volume of 100 ml with a current of 1·0 to 1·5 amp., a copper-plated platinum gauze cathode being used. Apply a potential difference to the electrodes before immersing in the electrolyte and immerse the copper-plated portion of the cathode completely during electrolysis.

RESULTS—

Typical results are shown in Table III.

TABLE III

RECOVERY OF ADDED COBALT FROM ADMIXTURE WITH MOLYBDENUM

Molybdenum taken, g	0·5	1·0	1·0	2·0	5·0	5·0
Cobalt added, g	0·0400	0·0477	0·0318	0·0318	0·0400	0·0159
Cobalt found, g	0·0402	0·0476	0·0313	0·0320	0·0415	0·0161
Cobalt added, %	8·00	4·77	3·18	1·59	0·80	0·32
Cobalt found, %	8·04	4·76	3·13	1·60	0·83	0·32

Copper

In the presence of molybdenum, copper is not readily separated quantitatively at the cathode during electrolysis or by the usual chemical means. The iodimetric titration of copper is possible however, even in the presence of up to 5 g of molybdenum, after solution in diluted aqua regia, when the usual precautions have been taken to remove nitrous acid with urea and to complex iron with fluoride. It is preferred to add an excess of iodide, so that the cuprous iodide re-dissolves to form the soluble double salt, thus avoiding the effect of adsorbed iodine.

PROCEDURE—

Weigh accurately an appropriate amount of sample into a 400-ml conical flask, add hydrochloric acid, sp.gr. 1·18, in the proportion of 10 ml of acid to each 1 g of alloy. Heat gently on the hot-plate with the addition of sufficient nitric acid, sp.gr. 1·42, to effect solution. Boil free from nitrous fumes, dilute to 50 ml with water and cool.

Add 10 ml of 10 per cent. w/v urea, 10 ml of 10 per cent. w/v sodium fluoride, and make alkaline with ammonium hydroxide, sp.gr. 0·880. Cool, if necessary, and make just acid with diluted acetic acid (1 + 1), adding about 5 drops in excess.

Add solid potassium iodide until a clear golden-brown solution is obtained and titrate with 0·02 *N* sodium thiosulphate, adding starch solution near the end-point in the usual way.

It has been found that at the end-point the solution is light brown in colour and is unaffected by further additions of sodium thiosulphate.

Standardisation of sodium thiosulphate—To determine the copper equivalent of the sodium thiosulphate, take 1 g of ammonium molybdate, add accurately a known amount of a standard copper solution (1 ml ≡ 0·001 g of copper) and proceed as described above, titrating to the same end-point as the sample.

RESULTS—

Typical results are shown in Table IV.

TABLE IV

TITRATION OF ADDED COPPER IN PRESENCE OF MOLYBDENUM

Molybdenum taken, g	5·0	2·0	2·0	1·0	1·0	0·5
Copper added, g	0·0010	0·0020	0·0050	0·0050	0·0100	0·0100
Copper found, g	0·0009	0·0019	0·0049	0·0050	0·0100	0·0098
Copper added, %	0·020	0·100	0·25	0·50	1·00	2·00
Copper found, %	0·018	0·095	0·25	0·50	1·00	1·96

Iron

The separation of this element from large amounts of molybdenum by re-precipitation from ammoniacal solution presents little difficulty, except that small amounts of ferric hydroxide are precipitated initially in a semi-colloidal form and require prolonged hot digestion before complete precipitation takes place. Direct methods of determination are vitiated by the fact that most reagents for the determination of ferric or ferrous iron also react with molybdenum. The volumetric finish described, involving titration with standard potassium dichromate after reduction of the iron by passing through a silver reductor, has been found to be a clean, rapid and very satisfactory method.

PROCEDURE—

Weigh accurately an amount of sample to give about 50 mg of iron (or 5 mg, if 0·01 *N* potassium dichromate is used for the final titration). Place in a 400-ml squat beaker, add 40 ml of hydrochloric acid, sp.gr. 1·18, and heat with sufficient nitric acid, sp.gr. 1·42, to effect solution.

Dilute to 100 ml with water, neutralise with ammonium hydroxide, sp.gr. 0·880, and add 10 ml in excess. Boil, filter on a Whatman No. 41 filter-paper and wash with warm diluted ammonium hydroxide (1 + 19).

Dissolve the precipitate by washing it with hot diluted hydrochloric acid (1 + 1), collecting the solution in the original beaker, boil, dilute to 100 ml and re-precipitate the iron as before. Filter on a Whatman No. 41 filter-paper and wash the precipitate with diluted ammonium hydroxide (1 + 19).

Re-dissolve the precipitate by washing the paper with hot diluted hydrochloric acid (1 + 1), collecting the solution in the original beaker, boil and dilute to give a solution containing 10 per cent. of hydrochloric acid. Cool.

Pass the cold solution through a silver reductor[3] that has been washed previously with diluted hydrochloric acid (1 + 9), collecting the liquid in a 500-ml conical flask containing 10 ml of phosphoric acid, sp.gr. 1·75, and 25 ml of diluted sulphuric acid (1 + 3). Wash the reductor four times with successive 20-ml portions of diluted hydrochloric acid (1 + 9).

To the flask containing the reduced iron and washings, add a few drops of 1 per cent. w/v barium diphenylaminesulphonate indicator solution and titrate with either 0·1 or 0·01 *N* potassium dichromate, depending on the amount of iron present.

RESULTS—

Typical results are shown in Table V.

TABLE V

RECOVERY OF ADDED IRON FROM ADMIXTURE WITH MOLYBDENUM

Molybdenum added, g	2·0	2·0	2·0	2·0	2·0	2·0
Iron added, g	0·0020	0·0050	0·0100	0·0200	0·0300	0·1000
Iron found, g	0·0018	0·0052	0·0100	0·0200	0·0288	0·0998
Iron added, %	0·10*	0·25	0·50	1·00	1·50	5·00
Iron found, %	0·09	0·26	0·50	1·00	1·44	4·99

* Colorimetric finish.

Manganese

An examination of the normal bismuthate method showed that after preliminary hot bismuthate oxidation, followed by decomposition of the manganese dioxide with a little sulphurous acid, the resulting solution was seldom colourless, even after prolonged boiling. The light brown colour, considered to be associated with residual oxides of nitrogen, could easily be discharged by the addition of urea, which resulted in evolution of gas bubbles and the production of a colourless solution. Quantitative manganese recoveries were obtained in presence of urea, both with and without the preliminary hot bismuthate oxidation stage.

In the procedure to be described the factor weight of 1·1 g is used for alloys containing up to 2 per cent. of manganese, with appropriate fractions of this weight for those of higher manganese content.

PROCEDURE—

Weigh accurately an appropriate amount of sample into a 250-ml conical flask, add 40 ml of diluted sulphuric acid (1 + 3) and 20 ml of nitric acid, sp.gr. 1·42, and boil gently until dissolved and all nitrous fumes have been expelled. Dilute with 50 ml of cold water, add 1 g of urea and cool thoroughly.

Add sufficient sodium bismuthate to develop the colour completely, set aside for 3 minutes, filter through a sintered-glass funnel of porosity No. 4, and wash with diluted nitric acid (1 + 49) until the washings are colourless. Remove the flask from the water-pump and the funnel from the mouth of the flask, add an excess of 0·1 *N* ferrous ammonium sulphate and titrate the excess with potassium permanganate in the usual way.

RESULTS—

Typical results are shown in Table VI.

TABLE VI

TITRATION OF ADDED MANGANESE IN PRESENCE OF MOLYBDENUM

Molybdenum taken, g	1·1	1·1	1·1	1·1	1·1	1·1
Manganese added, g	0·0011	0·0033	0·0055	0·0110	0·0220	0·0330
Manganese found, g	0·0010	0·0033	0·0055	0·0110	0·0197	0·0334
Manganese added, %	0·10	0·30	0·50	1·00	2·00	3·00
Manganese found, %	0·09	0·30	0·50	1·00	1·97	3·04

Nickel

Nickel presents much the same problem chemically as does cobalt, and the remarks cited under cobalt apply equally here. Although the electrolytic method of determination has been described below, no reason exists why the second dimethylglyoxime precipitation should not be used for the classical gravimetric finish, the usual precautions concerning the excess of reagent being borne in mind.

PROCEDURE—

To an appropriate weight of alloy in a 400-ml squat beaker add 40 ml of hydrochloric acid, sp.gr. 1·18, and heat with sufficient nitric acid, sp.gr. 1·42, to effect solution.

When solution is complete, cool, add 10 ml of 50 per cent. w/v citric acid and sufficient ammonium hydroxide, sp.gr. 0·880, to make the solution just alkaline. Heat to about 70° C, add 25 ml of a saturated solution of dimethylglyoxime in 95 per cent. ethanol, heat to boiling and then on a steam-bath for about 30 minutes or until the precipitate has coagulated.

Cool thoroughly, pass the solution through a pulp filter and wash the precipitate well with water containing a few drops of ammonium hydroxide, sp.gr. 0·880. Dissolve the precipitate in hot diluted hydrochloric acid (1 + 1) into the original precipitation beaker. Dilute to about 200 ml with water, add 5 ml of 50 per cent. w/v citric acid solution and ammonium hydroxide until the solution is just alkaline. To ensure complete re-precipitation of the nickel, add 10 ml of dimethylglyoxime solution and heat to coagulate the precipitate. Cool, filter on a pulp pad and wash well with water.

Dissolve the washed precipitate as described above, add 20 ml of diluted sulphuric acid (1 + 3) and heat to fumes of sulphuric acid. Add a little nitric acid, sp.gr. 1·42, heat to destroy organic matter, and evaporate to fumes of sulphuric acid.

Cool, add 10 ml of water and again fume to expel nitric acid completely. Cool, dilute to 25 ml, add 25 ml of 20 per cent. w/v ammonium sulphate, make alkaline with ammonium hydroxide, sp.gr. 0·880, add 15 ml in excess and dilute to 100 ml.

Electrolyse with a current of 1·0 to 1·5 amp., using a copper-plated platinum gauze cathode; apply a potential difference to the electrode before immersing it in the electrolyte, and immerse the copper-plated portion of the cathode completely during electrolysis.

RESULTS—

Typical results are shown in Table VII.

TABLE VII

RECOVERY OF ADDED NICKEL FROM ADMIXTURE WITH MOLYBDENUM

Molybdenum taken, g	0·5	1·0	1·0	2·0	5·0	5·0
Nickel added, g	0·0650	0·0674	0·0337	0·0540	0·0675	0·0166
Nickel found, g	0·0650	0·0680	0·0334	0·0536	0·0670	0·0169
Nickel added, %	13·0	6·74	3·37	2·70	1·35	0·33
Nickel found, %	13·0	6·80	3·34	2·68	1·34	0·34

Silicon

This method is virtually the same as the one commonly used for silicon in steel, and no difficulty has been encountered in the following procedure.

PROCEDURE—

To an appropriate weight of sample in a 600-ml squat beaker add 80 ml of diluted sulphuric acid (1 + 3) and 25 ml of nitric acid, sp.gr. 1·42, a little at a time. Boil gently to effect solution and then evaporate to copious fumes of sulphuric acid. Cool, wash down the sides of the beaker with a little water and again evaporate to the fuming stage to expel nitric acid.

Cool, add cautiously 100 ml of water and boil gently until a clear solution is obtained. Filter off the silica on a small pulp filter, wash once with hot water then with hot diluted hydrochloric acid (1 + 1) and finally several times with hot water till the pulp is free from acid. Transfer the pulp and contents to a weighed platinum dish and ignite first at low temperature and finally at 900° to 1000° C for 30 minutes, cool and weigh.

Determine the silicon by the loss in weight after the usual hydrofluoric acid - sulphuric acid treatment.

RESULTS—

Typical results are shown in Table VIII.

TABLE VIII

RECOVERY OF ADDED SILICON FROM ADMIXTURE WITH MOLYBDENUM

Molybdenum taken, g	5·0	5·0	2·0	1·0	1·0	1·0
Silicon added, g	0·0005	0·0049	0·0098	0·0098	0·0196	0·0294
Silicon found, g	0·0005	0·0049	0·0096	0·0102	0·0194	0·0301
Silicon added, %	0·010	0·098	0·49	0·98	1·96	2·94
Silicon found, %	0·010	0·098	0·48	1·02	1·94	3·01

Tantalum and Niobium

The analytical chemistry of tantalum and niobium is complicated by the few specific reactions of these elements and their great tendency to form hydrolysis products.

The researches of Schoeller[4] and his co-workers on tannic acid precipitation is perhaps the greatest contribution to the analytical treatment of these metals and affords the best method for their determination.

So far as is known, there is little detail in the literature on the subject, including the work of Schoeller,[4] relating to the separation of tantalum and niobium from large amounts of molybdenum. Three possible methods have been considered and tried—

(*i*) the double ammonium hydroxide separation followed by a tannic acid precipitation,

(*ii*) cupferron precipitation after the removal of molybdenum as the sulphide by decomposition of thiomolybdate from a citrate-complexed solution, and

(*iii*) single ammonium hydroxide separation followed by sulphurous acid purification of the tantalum - niobium hydroxides.

The third method cited has been found to be the simplest and most satisfactory; the procedure is as follows.

PROCEDURE—

Weigh accurately an appropriate amount of sample, sufficient to give approximately 50 mg of tantalum or niobium oxide. Place it in a 400-ml squat beaker and dissolve it in 40 ml of hydrochloric acid, sp.gr. 1·18, with sufficient nitric acid, sp.gr. 1·42, to effect solution. Any minute metallic particles remaining unattacked are probably small metallic segregates and their solution is not essential as they will be filtered off at a later stage and ignited to oxide.

Dilute the solution to 100 ml, neutralise with ammonium hydroxide, sp.gr. 0·880, and add 10 ml in excess. Add a little ash-free paper pulp and digest on the hot-plate for 1 hour. Filter on a pulp pad and wash with 5 per cent. w/v ammonium chloride solution containing about 1 per cent. of ammonium hydroxide, sp.gr. 0·880.

Transfer the pulp and contents to a 250-ml squat beaker, add 100 ml of diluted hydrochloric acid (1 + 19) and 25 ml of water saturated with sulphur dioxide. Boil for 2 to 3 minutes then digest for 15 minutes at the side of the hot-plate.

Filter off and wash well with diluted hydrochloric acid (1 + 49), transfer to a weighed platinum dish and ignite at 1000° C. Remove the silica by the usual sulphuric - hydrofluoric acid treatment and finally ignite at 1000° C to constant weight.

If both tantalum and niobium are present together in the alloy, separation is effected by fusing the mixed oxides obtained as above with sodium bisulphate, leaching out, and precipitating with tannic acid according to the fractional precipitation method of Schoeller.

RESULTS—

Typical results are shown in Tables IX and X.

TABLE IX

RECOVERY OF ADDED TANTALUM FROM ADMIXTURE WITH MOLYBDENUM

Molybdenum taken, g	5·0	5·0	2·0	1·0	1·0	0·5
Tantalum added, g	0·0390	0·0772	0·0448	0·0324	0·0431	0·0319
Tantalum found, g	0·0390	0·0772	0·0456	0·0322	0·0432	0·0314
Tantalum added, %	0·78	1·54	2·24	3·24	4·31	6·38
Tantalum found, %	0·78	1·54	2·28	3·22	4·32	6·28

TABLE X

RECOVERY OF ADDED NIOBIUM FROM ADMIXTURE WITH MOLYBDENUM

Molybdenum taken, g	2·0	2·0	2·0	1·0	1·0	1·0
Niobium added, g	0·0052	0·0110	0·0154	0·0124	0·0256	0·0542
Niobium found, g	0·0054	0·0110	0·0154	0·0128	0·0254	0·0546
Niobium added, %	0·26	0·55	0·77	1·24	2·56	5·42
Niobium found, %	0·27	0·55	0·77	1·28	2·54	5·46

Titanium

Precipitation of titanium by means of ammonium hydroxide results in occlusion of some molybdenum, and even after a double ammoniacal separation the titanium has to be freed from remaining molybdenum by cupferron precipitation. A final correction is made for the trace of iron present. Direct precipitation by cupferron is not possible owing to the occlusion of the molybdenum; similarly a direct colorimetric method with hydrogen peroxide is vitiated owing to the colour developed with molybdenum.

PROCEDURE—

Weigh accurately an appropriate amount of sample into a 400-ml squat beaker, add 50 ml of hydrochloric acid, sp.gr. 1·18, and heat gently on the hot-plate, with the addition of sufficient nitric acid, sp.gr. 1·42, to effect solution.

When dissolved, dilute with 100 ml of water and neutralise with ammonium hydroxide, sp.gr. 0·880, adding 10 ml in excess. Boil for a few minutes, filter on a Whatman No. 41 filter-paper and wash with warm diluted ammonium hydroxide solution (1 + 49). Dissolve the precipitate in hot diluted hydrochloric acid (1 + 1) into the original beaker, dilute, and re-precipitate the titanium; filter and wash as described earlier.

Re-dissolve the precipitate with hot diluted hydrochloric acid (1 + 1) into the original beaker and dilute the solution to give a concentration of hydrochloric acid of 10 per cent. v/v in a volume of 200 ml. Cool thoroughly, add a little paper pulp and precipitate the titanium by the addition of an excess of a 6 per cent. w/v aqueous solution of cupferron. Stand the beaker and contents at room temperature for 15 minutes, filter on a small pulp pad and wash with diluted hydrochloric acid (1 + 9) containing a small quantity of cupferron. Finally suck the pulp dry at the water-pump, ignite in a silica crucible, gently at first, later at 1000° C and weigh as titanium dioxide.

NOTES—

1. Since iron is always present in small amounts as an impurity and will be included in the titanium determined as described above, a correction must be applied by determining the iron colorimetrically after fusion of the weighed titanium dioxide with potassium pryosulphate.

2. When the amount of titanium is small, the final determination may be carried out by fusing the residue after ignition with potassium pyrosulphate, leaching out with diluted sulphuric acid (1 + 3) and determining the titanium colorimetrically by the addition of hydrogen peroxide.

RESULTS—

Typical results are shown in Table XI.

TABLE XI

RECOVERY OF ADDED TITANIUM FROM ADMIXTURE WITH MOLYBDENUM

Molybdenum taken, g	2·0	2·0	2·0	2·0	2·0	2·0
Titanium added, g	0·0020	0·0050	0·0090	0·0198	0·0298	0·0321
Titanium found, g	0·0019	0·0050	0·0089	0·0198	0·0294	0·0324
Titanium added, %	0·10	0·25	0·45	0·99	1·49	1·61
Titanium found, %	0·095	0·25	0·45	0·99	1·47	1·62

Tungsten

Most references dealing with separation of tungsten from molybdenum are concerned with the determination of major amounts of tungsten in the presence of relatively small quantities of molybdenum.[5,6,7] The first method to be considered involved the precipitation of tungsten with tannic acid after its separation from molybdenum by decomposition of thiomolybdate with sulphuric acid.

Satisfactory results were obtained with this method by experienced analysts, but it was found that the method was susceptible to slight changes in operational conditions, and that low results could be obtained owing to co-precipitation of tungsten sulphide with molybdenum sulphide.

As an alternative to the separation of molybdenum by decomposition of the thiomolybdate, Hamence's acid-precipitation procedure was tried.[8] By precipitating 0·1 g of molybdenum under these conditions, it was found that the filtrate still contained up to 0·40 mg of molybdenum.

The Methods of Analysis Committee of the Iron and Steel Institute[9] describe a method for a colorimetric determination of tungsten based on that of Westwood and Mayer,[10] in which use is made of the colour developed with tungsten and ammonium thiocyanate, any colour due to small amounts of molybdenum being suppressed by reduction with titanous chloride. By using this method for the determination of tungsten, after separation of molybdenum from tungsten under the conditions described by Hamence, it was found that the residual molybdenum present gave a red colour with thiocyanate, which on reduction with titanous chloride faded to a pale yellow and gave only a slight absorption at 400 mμ, the wavelength used for the determination of tungsten. This absorption was very weak, 0·4 mg of molybdenum producing a solution with an optical density of only 0·005. This value represented the maximum interference by molybdenum found in many determinations carried out.

The tungsten thiocyanate colour developed varied in intensity with the amount of ammonium thiocyanate used and it was, therefore, of importance to measure this reagent accurately. The calibration curve showing the linear relationship between the optical density and tungsten content would become invalid should the thiocyanate solution vary in composition after preparation of the curve.

It is for this reason that the amount of tungsten present was determined by proportionality from the optical density of the tungsten thiocyanate solution and standard solution of known tungsten content treated at the same time and under the same conditions as the sample. It is important that the volume of solution immediately before the addition of stannous chloride does not exceed 5 ml, since the reduction of the tungsten by this reagent must take place in an acid of concentration not less than 80 per cent. v/v of hydrochloric acid.

This method also made some correction for the effect of the small amount of molybdenum that was not precipitated under the conditions of the method.

PROCEDURE—

To 1 g of sample in a 250-ml squat beaker add 50 ml of hydrochloric acid, sp.gr. 1·18, and sufficient nitric acid, sp.gr. 1·42, to effect solution. Fit a cover-glass to the beaker, heat gently until solution is complete, remove the cover-glass and evaporate the solution just to dryness on an asbestos pad. Cool, add 50 ml of 20 per cent. w/v sodium hydroxide, heat until solution is complete, cool to room temperature and dilute to 200 ml in a calibrated flask.

To a carefully measured 20-ml aliquot of the solution in a 300-ml conical flask add 10 ml of 20 per cent. w/v tartaric acid, make just acid to litmus with diluted sulphuric acid (1 + 3) and add 3 ml in excess. Dilute to about 150 ml, heat to 60° C (measured) and saturate with a rapid stream of hydrogen sulphide gas until the solution appears to be colourless between the flocks of the precipitate. Heat just to boiling point and cool immediately in a water-bath to room temperature.

Filter through a fairly tight pulp filter into a 600-ml tall beaker, first decanting the supernatant liquid and then the precipitate admixed with a little paper pulp. Wash the beaker and precipitate at least six times with diluted sulphuric acid (1 + 99) and discard the pulp and precipitate. Boil the solution until it is free from hydrogen sulphide, cool it and treat it as follows—

0 to 1·0 per cent. of tungsten	..	Use all the solution.
1·0 to 5·0 per cent. of tungsten	..	Dilute to 250 ml and take a 50-ml aliquot.
5·0 to 10·0 per cent. of tungsten	..	Dilute to 250 ml and take a 25-ml aliquot.
10·0 to 15·0 per cent. of tungsten	..	Dilute to 500 ml and take a 25-ml aliquot.

Evaporate the solution or an aliquot to about 5 ml in a 200-ml wide-mouthed conical beaker,* add a few millilitres of a saturated solution of bromine in water, again evaporate to 5 ml, determined by a mark on the beaker corresponding to the liquid level when the beaker is tilted on an angle block of 60°, cool, add 20 ml of 12·5 per cent. w/v stannous chloride solution in hydrochloric acid, sp.gr. 1·18, 10 ml of 7·5 per cent. w/v ammonium thiocyanate, accurately measured from a burette, and from a second burette 0·5 ml of 10 per cent. w/v titanous chloride. Mix well, transfer to a clean 50-ml calibrated flask, dilute to the mark and again mix well. Set aside for at least 10 minutes, transfer to a clean dry 10-mm silica cell and, using a Unicam SP500 spectrophotometer, measure the optical density at a wavelength of 400 mμ.

Determine the amount of tungsten present by proportionality from the optical density of the solution and that of a solution obtained by carrying out the procedure described with a solution containing the equivalent of 1 g of molybdenum to which has been added a known amount of tungsten.

RESULTS—

Typical results are shown in Table XII.

TABLE XII

RECOVERY OF ADDED TUNGSTEN FROM ADMIXTURE WITH MOLYBDENUM

Molybdenum taken, g	1·0	1·0	1·0	1·0	1·0	1·0	1·0	1·0
Tungsten added, g ..	0·0080	0·015	0·0250	0·0500	0·0750	0·0950	0·0125	0·0150
Tungsten found, g ..	0·00795	0·0148	0·0251	0·0492	0·0752	0·0950	0·01243	0·01499
Tungsten added, %..	0·80	1·50	2·50	5·00	7·50	9·50	12·50	15·00
Tungsten found, %..	0·795	1·48	2·51	4·92	7·52	9·50	12·43	14·99

Vanadium

The method published by Ridsdale[11] for vanadium in steel has been found to give satisfactory results on synthetic mixtures of molybdenum and vanadium. The following method is an abridged version and is applicable in the presence of most elements likely to be found associated with molybdenum, *e.g.*, aluminium, chromium, cobalt, copper, iron, manganese, nickel, silicon, tantalum, niobium, titanium, tungsten and zirconium. In the presence of tantalum, niobium, silicon and tungsten it will be necessary to add a mixed

* When evaporating the solution from a sample in the 0 to 1·0 per cent. of tungsten range, use first a 600-ml tall beaker and transfer to a 200-ml wide-mouthed conical beaker when the volume is approximately 50 ml.

"Analoid" No. 317, which contains 4 g of sodium fluoride and 2 g of potassium nitrate, during solution of the alloy.

PROCEDURE—

To 1 g of sample in a 600-ml squat beaker with marks at 100-ml and 170-ml levels add 50 ml of diluted sulphuric acid (1 + 4) and heat with sufficient nitric acid, sp.gr. 1·42, to effect complete solution. Dilute to the 100-ml mark with hot water and oxidise any organic matter by boiling for 10 minutes with 0·75 g of ammonium persulphate ("Analoid" No. 8b).

Dilute the solution to the 170-ml mark with cold water and add a definite excess (about 5 ml) of 0·1 *N* ferrous ammonium sulphate solution, cool to room temperature and titrate slowly with 0·1 *N* potassium permanganate until a definite pink colour persists for about 1 minute, at which stage all the vanadium has been oxidised.

Destroy the excess of permanganate by dropwise addition of 0·35 per cent. w/v sodium nitrite solution until the pink colour fades and add 3 drops in excess. Add 1·4 g of urea (two No. 31 "Analoids"), and allow the solution to stand for 5 minutes to destroy the excess of nitrite.

Add one "Analoid" No. 32 of barium diphenylaminesulphonate, stir until dissolved, leave for 5 minutes for the full colour to develop, and titrate with 0·025 *N* ferrous ammonium sulphate to a brownish-green end-point. The usual indicator blank must be deducted from the final titration.

The apple-green end-point with steel analysis is not attainable, particularly with high vanadium concentration; further, the end-point tends to lag a little, so that the final additions of the titrant should be made slowly.

RESULTS—

Typical results are shown in Table XIII.

TABLE XIII

TITRATION OF ADDED VANADIUM IN PRESENCE OF MOLYBDENUM

Molybdenum taken, g	1·00	1·00	1·00	1·00	1·00	1·00
Vanadium added, g	0·0005	0·0010	0·0018	0·0049	0·0098	0·0196
Vanadium found, g	0·0005	0·0010	0·0017	0·0048	0·0097	0·0198
Vanadium added, %	0·05	0·10	0·18	0·49	0·98	1·96
Vanadium found, %	0·05	0·10	0·17	0·48	0·97	1·98

Zirconium

When zirconium is precipitated with cupferron, after two preliminary separations with ammonium hydroxide, the zirconium oxide obtained still contains various amounts of molybdenum in addition to any iron present in the sample. Whilst the iron and molybdenum present in the precipitate can be determined and the necessary corrections made, a single ammonium hydroxide separation followed by removal of molybdenum as sulphide, as described below, was found to be quicker and has the added advantage that no correction other than for iron is required.

PROCEDURE—

Weigh accurately an appropriate amount of sample into a 400-ml squat beaker, add 50 ml of hydrochloric acid, sp.gr. 1·18, and heat gently on the hot-plate with the addition of sufficient nitric acid, sp.gr. 1·42, to effect solution.

When dissolved, add 150 ml of water, boil free from nitrous fumes, and add 5 g of ammonium chloride. Make the solution alkaline with ammonium hydroxide, sp.gr. 0·880, add 10 ml in excess, heat to boiling and continue to boil for 3 minutes. Allow to settle for 10 to 15 minutes, filter on a Whatman No. 41 filter-paper or a small pulp pad, and wash several times with a warm 5 per cent. w/v solution of ammonium chloride that has been made just alkaline with ammonium hydroxide.

Re-dissolve the precipitate into the original beaker, using hot diluted hydrochloric acid (1 + 1), and wash well with hot water. To the solution add 5 ml of 50 per cent. w/v citric acid, ammonium hydroxide, sp.gr. 0·880, in slight excess, and saturate with hydrogen sulphide.

Add diluted sulphuric acid (1 + 3) until the colour fades or until no yellow colour can be seen between the flocks of the precipitate. Add sufficient acid to give a 1 per cent. w/v excess, heat to boiling to coagulate the molybdenum sulphide and cool.

Filter through a small pulp pad into a 600-ml squat beaker, wash with 5 per cent. w/v ammonium nitrate solution and reject the precipitate. Boil the filtrate free from hydrogen sulphide and add a few drops of bromine water to destroy the last traces. Cool and dilute to 200 ml.

To the cold solution add 40 ml of diluted sulphuric acid (1 + 3) and a slight excess of 6 per cent. w/v cupferron solution down the side of the beaker with vigorous stirring. When the precipitate settles, test for excess of reagent by the addition of a little cupferron to the supernatant liquid.

Allow to stand at room temperature for about 15 minutes, filter off on a small paper pulp and wash well with diluted hydrochloric acid (1 + 19) containing a little of the reagent.

Ignite gently until all the organic matter has been destroyed, then strongly at 1000° C for 30 minutes and weigh as zirconium oxide.

Correct for the iron present as an impurity in the alloy, and included in the zirconium oxide precipitate, by fusing the oxide with potassium pyrosulphate, dissolving the melt in hydrochloric acid and determining the iron colorimetrically by means of potassium thiocyanate.

RESULTS—

Typical results are shown in Table XIV.

TABLE XIV

RECOVERY OF ADDED ZIRCONIUM FROM ADMIXTURE WITH MOLYBDENUM

Molybdenum taken, g	.. ..	5·0	5·0	5·0	5·0	5·0	5·0
Zirconium added, g	.. ..	0·0019	0·0048	0·0096	0·0191	0·0334	0·0478
Zirconium found, g	.. ..	0·0019	0·0046	0·0098	0·0197	0·0340	0·0492
Zirconium added, %	.. ..	0·038	0·096	0·19	0·38	0·67	0·96
Zirconium found, %	.. ..	0·038	0·092	0·20	0·39	0·68	0·98

The authors are indebted to Mr. F. W. Box of this Establishment for preparing the numerous "unknown" mixtures. Reproduced with the permission of the Controller of H.M. Stationery Office.

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A Rapid Method for the Determination of Microgram Quantities of Germanium

By E. H. STRICKLAND

A rapid and accurate method for the determination of germanium in ores is presented. The sample is brought into solution with hot concentrated phosphoric acid. After the addition of concentrated hydrochloric acid the germanium is extracted into carbon tetrachloride and finally recovered as tri-oxalatogermanic acid. The oxalates are destroyed and the germanium is determined colorimetrically with quinalizarin acetate in an ammonium oxalate - oxalic acid solution buffered to pH 5.

The method is virtually free from interferences, and determinations can be conducted in under 2 hours.

GERMANIUM is known to occur in substantial amounts in only three rare minerals, namely, germanite from Tsumeb, South-West Africa, argyrodite and the recently discovered renierite from the Belgian Congo. In these minerals the germanium content may be in excess of 10 per cent. More frequently it occurs in quantities in the neighbourhood of 0·01 to 0·0001 per cent. or less in most zinc ores, descloizite, vanadinite, cassiterite, euxenite, many coals and some iron ores.

A rapid and accurate method for the determination of this valuable element is highly desirable, not only on account of its intrinsic value *per se*, but also in that it might stimulate further investigation into possible sources thereof.

Current methods invariably necessitate a distillation from interfering elements as the volatile germanium tetrachloride. This distillation is not quantitative in the presence of insoluble matter, silica and lead sulphate being particularly undesirable. Silica may be removed with hydrofluoric acid, but lead is objectionable. It was found that all zinc, copper, lead and vanadium minerals, together with wolfram, were completely soluble in hot concentrated orthophosphoric acid and nitric acid without any loss of germanium, provided that chlorides were absent. The decomposition is rapid and complete. Subsequent dilution with concentrated hydrochloric acid yields a virtually clear solution from which the germanium can be quantitatively extracted with carbon tetrachloride. In 1939, Tchakirian,[1] working upon South-West African germanite, exploited the readiness with which germanium formed a complex oxalatogermanic acid, and it was found that the germanium could be rapidly extracted from the carbon tetrachloride with an ammonium hydrogen oxalate solution. This reduced the time taken by Newcombe, Beamish, McBryde and Bartlett.[2]

Before publications by Nair and Gupta,[3,4] after the destruction of the oxalates the germanium was determined colorimetrically with quinalizarin in a buffered acetate solution. With quinalizarin it was found that, whilst mean values were satisfactory, there was a certain lack of precision, and upon occasion a highly erratic result was obtained. This was attributed to the extreme versatility and sensitivity of the reagent. Nair and Gupta[3,4] acetylated quinalizarin and found that the introduction of the acetyl group, presumably by blocking an –OH group, reduced the sensitivity to numerous interfering elements without impairing the sensitivity to germanium. The reagent, sensitive to 1 in 36,000,000, proved superior to quinalizarin and was employed in the subsequent determinations.

In addition to using quinalizarin acetate, the phenylfluorone method developed by Cluley[5] was used as a comparison together with the oxidised hematoxylin method of Newcombe, Beamish, McBryde and Bartlett.[2] In using the latter method, before distillation the silica was removed from the hemimorphite and willemite with hydrofluoric acid. In the determination of the germanium in descloizite and vanadinite the ores were added directly to the distillation flask containing concentrated hydrochloric acid, in which they are soluble.

METHOD

REAGENTS—

Ammonium oxalate - oxalic acid solution—Weigh out 5·0 g of ammonium oxalate and 5·0 g of oxalic acid into a 600-ml beaker. Dissolve in 500 ml of water and make up to 1 litre in a calibrated flask. Adjust the pH to 5.

Gelatin solution—Dissolve 0·5 g of best quality gelatin in 100 ml of water. Filter if necessary.

Quinalizarin acetate solution—Dissolve 0·1 g of quinalizarin acetate in 100 ml of methanol. Filter.

Phenylfluorone solution—Dissolve 0·05 g of phenylfluorone in 100 ml of 95 per cent. ethanol. Filter.

PREPARATION OF QUINALIZARIN ACETATE (FROM NAIR AND GUPTA[3,4])—

Heat under reflux 1·0 g of quinalizarin with 10 ml of acetic anhydride and 2·5 g of freshly fused anhydrous sodium acetate for 2 hours at 130° C. Cool the mixture and pour into 200 ml of iced water. Allow the solution to stand for 24 hours and filter. Wash with cold water, recrystallise from methanol and dry under reduced pressure at 60° C.

DECOMPOSITION PROCEDURE—

The method owes its rapidity to the facility with which willemite, hemimorphite, sphalerite (blende), smithsonite (calamine), galena, cerussite, anglesite, dioptase, chrysocolla, bornite, chalcocite, chalcopyrite, malachite, azurite, descloizite and slags are rendered soluble with orthophosphoric acid and nitric acid without loss of germanium, provided chlorides are absent.

However, with the silicates such as willemite, hemimorphite, dioptase, chrysocolla and slags, decomposition must not be prolonged beyond the period required to effect complete decomposition. On prolonged heating it would appear that there is an interaction between the silica and phosphoric acid, and a hard insoluble crust forms upon the bottom of the beaker. This vitiates the whole object of the decomposition procedure, which is to render all silica and lead soluble, so releasing any combined germanium.

In the silicates mentioned the silica is gelatinous and easily dissolved. Other refractory siliceous ores may be initially decomposed with 4·0 ml of 50 per cent. sulphuric acid, 5 ml of nitric acid and 5 ml of concentrated hydrofluoric acid, followed by the phosphoric acid treatment, without loss of germanium.

PROCEDURE—

Weigh out 0·25 to 0·50 g of sample into a 150-ml beaker. Add 5 ml of syrupy phosphoric acid and 5 ml of concentrated nitric acid.

Heat gently until all nitric acid is expelled, and transfer to an efficient bare hot-plate. Heat at the full temperature until the sample is completely decomposed, but not longer than necessary. Cool, and to the viscous semi-glassy mass add 25 ml of concentrated hydrochloric acid. Stir gently until dissolved and, with the aid of small portions of concentrated hydrochloric acid, transfer to a 75-ml separating funnel. Extract twice with 15-ml portions of carbon tetrachloride, shaking—with each extraction—for 2 minutes.

Transfer the carbon tetrachloride layers to another 75-ml separating funnel and again extract twice, for 2 minutes, with 10-ml portions of the ammonium oxalate - oxalic acid solution.

Transfer the oxalate extracts to a 250-ml beaker and add 20 ml of concentrated nitric acid. Cautiously evaporate to dryness, but do not bake. Cool and proceed according to (*i*) or (*ii*).

(*i*) To the cold residue add 5·0 ml of the ammonium oxalate - oxalic acid solution. Warm gently until solution is effected. Cool, add 1·0 ml of gelatin solution followed by 1·0 ml of quinalizarin acetate solution. Allow to stand for 30 minutes before dilution. With the aid of the ammonium oxalate - oxalic acid solution, transfer to a 25-ml calibrated flask and make up to volume. Determine the transmittance at 500 mμ.

(*ii*) To the residue add 5·0 ml of *N* sulphuric acid and evaporate to dryness. Cool and add a further 5·0 ml of the *N* sulphuric acid solution. Warm to effect solution, cool, and with the aid of *N* sulphuric acid transfer to a 25-ml calibrated flask. Add 1·0 ml of gelatin solution followed by 5·0 ml of phenylfluorone solution. Make up to 25 ml with *N* sulphuric acid and, after 30 minutes, determine the transmittance at 510 mμ.

PREPARATION OF CALIBRATION CURVES—

Weigh out 0·2000 g of pure dry germanium dioxide into a 250-ml beaker. Add 5·0 g of ammonium oxalate, 5·0 g of oxalic acid and 100 ml of water. Heat until all the germanium dioxide has dissolved. Cool and make up to 1 litre.

Transfer 5·0 ml of this solution to a 1-litre calibrated flask and make up to volume with a solution containing 5·0 g of ammonium oxalate and 5·0 g of oxalic acid per litre.

1·0 ml = 1·0 μg of germanium dioxide.

Into each of 11 small beakers add 0, 1, 2, 3 . . . 9 and 10 μg (ml) of the germanium dioxide solution. Add 25 ml of concentrated nitric acid and evaporate to dryness, but do not bake. Proceed according to procedure (*i*) or (*ii*) described above. The colour developed with quinalizarin acetate obeys Beers law.

RESULTS

Four common zinc minerals and a sample of descloizite were assayed by the proposed method, both quinalizarin acetate and phenylfluorone being used. As a check, the samples

TABLE I

DETERMINATION OF GERMANIUM BY VARIOUS METHODS

Sample	Germanium added, % of GeO_2	Quinalizarin acetate method: Found, % of GeO_2	Quinalizarin acetate method: Present, % of GeO_2	Phenylfluorone method: Found, % of GeO_2	Phenylfluorone method: Present, % of GeO_2	Oxidised hematoxylin method: Found, % of GeO_2	Oxidised hematoxylin method: Present, % of GeO_2
Hemimorphite, $2ZnO.SiO_2.H_2O$	Nil	0·0027	0·0027	0·0025	0·0025	0·0025	0·0025
	0·0010	0·0035	0·0025	0·0034	0·0024	0·0037	0·0027
	0·0025	0·0053	0·0028	0·0053	0·0028	0·0051	0·0026
		Mean ..	0·0027	Mean ..	0·0026	Mean ..	0·0026
Willemite, $2ZnO.SiO_2$ ..	Nil	0·0016	0·0016	0·0018	0·0018	0·0018	0·0018
	0·0010	0·0026	0·0016	0·0022	0·0012	0·0025	0·0015
	0·0025	0·0042	0·0017	0·0040	0·0015	0·0039	0·0014
		Mean ..	0·0016	Mean ..	0·0015	Mean ..	0·0016
Smithsonite, $ZnCO_3$ ..	Nil	0·0004	0·0004	0·0004	0·0004	0·0003	0·0003
	0·0010	0·0013	0·0003	0·0015	0·0005	0·0014	0·0004
	0·0025	0·0030	0·0005	0·0031	0·0006	0·0031	0·0006
		Mean ..	0·0004	Mean ..	0·0005	Mean ..	0·0004
Sphalerite, ZnS ..	Nil	0·0002	0·0002	0·0002	0·0002	0·0002	0·0002
	0·0010	0·0012	0·0002	0·0013	0·0003	0·0013	0·0003
	0·0025	0·0028	0·0003	0·0027	0·0002	0·0028	0·0003
		Mean ..	0·0002	Mean ..	0·0002	Mean ..	0·0003
Descloizite, $2PbO.2ZnO.V_2O_5.H_2O$	Nil	0·0007	0·0007	0·0006	0·0006	0·0007	0·0007
	0·0010	0·0016	0·0006	0·0016	0·0006	0·0017	0·0007
	0·0025	0·0031	0·0006	0·0033	0·0008	0·0032	0·0007
		Mean ..	0·0006	Mean ..	0·0007	Mean ..	0·0007

were also examined by the oxidised hematoxylin method. In addition to determining the germanium dioxide content of the minerals as received, to each was added an equivalent of 0·0010 and 0·0025 per cent. of germanium dioxide. To ensure that the percentage of germanium dioxide was within the range of the calibration graph suitable aliquots were taken. The results are given in Table I, all values being expressed as percentage of germanium dioxide.

DISCUSSION OF RESULTS

A sample of vanadinite was also assayed, but the use of both quinalizarin acetate and phenylfluorone resulted in extremely low results as compared with the oxidised hematoxylin

method. The sample was found to contain 2·48 per cent. of chlorine. In these circumstances the phosphoric acid decomposition resulted in a loss of germanium as germanium tetrachloride. The method of decomposition needs modification if substantial amounts of chlorides are present.

It does not appear to matter whether the final determination is made with quinalizarin acetate or phenylfluorone. The blende was known to contain 0·01 per cent. of gallium, 0·04 per cent. of antimony and 0·45 per cent. of arsenic and all samples contained iron and arsenic, but no interferences were encountered.

Although phenylfluorone is relatively insensitive to iron and arsenic, in an oxalate solution at pH 5 quinalizarin acetate gives no colour reaction with arsenic, iron, aluminium, molybdenum, manganese, zinc, vanadium, uranium, phosphorus and silica.

Quinalizarin acetate is more sensitive than either of the other two reagents, a difference of 1 μg of germanium dioxide in 25 ml being detectable.

Should substantial amounts of iron, antimony, arsenic and oxidising agents find their way into the solution they would affect the phenylfluorone determination. An oxalate solution has the additional advantage that arsenic and antimony in such a solution may be removed by hydrogen sulphide with no loss of germanium. Similarly, iron may be precipitated as hydroxide from an oxalate solution without co-precipitation of germanium. A determination of macro quantities of germanium, based upon these facts, will be the subject of further work.

As the quinalizarin acetate is laboratory prepared, variations in batches might necessitate a new calibration curve for each batch prepared. It is therefore advisable to prepare several batches at once and to mix these well before use.

In conclusion, the author thanks the Management of The South-West Africa Company Limited, Grootfontein, S.-W.A., for permission to publish this paper, which is the outcome of work undertaken in the laboratory of the Abenab West Mine.

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Determination of Amides in Aqueous and Non-aqueous Solution by the Conway Diffusion Technique

By H. E. HALLAM*

A rapid method is described for the determination of amide nitrogen to an accuracy of ±0·2 per cent. It is shown that solutions of hygroscopic amides cannot be made up precisely by weight. Silicone grease is suggested as a fixative in the Conway technique.

STUDIES on the distribution coefficients of amides necessitated a rapid analytical technique capable of an accuracy better than 0·5 per cent. At first a semi-micro ammonia distillation technique with a modified Parnas - Wagner assembly[1,2] was used. With this a determination of either the aqueous or non-aqueous layer could be carried out in 1 hour with an accuracy of 0·2 per cent. on a sample containing approximately 40 mg of nitrogen.

More recently[3] this micro-technique was developed, based on Conway's principle of micro-diffusion,[4,5] in which the effective time per analysis is about 15 minutes. This method of ammonia determination was introduced by Conway and Byrne[4] and is well summarised by Conway.[5] In the technique distillation is avoided, the transfer of ammonia taking place by diffusion from a solution to which alkali is added, in one chamber of a Conway unit, in which it exerts a vapour pressure, into standard acid in another chamber in which its pressure is reduced to zero, followed by titration.

Experimental

Standard No. 1 Conway units were used. Deliveries were made with 1-ml tube pipettes, prepared as detailed by Conway.[5] The latter were cleaned by daily immersion in acid permanganate for several minutes.

The optimum sample was found to be 0·28 mg of nitrogen contained in 1 ml, *i.e.*, 0·02 *N*. Approximately 1 ml of 10 *N* potassium hydroxide was used to liberate the base, which was absorbed in about 1 ml of 1·5 per cent. boric acid, with methyl red - bromocresol green mixed indicator incorporated. After oscillation on a Gallenkamp oscillating table, the ammonia or methylamine was titrated directly with 0·02 *N* hydrochloric acid from a 2-ml Bang-type burette, graduated in 0·01 ml and previously calibrated at 0·1-ml intervals.

Materials—

Boric acid absorbent—Prepared as detailed by Conway.[5] Fixative was high-grade vaseline in the case of aqueous solutions and silicone grease for the non-aqueous solutions.

Formamide—Kindly supplied by Dr. T. G. Cleasby. It had been distilled under reduced pressure and was kept over phosphorus pentoxide; $n_D^{26°C} = 1{\cdot}4448$; $n_F^{26°C} - n_C^{26°C} = 1{\cdot}175 \times 10^{-2}$.

Acetamide—The same sample as used previously[2]. It had been kept over phosphorus pentoxide for 4 years.

N-Methylacetamide—The British Drug Houses reagent, vacuum-distilled, b.p. 104·0° C at 15 mm. Crystallised in long white needles, m.p. 27·9° to 28·1° C (corr.); kept over phosphorus pentoxide.

Methyl carbamate—The British Drug Houses product recrystallised from analytical-reagent grade carbon tetrachloride as long thin white needles, which were freed from solvent by repeated evacuation in a desiccator containing silica gel and paraffin wax. The crystals, m.p. 55·5° to 56·0° C (corr.), had a faint almond odour.

Urethane—The British Drug Houses reagent was recrystallised from analytical-reagent grade benzene and freed from solvent as for methyl carbamate, m.p. 48·0° to 48·7° C.

N-Methylurethane—The British Drug Houses product was vacuum-distilled to give a colourless liquid with a fruity ester odour, b.p. 77·5° C at 16 mm; $n_D^{26°C} = 1{\cdot}4157$; $n_F^{26°C} - n_C^{26°C} = 8{\cdot}44 \times 10^{-3}$.

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CLEANING OF UNITS—

The success of the method very largely depends on having meticulously clean units, *i.e.*, clean in an acid - alkali sense and free from grease. If the units have the slightest trace of grease, the liquids will not cover the surfaces thoroughly and the absorption times will be appreciably longer than expected. The following cleaning routine was developed and strictly adhered to—

(*a*) thoroughly wash units under the cold-water tap,
(*b*) wipe off surplus grease with a piece of cotton-wool,
(*c*) rinse in approximately 0·005 *N* sulphuric acid,
(*d*) re-wash under tap and immerse in light petroleum for 15 minutes,
(*e*) re-wash and immerse in a detergent solution (Teepol) for 15 minutes,
(*f*) thoroughly re-wash, finally with distilled water, and
(*g*) oven-dry, or immerse in rectified spirit and air-dry.

DETERMINATIONS IN AQUEOUS SOLUTION—

A thorough investigation was made of the absorption conditions of aqueous solutions of formamide, acetamide, N-methylacetamide and urethane at room temperature. One-millilitre volumes of 0·02 *M* solutions (*i.e.*, a sample containing 0·28 mg of nitrogen) were taken and determined, with and without oscillation; the resultant absorption curves are shown in Figs. 1 and 2.

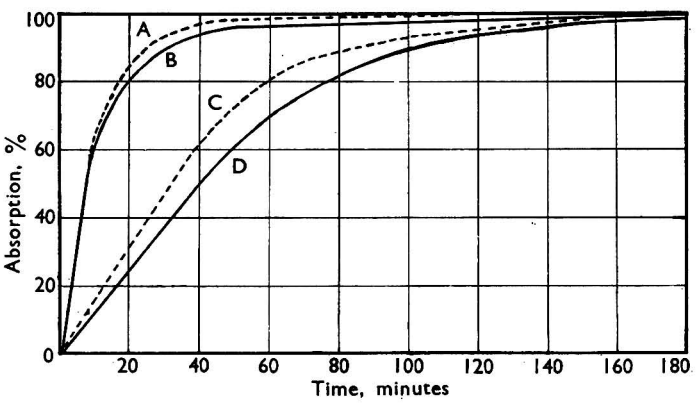

Fig. 1. Ammonia absorption curves at 31° C from 1 ml of 0·02 *M* aqueous solution of: A, formamide, with oscillation; B, formamide, without oscillation; C, acetamide, with oscillation; D, acetamide, without oscillation

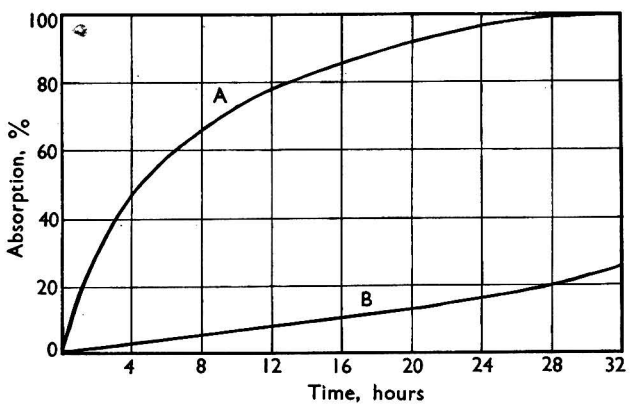

Fig. 2. A, absorption curve for methylamine from 1 ml of a 0·02 *M* aqueous solution of N-methylacetamide; temperature 30° C. B, absorption curve for ammonia from 1 ml of a 0·02 *M* aqueous solution of urethane; temperature 32° C

It was found that formamide can be determined to an accuracy of ±0·2 per cent. in 3 hours without oscillation, 99 per cent. of the ammonia being absorbed after 2 hours. With oscillation the 100 per cent. absorption time is reduced to 110 minutes. Acetamide can also be determined with equal accuracy in 5 hours without oscillation, the 99 per cent. absorption taking 2¾ hours. With oscillation absorption is complete in 3 hours.

The two curves differ markedly from those of Conway[5] (p. 15) for ammonia absorptions from nitrogen fixed as an ammonium salt, in having a long period between 99 and 100 per cent. absorption. This is due to the slower rate of ammonia evolution from the amides, the marked difference between the formamide and acetamide curve being due to the slower rate of hydrolysis of the latter. Also, the speeding-up effect of oscillation is much more pronounced in the case of the amides.

For N-methylacetamide the same accuracy was again achieved, the complete absorption taking 32 hours, presumably owing to the slow rate of methylamine absorption (*op. cit.*, p. 178) rather than a slow rate of hydrolysis. Oscillation was not attempted here, as Conway (*op. cit.*, p. 22) has found that oscillation does not notably increase the rate of a slow absorption.

In the case of urethane the method proved useless, only 44 per cent. of the ammonia being absorbed after 43 hours, owing to the very slow rate of hydrolysis.[6]

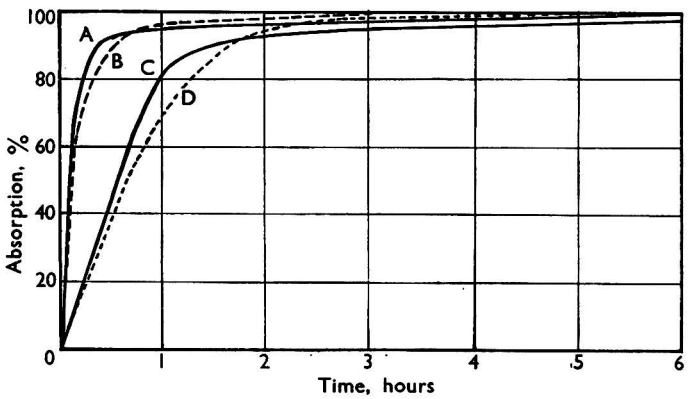

Fig. 3. Ammonia absorption curves at 31° C from 1 ml of 0·02 *M* chloroform solutions of: A, formamide; and C, acetamide, both without oscillation (curves B and D are reproduced from Fig. 1, curves B and D, respectively, for comparison, *i.e.*, aqueous solutions under identical conditions)

DETERMINATIONS IN NON-AQUEOUS SOLUTION—

A similar investigation was made for 0·02 *M* solutions of formamide and acetamide in chloroform and N-methylacetamide in carbon tetrachloride. It was found that the first two can be determined to ±0·2 per cent., although the difficulties involved are appreciable. The greatest of these is the high vapour pressure of the solvent, which causes it to condense on the lids and also to "creep" up the sides of the unit. Sometimes droplets of condensate fell into the central compartment and when this occurred the titre was always absurdly high, owing to the condensate carrying over traces of alkali into the boric acid.

The high volatility also caused the lids to blow off unless weighted; 2-lb. weights were found to be suitable, but were too clumsy to allow the units to be oscillated. To facilitate this some type of screw clamp arrangement fitted to the oscillating table to keep the lids down is required.

To overcome these difficulties analyses were attempted by first allowing the solvent to evaporate before covering up the unit and adding alkali. Results from these were neither accurate nor consistent, owing to evaporating solvent "creeping" up the sides of the unit and depositing the amide, in various amounts, high up on the sides, where the alkali was unable to attack it.

It was thus necessary to face the difficulties caused by the presence of the solvent. The lids were liberally coated with vaseline in the middle, in addition to the film of silicone grease fixative on the edges, in order to absorb as much as possible of the condensate. A film

of silicone grease was also spread on the top of the wall of the central compartment to prevent direct "creep" over. In spite of these precautions contamination still occurs in about one unit in three. Contaminated units are immediately obvious on opening, owing to the presence of blobs of grease floating on the boric acid, and are rejected. It must be emphasised that the units in which solvent does not pass into the central chamber present no trouble at all and the end-points are quite as sharp and accurate as those obtained from aqueous solutions. The procedure eventually adopted was to set up double the normal number of three units per determination to have sufficient to allow for the rejection of the ruined ones. This involves about an extra 10 minutes.

It must be pointed out that in a laboratory with a temperature considerably lower than 30° C condensation would probably not be serious.

The absorption curves (Fig. 3) differ in two respects from those obtained with aqueous solutions. First, the initial ammonia evolution is greater, probably owing to the non-dilution of the potassium hydroxide by the immiscible solvent. Secondly, the final 5 per cent. takes appreciably longer to be evolved and absorbed, owing to slow equilibration between the two layers. If oscillation were possible, distribution would be effected far more rapidly and the 100 per cent. absorption time considerably reduced. Without oscillation the complete times are double those for the aqueous solutions under similar conditions, *i.e.*, 6 hours for formamide and 10 hours for acetamide.

The determination of N-methylacetamide in carbon tetrachloride was found to be impossible because of the 32-hour absorption time required for methylamine. Practically all units become contaminated when left for longer than 12 hours.

Determinations with initial sulphuric acid digestion—

Amides which cannot be determined as above (*e.g.*, urethanes and N-methylacetamide in carbon tetrachloride) necessitate the normal Kjeldahl procedure of initial sulphuric acid digestion. Since this is one of the few recorded instances when the Conway technique has been applied to a macro nitrogen determination—by appropriate dilution—it seems worth mentioning briefly.

Samples, aqueous and non-aqueous, containing at least ten times the optimum sample of 0·28 mg of nitrogen, are delivered into a 100-ml round-bottomed flask and 10 ml of sulphuric acid are added. The flask is then fitted with a 12-inch reflux condenser and heated on an electric hot-plate for 2 hours for N-methylacetamide, 12 hours for methyl carbamate and urethane and 24 hours for N-methylurethane. These times were shown to be approximately 1½ times the minimum required for complete digestion under these conditions. In the case of the non-aqueous solutions the solvent was allowed to distil off after 2 hours' digestion, by removing the condenser for a few minutes. Blackening, sometimes very severe, occurred with the non-aqueous solutions, owing to the decomposition of the solvent; this had no effect on the determination.

After the digestion the flask is allowed to cool and the contents are quantitatively transferred to a calibrated flask of appropriate size for the resultant solution to be approximately 0·02 *N* with respect to nitrogen; 1 ml of this solution is delivered into a Conway unit and determined as before. An accuracy of ±0·2 per cent. is readily achieved.

Results

Table I summarises the total nitrogen recoveries obtained by the various methods, all values being the mean of at least six, and usually ten, determinations. The values for the direct micro Kjeldahl method were obtained from a 40-mg sample in 10 ml of aqueous solution by the modified Parnas - Wagner micro Kjeldahl method previously described.[2] For the macro Kjeldahl on a sulphuric acid digested sample, a 0·5-g sample was taken.

The same recoveries were obtained even when the Conway units were left standing for up to 48 hours. The results from all solutions, by all methods, are extremely consistent, so that the low values for formamide, acetamide and N-methylacetamide are attributed to traces of moisture remaining in the samples owing to their extremely hygroscopic nature. Acetamide shows no sign of giving up its 0·5 per cent. of water, as the sample still gives the same percentage of nitrogen after keeping for more than 4 years over phosphorus pentoxide with frequent evacuation. The absorption curves have, therefore, been corrected by these factors to show 100 per cent. absorption.

TABLE I

DETERMINATION OF AMIDE NITROGEN

Method	Nitrogen recovery from: Formamide, %	Acetamide, %	N-Methyl-acetamide, %	Methyl carbamate, %	Urethane, %	N-Methyl-urethane, %
Direct Conway, aqueous solution	98·2	99·5	97·5	—	—	—
Sulphuric acid digested, Conway, aqueous solution	98·2	99·5	97·5	100	100	100
Direct Conway, non-aqueous solution	98·1	99·4	97·6	—	—	—
Sulphuric acid digested, Conway, non-aqueous solution	98·3	99·5	97·5	100	100	100
Direct micro Kjeldahl, aqueous solution	98·2*	99·5	97·5	—	—	—
Sulphuric acid digested, micro Kjeldahl, aqueous solution	98·2	99·5	97·5	100	100	100
Direct micro Kjeldahl, non-aqueous solution	98·2*	99·4	97·4	—	—	—
Sulphuric acid digested, micro Kjeldahl, non-aqueous solution	—	99·6	97·4	100	100	100
Macro Kjeldahl, sulphuric acid digested, aqueous solution	98·2	99·5	97·5	—	—	—

* Performed by Dr. T. G. Cleasby.

These results show that solutions of these hygroscopic amides of known concentration cannot be made up by weight and that analysis gives the correct concentration.

CONCLUSIONS

Provided that the amides hydrolyse at room (or incubation) temperature in less than 48 hours, they can be determined directly in a Conway unit. If the time exceeds this, they have first to be digested with sulphuric acid.

One ammonia determination—starting from the ammonium sulphate solution—takes about 2¼ hours and a triplicate determination on the same solution, about 2¾ hours. This, at first sight, seems to be of little advantage compared with the 1 hour per determination required for the micro Kjeldahl method. However, in serial analyses the 2 hours spent on the oscillating table does not enter into consideration and the effective time per determination becomes 15 minutes.

This work fully confirms Conway's claim that the micro-diffusion technique can give an accuracy as great as the classical macro Kjeldahl procedure conducted at the 25 to 50-ml level. The method appears to be the simplest possible when large numbers of nitrogen determinations have to be performed. Further, the diffusion procedure is much less liable to error than the distillation methods and goes automatically without any observation being required during the process. The method is strongly recommended when vast numbers of Kjeldahl determinations have to be carried out, as not only is there a large saving in labour and time but also in chemicals.

I thank Prof. A. J. Henry for suggesting the use of the Conway technique.

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UNIVERSITY COLLEGE OF KHARTOUM
KHARTOUM, SUDAN

January 18th, 1955

Design and Operating Technique of a Vacuum Drying Oven

Part III.* Solids in *(a)* Mixtures of Cane and Beet Molasses and *(b)* Beet Molasses

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

The method described in Part II for the determination of true solids in cane molasses by vacuum drying, or alternatively by refractive index corrected for ash and invert, has been extended to include the determination in beet molasses and mixtures of cane and beet molasses. In the case of beet molasses, an equation relating true solids to refractive index solids by sucrose tables, invert sugar and sulphate ash has been derived. Unlike cane molasses, separate determinations of potassium and sodium were not found necessary for greatest accuracy. Mixtures of cane and beet molasses require corrections to the refractive index figures, and these corrections are tabulated.

Parts I and II of this series[1,2] described a method for determining true solids in cane molasses by vacuum drying, from the results of which refractometer corrections for sulphate as hand ash constituents were evolved. It is now shown that an ash correction factor of different sign is required for beet molasses and the correction has also been extended for mixtures of beet and cane molasses. The value of this ash factor, which is positive for 100 per cent. beet molasses, decreases as the amount of cane molasses in the mixture increases and becomes zero at 70 per cent. beet molasses content. It then increases on the negative side as the percentage of the latter decreases, and reaches the factor previously established for 100 per cent. cane molasses. In the light of present knowledge we conclude that the cane ash factor, shown in Part II,[2] is better expressed negatively to prevent confusion with the beet ash factor.

Experimental

Determination of true solids—

Solids in (*a*) mixtures of cane and beet molasses and (*b*) beet molasses were determined by the vacuum oven drying method[2] and termed "true solids." A slight modification was made in recording the weights at the following times: after 45 minutes, the temperature having risen to 70° C; after 5 hours; at 17 to 20 hours; at 42 to 45 hours; and at 67 to 70 hours. The drying curve showed less decomposition than that for cane molasses, concordant with the lower fructose content of beet molasses, and the bend was less pronounced, so necessitating these longer weighing intervals. The fructose content is not exactly one-half of the invert sugar found for, according to chromatographic estimations in beet molasses by Freed and Hibbert,[3] the ratio of dextrose to fructose is about 0·42. To ensure first that all the water was removed and, secondly, that a steady state of decomposition was reached, drying was continued for 70 hours.

True solids in nineteen samples, all free from undissolved crystals, were determined by vacuum drying, and the results tabulated in the order of increasing beet molasses content are shown in Table I.

Beet molasses content was calculated from invert sugar content, 2 per cent. and less corresponded to all-beet and 21 per cent. to all-cane molasses. The latter figure was obtained from Table I in the previous article and was the average of all the invert sugar determinations. Fig. 1 shows a typical beet molasses drying curve. Point A is the percentage loss of weight figure corrected for decomposition. All test samples were weighed out in duplicate and were dried to within ±0·05 per cent. loss of weight. Samples numbered 9, 12, 13 and 14 were re-determined completely, which involved separate dilution and drying operations, these results agreeing to within ±0·10 per cent. loss. Occasionally the bend of the drying

* For particulars of Part II of this series, see reference list, p. 561.

curve was less pronounced than usual, and this was attributed to colloidal substances in the beet molasses retarding drying.

TABLE I

DETERMINATION OF ASH CORRECTION FACTORS FROM SULPHATE ASH CONTENT

Sample	Beet molasses content, %	Solids by vacuum oven drying, %	Solids by refractive index, %	Sulphate ash, %	Invert sugar, %	Ash correction factor
1	38	77·54	78·43	11·10	13·82	−0·0014
2	46	77·17	78·11	12·08	12·30	−0·0012
3	51	75·83	76·24	11·26	11·37	−0·0007
4	62	78·62	78·69	12·52	9·30	−0·0002
5	84	79·61	79·25	12·70	5·21	0·0002
6	85	79·72	79·29	12·86	5·02	0·0003
7	91	79·31	78·60	12·50	3·94	0·0006
8	93	78·94	78·22	11·92	3·4	0·0007
9	95	79·86	79·31	12·77	3·0	0·0005
10	97	79·34	78·48	12·24	2·6	0·0008
11	100	79·64	78·47	12·52	1·8	0·0011
12	100	79·49	78·49	12·20	1·2	0·0011
13	100	78·71	78·03	11·79	1·4	0·0007
14	100	79·19	78·21	12·14	1·8	0·0010
15	100	78·93	78·49	10·77	1·6	0·0005
16	100	77·24	76·79	10·45	1·8	0·0005
17	100	77·02	76·28	11·72	0·4	0·0009
18	100	77·66	77·03	10·43	1·3	0·0008
19	100	75·15	74·58	11·49	0·7	0·0006

ASH CORRECTIONS BASED ON SULPHATE ASH CONTENT—

Further determinations were made of (*a*) apparent solids by refractometer and the use of sucrose conversion tables, (*b*) invert sugar and (*c*) sulphate ash (without deduction of one-tenth). Pre-defecation, by potassium oxalate solution and alumina cream, for invert sugar determination was omitted with samples which originally contained less than 5 per cent. of invert sugar. The sulphate ash correction factors were calculated for all samples and are shown in the last column of Table I. Those for samples 1 to 4 are negative and the remainder are positive. These factors were plotted against beet molasses content and from the graph ash factors, as shown in Table II, were derived. The ash correction constitutes the difference between refractometer solids corrected for invert sugar and solids by drying and is equal to the ash correction factor × true solids × sulphate ash. For all the beet

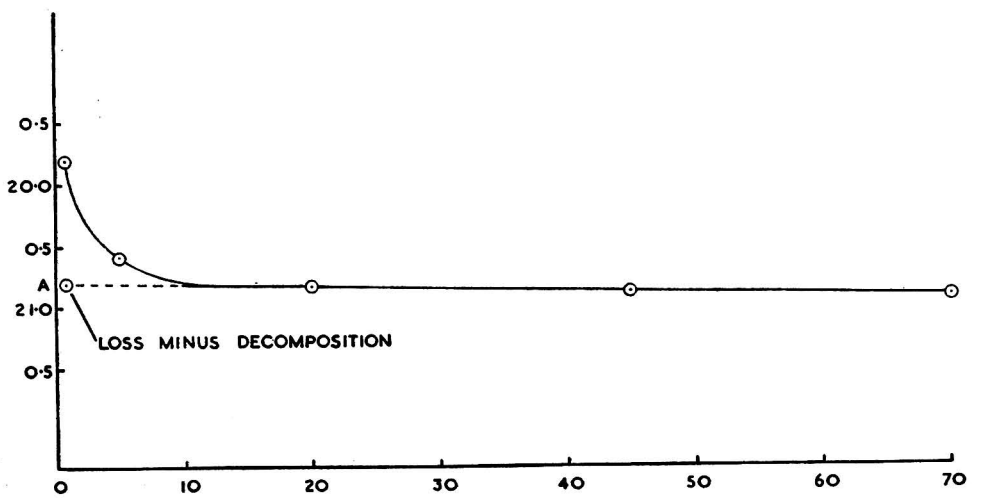

Fig. 1. Typical drying curve for beet molasses at a temperature of 69° to 70° C

molasses samples examined, this ash factor was found to be +0·0008. By combining this ash correction with that for invert sugar the following equation is obtained—

$$\text{True solids (beet molasses)} = \frac{\text{refractometer solids (sucrose tables)}}{1 - 0{\cdot}00025 \text{ invert sugar} - 0{\cdot}0008 \text{ sulphate ash}}$$

TABLE II

SULPHATE ASH CORRECTION FACTORS FOR BEET MOLASSES CONTENT IN MIXED MOLASSES

Beet molasses content, %	Ash correction factor	
0 (All-cane molasses)	−0·0043	Ash correction to be subtracted
10	−0·0034	
20	−0·0026	
30	−0·0019	
40	−0·0013	
50	−0·0008	
60	−0·0004	
70	Nil	
80	0·0003	Ash correction to be added
90	0·0006	
100 (All-beet molasses)	0·0008	

Table III shows the difference between vacuum drying solids and refractometer solids corrected for invert sugar and ash. It was convenient to add the invert sugar correction to the refractometer solids and then to add or subtract the ash correction to this figure. The appropriate ash correction factors were obtained from Table II, the beet content of the samples being taken to the nearest 5 per cent.

TABLE III

COMPARISON OF VACUUM OVEN DRYING SOLIDS WITH CORRECTED REFRACTOMETER SOLIDS

		Ash correction factors from Table II			
		Refractometer solids			
Sample	Solids by vacuum drying, % (*a*)	From sucrose tables, % (*b*)	Corrected for invert, % (*c*)	Corrected for invert and ash, % (*d*)	Difference, % (*a* − *d*)
1	77·5	78·4	78·7	77·6	−0·1
2	77·2	78·1	78·3	77·3	−0·1
3	75·8	76·2	76·4	75·7	0·1
4	78·6	78·7	78·9	78·5	0·1
5	79·6	79·3	79·4	79·9	−0·3
6	79·7	79·3	79·4	79·9	−0·2
7	79·3	78·6	78·7	79·3	0·0
8	78·9	78·2	78·3	79·0	−0·1
9	79·9	79·3	79·4	80·1	−0·2
10	79·3	78·5	78·6	79·3	0·0
11	79·6	78·5	78·5	79·3	0·3
12	79·5	78·5	78·5	79·3	0·2
13	78·7	78·0	78·0	78·7	0·0
14	79·2	78·2	78·2	79·0	0·2
15	78·9	78·5	78·5	79·2	−0·3
16	77·2	76·8	76·8	77·4	−0·2
17	77·0	76·3	76·3	77·0	0·0
18	77·7	77·0	77·0	77·6	0·1
19	75·2	74·6	74·6	75·3	−0·1

COMPOSITION OF SULPHATE ASH IN CANE AND BEET MOLASSES—

Flame photometer determinations were made on diluted solutions of the sulphate ashes. The ashes were extracted first with dilute hydrochloric acid, then taken to dryness, redissolved in distilled water and the solutions filtered. Sulphate ashes of three samples were

further analysed. In sample 3 calcium and magnesium sulphates, and silica by loss with hydrofluoric acid, were determined gravimetrically, and sodium and potassium sulphates were determined by flame photometer. The total of these determinations agreed approximately with the total sulphate ash figure. In samples 1 and 9 calcium sulphate as well as sodium and potassium sulphates were determined by flame photometer, and silica as before. Magnesium sulphate was obtained by the difference between the total sulphate and the sum of the other determined constituents. The results in Table IV showed the degree of reliability of the flame-photometer determinations.

Table IV

Composition of sulphate ash in molasses

	100 per cent. cane molasses No. 3, %	38 per cent. beet molasses No. 1, %	95 per cent. beet molasses No. 9, %
Sodium sulphate	0·3	1·1	1·4
Potassium sulphate	5·2	6·9	9·6
Calcium sulphate	2·3	2·2	1·0
Magnesium sulphate	1·6	0·7	0·7
Silica	0·8	0·2	0·1
Sulphate ash	10·0	11·1	12·8

Applicability of modified ash corrections—

Table V shows that in sulphate ash of beet molasses the total potassium and sodium sulphates was at least twice that found previously in cane molasses. (See Table II in Part II[2] of this series.) The total calcium and magnesium salts was in consequence much reduced and could be determined with a lesser degree of precision.

Modified ash corrections based on sulphate ash minus potassium and sodium sulphates were derived, but unlike those for cane molasses these proved less accurate than direct sulphate ash corrections, and were discarded in favour of the latter.

Table V

Calcium and magnesium salts in sulphate ash of molasses by the determination of potassium and sodium constituents

Sample	Beet molasses content, %	Sulphate ash, % (*a*)	Sodium sulphate, % (*b*)	Potassium sulphate, % (*c*)	Calcium and magnesium sulphates by difference, % [*a* − (*b* + *c*)]
1	38	11·10	1·1	6·9	3·1
2	46	12·08	1·2	7·8	3·1
3	51	11·26	1·1	7·3	2·9
4	62	12·52	1·2	8·5	2·8
5	84	12·70	1·8	9·4	1·5
6	85	12·86	1·7	9·7	1·5
7	91	12·50	1·8	9·6	1·1
8	93	11·92	2·0	8·5	1·4
9	95	12·77	1·4	9·6	1·8
10	97	12·24	1·9	8·4	1·9
11	100	12·52	1·9	9·8	0·8
12	100	12·20	1·5	9·4	1·3
13	100	11·79	1·3	9·3	1·2
14	100	12·14	1·6	8·6	1·9
15	100	10·77	1·5	8·7	0·6
16	100	10·45	1·6	8·7	0·2
17	100	11·72	2·3	8·9	0·5
18	100	10·43	1·7	8·0	0·7
19	100	11·49	2·1	8·5	0·9

Note—The last column includes trace elements.

Discussion of results

A large number of refractometer tests were made, as previously with cane molasses, to determine with sufficient degree of precision the solids content of the samples. Only the average results are shown in Table I.

The pH values of diluted molasses ranged from 5·5 to 7·3 except number 17, which was 9·3. Drying tests were made with solutions of potassium hydroxide and aconitic acid at pH values of 7·2 to 9·3, so extending the range already checked during the cane molasses determinations. The tests indicated that, as in the case of cane molasses, aluminium powder was just as inert as white quartz sand at these higher pH values.

Rundell determined raffinose pentahydrate in fourteen samples by paper chromatography,[4] and the results showed from 1·0 to 1·7 per cent. of raffinose. Two of these samples were checked by Runeckles by the double enzyme method[5] and close agreement was obtained. To determine whether retention of water of hydration of raffinose occurred on drying, tests were made by the vacuum oven drying method on two dilute solutions of mixtures of raffinose pentahydrate and sucrose. One solution was equivalent to 1·66 per cent. of raffinose hydrate and the other to 35·15 per cent., both calculated to 80 per cent. of solids, *i.e.*, normal molasses weight. All determinations were made by weight. It was already known that raffinose pentahydrate would lose all of its water of hydration when heated as a powder in the vacuum oven at 69° to 70° C, but it was found that this does not occur in a solution of raffinose and sucrose. Although these two tests showed that 0·18 per cent. was retained after 70 hours of heating, it was not assumed that this retention of water of hydration would occur in beet molasses. Further investigation would be necessary before a definite conclusion could be drawn with regard to the nature of this small but obstinate retention of water.

Retention of water in sucrose solutions was noticed by Mitchell[6] in steam-oven experiments, and was attributed to possible inversion increasing the solids content. Conversely, using vacuum-oven techniques, he removed these obstinate traces of water, but only to within ±0·1 per cent. There is no doubt that when drying is required to a greater degree of precision, consideration must be given to perfecting the procedure to enable these last traces of water to be driven off. As the need for such care was realised, all samples of sucrose crystals used by the authors were finely powdered before drying at 105° C for 30 minutes, to facilitate the removal of water otherwise occluded by the crystals. Hence, as in Part II[2] of this series, standard solutions of sucrose prepared from this dried crushed sucrose were evaluated to ±0·01 per cent.

Conclusion

The correction based on sulphate ash is convenient. It is not suggested that salts alone are responsible for the effect on refractive index, as other non-sugars are known to refract differently from sucrose and it is possible that the explanation of the change in sign of correction between cane and beet molasses depends upon this. An additional simplification is that the use of a flame photometer to determine potassium and sodium constituents is not required.

The authors express their thanks to the Directors of Tate and Lyle Limited for permission to publish this work.

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NOTE—References 1 and 2 are to Parts I and II of this series.

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Stability of Alkaline Solutions of Haematin

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The method of determining haemoglobin and myoglobin by conversion to alkaline haematin has been critically examined. Conditions affecting the behaviour of alkaline solutions of haematin have been investigated. Light, heat, agitation with air and the addition of copper were found to decrease the stability of such solutions.

THE conversion of haemoglobin to alkaline haematin is a relatively simple matter, and consequently was proposed[1] as a routine clinical method for its determination. This method has also been conveniently applied to the determination of total muscle pigment.[2] However, certain disadvantages of this method have precluded its general adoption.

Crystalline haemin dissolved in a solution of sodium hydroxide is generally used as a standard in this method, but as a reference standard it suffers from two serious disadvantages, which have tended to prevent its general adoption. First, the colour density, and to some extent the spectral absorption, of the solutions are different from those obtained if the haematin is prepared directly from a biological material such as haemoglobin. Secondly, such standard solutions are not sufficiently stable to be kept as reference standards for comparisons made with visual colorimeters.[3] To overcome these difficulties in clinical practice either a standardised glass filter transmitting a band of light in the region of the visible absorption band of alkaline haematin at 580 mμ is used, or artificial standards are prepared from solutions of coloured salts.

For more precise determinations of haemoglobin other colorimetric methods are available, such as conversion to pyridine haemochromogen.[4] This method is claimed to give values agreeing to within 1 per cent. with those obtained from estimating the total iron content of the blood. Stable coloured solutions can be obtained by converting haemoglobin to cyanhaemiglobin (cyanmethaemoglobin),[5,6] when comparison against a cyanhaematin standard is made. As with alkaline haematin, however, the values are not identical, and a conversion factor for the two solutions must be determined.[4]

The present investigation has revealed some of the conditions which affect the behaviour of alkaline haematin, both as a reference substance and in the presence of other biological materials, as when haematin is formed from blood. This has led to modifications in the normal alkaline haematin method[1] to make it suitable as a comparative method of analysis for either blood haemoglobin or tissue myoglobin. By using a spectrophotometer the determinations are made at 380 mμ, which is the peak of the intense Soret band of alkaline haematin. At this wavelength reliable and reproducible results are obtained.

Experimental

Reagents—

Preparation of haemin—Haemin was prepared from ox blood[7] and purified by crystallisation.[8]

Standard haematin solutions—A stock solution was prepared by dissolving 40 mg of haemin in 100 ml of *N* sodium hydroxide solution containing 50 mg of EDTA (disodium salt of ethylenediaminetetra-acetic acid). All glassware was the special "low-actinic lifetime red" (Corning). Dilutions were made with *N* sodium hydroxide solutions, and the solutions were kept cool before use.

Procedure for determination of blood haemoglobin—

The method used was essentially that of other workers,[1] which determines all the haematin-containing pigments present. Whole blood (oxalated or heparinised) was diluted 1 to 500 with water and 5 ml were added to 5 ml of 2 *N* sodium hydroxide solution contained in a "red" test tube calibrated at 10 ml. After being heated for 5 minutes in a boiling-water bath, the tube was cooled, the solution was adjusted to the mark, and the optical density determined at 380 mμ.

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PROCEDURE FOR DETERMINATION OF TOTAL MUSCLE PIGMENTS—

The method of pigment extraction was a modification of a preparative method.[9] A 1 to 2-g portion of fresh muscle was homogenised in a Marsh - Snow homogeniser[10] with 3 ml of 10 per cent. w/v lead acetate solution, followed by the addition of 6 to 7 ml of 0·1 *M* phosphate buffer (pH 6·5) and re-homogenising. After centrifugation, the supernatant liquid was filtered into a 25-ml calibrated flask, and the residue was re-homogenised with buffer solution. Two extractions with 5 ml of buffer solution were sufficient to remove the pigment almost completely from the tissue. The combined extracts were allowed to stand for approximately ½ hour, during which a protein precipitate appeared. This was particularly noticeable in extracts from pale muscles, such as those of the pig. The solution was cleared by centrifuging. To 5 ml of extract in a "red" test tube were added 50 mg of EDTA followed by 5 ml of 2 *N* sodium hydroxide and the mixture was heated for at least 10 minutes in a boiling-water bath. After the solution had cooled, its optical density was measured at 380 mμ.

PROCEDURE FOR DETERMINATION OF TOTAL TISSUE IRON—

Approximately 0·5 g of fresh muscle was wet-ashed with sulphuric - perchloric - nitric acid mixture and the iron determined colorimetrically.[7]

RESULTS

The behaviour of alkaline solutions of haematin has been shown to be influenced by the pH of the solution.[11] The present investigation has shown that in approximately 4 *N* sodium hydroxide solution haemin is hardly soluble, whilst in weaker solutions (0·1 *N*) the golden-brown colour of alkaline haematin is darker. Accordingly, all determinations were made with *N* alkali, in which haemin is in true solution. The colour of the brown solution in 0·1 *N* sodium hydroxide is deeper than that of a similar solution in *N* sodium hydroxide. This is true for haematin prepared either from crystalline haemin or blood.

Solid commercial haematin is not suitable for use as a standard, since solutions prepared from it are unstable both in spectral character and in optical density. It is, however, readily

TABLE I

FADING OF ALKALINE HAEMATIN SOLUTIONS

Haemin, 1 mg per 100 ml of *N* sodium hydroxide; 380 mμ; 1-cm cells

Initial density (fresh solution), 0·68

	Optical density						
	In the dark				In the light		
Time	Standard	Untreated	Air	100° C	Untreated	EDTA, 5 mg per ml	Copper, 2 μg per ml
½ hour	0·68	—	—	0·38	—	—	—
2 hours	0·68	0·66	0·65	—	0·63	0·65	0·60
24 hours	0·67	0·63	0·61	—	0·59	0·63	0·57
7 days	0·59	—	—	—	—	—	—

These figures are averages of four determinations.

soluble in borate buffers of pH 9·2 to 9·8 to give a yellow solution resembling that of alkaline haematin prepared from natural sources. The optical density, however, is not as great as that of an equivalent amount of haemin dissolved in alkali, and, moreover, such solutions were found to be unstable and greatly influenced by the pH of the buffer. The use of haemin in borate buffer of pH 9·4 has been recommended as a standard for haemoglobin determinations.[12] There is, however, evidence for haematin - borate complex formation.[13] The present investigation has revealed that such solutions are liable to fade appreciably, especially when dilute, *i.e.*, of the order of 1 mg of haemin per 100 ml.

Standard solutions of haemin dissolved in alkali are not stable, but show a progressive fading.[4] The present investigations into this effect have shown that it is accelerated by exposure of the solution to light, by heating, by agitation with air and by the presence of traces of copper. An evaluation of these effects is given in Table I.

As demonstrated above, the preparation and handling of such solutions of haematin require the observance of certain precautions if fading of the colour is to be avoided.

Accordingly, they are made up in "low-actinic lifetime red" (Corning) glass vessels with the addition of EDTA and kept in a cool place. Such stock solutions were stable for several days and fading was less marked than in the standard solutions shown in Table I. The absorption maximum of the solutions is at 380 mμ, and they obey Beer's law in concentrations as high as 1 mg per 100 ml at this wavelength.

The addition of sodium hydrosulphite to such standard solutions of haematin to avoid oxidation was found to be undesirable, since, apart from its high absorption at this wavelength, hydrogen peroxide is amongst the products of its autoxidation. It was found that at this wavelength the blank containing EDTA showed a slight absorption.

Solutions of haemoglobin in alkali exhibit the typical absorption spectrum of methaemoglobin hydroxide, with the Soret band maximum at 420 mμ. On heating for a few minutes this shifts to 385 mμ, the position of the alkaline haematin band. It is claimed[1] that 5 minutes' heating is sufficient for this change. In solutions of alkali stronger than 4 *N* a protein precipitate appears on heating or on long standing and adsorbs haematin from solution, so invalidating the results. With solutions of myoglobin the effect is manifest even with 0·1 *N* sodium hydroxide. This precipitation is prevented by the addition of EDTA to the solutions before the addition of the alkali. The conversion of myoglobin to alkaline haematin, moreover, requires a longer period of heating than with haemoglobin, and 10 to 15 minutes' heating is necessary for the complete transformation.

In 0·1 *N* sodium hydroxide it has been shown[1] that blood gave a greater absorption than did the equivalent amount of haemin. This discrepancy was of the order of 30 per cent. for mercury green illumination (546 mμ). An investigation of this effect has shown that it is dependent upon the wavelength, as exemplified in Table II.

Table II

Comparison of the extinction coefficients for alkaline haematin and alkaline solutions of blood haematin

Blood, 14·4 g of haemoglobin per 100 ml, diluted 1 to 100 with 0·1 *N* sodium hydroxide

Haemin, 5 mg per 100 ml in 0·1 *N* sodium hydroxide

Extinction coefficient calculated for 1 mg of iron per 100 ml

Wavelength, mμ ..	380	450	500	546	600	625	650	700
Blood	9·48*	2·42	1·37	1·10	1·08	0·67	0·35	0·19
Haemin	9·56*	1·48	1·10	0·76	0·82	0·56	0·19	0·09

* Haemin, 0·5 mg per 100 ml; blood diluted 1 to 500 in 0·1 *N* sodium hydroxide.

The extinction coefficients of Table II were calculated for 1 mg of iron per 100 ml, the theoretical iron value of 8·57 per cent. for haemin and the value of 0·34 per cent. of iron for haemoglobin being used.[14] The haemoglobin was determined by oxygen capacity. With stronger alkali solutions (*N*), the absorption of both haematin standard solutions and of blood haematin were lower than the corresponding solutions in 0·1 *N* sodium hydroxide. At 380 mμ the extinction coefficients for these solutions were, respectively, 7·93 and 7·74. Alkaline haematin solutions at this wavelength are thus 1·03 times more intense in colour than alkaline haematin derived from blood.

An attempt to compare muscle myoglobin and alkaline haematin on the basis of total tissue iron gave unreliable results, and accordingly in the calculation of the myoglobin content of tissues the extinction coefficient for haemoglobin-derived haematin (7·74) has been used.

By using the above procedure the values obtained for the myoglobin content of the skeletal muscles of cattle and pigs are in general agreement with those obtained by other workers by different procedures.[2,15] The myoglobin contents of ox muscle were in the range of 0·5 to 0·7 per cent. of the wet weight, whilst pig muscle (*longissimus dorsi*) contained 0·1 to 0·2 per cent. of myoglobin, for adult animals.

Discussion of results

Some of the conditions affecting the stability of alkaline haematin solutions have been investigated in this work and measures devised to allow the preparation of a stable coloured

solution to serve as a standard for a reasonable period of time. The method is suitable for use with spectrophotometric instruments, since the absorption of the Soret band of alkaline haematin is approximately ten times that of its visible absorption band in the green.

The observations of other workers, however,[1,16] that alkaline haematin derived from natural sources, such as blood, differs quantitatively, and to some extent qualitatively, from standards made up from crystallised haemin has been substantiated. Moreover, this variation has been shown to be dependent upon the concentration of alkali in the solution and upon the wavelength of the light used for the measurement. The reason for this difference seems not to have been clearly defined, but presumably lies in the presence of the globin in the solution. It has been shown[17] that parahaematin from denatured globin was stable only at or near the neutral point and dissociated into haematin and denatured globin in acid or alkaline solution. The spectral change on adding alkali is possibly similar to the change haemichrome to haemichrome hydroxide, and not to the degree of dispersion.[11] However, the presence of globin shifts the absorption maximum from 380 mμ for purified haematin to 385 mμ for haematin derived from both haemoglobin and myoglobin. As shown in Table II, qualitative differences are also apparent in the visible region of the spectrum. Moreover, comparisons of the alkaline haematin method against iron analyses for blood haemoglobin[1] led to the proposing of a conversion factor of 30 per cent. for the alkaline haematin method when 0·1 *N* sodium hydroxide was used and illumination was by means of a mercury lamp in the green region at 546 mμ.

The present investigation has substantiated the findings of other workers[1] with regard to this quantitative difference between purified crystalline haematin and haematin derived from blood. The value of this inequality has been shown to depend on the wavelength of the light used for making the measurement.

In weakly alkaline solutions haematin exists as an uncharged molecule and tends to aggregate and precipitate from solution, with polymerisation and the formation of partial or full anhydrides.[18] In strongly alkaline solutions haematin exists in true solution and has a golden-brown colour. In 0·1 *N* sodium hydroxide solution the colour is brown and optically more dense, an effect which is possibly related to particle size. This observation holds for both haematin standard solutions and for those derived from haemoglobin or myoglobin.

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Notes

THE ABSORPTIOMETRIC DETERMINATION OF TIN BY MEANS OF DITHIOL

In an earlier publication[1] we reported that certain modern dispersants could be used successfully to permit normal absorptiometric techniques to be employed for determining small concentrations of tin by Clark's method[2,3] with dithiol. Hitherto, dispersants such as agar and gum arabic had been used, and the red suspension was compared with standards by reflected light.

The dispersants originally selected by us, Belloid T.D. and Dispersol L, had to be added immediately after the formation of the red suspension, because, if added before, the reaction was partly inhibited. Since then, Williams and Whitehead[4] have used Teepol X and Farnsworth and Pekola[5] have used Santomerse S, each of which may be present during the reaction. We have since been able to try several more dispersants, and have concluded that the most satisfactory of these for this application is Lorol (sodium lauryl sulphate).

The conditions for optimum colour development are not particularly critical, but we had evolved a procedure which has since been used as the basis of a Lovibond Comparator method, and a disc with nine colour standards, for 1, 2, 3, 4, 5, 6, 8, 10 and 12 p.p.m. of tin, has been made available by The Tintometer Limited for use with 13·5-mm comparator cuvettes. In brief, a 10-ml aliquot of the sample solution, which should be 0·5 *N* in terms of hydrochloric acid and should contain not more than 0·25 mg of tin, is placed in a 20-ml calibrated flask and to this are added, in the order given, 1 drop of thioglycollic acid, 2·0 ml of concentrated hydrochloric acid, 0·5 ml of 1 per cent. sodium lauryl sulphate solution and 1 ml of dithiol reagent (0·125 per cent. w/v solution in 1·0 per cent. w/v aqueous sodium hydroxide—freshly made up as required), with thorough mixing after each addition. The flask is then placed in a water-bath at 60° C for 10 minutes and, after being cooled, the contents are diluted to the mark. A reagent blank is made up in the usual way to permit any necessary correction to be made.

Choice of dispersant

A less turbid system would be expected to result from having the dispersant present in the solution when the dithiol is added than from adding it after the formation of the red suspension. This has proved to be true for Teepol and Lorol, neither of which interfere with the reaction. Belloid T.D., however, cannot be added before the dithiol because it largely inhibits the reaction, although once the dithiol has been added it will not then affect the colour. These effects are illustrated by an experiment in which these three dispersants were added respectively to three pairs of test solutions containing known amounts of tin. In one set, A, the dispersant was added before the dithiol and in the other set, B, it was added immediately afterwards (but before heating to 60° C), all other reagents having been added in correct order. The optical densities of the resulting systems (measured at 545 mμ) are shown in Table I.

Table I

Optical densities at 545 mμ

Tin added, mg	Lorol		Teepol		Belloid	
	A	B	A	B	A	B
0·05	0·132	0·205	0·130	0·205	0·008	0·195
0·10	0·265	0·415	0·265	0·430	0·053	0·400
0·15	0·402	0·615	0·405	0·630	0·258	0·600

It can be seen that in set B the optical densities are higher, in each case being reasonably proportional to the tin content and almost independent of the dispersant used. The higher values for set B are due to extra light blocking by the suspensions, the particles presumably having grown to a larger size in the absence of the dispersant. Unfortunately the apparently greater sensitivity indicated by the higher optical densities of set B cannot be exploited, because the amount of this extra turbidity is not readily controlled, and this accounts for the noticeable improvement in the precision indicated by the results of set A for Lorol and Teepol.

Preference was given to Lorol because it was found that it was active at much lower concentrations than Teepol for the present purpose. The actual amount of Lorol added is not at all critical, and a large variation in its strength can be tolerated.

Spectrophotometric examination of the tin - dithiol complex

The absorption spectrum of the tin - dithiol complex, formed in the presence of Lorol and measured against a blank containing all reagents but no added tin, is shown in Fig. 1 as curve A. An absorption maximum occurs at 533 mμ and the specific extinction coefficient at this wavelength is 0·061 per cm per p.p.m. of tin (or 7100 per cm per g-atom of tin per litre).

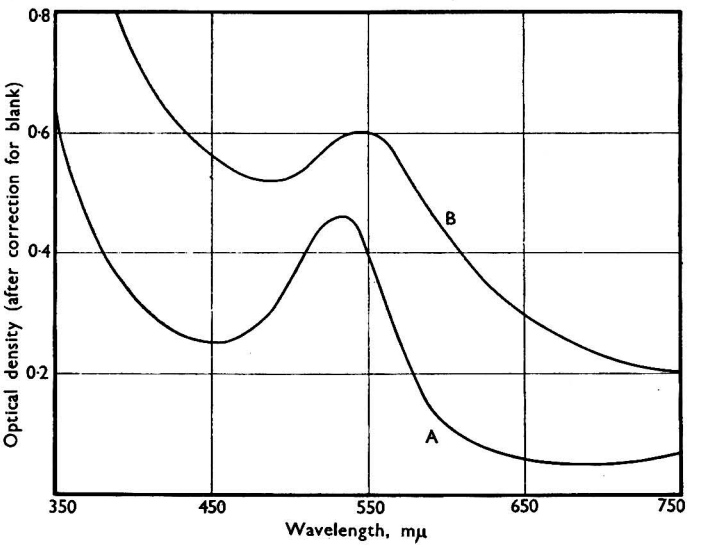

Fig. 1. Absorption spectra relating to the tin - dithiol system

By contrast, curve B in Fig. 1 shows the absorption spectrum of the same system when the Lorol is added immediately after the addition of the dithiol. The effect of the extra turbidity (light blocking) is clearly demonstrated, and an apparent absorption maximum occurs at 545 mμ.

An attempt was made to deduce the composition of the tin - dithiol complex by the method of continuous variations. A fresh batch of dithiol was used for this purpose, but even so it was known to fall somewhat short of 100 per cent. purity. On the assumption that it was 100 per cent. pure the results obtained from measurements at 500, 533 and 560 mμ indicate a 1 to 2·4 ratio for tin to dithiol. This ratio would become 1 to 2 if the dithiol had been 83 per cent. pure, which would seem to be a reasonable possibility. The evidence appears, therefore, to favour a 1 to 2 complex. Feigl[6] has suggested a 1 to 1 complex, although he did not rule out the possibility of further addition of the dithiol, in analogy to the production of sulpho salts with hydrogen sulphide reactions. Unfortunately we have not been able to pursue this study more thoroughly.

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THE EXAMINATION OF COMMERCIAL SORBITOLS, WITH SPECIAL REFERENCE TO THEIR SUITABILITY FOR DIABETIC DIETS

SORBITOL is on the threshold of universal acceptance as a valuable adjunct to the diabetic diet. Naturally, therefore, its contamination with unreduced glucose is undesirable; not only does it introduce an unwanted and unknown amount of glucose into a diet for which precise knowledge of carbohydrate contents is required, but the presence of a small amount of glucose apparently also increases the possibility of enhanced sorbitol absorption.

Sorbitol is most effectively produced at present by the reduction of starch hydrolysates; unfortunately this reduction and hydrolysis is usually not complete.

We must confess that we did not at first question the purity of commercially available sorbitols and that it was during *in vitro* work on the metabolic fate of sorbitol that the presence of extensive amounts of saccharide impurities was discovered.

The sorbitols used for this work were originally in the state of approximately 70 per cent. w/v syrups and, although pyridine number and PZ (phenylhydrazine complex) techniques were used in checking the amount of sorbitol present, results on these were consistently lower than refractometric readings given by pure syrup prepared from analytical grade sorbitol made from pure glucose.

It was decided to apply chromatographic techniques to investigating the result of enzymic work, and substrates were studied by the method of multi-development ascending paper chromatography, *n*-butanol - acetic acid - water being utilised as solvents and aniline hydrogen phthalate as a spraying agent. It was only when spots giving an R_F similar to those of glucose were found that it was decided to check the sorbitol initially. Further chromatographic work on sorbitol suggested the presence of maltose and, after hydrolysis, this was corroborated by a more intense spot from glucose. It was thought that the sample considered at that time contained incompletely hydrolysed starch and that maltose and related saccharides were present, including glucose from incomplete reduction.

In order to examine the sorbitol quantitatively various methods were adapted; we considered that the most generally satisfactory method was an adaptation of the Sichert and Bleyer[1] modification of the Bertrand method.

METHOD

Weigh 10 g of sorbitol into a 400-ml round-bottomed flask, and add 100 ml of distilled water and 10 ml of concentrated hydrochloric acid. Reflux for 1 hour, cool and neutralise with sodium hydroxide. Wash the contents of the flask into a 250-ml calibrated flask and dilute to the mark with distilled water; with a pipette place a 25-ml aliquot in a Taylor flask and add 25 ml each of Fehling's I and II solutions; heat the whole to boiling as rapidly as possible and boil for 3 minutes.

Separate the precipitated cuprous oxide by filtering the hot mixture through a sintered-glass crucible (K3), and examine the filtrate to check that no particles have passed through. Wash the crucible and filter-flask with water, and then dissolve the precipitate in hot ferric alum solution (10 per cent. of ferric alum in 10 per cent. sulphuric acid).

Cool the filtrate and titrate with 0·1 *N* potassium permanganate; calculate the amount of available reducing sugars from the tables prepared by Sichert and Bleyer for glucose, maltose and dextrin.

RESULTS

This method was checked by recovery tests on maltose and was shown to be consistently accurate to 2 per cent. of original material.

The application of this method to a number of commercially available sorbitol syrups is shown in the following results, which show the variable quality of sorbitols from different sources—

Soluble solids of syrup, per cent.	67·0	70·2	73·0	69·0	67·8	62·5	69·2
Initial sugars, per cent.	0·69	0·7	0·37	0·052	0·107	0·15	0·23
Final sugars, per cent.	5·2	1·00	1·74	0·42	8·85	0·52	2·70

REFERENCE

1. Sichert, K., and Bleyer, B., *Z. anal. Chem.*, 1936, **107**, 328.

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A. J. KENNEDY-RIPON
R. E. A. MAPES

December 16th, 1954

IMPROVEMENTS TO THE SILVER COBALTINITRITE METHOD FOR THE DETERMINATION OF POTASSIUM

In a Note[1] under the above heading, there occurs (line 7, p. 103) the following sentence: "When cool, add 5 ml of ammonium thiocyanate reagent for each 340 μg of potassium present." Correspondence with Dr. E. C. Wood indicates that this may be ambiguous. The volume of reagent added should be 5 ml for up to 340 μg, 10 ml for 340 to 680 μg, and 15 ml for 680 to 1020 μg of potassium. The volume of 2 *N* sulphuric acid is so small that it can be ignored. The calibration graph is prepared by using 5 ml of reagent and up to 340 μg of potassium; for the two higher ranges the results are read from the same graph, but are multiplied by 2 or 3, respectively.

REFERENCE

1. E. M. Chenery, *Analyst*, 1952, **77**, 102.

AGRICULTURAL RESEARCH STATION
KAWANDA, KAMPALA
UGANDA, B.E.A.

E. M. CHENERY
March 8th, 1955

British Standards Institution

AMENDMENT SLIP*

A PRINTED slip bearing amendments to a British Standard has been issued by the Institution, as follows—PD2191—Amendment No. 1 (May, 1955) to B.S. 2511 : 1954. Determination of Water by the Karl Fischer method.

Book Reviews

OXINE AND ITS DERIVATIVES. Volumes I and II: OXINE—PARTS 1 AND 2. By R. G. W. HOLLINGSHEAD, M.A. Pp. xx + 322; xxiv + 323–616. London: Butterworths Scientific Publications. 1954. Price 42s.; 42s.

The first comprehensive study of the metal derivatives of 8-hydroxyquinoline was published in 1910 by J. J. Fox, who did not, however, realise their potential analytical use. These began to be developed about 16 years later, mainly by Berg, who published in 1933 a monograph on the subject running to a mere 30 pages. Since that time, thousands of publications have appeared from sources all over the world, mainly but not exclusively on the analytical side; even since the main text of Mr. Hollingshead's books went to press, 58 further papers, listed in an appendix, have been issued, and current numbers of *Analytical Abstracts* generally include at least half a dozen relevant items. To deal with this mass of material, the author was faced with various courses of action, and he has chosen to cast a wide, rather than a critical, net. In this way, publications in less accessible journals and in less familiar languages are made available. There are blatant discrepancies in these; they are pointed out. For the rest, the arrangement will assist the analyst to exercise his judgment in assessing the merits of possible doubtful statements.

The opening chapters deal with the preparation of oxine, its physical and chemical properties and a general survey of its analytical uses, *e.g.*, pH ranges for precipitation of metals, stability of the complexes, methods of determining oxine, chromatographic techniques, reactions in molten oxine and even its recovery. Then follows the main subject-matter of these two volumes, the reaction of oxine with various metals; apart from two groups—the alkaline earths and the rare earths—the metals are dealt with in alphabetical order—aluminium, antimony, bismuth, cadmium, chromium, cobalt, copper, gallium, indium, iron, lead, magnesium, manganese, molybdenum, nickel, scandium, silver, thallium, thorium, titanium, tungsten, uranium, vanadium and zinc—and are thus readily located. It is interesting to note the various demands for space; the average number of pages required for most of the metals is eleven (extremes: antimony three; zinc twenty-three), but aluminium and magnesium occupy about one hundred pages each. The treatment is conveniently summed up by the author's observation that "the determination [of aluminium] in a host of materials in the presence of interfering ions has been successfully accomplished by the use of oxine," for, apart from general notes on conditions for precipitation and methods of determination, special attention is given to separations and to the practical applications arising therefrom. Conversely, "conflicting information appears in the literature relating to the determination of antimony as the oxinate."

* Obtainable from the British Standards Institution, Sales Department, 2 Park Street, London, W.1.

In a short chapter is discussed the reaction of oxine with miscellaneous metals, *e.g.*, the alkali metals, germanium, mercury and plutonium; in another, the determination of phosphate and silica is reviewed and in a third the separation of interfering metals in the determination of beryllium. The non-analytical uses of oxine are concerned chiefly with its fungicidal and bactericidal properties, but it has been investigated from other aspects, *e.g.*, its uses in preventing discoloration, as a stabiliser for peroxides and as a flotation collector. No mention is made here of its use as a coupling component in the manufacture of azo-dyes, *e.g.*, for some members in the Coprantine range; possibly these may be considered in Volume III, due for publication in 1955, which will deal with the derivatives of oxine.

In a way, it must be rather disheartening to write a book while new matter is appearing almost daily. There is, however, a solid foundation of well established fact, even though certain facets of the decoration may be of somewhat dubious authenticity. The author must be congratulated on having undertaken a heavy task and on the orderly manner in which he has presented his material.

B. A. ELLIS

COLORIMETRIC METHODS OF ANALYSIS. Volume IV. ORGANIC, II. By FOSTER D. SNELL, Ph.D., and CORNELIA T. SNELL, Ph.D. Third Edition. Pp. viii + 676. New York: D. Van Nostrand Co. Inc.; London: Macmillan & Co. Ltd. 1954. Price 93s. 6d.

With this volume the great task undertaken by the authors is brought to a close. Reviews of the first three volumes have already appeared in *The Analyst*, Volume I (Theory - Instruments - pH), 1948, **73**, 641; Volume II (Inorganic), 1951, **76**, 183; and Volume III (Organic, I), 1955, **80**, 405.

The scope of the book before us is best defined by listing the headings of the sixteen chapters as follows: Nitrites, Nitrates and Nitro Compounds; Aliphatic Amines and Amides; Amino-Acids; Proteins; Aromatic Primary, Secondary and Tertiary Amines; Azo Compounds, Nitrogen-containing Cycles, etc.; Urea and Related Compounds, Compounds with Inorganic Radicals; Sterols; Hormones; Alkaloids; Enzymes; Antibiotics; Haemoglobin and Related Compounds; Natural Pigments; Colour of Liquids.

There is little to add to the observations already made in connection with the earlier volumes. As in the rest of the work, one could wish for a more critical approach and more practical details in the preliminary preparation of samples and materials. Again, one questions whether the 22-page chapter on Antibiotics is justified; the reviewer, at any rate, would prefer to pin his faith to microbiological assays rather than colorimetric determinations for the assessment of these highly important substances.

One wonders if the day has not already passed when it is practicable to embody the whole subject of colorimetric analysis in four volumes of reasonable size; the subject has become too vast, and when one considers that a quarter of all papers on analytical chemistry now published throughout the world are concerned with colorimetric methods, it is obvious that no collected work, however ambitious, can be complete. No one would think of attempting the corresponding task in relation to gravimetric analysis, and it would seem that in future, if the problem is dealt with at all, there will have to be a number of books written by specialists each dealing with a restricted range of products.

In conclusion, it should be emphasised that it is the merit of this work that predominates, not the shortcomings, and there can be no doubt that analysts throughout the world will have every reason to be grateful to the authors for their skill and perseverance, patiently applied, in the fulfilment of a work of such distinguished magnitude.

N. L. ALLPORT

ORGANIC SYNTHESES. An Annual Publication of Satisfactory Methods for the Preparation of Organic Chemicals. Volume 34. Editor-in-Chief: W. S. JOHNSON. Pp. vi + 121. New York: John Wiley & Sons Inc.; London: Chapman & Hall Ltd. 1954. Price 28s.

This hardy annual differs from the commoner garden varieties in that its produce is always different, whatever the superficial similarities. This year's crop covers 1:2:3-trimethylbenzene, diphenylacetylene and 9:10-dihydrophenanthrene (with methods of purifying commercial phenanthrene); $\alpha\alpha'$-dibromo-*o*-xylene, styrene-β-sulphonyl chloride, toluene-*p*-sulphinyl chloride, 1:4-dinitrobutane and 4:4'-dinitrodibenzyl; 3-methylpentane-1:5-diol, *o*-methylbenzyl alcohol and 1-tetralyl hydroperoxide; ethoxyacetylene and 2-chloro-1:1:2-trifluorodiethyl ether; phthalaldehyde, cycloheptanone (by two different methods of ring enlargement), 4'-acetyl-2:5-dihydroxydiphenyl and phenanthraquinone; *o*-carboxycinnamic acid and its dihydro derivative; ethyl chlorofluoro-acetate and $\beta\beta$-pentamethyleneglycidate, diethyl hexadecylmalonate and heptoylsuccinate,

di*tert.*-butyl malonate (by a method different from that given in the previous volume) and diphenyl succinate; azelaonitrile, NN-dimethyl-2-methylbenzylamine, N-1:2-diphenylethylmethylamine, toluene-*p*-sulphon-N-methylnitrosamide (a convenient source of diazomethane) and O-methyl-uronium chloride; dihydro-2-methoxy-4-methylpyran, dimethylfurazan, 3-methylthiophen and N-phenylpiperidine.

In the customary manner, some of the monographs include accounts of preliminary or analogous preparations.

B. A. ELLIS

THE VITAMINS: CHEMISTRY, PHYSIOLOGY, PATHOLOGY. Volume III. Edited by W. R. SEBRELL, jun., and R. S. HARRIS. Pp. xiv + 665. New York: Academic Press Inc.; London: Academic Books Ltd. 1954. Price $15.00; 120s.

The contents of Volume III follow the same general pattern as those of Volumes I and II and comprise chapters on *p*-aminobenzoic acid, pteroylglutamic acid, pyridoxine and related compounds, riboflavin, thiamine, the tocopherols and "new and unidentified growth factors." Each chapter deals systematically with the chemistry, industrial preparation, biochemistry, determination, occurrence, effects of deficiency, pharmacology and requirements of each vitamin.

As might be expected from the fact that different authors specialising in different fields have written them, there is a certain unevenness of treatment in the way in which different sections are handled, but on the whole a reasonable degree of co-ordination has been secured. As in the previous volumes, the sections on determination are not intended to be in any way exhaustive, but rather to indicate where more detailed information can be found. Two exceptions are provided in the present volume, however, namely in the chapters on pteroylglutamic acid and vitamin B_6, both of which happen to give rise to particularly complicated problems. Thus, pteroylglutamic acid may exist in foodstuffs in the form of conjugates that have to be broken down before assay, and the existence of the citrovorum factor, a close relative of pteroylglutamic acid, complicates still further the estimation of the latter. Similarly vitamin B_6 may occur in foodstuffs in three different forms, pyridoxin, pyridoxal and pyridoxamine, which are known to have different biological activities for different animal species, and two of these forms of vitamin B_6 also exist as phosphates. Drs. E. L. R. Stokstad, H. Sherman and E. E. Snell, the authors of these two sections, make quite clear the fundamental problem in determining such substances, namely, that of ensuring that the results obtained by chemical or microbiological assays are a measure of the biological activity of the vitamin present. Unless selective methods of assay are available for each form and the biological value of each form for man is known, the result can obviously only be an approximate one.

In the chapter on pteroylglutamic acid it is pointed out that most of the figures given in the literature for the pteroylglutamic acid content of foodstuffs were obtained before the existence of the citrovorum factor was recognised, and that the values recorded therefore probably represent the sum of the pteroylglutamic acid and citrovorum factor contents. Since the book under review was published, however, further compounds of the same type have been recognised, which make the problem still more complicated.

In the vitamin-B_6 chapter, Drs. E. E. Snell and C. S. Keevil have tabulated the figures recorded by various workers for the amounts present in foodstuffs and have gone to the trouble of indicating in each instance the method used for the assay. It will be seen by reference to this table how greatly the results can differ when two different methods of assay are used. When it is borne in mind that several chemical methods of determining pyridoxin and related compounds are available and that 13 different hydrolytic procedures have been used for releasing the vitamins from their combined forms, it is perhaps surprising that the variations are not even greater.

By comparison, the chapters on riboflavin and thiamine are relatively simple, and the most interesting sections in these two chapters are probably those relating to the biochemical properties of the two vitamins. The chapter on vitamin E is of particular interest in view of the controversy that has raged in recent years over the status of this vitamin, and the section in which Dr. Karl Mason discusses the effects of vitamin-E deficiency is to be commended for its critical outlook and for the clear statement made that vitamin E is of no real value in any known human disorder in spite of the claims that have been made from time to time and the vast literature that has accumulated on the subject. Analysts will be particularly interested to see that the main function of vitamin E is apparently that of a biological antioxidant and that a knowledge of the amount present in foodstuffs may be of importance in indicating the extent to which other vitamins may be protected from oxidation.

Chemists concerned with the determination of vitamins in foodstuffs already have available a number of specialist volumes, but the three volumes comprising the present work are to be commended as one of the most complete sources of general knowledge about the vitamins at present available. It is unfortunate that the price will put them beyond the reach of most individuals, but no scientific library should be without these volumes. F. A. ROBINSON

NEW INSTRUMENTAL METHODS IN ELECTROCHEMISTRY. THEORY, INSTRUMENTATION AND APPLICATIONS TO ANALYTICAL AND PHYSICAL CHEMISTRY. By P. DELAHAY. Pp. xviii + 437. New York and London: Interscience Publishers Inc. 1954. Price $11.50; 92s.

In this book all the newer instrumental methods of electrochemistry, together with their applications in analysis or physical chemistry are discussed, and in his presentation the author has achieved an excellent balance between theory and practice. The book is divided into four parts, dealing in turn with voltammetric methods, coulometry and electrolytic methods, high-frequency methods and instrumentation. All the important techniques of electrochemistry are reviewed, with descriptions sufficient for them to be understood and for their relative merits to be appraised. However, the reader will not find details for experimental procedures. For these he must use the well-chosen bibliographies of original sources.

The section on high-frequency methods occupies more space than its usefulness justifies. Possibly it would have been put in proper perspective if Dr. Delahay had written it himself. The one or two minor blemishes of error or misprint that usually mar a first edition have not been found. The book is well printed and bound, but its price will deter many from buying personal copies. A. J. LINDSEY

Publications Received

ORGANIC SOLVENTS: PHYSICAL PROPERTIES AND METHODS OF PURIFICATION. Second Edition by JOHN A. RIDDICK and EMORY E. TOOPS, jun., based on the First Edition by Arnold Weissberger and Eric S. Proskauer. Pp. viii + 552. New York and London: Interscience Publishers Inc. 1955. Price $8.50; 68s.

CHEMISTRY OF CARBON COMPOUNDS. Volume III, Part A. AROMATIC COMPOUNDS. Edited by E. H. RODD, D.I.C., D.Sc., F.C.G.I., F.R.I.C. Pp. xxiv + 685. Amsterdam and New York: Elsevier Publishing Co. Ltd.; London: Cleaver-Hume Press Ltd. 1954. Price 115s.

SYMPOSIUM ON SEDATIVE AND HYPNOTIC DRUGS, HELD UNDER THE AUSPICES OF THE MILE-AMES RESEARCH LABORATORY AND SUMNER RESEARCH LABORATORY, ELKHART, INDIANA. Pp. xix + 111. Baltimore: The Williams and Wilkins Company; London: Baillière, Tindall & Cox Ltd. 1954. Price 27s. 6d.

ORGANIC SYNTHESES. Collective Volume 3. Editor-in-Chief: E. C. HORNING. Pp. x + 890. New York: John Wiley & Sons Inc.; London: Chapman & Hall Ltd. 1955. Price $15.00; 120s.

A revised edition of Annual Volumes 20 *to* 29.

POLAROGRAPHIC TECHNIQUES. By LOUIS MEITES. Pp. xiii + 317. New York and London: Interscience Publishers Inc. 1955. Price $6.00. 48s.

LEICHTMETALLANALYSE. By H. GINSBERG. Third Edition. Pp. xix + 285. Berlin: Walter de Gruyter & Co. 1955. Price DM 24.80.

ANALYSIS OF INSECTICIDES AND ACARICIDES. By FRANCIS A. GUNTHER and ROGER C. BLINN. Pp. xi + 696. New York and London: Interscience Publishers Inc. 1955. Price $14.00; 112s.

EXPERIMENTAL CHEMISTRY.* Part I. QUALITATIVE AND VOLUMETRIC ANALYSIS. By J. E. GARSIDE, Ph.D., M.Sc.Tech., Assoc.M.C.T., F.R.I.C., F.I.M., M.Inst.F., and P. A. CLARET, B.Sc., A.R.I.C. Pp. vi + 93. London: Sir Isaac Pitman & Sons Ltd. 1955. Price 9s.

* *Intermediate Science Series*

Erratum

MAY (1955) ISSUE, p. 348, line 28. *For* "0·578 mg" *read* "5·3 mg."

BRITISH COMPANY requires Inorganic or Metallurgica Chemist for research development and control in modern and well-equipped laboratory. This post offers advancement to the position of Chief Chemist in five years, to a man who proves his initiative and ability. Necessary qualifications, B.Sc. or A.R.I.C. Compulsory contributory pension scheme. Give full details of education and experience and salary required to Box No. 3900, THE ANALYST, 47 Gresham Street, London, E.C.2.

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J. K. HOPE,
Clerk of the County Council.

CHEMISTS, physico-chemical bias, interested in design, development and/or selling of equipment for laboratory use, particularly Vapour Phase Chromatography apparatus. Detailed applications to Technical Director, Griffin & George Ltd., 285, Ealing Road, Alperton, Wembley, Middx.

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