THE ANALYST

Analytical Methods Committee

REPORT PREPARED BY THE ESSENTIAL OILS SUB-COMMITTEE

Drying Agents for Essential Oils

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

REPORT

It is frequently necessary to dry a sample of essential oil for analysis, and a comparison has been made of the relative efficiencies for this purpose of (*a*) anhydrous sodium sulphate, (*b*) anhydrous magnesium sulphate and (*c*) commercial anhydrous silica gel. If there is no chemical reaction, then theoretically the minimum water content of the oil that can be attained with a given drying agent is a function of the water vapour pressure in equilibrium with the drying agent in the absence of the oil. These values are recorded in the literature, but they are equilibrium values obtained on prolonged contact.

The analyst is also interested in the rate of drying as well as the ultimate water content, and measurements have been made of the rate at which various essential oils can be dried. A coarsely powdered drying agent would not be effective under routine analytical conditions, even though it had a low water vapour pressure. Consideration of the problem shows that particle size affects the rate in two ways, since it influences the rate of settling as well as the surface area available for absorption.

EXPERIMENTAL

DRYING AGENTS USED—

(*a*) *Anhydrous sodium sulphate*—The hydrate, $Na_2SO_4.10H_2O$, was heated in an oven for 4 hours at 110° C.

(*b*) *Anhydrous magnesium sulphate*—The hydrate, $MgSO_4.7H_2O$, was calcined by heating it in a muffle furnace for 4 hours at 350° C.

(*c*) *Anhydrous magnesium sulphate*—As purchased.

(*d*) *Silica gel*—This was used in the dehydrated form as purchased (Hopkin & Williams Ltd.); particle size, 10 to 20 mesh.

PROCEDURE—

The essential oil (100 ml) was dehydrated over 15 g of the drying agent, the following different procedures being used—

(*i*) One minute of continuous shaking immediately after mixing.

(*ii*) One hour with seven shakings of one minute's duration at approximately 10-minute intervals.

(*iii*) Twenty-four hours with shaking for one minute at zero time and at 2, 4, 5, 21, 23 and 24 hours after mixing.

RESULTS—

The results are shown in Table I. The water contents were measured by the Dean and Stark method, with an additional check in some instances by Karl Fischer titration. Values are given for the drying of oil of clove leaf, oil of rosewood and oil of palmarosa. It can be seen that in every example the magnesium sulphate is superior to sodium sulphate,

both in the rate of drying and the ultimate water content of the sample. It is interesting to note that measurements of the equilibrium vapour pressure of water over magnesium sulphate and sodium sulphate were made over 50 years ago.[1] The results confirm the above findings, and it is recommended that calcined magnesium sulphate should be used instead of the commonly used anhydrous sodium sulphate. The drying time, with intermittent shaking, should be at least 2 hours.

TABLE I

EFFECTS OF DIFFERENT DRYING AGENTS ON THE DRYING OF ESSENTIAL OILS

		Residual water content, per cent., over—			
	Drying time	anhydrous sodium sulphate (*a*)	anhydrous magnesium sulphate (*b*)	anhydrous magnesium sulphate (as purchased) (*c*)	silica gel (as purchased) (*d*)
Oil of clove leaf ..	0	1·43	1·43	—	1·43
	1 minute	0·71	0·63	—	0·80
	1 hour	0·59	0·45	—	0·57
	24 hours	0·56	0·36	—	0·54
Oil of rosewood ..	0	2·2	2·2	2·2	—
	1 minute	1·6	0·8	1·0	—
	1 hour	1·4	0·6	0·8	—
	24 hours	1·2	0·5	0·6	—
Oil of palmarosa ..	0	2·2	2·2	2·2	—
	1 minute	1·6	1·0	1·4	—
	1 hour	1·4	0·8	1·0	—
	24 hours	1·2	0·6	0·8	—

REFERENCE

1. Foote, H. W., and Scholes, S. R., *J. Amer. Chem. Soc.*, 1911, **33**, 1309.

Diffusion-controlled Oxidation of Carbon in Nickel and Other Metals as a Basis of Analysis

By E. F. RICKARD

(*Engineering Department, G.P.O. Research Station, Dollis Hill, London, N.W.2*)

In the determination of small amounts of carbon in metals, the limits of discrimination are set by error variations arising from the combustion process. The method described avoids this source of error. With commercially pure nickel, all the carbon present is recoverable as carbon dioxide by surface oxidation, the carbon dioxide being evolved at a rate governed by the diffusion of carbon in nickel. This principle has been satisfactorily used for studying the variation of carbon content in components of thermionic valves, where sample weights are small, carbon contents low and replication impossible. Measurement of the carbon dioxide content has been made by using the conductimetric method of Still, Dauncey and Chirnside, and a brief account is given of some sources of variation in cell response noted in the course of the work. The method has also been applied to cast iron, mild steels and some other metals. For such materials, an oxidising atmosphere containing 7 to 8 per cent. v/v of oxygen in argon prevents combustion of the sample, but necessitates the use of a post-furnace oxidation catalyst to ensure complete conversion of carbon to carbon dioxide. The advantages and limitations of this and other methods are briefly discussed.

STILL, Dauncey and Chirnside[1] have described a conductimetric method for estimating carbon dioxide and its application to the determination of carbon in metals. When apparatus to their design became commercially available, the method was widely adopted as it promised to combine the simplicity and rapidity necessary for works-laboratory conditions with sensitivity and precision equal to that of the best absolute methods available. More recent developments of the method have been described by Green, Still and Chirnside.[2]

The two papers quoted above deal comprehensively with problems of combustion of metals as they effect carbon recoveries. The method described in this paper makes use of the same basic apparatus, but does not involve rapid total combustion of the metal sample. Even with the best techniques, there are variable blank values that occur as a result of the combustion process,[1,2,3,4] and a method that avoids variable blank values should give improved precision at low carbon levels. The need for better precision arose in the Research Branch of the G.P.O. Engineering Department when it was desired to determine the carbon contents of single nickel components that had been removed from thermionic valves. In this instance, carbon contents were sometimes as low as 20 p.p.m. and sample weights were about 0·3 g, permitting only single determinations. A method was required that would be applicable to this situation and at the same time permit the simple and rapid determination of the carbon contents of nickel-strip supplies for components before use. In the latter instance replication was possible and precision was less important.

It was already known that commercially pure nickel strip or wire could be de-carbonised at relatively low temperatures, the rate of carbon loss being governed by the known diffusion rate of carbon in nickel. Calculation showed that, in specimens having the dimensions of the components in question, more than 99 per cent. of the carbon would be lost within 20 minutes in an oxidising atmosphere at 900° to 1000° C. In this temperature range, non-porous boats and tubes made of fused silica could be used. Since combustion of the nickel was not required, fluxes and igniters could be eliminated. The effects of uncontrolled temperature variations during combustion on the size of the blank values derived from sample, boat and furnace tube would likewise be eliminated.

These principles have been satisfactorily applied to the problem of analysis of carbon in nickel and subsequently, with modifications, to other materials including mild and low-alloy steels.

General description of method

For practical reasons connected with the total times involved, the method is limited to instances in which at least one dimension of the metal specimens does not exceed about 400 microns (0·015 to 0·016 inch). It is thus applicable to metal in powder form, to thin sheet or wire and to drillings or turnings from cast specimens. Various methods of de-greasing have been used, and none gives as uniformly good results as a Soxhlet extraction with acetone. Even with this, trouble can be experienced from strong adsorption at surfaces of fine particles. It is best not to de-grease powders, and to sieve out the finest particles from drillings, etc., before de-greasing. Trouble is experienced in distinguishing between firmly bound surface contaminant and fast-diffusing carbon from surface layers of extremely small particles.

The gas train, conductimetric cell and measuring bridge used were basically the same as that described by Still, Dauncey and Chirnside.[1] The special furnace consisted of a transparent silica tube (30 cm long × 18-mm bore), with B24 quartz cone terminations and carrying its own heater winding. Pyrex sockets were used as inlet and outlet connections, and the joints were sealed with a narrow band of vacuum grease that had previously been heated for long periods in air to remove traces of volatile matter. Fused-silica boats (about 4 cm × 1·5 cm) were used, and were made in the laboratory from halved silica tubing. Since there is no sudden oxygen demand of the sort that occurs when combustion methods are used, no taps were included in the furnace leads, but a length of capillary tubing was inserted between the reservoir and furnace. The capillary limited the flow, even when the furnace was opened to admit the sample, and the main flow-control valve could be left unaltered during a sequence of runs. Samples were inserted into the cold furnace tube, which was then heated to about 400° C. With a gas flow-rate of 3·5 litres per hour, surface-adsorbed gases on the sample, boat and tube surfaces, were expelled in a few minutes. When evolution of carbon dioxide from this source ceased, the furnace temperature was raised to about 1000° C in the shortest possible time and maintained at this temperature until evolution of carbon dioxide from the sample was complete (usually within 15 to 20 minutes).

It was found that new sources of error, caused by changes in the furnace temperature, were involved in temperature changes of the cell solution. Heat exchangers were incorporated in the gas-flow line between the furnace and the cell, of which the more important was a 5-foot length of $\frac{1}{4}$-inch copper tubing, formed in a helix and mounted in the thermostat bath so that the cell and helix were coaxial. The efficiency of the gas stream as a heat-transfer medium proved to be far greater than that of the Perspex walls of the cell and, although the effects of fluctuations in the temperature of the furnace were eliminated, the cell now responded rapidly to fluctuations of bath temperature (a time lag of 15 to 20 seconds instead of several minutes). At first, the difficulty was countered by reading the bath temperatures to the nearest 0·005° C at the beginning and end of a run and correcting the conductance changes accordingly. Attempts were made later to run a duplicate cell alongside the measuring cell in a slightly enlarged thermostat bath. Oxygen was passed from the reservoir to this cell, through a duplicate heat-exchange coil. It was hoped that such a scheme would provide full automatic correction for the combined effects of temperature fluctuation and loss by evaporation. This hope was not fulfilled for reasons described below, and, in the final form of the method, one cell was used in a large thermostat bath whose temperature fluctuated by $\pm$0·003° C around the mean at 25° C. (This corresponds to uncertainties of $\pm$0·5 μg of carbon in the results obtained.) With temperature variations minimised, it became practicable to extend conductance readings at the beginning and end of each run, and to establish the rise in conductance caused solely by evaporation. The appropriate correction is important for accurately determining small amounts of carbon, particularly when diffusion is slow, but this technique considerably increases the time necessary for the determination.

Sources of variability

The attempt to use one cell as an automatic compensator for temperature and evaporation effects in the measuring cell led indirectly to the observation of several minor sources of variation, which are briefly summarised here.

It was found that even purely thermal effects were not identical in the two cells, one having a more rapid response than the other. This is presumed to be caused by minor dimensional differences that were also thought to be partly responsible for unequal evaporation effects. Both the rate of loss of water and the proportionate effect on the conductance of

the cell were found to differ in the two cells. A further cause of variation in the rate of evaporation was the difficulty of ensuring identical bubble-size distribution and surface tension of the solutions, and this affects the surface area from which evaporation occurs. A stable flow of bubbles and uniform size distribution were also found to be important for repeatable conductance measurements. When the cell liquid is circulating, the absorption path provides a conductance path in parallel with the formal inter-electrode gap, and fluctuations in the effective length of this parallel path may produce fluctuations in the conductance of the cell amounting to a few parts per ten thousand, equivalent to 1 to 2 μg of carbon. The reduction of this source of error to negligible proportions may need preliminary stabilisation of the gas flow-rate in the cell for periods as long as two hours, when a freshly filled cell is used.

All these individual sources of variability are small in themselves, but may augment or cancel each other at random, producing a scatter in the results.

One further observation is worth recording. There was a tendency for cell factors determined at the beginning of a working day to be higher than those determined later in the same day. The reason for this is not definitely known, but two interacting effects were suspected. When the apparatus was in frequent use, it was the custom to reduce the gas flow-rate to 0·5 litres per hour overnight, sufficient to keep a slight oxygen pressure in the system, but insufficient to circulate the cell liquid. In these circumstances there seemed to be a change in the surface tension of the liquid, and when the flow-rate was restored to 3·5 litres per hour each morning, bubble-size distribution was unstable for periods up to half an hour. Even after this there may have been changes, *e.g.*, in the rate of evaporation or in the effective length of the conduction path, that affected the cell response. The second effect may have been associated with carbon dioxide adsorption on the manganese dioxide surfaces. It was known that some adsorption and desorption occurred, since the time lag between furnace-temperature changes and cell response varied with gas flow-rates and the amount of manganese dioxide used. In the earlier form of the method (where conductance changes less than 1 μmho in 2 minutes were ignored), a small primary coverage of carbon dioxide may always have been left in a state of quasi-equilibrium. If this were removed in the slow purging carried out overnight, the first sample or two of the day may have lost a small proportion of carbon dioxide in restoring this primary cover. The delayed desorption of carbon dioxide from manganese dioxide, etc., has since been noted by Peterson.[5]

Experimental results

Results for nickel—

The method was developed in two phases. The ultimate aim was to achieve a high degree of confidence on single, small samples, but most of the experimental effort was devoted to establishing the use of diffusion-controlled oxidation as an alternative to combustion, in which freely available material was used. This was the first phase and was concerned with reproducibility in what is referred to as the rapid method.

The nickel used was of the relatively pure commercial grades widely used in the thermionics industry, usually containing carbon in the range 0·005 to 0·05 per cent. The most important impurities were magnesium, occurring up to 0·15 per cent., and silicon, occurring up to 0·05 per cent. Iron and cobalt occasionally exceeded 0·2 per cent., but all other elements were present at much lower concentrations.

Results were obtained by comparing the diffusion method with results obtained from total-combustion methods. A length of nickel strip, 0·006 inch thick, was stamped out in discs weighing 5 mg, which were aggregated to form a mixed sample. Spot checks along the strip indicated a carbon content varying between 0·033 and 0·040 per cent. (330 to 400 p.p.m.). The co-operation of the National Physical Laboratory and the Research Laboratories of the General Electric Company Ltd. was sought and acknowledgment is made of help received. The results obtained are set out in Table I.

It can be seen that the diffusion method is as sensitive and reliable for nickel as the total-combustion method, and it is emphasised that the results above were obtained before the proposed method had been fully refined. This partly explains the marked deterioration in precision at low sample weights. In part, however, the increased scatter can be attributed to variation of carbon content within the material. For similar sample weights, the conductimetric methods give standard deviations comparable with that for low-pressure

manometry, generally regarded as the most precise of the absolute methods available. The gravimetric determination gave a larger standard deviation despite the larger samples used. The conductimetric results were significantly higher than those obtained from conventional measurement methods. This could conceivably be caused by impurities in the chemicals used for determining the cell factor, or to imperfect recovery of carbon dioxide in the conventional methods or to both.

TABLE I: COMPARISON OF RESULTS OBTAINED FOR THE CARBON CONTENT OF NICKEL STRIP BY VARIOUS METHODS

Laboratory	Oxidation method	Measurement method	Sample weight, g	Number of determinations	Mean value, p.p.m.	Standard deviation, p.p.m.
N.P.L.	Combustion	Gravimetric	3·0	4	354	10
N.P.L.	Combustion	Manometric	0·5	4	355	5
G.E.C.	Combustion	Conductimetric	1·0 and 2·0	5	366	3·5
G.P.O.	Diffusion	Conductimetric	0·5 to 1·0	7	363	5
G.P.O.	Diffusion	Conductimetric	0·2	4	369	28

In the analysis of valve components, the precision achieved at low carbon levels revealed small-scale variations of carbon content that were of considerable technological interest. One anode, before assembly, had a content of approximately 2 p.p.m., though made from strip containing an average of 130 p.p.m. This anode contained oxide inclusions that had reacted with the carbon during de-gassing under vacuum. Another piece of nickel strip was found to contain a region in which the carbon content varied over a short distance from 53 to 84 p.p.m., though the rest was more or less uniform at 112 p.p.m. This also was probably owing to reaction with oxide inclusions during the annealing of the strip.

DISCUSSION OF THE METHOD AS APPLIED TO NICKEL—

The results quoted in Table I relate to the rapid method, in which the cell temperature varied by $\pm 0{\cdot}03^\circ$ C and was not known to nearer than $0{\cdot}005^\circ$ C. The effect of evaporation was ignored, and the initial and final measurements were made when the conductance change did not exceed 1 μmho in a period of two minutes. The limit of detection given by the cell and bridge combination was about 0·3 μg in the most favourable conditions, but the discrimination obtainable between samples was limited by the uncertainty arising from the temperature and evaporation effects. A study of many results on virtually uniform material indicated that, if M was the measured carbon content and C the true carbon content, then—

$$M = C \pm aC \pm b$$

where a was approximately 0·01 and b was about 3 μg. The factor a must have included any small variations in material and probably any time-dependent errors, since these would show an apparent dependence on the carbon content when working at a fixed diffusion temperature. Random temperature effects in the cell and any random errors in carbon dioxide recovery from the sample or furnace train are included in b.

From the known limits of temperature uncertainty and of rates of evaporation it may be concluded that the uncertainty due to these causes alone was in the range 1 to 2 μg. As mentioned earlier, larger sources of relatively constant error, such as contamination of the gas stream, boats and contents by atmospheric gas, had all been eliminated, and the remaining errors tended to support or cancel each other at random. Thus, it is not possible to make a clear statement of blank-value levels.

The effect of later improvements in method, *viz.*, improved temperature control and the continuance of measurements until the normal rise of conductance, due to evaporation, is restored, have not been systematically assessed. Results indicate that both a and b were reduced to about half the values quoted above. This gave an uncertainty on single determinations of about 2 per cent. on carbon amounts exceeding 100 μg, but amounts less than 10 μg could only be confidently quoted to within some 20 per cent. of the true value. The practical significance of this uncertainty varied with the type and size of sample.

RESULTS FOR 3½ PER CENT. TUNGSTEN - NICKEL ALLOY—

This material was prepared by the techniques of powder metallurgy and was low in carbon content. Because of uncertainty about the effects of tungsten on the carbon recovery by the

diffusion method, comparison was again made with combustion methods. In this work the co-operation of the Research Laboratories of the General Electric Company Ltd. and those of the Standard Telephones and Cables Ltd., Valve Division, is acknowledged. It was in the course of this study that the need to continue conductance measurements, until the normal conductance rise due to evaporation from the cell is fully restored, became apparent. Carbon recoveries from strip, heavily carburized at 1200° C, seemed in every way normal (the time - loss curves were similar to those for pure nickel), but recoveries of carbon present at the 20 p.p.m. level or less were low compared with those obtained by using the total-combustion method. When the change of technique just mentioned was made, results agreed with those obtained by using combustion methods. It also became apparent that the last traces (say 0·0005 per cent.) of carbon were diffusing more slowly than for pure nickel. This effect was later noticed in low-alloy steels and suggests that there may be two diffusion mechanisms in such instances, of which the predominant one is that pertaining to the pure metal. No reason is apparent why such a small proportion of the carbon should be affected, if tungsten - carbon compound formation were involved in the presence of excess of tungsten.

RESULTS FOR MILD STEEL—

During the development of the diffusion method for nickel it was envisaged that it would be inapplicable where the diffusion rate for carbon was low, or for materials where the rates of recovery of carbon were controlled by slow reaction rates involving refractory carbides or graphite inclusions. Various steel samples were examined to assess the wider usefulness of the technique. Preliminary tests with a plain mild steel (British Chemical Standard No. 264) showed that in pure oxygen, combustion was so violent that the oxide was fused even though the furnace temperature did not exceed 950° C. Replacement of oxygen by air reduced the rate of combustion and gave a densely sintered oxide residue. Further reduction in oxygen content was achieved by using a gas stream containing 7 to 8 per cent. v/v of oxygen in argon, and at 950° C this gave a porous, lightly sintered oxide residue. The pattern of carbon dioxide recovery with time was similar to that for nickel, and suggested that in these circumstances the evolution was again diffusion controlled.

Carbon recoveries were lower than with pure oxygen, and it was suspected that some carbon monoxide was produced at the lower concentration of oxygen. The guard tube was modified to contain a plug of granular manganese dioxide as an absorbent for acidic gases, and then a plug of Körbl's catalyst.[6,7] An electric heating tape was used for keeping the catalyst at a temperature between 200° and 250° C. The change in technique gave recoveries 2 to 3 per cent. higher, tending to confirm the suspicion that some carbon monoxide was produced.

A study of the effect of temperature on recovery was made on a sample of British Chemical Standard No. 224. This steel contains 1·46 per cent. of chromium, 0·24 per cent. of vanadium, 0·31 per cent. of silicon, 0·70 per cent. of manganese and smaller amounts of other impurities. The carbon contents reported by the eight standardising laboratories range from 0·380 to 0·400 per cent., with an average of 0·390 per cent.

The results of four determinations, made with sample weights of 0·5 g at each of four different temperatures, are given in Table II.

TABLE II: PERCENTAGE CARBON RECOVERED FROM A STEEL SAMPLE BY OXIDISING IT AT VARIOUS TEMPERATURES

British Chemical Standard No. 224: nominal carbon content, 0·390 per cent.
Standardising range, 0·380 to 0·400 per cent.

Oxidation temperature, °C	Range, per cent. carbon	Mean, per cent.
750	0·383 to 0·396	0·388
900	0·372 to 0·403	0·382
1050	0·378 to 0·385	0·381
1100	0·373 to 0·415	0·388

The conductance - time graphs for two experiments at 750° and 1050° C are shown in Fig. 1. The average values obtained at 750° and 1100° C agree well with the average of the results obtained by the standardising laboratories. The spread of individual results is not surprising since sample weights were approximately 0·5 g. It is unlikely that less than 2-g samples were used in the standardisation, and the effect of variation within the sample would have

been less than in the present work. The graphs in Fig. 1 reveal that, when long evolution times are involved, the rise in conductance caused solely by evaporation needs to be evaluated. However, it is clear that the conventional technique of using high temperatures to obtain a fluid slag can be replaced by careful oxidation at much lower temperatures, provided that time-dependent errors are known with the required precision, and provided that a closely sintered oxide residue can be avoided.

RESULTS FOR CAST IRON—

Diffusion-controlled oxidations were carried out at different temperatures on a sample of grey cast iron containing 2·91 per cent. of free carbon and 0·43 per cent. of combined carbon. The silicon content was 2·3 per cent.

There was a progressively greater recovery as the oxidation temperature was raised and, in one experiment in which the carbon dioxide recovery was plotted against a stepwise increase in sample temperature, there was a hint of a distinctive change in the rate of carbon release between 950° and 1050° C. Full recovery was only obtained at temperatures of 1000° C and above. This contrasts with the behaviour of the British Chemical Standard steels, and is probably associated with relatively massive graphitic or carbide inclusions. Koch and Malissa[8] have described how some specimens of grey cast iron can be "burnt" in oxygen to give a separate release, first of the free carbon (600° to 700° C) and then of the combined

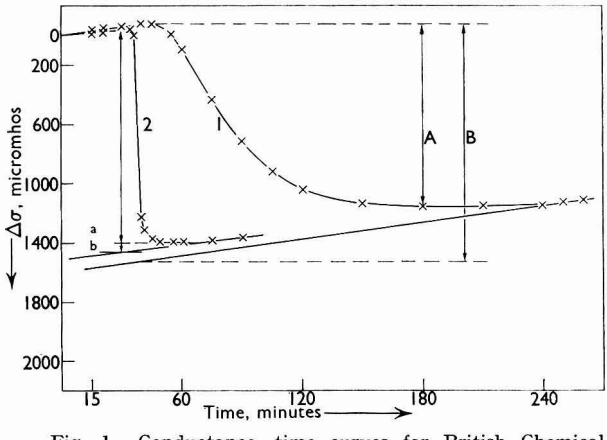


Fig. 1. Conductance - time curves for British Chemical Standard No. 224, showing influence of evolution rate on error of end-point determination: A and a, rapid method end-points; B and b, true end-points. Curve 1, 750° C; curve 2, 1050° C. (Sample weight, approximately 0·5 g)

carbon (above 800° C); they found others in which the separation was not precise. The present results give no hint of a separate release step in the neighbourhood of the free-carbon content. For such discrimination, oxidation seems to be much less reliable than the selective hydrogenation at relatively low temperatures described by Marion.[9]

RESULTS FOR OTHER METALS—

Satisfactory results have been obtained by the diffusion method on nickel - manganese alloys, tungsten, molybdenum and niobium. "Difficult" metals such as beryllium, magnesium, titanium and zirconium have not been examined. Within the limits of experiments made, the method has been found useless for only two alloys. One was an iron - 16 per cent. aluminium alloy and the other a nickel - iron - 16 per cent. chromium alloy. In both instances, only a small fraction of the total carbon was recoverable by diffusion-controlled oxidation at 1100° C. This may have been owing to stability of carbides, but it is also likely that stable, impervious oxide films were involved.

GENERAL DISCUSSION

The results show that full recovery of carbon from metallic samples can be achieved at temperatures below those necessary for combustion and formation of a fluid slag, provided

that the metal composition permits rapid diffusion of carbon without the formation of an impervious oxide. Nickel or iron alloys in general can be satisfactorily treated, but only if the aluminium or chromium content does not exceed 1 or 2 per cent. One might postulate similar limits for other alloying elements, such as titanium and silicon, that are capable of forming stable carbides and impervious oxide films.

The difficulties associated with too rapid an evolution of the heat of formation of the oxide can be avoided by using an argon - 8 per cent. oxygen gas stream, but if this gas is used a post-furnace oxidation catalyst is necessary to ensure complete conversion of carbon to carbon dioxide. Though independently adopted here, the use of argon is not new. Wood and Williams[10] secured controlled combustion of titanium and zirconium by flushing the furnace tube with argon and slowly displacing with oxygen. There are probably practical advantages during a conductimetric assay in keeping the composition of the gas stream constant, as in this work, but the point has not been examined.

The primary reason for investigating the diffusion technique was the requirement to improve precision at low carbon levels by avoiding sources of blank values arising from the combustion process. This was a specialised requirement and may not be of widespread interest. (In many laboratories, extremely small samples are useless, since the materials studied are heterogeneous.) To make full use of the advantages of the method it has been found necessary to take rather elaborate steps to control the variations of temperature in the cell, and the need for elaboration arises from the use of a small, quick-response furnace that gives the gas stream wide temperature fluctuations. However, for laboratories interested in extremely small samples, where replication is impossible, the techniques described are believed to be as precise and sensitive as the best absolute methods, and require no vacuum equipment or high-frequency source of power.

A form of the method that may have a wide usefulness has been described in a report from the laboratories of the Valve Division of Standard Telephones and Cables Ltd. Acknowledgment is made of permission to refer to it here. James and Ogden (Unpublished Laboratory Report, Standard Telephone and Cables Ltd., Valve Division, 1958) used the apparatus of Still, Dauncey and Chirnside,[1] but operated the furnace at 1060° C whenever appropriate (*e.g.*, for all nickel specimens). They found that blank values caused by permeation of the atmosphere through the furnace tube were much reduced at this lower temperature, and those caused by igniters and fluxes were eliminated. By working with a fixed furnace temperature, the small and simple thermostat[1] remained adequate. It is possible that such a technique, perhaps modified by the use of an argon - 8 per cent. oxygen mixture and a Körbl catalyst, could be of interest to many laboratories requiring good precision in the determination of carbon in small, single samples of nickel or iron alloys, since the equipment is also capable of giving the higher temperature (in pure oxygen) needed for total combustion. The methods of most general applicability are those in which induction heating in a silica tube furnace is used, such as that described by Green, Still and Chirnside,[2] with conductimetric or low-pressure manometric determination of carbon dioxide produced by total combustion of the sample to a fluid slag. These methods, however, require facilities not present in every laboratory.

I gratefully acknowledge the co-operation received from the other laboratories mentioned in the text and the experimental assistance given by Messrs. D. G. Fiddyment and J. S. Smith in studying sources of variability. Acknowledgment is made to the Engineer-in-Chief, G.P.O., for permission to publish this paper.

References

1. Still, J. E., Dauncey, L. A., and Chirnside, R. C., *Analyst*, 1954, **79**, 4.
2. Green, I. R., Still, J. E., and Chirnside, R. C., *Ibid.*, 1962, **87**, 530.
3. Dailly, D. F., and Elliott, T. A., *J. Chem. Soc.*, 1956, 3398.
4. Fryxell, R. E., *Anal. Chem.*, 1958, **30**, 273.
5. Peterson, W. M., *Ibid.*, 1962, **34**, 575.
6. Körbl, J., *Mikrochim. Acta*, 1956, 1706.
7. Horáček, J., and Körbl, J., *Ibid.*, 1959, 303.
8. Koch, W., and Malissa, H., *Arch. Eisenhutienw.*, 1956, **27**, 695.
9. Marion, F., *Bull. Soc. Chim. France*, 1958, 1187.
10. Wood, D. F., and Williams, M., *Metallurgia*, 1958, **58**, 47.

Received *May 17th*, 1963

The Analytical Uses of Metallic Silver

Part I.* The Qualitative Analysis of those Group 2 Elements that can be Precipitated by Silver

By A. J. HENRY

(*Chemistry Department, Fourah Bay College, Freetown, Sierra Leone*)

Salts of gold, platinum, palladium and mercury can be reduced to the free elements, and selenous and tellurous acids can be reduced to silver selenide and silver telluride, respectively, by silver powder in 3 N hydrochloric acid at room temperature.

In the presence of excess of iodide and 5·5 N hydrochloric acid at 100° C, selenic acid is reduced to selenium, and arsenic and telluric acids are reduced to arsenous and tellurous acids, respectively. If the solution is then shaken at room temperature with silver powder, arsenic, antimony, bismuth, tellurium (from tellurates) and copper are precipitated.

A scheme for the qualitative analysis of the classical group 2 elements, based on these reductions, has been shown to give satisfactory separation and identification of all those elements that can be precipitated, and to yield a solution in which the detection of the remaining elements of group 2 and those of subsequent groups may be effected.

An approximately quantitative thermodynamic discussion of the reducing action is given.

The reducing actions of branched-chain alcohols on the elements that can be precipitated by silver have been investigated.

METALLIC silver has gained little acceptance as an analytical reagent, except in the form of the silver reductor.[1] Gooch and Perkins[2] used silver powder for the gravimetric estimation of free iodine, and Perkins[3,4] extended the estimation of free iodine by means of metallic silver to the indirect estimation of other substances that liberate iodine from potassium iodide, and showed that accurate estimations of selenite, tellurite, vanadate, molybdate, iron^{III}, dichromate, permanganate and hydrogen peroxide could be carried out by shaking a known volume of the acidified solution with a known amount, in excess, of silver powder and an excess of iodide, and determining the increase in weight of the final residue (silver *plus* silver iodide *plus* other element, if any, precipitated) over the weight of silver used. Tellurous acid, however, does not liberate free iodine from hydriodic acid, but it is reduced by silver under these conditions and the tellurium is deposited. Tellurous acid is reduced by silver in the presence of hydrochloric acid only. This point is important because tervalent arsenic, antimony and bismuth can also be reduced and deposited by silver in presence of iodide and sufficient concentration of acid, although no liberation of iodine occurs.

For some years we have used metallic silver for reducing organic poly-iodides and for completely removing gold and platinum from organic chloroaurates and chloroplatinates. For these purposes, silver has the great advantages that nothing enters the solution to replace the constituent removed, the reactions are carried out at room temperature and the precipitated silver salt, and the gold or platinum, adhere to the excess of silver in a compact form that can readily be filtered off, so that separations are eminently clean. This is in sharp contrast with the difficulty found when precipitating gold or platinum completely from solution by means of hydrogen sulphide, which can further cause trouble owing to oxidation, particularly for gold,[5] of some of the hydrogen sulphide to sulphuric acid, with consequent risk of precipitating the alkaline-earth elements in group 2, or of charring an organic base.

We have considerably extended the gravimetric applications of metallic silver, and hope to present these results in another paper. We have based an entirely new approach to the

* For details of Part II of this series, see reference list, p. 254.

problems of the semi-micro qualitative analysis of the classical group 2 elements on the use of metallic silver; the proposed analytical scheme is of special value when the presence of the rarer elements must also be taken into account. This forms the subject of Part I of this Paper. Its adaptation to the qualitative analysis of the common elements of group 2 has led to a proposed revision of existing schemes of analysis of the common elements as a whole, and forms the subject of Part II[6] of this Paper.

Discussion

Owing to the position of silver in the electrochemical series, its reducing reactions in solution cannot normally proceed to any great extent unless the resulting concentration of silver ions in the system is automatically maintained at a low value by precipitating them with a suitable anion, X^-, present in excess in the solution. By appropriate choice of the nature and concentration of the anion X^-, the electrode potential of the silver - silver ion system may be maintained at approximately constant values that may lie in a considerable range, so that the reducing power of the metal may be altered by varying the electrode potential.

The reactions that have to be considered can be divided into two groups. In Group A, the reductions are formally represented as involving only simple transfer of electrons from the silver atoms to the oxidising agent, although, even in this group, complex halogeno-anion formation plays a considerable part, which is of particular importance for bismuth. Even though they do not strictly belong to this group, the cupric ion - cuprous halide systems, in which halide ions play an essential part in the correct interpretation of the reductions, are also included. Group B consists of the oxy-acids and oxy-ions, the reduction of which, besides involving transfer of electrons, depends on hydrogen-ion concentration.

The thermodynamic data used in the discussion given below were obtained from "Selected Values of Chemical Thermodynamic Properties,"[7] "Comprehensive Analytical Chemistry"[8] and "Oxidation Potentials."[9]

Group A—

The reactions may be represented by the general equations—

(*a*) Oxidised form (Ox.) + $ze \rightleftharpoons$ Reduced form (Red.) (E_1^0)

(*b*) $z\mathrm{Ag}^+ + ze \rightleftharpoons z\mathrm{Ag}^0$ $(E_2^0 = +0{\cdot}80\ \mathrm{V})$.

The corresponding thermodynamic equations* at 25° C are—

(*c*) $$E_1 = E_1^0 + \frac{0{\cdot}059}{z} \log \frac{(a_{\mathrm{Ox.}})}{(a_{\mathrm{Red.}})}$$

(*d*) $$E_2 = E_2^0 + \frac{0{\cdot}059}{z} \log \frac{(a_{\mathrm{Ag}^+})^z}{(a_{\mathrm{Ag}^0})^z}$$

$$= E_2^0 + \frac{0{\cdot}059}{z} \log (a_{\mathrm{Ag}^+})^z, \text{ since } (a_{\mathrm{Ag}^0}) = 1.$$

At equilibrium $E_1 = E_2$, and by replacing activities† by concentration terms we get—

$$E_2^0 - E_1^0 = \frac{0{\cdot}059}{z} \log \frac{[\mathrm{Ox.}]}{[\mathrm{Red.}]} - \frac{0{\cdot}059}{z} \log [\mathrm{Ag}^+]^z$$

Thus—

$$\log \frac{[\mathrm{Ox.}]}{[\mathrm{Red.}]} = \frac{z(E_2^0 - E_1^0)}{0{\cdot}059} + z \log [\mathrm{Ag}^+] \quad \ldots\ \ldots\ \ldots\ \ldots\ \ldots \quad (1)$$

Some electrochemical values, calculated by using equation (1), are incorporated in Table I.

* Few electrode systems are strictly reversible thermodynamically,[8] so that application of the Nernst equation is only approximately quantitative.

† Activities of the various species in the rather concentrated solutions are unknown, and there is no alternative to the use of concentration terms, which, however, are themselves uncertain, owing to complex formation.

TABLE I

ELECTROCHEMICAL VALUES CALCULATED BY USING EQUATION (1)

$E_2^0 = E^0_{Ag} = +0{\cdot}80$ V: concentration of silver ions in M chloride solutions $= 10^{-10}$ M, and in M iodide solutions $= 10^{-16}$ M

Reduced form	Reaction	E_1^0, volts	$E_2^0 - E_1^0$, volts	Equilibrium ratio $\frac{[Ox.]}{[Red.]}$ in M chloride	Equilibrium ratio $\frac{[Ox.]}{[Red.]}$ in M iodide
Gold	$Au^{3+} + 3e \rightleftharpoons Au$	+1·50	−0·70	10^{-65}	—
Platinum	$Pt^{4+} + 4e \rightleftharpoons Pt$	+0·86	−0·06	10^{-44}	—
Palladium ..	$Pd^{2+} + 2e \rightleftharpoons Pd$	+0·99	−0·19	10^{-26}	—
Mercury	$Hg^{2+} + 2e \rightleftharpoons Hg$	+0·85	−0·05	$2{\cdot}5 \times 10^{-22}$	—
Bismuth	$Bi^{3+} + 3e \rightleftharpoons Bi$	+0·28	+0·52	$2{\cdot}5 \times 10^{-4}$	$2{\cdot}5 \times 10^{-22}$
Iron^{II}	$Fe^{3+} + e \rightleftharpoons Fe^{2+}$	+0·76	+0·04	5×10^{-10}	5×10^{-16}
Ferrocyanide ..	$Fe(CN)_6^{3-} + e \rightleftharpoons Fe(CN)_6^{4-}$	+0·36	+0·44	$2{\cdot}5 \times 10^{-3}$	$2{\cdot}5 \times 10^{-9}$
Cuprous chloride	$Cu^{2+} + Cl^- + e \rightleftharpoons CuCl$	+0·54	+0·26	$2{\cdot}5 \times 10^{-6}$	—
Cuprous iodide ..	$Cu^{2+} + I^- + e \rightleftharpoons CuI$	+0·85	−0·05	—	$1{\cdot}6 \times 10^{-17}$

NOTES—

1. In those reactions in which the reduced form is precipitated (this include scuprous iodide, but not cuprous chloride) and is therefore in its standard state, the ratio $\frac{[Ox.]}{[Red.]}$ is equal to the concentration of the oxidised form remaining in the solution.

2. The formation of complex bismuth chloro-anions prevents the reduction of the element in chloride solution. The stability of the complex iodides is insufficient to prevent reduction and precipitation in the presence of iodide ions.

3. The presence of iodide or bromide ions is required for the quantitative reduction of ferricyanide.

GROUP B—

These reactions may be represented by the general equations—

(*a*) $Ox. + nH^+ + ze \rightleftharpoons Red. + mH_2O \qquad (E_1^0)$.

(*b*) $zAg^+ + ze \rightleftharpoons zAg^0 \qquad (E_2^0 = +0{\cdot}80$ V).

The corresponding thermodynamic equations at 25° C are—

(*c*) $$E_1 = E_1^0 + \frac{0{\cdot}059}{z} \log \frac{(a_{Ox.})}{(a_{Red.})} + \frac{0{\cdot}059}{z} \log (a_{H^+})^n$$

(*d*) $$E_2 = E_2^0 + \frac{0{\cdot}059}{z} \log (a_{Ag^+})^z$$

At equilibrium, $E_1 = E_2$, so that, replacing activities by concentration terms, we have—

$$E_2^0 - E_1^0 = \frac{0{\cdot}059}{z}\left(\log \frac{[Ox.]}{[Red.]} + \log [H^+]^n - \log [Ag^+]^z\right).$$

Thus—

$$\log \frac{[Ox.]}{[Red.]} = \frac{z(E_2^0 - E_1^0)}{0{\cdot}059} - n \log [H^+] + z \log [Ag^+] \qquad .. \quad .. \quad .. \quad (2)$$

For those instances in which the reduced form remains in solution, the values of 10^{+2} and 10^{-4} for the ratio $\frac{[Ox.]}{[Red.]}$ may be accepted as indicative of the initial (1 per cent.) reduction and of the final, quantitative reduction, respectively. By inserting these figures, and the other known data, into equation (2) the theoretical hydrogen-ion concentrations necessary for the initial and quantitative reductions may be calculated. For those instances in which

the reduced form is deposited (and is therefore in its standard state, with unit activity) the ratio $\frac{[Ox.]}{[Red.]}$ is equal to the concentration of the oxidised form in equilibrium with the solid reduced form in any set of conditions. By inserting appropriate values for [Ox.] and other known data in equation (2), the theoretical minimum hydrogen-ion concentrations required for the initial and quantitative reductions (ratio = 10^6) for any given initial concentration (10^{-2} M has been taken as standard) may be calculated.

The values of the limiting hydrogen-ion concentrations in molar chloride and molar iodide solutions for many of the common oxy-ions and oxy-acids, calculated by using equation (2), are given in Table II.

TABLE II

ELECTROCHEMICAL VALUES CALCULATED BY USING EQUATION (2)

$E_2^0 = E_{Ag}^0 = +0{\cdot}80$ V: concentration of silver ions in M chloride solutions = 10^{-10} M, and in M iodide solutions = 10^{-16} M

Reduced form	Reaction	E_1^0, volts	$E_2^0-E_1^0$, volts	Hydrogen-ion concentration for M chloride solution: Initial reduction	M chloride solution: Quantitative reduction	M iodide solution: Initial reduction	M iodide solution: Quantitative reduction
U^{4+}	$UO_2^{2+} + 4H^+ + 2e \rightleftharpoons U^{4+} + 2H_2O$	+0·33	+0·47	3×10^{-2}	$1{\cdot}0 \times 10^{0}$	$2{\cdot}5 \times 10^{-5}$	$1{\cdot}0 \times 10^{-3}$
VO^{2+}	$VO_3^- + 4H^+ + e \rightleftharpoons VO^{2+} + 2H_2O$	+1·10	−0·30	5×10^{-5}	$1{\cdot}8 \times 10^{-3}$	$2{\cdot}0 \times 10^{-6}$	$5{\cdot}4 \times 10^{-5}$
V^{3+}	$VO^{2+} + 2H^+ + e \rightleftharpoons V^{3+} + H_2O$	+0·40	+0·40	$2{\cdot}5 \times 10^{-3}$	$3{\cdot}2 \times 10^{0}$	$2{\cdot}5 \times 10^{-6}$	$2{\cdot}3 \times 10^{-3}$
Ti^{3+}	$TiO^{2+} + 2H^+ + e \rightleftharpoons Ti^{3+} + H_2O$	+0·10	+0·70	$8{\cdot}0 \times 10^{-1}$	$1{\cdot}0 \times 10^{3}$	$1{\cdot}0 \times 10^{-3}$	$1{\cdot}0 \times 10^{0}$
H_3AsO_3	$H_3AsO_4 + 2H^+ + 2e \rightleftharpoons H_3AsO_3 + H_2O$	+0·49	+0·31	—	$1{\cdot}7 \times 10^{-3}$	—	—
As	$H_3AsO_3 + 3H^+ + 3e \rightleftharpoons As + 3H_2O$	+0·24	+0·56	$1{\cdot}4 \times 10^{0}$	$3{\cdot}4 \times 10^{1}$	$1{\cdot}8 \times 10^{-6}$	$3{\cdot}0 \times 10^{-5}$
H_3SbO_3	$H_3SbO_4 + 2H^+ + 2e \rightleftharpoons H_3SbO_3 + H_2O$	+0·75	+0·05	—	$7{\cdot}0 \times 10^{-8}$	—	—
Sb	$H_3SbO_3 + 3H^+ + 3e \rightleftharpoons Sb + 3H_2O$	0·0	+0·80	$1{\cdot}7 \times 10^{4}$	—	$2{\cdot}0 \times 10^{-2}$	$3{\cdot}0 \times 10^{-1}$
H_2SeO_3	$H_2SeO_4 + 2H^+ + 2e \rightleftharpoons H_2SeO_3 + H_2O$	+1·20	−0·40	—	$1{\cdot}6 \times 10^{-15}$	—	—
H_2Se (Ag salt)	$H_2SeO_3 + 6H^+ + 6e \rightleftharpoons H_2Se + 3H_2O$	+0·37*	+0·43	$4{\cdot}0 \times 10^{-10}$	$4{\cdot}0 \times 10^{-9}$	—	—
H_2TeO_3	$H_2TeO_4 + 2H^+ + 2e \rightleftharpoons H_2TeO_3 + H_2O$	+1·02	−0·22	—	$1{\cdot}2 \times 10^{-12}$	—	—
H_2Te (Ag salt)	$H_2TeO_3 + 6H^+ + 6e \rightleftharpoons H_2Te + 3H_2O$	+0·12*	+0·68	$3{\cdot}0 \times 10^{-6}$	$3{\cdot}0 \times 10^{-5}$	—	—
MoO^{3+}	$MoO_3 + 4H^+ + e \rightleftharpoons MoO^{3+} + 2H_2O$	+0·50	+0·30	$2{\cdot}0 \times 10^{-2}$	$6{\cdot}0 \times 10^{-1}$	$6{\cdot}0 \times 10^{-4}$	$2{\cdot}0 \times 10^{-2}$
NO	$HNO_2 + H^+ + e \rightleftharpoons NO + H_2O$	+0·98	−0·18	$1{\cdot}0 \times 10^{-14}$	$1{\cdot}0 \times 10^{-9}$	—	—
NO	$NO_3^- + 4H^+ + 3e \rightleftharpoons NO + 2H_2O$	+0·99	−0·19	$3{\cdot}0 \times 10^{-11}$	$1{\cdot}0 \times 10^{-9}$	—	—

* Calculated by using the equations for the partial reactions—

$H_2MO_3 + 4H^+ + 4e \rightleftharpoons M + 3H_2O$ and $M + 2H^+ + 2e \rightleftharpoons H_2M$,

for which standard electrode potentials are available.

NOTES—

1. Reduction of metavanadate to vanadyl^{IV} closely conforms with theoretical predictions, but further reduction to vanadium^{III} is more difficult than might be expected.

2. Antimony^{V} and molybdenum^{VI} are readily reduced to antimony^{III} and molybdenum^{V}, respectively, in 2 M hydrochloric acid, but arsenic^{V}, selenium^{VI} and tellurium^{VI} are not.

3. The reduction of antimony^{III} in the presence of iodide ions is more easily effected than is that of arsenic^{III}, although theory predicts the reverse.

4. The presence of small amounts of nitric acid does not interfere with the estimation of copper in a solution of cupric nitrate.

5. Nitrous acid should be completely removed from the system, especially in quantitative work in the presence of iodide ions.

Further quantitative study of all of these reactions is desirable, but interference through hydrolysis is likely to be extensive in some instances.

Reduction of Selenous and Tellurous Acids to Silver Selenide and Telluride

Solubility Products of Silver Selenide and Silver Telluride—

Approximate values for these quantities, which are not available in the literature, were calculated by the procedure given below, for which I thank Mr. G. R. Martin, Reader in Radiochemistry, Londonderry Laboratory of Radiochemistry, University of Durham.

Silver selenide—Inspection of the standard entropies of the halides of univalent copper, silver and thallium shows an average increase of 2 cal. per degree per mole for the bromide over the chloride. It is reasonable to assume a similar increase in entropy of the selenide over the sulphide, so that the standard entropy of silver selenide is given by—

$$S^0_{Ag_2Se} = S^0_{Ag_2S} + 2 = 34{\cdot}8 + 2 = 36{\cdot}8 \text{ cal. per degree per mole.}$$

From data tables, $S^0_{Ag} = 10{\cdot}21$ and $S^0_{Se} = 10{\cdot}0$.

Thus—

$$\begin{aligned}\Delta S^0_f &= 36{\cdot}8 - (2 \times 10{\cdot}21 + 10{\cdot}0)\\ &= 6{\cdot}38 \text{ cal. per degree per mole.}\end{aligned}$$

But, in general—

$$\Delta G^0_f = \Delta H^0_f - T\Delta S^0_f$$

From data tables, ΔH^0_f for silver selenide $= -2{\cdot}9$ kcal. per mole. Thus, we have—

$$\begin{aligned}\Delta G^0_f &= -2{\cdot}9 - 298 \times 6{\cdot}38 \times 10^{-3}\\ &= -4{\cdot}8 \text{ kcal. per mole.}\end{aligned}$$

The solubility product of silver selenide may be calculated from the change in free energy of the reaction—

$$Ag_2Se = 2Ag^+ + Se^{2-}$$

This change in free energy may be calculated from the free-energy changes of the three processes involved; these processes are given below, together with the corresponding changes in free energy—

(*i*) $-(2Ag + Se = Ag_2Se)$	$\Delta G^0 = -(-4{\cdot}8)$ kcal. per mole.
(*ii*) $2Ag = 2Ag^+ + 2e$	$\Delta G^0 = 36{\cdot}86$ kcal. per mole.
(*iii*) $Se + 2e = Se^{2-}$	$\Delta G^0 = 37{\cdot}2$ kcal. per mole.

Summing, we have—

$$Ag_2Se \rightleftharpoons 2Ag^+ + Se^{2-} \qquad \Delta G^0 = 78{\cdot}86 \text{ kcal. per mole.}$$

But—

$$\Delta G^0 = -RT\ln K_s$$

where K_s is the solubility product.

Thus, for silver selenide—

$$\log K_s = -\frac{78{\cdot}86}{1{\cdot}364}$$

$$\text{and } K_s = 10^{-57{\cdot}8}$$

For comparison, the solubility products of mercuric selenide and cupric selenide are 10^{-59} and 10^{-49}, respectively.

Silver telluride—The standard free-energy change of the reaction—

$$Ag_2Te \rightleftharpoons 2Ag^+ + Te^{2-}$$

may be calculated in a similar manner, if approximate values of the standard entropy ($S^0 = 38{\cdot}8$ cal. per degree per mole) and standard heat of formation ($\Delta H^0_f = -1{\cdot}9$ kcal. per mole) are used. From the value of ΔG^o, the solubility product of silver telluride may be calculated (10^{-67}).

THERMODYNAMIC EQUATIONS FOR REDUCTION TO THE SELENIDE AND TELLURIDE—

The concentration of silver ions in M chloride solutions is 10^{-10} M, and in M iodide solutions is 10^{-16} M.

Reduction of selenous acid in M *chloride solution*—By applying equation (2) to the reaction—

$$H_2SeO_3 + 6H^+ + 6e \rightleftharpoons H_2Se + 3H_2O \qquad (E_1{}^o = +0{\cdot}37 \text{ V})$$

we have—

$$\log \frac{[H_2SeO_3]}{[H_2Se]} = \frac{6 \times (0{\cdot}80 - 0{\cdot}37)}{0{\cdot}059} - 6 \log [H^+] + 6 \log [Ag^+].$$

The equilibrium constant, $K_{\text{equil.}}$, for the reaction—

$$H_2Se \rightleftharpoons Se^{2-} + 2H^+$$

is given by—

$$K_{\text{equil.}} = \frac{[Se^{2-}]\,[H^+]^2}{[H_2Se]}$$

For hydrogen selenide, $K_{\text{equil.}} = 10^{-14{\cdot}8}$

Therefore—

$$\log \frac{[H_2SeO_3] \times 10^{-14{\cdot}8}}{[Se^{2-}]\,[H^+]^2} = 43{\cdot}7 - 6 \log [H^+] + 6 \log [Ag^+]$$

and—

$$\log \frac{[H_2SeO_3]}{[Se^{2-}]} = 58{\cdot}5 - 4 \log [H^+] + 6 \log [Ag^+].$$

It can be calculated from the value of the solubility product of silver selenide that the concentration of selenide ions in equilibrium with silver ions in M chloride solution is 10^{-38} M.

Thus—

$$\log \frac{[H_2SeO_3]}{10^{-38}} = 58{\cdot}5 - 4 \log [H^+] + 6 \log [10^{-10}]$$

and—

$$-39{\cdot}5 - \log [H_2SeO_3] = 4 \log [H^+] \quad .. \quad .. \quad .. \quad .. \quad .. \quad (3)$$

Reduction of tellurous acid in M *chloride solution*—By using the appropriate figures ($E^0_1 = +0{\cdot}12$ V, $K_{\text{equil.}} = 10^{-13{\cdot}6}$ and concentration of telluride ions in equilibrium with silver ions in M chloride solution $= 10^{-47}$ M) and by using similar logic, we get—

$$-24{\cdot}1 - \log [H_2TeO_3] = 4 \log [H^+] \quad .. \quad .. \quad .. \quad .. \quad (4)$$

Equations (3) and (4) were used for calculating the values of hydrogen-ion concentration given in Table II. Preliminary experiments indicate that the behaviour of selenous acid conforms with that predicted, to the extent that slight reduction occurs at a pH value of about 6. The reduction of tellurium is complicated by the low solubilities of the dioxide and of tellurous acid.

EXPERIMENTAL

The equations given below represent the principal reductions that enter into the qualitative analytical schemes (p denotes a precipitate)—

$$M^{n+} + n\text{Ag}^o + n\text{X}^- = n\text{AgX (p)} + \text{M}^o \text{ (p)} \quad .. \quad .. \quad .. \quad (i)$$

where M can be gold, platinum, palladium or mercury and X can be chloride, bromide or iodide.

$$M^{3+} + 3\text{Ag}^o + 3\text{I}^- = \text{M}^o \text{ (p)} + 3 \text{ AgI (p)} \quad .. \quad .. \quad .. \quad .. \quad (ii)$$

where M can be arsenic, antimony or bismuth.

$$Cu^{2+} + Ag^{o} + 2I^{-} = CuI\ (p) + AgI\ (p) \quad .. \quad .. \quad .. \quad .. \quad (iii)$$

$$MO_4^{x-} + 2HCl = MO_3^{x-} + H_2O + Cl_2 \quad .. \quad .. \quad .. \quad .. \quad (iv)$$

where M can be arsenic, selenium or tellurium.

$$MO_3^{2-} + 6Ag^{o} + 4Cl^{-} + 6H^{+} = Ag_2M\ (p) + 4AgCl\ (p) + 3H_2O \quad (v)$$

where M can be selenium or tellurium.

$$SeO_3^{2-} + 4I^{-} + 6H^{+} = Se^{o}\ (p) + 2I_2 + 3H_2O \quad .. \quad .. \quad .. \quad (vi)$$

$$TeI_6^{2-} + 4Ag^{o} = Te^{o}\ (p) + 4AgI\ (p) + 2I^{-} \quad .. \quad .. \quad .. \quad (vii)$$

$$Fe^{3+} + Ag^{o} + I^{-} = Fe^{2+} + AgI\ (p) \quad .. \quad .. \quad .. \quad .. \quad (viii)$$

The scheme of qualitative analysis of the classical group 2 elements now proposed assumes that the classical group 1 elements have been removed by hydrochloric acid. Allowance must be made for incomplete removal of lead in group 1. If arsenic, selenium and tellurium have previously been reduced to their lower valency states, all of the group 2 elements except lead, cadmium, tin and molybdenum are deposited from a 5·5 N hydrochloric acid solution when the solution is shaken at room temperature with excess of iodide and of silver powder. Under these conditions some of the silver iodide produced during the reduction reactions remains in solution, but, when the solution is substantially diluted and again shaken, silver iodide is deposited. The precipitations are complete, to the extent that the separated solution contains no detectable traces of those elements that are deposited, nor of silver. The insoluble residue obtained by this treatment consists of excess of silver, silver iodide and any or all of the elements listed below: gold, platinum, palladium, mercury, selenium, tellurium, arsenic, antimony, bismuth and copper (as cuprous iodide). The solution contains lead, cadmium, tin, molybdenum and any elements of group 3 and subsequent groups, some of which undergo partial reduction. Because of the strongly acid conditions under which the precipitated elements are deposited and the nature of the deposition processes, which are essentially electrolytic in most cases, it is unlikely that there is any appreciable tendency for non-precipitated elements to be carried down in the residue. In view of the difficulties attending the usual group 2 separations, it seemed that much improvement could be obtained by basing a new scheme of analysis on these precipitations. The success of such a scheme depends on ability to separate and identify the possible constituents of the complicated mixture that can comprise the insoluble residue of the single-stage precipitation, and this has so far proved too difficult, even on the semi-micro scale. Instead, a three-stage procedure has been evolved. Not only are the separations easier to effect, but precise information can be obtained on the oxidation state or states in which selenium and tellurium are present. Unfortunately, this does not apply to arsenic and antimony.

In Stage I of the three-stage procedure, the precipitation of gold, platinum, palladium and mercury as the free elements, and of selenium and tellurium from selenous and tellurous acids as silver selenide and telluride, respectively, is effected by shaking the solution at room temperature with silver powder in presence of 3 N hydrochloric acid, and separating the residue. In Stage II, the solution obtained from Stage I is further acidified to 5·5 N, excess of ammonium iodide added and the solution heated at 100° C for one hour under reflux, under which conditions selenic acid is reduced to the free element and precipitated, accompanied by a little copper, if present, and telluric and arsenic acids are reduced to tellurous and arsenous acids, respectively. The precipitated selenium (plus copper) is separated and the solution used for Stage III, in which the solution is shaken with silver powder at room temperature, and arsenic, antimony and bismuth are deposited as the free elements, and tellurium (from tellurate) is deposited as a mixture of free element and silver telluride. The behaviour of copper is not yet fully understood. It is reduced to the cuprous state in Stage I, but remains in solution as complex chloro-anions. Its failure to precipitate other than partially in Stage II along with selenium (from selenate), is probably due to its conversion to iodocuprous acid under the high acid and iodide concentrations used. During Stage III there is little change in the concentration of acid, but there is a considerable drop in that of iodide if other reducible elements are present in substantial proportions. This factor may be of some importance, but it is thought that precipitation as silver iodocuprite might be of major importance. At much lower acid concentration, copper is precipitated completely as cuprous iodide without difficulty.

FORMS IN WHICH THE ELEMENTS ARE PRECIPITATED—

Gold, platinum, palladium and mercury—These are all precipitated as the free elements; see reaction (*i*).

Selenium and tellurium—These elements are deposited from selenous and tellurous acids in 3 N hydrochloric acid as silver selenide and telluride, respectively. Tellurium is precipitated, partly as the free element and partly as silver telluride, from 5 N hydrochloric acid in the presence of excess of iodide. These conclusions are derived from the results of experiments, in which suitably acidified solutions were shaken in the dark with silver foil to permit recovery of the deposit free from metallic silver to be effected. In all experiments, deposits adhered firmly to the foil. This allowed complete separation from the solution to be effected, but made it necessary for the foil *plus* deposit to be digested with concentrated ammonia solution to dissolve co-deposited silver chloride, or with concentrated sodium thiosulphate solution to dissolve co-deposited silver iodide, which in each instance cemented the deposit to the foil. After these treatments the residual deposit could readily be detached from the foil, and analysed after it had been suitably washed and dissolved in nitric acid (1 + 1). Results obtained by using this method are given in Table III.

TABLE III

RESULTS OF ANALYSES OF RESIDUES SEPARATED FROM SILVER FOIL

Conditions of reaction	Weight composition of separated residue, mg	Atomic ratio of separated residue
K_2SeO_3 + 3 N HCl + silver foil	Ag, 3·8; Se, 1·3	$\frac{Ag}{Se} = 2{\cdot}0$
K_2TeO_3 + 3 N HCl + silver foil	Ag, 6·1; Te, 3·5	$\frac{Ag}{Te} = 2{\cdot}0$
K_2TeO_3 + 5 N HCl + excess of KI + silver foil ..	(i) Ag, 1·7; Te, 4·9*	$\frac{Ag}{Te} = 0{\cdot}42$
	(ii) Ag, 0·7; Te, 1·7	$\frac{Ag}{Te} = 0{\cdot}50$

* In this experiment there was insufficient potassium iodide to keep all the tellurium in solution as the complex halide, but no difficulty was experienced in separating the silver foil, with its deposit, from the precipitated tellurium tetra-iodide.

Arsenic, antimony and bismith—These elements are deposited only in presence of excess of iodide, and are all deposited as the free elements (see reaction (*ii*)), as shown by the results of experiments in which solutions, 5 N with respect to hydrochloric acid and containing excess of iodide, were shaken with silver foil. Deposited antimony and bismuth could readily be removed from the foil after it had been shaken with concentrated sodium thiosulphate to dissolve silver iodide. When the deposits had been analysed, after they had been washed and dissolved in diluted nitric acid (1 + 1), the deposits were found to be free from silver. Deposition of arsenic was slow at ordinary temperatures. Some deposit was obtained after the solution had been boiled for two hours, but it was difficult to remove from the foil. The deposit finally isolated was virtually free from silver.

THE THREE-STAGE SEMI-MICRO ANALYTICAL PROCEDURE

The essential steps of the three-stage procedure for isolating and identifying the elements, including the less common ones, precipitable by metallic silver, are incorporated in Table IV. Any preferred test may be used for confirming the presence of the elements. The separation and identification of the non-precipitable elements of group 2 (lead, cadmium, molybdenum and tin) are dealt with in Part II of this paper, which also gives a simplified scheme for separating and identifying the common elements that can be precipitated by silver in presence of iodide, as part of a wider survey of the problems of the qualitative analysis of the common elements, including molybdenum.

REDUCTION OF OXY-IONS AND OXY-ACIDS

REDUCTION OF METAVANADATE TO VANADYL^{IV}—

A 25-ml portion of M sodium chloride solution, containing 0·2340 g of ammonium metavanadate (0·08 M) and 3·51 ml of 2·278 N hydrochloric acid (the theoretical amount) was

TABLE IV

ANALYSIS OF THE GROUP 2 ELEMENTS (SILVER GROUP)

Elements involved are gold, platinum, palladium, mercury^{II}, selenium^{IV}, selenium^{VI}, tellurium^{IV}, tellurium^{VI}, arsenic, antimony, bismuth and copper.

STAGE I

Separation of gold, platinum, palladium, mercury, selenium^{IV} and tellurium^{IV}

Adjust the acidity of the filtrate from group 1 of the classical analysis procedure to about **3** N by adding concentrated hydrochloric acid. Insert the tube containing the solution into a rubber tube, to exclude light. Add an excess of silver powder, and shake the tube vigorously for 5 minutes. Spin the tube in a centrifuge.

Filtrate (F1). Keep for Stage II: contains selenate, tellurate and all remaining elements.

Residue. Contains silver, silver chloride, gold, platinum, palladium, mercury, silver selenide and silver telluride. Wash the residue thoroughly with **3** N hydrochloric acid and with water in a centrifuge tube, and discard the washings. Shake the residue twice with concentrated ammonia solution, to dissolve silver chloride, and discard the washings.

Residue. Contains silver, gold, platinum, palladium, mercury, silver selenide and silver telluride. Wash the residue until it is free from ammonia, then wash it with ethanol and acetone and dry it at 80° C in an oven. Add 8 to 10 times its bulk of anhydrous (freshly heated) sodium carbonate, mix it intimately by means of a platinum wire and heat it gently to sublime any **mercury**, which may be detected as globules when viewed through a microscope. If mercury is confirmed, expel it by heating the tube in a fume cupboard. (The sodium carbonate retains, as selenite or tellurite, any selenium dioxide or tellurium dioxide formed by air oxidation of silver selenide or telluride during the heating.

Residue. Contains silver, gold, platinum, palladium, silver selenide and silver telluride. Extract the residue with two 1-ml portions of water, and combine the extracts.

Extracts. Combine, and carefully acidify with hydrochloric acid, noting any precipitation of tellurous acid. Pass hydrogen sulphide through the solution, and wash any precipitated selenium or tellurium. Reserve the precipitate for incorporation in later selenium and tellurium precipitates.

Residue. Contains silver, gold, platinum, palladium, silver selenide and silver telluride. Digest the residue in a water-bath twice with 2 N nitric acid, and combine the extracts. (Nitric acid of this strength appears to exert no solvent action on the platinum; if the strength of the acid is appreciably increased, some platinum may be dissolved.)

Extract. Contains dissolved silver, most of the palladium and the remaining selenium and tellurium. Dilute the extract with an equal volume of water and add to the cold solution a small excess of a 1 per cent. solution of dimethylglyoxime in ethanol. Separate the precipitate.

Filtrate. Dilute the filtrate with an equal volume of water. Add a small excess of dilute hydrochloric acid and filter off, and discard, the precipitated

Precipitate. A yellow crystalline precipitate

Residue. Contains gold, platinum and some palladium. Digest the residue with aqua regia. Dilute the mixture and filter off the silver chloride. Add concentrated hydrochloric acid to the filtrate and evaporate it. Adjust the hydrochloric acid concentration to N, and add an excess of dimethylglyoxime solution. Separate the precipitate.

Filtrate. Heat the filtrate with aqua regia to destroy the excess of dimethylglyoxime. Add one drop of 10 per cent. potassium chloride

Precipitate. A yellow crystalline precipitate

TABLE IV, STAGE I—*continued*

		silver chloride. Pass hydrogen sulphide into the filtrate, separate any precipitate and combine it with the selenium and tellurium sulphide precipitates obtained earlier. Dissolve the precipitate in the minimum volume of diluted nitric acid (1 + 1), dilute and filter off any precipitated sulphur. Add 3 drops of 5 N sulphuric acid to the filtrate, and evaporate it on a water-bath until all the nitric acid has been expelled. Add 1 ml of concentrated hydrochloric acid, and transfer the solution to a test-tube. Saturate the solution with sulphur dioxide, stopper the tube and set it aside while the precipitate coagulates. Separate the precipitate. (In the absence of added sulphuric acid, expulsion of nitric acid is incomplete in the presence of tellurium owing to the formation of the basic nitrate. When concentrated hydrochloric acid is added, the retained nitric acid forms aqua regia, which can completely inhibit the test for selenium if much tellurium is present.)		indicates the presence of **palladium.**	solution, and evaporate the solution to a low volume in a test-tube. Add acetone, shake the solution and separate the precipitate.	indicates the presence of **palladium.**
		Filtrate. Dilute the filtrate to three times its volume with water and heat it. A black precipitate indicates the presence of **tellurium** from **tellurite**.	*Precipitate.* A red precipitate that becomes dark grey when heated indicates the presence of **selenium** from **selenite**.		*Filtrate.* A yellow acetone solution indicates the presence of **gold.** Confirm by any standard test.	*Precipitate.* A yellow crystalline precipitate indicates the presence of **platinum.**

STAGE II

Separation of selenium[VI]

To the filtrate, F1, from Stage I of the analytical procedure, add sufficient concentrated hydrochloric acid to raise the acid concentration to 5·5 N, and add 0·2 ml of freshly prepared 100 per cent. w/v ammonium iodide solution. Note any changes of colour, or the formation of a precipitate (e.g., a precipitate of arsenic tri-iodide). Heat the solution under reflux in a water-bath for 1 hour. (The heating with ammonium iodide and hydrochloric acid also reduces arsenic and telluric acids to arsenous and tellurous acids, respectively, which can then be further reduced by silver in Stage III.) Separate the precipitate.

Filtrate (F2). Keep for Stage III: contains tellurate, arsenic, antimony, bismuth and copper.

Residue. Contains selenium from selenate, some of the copper and, possibly, some arsenic tri-iodide. Wash the residue with a few drops of **3** N hydrochloric acid, and add the washings to filtrate F2. Wash the residue again with **3** N hydrochloric acid, and then with water and discard the washings. Dissolve the residue in a few drops of hot, diluted nitric acid (1 + 1), dilute the solution and make it alkaline with ammonium hydroxide. Pass hydrogen sulphide through the solution and separate any black precipitate that forms.

Filtrate. An amber colour indicates the presence of **selenium** from **selenate**. Acidify the solution with concentrated hydrochloric acid and separate any precipitate. Wash the precipitate well with water, and dissolve it in hot, diluted nitric acid (1 + 1). Evaporate the solution to dryness on a water-bath, add concentrated hydrochloric acid and pass sulphur dioxide through the solution. A red precipitate indicates the presence of **selenium** from **selenate**.

Precipitate. This will be copper sulphide. Wash the precipitate and then dissolve it in two drops of hot, diluted nitric acid (1 + 1). Test for **copper** by any standard procedure.

STAGE III

Separation of tellurium[VI], arsenic, antimony, bismuth and copper

Shake the filtrate, F2, from Stage II of the analytical procedure with an excess of silver at room temperature for 5 minutes. Dilute the solution with twice its volume of water, shake it thoroughly, spin it in a centrifuge, separate the precipitate and filter the solution.

Filtrate (F3). Contains lead, cadmium, molybdenum, tin and all elements of later groups. If only the common elements are present at this stage in the procedure, this filtrate may be examined by using that part of the analytical scheme, given in Part II of this Paper, that follows the silver stage.

Residue. Contains tellurium, arsenic, antimony, bismuth, cuprous iodide, silver iodide and silver. Wash the residue with 2 N hydrochloric acid, and then with water until all the free acid has been removed, and discard the washings. Shake the residue with two 1-ml portions of concentrated sodium thiosulphate solution, and combine the extracts.

Extract. Contains cuprous and silver ions as complex thiosulphates. Make the extract alkaline with ammonium hydroxide, and pass hydrogen sulphide through the solution. Separate the black precipitate of silver sulphide and cuprous sulphide, wash it and dissolve it in diluted nitric acid (1 + 1). Dilute the solution, and add an excess of dilute hydrochloric acid. Separate the precipitate. (Hydrogen sulphide causes cuprous sulphide to be precipitated from alkaline thiosulphate solutions, but not from neutral thiosulphate solutions.)

Filtrate. Add an excess of ammonium hydroxide to the filtrate. A blue colour indicates the presence of **copper**. Confirm by any standard test.

Precipitate. Contains silver chloride and sulphur: discard it.

Residue. Contains tellurium, arsenic, antimony, bismuth, silver and some silver iodide. Wash the residue thoroughly with water, and then digest it with diluted nitric acid (1 + 1) until the reaction ceases. Dilute the mixture, spin it in a centrifuge and separate the nitric acid extract. Heat the residue, which consists of hydrated antimony pentoxide and silver iodide, with 3 drops of concentrated sulphuric acid until reaction ceases and iodine and sulphur have been expelled from the tube. Dilute the solution, and add it to the nitric acid extract. Make the combined extract strongly alkaline with ammonium hydroxide, and saturate it with hydrogen sulphide. Shake the mixture thoroughly, and separate the sulphide precipitate.

Filtrate. Contains arsenic, antimony and tellurium as thio-salts. Acidify the filtrate with concentrated hydrochloric acid and filter off the precipitated sulphides. Wash the precipitate with water, and discard the washings and the filtrate. Digest the precipitate with 6 N hydrochloric acid on a water-bath, and separate the sulphide precipitate.

Filtrate. Contains antimony. Dilute the filtrate to three times its volume with water, and pass hydrogen sulphide through it. An orange precipitate indicates the presence of **antimony**.

Residue. Contains tellurium and arsenic. Digest the residue with concentrated hydrochloric acid on a water-bath, to ensure complete removal of antimony. Separate the residue, wash it thoroughly with water and digest it with diluted nitric acid (1 + 1) on a water-bath. Dilute the mixture with water, filter off precipitated sulphur and evaporate the solution to dryness on a water-bath. Dissolve the residue in 3 N hydrochloric acid, and pass sulphur dioxide through the solution. Separate the precipitate.

Filtrate. Boil off sulphur dioxide, and pass hydrogen sulphide through the solution. A yellow precipitate indicates the presence of **arsenic**.

Precipitate. A black precipitate indicates the presence of **tellurium** from **tellurate.**

Residue. Contains bismuth trisulphide and silver sulphide. Digest the residue with the minimum volume of diluted nitric acid (1 + 1), dilute the solution and add an excess of dilute hydrochloric acid. Filter off the silver chloride, and add an excess of ammonium hydroxide to the filtrate. A white, flocculent precipitate indicates the presence of **bismuth**. Confirm by any standard test.

shaken with 0·8079 g of silver powder for 20 minutes. The colour of the solution was dark green, probably owing to the hydrolysis of vanadyl^{IV} ions. After the addition of a further 0·05 ml of the hydrochloric acid solution and after a further 5 minutes shaking, the colour rapidly changed to the pure blue of the vanadyl^{IV} ion. The solution was filtered through a dry filter-stick (porosity 4) and the filtrate had a pH value of 1·89. The washed residue showed an increase in weight of 0·0687 g, due to the precipitation of chloride ions, corresponding to a 97 per cent. reduction. The final pH value of the filtrate, calculated from the hydrogen-ion concentration of the acid added and the theoretical acid consumption for complete reduction, should have been 2·35. The final pH value of the filtrate, calculated for 97 per cent. reduction is 1·85. The theoretical maximum pH value for quantitative reduction in M chloride solution is 2·75 (see Table II).

REDUCTION OF SELENITE—

Portions of 0·05 M potassium selenite solutions in M potassium chloride had their pH values adjusted to: (*a*) 2·5, (*b*) 3·7, (*c*) 5·2 and (*d*) 8·2. The solutions were each shaken for several hours with 0·5 g of silver powder, and then set aside in the dark for a fortnight. The final pH values of the solutions were: (*a*) 4·24 (rapid darkening of the silver occurred), (*b*) 4·5 (definite, but slow darkening occurred), (*c*) 6·6 (darkening was doubtful) and (*d*) 8·16 (no darkening occurred).

REDUCTIONS IN THE PRESENCE OF ISOPROPANOL AND t-BUTANOL—

An acceptor of free chlorine that could be added to the reaction mixture without introducing any undesirable substance, was needed to permit the reduction of arsenate, selenate and tellurate to arsenite, selenite and tellurite, respectively, by heating with hydrochloric acid (reaction (*iv*)) to proceed to completion in a closed vessel (to avoid loss of arsenic and selenium). Isopropanol and t-butanol were chosen because they are water-soluble and reactive, and their effect on the reaction was investigated.

Isopropanol—In 3 N hydrochloric acid at 100° C, the reduction of arsenate and selenate to arsenite and selenite, respectively, was complete in 45 minutes; that of tellurate to tellurite was incomplete after 1 hour. Other elements that can be precipitated by silver were not reduced.

In 6 N hydrochloric acid at 100° C, both selenate and selenite were reduced to the free element; within 30 minutes the element was precipitated in a dark grey form that could be filtered easily. This reduction appears to be specific for selenium. Tellurate was completely reduced to the tellurite within 1 hour.

t-Butanol—In 3 N hydrochloric acid at 100° C, selenate and selenite were almost completely reduced to the free element in 30 minutes; after 1 hour, tellurate was reduced to the tellurite. Tellurite in the initial reaction mixture was partially reduced to the free element after 1 hour. Arsenate was completely reduced to arsenite in 30 minutes, and chloro-auric acid was reduced to the free element in 15 minutes. Palladium, platinum, mercury, antimony^{III} and bismuth^{III} were not reduced.

I thank Dr. E. Downing, Head of the Chemistry Department, for checking all thermodynamic considerations and calculations, for pH measurements and for continued interest and valuable discussions and suggestions throughout. I also thank the 1960–61 Final-year Degree students of Fourah Bay College for their collaboration in the early stages of this work.

REFERENCES

1. Walden, G. H., Hammett, L. P., and Edmonds, S. M., *J. Amer. Chem. Soc.*, 1934, **56**, 350.
2. Gooch, F. A., and Perkins, C. C., *Z. anorg. Chem.*, 1909, **63**, 318.
3. Perkins, C. C., *Ibid.*, 1910, **66**, 432.
4. ——, *Ibid.*, 1910, **67**, 361.
5. Mellor, J. W., "A Comprehensive Treatise on Inorganic and Theoretical Chemistry," Longmans, Green & Co. Ltd., London, New York and Toronto, 1923, Volume III, p. 597.
6. Henry, A. J., *Analyst*, 1964, **89**, 255.
7. "Selected Values of Chemical Thermodynamic Properties," Circular of the National Bureau of Standards, No. 500, Washington, D.C., 1952.
8. Wilson, C. L., and Wilson, D. W., *Editors*, "Comprehensive Analytical Chemistry," Elsevier Publishing Company, Amsterdam, London, New York and Princeton, N.J., Volume IB, chapter VII.
9. Latimer, W. M., "Oxidation Potentials," Second Edition, Prentice-Hall Inc., Englewood Cliffs, N.J., and London, 1952.

NOTE—Reference 6 is to Part II of this series.

First received *July 31st*, 1962
Amended *June 10th*, 1963

The Analytical Uses of Metallic Silver

Part II.* A Revised Scheme for the Semi-micro Qualitative Analysis of the Common Elements

By A. J. HENRY

(*Department of Chemistry, Fourah Bay College, Freetown, Sierra Leone*)

A general survey of the problems of the qualitative analysis of the common elements is given, and a revised scheme of analysis is proposed, in which the reducing action of silver powder in presence of iodide and hydrochloric acid plays a prominent part.

Some theoretical considerations that determine the reducing powers of metallic silver under various conditions are given in Part I[1] of this paper, in which a three-stage procedure based upon these reductions is described for the qualitative analysis of the classical group 2 elements that can be precipitated by silver, when the less common ones are also included. Lead, cadmium, molybdenum and tin are not deposited under the conditions used, but molybdenum and tin are reduced to lower oxidation states. Among the common cations of the later groups, the only other partial reduction is that of $iron^{III}$ to $iron^{II}$, but chromate and permanganate are also reduced, to the most stable oxidation state of the metal in each instance.

Even under the most favourable conditions, precipitation of lead in group 1 is incomplete; the amount remaining in solution in the cold is about 1 g per litre, which is comparable with the concentrations recommended in the present scheme of analysis. The inclusion of molybdenum among the common elements for purposes of qualitative analysis is desirable, but it is usually omitted because of the difficulty of effecting its complete precipitation as sulphide from acidic solution. Complete precipitation can, however, be effected by passing hydrogen sulphide into an ammoniacal solution (producing the amber thiomolybdate ion) and subsequently acidifying the solution, and it was expected that this procedure could be made the basis of a scheme of analysis for all of the common elements that are not precipitated by silver. When lead, cadmium, molybdenum and tin^{IV} are the only such elements present, excellent separations result.

In the absence, or after the removal, of phosphate it was hoped that all the common elements remaining after the reduction with silver could be separated in the first instance into those precipitable as sulphides or hydroxides from weakly ammoniacal solution in the presence of ammonium chloride (*viz.*, lead, cadmium, iron, aluminium, chromium, cobalt, nickel, zinc and manganese) and those remaining in solution either unchanged or as thio-salts (*viz.*, molybdenum, tin, calcium, strontium, barium, magnesium, sodium and potassium). The heating necessary to exclude air (to avoid loss of alkaline-earth elements as sulphates) and to ensure complete precipitation of aluminium and chromium as their hydroxides, proved to be incompatible with the retention of molybdenum and tin in solution as their thio-salts, and this approach was abandoned.

* For details of Part I of this series, see reference list, p. 260.

TABLE I

ANALYSIS OF THE GROUP 2 ELEMENTS (SILVER GROUP)

Elements involved are mercury, arsenic, antimony, bismuth and copper

Take the solution from group 1 of the classical analytical scheme, whose volume should be about 1 ml and whose acidity should be about 2 N with respect to hydrochloric acid, and add an equal volume of concentrated hydrochloric acid. Add 0·2 ml of a freshly prepared 100 per cent. w/v solution of ammonium iodide and note any change in colour, or the formation of a precipitate that, on mixing, may re-dissolve as a result of complex-ion formation, or may be permanent, *e.g.*, arsenic tri-iodide (orange-yellow). Add an excess of silver powder, stopper the tube with a closely fitting rubber bung and shake it vigorously for 5 minutes, preferably in a mechanical shaker. Note any change in the solution or in the residue. Dilute the solution to 2·5 times its volume with water to precipitate dissolved silver iodide, shake the solution and spin it in a centrifuge.

Filtrate (F1). Reserve this filtrate for analysis of subsequent groups.

Residue. Contains silver, silver iodide, mercury, arsenic, antimony, bismuth and cuprous iodide. Wash the residue twice with 2 N hydrochloric acid and then with water until the free acid has been completely removed, and discard the washings. Shake the residue with 1 ml of concentrated sodium thiosulphate solution at room temperature for 5 minutes. Spin the mixture in a centrifuge, and decant the supernatant liquid. Repeat this extraction, and combine the extracts.

Extract. Contains silver and copper[I] as complex thiosulphates. Make the solution alkaline with ammonium hydroxide, and pass hydrogen sulphide through it. A black precipitate of silver sulphide and copper[I] sulphide is formed. Separate the precipitate, wash it well and dissolve it in diluted nitric acid (1 + 1). Dilute the solution, add an excess of dilute hydrochloric acid and filter off the mixed silver chloride - sulphur residue. Add an excess of ammonium hydroxide to the solution. A blue colour indicates the presence of **copper.** Confirm by any standard test. (Hydrogen sulphide causes cuprous sulphide to be precipitated from alkaline thiosulphate solutions, but not from neutral thiosulphate solutions.)

Residue. Contains silver, mercury, arsenic, antimony, bismuth and some silver iodide. Wash the residue thoroughly with water, and then with ethanol and acetone. Dry it at 80° C for 5 minutes in an oven. Add 3 to 4 times its bulk of anhydrous (freshly heated) sodium carbonate through a clean dry funnel, mix well, hold the tube horizontally and heat it carefully in a flame. A grey sublimate, coalescing to form globules that may be detected under a microscope, indicates the presence of **mercury**. If mercury is confirmed, expel it by heating the tube in a fume cupboard. (The sodium carbonate retains as arsenite and antimonite any arsenic trioxide or antimony trioxide formed by air oxidation during the heating and which might be lost while expelling the mercury. These oxides interfere with the detection of mercury.)

Residue. Contains silver, arsenic, antimony, bismuth, sodium carbonate and some silver iodide. Add carefully an excess of diluted nitric acid (1 + 1) to the residue, and heat the mixture in a water-bath until the reaction ceases. Dilute the mixture, spin it in a centrifge and separate, and retain, the nitric acid solution. Heat the residue, which consists of silver iodide and hydrated antimony pentoxide, with 3 drops of concentrated sulphuric acid until the reaction ceases and iodine and sulphur have been expelled from the tube. Dilute the solution and add it to the nitric acid extract. Make the combined extracts strongly alkaline with ammonium hydroxide, and then saturate them with hydrogen sulphide. Shake the mixture thoroughly, and separate the sulphide precipitate.

Filtrate. Contains antimony and arsenic as thio-salts. Acidify the filtrate by the dropwise addition of concentrated hydrochloric acid, and separate off the precipitate. Wash it and digest it in a water-bath with 6 N hydrochloric acid. Separate the precipitate.

Filtrate. Contains antimony. Dilute the filtrate

Residue. Contains arsenic trisulphide. Heat the residue with concentrated

Residue. Contains silver sulphide and bismuth trisulphide. Dissolve the residue in the minimum volume of diluted nitric acid (1 + 1), dilute the solution and add an excess of dilute hydrochloric acid. Filter off the precipitated silver chloride.

TABLE I—*continued*

		to 3 times its volume and pass hydrogen sulphide through it. An orange precipitate indicates the presence of **antimony**. Confirm by any standard test.	hydrochloric acid to free it from antimony. Wash it well, and then dissolve it in the minimum volume of diluted nitric acid (1 + 1). Dilute the solution, and filter off the precipitated sulphur. Add an excess of ammonium hydroxide to the filtrate, and pass hydrogen sulphide through it. Acidify the solution and heat it on a water-bath. A bright yellow precipitate, forming rather slowly, indicates the presence of **arsenic.** Confirm by any standard test.	Add an excess of ammonium hydroxide to the filtrate. A white flocculent precipitate indicates the presence of **bismuth**. Confirm by any standard test.

TABLE II

ANALYSIS OF THE GROUP 3 ELEMENTS

Elements involved are lead, calcium, strontium and barium

The filtrate, F1, from the analysis of the group 2 elements (see Table I) contains substantial amounts of ammonium iodide, a little silver iodide and all the remaining elements, and is approximately 2 N with respect to hydrochloric acid. Shake the filtrate at room temperature with a small excess of freshly prepared silver oxide, separate, and retain, the solution and wash the residue of silver iodide with 2 N hydrochloric acid. Add the washings to the main solution. Add a small excess of bromine water to the solution, to ensure complete oxidation of tin, iron and molybdenum, and filter off the precipitated silver bromide on a Whatman No. 5 filter-paper. Evaporate the filtrate almost to dryness, and dissolve the residue in 1 ml of 0·5 N hydrochloric acid. This solution contains ammonium chloride and all elements not removed in groups 1 and 2. Add to it an equal volume of ethanol and then 4 drops of 5 N sulphuric acid. Stir the mixture well, stopper the tube and set it aside at room temperature for as long as possible, preferably overnight. Separate the precipitate.

Filtrate (F2). Reserve this filtrate for analysis of subsequent groups.	*Residue.* Contains lead, calcium, strontium and barium. Wash the sulphate residue with 2 ml of ethanol that has been acidified with one drop of 5 N sulphuric acid, and then with 2 ml of acetone. Dry the residue in an oven, and then mix it intimately with 6 to 8 times its bulk of anhydrous fusion mixture and fuse it until the melt is clear. Extract the cold melt twice with hot water, and discard the extracts. Dissolve the residue in hot, dilute acetic acid and test the solution for **lead, calcium, strontium** and **barium** by standard procedures.[3]

The great advantage of this method is that the alkaline-earth elements are separated before there is any risk of loss as sulphates or carbonates. This permits better control of the conditions for precipitation of the cadmium - molybdenum group.

TABLE III

ANALYSIS OF THE GROUP 4 ELEMENTS

Elements involved are lead, cadmium, molybdenum and tin

The filtrate, F2, contains all the elements not removed in groups 1, 2 and 3 (see Tables I and II). Evaporate the filtrate on a water-bath until all free acid, except sulphuric acid, has been expelled. Dilute the solution with 1 ml of water, spin it in a centrifuge and discard any residue. Add dilute ammonium hydroxide until the solution is just alkaline (or until there is a slight, but permanent, precipitate), and then add 0·25 ml of 2 N hydrochloric acid. Heat the solution in a water-bath, and then pass hydrogen sulphide through the liquid until it is cold. Add an equal volume of water, heat the solution and again pass hydrogen sulphide through the solution until it is cold. Separate the residue, and filter the solution. (If there is 1 mg each of lead, cadmium, molybdenum and tin present in 1 ml of solution, then the normality of the free acid increases by a factor corresponding to 0·12 N when the metals are precipitated as sulphides. The dilution of the solution compensates for this increase.)

Filtrate. (F3). Reserve this filtrate for analysis of subsequent groups.

Residue. Contains lead, cadmium, molybdenum and tin. Wash the residue twice with a 1 per cent. solution of ammonium chloride that has been slightly acidified with hydrochloric acid, and discard the washings. Extract the residue, in the cold, twice with 0·5 ml of 0·75 N potassium hydroxide, and combine the extracts. Note any amber colour due to the thiomolybdate (MoS_4^{2-}) ion.

Extract. Contains molybdenum and tin^{IV} as thio-salts. Make the extract just acidic by adding hydrochloric acid. Separate the precipitate, wash it once with water and digest it in a water-bath with 3 N hydrochloric acid. Separate the residue and filter the solution.

Residue. Contains cadmium sulphide, molybdenum trisulphide and some lead sulphide. Wash the residue in water, and then dissolve it in the minimum volume of diluted nitric acid (1 + 1). Dilute the solution and filter off the precipitated sulphur. Make the solution alkaline with ammonium hydroxide, and separate the precipitate.

Filtrate. Contains **tin.** Confirm by any standard test.

Residue. If molybdenum is present, the residue will be dark brown. Wash the residue with water, add 6 to 8 drops of concentrated nitric acid and boil vigorously. Transfer the nitric acid solution to a porcelain crucible and evaporate it to dryness on a water-bath. A deep blue colour when the residue is hot indicates the presence of **molybdenum.** Add water to the residue (the blue colour disappears), and then add potassium ferrocyanide solution. A red-brown colour or precipitate confirms the presence of molybdenum. (Oxidation of the molybdenum sulphide in boiling nitric acid produces sufficient sulphuric acid to give the molybdenum-blue test when the solution is evaporated to dryness. If a dark-brown residue has been obtained, a confirmatory test must be performed, especially if the molybdenum-blue test is negative or inconclusive.)

Filtrate. Render the filtrate just acidic with hydrochloric acid, and pass hydrogen sulphide through it. Separate the precipitate, and digest it with N hydrochloric acid in a water-bath. Separate the solution from any dark-brown residue of molybdenum trisulphide, and make it just alkaline with ammonium hydroxide. Make the solution just acidic with hydrochloric acid, and pass hydrogen sulphide through it. A bright yellow precipitate, readily soluble in concentrated hydrochloric acid, indicates the presence of **cadmium**.

Residue. This will be lead hydoxide. Test for **lead** by any standard test.

TABLE IV

ANALYSIS OF THE GROUP 5 ELEMENTS

Elements involved are iron, aluminium and chromium

The filtrate, **F3**, from the analysis of the group 4 elements (see Table III) contains iron, aluminium, chromium, cobalt, nickel, zinc, manganese, magnesium, sodium and potassium. Evaporate the filtrate to a low bulk, to remove hydrogen sulphide, and then oxidise $iron^{II}$ to $iron^{III}$ with nitric acid. Ammonium salts are already present. Almost neutralise the solution with concentrated ammonium hydroxide, and heat it for 15 minutes in a water-bath to de-aerate it. Make the solution slightly alkaline by adding dilute ammonium hydroxide to the hot solution. Boil the solution until the smell of ammonia is barely detectable, and then separate the precipitate from the hot solution. (This procedure ensures that the precipitation of aluminium and chromium is complete, and that the risk of oxidation and precipitation of manganese is kept to a minimum.)

Filtrate. (F4). Reserve this filtrate for the analysis of subsequent groups.	*Residue.* Examine the residue for **iron, aluminium** and **chromium** by standard procedures.

TABLE V

ANALYSIS OF THE GROUP 6 ELEMENTS

Elements involved are cobalt, nickel, zinc and manganese

The filtrate, **F4**, from the analysis of the group 5 elements (see Table IV) contains cobalt, nickel, zinc, manganese, magnesium, sodium and potassium, and is alkaline. Add sufficient glacial acetic acid to raise the acid concentration to N, and pass hydrogen sulphide through the solution. Zinc is precipitated as the white sulphide, although the colour of the precipitate may be pale yellow (owing to the presence of cadmium sulphide not precipitated in group 4), or grey (owing to the presence of a little molybdenum trisulphide). Separate the precipitate and filter the solution.

Filtrate. Contains cobalt, nickel, manganese, magnesium, sodium and potassium. Add an excess of ammonium hydroxide, and pass hydrogen sulphide through the solution. Heat the solution to boiling, to render cobalt sulphide and nickel sulphide insoluble, and separate the precipitate.

Residue. Wash the residue with a 1 per cent. solution of ammonium chloride, and discard the washings. Digest the residue with N hydrochloric acid, and filter off any black residue of molybdenum trisulphide. Adjust the acid concentration of the solution to 0·2 N. Pass hydrogen sulphide through the solution, and separate the precipitate.

Filtrate (F5). Reserve this filtrate for the analysis of group 7.	*Residue.* Contains cobalt, nickel and manganese as sulphides. Wash the residue once with a 1 per cent. solution of ammonium chloride, and discard the washings. Add 1 ml of water to the residue and make the mixture weakly acidic with acetic acid. Stir the mixture well, and separate the precipitate.		*Filtrate.* Add sodium acetate solution to the filtrate. A white precipitate indicates the presence of **zinc.** Confirm by any standard test.	*Residue.* A yellow precipitate indicates the presence of **cadmium**. Confirm by any standard test.
	Filtrate. Examine the filtrate for **manganese** by any standard test.	*Residue.* Examine the residue for **cobalt** and **nickel** by standard procedures.		

TABLE VI

ANALYSIS OF THE GROUP 7 ELEMENTS

Elements involved are magnesium, sodium and potassium

The filtrate, F5, from the analysis of the group 6 elements (see Table V) contains magnesium, sodium and potassium. Destroy the ammonium salts, and dissolve the residue in 0·5 ml of 2 N hydrochloric acid. Add a trace of concentrated nitric acid and 0·05 ml of a 5 per cent. solution of chloroplatinic acid, and evaporate the solution to low bulk. Cool the solution, dilute it to 0·5 ml with water, and separate the precipitate. The solution should be yellow.

Filtrate. Evaporate the solution almost to dryness on a water-bath. Dissolve the residue in 0·5 ml of water, and stir the mixture with silver powder until the solution is colourless. Filter off the residue, and test the solution for **sodium** and **magnesium** by standard procedures.	*Residue.* A yellow crystalline residue indicates the presence of **potassium**. Confirm by any standard test.

The main difficulties encountered in existing schemes of qualitative analysis arise from the factors listed below—

1. The lack of an entirely satisfactory method for separating the group 2 elements into sub-groups 2A and 2B.[2]

2. The difficulty of securing complete precipitation of cadmium and molybdenum as sulphides in group 2.

3. The high acidity required for precipitating arsenic as sulphide when present as arsenate, without resorting to a separate reduction procedure.

4. The loss of alkaline-earth metals through (*a*) the oxidation by air or nitric acid of hydrogen sulphide to sulphuric acid and (*b*) the absorption of carbon dioxide by ammonia solution.

5. The tendency for manganese to be precipitated with the iron group owing to its oxidation by air to hydrated manganese dioxide.

The reducing action of silver simplifies the analysis of the classical group 2 elements, and its incorporation into a complete scheme for the qualitative analysis of the common elements has led to a review of existing schemes of analysis with the object of devising one in which the difficulties listed above are largely eliminated. It is considered that the scheme outlined in Tables I to VI achieves a substantial measure of success in this object; it presents no difficulties in execution, and has been shown to yield reliable results. The relevant equations for the reductions by silver are given in Part I of this Paper.

EXPERIMENTAL

The present scheme for the semi-micro qualitative analysis of the common elements assumes the prior removal of silver and mercury[1] (and perhaps some lead) as chlorides in group 1, and of any "insoluble material." The concentration of hydrochloric acid should be about 2 N, which has only a small solvent action on silver chloride, retains in cold solution approximately 1 mg of lead per ml of solution and prevents hydrolysis of antimony, bismuth and tin compounds. The weight of any element present in the analysis sample should preferably not exceed 1 mg. The proposed analytical procedures are set out in the Tables.

I thank Dr. E. Downing, Head of the Chemistry Department, for his continued interest and for valuable discussions and suggestions during the course of this work. I also thank the Committee of the Midlands Association for Qualitative Analysis for permission to incorporate the essentials of their methods[3] into Table II of the proposed analytical scheme.

REFERENCES

1. Henry, A. J., *Analyst*, 1964, **89**, 242.
2. James. C. F., and Woodward. P., *Analyst*, 1955, **80**, 825.
3. **The Midlands Association for Qualitative Analysis, "Semi-micro Qualitative Inorganic Analysis,"** Stanford and Mann Ltd., Birmingham, various Editions.

NOTE—Reference 1 is to Part I of this Series.

First received *July* 31*st*, 1962
Amended *June* 10*th*, 1963

The Photometric Determination of Excess of Zinc in Zinc Oxide

By V. J. NORMAN

(*Australian Defence Scientific Service, Defence Standards Laboratories, Department of Supply, Adelaide*)

A rapid photometric method is described, based on the reduction of dichromate, for determining the excess of zinc in zinc oxide.

When the sample is dissolved in acid in the presence of standardised potassium dichromate solution, the dichromate is reduced by excess of metal in the zinc oxide, and the residual sexavalent chromium is estimated photometrically with diphenylcarbazide as a colouring reagent. The necessary compensation for oxidisable matter present in the sample and reagents is achieved by means of a reference, in which the dichromate solution is added only after dissolution of the sample in acid has been completed.

The excess of zinc in a number of samples of analytical-reagent grade zinc oxide ranged from 1·0 to 7·3 p.p.m. by weight.

The method gives good reproducibility and is sensitive to 0·1 p.p.m. of excess of zinc by weight.

THE determination of excess of zinc in zinc oxide by the reduction of permanganate or dichromate has been undertaken previously by Ehret and Greenstone[1] and by Von Wartenburg, whose work has been reported by Mollwo and Stockmann.[2] Ehret and Greenstone found that samples of red zinc oxide, when examined for reducing power by means of standardised dichromate or permanganate, gave a "free zinc" content of approximately 0·02 per cent., but supplied no further details of the analytical procedure used nor any indication whether the effect of oxidisable matter present in the samples and reagents, other than nitrite, had been taken into consideration. Von Wartenburg, using the reduction of potassium permanganate, found an excess of zinc of 0·5 to 1 per cent. by weight in samples of yellow zinc oxide.

The excess of zinc in the yellow and red varieties of zinc oxide reported by these workers is several orders higher than that of analytical-reagent grades of zinc oxide, which are being used for photo-conductivity and semi-conductivity studies, and Allsopp[3] has shown that for samples of zinc oxide in which departure from stoicheiometry is small, the method of Von Wartenburg, based on the reduction of permanganate, is both erratic and lacking in sensitivity. Although both Von Wartenburg and Allsopp carried out dissolution of the zinc oxide and back-titration of the permanganate under an inert atmosphere, neither compensated for oxidisable matter, significant amounts of which may have been present in the sample itself. By using a hydrogen-evolution technique having an estimated sensitivity of 0·1 p.p.m., Allsopp has estimated the excess of zinc in samples of zinc oxide used for semi-conductivity studies and has reported results within the range 0·2 to 18 p.p.m.

In view of the importance of excess of zinc on the semi-conductivity and sintering properties of zinc oxide, the development of a rapid and accurate method of adequate sensitivity and requiring no complicated vacuum equipment was considered worthwhile.

EXPERIMENTAL

In methods that are based upon the reducing effect of excess of zinc on an acidified solution of standardised oxidant, the amount of oxidisable matter in the sample, other than free metal, is of prime importance, and, unless accurately compensated for, must give rise to high results, especially where the amount of excess of zinc is small.

Since even analytical-reagent grades of zinc oxide, in the as-received condition, contain significant amounts of oxidisable matter, it was apparent from the outset that the development of a rapid, accurate and reliable method for determining the excess of zinc in zinc oxide, based upon the reduction of either permanganate or dichromate, would depend on the development of a technique, whereby the effect of this oxidisable matter in the sample and in the reagents would be automatically compensated for by means of a reference solution. Preliminary experiments were, therefore, directed towards this end. The general system given below was finally adopted and used throughout the investigation.

SAMPLE SOLUTION—

A fixed volume of standardised oxidising reagent (potassium permanganate or potassium dichromate) was added to a weighed sample of zinc oxide, and then a fixed volume of acid was added to effect dissolution.

REFERENCE SOLUTION—

A fixed volume of acid was added to a sample of zinc oxide of the same weight as that used for the sample solution. After dissolution was complete, a fixed volume of standardised oxidising reagent was added.

PROCEDURE—

The optical densities of both the sample and the reference solutions were measured.

Reduction of the standardised oxidising reagent in the sample solution was thus effected by both oxidisable matter in the zinc oxide and reagents, and by excess of metal in the sample; the oxidising reagent in the reference solution was reduced only by the oxidisable matter in the zinc oxide and reagents. The difference between the optical densities of the two solutions is, therefore, proportional to the net reduction due to an excess of metal in the sample. It was confirmed experimentally that the oxidising reagent in the reference solution was not reduced by dissolved hydrogen. The optical densities of permanganate and dichromate reference solutions were not affected when additions of zinc dust were made to samples of zinc oxide.

POTASSIUM PERMANGANATE METHOD—

Preliminary work on the potassium permanganate method of Von Wartenburg confirmed the finding of Allsopp that results obtained by this method, when determined either volumetrically or photometrically, were erratic and lacked the sensitivity necessary for determining an excess of zinc of the order of 1 p.p.m. Moreover, the results were invariably higher than those obtained by a method based on the reduction of potassium dichromate. It was noted that the average results reported by Ehret and Greenstone[1] for free zinc in red zinc oxide determined by the permanganate method exceeded those obtained by the dichromate method by approximately 40 p.p.m.

It was felt that little could be done to increase the sensitivity of the permanganate method to the extent required. Nevertheless, it was considered desirable to investigate further the cause of the high and erratic results obtained by this method.

In preliminary tests it was found that appreciable reduction of stabilised 0·0004 N potassium permanganate, indicated by a decrease in optical density, was effected by digestion of the solution with zinc oxide. This was attributed to the presence of oxidisable matter in the sample. Further experiments, however, showed that oxidisable matter in the zinc oxide was not solely responsible for this reduction, since the effect was still apparent, though less pronounced, when permanganate solution was digested with samples of zinc oxide that had been ignited for several hours at 650° C. Digestion with calcined magnesium oxide, with calcined calcium sulphate and with calcined aluminium oxide also resulted in a significant reduction of the permanganate solution.

This decrease in optical density of permanganate solutions after contact with insoluble powders containing no organic or other oxidisable matter varied from 5 to 25 per cent., and appeared to be influenced by the particle size of the powder; the finer the powder the greater the reduction effected.

It could only be assumed that solid particles in an extremely fine state of sub-division initiated a break-down of extremely dilute permanganate solutions to manganese dioxide, which itself promoted further catalytic auto-reduction of permanganate. This effect would explain the erratic nature of the results obtained by the permanganate method and account for the fact that these results were higher than those obtained by methods involving reduction of dichromate.

Even by using the system of sample and reference solutions detailed above, compensation for this effect was not feasible, since solid zinc oxide was in contact with permanganate only in the sample solution, which, consequently, was the only solution to be disproportionately reduced.

DICHROMATE METHOD—

Attention was next directed towards a method based on the reduction of potassium dichromate, which, being an extremely stable primary standard, was not subject to the catalytic breakdown experienced with permanganate.

In order to obtain the necessary sensitivity, the amount of sexavalent chromium remaining in the solution after reduction by the sample was estimated photometrically with diphenylcarbazide as a colouring reagent.

EFFECT OF ACID CONCENTRATION AND STABILITY OF COLOUR—

It was found that the lower the sulphuric acid concentration at the time of development the greater the optical density of the diphenylcarbazide - chromate complex; the amount of sulphuric acid was, therefore, initially kept to the minimum for the convenient dissolution of the sample. Under these conditions, however, the colour complex developed was unstable; significant fading of the colour occurred with time. This was overcome by adding orthophosphoric acid, which had the effect of stabilising the colour for at least 3 hours after development.

EFFECT OF TEMPERATURE—

Under the conditions of development the optical density of the diphenylcarbazide - chromate complex varied slightly with temperature. However, the difference between the optical densities of the reference and sample solutions was constant, at least within the temperature range of 12° to 27° C, so that, provided both these solutions were maintained at the same temperature, it was unnecessary to measure their optical densities at the temperature of calibration.

EFFECT OF MOISTURE—

It was found that the results obtained for the excess of zinc in any particular sample of zinc oxide decreased markedly when the sample was permitted to come in contact with moisture for any length of time before the determination was carried out. This was first noticed when samples of zinc oxide were allowed to stand in undried beakers before dissolution. For this reason, in all subsequent work, samples of zinc oxide were introduced into an already prepared mixture of acid and water, or acid and dichromate immediately after weighing.

EFFECT OF ZINC SULPHATE—

Zinc sulphate in solution slightly reduced the intensity of the colour complex; the optical density of a solution containing 5·0 g of zinc oxide dissolved in the specified acid mixture was 0·050 on the drum scale less than one containing no zinc sulphate. However, since the repressing effect of zinc sulphate on the optical density of the complex was the same in both sample and reference solutions, no significant error resulted from failure to correct for the slight change of slope of the calibration graph for different sample weights. The 5·0-g calibration curve could be used for lower weights of zinc oxide sample provided that the amount of acid mixture used was correspondingly reduced.

METHOD

APPARATUS—

Spekker absorptiometer—Hilger & Watts Ltd.

REAGENTS—

Acid mixture—Mix 165 ml of sulphuric acid, sp.gr. 1·84, and 65 ml of orthophosphoric acid, sp.gr. 1·75, and dilute the mixture with water to 1 litre.

Standard potassium dichromate, 0·0001 N.

Diphenylcarbazide—A 0·25 per cent. solution in acetone.

PROCEDURE—

(*i*) *Reference solution*—Place a beaker containing 20 ml of acid mixture, diluted with 25 ml of water, in a cooling-bath, weigh 5·0 g of zinc oxide and add it immediately to the acid solution. Effect dissolution of the zinc oxide as rapidly as possible, and allow the solution

to remain in the cooling-bath until it reaches room temperature. Add 25 ml of standard dichromate solution, and transfer the mixture to a 100-ml calibrated flask. Dilute the solution to approximately 90 ml with water, add 5 ml of diphenylcarbazide solution, and dilute to the mark immediately.

(*ii*) *Sample solution*—Place a beaker containing a mixture of 25 ml of standard dichromate solution and 20 ml of acid mixture in a cooling-bath, weigh 5·0 g of zinc oxide, and add it immediately to the acid solution. Effect dissolution of the zinc oxide as rapidly as possible, and allow the solution to remain in the cooling-bath until it reaches room temperature. Transfer the mixture to a 100-ml calibrated flask, dilute it to approximately 90 ml with water, add 5 ml of diphenylcarbazide solution and dilute to the mark immediately.

(*iii*) *Blank solution*—Dissolve 5·0 g of zinc oxide in 20 ml of acid mixture. Transfer the solution to a 100-ml calibrated flask, dilute it to approximately 90 ml with water, add 5 ml of diphenylcarbazide solution and dilute to the mark.

Place the flasks in a water-bath for 10 minutes to allow complete development of the colour and to ensure that both reference and sample solutions are at the same temperature.

Measure the optical density of the solutions in 4-cm cells, at 520 mμ, by using green filters and a water-setting of 1·0.

From the calibration graph read the volume of 0·001 N dichromate equivalent to the difference in optical density between the blank and reference solutions (*a*) and between blank and sample solutions (*b*).

Then (*a*–*b*) ml is equivalent to the volume of 0·0001 N dichromate reduced by the excess of zinc in 5·0 g of the sample of zinc oxide.

Then—

$$\text{Excess of zinc, p.p.m.} = (a-b) \times 32{\cdot}7 \times 10^{-3} \times 10^{-4} \times 0{\cdot}2 \times 10^{6}$$
$$= 0{\cdot}654\ (a-b).$$

NOTES—

1. It is essential that all glassware used throughout be thoroughly cleansed to ensure freedom from grease or oxidisable matter.

2. The optical density of solution (*iii*) is small, and it is unnecessary to run a blank solution with every determination. It is advisable, however, to check periodically the optical density of the blank solution, particularly when a new solution of colouring reagent has been prepared.

CALIBRATION—

Prepare the calibration graph by making appropriate additions of standard potassium dichromate solution to samples of zinc oxide that are known to be free from oxidisable matter: this makes the calibration graph applicable to zinc oxide specimens containing indeterminate amounts of oxidisable matter. Any analytical-reagent grade of zinc oxide that has been heated for several hours at 650° C, with occasional raking, is suitable for use in calibration.

CALIBRATION PROCEDURE—

By using 5·0-g samples of zinc oxide that have previously been ignited at 650° C, and proceeding exactly as outlined under "Reference Solution" in the method (except for the volume of standard dichromate solution added), prepare five "ignited references" containing 5, 10, 15, 20 and 25 ml of dichromate, respectively. Prepare a blank solution as outlined in the method. Draw the calibration graph by plotting the difference between the optical densities of the blank solution and "ignited references" *versus* volume of 0·0001 N dichromate used.

RESULTS AND DISCUSSION

The results of analysis of several samples by zinc oxide by the proposed method are shown in Table I.

The results in Table I show that the proposed method is sensitive to at least 0·1 p.p.m. of excess of zinc by weight, and that the agreement between duplicate analyses, with most samples, is good.

The difference between the optical density of the "ignited reference" (which is constant for all samples) and the optical density of the reference solutions prepared from samples of zinc oxide in the as-received condition represents a measure of oxidisable matter present in the particular sample in terms of 0·0001 N dichromate.

The fact that the degree of correspondence between replicate analyses varies from sample to sample when the hydrogen-evolution method is used, has been attributed by Allsopp and Roberts[4] to more uniform distribution of excess of zinc in some powders than in others. The variation between the optical densities of replicate reference solutions shown in Table I indicates, moreover, that the samples are not homogeneous with respect to oxidisable matter. These variations could not be attributed to uncontrollable processes in the dissolution of the samples, since excellent agreement was obtained between replicate determinations on "ignited references." That the method outlined gives better duplication for analytical-reagent grades of zinc oxide than that reported by Allsopp and Roberts is caused, at least in part, by the larger sample weights used, with consequent levelling of random compositional variations.

TABLE I

RESULTS BY THE PROPOSED METHOD

Optical density of blank solution .. = 0·980
Optical density of "ignited reference" = 0·177
Net optical density .. .. .. = 0·803 (equivalent to 25 ml of 0·0001 N dichromate)

Sample	Reference, net optical density	Equivalent dichromate, *a* ml	Colour, net optical density	Equivalent dichromate, *b* ml	*a* − *b*, ml	Excess of zinc, p.p.m.
A 1	0·669	20·8	0·346	10·8	10·0	6·5
	0·669	20·8	0·346	10·8	10·0	6·5
A 2	0·748	23·2	0·480	14·9	8·3	5·4
	0·752	23·3	0·488	15·2	8·1	5·3
	0·757	23·4	0·496	15·5	7·9	5·2
	0·742	23·0	0·485	15·1	7·9	5·2
A 3	0·652	20·3	0·339	10·5	9·8	6·4
	0·628	19·5	0·332	10·3	9·2	6·0
	0·665	20·6	0·350	10·9	9·7	6·3
A 4	0·488	15·2	0·152	4·7	10·5	6·9
	0·514	15·7	0·148	4·6	11·1	7·3
B 1	0·792	24·5	0·721	22·4	2·1	1·4
	0·792	24·5	0·722	22·4	2·1	1·4
	0·778	24·1	0·711	22·1	2·0	1·3
	0·775	24·0	0·725	22·5	1·5	1·0
	0·768	23·7	0·685	21·3	2·4	1·6
B 2	0·765	23·8	0·690	21·4	2·4	1·6
	0·779	24·1	0·696	21·6	2·5	1·6
	0·778	24·1	0·688	21·4	2·7	1·8
C 1	0·760	23·6	0·595	18·5	5·1	3·3
	0·762	23·6	0·619	19·2	4·4	2·9
	0·779	20·1	0·622	19·3	4·8	3·1

Allsopp and Roberts have drawn attention to the fact that, in their analyses, the amount of hydrogen evolved may be taken as a measure of non-stoicheiometry only if the presence of free zinc or other free metal capable of displacing hydrogen can be excluded, and it is pointed out that this proviso also applies to the present method. Whilst the possibility of free metals being present in indirect-process powders cannot be excluded, their presence in analytical-reagent grades of zinc oxide is considered unlikely.

This paper is published by permission of the Chief Scientist, Australian Defence Scientific Service, Department of Supply, Melbourne, Victoria, Australia.

REFERENCES

1. Ehret, W. F., and Greenstone, A., *J. Amer. Chem. Soc.*, 1943, **65**, 872.
2. Mollwo, E., and Stockman, F., *Ann. Phys.*, 1948, (**6**) **3**, 226.
3. Allsopp, H. J., *Analyst*, 1957, **82**, 474.
4. Allsopp, H. J., and Roberts, J. P., *Trans. Faraday Soc.*, 1959, **55**, 1386.

Received *July 29th*, 1963

The Determination of Calcium and Magnesium in Biological Material by Radioactivation Analysis

By H. J. M. BOWEN, P. A. CAWSE and Mrs. M. DAGLISH
(*Wantage Research Laboratory (A.E.R.E.), Wantage, Berkshire*)

Neutron-activation analysis has been used for determining calcium and magnesium in biological material. The calcium was determined either as 8·8-minute calcium-49 or as its daughter, 58-minute scandium-49, and the magnesium as 9·5-minute magnesium-27. The limits of sensitivity were about 3×10^{-6} g for calcium and 2×10^{-7} g for magnesium. Manganese and phosphorus interfered, but there was no mutual interference between calcium and magnesium.

Calcium and magnesium are important constituents of biological tissues and there are many analytical methods available for determining their presence in tissues. In most vegetable tissues and in mammalian bone, calcium is the more abundant element, but in seeds, animal soft-tissues and especially in red blood-cells the reverse is true. Since the two elements have rather similar chemical properties, their analysis can be difficult, especially when determining the less abundant of the two.

When calcium is the more abundant element, it is probably best determined by precipitation as the oxalate. This procedure eliminates interference from magnesium and phosphate and is satisfactory for amounts of calcium greater than 100 μg. For smaller amounts, down to 1 μg, colorimetric,[1,2] spectrometric,[3] or flame-photometric techniques[4] are available, but all these are subject to interference by phosphate and often by metallic ions as well. This may account for the fact that only two analyses of calcium in red blood-cells appear in the literature and that these are in poor agreement one with another.[5]

Volumetric procedures for magnesium are less satisfactory, in that the element is generally determined by difference. Calcium may be determined by titration in alkaline solution with murexide indicator, and calcium *plus* magnesium with Eriochrome black T as indicator.[6,7] This is obviously inapplicable when magnesium is much less abundant than calcium, as in bone. Magnesium may also be determined by colorimetry[2] (though the Titan yellow method is now regarded with some disfavour[8]), spectrometry[9] or flame photometry.[10]

Neither polarography nor X-ray fluorescence techniques have found much application to these elements in view of their single valency state and low atomic weights, respectively. Neutron activation to calcium-45, with a half-life of 165 days, has been used by Brune, Frykberg, Samsahl and Webster[11] and by Samsahl[12] for determining calcium in blood and teeth, respectively. In this work we have explored the possibility of using activation of short-lived nuclides for determining both calcium and magnesium. Magnesium yields the 9·5-minute magnesium-27, which emits both β- and γ-rays. Bethard, Schmidt and Olehy[13] have used this nuclide in the analysis of magnesium in blood. Calcium gives rise to 8·8-minute calcium-49, which decays to 58-minute scandium-49; both these nuclides are potentially suitable for analytical purposes, though only calcium-49 is a γ-ray emitter (see Table I).

Table I

Comparison of nuclides potentially useful for analysis

Nuclide	Half-life, minutes	Maximum β-ray energy, MeV	γ-Ray energies, MeV	mC per g*
Magnesium-27	9·5	1·75	0·83, 1·02	0·95
Calcium-49	8·8	2·00	3·10, 4·05, 4·68	0·35
Scandium-49	58	2·05	none	0·1

* Measured after exposure to a neutron flux of 10^{12} per sq. cm per second.

Experimental

Irradiation—

Samples were sealed in small polythene tubes and irradiated for 10 minutes in a flux of about $1{\cdot}5 \times 10^{12}$ neutrons per sq. cm per second in the Harwell reactor, BEPO. Standards were prepared by dissolving Specpure magnesium oxide and calcium carbonate in nitric acid distilled in an apparatus of silica, and diluting the solution so that it contained about 1 mg per ml. Weigh portions of the standard solutions were activated in sealed polythene tubes along with the samples.

Reagents—

All reagents were of analytical-reagent grade.
Perchloric acid, 70 *per cent. w/v.*
Nitric acid, 24 N.
Hydrochloric acid, 6 N.
Ammonia solution, 15 N.
Sodium chlorate solution, 10 *per cent. w/v, aqueous.*
Ammonium acetate solution, 50 *per cent. w/v, aqueous.*
Ammonium oxalate solution, 4 *per cent. w/v, aqueous.*
Ammonium dihydrogen orthophosphate solution, 7·2 *per cent. w/v, aqueous.*
Ferric chloride solution, 5·8 *per cent. w/v, aqueous.*
Disodium hydrogen orthophosphate solution, 10 *per cent. w/v, aqueous.*
Bromine.
Hydrogen sulphide—Generated in the laboratory.
Calcium carrier solution—Dissolve 4·995 g of calcium carbonate in 6 N hydrochloric acid and dilute the solution to 100 ml with water.

1 ml of solution ≡ 20 mg of calcium.

Magnesium carrier solution—Dissolve 17·638 g of magnesium acetate tetrahydrate in water and dilute the solution to 100 ml.

1 ml of solution ≡ 20 mg of magnesium.

Manganese carrier solution—Dissolve 7·205 g of manganous chloride tetrahydrate in water and dilute the solution 100 ml.

1 ml of solution ≡ 20 mg of manganese.

Chemical separation—

It became apparent that the radioactive contaminants most difficult to remove were phosphorus-32 and manganese-56; sodium-24 and potassium-42 were also produced in large amounts but were relatively easy to separate. Two rapid separation schemes were evolved for removing manganese or phosphorus.

Method A (For eliminating manganese-56.)—Immediately they returned from the reactor, samples or standards were placed in 150-ml beakers containing 1 ml of calcium carrier solution, 0·5 ml each of magnesium and manganese carrier solutions, 0·5 ml of perchloric acid and 1 ml of nitric acid. After heating to destroy organic matter, 0·5 ml of sodium chlorate was added, together with more nitric acid if necessary, to precipitate manganese dioxide. The slurry was washed into a 50-ml centrifuge tube with water, and spun. The supernatant liquid was poured into a fresh centrifuge tube containing 4 ml of ammonium acetate and 0·5 ml of manganese carrier solution. Sufficient ammonia solution was then added to render this alkaline (about 4 ml) and hydrogen sulphide was bubbled through the solution, which was placed in a boiling-water bath, for 30 seconds. The solution was spun in a centrifuge and the manganese sulphide rejected. This step was repeated, with further portions of manganese carrier solution, up to three times, depending on the counting technique used.

The final supernatant liquid was poured into a fresh centrifuge tube containing 4 ml of ammonium oxalate and spun. The resulting calcium oxalate precipitate was dissolved in 6 N hydrochloric acid and re-precipitated with ammonia solution. It was washed twice with water and once with acetone, and finally transferred to a weighed aluminium counting tray as a slurry with acetone. When dry, its radiation was counted with the minimum of delay and it was subsequently weighed. The mean chemical yield for calcium was 85 per cent.

The supernatant liquid from the calcium oxalate precipitation stage was poured into a fresh centrifuge tube containing 4 ml of disodium hydrogen orthophosphate. The tube was spun in a centrifuge, ammonium magnesium orthophosphate precipitate was collected, then dissolved in 6 N hydrochloric acid, re-precipitated with ammonia solution and washed and dried, as described for calcium oxalate. This washing removes sodium-24 and potassium-42. The mean chemical yield for magnesium was 75 per cent.

Method B (For eliminating phosphorus-32.)—Active samples or standards were placed in 150-ml beakers containing 1 ml each of calcium carrier solution and ferric chloride, and 0·5 ml each of magnesium carrier solution and ammonium dihydrogen orthophosphate, 0·5 ml of perchloric acid and 1 ml of nitric acid. After heating to destroy organic matter, the contents of the beaker were poured into a 50-ml centrifuge tube containing 4 ml of ammonium acetate. Sufficient ammonia solution was then added to bring the solution to pH 5, and the solution was spun in a centrifuge and the ferric orthophosphate precipitate rejected. The supernatant liquid was poured into a fresh tube containing 1 ml of ferric chloride, 0·5 ml of ammonium dihydrogen orthophosphate and 0·5 ml of nitric acid. It was again brought to pH 5 with ammonia solution; spun in a centrifuge, and the ferric orthophosphate was rejected. The supernatant liquid was poured into a fresh tube containing 1 ml of manganese carrier solution, and manganese dioxide was precipitated by carefully adding about 0·5 ml of ammonia solution and 1 ml of bromine. The solution was spun in a centrifuge and the residue was rejected. The supernatant liquid was treated successively with oxalate and sodium orthophosphate to precipitate calcium and magnesium, as before.

Table II lists the time taken by these operations. In practice, it was possible to begin counting 20 to 25 minutes after the removal of four samples from the reactor, with two analysts performing the chemical manipulations.

TABLE II

TIME REQUIRED FOR UNIT PROCESSES IN THE CHEMICAL SEPARATION

Operation	Time required, minutes
Opening samples	0·5
Ashing samples	3
Precipitating MnO_2, $FePO_4$ or MnS	2
Precipitating calcium oxalate and $MgNH_4PO_4$	2
Washing final precipitates	6
Transferring precipitates to counting-trays	0·5
Drying final precipitates	2

DETERMINATION OF RADIOACTIVITY—

The β-particle emissions of the precipitate were counted with a 2B2 end-window Geiger - Müller counter of approximately 40 per cent. efficiency. Radiochemical purity was checked by comparing the decay curves of samples and standards over several half-lives. For magnesium, which has a simple decay curve, long-lived activities with half-lives of 2·6 hours and 14 days were sometimes found. These were shown to be caused by manganese-56 and phosphorus-32, respectively. The manganese contaminant was not found when three manganese sulphide precipitations were made, and the phosphorus-32 seldom exceeded 1 per cent. of the initial count-rate from magnesium -27.

Calcium-49 has a complex decay curve, and it was not found practicable to count the β-particles emitted from this nuclide after the chemical separation. Instead, the β-particles emitted from its daughter product, scandium-49, were counted after a delay of 2·5 hours to allow all the parent calcium-49 to decay.

The γ-ray emissions of the calcium and magnesium precipitates were measured with a scintillation counter coupled to a single-channel analyser focused on the 3·10 and 0·83-MeV peaks, respectively. There was no difficulty in counting the calcium-49 apart from its short half-life, but in counting magnesium it was impossible to discriminate against the 0·84-MeV peak of manganese-56, so that it was again necessary to remove all manganese in the chemical procedure. The γ-rays from magnesium samples were counted through an aluminium shield of 850 mg per sq. cm to reduce any contribution to the count of β-particles from phosphorus-32.

Discussion of the method

Table III shows recovery results for known amounts of calcium and magnesium placed on a 1-sq. cm filter-paper. All counts have been corrected for chemical yield, filter-paper blank value and radioactive decay.

Table III

Recovery results for calcium and magnesium

Element	μg Taken	β Emission, counts per minute	γ Emission, counts per minute
Calcium	5000	40,640	5550
Calcium	500	3880	524·5
Calcium	50	406·6	54·6
Calcium	5	42·6	—
Magnesium	1000	—	5851
Magnesium	100	14,738	578·5
Magnesium	10	1493	58·0
Magnesium	1	151·5	—

All counts have been re-calculated to an arbitrary time 25 minutes after removal of the samples from the reactor, apart from the β-counts for calcium, which have been re-calculated to 150 minutes after removal from the reactor. It can be seen that the counts increase almost linearly with the weight of the element added, and that the precision is of the order of ±5 per cent. for calcium and ±2 per cent. for magnesium. The limit of sensitivity is about 3 μg for calcium and 0·2 μg for magnesium with β-particle counting, or 3 and 8 μg, respectively, with γ-ray counting. These limits could be lowered (*a*) by increasing the neutron flux, (*b*) by speeding up the chemical separation and (*c*) in the instance of calcium, determined by β-counting scandium-49, by increasing the activation time to 1 hour, and separating scandium rather than calcium for counting.

Testing the radiochemical procedures—

Method A was tested with portions of radiochemically pure bromine-82, calcium-49, copper-64, magnesium-27, manganese-56, phosphorus-32, scandium-46, sodium-24, strontium-87 and zinc-69. Each step was tested for the percentage de-contamination that could be achieved under the hurried conditions necessary for separating short-lived nuclides. The results are summarised in Table IV.

Table IV

Percentages of ten elements in radiochemical fractions

Element	Br	Ca	Cu	Mg	Mn	P	Sc	Na	Sr	Zn
Loss on ashing ..	95·41	0	0	0	0	0	0	0	0	0
MnO_2 ppt. ..	0·056	2·36	3·45	2·55	98·84	21·33	0·74	1·2	0·85	0·33
MnS ppt. ..	0·19	0·84	84·44	0·77	1·08	10·78	99·00	0·88	0·85	92·69
Second MnS ppt.	0·18	0·83	10·22	0·76	0·074	7·32	0·26	0·87	0·84	6·47
CaC_2O_4 ppt. ..	<0·0001	94·43	0·0013	0·17	0·0013	6·41	0·00082	0·70	93·30	0·054
$MgNH_4PO_4$ ppt...	0·00043	1·20	0·25	91·91	0·0011	5·10	0·00044	0·35	1·72	0·027
Final spt. ..	4·16	0·34	1·64	3·84	0·0031	49·06	0·00134	97·74	2·44	0·43

Table IV shows that bromine, copper, manganese, sodium and zinc should not interfere if method A is used. Manganese is potentially the most serious radiochemical contaminant because of its high cross-section. Phosphorus contaminates both the final calcium and magnesium precipitates, but it is activated little during the ten minutes in the reactor and only requires a minor correction when β-particles are counted. The correction can be eliminated by using method B, since ferric orthophosphate carries down at least 98·5 per cent. of the phosphorus. Scandium is effectively scavenged during the manganese sulphide precipitation; scandium-49 begins to grow again as soon as this stage is completed. Strontium follows calcium closely in the chemistry of the elements, but it can be neglected in practice, since it is much less abundant in nature. The predominant radionuclide produced in strontium activated for a short period is 2·8-hour strontium-87, which emits a γ-ray of 0·39 MeV, and so would not be readily counted in our procedure. It could readily be separated by the insolubility of strontium nitrate in 80 per cent. nitric acid.[14]

In the course of the radiochemical work, several separation techniques were tried and abandoned. These included (*i*) extraction of magnesium sulphate from sulphated ash with methanol, (*ii*) extraction of magnesium perchlorate from perchlorated ash with n-butanol, (*iii*) solvent extraction of magnesium at pH 10 with oxine in chloroform *plus* 1-butoxy-ethanol.[15] In addition, it was found impracticable to precipitate magnesium with oxine when working at high speed.

ERRORS AND ACCURACY—

Errors in the activation process are unlikely to be serious. The flux gradient in the "Rabbit" used for activation was less than 2 per cent. Calcium-49 is not produced by any nuclear reaction on adjacent elements, but scandium-49 could be produced by the reaction—

$$^{49}\mathrm{Ti}\ (\mathrm{n,p})\ ^{49}\mathrm{Sc}.$$

By activating 0·1 g of Specpure titanium dioxide, it was shown that 1 μg of titanium (the approximate amount in 1 g of biological material) would be equivalent to $5{\cdot}3 \times 10^{-11}$ g of calcium in the production of scandium-49. Titanium therefore gives negligible interference.

Magnesium-27 is produced by the reactions—

$$(i)\ ^{27}\mathrm{Al}\ (\mathrm{n,p})\ ^{27}\mathrm{Mg}$$
$$(ii)\ ^{30}\mathrm{Si}\ (\mathrm{n},\alpha)\ ^{27}\mathrm{Mg}.$$

By activating 0·01 g of Specpure aluminium oxide and 0·1 g of Specpure silica, it was shown that 1 μg of aluminium and 1 μg of silicon were equivalent to $1{\cdot}52 \times 10^{-9}$ g and $2{\cdot}8 \times 10^{-11}$ g of magnesium in the production of magnesium-27. These interferences are therefore negligible in biological material, but could be serious in the analysis of geological material or aluminium alloys.

Errors in the chemistry have already been discussed, and it appears that manganese-56, phosphorus-32 and strontium-87 are the most likely radiochemical contaminants. They are reduced to tolerable levels by the chemical procedure suggested above, which would have to be modified for samples containing large amounts of these three elements.

Counting errors are probably the most serious of all errors in determining short-lived nuclides, since it is not possible to increase the counting period indefinitely to obtain better statistical accuracy. It is also important to record the exact times at which counts are carried out, since an error of 10 seconds corresponds to 1·8 and 1·3 per cent. corrections for radioactive decay for calcium and magnesium, respectively.

RESULTS

Calcium and magnesium were determined in Whatman No. 541 ashless filter-paper, tomato seeds, human red blood-cells and shells of marine mollusc (*Solen marginatus*). The results, which are the means of four replicate analyses, are shown in Table V.

TABLE V

AMOUNT OF CALCIUM AND MAGNESIUM IN VARIOUS SAMPLES

Sample	Weight, g	Calcium, μg per g	Magnesium, μg per g
Whatman No. 541 filter-paper	2	4·1 ± 3	6·4 ± 1·0
Tomato seeds	0·5	520 ± 25	3520 ± 250
Human red blood-cells (1 individual) ..	1	<5·5	55 ± 4
Shell of *Solen marginatus*	0·1	385,000 ± 10,000	2240 ± 150

The samples were chosen to span the range of ratios of calcium to magnesium found in biological material. Method A was satisfactory for all these samples except red blood-cells, which contain relatively little manganese and large amounts of phosphorus; method B is more suitable for these samples. It was not possible to obtain more than an upper limit for the calcium in red blood-cells. Good agreement was obtained between results obtained by the different counting procedures, but it was often impossible to count the short-lived calcium-49 with sufficient accuracy for analytical work.

Discussion

The technique of activation analysis should prove useful for determining traces of magnesium in biological materials, since it is more sensitive than many other techniques and not subject to interference by calcium. Interference by manganese and phosphorus is potentially serious but can be eliminated by careful chemistry, and the only disadvantage of the method is the necessity for working fast.

The determination of calcium by activation, either to calcium-49 or its daughter scandium-49, does not seem to have any major advantages over established techniques. Activation is not a sensitive method for calcium, and the short half-life of calcium-49 makes separation from other activities difficult.

The results for tomato seeds and human red blood-cells agree with those found by other methods.[5,16] For example, a recent review[5] gives mean values of 61 μg of magnesium per ml for red blood-cells (235 samples) and 5·4 μg of calcium per ml for red blood-cells (11 samples), but the figure for calcium requires confirmation.

References

1. Natelson, S., and Penniall, R., *Anal. Chem.*, 1955, **27**, 434.
2. Hunter, G., *Analyst*, 1959, **84**, 24.
3. David, D. J., *Ibid.*, 1959, **84**, 536.
4. Toribara, T. Y., Dewey, P. A., and Warner, H., *Anal. Chem.*, 1957, **29**, 540.
5. Bowen, H. J. M., *U.K. Atomic Energy Authority Report* AERE–R 4196, Harwell, 1963.
6. Zak, B., Hindman, W. M., and Baginski, E. S., *Anal. Chem.*, 1956, **28**, 1661.
7. Carlson, R. J., and Johnson, C. M., *J. Agric. Food Chem.*, 1961, **9**, 460.
8. Kolthoff, I. M., Elving, P. J., and Sandell, E. B., *Editors*, "Treatise on Analytical Chemistry," Part II, Interscience Publishers Inc., New York and London, 1961, Volume III, p. 43.
9. Herring, W. B., Leavell, B. S., Paixao, L. M., and Yoe, J. H., *Amer. J. Clin. Nutrition*, 1960, **8**, 846.
10. Vallee, B. L., and Bartholomay, A. F., *Anal. Chem.*, 1956, **28**, 1753.
11. Brune, D., Frykberg, B., Samsahl, K., and Webster, P. O., *Aktiebolaget Atomenergi, Report* AE–60, Stockholm, 1961.
12. Samsahl, K., *Aktiebolaget Atomenergi Report* AE–61, Stockholm, 1961.
13. Bethard, W. F., Schmitt, R. A., and Olehy, D. A., *U.S. Atomic Energy Commission General Atomic Report* GA–2803, San Diego, California, 1962.
14. Sunderman, D. N., and Townley, C. W., *U.S. Atomic Energy Commission Report* NAS–NS–3010, U.S. Department of Commerce, Office of Technical Services, Washington, D.C., 1960.
15. Morrison, G. H., and Freiser, H., "Solvent Extraction in Analytical Chemistry," John Wiley & Sons Inc., New York and Chapman & Hall Ltd., London, 1957, p. 216.
16. Bowen, H. J. M., and Cawse, P. A., *Radiation Botany*, 1962, **1**, 215.

Received *July 22nd*, 1963

An Apparatus for Determining Small Amounts of Alchohol in Sour Milk and Urine

By R. E. S. ANDREWS and P. J. COOPER

(*Department of Scientific and Industrial Research, Laboratory of the Government Chemist, Cornwall House, Stamford Street, London, S.E.*1)

A compact apparatus is described for steam-distilling small amounts of alcohol from samples of sour milk and urine. The alcohol is subsequently determined by oxidation with acidic potassium dichromate solution. Recoveries, at different concentrations, from water, sour milk and urine are given. Possible interference from acetone is prevented by using an alkaline mercuric oxide trap.

In order to determine the solids-not-fat content of samples of sour milk, it is necessary to correct for losses due to the souring. Of these, the most important correction is that due to the formation of alcohol from lactose. Bell[1] used a time factor to make this correction, and although Stokes[2] confirmed the necessity for such a correction, he showed that one based on time was not valid. The necessity for the correction for alcohol was demonstrated by Thorpe,[3] who also established the fact that it was the largest of the corrections necessary for the solids-not-fat content of sour milk.

The determination of alcohol in sour-milk samples, received under the Food and Drugs Act, was formerly carried out by double distillation of the sample followed by a densitometric estimation. The amount of sample required for this densitometric method, usually 55 g, was so large in relation to the total amount of sample available that duplicate determinations of the alcohol content were not always possible. Occasionally the method gave a nil or even an apparently negative result on samples of sour milk when some alcohol would have been expected. There is reason to suppose that the natural acidity of the sour-milk sample does not entirely prevent the distillation of volatile organic bases. In the method described, the presence of the sulphuric acid certainly does so.

For the reasons given above, the method of Kozelka and Hine[4] for determining alcohol in biological fluids was introduced incorporating a modified form of apparatus which is more compact and convenient to use and which requires only 5 g of the sample. The modified apparatus also has the advantage of reduced dead-space, which should help to avoid the small systematic negative error found by Kent-Jones and Taylor for urine.[5] Kent-Jones and Taylor also recommended the Kozelka and Hine method in a slightly modified form as an official method for determining alcohol in blood and urine.[5] The official French method for determining alcohol in blood[6,7] is based on a similar principle.

Experimental

In the proposed method, alcohol is steam-distilled from the sample, and the distilled alcohol is subsequently oxidised in a closed flask with a known volume of standardised potassium dichromate solution. The excess of potassium dichromate solution, after reaction, is determined iodimetrically and hence the alcohol content of the sample calculated. Ketonic and acidic bodies are prevented from interfering by using an alkaline mercuric oxide trap.

Method

Reagents—

All reagents should be of analytical-reagent grade whenever possible.

Potassium dichromate, 0·1 N.

Sodium thiosulphate, 0·1 N.

Potassium iodide—Free from iodate.

Sodium starch glycollate solution, 1 *per cent.*—B.D.H. iodine indicator may be used instead.

Sulphuric acid, sp.gr. 1·84, *and approximately* 5 N.
Mercuric chloride, saturated aqueous solution.
Sodium hydroxide pellets.
Sodium tungstate solution, 10 *per cent. w/v, aqueous.*

PROCEDURE—

Set up the apparatus as shown in Fig. 1. Place 100 ml of water into the steam-generator, C, and close the side arm. Place 5 ml of sample, 1 ml of sodium tungstate solution and 2 ml of 5 N sulphuric acid into the tube, A, and fit the tube into flask C. Fit the tube, B, into tube A and then place 2 g of sodium hydroxide pellets and 3 ml of saturated mercuric chloride

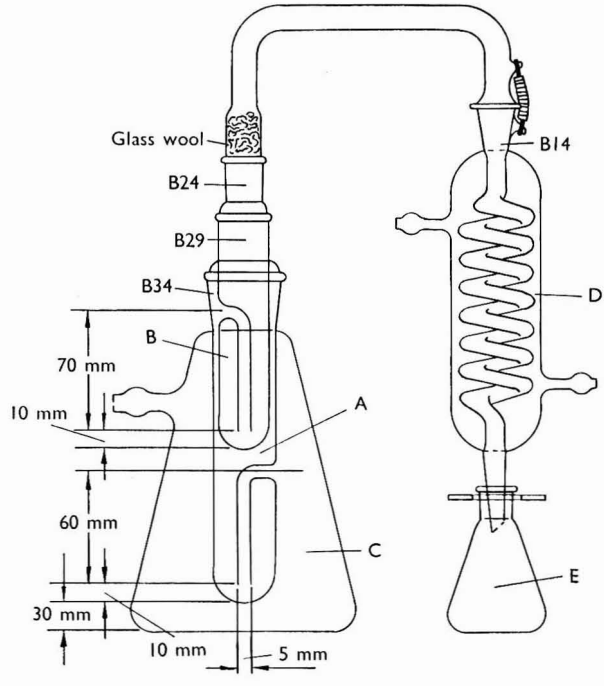


Fig. 1. Diagram of the distillation apparatus. All ground-glass joints are coated with polytetrafluoroethylene

solution into tube B. Fit the flask, E, to the condenser, D, screening it from the source of heat. Boil the water in flask C, and collect 25 ml of distillate. Open the side arm on flask C to release the steam and to prevent the contents of tubes A and B from sucking back into the steam-generator on cooling down. Add 10 ml of 0·1 N potassium dichromate to the distillate, and then 5 ml of sulphuric acid, sp.gr. 1·84. Replace the stopper quickly before mixing the contents, and use strong springs to keep the stopper in place during the reaction. Heat the flask and contents on a steam-bath for 20 minutes. Cool the flask and dilute the contents to 150 ml with water. Add 2 g of potassium iodide, and stopper and shake the flask. Titrate the mixture with 0·1 N sodium thiosulphate, and add 1 ml of starch solution or 0·2 g of B.D.H. iodine indicator just before the end-point is reached. Determine the

value of the blank solution on 25 ml of water by using exactly the same procedure, but omitting the sample. The difference between the titres is proportional to the amount of alcohol present, calculated as ethanol—

1 ml of 0·1 N potassium dichromate ≡ 0·00115 g of ethanol.

Results

Recovery from water—

Results for recovery of alcohol from water with the modified apparatus are shown in Table I.

Table I

Recovery of alcohol from water

Alcohol added, mg	Alcohol recovered, mg	Recovery, per cent.	Alcohol added, mg	Alcohol recovered, mg	Recovery, per cent.
1·57	1·68	107	4·11	3·98	97
2·35	2·33	99	4·13	3·97	96
2·35	2·38	101	4·27	4·23	99
3·05	2·95	97	5·05	4·85	96
3·05	3·00	98	5·05	5·02	99·5
3·14	3·08	98·5	5·05	5·23	103
3·14	3·22	102·5	6·03	5·93	98·5
3·39	3·35	99	6·03	5·94	98·5
3·70	3·86	104	8·02	7·38	92
3·70	3·89	105	8·02	7·87	98
3·92	3·92	100			

Mean recovery, 99·5 per cent.

The variation in the blank value expressed in terms of alcohol is approximately 0·1 mg or about 5 per cent. possible variation in recovery at the 2-mg level. In view of this, the recoveries shown above are considered to be satisfactory.

Determination of alcohol in sour milk—

Recovery experiments with alcohol added to sour milk have been carried out on similar lines to those for water. The range of alcohol concentrations (as determined by densitometric methods) encountered in the past has usually been 0·00 to 0·10 per cent. w/v. This range corresponds to an alcohol content of 0 to 5 mg in a 5-ml sample. The results obtained are shown in Table II.

Table II

Recovery of alcohol from sour milk

Alcohol added, mg	Alcohol recovered, mg	Recovery, per cent.	Alcohol added, mg	Alcohol recovered, mg	Recovery, per cent.
1·22	1·42	116	2·44	2·30	94
1·22	1·22	100	4·88	3·81	78
1·22	1·35	111	4·88	4·71	97
1·22	1·09	90	4·88	4·27	88
2·44	2·51	103	4·88	4·39	90
2·44	1·88	77	5·05	4·98	99

Mean recovery, 95 per cent.

The variation in recovery is substantially greater than that for plain water. Incidental determinations of the alcohol content of the sample of sour milk used for the recovery experiments in Table II gave the following results: 0·033, 0·036, 0·041, 0·038, 0·040, 0·040, 0·038, 0·038, 0·045 and 0·042 per cent. w/v over a 14-day period; a mean alcohol content of 0·039 per cent. After a further 2 weeks storage at 0° C, the alcohol content was unchanged.

Determination of alcohol in urine—

The effectiveness of the apparatus in preventing interference from acetone was checked by recovery experiments in which acetone was added to solutions of alcohol in water and urine. The results in Table III show the recoveries from 5-ml aqueous samples containing 6 mg of added acetone.

TABLE III

RECOVERY OF ALCOHOL FROM WATER IN PRESENCE OF ACETONE

Alcohol added, mg	Alcohol recovered, mg	Recovery, per cent.	Alcohol added, mg	Alcohol recovered, mg	Recovery, per cent.
1·90	1·83	96	5·79	5·53	96
1·86	1·88	101	7·93	7·57	95
1·86	1·99	107	7·93	7·61	96
1·86	1·93	104	7·93	7·90	100
3·84	3·78	98	7·93	7·29	92
3·84	3·77	98	7·87	7·59	96
3·88	3·91	101	7·87	7·75	98
3·88	3·58	92	8·02	7·64	95
5·79	5·74	99	8·02	7·79	97

Mean recovery, 98 per cent.

Results for recoveries of alcohol from urine solutions are shown in Table IV, and from urine solutions containing 5·7 mg of acetone per sample in Table V.

TABLE IV

RECOVERY OF ALCOHOL FROM URINE

Alcohol added, mg	Alcohol recovered, mg	Recovery, per cent.	Alcohol added, mg	Alcohol recovered, mg	Recovery, per cent.
0·97	1·00	103	7·44	7·18	96
0·97	0·92	95	7·44	7·03	95
3·93	3·72	95	7·38	7·32	99
3·93	3·76	96	7·38	7·21	98

Mean recovery, 97 per cent.

TABLE V

RECOVERY OF ALCOHOL FROM URINE IN PRESENCE OF ACETONE

Alcohol added, mg	Alcohol recovered, mg	Recovery, per cent.	Alcohol added, mg	Alcohol recovered, mg	Recovery, per cent.
0·97	1·06	109	3·93	3·95	100
0·97	0·98	101	7·44	7·32	98
0·97	0·91	94	7·44	7·01	94
3·93	3·91	100	7·38	7·35	100
3·93	3·90	99			

Mean recovery, 99 per cent.

The results given in Table II confirm that the modified Kozelka and Hine apparatus is suitable for determining alcohol in sour milk. Its advantages over the densitometric method are greater ease of determination, greater analytical accuracy at the low concentrations of alcohol encountered, and the use of much smaller sample volumes.

The apparatus is also suitable for determining alcohol in urine. Acetone does not interfere in the determination.

We thank the Government Chemist for permission to publish this paper.

REFERENCES

1. Bell, J., *Analyst*, 1883, **8**, 141.
2. Stokes, A. W., *Ibid.*, 1887, **12**, 226.
3. Thorpe, T. E., *Ibid.*, 1905, **30**, 197.
4. Kozelka, F. L., and Hine, C. H., *Ind. Eng. Chem., Anal. Ed.*, 1941, **13**, 905.
5. Kent-Jones, D. W., and Taylor, G., *Analyst*, 1954, **79**, 121.
6. Truffert, L., *Ann. Falsif.*, 1957, **50**, 59.
7. Kohn-Abrest, E., *Ann. Falsif. Exp. Chim.*, 1963, **56**, 85.

Received *October 22nd*, 1963

The Colorimetric Determination of Total Nitrogen in Feeding Stuffs

By P. C. WILLIAMS

(*Department of Agriculture, Agricultural Research Institute, Wagga Wagga, New South Wales, Australia*)

A colorimetric procedure is described for determining total nitrogen in feeding stuffs. The material to be analysed is subjected to a Kjeldahl-type oxidation, and the diluted digest subjected to direct Nesslerisation with an improved Nessler's reagent. Recovery of nitrogen is excellent, and compares favourably with that obtained by the standard Kjeldahl procedure. The proposed procedure is considered to be technically and economically superior to standard Kjeldahl procedure, in that it obviates the use of large volumes of highly caustic solutions.

THE demand for a rapid, accurate and economical method for the analytical determination of total nitrogen has for some 80 years been fulfilled by the Kjeldahl process, now modified and streamlined into the familiar pattern whereby hundreds of thousands of analyses are completed annually throughout the world. The Nessler test, however, is nearly 30 years older than the Kjeldahl test, having been first described by Julius Nessler in 1856.[1] Nessler himself refers to an earlier use of an alkaline solution containing mercuric iodide for detecting alkaloids, and there is evidence that the reaction had already attracted the attention of other workers for at least 30 years before Nessler published his findings. The possibility of using a modified Kjeldahl procedure has been examined in this laboratory, where the water supply is subject to periodical failure during the summer months. The literature since 1856 contains references to many facets of the reaction and its practical application. One of the most important factors has been found to be the constitution of the reagent.

Experimental

Recipes for the preparation of "Nessler's Reagent" are legion. Besides the original solution used by Nessler himself, different formulations have been prepared by Folin and Denis[2] (modified by Koch and McMeekin[3]), Wicks,[4] Bear,[5] Treadwell,[6] Vanselow,[7] Jackson[8] and more recently by Middleton,[9] and Bucki, Alther and Soliva[10] refer to a further eight methods of preparation. Several of these workers have used different starting materials for the preparation of the reagent, as well as different concentrations of these materials. Others advocated the use of separate solutions of potassium mercuric iodide and alkali. If the mercury content of the various compounds is referred to on a basis of mercuric iodide, however, practically all of the more successful reagents contain potassium iodide and mercuric iodide in the ratio of 0·73 to 0·75, whereas the theoretical molar ratio of potassium iodide to mercuric iodide in the hypothetical double iodide, K_2HgI_4, is 0·73. A series of reagents was prepared having a constant ratio of potassium iodide to mercuric iodide of 0·75, the concentrations of the two salts being increased from 0·188 per cent. of potassium iodide and 0·25 per cent. of mercuric iodide to 7·5 and 10 per cent. of these salts, respectively. In order to determine the optimum constitution of the reagent, tests were carried out on several feeding stuffs and two standard solutions containing a fixed amount of ammonium sulphate. The nitrogen content of these samples was determined by using reagents containing different concentrations of mercuric iodide and measuring the transmission of the solutions with an absorptiometer. The results are shown in Fig. 1. A constant ratio of potassium iodide to mercuric iodide of 0·75 was maintained throughout the tests. In each experiment the volume of test solution was 50 ml, and 4 ml of reagent were added. A reagent solution containing 5·5 per cent. of mercuric iodide was found to be satisfactory for the Nesslerisation of solutions containing up to 400 μg of nitrogen per 50 ml of final solution. Reagents containing lower concentrations of mercuric iodide showed a tendency to develop turbidity, particularly at higher concentrations of nitrogen. The reagent, in the proposed constitution, will detect 0·002 p.p.m. of nitrogen and therefore may be used for detecting minute amounts of nitrogen.

The intensity of the colour produced by the reagent varies directly with the nitrogen content up to a concentration of 80 μg per 50 ml. Above this concentration the yellow colour progressively assumes a reddish-orange tinge. The accompanying shift in wavelength results in considerable discrepancies in the subsequent computation of the results. Consequently it was found that, for concentrations up to 80 μg of nitrogen per 50 ml, the optimum wavelength for optical-density measurements was 430 mμ, and, for concentrations between 80 and 400 μg, the optimum wavelength was 520 mμ. Colour development is complete after 20 minutes and is perfectly stable for several hours thereafter.

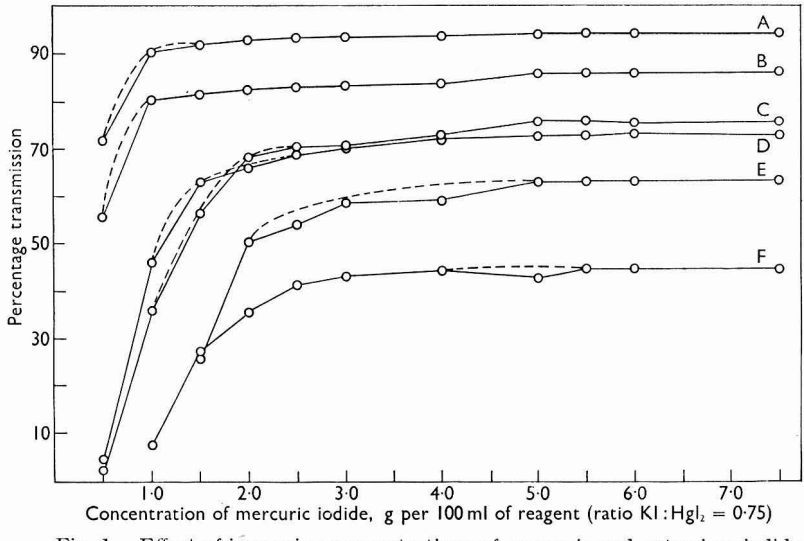


Fig. 1. Effect of increasing concentrations of mercuric and potassium iodides in the reagent on colour development, with: curve A, wheat straw; curve B, low-protein wheat flour; curve C, high-protein wheat grain; curve D, ammonium sulphate solution containing 200μg of nitrogen per 50 ml; curve E, dried green lucerne; curve F, ammonium sulphate solution containing 400 μg of nitrogen per 50 ml

Method

Reagents—

Use analytical-reagent grade material whenever possible.

Nessler's reagent—Prepare the reagent by dissolving 55 g of red mercuric iodide and 41·25 g of potassium iodide in about 250 ml of water. Dissolve 144 g of sodium hydroxide pellets in 500 ml of water, cool the solution and stir it into the iodide solution. Adjust the volume to 1 litre and store the reagent in a dark bottle. The solution is ready for immediate use.

Digestion mixture—Intimately mix potassium sulphate and powdered selenium in the ratio 100 to 1.

Sulphuric acid, sp.gr. 1·84.

Standard nitrogen solution—Dissolve 4·7162 g of ammonium sulphate in water and dilute to 1 litre.

1 ml of solution $\equiv$ 1 mg of nitrogen.

Preparation of calibration graphs—

Prepare solutions containing 0 to 80 μg of nitrogen by diluting appropriate portions of the standard nitrogen solution with water to approximately 30 ml in 50-ml calibrated flasks. Add 2 ml of Nessler's reagent to each and make up to the mark. Shake well and measure the optical density of each solution at 430 mμ by using an absorptiometer. Plot a graph of optical density *versus* nitrogen concentration.

TABLE I

COMPARISON OF THE NITROGEN CONTENT OF TEN FOOD SUBSTANCES AS DETERMINED BY THE PROPOSED METHOD AND THE STANDARD KJELDAHL METHOD

Sample	Analytical method	Percentage nitrogen found, in six replicate determinations						Mean percentage found	Difference between mean of the two methods, per cent.	Standard deviation
Wheat straw	Proposed*	0·340	0·342	0·342	0·340	0·344	0·342	0·342		0·002
	Standard Kjeldahl	0·343	0·345	0·342	0·346	0·343	0·342	0·344	−0·6	0·002
Ground whole oats	Proposed*	1·079	1·083	1·081	1·081	1·081	1·083	1·081		0·002
	Standard Kjeldahl	1·080	1·081	1·078	1·077	1·082	1·081	1·080	+0·1	0·002
Rye-grass hay	Proposed*	1·374	1·380	1·374	1·377	1·374	1·377	1·376		0·003
	Standard Kjeldahl	1·375	1·382	1·380	1·378	1·382	1·378	1·379	−0·2	0·003
Dried green lucerne ..	Proposed†	4·372	4·368	4·361	4·370	4·366	4·362	4·367		0·004
	Standard Kjeldahl	4·359	4·368	4·371	4·366	4·360	4·366	4·365	0·0	0·005
Peas	Proposed†	4·024	4·020	4·029	4·024	4·024	4·020	4·024		0·004
	Standard Kjeldahl	4·028	4·031	4·032	4·030	4·027	4·030	4·030	−0·1	0·002
Rapeseed meal	Proposed†	2·878	2·878	2·868	2·872	2·876	2·878	2·875		0·004
	Standard Kjeldahl	2·880	2·872	2·877	2·879	2·881	2·880	2·876	0·0	0·004
Low-protein wheat flour ..	Proposed*	1·330	1·333	1·328	1·333	1·330	1·334	1·331		0·002
	Standard Kjeldahl	1·329	1·327	1·328	1·333	1·331	1·329	1·330	+0·1	0·002
High-protein wheat grain ..	Proposed†	3·166	3·171	3·168	3·171	3·171	3·168	3·169		0·002
	Standard Kjeldahl	3·171	3·173	3·175	3·170	3·169	3·172	3·172	−0·1	0·002
Low-protein wheat grain ..	Proposed*	1·436	1·431	1·434	1·434	1·431	1·436	1·434		0·002
	Standard Kjeldahl	1·440	1·432	1·434	1·432	1·434	1·436	1·435	0·0	0·003
Meat meal	Proposed†	12·426	12·418	12·426	12·418	12·430	12·424	12·424		0·005
	Standard Kjeldahl	12·424	12·428	12·418	12·416	12·411	12·421	12·420	0·0	0·007

* The optical density was measured at 430 mμ after 2 ml of reagent had been added.

† The optical density was measured at 520 mμ after 4 ml of reagent had been added.

TABLE II

RECOVERY OF NITROGEN ADDED TO RYE-GRASS HAY*

Addition	Analytical method	Percentage nitrogen found, in six replicate determinations						Mean percentage found	Theoretical percentage present	Recovery of added nitrogen, per cent.
10 mg ammonium sulphate	Proposed†	1·588	1·586	1·590	1·591	1·586	1·592	1·589	1·588	100·3
	Standard Kjeldahl	1·582	1·580	1·582	1·579	1·581	1·583	1·581	1·588	97·2
50 mg ammonium sulphate	Proposed†	2·430	2·429	2·430	2·425	2·431	2·430	2·429	2·436	99·4
	Proposed‡	2·426	2·424	2·420	2·424	2·421	2·423	2·423	2·436	98·7
	Standard Kjeldahl	2·432	2·430	2·426	2·428	2·429	2·431	2·429	2·436	99·4
100 mg ammonium sulphate	Proposed‡	3·470	3·471	3·468	3·471	3·472	3·476	3·471	3·496	98·9
	Standard Kjeldahl	3·470	3·467	3·469	3·468	3·470	3·472	3·470	3·496	98·8
10 mg glycine	Proposed†	1·556	1·550	1·558	1·553	1·557	1·558	1·555	1·563	96·2
	Standard Kjeldahl	1·563	1·560	1·558	1·561	1·563	1·560	1·561	1·563	99·1
50 mg glycine	Proposed†	2·300	2·298	2·306	2·297	2·297	2·303	2·300	2·309	99·0
	Proposed‡	2·294	2·297	2·293	2·294	2·294	2·297	2·295	2·309	98·5
	Standard Kjeldahl	2·300	2·297	2·300	2·295	2·296	2·299	2·298	2·309	98·8
100 mg glycine	Proposed‡	3·216	3·220	3·214	3·216	3·215	3·218	3·217	3·242	98·6
	Standard Kjeldahl	3·224	3·228	3·230	3·221	3·227	3·227	3·226	3·242	99·2

* The rye-grass hay used contained 1·376 per cent. of nitrogen.
† The optical density was measured at 430 mμ after 2 ml of reagent had been added.
‡ The optical density was measured at 520 mμ after 4 ml of reagent had been added.

Similarly, prepare solutions containing 0 to 400 μg of nitrogen. Treat the solutions by the above procedure, but add 4 ml of Nessler's reagent instead of 2 ml, and measure the optical density of each solution at 520 mμ. Plot a graph of optical density *versus* nitrogen concentration.

PROCEDURE—

Digest 0·1 to 1·0 g of the finely powdered sample in a 300-ml Kjeldahl flask with 20 ml of sulphuric acid, sp.gr. 1·84, and 10 to 12 g of digestion mixture. Continue digestion for 30 minutes after the solution has cleared. Cool the flask, transfer the contents to a 200-ml calibrated flask and dilute them to the mark with water. (If fibrous or calcareous material is present, filtration through a Whatman No. 40 or 42 filter-paper is necessary at this stage.) Transfer a 1-ml portion to a 50-ml calibrated flask and dilute it with 20 to 30 ml of water. Add 2 ml of Nessler's reagent (4 ml if the expected concentration is between 80 and 400 μg per 50 ml of final solution) and dilute the mixture to the mark. Shake the mixture well, and measure the optical density at 430 mμ (520 mμ if the expected concentration is between 80 and 400 μg per 50 ml of final solution) by using a suitable absorptiometer.

Calculate the amount of nitrogen present by reading off from the appropriate calibration graph, prepared as described above.

RESULTS

The Kjeldahl test as prescribed by Winkler,[11] but with metallic selenium as the catalyst, was used as a reference standard throughout these experiments. Several organic food materials were analysed by using both the proposed and Kjeldahl methods. The results are given in Table I. One of these materials was also analysed after the incorporation of 10, 50 and 100 mg of both ammonium sulphate and glycine. The figures in Table II show that the recovery of nitrogen was excellent, and compared favourably with those determined by the standard Kjeldahl method.

DISCUSSION

In the course of this study of the Nessler test, colour development was found to commence at an alkalinity as low as 0·01 N in the final 50 ml of solution, and that there was little increase in colour intensity beyond 0·07 N. To ensure the adequacy of 2 ml of Nessler's solution for complete colour development in the lower nitrogen range, the acidity of the final 50 ml of solution was measured volumetrically before and after the addition of reagent. In each experiment, the acidity of the test solutions was less than that theoretically predicted, owing to the use of some of the sulphuric acid during oxidation; the amount of acid used was appreciably greater with fibrous material. Consequently, the final alkalinity was higher than that theoretically predicted and was always in excess of 0·07 N, even when only 2 ml of Nessler's reagent was used.

Further experiments led to the conclusion that the procedure was unaffected by increasing the concentration of alkali in the reagent to 4 N. The use of separate solutions of potassium mercuric iodide and alkali had no noticeable effect on the results obtained, but simply involved an additional unnecessary step in the procedure.

Additional factors affecting the test include temperature, which has quite a remarkable effect. A linear relationship between temperature and colour intensity was found to exist up to 35° C, above which there was evidence of the development of turbidity, particularly at high concentrations of nitrogen. Consequently it is recommended that all test solutions are prepared at the same temperature as that of the appropriate standards. Extraneous materials such as traces of organic solvents produce turbidity and affect the quality of the colour, and the harmful influence of certain metallic and acid radicals is discussed in the literature. The Group VIII metals appear to be singularly malignant in this respect, although their concentration in feeding stuffs rarely attains significant proportions. The cupric ion has adverse powers, which are sufficient to preclude its use as a catalyst. Selenium, on the other hand, has no such influence in concentrations of up to 20 p.p.m. in the final 50 ml of solution, which permits the use of 200 mg of catalyst in a determination if it is needed. The incorporation of 1 ml of gum arabic solution prepared as described by Chiles[12] and Middleton,[9] together with 5 ml of M sodium tartrate or M sodium potassium tartrate was found to be

of some use in combating all but the more serious interferences by extraneous ions. These solutions were, however, of much less value in preventing turbidity caused by high temperature.

I gratefully acknowledge the technical assistance of Miss Nola McEwin throughout this series of experiments.

References

1. Nessler, J., *Chem. Centr.*, 1856, **27** (neue Folge **1**, Band **I**), 529.
2. Folin, O., and Denis, W., *J. Biol. Chem.*, 1916, **26**, 473.
3. Koch, F. C., and McMeekin, T. L., *J. Amer. Chem. Soc.*, 1924, **46**, 2066.
4. Wicks, L. F., *J. Lab. Clin. Med.*, 1941, **27**, 118.
5. Bear, F. E., "Chemistry of the Soil," *American Chemical Society Monograph No.* 126, Reinhold Publishing Corp., New York, 1955, p. 360.
6. Treadwell, F. P., *translated and revised* Hall, W. T., "Analytical Chemistry," Ninth English Edition, Chapman & Hall Ltd., London, 1937, Volume I, p. 91.
7. Vanselow, A. P., *Ind. Eng. Chem., Anal. Ed.*, 1940, **12**, 516.
8. Jackson, D. D., "Standard Methods for the Examination of Water and Sewerage," Seventh Edition, American Public Health Association, New York, 1933.
9. Middleton, K. R., *J. Appl. Chem.*, 1960, **10**, 281.
10. Bucki, J., Alther, R., and Soliva, M., *Pharm. Acta Helvet.*, 1953, **28**, 237.
11. Winkler, L. W., *Z. angew. Chem.*, 1914, **27**, 630.
12. Chiles, H. M., *J. Amer. Chem. Soc.*, 1928, **50**, 217.

First received *March 9th*, 1962
Amended *April 16th*, 1963

The Determination of Betaine in the Vinasses of Beet Molasses

BY S. GÖRÖG AND E. EZER

(*Chemical Works of G. Richter, Ltd., Budapest, Hungary*)

Betaine may be precipitated as a tetraphenylboron derivative in an acid medium. A gravimetric method for determining betaine has been developed, based on weighing the well defined, crystalline precipitate. Other compounds are also precipitated with sodium tetraphenylboron, but the method may be made specific for betaine by varying the pH at which the precipitation is carried out. The method has been successfully applied to the determination of betaine in the vinasses of beet molasses.

THE hydrochloride of betaine is extensively used in therapeutics as a substitute for gastric acid. It is produced on an industrial scale from the molasses obtained as a by-product in the manufacture of sugar from sugar-beet.[1] The sugar content of molasses is converted into alcohol by fermentation. The vinasses (malt returns) of beet molasses, obtained after distilling the alcohol and concentrating the solution, serve as raw material for the production of betaine. They also contain large amounts of inorganic salts, amino-acids, humic acids and several other organic compounds besides betaine.

It is essential to know the exact betaine content of the vinasses of beet molasses to assess the completeness of recovery of betaine when vinasses are processed. The analytical determination presents a major problem because of the number of accompanying substances, often chemically similar to betaine, present in large amounts.

The solution of this problem has been discussed in earlier work. Two methods have been widely used—

(*i*) Betaine is isolated as its insoluble periodide, and the determination can be performed iodimetrically[2] after the complexed iodine has been liberated, or spectrophotometrically.[3] After the precipitate had been suitably treated, the nitrogen was determined by distilling it as ammonia.

(*ii*) Betaine is precipitated on a larger scale and separated as its reineckate. The determination can be performed titrimetrically[6,7,8,9] or colorimetrically.[8,10]

Since these two general methods are not specific for betaine, recent analytical methods have involved purification by chromatography[5] or ion exchange.[9,11]

Owing to the insufficiently specific nature of the methods and the imperfection of the isolations, the problem does not as yet appear to have been solved. It is extremely difficult to obtain reproducible results with these methods, and totally contrasting results can be found even in recent literature.

Our object has been to develop a method that permits a degree of control such that—

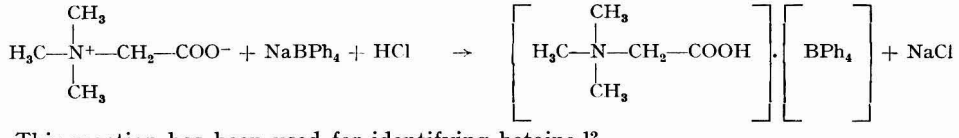
(*a*) all the betaines are measured by the proposed method and

(*b*) in the presence of other substances, betaine is measured preferentially.

From this view-point, the gravimetric method must naturally be considered as the first alternative. Therefore we have attempted to solve the problem by gravimetrically determining the betaine in the form of its tetraphenylboron derivative.

EXPERIMENTAL

Betaine, as a quaternary ammonium compound, will react with sodium tetraphenylboron ($NaBPh_4$) in an acidic medium to yield a precipitate possessing a well defined melting-point, in accordance with the reaction scheme given below—



This reaction has been used for identifying betaine.[12]

According to our tests, the reaction is suitable for the quantitative determination of betaine or, under proper conditions, for the control of the betaine content of the vinasses from beet molasses.

In the latter instance betaine must be determined in the presence of several foreign substances, and a separation is required before precipitation can take place. Betaine can be quantitatively isolated after separation in the form of its tetraphenylboron derivative; this has been established by using a radioactive method with betaine labelled with carbon-14.* The procedure adopted was as follows. Carbon-14-labelled betaine of known activity was added to the samples at the start of tests. The activity of filtrates and precipitates was determined at the appropriate stages by using an end-window Geiger - Müller counter in conjunction with an E.M.G. decimal scaler (made by Elektrónikus Müszerek Gyára—Works of Electronic Measuring Instruments) after it had been dried on an aluminium cup.

The first separation process, after precipitating betaine from the alcohol, was designed to remove amino-acids and the brown substances of humic acid character. If ethanol is used to effect precipitation, there may be extensive precipitation of impurities. However, according to isotope measurements, the resinous precipitate included 5 to 10 per cent. of betaine even after the most thorough attempts at removing the liquor. By using methanol, a powder-like precipitate that did not include betaine was obtained. This method, however, resulted in loss of efficiency of precipitation. The best method was to inititate the precipitation with methanol and then to dilute the mixture with ethanol. In this way a powder-like precipitate that lent itself to filtration was obtained, and, according to isotope measurements, it contained no betaine. The filtrate was evaporated to dryness, and the residue was dissolved in water. The solution still contained impurities that would yield a precipitate with sodium tetraphenylboron, and the betaine tetraphenylboron would consequently still be contaminated after separation. The reaction scheme given above suggested that betaine could be separated from the solution in the form of its tetraphenylboron derivative only if acid were present. It was found that the separation of the precipitate begins at about pH 4 and becomes complete only from strongly acidic solutions. Since impurities also yield precipitates from the solution at pH values of 4 and above, if the pH of the aqueous solution is adjusted to about 4·5, it should be possible to precipitate the impurities by means of an excess of sodium tetraphenylboron. This was indeed so, and the precipitate was removed by filtration after flocculating it with aluminium chloride. It was verified by isotope measurement that the precipitate, which was mainly potassium tetraphenylboron, did not contain betaine.

The filtrate was acidified, and the betaine tetraphenylboron readily separated from the solution in the form of a white, crystalline precipitate. The success of the analysis is determined fundamentally by the pattern of acidification. If a weak acid, *e.g.*, acetic acid, is used, the separation will not be quantitative, whereas with strong mineral acid, *e.g.*, hydrochloric acid, the separation will be quantitative. However, the effect of the excess of hydrochloric acid immediately causes acid-catalysed hydrolysis of sodium tetraphenylboron, which results in the formation of a substance that is initially solid, but then becomes oily. This problem has been overcome by using an acid of medium strength. A dilute solution of oxalic acid gave a quantative separation. The precipitate obtained in this way could be readily filtered, dried and weighed after it had been washed with dilute acetic acid.

To confirm that the precipitate obtained was pure betaine tetraphenylboron, a pure betaine hydrochloride was prepared from a sample of betaine tetraphenylboron that could be prepared as a standard. The melting points, elementary analyses and tetraphenylboron component contents of the two materials were compared. The tetraphenylboron content was determined in an acetone - water mixture by using potentiometric end-point indication with a silver - calomel electrode system. The results, on comparing the theoretical and reference values, are presented in Table I.

* The betaine labelled with carbon-14 in the *N*-methyl group used for the tests was produced by the Isotope Institute of the Hungarian National Atomic Energy Commission.

TABLE I

ANALYSIS OF TETRAPHENYLBORON PRECIPITATES

	Melting-point, °C	Results of analysis, per cent.— C	H	N	Tetraphenylboron
Betaine tetraphenylboron separated from vinasses	118 to 121	80·20	7·52	3·40	71·87
Standard betaine tetraphenylboron	119 to 121	79·82	7·51	3·34	72·51
Theoretical and reference values	117 to 118·5[12]	79·64	7·38	3·21	72·89

As can be seen from Table I, the values of the standard sample and those of the sample separated from the vinasses of beet molasses were in good agreement. A fair agreement with the theoretical and reference values is also evident. A mixture of the two substances did not show melting-point depression.

These results compare favourably with the results obtained by radiochemical methods.

METHOD

REAGENTS—

Use analytical-reagent grade material wherever possible.

Sodium tetraphenylboron solution, 2 per cent. w/v, aqueous—Dissolve 2 g of sodium tetraphenylboron in 100 ml of water. Add 0·5 g of aluminium hydroxide, mix and shake the solution thoroughly. Use within two weeks.

Acetic acid solution, 1 per cent. w/v, aqueous.

Aluminium chloride solution, 1 per cent. w/v, aqueous.

Oxalic acid, saturated aqueous solution.

Hydrochloric acid, 0·1 N.

Ethanol—Use material that has a water content of less than 1 per cent.

Methanol—Use material that has a water content of less than 1 per cent.

BETAINE HYDROCHLORIDE TEST—

Dissolve about 0·05 g of betaine hydrochloride in 20 ml of water. Add 2 ml of 0·1 N hydrochloric acid (at such a low concentration, hydrochloric acid will not cause any difficulty; oxalic acid need not be used under these conditions) and 10 ml of 2 per cent. sodium tetraphenylboron solution dropwise with continuous stirring. Set the solution aside for two hours and then filter off the precipitate on a G-3 glass filter. Wash the residue with three successive 5-ml portions of 1 per cent. acetic acid and dry it for three hours at 80° C. Weigh the residue. Then—

1 mg betaine tetraphenylboron ≡ 0·351 mg betaine hydrochloride.

DETERMINATION OF BETAINE IN THE VINASSES OF BEET MOLASSES—

Weigh between 0·4 and 0·5 g of vinasses into a 50-ml beaker. Add 5 ml of methanol, mix the solution thoroughly, and then add 15 ml of ethanol with continuous stirring. Filter the finely divided precipitate off on a suitable filter-paper, and wash it with two successive 5-ml portions of ethanol. Evaporate the combined filtrate and washings to dryness on a water-bath. Dissolve the residue in 15 ml of water and add 1·5 ml of 1 per cent. acetic acid. To this solution, slowly add 25 ml of 2 per cent. sodium tetraphenylboron solution and flocculate the precipitate formed by adding 2 ml of 1 per cent. aluminium chloride solution. Set the solution aside for 5 minutes and filter the precipitate off on a G-4 glass filter. Wash it with three 3-ml portions of water. Wash the combined filtrate and washings from the suction flask into a 100-ml beaker, and add 2 ml of saturated oxalic acid solution dropwise with vigorous stirring. Agitate the solution for a few minutes. Set the mixture aside for 30 minutes, and collect the precipitate on a G-3 glass filter. Wash it with three 5-ml portions of 1 per cent. acetic acid, dry it for three hours at 80° C and weigh the residue. The betaine content of the sample is given by—

$$\text{Betaine content} = \frac{100\ W \times \text{Molecular weight of betaine}}{\text{Molecular weight of betaine tetraphenylboron} \times B}$$

$$= 26{\cdot}8\ \frac{W}{B}\ \text{per cent.}$$

where W is the weight of the precipitate, and B is the weight of the sample.

Results

It was established from several betaine hydrochloride tests that the standard deviation of the results was within $\pm 0{\cdot}5$ per cent. These compare favourably with those obtained by acidimetric titration.[13]

The results obtained by testing several samples of vinasses from beet molasses are shown in Table II.

Table II

Betaine content of five samples of vinasses

Vinasses sample	Betaine content, per cent.	Mean value, per cent.
A	9·81, 9·81, 9·67	9·76
B	9·52, 9·66, 9·50	9·65
C	9·54, 9·20, 9·14	9·29
D	9·82, 10·06, 10·10	9·99
E	8·94, 9·07, 9·11	9·04

It is evident that the relative deviation of the single values from the mean value is within ± 2 per cent.

Recovery tests—

Recovery tests were carried out to determine the accuracy of the proposed method. Known amounts of pure betaine were added to the vinasses under examination, and the resultant mixtures from this procedure examined and their betaine content determined. The results are given in Table III.

Table III

Recovery of added betaine from samples of vinasses

Original amount of betaine found, g	Amount found after addition of betaine, g	Calculated content, after addition of betaine, g
0·0330	0·0490	0·0494
0·0278	0·0530	0·0525
0·0274	0·0593	0·0603

As can be seen from Table III, the results obtained were fairly good.

The proposed method has been successfully used for testing raw materials and intermediate products for betaine in the industrial production of betaine.

We wish to express our appreciation of the valuable suggestions made by Mr. I. Gyenes and our thanks to Mrs. I. Füvessy for the micro-analyses and to Mrs. Gy. Berhidai for her assistance in the tests.

References

1. "Ullmans Encyklopädie der technischen Chemie," Fourth Edition, Urban-Schwarzenberg, Munich and Berlin, 1953, p. **334**.
2. Blood, J. W., and Cranfield, H. T., *Analyst*, 1936, **61**, 829.
3. Wall, J. S., Christianson, D. D., Dimler, R. J., and Senti, F. R., *Anal. Chem.*, 1960, **32**, 870.
4. Stanek, V., *Z. Zuckerind. in Böhmen*, 1904, **28**, 578; *Biochem. Centralbl.*, 1905, **III**, 471.
5. Benin, G. S., and Shnaïder, E. E., *Sakhar. Prom.*, 1951, **25**, 44; *Chem. Abstr.*, 1954, **48**, 4092.
6. Walker, H. G., jun., and Erlandsen, R., *Anal. Chem.*, 1951, **23**, 1309.
7. Cromwell, B. T., and Rennie, S. D., *Biochem. J.*, 1953, **55**, 189.
8. Simenauer, A., *Bull. Soc. Chim. France*, 1958, 294.
9. Carruthers, J. F., Oldfield, J. F. T., and Teague, H. J., *Analyst*, 1960, **85**, 272.
10. Focht, R. L., Schmidt, F. H., and Dowling, B. B., *J. Agric. Food Chem.*, 1956, **4**, 546; *Anal. Abstr.*, 1957, **4**, 681.
11. Szabolcs, O., and Prey, V., *Z. Zuckerind.*, 1959, **9**, 517; *Anal. Abstr.*, 1960, **7**, 3497.
12. Crane, F. E., jun., *Anal. Chem.*, 1956, **28**, 1794.
13. British Pharmaceutical Codex, 1949, p. 148.

First received *May 1st*, 1963
Amended *November 11th*, 1963

SHORT PAPERS

Interference by Extractives in the Determination of Malathion Residues in Rice Bran

By ANGELA N. BATES* and D. G. ROWLANDS
(*Agricultural Research Council, Pest Infestation Laboratory, Slough, Bucks*)

The method of Norris, Easter, Fuller and Kuchar[1] for the colorimetric analysis of malathion proved unreliable when applied to residues in rice bran, unless chromatographic purification of the extracts on Fuller's earth was introduced.[2] It has been noted, however, in this and other laboratories (private communication from Border and Hill) that, without this chromatographic step, the divergence between the amount of insecticide found and that actually present varied markedly with different samples of rice bran (see Table I). As previously reported,[2] low recoveries were recorded when considerably less than the theoretical amount of 6 N hydrochloric acid (1 ml) was required for neutralisation after alkaline hydrolysis during the analysis.

Table I

Recovery of malathion from rice bran without purification of the extract

Origin of bran			Malathion analysis: Recovery of insecticide from 25-g samples		Malathion analysis: Volume of 6 N HCl required during analysis	
Source	Paddy parboiled before milling	Bran extracted with a solvent after milling	On receipt of sample, per cent.	After storage,* per cent.	On receipt of sample, ml	After storage,* ml
British Guiana ..	Yes	No	93	86	0·8	0·8
Burma	†	Yes	114	96	0·8	0·8
England	†	†	55	Nil	0·2	<0·1
Italy	No	No	67	Nil	0·2	<0·1
United States ..	†	†	Nil	Nil	Nil	Nil

* Storage periods are given in Table II.
† History of sample unknown.

Rice bran, unless extracted with a solvent, contains up to 15 per cent. of an oil that is rapidly hydrolysed under normal storage conditions. If the bran is produced from parboiled paddy, breakdown of this oil is inhibited. Loeb, Morris and Dollear[3] found that over 50 per cent. of the oil in rice bran was converted to free fatty acid and glycerol in the first 10 days of storage at 25° C, but, in bran prepared from parboiled paddy, hydrolysis of only 10 per cent. of the oil occurred in 6½ months.

Table II

Oil and free fatty acid content of rice brans after storage

Source of bran	Length of storage in the laboratory, weeks	Oil content, per cent.	Free fatty acid content, calculated as oleic acid: in oil, per cent.	Free fatty acid content, calculated as oleic acid: in bran, per cent.
British Guiana	13	15·3	4·1	0·63
Burma	17	1·4	60·3	0·84
England	26	15·1	67·9	10·26
Italy	6·5	14·1	66·0	9·33
United States	17	11·9	77·8	9·22

The oil contents of bran samples available in this laboratory were determined by extraction with light petroleum, boiling-range 40° to 60° C, and the extent of production of fatty acids was measured by titration with sodium hydroxide solution. Table II shows that high contents of

* Present address: Benenden School, Cranbrook, Kent.

free fatty acids were found in the English, Italian and United States brans, from all stored samples of which no malathion could be recovered unless chromatographic purification was included in the analytical procedure. The British Guiana bran, prepared from parboiled paddy, contained only a small amount of free fatty acids; satisfactory recoveries were recorded when malathion was added in in small amounts up to 10 p.p.m., and when the samples were subsequently analysed without chromatographic purification. The rice bran from Burma, having been extracted with a solvent in the country of origin, contained a low percentage of oil. This oil had been hydrolysed to about the same extent as that in the English, Italian and United States brans, but the weight of fatty acids produced, relative to that of the bran, was low. Determinations for malathion made on extracts of this bran did not need the chromatographic step.

The change with time in free fatty acid content and insecticide recovery was investigated on Italian rice bran whose milling date was known. The free fatty acids increased over the period studied, whereas malathion recoveries from unpurified extracts fell from 67 per cent. to zero (see Table III).

TABLE III

EFFECT OF STORAGE ON ITALIAN RICE-BRAN

Length of storage after milling, days	Free fatty acid content, calculated as oleic acid		Determination of malathion	
	in oil, per cent.	in bran, per cent.	Recovery of insecticide from 25-g samples, per cent.	Volume of 6 N HCl required during analysis, ml
7	39·2	5·84	67	0·2
13	52·4	7·64	—	—
45	66·0	9·33	Nil	<0·1

Oleic acid, the major acid component of rice-bran oil,[4] equivalent to the total weight of free acids found in 25-g samples of Italian rice bran after 45 days storage, was added to 500 μg of malathion in carbon tetrachloride solution. Analysis by the standard colorimetric method[1] for malathion, without chromatography, gave zero recovery of insecticide.

It appears probable, therefore, that interference in the colorimetric determination of malathio residues in rice bran is caused mainly by the free fatty acids neutralising the alkali added during the analysis to effect hydrolysis of malathion to dimethyl phosphorodithioate, thereby inhibiting the production of the chelate complex with cupric ions. These fatty acids are presumed to arise from enzymatic hydrolysis of the rice-bran oil. Solvent-extracted bran or that prepared from parboiled paddy is not likely to contain a high proportion of free fatty acids, and difficulty with malathion determination should not be experienced. Freshly milled bran may also be analysed without preliminary purification, but interference is likely to appear after only brief storage. Interference should be suspected when malathion residues are determined in any product of high oil content that might be expected to accumulate more than 2 per cent. of free fatty acids during storage, and the need for chromatographic purification before analysis is indicated by a decrease in the volume of 6 N hydrochloric acid used in the analytical procedure.

We thank Dr. E. A. Parkin for his valuable suggestions and interest in this work. We are indebted to Messrs. J. Bibby & Sons Ltd., and to the Thames Rice Milling Co. Ltd., for rice-bran samples.

REFERENCES

1. Norris, M. V., Easter, E. W., Fuller, L. T., and Kuchar, E. J., *J. Agric. Food Chem.*, 1958, **6**, 111.
2. Bates, A. N., Rowlands, D. G., and Harris, A. H., *Analyst*, 1962, **87**, 643.
3. Loeb, J. R., Morris, N. J., and Dollear, F. G., *J. Amer. Oil Chem. Soc.*, 1949, **26**, 738.
4. Murti, K. S., and Dollear, F. G., *Ibid.*, 1948, **25**, 211.

Received *July 25th*, 1963

The Recovery of Malathion from a Range of Stored Products

By ANGELA N. BATES* and D. G. ROWLANDS
(*Agricultural Research Council, Pest Infestation Laboratory, Slough, Bucks*)

In a recent paper,[1] we announced our intention of investigating a number of cereals and oil seeds for the presence of plant extractives interfering in the analytical procedure of Norris, Easter, Fuller and Kuchar[2] for determining malathion residues. The results of this study are presented in Table I.

TABLE I

MALATHION RECOVERIES FROM PLANT PRODUCTS

	Malathion analysis				
		Without preliminary chromatography of plant extracts		With preliminary chromatography of plant extracts	
Plant material	Sample size, g	Malathion added, p.p.m.	Recovery,* per cent.	Malathion added, p.p.m.	Recovery,* per cent.	Chromatographic adsorbent
†Citrus pulp	50	5	88	5	90	Acid alumina
Cocoa	50	5	94	—	—	
†Coconut meal ..	50	5	89	5	92	Acid alumina
†Copra	50	5	97	5	91	Acid alumina
Groundnuts ..	50	5	82‡	5	100	Silica gel
Maize	25	10	99	—	—	—
Pea beans	25	10	97	—	—	—
Rice products—						
Husk	40	5	93	—	—	—
Paddy	50	5	97	—	—	—
Polished rice ..	50	5	94	—	—	—
Sorghum	50	5	101	—	—	—
†Soya-bean meal ..	50	5	75	5	81	Acid alumina
Wheat products—						
Bran	25	10	83‡	10	93	Silica gel
†Wholemeal flour ..	25	10	88	10	96	Acid alumina
†White flour	25	10	86	10	96	Acid alumina

* Recovery figures represent an average of 2 to 4 determinations and are corrected for the apparent malathion content of plant material not treated with insecticide.
† Troublesome emulsions are produced if chromatography is omitted.
‡ Volume of 6 N hydrochloric acid required during analysis was considerably less than 1 ml.

When colorimetric analysis was carried out without the preliminary use of adsorbent columns to clean up the plant extracts containing the insecticide, recoveries of malathion were acceptable from all the commodities studied. The introduction of the chromatographic stage is, however, recommended when troublesome emulsions are encountered with the unpurified plant extract, or when the recovery of insecticide is appreciably raised by this means.

Experimental details for the chromatography on acid-washed alumina are given in our previous paper.[1] Silica gel, of chromatographic-adsorption quality, was activated for 2 hours at 240° C, and was stored thereafter in a screw-topped jar. The silica gel was used in a manner exactly analogous to that described for the alumina, except that 15 g of adsorbent was used in each column.

We thank Dr. E. A. Parkin for his interest in this work and Miss J. E. James for technical assistance.

REFERENCES

1. Bates, A. N., Rowlands, D. G., and Harris, A. H., *Analyst*, 1962, **87**, 643.
2. Norris, M. V., Easter, E. W., Fuller, L. T., and Kuchar, E. J., *J. Agric. Food Chem.*, 1958, **6**, 111.

Received *August 29th*, 1963

* Present address: Benenden School, Cranbrook, Kent.

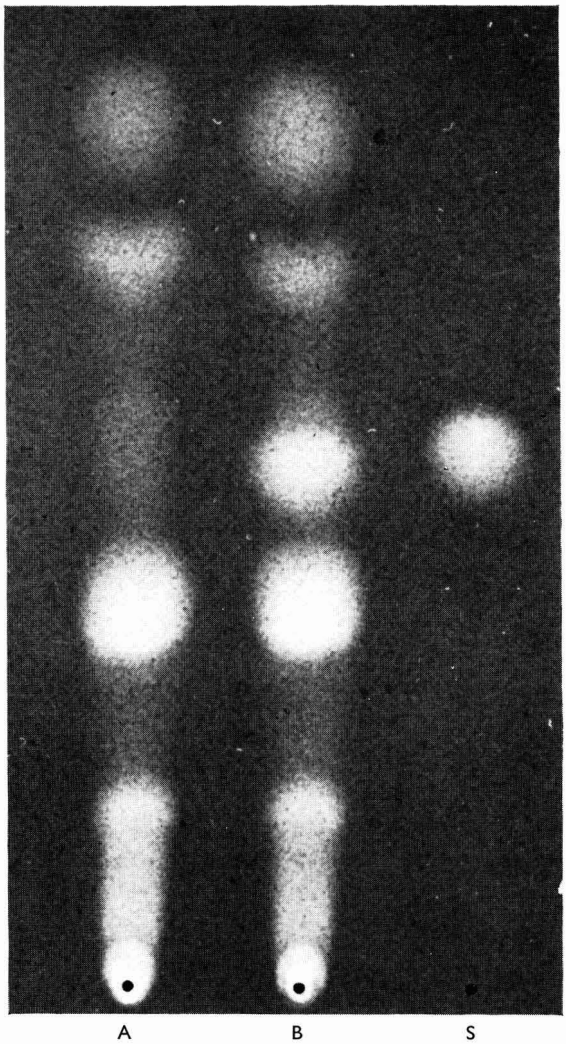


Fig. 1. Thin-layer chromatograms of the unsaponifiable fraction of bread lipids photographed in ultraviolet light. Chromatogram A, control bread containing no stearyl tartrate; chromatogram B, bread containing 10 mg of stearyl tartrate in 100 mg of dry bread; chromatogram S, single standard of stearyl alcohol

To face p. 289]

The Detection and Approximate Determination of Stearyl Tartrate in Bread

By E. I. WILLIAMS

(*Spillers Limited, Central Laboratory, Station Road, Cambridge*)

Although stearyl tartrate is included in the list[1] of emulsifiers permitted for use in bread, no method for its detection or estimation appears to have been published.

Commercial stearyl tartrate yields on saponification approximately 80 per cent. of stearyl alcohol* and 24 per cent. of tartaric acid. When it is present in fatty material, stearyl tartrate is probably best determined through its tartaric acid component, provided that a reasonably large proportion is present and that evidence is produced to show that the tartaric acid is in fact derived from stearyl tartrate and not from some other permitted emulsifier containing that acid.

When stearyl tartrate is used in bread, the normal amount present is of the order of 0·025 lb per 280 lb of flour. This would be equivalent to about 10 mg in 100 g of dry bread. At this level, the fat extracted from an average type of bread would contain approximately 0·5 per cent. of stearyl tartrate, equivalent to 0·12 per cent. of tartaric acid. The determination of this proportion of tartaric acid in the fat extracted from a reasonably sized sample of bread, say 50 g, would not be easy. In this particular instance it was thought that a better approach would be through the stearyl alcohol, since this component could be concentrated in the unsaponifiable fraction of the bread extract. Further, of the permitted emulsifiers, stearyl tartrate is the only one that yields stearyl alcohol on saponification.

Since lipids extracted from bread (whether made with stearyl tartrate or not) contain a significant and variable proportion of unsaponifiable matter, direct estimation of stearyl tartrate from a measure of this characteristic is not possible. However, separation of the stearyl alcohol from the other materials present in the unsaponifiable matter by thin-layer chromatography has given satisfactory results.

Analytical method

Fifty grams of air-dried and ground bread crumbs, of known moisture content, are extracted with a solvent after acid hydrolysis as described by the Association of Official Agricultural Chemists.[2]

The lipid material is then saponified, and the unsaponifiable matter isolated in the usual way.[3]

The unsaponifiable components are then dissolved in a known volume of chloroform (usually about 1 ml), and 20 μl of this solution are placed on a chromatographic plate that has been coated with silicic acid. A series of suitable standards, usually ranging from 1 to 20 μl of a 0·7 per cent. solution of stearyl alcohol in chloroform, is also placed on the plate.

Separation is effected by using a diethyl ether - light petroleum (boiling-range 40° to 60° C) mixture (9 + 1), the plate is sprayed with a 0·2 per cent. solution of dichlorofluorescein in alcohol, and the developed plate is examined in ultraviolet light. The general appearance of the chromatogram and position of the separated stearyl alcohol can be seen in Fig. 1.

An estimate of the amount of stearyl alcohol in the test solution can be made by direct visual assessment. If necessary, for greater accuracy, the separation can be repeated with standards nearer to the estimated amount present.

The proportion of stearyl alcohol, and from it, the amount of stearyl tartrate associated with a known weight of bread, can be readily calculated.

Results

Recovery from breads of known composition—

Bread was prepared with various stearyl tartrate - fat mixtures by using the following basic formula—

Flour	56 oz
Yeast	0·75 oz
Salt	1 oz
Fat mixture	0·4 oz
Water	31 oz

* Cetyl alcohol is likely to be associated with stearyl alcohol in some commercial products, but, as far as the work here is concerned, both alcohols behave similarly and are therefore referred to in the text simply as stearyl alcohol.

The stearyl tartrate content of the fat mixture for four breads is as under—

Bread reference	A	B	C	D
Stearyl tartrate in the fat mixture, %	Nil	1·25	0·75	1·75

The results, as stearyl tartrate in 100 g of dry bread, are given below—

	A	B	C	D
Calculated, mg	Nil	10·0	6·0	14·0
Found, mg	Trace	8·0–9·5	5·0–6·0	11·5–13·5

The moisture contents of the various ingredients were taken into account when the theoretical stearyl tartrate content in the bread samples was calculated.

The results represent the maximum and minimum results obtained after the assessment of duplicate plates by three persons.

As shown in Fig. 1, the control bread gave a faint trailing spot partially overlapping the stearyl alcohol position. The intensity of this spot is, however, such that it does not cause a significant error in the estimation of stearyl tratrate when the latter is present in normal amounts.

EXAMINATION OF COMMERCIAL LOAVES—

Three commercial loaves, from bakeries associated with us, and known to contain no stearyl tartrate, gave apparent stearyl tartrate figures of less than 1 mg per 100 g of dry bread.

Four loaves from other producers, and of unknown composition, gave figures of 1·5, 6·0, 6·5 and 7·5 mg of stearyl tartrate for 100 g of dry bread.

DISCUSSION

The detection of normal amounts of stearyl tartrate in bread can readily be accomplished by means of the technique described. From the quantitative aspect the method has its limitations, but, in practice, the accuracy achieved by visual assessment of spot intensities is considered to be good. At most there can be an under-estimate of the added amount by not more than 20 per cent.

I thank the Directors of Spillers Limited for permission to publish this paper, Dr. J. Williams for his interest and Mr. A. W. Hartley for producing the photograph.

REFERENCES

1. Emulsifiers and Stabilisers in Food Regulations, 1962.
2. Association of Official Agricultural Chemists, "Official Methods of Analysis," Ninth Edition, A.O.A.C., Washington, D.C., 1960, Method No. 13.079.
3. British Pharmacopoeia, 1958, p. 875.

Received *August 8th*, 1963

The Flame-photometric Determination of Calcium in Industrial Phosphoric Acid

BY I. JOHNSTON AND M. STOW

(*Department of Chemistry, Levington Research Station, Levington, Ipswich, Suffolk*)

THE filtration properties of the gypsum produced in the manufacture of phosphoric acid from rock phosphate and concentrated sulphuric acid, and usually referred to as "wet-process" acid, varies with the free sulphuric acid content of the acid. Control of the sulphate content is normally obtained by titration with barium chloride with sodium rhodizonate as external indicator. The phosphoric acid, however, is saturated with respect to calcium sulphate, and the total sulphate values obtained require correcting by an amount equivalent to the calcium in order to obtain the free sulphuric acid content.

The volumetric determination of calcium as oxalate is lengthy and unsuitable for purposes of plant control. Banerjee, Budke and Miller[1] have published a spectrophotometric method for determining calcium in wet-process phosphoric acid with sodium naphthalhydroxamate, and claim to be able to analyse 6 samples in 1½ hours.

For control purposes, analytical results are required quickly, and the aim of this investigation was to produce a method capable of giving results in less than half an hour if possible.

Flame photometry seemed a reasonable approach to the problem, if the interference due to the phosphate and other interfering ions present in the phosphoric acid could be overcome. Kramer[2] describes a radiation buffer to be used for the flame-photometric determination of calcium in phosphate, carbonate and silicate rocks. This gives reasonable results for single superphosphate and ammonium dihydrogen orthophosphate but only a 30 per cent. recovery with phosphoric acid.

Brabson and Wilhide[3] use an ion-exchange resin for extracting the calcium from the phosphoric acid. The calcium-containing resin is washed free of phosphate, ignited and the calcium determined on the residue by flame photometry. The time required for analysis in this instance is 1 hour.

The composition of wet-process phosphoric acid will vary with the rock phosphate used and the concentration of acid produced. The following analysis for 30 per cent. acid serves as a useful guide when considering methods of analysis—

Substance ..	P_2O_5	Total SO_4^{2-}	Free H_2SO_4	CaO	Fe_2O_3	Al_2O_3	SiO_2	F	MgO
Per cent. w/w ..	30	2·9	2·3	0·3	0·3	0·4	0·5	1·2	0·3

There are three possible methods of approach for overcoming the interference by other elements with the flame-photometric method.

(*i*) The calcium may be isolated from the acid.

(*ii*) The acid may be used as its own radiation buffer and the calcium content obtained by extrapolation.

(*iii*) The interference due to other ions present in the acid may be studied and a radiation buffer formulated to compensate for these; the radiation buffer being added to both sample and standards. The time factor precludes methods (*i*) and (*ii*).

The ions studied together with their maximum concentration are shown below—

Ion	PO_4^{3-}	SO_4^{2-}	Al^{3+}	SiF_6^{2-}	F^-	Fe^{3+}	Na^+
Maximum concentration	0·5 M	0·5 M	50 p.p.m.	100 p.p.m.	80 p.p.m.	100 p.p.m.	100 p.p.m.

In all instances the readings were corrected for background emission. Phosphate, sulphate, aluminium and fluorosilicate all suppressed the flame emission, whereas sodium, iron and fluoride enhanced the radiation. A graph of percentage emission *versus* concentration of the interfering element showed plateau regions in every instance in agreement with the references cited. By using the results of this study a radiation buffer was formulated.

Method

Reagents—

Standard calcium stock solution—Dissolve 2·4980 g of analytical-reagent grade calcium carbonate, previously dried at 105° C, in 10 ml of 50 per cent. v/v nitric acid. Transfer the solution to a 1-litre calibrated flask and dilute to the mark with de-ionised water. This solution contains 1,000 p.p.m. of calcium.

Standard calcium solution—Transfer 50 ml of the standard calcium stock solution into a 500-ml calibrated flask and dilute to the mark with de-ionised water.

Buffer solution A—Dissolve 2·97 g of anhydrous aluminium chloride, 1·62 g of ferric nitrate monohydrate, 4·62 g of analytical-reagent grade sodium nitrate, 1·09 g of analytical-reagent grade ammonium fluoride and 112 ml of sulphuric acid, sp.gr. 1·84, in water and dilute the solution to 1 litre. Store in a polythene bottle.

Buffer solution B—Mix 75 ml of orthophosphoric acid, sp.gr. 1·75, and 27·5 ml of 30 per cent. w/w technical fluorosilicic acid with water and dilute the solution to 1 litre. Store in a polythene bottle.

Sample of industrial phosphoric acid—Filter the slurry of phosphoric acid and gypsum through two Whatman No. 541 filter-papers and a warm dry Buchner flask and funnel. Take approximately 20 g, weighed to the nearest milligram, of the filtered acid and transfer it to a 500-ml calibrated flask. Dilute the acid to the mark with water and mix it thoroughly.

Apparatus—

Use a Unicam SP900 flame spectrophotometer, or similar instrument. Switch on the SP900 flame spectrophotometer and ignite the burner at least 30 minutes before use.

CALIBRATION—

Calibrate the instrument daily. By using a burette measure 10, 12, 15 and 17 ml of the diluted calcium stock solution into separate 100-ml calibrated flasks. To each flask add 4 ml of radiation buffer solution A and 25 ml of radiation buffer solution B. Dilute each solution to the mark with water and mix each one thoroughly. These solutions contain 10, 12 15 and 17 p.p.m. of calcium, respectively. Spray the solutions into the flame of the Unicam SP900 flame photometer. Use a wavelength of 422·7 mμ and a slit width of 0·05 mm. Set the zero reading with the 10 p.p.m. of calcium solution, and adjust the instrument to read about 60 on the galvanometer scale by using the 17 p.p.m. of calcium standard. Plot a graph of p.p.m. of calcium *versus* the galvanometer reading.

SAMPLE ANALYSIS—

Transfer a suitable portion of the solution of industrial phosphoric acid into a 100-ml calibrated flask. (For samples of industrial acid containing 0·4 to 0·5 per cent. of calcium take a 7-ml portion, and for those containing 0·2 per cent. of calcium take a 10-ml portion.) Add 4 ml of radiation buffer solution A, 25 ml of radiation buffer solution B and dilute the solution to the mark. Spray the sample into the flame of the SP900 flame spectrophotometer. Check the zero reading, and the setting of 60 after every third spraying, by using the procedure described under "Calibration." Calculate the percentage calcium in the original acid from the calibration curve.

RESULTS—

TABLE I

COMPARISON OF RESULTS OBTAINED BY THE FLAME-PHOTOMETRIC METHOD WITH THOSE OBTAINED BY THE VOLUMETRIC OXALATE - PERMANGANATE METHOD

	Per cent. calcium found by using—			Per cent. calcium found by using—	
Sample	Flame-photometric method	Oxalate method	Sample	Flame-photometric method	Oxalate method
1	0·24	0·28	19	0·46	0·47
2	0·38	0·37	20	0·42	0·45
3	0·42	0·42	21	0·46	0·45
4	0·34	0·37	22	0·43	0·46
5	0·39	0·36	23	0·45	0·47
6	0·41	0·42	24	0·68	0·70
7	0·22	0·21	25	0·68	0·72
8	0·21	0·18	26	0·21	0·25
9	0·22	0·20	27	0·20	0·23
10	0·39	0·43	28	0·22	0·24
11	0·38	0·40	29	0·21	0·24
12	0·43	0·45	30	0·25	0·27
13	0·46	0·49	31	0·25	0·26
14	0·38	0·40	32	0·24	0·24
15	0·45	0·47	33	0·26	0·28
16	0·23	0·24	34	0·23	0·24
17	0·24	0·25	35	0·21	0·22
18	0·25	0·28			
			Mean result:	0·340	0·355

DISCUSSION

It was originally intended to use the flame photometer by differentially setting the zero with a 10 p.p.m. calcium solution and the full-scale deflection with the 20 p.p.m. solution. In practice it was found that the calibration was not linear above 17 p.p.m. For this reason results were not accepted from solutions containing more than 17 p.p.m. However, it is possible to obtain an approximate value for samples richer in calcium than expected, and hence calculate the dilution factor required to obtain a more precise answer.

The suppression of the flame emission of calcium by phosphate, sulphate and aluminium has been studied by several workers.[5,6,7] In general, however, they have studied relatively dilute solutions. Yofè and Finkelstein[5] have shown the existence of a plateau region in the interference by phosphate and sulphate on the flame emission of calcium. The maximum ratio of phosphate to calcium they studied was 8, equivalent to a maximum phosphoric acid molarity of 0·006. The

ratio of phosphate to calcium for wet-process phosphoric acid may vary between 80 to 1 and 200 to 1 and even on dilution to obtain a solution containing only 10 to 20 p.p.m. calcium, the phosphoric acid has a molarity between 0·02 and 0·04. At this concentration the variation of flame emission with phosphate content is pronounced. Yofè and Finkelstein suggest that interference is due to the formation of calcium pyrophosphate in the flame, whereas Dinnin[7] postulates that reactions occur in the droplet as it evaporates in the flame, orthophosphate being produced. Aluminium reacts to form calcium aluminate, $Ca(AlO_2)_2$, an anhydrous refractory spinel.

The idea that ion-exchange reactions occur in the solution phase of the droplet as evaporation occurs is an important one, permitting some comprehension of the mechanism by which radiation buffers may work. The releasing effect of lanthanum, iron,[5] strontium and the rare-earth elements[7] is one of preferential precipitation of phosphate and sulphate, leaving the calcium in solution in the form of a readily volatile salt.

The use of releasing agents is not applicable to wet-process phosphoric acid because of the large amount of releasing agent required and the variety of interfering radicals.

The proposed radiation buffer does not release calcium emission, rather it swamps the sample to such an extent that interferences indigenous to the sample are negligible; even so, the sensitivity is satisfactory, 1 p.p.m. of calcium being equivalent to approximately 10 divisions on the galvanometer when the differential technique described is used.

We thank the Directors of Fisons Fertilisers Limited for permission to publish this paper.

References

1. Banerjee, D. K., Budke, C. C., and Miller, F. D., *Anal. Chem.*, 1962, **34**, 440.
2. Kramer, H., *Anal. Chim. Acta*, 1957, **17**, 521.
3. Brabson, J. A., and Wilhide, W. D., *Anal. Chem.*, 1954, **26**, 1060.
4. Baker, G. L., and Johnson, L. H., *Ibid.*, 1954, **26**, 465.
5. Yofè, J., and Finkelstein, R., *Anal. Chim. Acta*, 1958, **19**, 166.
6. West, A. C., and Cooke, W. D., *Anal. Chem.*, 1960, **32**, 1471.
7. Dinnin, J. I., *Ibid.*, 1960, **32**, 1474.

Received *October 9th*, 1963

A Rapid Method for the Determination of Indium in Cyanide Indium Plating Solutions

By J. METCALFE and C. J. KNOWLES*

(*Chemical Laboratory, Ferranti Ltd., Moston, Manchester* 10)

Indium is frequently used as an alloying metal in the electronics industry, and we had an experimental project in hand that necessitated indium plating small components. A rapid method of analysis of the plating solution was thus required. The recommended gravimetric and electrolytic methods,[1] while being straightforward, were considered rather time consuming, and a volumetric procedure was considered more desirable.

The direct titration of indium with disodium ethylenediaminetetra-acetate (EDTA) has been carried out at boiling-point by Flaschka and Amin,[2] and a back-titration procedure with zinc as the titrant and zincon as indicator has been suggested.[3] The back-titration method appeared to be preferable but zinc could not be used as titrant because of cyanide in the samples. As an alternative it was decided to use magnesium as titrant. Eriochrome black T has been used most frequently in magnesium - EDTA titrations, but the stability of aqueous solutions of this indicator is uncertain. Diskant[4] has shown that a solution of the indicator in triethanolamine is stable indefinitely. A solution in triethanolamine diluted with absolute ethanol is stable for about two months, and this solution was chosen because of its less viscous nature.

Experimental

The plating solution contains nominally 20 g of indium, as the sulphate, 150 g of potassium cyanide, 35 g of dextrose and 35 g of potassium hydroxide per litre. None of these other compounds in the solution was expected to interfere with the determination of indium and the results in Table I confirm this opinion.

* Present address: Chemistry Department, University of Leicester.

The reaction between indium and EDTA in a solution buffered with ammonia - ammonium chloride solution was found to be instantaneous. No difference was recorded in titrations carried out immediately, and after fixed periods of up to 20 minutes after the addition of EDTA.

TABLE I

RECOVERY OF INDIUM IN THE PRESENCE OF OTHER COMPONENTS OF THE PLATING SOLUTION

Component present	Indium added, mg	Indium found, mg
Dextrose, 0·35 g	199·6	199·0
Dextrose, 0·35 g + Potassium hydroxide, 0·35 g	199·6	199·0
Dextrose, 0·35 g + Potassium hydroxide, 0·35 g + Potassium cyanide, 1·5 g	199·6	199·0

Indium can be plated on to brass, silver, gold and possibly cadmium-plated articles. The effect of these metals on the proposed method was investigated because inevitably they will give rise to some build-up of impurity in the plating bath. The levels of impurity would never be expected to approach the levels investigated, but the proposed method may possibly be of use in the analysis of plated deposits that have been alloyed with the base metal. Table II shows the recovery of indium in the presence of copper, zinc, silver, cadmium and gold. The solutions also contained dextrose, potassium hydroxide and potassium cyanide.

TABLE II

RECOVERY OF INDIUM IN THE PRESENCE OF OTHER METALS

Indium added, mg (assuming 100 per cent. purity)	Copper, mg (as $CuSO_4$)	Zinc, mg (as $ZnCl_2$)	Silver, mg (as $AgNO_3$)	Cadmium, mg (as $CdSO_4$)	Gold, mg (as $AuCl_3$)	Indium found, mg
221·5	—	—	—	—	—	220·7, 220·9
221·5	21·6	—	—	—	—	220·8, 220·4
221·5	43·2	—	—	—	—	220·7, 220·6
221·5	86·3	—	—	—	—	220·4
221·5	—	31·6	—	—	—	220·4, 221·4
221·5	—	63·2	—	—	—	220·8
221·5	—	126·4	—	—	—	221·6
221·5	—	—	25·5	—	—	220·8
221·5	—	—	51·0	—	—	220·8
221·5	—	—	102·0	—	—	221·4
221·5	—	—	—	25·0	—	220·8
221·5	—	—	—	50·0	—	220·8, 219·8
221·5	—	—	—	100·0	—	222·8, 219·8
223·1	—	—	—	—	23·7	221·8, 221·8
223·1	—	—	—	—	47·4	222·6, 222·6
223·1	—	—	—	—	94·8	221·8
221·5	32·4	18·9	—	—	—	220·7
221·5	10·8	31·6	25·5	25·0	—	220·6, 221·4

The end-point of the titration was rather prolonged when the larger amounts of impurities were present. However, the results show that no serious interference can be expected with the ions tested and the large amount of potassium cyanide in the samples serves as an excellent masking agent.

METHOD

REAGENTS—

Reagents should be of analytical-reagent grade whenever possible.

Standard magnesium solution, approximately 0·1 M—Dissolve about 2·4 g of pure magnesium metal (99·99 per cent.) that has been previously cleaned, dried and accurately weighed, in the minimum volume of hydrochloric acid, sp.gr. 1·18, and dilute the solution to 1 litre with water. Calculate the exact molarity from the weight of metal taken.

EDTA, approximately 0·1 M—Dissolve about 37 g of EDTA in water and dilute the solution to 1 litre. Standardise it against the magnesium solution with Eriochrome black T as indicator, in a solution buffered with ammonia - ammonium chloride solution.

Ammonia - ammonium chloride buffer solution—Dissolve 70 g of ammonium chloride in water, add 570 ml of ammonia solution, sp.gr. 0·88, and dilute the solution to 1 litre with water.

Eriochrome black T indicator—Dissolve 0·2 g of Eriochrome black T in 15 ml of triethanolamine and add 5 ml of absolute ethanol. This solution should be stable for two months.

PROCEDURE—

Dilute a 10-ml sample of plating solution to about 50 ml with water. Add 25 ml of EDTA, and mix the solution. Add 10 ml of ammonia - ammonium chloride buffer solution and 2 drops of Eriochrome black T indicator. Titrate the solution with the standard magnesium solution until the indicator changes from green to bright violet-pink.

RESULTS

The results obtained by the proposed procedure are compared with gravimetric results in Table III. Gravimetric determinations were carried out by precipitation of indium with ammonia solution after organic matter and cyanide had been destroyed with acid. The hydroxide was then ignited at 800° C and weighed as indium oxide, In_2O_3.

TABLE III

COMPARISON OF VOLUMETRIC AND GRAVIMETRIC RESULTS

Sample solution	Indium, mg by volumetric procedure	Indium, mg by gravimetric procedure
A	198·4, 198·6, 199·7 Mean 198·9	199·1, 199·1 Mean 199·1
B	168·6, 168·7, 168·5 Mean 168·6	168·3, 167·9 Mean 168·1
C	199·5, 198·5, 199·0 199·0, 199·5 Mean 199·1	198·8, 199·0, 199·2 198·7, 198·7 Mean 198·9

We thank the Chief Chemist, Ferranti Limited, for permission to publish this paper.

REFERENCES

1. Langford, K. E., "Analysis of Electroplating and Related Solutions," Electroplating and Metal Finishing, Teddington, Middlesex, 1951, p. 201.
2. Flaschka, H., and Amin, A. M., *Z. anal. Chem.*, 1953, **140**, 6.
3. Kinnunen, J., and Merikanto, B., *Chemist Analyst*, 1955, **44**, 50.
4. Diskant, E. M., *Anal. Chem.*, 1952, **24**, 1856.

Received *September 30th*, 1963

Rapid Elimination of Water in the Gas-chromatographic Determination of Chloroform in Aqueous Solutions

BY D. W. S. EVANS*

(*Physical Assay Division, Standards Department, Boots Pure Drug Co. Ltd., Station Street, Nottingham*)

IN estimating chloroform in largely aqueous solutions by the method of Brealey, Elvidge and Proctor,[1] slow elution and tailing of the final water peak gives rise to a delay of up to 30 minutes before a subsequent injection can be made.

Elimination of this time-lag by initial drying, or by allowing the samples to react with carbide in a pre-column[2] is impracticable because of the large percentages of water present, whereas back-flushing[3] presents no advantage over forward flushing since the (negative) water peak is normally eluted immediately after that of the n-propanol internal standard.

* Present address: "Avallon," 322a, Hucknall Road, Sherwood, Nottingham.

Bouthilet, Caputi and Ueda[4] obtained successful forward flushing of water from wine samples by inserting a three-way tap and two coiled capillaries of unequal length after the needle valve in the inlet carrier-gas stream, but I have found that a simple on - off by-pass tap is equally effective (see Fig. 1).

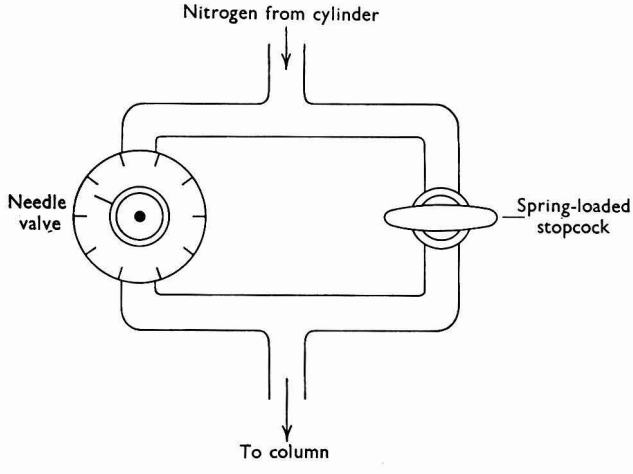


Fig. 1. Diagram of the apparatus

The above arrangement possesses several advantages—

(*i*) Only one single-bore glass stopcock and two T-pieces are needed. Little space is occupied, and the tap, which should be spring-loaded and well lubricated with silicone grease, may be conveniently mounted next to the needle valve.

(*ii*) With a 1-mm bore tap, 15 μl of water is removed almost completely from the column in about 45 seconds, this being indicated by a positive movement of the recorder pen. After a further 20 seconds the flush is turned off, and the next sample is injected. It is found in practice that the setting of the needle valve, and hence the gas flow-rate, is not altered perceptibly, and that the recorder base-line settles down before the first (ethanol) peak appears.

(*iii*) The device is useful also for rapid drying of the contents of freshly inserted pre-columns, and for removing the results of faulty injections from the main column.

The system has been in daily use in our laboratory for eighteen months, and has been applied successfully to other gas-chromatographic analyses in which katharometer detectors are used. In the determination of water[5] in mixtures containing ethylene glycol, it permits rapid removal of the latter strongly held compound, whereas for oil analyses with non-polar (squalane columns), *e.g.*, in the assay of cineole in oil of rosemary, a similar saving of time may be effected by flushing the late peaks from the column. There appears to be no reason why it should not be applied to the analysis of non-aqueous mixtures with detection by β-ionisation. For flame ionisation, an additional change-over device would be required between the column and the detector, thus protecting the flame from sudden changes in gas pressure.

I thank Mr. K. Calvert for practical assistance.

References

1. Brealey, L., Elvidge, D. A., and Proctor, K. A., *Analyst*, 1959, **84**, 221.
2. Kung, J. T., Whitney, J. E., and Cavagnol, J. C., *Anal. Chem.*, 1961, **33**, 1505.
3. Lichtenfels, D. H., Fleck, S. A., Burow, F. H., and Coggeshall, N. D., *Ibid.*, 1956, **28**, 1376.
4. Bouthilet, R. J., Caputi, A., and Ueda, M., *J. Ass. Off. Agric. Chem.*, 1961, **44**, 410.
5. Elvidge, D. A., and Proctor, K. A., *Analyst*, 1959, **84**, 461.

Received *August 8th*, 1963

An Automatic Starter for Paper Chromatography

By G. W. PEROLD,

(*Department of Chemistry, University of South Africa, P.O. Box* 392, *Pretoria, South Africa*)

H. J. JACOBS and J. M. C. VAN STADEN

(*Research & Process Development, S.A. Iron and Steel Industrial Corp., P.O. Box* 450, *Pretoria, South Africa*)

In the normal practice of paper chromatography, equilibration of the prepared sheet of paper with the vapour of the chromatographic tank before elution, is required for best results. When equilibration is feasible overnight and the elution time necessary is only a few hours, this permits subsequent drying, detection and evaluation of the chromatogram during the following working day so that the next run may be carried out in the same manner during the next 24-hour period. When longer elution times are necessary, particularly when impregnated paper is being used, this is no longer feasible. Such considerations led Husband[1] to the design of an automatic starter in which a solenoid-operated glass valve and an electric timer were used. Our own approach led to a simple mechanical starter based on an alarm clock and an automatic primer for a siphon delivering the chromatographic solvent to the trough.

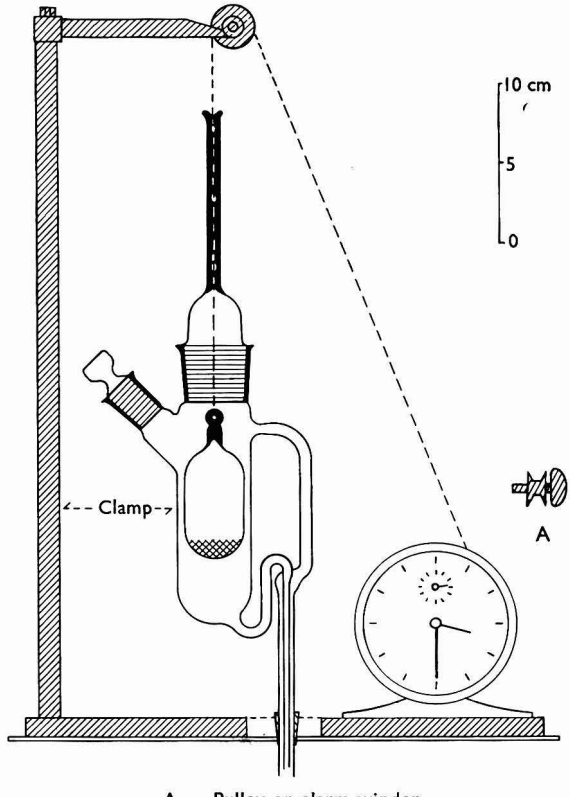


Fig. 1. Diagram of the apparatus

The automatic starter described here allows prepared sheets of paper to be equilibrated for any period up to 11 hours, commencing at the end of the working day, and then starts the elution 4 to 7 hours before the beginning of the next working day. Elution may then take place for up to 12 hours and still allow sufficient time for all manipulations during a 24-hour cycle.

An enclosed glass siphon (see Fig. 1), which accommodates 10 to 80 ml of solvent, is provided with a sealed glass plunger weighted with mercury. A strong, thin cord running over a pulley carries the plunger, passing into the siphon body through a capillary entry with three baffle spaces; the other end is wound for a few turns round a small brass pulley (separately shown at A in Fig. 1) that is fitted to the winding key of an ordinary alarm clock. The alarm clock is mounted on a Bakelite baseboard (1·2 cm × 11 cm × 28 cm) provided with a rod that carries the free-running pulley as shown. The delivery tube of the siphon passes through a 4-cm hole in the baseboard and fits into an orifice in the glass lid of the chromatographic tank through a cork stopper so that it is positioned directly above the solvent trough. In this manner the solvent is available for delivery while the tank is adequately closed against the outside atmosphere even when volatile solvents are used. The body of the siphon is held in a clamp (indicated but not shown in Fig. 1) so that the whole device is a self-contained and portable unit that can be readily positioned on the lid of the chromatographic tank.

To operate the starter the alarm clock is wound up with the cord carrying the plunger winding on the pulley of the modified winding key of the alarm clock, so that the plunger will descend when the alarm goes off and the alarm spring unwinds. The charged siphon is thus started and empties the solvent charge into the solvent trough so that elution proceeds.

We have been using this device for some years already, and have found it to be trouble-free and reliable in operation.

REFERENCE

1. Husband, L. J. B., *Chem. & Ind.*, 1954, 776.

Received *August 26th*, 1963

Book Reviews

GAS PHASE CHROMATOGRAPHY. VOLUME 1: GAS CHROMATOGRAPHY; VOLUME 2: CAPILLARY CHROMATOGRAPHY; VOLUME 3: TABLES FOR GAS CHROMATOGRAPHY. By RUDOLF KAISER. Translated by P. H. Scott. Pp. vii + 199; x + 120; x + 162. London: Butterworth & Co. (Publishers) Ltd. Price 42s.; 35s.; 40s.

Volumes 1 and 2 are intended to cater for the newcomer to gas chromatography, whereas Volume 3 is a reference book of gas-chromatographic data.

VOLUME 1—

Volume 1 is divided into three main parts: Theory, Apparatus and The Analytical Result. The first part starts by attempting to describe the chromatographic process by a somewhat juvenile analogy involving boats drifting down a river and then goes straight into the mathematics of the theory of gas chromatography. Heavy weather is made of the dependence of measured retention volumes on pressure drop along the column without pointing out that *relative* retention volumes are independent of operating parameters other than temperature and stationary phase. Even in Part 3 "relative retention values" are merely referred to in passing, without any previous definition or explanation.

Part 2 is subdivided into sections dealing respectively with columns, the carrier gas, sample injectors, detectors, recorders and temperature considerations. This part is a curious mixture of theory and inadequate practical details and in many places great stress is placed on points that are obvious, *e.g.*, with reference to the carrier gas (p. 65), "its physical and chemical state should, to the highest possible degree, be kept constant while it is flowing through the column and the detector." I like the technique described for impregnating solid support with fixed-phase liquid (p. 56), but the pre-treatment of the solid support gets merely a passing reference (p. 58). The types of sample injector and detector also are, I think, dismissed too briefly, as also is the technique of temperature programming. The statement (p. 74) that "a gas cylinder will, of course, provide a practically constant pressure source over a more or less long period of time, since normally only a little gas is removed" is vague and misleading; such a sentence might be expected to be written in a fourth-form examination paper rather than a textbook.

Part 3, which deals with the qualitative and quantitative interpretation of chromatograms, is adequate. The main defect of this part is one from which the whole book suffers, *i.e.*, an English style that is not easy to read (though this frequently happens with translated works), including

a few examples of what I described as "Pen of my Aunt" English, and a too-generous sprinkling of the exclamation marks so beloved by the Germans. Details, possibly, but these add up to a real irritation to the reader.

VOLUME 2—

This volume, again, is divided into three parts of similar scope to those of Volume 1 with reference specifically to capillary columns.

Part 1, "Theory," closely follows the general plan of Part 1 of Volume 1, even including the "boats on the river"!

Part 2, "Methods and Apparatus," is arranged similarly to its counterpart in Volume 1, the section on the coating of capillaries (pp. 49 to 56) being particularly well done. The methods of sample injection are reasonably adequately dealt with, as also are the flame-ionisation and β-ray detectors.

Part 3, "Applications," is almost a repetition of Part 3 of Volume 1; this is to be expected as it deals with the interpretation of chromatograms and the author intends Volume 2 to be usable without constant cross-reference to Volume 1.

VOLUME 3—

This volume contains the following tables—

(*a*) Column packings for given separations, the information being given not directly, but in the form of a reference to the bibliography.

(*b*) Stationary phases and their main applications.

(*c*) Retention data: given, not as relative retention times referred to specified standard substances, but calculated as the Retention Indices of Wehrli and Kovāts (*Helv. Chim. Acta*, 1959, **42**, 2709). This system would have advantages if generally adopted, but it is not possible to calculate these Retention Indices back to the relative retention volumes as used by most analysts.

(*d*) Peak-area factors for use in quantitative calculations.

(*e*) Other useful data.

GENERAL OBSERVATIONS—

The irritations commented on in connection with Volume 1 are repeated in Volumes 2 and 3. I think Volume 3 might be the most useful of the three. Apart from the inevitable overlapping of Volumes 1 and 2, I consider the price excessive for the information in them, especially in view of the difficulty with which the reader will extract it; in fact, this work falls surprisingly below the high standard we have come to expect from Butterworths. B. A. ROSE

STANDARD METHODS OF CHEMICAL ANALYSIS. Sixth Edition. Volume II. INDUSTRIAL AND NATURAL PRODUCTS AND NONINSTRUMENTAL METHODS. PARTS A AND B. Edited by FRANK J. WELCHER, Ph.D. (Part A), pp. xiv + 1282; (Part B), pp. x + 1283–2613. Princeton, N.J., New York, Toronto and London: D. Van Nostrand Company Inc. 1963. Price (both parts) £9 9s.

This new edition of Volume II of this well known work has been almost completely revised; new chapters have been added, and its size is approximately double that of the fifth edition. The decision to produce it in two parts, bound separately, is therefore a wise one.

The general arrangement of material is as before. However, an extensive new part, "Apparatus, General Operations and Reagents" (pp. 1 to 532) has been added. This consists of 21 chapters, 13 of which are new, namely, Standard Laboratory Apparatus; Detection of the Cations and Anions; Mechanical Separation; Separation by Precipitation; Separation by Electrolysis; Solvent Extraction; Separation by Distillation and Evaporation; Chromatography; Ion-Exchange Methods in Analysis; Final Gravimetric Treatment; Acid - Base Titration in Non-Aqueous Solvents; Statistical Interpretations; and Quantitative Organic Analysis. Here a wealth of information, concisely presented, is to be found. However, in some of the chapters, little critical selection is evident, and in others much is left unsaid. Thus in the section on volumetric apparatus, the treatment of drainage errors is inadequate; there is useful guidance on setting and reading the meniscus of a pipette, but none on how to treat the tip after delivery. In the section

on chromatography, there is no reference to thin-layer methods. In the chapter on final gravimetric treatment, the usual misleading thermogravimetric data on Al_2O_3 are perpetrated, but $Mg_2P_2O_7$ is not mentioned. The section on electrolysis does not introduce the concept of a limiting current density; the author also makes the common American mistake of referring to International Critical Tables for information on hydrogen overpotential; we are therefore yet again referred to the archaic (1923) and misleading work of Knobel, Caplan and Eiseman. Elsewhere, the selection and presentation of information deserves much praise and perhaps it is not surprising that the most successful chapters are those dealing with modernised classical methods.

Part Two deals with "Special Techniques for Industrial Products and Other Special Substances." This consists of 29 chapters of which 10 are new, namely, Air Pollutants; Amino Acid Analysis of Protein Hydrolysates; Chemical Analysis in Clinical Medicine; Fertilizers; Gas Analysis - Vacuum Techniques; Pesticides; Plastics; Silicates; Glasses, Rocks and Ferrous Slags; Soils; and Vitamins. Many of the chapters are résumés of standard methods laid down by such bodies as A.S.T.M., A.O.A.C., and the American Oil Chemists' Society. Others are more personal collections. The chapter on amino-acids, which is reprinted from elsewhere, gives a good account of chromatographic methods, but the statement (p. 950) that these have largely supplanted microbiological methods would be challenged by many. The same aversion to microbiological methods is shown in the chapter on Vitamins; only for biotin is chemical defeat admitted! The chapter on clinical analysis brings together methods that would otherwise be difficult to find. However, despite the introduction of new chapters, one major omission is still evident—the analysis of foods.

There is a comprehensive index at the end of Volume II, Part B, and the printing and binding are of high standard. This new edition of "Scott" is becoming extremely bulky and one wonders if this is the last time it can keep its unique place, intermediate between the single-volume texts and the encyclopaedic treatises on chemical analysis. J. F. HERRINGSHAW

MODERN METHODS OF ANALYSIS OF COPPER AND ITS ALLOYS. By CHARLES M. DOZINEL. Second, Revised Edition. Pp. xviii + 287. Amsterdam, London and New York: Elsevier Publishing Company. 1963. Price 80s.

It is surprising how many laboratories *outside* the non-ferrous industry analyse copper-base materials. In view of this, and because of the large number of laboratories engaged exclusively in the analysis of copper and copper alloys, why is it that so few books on this subject have appeared in recent years? This in no way reflects on the analysts in question, because a glance at the contents page of this book will show the extent to which procedures, such as those based on polarography, spectrophotometry, including atomic absorption, are widely used, and how the more established procedures, based on emission spectroscopy have been brought up-to-date and extended, for example, by the use of direct-readers.

The author has made an extensive collection of published analytical procedures pertinent to the title of his book with a view to presenting this information in perspective. Hence the book draws heavily on the work of other analysts, with clear indications in several places that the author is not quoting from his own experience.

How far his objective has been achieved is not clear. For example, I was perplexed by the contents of the first paragraph in the first chapter which reads "*Before we deal with this subject, an important observation should be made. As chemists, we have studied analytical wet methods utilising purely chemical means (classical methods) or those involving physico-chemical techniques (modern methods). The spectrographic method is a physical method and should be not then be dealt with in this book.*" This hardly justifies an apology on the part of the author, or further comment by me.

The book cannot be claimed to be complete in itself because over 1100 references are given. In many respects this is a commendable feature, although a brief indication of what the references contain would have added considerably to their usefulness.

Anyone experienced in this field of analysis must agree that the book contains a wealth of information, but it is doubtful whether, in certain sections, the information has been presented in the best possible way, and the inexperienced analyst will have to resort to careful reading of other published work in order to make a reliable appraisal of some of the information.

The book is divided into several chapters; the first, occupying about 43 pages, comes under the heading "*Spectrographic Method*" and Chapter 2 "*Chemical Wet Methods (General).*" This takes the reader up to about page 86 and thereafter separate chapters deal with specific typical determinations, under such sub-headings as *Gravimetry*, *Titrimetry*, *Polarography* and *Colorimetry*.

Chapters 26 onwards deal with "*Determination of Special Elements (References)*," "*Inclusions—Gases*," "*Combined Procedures*," and a final chapter under the heading "*Miscellaneous*."

For a book of this coverage, the price is not unreasonable, and anyone with a detailed, or even a passing interest in the analysis of copper and its alloys will find the book, by present monetary standards, value for money.

W. T. ELWELL

ANALYTICAL CHEMISTRY 1962. Edited by PHILIP W. WEST, A. M. G. MACDONALD and T. S. WEST. Pp. xii + 411. Amsterdam, London and New York: Elsevier Publishing Company. 1963. Price 90s.

This is the volume of papers read at the Professor Fritz Feigl 70th Birthday Commemoration Symposium, held at Birmingham University in April 1962. The meeting was international in character: it was organised by the Midlands Section of the Society and was supported by I.U.P.A.C. Those of us who attended will remember with pleasure the occasion, so well organised and full of interest. It was an occasion not only for paying homage to the doyen of microchemistry, who attended in person, but also (as with most modern symposia) it was an excellent opportunity for meeting and discussing analytical ideas and problems with friends and colleagues from here and overseas.

The papers read were generally of a high standard, covered a wide range of analytical technology and invariably evoked much stimulating discussion. Many eminent international figures in the world of analytical chemistry attended as delegates or read papers, a fitting tribute of the esteem held for Fritz Feigl. In all, some 400 delegates from 28 countries attended, and some 60 papers were read.

The editors have arranged these papers in workman-like fashion, and as a result we are presented with a treatise filled with contributions of topical and lasting value. Most of the contributions are quite short, the speakers generally being rationed to 20 minutes. Consequently, there is usually much substance packed into a few lines.

Since the lecturers were from both academic circles and industry, the papers included contributions fundamental to analytical chemistry, and also applications of analytical technique to specific practical problems. This mingling of interests was one of the most useful aspects of the Symposium, the results of which are now enscribed in this permanent record of the proceedings.

The arrangement of the papers delivered falls into three classes: qualitative analysis with particular reference to spot-tests; organic reagents and their applications; and various instrumental methods of analysis. This is also the arrangement that has been followed by the editors in compiling this volume.

Feigl's plenary lecture on the "Spot-test Analysis" is the longest contribution, occupying 9 pages: most of the others are limited to 3 or 6 pages, and the whole 60 contributions are contained in 408 pages. With a collection of papers covering so wide a field, it is pointless to single out any for special mention in a short review. Suffice it to say that those who attended will certainly wish to possess this record, and those analysts unable to participate are recommended to obtain it as a useful guide to the present position in fundamental and applied analytical chemistry.

R. F. MILTON

CHROMATOGRAPHIC REVIEWS: PROGRESS IN CHROMATOGRAPHY, ELECTROPHORESIS AND RELATED METHODS. Volume 5. Edited by MICHAEL LEDERER. Pp. x + 244. Amsterdam, London and New York: Elsevier Publishing Company. 1963. Price 60s.

Continuing the practice of recent years, *Chromatographic Reviews*, Volume 5, contains some previously unpublished reviews along with others, in this instance an equal number, reprinted or translated from the *Journal of Chromatography*. In future, however, *Chromatographic Reviews* will become the exclusive medium for reviews, none of which have been published elsewhere. This separation is to be welcomed, and librarians can no longer doubt whether both publications are really necessary. Almost every reader must have impatiently thumbed through the pages for a reference, and then looked up one belonging to the following chapter. Here a welcome innovation obviates this frustrating experience; it is simply an unobtrusive footnote on every page saying "references, p. ..."

The first review by Waldman-Meyer deals courageously with the theoretical background of zone electrophoresis, after quoting, "Probably no physico-chemical method has ever been used

so extensively with so little knowledge of its fundamentals. But to obtain true mobilities, so many disturbing factors need to be taken into consideration, corrections for some of which remain uncertain, that most workers will continue to use the technique as an invaluable empirical tool. In complete contrast there follows a short paper by von Arx and Neher on the design of the Chromatography Laboratory of the Pharmaceutical Research Division of CIBA. It contains little text beyond detailed lists of fittings and equipment, clearly related by code numbers to layout diagrams, and illustrated by 12 photographs of the various rooms. This should prove very helpful to anyone needing to plan for a considerable volume of chromatographic work. The next review on paper chromatography and chemical structure by Green, Marcinkiewicz, and (in part) McHale is divided into 8 sections and occupies nearly half the volume. It opens with the startling notion that present techniques are incapable of yielding R_F values precise enough to form a basis for valid calculations. One can only escape from the uncertain dis-equilibrium between vapour and hydrophilic cellulose by abolishing both! The answer is reversed-phase tankless paper chromatography between sheets of metal foil, which gives R_F values reproducible to 0·01 or less. Under these conditions Martin's theoretical predictions of constant increment in the R_M values for homologous series are readily verified. With aromatic and polycyclic compounds the value of ΔR_M for a CH_2-group is altered by constitutive effects of various kinds, such as proximity to the ring, ortho effects and so on. Nevertheless, atomic R_M values can be calculated conveniently if all these effects are ascribed not to carbon but to hydrogen in different positions. The authors have achieved remarkable success in extending their calculations to such complex molecules as the tocopherols and ubiquinones. This approach has potential application in structure determination. In subsequent sections they show how to cope with intramolecular hydrogen bonding, tautomerism and hyperconjugation, and discuss the reasons why some *m*- and *p*-isomers can be separated chromatographically.

Counter-current distribution of organic substances is exhaustively and tersely treated by Casinovi in a 40-page review with nearly 500 references. This should prove extremely helpful when looking for likely solvent systems for a new separation problem. Oakey provides a brief but adequate review on paper chromatography of oestrogens and derivatives, with numerous tables of R_F values: special consideration is given to separation of epimeric and recently isolated oestrogens. Finally, Tadmor has surprisingly found it possible to write a dozen pages with over 100 references on gas chromatography of inorganic compounds. The international flavour of these volumes has been maintained by contributions from five countries, nicely balanced between academic and industrial institutions. The quality of material and production is well up to the standard of previous volumes.

E. LESTER SMITH

Errata

JANUARY (1964) ISSUE, p. 19, equation (4.4). Insert a rule between the numerator and denominator in right-hand brackets.

IBID., p. 20, equation (7·2) denominator of the fraction in the centre brackets. *For* "$\bar{N}$" *read* "$\bar{N}w$".

IBID., p. 21, 12th line reference at end of line. *For* "2" *read* "4".

IBID., p. 25, 3rd line from foot of page. *For* "functions" *read* "fractions".

IBID., p. 30, reference 2. *For* "670" (the page number of the reference) *read* "81".

IBID., p. 30, reference 4. *For* "81" (the page number of the reference) *read* "682".

IBID., p. 80. *Review of* BRITISH PHARMACOPOEIA 1963.

An inadvertent transposition led to the inclusion in this Review of a statement that biological assay was used for thyroid. Thyroid is in fact assayed by an oxygen-flask method. The fourth paragraph of the Review should be amended to read—

"It is interesting to note that in some instances (benzylpenicillin, benzathine penicillin and procaine penicillin) it has been possible to replace biological assay with chemical tests and assays."

FEBRUARY (1964) ISSUE, p. 121, reference 6. This should read "Merck Index, Sixth Edition, Merck & Co. Inc., Rahway, N.J., U.S.A., 1952, p. 895".

Notice to Authors

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

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

Every paper will be submitted to at least two referees, by whose advice the Editorial Committee of *The Analyst* will be guided as to its acceptance or rejection. Papers that are accepted must not be published elsewhere except by permission of the Committee. Submission of a manuscript will be regarded as an undertaking that the same material is not being considered for publication by another journal.

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

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

Proofs—The address to which proofs are to be sent should accompany the paper. Proofs should be carefully checked and returned within 48 hours of receipt.

Reprints—Twenty-five reprints, or a maximum of fifty if there is more than one author, are supplied gratis. Additional reprints may be obtained at cost if ordered directly from the printers, W. Heffer & Sons Ltd., Hills Road, Cambridge, at the time of publication. Details are sent to authors with the proofs.

NOTES ON THE WRITING OF PAPERS FOR *The Analyst*

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

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

The recommended order of presentation is as indicated below—

(*a*) Synopsis.

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

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

(*d*) Presentation of results.

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

(*f*) Discussion of scope and validity.

(*g*) Summary and conclusions.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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