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No. 878, Pages 285—332

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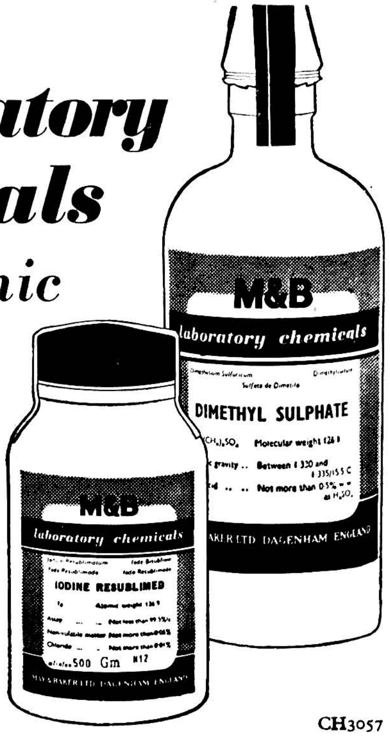
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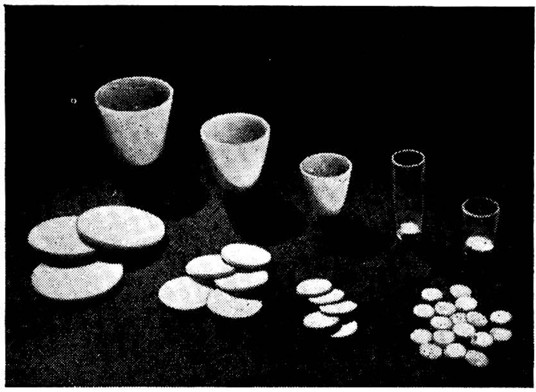

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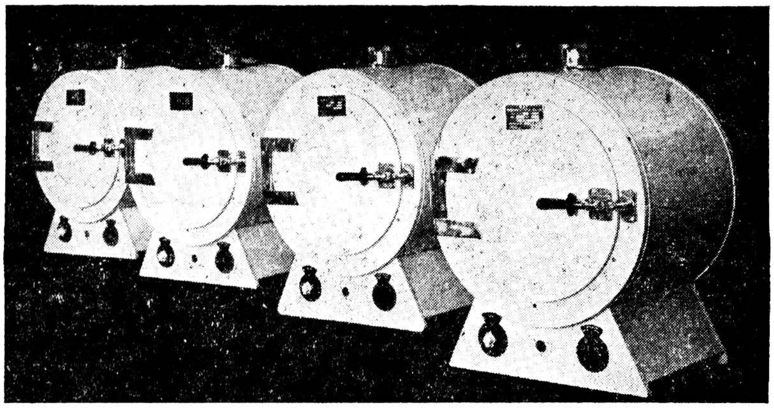

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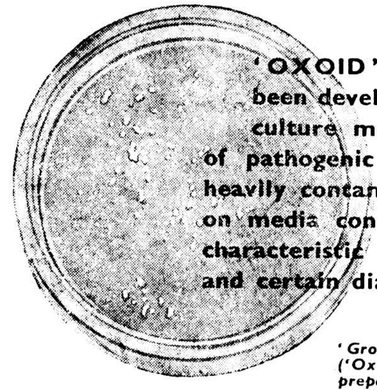

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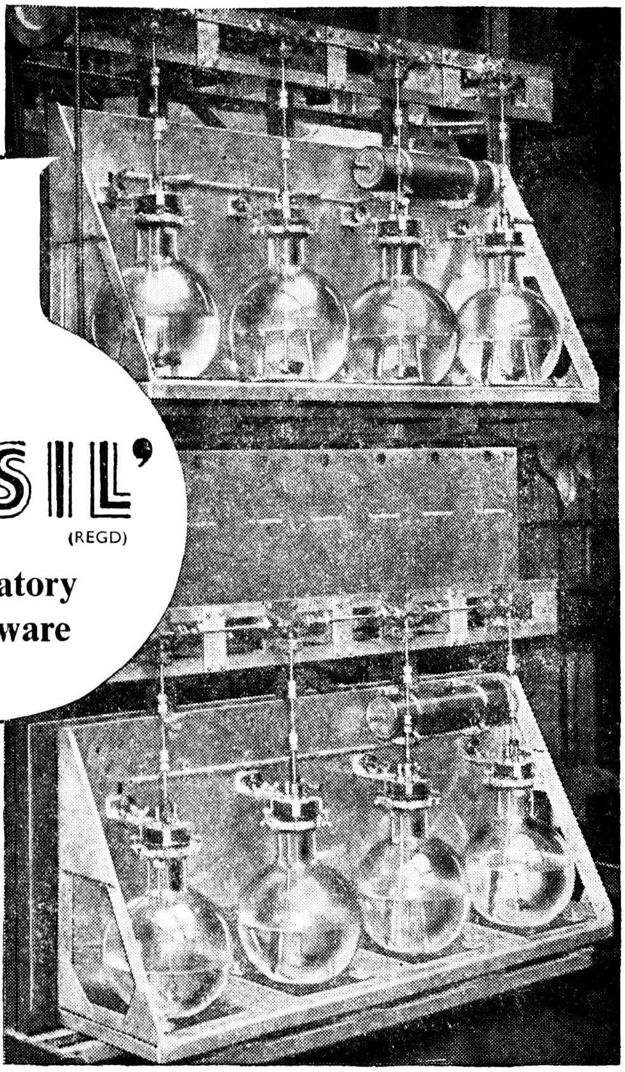

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THE ANALYST

PROCEEDINGS OF THE SOCIETY OF PUBLIC ANALYSTS
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AN Ordinary Meeting of the Society was held at 7 p.m. on Wednesday, May 4th, in the Hall of the Royal Society of Tropical Medicine and Hygiene, Manson House, 26 Portland Place, London, W.1, with the President, Mr. George Taylor, in the chair. The following papers were presented and discussed: "The Colorimetric Determination of Streptomycin B (Mannosido-streptomycin)," by W. B. Emery, B.Sc., F.R.I.C., and A. D. Walker, B.Sc., A.R.I.C.; "The Chemical Determination of Nicotinic Acid in Food Products," by P. O. Dennis, B.Sc., A.R.I.C., and H. G. Rees, B.Sc., Ph.D., A.R.C.S., F.R.I.C.; "The Reduction of Antimonial Tin Solutions with Metallic Nickel and Cobalt," by H. Holness, M.Sc., F.R.I.C.

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The Polarographic Analysis of Light Metals and Alloys: A Survey

BY W. STROSS

(Lecture delivered at a meeting of the Physical Methods Group organised by the Polarographic Discussion Panel on April 9th, 1948, at Leeds)

NO clear definition seems to exist as to where lightness of metals ends and where heaviness begins. There is hardly any doubt that aluminium is a light metal and zinc is not, but no rule or even usage seems to exist about metals with specific gravities between these two. In the following, the limit will be drawn at the specific gravity of barium, 3·5, but a few remarks about the rare earth metals will be included.

It is intended to concentrate upon less known and less accessible publications; a number of well known monographs[7,29,40] and papers,[18,27,30] and the writer's own papers[31,32,33,34] on the determination of zinc and lead in aluminium alloys will be referred to only in the bibliography.

In this survey of methods for the analysis of light metals and alloys the determination of alloying constituents and impurities alone is being considered, not the determination of the base metal. Thus the section "Magnesium" deals with the determination of impurities or alloying constituents, which themselves may be light or heavy metals, in magnesium metal or magnesium-based alloys.

The estimation of the base metal is rarely carried out in metallurgical practice, even by gravimetric or volumetric methods; polarographic methods are, generally speaking, less suitable, and therefore still less frequently used. An exception is the determination of copper and zinc in brass,[35] but this does not come within the scope of the present survey, in which (in addition to its limitation to light metals and alloys) emphasis is placed on applications of polarography that have an obvious advantage over other methods.

Although the survey deals with metals in the first place, much of it will apply to the testing of salts (chemicals) as well; for the metals are tested in the form of salt solutions during the polarographic procedure. This should be emphasised, as the polarograph does not seem to have found the wide application it deserves in the testing of chemicals for impurities.

1. THE ALKALI METALS

For general information about the determination of the various alkali metals themselves, see Heyrovsky's[7] or Kolthoff and Lingane's monographs[40]; as explained before, their determination only comes within the scope of this section if one of them should be a constituent or an impurity of a metal or alloy of which another one is the base. In view of the coincidence of the half-wave potentials of sodium, potassium, rubidium and caesium this only offers any promise with lithium metal or lithium based alloys, and even there the distance between the half-wave potentials is only about 0·15 v., rather smaller than is desirable.

The alkali metals have a very negative half-wave potential; they show little tendency to hydrolyse or to precipitate in the course of pH adjustment or on mixing with the usual ground solutions; the polarographer can therefore expect to have a free hand in obtaining satisfactory waves of a variety of components or impurities. Many of the difficulties encountered in the analysis of, say, aluminium and ferrous alloys, would not arise here at all.

In spite of these very favourable conditions, surprisingly little has been published on the subject; an old paper by Hohn[1] on impurities in lithium was all that could be found in the literature. As lithium has the most negative half-wave potential of all the alkali metals, it is possible to determine other alkali metals (or at least their sum) in presence of lithium, apart from all the nobler metals.

2. ALKALINE EARTH METALS

The remarks above about alkali metals—favourable conditions and yet little research work done—apply almost as much to the alkaline earth metals (except magnesium and magnesium alloys).

The reason obviously lies in the comparatively small practical importance of these metals, but again it should be pointed out that polarographic methods can be applied to salts, *i.e.*, to chemicals as well as to metals. It should be of some practical importance that under suitable conditions calcium, barium and strontium can be distinguished from each other polarographically. According to the sequence of their half-wave potentials,[2] a small content of calcium could not be determined polarographically (at least not without resorting to chemical separation) in a base of barium or strontium; but the determination of barium or strontium in calcium metal or salts should be nearly as easy as that of any of the "classical" objects of polarographic technique, say cadmium or zinc.

It may be worth while pointing this out, as alloys of barium and aluminium in a calcium base find practical application as "getters" in radio and vacuum technique; it appears that so far no use has been made of this possibility and a recent monograph about the metallurgy and technology of calcium* does not seem to contain the word "polarography" at all.

A paper on the determination of strontium as impurity in calcium carbonate has been published in Germany[3] during the war.

Further data are contained in Heyrovský's book.[4]

Before going over to those light metals that are usually thought of when this term is used, *i.e.*, magnesium, aluminium and beryllium, a few short remarks may be made about the rare earth metals.

3. RARE EARTH METALS

These metals are included in this survey for two reasons. One is sentimental, or historical: it is interesting that the only polarographic experiment that could be found about one of the most important elements of this group dates back to one of the very first papers in which the polarograph was described by Heyrovský and Shikata[37] and the word "polarography" was coined. One of the examples given there of the possible applications of the new technique is a beautiful wave of lead as impurity in cerium metal, and it becomes clear from this graph that here, as with the alkali and alkaline earth metals, practically the entire voltage range is available for the determination of whatever other elements may need to be determined. This similarity is the second reason for including the rare earth group in this survey, although only some of its members have a specific gravity smaller than that of zinc.

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* *Calcium, Metallurgy and Technology*, by C. L. Martell and C. Hardy, Reinhold Publishing Corporation, New York, 1945.

There is, however, one rare earth metal that has not a very negative reduction potential—europium, with a half-wave potential of only $-0{\cdot}77$ v.—a circumstance that has been utilised for determining this element in presence of others of the same group[38]; the same paper also deals with the determination of zinc in presence of rare earth metals other than europium.

4. MAGNESIUM

Magnesium and its alloys are of much greater practical importance than the metals previously referred to.

In Gull's well known method,[5] the metal is dissolved in hydrochloric acid and the pH is adjusted to the lowest acidity at which aluminium will remain unprecipitated, bromophenol blue being used as indicator.

Lead, cadmium, zinc and aluminium, if present in suitable proportions, can be determined in one operation, and as the existing commercial alloys fulfil this condition the method is very useful. It should even be possible to obtain at the same time a semi-quantitative determination of small quantities of copper and iron; but owing to the great ease with which these elements can be determined photometrically, this point has not been followed up.

The size of sample recommended by Gull (80 mg. per 200 ml. of final solution)—unless this figure is a misprint—seems rather small; it may be large enough for the aluminium in alloys containing 5 to 10 per cent. of this metal, but a concentration about 10 times greater (100 to 200 mg. per 25 ml. of final solution) seems preferable in order to obtain waves of suitable size for the other elements, particularly lead. This requires, of course, an adequate increase of the quantity of hydrochloric acid used.

With very high aluminium contents (15 per cent. and more) the use of the lowest sensitivity of the instrument and possibly a slight dilution of the solution may be necessary.

Gull's method would fail if the nobler metals were present in much larger quantity, and difficulties would also arise in presence of substantial amounts of tin or nickel, as the wave of bivalent tin coalesces with that of lead and the wave of nickel with that of zinc.

In commercial alloys these two elements are rarely present in alloying quantities (though Specification DTD350A does call for 4·5 to 7·5 per cent. of tin) and it is easy to test for their presence by well known methods; the photometric and volumetric methods in general use for determining nickel and tin in aluminium alloys can be applied without substantial modification to magnesium alloys.

With uncommon alloys or "freaks," encountered occasionally, particularly among swarfs and similar material, it is useful to apply the method published by the writer for the determination of lead in aluminium alloys.[33] The use of this procedure, which is a simplification of Kolthoff and Matsuyama's technique,[18] will overcome a number of possible difficulties: copper will be precipitated as the thiocyanate and iron will be reduced; tin will be oxidised to the quadrivalent form, in which it does not give a wave at all at the pH of the solutions; the waves of nickel and zinc are well separated in this medium. No interference from any of these elements is therefore to be expected in the determination of lead and zinc.

If this method[33] is to be applied to magnesium alloys, it is necessary to modify the procedure slightly in order to obtain the required pH. A short description of the two techniques is given below for greater clarity. (A) is essentially Gull's technique, with minor modifications. (B) is the writer's.

(A) *Copper, tin and nickel absent*—Attack a weighed sample, 100 to 200 mg., in a 25-ml. volumetric vessel (a Pyrex boiling tube, 6 in. by 1 in., carefully calibrated at 25 ml. and 40 ml., is very suitable), with 2 ml. of 5 *N* hydrochloric acid and a little water. Complete the reaction by immersing the vessel in hot water. Cool and add 0·1 ml. of 0·04 per cent. bromophenol blue solution containing 1·5 ml. of 0·1 *N* sodium hydroxide per 100 mg. of the dye. Then add, dropwise and with shaking, 1 *N* sodium carbonate* until the colour changes to green or blue. (The dye is bright yellow up to pH 2·8 and blue to violet beyond 4·6; green is the intermediate colour. As the solution is not strongly buffered if little aluminium is present, 1 drop of 1 *N* alkali easily makes the colour change from yellow to blue without any green becoming apparent.) Adjust the colour to green by adding, dropwise and with shaking, 0·1 *N* hydrochloric acid. Add water nearly to the 25-ml. mark. Allow to stand for several hours, to ensure that all the aluminium is in solution even if it has been temporarily precipitated, and observe the colour from time to time, adding some more indicator, if required, and adjusting to green, if necessary, by adding a drop or two of approximately 0·1 *N* sodium carbonate or hydrochloric acid. Add 0·5 ml. of 0·25 per cent. gelatin solution and make up to the mark. Mix and take a portion for the polarographic test, or, if a silver wire anode be used[39] (this is particularly advantageous in combination with the use of the above-mentioned calibrated boiling tubes), de-aerate and polarograph directly in the tube. This latter technique has the further advantage that, after the steps of lead and zinc have been recorded, the solution can very simply be adjusted to 40 ml. for further dilution should it be found too concentrated to give a suitable aluminium step with alloys containing 10 per cent. or more of this element. It is obvious that in this event a calibration must be made under the same conditions.

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* The author prefers sodium carbonate to caustic soda, recommended by Gull, as with the former it is easier to avoid temporary precipitation of aluminium.

(B) *Copper, iron, tin or nickel present in substantial quantity*—For determining lead and zinc in presence of the above metals, proceed as described for the determination of lead in aluminium alloys,[33] but before adding hydroxylamine and thiocyanate (for the purpose of reducing the iron and precipitating the copper), adjust the reaction as described in (A) above.

This procedure is, however, not suitable for the simultaneous determination of aluminium; although aluminium gives in this medium a wave *qualitatively* not inferior to that obtained by Gull's method, the results are low if the alloy contains substantial quantities of copper, probably owing to co-precipitation of aluminium with the copper at the low acidity necessary for precipitating the copper and reducing the iron by the combined action of hydroxylamine and potassium thiocyanate. In such circumstances a second sample has therefore to be processed by Gull's method, or the modification described above in (A), for the determination of aluminium; as the alloys in question practically always contain much more, or at least not less, aluminium than copper, iron, zinc, tin, lead or cadmium, none of these elements will cause any difficulty in the determination of the aluminium, although their waves precede that of aluminium. It is worth noting that the polarographic method is suitable only for comparatively large aluminium contents (say 1 per cent. and more) because of the hydrogen wave, which immediately precedes that of aluminium. As complex-formers inhibit the aluminium wave altogether and as lowering the acidity leads to hydrolysis (with deterioration of the wave), a compromise must be found, *viz.*, moderate acidity and high aluminium concentration. At the low galvanometer sensitivity required, the hydrogen wave does not then interfere. To process a second sample according to Gull's technique in such cases has a further advantage. Whilst in the thiocyanate medium the waves of nickel and zinc are separated, the nickel wave coalesces with that of cadmium; this is not so under Gull's conditions. The use of both techniques will therefore also contribute to clarify the situation if cadmium, nickel *and* zinc should all three be present; this, however, will be a very rare occurence.

Semerano and Ronchi[8] have severely criticised the reproducibility of results by Gull's method. The writer is disposed to think from his experience that their criticism is too severe.

Semerano and his school have published three different techniques for the polarographic analysis of magnesium.[8–10]

The first is very similar to that of Gull, except for the use of a different indicator: a mixture of methylene blue and methyl yellow, claimed to give a particularly sharp colour change, from violet to green, at pH 3·25.

The second method also begins by dissolving the alloy (0·1 g.) in hydrochloric acid but continues by separating the alloying elements from the bulk of the magnesium; this is effected by carefully neutralising to methyl red with dilute ammonia solution and then adding ammonium sulphide solution and centrifuging. The precipitate is washed by centrifuging, lithium chloride being added to the washing water to prevent any peptisation of aluminium, and the residue is dissolved in diluted hydrochloric acid (1 + 1). The solution is neutralised with lithium hydroxide, using the mixed indicator mentioned above, and made up to volume. After recording the wave of aluminium, methyl red is added and then some more lithium hydroxide, until the methyl red becomes yellow; the other components are then recorded, with adequate sensitivity, starting from zero potential. Owing to the very negative deposition potential of lithium, the diffusion current of the aluminium is very well defined under these conditions.

The third technique, dealing with impurities in pure magnesium metal, is not accurately described; it seems to consist in dissolving so much metal in hydrochloric acid that a practically saturated solution is obtained.

The work of Semerano and his colleagues has also been extended to the determination of traces of sodium in magnesium (further data about this have also been published by Heyrovský[6,7]) and further to the determination of antimony,[11] which seems to be a constituent of Italian magnesium alloys. In this method 0·5 g. of the alloy is dissolved in hydrochloric acid, with addition of a few drops of concentrated nitric acid, and then twice evaporated to dryness with addition of some hydrochloric acid, to drive off the nitric acid; the residue is taken up in Hohn's ground solution E (hydrochloric acid with gelatin) and antimony and lead are determined. An aliquot of this solution is then evaporated to dryness and the residue taken up in Hohn's ground solution C (ammoniacal ammonium chloride solution with tylose) for the determination of zinc and manganese. Aluminium and iron can eventually be determined by dissolving in hydrochloric acid the residue that remains undissolved in the ammoniacal ground solution C and neutralising with lithium hydroxide as described before.

The writer cannot claim experience with any of Semerano's methods.

The determination of copper, zinc and aluminium is also described in a report by Payne.[13]

5. ALUMINIUM

For practical purposes this is by far the most important of the light metals and it has accordingly been the subject of the greatest volume of published work, including a well known monograph,[29] and a comprehensive scheme,[18] which cannot be discussed here in detail for reasons of space.

In the writer's opinion the methods for zinc and lead deserve—at least at present—primary consideration, as for them the polarographic technique seems to offer greater advantage, in comparison with the other available "wet" methods, than it does in respect of the other elements (such as copper, iron and nickel) that also *can* be determined polarographically, but for which very rapid and good photometric methods are available.

A. *Determination of zinc*—Various methods have been proposed, many of which[18,19,29] were reviewed in the writer's papers[31,32] describing his own method.* About the latter method it may suffice to say that it has found large-scale routine application over a number of years and has proved to be reliable, rapid[34,36] and applicable to a very wide range of alloys.

Attention should be drawn to a method,† hitherto unpublished, by H. C. Davis,[28] from the Royal Aircraft Establishment, Farnborough. It begins with *aqua regia* attack, followed by neutralisation with ammonia in presence of citrate.

If much copper is present it is separated by addition of some sodium sulphide in presence of excess of 1 *N* sulphuric acid, followed by filtration. An aliquot of the filtrate is neutralised and excess of formic acid and again some sodium sulphide are added. In presence of only small amounts of copper, the first separation is unnecessary and the combined sulphides of copper and zinc are precipitated in the formic acid medium.

The sulphide precipitate is extracted with a measured quantity of 2·5 *N* sulphuric acid, excess of sulphide is destroyed by bromine, the excess of which is boiled off, and the solution is then poured into an excess of sodium hydroxide solution so adjusted that the final concentration becomes 0·1 *N*. After addition of a standard solution of lead and enough gelatin to produce a final concentration of 0·002 per cent. the solution is made up to the mark and the polarogram recorded. The lead addition serves as internal standard or "pilot-ion."

The "pros" and "cons" of this procedure cannot be discussed here in detail, but it should be pointed out that the separation from the bulk of the aluminium makes it technically easy to restrict the final alkalinity to 0·1 *N*, which produces very well defined graphs, as illustrated in Fig. 1.

The same principle of separation from the bulk of the metal, *i.e.*, from the aluminium, by means of sulphide, has been used by Semerano and Capitanio[24–26] for the determination of impurities in "super-pure" aluminium. In order to attain great sensitivity, these authors begin with a 5-g. sample. After an alkaline attack, using very pure potassium hydroxide and redistilled water, a small quantity of hydrogen sulphide is passed slowly into the alkaline solution and the precipitate is collected by centrifuging and washed with dilute sulphide solution. The residue is taken to dryness with nitric acid. From this residue copper, cadmium, nickel and zinc are extracted with 1 ml. of an ammoniacal solution, which is 0·5 *N* in ammonia, 0·1 *N* in ammonium carbonate and 1·5 *N* in ammonium chloride and contains 5 per cent. of sodium sulphite and 0·005 per cent. of gelatin. After centrifuging, the polarogram is recorded in a suitable cell. The elements mentioned above can all be recorded in one graph. The residue in the centrifuge tube is washed, dissolved in hydrochloric acid and taken to dryness. The residue is extracted with 1 ml. of a solution similar to Hohn's ground solution P (9 parts of saturated ammonium chloride solution, 10 parts of 2 per cent. tylose solution and sufficient hydrochloric acid to make the mixture 0·1 *N*) and then the waves of iron and lead can be recorded.

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* After this paper went to print a paper by B. A. Scott appeared (*Analyst*, **1948**, **73**, **613**). The method described, however, is applicable only if the alloy does not contain much more nickel than zinc, and therefore fails with many important alloys.

† Thanks are due to the Ministry of Supply and to Mr. Davis for permission to discuss the method here.

Owing to the size of the sample taken and the small volume of the final solution, as little as 0·00003 per cent. of the elements mentioned is claimed to be measurable. Possible contamination by the reagents must be allowed for.

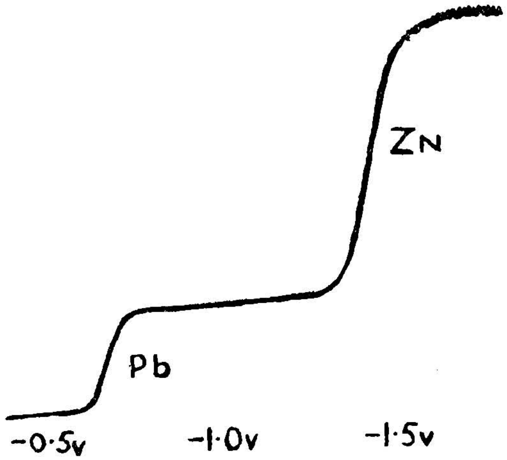

Fig. 1

Steps for lead and zinc in 0·1 *N* NaOH

Zn 0·02 g./litre; Pb 0·02 g./litre; gelatin 0·02 g./litre. Sensitivity 1/10

(From Report MET 19, by courtesy of the Ministry of Supply and Mr. H. C. Davis)

For the commercially pure aluminium the same authors propose a rapid method[23] similar to the well-known old method of Hohn,[36] but avoiding the excessive concentration used by Hohn, which leads to losses by adsorption on the undissolved aluminium hydroxide. Iron, copper and lead can be determined as well as zinc, and the lower limits claimed are 0·015 per cent. of iron, 0·006 per cent. of copper, 0·01 per cent. of lead and 0·015 per cent. of zinc. The method is not suitable for alloys.

The most recent paper is that by M. Spalenka,[20] which describes methods for determining manganese and copper as well as zinc. The procedure for zinc differs according to the composition of the alloy. (*a*) For the determination of copper in all alloys, and of zinc in alloys containing little copper and nickel, 0·3 g. of alloy is dissolved in hydrochloric acid (with addition of a little nitric acid or hydrogen peroxide to dissolve the copper); excess of ammonia is added and, after cooling, 1 ml. of saturated sodium sulphite solution and some gelatin are added and the solution is made up to 100 ml. (*b*) Alloys rich in copper and nickel are attacked with caustic soda (0·5 g. of alloy and 10 ml. of 3·5 *N* sodium hydroxide). When the reaction is complete, 10 ml. of water and, after cooling, 15 ml. of a solution containing 45 g. of ammonium chloride and 350 ml. of concentrated ammonium hydroxide solution per litre are added, followed by 1 ml. of saturated sodium sulphite solution and some gelatin. The solution is made up to 50 ml. and polarographed.

It is admitted by Spalenka that in procedure (*a*) the precipitated aluminium hydroxide adsorbs some copper and it is well known[29,31,32] (although not mentioned by Spalenka) that an alkaline attack does not extract the total zinc from an alloy containing considerable quantities of copper and other alkali-insoluble constituents. It is thought by Spalenka that these sources of error can be eliminated by calibrating under the same conditions, when an identical loss will occur. The writer thinks, however, that this is a dangerous principle, as it may be difficult to ensure that the same factors act each time to the same extent.

The addition of ammonium chloride - ammonia solution improves the shape of the zinc wave, compared with that obtained in pure sodium hydroxide solution.

For the determination of manganese, 0·5 g. of the metal is dissolved in hydrochloric acid and a few drops of hydrogen peroxide. Rochelle salt, sulphite and a sodium hydroxide solution containing potassium cyanide are added and, before the cyanide, some *p*-methylaminophenol sulphate (Metol), to prevent the manganese precipitating at alkaline reaction.

B. *Determination of lead*—The method of Kolthoff and Matsuyama[19] has been simplified by the writer.[33] This procedure is applicable to all usual types of alloys and is simple and reliable, whilst other procedures[19,22,23,24,27,29,30] are either slower or applicable only to a restricted range of alloys, mainly to pure aluminium.

C. *Determination of sodium*—Several methods have been proposed[14–17] which should be able to compete with existing "wet" methods; the writer has, however, no personal experience of them. The method of Urech and Sulzberger is based on separation from the bulk of the aluminium by saturating with hydrogen chloride gas.

6. BERYLLIUM

Very little work has been published about the application of polarography to this metal and nothing, to the writer's knowledge, about polarographic determinations in beryllium-based alloys. It seems likely, in view of the general properties, that the methods developed for aluminium alloys can be applied to beryllium as well. Whether beryllium itself can be determined—in a similar manner to the determination of aluminium in magnesium alloys—is not clear, but is probably of no practical importance, as what would most likely be needed is differentiation between aluminium and beryllium, which does not appear to be practicable.

The permission of the Directors of International Alloys Ltd. to publish this survey is gratefully acknowledged.

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21.	„	Sb, Zn, Pb, Sn	Payne, S. T., see ref. 13 above.

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* Lithoprinted by Edwards Brothers Inc., Ann Arbor, Michigan, U.S.A., by authority of the Alien Property Custodian.

22.	”	Zn, Pb, Cd	Ginsberg, H., *Leichtmetallanalyse*, printed as a MS. in restricted numbers in Oslo, **1945**, p. 236.
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24.	High pur. Al	Cu, Ni, Zn, Cd, Pb	Capitanio, V., *Ibid.*, 1940-41, **100**, II, 419.
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Polarographic Determination of Thiomersalate

BY J. E. PAGE AND J. G. WALLER

IN view of the difficulty of measuring small amounts of thiomersalate (sodium ethyl mercurithiosalicylate) in pharmaceutical preparations, we have developed a polarographic method for its determination. Herasymenko, Heyrovský and Tančakivský,[1] and Kolthoff and Miller[2] showed that mercurous and mercuric ions yield well defined diffusion currents that are directly proportional to the concentration of mercurous or mercuric salt, but very little information has been published on the polarographic behaviour of mercury complexes. Recently, Poma[3] described the behaviour of mercury solutions containing sodium perchlorate as the indifferent electrolyte and Malyugina, Shchennikova and Korshunov[4] and Shchennikova and Korshunov[5] have studied mercuric iodide complexes.

TABLE I

EFFECT OF HYDROGEN ION CONCENTRATION ON POLAROGRAMS GIVEN BY 0·02 PER CENT. THIOMERSALATE SOLUTIONS

		First step		Second step	
Indifferent electrolyte	pH value	Half-wave potential, v.	Step height in microamp.	Half-wave potential, v.	Step height in microamp.
5·0 *N* Hydrochloric acid . .	—	—	0	−0·50	1·25
1·0 *N* ” . .	—	—	0	−0·48	1·35
0·1 *N* ” . .	0·9	−0·10	1·15	−0·46	1·30
0·01 *N* ” . .	1·7	−0·10	0·75	−0·53	1·00
0·025 *M* Potassium hydrogen phthalate	4·0	−0·10	0·75	−0·63	1·20
0·025 *M* Sodium phosphate mixture	7·0	−0·10	0·70	−0·80	0·95
0·025 *M* Sodium borate . .	9·2	−0·16	1·55	−0·95	0·95
0·1 *N* Sodium hydroxide . .	12·0	−0·31	0·55	−0·82	0·50

We found that thiomersalate in either acid or neutral solution gave a characteristics polarogram with two well defined steps (Fig. 1). The position and height of the steps formed by a 0·02 per cent. solution of thiomersalate varied considerably with change in hydrogen ion concentration and chemical composition of the solution (Table I). The first step was not formed in strongly acid solutions: the second step was the more suitable for the determination of thiomersalate.

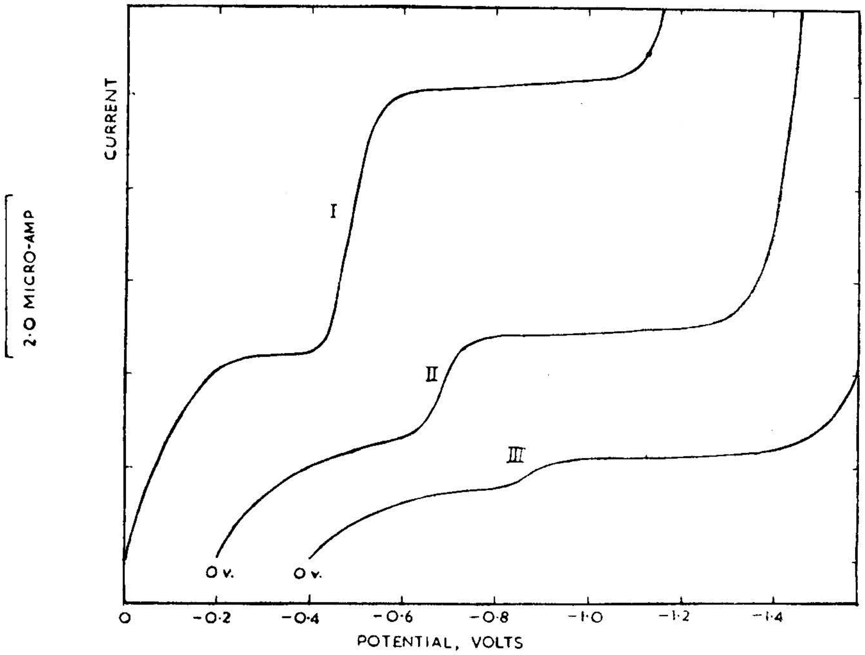

Fig. 1. Polarograms for thiomersalate in 1·0 *N* hydrochloric acid containing 0·01 per cent. of gelatin: curve I 0·05 per cent.; curve II 0·02 per cent.; curve III 0·005 per cent.

Thiomersalate in 0·1 *N* and 1·0 *N* nitric and perchloric acids gave similar steps to those produced in the same concentrations of hydrochloric acid.

EXPERIMENTAL

A Cambridge recording polarograph calibrated to read in microamps. was used in this investigation. All potentials were measured directly against the saturated calomel electrode. The dropping mercury capillary had the following characteristics. At a pressure of 55·5 cm. of mercury the drop-time (t) on open circuit in 0·1 *N* potassium chloride at 25° C. was 3·13 sec., the weight of mercury dropping per sec. (m) was 1·82 mg., and $m^{2/3} t^{1/6}$ was 1·80.

Our recommended procedure for the determination of thiomersalate in a solution is as follows.

Add 1·0 ml. of concentrated hydrochloric acid and 1·0 ml. of 0·1 per cent. gelatin solution to a volume of solution containing between 0·1 and 1·0 mg. of thiomersalate and dilute the mixture to 10 ml. Transfer a 3-ml. portion of the diluted solution to the polarograph cell, bubble nitrogen through it for 10 minutes to remove oxygen and examine it over the potential range 0 to —0·8 v. Measure the height of the step appearing at about —0·5 v.

For solutions containing suspended matter (*e.g.*, alum-precipitated vaccines), either the precipitate was filtered off before addition of acid or the final solution containing suspended matter was examined in a polarograph cell connected through an agar bridge to a saturated calomel electrode, the latter being used as anode. Determinations by the two procedures on vaccines containing 0·01 per cent. of thiomersalate agreed to within 10 per cent.

Some typical polarograms for thiomersalate in acid solution are shown in Fig. 1. The results set out in Table II show that the diffusion current of the second step formed in 1·0 *N* hydrochloric acid is proportional to concentration over the range 0·002 to 0·05 per cent. of thiomersalate.

TABLE II

RELATION BETWEEN DIFFUSION CURRENT AND CONCENTRATION OF THIOMERSALATE IN 1·0 *N* HYDROCHLORIC ACID

Percentage of thiomersalate C	Diffusion current in microamp. *i*	*i*/C
0·002	0·15	75
0·005	0·30	60
0·010	0·65	65
0·020	1·30	65
0·050	3·50	70

Experiments on the recovery of added thiomersalate from pharmaceutical preparations are summarised in Table III. The recoveries from vaccine preparations are very good, but those from a crude prolactin preparation and from liver extract preparations are less satisfactory. For vaccines containing about 0·01 per cent. of thiomersalate the recovery was 90 per cent. or more.

TABLE III

RECOVERY OF THIOMERSALATE FROM PHARMACEUTICAL PREPARATIONS

Preparation	Thiomersalate added to 100 ml. of preparation mg.	Thiomersalate found in 100 ml. of preparation mg.	Recovery of thiomersalate %
Whooping cough vaccine	2·0	1·8	90
" "	10·0	9·5	95
" "	50·0	45·0	90
" "	100·0	95·0	95
Prolactin preparation ..	10·0	8·5	85
" " ..	15·0	13·5	90
Liver extract	10·0	8·0	80

Antiseptics of the phenol type, such as *p*-chloro-*m*-cresol, most organic substances and metallic ions (except those of antimony, arsenic, bismuth, cadmium, tin, titanium and vanadium) do not interfere with the polarographic procedure for the determination of thiomersalate. Thus thiomersalate can be determined in vaccine preparations, but not in whole blood or in ter- and quinquevalent antimony preparations, such as stibophen and sodium antimony^{V} gluconate. Determinations on liver extracts containing about 0·01 per cent. of thiomersalate were usually about 20 per cent. low. Since this loss is consistent, a calibration curve can be constructed to allow for it.

BEHAVIOUR OF THIOMERSALATE IN AMMONIACAL COBALT BUFFER SOLUTIONS—

In view of the interest attaching to the catalytic steps formed by sulphydryl-containing amino acids (such as cysteine) in ammoniacal cobalt buffer solutions,[6] we have studied the behaviour of thiomersalate in similar solutions.

Ten ml. of a 0·01 per cent. thiomersalate solution were added to a mixture of 2·0 ml. of 1·0 *N* ammonium chloride, 2·0 ml. of 0·02 *M* cobalt chloride and 2·0 ml. of 1·0 *N* ammonium hydroxide, the solution was made up to 20 ml. with distilled water and an aliquot portion was polarographed over the potential range −0·8 to −2·0 v.

The polarograms obtained in this way showed steps at about −1·5 v.; these were surmounted by catalytic maxima at about −1·9 v. Although 0·01 per cent. of gelatin suppressed the maximum on top of the cobalt step at −1·1 v., it did not affect the catalytic maximum due to the thiomersalate. These catalytic steps were somewhat similar in shape to those formed by cysteine under the same conditions; they are illustrated in Fig. 2.

For solutions containing more than 0·001 per cent. of thiomersalate, the height of the catalytic step was roughly proportional to concentration and was about one-fiftieth that of the corresponding step formed by a cysteine solution of the same molar concentration. During the contact with ammoniacal cobalt buffer solution the height of the catalytic step increased slightly, whereas such contact tends to reduce the height of the step formed by cysteine. Although the catalytic step formed by thiomersalate is not likely to be of practical importance, it is of considerable theoretical interest.

POLAROGRAPHIC BEHAVIOUR OF OTHER MERCURY PREPARATIONS—

A number of mercury preparations were examined in acid solution at negative potentials (Table IV); of those tested only phenylmercuric acetate and nitrate formed distinctive steps (Fig. 3), the second being the more satisfactory. Unfortunately, owing to the low solubility of these salts in acid solution, it was not possible to study them at concentrations greater than 0·01 per cent. Mercuric and mercurous nitrate in 0·1 N nitric acid yield reduction waves at about +0·42 v. (Kolthoff and Miller[2]) and not at negative potentials.

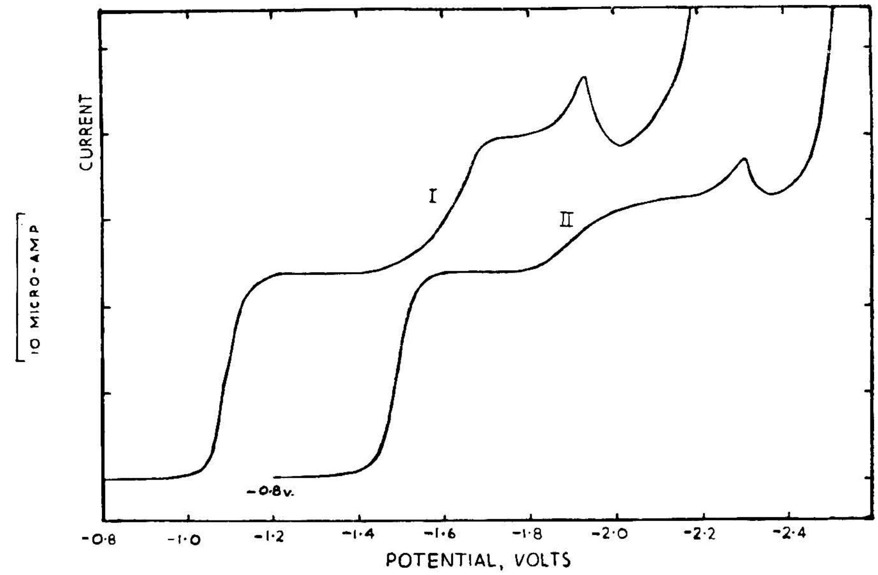

Fig. 2. Polarograms for thiomersalate in ammoniacal cobalt buffer solution containing 0·01 per cent. of gelatin: curve I 0·01 per cent.; curve II 0·005 per cent.

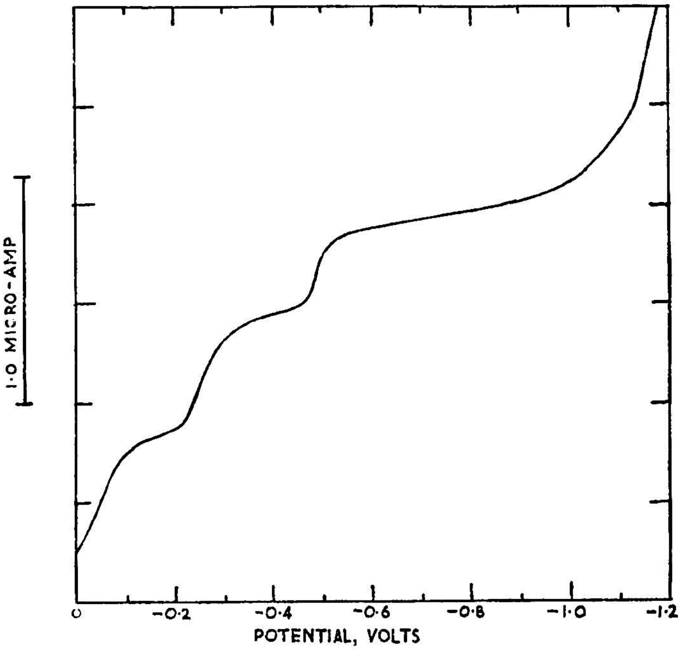

Fig. 3. Polarogram for $1{\cdot}0 \times 10^{-4}$ phenylmercuric nitrate in 1·0 N hydrochloric acid.

The steps shown by thiomersalate and phenylmercuric salts are probably formed by non-ionising mercury complexes and not by free mercury ions.

TABLE IV

POLAROGRAMS OF OTHER MERCURY COMPOUNDS

Mercury preparation	Concn. of mercury preparation %	Indifferent electrolyte	First step		Second step	
			Half-wave potential, v.	Diffusion current, microamp.	Half-wave potential, v.	Diffusion current, microamp.
Phenylmercuric nitrate	0·010	1·0 M HNO_3	−0·08	0·2	−0·62	0·6
„	0·004	1·0 M HCl	−0·25	0·3	−0·47	0·2
„	0·021	Sodium phosphate 0·025 M (pH 7·0)	−0·12	0·5	−1·15	0·6
Phenylmercuric acetate	0·0035	1·0 M HCl	−0·25	0·4	−0·50	0·2
Mercurochrome ..	0·01	1·0 M HCl	—	0	—	0
Mercuric nitrate ..	0·01	1·0 M HNO_3	—	0	—	0
Mercuric chloride ..	0·01	1·0 M HCl	—	0	—	0

SUMMARY

In 1·0 N hydrochloric acid solution, thiomersalate gives a well defined step at −0·48 v. *versus* the saturated calomel electrode; this can be used for the determination of the antiseptic in vaccines and pharmaceutical preparations. Phenylmercuric acetate and nitrate, but not mercuric nitrate and chloride, form similar steps. In an ammoniacal cobalt buffer solution, thiomersalate gives catalytic steps of similar shape to, but of a much lower height than, those given by cysteine.

The authors wish to thank Mr. M. Robson for technical assistance.

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RESEARCH DIVISION
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July, 1948

Estimation of Aluminium in Beer

BY J. W. TULLO, W. J. STRINGER AND G. A. F. HARRISON

ALUMINIUM is usually present in beer in minute quantities and its accurate estimation is a matter of considerable difficulty. Much research has been carried out in this Laboratory on colorimetric methods employing aurine tricarboxylic acid, but no method involving this reagent was found to give accurate and reproducible results for beer. The most difficult part of the method is the separation of iron from aluminium.

The method of Gentry and Sherrington[1] for the "Direct Photometric Determination of Aluminium with 8-Hydroxyquinoline" was tried without success. Iron, and possibly some other elements, caused an interference which could not be completely eliminated. Gentry and Sherrington, however, recorded the fact that the chloroform solution of aluminium 8-hydroxyquinolinate "gave an intense greenish-yellow fluorescence on illumination with ultra-violet light," and suggested that this "might form the basis of a fluorimetric method for the determination of aluminium."

Following this suggestion a fluorimetric method has now been developed which is remarkable for its simplicity. The method would also appear to be highly specific for aluminium.

METHOD

OXIDATION OF SAMPLE—

Twenty-five ml. of beer are evaporated to a syrup in a silica Kjeldahl flask. Nitric acid is added and the flask gently warmed. About 4 ml. of sulphuric acid are then added and the flask is more strongly heated until signs of charring are noted. Nitric acid is added drop by drop to clear the dark colour, and this procedure continued until the sample will no longer char. The flask is then heated until sulphuric acid fumes are evolved, when about 1 ml. of nitric acid is added. When all the brown fumes have disappeared the sulphuric acid residue should appear colourless on cooling. About 0·5 g. of ammonium sulphate is added and washed in with a little distilled water, and the contents of the flask are again heated to fuming for about 5 minutes. This method, due to Pelouze,[2] is to ensure the complete removal of nitric acid.

(The nitric and sulphuric acids used were obtained by distillation of the "pure" acids from Pyrex glass and silica stills respectively.)

The time taken for oxidation is about 20 minutes. Ashing beer at 500° to 550° C. in a muffle furnace required a much longer time and resulted in the loss of about 20 per cent. of the aluminium present.

EXTRACTION OF ALUMINIUM—

No fixed concentration of 8-hydroxyquinoline in chloroform can be recommended for all circumstances, but it was found that 10 ml. of a 0·2 per cent. solution w/v always gave complete extraction of aluminium from 25 ml. of oxidised beer. Ten ml. of a 0·1 per cent. solution gave complete extraction for some beers, but when the aluminium or iron content of the beer was high (*i.e.*, more than about 3 parts per million), aluminium was not completely extracted. The procedure finally adopted for the extraction of aluminium from oxidised beer is as follows.

The sulphuric acid residue from the oxidation of 25 ml. of beer is diluted with about 40 ml. of distilled water, cooled and transferred with washing to a 100-ml. or 150-ml. separating funnel. To this, 10 ml. of a 0·2 per cent. solution of 8-hydroxyquinoline in chloroform are added. After a few seconds shaking, ammonia is added to adjust the pH to between 8 and 11. The separating funnel is then vigorously shaken for about 30 seconds, and the chloroform layer on settling is run off into a 20-ml. graduated flask. The aqueous layer is extracted with two 4-ml. portions of chloroform, which are added as washings to the flask, and the volume adjusted to 20 ml. with chloroform.

The extract is poured into a small Erlenmeyer flask and clarified by addition of about 1 g. of anhydrous sodium sulphate. This extract, which contains all the aluminium as the 8-hydroxyquinolinate, and also the 8-hydroxyquinolinates of iron and other elements, is then diluted 4-, 10- or 20-fold with chloroform (*i.e.*, 5, 2, or 1 ml. made up to 20 ml.).

If the undiluted extract is illuminated with ultra-violet light in the fluorimeter, a relatively intense fluorescence appears at the bottom of the cell, progressively weakening towards the top of the cell, owing to absorption of ultra-violet light. A chloroform solution of 8-hydroxyquinoline of concentration greater than about 0·05 per cent. w/v appreciably absorbs ultra-violet light. This effect is eliminated when the extract is diluted to 4 times its volume with chloroform. Similarly, if the extract is dark coloured (owing to the presence of iron or other 8-hydroxyquinolinates), dilutions of 10- or 20-fold may be employed to reduce the colour to a point at which no interference is caused.

MEASUREMENT OF INTENSITY OF FLUORESCENCE—

The diluted extract as obtained above is exposed to ultra-violet light (filtered through Wood's glass) in the Spekker fluorimeter, a Hilger No. 5 (green) filter being placed in front of the measuring photo-cell. With the chloroform solution in position and the drum set at zero the photo-cells are balanced. A solution containing 200 μg. of fluorescein per 150 ml. of distilled water is next placed in position, and the drum rotated to obtain a balance. The drum reading R is then noted and the value of the antilog of (2 — R) is calculated. From this value the amount of aluminium in the extract may be found by reference to the appropriate standard curve.

This method of using the Spekker fluorimeter has been found very convenient. The drum scale of the instrument is logarithmic, and is such that the reading $R = \log a$, where $1/a$ is the fraction of the full aperture. The graph of $1/a$ against the concentration of fluorescent substances is approximately linear for dilute solutions. It is more convenient to graph 100/*a*, *i.e.*, antilog (2 — R), whereby small decimal quantities are avoided.

STANDARD CURVES—

Standard curves are made by extracting 0 to 80 μg. of aluminium in solution under the same conditions as in the test (*i.e.*, ammonium sulphate - ammonia solution, pH 8 to 11) with 10 ml. of a 0·2 per cent. solution of 8-hydroxyquinoline in chloroform. The extracts are adjusted to 20 ml. volume, and diluted 4-, 10- and 20-fold with chloroform, and the intensity of fluorescence of these diluted solutions measured in the fluorimeter against a fluorescein standard as described above. The results obtained are shown in Table I.

TABLE I

FLUORESCENCE OF STANDARD ALUMINIUM SOLUTIONS

μg. Aluminium per 20 ml. of undiluted extract	Dilution ×4		Dilution ×10		Dilution ×20	
	Drum reading R*	$\frac{100}{a}$	Drum reading R*	$\frac{100}{a}$	Drum reading R*	$\frac{100}{a}$
0	1·55	2·8	1·75	1·8	2·0	1·0
5	0·54	28·8	—	—	—	—
10	0·27	53·7	0·57	26·9	0·815	15·3
15	0·135	73·3	—	—	—	—
20	0·033	92·6	0·30	50·1	0·56	27·5
30			0·15	70·8	0·38	41·7
40			0·05	89·1	0·28	52·5
50					0·19	64·6
60					0·12	75·9
70					0·068	86·5
80					0·015	96·7

* R = log *a*.

INTERFERENCE—

Iron and other elements giving coloured 8-hydroxyquinolinates extractable by chloroform at pH 8 to 11 may interfere by absorbing the ultra-violet or fluorescent light, or both.

The removal of iron from solution by adjusting the pH to 2·0 and extracting with an 8-hydroxyquinoline solution in chloroform, as suggested by Gentry and Sherrington,[1] was found to be satisfactory for solutions containing only calcium, magnesium, potassium, ammonium, chloride, sulphate and phosphate ions. Iron was not, however, completely removed from oxidised beer by this method.

For the fluorimetric method it has not so far been found necessary to remove iron or other elements from any samples of beer examined. The ratio of iron to aluminium present may exceed 10 to 1 without causing interference. The sensitivity of the method is sufficiently great to permit of diluting the fluorescent solution to an extent that eliminates interference by iron or other metals giving coloured 8-hydroxyquinolinate soluble in chloroform. The requisite dilution still gives an intensity of fluorescence measurable by the Spekker fluorimeter.

SPECIFICITY—

In testing the method for specificity, solutions containing 50 μg. of different metals in about 100 ml. of an aqueous ammonium sulphate - ammonia solution of pH 8 to 11, were extracted with a 0·2 per cent. chloroform solution of 8-hydroxyquinoline as in the test, and the extracts examined in ultra-violet light. None of the following metals gave a fluorescent extract under these conditions: bismuth, calcium, cerium, chromium, cobalt, copper, iron, lead, magnesium, manganese, mercury, molybdenum, nickel, silver, thallium, thorium, tin, tungsten, uranium, vanadium.

With cadmium the extract exhibited a faint yellow fluorescence, which diminished in intensity as a precipitate formed. The extract from a zinc solution showed a faint greenish-yellow fluorescence, which also diminished in intensity with the formation of a precipitate. The fluorescence given by each of these is readily distinguishable from that of the aluminium 8-hydroxyquinolinate. If measured in the fluorimeter as being aluminium, 50 μg. of either metal would correspond to about 2 μg. of aluminium.

The extract from a solution of 50 μg. of beryllium (as beryllium sulphate) showed a similar fluorescence to that given by aluminium, and on measurement in the fluorimeter corresponded to the presence of 8·6 μg. of aluminium. The beryllium sulphate was then treated according to the method of Haber and Van Oordt[3] and a purified specimen of the acetate, $BeO.3Be(C_2H_3O_2)_2$, prepared. From this a further solution containing 50 μg. of beryllium was made up, which on extraction in the usual manner gave a faint greenish-yellow fluorescence, corresponding in the fluorimeter to 2·0 μg. of aluminium.

After a second purification the fluorescence was reduced to an extent corresponding to the presence of 0·4 μg. of aluminium in 50 μg. of beryllium. It would therefore appear that beryllium itself does not yield a fluorescent 8-hydroxyquinolinate, the fluorescence obtained here being due to aluminium present as an impurity.

ALUMINIUM IN BEER—

The method described has been applied successfully to the estimation of aluminium in beer. For beer of normal iron content (up to 1 part Fe per million), 4- or 10-fold dilution of the chloroform extract has been found to eliminate all interference, but for beer of high iron content a 20-fold dilution may be necessary. Aluminium added to beer was completely recovered. Table II shows four examples of recoveries obtained.

TABLE II

RECOVERY OF ALUMINIUM ADDED TO BEER

Al in 25 ml. beer μg.	Al added μg.	Total Al found μg.	Added Al recovered μg.	Recovery %
4·3	20	25·0	20·7	104
51·0	30	80·0	29·0	97
9·4	16	25·6	16·2	101
4·3	20	23·0	18·7	94

Duplicate analyses gave good agreement with one another. Seven examples of this are shown in Table III.

TABLE III

DUPLICATE ANALYSIS OF BEER SAMPLES

	μg. Al in 25 ml. beer		Al parts per million	
Sample No.	Test 1	Test 2	Test 1	Test 2
1	1·9	1·7	0·08	0·07
2	1·6	1·5	0·06	0·06
3	1·8	2·1	0·07	0·08
4	2·7	2·4	0·11	0·10
5	1·2	1·3	0·05	0·05
6	1·3	1·4	0·05	0·06
7	0·6	1·1	0·02	0·04

The method is capable of measuring as little as 0·01 parts per million of aluminium in beer.

SUMMARY

A simple and specific method for the fluorimetric estimation of traces of aluminium as the 8-hydroxyquinolinate has been described. Good recoveries have been obtained for aluminium added to beer, and duplicate analyses have agreed well. The method is very sensitive, and the presence of iron does not cause any interference.

The Directors of Messrs. Arthur Guinness Son & Co. Ltd. have kindly granted permission for the publication of this work.

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CHEMIST'S LABORATORY
ST. JAMES'S GATE BREWERY
DUBLIN

July, 1948

A Modification of the Micro-Analytical Test for Purity in Food for use in the Examination of Dried Fruits

By R. C. A. BRADSHAW and J. B. M. COPPOCK

In a previous paper describing the micro-analytical test for purity in food,[1] it was pointed out that the process of acid hydrolysis followed by pancreatin hydrolysis was not readily applicable to dried fruits. Moreover, a survey of the American literature on the subject has failed to reveal a suitable technique for dealing with such materials.[2] Past experience has shown that difficulties inherent in the hydrolytic method result in the filter paper upon which the extraneous materials are ultimately collected being deeply covered with fragments of skin from the fruit. These fragments have to be explored with a probe to allow adequate microscopic examination, with the result that an accurate determination of the rodent and other filth present becomes difficult or even impossible.

The prospect of applying this procedure to the extent likely to result from increasing analytical control of extraneous materials in foodstuffs was by no means attractive, and a method was evolved, therefore, using air and water agitation, by which the dried fruit could be vigorously scrubbed free from extraneous materials and these subsequently collected at an aviation spirit - water interface. This system was chosen because it was found that emulsions were formed if the scrubbing was carried out in the presence of both water and aviation spirit. Moreover, in such emulsions fleshy particles from the fruit were found to be present and these apparently contained a stabilising agent, which made the emulsion extremely difficult to break.

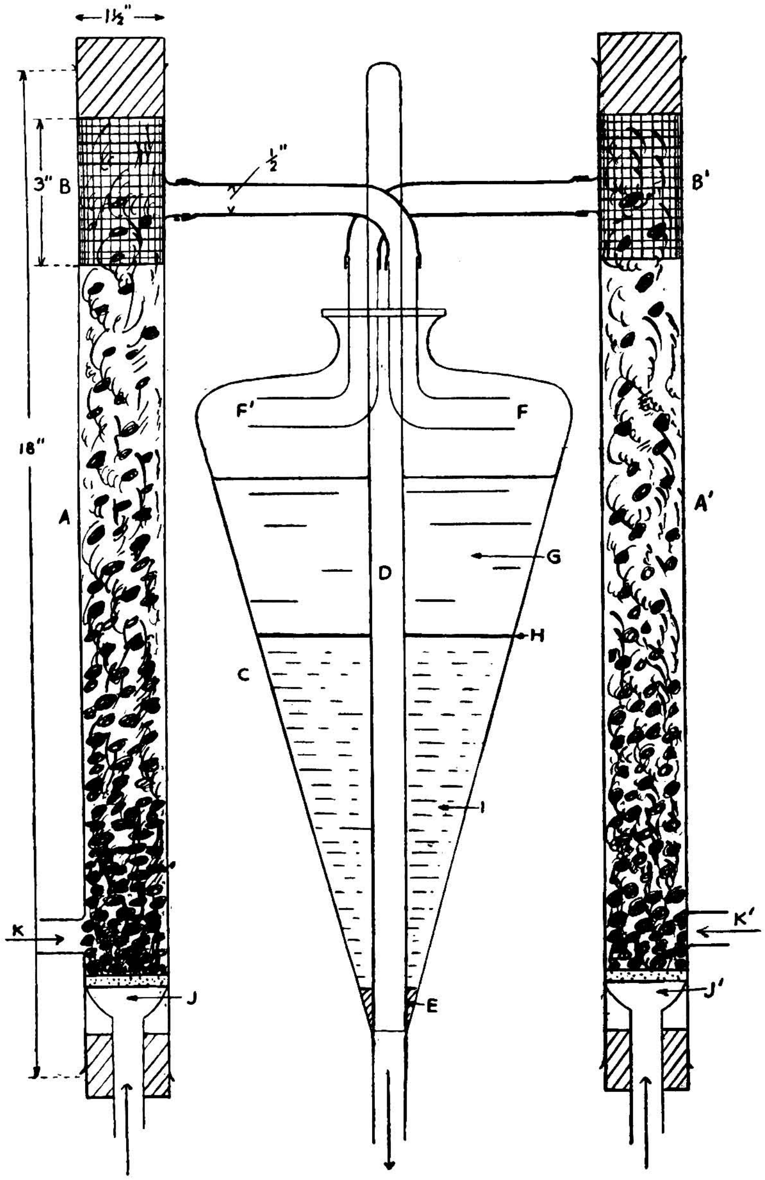
METHOD

The apparatus developed for the removal of the extraneous materials from fruit is illustrated in Fig. 1. In a convenient form of construction, slightly modified Liebig condenser jackets, A and A′, are fitted at one end with gauzes, B and B′ (mesh 10 per inch), so that large fleshy particles cannot enter and choke the upper side tubes. At the lower ends of the main tubes are fitted sintered filter-sticks, J and J′, of equal porosity and of the coarsest pore size available; these are connected to an air supply. By means of rubber tubes the upper side tubes are connected to glass angle tubes, F and F′, which deliver into a 2-litre separating funnel, C. The outlet of this is closed by a glass rod, D, fitted with a rubber seating, E. The separating funnel contains 800 ml. of aviation spirit, G, and, initially, 900 ml. of water, I, although this latter quantity may fall slightly in the course of the experiment.

The method of operation is to place 50 g. of the dried fruit in each vertical column. The side tubes, K and K′, are connected to the water supply and the water is allowed to fill approximately half of each column. It is important that the water be fed equally into the two columns; this may be accomplished by a suitable arrangement of rubber tubes and clips. The delivery of air to the filter-sticks is then begun. Equal amounts of air should be delivered to both columns; this may be judged by the relative bubbling that occurs. The amount of air required for the two columns is about 6 litres per minute at a pressure of 8 cm. of mercury. By suitable adjustment of the flow of air and water thorough agitation of the dried fruit may be produced and a frequent, though intermittent, delivery of water into C may be obtained.

This intermittent delivery is regarded as necessary as it has a flushing action and clears the delivery system. The glass angles, F and F′, are bent to accentuate the flushing action and also to direct the force of the jet at the moment of delivery on to the walls of the separating funnel. The water containing the extraneous material then slowly settles through the aviation spirit and the filth is held at the interface. If the rod, D, is eased, the water will slowly drain past E, so that the aviation spirit - water interface, H, remains approximately at the same level. The scrubbing of the fruit is continued for 30 minutes, unless the water in the column still remains cloudy after that time, in which event it is continued until the water clears. The supply of air is then stopped and the whole apparatus flushed with water for 10 minutes. The glass angle tubes are removed and most of the water is allowed to flow away. The small amount of the residual water and the whole of the aviation spirit is then poured on to a filter paper in the usual way and the filter paper is examined under a microscope.

Although in the illustration the water inlets, K and K′, are shown above the sintered stick, it is often more convenient to raise these sticks so that the water enters below the surface of the sintered discs. If this is done care should be taken to ensure that there is a sufficient annular space between the sintered disc and the condenser jacket to allow free passage for the water.



Results

Before this new technique was adopted as routine a comparison was made with the conventional hydrolytic process. The results obtained are shown in Table I, which indicates the reliability and increased efficiency of the new technique. Several qualities of dried fruit from different sources were then examined by the method here described and some typical results are given in Table II. It is possible that, besides being applicable to dried fruit, this method may also be used for the removal of extraneous materials from whole wheat, nuts and the fruit in baked products. These possibilities are at present under examination.

TABLE I

COMPARISON OF METHODS

A = Water and air scrubbing method
B = Acid - pancreatin method

100-g. samples used in all experiments

Sample No.	Description of fruit	Method	Rodent hairs (RH) No.	Rodent hairs (RH) Length mm.	Other animal hairs (AH) No.	Other animal hairs (AH) Length mm.	RH + AH Total No.	RH + AH Total length mm.	Insect fragments	Bird fluff No.	Bird fluff Length mm.
(i)	Sultanas	A	6	11·5	2	4·0	8	15·5	0	1	2·0
(i)	,,	B	4	1·5	3	1·8	7	3·3	1	5	7·9
(i)	,,	A	8	9·7	1	1·0	9	10·7	0	3	4·5
(i)	,,	B	4	1·5	3	1·8	7	3·3	1	5	7·9
(ii)	Currants	A	1	15·2	—	—	1	15·2	10	—	—
(ii)	,,	B	1	1·7	—	—	1	1·7	1	—	—
(iii)	,,	A	1	0·1	—	—	1	0·1	1	1	0·5
(iii)	,,	B	—	—	—	—	—	—	2	—	—

TABLE II

FILTH ESTIMATIONS ON CURRANTS AND SULTANAS

Method—Continuous scrubbing with air and water

All 100-g. samples

Sample No.	Description of fruit	Rodent hairs No.	Rodent hairs Length mm.	Animal hairs No.	Animal hairs Length mm.	Insect fragments	Bird fluff No.	Bird fluff Length mm.	Total of all hairs Total No.	Total of all hairs Total length mm.
1	Sultanas	4	5·4	2	2·8	0	1	0·4	6	8·2
2	,,	4	2·0	1	1·0	0	3	1·8	5	3·0
3	,,	6	11·5	2	4·0	0	5	7·9	8	15·5
4	,,	3	5·6	1	0·5	1	2	2·3	4	6·1
5	,,	3	1·3	3	4·3	1	4	1·6	6	5·6
6	,,	2	0·3	2	0·8	1	5	5·5	4	1·1
7	,,	1	4·5	3	35·9	0	3	4·7	4	40·4
8	,,	2	1·7	4	10·1	0	8	8·3	6	11·8
9	,,	2	2·0	3	5·0	1	1	3·0	5	7·0
10	Currants	3	4·9	1	0·4	1	1	1·1	4	5·3
11	,,	2	2·9	0	0	0	1	1·1	2	2·9
12	,,	2	2·2	1	1·3	0	1	2·0	3	3·5
13	,,	1	1·3	1	1·0	7	1	1·1	2	2·3
14	,,	1	0·1	0	0	2	1	0·5	1	0·1
15	,,	0	0	1	1·4	11	0	0	1	1·4
16	,,	1	15·2	0	0	10	0	0	1	15·2
17	,,	2	23·8	1	1·2	8	0	0	3	25·0

SUMMARY

A method is described for the removal of filth from dried fruits by air and water scrubbing. A comparison with the existing method is made and data are recorded indicating the amount of contamination with extraneous materials likely to be encountered in the examination of present-day dried fruits.

Thanks are due to the Council of the Association and the Department of Scientific and Industrial Research for permission to publish this work.

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THE BRITISH BAKING INDUSTRIES RESEARCH ASSOCIATION
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October, 1948

The Determination of Aneurine in Pharmaceutical Products

By RITA PATRICK AND J. F. H. WRIGHT*

THE assay of aneurine in pharmaceutical products by the thiochrome method of Jansen is lengthy and the precision is not high.[1,2] In a method for the estimation of aneurine in oats, Holman[3] substituted mercuric oxide for potassium ferricyanide as oxidising agent, the measurement of fluorescence being made in aqueous acetone solution. The application of this method to pharmaceutical products has been investigated and a modification having several advantages over the Jansen method has been developed. The technique is simpler and the precision and accuracy are higher. All reagents are stable for long periods and the tedious purification of *iso*-butanol is eliminated. The acetone used is prepared by merely heating the commercial product with activated carbon under reflux and distilling.

This method has been applied to routine analyses, the products including aneurine tablets, vitamin "B group" tablets, injectable "B group" solutions, a multi-vitamin concentrate in aqueous alcohol and chocolate malt based granules containing vitamins A, B_1, C and D and vanillin. For the last product a method for the separation of aneurine from interfering substances by adsorption on synthetic zeolite, based on that of Hochberg, Melnick and Oser,[4] has been developed.

EXPERIMENTAL

In the earlier part of this investigation fluorescence was measured by means of a Hilger Spekker fluorimeter; this was later replaced by a Klett instrument (Model 2070). Most of the results reported are based on measurements made with the latter, using a Corning No. 586 filter in the exciting beam and Corning Nos. 430 and 038 filters in front of the photo-cell. An aqueous solution of 1-methyl-5-aminoacridine hydrochloride was preferred to quinine sulphate as standard, because of its greater stability. Fluorimeter readings were found to be directly proportional to aneurine concentrations over the range used. The day-to-day drift in the calibration line was very slight.

In preliminary experiments with aqueous aneurine solutions, precipitation of mercuric oxychloride (as mentioned by Holman) sometimes occurred. Although the precipitate dissolved after the addition of acetone, results in these instances were low and erratic. Occasionally mercuric oxide was also precipitated. Both these difficulties were overcome by reducing the concentration of potassium hydroxide from 0·06 *N* to 0·04 *N*, and that of the mercuric chloride solution from 1·0 to 0·8 per cent. With these modifications highly reproducible results were obtained, sixty determinations giving a coefficient of variation of 1·0 per cent.

Reproducible results were obtained with dilute hydrochloric acid extracts of tablets. Eleven assays of samples from one batch of B group tablets carried out over a period of 6 weeks gave results as follows:—

Mean weight of aneurine per tablet	0·958 mg.
Range	0·94 to 0·98 mg.
Coefficient of variation	1·6 per cent.

Satisfactory precision was also obtained with the multi-vitamin concentrate, the aqueous alcoholic solution being first diluted with water and extracted with carbon tetrachloride to remove vitamins A and D.

To test the accuracy of the method extracts from a B group tablet granule without aneurine were prepared for assay in the usual way and measured volumes of standard aneurine solution were added before oxidation. Ten assays were carried out with the following results:

Mean recovery	98·8 per cent.
Range	98 to 102 per cent.
Coefficient of variation	1·6 per cent.

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With the chocolate-malt granules very poor results were obtained by simple acid extraction. Separation of the aneurine from interfering substances by adsorption on synthetic zeolite was therefore investigated.

ADSORPTION OF ANEURINE ON SYNTHETIC ZEOLITE—

The most suitable adsorbent obtainable was a siliceous synthetic zeolite, as used for water purification. It was found that preliminary treatment with 3 per cent. acetic acid, as usually described for Decalso, was not sufficient. Iron, the main impurity, interfered with the assay, because it was precipitated when the solution was made alkaline for oxidation. Removal of the iron by heating under reflux with hydrochloric acid was investigated; but, although all iron could be removed with normal or more concentrated acid, the adsorptive capacity of the zeolite was considerably reduced. Eventually it was found best to activate the zeolite in the columns, by washing alternately with boiling 3 per cent. acetic acid and with a 25 per cent. solution of potassium chloride in 0·1 *N* hydrochloric acid until no precipitate appeared when the potassium chloride washings were made alkaline.

On following the method of Hochberg *et al.*[4] with columns 120 mm. long and 6 mm. in internal diameter, recoveries were found to be low. Investigation proved that aneurine was completely removed from solutions passed through the columns, suggesting incomplete elution. On increasing the volume of eluant to 40 ml. and insulating the columns with asbestos string, satisfactory recoveries were obtained.

To test the performance of these columns with extracts from the chocolate-malt granules, a laboratory batch containing exactly 0·1 mg. of aneurine per gram was prepared. Nine samples were heated under reflux with 0·05 *N* sulphuric acid, the vitamin A, other fatty substances and vanillin being extracted with carbon tetrachloride, and from each extract duplicate aliquots were analysed, with the following results:—

Mean weight of aneurine per gram	0·0995 mg.
Range	0·095 to 0·102 mg.
Coefficient of variation	2·0 per cent.

Columns of this type have been in routine use for several months and continue to function satisfactorily.

METHOD FOR SOLUTIONS AND TABLETS

REAGENTS—

(1) Potassium chloride solution, 25 per cent. w/v in water.

(2) Mercuric chloride solution, 0·8 per cent. w/v in water.

(3) Potassium hydroxide solution, 0·04 *N*.

(4) Acetone, commercial, heated with activated carbon under reflux and distilled in an all-glass apparatus.

(5) 1-Methyl-5-aminoacridine hydrochloride solution, 0·20 µg. of the monohydrate per ml. in water. This solution is prepared, approximately weekly, from one containing 100 µg. per ml. in water. The latter solution, stored in the dark, has so far been stable for two years. The dilute solution in the fluorimeter cell should be changed after about 1 minute total exposure.

(6) Aneurine standard solution, 2·0 µg. per ml. in water. This solution is prepared daily from one containing 100 µg. per ml. in 25 per cent. potassium chloride solution in 0·1 *N* hydrochloric acid. Aneurine is recrystallised from aqueous alcohol acidified with hydrochloric acid, and dried under vacuum at approximately 75° C.

PREPARATION OF SOLUTION—

(*a*) *Solutions*—Pipette a suitable volume of sample and dilute to give a final aneurine concentration of 0·5 to 2·0 µg. per ml. in 0·01 *N* hydrochloric acid.

(*b*) *Tablets*—Weigh a quantity of finely powdered sample containing about 1 mg. of aneurine and shake with 0·01 *N* hydrochloric acid in a 1-litre standard flask. Make to volume with 0·01 *N* hydrochloric acid and allow insoluble matter to settle. If necessary, centrifuge a small volume to obtain a clear solution.

It may sometimes be necessary to weigh a larger sample and use two dilution stages.

DETERMINATION—

Pipette 1 ml. of solution prepared as above, and aliquots of standard solution containing 0·5 to 2·0 µg. of aneurine, into 25-ml. glass-stoppered measuring cylinders or standard flasks.

Make up the volume to 7·5 ml. with 25 per cent. potassium chloride solution. Add 1 ml. of 0·8 per cent. mercuric chloride solution and mix well. Add 4 ml. of 0·04 *N* potassium hydroxide and mix again. (It is important to mix well before and after addition of the alkali.) Stopper the cylinders or flasks and heat in a water-bath at 40° C. for 15 minutes.

Cool and make up the volume to 25 ml. with acetone. Measure the fluorescence of the solutions, using 1-methyl-5-amino-acridine hydrochloride solution as standard.

METHOD FOR PREPARATIONS CONTAINING CHOCOLATE, MALT EXTRACT, VANILLIN, ETC.

ADDITIONAL REAGENTS—

(1) Potassium chloride in hydrochloric acid solution, 25 per cent. w/v of potassium chloride in 0·1 *N* hydrochloric acid.

(2) Sulphuric acid, 0·05 *N*.
(3) Sodium acetate solution, 1·8 *M*.
(4) Potassium hydroxide solution, 0·1 *N*.
(5) Carbon tetrachloride, redistilled.
(6) Synthetic zeolite (supplied by Fletcher Chemical Co. (Aust.) Pty. Ltd., Melbourne).
(7) Acetic acid, 3 per cent. w/v.

Adsorption apparatus as described by Hochberg *et al.*[4], the columns being insulated with three layers of asbestos string.

PREPARATION AND ACTIVATION OF ZEOLITE—

Grind and sieve a sample of zeolite and collect the 44–85 B.S.S. fraction. Soak the adsorption tube in chromic acid solution for several hours and rinse and dry it thoroughly. Place a small wad of glass wool at the bottom of the column. Fill the dry zeolite carefully into the column, tapping gently to ensure even packing, and wash with water until the washings are clear. Pour 50 ml. of boiling 3 per cent. acetic acid through the column, followed by 100 ml. of 25 per cent. potassium chloride solution in 0·1 *N* hydrochloric acid (approximately 3 drops per second). Repeat this process until there is no precipitate when the KCl + HCl washings are made alkaline. (Approximate volumes required: 250 ml. of the 3 per cent. acetic acid and 500 ml. of the KCl + HCl solution.)

PREPARATION OF EXTRACT—

Weigh a portion of the ground sample containing about 10 μg. of aneurine and heat under reflux with 150 ml. of 0·05 *N* sulphuric acid for 30 minutes. Adjust the solution to pH 4·0 to 4·5 with 1·8 *M* sodium acetate solution. Make up the volume to 200 ml. and heat the solution under reflux for a further 30 minutes with carbon tetrachloride to remove vitamin A concentrate, cocoa fat and vanillin. Then centrifuge at least 50 ml. of the aqueous layer until clear.

ADSORPTION AND ELUTION OF ANEURINE—

Pass 50·0 ml. of the clear solution through the column at room temperature (not more than 3 drops per second). Pass steam through the jacket and fill the column with boiling water. Allow to heat for ½ minute and then draw through rapidly. Repeat this heating process three times. Elute the aneurine immediately with 40 ml. of boiling 25 per cent. potassium chloride solution in 0·1 *N* hydrochloric acid (not more than 2 drops per second). Dilute the eluate to 50 ml. with water.

Wash the column with 200 ml. of boiling water, followed by 50 ml. of cold water. It is then ready for the next sample.

ASSAY—

Titrate 5·0 ml. of the prepared solution with 0·1 *N* potassium hydroxide, to the methyl red end-point. Note the volume, *v* ml., of alkali required. Pipette another 5·0 ml. of solution into a 25-ml. stoppered cylinder or standard flask. Add (4·9 — *v*) ml. of 25 per cent. potassium chloride solution and 1 ml. of 0·8 per cent. mercuric chloride solution. Mix well. Add (*v* + 1·6) ml. of 0·1 *N* potassium hydroxide and mix again. Complete the assay as described under "Method for Solutions and Tablets."

SUMMARY

The method of Holman,[4] using mercuric oxide as oxidant, has been adapted for the fluorimetric assay of aneurine in pharmaceutical products. With a preparation containing

chocolate, malt extract and vanillin the aneurine is first separated from interfering substances by adsorption on synthetic zeolite. The method described is simpler, quicker and of greater precision and accuracy than those based on the conventional ferricyanide oxidation.

The authors wish to thank the Directors of Nicholas Pty. Ltd., Melbourne, for permission to publish this paper and Mr. D. C. M. Adamson for reading the proof.

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NICHOLAS PTY., LTD.
MELBOURNE, AUSTRALIA

September, 1948

A Micro Method for the Determination of Unsaturation

BY MISS W. M. PHILLIPS AND W. C. WAKE

(*Read at the meeting of the Society on November 3rd*, 1948)

ALTHOUGH most of the techniques used in chemical analysis have been adapted for use with small or very small quantities of material, no such adaptation of the Wijs procedure for determining unsaturation appears to be recorded. The work described in this paper examines the determination of unsaturation on the micro scale both with iodine chloride and with bromine, and with particular reference to *cyclo*hexene as a standard substance; some values obtained for linseed and castor oils are also recorded. The use of a specially designed "iodine flask" was found necessary and it may be noted that this piece of apparatus may also be used with advantage on the macro scale.

EXPERIMENTAL

The essentials of most halometric methods for measuring unsaturation involve a final titration with sodium thiosulphate of iodine liberated from potassium iodide solution. Attention was, therefore, first concentrated on the estimation of iodine chloride dissolved in carbon tetrachloride, by means of aqueous sodium thiosulphate solution. On the normal scale of working, adequate interaction between the two phases in the course of the titration is usually accomplished by re-stoppering the flask after each addition of sodium thiosulphate and shaking vigorously to re-establish equilibrium between the aqueous and non-aqueous phases. This is not possible on the micro scale because of the necessity, when titrating small volumes, of keeping the tip of the burette below the surface of the aqueous layer while adding solution from the burette. The use of a nitrogen stream, as frequently recommended for micro-titrations, is impracticable because of volatilisation of iodine. The method finally adopted was to use glass beads in the flask, the beads being of such size that, although resting on the bottom of the flask, they projected through the interface of the two phases. It was found that the liquid in the flask could be swirled round sufficiently rapidly for the rolling beads to cause turbulence at the interface, and trials showed that they considerably shortened the time required for the titration.

Comparison was made of the standardisation of iodine chloride in carbon tetrachloride on the macro and on the micro scale. The mean of four micro-determinations was 0·1954 *N* with a coefficient of variation[*] of 1·7 per cent., whilst four macro-determinations gave 0·1967 *N* with a coefficient of variation of only 0·34 per cent. Further experiments showed differences between the results obtained by carrying out the titration immediately and those obtained after the solution had been allowed to stand in the dark for 1 hour. To investigate the cause of this difference, six titrations were made, three without standing and three after allowing to stand for 1 hour, each set of three consisting of a series in which the volume of diluting solvent (carbon tetrachloride) was 0, 25, and 100 ml. respectively. The addition of carbon tetrachloride had no effect on those titrated immediately, but in those left for 1 hour there was an apparent loss of iodine chloride (*i.e.*, a decrease in normality) greatest for that to which the least solvent had been added. The series was repeated except that, before titrating but after standing, the flasks were cooled in ice to reduce the vapour pressure in them. In this way potassium iodide solution around the stopper was allowed to run in without apparent displacement of vapour. As a result, the loss of iodine was considerably reduced, although some still occurred where no additional carbon tetrachloride had been added.

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[*] The coefficient of variation is the standard deviation expressed as a percentage of the mean value of the quantity determined.

These experiments pointed to a loss of iodine chloride as vapour, probably by diffusion from the flask during the operation of adding the potassium iodide solution, the higher loss after standing being due to the extra time, which allowed an equilibrium partial pressure of vapour to establish itself. To overcome this a special micro iodine flask was designed as shown in the Figure. In addition to the usual wide lip, A, for sealing with potassium iodide solution, a centrally placed thistle funnel, B, with a single-turn trap for liquid is added to the stopper. The ground part of the seating for the stopper contains an indentation, C, and the stopper has a hole, D, which can be brought opposite this indentation. When carrying out a determination, after the necessary standing period, the stopper is turned so that the hole and the indentation register, and excess of potassium iodide solution is added through the funnel, any vapour displaced being washed by bubbling through the potassium iodide solution in the lip of the flask. Once there is an excess of potassium iodide in the flask a few minutes suffice for the absorption of any iodine chloride vapour. Experiments showed that no loss occurred on standing and the spread of the titrations was much reduced, as is shown by Table I.

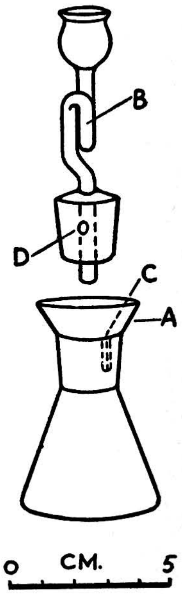

TABLE I

NORMALITY OF IODINE CHLORIDE DETERMINED BY MICRO-TITRATION

Each figure is based on four determinations

Flask used	Normality	Coefficient of variation of normality determination %
Ordinary flask	0·1954 N	1·7
Special iodine flask ..	0·00990 N	0·9
	0·0159 N	0·5
	0·3676 N	0·2

The titrations were carried out with 0·1 N sodium thiosulphate contained in a horizontal burette about 30 inches long, with a capacity of 1·6 ml. The iodine chloride was, owing to supply difficulties, prepared from its elements in the laboratory.

PROCEDURE ADOPTED

*Cyclo*hexene, which was adopted as a convenient standard, was passed down an alumina column under purified nitrogen to remove peroxide. The chromatographed material was tested for peroxide by the method of Yule and Wilson[1] and glass capillaries were filled in the usual way. The capillaries, containing about 6 mg., were crushed with a glass rod under about 3 ml. of carbon tetrachloride in the iodine flask, which already contained the glass beads needed during the titration. A further 2 ml. of carbon tetrachloride was used to rinse the rod, and the calculated quantity of iodine chloride or bromine solution was added from an automatic burette, a standard piece of apparatus, the graduated portion, 25 ml. in capacity, being fitted on to a 1-litre reservoir by a ground glass joint; solution from the reservoir was pumped up into the burette by means of compressed air. This piece of apparatus was probably the limiting factor in accuracy, as its graduations were 0·1 ml. It is essential to use such a burette for the iodine chloride or bromine solution, not only because of the high vapour pressure of the solution but also because of the need to exclude water vapour.

After the addition of the halogen solution, the stopper, already having some 10 per cent. potassium iodide solution in the liquid trap, was placed in position, and further potassium iodide solution was added to the rim. The sealed flask was then placed in a cupboard away from light for the period required for the reaction. After this prescribed period, the funnel of the flask was filled with potassium iodide solution and the stopper turned so that the solution entered slowly, the displaced air and vapour issuing, a bubble at a time, through the iodide seal in the rim. The flask was then allowed to stand for a minute before the stopper was removed and the titration carried out. After the completion of the titration, potassium iodate solution was added and the titration continued if there was any liberation of iodine. This enabled the occurrence of substitution to be tested and, if necessary, allowed for, since halogen acid would be formed in the substitution reaction in the proportion of 1 equivalent to half an equivalent of the iodine chloride or bromine reagent.[2]

BROMINE VALUES OF *cyclo*HEXENE—

The accuracy of the titration can be assessed from examination of the coefficients of variation for "blank" titrations on the bromine solutions used. These are given in Table II.

TABLE II

Mean normality found	Coefficient of variation %	Number of determinations
0·05011	0·5	3
0·07521	0·2	4
0·03125	1·0	3
0·1181	0·5	4
0·03251	0·5	4
0·03236	0·7	4
0·03228	0·6	3
Mean	0·6	

For a standard reaction period of 1 hour and about 100 per cent. excess of bromine (this ranged from 61 to 112 per cent.), a series of eight determinations was made. No substitution occurred. The values obtained were: 197·0, 194·4, 194·3, 193·0, 194·9, 198·7, 194·1 and 193·3, having a mean of 195·0 compared with the theoretical value 194·6. The coefficient of variation was 1 per cent., showing an experimental error slightly larger than that of the blank determinations. In corresponding determinations on the macro scale two observers found respectively 193·6 and 193·1 for the mean and 0·7 and 1·2 per cent. for the coefficient of variation, based on 7 and 8 determinations respectively.

The effects of variation in time of reaction and excess of reagent were investigated, and it was found that low values are associated only with times as short as 5 minutes and excesses of bromine less than 50 per cent. It would appear that with 100 per cent. excess of reagent the addition reaction occurs so rapidly that it is complete before substitution is perceptible. When bromine is present only in small excess, the reaction slows down as the concentration of the reagent falls, and the velocity of the substitution reaction becomes appreciable compared with that of addition. Even so, such substitution as occurs is equivalent to only 2 or 3 units of the bromine value.

The best conditions are, therefore, a period of, say, 15 minutes with an excess of reagent of the order of 80 to 100 per cent.

IODINE VALUES OF *cyclo*HEXENE—

The iodine values of *cyclo*hexene determined by means of a 100 per cent. excess of a solution of the reagent in glacial acetic acid were found to increase linearly with the length of time of standing. As correction for substitution is not possible in acetic acid medium, further work on this procedure was abandoned and use made only of the reagent in carbon tetrachloride solution.

As with the bromine values, the accuracy of the titration can be assessed from the variation of "blank" values, and this is given in Table III.

The mean coefficient of variation is identical with that obtained for bromine titrations.

Experiments showed that a standard period of 1 hour with 100 per cent. excess of reagent produced substitution, and that 15 minutes with about 60 per cent. excess of reagent was a suitable procedure. Values thus obtained were: 309·6, 309·8, 306·2, 308·4, 309·9 and 308·9, of which the mean value is 308·8 compared with the theoretical figure 309·7. The coefficient of variation was 0·5 per cent. and the agreement of this figure with the corresponding figure for the blanks (see Table III) shows that the precision of the method is largely a function of the measurement and titration of the reagent.

TABLE III

Mean normality found	Coefficient of variation %	Number of determinations
0·02669	0·8	5
0·02619	0·7	4
0·02593	0·4	3
0·02616	0·4	5
0·03170	0·4	4
0·03057	1·1	4
0·1925	0·3	4
0·1937	0·6	4
Mean	0·6	

Attempts were made to use the titrations to investigate the kinetics of the reaction, but the rate of reaction is obviously too great for such simple technique. Table IV shows the values obtained for short reaction times and small excesses of reagent.

TABLE IV

Reaction period, minutes	Excess of reagent %	Iodine value
2	22·5	308·7
2	23·0	308·0
2	20·0	306·9
4	22·5	306·7
4	24·0	308·9
8	23·0	310·7
8	24·7	306·5

IODINE VALUES OF UNSATURATED OILS—

In order to provide evidence of the general applicability of the micro method the iodine values of castor and blown linseed oils were determined under conditions of period of reaction and amount of reagent recommended in a standard work on the subject.[3] With the micro method, carbon tetrachloride was used as solvent, thus permitting correction for substitution, but in some corresponding determinations made by macro procedure the recommendations were strictly adhered to and glacial acetic acid was used as the medium. The results, given in Table V, show reasonable agreement between micro and macro results for castor oil, but for the blown linseed oil this is only true of the total iodine value, for, when corrected for substitution, the micro values are significantly lower than the macro values. The spread of results has not been investigated, nor has the question of removing peroxides.

TABLE V

Material	Reaction period	Excess of reagent %	Iodine value (macro method in acetic acid)	Total iodine value (micro in CCl_4)	Iodine value (micro after correction for substitution)
Castor oil (iodine value expected, *c.* 84)	60 min.	*c.* 100	82·6	88·0	No substitution
			84·5	87·1	
			84·5	85·9	
Means	..	.. ..	83·8	87·0	
Blown linseed oil (iodine value expected, less than 120)	3 hr.	*c.* 100	107·2	105·3	98·2
				106·8	103·5

CONCLUSIONS

The use of special iodine flasks has enabled iodimetric titrations to be carried out with a horizontal micro-burette and about 1 ml. of sodium thiosulphate solution. The application of this to the bromine and iodine values of *cyclo*hexene demonstrates the accurate determination of iodine values with samples of a few milligrams, the accuracy obtained with iodine chloride being that of the volume measurements. Conditions under which full saturation of *cyclo*hexene is achieved without substitution have been found, but these may not be suitable for other olefines. Of more general interest is the application to unsaturated oils, where agreement is obtained with macro procedure if the total iodine value is accepted, but it is shown that with a blown linseed oil some substitution occurs.

The authors express their thanks to Miss S. M. Lanham for carrying out many of the titrations and to the Research Association of British Rubber Manufacturers for permission to publish this paper.

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3. Dean, H. K., *The Utilisation of Fats*, London, 1938.

THE RESEARCH ASSOCIATION OF BRITISH RUBBER MANUFACTURERS
105–107 LANSDOWNE ROAD
CROYDON, SURREY

May, 1948

DISCUSSION

The PRESIDENT congratulated the authors on their paper and enquired whether they had made any comparison with micro-determinations using iodine chloride dissolved in glacial acetic acid.

Mr. C. L. HINTON pointed out that there were already in existence a large number of figures for fatty oils, published and unpublished, that had been obtained by the traditional method; even if these should not be strictly accurate, an undesirable confusion might arise if different figures, obtained by a micro method, should come into use.

Mr. K. A. WILLIAMS asked if a sufficient excess of the reagent was used to leave more unabsorbed at the end of an experiment than was consumed by the fat.

Dr. ROMAN asked whether the chloride ion formed would interfere with the iodate titration.

Mr. WAKE, in reply, thanked the President for his remarks and said that comparative figures with glacial acetic acid in the micro method had not been obtained owing to the uncertainty whether substitution would occur. He agreed that such comparison should have been made with castor and linseed oils. In reply to Mr. Williams, he said that the excess used would leave about an equal quantity. In reply to Dr. Roman, he said that as far as he knew the standardisation of hydrochloric acid by iodate was satisfactory and hence one would not expect interference by the chloride ion.

A Colorimetric Method for the Estimation of Amyl Acetate Vapour in the Air

BY H. M. CUSTANCE AND M. HIGGINS

A CONVENIENT and simple method was required to estimate the amyl acetate content of air in a room used for the extraction of penicillin. Most of the methods previously described entailed absorbing the amyl acetate in ethyl alcohol, hydrolysing and then titrating with standard sulphuric acid the excess of sodium hydroxide used in the hydrolysis.[1,2,3] This type of method is tedious, is not specific and would not in general be capable of giving accurate results in the routine determination of low concentrations of ester vapour. Methods applicable generally to solvent vapours can be used,[1] but are even less specific then the saponification procedure.

Several colour tests for amyl acetate have been described,[1] the best known of which is the Komarowsky reaction; this has been used for the colorimetric determination of amyl acetate and of higher alcohols in fusel oil.[4,5] This method is based on the formation of coloured products through the interaction of higher alcohols and esters with cyclic aldehydes in the presence of concentrated sulphuric acid. The aldehydes which have been employed as reagents include salicylaldehyde, benzaldehyde, *p*-dimethylaminobenzaldehyde, furfural, veratric aldehyde and vanillin. Komarowsky's was considered the most promising method for rapid and convenient determination of amyl acetate in the air. Snell[4] describes the application of the method and includes a comprehensive bibliography. Its application to fusel oil determinations is discussed at some length by Penniman, Smith and Lawshe.[5] Nowhere however are sufficiently detailed figures available for accurately defining the conditions under which the determination of amyl acetate in alcoholic solution may best be made.

We have therefore tested various aldehydes as to their suitability and have developed a method using *p*-dimethylaminobenzaldehyde. The optimum conditions for making determinations by this method have been established and figures compiled for estimating the efficiency of ethyl alcohol as an absorbent for amyl acetate.

The experimental investigations of the conditions for carrying out the method were all made with pure *iso*-amyl acetate prepared from *iso*-amyl alcohol.

DEVELOPMENT OF THE COLOUR—

Selection of suitable solvent—In testing the suitability of a solvent, 2 ml. of a solution containing 0·5 mg. of amyl acetate per ml. of the solvent were treated with 2 ml. of water, 10 ml. of concentrated sulphuric acid and 2 ml. of a 2 per cent. *p*-dimethylaminobenzaldehyde solution in the same solvent. The colour was read in a Spekker absorptiometer after 30 minutes. The choice of solvent was restricted to liquids miscible with water and not decomposed by sulphuric acid. *Cyclo*hexanol and dioxan were unsuitable because they formed a black colour with the reagents. Of the range of solvents tested, ethyl alcohol and methyl alcohol were found the most satisfactory. With alcohols of ordinary laboratory-reagent quality the "blanks" were very high, owing, no doubt, to the presence of higher alcohols. Although a single straight distillation did not improve the solvents in this respect, blanks as low as 0·06 (Spekker reading) with methyl alcohol and 0·33 with ethyl alcohol were obtained by careful fractionation of the solvent. Ethyl alcohol was eventually chosen in preference to methyl alcohol, as its lower volatility made it more suitable for long sampling periods.

Selection of a suitable aldehyde—Two ml. of a solution containing 0·5 mg. of amyl acetate per ml. of alcohol were treated with 2 ml. of water, 10 ml. of concentrated sulphuric acid, and 2 ml. of a 2 per cent. solution of various aldehydes in fractionated ethyl alcohol (see page 310) and the colour was read after 30 minutes. A blank was prepared and read at the same time. With salicylaldehyde, crotonaldehyde and paraldehyde, the blank was too dense to be read with the Spekker absorptiometer. When benzaldehyde was used the solution was cloudy. With vanillin the readings for the blank and standard solutions were respectively 0·50 and 1·45, and with *p*-dimethylaminobenzaldehyde 0·34 and 1·70. The latter substance was selected.

Optimum concentrations—The colour intensity was found to be reduced by water, but with the water content below a certain amount the colour of the blank became too intense. The addition of 2 ml. of water to 2 ml. of sample solution gave the best results. Alcohol also reduced the colour intensity, so that an optimum value existed for the amount of sample solution to be employed; this was found to be 2 ml. A 2 per cent. solution of *p*-dimethylaminobenzaldehyde gave the most satisfactory results.

TABLE I

EFFECT OF TEMPERATURE ON THE COLOUR REACTION

Type of reaction vessel	conical flask, 50 ml.	conical flask, 150 ml.	conical flask, 250 ml.	Test tube, 9 × 1 in.
Maximum temperature	84° C.	74° C.	58° C.	82° C.
Average temperature during 30 minutes	40° C.	34° C.	29° C.	44° C.
Absorptiometer reading after 30 minutes:				
Total reading for 0·3 mg. of amyl acetate	0·69	0·40	0·28	0·71
Blank reading	(0·35)	(0·29)	(0·20)	(0·32)
Net reading	0·34	0·11	0·08	0·39
Absorptiometer reading after holding for 30 minutes in a water-bath at 60° C.:				
Total reading for 0·3 mg. of amyl acetate	1·03	—	—	1·07
Blank reading	(0·59)	—	—	(0·59)
Net reading	0·44			0·48

As the volume of sulphuric acid added was increased, so the colour intensity rose to a maximum at 15 ml., beyond which amount it slowly decreased. The volume selected was 10 ml., as it was considered desirable to keep the volume of the reagents small, and the sensitivity of the method was such that the increase in colour of about 50 per cent. that would occur if 15 ml. were used instead of 10 ml. was not of practical importance.

Effect of temperature—The early determinations were carried out by adding the reagents to the alcoholic solution of amyl acetate in 50-ml. conical flasks, allowing them to cool and then making the absorptiometer readings. Consistent results were difficult to obtain, particularly when flasks of different dimensions were used. This was traced to differences in temperature produced in flasks of different sizes when the sulphuric acid was added to the mixture.

Table I shows these different temperatures, together with two sets of absorptiometer readings, the first taken after the reaction vessels had been allowed to stand for 30 minutes, the second after they had been held for 30 minutes in a water-bath at 60° C. instead.

As high temperatures led to very high blanks it was decided to hold the vessels in a water-bath at 60° C. The time of heating, while not particularly critical, did affect the results. The extent of this effect can be seen from Fig. 2, in which time of heating at 60° C. is plotted against absorptiometer readings, both for a blank and for a solution of 0·5 mg. of amyl acetate per ml. of alcohol. Extending the time of heating beyond 30 minutes not only rendered the method more inconvenient but also increased the blank reading without appreciably increasing the sensitivity of the method. If a quicker procedure were desired, satisfactory results could be obtained after 10 minutes' heating.

Stability of colour—The reaction was carried out as outlined below (page 313), and after the heating at 60° C. for 30 minutes, the solution was cooled to room temperature and readings of the colour were then made at various intervals. The results in Table II show that the colour is stable for at least 2½ hours, and that the readings may be made within 5 minutes of removal from the water-bath.

TABLE II

STABILITY OF COLOUR

Time after removal from bath, min.	Readings for 0·3 mg. of amyl acetate present	Blank readings
5	1·26	0·70
30	1·28	0·72
60	1·29	0·72
90	1·29	0·72
150	1·29	0·72

Details of the method finally adopted for determining amyl acetate in air are set out below. The amyl acetate is absorbed in fractionated ethyl alcohol and determined by the colorimetric procedure worked out as described above.

METHOD

APPARATUS—

The amyl acetate vapour was absorbed by means of two simple all-glass bubblers (6 in. by 1 in.), of the design shown in Fig. 1, connected in series. An electric pump and rotameter were used for drawing air through these bubblers at a rate of 0·5 litre per minute.

A Spekker absorptiometer with 1-cm. cells and equipped with Ilford 603 filters was used for measuring the coloured solutions.

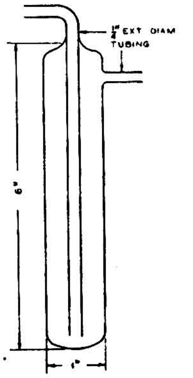

Fig. 1. Absorption Bubbler

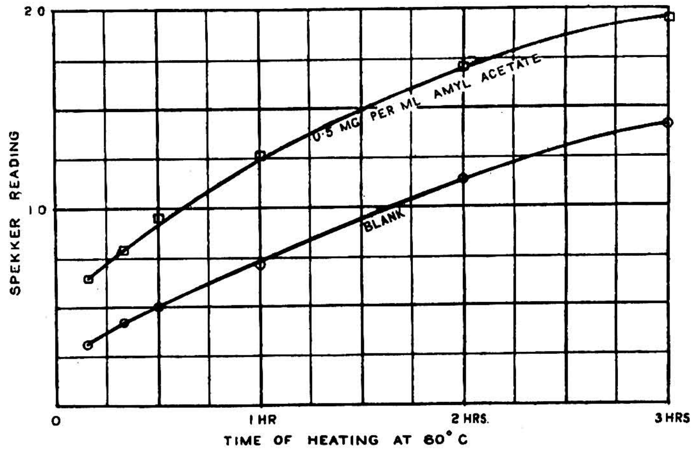

Fig. 2

REAGENTS—

Fractionated absolute alcohol—Absolute ethyl alcohol fractionated through a 3-ft. Stedman column or its equivalent.

Reagent solution—2 g. of *p*-dimethylaminobenzaldehyde in 100 ml. of fractionated absolute alcohol.

Sulphuric acid—C.P. sulphuric acid (about 96 per cent.).

Standard solution of amyl acetate—0·50 g. of amyl acetate made up to 1 litre with fractionated absolute alcohol. The amyl acetate used for making up this standard should be obtained from the same source as that producing the atmospheric contamination.

PROCEDURE—

The air to be tested was drawn through the bubblers, each charged with 10 ml. of the fractionated absolute alcohol, at a rate of 0·5 litre per minute for a sampling period of 30 minutes. The bubblers were then washed out separately with the fractionated absolute alcohol and each solution was made up to a volume of 15 ml.

To 2 ml. of the resulting solution from a bubbler were added 2 ml. of water, 2 ml. of the reagent solution and 10 ml. of the sulphuric acid in a 1-in. by 9-in. test tube. The test tube was kept in a water-bath at 60° C. for 30 minutes and then cooled for 5 minutes and the colour read within 2 hours, with distilled water as the comparison liquid.

Standard curves were prepared each day by carrying out the reaction with 0, 0·1, 0·5, 1·0, 1·5, and 2·0 ml. of the standard solution of amyl acetate, to each of which the required amount of the fractionated absolute alcohol to bring the volume to 2·0 ml. was added.

RESULTS

EFFICIENCY OF ETHYL ALCOHOL AS AN ABSORBENT—

A large number of determinations of amyl acetate in rooms used for extracting penicillin have been made by means of this standardised method. From the separate analytical results from each of the two bubblers it was possible to estimate the suitability of ethyl alcohol as an absorbent for amyl acetate. Typical figures are given in Table III. It appears that in the range of concentrations encountered satisfactory absorption took place in two bubblers, since on the average, 91 per cent. of the total amount collected by the two bubblers was collected in the first.

TABLE III

ABSORPTION OF AMYL ACETATE

Weight in first bubbler mg.	Weight in second bubbler mg.	Concentration calculated from analytical figures mg. per cubic metre of air	Percentage of total amount absorbed in first bubbler
53·1	4·8	3860	92
52·8	4·8	3830	92
50·8	3·9	3640	93
49·7	3·9	3560	93
16·4	1·8	1210	90
15·0	1·2	1078	93
13·8	1·5	1018	90
13·1	1·4	965	90
12·5	1·3	920	90
12·1	1·3	892	90
8·55	0·72	617	92
7·97	0·91	591	90
7·40	1·03	561	88
7·12	0·99	540	88
7·05	0·65	513	92

APPLICATION RANGE—

A standardisation curve from 0 to 0·4 mg. per ml., covering the range of readings on the Spekker absorptiometer from 0·3 (the blank) to 1·4, is a straight line. The blank has a rather high value and has been found to vary between 0·3 and 0·7, depending on the ethyl alcohol used. By careful fractionation it can be kept at the lower figure, and for a given batch of alcohol is closely reproducible. The figures in Table IV give the results of five series of seven determinations made with known solutions on different days, all based on the same calibration curve. It can be seen that the method is very satisfactory for concentrations down to 0·05 mg. per ml. of solution.

TABLE IV

ANALYSIS OF KNOWN SOLUTIONS OF AMYL ACETATE

Concentrations of solution mg./ml.	Averages of seven determinations mg./ml.	Standard deviation
0·01	0·017	± 0·005
0·05	0·051	± 0·003
0·10	0·098	± 0·005
0·20	0·199	± 0·006
0·30	0·297	± 0·005

With the recommended procedure, 0·05 mg. per ml. corresponds to a concentration in the air of 50 mg. per cubic metre, or 10 parts per million.

COMPARISON WITH SAPONIFICATION METHOD—

Thirty-seven pairs of determinations were carried out with air containing varying concentrations of amyl acetate. Of each pair, one determination was made by the colorimetric and the other by the saponification method.[1] A statistical analysis was made of these results and, as indicated in Table V, the "t" test showed that there was no significant difference between the means.

TABLE V

DETERMINATIONS OF AMYL ACETATE IN AIR

Method	Number of determinations	Mean	Standard deviation	*t*	Value of *t* for probability of 0·05 against
Colorimetric	37	889·5	72·38	0·674	2·03
Saponification	37	855·3	73·62		

A series of alcoholic solutions of amyl acetate of known concentration was prepared and each solution analysed by both procedures. Twenty determinations were made by each method on each of the solutions, and a summary of the results is given in Table VI.

TABLE VI

COMPARISON WITH SAPONIFICATION METHOD

Concentration of amyl acetate taken, mg. per ml.	Found by saponification method		Found by colorimetric method		Comparison between saponification and colorimetric methods	
	Mean mg. per ml.	Standard deviation	Mean mg. per ml.	Standard deviation	Difference between means	*t*
2	1·89	0·022	1·92	0·081	0·03	0·81
1	1·01	0·020	1·01	0·031	nil	nil
0·5	0·484	0·021	0·490	0·013	0·006	1·9
0·2	0·190	0·020	0·195	0·007	0·005	2·1

For 38 degrees of freedom and $p = 0{\cdot}05$ .. .. 2·0
For 38 degrees of freedom and $p = 0{\cdot}02$ .. .. 2·4

It is obvious from these results that the colorimetric procedure was not subject to any systematic error. Whilst at concentrations of 1·0 mg. per ml. and higher, results obtained by saponification showed a lower standard deviation than those obtained by colorimetric analysis, the latter method was superior at lower concentrations.

APPLICATION TO *n*-BUTYL ACETATE—

When the procedure for amyl acetate was applied directly to *n*-butyl acetate it was found that approximately 100 times the amount of *n*-butyl acetate was required to give colours of the same density as with amyl acetate. This means that the minimum concentration of *n*-butyl acetate that could be determined with any accuracy by this method would be of the order of 5000 mg. per cubic metre of air or 1000 parts per million. This renders the method unsuitable for the determination of *n*-butyl acetate. It is also apparent that small amounts of *n*-butyl acetate would not affect the determination of amyl acetate.

DISCUSSION AND CONCLUSIONS

According to Snell,[4] the reaction is given, in general, by higher aliphatic alcohols and their esters, hydro-aromatic alcohols, phenols and straight-chain ethylene compounds. However, the amount of interference depends upon the behaviour of the compound under the conditions of the procedure as developed here. From the information given by Penniman, Smith and Lawshe,[5] it would appear that *n*-butyl, *iso*-propyl and *n*-propyl alcohols are unlikely to give serious interference if present in small quantities, but that *n*-amyl and *iso*-butyl alcohols may very well do so, since they are recorded as giving colours of the same intensity as *iso*-amyl alcohol.

The method is capable of determining accurately concentrations of amyl acetate in the air down to 10 parts per million, and is in this respect superior to the saponification procedure. Furthermore, although not completely specific for amyl acetate, the method compares favourably with any other available procedure from the point of view of specificity. Extensive use has confirmed the value of the method and proved it to be convenient and accurate.

SUMMARY

A colorimetric method, based on the colour given by the reaction of amyl acetate with sulphuric acid and *p*-dimethylaminobenzaldehyde in ethyl alcohol solution, has been applied to the quantitative determination of amyl acetate vapour in the air. The optimum conditions for the application of this method have been established, and comparisons made between it and the saponification procedure. For low concentrations the colorimetric procedure was found preferable. As an absorbent for the amyl acetate, ethyl alcohol proved satisfactory.

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May, 1948

Determination of the Iron Content of Blood Serum

BY S. L. TOMPSETT AND R. A. McALLISTER

NORMAL blood serum contains from 50 to 150 μg. of iron per 100 ml. Larger or smaller values may be encountered in disease. This iron appears to exist as a labile compound with protein, as distinct from the more firmly bound iron of the haemoglobin present in the red blood cells. Owing to the small amount of serum usually available for analysis, it is necessary to be in a position to estimate quantities of iron of the order of 1 to 3 μg. with some degree of accuracy. Methods involving a preliminary ashing are to be avoided because serum usually contains some haemoglobin from haemolysed red blood cells. By the use of appropriate techniques, serum iron can be recovered quantitatively in trichloroacetic acid extracts of serum, haemoglobin iron being unextracted (Barkan[1], Barkan and Walker,[2] Tompsett[3]). Such iron may be determined directly in these extracts. One of us[3] employed the violet colour produced with thiolacetic acid and ammonia in conjunction with visual colorimetry. Barkan and Walker[2] described a method in which the depth of colour produced with *o*-phenanthroline was determined in a visual colorimeter. The depth of colour was determined after the reactants had stood at room temperature for at least 1 hour.

The present writers found that colour development with *o*-phenanthroline was exceedingly irregular at room temperature. At 37° C., however, colour development was found to be more regular, and reproducible results could be obtained after 1 hour.

The following is a description of the method now adopted.

METHOD

All glassware must be washed with dilute hydrochloric acid and then with glass-distilled water and dried.

Collection of blood—

About 10 ml. of blood are collected in a centrifuge tube and centrifuged as soon as possible and the serum is separated. An all-glass syringe fitted with a stainless steel needle should be used for collecting the blood.

Reagents—

(1) Hydrochloric acid, 1·2 per cent.

(2) Trichloroacetic acid, 20 per cent. solution.

(3) Sodium acetate solution, saturated.

(4) 2 *M* Acetate buffer solution, pH 4·5. This is prepared by mixing 90 volumes of 2 *M* sodium acetate and 110 volumes of 2 *M* acetic acid.

(5) Hydrazine sulphate, 1 per cent. solution in 2 *M* acetate buffer of pH 4·5. This reagent must be freshly prepared before use.

(6) *o*-Phenanthroline, 0·1 per cent. solution in water.

All solutions must be prepared with glass-distilled water and filtered.

Procedure—

Place a mixture of 4 ml. of the serum and 2 ml. of 1·2 per cent. hydrochloric acid in an incubator at 37° C. for at least 1 hour. After cooling to room temperature, precipitate proteins by addition of 2 ml. of 20 per cent. trichloroacetic acid solution and centrifuge the mixture. As the supernatant fluid tends to be slightly turbid, pass it through a No. 42 Whatman filter paper of minimal dimensions which has been washed with dilute hydrochloric acid and water and dried. To 4 ml. of the filtrate add, in order, 1 ml. of saturated sodium acetate solution, 1 ml. of buffered 1 per cent. hydrazine sulphate solution, 1 ml. of 0·1 per cent. *o*-phenanthroline solution and 3 ml. of water.

Place the mixture in an incubator at 37° C. for 1 hour and evaluate the depth of colour in a Spekker absorptiometer, using Ilford Spectrum Green Filters No. 604.

When 1-cm. cells were used it was found that drum readings (less blank) ranging from 0 to 0·08 were proportional to quantities of iron from 0 to 5·8 μg. in the 2 ml. of serum used in the general analysis.

Notes—A blank should register less than 1 μg. of iron. No difficulty has been experienced when current chemicals have been employed—no additional purifications being required. Anti-coagulants should be avoided, since they may cause interference with the colour development.

Results—

Recoveries of iron added to serum are shown in Table I.

TABLE I

RECOVERY OF IRON ADDED TO SERUM

Results expressed in micrograms of iron per 100 ml. of serum

	initial iron content	Iron added	Total iron determined	Added iron recovered	
	μg.	μg.	μg.	μg.	%
1	66	58	115	55	95
2	60	117	185	125	107
3	60	146	215	155	106
4	105	58	170	65	112
5	105	117	230	125	107
6	105	146	260	155	106

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INSTITUTE OF PATHOLOGY
ROYAL INFIRMARY
GLASGOW

February, 1948

Notes

THE QUANTITATIVE SEPARATION OF BERYLLIUM FROM ALUMINIUM

(*Read at the meeting of the Society, on Wednesday, February 3rd,* 1949)

THE generally accepted standard method for determining beryllium in presence of excess of aluminium is the fusion of the mixed oxides with sodium carbonate.[1,2] This is based on conversion of alumina to soluble aluminate whilst the beryllia remains unaffected and insoluble on subsequent leaching.

Paradoxically, the usual method for decomposition of beryl is also a sodium carbonate fusion. Osborn,[3] who has recently investigated the reaction, showed that whereas complete decomposition is possible with a beryl to sodium carbonate ratio of 1 : 2, complete insolubility of the beryllium is not attained in any circumstances. In addition, it is well known that the aluminium is not always completely soluble and a second treatment of the insoluble residue with sodium carbonate is generally recommended. In view of these difficulties, an alternative method of separation seems desirable.

PRECIPITATION OF BERYLLIUM FROM CAUSTIC ALKALINE SOLUTION—

The reaction that led to the discovery of beryllium by Vauquelin in 1798 depends on the relative instability of the beryllate. Whereas an alkaline solution of sodium or potassium aluminate is stable on boiling, a solution of the beryllate becomes decomposed with precipitation of beryllia. In 1921, Britton[4] showed that this reaction could be applied quantitatively, but it has never come into general use and is not mentioned in Schoeller and Powell's treatise. To obtain quantitative precipitation of the beryllium the concentration of free alkali must be very small and Britton's method was to add strong sodium hydroxide solution to an acid solution of the aluminium and beryllium until the precipitated hydroxides just redissolved; the solution was then diluted and boiled. Our experience is that this method yields very erratic results, the difficulty lying in the fact that the hydroxides will not dissolve very easily in a small excess of alkali, and unless great care is taken more than the optimum amount will be added. It was noticed, however, that on neutralising caustic alkali solutions of aluminium and beryllium with hydrochloric acid, the beryllium begins to precipitate in an appreciably more alkaline solution than the aluminium. If a solution is known to contain both metals, therefore, the alkalinity can be adjusted by adding sufficient acid to cause the first precipitation of beryllium hydroxide. Subsequent boiling completes the precipitation of the beryllium leaving the aluminium in solution.

Although this method is suitable for solutions that are known to contain beryllium, it is clearly desirable with unknown solutions to have an indicator that gives a colour change at the correct conditions for precipitation of beryllium. Of a number of indicators examined, indigo carmine, although not ideal, was found to be the most suitable. In strongly alkaline solution this dye gives a yellow colour, and on neutralising with acid the colour changes through green to blue. The green-blue colour change is fairly sharp at pH 11·2, but the change from yellow to green is more gradual. It was found that while beryllium hydroxide precipitates in the greenish-yellow solution, aluminium is not precipitated until the blue colour is reached. With unknown solutions a few drops of an aqueous solution of indigo carmine should be added and the solution titrated with hydrochloric acid until there is a slight precipitate or until the solution assumes a definite green colour, whichever occurs first. If on boiling the green solution, no precipitate appears, then beryllium is absent.

EXPERIMENTAL—

Different amounts of a standard beryllium sulphate solution were mixed with 50 mg. of aluminium, added in the form of potassium alum solution, treated with 5 g. of sodium hydroxide and diluted to 200 ml. A few drops of 1 per cent. indigo carmine solution were added and diluted hydrochloric acid (1 + 1) was run in from a burette until a slight permanent precipitate appeared in the yellowish-green solutions. After boiling for 30 minutes the precipitates were filtered off and washed with 1 per cent. sodium chloride solution to which sodium hydroxide solution had been added dropwise until a green colour was produced with indigo carmine. The precipitates were then redissolved in hydrochloric acid and precipitated with ammonia, using litmus paper as indicator (methyl red, which is commonly recommended, does not ensure complete precipitation). The reprecipitated beryllium hydroxide was filtered and washed with dilute ammonium chloride solution to remove any sodium salts and finally ignited to BeO.

Recovery of beryllium was quantitative, as the following results show.

PRECIPITATION OF BERYLLIUM IN PRESENCE OF 50 MG. OF ALUMINIUM

BeO added, mg.	BeO recovered, mg.
140·5	140·0
70·2	70·0
35·1	34·8
14·0	13·9

In presence of larger amounts of aluminium, results are somewhat high with one precipitation from caustic soda solution and a double precipitation is necessary for complete separation. This is illustrated by the following figures, which were obtained by precipitating beryllia from solutions containing 1·0 g. of Hilger H.H.P. aluminium.

PRECIPITATION OF BERYLLIUM IN PRESENCE OF 1·0 G. OF ALUMINIUM

BeO added, mg.	BeO recovered, mg.	
	1 precipitation	2 precipitations
145·0	147·0	144·0
72·5	76·0	71·6

The results shown in the last column were obtained by dissolving the first precipitate in hydrochloric acid, adding excess of sodium hydroxide and precipitating again in the same way. After filtering, a final reprecipitation with ammonia was carried out to eliminate sodium chloride.

SUMMARY—

When a caustic alkaline solution of beryllium and aluminium is neutralised with dilute acid, the beryllium hydroxide starts to precipitate first, and on subsequent boiling precipitation of beryllia is quantitative whilst aluminium remains in solution. Indigo carmine may be used as indicator to show the correct alkalinity. With a large excess of aluminium double precipitation from the caustic alkaline solution is necessary.

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DISCUSSION

Mr. W. H. BENNETT asked if the method had been tested for the separation of small quantities of beryllium of the order of 1 mg., such as would be likely to be encountered in the analysis of rocks. Would the author expect any difficulty in filtering and washing small amounts of precipitate, owing to colloidal effects, and did phosphates interfere with this separation?

Mr. G. H. OSBORN asked whether the author had tested for aluminium in the beryllium precipitate, and for beryllium in the aluminium precipitate. He congratulated the author on what was apparently a very useful method of separation of the two elements.

Mr. COPPINS, in reply, said that he had not used the method for separation of less than about 5 mg. of beryllium and considered that quantities of the order of 1 mg. would be better determined by a photometric method which did not involve separation from aluminium. Apart from the usual difficulties of dealing with a very small precipitate, he would not expect any trouble from colloidal effects, as the α-hydroxide of beryllium which is precipitated from alkaline solution is quite granular in character. Difficulty from this source was more likely during the reprecipitation with ammonia, but provided adequate time was allowed before filtering it should not be serious.

As he was more interested in alloys than in minerals, he had not had occasion to investigate the effect of phosphates.

Some of the beryllia precipitates had been examined spectrographically and no aluminium was detected.

ROTARY STIRRING DEVICES FOR MICRO-TITRATION

As with the reciprocating stirrers previously described,[1] the device shown in Fig. 1 is operated by a filter pump. Although the torque is not great, the high speed of rotation produces excellent mixing in aqueous or similar media.

The T-piece A, forming the body, is about 60 mm. long and has 15 mm. inside diameter. Both ends are closed by corks, the lower of which carries bearing B. This is a 40-mm. length of glass tubing, the ends of which are slightly constricted in the flame until spindle C will just not enter. The spindle, which is half of a No. 15 steel knitting needle, is then ground into the bearing with extra fine emery paste until an easy running fit is obtained. Stop D, which is of 6-mm. outside diameter glass tubing, is closed and flattened at the lower end and a small hole is blown in the wall as shown.

Rotor E, which is cut from an ordinary tapering cork, is about 12 mm. long and of 13 mm. diameter at the larger end. By means of a razor blade, about twelve teeth are cut lengthwise so that a "rachet-wheel" section as shown at (*a*) is produced. The pointed end of the spindle, C, is then thrust axially through the rotor as shown; reasonable errors either in alignment or in tooth-shape have little effect upon efficiency. Before insertion into the bearing, a small brass washer, F, is slipped on the spindle beneath the rotor, to prevent sticking.

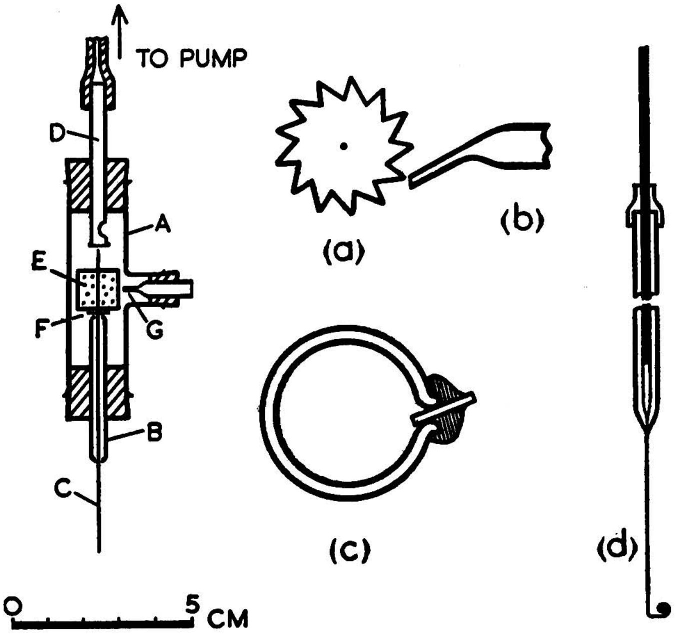
TO PUMP
D
A
E
F
G
B
C
(a)
(b)
(c)
(d)
0
5
CM

Fig. 1

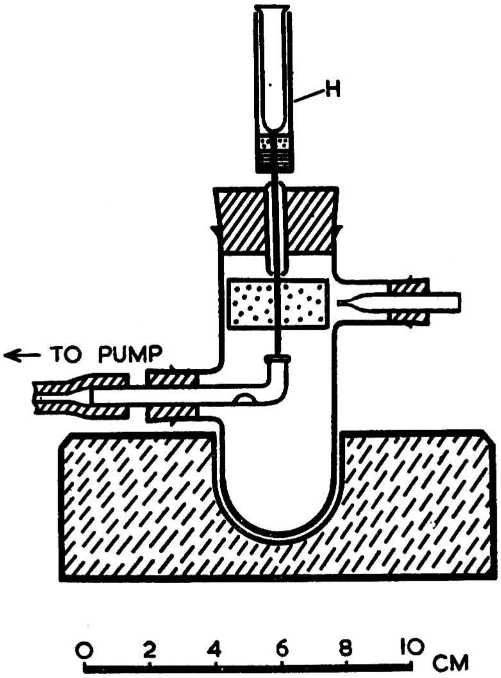
H
TO PUMP
0
2
4
6
8
10
CM

Fig. 2.

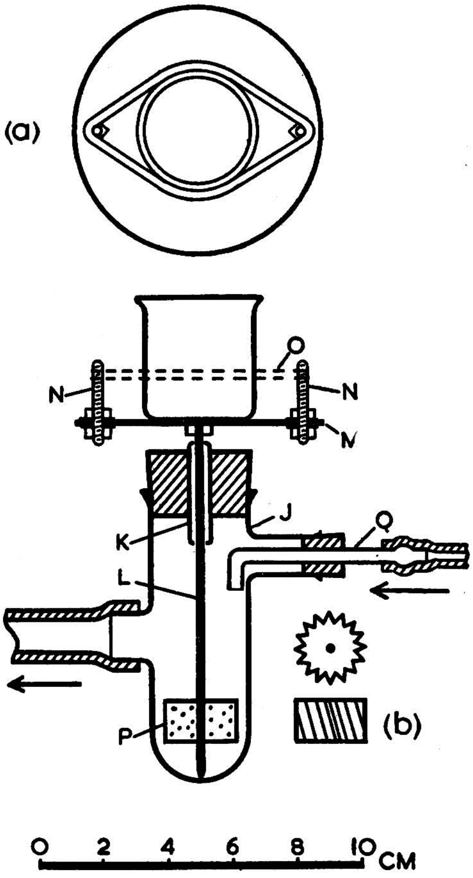
(a)
O
N
N
M
J
K
Q
L
P
(b)
0
2
4
6
8
10
CM

Fig. 3.

Jet G is drawn from 4-mm. outside-diameter tubing to give an orifice of about 1-mm. diameter. It is bent as shown at (*b*), the optimum angle being obtained by trial. An alternative arrangement is to use as a body a piece of glass tubing with a small hole blown midway in the wall; the jet is then cemented in the hole by sealing wax, as shown at (*c*), adjustments being made while the wax is still soft.

The parts are assembled so that the distance between the jet-tip and rotor is 1 to 2 mm., clearance between the pointed upper end of the spindle and the stop being about the same. On connecting to a filter pump, the stream of air drawn in through the jet causes the spindle to rotate rapidly. At the same time, atmospheric pressure slightly lifts the spindle assembly, thus greatly reducing friction.

Interchangeable stirrer-heads as shown at (*d*) are made from wide melting-point tubes, into which the lower end of the spindle slips comfortably. Retention is by a sleeve of cycle valve tubing.

When titrating in narrow vessels, or when, as in potentiometric micro-titration, several pieces of apparatus dip into the solution, a convenient method of stirring is by rotating the vessel.[2,3] For thus performing titrations in a micro test tube, the device shown in Fig. 2 is satisfactory. Construction is along the same lines as with the first device. The rotor, which has about twenty-four teeth, is 30 mm. in diameter. Holder H, a 40-mm. length of glass tubing, is mounted truly on the upper end of the spindle by means of a cork reinforced by sealing wax. The bore of the holder should snugly accommodate the titration vessel, the contents of which may be kept under observation during titration.

For larger quantities of liquid (*i.e.*, from 2 to 25 ml.) a micro-beaker of appropriate size is a more convenient titration vessel. In this case the inherently high speed of these turbine devices is disadvantageous, centrifugal action causing loss of liquid. Some improvement may be achieved by driving by water power instead of air; at low speeds, however, torque is very poor and highly sensitive to mains fluctuations.

The submerged rotor device shown in Fig. 3 was designed to permit steady rotation of micro-beakers at 100 to 150 r.p.m. Container J, of heavy glass, has 27 mm. inside diameter. A rubber stopper carries bearing K, into which spindle L is ground to an easy running fit as described above. The spindle is of 3/32-inch diameter brass rod and is tapered to a rounded point at the lower end. Brass platform M, which is 75 mm. in diameter and about 1 mm. thick, is soldered to the upper end of the spindle, the joint being strengthened by a small brass boss. Two pillars, NN, of No. 4 B.A. brass screw rod, are attached to the platform as shown. A rubber band, O, conveniently made by joining the ends of a length of cycle valve tubing with a short piece of glass rod, is stretched between the pillars. Micro-beakers of different size are held on the platform merely by being slipped within the band, as shown at (*a*).

The cork or rubber rotor P is 23 mm. in diameter and 12 mm. long. It has sixteen teeth, of section as shown at (*b*), cut at an angle of about 15 degrees to the length, and is positioned on the spindle as shown. Jet Q is of 3-mm. bore glass tubing, the end being bent at right angles as shown, but not constricted. The stream of water should be projected vertically downwards as close as possible to the container wall.

In operation the container becomes filled with water so that the rotor is submerged. Liquid drag, which increases rapidly with speed, then hinders rapid revolution. It is peculiar that the rotor turns the opposite way to that expected. The jet of water appears to pass downwards through the annulus formed by container and rotor, so that it is the rising water which causes the rotor to turn. Nevertheless, the direction of rotation may be reversed by turning the stem of the jet through about 45 degrees, thus giving the water a spiral, turbulent path. Under such conditions, however, rotation is more rapid and less uniform. Raising the rotor on the spindle also reduces uniformity of rotation.

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August, 1948

ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

Food and Drugs

The Official Assay of Ammoniated Mercury Ointment. M. A. Ellis (*Pharm. J.*, 1948, [iv], **107**, 400)—Because of indefinite end-points and consequent variable, low results, a source of error was sought in the B.P. directions for the assay of ammoniated mercury ointment. Difficulty in dissolving all the ammoniated mercury in the prescribed volume of 10 per cent. potassium iodide solution suggested that this quantity is insufficient. For the assay of ammoniated mercury itself it is directed that 0·25 g. of the powder be dissolved in water containing 3 g. of potassium iodide; the ointment contains 2·5 per cent. of medicament so that by analogy, for a 5-g. sample, 1·5 g. of potassium iodide should be required, whereas only 2 ml. of solution (0·2 g.) is stated to be required.

When assays were performed on different samples of ointment, both by strict adherence to the official directions and by addition of 1·5 g. of potassium iodide, the results obtained by the latter method were concordant and reproducible.

The amount of potassium iodide should therefore be increased, probably to 1·5 g. A. H. A. Abbott

Purification of Paraldehyde. C. H. Hunt and F. H. Reuter (*Aust. Chem. Inst. J. and Proc.*, 1948, **15**, 122–125)—Paraldehyde stored in incompletely filled or improperly closed containers is very susceptible to the formation of peroxide impurities, which, in turn, decompose to aldehydes and acids. For 5-ml. samples of paraldehyde the B.P. 1932 gives limits of acid and peroxide impurities as 1·5 ml. of 0·1 *N* sodium hydroxide and 2·0 ml. of 0·1 *N* sodium thiosulphate, respectively, under the conditions of the tests. Samples that do not comply with these requirements can be purified by the destruction of peroxides by heat, extraction with strong caustic soda solution to remove organic acids, and fractionation.

The following procedure, applied to a sample that required 9·7 ml. of 0·1 *N* sodium hydroxide and 1·9 ml. of 0·1 *N* sodium thiosulphate for the acid and peroxide limit tests, resulted in a yield corresponding to 85·5 per cent. of paraldehyde that conformed to the limits laid down by the B.P., in the original material.

Nine litres of the paraldehyde were boiled under a reflux condenser for 2 hr., cooled quickly, and extracted with 500 ml. of a mixture of 1000 ml. of brine and 300 ml. of 10 *N* sodium hydroxide. After washing with two successive 500-ml. portions of brine and allowing the neutral product to stand overnight in a filled closed container it was distilled, using a short fractionating column, and collected in the following fractions:—(1) b.p. 84° to 121° C., (2) b.p. 121° to 123° C., (3) b.p. 123° to 124·5° C., and (4) b.p. 125° C. Fractions (2) to (4) conformed with the B.P. requirements and were mixed and preserved in filled bottles with well-fitting glass stoppers.

Samples stored as described for 6 years showed no appreciable deterioration on re-test.

Samples with initially different acid and peroxide contents may require final collection in different fractions and each fraction should be tested for conformity to the B.P. tests before blending.

A. H. A. Abbott

Biochemical

Colour Test for Tryptophan. H. Tauber (*J. Amer. Chem. Soc.*, 1948, **70**, 2615)—*Procedure*—Suspend 0·5 mg. of tryptophan or 10 mg. of a tryptophan-containing protein in 1 ml. of water, add 3 ml. of perchloric acid (70 to 72 per cent.), and mix well. If a stable, intense yellowish-green colour and fluorescence develop within a few minutes, tryptophan is present. To confirm, add 0·1 ml. of a 1 per cent. aqueous solution of ferric chloride, and note that the yellowish-green colour changes to reddish-orange.

The following tryptophan-containing materials give the reaction: casein, albumen, human blood serum, pepsin, and crystalline soya bean trypsin inhibitor.

The following amino acids do not give the reaction: glycine, alanine, leucine, *iso*leucine, valine, phenylalanine, tyrosine, cysteine, cystine, methionine, threonine, proline, hydroxyproline, histidine, arginine, lysine, serine, aspartic acid, glutamic acid, and *p*-aminobenzoic acid. Indolyl acetic acid, however, gives a slight pink colour and a slight fluorescence. A. H. A. Abbott

Estimation of Leucine in Complex Mixtures of Amino Acids by Reaction with Sodium Hypochlorite. J. Asselineau (*Bull. Soc. Chim.*, 1947, 1065–1068 M)—Earlier work (Aubel and Asselineau, *Ibid.*, 1947, **41**, 689) on the determination of leucine, alanine, and valine by reaction with sodium hypochlorite to form aldehydes is summarised, and the determination of leucine in mixtures of amino acids is described.

Leucine in presence of alanine and valine—Treat the solution, containing about 30 mg. of amino acids, at 0° C. with an excess of *N* to 0·2 *N* sodium hypochlorite. After 10 min. add freshly-prepared 10 per cent. urea solution to remove the excess of hypochlorite. Stir and cool in ice for 10 min. Add the solution of chloramine derivatives dropwise to 60 ml. of boiling 0·1 *M* phosphate buffer (pH 8·5), and remove the aldehydes by a stream of air into bisulphite solution. An apparatus similar to that used for the determination of lactic acid is recommended (*cf.* Lieb and Zacherl, *Z. Physiol. Chem.*, 1932, **211**, 211). Determine the mixed aldehydes (valeric aldehydes, acetaldehyde, and *iso*butyraldehyde, respectively) by titration with 0·01 *N* iodine in the usual way.

The acetaldehyde, representing the alanine, is determined colorimetrically by means of sodium nitroprusside and piperazine.

The *iso*butyraldehyde, representing the valine, is determined (*loc. cit.*) on a new portion of the solution by sweeping the aldehydes into carbon tetrachloride absorbers and heating the carbon tetrachloride solution under a fractioning column with bisulphite absorbers at the top.

The valeric aldehydes, representing the leucine, are calculated by difference.

Application to a mixture of amino acids—Phenylalanine and methionine give aldehydes, but the formation is not quantitative. These amino acids are removed by adsorption on a column of freshly-precipitated silver sulphide. With mixtures of amino acids an alumina column is used before the silver sulphide to remove amino acids which, although not interfering with the estimation, tend to saturate the silver sulphide and prevent complete adsorption of the phenylalanine.

Glycine gives rise to formaldehyde, which is removed by addition of 10 ml. of 1 per cent. asparagine solution to the phosphate buffer.

The method provides for rapid estimation of leucine in mixture of amino acids with an accuracy of about 90 per cent. Alcohol and other substances oxidisable to aldehydes must be absent. Leucine and *iso*leucine are both determined as leucine and cannot be distinguished. V. M. BOND

Determination of Mannitol in Plasma and Urine. A. C. Corcoran and I. H. Page (*J. Biol. Chem.*, 1947, **170**, 165–171)—The principle of the method is the oxidation of mannitol to formic acid and formaldehyde by periodic acid. The conditions of the oxidation are such that glucose produces little formaldehyde. The periodic acid is reduced to iodine by stannous chloride and the formaldehyde produced is determined by the method of MacFadyen (*Ibid.*, 1945, **158**, 107).

Reagents—Periodic acid reagent—0·03 M potassium periodate in 0·25 M sulphuric acid. *Stannous chloride*—Prepare freshly each day and make approximately 0·125 M in 0·3 N hydrochloric acid. Immediately before use take 10 ml. of stannous chloride, add 5 ml. of concentrated hydrochloric acid and titrate against the periodic acid reagent, using starch as indicator. Dilute the stannous chloride so that 10 ml. ≡ 10·2 ml. of the periodic acid reagent. *Chromotropic acid*—Dissolve 0·2 g. of chromotropic acid (1 : 8 dihydroxynaphthalene-3 : 6-disulphonic acid) in 4 ml. of water in a 100-ml. flask and dilute to volume with 15 M sulphuric acid.

Procedure—For plasma samples prepare a 1 : 20 dilution by the method of Somogyi (*Ibid.*, 1945, **160**, 69). Filtrates made with zinc sulphate and sodium hydroxide or cadmium sulphate and sodium hydroxide are equally satisfactory. From a filtrate of mannitol-free plasma prepare two blanks; treat one (oxidised blank) as a sample and treat the other (unoxidised blank) with stannous chloride before adding the periodic acid.

Dilute urine samples with water.

Into a tall test tube graduated at 25 ml., pipette 2 ml. of plasma filtrate or diluted urine containing 10 to 60 µg. of mannitol and not more than 140 µg. of glucose. Use 2 ml. of water in another tube to give a reagent blank. Add 0·5 ml. of periodic acid reagent and allow to stand at room temperature for 8 to 10 min. Add 0·5 ml. of the stannous chloride solution and shake well. Stannic acid is precipitated, and iodine may be formed momentarily. Add 5 ml. of the chromotropic acid reagent from an automatic pipette with free delivery, shaking well. The stannic acid should have dissolved on addition of this reagent. Place the tube in boiling water for 30 min. Remove the tube, cool, make up to 25 ml. and allow to stand at 25° C. in a water-bath. The colour is stable for several hours at this temperature, and the colour intensity changes by about 1 per cent. per ° C. Read the optical density (D) at 570 mµ. in a No. 6–300 cell of a Coleman 6A clinical spectrophotometer, setting the galvanometer at zero with air in the cuvette.

For urine and water samples, find ΔD as:—

$\Delta D = D$ (sample) $-$ D (reagent blank).

For plasma samples calculate:—

$\Delta D_1 = D$ (sample) $-$ D (unoxidised blank)

$\Delta D_2 = D$ (oxidised blank) $-$ D (unoxidised blank).

Read off from a calibration curve the mannitol concentrations corresponding to the ΔD's, and for plasma samples take the mannitol concentration as the difference in the mannitol concentrations corresponding to ΔD_1 and ΔD_2 respectively.

With careful control of experimental technique, the recovery of mannitol with water and urine dilutions is complete with an error of less than 2 per cent. The recovery from plasma filtrates is 98 to 100 per cent. when the glucose content is within the specified limits.

Fructose, α-glycerophosphate, ethylene and propylene glycols, and most compounds containing a –CHOH–CH_2OH or a –CO–CH_2OH grouping would interfere by producing formaldehyde, if they were present in large enough concentrations. Substances with a –CHOH–CHOH– or a –CO–CHOH– or a –CO–CO– group might interfere by using up periodic acid reagent, but would not produce formaldehyde. The interference from glucose is small because in acid solution glucose exists mainly as the pyranose form, which does not have a free –CHOH– group adjacent to the terminal –CH_2OH group. W. S. WISE

Determination of Micro-Quantities of Citric Acid in Biological Fluids. S. Natelson, J. K. Lugovoy, and J. B. Pincus (*J. Biol. Chem.*, 1947, **170**, 597–605)—Hitherto there has been no method of determining citric acid sufficiently sensitive to be applied to less than 20 ml. of blood. The most promising method depends on bromination of citric acid to yield pentabromoacetone which, after extraction with light petroleum, forms a colour when added to sodium sulphide solution. The optimal conditions for this estimation have been determined; 2 to 60 µg. of citric acid in 0·3 ml. of whole blood can be estimated.

The absorption curve of the colour shows a maximum at 450 mµ. This value is different from the values adopted by various other investigators who did not report the absorption curve.

The colour fades with time but the fading is greatly reduced by lowering the temperature to about 0° C. The colour then takes about 15 min. to develop. The addition of pyridine to stabilise the colour is unnecessary.

At 650 mμ., where the absorption should be zero, there is an opalescence due apparently to emulsification of the light petroleum with the aqueous layer. This effect could be eliminated by centrifuging.

The separating funnels of the original method can conveniently be replaced by glass-stoppered test tubes. The phases were separated by centrifuging, and aliquots taken for the next stage. This procedure ensures good separation of the phases so that only one extraction is necessary. This technique could be applied to other methods where separating funnels are used, and washing is necessary.

Reagents—Use A.R. reagents throughout. (1) Standard solution of 1 mg. of anhydrous citric acid per ml. (2) Six per cent. hydrogen peroxide solution—Dilute 10 ml. of 30 per cent. hydrogen peroxide to 50 ml. with water. Keep in a refrigerator. (3) Four per cent. sodium sulphide solution. Dissolve 40 g. of sodium sulphide crystals ($Na_2S.9H_2O$) in 1 litre of water. Discard this reagent if the solution shows a transmission less than 95 per cent. of that of water at 450 mμ. Store the solution in a refrigerator. (4) Light petroleum, b.p. 90° to 100° C. The commercial product must be purified before use. Shake about 700 ml. with 100 ml. of concentrated sulphuric acid in a separating funnel and set aside overnight. Remove the petroleum layer and wash it three times with 50-ml. portions of sulphuric acid, then several times with 100 ml. of water, and finally with a saturated solution of potassium permanganate in $0{\cdot}5\,N$ sulphuric acid. Allow to stand for about 30 min. Wash again with water to remove permanganate. Test a small portion with concentrated sulphuric acid. If a colour develops repeat the treatment with sulphuric acid and permanganate. Dry the purified petroleum over anhydrous potassium carbonate and filter through glass-wool into a distilling flask. Distil and collect the 90° to 100° C. fraction.

Commercial *n*-heptane, b.p. 96° to 97° C., can be used without purification.

Procedure—Pipette 1 ml. of blood, plasma, or serum, or 0·02 ml. of urine into a 15-ml. centrifuge tube. Add rapidly 5 ml. of 10 per cent. trichloroacetic acid by blowing down the pipette, allowing to drain at the end. This is to precipitate the protein as fine particles. Shake and then set aside for 10 min. Centrifuge and place a 5-ml. aliquot of the supernatant fluid in a 15-ml. glass-stoppered graduated centrifuge tube. Add 0·2 ml. of 18 N sulphuric acid and shake the tube. Put two hollow glass beads in the tube and place it in an oil-bath at 110° to 120° C. until the volume is reduced to about 2 ml. Cool to room temperature and add 0·2 ml. of saturated bromine water. Allow to stand for a few minutes. The colour due to the excess of bromine should be present. Add 0·2 ml. of 1 M potassium bromide, make up to 3 ml. with water, and then add 1 ml. of 1 N potassium permanganate. Allow to stand at room temperature for 10 min. and then cool to 10° C. Keeping the tube cold, remove the colour with the minimum amount of 6 per cent. hydrogen peroxide. Dilute to 5 ml. with water. Add exactly 5 ml. of the light petroleum, stopper the tube and shake for 5 min. in a machine. Then centrifuge for 5 min. at 2000 r.p.m. Take a 4-ml. aliquot without disturbing the aqueous phase. Place the aliquot in a 10- or 15-ml. glass-stoppered centrifuge tube and cool in a refrigerator. This solution can be kept overnight in the refrigerator. After this point keep all solutions below 15° C. Add exactly 3·5 ml. of 4 per cent. sodium sulphide solution. Stopper the tube, shake for 1 to 2 min. and then centrifuge for 3 min. at 2000 r.p.m. Pack the centrifuge tube in ice to keep the temperature low. Aspirate off the light petroleum and take about 3 ml. of the coloured aqueous phase, which should fill the cuvette. Read the transmission in a Coleman spectrophotometer at 450 and 650 mμ. from 15 to 35 min. after the sodium sulphide solution was added. Read the colour against the sodium sulphide solution. Subtract the optical density at 650 mμ. from the optical density at 450 mμ.

Prepare a standard curve with dilutions containing 10 to 60 μg. of citric acid per ml. Use 1 ml. of each solution. Prepare a new curve for each new batch of sodium sulphide solution. Use the same procedure as for blood, starting with the addition of sulphuric acid. Omit the preliminary boiling and treatment with bromine water.

The standard curve shows a slight deviation from Beer's law; 2 to 60 μg. of citric acid can be determined with an accuracy to within 5 per cent.

With blood samples, nearly all the citric acid is in the plasma or serum and the clotting mechanism does not remove a measurable amount of citric acid from the serum. Serum should be used in routine determinations. W. S. Wise

Effect of Temperature on the Fading of the Carr - Price Colours of Vitamin A and Common Carotenoid Pigments. M. J. Caldwell and J. S. Hughes (*J. Biol. Chem.*, 1947, **170**, 97–103)—The well known transient blue colour produced by a chloroform solution of antimony trichloride (Carr - Price reagent) with vitamin A and various carotenoid pigments has been investigated.

Vitamin A was used as the alcohol, the crystalline acetate, and the liquid concentrate of the natural esters. There were no major differences in the initial intensity or rate of fading with the various forms used. The maximum colour was reached almost instantaneously and was independent of the temperature. The rate of fading was greatly increased by a rise in temperature. The usual variations in room temperature would probably not greatly affect the Carr - Price determination of vitamin A when the colour is measured within a few seconds of mixing the reactants.

The pigments investigated were α-, β-, and γ-carotene, lycopene, cryptoxanthin, lutein, and zeaxanthin. With these, there was a slower colour development and also a colour fading with time. The rapidly increasing portion of several of these curves coincided with the time of colour measurement in the Carr - Price determination of vitamin A. A high concentration of carotenoid pigments would thus interfere with the estimation. The rate of colour formation from the carotenoid pigments and the Carr - Price reagent is decreased by lowering the temperature and this might be used in a method of estimating vitamin A in presence of a large concentration of carotenoid pigments.

W. S. WISE

Critical Study of the Proposed Modifications of the Roe and Kuether Method for the Determination of Ascorbic Acid, with further contributions to the Chemistry of this Procedure. M. B. Mills and J. H. Roe (*J. Biol. Chem.*, 1947, **170**, 159–164)—Bolomey and Kemmerer (*Ibid.*, 1946, **165**, 377) proposed using glacial acetic acid instead of 85 per cent. sulphuric acid as a reagent to develop colour in the Roe and Kuether method of estimating ascorbic acid (*Ibid.*, 1943, **147**, 399). The use of acetic acid, however, may lead to serious error, owing to the fact that substances other than dehydro-ascorbic acid, or closely related compounds, can couple with the 2 : 4-dinitrophenylhydrazine reagent and produce an interfering colour. With sulphuric acid, these interfering colours fade and after 30 min. are negligible. In acetic acid solution, however, this fading does not occur.

Bolomey and Kemmerer failed to control the redox potential with, *e.g.*, thiourea as proposed in the original method. This accounts for some anomalies observed by them.

The 540-mμ. filter is better for general work, but absorption is greater at 520 mμ. and this wavelength could be used when little or no interfering substance is present, *e.g.*, in determining ascorbic acid in blood.

W. S. WISE

Estimation of Potassium in Composts and Sewage Sludges. P. Garrick (*Nature*, 1947, **160**, 434)—Several methods for the extraction of potash in organic substances containing large quantities of siliceous matter have been tested. The only method found to be satisfactory was to ignite the material and sinter the ash by the Lawrence - Smith method. The potash was then separated by one of the standard methods. In the estimation of potash in sewage sludges and composts very low results may be obtained if a procedure other than that indicated above is followed.

B. ATKINSON

Determination of Potassium in Soil and Plant Material by the Cobaltinitrite Method. J. ten Have (*Chem. Weekblad*, 1948, **44**, 484–488)—In this determination the precipitate contains both sodium and potassium in proportions that vary with the conditions of precipitation, although all the potassium is present in the precipitate. In order to know the correct factor, the conditions of precipitation, in particular the temperature, must be carefully observed. The method recommended is as follows.

Procedure for soil extracts—Dry down a portion of the extract with 4 ml. of 20 per cent. ammonium nitrate solution, and ignite to remove organic matter. Dissolve the residue in hydrochloric acid, evaporate the solution to dryness again, and redissolve with addition of a few drops of hydrochloric acid. Treat the solution with ammoniacal ammonium carbonate solution to remove alkaline earths, etc., evaporate the filtrate to dryness, and remove ammonium salts by heating the residue. Dissolve in warm water, filter through a small filter, and evaporate to dryness in a 50-ml. beaker. To the residue add 10 ml. of water and 5 ml. of saturated sodium chloride solution, place the solution in a thermostat at 25° C., and, after 30 min., add 5 ml. of cobaltinitrite reagent. Keep in the thermostat overnight, and then filter through a sintered-glass crucible (porosity G4), washing with the minimum quantity of 10 per cent. acetic acid, and then once with water. The total volume of washing liquor should be 10 to 12 ml. Wash twice with small quantities of 96 per cent. alcohol and twice with acetone, and dry for 30 min. at 105° C.; 100 mg. of precipitate are equivalent to 17·95 mg. of K_2O. If the result is to be obtained by titration, then transfer the crucible containing the precipitate (not washed with alcohol or acetone) to a 250-ml. beaker containing 50 ml. of water, add 5 ml. of 10 per cent. sulphuric acid, followed immediately by an excess of potassium permanganate solution. Heat to boiling and allow to stand for 10 min. covered with a clock-glass, then add an amount of oxalic acid solution equivalent to the permanganate added. Heat to boiling again, and titrate the excess of oxalate with permanganate. The factor varies somewhat with the quantity of potassium; thus 1 ml. of 0·1 *N* potassium permanganate solution corresponds to 0·770 mg. of K_2O if the total quantity of the latter is 15·79 mg.; and to 0·747 mg. for a total of 3·16 mg.

Procedure for plant material—Ash 5 g. of material in a platinum dish at 300° C. Dissolve the ash in hydrochloric acid, transfer to a 100-ml. measuring flask, and heat the solution to boiling. Add milk of lime until the reaction is alkaline, and place in boiling water for 1 hr. After cooling, dilute to 100 ml. with water and filter. Take 20 ml. of the filtrate in a 50-ml. beaker, acidify with acetic acid, and evaporate to dryness. Dissolve the residue in 10 ml. of water and treat as in the previous method.

Preparation of the reagent—*Solution A*—Dissolve 50 g. of hexahydrated cobalt nitrate in 100 ml. of water and 25 ml. of glacial acetic acid. *Solution B*—Dissolve 50 g. of sodium nitrite (potassium-free) in 100 ml. of water. Prepare the reagent by mixing 3 parts of A and 5 parts of B, drawing air through the mixture for at least 90 min., and, on the following day, filtering.

G. MIDDLETON

Water Analysis

Reduction of Potassium Permanganate in the Presence of Glass. C. B. Taylor (*Nature*, 1947, **160**, 56–57)—While performing experiments involving the standard test for decomposable organic matter (*Methods of Chemical Analysis as Applied to Sewage and Sewage Effluents*, H.M. Stationery Office, 1929), it was found that larger amounts of acidified potassium permanganate were reduced in presence of glass beads than in their absence. For investigating this reduction, $N/80$ potassium permanganate containing 25 per cent. by volume of sulphuric acid was used and the procedure of the standard test was followed. With 100-g. quantities of plate, bottle, and Pyrex glass, and silica, crushed to approximately the same size range and dried under identical conditions, amounts of oxygen of the order of 0·7 p.p.m. were absorbed. The amount absorbed was proportional to the weight of glass added. Glass that had been used in the test and then washed with distilled water, but not dried, was not active, but drying at 140° C. or at 30° C. re-activated the glass. Water that had been in contact with active glass for not less than 20 min. reduced potassium permanganate in absence of the glass. B. Atkinson

Organic

Quantitative Determination of Halides in Organic Compounds by the Action of an Alkali Metal in the Medium of an Indifferent Solvent. A. K. Ruzhentseva and V. S. Letina (*J. Anal. Chem. Russ.*, 1948, **3**, 139–146)—For the determination of halogens in organic compounds, neither the original Stepanov method (*J. Russ. Phys.-Chem. Soc.*, 1905, **37**, 12) nor any of the numerous later modifications, *e.g.*, those based on changes in the amounts of alkali metal and alcohol (Bacon, *J. Amer. Chem. Soc.*, 1909, **31**, 49, etc.) and those based on the use of another solvent (Landis and Wichmann, *Ind. Eng. Chem., Anal. Ed.*, 1930, **2**, 394; Umhoefer, *Ibid.*, 1943, **15**, 383, etc.) can be considered as generally applicable. In most cases the method of Landis and Wichmann gives theoretical results, but discrepancies have been found with dichloroethane (12 per cent. low), butyl chloride (15 per cent. low), *p*-chlorophenol (20 per cent. low), and other compounds. The indifferent solvent introduced by these authors was supposed by them to assist in the removal of hydrogen bubbles from the surface of the metallic sodium and thus to maintain its reducing activity. Nevertheless the main reaction is probably not a reduction but an elimination reaction. Hence the possibility of applying the Wurtz - Fittig reaction was studied.

With dibromoethane, 0·1 to 0·3 g., in 50 ml. of xylene, boiled under a reflux condenser for 2 to 3 hr. with 2 to 3 g. of metallic sodium, theoretical results were obtained for the bromine content after addition of alcohol, and water, to the reaction products, removal of xylene, and titration by Volhard's method; but with 1-chloro-2-bromoethane, and dichloroethane, the results were low, as occurred also when a solvent of higher boiling-point was used. Further experiments showed that the sodium (or potassium) must be present in a very finely divided form. Results then obtained for 26 aliphatic, 23 aromatic, and 2 alicyclic compounds were close to the theoretical. In four cases only was a slight modification (see below) necessary.

Procedure—Preparation of finely-divided sodium—Place 30 ml. of anhydrous xylene and 2 to 3 g. of sodium, in small pieces, in a pear-shaped flask, insert a cork stopper carrying a glass tube 50 to 70 cm. long, and heat the flask until the xylene begins to boil. Then wrap the flask in a cloth and shake vigorously, using a circular motion until the sodium is reduced to powder.

General procedure—In a 200-ml. pear-shaped flask with a ground joint for a reflux condenser place 2 to 3 g. of sodium or potassium in powder form and 50 ml. of anhydrous xylene. Weigh into a weighing bottle 0·1 to 0·3 g. of the organic substance and introduce the bottle into the flask, rapidly removing the stopper from the bottle and inserting the reflux condenser, the joint being greased with lanoline. No particles of sodium should appear in the neck of the flask. Boil on an electric hot-plate for 2 to 3 hr., cool, and then pour several small portions (3 to 5 ml.) of alcohol down the condenser to dissolve the residual sodium, followed by 20 to 30 ml. of water. Transfer the contents of the flask to a separating funnel, neutralise to phenolphthalein by means of nitric acid to avoid the formation of an emulsion in the subsequent shaking, separate the water layer, wash the xylene layer several times with water, and determine halide in the combined water layers by Volhard's method. Alternatively, when the solution is highly coloured or contaminated distil off the xylene with steam instead of using a separating funnel.

When, besides halides, other functional groups capable of reacting with sodium are present in the molecule, increase the amount of sodium used correspondingly.

Compounds not amenable to the above-given treatment—With methylene chloride, trichloroethylene, heptachlorotoluene, and 2 : 5-dichloronitrobenzene, use ether initially in place of xylene, boil for 1 to 1·5 hr., then add xylene and boil to complete the reaction. A mixture of ether and xylene at the start or ether alone throughout gives results no better than with xylene alone. The process gives also a less highly coloured product than with xylene alone and facilitates the titration.

In presence of sulphur compounds, *e.g.*, dichlorodiphenylsulphone silver sulphide would appear in the Volhard titration. In such cases precipitate silver chloride and sulphide together, extract the chloride by means of ammonia solution, and re-precipitate the chloride by means of nitric acid, completing the determination gravimetrically.

Determination of chlorine, bromine, and iodine present together—Heat the substance with metallic sodium and xylene as described in general procedure, remove the xylene, and then neutralise the water layer with 20 per cent. sulphuric acid solution. Make up the solution to 250 ml. with water, pipette 50 ml. into a wide 200-ml. beaker, add to it 50 ml. of 10 per cent. magnesium sulphate solution, and 1 to 2 ml. of 20 per cent. sulphuric acid solution, and titrate potentiometrically with 0·1 N silver nitrate.

Determination of fluorine—Treat with sodium as for the other halides and determine fluorine by the lead chlorofluoride method. Ethylene fluorhydrin, fluorochloroethane, fluorobenzene, and benzotrifluoride were tested by this method and gave theoretical results. G. S. Smith

Determination of Glycerol in Soap. Rapid Cerate Procedure. L. Silverman (*J. Amer. Oil Chem. Soc.*, 1947, **24**, 410–411)—The perchloratocerate procedure has been applied to the determination of glycerol in soap. Fatty acids, proteins, and chlorides are first removed and an aliquot of the residual solution is treated with perchloric acid and cerate, and the excess of cerate titrated with oxalate solution.

Procedure—Weigh accurately about 20 g. of dry soap, dissolve in 300 ml. of hot water, and add 1 drop of methyl orange indicator (prepared without alcohol). Carefully add 25 per cent. sulphuric acid solution until the liquid is coloured red and add 1 ml. in excess. Maintain the solution at 80° C. until the fatty acids separate on the surface and then cool until the fatty acids solidify. Filter the cold solution, wash the residue with cold water, and discard the fatty acids. To the combined filtrate and washings add, in 1-ml. portions, a 10 per cent. solution of lead acetate previously boiled with lead oxide, until no further precipitation of protein occurs. Dilute the solution to 500 ml., mix well and set aside until the supernatant liquid is clear. Transfer 50 ml. of the solution (2·0 g. of soap) to a beaker and add 25 ml. of water and 25 ml. of 72 per cent. reagent-grade perchloric acid. Add exactly 20 ml. of a solution containing 0·5 N ammonium cerate in 6 N perchloric acid, heat the mixture to 50° C., and maintain at this temperature for 12 to 13 min., stirring occasionally. Add 100 ml. of cold water, set aside for at least 5 min., and slowly titrate the excess of cerate with 0·2 N sodium oxalate, using nitroferroin as indicator. The blue colour disappears about 0·1 ml. short of the end-point and changes to pink at the end-point. The percentage of glycerol in the sample can be calculated from the expression:—

$$\text{Glycerol per cent.} = [(\text{ml. of cerate as } N) - (\text{ml. of } 0{\cdot}2\ N \text{ oxalate})] \times 0{\cdot}0023/\text{weight of sample}$$

[ABSTRACTOR'S NOTE—Full details of the preparation of ammonium perchloratocerate reagent are given in a free publication to be obtained from The G. F. Smith Chemical Co., Columbus, Ohio.]

A. H. A. ABBOTT

Polarographic Method of Determining α-Nitronaphthalene in Presence of α-Naphthylamine. Yu. I. Vayshteyn (*Zavod. Lab.*, 1948, **14**, 517–519)—In a de-oxygenated mixture (1 : 1) of methyl alcohol and water containing potassium chloride in 0·1 N concentration as supporting electrolyte and 3 drops of 0·5 per cent. gelatin solution per 10 ml. as maximum suppressor, α-nitronaphthalene gives a well defined polarographic wave with a half-wave potential of −0·26 v. *versus* the normal calomel electrode, and α-naphthylamine gives no wave and has no effect on the wave of the nitro-compound even when its concentration is fifty times that of the latter. The wave-height is strictly proportional to concentration over the range 20 to 200 mg. per litre. In presence of benzene in concentrations up to 2 per cent., similar results are obtained if the alcohol concentration is increased to 80 per cent. Thus, the polarographic method may be used for production control of α-naphthylamine reduced in benzene solution from α-nitronaphthalene. Other nitro-derivatives of naphthalene, *e.g.*, 1 : 8-dinitronaphthalene, behave like α-nitronaphthalene.

G. S. SMITH

Polarographic Study of Certain Anthraquinones. N. H. Furman and K. G. Stone (*J. Amer. Chem. Soc.*, 1948, **70**, 3055–3061)—The polarographic behaviour of a large number of anthraquinone derivatives has been studied. All the compounds examined give well defined reduction steps suitable for analytical purposes. Buffered solutions of pH 9·0 to 10·0 should be used and the concentration of reducible material should be 1·0 to 2·0 × 10^{-3} M. Under these conditions the half-wave potentials are all about −0·6 to −0·7 v. *versus* the saturated calomel electrode. For the less soluble compounds a solvent containing 40 per cent. by volume of dioxan is used.

J. G. WALLER

Estimation of Hemicelluloses in Holocellulose from Non-woody Plant Material. E. Bennett (*Anal. Chem.*, 1948, **20**, 642–643)—*Procedure*—To isolate holocellulose from beet, citrus or cranberry pulps, cornstalks, oat straw or mixed hay, extract the sample (ground to pass a 25- but not a 50-mesh sieve) with a mixture of alcohol and benzene (1 : 2) for 6 to 8 hr., and then twice with 0·5 per cent. ammonium citrate solution at 80° C. for about 5 and 19 hr., respectively. After the usual treatment with sodium chlorite and acetic acid, filter off the holocellulose on a cloth, allow it to dry in air, and treat 0·2 g. of it with 20 ml. of 12 per cent. sodium hydroxide solution at 20° C. for 10 min. Add 20 ml. of water at 20° C., and maintain at 20° C. for 90 min., agitating the mixture once during this period. Dilute to 100 ml. in a graduated flask, mix well, filter through glass wool, and boil 25 ml. of the filtrate with 5 ml. of water, 30 ml. of concentrated sulphuric acid, and 5 ml. of 1·0 N potassium dichromate for 2 min. at 140° to 150° C., air being passed through slowly to facilitate the boiling. Cool rapidly, and measure the transmittance of the solution in a Coleman Universal Spectrophotometer, with distilled water as standard, and using a wavelength of 600 mμ. and a PC-4 filter. Establish a curve relating glucose and the reduced dichromate by oxidising (as described above) a 25-ml. aliquot of each of 8 solutions containing 0 to 140 mg. of glucose per 100 ml., and plotting the transmission values against the number of milligrams of anhydroglucose used. The curve shows a slight tendency to deviate from Beer's law at the higher concentrations. Report the results on an ash-, moisture- and nitrogen-free basis. In general, agreement within any one set of determinations is better than that between sets of duplicates or triplicates, because the control of the procedure for the isolation of the holocellulose is not so good as that for the subsequent hemicellulose determination. Average values obtained are, for beet pulp 5·9 ± 0·8, citrus pulp 9·2 ± 0·5, cranberry pulp 13·1 ± 0·4, cornstalks 26·5 ± 1·4, oat hay 27·5 ± 1·4, and mixed hay 23·1 ± 1·0 per cent.

J. GRANT

Replacement of Rochelle Salt by Glycerol in the Determination of Sugar. S. N. Lutokhin and O. A. Kuzmenko (*J. Anal. Chem. Russ.*, 1948, **3**, 196–197)—Previous workers who have used glycerol in place of Rochelle salt in Fehling's solution have found that the normal tables could not be used in the higher range of concentrations of sugar. With the composition given below the iodimetric method of Fresenius (*Anleit. z. chem. Anal. d. Weines*, 1922) gives correct results with concentrations not exceeding 5 g. per litre and by using Fresenius's tables.

Procedure—To 10 ml. of the sugar solution add 3 ml. of glycerol, 9 ml. of 20 per cent. potassium hydroxide solution, 10 ml. of 4 per cent. copper sulphate solution, and 10 ml. of water. Then proceed as described in Fresenius's method.

G. S. Smith

Colour Reagents for the Paper Chromatography of Sugars. W. G. C. Forsyth (*Nature*, 1948, **14**, 239–240)—The application of paper chromatography to the identification of sugars has been described (Partridge, *Ibid.*, 1946, **158**, 270). The tests may be made more selective by using resorcinol and naphthoresorcinol as test reagents in addition to the usual ammoniacal silver nitrate reagent.

Procedure—Place three spots of a 1 per cent. solution of the sugar on a filter paper and, alternately, three spots of a standard mixture of sugars, and dry in the usual manner. Cut the paper into three strips and spray each strip, one with ammoniacal silver nitrate solution, one with resorcinol solution, and one with naphthoresorcinol solution. Prepare the phenol solutions by adding 10 ml. of a 1 per cent. solution of the phenol to 90 ml. of 2 *N* hydrochloric acid. Dry the papers sprayed with the phenol solutions at 85° to 95° C. for 10 min. Colours given by the phenols with sugars are—with resorcinol: fructose, red; xylose and arabinose, blue; rhamnose, yellow; with naphthoresorcinol: galactose, glucose, and mannose, grey; fructose, brown; xylose and arabinose, blue; rhamnose, green. Non-reducing sugars also give a positive reaction.

B. Atkinson

Inorganic

New Qualitative Reaction for Boric Acid and its Salts. A. P. Kreshkov and S. S. Vilborg (*J. Anal. Chem. Russ.*, 1948, **3**, 172–175)—Boric acid reacts with ethyl orthosilicate or its polymers to give a volatile compound that burns with the characteristic green flame. This reaction is preferable to the normal flame test which requires heating the substance with concentrated sulphuric acid and alcohol and may be dangerous, and to certain colour reactions, *e.g.*, with quinalizarin, etc., since oxidising agents and fluorides do not interfere.

Procedure—Gently heat the dried material under test with a few drops of ethyl orthosilicate or its polymers in a crucible, and apply a burner to the vapour, or heat the mixture in a test tube fitted with an outlet tube.

With 3 to 4 drops of the ester the minimum amount of boric acid detectable is 0·6 to 1 mg.

Preparation of ethyl orthosilicate—Place 2·2 g.-mol. of absolute alcohol in a round flask with three necks, carrying a stirrer, reflux condenser, dropping funnel, and capillary for passage of dry air. The condenser is fitted with a calcium chloride tube. Place the flask in a mixture of ice and salt, and introduce through the dropping funnel 0·5 mol. of freshly distilled silicon tetrachloride dropwise with constant stirring. Remove the calcium chloride tube and connect the end of the condenser to a vacuum pump and suck dry air through the reaction mixture for 1 hr. at room temperature and 2 hr. at 80° to 90° C. Then transfer the contents to a Wurtz flask and distil from a glycerol bath at normal pressure.

Preparation of ethyl orthosilicate polymers—To 230 g. of the ester, add, during 2 hr., 100 g. of 80 per cent. by weight ethyl alcohol with constant stirring and cooling. Leave for 2 hr. at room temperature, then heat under a reflux condenser at 100° C. for 2 hr., leave overnight, and finally distil off the alcohol.

G. S. Smith

Colorimetric Determination of Copper by Means of Dimethylglyoxime. V. M. Peshkova, M. E. Levontin, and K. I. Litvin (*J. Anal. Chem. Russ.*, 1948, **3**, 161–166)—A study of the colorimetric determination of copper based on the colour obtained with dimethylglyoxime, pyridine, and ammonium persulphate has been made. The method described by Clarke and Jones (*Analyst*, 1926, **54**, 333) is preferable to that of Goethals (*Z. anal. Chem.*, 1936, **104**, 170) since the colour intensity is more stable. The former method may be improved by reducing the amount of sulphuric acid, and also by adding alkali, which gives a more stable colour, though with somewhat reduced sensitivity.

Tests designed to discover if in absence of an oxidant a compound containing both dimethylglyoxime and pyridine, analogous to that found for iron by Chugaev could be obtained, yielded only the dark-lilac compounds $CuSO_4.3{\cdot}5C_5H_5N$ and $CuSO_4.4C_5H_5N$, and the reaction was sensitive to not less than 2 mg. of copper per 100 ml.

Clarke and Jones supposed that the function of the pyridine was to give the necessary alkalinity, but it is most probable that pyridine enters into the composition of the coloured compound. A buffer solution of pH 5 treated with a solution of copper and all the reagents except pyridine remains colourless, but at once assumes the characteristic colour when pyridine is added.

The oxidising agent appears to act not on copper ions, by analogy with the nickel - dimethylglyoxime reaction, but on the dimethylglyoxime. Addition of a nickel salt after various intervals of time showed that the amount of the nickelous-dimethylglyoxime compound that could be precipitated progressively decreased, indicating that oxidation of dimethylglyoxime was taking place.

The most stable colour is obtainable with a 12- to 15-fold excess of dimethylglyoxime.

Procedure—To 50 ml. of the test solution, free from heavy metal ions other than copper and from chlorides, and neutral to litmus, add 1·5 ml. of 0·5 *N* sulphuric acid, 1 g. of dry ammonium persulphate, 1 ml. of 1 per cent. alcoholic dimethylglyoxime solution, 0·5 ml. of 0·5 per cent. silver nitrate solution, 2 ml. of 10 per cent. pyridine solution, and 3·3 ml. of 0·5 *N* sodium hydroxide, adding the reagents in the order given, and the last one gradually with stirring; then dilute to 100 ml., and after 20 to 25 min. compare the colour with a standard treated in exactly the same way at the same time. If no colour appears the amount of copper is less than 0·04 mg. in the 100 ml. In this case repeat the test without the alkali addition, but compare the colour, within 5 min. of adding the reagents, with a standard simultaneously prepared. Alternatively, with 0·05 to 0·6 mg. of copper, the range over which Beer's law is obeyed, use a photo-colorimeter and a calibration curve applicable to the colour intensity obtained after 20 to 25 min., or use two standards and apply Strelkov's formula

$$C_x = C_1 + \frac{(C_2 - C_1)}{(R_2 - R_1)} (R_x - R_1)$$

where C_1 and C_2 are the concentrations of the standard solutions, C_x is the concentration of the test solution, R_1 and R_2 are the drum readings for solutions C_1 and C_2, and R_x is the reading for the test solution.

The method of colorimetric titration suggested for this reaction by Ayzenberg and Menshikova (*Zavod. Lab.*, 1946, **12**, 673) can be used when the amount of copper is less than 0·1 mg. With higher amounts the titration is too prolonged.

Alkali and alkaline earth metals do not interfere with the reaction if a blank consisting of an aqueous solution of the salts present is used. If aluminium and zinc are present copper must be separated first. Any metals that react with the products of oxidation of dimethylglyoxime must be removed.

G. S. SMITH

Colorimetric Determination of Small Amounts of Copper in Aluminium. G. I. Ayzenberg and E. M. Menshikova (*Zavod. Lab.*, 1946, **12**, 673–674)—The method described by Clarke and Jones (*Analyst*, 1926, **54**, 333) is modified to allow of a colorimetric titration of small amounts of copper in aluminium.

Procedure—Treat 5 g. of aluminium with 50 to 60 ml. of 20 per cent. sodium hydroxide solution added in small portions. Heat on a sand-bath to complete dissolution, add 20 ml. of 20 per cent. sodium sulphide solution and 350 ml. of water, heat without boiling for 1 hr., and leave overnight. Filter off the precipitate, wash first with 1 per cent. sodium sulphide solution, and then with 5 per cent. sulphuric acid solution to remove zinc. Place the washed precipitate in the original flask, dissolve in 20 to 30 ml. of hot nitric acid, precipitate the sesquioxides with ammonia, filter off the precipitate and wash it, and treat the filtrate with a few drops of perhydrol. Boil to remove coloured products of oxidation of the paper formed by nitric acid, adding more perhydrol and boiling if necessary. Finally, boil to ensure complete decomposition of the perhydrol. Cool, make up the solution to 200 ml. in a graduated flask, transfer 50 ml. to a small flask and just acidify to litmus paper with diluted sulphuric acid (1 + 10). In another similar flask place 50 ml. of water and acidify with 1 drop of acid. To each flask add 2 ml. of 0·5 per cent. silver nitrate solution, 1 ml. of 10 per cent. pyridine solution, 1 ml. of saturated alcoholic dimethylglyoxime solution, and 0·5 g. of ammonium persulphate. If copper is present in the test solution a raspberry colour appears. To the contents of the second flask, add dropwise from a microburette a copper sulphate solution containing 0·1 mg. of copper in 1 ml. until the solutions are of the same colour.

The method is most suitable for the determination of 0·01 to 0·1 mg. of copper in the 50-ml. aliquot portion.

G. S. SMITH

Precipitation of Mono- and Di-Zirconyl Phosphate and the Use of Zirconyl Salts for the Separation and Determination of Phosphoric Acid. R. Stumper and P. Mettelock (*Bull. Soc. Chim.*, 1947, 674–676 M)—Phosphoric acid is precipitated quantitatively from a hydrochloric acid solution of a simple salt or of a slag by adding an excess of zirconium oxychloride solution. The precipitate forms dizirconyl phosphate, $2ZrO_2.P_2O_5$, on ignition. Monozirconyl phosphate is obtained if phosphoric acid is in excess, or if precipitation is carried out in sulphuric acid solution. The dizirconyl phosphate is less soluble and less readily hydrolysed than is the monophosphate. The procedure given below can be used for determining phosphate or for removing phosphate before the precipitation of the third group of metals in qualitative analysis.

Procedure—Prepare a solution of the phosphate containing about 0·1 g. of phosphorus pentoxide in a mixture of about 40 ml. of water and 20 ml. of hydrochloric acid (d. 1·07). Add a 200 per cent. excess of a 10 per cent. solution of zirconyl chloride, evaporate to dryness, and digest the residue at 130° C. with 20 ml. of hydrochloric acid (d. 1·07) for 1 hr. Filter the liquid and wash the precipitate with warm, diluted hydrochloric acid (1 + 10). Ignite the precipitate at 1050° C. for 1 hr. and weigh as $2ZrO_2.P_2O_5$. The method has been used for determining phosphorus in slags and in steels.

B. ATKINSON

Micro-Determination of Bromides and Iodides in Presence of Chlorides. I. Bellucci (*Gazz. Chim. Ital.*, 1942, **72**, 501–507)—The method is intended for the determination of iodine and bromine in blood and other biological material. The organic matter is destroyed by careful incineration in presence of sodium hydroxide according to the process previously described by the author (*Analyst*, 1935, **60**, 263), the determination being carried out on the resultant ash.

Procedure—Extract the ash with 95 per cent. alcohol, thereby dissolving part of the chloride and the whole of the bromide and iodide present. Evaporate the solution to dryness and re-dissolve the residue in 15 to 20 ml. of water. Place the solution in a 50-ml. separating funnel and add a few drops of diluted sulphuric acid (1 : 10) and 3 to 4 ml. of carbon tetrachloride, followed by 2 ml. of a 1 per cent. solution of sodium nitrite. Separate the tetrachloride layer containing the liberated iodine and extract the aqueous layer three or four times with further small quantities of tetrachloride. To the total extract add, drop by drop with continuous shaking, 10 per cent. sodium hydroxide solution until the colour is completely discharged, and evaporate to dryness on the water-bath. Re-dissolve in about 10 ml. of water, add a crystal of potassium permanganate, and heat to incipient boiling. Destroy the excess of permanganate with a few drops of alcohol, filter on a small filter, and wash with boiling water, keeping the volume as small as possible. Evaporate to about 10 ml. Remove any excess of nitrous acid by adding 0·1 g. of urea and 10 drops of acetic acid and boiling for 3 to 4 min. Cool, add a few centigrams of sodium iodide and 2 to 3 drops of dilute sulphuric acid, and titrate the liberated iodine with 0·002 N thiosulphate.

Make the aqueous solution containing the chloride and bromide slightly alkaline by adding sodium hydroxide, and evaporate to dryness on the water-bath. Re-dissolve in 20 to 25 ml. of water and transfer to a small flask. Add 0·5 g. of pure sodium chloride, 5 ml. of N sodium hydroxide, 30 ml. of freshly prepared chlorine water containing not less than 5 mg. of chlorine per ml. and 1 g. of boric acid. Heat for 10 min. over boiling water to oxidise the bromide to bromate. To the hot liquid add 2 ml. of 10 per cent. sodium formate solution and boil on an asbestos-covered gauze to eliminate the excess of hypochlorite, continuing until the volume is reduced to about 15 ml. Cool, add 3 drops of 5 per cent. potassium iodide solution, 2 ml. of starch solution, 1 drop of 5 per cent. sodium molybdate, and 3 ml. of 2 N hydrochloric acid. After 4 to 5 min. standing, sheltered from light, titrate with 0·004 N thiosulphate. Make blank tests on the reagents.

A. H. Bennett

Co-precipitation of Uranous Sulphate with Rare-Earth Double Sulphates. A. W. Wylie (*Nature*, 1947, **160**, 830)—Uranous ions in acid solution are co-precipitated with sodium cerium sulphate and the sodium rare-earth sulphates. Almost complete removal of uranous ions from solution is obtained by treating an acid solution containing not less than 30 g. per litre of rare-earth oxides and about 3 g. per litre of uranous oxide with sufficient sodium sulphate to precipitate the rare earths completely. Uranyl ions are not co-precipitated under these conditions.

B. Atkinson

New Dry Test for Gold. R. C. Mehrotra (*Nature*, 1948, **161**, 321)—To the gold salt in a porcelain dish add a quantity of concentrated hydrochloric acid and a speck of zinc. Insert a test tube full of water into the solution over the point where the hydrogen is bubbling up. Hold the test tube in the hottest part of a bunsen flame. If gold is present a brilliant green mantle forms round the bottom of the test tube. The test is sensitive to 0·1 mg. of gold in 1 ml. of hydrochloric acid. Copper interferes and, in presence of tin, the gold green mantle is replaced by the blue mantle given by tin.

B. Atkinson

Improvement of Analytical Control for Silica Brick. J. T. Rozsa (*J. Amer. Ceram. Soc.*, 1948, **31**, 280–283)—In general, the preparation of a representative sample of silica bricks is difficult. The author takes 20 to 50 bricks from a car-load, chips a small portion from each and grinds the composite sample, finally sampling in the usual way. After mixing with an equal weight of pure carbon dust, the sample is completely consumed in a D.C. arc, placed 110 cm. from the slit of the spectrograph to minimise the effect of wandering of the discharge. Standards of the same approximate composition as the sample to be analysed are photographed on each plate and allow the oxides Al_2O_3, MgO, Fe_2O_3, CaO, Na_2O, TiO_2, K_2O, ZrO_2, MnO_2, and Li_2O to be estimated. As the silica content is fairly constant this material is used as an internal standard in preference to an added material, such as copper oxide. Using the method as described, an accuracy to within ±5 per cent. of the amount of minor constituent is obtainable in routine practice. The time of analysis is about 1 hr., 40 min. of which are taken in preparing the sample.

H. R. Clayton

Calculation of the Content of Volatile Matter in Coal to Ash- and Water-free Coal. J. Hamaker (*Chem. Weekblad*, 1948, **44**, 517–525)—In computing the content of volatile matter in coal to an ash- and water-free basis there are considerable uncertainties. The loss in weight resulting from decomposition of the mineral matter is unknown owing to uncertainty in the water content of the shale and in the reactions that occur. It is possible to separate the coal fraction from a powdered coal by treatment with a mixture of benzene and carbon tetrachloride of density 1·4, and the content of volatile matter can then be determined on this fraction. This method is quicker than the indirect one. That the treatment does not fractionate the coal is shown by the constancy in calorific value and organic sulphur content, when calculated on dry mineral-free basis. This separation is necessary when the content of volatile matter is used as a basis for classifying coal, and it should be used for all coals high in carbonate. The A.S.T.M. Designation D388–38 does not take into account the volatile matter resulting from decomposition of the mineral matter.

G. Middleton

Physical Methods, Apparatus, etc.

Oscillographic Polarography with Periodical Triangular Voltage. A. Sevcik (*Coll. Czech. Chem. Comm.*, 1948, **13**, 349–377)—An equilateral triangular, periodical voltage is used to charge and discharge the dropping mercury electrode linearly with time, and current - voltage curves are recorded with a cathode-ray oscillograph.

The reduction processes for cadmium, plumbic, and thallous ions have been shown to follow the equations calculated for reversible reactions. The maximum of the electrolytic current is proportional to concentration in solutions that are less than 10^{-3} N with respect to the reducible ions.

J. G. Waller

Application of Corrections in Viscometry of High-Polymer Solutions. R. H. Wagner (*Anal. Chem.*, 1948, **20**, 155–157)—This paper discusses the importance and magnitude of corrections for density and kinetic energy in the determination of relative and hence of intrinsic viscosities.

If t/t_0 is the ratio of the efflux times of a solution and pure solvent of densities d and d_0, respectively, the true relative viscosity η_r is given by—

$$\eta_r = \frac{dt}{d_0 t_0} + \frac{mVd}{8\pi l \eta_0 t} \times \left[\left(\frac{t}{t_0}\right)^2 - 1\right].$$

Putting $d/d_0 = \beta$, and $t/t_0 = \alpha$, and $\dfrac{Vd_0}{8\pi l \eta_0 t_0} = \mathrm{K}$, we may write $\eta_r = \alpha\beta + \beta\mathrm{K} \times \dfrac{\alpha^2 - 1}{\alpha}$, where V is the volume of the measuring bulb, l the capillary length, and m a coefficient, here assumed to be unity. If C is the concentration of the solution in grams per 100 ml., then the Inherent Viscosity $\{\eta\} \equiv \dfrac{\log_e \eta_r}{C}$, which should not be confused with the Intrinsic Viscosity $[\eta] \equiv \underset{C \to 0}{\mathrm{Lt}}\{\eta\}$. The inherent viscosity is a function of the concentration, whereas the intrinsic viscosity, obtained by graphical extrapolation of the inherent viscosity to $C = 0$, is not.

Contribution of the density factor—The error produced in the inherent viscosity $\{\eta\}$ due to the departure of β from unity ($\Delta\beta$) is given by $\Delta\beta/C$. This correcting term may be ignored if the allowable uncertainty in $\{\eta\}$ is 0·01 unit or more.

Contribution of the kinetic energy factor—The correcting term due to the kinetic energy is given by $\Delta\{\eta\}_{\mathrm{kc}} = \dfrac{\mathrm{K}(\alpha^2 - 1)}{\alpha^2 C}$ if second magnitudes are ignored. A nomogram is given to facilitate calculation of this correcting factor or to establish conditions such as solvent density and viscometer constants necessary to keep the correction within specified limits. The fully corrected value of the inherent viscosity is

$$\{\eta\} = \frac{\log_e \alpha}{C} + \frac{\Delta\beta}{C} + \frac{\mathrm{K}(\alpha^2 - 1)}{\alpha^2 C}$$

W. C. Wake

Capillary Tube Viscometer for Routine Measurements of Dilute High-Polymer Solutions. R. H. Wagner and J. Russell (*Anal. Chem.*, 1948, **20**, 151–155)—The viscometer is particularly suited for routine measurements of dilute high-polymer solutions such as are required for determining molecular weights from the Staudinger equation.

Apparatus—The complete apparatus is shown in Fig. 1, and comprises a viscometer, A, and a thermometer and thermometer jacket, B. The viscometer itself is shown in Fig. 2, and consists of an inner and outer assembly, the former having a measuring bulb, J, and capillary, M, of 0·5-mm.

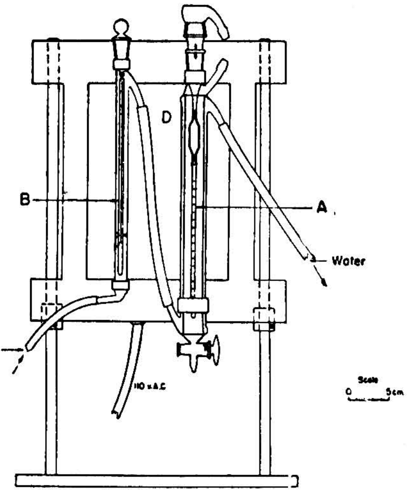


Fig. 1

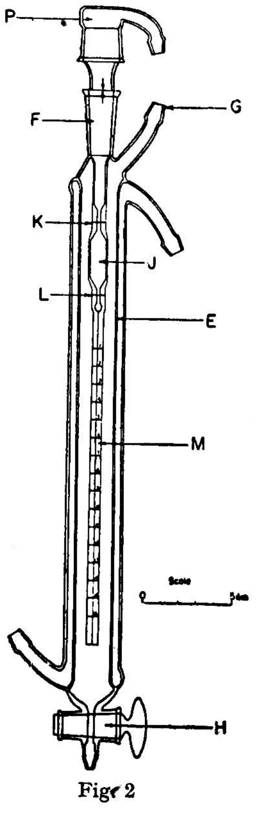


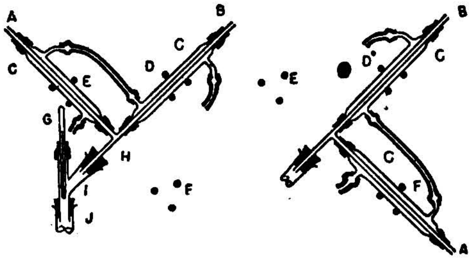
Fig. 2

precision-bore tubing of 190-mm. length. The bulb has a volume (between marks K and L) of about 1 ml. From 3 cm. below the lower transit mark, filling marks are engraved on the capillary at 16 intervals of 1 cm. These enable different average internal heads of liquid to be used and, consequently, the outflow time to be adjusted.

Procedure—Pour sufficient liquid into the outside jacket to come above the desired filling mark. Insert the inner assembly, and, after allowing time for thermal equilibration and drainage, adjust accurately to the filling mark. Fill the measuring bulb, J, by application of pressure at G and obtain the time for outflow of the volume of liquid contained between the transit marks. It is convenient to determine outflow times at various filling levels for solvents to be used, and, apart from occasional checks, these may be taken as standard, only the polymer solution viscosities being normally measured. The relative viscosity is assumed to be the ratio of the outflow times of the solution and solvent. The density and kinetic energy corrections are small and may usually be ignored. Instructions for calculating these corrections, together with a nomogram, are given for use where great accuracy is desired (see preceding abstract, Wagner, *Ibid.*, 155–157). W. C. Wake

Simple Still-Head. R. W. Hakala (*J. Chem. Educ.*, 1948, **25**, 465)—The apparatus, which has no stopcock, is kept in the reflux position during operation until the vapours reach a constant temperature when the fraction is collected by rotating the reflux arm into the "take-off" position until the temperature again begins to rise, when the arm is returned to the reflux position.

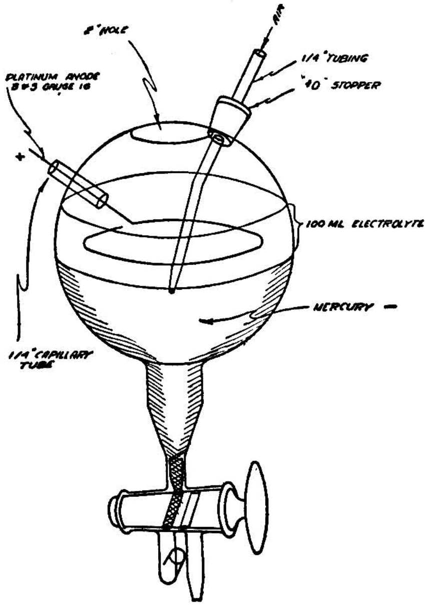
M. E. Dalziel



Reflux position. Take-off position.

A. Angular reflux arm.
B. Stationary reflux arm.
C. Micro Liebig condenser jacket (used when necessary).
D. Clamp.
E. Clamp for A in reflux position.
F. Clamp for A in take-off position.
G. Thermometer.
H. Stopper to hold T-tube; with hole large enough to allow rotation of T-tube, but snug enough to be gas-tight; lubricated with glycerol or stopcock grease.
I. Addition tube.
J. Fractionating column.

Mercury Cathode Cell for Rapid Electrolysis. F. T. Rabbitts (*Anal. Chem.*, 1948, **20**, 181–182)—The cell is made from a 700-ml. Pyrex Florence flask modified as shown in the figure. The platinum wire anode has a diameter of 10 cm. and lies horizontally about 0·5 cm. above the mercury surface. The air-inlet tube through which a slow current of air is passed dips 1 cm. below the centre of the mercury surface. A rubber stopper fitting into the 2-in. hole at the top of the cell carries a small U-tube designed to contain 5 or 6 ml. of distilled water to remove spray from the gases leaving the flask. A 300-ml. flask is used as a mercury levelling bottle. Electric contact with the mercury is made by a length of platinum wire held in place by a rubber stopper containing an air vent. A current supply of up to 7 amp. at 6 v. is required.



Procedure—Electrolysis is usually performed in 0·3 *N* sulphuric acid solution. Electrolyse for 30 to 40 min. with a current of not less than 6 amp. Lower the levelling bottle and, as soon as the mercury has drained from the cell, run the solution into a 400-ml. beaker. Rinse the spray trap and add the washings to the beaker. If the solution contains a suspension, add filter pulp and filter the liquid through a No. 30 Whatman paper. Add 30 ml. of 2 per cent. sulphuric acid solution to the cell, raise the levelling bottle, and continue electrolysis for 5 min. Run off the wash liquid, rinse the spray trap again, and filter the combined washings through the original paper. B. Atkinson

Review

INORGANIC CHEMISTRY. FRITZ EPHRAIM. Fifth English Edition. Revised. By P. C. L. THORNE, M.A., M.Sc., Ph.D., F.R.I.C., and E. R. ROBERTS, A.R.C.S., Ph.D., D.I.C. Pp. xii + 939. London: Gurney & Jackson. 1948. Price 32s.

The four previous English editions of Fritz Ephraim's well-known book were favourably reviewed in *The Analyst* (1926, **51**, 651; 1934, **59**, 309; 1940, **65**, 584; 1944, **69**, 139). In these reviews, written by able chemists and experienced analysts, will be found all that can be said about this book; indeed, to paraphrase the suggestion of one writer, who thought that the only adequate method of reviewing it would be to issue an order that it should be read by all chemists, one might, with some excuse, suggest that anyone wishing for a considered opinion on the fifth edition should look up the reviews of the previous four and not trouble to read this review any further; but as two of the previous reviews appeared in what are now becoming scarce war-time issues of *The Analyst*, there is perhaps some excuse for writing another, even at the risk of incurring a charge of plagiarism.

The outstanding feature of the book is the arrangement of the subject-matter; this has been done as though a full-scale modern textbook of advanced inorganic chemistry of the classical type, which treats each element and its compounds under a separate heading, had been cut loose from its binding, had had all the pages carrying information of a chemically related nature sorted into separate groups and then, with the additional matter necessary to make a continued story, had been rebound into a new volume. There is, of course, rather more to it than that. There is the critical acumen that has gone to the collection and selection of material for inclusion, the clarity of the style in which it is written and the quality of the typography, all of which make for ease in the reading of a subject that is, in many parts, by no means too easy. Nevertheless, the value of the book and its absorbing interest to the reader lie very largely in this ingenious grouping of related chemical facts and information. In pursuing this method of arrangement the author takes the same cross-sectional view of chemistry that an examiner so often takes in setting a certain type of question; for instance, the answer to a question such as, "Exemplify and account for the various methods that are used in the extraction of metals from their ores," will, in "Ephraim," be found complete in one place, instead of scattered over several hundred pages as it is in the classical inorganic texts. This method of imparting collateral information in juxtaposition will appeal to students working for the higher examinations in chemistry; it also makes a very readable book for those to whom examinations are no longer of pressing importance, but who may wish to keep in touch with modern thought in inorganic chemistry; for it enables the reader to review the existing collection of chemical facts and theories as a whole and to appreciate the interconnection and relative importance of the various parts. Modernity is the key-note of the book; its chemistry begins with the Rutherford - Bohr atom and the co-ordination theory; in it there is to be found neither history nor human interest. This must surely be the only book on general inorganic chemistry in the English language that contains no mention of John Dalton. In this strict adherence to a set plan much that is of interest is lost, but the gain is great and more would certainly have been lost had the inclusion of other matter been allowed to interfere with the consistent scheme of the book.

The revision of this fifth edition has been thorough; there is evidence that consideration has been given to errors noted in previous reviews, but an implication that copper is not precipitated in an alkaline tartrate solution by ammonium sulphide remains, on page 469, to surprise an analyst. F. L. OKELL

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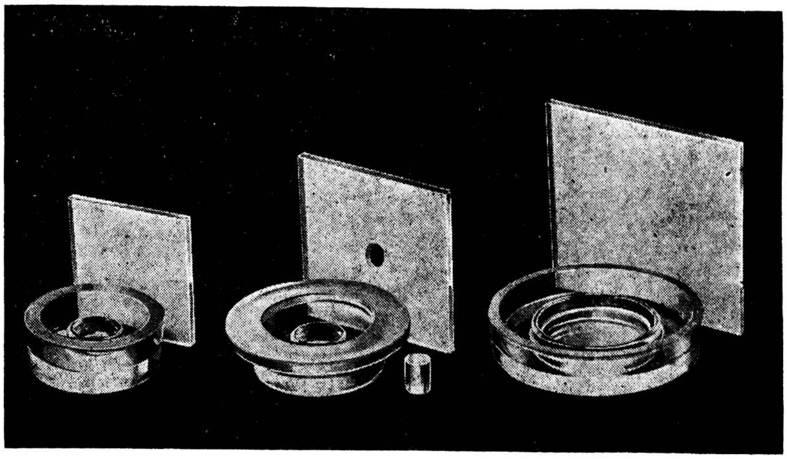

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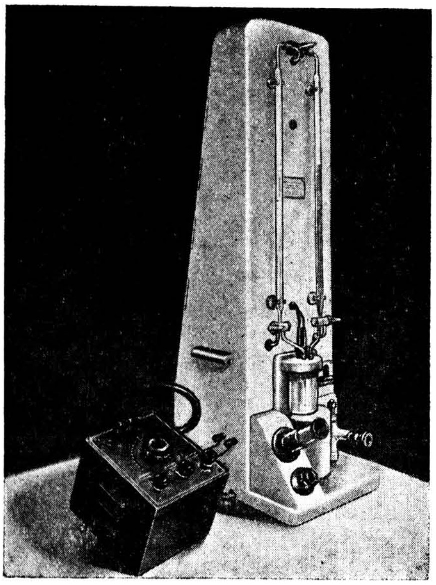

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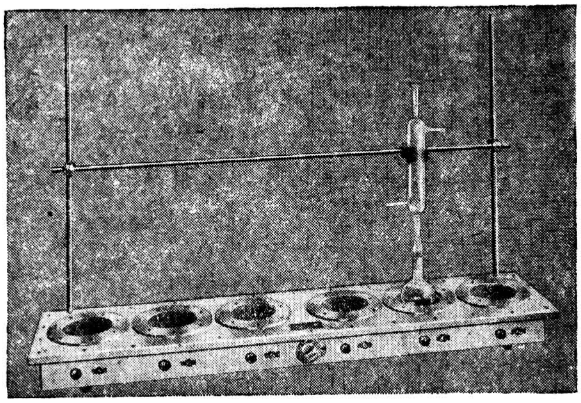

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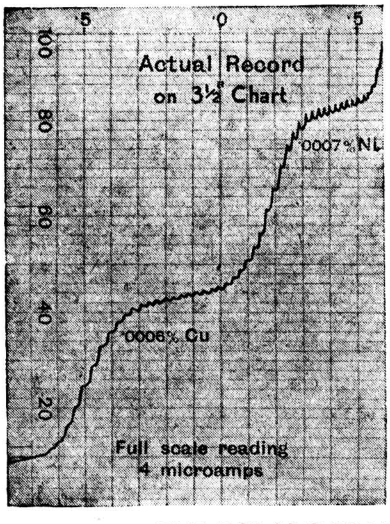

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Printed and Published for the Society of Public Analysts and other Analytical Chemists by W. Heffer & Sons Ltd., Cambridge, England. **Communications to be addressed to the Editor, J. H. Lane, 7–8, Idol Lane, London, E.C.3.** Enquiries about advertisements should be addressed to Walter Judd Ltd., 47, Gresham Street, London, E.C.2

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