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THE ANALYST

EDITORIAL

In this issue *The Analyst* has departed from its general principle of selecting for publication only those papers that contain, for the most part, original and previously unpublished matter of analytical interest.

The subject of this exception to the general rule is a very full account of a scheme for the standardisation of a complete set of volumetric solutions that has been devised by the Analytical Chemists' Committee of Imperial Chemical Industries Limited.

An outline of the scheme was given by the late Edward Hinks, a public analyst, in a Presidential Address to the Society in 1930.* In describing it as fundamental work of the highest kind, he said that it dealt with a matter that concerned almost all analytical chemists and that it reflected great credit on the analysts who had devised it.

Although this paper consists almost entirely of information that has previously been recorded in journals and textbooks, its value to chemists in general and the manner and order of its presentation are of sufficient merit to make it desirable that it should be published somewhere in a form that will make it easily accessible to all practising analysts. That being so, *The Analyst* would appear to be the most suitable journal for its appearance in print. It contains the results of many years' work by a number of collaborators who, starting with a scheme for standardising volumetric solutions based on silver as a primary standard that was devised by the late William Rintoul of Nobel's Explosive Company in 1912, have brought it to its present state of perfection.

To an analytical chemist, the value of this scheme lies in the lengthy experience that has been gained in its use by a large number of chemists engaged in the scientific control of industrial manufacturing processes. This experience has, down the years, made it possible to discover any weak points in the scheme or the cause of any discrepancies in the results by different workers or between different laboratories; and so, by a gradual process of evolution, to select the best methods and submit them to an extended trial.

The methods used for the purification of the reagents, the preparation of the standard solutions from them and also the work of standardisation are described in full working detail. To have attempted any condensation or abridgement would, by spoiling this continuity, have deprived the scheme of most of its value to a practical analyst.

By printing this paper in full, *The Analyst* pays a tribute to the chemical acumen of a former President and makes available to chemists in general the labours of all those, unnamed but not unhonoured, who have worked towards its perfection and made its publication possible.

* *Analyst*, 1930, **55**, 238.

PROCEEDINGS OF THE SOCIETY OF PUBLIC ANALYSTS AND OTHER ANALYTICAL CHEMISTS

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ELECTED JUNE 14TH, 1950

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ELECTED SEPTEMBER 6TH, 1950

Leslie Airey; Fazlur Rahman Mahmood Alvi, M.Sc. (Punjab), A.R.I.C.; Malcolm Cornelius Bourne, B.Sc. (Adelaide), R.A.C.I., A.S.A.S.M.; George Hope, B.Sc. (Lond.); William Lewis Jones, M.Sc. (Minnesota), A.A.C.I.; Peter Douglas Ridsdale, B.Sc. (Leeds).

Analytical Methods Committee

REPORT PREPARED BY THE CAROTENE PANEL OF THE SUB-COMMITTEE ON VITAMIN ESTIMATIONS

The Determination of Carotene in Green-Leaf Material

Part I. Fresh Grass

THE Analytical Methods Committee has received the following Report from the Carotene Panel of the Sub-Committee on Vitamin Estimations, and its publication has been duly authorised.

PROLEGOMENON

In 1948 the Sub-Committee on Vitamin Estimations appointed a Panel "to investigate the application to other green-leaf materials of the method outlined by Dr. W. A. G. Nelson at the April, 1947, meeting of the Society for the estimation of carotene in dried grass, and to advise the Sub-Committee on the practicability of extending the method to animal and vegetable food products generally," under the Chairmanship of Mr. A. L. Bacharach. The other members were Dr. R. G. Booth, Dr. V. H. Booth, Dr. J. Green, Mr. N. T. Gridgeman (Hon. Secretary), Mr. T. Barton Mann, Dr. F. E. Moon, Professor R. A. Morton, Dr. W. A. G. Nelson and Dr. S. Y. Thompson. Mr. D. C. M. Adamson served for a while but was unable to continue.

The method of Nelson being inapplicable as it stands to fresh green-leaf material (for which it was not designed), a new method has been derived from the work of V. H. Booth (*J. Soc. Chem. Ind.*, 1945, **64**, 162) and T. Barton Mann (*Analyst*, 1944, **69**, 34). This method is based solely on experimental work with fresh grass, which was used because of its interest *per se*, its constant availability and the comparatively small sampling error involved in analysing it. It is hoped to consider later any special problems that may arise in estimating the carotene content of certain green leaves in common human consumption, such as cabbage and lettuce, though it is realised that they present formidable sampling difficulties and that any figure purporting to express the carotene content of, for example, a whole cabbage or a whole lettuce is of doubtful practical and scientific value. Meanwhile, there is no evidence for believing that the analytical method for determining carotene in the fresh grass will not, apart from sampling difficulties, apply to green-leaf material generally.

GENESIS OF METHOD

The carotenoids that, with chlorophyll, make up the pigment of green-leaf material, are divisible into two groups according as they are biologically inert or exhibit provitamin A activity. The former comprise the carotenols and their esters; the latter are dominated by

β-carotene, and usually include small quantities of congeners. Separation of these allied hydrocarbons from β-carotene is difficult; fortunately the relative quantities (in green leaf) justify their inclusion and estimation together with β-carotene as simply "carotene," and this is normal practice.

Until about 1940 the pigments were usually partitioned by phase separation; subsequently the more selective method of chromatography has replaced it. Numerous variants of this method have appeared, all the more important of which have been studied, and many tried, by the Panel, which now recommends two alternative adsorbents. Efficient use of either demands, however, careful working, as indeed does the whole process of estimation, although it is in essence simple. Some forethought is needed for the drawing of the sample, which must be fresh and must be processed without delay, for the cutting of tissue initiates enzymic destruction of carotene; and throughout the operations the worker must be alert to the fact that excessive heat, light and air are inimical to accuracy. To avoid delays and consequent losses, it is advisable to mobilise all apparatus and reagents beforehand.

There are four stages in the method: (1) comminution-extraction in cold light petroleum and acetone; (2) removal of acetone from the extract by washing with water; (3) chromatographic separation of the carotene on a column of bone-meal or alumina; (4) photometric or tintometric estimation of the carotene in solution. Three not uncommon features of other carotene methods have been avoided: mechanical homogenisation, which needs special apparatus and is more efficient than the method here recommended; alkali treatment, which promotes formation of epiphasic pigments; and heat, which fosters isomerisation.

The choice of solvents requires explanation. Acetone, one of the best extractors of carotene from moist vegetable matter, tends to hold particles in suspension; this necessitates the operation, undesirable in this context, of filtration; furthermore, acetone is unsuitable in chromatography. Light petroleum, although itself an inefficient extractor, yields clean decantable solutions and is eminently suitable for chromatography. It has been found that a mixture of equal parts of acetone and light petroleum retains the advantages of both components and the disadvantages of neither, provided the acetone is removed before chromatography.

Apparatus

Pestles—Pestles 12 cm. long, made from 8-mm. glass rod flattened at one end, and provided with handles made from corks (see Fig. 1).

Squat beakers—Heavy-gauge squat beakers, 50 to 60 ml., to serve as mortars. The beaker bottoms must have plane inside surfaces.

Separating funnels—About 250-ml. capacity, of narrow conical type with outlet tubes 7 cm. long. The taps should be ungreased or lubricated with a non-fatty compound. It is wise to cover the sides with opaque paper.

Continuous washing device (see Fig. 2)—The glass drip tube is sleeve-joined with heavy-gauge rubber tubing—clamps being thereby obviated—to the outlet tube of a separating funnel. A bigger separator, or other suitable reservoir, steadily releases large drops of water.

Chromatograph tubes (see Figs. 3 and 4)—The recommended dimensions are approximate.

Water pump, Buchner flask, measuring cylinders, etc.

A *spectrophotometer* or *absorptiometer* or *colorimeter*.

Reagents, etc.

Light petroleum—Boiling range 40° to 60° C.

Acetone—Redistilled.

Quartz powder—"Medium fine," average particle size 0·17 mm.

Quinol.

Sodium sulphate—Anhydrous.

β-Carotene standard—For calibration of absorptiometer or colorimeter. For routine purposes commercial crystalline carotene is satisfactory.

Bone-meal—Bone-meal specially prepared for carotene chromatography can now be purchased. It is advisable to ensure that bought material contains nothing that will pass a 120-mesh sieve, because "fines" retard the flow of carotene.

Preparation, from steamed bone flour (*synonyms*, sterilised bone-meal, feeding bone flour) passing 80-mesh, but retained by 120-mesh sieves, is not difficult. Extract by boiling under

reflux about 100 g. of the sieved meal in a Tate tube for about 24 hours with a 3 + 1 + 1 mixture of light petroleum, acetone and ether (ether meth. sp.gr. 0·720); dry overnight at 100° C.; immediately before use wash with light petroleum to remove any traces of the mixed solvents.

Alumina and sodium sulphate—This mixture is an alternative to the bone-meal.

Mix equal weights of alumina (as sold for chromatography; 200 mesh) and anhydrous sodium sulphate (100 mesh) and dry in 1 to 1½-cm. layers, at 150° C. for 12 to 16 hours. The heating conditions may have to be altered for particular materials—brands differ in adsorptive properties. A good preparation should (*a*) allow light petroleum to percolate evenly down a standard column at a rate of about 1 cm. per second—slower flow is usually due to the presence of too many fines, and faster or uneven flow to too much coarse material (which

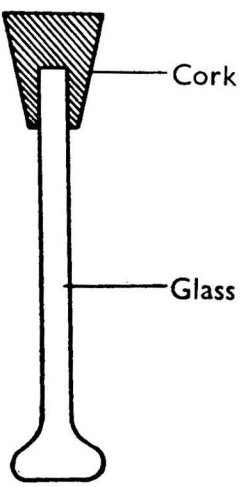

Fig. 1. Pestle

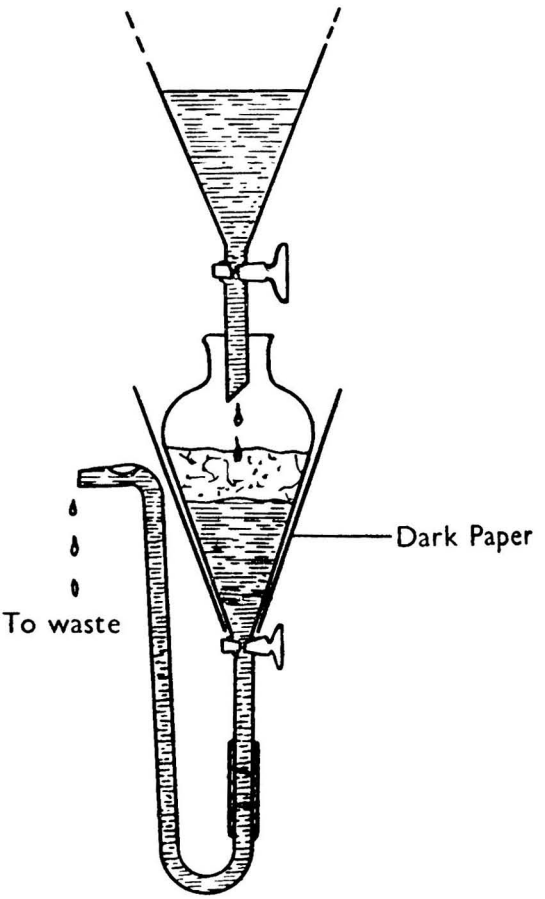

Fig. 2. Continuous washing apparatus

grinding will remedy), and (*b*) adsorb as a band at the top of a standard column all the pigment from a light-petroleum extract of 0·5 g. of green leaves; from this band the carotene should be elutable with 15 ml. of a 2 + 98 mixture of acetone and light petroleum as a well-defined evenly descending orange zone. The drier the adsorbent, the poorer and slower will elution be. Slight over-dryness can be compensated by the use of more acetone, *e.g.*, a 3 + 97 mixture, but a considerably over-dry adsorbent will have to be diluted with unheated sodium sulphate and alumina. The wetter the adsorbent the greater the likelihood of elution of non-carotene pigments with the carotene as a thin sharp line; over-wet material must be further heated. Once prepared and tested, a batch of the adsorbent can be stored in tightly-stoppered bottles and used at any time without further treatment.

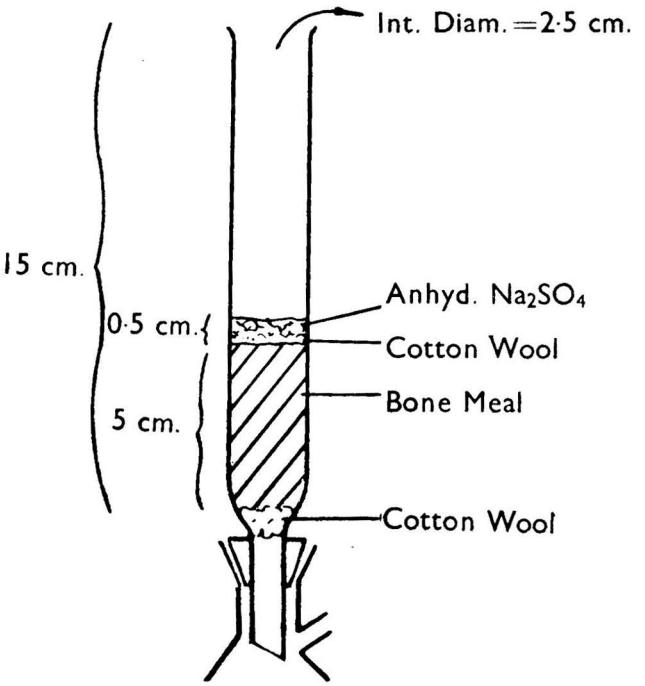

Fig. 3. Bone-meal column

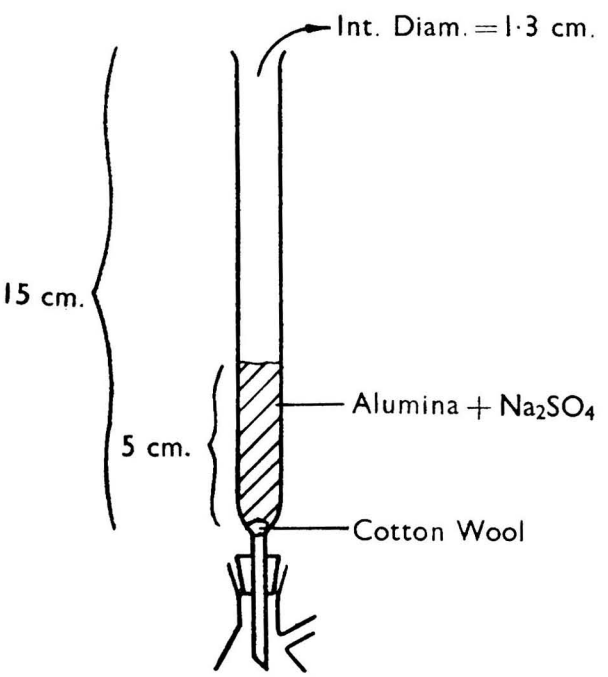

Fig. 4. Alumina column

Sampling and preparation

Quantities of the order of 1 to 2 g. are required for one determination. Not less than two, and preferably three, simultaneous samples should be taken and assayed independently. Care should be taken to minimise leaf damage.

Weigh a grinding beaker. If the leaves are wet, gently press them between blotting- or filter-paper to remove extraneous moisture, quickly cut off pieces or strips from various parts or bunches, drop them into the beaker, and weigh. Add approximately the same weight of quartz, and at once cover with 5 to 8 ml. of a mixture consisting of equal parts of acetone and light petroleum and containing 0·1 per cent. of quinol.

Extraction

Firmly grind the mixture in the solvent for a minute or so, allow to settle, and then decant into the separator containing water. Add fresh solvent to the grist, regrind, settle, and decant as before. Repeat until no more colour can be extracted. Not less than five, and sometimes up to ten, extractions will be required. If in doubt about completion, decant the last extract into a small separator half-full of water and shake gently; any pigment will then easily be visible through the width of the thin layer.

If any pigment collects below the spout of the beaker it can be recovered on a scrap of filter-paper, which is subsequently placed in the beaker. If the grist becomes dehydrated during extraction, the efficiency of extraction and settlement of the particles will be impaired; a drop or two of water should therefore be added.

Removal of acetone

The separator containing the extract, over water, now takes its place as the lower, paper-covered separator in the washing apparatus shown in Fig. 2. Adjust the tap of the upper and larger "water reservoir" separator to deliver 100 to 200 large drops of ordinary tap water per minute directly on to the solution surface; if the water runs down the inside of the funnel the washing action is lessened. Open the lower tap fully and slowly oscillate the separator in the plane of the S-tube (whose top should reach just over half-way up the separator bowl) until the tube is full of water. Surplus water will overflow, taking with it acetone, quinol and other water-soluble substances. About $1\frac{1}{2}$ litres of water will be needed to remove all the acetone, any trace of which will interfere with the subsequent chromatography. The depth of water below the solvent level must at all times be sufficient to prevent mechanical loss of pigmented layer; about 6 to 8 cm. is usually necessary.

Chromatography

Either a bone-meal column (Fig. 3) or an alumina-Na_2SO_4 column (Fig. 4) may be employed.

With bone-meal, use a "straight-through" method, *i.e.*, pass the light-petroleum solution of the pigments through the column (previously moistened with light petroleum) by gentle suction; the carotene will elute directly, leaving all other fractions in a zone at the top. Colourlessness of the eluate indicates completion; in case of difficulty of decision on the end-point, a test tube placed in the collecting flask, after most of the carotene has obviously come through, can be used to collect fractionally; bulkiness of the final solution can thus be avoided. The column can be used many times without cleaning. When the adsorbed chlorophylls and irrelevant carotenoids eventually choke the column its useful life may be extended as follows: wash out with acetone and remove the acetone with light petroleum; the column is then ready for further use.

With the alumina column use an adsorption-elution procedure. Moisten the column with light petroleum, pour the solution of the pigments on top, rinse with small amounts of the same solvent, applying suction as required. All the pigments should adsorb at the top of the column. Suck through 10 to 25 ml. of a solution of 2 per cent. of acetone in light petroleum; carotene will be seen to free itself from the other pigments and to descend the column. The trailing edge should be fairly clear; a diffuse band indicates that the adsorbent is too strong (possibly having caused isomerisation) and that satisfactory elution of the carotene cannot be ensured. If the adsorbent is too moist, or if the acetone has been incompletely removed, elution will be too rapid and unspecific. To keep down the bulk of the final solution do not begin to collect the eluate until the leading edge of the descending carotene zone is near the bottom of the column.

Estimation

If a spectrophotometer is used, work on the basis of $\lambda_{max.} = 450$ mμ., at which $E^{1\%}_{1cm.} \times 400$ = p.p.m. If a light-filter absorptiometer or a colorimeter is used, calibrate with a solution of the carotene standard in light petroleum.

Carotene obtained by the bone-meal method appears to be all-trans β-carotene. On the reasonable assumption that the alumina product is similar, both results may alternatively or additionally be expressed in the form of International units of Provitamin A per gram; one such unit is 0·6 μg. of β-carotene.

Precision

Some typical duplicate results on ten pairs of samples of fresh grass are given below—

Laboratory	Instrument	Carotene, p.p.m.
A	Lovibond colorimeter	110, 112; 112, 115
B	Pulfrich absorptiometer	117, 118; 119, 123
C	Spekker absorptiometer	105, 120; 107, 112
D	Visual spectrophotometer	128, 132; 144, 145
E	Photo-electric spectrophotometer ..	131, 132; 144, 153

N.B.—The ten samples are all different.

From these and all other available replicated results the coefficient of variation of one determination was found to be approximately 4. This means that in any given set of replicated results, two-thirds of them will lie within the range 96 to 104 per cent. of the over-all mean, and only one result in twenty will be more than ± 8 per cent. from the over-all mean.

This does not, however, take into account inter-laboratory differences, which mainly arise from imperfect standardisation of instruments rather than manipulative errors. No comparisons between laboratories were carried out in the trials on which this report is based, because of the difficulty of transporting fresh grass samples without loss of moisture or stimulation of enzymic action. Evidence from another comparable investigation may, however, be quoted. The Carotene Committee of the Grass Driers' Association recently organised collaborative trials of a method of determining carotene in dried grass, and the variation between laboratories was found to average 4·6 (coefficient of variation). Those laboratories that used spectrophotometers showed better agreement than those that used absorptiometers or colorimeters—which is to be expected. Taking these things into consideration it can be said that the coefficient of variation of single estimates of the carotene content of fresh grass is probably about 6, and that of duplicate estimates about 4·5.

Bibliography on Carotene Estimation, with Special Reference to Green-Leaf Material

PREPARED BY N. T. GRIDGEMAN FOR THE CAROTENE PANEL OF THE SUB-COMMITTEE ON VITAMIN ESTIMATIONS OF THE ANALYTICAL METHODS COMMITTEE OF THE SOCIETY

THIS list does not pretend to be exhaustive, but it is hoped that there are no serious omissions. A few items may appear from their titles to be only remotely relevant; they will nevertheless be found to contain information pertinent to some aspect of the subject. As a rough guide to the several aspects, the following sub-division index may be helpful—

Surveys and Reviews:	4, 5, 68, 69, 71.
Sampling and Preparation:	4, 29, 88.
Stability and Precautions:	14, 18, 53, 54, 55, 65.
Special Apparatus:	13, 15, 34, 60.
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Chromatography:	Magnesia—3, 4, 5, 19, 22, 26, 27, 33, 44, 51, 55, 56, 71, 72, 73, 74, 79, 80, 84, 89, 90, 97; Magnesium carbonate—2, 27, 28; Dicalcium phosphate—1, 2, 12, 58, 59, 60, 69, 73, 76; Alumina—13, 14, 61, 73, 78, 85; Lime—11, 23, 41; Calcium carbonate—8; Bone-meal—50, 63, 97; Miscellaneous—20, 21, 37, 38, 40, 45, 91.
Instrumentation:	4, 16, 24, 86, 95.
Carotene:	Characteristics—6, 7, 10, 42, 50, 64; Isomerisation—6, 7, 11, 17, 19, 30, 31, 36, 50, 79, 94; Distribution in plants—43, 44, 82.

It should be noted that only the post-1945 reports of the Association of Official and Agricultural Chemists are itemised, the conclusions of all earlier work having been embodied in ref. 1. This restriction does not of course apply to the ordinary contributed papers in the journal of the A.O.A.C.

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The Standardisation of Volumetric Solutions*

BY THE ANALYTICAL CHEMISTS' COMMITTEE OF IMPERIAL CHEMICAL INDUSTRIES LIMITED

THE methods employed by Imperial Chemical Industries to standardise volumetric solutions have been in use for a number of years, and it is thought that it is now opportune to publish a full description of them.

An outline of the scheme was given by Edward Hinks[1] in 1930, but this did little more than mention the relationships that are employed. It is hoped that the following detailed description of Imperial Chemical Industries' practice will be of service to all analysts.

SYSTEM OF STANDARDISATION

The standardisation system is based on pure electrolytic silver as ultimate standard, with iodine, sodium carbonate and sodium chloride as working standards and arsenious oxide and potassium dichromate as secondary working standards.

The principle underlying the system is the adoption of a single ultimate standard of reference upon which the titre of all the principal laboratory standard solutions is based. The working standards are used as intermediaries between the ultimate standard and the volumetric solutions, but where the latter are not directly referable to the working standards, reference is made through the secondary working standards. The whole scheme is given in outline in Fig. 1.

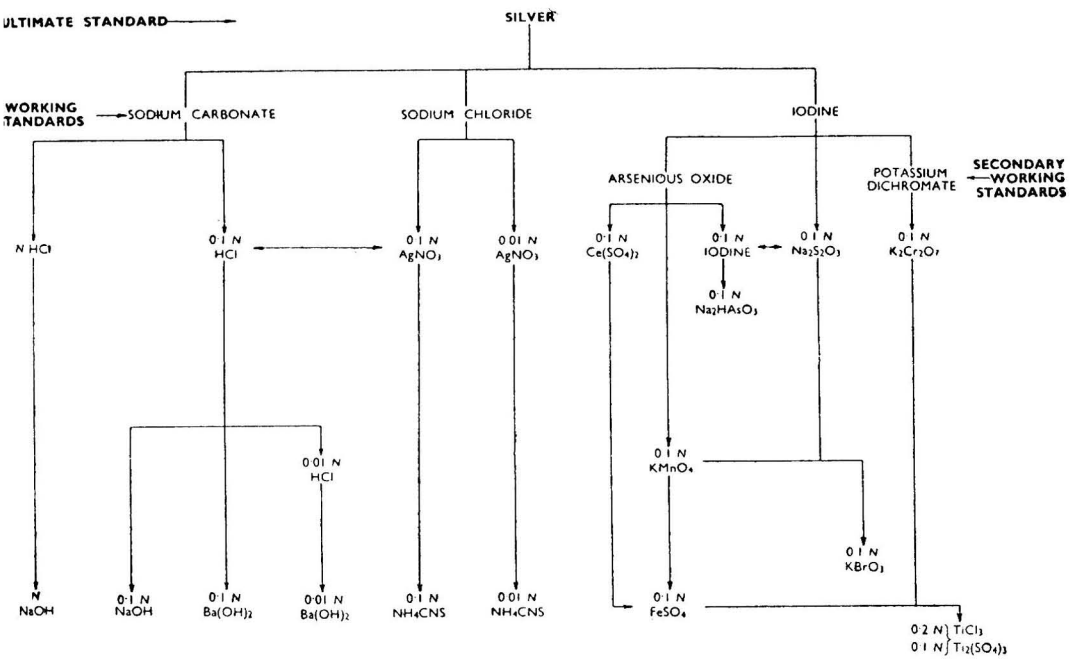


Fig. 1. Scheme of standardisation

It should be mentioned that the scheme outlined in Fig. 1 does not include some of the less frequently used secondary working standards, such as antimony (pure metal), copper foil (pure electrolytic), *p*-nitroaniline and picric acid.

* The bulk of the work involved in devising the system described in this paper was carried out in the laboratories of the Nobel Division under the direction of the late Mr. W. Rintoul.

INDICATORS AND OTHER REAGENTS

WATER—

(*a*) Where reference is made to water, ordinary distilled water is intended.

(*b*) "Boiled-out water" is defined as distilled water that has been boiled until it is free from ammonia and carbonate, and cooled out of contact with the air.

(*c*) The specially purified, or specially pure, water that is used in the reference of working standards to ultimate standard silver is prepared as follows—

Place 2 litres of distilled water in a quartz or hard-glass flask and add 2 to 3 g. of A.R. quality sodium carbonate and 2 to 3 g. of A.R. quality potassium permanganate. Distil the water through a quartz or block tin condenser and reject the first 500 ml. If the distillate no longer contains chlorides or ammonia, collect and store the next 1000 ml. in a quartz or copper flask.

INDICATORS—

Methyl red—Dissolve 0·5 g. of water-soluble methyl red in water and dilute the solution to a litre.

Methyl orange—Dissolve 0·5 g. of the dye in water and dilute the solution to a litre.

Bromophenol blue—Triturate 0·4 g. of the dye in an agate mortar with 6·0 ml. of 0·1 *N* sodium hydroxide and dilute the resulting solution to a litre with 20 per cent. v/v alcohol prepared by the appropriate dilution of industrial methylated spirit.

Phenolphthalein—Dissolve 0·5 g. of pure phenolphthalein in a litre of 50 per cent. v/v alcohol that has previously been neutralised to the indicator. Industrial methylated spirit may be used for the preparation of this reagent.

Starch—Triturate 5 g. of pure starch and 0·01 g. of mercuric iodide with 30 ml. of water in a mortar. Pour the resulting cream into 1 litre of boiling water, boil for 3 minutes, allow the solution to cool and, when the sediment has settled, decant off the clear liquid.

Ferroin (*o-phenanthroline ferrous sulphate*)—Dissolve 0·695 g. of A.R. grade ferrous sulphate, $FeSO_4.7H_2O$, in 100 ml. of warm water, then add 1·48 g. of *o*-phenanthroline and shake the vessel until the reagent dissolves. Immediately before use, transfer 10 ml. to a 400-ml. beaker and titrate it, drop by drop, with 0·1 *N* ceric sulphate solution. As the oxidised form of the indicator in concentrated solution is blue-green in colour, the solution darkens and becomes brown. Titrate just to the disappearance of the red colour; the end-point is sharp to 1 or 2 drops. The oxidised indicator does not keep well.

Diphenylamine—Dissolve 5 g. of diphenylamine in a litre of A.R. grade concentrated sulphuric acid, of density 1·836 at 20° C.

Ferric ammonium alum—Dissolve 140 g. of the A.R. grade salt in water. Slowly add sufficient concentrated nitric acid to change the colour of the solution from red-brown to yellow. Dilute to 1 litre with water.

Potassium chromate—Dissolve 100 g. of chloride-free A.R. quality potassium chromate in water and dilute the solution to 1 litre.

Potassium thiocyanate—Dissolve 20 g. of the A.R. quality salt in water and dilute the solution to 100 ml.

Ammonium thiocyanate—Dissolve 20 g. of the A.R. quality salt in water and dilute the solution to 100 ml.

REAGENTS—

Osmic acid solution—Dissolve 0·25 g. of osmium tetroxide, OsO_4, in 0·1 *N* sulphuric acid and dilute the solution to 100 ml. with 0·1 *N* sulphuric acid.

Sulphuric - phosphoric acid reagent—Mix 150 ml. of A.R. grade concentrated sulphuric acid, density 1·84 at 20° C., with 150 ml. of A.R. grade phosphoric acid, density 1·70 at 20° C.; add the mixture cautiously to 500 ml. of water. Cool the solution and dilute it to 1 litre.

ULTIMATE STANDARD

The ultimate standard silver is prepared from fine silver by electrolytic deposition. It is kept in sealed tubes in the form of buttons weighing from 9 to 10 g. each. The purity of the ultimate standard silver is 99·995 per cent.

WORKING STANDARDS

Sodium carbonate

The standard sodium carbonate is prepared by heating sodium sesquicarbonate to 270° ± 10° C. until it is constant in weight. The sesquicarbonate is prepared from a selected sample of sodium bicarbonate which has been shown to be of very high purity by exhaustive qualitative tests. Suitable tests are given by Rosin.[2]

Preparation of sodium sesquicarbonate—

Add 1025 g. of the selected sodium bicarbonate gradually and with frequent stirring to 4000 ml. of hot water in a hard-glass beaker, the mixture being heated to 86° ± 1° C. and maintained at this temperature until the whole of the bicarbonate has been added. Filter the resulting solution while still hot through a close-grained filter-paper and cool the filtrate quickly. When the separation of crystals of sesquicarbonate is complete, pour off the mother liquor and break up the crystalline mass. Transfer the wet crystals to a large funnel fitted with a perforated porcelain disc and remove as much as possible of the mother liquor by suction. Transfer the crystals to a porcelain basin, dry them in an electrically heated boiling water oven, grind them to a powder and store in a glass-stoppered bottle.

Repeat this process until the desired quantity of sesquicarbonate has been obtained; then blend the various batches together thoroughly, weigh off 100-g. portions of the product into hard-glass bottles and seal the bottles by drawing out the necks in a blowpipe flame. Use a glass funnel in filling the bottle to avoid contamination of the neck and consequent difficulties in the sealing operation.

Method for referring the working standard sodium carbonate to the ultimate standard silver—

Accurately weighed quantities of the working standard sodium carbonate are titrated from a Ripper's weighing burette with *N* hydrochloric acid that has been previously standardised gravimetrically with the ultimate standard silver. The procedure involves the conversion of sodium sesquicarbonate to sodium carbonate, and for this purpose a temperature of 270° ± 10° C. is maintained and crucibles of platinum, or preferably silver, are used.

Because of the ease with which sodium carbonate absorbs traces of sulphur oxides, precautions must be taken to avoid its exposure to the products of combustion of coal gas. An electrically heated oven should, therefore, be used. The time required for the conversion may be as little as 2 hours, provided that the material is stirred from time to time and that the oven is well ventilated. However, 6 to 8 hours may be necessary before constant weight is obtained if the oven is badly ventilated and the material is not stirred. Fit a thermometer in the oven with its bulb in the vicinity of the crucible. It is advisable to determine the time required for the conversion with the particular apparatus employed and under the conditions of heating and stirring that are used. By heating for half an hour longer in subsequent experiments with the same apparatus, confidence may be placed in the success of the operation. The details are given below.

Preparation of pure hydrochloric acid—Prepare an aqueous solution of hydrogen chloride, free from other halogen acids and free chlorine, by dropping concentrated sulphuric acid into hydrochloric acid in the apparatus shown in Fig. 2. The sulphuric acid used for this purpose must be free from nitrogen and halogens, and the hydrochloric acid must have been boiled thoroughly after addition of a small quantity of potassium permanganate. Wash the gas with a solution of cuprous chloride in hydrochloric acid and with boiled-out water. Conduct the washed gas through a hard-glass tube into 400 ml. of boiled-out water contained in a 600-ml. silica flask covered with a piece of platinum foil. Boil the resulting hydrochloric acid, dilute it with boiled-out water until it is of approximately normal strength and then mix. Preserve the solution in the dark in a hard-glass bottle that has been well steamed out and washed with water.

Standardisation of hydrochloric acid with the ultimate standard silver—Weigh accurately a button (9 to 10 g.) of the standard silver, which has previously been scrubbed with sand, boiled successively in dilute hydrochloric acid, dilute ammonium hydroxide and water, and finally dried. Place the button in a 2-litre hard-glass conical flask and add 20 ml. of chloride-free 40 per cent. nitric acid. Remove the nitrous fumes by boiling, then cool and dilute the solution to 900 ml. with specially pure water.

Weigh from a weighing burette a quantity of the normal hydrochloric acid slightly less than that required to precipitate the whole of the silver, and dilute it to 900 ml. with water. Add the silver nitrate solution slowly to the diluted hydrochloric acid and wash the flask which contained the silver nitrate with water, adding the washings to the united solution. Mix the liquid well, but do not shake it violently, and set it aside overnight in a dark place. Filter the liquid through a filter-paper that has previously been washed free from chloride, and wash the precipitated silver chloride six times by decantation with specially pure water, shaking vigorously each time, and pour the washings through the filter. Transfer the precipitate to the filter and wash it thoroughly. Reject the precipitate. Evaporate the

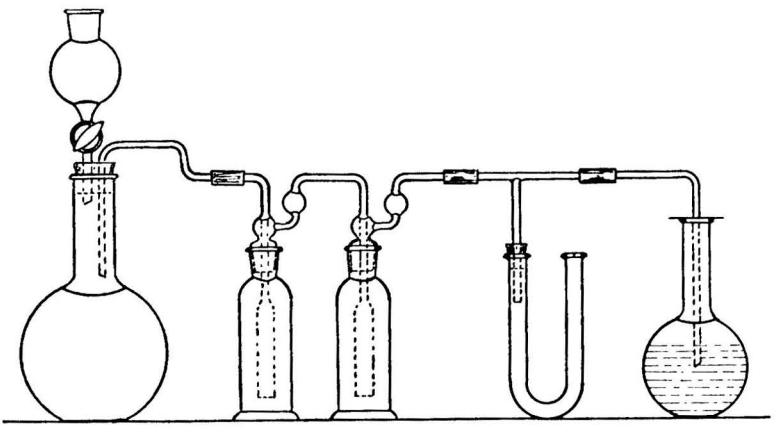


Fig. 2. Apparatus for preparation of pure hydrochloric acid

filtrate and washings to dryness in a porcelain basin. Dissolve the residue in a small amount of hot water and, after the addition of 1 ml. of 3 *M** nitric acid, allow the mixture to stand in the dark for at least 12 hours. Filter the solution through a close-grained filter-paper previously washed with specially pure water, and wash the precipitate of silver chloride with 0·01 *M* nitric acid. Reject the precipitate.

Add an excess of 0·1 *M* hydrochloric acid to the combined filtrate and washings and allow the mixture to stand in the dark for 12 hours. Collect the precipitated silver chloride in a tared Gooch - Munroe crucible and wash it with 0·1 *M* hydrochloric acid. Dry the crucible and its contents to constant weight at 130° C. Calculate the concentration of the hydrochloric acid from the weight of the acid corrected for displacement of air, the weight of the silver and the excess of silver weighed as silver chloride, the weight of silver being corrected for air displacement (see Table I, p. 587).

Note on the use of weighing burettes—Where a weighing burette is used in standardisation work it should be allowed to remain in the balance case for 20 minutes before each weighing.

Standardisation of sodium carbonate with the standardised hydrochloric acid—Weigh from a weighing burette a quantity of the standardised hydrochloric acid in the same way as for the standardisation of the acid. Weigh in a tared silver crucible a quantity of the sodium sesquicarbonate slightly less than that required to neutralise the weighed quantity of the acid. Heat the sesquicarbonate in an electrically heated air-bath maintained at 270° ± 10° C. until the salt is constant in weight; 8 hours is usually sufficient. During weighing operations place the crucible containing the carbonate in a well stoppered weighing bottle. Transfer the anhydrous sodium carbonate to a silica flask and reweigh the empty crucible; then dissolve the salt in ammonia-free water. To this solution slowly add the hydrochloric acid, pouring it through a funnel which has been drawn out to a capillary. After the acid has been added and washed into the flask, boil the solution until all carbon dioxide is expelled, and then cool. Titrate the excess of acid with 0·01 *N* barium hydroxide, methyl red being used as indicator. Calculate the result as a percentage of sodium carbonate in the heated salt, the weight of sodium carbonate being corrected for air displacement.

* In this paper the designation molar (*M*), with or without modifying figures, signifies that the solution need not be accurately made up or standardised, in contrast to those designated by *N*.

The 0·01 *N* barium hydroxide used in the above procedure should be standardised by means of 0·01 *N* hydrochloric acid, which has been prepared by dilution from the standardised *N* hydrochloric acid, with methyl red as indicator.

Iodine

Preparation of iodine—

Iodine is purified by sublimation from potassium iodide and calcium oxide. The potassium iodide retains any chlorine or bromine that may be present and the lime combines with water and with any acid such as hydriodic acid.

Reduce some re-sublimed iodine to powder and mix it intimately with 10 per cent. of its weight of powdered potassium iodide and 5 per cent. of ground calcium oxide. Sublime the mixture slowly in an apparatus of the form shown in Fig. 3.

Place the mixture in the beaker and heat by an electric lamp placed below, the whole being surrounded by an aluminium sheath covered with asbestos mill-board. The iodine sublimes in a thick crust and adheres to the projecting part of the covering flask, which is kept cool by allowing tap water to flow through it. Detach the iodine by filling the flask with ice-cold water, break up the iodine and re-sublime it in the same apparatus after removal of the residue from the first operation. This second sublimation, which should be carried out at as low a temperature as possible, separates any small traces of potassium iodide or lime that may have been carried up mechanically in the first operation.

Powder the purified iodine and dry it over phosphorus pentoxide in a desiccator, the rim of which should be lubricated with the minimum amount of grease, which should be unreactive in nature.* Transfer 10-g. portions to hard amber glass tubes and seal the latter in a blow-pipe flame. Use a glass funnel to obviate contamination of the upper part of the tube and consequent difficulties in the sealing operation.

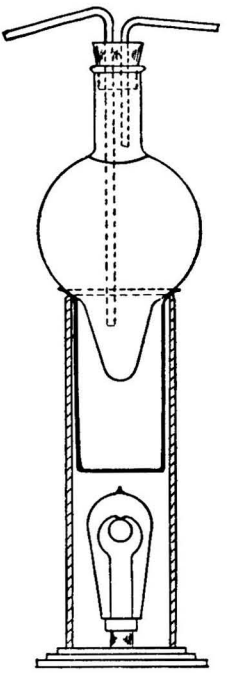


Fig. 3. Apparatus for sublimation of iodine

Standardisation of iodine with the ultimate standard silver—

Weigh accurately about 3 g. of the ultimate standard silver that has been previously scrubbed with sand, boiled successively in dilute hydrochloric acid, dilute ammonium hydroxide and water, and finally dried. Transfer the button to a 1-litre conical flask; add 6 ml. of chloride-free nitric acid (density 1·415 at 20° C.) and 5 ml. of specially pure water, and evaporate the solution to dryness on a water-bath in order to expel the excess of acid. Dissolve the residue in 750 ml. of specially pure water.

Weigh accurately in a small well-stoppered weighing bottle a quantity of the iodine half a milligram less than that theoretically required to react with the whole of the silver. Withdraw the stopper from the weighing bottle and immediately drop both stopper and bottle into a mixture of 3 ml. of 1 + 1 solution of hydrazine hydrate in water and 15 ml. of 3 *M* ammonium hydroxide dissolved in 250 ml. of specially pure water contained in a 2-litre hard-glass flask fitted with a reflux condenser ground into the neck; the condenser prevents mechanical loss arising from the effervescence which occurs at this stage. When the reduction of the iodine is complete, dilute the solution to 750 ml. with specially pure water added through the condenser, and acidify with dilute nitric acid which has been well boiled to remove nitrous acid. Cover the flask with brown paper to protect the contents from light and slowly add the solution of silver nitrate through a funnel with occasional gentle agitation. When all the solution has been added, wash the last traces of silver nitrate into the iodide solution with several small portions of specially pure water. Mix the liquid thoroughly and allow it to settle in the dark overnight, or until the supernatant liquid is clear. Filter the solution through a close-grained filter-paper that has been well washed with specially pure water to remove chloride. Wash the precipitated silver iodide by decantation with pure water. Boil

* The following composition has been found to be efficacious: Dissolve 35 g. of ceresine wax in 65 g. of B.P. quality liquid paraffin by heating the mixture in a water-bath until it is homogeneous. Stir the mixture until it cools to a uniform mass.

the precipitate for 5 minutes with water containing 1 per cent. of ammonia, cool, acidify with dilute nitric acid and filter it. Finally wash the silver iodide thoroughly with boiling water. Discard the residue and evaporate the combined filtrate and washings to dryness in a porcelain basin. Extract the residue with 0·03 *M* nitric acid, filter if necessary, and add 10 ml. of 0·1 *M* hydrochloric acid. Allow the mixture to stand in the dark overnight. Collect the precipitated silver chloride in a tared Gooch - Munroe crucible, wash with 0·1 *M* hydrochloric acid and dry to constant weight in an air oven at 130° C.

Calculate the purity of the iodine from the weight of silver combined with the iodine, the necessary corrections for air displacement being applied. It is of the utmost importance that the distilled water, the reagents and the apparatus shall be free from traces of chloride. Specially pure water should be used throughout, and the filter-papers and apparatus should be washed with it before they are used.

METHOD FOR THE COMPARISON OF DIFFERENT BATCHES OF WORKING STANDARD IODINE—

Different batches of working standard iodine are compared by weight titration with 0·5 *N* sodium thiosulphate that has been prepared by the dilution of a well-matured strong solution of the pure salt with freshly boiled-out water. A small excess of the sodium thiosulphate is used and the excess titrated with 0·01 *N* iodine, starch solution being used as indicator. The details of the procedure are as follows—

Place 5 g. of potassium iodide and 0·5 ml. of water in a small well-stoppered weighing bottle. Put the whole inside the balance case for half an hour and then weigh it accurately. Add 2 to 3 g. of the iodine, again place the vessel inside the balance case for half an hour and weigh it accurately. Rotate the bottle to ensure that all the iodine is dissolved. Withdraw the stopper and immediately drop the bottle and the stopper into 200 ml. of water contained in a 1-litre conical flask. From a weighing burette add a quantity of the 0·5 *N* sodium thiosulphate slightly in excess of that required to reduce the whole of the iodine, and dilute the clear solution to 750 ml. with water. Titrate the small excess of sodium thiosulphate with 0·01 *N* iodine, starch solution being used as indicator, until a pale purple-pink colour is obtained. Prepare the 0·01 *N* iodine by accurately weighing about 0·64 g. of working standard iodine in a small stoppered weighing bottle containing 2 g. of moistened iodate-free potassium iodide; take care to ensure that the weighing bottle and its contents are at the same temperature as the air inside the balance case. Dissolve the iodine and the potassium iodide in boiled-out water, dilute the solution to 500 ml. and calculate the normality factor.

In this comparison there is no need to correct the weighings for air displacement.

SODIUM CHLORIDE

THE STATUS OF SODIUM CHLORIDE AS A STANDARD CHEMICAL—

Richards and Wells, in their classical work "A Revision of the Atomic Weights of Sodium and Chlorine,"[3] showed the identity of many specimens of sodium chloride prepared in various ways from materials of widely different origin. The method of purification adopted by them was the familiar one of precipitation by hydrochloric acid gas followed by recrystallisation from water. It is very doubtful if sodium chloride can be completely dried at any temperature below its melting-point, but the authors show (*ibid.*) that the expulsion of water upon fusion of sodium chloride is attained without change in its composition and that "probably not enough gas is absorbed during fusion of the salt in air sensibly to alter the apparent combining weight." They state "that sodic chloride is among the substances whose preparation in a pure state is an easy problem." In view of the foregoing, and in view of the fact that the technique of the method of referring sodium chloride to silver has been elaborated to the last detail by many notable workers in the field of atomic weight determination, it is concluded that sodium chloride satisfies all the criteria usually demanded of a working standard chemical. It is superior to sodium carbonate in that it can be directly referred to silver, and superior to iodine in that the technique of its reference to silver is not attended by the difficulties that accompany the reference of iodine to silver.

PREPARATION OF THE SODIUM CHLORIDE—

The working standard sodium chloride is prepared from purified brine containing about 30 g. of sodium chloride per 100 ml. and not more than traces of calcium and magnesium. Alternatively, a solution of pure sodium chloride, A.R. quality, may be used.

Into a convenient volume of the brine, pass hydrogen chloride gas until the acidity is about 5 *N*. The hydrogen chloride gas used for this purpose should be prepared by the slow addition of sulphuric acid to fragments of clean rock salt, the gas being scrubbed by passage through a gas-washing bottle containing concentrated hydrochloric acid.

Filter the precipitated salt by suction through prepared "Tarantulle"* cloth in a porcelain Büchner funnel. The Tarantulle is prepared by steeping it in concentrated hydrochloric acid and washing it with specially pure water.

Wash the salt on the filter with a little specially pure water, then mix it with a little of the water into a slurry and again filter by suction through Tarantulle.

Recrystallise the washed salt twice from pure water in porcelain vessels, filtering the solutions through Tarantulle, and concentrating the filtrates by evaporation so that a yield of about 60 per cent. is obtained in each crystallisation.

Collect the final crystals on Tarantulle and dry them as completely as possible by suction. Reject the layer of crystals in contact with the cloth. Dry the remainder at 105° C. in an electrically heated air oven and, after cooling, mix well.

During the preparation care must be taken to exclude dust, and vessels of porcelain should be used throughout.

Before proceeding with the standardisation, verify the purity of the salt according to the directions given by Rosin.[2]

DETERMINATION OF MOISTURE IN SODIUM CHLORIDE—

Grind about 5 g. of the sodium chloride to fine powder in an agate mortar, transfer it to a tared platinum crucible fitted with a lid and weigh the whole accurately. Dry to constant weight in an electrically heated air oven at 270° ± 10° C., the lid of the crucible being removed during the drying. Note the loss of weight. Transfer the crucible with the lid in position to an electrically heated muffle furnace, gradually raise the temperature to 820° to 830° C., and maintain the furnace at a temperature not exceeding 830° C. for 10 minutes. Remove the crucible from the furnace, cool it in a desiccator and weigh it.

The fused salt should be free from colour, and the difference between the total percentage loss of weight and the percentage loss of weight at 270° C. should not exceed 0·1.

STANDARDISATION OF SODIUM CHLORIDE WITH THE ULTIMATE STANDARD SILVER—

Weigh accurately a button (9 to 10 g.) of the standard silver, which has previously been scrubbed with sand, boiled successively in dilute hydrochloric acid, dilute ammonium hydroxide and water, and finally dried. Place the button in a hard-glass flask and add 20 ml. of chloride-free 40 per cent. nitric acid; insert a funnel in the neck of the flask and warm the vessel on a water-bath to aid solution. Boil carefully to expel nitrous acid and add 500 ml. of pure water, washing the funnel inside and out with the water.

Grind 6 to 7 g. of the standard sodium chloride to a fine powder in an agate mortar and dry it to constant weight in an electrically-heated air oven at 270° ± 10° C. Weigh accurately a quantity of the dried salt sufficient to precipitate all but a milligram or so of the silver, and dissolve it in 500 ml. of specially pure water in a hard-glass flask. Add the silver nitrate solution slowly to the salt solution with gentle rotatory agitation and wash the last traces of the silver solution into the salt solution with several small portions of specially pure water. Stopper the flask, mix the contents thoroughly by gentle rotation and set aside in the dark overnight, or until the supernatant liquid is clear. Decant the clear liquid through a filter-paper previously washed free from chloride with specially pure water. Wash the silver chloride six times by decantation with 100 ml. of specially pure water, shaking vigorously each time, transfer it to the filter and wash it with specially pure water, filling the filter six times. Reject the precipitate. Evaporate the combined filtrate and washings to dryness in a porcelain basin over a water-bath in a dust-free atmosphere. To the residue add 5 ml. of 0·1 *M* nitric acid and 50 ml. of specially pure water, and filter through a small filter-paper previously washed with specially pure water, collecting the filtrate and washings in a hard-glass flask. Add 10 ml. of 0·1 *M* hydrochloric acid and set the mixture aside in the dark overnight or until the supernatant liquid is clear. Collect the precipitate of silver chloride in a prepared Gooch - Munroe crucible and wash it with 0·1 *M* hydrochloric acid. Finally, dry the crucible to constant weight at 130° C.

* Tarantulle is a high grade cotton cloth manufactured by Tootal Broadhurst Lee Co., Ltd. It is free from filling materials and has a very low ash content.

From the weight of silver chloride thus obtained calculate the corresponding weight of silver. Deduct this from the weight of silver taken and calculate from the difference the titre of the standard sodium chloride. Correct the various weighings for air displacement, by means of the values given in Table I (p. 587).

SECONDARY WORKING STANDARDS

Arsenious oxide

THE STATUS OF ARSENIOUS OXIDE AS A STANDARD CHEMICAL—

Arsenious acid was first used as a volumetric standard by Gay Lussac in chlorimetry. Penet improved the original method and later Mohr adapted it to iodimetry. The first critical study of the theory and practice of the method, however, was made by Washburn,[4] who clearly explained the part played by the sodium bicarbonate in the reaction, determined the upper and lower limits of the pH range permissible and showed that sodium phosphate or borax - boric acid could replace the sodium bicarbonate buffer with advantage for certain purposes. He also showed that, if a high degree of accuracy was to be attained, the pH value of the reaction mixture at the end of the titration must be about 7, *i.e.*, the solution must be neutral. His final conclusion was that "arsenious acid is the most convenient and accurate standard for iodimetry." Menzies and McCarthy[5] advocate the use of arsenious acid as an alkalimetric standard. For this purpose a known weight of arsenious oxide is oxidised with nitric acid, the excess of the latter is removed by evaporation and the residue of arsenic acid is titrated with the alkaline volumetric solution. Chapin[6] elaborated a laboratory method for the preparation of pure arsenious acid and methods for detecting likely impurities, *e.g.*, antimonious oxide. The same author[7] established the reliability of properly purified arsenious oxide as a standard and, with borax - boric acid as a buffer in a series of weight titrations, he obtained a mean figure for the iodine to arsenious acid ratio that differed from the theoretical by less than 1 part in 3600. Gooch[8] recommends the use of arsenious acid as an iodimetric and as an oxidimetric standard. For the latter a standard solution of arsenite is prepared and used for titrating the iodine liberated from an iodide by a known volume of permanganate solution in presence of acid. Thus, there is a large body of evidence to support the use of arsenious oxide as a standard in volumetric analysis.

SELECTION OF MATERIAL—

The standard arsenious oxide is selected from the purest available material of A.R. quality, the selection being based on the results of tests for purity applied according to the specification of the Committee on Analytical Reagents of the American Chemical Society.[9]

STANDARDISATION OF ARSENIOUS OXIDE WITH WORKING STANDARD IODINE—

Place 5 g. of pure potassium iodide and 0·5 ml. of water in a small glass-stoppered weighing bottle, allow the mixture to stand inside the balance case for half an hour and then weigh accurately. Add about 3 g. of the working standard iodine to the mixture, allow the whole to stand for half an hour in the balance case and weigh accurately. Rotate the bottle gently to ensure that all the iodine is dissolved. Weigh accurately a quantity of the arsenious oxide, previously dried to constant weight at 105° C., that would reduce 1 mg. more than the weight of iodine taken. Transfer the arsenious oxide to a 1-litre conical flask and dissolve it in 50 ml. of *N* sodium hydroxide, warming the flask to aid solution. When solution is complete, cool the liquid to room temperature, add 50 ml. of *N* hydrochloric acid to neutralise the alkali, dilute the solution to 650 ml. and add sufficient of a 5 per cent. solution of sodium bicarbonate to give 2 g. of the bicarbonate for every gram of iodine taken. Withdraw the stopper from the weighing bottle and gently drop both stopper and bottle into the flask. Shake the flask gently and, as soon as the whole of the iodine is reduced, add 5 ml. of starch solution and titrate the mixture at once with 0·01 *N* iodine until a pale purple-pink colour is obtained. Perform a blank titration with the 0·01 *N* iodine on a solution containing the following, mixed in the order given—

50 ml. of *N* sodium hydroxide
55 ml. of *N* hydrochloric acid
550 ml. of water
100 ml. of 5 per cent. sodium bicarbonate solution
9 g. of potassium iodide
5 ml. of starch solution.

Deduct the volume of 0·01 *N* iodine required to reproduce the end-point in the blank, usually about 0·2 ml., from the volume used in the titration of the arsenious oxide.

Correct the weights of arsenious oxide and iodine for air displacement and calculate the titre of the arsenious oxide.

Potassium dichromate

The status of potassium dichromate as a standard chemical—

The chief advantages of potassium dichromate as a standard chemical are the ease with which it can be obtained in a high state of purity and the permanence of its aqueous solution. Its use as a standard for oxidation processes was placed on a firm basis by Brühns.[10] For a discussion on the reliability of potassium dichromate as a primary standard, reference may be made to Kolthoff and Furman.[11] One of the difficulties of its use in the standardisation of solutions of sodium thiosulphate is the colour imparted by the chromium salt to the solution. Thus the advantage of the use of starch solution as indicator is somewhat impaired. These difficulties are overcome if the liberated iodine is distilled into a solution of potassium iodide in a stream of carbon dioxide. The titration with sodium thiosulphate may then be carried out on the distillate with the high degree of accuracy associated with this titration.

Preparation—

Recrystallise A.R. quality material from water until the product after drying at 150° C. complies with the specification of the Committee on Guaranteed Reagents of the American Chemical Society.[12]

Standardisation of potassium dichromate with working standard iodine—

The reference of potassium dichromate to iodine involves an accurate determination of two ratios, (1) potassium dichromate to 0·5 *N* sodium thiosulphate, and (2) 0·5 *N* sodium thiosulphate to working standard iodine.

Step 1. Determination of the ratio potassium dichromate to 0·5 *N* sodium thiosulphate—The following reagents and apparatus are required—

Sodium thiosulphate—0·5 *N* solution, prepared by dissolving 313 g. of the pure salt in 2½ litres of freshly boiled-out water. Allow the solution to stand for at least 14 days, then decant or siphon off the clear liquid into an amber glass bottle fitted with a siphon and soda-lime tube.

Boiled-out water—Oxygen free.

Potassium iodide—20 per cent. solution, freshly prepared from pure iodate-free potassium iodide of A.R. quality and boiled-out water.

Hydrochloric acid—Hydrochloric acid of density 1·1 at 20° C., chlorine-free, freshly prepared by boiling 50 g. of pure concentrated hydrochloric acid in a small open hard-glass flask until only 25 g. remain.

Starch solution.

Iodine solution—0·01 *N*, prepared as described above (p. 582).

A Gooch distillation apparatus (Fig. 4)—The tap and joints of this should be lubricated with syrupy phosphoric acid.

A Kipp's or other suitable *carbon dioxide generator.*

Procedure—Weigh accurately about 1 g. of the potassium dichromate, previously dried over phosphorus pentoxide, and dissolve it in 50 ml. of boiled-out water. Transfer the solution to flask A of the Gooch distillation apparatus (Fig. 4) through the tap funnel; wash the whole of the solution into the flask with several small portions of boiled-out water, using 20 ml. in all. Place 100 ml. of the potassium iodide solution in the receiver, B, and a few ml. in the trap, C. Loosely close the exit tube of the trap with a plug of glass wool, D, to prevent loss of spray. Dilute the solution in the receiver, B, with sufficient boiled-out water to trap gases entering from A. Remove air from the apparatus by passing a stream of carbon dioxide for 20 minutes. Add 15 ml. of the chlorine-free hydrochloric acid to the flask, followed by sufficient of the 20 per cent. solution of potassium iodide to give 3·75 g. of potassium iodide for each gram of potassium dichromate taken. Wash down the tap funnel with 20 ml. of oxygen-free water, running the washings into the flask and taking care to exclude air.

Distil the liberated iodine in a slow stream of carbon dioxide; continue the heating until the whole of the iodine has passed over into the receiver, which should be cooled by immersion in a constant-level water-bath through which cold water is circulated. At the end of the distillation allow the apparatus to cool, but maintain the stream of carbon dioxide; then disconnect the distilling flask. Transfer the potassium iodide solution from the trap to the receiver, wash the trap and glass wool plug with boiled-out water and add the washings to the receiver.

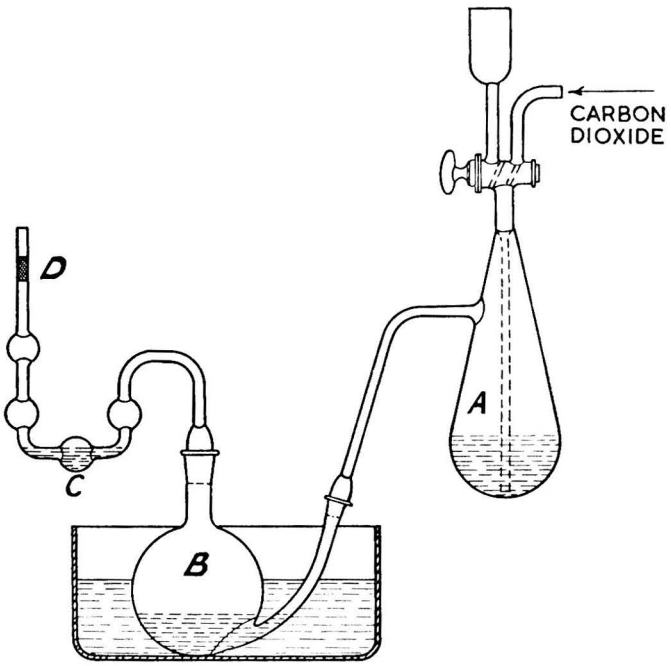


Fig. 4. Gooch distillation apparatus

Add from a weighing burette a slight excess of 0·5 *N* sodium thiosulphate. Transfer the decolorised solution to a 1-litre conical flask, wash the receiver with several small portions of boiled-out water and add the washings to the flask. Add 5 ml. of starch solution, dilute to 750 ml. with boiled-out water and titrate with 0·01 *N* iodine until a pale purple-pink colour is obtained.

Correct the volume of 0·01 *N* iodine used for that required to produce the end-point tint in 750 ml. of boiled-out water containing 14·6 g. of potassium iodide and 5 ml. of starch solution.

Calculate the weight of potassium dichromate equivalent to 100 g. of 0·5 *N* sodium thiosulphate.

STEP 2. DETERMINATION OF THE RATIO 0·5 *N* SODIUM THIOSULPHATE TO WORKING STANDARD IODINE—Place 5 g. of potassium iodide and 0·5 ml. of water in a small well-stoppered weighing bottle, allow the bottle and contents to remain inside the balance case for half an hour and then weigh it accurately. Add 2 to 3 g. of the working standard iodine, again place the vessel inside the balance case for half an hour and weigh it accurately. Rotate the bottle gently to ensure that all the iodine is dissolved. Withdraw the stopper and immediately drop the bottle and the stopper into 200 ml. of boiled-out water contained in a 1-litre conical flask. From a weighing burette add a quantity of the 0·5 *N* sodium thiosulphate slightly in excess of that required to reduce the whole of the iodine, and dilute the clear liquid to 750 ml. with boiled-out water. Add 5 ml. of starch solution and titrate the excess of sodium thiosulphate with 0·01 *N* iodine until a pale purple-pink colour is obtained.

Correct the volume of 0·01 *N* iodine used for that required to produce the end-point tint n 750 ml. of boiled-out water containing 5 ml. of starch solution and 9 g. of pure potassium

iodide. Calculate the weight of iodine equivalent to 100 g. of 0·5 *N* sodium thiosulphate and, from the result so obtained and that obtained in Step 1, calculate the titre of the potassium dichromate in terms of working standard iodine. In this calculation corrections for air displacement, shown in Table I, should be applied to the weighings of iodine and potassium dichromate, but they need not be applied to the weighings of the sodium thiosulphate solution.

TABLE I

CORRECTIONS FOR AIR DISPLACEMENT IN GRAMS PER GRAM OF SUBSTANCE WEIGHED

	g. per ml.
Density of air, assumed	= 0·0012
Density of brass, assumed ..	= 8·4
Density of platinum, assumed ..	= 21·55
Density of aluminium, assumed ..	= 2·67

Substance weighed	Assumed density	Brass weights	Platinum weights	Aluminium weights
Arsenious oxide	3·7	+0·00018	+0·00027	−0·00012
Iodine	4·93	+0·00010	+0·00019	−0·00021
Potassium dichromate	2·69	+0·00030	+0·00039	+0·00000
Silver	10·49	−0·00003	+0·00006	−0·00033
Sodium carbonate	2·533	+0·00033	+0·00043	+0·00002
Sodium chloride	2·161	+0·00041	+0·00050	+0·00010

Note—For ordinary sets of analytical weights calibrated on a "weight in air" basis, use the correction given in the column headed "Brass weights" for all the weights in the set. For special sets of weights calibrated on a "mass" basis, the corrections given in the columns headed "Platinum weights" and "Aluminium weights" must be used for the corresponding fractional weights.

STANDARD SOLUTIONS

A standard solution is one that contains an exactly known quantity of any reagent in unit volume. Sometimes a standard solution is designated by its percentage content, but more often in terms of its relation to a normal solution.

NORMAL SOLUTION—

A solution is stated to be normal when it is of such strength that 1 litre of it contains, in available form, 1 gram-equivalent of the essential component. The gram-equivalent is that quantity which can theoretically combine with, or replace, 8·000 g. of oxygen or 1·008 g. of hydrogen.

A solution which is weaker or stronger than normal is designated by the fraction, or multiple, of its normality; for instance, a solution of one-tenth normal strength is designated as 0·1 *N* and a solution twice normal strength as 2 *N*.

FACTOR OF A STANDARD SOLUTION—

Standard solutions are made as nearly the required normality as is conveniently possible, and the strength is accurately determined. The variation from the normality is expressed in the form of a factor by which the volume of the solution actually used must be multiplied in order to ascertain the equivalent volume of a solution of exact normality.

The following example shows how this factor is determined. Assuming that it has been found that 2·500 g. of pure sodium carbonate require 48·00 ml. of approximately normal hydrochloric acid for neutralisation, the factor will have to be such that when 48·00 ml. is multiplied by it, the result will be 47·17 ml., the exact quantity which would have been required had the hydrochloric acid been strictly normal. In other words, the factor would be in this case—

$$\frac{47{\cdot}17}{48{\cdot}00} = 0{\cdot}9827$$

This hydrochloric acid would be labelled thus—

N Hydrochloric acid, factor 0·9827.

In the same way an 0·1 *N* potassium permanganate solution having a factor of 1·023 would be labelled—

0·1 *N* Potassium permanganate, factor (acid solution) 1·023.

Storage and protection of solutions

Glass bottles for the storage of standard solutions should be thoroughly cleaned with chromic acid and then well washed with tap water and distilled water. Solutions must be protected from contamination, and on no account should an unused portion be returned from the burette to the stock bottle. The stock bottle should not be exposed to direct sunlight and the solution should be well mixed before portions are withdrawn. The stopper and neck of the bottle should be wiped with a clean cloth immediately after use.

Standard temperature

As the temperature of reference for graduated volumetric glassware is 20° C., standard volumetric solutions should be made up at this temperature. If work at this temperature is impossible, an allowance must be made in the final adjustment of the volume of the solution so that when the solution is used at 20° C. the normality is correct.

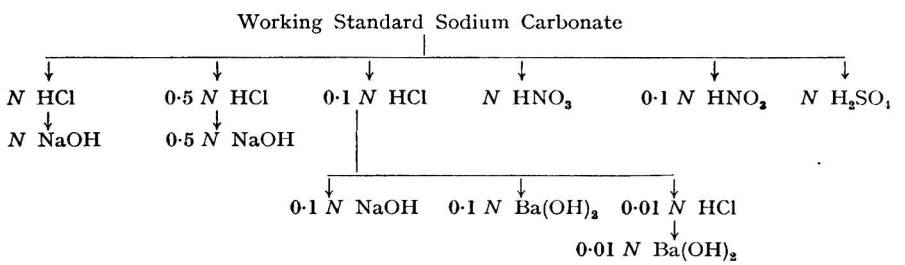
Table IV may be used for calculating the necessary corrections; for 0·1 *N* solutions not mentioned in the table the corrections for 0·1 *N* hydrochloric acid should be used.

STANDARDISATION OF SOLUTIONS

ACIDIMETRY AND ALKALIMETRY

The scheme for referring standard solutions of acids and alkalis to the working standard sodium carbonate is outlined below—

Outline of standardisation scheme for acids and alkalis



Standard acids

The preparation of standard acids—

Only pure concentrated acids of reagent quality should be used in the preparation of standard acids, and the dilution should be made with distilled water of good quality that has been well boiled to free it from carbon dioxide.

N acids from concentrated acids—Hydrochloric acid is recommended in preference to sulphuric acid because with certain indicators the end-point is more sharply defined. The approximate quantities of concentrated acids required for the preparation of various normal standard acids are given in Table II.

By means of a British Standard density hydrometer, determine the density at 20° C. of the concentrated acid to be used and calculate from the above data the quantity of acid required to produce the desired volume of normal solution.

Standard solutions of nitric acid should be protected from light; they are best stored in bottles of amber glass.

0·1 *N acids*—Prepare 0·1 *N* acids by dilution of accurately known amounts of *N* acids so that the calculated value of the factor may serve as a check upon the determined value. Large batches are best prepared by dilution on a weight basis, use being made of the expression

$$W = \frac{\frac{V \times F \times b}{a}}{1{\cdot}00283} - V$$

where—

W = weight of water in grams, weighed in air, to be added to V ml. of the given standardised solution
V = volume in ml. at 20° C. of the given standardised solution to be diluted
F = factor of N/a
a = value of a in the expression of the normality of the given standardised solution, as N/a
b = value of b in the expression of the normality of the required diluted solution, as N/b
1·00283 = volume in ml. at 20° C. of 1 g. of water weighed in air.

The following example will make the use of this formula quite clear. It is required to prepare 0·05 N hydrochloric acid from 2000 ml. of 0·5 N hydrochloric acid of factor 0·9965. The weight of water W to be added to the 2000 ml. of 0·5 N acid is as follows—

$$W = \frac{\frac{2000 \times 0{\cdot}9965 \times 20}{2} - 2000}{1{\cdot}00283}$$

$$= 17879{\cdot}4 \text{ g.}$$

0·01 N *acids*—Prepare 0·01 N acids by dilution from accurately known amounts of 0·1 N acids. In this case the purity of the distilled water is of particular importance and it is essential that ammonia and carbon dioxide should be absent.

TABLE II

DATA FOR USE IN THE PREPARATION OF NORMAL SOLUTIONS OF ACIDS

Acid	Density of acid at 20° C., g. per ml. (D)	Concentration of acid corresponding to D, %	Quantity of acid required for dilution to 1 litre of approximately normal strength	
			g.	ml.
HCl	1·140	28·2	129·5	113·6
	1·145	29·1	125·1	109·3
	1·150	30·1	121·0	105·2
	1·155	31·1	117·1	101·4
	1·160	32·1	113·5	97·8
	1·165	33·2	110·0	94·4
	1·170	34·2	106·7	91·2
	1·175	35·2	103·6	88·1
	1·180	36·2	100·7	85·3
HNO_3	1·405	68·1	92·6	65·9
	1·410	69·1	91·2	64·7
	1·415	70·4	89·5	63·3
	1·420	71·6	88·1	62·0
	1·425	72·8	86·6	60·8
H_2SO_4	1·395	50·0	98·0	70·3
	1·520	62·0	79·0	52·0
	1·834	95·1	51·5	28·09
	1·834	99·1	49·4	26·96
	1·835	95·6	51·3	27·93
	1·835	98·7	49·6	27·05
	1·836	96·4	50·8	27·68
	1·836	98·1	50·0	27·21

STANDARDISATION OF NORMAL AND DECINORMAL ACIDS—

Several methods are available for the standardisation of acids with sodium carbonate, but the conversion of the sesquicarbonate into anhydrous sodium carbonate is the first step and is common to all methods (see p. 579).

Transfer the crucible containing the anhydrous carbonate while still hot to a desiccator containing a stoppered weighing bottle of suitable size. When it is cold, place the crucible in the bottle and transfer the latter to the balance case and weigh it. In order to obtain equilibrium between the air in the balance case and the surface of the bottle, half an hour should elapse before weighing.

Solution of the carbonate—Place the crucible well inside the neck of a dry conical flask of hard glass and empty the carbonate into the flask, care being taken to avoid loss of fine particles of the carbonate dust. Wear finger-stalls of thin rubber whilst handling the crucible. Immediately return the empty crucible to the weighing bottle and weigh it after an interval of 15 minutes in the balance case.

Add about 50 ml. of water to the flask, taking care to avoid loss of fine particles of the carbonate, and promote solution by swirling the liquid gently.

The titration—The choice of weight or volume titration depends chiefly upon the precision sought. Where a high degree of precision is desired, it is imperative to employ weight titration. The density of the acid must then be known and it is necessary to correct the weighings for air displacement. The error of the weight titration method for the standardisation of *N* hydrochloric acid in experienced hands may be as low as ± 3 in 10,000 for a single titration. Under the best conditions and with a 50-ml. burette the error of the volumetric titration method is at least three times as great as this, *i.e.*, the error of a single titration is of the order of 1 in 1000. This error is unavoidable and is due to uncertainty in the calibration and reading of the burette. It may be reduced considerably, however, by using the 105-ml. bulb burette referred to below in place of the 50-ml. burette. If an accuracy of not less than 99·9 per cent. is required in the determination of alkalis by volumetric titration with *N* acid, the acid must be standardised by weight titration, or by titration from the 105-ml. bulb burette. If a lower degree of accuracy suffices, the standardisation may be carried out volumetrically with a 50-ml. burette.

Care must be taken to avoid loss of sodium carbonate due to spraying, *e.g.*, by placing a funnel in the neck of the titration flask; if a funnel is used, it will be necessary to rinse it inside and out with water before completing the titration.

Only burettes that have been carefully calibrated should be used in standardisation work, and titrations should be performed at or near the temperature of reference, *i.e.*, 20° C.

Greater accuracy is obtained in the volumetric titration by the employment of a 105-ml. bulb burette, the stem of which is calibrated from the 80-ml. to the 105-ml. mark in ml. and twentieths. These burettes must be fitted with jets such as would be fitted to the corresponding 25-ml. burettes if drainage errors are to be avoided. See the British Standard Specification for bulb burettes.[13]

Choice of conditions—The conditions of standardisation, including the choice of an indicator, are almost invariably governed by the rule that the method of use of a volumetric solution should be comparable in all respects with the method of standardisation.

PROCEDURE—For the standardisation of *N*, 0·5 *N* and 0·1 *N* acids the quantities of sodium sesquicarbonate shown in Table III are recommended. Weigh the quantity of working

TABLE III

WEIGHT OF SODIUM SESQUICARBONATE FOR USE IN THE STANDARDISATION OF *N*, 0·5 *N* AND 0·1 *N* ACID

	Sodium sesquicarbonate required for	
	Weight titration using a 60-g. Ripper's burette, g.	Volumetric titration using a 105-ml. bulb burette graduated from 80 ml. to 104 ml. in 0·05 ml., g.
1·0 *N*	5·0 (= 3·4 g. of Na_2CO_3)	7·1 (= 4·8 g. of Na_2CO_3)
0·5 *N*	2·5 (= 1·7 ")	3·6 (= 2·4 ")
0·1 *N*	0·50 (= 0·34 ")	0·71 (= 0·48 ")

standard indicated in the table into a silver or platinum crucible and heat at 270° ± 10° C. in an electrically heated air oven until it is constant in weight, stirring the sodium carbonate occasionally with a stout silver or platinum wire. Transfer the crucible to a desiccator containing a stoppered weighing bottle of suitable size and, when cold, place the crucible in the bottle. Transfer the bottle to the balance case and weigh accurately after an interval of half an hour. Empty the contents of the crucible into a dry 500-ml. conical flask of hard glass, placing the crucible well inside the neck of the flask in order to avoid loss of material. Immediately replace the crucible in the bottle, transfer the whole to the balance case and weigh after half an hour.

Dissolve the sodium carbonate in 50 ml. of water that has previously been neutralised to pH 3·9,* washing down any carbonate adhering to the neck and side of the flask, and add 4 to 5 drops of bromophenol blue solution.

Weight titration—Put into a 60-g. weighing burette a quantity of the standard acid slightly greater than that required to neutralise the sodium carbonate, and weigh accurately. Titrate the solution until a greenish-blue colour is obtained, or until the colour of the solution matches that of a standard buffer solution of pH 3·9 containing the same relative amount of the indicator. At the end of the titration again weigh the burette.

Determine the density of the acid solution in grams per ml. at 20° C. by means of a Regnault pyknometer.

From the weight of acid required in the titration calculate the normality factor, x, of the acid at 20° C. by means of the expression—

$$x = \frac{A \times \frac{B}{100} \times C}{D \times E \times F}$$

where

A = weight in grams of sodium carbonate taken
B = percentage purity of the sodium carbonate
C = density of the hydrochloric acid in grams per ml. at 20° C.,
i.e., C = 1·0165 for N HCl
= 1·0072 for 0·5 N HCl
= 1·0004 for 0·1 N HCl
D = weight in grams of hydrochloric acid used in the titration
E = grams of Na_2CO_3 equivalent to 1 ml. of solution
i.e., E = 0·053 for N solution
= 0·0265 for 0·5 N solution
= 0·0053 for 0·1 N solution and so on
F = correction factor for air displacement of the hydrochloric acid, *i.e.*, 1·00105.

Volumetric titration—Fill a standardised 105-ml. bulb burette (see p. 590) with the acid, set the meniscus accurately on the zero of the scale and titrate the sodium carbonate solution until the colour changes to greenish-blue, or until it matches a standard buffer solution of pH 3·9 containing the same relative amount of indicator. At the end of the titration read the burette. Note the temperature of the room and, if it differs from the reference temperature (20° C.), correct the volume of standard acid used accordingly with the aid of Table IV.

From the corrected burette reading and the weight of pure sodium carbonate taken, calculate the strength of the acid in grams per ml. and thence express the result as a factor of N, 0·5 N or 0·1 N as appropriate.

STANDARDISATION OF 0·01 N HYDROCHLORIC ACID—

The standardisation of 0·01 N hydrochloric acid is only carried out as a check on the calculated factor. Measure accurately from a 5- or 10-ml. burette 4·50 ml. of standard 0·1 N alkali into a 100-ml. hard-glass flask. Add 4 drops of phenolphthalein solution and titrate rapidly with the 0·01 N acid from a 50-ml. burette until the pink colour just disappears. Calculate the factor of the acid by use of the phenolphthalein factor of the standard 0·1 N alkali. The factor so obtained should agree with the calculated factor.

STANDARD ALKALIS

PREPARATION OF STANDARD ALKALI—

N sodium hydroxide—Standard solutions of sodium hydroxide are best prepared from a solution having a density of 1·525 g. per ml. at 20° C. and containing 50 per cent. of sodium hydroxide. The solubility of sodium carbonate in this solution is almost negligible. Solutions of this strength can be filtered through ordinary stout filter-paper and yield a clear filtrate. If the 50 per cent. solution is not available, dissolve 106 parts by weight of commercially

* For purposes where the highest accuracy is required, it is necessary to use for both the determination and the standardisation water that has previously been brought to the mid-point of the pH range of the indicator employed.

pure powdered sodium hydroxide in 100 parts by weight of water in an enamelled-iron, nickel, silver or stainless steel pan. Allow a stock solution prepared in this way to stand for a few days, and filter it through paper into hard-glass bottles. About 53 ml. of this solution are required for a litre of *N* sodium hydroxide.

Use boiled-out water for the dilution to normal strength, and set the resulting solution aside for several days to allow traces of iron and manganese to settle as a precipitate of hydroxides to the bottom of the bottle. Siphon off the clear solution into storage bottles fitted with rubber bungs of good quality.

0·1 *N sodium hydroxide*—Prepare 0·1 *N* sodium hydroxide by diluting the *N* solution with boiled-out water.

0·1 *N and* 0·01 *N barium hydroxide*—For most purposes 0·1 *N* and 0·01 *N* barium hydroxide are preferable to the corresponding solutions of sodium hydroxide.

Prepare standard solutions of barium hydroxide from a stock solution saturated with pure crystalline barium hydroxide. Store the stock solution undisturbed for several days and siphon off the clear liquid as required. The saturated solution has an approximate strength of 0·4 *N*.

Prepare the standard solutions with boiled-out water in an atmosphere as free as possible from carbon dioxide, preferably in the open air, and allow them to stand several days to deposit any traces of barium carbonate.

Fit soda-lime guard tubes to all containers from which standard solutions of alkalis are to be siphoned.

STANDARDISATION OF ALKALIS—

N and 0·5 *N sodium hydroxide*—Standardise *N* and 0·5 *N* sodium hydroxide by titrating with an accurately measured volume, about 90 ml., of the standard acid of corresponding normality, using a 105-ml. bulb burette, graduated from 80 to 105 ml. in 0·05 ml., and bromophenol blue as indicator. The acid and alkali must be at the same temperature. A standard buffer solution of pH 3·9 containing the same relative amount of indicator may be used to facilitate the determination of the end-point. When the phenolphthalein factor of the standard alkali is required it is necessary to remove carbon dioxide from the standard acid. For this take an accurately measured volume of the standard acid, dilute it to 200 ml. with water, boil for 5 minutes to expel carbon dioxide, cool the solution out of contact with atmospheric carbon dioxide and titrate with the alkali, with phenolphthalein as indicator.

0·1 *N sodium hydroxide*—Standardise 0·1 *N* sodium hydroxide by titration of accurately measured volumes of about 90 ml. of standard 0·1 *N* acid, using as indicators bromophenol blue, methyl red or phenolphthalein.

When more than one indicator is used, factors are given for each indicator. It must be borne in mind that carbon dioxide begins appreciably to affect indicators that give an end-point above pH 4·0 and consequently carbon dioxide must be removed from the standard acid where an accurate methyl red or phenolphthalein factor is required.

0·1 *N and* 0·01 *N barium hydroxide*—Standardise 0·1 *N* and 0·01 *N* barium hydroxide by titrating accurately measured volumes, about 90 ml., of the corresponding standard hydrochloric acid solutions which have been freed from carbon dioxide by boiling after dilution with water. Use phenolphthalein as indicator. Re-standardise 0·1 *N* and 0·01 *N* alkalis at comparatively frequent intervals if reliance is to be placed on results obtained with them.

CORRECTIONS OF VOLUME OF STANDARD SOLUTIONS OF ACIDS AND ALKALIS FOR A REFERENCE TEMPERATURE OF 20° C.—

Where the measurement of volumetric solutions of acids of alkalis at temperatures other than the normal reference temperature (20° C.) is unavoidable, adjust the observed volume at temperature *t*° C. by means of the corrections given in Table IV.

OXIDIMETRY

The relationship between the solutions used in oxidimetry is shown in the scheme in Fig. 1 (p. 577).

SODIUM THIOSULPHATE

GENERAL REMARKS—

Several methods are available for the standardisation of sodium thiosulphate solutions, but a direct titration with standard iodine (method 1) is most frequently used. When the

solution is used for the determination of copper, standardisation with pure copper (method 2) is to be preferred. Other methods that may be employed in special circumstances involve (3) a direct reference to secondary standard potassium dichromate by titration, the dichromate being used for the liberation of iodine from potassium iodide, (4) titration of the iodine liberated from an acid solution of potassium permanganate to which potassium iodide has been added,

TABLE IV

CORRECTIONS IN ML. TO BE APPLIED TO REDUCE 1000 ML. MEASURED AT $t°$ C. TO VOLUME AT 20° C. (SCHLOESSER[14] AND SCHOORL[15])

	Correction in ml.					
	Hydrochloric acid			Sodium hydroxide		Nitric acid, sulphuric acid and sodium hydroxide
Temperature, t °C.	0·1 *N*	0·5 *N*	*N*	0·1 *N*	0·5 *N*	*N*
12	1·1	1·4	1·6	1·3	1·7	2·0
13	1·0	1·2	1·4	1·1	1·5	1·8
14	0·9	1·1	1·2	1·0	1·3	1·6
15	0·8	0·9	1·0	0·9	1·1	1·3
16	0·6	0·8	0·8	0·7	0·9	1·1
17	0·5	0·6	0·6	0·6	0·7	0·8
18	0·3	0·4	0·4	0·4	0·5	0·6
19	0·2	0·2	0·2	0·2	0·2	0·3
20	0·0	0·0	0·0	0·0	0·0	0·0
21	−0·2	−0·2	−0·2	−0·2	−0·2	−0·3
22	−0·4	−0·4	−0·5	−0·4	−0·5	−0·6
23	−0·6	−0·7	−0·7	−0·6	−0·8	−0·9
24	−0·8	−0·9	−1·0	−0·9	−1·0	−1·2
25	−1·0	−1·1	−1·2	−1·1	−1·3	−1·5
26	−1·3	−1·4	−1·4	−1·4	−1·5	−1·8
27	−1·5	−1·7	−1·7	−1·7	−1·8	−2·1
28	−1·8	−2·0	−2·0	−2·0	−2·1	−2·4
29	−2·1	−2·3	−2·3	−2·3	−2·4	−2·8

the potassium permanganate solution having been referred to secondary standard arsenious oxide, or (5) titration of a solution of iodine which has been referred to secondary standard arsenious oxide. The stoicheiometric relations involved in the above reactions may be expressed as follows—

(1) $2Na_2S_2O_3 + I_2 = 2NaI + Na_2S_4O_6$
(2) $2Cu(NO_3)_2 + 4KI = I_2 + 4KNO_3 + Cu_2I_2$
(3) $K_2Cr_2O_7 + 6KI + 14HCl = 3I_2 + 8KCl + 2CrCl_3 + 7H_2O$
(4) $2KMnO_4 + 10KI + 16HCl = 5I_2 + 12KCl + 2MnCl_2 + 8H_2O$
(5) $2I_2 + As_2O_3 + 2H_2O = As_2O_5 + 4HI$.

When a solution of thiosulphate is allowed to stand and the titre is examined at intervals, it is sometimes observed that there is an initial rise in the value, followed by a gradual fall with the simultaneous deposition of sulphur. This increase in titre is attributed to the conversion of polythionate, present as an impurity, into thiosulphate—

$$4S_4O_6'' + 6OH' = 5S_2O_3'' + 2S_3O_6'' + 3H_2O.$$

The subsequent decrease in the titre is ascribed to such influences as (*a*) light and (*b*) micro-organisms, the influence of which may be reduced to some extent by the use of salt of high purity, by storing the solution in dark-coloured glass bottles and by the preparation of the solution with water free from carbon dioxide.

The effect of micro-organisms, particularly sulphur bacteria, can be further reduced by taking care to boil thoroughly in water any rubber bungs and rubber tubing that are to be used with solutions of sodium thiosulphate.

Sodium thiosulphate solution does, however, deteriorate slowly, and it is advisable to re-standardise at weekly intervals any solutions that are intermittently exposed to the air. If the solution is protected from carbon dioxide, less frequent checking is necessary.

PREPARATION OF 0·1 *N* SODIUM THIOSULPHATE—

Weigh 25 g. of crystallised sodium thiosulphate for each litre of 0·1 *N* solution required. Dissolve the salt in water free from carbon dioxide and dilute to the required volume. Allow the solution to stand in the dark for at least a fortnight and, at the end of this period, siphon off the supernatant liquid without disturbing the deposit of sulphur. Determine the strength of the solution by titration with 0·1 *N* iodine and, if necessary, add sufficient water to give a solution which is as nearly as possible 0·1 *N*. Shake the solution well and proceed with the standardisation as follows.

STANDARDISATION OF 0·1 *N* SODIUM THIOSULPHATE—

Place approximately 5 g. of pure potassium iodide, free from iodate, and 0·5 ml. of water in a small weighing bottle. Insert the stopper in the weighing bottle, place the whole in a balance case for half an hour and weigh. Remove the stopper from the bottle and with the least possible delay add 1·1 to 1·2 g. of working standard iodine. Replace the stopper and weigh the whole again. Rotate the bottle gently to ensure that all the iodine is dissolved. Place in a 500-ml. conical flask 100 ml. of boiled-out water and add 85 ml. of the 0·1 *N* sodium thiosulphate from a 105-ml. bulb burette. Hold the flask in an inclined position, loosen the stopper of the bottle containing the iodine and allow the bottle and stopper to drop into the liquid. Promote solution of the iodine by swirling the liquid gently and then add more of the thiosulphate solution until the liquid in the flask is pale yellow. Add 2 to 3 ml. of starch solution and continue the titration slowly, shaking well after each addition of reagent, until the blue colour has just disappeared.

0·01 *N* SODIUM THIOSULPHATE—

Prepare the solution by accurate dilution of 0·1 *N* sodium thiosulphate with water free from carbon dioxide, and calculate the factor. Check this factor by titration, as follows.

Introduce 1 g. of potassium iodide into a 200-ml. conical flask. Add from a 5- or 10-ml. burette 4·50 ml. of standard 0·1 *N* iodine accurately measured; titrate the solution immediately with 0·01 *N* sodium thiosulphate, delivered from a 50-ml. burette, until the colour of the iodine has nearly disappeared. Add 2 ml. of starch solution and continue the titration, shaking the liquid vigorously after each addition, until the blue colour is just discharged. The factor so obtained should agree with the calculated factor.

IODINE

GENERAL REMARKS—

Standard solutions of iodine deteriorate slowly owing to volatilisation of iodine, and in order to reduce this effect to a minimum it is necessary to have a concentration of at least 2 per cent. of potassium iodide in the diluted solution. Solutions of iodine are affected (*a*) by light, so they should therefore be stored in dark-coloured glass bottles, and (*b*) by organic matter, hence contact with cork or rubber in any form should be avoided.

Iodine solution is standardised with standard arsenious oxide. It is necessary in the titration of arsenious acid with iodine to keep the solution approximately neutral to phenolphthalein, and this is accomplished by the addition of sodium bicarbonate. The stoicheiometric relation may be expressed as follows—

$$As_2O_3 + 2I_2 + 8NaHCO_3 = 2Na_2HAsO_4 + 4NaI + 3H_2O + 8CO_2.$$

PREPARATION OF 0·1 *N* IODINE—

Weigh roughly 12·7 g. of re-sublimed iodine, mix it with 26 g. of potassium iodide that is free from iodate, add 30 ml. of water and allow the whole to stand until solution of the iodine is complete. Finally dilute to 1 litre with water and mix well.

STANDARDISATION OF 0·1 *N* IODINE—

Weigh accurately 0·42 to 0·45 g. of secondary working standard arsenious oxide, previously dried to constant weight at 105° C.; transfer it to a 500-ml. conical flask, add 20 ml. of *N* sodium hydroxide and warm the flask to aid solution. Cool to room temperature, add 15 ml. of *N* hydrochloric acid to neutralise the excess of alkali, dilute to 200 ml. and add 20 ml. of a saturated solution of sodium bicarbonate. Titrate the solution with iodine solution, which

should be added from a 105-ml. bulb burette; towards the end of the titration add 2 to 3 ml. of starch solution as indicator, and continue to add the iodine until a pale purple-pink colour is obtained throughout the solution.

0·01 *N* IODINE—

Prepare the solution by accurate dilution of 0·1 *N* iodine and calculate the factor. This factor may be checked by means of 0·1 *N* sodium thiosulphate as follows.

Place 1 g. of potassium iodide that is free from iodate, 50 ml. of water and 2 ml. of starch solution in a 250-ml. conical flask, and add 4·50 ml. of standard 0·1 *N* sodium thiosulphate accurately measured from a 5- or 10-ml. burette. Titrate the solution with 0·01 *N* iodine, added from a 50-ml. burette, until the liquid becomes pale blue. The factor found in this way should agree with the calculated factor.

SODIUM ARSENITE

PREPARATION OF 0·1 *N* SODIUM ARSENITE—

Weigh 4·948 g. of sublimed arsenious oxide, transfer it to a 500-ml. beaker, add 200 ml. of *N* sodium hydroxide and warm until the solid is completely dissolved. Then add 100 ml. of *N* hydrochloric acid to neutralise the excess of alkali, cool the solution to room temperature, transfer it to a 1-litre graduated flask, dilute to the mark and shake well.

STANDARDISATION OF 0·1 *N* SODIUM ARSENITE—

Measure 85 ml. of the solution from a bulb burette into a conical flask containing 2 g. of iodate-free potassium iodide, 100 ml. of water and 20 ml. of a saturated solution of sodium bicarbonate. Add 2 to 3 ml. of starch solution as indicator and titrate with standard 0·1 *N* iodine until a pale purple-pink colour is obtained throughout the solution.

POTASSIUM PERMANGANATE

GENERAL REMARKS—

Potassium permanganate may serve as an oxidising agent either in acid or alkaline solution as indicated by the following equations—

$$\text{(Acid medium)}\quad 2KMnO_4 = K_2O + 2MnO + 5O$$

$$\text{(Alkaline medium)}\quad 2KMnO_4 = K_2O + 2MnO_2 + 3O$$

Oxidation in acid medium in which the permanganate is reduced to the manganous state is nearly always employed in analysis and is, therefore, used throughout the methods of standardisation.

Potassium permanganate solutions are conveniently standardised by means of secondary standard arsenious oxide according to the following equation—

$$4KMnO_4 + 5As_2O_3 + 6H_2SO_4 = 2K_2SO_4 + 4MnSO_4 + 5As_2O_5 + 6H_2O.$$

An alternative method that is given is based on the liberation of the equivalent of iodine in an acid solution of potassium iodide, followed by titration with standard sodium thiosulphate solution. According to MacBride[16] and Popoff and Whitman,[17] the order of mixing the reagents is a factor which should be carefully observed, since it has been shown that oxygen is lost when permanganate is allowed to accumulate in an acid solution. For this reason, the conditions recommended in such titrations are as follows.

The potassium permanganate solution is added to an acidified solution of potassium iodide, the liberated iodine being subsequently titrated with thiosulphate solution.

Potassium permanganate solutions are very susceptible to the influence of impurities such as manganese dioxide, which results in progressive decomposition of the solution, and organic matter. In view of this, the following precautions should be taken: (*a*) the solution should be filtered through a plug of glass wool or purified asbestos or a No. 4 sintered glass filter, (*b*) dark-coloured (amber) glass bottles should be used for storage and (*c*) the solution should be allowed to mature for two or three weeks before standardisation.

PREPARATION OF 0·2 *N* POTASSIUM PERMANGANATE—

Weigh 6·4 g. of recrystallised potassium permanganate for each litre of 0·2 *N* solution required, transfer the salt to a large porcelain mortar, add sufficient water to cover it and

triturate the mixture with a pestle. Half fill the mortar with water, stir well, allow the undissolved permanganate to settle and filter the solution through a plug of glass wool or a No. 4 sintered glass filter into the stock bottle. Continue to triturate the residue in the mortar, diluting with water and decanting the supernatant liquid as before until solution is complete. Dilute the bulk solution to the correct volume, shake it well and allow it to stand in the dark for a fortnight. At the end of this period, siphon off the solution without disturbing the sediment formed by the slight decomposition of the permanganate. Determine the titre as described below and, if necessary, add sufficient water to give a solution that is as nearly as possible one-fifth normal. Shake the solution well and proceed with the standardisation.

STANDARDISATION OF 0·2 *N* POTASSIUM PERMANGANATE WITH SECONDARY WORKING STANDARD ARSENIOUS OXIDE[18]—

Accurately weigh 0·46 to 0·48 g. of arsenious oxide that has recently been dried at 105° C. and transfer it to a 600-ml. beaker. Add 20 ml. of a cold 20 per cent. solution of sodium hydroxide that is free from oxidising or reducing substances.

Allow the whole to stand for 8 to 10 minutes with occasional stirring. When all the arsenious oxide is dissolved, add 200 ml. of water, 20 ml. of 10 *M* hydrochloric acid, and 1 drop of 0·0025 *M* potassium iodate or potassium iodide solution.

Titrate with the permanganate solution until a faint pink colour persists for 30 seconds. Add the last 1·0 to 1·5 ml. dropwise, allowing each drop to become decolorised before the next is introduced.

Determine the volume of permanganate required to duplicate the faint pink colour of the end-point. This is done by adding permanganate to a solution containing the same amounts of alkali, acid and catalyst as were used in the test. The correction should amount to less than 0·03 ml.

Calculate the factor from the relation—

$$1{\cdot}000 \text{ g. of } As_2O_3 \equiv 101{\cdot}10 \text{ ml. of } 0{\cdot}2\ N\ KMnO_4.$$

PREPARATION OF 0·1 *N* POTASSIUM PERMANGANATE—

Weigh 3·2 g. of recrystallised potassium permanganate for each litre of 0·1 *N* solution required and proceed as above under the heading of 0·2 *N* potassium permanganate.

STANDARDISATION OF 0·1 *N* POTASSIUM PERMANGANATE—

(*a*) *With secondary working standard arsenious oxide*[18]—Accurately weigh 0·23 to 0·24 g. of arsenious oxide that has recently been dried at 105° C. and transfer it to a 400-ml. beaker. Add 10 ml. of a cold 20 per cent. solution of sodium hydroxide that is free from oxidising or reducing substances. Allow the whole to stand for 8 to 10 minutes, with occasional stirring. When all the arsenious oxide is dissolved, add 100 ml. of water, 10 ml. of 10 *N* hydrochloric acid and 1 drop of 0·0025 *M* potassium iodate or potassium iodide solution.

Titrate with the permanganate solution until a faint pink colour persists for 30 seconds. Add the last 1·0 to 1·5 ml. dropwise, allowing each drop to become decolorised before the next is introduced.

Determine the volume of permanganate required to duplicate the faint pink colour of the end-point. This is done by adding permanganate to a solution containing the same amounts of alkali, acid and catalyst as were used in the test. The correction should amount to less than 0·03 ml.

Calculate the factor from the relation—

$$1{\cdot}000 \text{ g. of } As_2O_3 \equiv 202{\cdot}20 \text{ ml. of } 0{\cdot}1\ N\ KMnO_4.$$

(*b*) *With* 0·1 *N sodium thiosulphate*—Place 100 ml. of boiled-out water in a 500-ml. glass-stoppered flask and add 15 ml. of 10 per cent. hydrochloric acid and 3 g. of potassium iodide. Run into the flask an accurately measured volume of about 85 ml. of potassium permanganate solution from a 105-ml. bulb burette, and then titrate the liberated iodine with standard 0·1 *N* sodium thiosulphate (recently standardised with working standard iodine) until a pale yellow colour is obtained. Add 2 to 3 ml. of starch solution and continue the titration, shaking vigorously after each addition, until the blue colour has just disappeared.

0·1 *N* CERIC SULPHATE

GENERAL REMARKS—

Ceric sulphate[19,20] behaves as an oxidising agent in acid solution as indicated by the following equation—

$$2Ce(SO_4)_2 + H_2O = Ce_2(SO_4)_3 + H_2SO_4 + O.$$

Solutions of ceric sulphate possess certain characteristics. They are quite stable, they have a high oxidation potential, and many of the reactions in which they are employed take place instantaneously and in the cold. For these reasons standard ceric sulphate can be employed with advantage in place of standard potassium permanganate in most, if not all, determinations where the latter is customarily used. The colour change corresponding to the reduction of the ceric to the cerous state is not very marked, but this difficulty is readily overcome by the use of an appropriate oxidation - reduction indicator; *o*-phenanthroline ferrous sulphate ("Ferroin") is generally the most valuable indicator for the purpose. N-phenyl anthranilic acid is also useful when ceric sulphate solutions are titrated with ferrous salts.

Solutions of other ceric salts, in particular ceric ammonium nitrate, are useful for certain determinations. Their preparation is described in the literature,[19,20] to which reference should be made; they are standardised against arsenious oxide in the presence of iodine monochloride or in the presence of osmic acid as described below.

The stoicheiometric relationship involved in the standardisation of ceric sulphate by means of arsenious oxide is expressed as follows—

$$As_2O_3 + 4Ce(SO_4)_2 + 2H_2O = As_2O_5 + 2Ce_2(SO_4)_3 + 2H_2SO_4.$$

PREPARATION OF SOLUTION—

Add 600 ml. of concentrated sulphuric acid slowly to 600 ml. of water contained in a 4-litre beaker and stirred with a large mechanical stirrer; immerse a thermometer in the solution.

When the temperature of the dilute acid falls to 100° C., add 420 g. of technical quality ceric oxide in quantities of a few grams at a time, and at such a rate that the temperature remains between 95° and 105° C.; the reaction is exothermic.

When all the ceric oxide has been added, allow the temperature to fall to about 90° C., add about 2 litres of water and stir the solution for a further 15 to 20 minutes; finally divide it into two equal portions, dilute each to about 4 litres, stir each for about 15 minutes and allow the insoluble matter to settle.

Prepare a 12·5-cm. Büchner funnel as follows. Place a Whatman No. 541 filter-paper in the funnel, fit the funnel to a large filter flask and add sufficient good quality diatomaceous earth in the form of a slurry to produce a thin layer on the surface of the paper and act as a "filter-aid." Suck the excess water from the funnel and wash the layer with water until the filtrate is free from fine particles. Discard the washings and rinse the flask thoroughly.

Filter the ceric sulphate solution through the prepared paper, transfer the insoluble matter to the filter and wash it several times with water. Finally transfer the filtrate to a graduated 10-litre aspirator and mix the solution well.

STANDARDISATION—

Dry a sufficient amount of arsenious oxide at 105° C. and cool it in a desiccator. Weigh out 200 to 240 mg. with an accuracy of 0·1 mg. and transfer it to a 250-ml. beaker. Add approximately 1 g. of A.R. grade sodium hydroxide and 10 ml. of water. Warm the mixture slightly and stir it until the arsenious oxide has dissolved completely; then add 30 ml. of water and 10 ml. of 9 *M* sulphuric acid, stir the liquid and add a further 50 ml. of water. Add 3 drops of a 0·01 *M* solution of osmium tetroxide in 0·1 *N* sulphuric acid and 1 drop of Ferroin indicator, and add the ceric sulphate from a 50-ml. bulb burette until the colour changes from red to colourless.

Calculate the normality of the ceric sulphate solution and, if necessary, adjust it by dilution with 2 *N* sulphuric acid; re-standardise it as above.

POTASSIUM DICHROMATE

GENERAL REMARKS—

The chief use of 0·1 *N* potassium dichromate is in the determination of ferrous iron. The reaction, which takes place readily at ordinary temperature in presence of either sulphuric or hydrochloric acid, is expressed as follows—

$$6FeSO_4 + K_2Cr_2O_7 + 7H_2SO_4 = 3Fe_2(SO_4)_3 + Cr_2(SO_4)_3 + K_2SO_4 + 7H_2O.$$

The salt is easily obtained in a state of high purity by recrystallisation from water, and material of A.R. quality that has been ground to powder and dried to constant weight at 150° C. is usually suitable for making the standard solution; the criterion of suitability is the comparison with the secondary working standard potassium dichromate by the methods given on p. 585 for the standardisation of the latter.

The solution, which is quite stable, is best made from an accurately weighed amount of the purified salt diluted to an exact volume, because the calculated concentration is more reliable than the figure obtained by standardisation. Should the concentration be in doubt, however, it may be verified by titration of a solution of ferrous sulphate the strength of which has recently been determined by reference to working standard potassium dichromate.

Diphenylamine is a satisfactory internal indicator for use with potassium dichromate and has almost completely superseded potassium ferricyanide, which can only be used externally. It should be noted that diphenylamine may be used when the iron has been reduced to the ferrous state by means of stannous chloride and the excess of the latter removed by addition of mercuric chloride. Knop[21] has shown that if phosphoric acid is added the titration is reversible.

PREPARATION OF 0·1 *N* POTASSIUM DICHROMATE—

Weigh accurately 4·9035 g. of pure potassium dichromate, previously dried to constant weight at 150° C., for each litre of solution required, dissolve the salt in water, dilute the solution to the correct volume and mix it well.

VERIFICATION OF CONCENTRATION OF 0·1 *N* POTASSIUM DICHROMATE—

The method which follows is to be used where it is necessary to verify the factor of 0·1 *N* potassium dichromate.

Measure accurately about 85 ml. of 0·1 *N* ferrous sulphate solution into a conical flask. Add 50 ml. of sulphuric - phosphoric acid reagent and 0·2 ml. of diphenylamine indicator. Dilute the solution to about 200 ml., and titrate it with the potassium dichromate until the green colour deepens to an intense blue-violet that remains permanent after shaking.

Determine the titre of the 0·1 *N* ferrous sulphate by carrying out a similar titration with a solution prepared by dilution of an accurately weighed amount of the secondary working standard potassium dichromate. From the results thus obtained calculate the factor of the potassium dichromate solution under test.

FERROUS AMMONIUM SULPHATE

GENERAL REMARKS—

Solutions of ferrous sulphate gradually deteriorate owing to the absorption of atmospheric oxygen; because of this they require to be standardised immediately before use. The relatively more stable double salt, ferrous ammonium sulphate, is used in the preparation of the solution, and its hydrolysis is prevented by using dilute sulphuric acid as solvent.

PREPARATION OF 0·1 *N* FERROUS AMMONIUM SULPHATE—

Weigh 39·2 g. of ferrous ammonium sulphate hexahydrate and dissolve it in boiled-out water to which 100 ml. of 50 per cent. sulphuric acid have been added. Transfer the solution to a 1-litre graduated flask, dilute to the mark with boiled-out water and mix well.

STANDARDISATION OF 0·1 *N* FERROUS AMMONIUM SULPHATE—

Decinormal ferrous ammonium sulphate is standardised by means of 0·1 *N* potassium dichromate as follows.

Measure accurately about 85 ml. of the 0·1 *N* ferrous ammonium sulphate solution into a conical flask. Add 50 ml. of sulphuric - phosphoric acid reagent and 0·2 ml. of diphenylamine indicator. Dilute to about 200 ml. with boiled-out water, and titrate it with 0·1 *N* potassium dichromate until the green colour changes to an intense blue-violet, which remains permanent after shaking.

TITANOUS CHLORIDE AND TITANOUS SULPHATE

PREPARATION OF 0·2 *N* SOLUTIONS—

(*a*) *Titanous chloride*—Add 200 ml. of commercial titanous chloride solution (15 to 20 per cent. of $TiCl_3$) to 200 ml. of concentrated hydrochloric acid, boil for 1 to 2 minutes in a flask, cool and dilute to 1000 ml. with boiled-out water.

Place the solution in the bottle, A, of the apparatus shown in Fig. 5. Siphon the solution over into the burette, B, by way of its two-way stopcock. Pour dilute sulphuric acid into the hydrogen generator, C, the bulb of which has been charged with granulated zinc. Turn the burette stopcock so as to run out the solution already in the burette and leave the stopcock

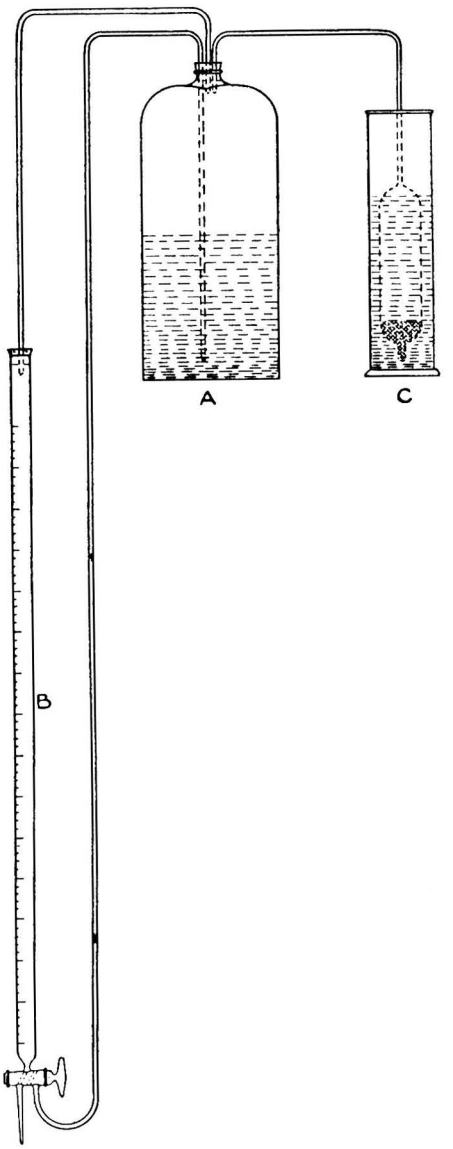


Fig. 5. Titanous sulphate titration apparatus

open until the air above the solution and in the burette has been replaced by hydrogen from the generator. The apparatus is then allowed to stand for one day with occasional shaking before using the solution.

(*b*) *Titanous sulphate*—Add 800 ml. of commercial titanous sulphate solution (15 per cent. of $Ti_2(SO_4)_3$) to 1000 ml. of dilute sulphuric acid consisting of 1 volume of 95 per cent. sulphuric acid to 4 volumes of water, and boil the mixture for 1 to 2 minutes. Cool to room temperature in a stream of hydrogen or carbon dioxide and dilute to 2000 ml. with boiled-out water. Store the solution under hydrogen as for the chloride.

STANDARDISATION OF 0·2 *N* SOLUTIONS—

The standardisation is carried out with 0·1 *N* potassium dichromate, using as intermediary 0·2 *N* ferric ammonium sulphate. As the titre of the latter remains constant, the concentration of the titanous solution is conveniently expressed as the volume in ml. equivalent to 1 ml. of 0·2 *N* ferric alum solution of known factor.

Measure into a 300-ml. conical flask 25 ml. of boiled-out water, 50·0 ml. of 0·1 *N* potassium dichromate and 25 ml. of 40 per cent. sulphuric acid. Fit the flask with a rubber bung bored with two holes, through one of which passes a leading tube reaching about 2 inches into the flask, and pass a rapid stream of carbon dioxide into the flask for several minutes in order to displace the air. Place the jet of the burette containing the titanous solution in the second hole of the rubber bung and, whilst continuing to pass carbon dioxide, run in 40·0 ml. of the titanous solution.

Mix the liquids by swirling the flask gently, and titrate the excess of titanous salt with 0·2 *N* ferric ammonium sulphate added from a 25-ml. burette, the jet of which is placed inside the second hole in the rubber bung. Towards the end of the titration, *i.e.*, when the dark colour due to the titanous salt has nearly disappeared, add 10 ml. of 10 per cent. ammonium or potassium thiocyanate and continue the addition of the iron solution until a pink colour is obtained that is permanent for 1 minute. Maintain a slow stream of carbon dioxide throughout the titration.

In the same way titrate 40·0 ml. of the titanous solution with the 0·2 *N* ferric ammonium sulphate, the conditions being exactly the same, except for (*a*) the omission of the 0·1 *N* potassium dichromate and (*b*) the use of a 50-ml. burette for the iron solution.

From the data thus obtained calculate the normality factor of the 0·2 *N* ferric ammonium sulphate and the volume of it that is equivalent to 1 ml. of the 0·2 *N* titanous solution.

PREPARATION OF 0·025 *N* SOLUTION OF TITANOUS CHLORIDE—

Add 25 ml. of commercial titanous chloride solution (15 to 20 per cent. of $TiCl_3$) to 50 ml. of concentrated hydrochloric acid, boil for 1 to 2 minutes in a flask, cool and dilute to 1000 ml. with boiled-out water. Store the solution under hydrogen as described above.

STANDARDISATION—

Carry out the standardisation exactly as described for 0·2 *N* titanous solutions, substituting 0·025 *N* titanous solution for the 0·2 *N*, 0·0125 *N* potassium dichromate for the 0·1 *N* and 0·025 *N* ferric ammonium sulphate for the 0·2 *N*.

FERRIC AMMONIUM SULPHATE

PREPARATION OF 0·2 *N*, 0·1 *N* AND 0·025 *N* FERRIC AMMONIUM SULPHATE—

Dissolve the quantity of ferric ammonium sulphate (iron alum) indicated below in 500 ml. of boiled-out water that has been acidified with 10 ml. of 50 per cent. sulphuric acid and dilute to 1000 ml. with boiled-out water.

For 0·2 *N*	use	96·44 g.	of ferric alum
For 0·1 *N*	»	48·22 g.	»
For 0·025 *N*	»	12·05 g.	»

STANDARDISATION OF 0·2 *N*, 0·1 *N* AND 0·025 *N* FERRIC AMMONIUM SULPHATE—

Carry out the standardisation of 0·2 *N* solutions by the method given above for 0·2 *N* titanous solutions. Standardise the 0·1 *N* and 0·025 *N* solutions in the same way, with modifications appropriate to the concentrations of the solutions.

POTASSIUM BROMATE

GENERAL REMARKS—

Potassium bromate is used as an oxidising agent and, in the presence of potassium bromide, as a brominating agent. In the former use the reducing agent to be titrated is acidified with hydrochloric acid and titrated hot with the bromate solution; any excess of bromate then reacts with the bromide produced in the reaction, equation (1), with liberation of bromine, equation (2), the free bromine being usually detected by the decolorisation of methyl orange, or potentiometrically—

(1) $KBrO_3 + 6HCl + 3SbCl_3 = 3SbCl_5 + KBr + 3H_2O$

(2) $KBrO_3 + 5KBr + 6HCl = 6KCl + 3H_2O + 3Br_2$.

When potassium bromate is used as a brominating agent, an excess of potassium bromide is added to the reaction mixture before adding the bromate solution. Equation (2) shows the reaction. It is usual to add an excess of the bromate solution and to determine the excess by addition of potassium iodide and titration with a standard solution of sodium thiosulphate.

PREPARATION OF 0·2 *N* AND 0·1 *N* POTASSIUM BROMATE—

Weigh 5·56 or 2·78 g., as appropriate, of potassium bromate for each litre of 0·2 *N* or 0·1 *N* solution required, dissolve the salt in cold water, transfer the solution to a litre flask, dilute to the mark and mix thoroughly. Standardise the resulting solution preferably by method (*a*), or, if it is to be used for the determination of arsenic or antimony, by method (*b*).

STANDARDISATION OF 0·2 *N* AND 0·1 *N* POTASSIUM BROMATE—

(*a*) *With 0·2 N and 0·1 N sodium thiosulphate respectively*—Measure accurately about 40 ml. of the bromate solution into a 500-ml. glass-stoppered flask or bottle containing 150 ml. of water. Cool the flask and its contents under the tap, then add 4 g. of potassium bromide, 4 g. of potassium iodide and 5 ml. of concentrated hydrochloric acid; stopper the flask, well mix the solution and allow it to stand for 15 minutes. At the end of this period titrate the liberated iodine with 0·2 *N* or 0·1 *N* sodium thiosulphate, according to the normality of the potassium bromate solution, at a rate of 10 ml. per minute until the end-point is almost reached; then add the sodium thiosulphate solution drop by drop. When the colour of the iodine is faint, add 2 or 3 ml. of starch solution and complete the titration, adding the thiosulphate solution drop by drop, with shaking, until the blue colour just disappears.

Calculate the normality factor of the bromate solution from the following equation—

$$KBrO_3 + 6KI + 6HCl = KBr + 6KCl + 3H_2O + 3I_2$$

(*b*) *With standard arsenious oxide*—Weigh accurately 0·40 g., or 0·20 g. for a 0·1 *N* solution, of secondary working standard arsenious oxide, previously dried to constant weight at 105° C., transfer to a 500-ml. conical flask and dissolve in 20 ml. of 1 *M* sodium hydroxide solution, warming to aid solution. When solution is complete add 25 ml. of water and 25 ml. of concentrated hydrochloric acid, density 1·16 at 20° C. Heat the solution almost to boiling and titrate while hot with the bromate solution, running the bromate in slowly with vigorous stirring until 36 ml. have been added. Then add 0·2 ml. of methyl orange solution and continue the titration until the colour of the indicator disappears.

Carry out a second titration in which the indicator is not added until the volume of bromate added is within 0·2 ml. of the volume previously used. Complete the titration after adding the methyl orange and use the value so obtained.

Calculate the normality factor of the bromate solution from the following equation—

$$2KBrO_3 + 3As_2O_3 = 2KBr + 3As_2O_5.$$

PREPARATION OF 0·2 *N* AND 0·1 *N* BROMATE - BROMIDE—

Weigh 5·56 g. of potassium bromate and 30 g. of potassium bromide, or 2·78 g. of potassium bromate and 15 g. of potassium bromide, for each required litre of the 0·2 *N* or 0·1 *N* solution respectively. Dissolve the salts in cold water, transfer the solution to a 1-litre flask, dilute to the mark and mix thoroughly. A standard solution of bromate - bromide that is 0·2 *N* or 0·1 *N* in respect of bromine may also be prepared and used for bromination.

STANDARDISATION OF 0·2 *N* AND 0·1 *N* BROMATE - BROMIDE SOLUTION—

Measure accurately about 40 ml. of the bromate - bromide solution into a 500-ml. glass-stoppered flask or bottle containing 150 ml. of water. Cool the flask and its contents under the tap, add 4 g. of potassium iodide and 5 ml. of concentrated hydrochloric acid, stopper the flask, mix the solution well and allow it to stand for 15 minutes. At the end of this period titrate the liberated iodine exactly as described above for the standardisation of 0·2 *N* and 0·1 *N* potassium bromate with standard arsenious oxide.

Calculate the normality factor of the bromate - bromide solution on the basis of the following equations—

$$KBrO_3 + 5KBr + 6HCl = 6KCl + 3H_2O + 3Br_2$$
$$3Br_2 + 6KI = 6KBr + 3I_2$$
$$3I_2 + 6Na_2S_2O_3 = 6NaI + 3Na_2S_4O_6.$$

PRECIPITATION REACTIONS

The relationship between the solutions used in precipitation reactions and the working standards is outlined in Fig. 1 (p. 577).

Silver nitrate

General remarks—

Standard solutions prepared from silver nitrate of good quality remain stable for long periods provided they are stored in well-stoppered dark-coloured bottles.

Preparation of 0·1 *N* silver nitrate—

Weigh 17·0 g. of recrystallised silver nitrate for each litre of solution required; dissolve the salt in water in a graduated measuring flask, dilute to the mark and mix well.

Standardisation of 0·1 *N* silver nitrate—

Transfer about 0·25 g. of working standard sodium chloride to a platinum crucible and dry it in an electrically heated air-bath at 270° C. until it is constant in weight. Use the weighed quantity of dried salt thus obtained for the standardisation by one of the following methods.

(*a*) *Mohr's method*—Transfer the sodium chloride to a 400-ml. conical flask, washing the last traces of salt into the flask with water. On the assumption that the silver nitrate is exactly 0·1 *N*, calculate the volume required to precipitate all the chloride and add 0·2 ml. less than this. Shake the mixture vigorously and filter it through an 11-cm. Whatman No. 1 filter-paper into another 400-ml. flask and wash the paper once with water. Add a few drops of 10 per cent. potassium chromate to the filtrate and continue the titration until a faint reddish-brown colour persists throughout the solution.

(*b*) *Drechsel's modification of Volhard's method*—Transfer the sodium chloride to a 350-ml. glass-stoppered flask, washing it in completely with a total of 130 ml. of water. Add 5 ml. of 1·5 *M* nitric acid. Calculate the volume of silver nitrate solution, assuming it to be exactly one-tenth normal, necessary to precipitate the sodium chloride, and add this amount, plus 0·2 ml. in excess, slowly and with vigorous shaking until the precipitate is coagulated. Filter the solution through a washed filter-paper and wash the precipitate by decantation several times, each time shaking the precipitate thoroughly with the washing water. Finally transfer the precipitate to the filter and complete the washing. To the combined filtrate and washings add 1 ml. of ferric ammonium alum solution for every 50 ml. of liquid present, and titrate the excess of silver nitrate with 0·01 *N* ammonium thiocyanate until a pale reddish-brown colour persists.

Note—The 0·01 *N* ammonium thiocyanate for this titration should be standardised in terms of the silver nitrate solution with which it is to be used.

(*c*) *Electrometric method*[22]—Transfer the weighed quantity of sodium chloride to a porcelain basin, and wash it in with 50 ml. of water. Add 5 ml. of 1·5 *M* nitric acid and place a silver wire electrode in the solution and connect it in series with a high-resistance galvanometer, a tapping key and a reference electrode. The latter consists of a silver wire coated electrolytically with silver chloride and immersed in 0·1 *N* nitric acid containing 0·05 ml. of 0·1 *N* silver nitrate per 100 ml. in a small wide-mouthed bottle. Make electrolytic contact between the titrating vessel and the reference electrode by means of an H-type salt-bridge, the limbs of which are filled with saturated potassium sulphate or ammonium nitrate solution. Titrate the prepared solution with the silver nitrate solution, and after each addition close the circuit momentarily by depressing the tapping key. Mechanical stirring of the liquid is advisable. The end-point is reached when the galvanometer needle remains stationary or the direction of its swing is reversed. Check the e.m.f. of the reference electrode at frequent intervals by means of a potentiometer.

Preparation of 0·01 *N* silver nitrate—

Prepare the solution by accurate dilution of 0·1 *N* silver nitrate and calculate the factor. The calculated factor may be checked by one of the three methods used for the standardisation of 0·1 *N* silver nitrate as follows.

STANDARDISATION OF 0·01 *N* SILVER NITRATE—

Transfer about 0·25 g. of working standard sodium chloride to a platinum crucible and dry it in an electrically heated air-bath at 270° C. until it is constant in weight. Transfer the sodium chloride to a 500-ml. graduated flask, washing it in completely with water; dilute the solution to the mark and mix well. Measure 50·0 ml. of this solution and titrate it with the 0·01 *N* silver nitrate by methods (*a*), (*b*) or (*c*) as given under 0·1 *N* silver nitrate. The factor so obtained should agree with the calculated factor.

AMMONIUM THIOCYANATE

GENERAL REMARKS—

Thiocyanate solutions are standardised by direct titration with standard silver nitrate; sufficient nitric acid is added to discharge the brown colour of the iron alum indicator. When thiocyanate is added to a solution of silver nitrate, white insoluble silver thiocyanate is precipitated in accordance with the equation—

$$AgNO_3 + NH_4CNS = NH_4NO_3 + AgCNS$$

When the silver has been completely precipitated, the excess of thiocyanate is indicated by the appearance of a red-brown colour due to the formation of ferric thiocyanate.

Ammonium thiocyanate is hygroscopic, so that the salt cannot be weighed out and dissolved to give a one-tenth normal solution with sufficient accuracy. It is, therefore, necessary to prepare a solution slightly stronger than decinormal in order to obtain an exact solution by final adjustment when the strength has been determined by titration with silver nitrate as described below. Thiocyanate solutions are not very stable.

PREPARATION OF 0·1 *N* AMMONIUM THIOCYANATE—

Weigh 8 g. of ammonium thiocyanate for each litre of solution required, dissolve the salt in water, transfer the solution to a graduated flask, dilute it to the mark and shake well.

STANDARDISATION OF 0·1 *N* AMMONIUM THIOCYANATE—

Measure accurately 40·0 ml. of the ammonium thiocyanate solution into a 450-ml. flask provided with a ground-glass stopper. Add 115 ml. of water and 5 ml. of 1·5 *M* nitric acid. Run into the flask, slowly and with vigorous shaking, a very slight excess of standard 0·1 *N* silver nitrate and shake the flask vigorously until coagulation of the precipitate is complete. Excess of silver nitrate is indicated by the fact that as long as ammonium thiocyanate is present the mixture remains milky, but as soon as the silver nitrate is in excess the precipitate coagulates and settles at once. Filter the solution through a washed filter-paper and wash the precipitate several times by decantation, each time shaking the precipitate thoroughly with the washing water. Finally transfer the precipitate to the filter and complete the washing. To the combined filtrate and washings add 1 ml. of ferric ammonium alum for every 50 ml. of liquid, and titrate the excess of silver with 0·01 *N* ammonium thiocyanate until a pale reddish-brown colour persists.

Calculate the factor by means of the following expression—

$$\text{Factor} = \frac{B - \frac{C}{10}}{A}$$

where
A = ml. of 0·1 *N* NH_4CNS taken
B = ml. of 0·1 *N* $AgNO_3$ added
C = ml. of 0·01 *N* NH_4CNS required.

PREPARATION OF 0·01 *N* AMMONIUM THIOCYANATE—

Prepare this solution by accurate dilution of 0·1 *N* ammonium thiocyanate and calculate the factor. This factor may be checked as follows.

STANDARDISATION OF 0·01 *N* AMMONIUM THIOCYANATE—

Accurately measure 4·50 ml. of 0·1 *N* silver nitrate from a 5- or 10-ml. burette into a 50-ml. stoppered flask, add 50 ml. of water, 5 ml. of 1·5 *M* nitric acid, and 2 ml. of ferric

ammonium alum solution and then titrate with the 0·01 *N* ammonium thiocyanate, shaking vigorously after each addition until a pale reddish-brown colour persists. The factor thus obtained should agree with the calculated factor.

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Nobel Division
Stevenston, Ayrshire

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The Analysis of Petrol - Kerosine Mixtures, with Specific Reference to the Boiling-point

By C. H. MANLEY

(*Read at the meeting of the Society on Wednesday, May 3rd*, 1950)

Synopsis—The value of the density determination, as well as that of the distillation range, as a means of detecting and determining the proportion of kerosine present in a petrol - kerosine mixture is discussed. A third method, giving results in close agreement with the first two methods, is described. This depends upon the determination of the boiling-point of the motor spirit under examination in an all-glass reflux apparatus, in which the boiling-points of mixtures of known composition have been previously ascertained.

Following the coming into force on June 1st, 1948, of the Motor Spirit (Regulation) Act and the Regulations made thereunder, as well as of the Motor Fuel (Control) Order, the Public Analyst found himself called upon not only to identify "commercial" petrol (with or without the red colour removed), but also to examine "private" petrol for the presence of kerosine. In this connection it is to be noted that the Motor Fuel (Control) Order, unlike the Motor Spirit (Regulation) Act, was made under the Defence (General) Regulations, 1939, with the result that the penalties involved for offences committed against it differ materially from those liable to be incurred under the Act. Further, Section 5 (5) of the

Order makes it an offence (except with the authority of a licence) to use any kerosine, whether or not in admixture, for supplying motive power to any motor vehicle chargeable with duty under Section 13 of the Finance Act, 1920, and Section 4 (2) of the Order states when motor spirit shall be deemed to contain kerosine in admixture. No methods, however, are given for calculating the proportion in which any kerosine so found is present. Section 4 (2) simply states that motor spirit or any other hydrocarbon oil shall be deemed to contain kerosine in admixture if, with a closed flash-point less than 73° F., the final boiling-point thereof exceeds 215° C., or if, with a closed flash-point greater than 150° F., not less than 20 per centum thereof distils at a temperature not greater than 210° C. The method I.P.—28/42 in the Institute of Petroleum publication, "Standard Methods for Testing Petroleum and its Products," only details apparatus and defines distillation procedure, and those standard textbooks that have been consulted only describe tests that are applicable to individual petroleum fractions. It is important therefore that, if marked discrepancy is to be avoided in the conclusions reached by two analysts examining portions of the same motor spirit, there should be some agreed methods of analysis. It became evident in due course that valuable use could be made of the fact that at least 40 per cent. of ordinary petrol (or gasoline) distils below 100° C. and that where the final boiling-point of a sample exceeded 200° C., and less than 40 per cent. distilled below 100° C., the amount of petrol present could be calculated by simple proportion, the difference between 100 per cent. and the figure for petrol being kerosine. A sample of which only 20 per cent. distilled below 100° C. therefore represented a 50 : 50 mixture of petrol and kerosine, or expressed in another way, contained not less than 50 per cent. of kerosine by volume.

To confirm this method, a mixture of petrol and kerosine in the proportions found in an adulterated sample of petrol was prepared and distilled, as in the example shown in Table I.

TABLE I

Sample	Final b.p.	Proportion distilling below 100° C., %	Calculated proportion of kerosine, %
P.260	272° C. (96%)	10	75
30% petrol : 70% kerosine.. ..	272° C.	11	72½

In examining a number of suspected samples received on one particular day, it was appreciated that valuable time could be saved in testing for samples of genuine petrol if two quick reliable tests could be applied.

DENSITY AND REFRACTIVE INDEX—

In the majority of the petrols examined, whether for the presence of kerosine or diphenylamine, either the specific gravity or the density (grams per millilitre) has been determined, measurements being originally made at 15·5° C., but later at the rather more convenient temperature of 20° C., at which all later density determinations have been carried out, 0·740 and 0·800 being taken as the densities of petrol and kerosine respectively, and the proportion of kerosine in a sample having a density in marked excess of 0·740 being calculated from the rise in density above 0·740. This was done on the assumption that the densities of mixtures of the two petroleum fractions concerned lie on a straight line, which was, in fact, borne out by direct measurement. As, moreover, it had been privately suggested that helpful indications might also result from a determination of the refractive index, this was ascertained for each of the mixtures examined, the values obtained also lying on a straight line and ranging from approximately 1·420 to 1·445.

The mixtures used were made by volume, the requisite proportions being measured at 20° C. in pipettes graduated at this temperature. The density was then determined in a stoppered 10-ml. graduated bottle correct at 20° C., and the refractive index in an Abbé instrument adjusted to 20° C.

BOILING-POINT—

Whilst still relying upon the distillation and density methods for ascertaining the proportion of kerosine in petrol - kerosine mixtures, consideration was given to other possible means of achieving this. Eventually, determination of the boiling-point of the sample under examination seemed a possibility, and this, together with the density, has proved

particularly useful, for when both density and boiling-point have been found to be normal, it has been considered unnecessary to proceed with distillation. As petrol and kerosine consist of mixtures of substances of similar chemical composition, it seemed reasonable to conclude that a steady rise in the boiling-point would be observed with increasing percentages of kerosine. Accordingly, boiling-point determinations were carried out on approximately 50-ml. quantities of (*a*) petrol, (*b*) kerosine and (*c*) various mixtures of these in a Quickfit and Quartz apparatus consisting of a 150-ml. flask connected to a reflux condenser (effective length, 8 inches) in which was held, by copper wire passed through the loop, a thermometer (range 0° to 250° C.), the bulb of which dipped into the liquid under examination. In order to maintain the composition of the boiling liquid as uniform as possible it was kept just gently boiling, thereby reducing to a minimum the proportion of the more volatile constituents vaporised into the reflux condenser.

On plotting the results, a slightly concave curve was obtained, the temperature range covered lying between 80° C. and 200° C. The figures shown in Table II were obtained for the mixtures studied, and the results were plotted.

TABLE II

DENSITY, REFRACTIVE INDEX AND BOILING-POINT OF PETROL - KEROSINE MIXTURES

Kerosine, %	Density at 20° C.	Refractive index at 20° C.	B.p., ° C.
0	0·741	1·421	80
10	0·747	1·423	83
20	0·753	1·425	85
30	0·757	1·429	93
40	0·763	1·430	97
50	0·769	1·434	102
60	0·773	1·436	115
70	0·779	1·437	131
80	0·784	1·440	147
90	0·790	1·442	166
95	0·791	1·443	179
100	0·795	1·444	195

The three methods involving density, distillation and boiling-point were applied with success to four samples in which appreciable proportions of kerosine had been mixed with petrol, P.259 to 261 concerning one defendant and P.274 another, as shown in Table III.

TABLE III

APPLICATION OF THE THREE METHODS FOR DETERMINATION OF KEROSINE

Sample	Density at 20° C.	Final b.p.	Proportion distilling below 100° C., %	B.p., ° C.	Proportion of Kerosine by: Density at 20° C., %	Distillation, %	B.p., %
P.259	0·772	246° C. (92%)	20	105	53	50	52
P.260	0·779	272° C. (96%)	10	133	65	75	72
P.261	0·764	264° C. (94%)	24	102	40	40	48
P.274	0·773	242° C. (95%)	18	118	55	55	64

The minimum proportions of kerosine certified for the purposes of the Court proceedings which followed were returned as 50, 65, 40 and 55 per cent. respectively, the two defendants concerned pleading guilty. Whilst the proportions deduced from the boiling-point in two of the examples quoted are higher than those obtained by the other two methods, it should be pointed out that appreciably higher figures would result from the distillation test if the probable average figure of 45 per cent. was used for the proportion of petrol distilling below 100° C., when the proportions of kerosine would become 56, 78, 47 and 60 per cent. in the preceding table, and higher still if the figure of 50 per cent. (sometimes encountered) was used as the basis of calculation.

As an example of a case in which suspicions proved unfounded we have that of the car owner who alleged that a garage proprietor had supplied him with petrol containing white

spirit. The police sample submitted on his behalf had a density at 20° C. of 0·734 and boiled at 78° to 79° C., both being figures normal to genuine petrol.

It is appreciated that whilst most samples of petrol have densities at 20° C. of approximately 0·740, a few reach 0·750 or even 0·760, and determinations of the boiling-point and distillation range may well afford useful information as to whether or not kerosine is present.

Finally, it should be possible to apply the boiling-point method to the elucidation of the approximate composition of other binary mixtures of petroleum products and to various binary mixtures of organic liquid compounds of similar composition.

CITY ANALYST'S LABORATORY
LEEDS

DISCUSSION

MR. P. MCGREGOR said that he had had considerable experience in examining samples of suspected petrol - kerosine mixtures taken from all parts of the country. When there had been an admixture the proportion of kerosine found was frequently less than 20 per cent. He would like to be able to estimate it by specific gravity or refractive index, but owing to the wide variation in the physical constants of petrol and kerosine, this would not be satisfactory.

Samples taken under the Motor Fuel (Control) Order, 1948, have to be examined by the prescribed methods. When using the standard method for the distillation (I.P.—28/42), it did not follow that, if a final boiling-point above 215° C. was obtained, kerosine was necessarily present. Similar effects could be obtained if gas oil or lubricating oil were added, but then the residue after distillation was greater than that normally obtained from petrol or petrol - kerosine mixtures. It was therefore necessary to take the nature and volume of the residue into account. An approximate idea of the amount of kerosine present could be obtained by assuming that at 200° C. all the petrol and half the kerosine had been distilled.

DR. E. J. MILLER said that he had listened with great interest to Mr. Manley's talk and to the subsequent discussion, as he and his colleagues, at the North-Eastern Forensic Science Laboratory in Wakefield, dealt with a steady stream of samples of the nature described.

Although they were primarily interested in the qualitative aspect of petrol - kerosine mixtures, nevertheless they felt that it was of great value to have a reasonably accurate knowledge of the quantities of each of the two "constituents" of the mixture.

Their method of determining this, which they had found to be sufficiently near the truth, was to assume that in a genuine petrol all of it (or at least 95 per cent.) would have distilled over at a temperature of 190° C.

They distilled the mixture as laid down in I.P.—28/42, and from the results they determined graphically the amount that had distilled over at this temperature. The difference represented the kerosine fraction. Any small amount of petrol that had been held back by the kerosine was compensated for by the small amount of kerosine which had distilled over at this temperature.

MR. J. HASLAM said that for the method to be valid it appeared that petrol and kerosine must be substances of reasonably constant composition. Mr. Manley had presumably examined several samples of petrol and kerosine in the first place, and Mr. Haslam asked what variations Mr. Manley had found on application of the tests he had described to these samples.

DR. J. H. HAMENCE said that he had listened to the paper with considerable interest, as he had been called upon to examine a number of samples of private petrol for the presence of kerosine. He would like to ask the author if he had considered the influence of benzene on the physical constants which formed the basis of his assessment of the proportion of kerosine. He understood on good authority that the proportion of benzene in private petrol varied considerably, and this was borne out by analysis of many hundreds of samples which he had made during the past two years. The presence of benzene had a profound effect on the specific gravity of the petrol, and also affected considerably the initial boiling-point. With these facts in mind, he was of the opinion that any calculations based on specific gravity or boiling-point might well be rendered completely erroneous if benzene in fair quantities were present. He pointed out that in some of the examples which had been quoted by the author the high specific gravity might equally well be due to the presence of benzene in the petrol, and not to kerosine. Dr. Hamence said that in his view, one of the most reliable methods of arriving at an assessment of the proportion of kerosine in a petrol - kerosine mixture was from a study of the boiling-point curve, particularly the fractions distilling above 190° C., as compared with the boiling-point curves given by mixtures containing known amounts of kerosine. He found that the presence of benzene did not affect the boiling-point range above 190° C., whereas it had a marked effect on many of the other physical constants.

MR. MANLEY, replying to Mr. McGregor, pointed out that, whilst it was true that the Motor Fuel (Control) Order, 1948, prescribed special methods and apparatus for determining whether or not motor spirit contained kerosine in admixture, the analyst was left free to use such methods as he deemed fit for determining the actual proportion of kerosine once its presence had been indicated. The chances of gas oil or lubricating oil being used were decidedly small, kerosine being the most likely alternative fuel available to the motorist; in fact, in none of the cases with which he had been concerned had the allegation that kerosine was the adulterant been contested. It was true that fuels other than kerosine had (in his own

experience) been used to supplement the petrol ration. Special Boiling-Point Spirits No. 2 and Aviation Spirit were examples, but their use was understandable by reason of their much lower distillation range.

Mr. Manley said that he agreed with Dr. Miller regarding the final boiling-point of petrol. He had attempted to calculate the percentage of kerosine present in petrol - kerosine mixtures by having regard to the proportion distilling above 190° C., but had found that this had led to a gross under-estimate. Later, as pointed out, results not far removed from the truth were obtained by having regard to the proportion distilling below 100° C. Moreover, it was necessary to emphasise the fact that qualitative findings alone singularly failed to impress a Court of Law, which always wanted to know how much of the offending ingredient was present, and took a lean view of an expert witness who, for instance, could not say whether the proportion was of the order of 5 or 50 per cent.

In reply to Mr. Haslam's observations, Mr. Manley said that he had examined a number of samples of both petrol and kerosine, and had found them to be of reasonably constant composition. Certain variations in distillation range and density were inevitable, just as there were variations within limits in the various chemical and physical values of the numerous foods examined by the public analyst, who both in his own interest and in fairness to others concerned was careful to consider these variations before issuing his report. A similar approach had been made to the petrol - kerosine problem.

Replying to Dr. Hamence, Mr. Manley stated that he had considered the possible influence of benzene on the physical constants studied inasmuch as he was aware that most of the various pool grades contained, in addition to paraffins and naphthenes, small percentages of aromatics, the proportion being characteristic of the particular grade. It was perhaps well to point out that there was no intention of condemning a product on density or boiling-point alone. In fact, when suspicion was thereby aroused, it was necessary to proceed to determine the distillation range in order to comply with Section 4 (2) of the Motor Fuel (Control) Order, 1948. If confirmation followed, then the method giving the lowest figure for the kerosine present would be used for purposes of certification. Density and boiling-point determinations were extremely useful for genuine petrols, for where normal figures were obtained, it was rarely necessary to proceed with distillation.

The Microbiological Assay of Riboflavine in Yeast and Yeast Products

Use of *Lactobacillus helveticus* in a 17-hour Titrimetric Method

By A. JONES and S. MORRIS

(*Read at the meeting of the Biological Methods Group on Tuesday, December 13th,* 1949)

Synopsis—A 17-hour titration method is proposed for the assay of riboflavine in yeast and yeast extracts by the use of *Lactobacillus helveticus*. A comparison has been made with the 72-hour assay, and the results obtained by the proposed method are satisfactory.

Until recently, only two methods of reasonably high accuracy existed for the assay of riboflavine, the chemical method using fluorimetry[1] and the microbiological method of Snell and Strong[2] using *L. helveticus*. In the latter, the organism is incubated for 72 hours before titration of the acid produced. Although the degree of accuracy obtained is good, the length of time required is frequently a disadvantage and may sometimes endanger the success of a commercial process. More recently, a 16 to 18-hour turbidimetric method involving *Leuconostoc mesenteroides* was published.[3] In this, however, specialised apparatus and assistance are both essential in order to attain a reasonable degree of accuracy and duplication of results. There is little doubt that a titrimetric method is in many ways preferable and Strong[4] has given some details of a 24-hour titrimetric method developed by Lipton[5] in which *L. helveticus* is used. Unfortunately, insufficient detail is given, and the present publication deals with an investigation into the possibilities of such an assay technique. Comparative results, using the 17-hour and the 72-hour techniques, are given to show the degree of accuracy that can be obtained.

Experimental

Assay organism—

The organism used in the whole series of experiments was *Lactobacillus helveticus* (*L. casei* ϵ).

CULTURE MEDIA—

Stab cultures of *L. helveticus* were maintained on either (*a*) a yeast-water - glucose - agar medium[6] or on (*b*) liver - tryptone - agar.[7] On the former, sub-cultures were made monthly; on the latter, at fortnightly intervals. The compositions of the stock culture media were—

(*a*) 0·5 g. of glucose, 100 ml. of yeast-water and 1·5 g. of agar, at pH 6·8; and

(*b*) 1·0 g. of tryptone (Difco), 1·0 g. of glucose, 0·2 g. of di-potassium hydrogen phosphate (K_2HPO_4), 0·3 g. of calcium carbonate, 10 ml. of liver extract, 0·5 ml. of salts A, 0·5 ml. of salts B, 1·5 g. of agar, and glass-distilled water to make 100 ml.

INOCULUM SUB-CULTURES—

For the yeast - agar cultures, the inoculum medium consisted of 5·0 ml. of the 72-hour basal medium, 1 μg. of riboflavine and 5·0 ml. of water. This was sterilised at 15 lb. pressure for 10 or 15 minutes. For the liver - tryptone - agar cultures, the medium consisted of 10·0 ml. of liver - tryptone broth[7] sterilised at 15 lb. pressure for 15 minutes.

After inoculation, the media were incubated at 37° C. for 18 hours in centrifuge tubes. They were kept, after incubation, in a refrigerator until required. In the 17-hour assay, it is essential that the organisms be grown in the inoculum medium for not more than 16 to 18 hours. Longer periods of incubation lead to erratic results in the assay.

For use, the medium containing the organisms was centrifuged and the organisms were washed twice with 10 ml. of sterile 0·9 per cent. saline. The organisms were finally suspended in 20 ml. of sterile saline and two drops of this suspension were used for the inoculation of each assay tube.

At this stage, stress must be laid on the effect of the age of the inoculating medium on the growth of the organisms and the final assay results. It has been confirmed on many occasions that, when the inoculating medium was more than one month old, either the organisms did not grow or the assay yielded extremely erratic results. For this reason the inoculating medium was kept for only two weeks, after which fresh supplies were made.

BASAL MEDIA—

The basal media for the 72-hour and the 17-hour assays are given in Table I. The former is a slight modification of that recommended in the Report of the Analytical Methods Committee of this Society,[6] the latter is based on the medium used by Lipton.[5]

TABLE I

BASAL MEDIA FOR 17-HOUR AND 72-HOUR ASSAYS

	17-hour assay	72-hour assay
Photolysed (sodium hydroxide treated) peptone solution (Difco Bacto-peptone or Oxo bacteriological peptone)	200 ml.	100 ml.
L-Cystine	0·1 g.	0·1 g.
DL-Tryptophan	0·2 g.	0·2 g.
Glucose	20·0 g.	20·0 g.
Yeast supplement, photolysed	50 ml.	—
Yeast supplement, lead precipitated	—	20 ml.
Sodium chloride	5·0 g.	5·0 g.
Ammonium sulphate	3·0 g.	3·0 g.
Xanthine	0·01 g.	0·01 g.
Adenine	0·01 g.	0·01 g.
Guanine	0·01 g.	0·01 g.
Uracil	0·01 g.	0·01 g.
p-Aminobenzoic acid	400 μg.	400 μg.
Pyridoxine	100 μg.	100 μg.
Calcium pantothenate	200 μg.	200 μg.
Nicotinic acid	100 μg.	100 μg.
Inorganic salt solution A	5·0 ml.	5·0 ml.
Inorganic salt solution B	5·0 ml.	5·0 ml.
L-Asparagine	0·25 g.	—
L-Glutamic acid	0·10 g.	—
Glass distilled water to	500 ml.	500 ml.
pH adjusted to	6·7–6·8	6·7–6·8

It will be seen that the two media are largely the same. The differences consist (*a*) in the replacement of lead-precipitated yeast supplement by photolysed yeast supplement, and (*b*) the addition of L-asparagine and L-glutamic acid to the 17-hour medium.

PREPARATION OF EXTRACTS—

A suitable weight of material, 0·2 g. for dried yeast, was autoclaved for 15 minutes at 15 lb. pressure with 50 ml. of 0·1 *N* hydrochloric acid. The solution was cooled, then adjusted to pH 4·5 with acetic acid and diluted to 100 ml. with glass-distilled water. It was then

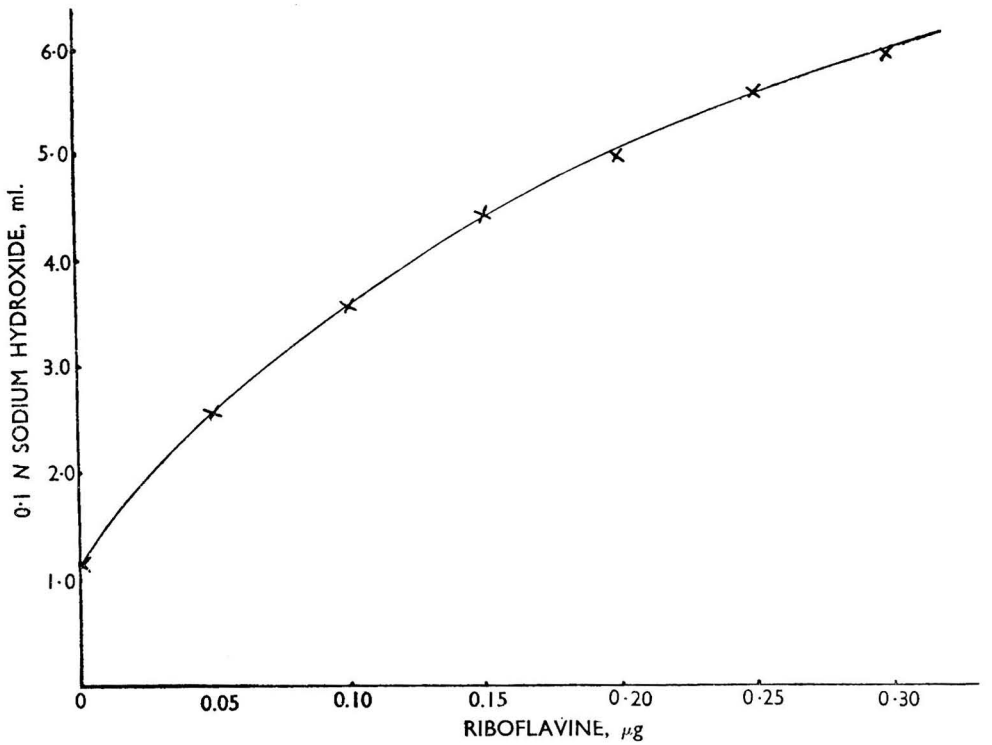

Fig 1. Typical standard curve for the 17-hour assay.

filtered, its pH was adjusted to 6·8, and it was finally diluted until each ml. contained 0·05 to 0·10μg. of riboflavine. For the assay, 1·0, 1·5, 2·0 and 2·5 ml. were taken.

PREPARATION OF STANDARD CURVE—

For the 72-hour assay, the range of the standard curve was 0 to 0·25 μg. of riboflavine, whereas for the 17-hour assay the range was 0 to 0·30 μg.

ASSAY PROCEDURE—

After addition of the extracts to the basal medium, dilution, sterilisation and inoculation, the tubes containing the solutions were incubated at 37° C. for 17 hours or 72 hours. In the former assay, the tubes were removed from the incubator after 17 hours and placed in the refrigerator to prevent further growth of the organisms. For titration, the racks containing the tubes were taken from the refrigerator as required. Titration was carried out with 0·1 *N* sodium hydroxide, bromothymol blue being the indicator.

For the 72-hour assay such precautions were unnecessary, since maximum growth and acid production had already been reached.

A typical standard curve for the 17-hour assay technique is given in Fig. 1. The slope-ratio method of computation cannot be applied to this assay. The vitamin content of the sample is directly read from the graph. The titration results in the present method are not so high as those obtained by Lipton[5] with the 24-hour assay. In view of the lack of detail given by Strong, it is impossible to state why this difference occurs.

RECOVERY OF RIBOFLAVINE—

The recovery of riboflavine added to yeast was investigated by the 17-hour assay technique. Table II gives the results, which show that the recovery is good. Further, good agreement was obtained in the riboflavine values of samples assayed at different levels.

TABLE II

RECOVERY OF RIBOFLAVINE ADDED TO YEAST

Yeast dilution taken, ml.	Riboflavine in yeast dilution,* μg.	Total riboflavine found, μg.	Difference, μg.	Riboflavine added, μg.	Recovery, %
2·0	0·125	0·169	0·044	0·050	88
3·0	0·188	0·237	0·049	0·050	98
4·0	0·250	0·300	0·050	0·050	100
3·0	0·094	0·200	0·106	0·100	106
4·0	0·125	0·234	0·109	0·100	108

* Found by 17-hour assay without added riboflavine.

ASSAY OF YEAST, YEAST EXTRACTS AND MEAT EXTRACT—

The 17-hour and 72-hour assay techniques have been compared, using yeast, yeast extracts and a meat extract. The results are given in Table III; they were obtained by

TABLE III

COMPARISON OF THE 17-HOUR AND 72-HOUR ASSAY METHODS

Sample	17-hour assay		72-hour assay	
	Riboflavine, μg. per g.	Mean value	Riboflavine, μg. per g.	Mean value
Dried brewer's yeast Y ..	75, 78, 84, 83, 80, 77, 78, 75, 74, 83, 79, 72, 73, 74, 73, 73, 73, 82, 81, 74	77·1	87, 88, 83, 84, 87, 80, 79, 80, 81, 80, 80, 84, 83, 80, 81, 85, 78, 80, 81, 79	82·1
Dried baker's yeast	41, 42	41·5	41, 42	41·5
Dried brewer's yeast X ..	82, 85, 81, 84, 85, 80, 78	82·1	82, 79, 78, 81, 80, 78, 79	79·4
Yeast extract (Oxo)	85, 99, 100, 87, 92, 103, 99, 100	95·6	96, 97, 92, 89, 94, 93, 99, 99	94·4
Meat extract (Oxo)	21·1, 22·7, 23·5, 22·3, 22·4, 23·9, 22·5, 20·8, 22·5, 20·8, 22·8, 22·5	22·3	32·6, 30·9, 32·3, 31·0, 31·8, 32·1, 31·3, 33·0, 32·9, 32·6, 30·0, 32·9	32·0

three operators, as it was thought that a better assessment of the methods would be so obtained. It will be seen that the maximum difference from the mean is never greater than ±10 per cent. by either method. Further, with yeast and yeast extracts, the values obtained by the two methods are almost identical. With the meat extract, on the other hand, the 72-hour assay technique yields significantly higher values, although occasionally these higher values can be obtained in the 17-hour assay. This difference may be due to the presence in the meat extract of a riboflavine precursor or substitute that requires more than 17 hours to be utilised by *L. helveticus* under the conditions of treatment given. For this reason, it must be stressed that, for the present and until various types of materials have been studied, the 17-hour assay technique can only be recommended for yeast and yeast extracts.*

COMPARISON OF ELECTROMETRIC (pH) METHOD AND INDICATOR TITRATION METHOD—

Comparisons have been made of the results by the titration method, as given above, and by the electrometric measurement of the pH of the same sample solutions. Whereas

* Since the submission of this paper for publication, further tests have shown that, by replacing the Difco peptone with Oxo peptone in the 72-hour medium, the same results can be obtained for meat extracts with either the 17-hour or 72-hour techniques.

in the 72-hour assay reasonably good agreement in assay results was obtained by the two methods, in the 17-hour assay the low acid production and the high buffering qualities of the medium combined to give extremely small differences in the pH values recorded. The pH method gave results of low precision that did not bear comparison with the titrimetric procedure.

COMPARISON OF THE PLATE ASSAY AND THE TITRIMETRIC ASSAY TECHNIQUES—

The plate assay technique[8,9] is quick and reasonably accurate for materials containing relatively large amounts of riboflavine. Comparisons of the plate method and the two titration methods have been carried out, and the results are given in Table IV.

TABLE IV

COMPARISON OF PLATE AND TITRIMETRIC ASSAY TECHNIQUES

Sample	Riboflavine, μg. per g.		
	Plate assay	17-hour assay, mean value	72-hour assay, mean value
Dried brewer's yeast Y	52, 52, 68, 30, 31, 38	77·1	82·1
Dried baker's yeast	16, 16, 16	41·5	41·5
Yeast extract (Oxo)	29	95·6	94·4
Meat extract (Oxo)	14, 17	22·3	32·0

In view of the relatively low potency of the materials used, large samples were necessary, and this gave rise to a corresponding difficulty of extraction. From Table IV it will be seen that, with the extraction methods used, low and erratic results were obtained.

CONCLUSIONS

From a general survey of the results, it is possible to draw certain conclusions about the value of the 17-hour assay technique for the assay of riboflavine, using *L. helveticus*. For yeast and yeast products, the results compare very favourably with those obtained by the 72-hour technique. From the results obtained with the meat extract it would appear that, with materials other than yeast, modifications in the technique are necessary either in the preparation of the sample or in the basal medium. Further investigations on a variety of foodstuffs would be essential before any definite conclusions and final method, applicable to all types of materials, could be formulated.

There are, however, certain salient facts in the 17-hour technique that cannot be ignored if a high degree of precision is to be maintained. Of these, possibly the most important are the growth period of the inoculum and the incubation period in the test. By a rigid control of these factors, an occasional irregularity in an assay can then be traced to an external source and can be readily remedied.

Finally, the 17-hour assay has been in use in these laboratories for more than 12 months, with frequent checks by the 72-hour assay. The precision of the results obtained has been high.

The authors thank Miss M. Copus and Miss A. Braddick for assistance during the course of these assays and Mr. S. A. Price for advice. The authors also thank the Directors of Beecham Research Laboratories, Ltd., for permission to publish this paper.

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Discussion

Mr. H. Pritchard said that Dr. Morris had indicated his lack of faith in turbidimetric measurements. This, he thought, was surprising—particularly in view of the fact that turbidity measurements were used in the recently published method for the assay of vitamin B_{12} with *Lactobacillus leichmannii*. The use of suitable instruments had surely led to a better expectation of accuracy. He enquired also why the tubes were placed in the refrigerator to arrest growth after 17 hours; he thought autoclaving would be more convenient.

Mr. J. S. Harrison agreed with Mr. Pritchard. On the basis of a large number of assays by both methods, he had found turbidimetric measurements were as accurate as titrimetric. In his experience, the main errors in microbiological assays occurred not at the final measurement, but during the preparation of dilutions for test. A possible reason for the differences between results by the 17-hour and 72-hour *L. helveticus* tests on meat extract was that 17 hours fell during, or shortly after, the active growth period. The acid produced was a measure of the mean population of bacteria during that time. If the growth rates, as opposed to the final growth, caused by the standard and test solutions were different (owing to interfering substances in the latter) the acid produced after 17 hours would not be in the same relation as the values obtained after a further 55 hours—during which time acid continued to be produced by the entire final population, while little growth was taking place. Thus a higher value for the sample in the 72-hour method would infer a slower growth-rate during the first 17 hours.

Mr. S. A. Price said that although he agreed that turbidimetric methods could be no less precise than titrimetric, they were often less convenient. Especially was this true if the extracts for assay were not crystal-clear or if they were dark in colour; it was then necessary to run additional "blanks" to correct for the turbidity or colour of the extracts.

Mr. N. T. Gridgeman asked whether it was known which of the results on meat extract were more nearly correct. Were the 17-hour results low or the 72-hour results high?

Dr. Morris, replying to the questioners, said that his laboratory had not the apparatus required for turbidimetric measurements, and, furthermore, turbidity readings required more technical assistance. He would like to emphasise that the 16 to 18-hour incubation period must be adhered to rigidly: a longer period gave most erratic results. It was imperative, too, that the inoculum medium should be freshly prepared every 2 weeks and that the assay tubes should be refrigerated after incubation. He, too, was puzzled by the results obtained with meat extracts, and he wished to make it clear that the assay method described was recommended for yeast and yeast products, for which it had been found successful; it must not be assumed that the procedure could be applied to other test materials without modification.

A 24-Hour Plate Assay Technique for the Vitamin B_6 Complex of Yeast

By A. Jones and S. Morris

(*Read at the meeting of the Biological Methods Group on Tuesday, December 13th, 1949*)

Synopsis—A plate assay technique for the vitamin B_6 complex has been developed using *Saccharomyces carlsbergensis*. For the complete utilisation of pyridoxamine in the plate assay, tryptophan appears to be an essential metabolite for the organism. Indications are also given that a fourth, hitherto unknown, factor of the B_6 complex may be present in certain yeasts.

In a recent publication[1] it was shown that, by certain modifications of the original method of Stokes, Larson, Woodward and Foster[2] in which *Neurospora sitophila* was used, a satisfactory degree of accuracy could be obtained for the assay of the vitamin B_6 complex in yeast and certain other foodstuffs. Unfortunately, the incubation period for the mould is 5 days, which entails considerable delay in obtaining the assay results. The turbidimetric method of Atkin, Schultz, Williams and Frey,[3] using *Saccharomyces carlsbergensis*, has the advantage of a short incubation period of 24 hours, but requires highly specialised apparatus. For these reasons, the possibility of obtaining a plate-assay technique, using *S. carlsbergensis*, was investigated. The plate assay has been used for other vitamins of the B complex[4,5,6,7] but, so far as is known, no attempt has been made to assay the vitamin B_6 complex by this method.

Throughout this study parallel assays were carried out, the mould technique being used for control purposes. In one case, with the assistance of Dr. E. R. Dawson of Distillers

Company, Ltd., Great Burgh, Epsom, a comparative assay was carried out using *S. carlsbergensis* and the technique in which the yeast is hydrolysed in 0·055 *N* sulphuric acid.

Throughout the present paper, the term vitamin B_6 is used to include the three known constituents pyridoxine, pyridoxal and pyridoxamine, and the assay values are stated as μg. of vitamin B_6 per g. or as μg. of pyridoxine, pyridoxal or pyridoxamine, not as their hydrochlorides.

Method of assay

Basal medium—

The basal medium (Table I) is essentially that used by Atkin *et al.*[3] with the addition of nicotinic acid[8] and tryptophan. Although the inclusion of 2·25 μg. each of nicotinic acid and calcium pantothenate per ml. has been recommended for the assay of vitamin B_6 using *S. carlsbergensis*, for the plate-assay technique 1000 μg. per 900 ml. of medium was found to be adequate.

The medium, with the addition of 2 per cent. of agar was filled into tubes in amounts of 20 ml., steamed for 20 minutes and stored at laboratory temperature until required.

Stock culture and preparation of the inoculum—

The organism, *S. carlsbergensis* 4228, was grown in 1-ounce screw-capped bottles. The growth medium was 4 per cent. malt - agar at a pH of 5 to 5·5, and incubation took place for 18 to 24 hours at 28° C. Sub-culturing was carried out every 14 days.

Table I

Basal medium

Glucose	50 g.
Potassium di-hydrogen phosphate	0·55 g.
Potassium citrate	5·0 g.
Citric acid	1·0 g.
Casein hydrolysate	4·0 g.
Nicotinic acid	1000 μg.
Aneurine hydrochloride	250 μg.
Calcium pantothenate	1000 μg.
Biotin	8 μg.
Inositol	0·025 g.
Salt solution*	25 ml.
DL-Tryptophan	0·1 g.
Volume made up to	900 ml.
pH adjusted to	4·6
Agar (Oxo)	18 g.

* The salt solution contains 1·70 g. of KCl, 0·5 g. of $CaCl_2.6H_2O$, 0·5 g. of $MgSO_4.7H_2O$, 0·01 g. of $FeCl_3.6H_2O$ and 0·01 g. of $MnSO_4.4H_2O$ per 100 ml.

To prepare the inoculum, the growth of yeast from the surface of a malt - agar slope was washed off with 10 ml. of sterile saline. The suspension was centrifuged and the yeast washed once with sterile saline and finally suspended in 10 ml. of saline for use as inoculum.

Sub-cultures older than 14 days were never used for preparation of the inoculum. With increasing age of the sub-culture, the zone of growth increased in size and diminished in clarity.

Preparation of the petri plates—

The Petri plates were prepared in a manner similar to that used for the assay of aneurine.[9] One millilitre of inoculum was used for each 20 ml. of medium. Preliminary drying of the plates and incubation with the lids raised, essential for the assay of aneurine, were found to be unnecessary for the assay of vitamin B_6.

Five tubes of medium were used for the standard and for each test sample. The contents were melted by immersing the tubes in a boiling water-bath. The tubes were cooled to a temperature of 48° to 50° C. and maintained at that temperature, and 1 ml. of inoculum was added to each tube. Immediately after addition of the inoculum, the contents of each tube were thoroughly mixed by rotation to distribute the bacterial suspension evenly throughout the medium. The contents of the tubes were poured severally into sterile Petri

dishes, 9 cm. in diameter, on a flat, even surface and allowed to set. Five holes were cut in each plate, each hole 10 mm. in diameter, the agar discs removed and the holes sealed with the addition of a drop of melted medium.

PREPARATION OF THE YEAST SAMPLES—

Two grams of dried yeast and 0·3 g. of takadiastase were added to 20 ml. of 1 per cent. sodium acetate buffer solution at pH 4·5. When necessary, the pH of the mixture was adjusted to 4·5, then 2 drops of benzene were added and the whole was incubated at 37° C. for 18 hours.

After incubation, the mixture was steamed for 10 minutes, cooled, diluted to 25 ml. and filtered. The filtrate was diluted 1 to 2, 1 to 4, 1 to 8 and 1 to 16 with glass-distilled water and 0·1 ml. of each dilution was put into the appropriate hole of the Petri plates. The Petri plates were finally incubated at 28° C. for 18 hours.

For the preparation of the standard curve, the assay range was 0·125, 0·25, 0·50, 1·0 and 2·0 μg. of pyridoxine, pyridoxal or pyridoxamine per ml. The mean values of the diameters in millimetres of the zones of growth were plotted against the logarithms of the concentrations of the standard pyridoxine, pyridoxal or pyridoxamine.

Under the test conditions, the effect of doubling the concentration of pyridoxine, pyridoxal or pyridoxamine was to increase the zone diameters by 2·5 mm. Since the activities of the three members of the vitamin B_6 complex were equal for *S. carlsbergensis* on this medium, pyridoxine was mostly used as a standard.

A series of different brewers' yeasts has been assayed by this method and, for comparative purposes, by the *Neurospora* technique. The results are shown in Table II, and a statistical evaluation of the results is given in Table III.

TABLE II

COMPARISON OF VITAMIN B_6 CONTENT OF YEASTS ASSAYED BY THE PLATE AND *Neurospora* METHODS

	Vitamin B_6			
	Neurospora method		Plate method	
Yeast	μg. per g.	Mean μg./g.	μg. per g.	Mean μg./g.
A	21·7, 22·5, 21·7, 23·0, 21·5, 21·4, 22·6, 20·8, 20·3, 20·2, 18·7	21·31	29·2, 31·2, 29·4, 26·0, 29·4	29·04
B	20·1, 20·5, 21·2, 20·9, 18·2	20·18	18·8, 20·6, 22·1, 18·0, 21·7, 21·4	20·43
C	18·8, 16·1, 17·0, 18·5, 17·6, 18·6, 23·5, 23·1, 23·0, 23·6, 24·1, 22·7, 23·3	20·76	24·4, 24·6, 24·5, 25·7, 24·0, 24·6, 25·4	24·74
D	17·4, 18·4, 16·9, 16·9, 17·3, 14·9, 18·6, 17·1, 19·4, 18·0, 20·5, 21·0, 20·0, 20·5	18·35	23·6, 21·7, 21·2, 21·2	21·92
E	17·0, 17·7, 20·1, 18·0, 17·9, 18·3, 18·3, 17·7, 18·3, 19·1	18·24	23·6, 22·2, 20·2, 20·4, 22·3	21·74
Y	19·8, 20·4, 20·8, 20·3, 21·3, 17·7, 19·1, 20·3, 19·5, 20·3, 18·1, 18·2, 19·1, 18·2, 17·1, 16·2	19·15	22·8, 23·8, 24·0, 24·4, 20·5, 20·5, 21·7, 20·5	22·27
449	17·7, 18·7, 16·9, 16·5, 17·2, 17·0, 19·8, 18·3, 19·1, 19·6, 18·1, 18·7, 19·7, 19·2, 18·5	18·33	28·8, 29·9, 29·8, 30·1, 29·8, 29·8, 29·8	29·71

YEAST 449. Assay carried out with *S. carlsbergensis* 4228—

100 mg. of dried yeast were treated with 25 ml. of 0·055 *N* sulphuric acid for 5 hours at 20 lb. pressure. The amounts of vitamin B_6 found were 33, 29 and 34 (mean 32) μg. per g.

THE EFFECT OF THE TRYPTOPHAN CONTENT OF THE BASAL MEDIUM ON THE ASSAY OF VITAMIN B_6—

The effect of the tryptophan content of the basal medium was examined by preparing two media, one with and the other without it. Otherwise the test was carried out as above.

Dose - response curves were obtained for pyridoxine, pyridoxal and pyridoxamine. Whereas the curves for the two former were identical, irrespective of the medium, those for

TABLE III

STATISTICAL EVALUATION OF THE RESULTS SHOWN IN TABLE II

Yeast	Method	No. of Samples	Mean value ± standard error	Coefficient of Variation
A	*Neurospora*	11	21·31 ± 0·377	5·9
	Plate	5	29·04 ± 0·842	6·5
B	*Neurospora*	5	20·18 ± 0·528	5·9
	Plate	6	20·43 ± 0·681	8·2
*C	*Neurospora*	13	(20·76 ± 0·828)*	(14·4)*
	Plate	7	24·74 ± 0·225	2·4
D	*Neurospora*	14	18·35 ± 0·469	9·6
	Plate	4	21·92 ± 0·571	5·2
E	*Neurospora*	10	18·24 ± 0·269	4·7
	Plate	5	21·74 ± 0·638	6·6
Y	*Neurospora*	16	19·15 ± 0·361	7·5
	Plate	8	22·27 ± 0·596	7·6
449	*Neurospora*	15	18·33 ± 0·277	5·9
	Plate	7	29·71 ± 0·158	1·4

* The results of the *Neurospora* assay on yeast C clearly fall into two groups. The first six samples have a range of 16·1 to 18·8, the second seven from 22·7 to 23·6. It is not permissible to treat all thirteen as a homogeneous group.

The second group of seven have a mean value of 23·33 with a standard error of ±0·173. (C.V. = 2·0.) Like the values for all other samples of yeast except B, this is significantly lower than that given by the plate assay. For sample 449 the plate assay gave a mean value more than 50 per cent. higher than the *Neurospora* method.

pyridoxamine varied markedly according to the medium. In the absence of tryptophan, the responses for pyridoxamine were always 50 to 60 per cent. lower than those for pyridoxine and pyridoxal. On the other hand, the activity of the pyridoxamine was constant throughout the assay range in terms of pyridoxine and pyridoxal. Further, the zones of growth obtained with pyridoxamine and with yeast samples on a tryptophan-deficient medium were not as sharp and clearly defined as those from pyridoxine and pyridoxal.

With the medium containing 0·1 g. of DL-tryptophan per 900 ml., the dose - response curve for pyridoxamine was identical with those for pyridoxine and pyridoxal (Fig. 1). The clarity and ease of measurement of the growth zones with pyridoxamine and yeast was improved by using the tryptophan medium.

Several attempts were made to raise the lower response with pyridoxamine on the tryptophan-deficient medium, various additions or substitutions being made to the basal medium.

TABLE IV

COMPARISON OF RESULTS OBTAINED BY SUBSTITUTING OTHER AMINO ACIDS FOR TRYPTOPHAN IN THE BASAL MEDIUM

	Zone diameters in mm.									
	Pyridoxine, μg./ml.					Pyridoxamine, μg./ml.				
	0·125	0·25	0·50	1·0	2·0	0·125	0·25	0·50	1·0	2·0
Tryptophan	24·3	26·9	29·4	31·9	34·4	24·8	27·3	29·8	32·5	**34·9**
Asparagine	23·4	26·4	29·1	31·9	34·4	20·8	24·5	26·1	28·6	**31·1**
Histidine	24·7	27·4	29·7	32·3	34·7	21·4	24·1	26·8	29·4	**31·9**
Arginine	23·2	25·7	28·2	30·7	35·2	20·9	23·2	26·0	28·4	**30·9**
Lysine	24·0	26·5	29·0	31·5	34·0	20·6	23·7	26·4	28·9	**31·4**
Phenylalanine	26·2	29·0	31·3	33·8	36·2	24·1	26·5	29·3	31·7	**34·7**
Valine	26·7	29·4	31·6	34·4	37·0	24·2	27·1	29·7	32·2	**34·8**
Serine	25·5	28·5	31·2	33·7	35·9	22·5	25·2	28·1	30·6	**33·3**
Aspartic acid	27·0	30·1	32·2	34·4	36·9	23·7	26·5	29·1	31·8	**34·1**
Glutamic acid	25·6	28·2	30·9	33·5	36·0	22·8	25·6	28·2	30·6	**33·6**
Tyrosine	26·5	29·0	32·1	34·5	36·6	23·3	25·9	28·4	30·9	**33·4**
Cystine	26·2	29·0	31·9	34·1	36·8	23·3	26·4	28·8	31·3	**33·8**

In Table IV are shown the results obtained by substituting other amino acids for tryptophan in the basal medium. In some experiments, the zone diameters obtained both with pyridoxine and with pyridoxamine were greater than those with the tryptophan medium. But in none was the zone diameter the same for pyridoxine and pyridoxamine; that for pyridoxine was always the greater.

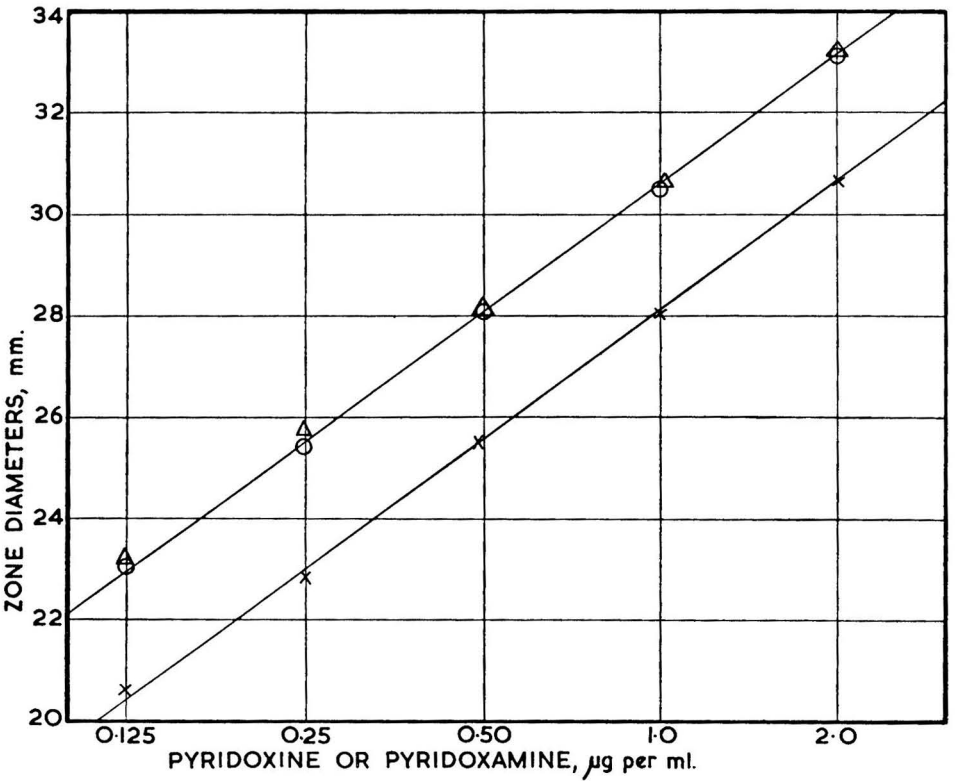

Fig. 1. Dose response curve for pyridoxine and pyridoxamine on basal media with and without tryptophan

○ = Pyridoxine on basal medium with or without tryptophan
× = Pyridoxamine on basal medium without tryptophan
△ = Pyridoxamine on basal medium with tryptophan

With certain amino acids, rather irregular increases in zone diameters with increasing levels of pyridoxine and pyridoxamine were found.

An enzymatically-prepared casein digest was substituted for the acid-hydrolysed casein, the nicotinic acid and calcium pantothenate contents of the medium were increased to 3·5 μg. per ml., five different samples of agar were used and finally the medium was used at double strength. On no occasion was the pyridoxamine response curve identical with those for the other vitamin B_6 members, and assays of yeast gave low values.

In this connection, it is interesting to note that Rabinowitz and Snell,[10] using the original yeast assay technique, found that the activity of pyridoxamine, in terms of pyridoxal or pyridoxine, was not constant over the assay range used. In these experiments the assay range was low, being 0·002 to 0·2 μg.

METHODS OF EXTRACTING VITAMIN B_6 FROM YEAST—

In a series of experiments investigating methods for the extraction of vitamin B_6 from yeast for assay purposes, Rabinowitz and Snell[11] found that the most effective method was to autoclave the yeast at 20 lb. pressure for 5 hours with 0·055 *N* hydrochloric acid. When 2 *N* acid was substituted for 0·055 *N*, lower results were obtained and could be increased by a second digestion with 0·055 *N* acid. Digestion with clarase also gave maximum extraction of vitamin B_6.

For the plate-assay technique, the minimum necessary amount of vitamin B_6 is much greater than for the method of Atkin, Schultz, Williams and Frey. For this reason the extraction of yeast samples containing 2 μg. of vitamin B_6 in 180 ml. of 0·055 *N* acid, as advocated by Rabinowitz and Snell, was not feasible. It was necessary to investigate other extraction methods. The results are given in Table V.

TABLE V

METHODS OF EXTRACTION OF VITAMIN B_6 FROM YEAST

	Extraction method	Extraction rate for: Medium without tryptophan, μg. per g.	Extraction rate for: Medium containing tryptophan, μg. per g.
Yeast 449	2 *N* HCl autoclaved for 5 hours at 20 lb.	20·3, 20·1, 23·3	23·0, 24·7, 23·9, 23·9, 22·9, 26·8, 28·2, 26·6, 28·0
	3 *N* HCl autoclaved for 5 hours at 20 lb.	18·8	—
	4 *N* HCl autoclaved for 5 hours at 20 lb.	19·2	—
	0·3 g. of takadiastase	—	28·8, 29·9, 29·8, 30·1, 29·8, 29·8, 29·8
Yeast Y	2 *N* HCl autoclaved for 5 hours at 20 lb.	18·9	19·7, 20·1, 25·2, 22·0, 22·8
	Takadiastase—		
	0·05 g.	6·8	17·9, 20·1
	0·10 g.	10·2	20·9
	0·15 g.	12·0	24·0
	0·20 g.		24·4
	0·30 g.	12·6, 14·9, 15·7, 15·1, 16·8	24·4, 23·8, 20·5, 20·5, 21·7, 20·5, 24·4, 24·0
	0·50 g.		24·4

By using takadiastase, higher values were obtained than by autoclaving the yeast for 5 hours with 2 *N* acid. Although acid treatment yielded values as high as those obtained with takadiastase in some assays, this did not occur regularly.

Different samples of takadiastase appeared to vary in their ability to release vitamin B_6 from yeast. For all samples tested, however, 0·3 g. of the enzyme was found to be adequate. The vitamin content of the takadiastase was always less than 0·2 μg. per g., it being impossible to assay this amount by either the plate or the mould technique.

It appeared possible that the lower values recorded after hydrolysis with 2 *N* acid were due to the excessive amount of sodium chloride present after neutralisation of the acid. The possible inhibitory effect of salt on *S. carlsbergensis* was studied, using a standard solution of pyridoxine and a takadiastase digest of yeast. Sodium chloride was added to these solutions to give final concentrations of 2·5, 5 and 10 per cent. On assay there were noted no differences in zone diameters from those obtained with the normal standard or yeast to which salt had not been added. It would appear that concentrations of sodium chloride up to 10 per cent. have no inhibitory action on the growth and activity of *S. carlsbergensis* on the solid medium.

THE EFFECT OF THE AGE OF THE CULTURE OF *S. carlsbergensis*—

In the assay of vitamin B_6 using *Neurospora sitophila*,[1] the mould was found to retain its activity up to at least 5 months. On the other hand, *Streptococcus faecalis* cultures, after sub-culturing on certain media, partly failed to respond to folic acid.[9]

For this reason it was decided to study the effect of the age of the *S. carlsbergensis* culture on the assay of vitamin B_6. Cultures on malt - agar were tested over a period of 5 months.

From the results given in Table VI it will be seen that, with cultures older than 2 weeks, the growth zones became progressively larger and less distinct. With cultures older than 2 months, no growth zones were obtained.

TABLE VI

EFFECT OF AGE OF CULTURE USED IN ASSAY

Age of culture	Zone diameters at concentrations of pyridoxine, μg./ml., of 0·125, mm.	0·25, mm.	0·50, mm.	1·0, mm.	2·0, mm.	Remarks
1 week	24·5	27·0	29·5	32·0	34·5	Sharp zones
2 weeks	24·5	27·0	29·6	32·0	34·5	
1 month	27·2	29·8	32·1	34·6	37·1	Zones not so sharp
2 months	25·0	29·0	36·0	39·0	44·0	Zones diffuse and irregular
3 months, 4 months	No zones of growth—only slight diffuse growth around the holes					

THE USE OF TAKADIASTASE FOR THE EXTRACTION OF VITAMIN B_6 IN THE ASSAY OF VITAMIN B_6 BY USE OF *Neurospora sitophila*—

It will be seen from Table III that, with the majority of yeasts, the mould method gave lower results than those found with *S. carlsbergensis*. Since takadiastase appeared to release maximum amounts of vitamin B_6 with the yeast-assay technique, it was decided to investigate the effect of this treatment on the results with *Neurospora sitophila*. In the latter method it was essential to destroy all the aneurine before assaying vitamin B_6. Three yeasts were treated for 18 hours with takadiastase and then sodium hydroxide was added to the mixture to give a final concentration of 4 per cent. The alkaline solution was autoclaved at 15 lb. pressure. The results are given in Table VII.

TABLE VII

COMPARISON OF VITAMIN B_6 ASSAY VALUES BY USE OF *Neurospora* OR *S. carlsbergensis*

	Assay and treatment: *Neurospora*; NaOH only. Vitamin B_6, μg. per g.	*Neurospora*; takadiastase followed by NaOH. Vitamin B_6, μg. per g.	*S. carlsbergensis* (plate assay); takadiastase. Vitamin B_6, μg. per g.
Yeast A	21·3	21·8	29·0
Yeast C	20·8	22·5	24·7
Yeast 449	18·3	23·9	29·7

Although treatment with takadiastase appears to increase the amount of vitamin B_6 found, the increase is very small and the results obtained were still below those found with *S. carlsbergensis*. It was possible that the treatment with sodium hydroxide had destroyed part of the vitamin B_6, although it is known[1,12] that pyridoxine, pyridoxal and pyridoxamine and their phosphates are unaffected by this treatment. Further evidence to support the view that alkaline hydrolysis may destroy a fraction of the vitamin comes from the results obtained after hydrolysis of yeast with sodium hydroxide followed by acid hydrolysis, or *vice versa*. There was no increase in the amount of vitamin B_6 found by these treatments.

To confirm this view, the effect on two yeasts of alkaline hydrolysis following treatment with takadiastase was observed, with *S. carlsbergensis* as the test organism. From the results, given in Table VIII, it would seem either that the *Neurospora* assay measures an entity that does not affect *S. carlsbergensis* in the plate assay, and is not destroyed by sodium hydroxide,

TABLE VIII

COMPARISON OF VITAMIN B_6 CONTENT OF YEASTS BY THE PLATE METHOD BEFORE AND AFTER TREATMENT WITH SODIUM HYDROXIDE

	Takadiastase treatment alone	Takadiastase followed by *N* NaOH
Yeast 449	29·7	16·6, 10·0
Yeast C	24·7	16·0

or, more probably, that an inhibiting agent for *S. carlsbergensis* is formed during treatment with sodium hydroxide. So far, it has been difficult to show which is correct. In the *Neurospora* assay, the aneurine must be removed without destroying part of the vitamin B_6 group, and in the plate assay, the higher level of testing limits the use of efficient extraction methods.

In a preliminary series of experiments, using a casein solution treated with sodium hydroxide as diluent for pyridoxine, pyridoxal and pyridoxamine at a high level (0·4 g. of treated casein per flask) in the *Neurospora* assay, growth appeared to be restricted—especially so with pyridoxamine, less so with pyridoxal, and least of all with pyridoxine. However, when the casein solution was added in smaller amounts corresponding to the protein level of yeast present in an assay, no effect upon the growth of *Neurospora* was found. But the same high levels added in the *S. carlsbergensis* plate assay appeared to have no effect.

Conclusions from results

From the results of the plate-assay technique with *S. carlsbergensis*, it appears that a satisfactory method has been devised, having a precision closely approaching that of the original yeast method of Atkin and his associates. The advantages of the plate method are that no specialised apparatus is necessary and that measurements of growth zones are more easily made and are as accurate as by turbidity measurements. It has the advantage over the *Neurospora* method of being much more rapid. That the results obtained with the plate technique are generally higher than with *Neurospora sitophila* is a finding discussed below.

In the plate-assay method, the effect of tryptophan on the assay of pyridoxamine is most marked. Tryptophan was found to be an essential growth factor in the assay of the vitamin B_6 complex using *Saccharomyces cereviseae*.[13] No distinction was made between the various members of the complex and it is probable that, had this been done, results similar to those recorded in the present paper would have been obtained. It is peculiar, however, that no such finding was noted by Snell and the various workers[8,10,14] who have used the original yeast method. In this connection the possibility exists that, owing to the very low assay level of the original method, sufficient tryptophan was present in the basal medium for assay purposes. On the other hand, Snell found that the pyridoxamine and pyridoxal growth - response curves were dissimilar, and this dissimilarity may be the expression, at the low level of assay, of a tryptophan deficiency.

In the present work, the effect of tryptophan appears to be highly specific in that absence of it affects only the utilisation of pyridoxamine by *S. carlsbergensis*. Further work is clearly essential to determine the exact role of tryptophan in the metabolism of pyridoxamine and whether the effect is specific for tryptophan.

Attention must be drawn to the marked discrepancy, which was found with some of the yeasts, between the results obtained by the mould and the yeast assay techniques. With certain yeast samples assayed, the mould, *Neurospora sitophila*, yielded results down to 30 per cent. lower than the yeast *S. carlsbergensis*. It has been shown[1] that pyridoxine, pyridoxal and pyridoxamine are equally effective for the growth of *Neurospora sitophila*. Further, the phosphates of pyridoxal and pyridoxamine are released from yeast by treatment with *N* sodium hydroxide, as prior treatment of the yeast with acid, known to release the phosphates, followed by treatment with alkali, does not yield higher results. It appears possible that the difference in the results obtained with the mould and yeast is due to the presence of a fourth member in the vitamin B_6 complex.

It seems that certain yeasts do not contain this factor in measurable quantities. Whether it is related to the known members of the vitamin B_6 complex cannot yet be shown. In this connection it is interesting to note that Melnick, Hochberg, Himes and Oser[15] mentioned the possible existence of a fourth factor in the B_6 complex, but it was shown that the idea arose from a faulty extraction of the complex.

Further work is being carried out with special reference to the extraction of this factor and to its effect on the growth and development of vertebrates. Preliminary chromatographic analyses confirmed Snell's thesis that most of the vitamin B_6 of yeasts was present as pyridoxamine. No trace of a possible fourth factor was noted when butanol - acetic acid was used as extractant.

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Discussion

Mr. J. S. Harrison and Mr. S. A. Price both expressed surprise at the statement that there was no loss on treatment with sodium hydroxide. Mr. Harrison had observed losses in the *Saccharomyces carlsbergensis* method of assay.

Mr. A. L. Bacharach said that surely there must be a more elegant method for removal of aneurine. What was the effect of nitrous acid?

Mr. S. A. Price wondered whether a thiaminase, *e.g.*, from carp viscera, could be used for removal of aneurine from extracts prepared by acid hydrolysis.

Dr. Morris said that vitamin B_6 in yeast was mainly in the form of pyridoxamine, which would be destroyed by nitrous acid.

Picrolonates and Calcium
Semi-Micro Volumetric Determination with Cetyl Pyridinium Bromide

By C. C. WASHBROOK

Synopsis—Picrolonates are determined by titration with cetyl pyridinium bromide solution by two partition methods, methylene blue and bromophenol blue being used as indicators. In the first method, the methylene blue - picrolonic acid complex is extracted by shaking with a mixture of nitrobenzene and trichloroethylene (1 : 4). On subsequent titration with cetyl pyridinium bromide solution the blue dye returns to the aqueous phase when the layers are shaken together. Complete transfer indicates the end-point. In the second method the aqueous picrolonate solution is titrated with cetyl pyridinium bromide in the presence of bromophenol blue and chloroform. The formation of a blue dye complex, soluble in chloroform, indicates the end-point. The titrations are stoicheiometric and the methylene blue procedure is recommended for the determination of calcium. This determination is made by the back titration of lithium picrolonate solution after precipitation and removal of the sparingly soluble calcium picrolonate.

Many of the methods available for the micro and semi-micro volumetric estimation of calcium depend to a greater or less degree on specialised manipulative techniques. These are not readily adaptable to routine work by operators unskilled in microchemical techniques nor suitable for use in laboratories without specialised equipment. A simple method that is free from these disadvantages has been described by Bolliger.[1] It is based on the back

titration of lithium picrolonate with methylene blue solution after precipitation of the sparingly soluble calcium picrolonate by addition of the corresponding lithium salt. The methylene blue - picrolonic acid complex is removed by extraction with chloroform, the end-point being indicated by the chloroform extracts becoming colourless. Experimental work has shown that this procedure is tedious and lengthy. A detailed investigation of the titration procedure has been made by Cohn and Kolthoff,[2,3] who devised suitable conditions for amperometric titration. A rapid and simple titration procedure has now been devised which is an improvement on the visual technique; it is based on the following considerations.

The ability of certain anionic surface-active substances to form chloroform-soluble complexes with methylene blue has been demonstrated by Jones,[4] and Auerbach[5] has described complexes, soluble in chloroform, formed by the union of bromophenol blue with paraffin chain cation-active substances. Simple methods for the direct titration of dilute solutions of anion-active substances with dilute solutions of cation-active substances have been described by Epton,[6] and by Barr, Oliver and Stubbings.[7] These methods use partition end-points in a mixture of chloroform and aqueous solution, methylene blue or bromophenol blue being used as indicator. Thus, chloroform solutions of the methylene blue anion-active complexes can be titrated with aqueous cetyl pyridinium bromide solution with consequent liberation of the methylene blue. Similarly, aqueous solutions of anion-active substances can be titrated with aqueous cation-active solutions in the presence of bromophenol blue, the end-point being indicated by the formation of the blue cation-active substance with the bromophenol blue.

Picrolonic acid and its salts form methylene blue complexes which are similarly soluble in organic solvents[8] and it has been found that a chloroform solution of the complex is decomposed by titration with an aqueous solution of cetyl pyridinium bromide. A superior solvent, however, is a mixture of nitrobenzene and trichloroethylene, which removes the dye complex in a single extraction; as this solvent separates readily from aqueous solutions, its use enables titrations to be carried out rapidly and the former tedious extraction procedure is avoided.

Two alternative procedures have been devised. In the first, the aqueous picrolonic acid solution is treated with an aqueous solution of methylene blue hydrochloride, and the dyestuff complex is separated by partition with the solvent. The mixture is titrated with aqueous cetyl pyridinium bromide solution and when sufficient has been added, the dyestuff is liberated and is transfered to the aqueous layer when the two phases are shaken together. The end-point, which is observed in the solvent phase, is indicated by the complete transfer of methylene blue to the aqueous phase. Detection of the transfer of the last traces of methylene blue may be aided by the addition of a small quantity of an oil-soluble red dyestuff to the solvent phase. In the second procedure an aqueous solution of picrolonic acid is titrated with cetyl pyridinium bromide solution to form a complex which is extracted with chloroform as a yellow solution. The end-point of the titration is then determined in the manner used by Barr *et al.*,[7] with bromophenol blue as the indicator. Thus, excess of cetyl pyridinium bromide forms a complex with the bromophenol blue and passes into the chloroform layer which then becomes green.

Determination of picrolonic acid

Reagents—

Picrolonic acid—0·002 *M* aqueous solution. Prepared from the "Spot Test" grade by recrystallisation from benzene containing a trace of methyl alcohol.

Cetyl pyridinium bromide—0·001 *M* or 0·005 *M* aqueous solution. Prepared from Fixanol C (Imperial Chemical Industries, Ltd.) twice recrystallised from methyl-ethyl ketone.

Methylene blue indicator—Methylene blue, zinc-free salt, 0·5 per cent. alcoholic solution, 6·0 ml.; 2 *N* sulphuric acid, 120 ml.; sodium sulphate, anhydrous A.R., 50 g.; distilled water to 1 litre.

Bromophenol blue indicator—Bromophenol blue, 0·1 per cent. alcoholic solution, 20 ml.; sodium sulphate, anhydrous A.R., 50 g.; sodium carbonate, anhydrous A.R., 5·0 g.; distilled water to 1 litre.

Solvent (*a*)—A mixture of trichlorethylene, 75 ml., freshly distilled nitrobenzene, 20 ml., and oil-soluble red dyestuff, colour index No. 258, 0·01 per cent. solution in trichlorethylene, 5·0 ml.

Solvent (*b*)—Chloroform B.P.

Method A

With methylene blue as the indicator—

Transfer a suitable volume of the picrolonic acid solution to a chemically-clean stoppered reagent bottle (all traces of any detergent used for washing must be removed); add 20 ml. of methylene blue solution, and 20 ml. of solvent. Shake the bottle vigorously with a rocking motion of the hand and then stand to allow the two phases to separate. At this stage the solvent should be a deep blue colour and the aqueous phase should be almost colourless. Titrate the mixture with cetyl pyridinium bromide solution; shake the mixture vigorously after each addition and stand to allow the phases to separate. As the end-point is approached the solvent layer becomes pale green and the methylene blue is concentrated in the aqueous layer; the end-point is indicated by a colour change from pale grey-green to amber in the solvent phase. Carry out a preliminary titration adding the cation-active solution in 1-ml. stages, followed by a second titration in which additions of 0·1 ml. or less are made as the end-point is approached.

Method B

With bromophenol blue as indicator—

Transfer the picrolonate solution to the stoppered reagent bottle, add 20 ml. of indicator solution and 20 ml. of chloroform. Titrate the mixture with cetyl pyridinium bromide solution in the manner described for use with methylene blue. The end-point is indicated by a change of colour of the chloroform phase from yellow to pale green; further additions of cation-active solution intensify the green colour.

Stoicheiometry of the titrations—Experimental work has indicated that the relationship between picrolonic acid and cetyl pyridinium bromide is virtually stoicheiometric. In this investigation a purified sample of picrolonic acid was used for checking the relationship between cetyl pyridinium bromide and picrolonic acid. A solution of the picrolonic acid of exactly 0·002 *M* concentration was prepared and its strength was assessed by two methods, *viz.*, (*a*) titration with carbonate-free sodium hydroxide using phenolphthalein indicator, (*b*) titration with cetyl pyridinium bromide by means of the two procedures described above. The titrations were made in replicate with 0·005 *M* cetyl pyridinium bromide and 0·005 *M* sodium hydroxide in aqueous solutions.

Table I

Concentration of 0·002 *M* picrolonic acid solution

	Titration with cetyl pyridinium bromide						Titration with Sodium hydroxide	
	(*a*) Methylene blue			(*b*) Bromophenol blue				
Picrolonic acid titrated, ml. ..	10·0	10·0	10·0	10·0	10·0	10·0	80·0	100·0
Titre, ml.	4·43	4·43	4·43	4·28	4·30	4·28	27·5	34·3
Concentration titrating solution $\times 10^{-3}$ *M*	4·63			4·63			5·807	
Picrolonic acid concentration $\times 10^{-3}$ *M*	2·05	2·05	2·05	1·98	1·98	1·98	1·98	1·99

Table I shows that the bromophenol blue method gives results that are strictly stoicheiometric and the methylene blue method gives results approximately 3 per cent. higher. These findings are comparable with those of Barr *et al.*,[7] who found that in determinations of anionic surface-active agents the methylene blue technique gave results varying from 102 to 106 per cent. of those obtained with the bromophenol blue method. They attributed this divergence to the necessity of establishing a minimum concentration of cation-active material by partition in the solvent phase before release of methylene blue occurs.

In the procedure described by Epton,[6] methylene blue is used as the indicator, and the end-point is taken when the dye concentration in each phase is equal. This technique is stated to give strictly stoicheiometric results, but it was not readily applicable to this investigation because of the differing hues of the two phases.

Reference standards—In the work described by Barr *et al.*,[7] highly purified anion-active substances, *e.g.*, potassium *p*-benzyl-*o*-sulphonate, were used to standardise the cetyl pyridinium bromide solution. For the present investigation the procedure described by Epton[6] has been used, whereby the cetyl pyridinium bromide solution is standardised against

potassium dichromate using sodium thiosulphate as an intermediate. The cetyl pyridinium bromide solution is treated with excess of potassium dichromate solution of a suitable concentration and the mixture is heated to coagulate the insoluble quaternary dichromate. This is removed by filtration and the concentration of dichromate in the filtrate determined iodimetrically.

The present work has shown a strictly stoicheiometric relationship between picrolonic acid and cetyl pyridinium bromide when the bromophenol blue titration procedure is used. Picrolonic acid is therefore an acceptable primary substance for the standardisation of cetyl pyridinium bromide solutions. Hence, the bromophenol blue technique forms a direct and analogous procedure for standardisation when the halide solution is to be used for partition titrations.

Determination of calcium

The titration procedures described above have been applied to the estimation of calcium with picrolonic acid. The general technique using lithium picrolonate described by Bolliger[1] has been used, and the concentration of the picrolonate has been determined by titration with cetyl pyridinium bromide. Experience has shown that for this application the methylene blue procedure is to be preferred, as the approach of the end-point is clearly indicated, and if necessary the determination may be made with a single titration. The higher values given by this procedure do not cause erroneous results for calcium, because the determination is based on the difference between two titrations. The interference of the alkali metals with the picrolonate procedure has been thoroughly investigated by Cohn and Kolthoff[3]; and the calcium solution should therefore be treated either to eliminate or reduce the concentration of alkali metal ions before the determination is made. Barium, strontium, lead, copper, thorium, iron, manganese, zinc and some organic bases form sparingly soluble picrolonates and should be absent from the test solution.

Method

Reagent—

Lithium picrolonate—0·05 *M* aqueous solution.

Procedure—

Adjust the concentration of the neutral calcium solution so that it does not exceed 40 mg. of calcium per litre. Add 2·00 ml. of lithium picrolonate solution to 25 ml. of calcium

Table II

Determination of known amounts of calcium by titration with 0·00464 *M* cetyl pyridinium bromide

Concentration of calcium solution $\times 10^{-3}$ *M*	Calcium taken, mg.	Calcium recovered, mg.	Volume of solution titrated, ml.	Titre difference, ml.	Error, %
1·04	1·04	1·01	5·0	2·02	−2·9
	1·04	1·02	5·0	2·04	−1·9
0·842	0·83	0·83	5·0	1·67	0
	0·83	0·82	5·0	1·64	−1·2
	0·81	0·81	5·0	1·63	0
0·886	0·65	0·66	5·0	1·33	+1·5
	0·65	0·65	5·0	1·30	0
0·510	0·49	0·49	10·0	1·93	0
	0·49	0·48	10·0	1·88	−2·1
	0·49	0·48	10·0	1·86	−2·1
0·328	0·31	0·31	15·0	1·86	0
0·166	0·16	0·16	15·0	0·93	0

solution, stopper the flask and allow to stand overnight in a refrigerator at 35° F. Immediately after removal from the refrigerator, filter the solution through a No. 3 sintered-glass filter-stick. Allow the filtrate to return to room temperature and remove an aliquot portion for titration by the methylene blue technique previously described. Simultaneously carry out a blank determination in which 25 ml. of distilled water are used instead of the calcium

solution. The difference between the blank and test titres is equivalent to the amount of calcium present.

The determination of known quantities of calcium was made by taking aliquot portions of a standard solution of calcium prepared by dissolving A.R. calcium carbonate in dilute acetic acid. The new technique was compared with the original Bolliger[1] titration procedure.

TABLE III

COMPARISON OF THE CETYL PYRIDINIUM BROMIDE TITRATION WITH BOLLIGER'S METHOD

Calcium taken, mg.	C.P.B. method Titration with C.P.B. Calcium recovered, mg.	Bolliger's method Titration with methylene blue Calcium recovered, mg.
1·00	0·99	1·01
1·00	1·00	0·99
0·63	0·63	0·64
0·63	0·63	0·63
0·80	0·81	0·80
0·80	0·80	0·79
0·49	0·49	0·49
0·16	0·16	0·16

The method has been applied to the determination of calcium in used lubricating oil. The oil is ashed and the ash is suitably treated to remove interfering metals using the A.S.T.M. procedure.[9] An alternative method involves the precipitation of lead and barium as sulphates followed by extraction of zinc, copper and iron as thiocyanates in amyl alcohol. This alternative method is to be described separately at a later date.

The conditions that usually apply necessitate the determination of amounts of calcium varying between 0·2 and 1 mg., and the method has been devised for this range. Typical values obtained and the effect of addition of specific quantities of calcium are shown in Table IV.

TABLE IV

DETERMINATION OF CALCIUM IN USED LUBRICATING OIL

Sample No.	Oil taken, g.	Calcium added, mg.	Calcium found, mg.	Calcium found, %
1	5·00	nil	0·32	0·0064
—	—	0·20	0·51	—
—	—	0·40	0·73	—
2	5·00	nil	0·54	0·011
—	—	0·30	0·84	—
—	—	0·50	1·02	—
3	5·00	nil	0·93	0·019
—	—	0·10	1·02	—
—	—	0·050	0·99	—

The errors involved are of the same order as those observed by Cohn and Kolthoff[2] and by Bolliger,[1] who recorded a maximum error of 3 per cent.

CONCLUSIONS

Picrolonic acid forms a complex with a paraffin chain cation-active substance, cetyl pyridinium bromide, that is soluble in both chloroform and a mixture of nitrobenzene and trichlorethylene.

Solutions of picrolonic acid and picrolonates can be directly titrated with cetyl pyridinium bromide solutions, with methylene blue or bromophenol blue as indicators in partition end-points.

The bromophenol blue titration procedure gives strictly stoicheiometric results, but the methylene blue method gives results approximately 3 per cent. too high.

The methylene blue method is conveniently applied to the volumetric semi-micro determination of calcium with lithium picrolonate. It is preferable to the bromophenol blue technique for this purpose, the approach of the end-point being more easily observed. This procedure is more convenient than the older extraction method.

Picrolonic acid is a convenient primary substance for standardisation of cetyl pyridinium bromide solution when the bromophenol blue titration procedure is used.

Acknowledgment is made to the Chief Scientist of the Ministry of Supply for permission to publish this communication.

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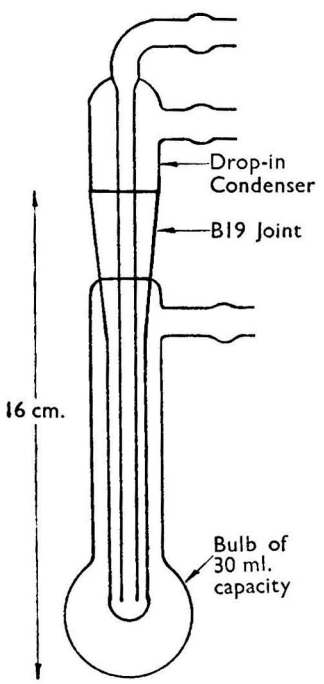
Ministry of Supply
Chemical Inspectorate

February, 1950

Notes

PREPARATION OF *p*-NITROBENZYL DERIVATIVES OF BARBITURIC ACIDS ON A SEMI-MICRO SCALE

Barbituric acids extracted from viscera during toxicological examinations are often exceedingly difficult to identify, because (*a*) they will not crystallise readily, *e.g.*, 5-allyl-5-*sec*amyl barbituric acid (seconal), and (*b*) they are not sufficiently pure to give good melting-points. Very often neither repeated vacuum sublimation, nor charcoal purification will give adequate purity. Then one course is the preparation and subsequent identification of some derivative from the barbituric acid. The *p*-nitrobenzyl derivatives seem best for this purpose as their melting-points, in general, are well spaced in the range 100° to 250° C.



However, the literature[1,2] describes their preparation on a large scale. About 0·5 g. is taken and the derivative isolated by washing with water, to free from inorganic material, followed by alcohol, to free from surplus *p*-nitrobenzyl chloride or bromide.

Owing to the solubility of the derivatives themselves in alcohol, the starting quantity cannot in general be reduced below 0·25 g. Unfortunately, quantities as large as this are rarely found during toxicological examinations.

Improved yield of derivative has been found to result by removal of the surplus *p*-nitrobenzyl chloride or bromide by vacuum sublimation followed by leaching of the derivative from the residual inorganic material.

The apparatus shown is useful for performing nearly all the steps in one vessel, thus avoiding losses due to transference.

Procedure—Heat the sodium salt of the acid with an alcoholic solution of *p*-nitrobenzyl chloride or bromide under reflux for 1 hour, with the condenser in and the side tube open. Then, with the condenser out and the side tube still open, dry the resulting mixture. Replace the condenser, connect the side tube to the vacuum line and carry out complete vacuum sublimation at 100° C. of the *p*-nitrobenzyl chloride or bromide. Leach out the residue with hot dry chloroform, filter the chloroform solution, and evaporate the filtrate to dryness. Recrystallise the derivative from a suitable solvent such as alcohol or an alcohol - benzene or alcohol - light petroleum mixture.

This method has been found to yield adequate amounts for identification with starting quantities of about 10 mg.

The writer is indebted to Mr. E. B. Parkes, Director of this laboratory, for permission to publish.

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April, 1950

ESTIMATION OF BROMIDES BY AN ACTIVATION - EXCHANGE METHOD

In the method of activation analysis,[1] the sample is bombarded by suitable particles and one or more of the original elements are identified or determined by the radiation characteristics of the unstable nuclides to which they give rise. The analysis of mixtures may be very difficult because of the complex nature of the decay schemes involved; their analysis requires many observations over a long period of time. This difficulty may be partly met by the addition of a carrier followed by separation and specific activity measurements of the radio-nuclide being determined. This separation may be tedious and unreliable on account of such dangers as co-precipitation of contaminating radio-activities out of all proportion to the chemical contamination.

If, however, the radio-nuclide A* to be determined, *e.g.*, radio-active bromide produced by the neutron activation of natural bromide in the Pile, will exchange isotopically with an added compound RA, *e.g.*, methyl bromide, which is a gas at room temperature and is therefore easily separated, then these difficulties are obviated. The exchange may be represented—

$$\underset{\substack{\text{(Unknown weight}\\ \text{to be determined)}}}{\text{A*}} + \underset{\substack{\text{(known weight}\\ \text{added)}}}{\text{RA}} \rightleftharpoons \text{RA*} + \text{A}$$

The asterisk denotes radio-active material. The experimental procedure for bromide in a mixture of salts is then as follows.

Procedure—Irradiate a known weight of the mixture (*e.g.*, 100 mg.) in the Pile at a known flux for a given time. On removal from the Pile, transfer the sample to a flask fitted with a head and vacuum tap, together with an ampoule containing a known weight of pure methyl bromide (*e.g.*, 500 mg.) and 1 ml. of aqueous acetone, which serves as a common solvent. Partly evacuate the flask, close the tap, break the ampoule and heat the mixture at 100° C. to facilitate exchange. Then connect the flask to a similar flask that has been evacuated. Open both taps so that some of the methyl bromide, which will now be radio-active according to the bromide content of the original sample, is drawn into the second flask. Decompose the methyl bromide sample by drawing a suitable reagent (*e.g.*, monoethanolamine) into the second flask, precipitate the bromide formed as silver bromide after acidification and measure its radio-activity under a thin end-window Geiger counter. When the ratio RA/A is large, it can be shown that if w be the weight in grams of element giving rise to A* under bombardment, then,

$$w = \frac{1{\cdot}67.a.d.e^{\lambda t}}{F.\sigma.\theta.(1 - e^{-\lambda t'})(1 - e^{-Kt''})}$$

where a is the atomic weight of the element being assayed, d the observed number of disintegrations per second due to A* in a separated fraction θ of the total weight of compound RA with which the sample was allowed to exchange after bombardment, λ is the disintegration constant in reciprocal seconds, t is the time in seconds between determining d and removing the sample from the target area, t′ is the irradiation time, t″ is the time allowed for exchange, F is the effective particle flux in particles per sq. cm. per second, σ is the particle capture cross-section in barns for the formation of A*, and K is the exchange velocity constant in reciprocal seconds. F and σ may not be accurately known in practice, in which case a second sample containing a known added amount of the required element is bombarded under identical conditions and used as a reference. K need not be known accurately provided the exchange is allowed to proceed to virtual equilibrium.

Starting with known concentrations of bromide (17 p.p.m.) in tobacco leaf the method has

been applied alternatively to the determination of K for the exchange between the bromide-82 formed by direct neutron irradiation of an untreated sample of the leaf and the bromine of pure methyl bromide subsequently applied; 8·2 and 28·0 per cent. respectively of the bromine-82 found had exchanged at 20° C. and at 35° C. respectively in 24 hours. Other experiments indicated that the exchange between radio-active bromides (and iodides) and the corresponding inactive methyl halides was virtually complete within an hour or so at 100° C. in acetone. The method appears therefore to be reasonably rapid, specific and sensitive; *e.g.*, assuming an available neutron flux of 5×10^{11} neutrons per sq. cm. per second it would be possible to determine 10^{-8} g. of bromine in the presence of any amount of the other halogens to within a few per cent. with typical counting equipment. The method should be similarly applicable to the ultra-micro determination of iodine and chlorine by making use of the Cl^{37} (n, γ) Cl^{38}, and the I^{127} (n, γ) I^{128} reactions, but then the assays would have to be completed within a few hours of removal from the Pile because of the short half-lives of these isotopes.

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F. P. W. WINTERINGHAM

February, 1950

THE DECIPHERING OF CHARRED DOCUMENTS

A PAPER by Grant[1] summarised the methods available for deciphering charred documents. Some further observations may be of interest to those who have to decipher documents damaged by fire.

The charred remains are carefully "painted" with a 25 per cent. solution of gum arabic and allowed to dry, after which it is lightly coated, by the aid of a soft camel hair brush, with a white paint made by ball-milling pure titanium oxide in 25 per cent. gum arabic solution. When dry, the document should appear a uniform light grey or white. It is then placed in a desiccator containing chlorine or bromine vapour, in heavy concentration, for about 30 minutes, after which it is removed and set aside in moist air for an hour. A "gassing" in a desiccator containing ammonium sulphide will cause the development of any iron left as residue of iron gall ink, and if the writing so produced is only faint, the bromine and moist air treatments should be repeated until a satisfactory development is obtained when the document may be photographed. If a permanent reproduction on the document is desired, the final ammonium sulphide treatment may be omitted and the document lightly "painted" with a 5 per cent. solution of potassium ferrocyanide containing 2 per cent. nitric acid, when the remains of iron gall ink writing develop on the pale background.

The effect of iron in the paper due to loading materials, etc., is reduced to a minimum and the contrast between the writing and the background increased to a maximum by this process.

A modification of Murray's method[2] has proved very successful with printing, typing, carbon copies, iron gall writing inks and lithographic prints. In the modified method, the 5 per cent. silver nitrate solution is treated with ammonia until the precipitate first formed is dissolved, and the charred or incinerated document is immersed in this solution, which is held at a temperature between 80° and 95° C. The actual temperature between these limits does not appear to be critical for obtaining good results. Within a few minutes a silvery outline of the printed, typed or written matter appears on the darker background of the rest of the document, or the writing, etc., may appear black on a silvery background. Whether the writing will appear as silver on black, or black on silver, appears unpredictable and fortuitous; in some instances, both the phenomena appear in different parts of the same document.

The reproduced matter is best read or photographed while the document is immersed under water. Address, stamp and cancellation marks, together with any printed matter, are readily reproduced on burnt envelopes and this modification appears to be more universal in its application than any so far examined, providing that the documents have been burnt with reasonably free access of air and not kept in the charred condition for more than two or three days. Documents charred whilst packed into a closed box, or those which have been lying in the charred state for more than three days have not been examined. The effect on pencil writing is very erratic; sometimes excellent results have been achieved, but on other occasions only fair or poor results have been attained.

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87, SCHOOL ROAD
TILEHURST
READING, BERKS.

J. A. RADLEY
April, 1950

ERRATUM: August (1950) issue, p. 445. Line 6 (Equation 3), *for* 1·2A — 0·069 B.I., *read* 1·2A + 0·069 B.I.

Ministry of Food

STATUTORY INSTRUMENT*

1950—No. 1430. The Chocolate, Sugar Confectionery and Cocoa Products (Amendment No. 3) Order, 1950. Price 1d.

This Order, which came into operation on 27th August, 1950, further amends the Chocolate, Sugar Confectionery and Cocoa Products Order, 1949, as amended by S.I., 1949 (Nos. 781 and 1524), II, pp. 292–305, and S.I., 1950, Nos. 190 and 766, by providing a new definition of sugar confectionery, as follows—

"Sugar confectionery" includes boiled sugar goods, toffees, panned goods, cream paste goods, liquorice, lozenges, pastilles, clear gums, chewing gum, medicated sugar confectionery, and any other goods that would be regarded in the confectionery industry as sugar confectionery; but does not include candy (or fairy) floss, any product of a description specified in the First Schedule to this Order (*The Chocolate, Sugar Confectionery and Cocoa Products Order, 1949, as amended*), or any product that would not be regarded in the confectionery industry as suitable for retail sale without further processing:

Provided that for the purposes of this definition sugar means sucrose, glucose, fructose, maltose, lactose, any starch conversion product capable of reducing Fehling's solution, or any mixture of such substances; and nothing shall be deemed not to be sugar confectionery by reason only of the fact that it does not contain any particular kind of sugar.

British Standards Institution

DRAFT SPECIFICATIONS

A FEW copies of the following draft specifications, issued for comment only, are available to interested members of the Society, and may be obtained on application to the Secretary, Miss D. V. Wilson, 7–8, Idol Lane, London, E.C.3.

Draft Specifications prepared by Technical Committee DS/13—Upholstery and Bedding Fillings.

CM(DS) 4759—Draft B.S. for Woollen Felt for Bedding Upholstery and similar purposes.
CM(DS) 4684—Revision of B.S. 1425, Cleanliness of Fillings and Stuffings for Bedding, etc.

Draft Specification prepared by Technical Committee LBC/13—Filtration Apparatus.

CM(LBC) 5402—Draft B.S. for Filters made of Sintered Materials.

* Obtainable from H.M. Stationery Office. Italics indicate changed wording.

Reviews

PHENOMENA, ATOMS, MOLECULES. By IRVING LANGMUIR. Pp. xi + 436. New York: The Philosophical Library, Inc. 1950. Price $10.00.

Dr. Langmuir's book seeks to do two things. The first is to set out the author's opinions on science and topics of related interest, such as scientific legislation, the provision of incentives to further progress in research, the limitation of the scientific method when applied to human affairs and the world control of atomic energy. The second is to present the important results of Dr. Langmuir's forty years of research, mainly by reprinting, with little alteration, his most significant papers.

The result is an uneven book, in which there is a certain amount of repetition, but also a most stimulating book, which its purchasers will value, and which many non-purchasers, who may be in this category on sheer grounds of price, will regret not having on their shelves.

To the specialist in the field of surface chemistry the book will be welcomed as a distillation of Dr. Langmuir's contribution to the subject, which is by no means solely of historic interest, though most of the work was published over twenty-five years ago. To the non-specialist, the volume will provide as good an introduction to the subject as is to be found. If some of the sections may be rather detailed for him, this will be more than offset by the clarity and simplicity of expression and thought that characterise Dr. Langmuir.

The general sections of the book, occupying one-tenth, are thought-provoking, as may best be shown by quoting some of the author's conclusions. "The net result of the modern principles of physics has been to wipe out almost completely the dogma of causation." "Human affairs are characterised by a complexity of a far higher order than encountered ordinarily in the field of science. I believe the field of application of science to such problems to be extremely limited." "In Russia they are frankly incorporating into their communist government the best features of our capitalist system, while we are tending to put into our democracy some of the worst features of communism." "I believe Russia is planning to embark on a programme of research and development greater than that contemplated by any other government."

The sections covering Dr. Langmuir's research, occupying some 350 pages, embrace a wide variety of topics in surface chemistry. His classical work on the interactions of gases with heated metal filaments, and on the adsorption of electropositive layers, such as caesium or thorium on tungsten, is described in detail. In addition, there are sections on molecular orientation in the surfaces of pure liquids, on intermolecular forces, on interactions in liquid - liquid mixtures, on monolayers adsorbed on water surfaces, on the condensation and evaporation of gas molecules, and of course on the familiar adsorption isotherm. In particular one admires the fruitful study of caesium layers on tungsten, which gave the roughness factor of the filament, the condensation coefficient of the caesium, the variation of the surface dipole with adsorbed amount and quantitative information on the surface migration of the caesium, and also showed that, except for a few atoms, the surface was uniform in adsorptive power. Altogether the reader is left with the feeling that Dr. Langmuir's work is of even greater weight than he had previously thought.

B. M. W. TRAPNELL

ELSEVIER'S ENCYCLOPAEDIA OF ORGANIC CHEMISTRY. Edited by F. RADT. Volume 12B, Part I. Series III, Carbo*iso*cyclic Condensed Compounds. Pp. xxx + 344 + index. New York and Amsterdam: Elsevier Publishing Co. London Distributors: Cleaver-Hume Press. 1948. Price £6 5s. for subscribers to the whole work.

The editorial aims of this encyclopaedia, the way in which they are being achieved and certain shortcomings were commented upon in connection with volume 13, the first to appear after the war (see *Analyst*, 1948, **73**, 53). The remarks then made still apply, save that the proposed interval of four years between literature references and publication has now been attained; as before, the text has been opened occasionally for the inclusion of additional information, especially where structure is concerned.

The present part deals with the hydrocarbons containing one naphthalene nucleus (including their hydrogenated derivatives) and the halogen derivatives (including iodoso- and iodoxy-compounds) of such hydrocarbons. A careful check on the many methylnaphthalenes has disclosed no omission of any reference of importance. The collection of references by years reveals that considerable interest in this group of compounds has been shown over the past thirty years; indeed, this interest is being maintained and more recent work will probably necessitate amendment of the data on physical properties attributed to some of the less simple naphthalene hydrocarbons.

In the accumulation of matter for a work of such expanse, occasional discrepancies in the literature are inevitably encountered. Part of the Elsevier service is to investigate these discrepancies and several reports on such have already appeared in *Rec. trav. chim.*; reprints of these are available for circulation with the appropriate volume if the results have not already been incorporated therein.

Finally, it may be added that the second part of Volume 12B, dealing with the nitrogen derivatives of the compounds appropriate to Part I, has now been published. B. A. ELLIS

MÉTHODES MODERNES D'ANALYSE QUANTITATIVE MINÉRALE. By G. CHARLOT and D. BÉZIER. Second Edition. Pp. viii + 685. Paris: Masson et Cie. 1949. Price 2000 fr.; 44s.

This is the second edition of a book first published in 1944 by Professor Charlot and his colleague of l'Ecole de Physique et de Chimie industrielles.

In the introduction, Professor Charlot states that the book has been entirely re-set in order to take note of various criticisms and suggestions that were made concerning the first edition. Its general character, however, remains the same and its declared objective is to serve as a guide rather than as an analytical treatise, something which he considers inevitable having regard to the breadth of the subject.

The book is divided into two main sections, the first headed "Généralités," comprises some 25 chapters; the second, "Dosage de Principaux Eléments," over 350 pages, gives in alphabetical order a survey of the methods available for the determination of the elements from aluminium to zirconium.

Section I covers a great deal of ground and it may be useful to itemise some of the main chapter headings. Beginning with a fairly elementary treatment of volumetric processes and techniques, the book goes on to deal with oxidimetry and oxidation - reduction processes. Chapter VI is on "Titrimétrie par formation de Complexes"; Chapter VII, "Titrimétrie par precipitation"; Chapter VIII, "Acidimétrie dans les solvants autres que l'eau." Chapter IX deals with reactions in the presence of two solvents and with extraction processes. In Chapter X there is a brief review of the apparatus and techniques of gravimetric analysis and a very short section on Sampling.

Chapter XI is a page and a half only of general discussion of the growing importance of Physical Methods, while XII and XIII deal with Absorption Spectroscopy and Emission Spectroscopy respectively. In the introduction to this edition, the authors say that they have given "une plus grande place" to instrumental methods, in particular to absorption spectrophotometry; it is therefore disappointing to find this section, and that on emission spectroscopy, so brief and superficial. Absorption in the ultra-violet and in the infra-red are each covered in six lines. Some of the information about photo-electric cells is out of date, no examples of the applications of the methods are given and there is hardly a reference to any British instrument. At the end of the chapter on Emission Spectroscopy, X-rays (presumably used for diffraction techniques) are dismissed in three lines.

Then follow chapters on Potentiometric Titration, Electrolysis, Polarography, and one on miscellaneous methods. This last comprises Conductimetry, Dielectric Constant (4 references only), Measurement of High Frequency Current, Absorption of Neutrons (1 reference), Chromatography (1 page), Capillary Analysis, Methods involving Radio-activity, Mass Spectrometry, Magnetic Permeability, Raman Spectra, and three or four more techniques, none of which receives more than a few lines. This section is almost useless and falls far short of what the authors promised.

The usefulness of the next chapter, on Organic Reagents, is increased by the references and the bibliography that are given; indeed, one of the good features of this book is the inclusion of a comprehensive bibliography and a number of references in each chapter. There is a chapter on the Properties of Precipitates and one on Separations, a short one on the Determination of Traces and a still shorter one on Semi-micro and Micro Determinations. Methods of Solution, Destruction of Organic Matter prior to Determination of Inorganic Constituents and Gas Analysis complete the first part.

In the second part there is a chapter for each element, and the general pattern of the work will be illustrated by quoting the section headings for Silver: Rappel de quelques propriétés, Séparations, Volumétrie, Gravimétrie, Potentiométrie, Électrolyse, Colorimétrie, Cas Particuliers, Dosage des Métaux précieux par voie seche.

This is the more useful section of the book and its value, as before, is enhanced by the number of up-to-date references. Some elements, unfortunately, receive very scant treatment; for rubidium and caesium, for example, there are only a few references. While other elements

are dealt with more comprehensively, there is sometimes a lack of critical selection and a number of methods given are described as "très rapide mais assez peu précise."

None of these criticisms should obscure the fact that the book, despite its title, is declared to be a guide rather than a treatise, and that in it is collected together a great deal of very useful information, not least the copious and up-to-date references and the general bibliography at the end. It is noted that Abstracts C does not appear in the list of "Périodiques donnant la bibliographie complète."

To sum up, this book disappoints in many respects, and yet contains so much that is valuable as perhaps to deserve a place on the bookshelf of the inorganic analyst. It attempts too much, so that inequality of treatment was bound to ensue.

One must be critical of the binding, as of most French bindings. The paper covers will not stand up to the amount of handling required even for review purposes, and the reader must also be prepared to cut his way into the pages, no mean task with a book of this length.

R. C. CHIRNSIDE

Publications Received

REPORTS ON BIOLOGICAL STANDARDS: VI. THE DESIGN OF TOXICITY TESTS. (Medical Research Council, Special Report Series No. 270.) By W. L. M. PERRY. Pp. vi + 51. London: H.M. Stationery Office. 1950. Price 1s. 6d.

THORPE'S DICTIONARY OF APPLIED CHEMISTRY. Fourth Edition. Vol. X: Plagioclase—Sodium. Pp. xi + 913. London: Longmans, Green & Co. 1950. Price £5.

COLONIAL PLANT AND ANIMAL PRODUCTS. Vol. I, No. 1, January - March, 1950. Editor: G. T. BRAY, F.R.I.C. Pp. vi + 94. London: H.M. Stationery Office. Price 5s.

This quarterly journal is the successor to the Plant and Animal Products Department section of the Bulletin of the Imperial Institute.

COLONIAL GEOLOGY AND MINERAL RESOURCES. Vol. I, No. 1, 1950. Editor: E. H. BEARD, B.Sc. Pp. xvi + 120. London: H.M. Stationery Office. Price 5s.

The quarterly bulletin of the Colonial Geological Surveys.

PRACTICAL SPECTROSCOPY. By G. R. HARRISON, Ph.D., Sc.D., R. C. LORD, Ph.D., and J. R. LOOFBOUROW, Sc.D. Pp. xiv + 605. London: Blackie & Son, Ltd. 1950. Price 35s.

STRUCTURAL CHEMISTRY OF INORGANIC COMPOUNDS. Vol. I. By W. HUCKEL, D.Phil. Pp. xii + 437. London: Cleaver-Hume Press, Ltd. New York and Amsterdam: Elsevier Publishing Co., Inc. 1950. Price 70s.; $9.00.

ANALYTICAL ABSORPTION SPECTROSCOPY. Edited by M. G. MELLON. Pp. vii + 618. London: Chapman & Hall. New York: John Wiley & Sons, Inc. 1950. Price 72s.; $9.00.

AN INTRODUCTION TO LABORATORY TECHNIQUE. Second Edition. By A. J. ANSLEY. Pp. xv + 288. London: Macmillan & Co., Ltd. 1950. Price 16s.

INTERIM REPORT OF THE MINISTRY OF HEALTH DEPARTMENTAL COMMITTEE ON DETERIORATION OF CAST IRON AND SPUN IRON PIPES. London: H.M. Stationery Office. 1950. Price 3s. 6d.

This Report covers the scientific and technical aspects of corrosion and the extent of the problem and its implications. It suggests measures for protecting pipes in areas where the soil formation is known to be corrosive.

THE 1951 PITTSBURGH CONFERENCE ON ANALYTICAL CHEMISTRY AND APPLIED SPECTROSCOPY

THIS conference will be held at the William Penn Hotel, Pittsburgh, Pennsylvania from March 5th to 7th, 1951. It is jointly sponsored by the Analytical Chemistry Group of the Pittsburgh Section of the American Chemical Society and the Spectroscopy Society of Pittsburgh. An Exposition of Modern Laboratory Equipment will be a feature of the conference.

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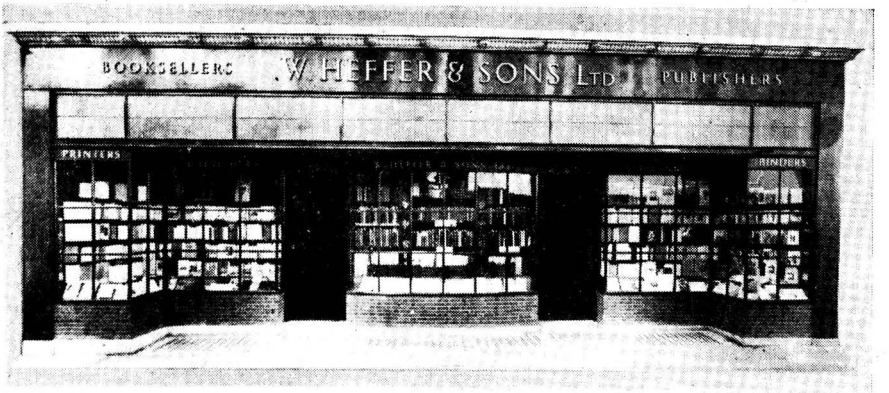

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