THE ANALYST

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SOCIETY OF PUBLIC ANALYSTS

AND OTHER ANALYTICAL CHEMISTS

MEETINGS OF CONTINENTAL SOCIETIES

AUTUMN, 1950

The Society has received notices of three meetings which will be held in Geneva, Milan and Paris in September and November next. Invitations have been extended to members of the Society to be present at these meetings, and it is hoped that some members will be able to attend one or more of them. The meetings are as follows:—

Schweiz. Gesellschaft für analytische und angewändte Chemie: Société Suisse de Chimie analytique et appliqué.

62nd Yearly Meeting of the Swiss Society for Analytical and Applied Chemistry, to be held in Geneva on the 15th and 16th September, 1950.

Société de Chimie Industrielle, 28 Rue Saint-Dominique, Paris.

XXIIIe Congres de Chimie Industrielle, Milan, 17th to 25th September, 1950.

Société de Chimie Industrielle, 28 Rue Saint-Dominique, Paris.

Journées Internationales de l'Analyse et des Essais Paris, 20th to 24th November, 1950.

Members who intend to attend any of the above meetings and who wish for further information about them should get into touch with me at 161-165, Rosebery Avenue, London, E.C.1.

K. A. WILLIAMS,
Honorary Secretary.

P.T.O.

THE SCIENCE MUSEUM

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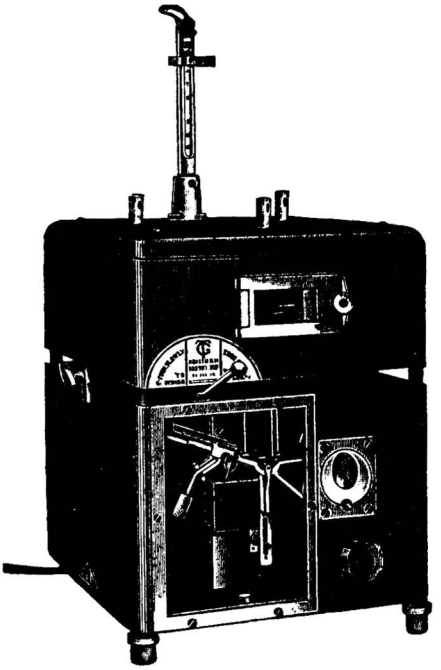
ANALYTICAL CHEMISTRY AT THE SCIENCE MUSEUM

From 5th August until 10th September the Science Museum in South Kensington will contain a special display of old and new apparatus used in chemical analysis. Its purpose is to show the development of modern refined methods of analysis and also the way in which new discoveries in other branches of science may be pressed into the service of the analytical chemist. Some of the apparatus will be working and in particular there will be a push-button demonstration of the principles of radio-chemical analysis in which visitors will be able to bring radioactive specimens in turn in front of a device for measuring their activity. Microbiological analysis (analysis of substances by means of their effects on the growth of bacteria and moulds) is another new technique, the principles of which will be explained with the aid of laboratory specimens.

This small exhibition was originally planned for the benefit of the two hundred analysts who will be attending the Royal Institute of Chemistry (London Section) Summer School in Analytical Chemistry which is to take place early in September. It was later decided, however, to present it in a form suitable also for the general public and the Science Museum has prepared the display with the co-operation of leading instrument manufacturers, Government departments and senior staff in some of London's technical colleges.

The Science Museum is open from 10 a.m. until 6 p.m. on weekdays and from 2.30 p.m. until 6 p.m. on Sundays.

P.T.O.



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THE ANALYST

PROCEEDINGS OF THE SOCIETY OF PUBLIC ANALYSTS AND OTHER ANALYTICAL CHEMISTS

Fluorescence Microscopy as an Aid to Food and Drug Analysis

BY J. KING AND R. E. WESTON

SYNOPSIS—A simple method has been devised for using fluorescence microscopy in food and drug analysis, the wavelength of 3650 A. being selected from the light given by a 250-watt compact-source high-pressure mercury-vapour lamp. The advantages over normal microscopy are discussed, and illustrations are given from the authors' experiences of the application of the method, with special reference to the detection of minute quantities of aneurine added in the form of a solution in fine droplets sprayed on to wheat flour for the purpose of "enrichment."

THE principle of fluorescence microscopy has been widely used in biology, and an excellent summary of the subject up to 1940 has been given by Ellinger.[1] A more recent review by White[2] and a new edition of Pringsheim's book on fluorescence[3] give useful references up to 1949. Unfortunately the apparatus used is expensive, difficult to procure and requires a skilled technique. It has been found, however, that fluorescence is frequently generated when light in the near ultra-violet region impinges on materials of plant or animal origin. The recent introduction of compact-source high-pressure mercury-vapour lamps induced us to investigate their potentialities in fluorescence microscopy as applied to food and drug analysis. The radiant power output per unit area of source in this type of lamp is extemely high; this enables an intense beam of radiation to be focussed on to the object to be examined whether by incident or transmitted light. The wavelength of 3650 A. has been selected for the following reasons. Extremely intense emission occurs in the near ultra-violet at this wavelength and this radiation can readily be isolated by filtering through Wood's glass which transmits about 65 per cent. of the light, or through a Wratten filter No. 18A. Light of this wavelength is transmitted freely by most optical glass and an ordinary microscope can be used without quartz condensers, slides, etc. It has the further advantage of being innocuous to the eyes, and so obviates the special precautions necessary with lower wavelengths. When examining the specimens by transmitted light it is an advantage, although not essential, to replace the normal silvered mirror by one made of "super-purity" aluminium, polished and anodised. Such a mirror reflects about 80 per cent. of the incident light of 3650 A. wavelength. When dark-ground illumination is required, an Abbé condenser can be used and at high magnifications (using objectives of shorter working distance than 4 mm.) this is necessary, as the angle of incidence is of necessity so small under these conditions that severe distortion of the image occurs. It is an advantage when using objectives of the order of 4 mm. to have the highest numerical aperture possible. If it is desired to use the shorter wavelengths of the mercury-vapour lamp, the condenser system described by

Smiles[4] and used with success by Barnard and Welch[5] is recommended; it is then necessary to use transmitted light. Recently, a type of glass that transmits light of shorter wavelength has become commercially available and from this lenses and prisms can be made. This should enable the optical system formerly made from quartz to be made much more cheaply, and greatly extend the use of fluorescence microscopy at the shorter wavelengths.

The essential details of the system adopted by us are shown in Fig. 1, in which A is a choke made for use with this type of lamp, furnished with tappings to match accurately the voltage of the A.C. supply when under load (the lamp is unsuitable for D.C.). The lamp housing should be well ventilated, in view of the heat to be dissipated, and constructed so as to prevent stray beams of ultra-violet light of various wavelengths escaping and affecting the eyes of the operator. The lenses at B are of any type of optical glass that will readily transmit light of wavelength 3650 A., and are spaced to enable the light to be focussed readily on to the stage of the microscope. It is an advantage to have these mounted on an optical bench, as this enables them to be adjusted so that when transmitted light is used a parallel beam may be reflected through the substage condenser. If light of shorter wavelength is required, these lenses must be of quartz or the special glass referred to above, as must also be the slip covering the preparation to be examined; C is a sheet of Wood's glass approximately 3 mm. in thickness, or a Wratten filter No. 18A. For some purposes it is essential to utilise the maximum intensity of ultra-violet light, and this is made possible by careful focussing on to a small sheet of paper impregnated with a dilute solution of acidified quinine sulphate and mounted on a glass slide in the usual position on the microscope stage. Unfortunately, the image is in the form of an elongated ellipse, which makes even illumination of the field impossible at the highest light intensities when using low-power objectives. Evenness of illumination is improved greatly by adopting an "out-of-focus" position, but at the expense of intensity. A compromise may be obtained by presenting the stage of the microscope at an appropriate angle to the incident light or by interposing a lens having a cylindrical face. The microscope slides and cover-slips may be of ordinary glass, but they should be examined for mechanical inclusions by observing them under ultra-violet light and rejecting any showing fluorescent patches or spots. These spots as well as any extraneous matter that may have settled on the top of the slide may be troublesome when working with objectives of greater power than 16 mm., and on all occasions when a hazy fluorescence is encountered it is essential to focus accurately on to the material responsible, so that its nature may be identified.

The appearance of objects seen under a powerful beam of ultra-violet light differs markedly from that under normal lighting and the microscopist will need to build up his own experience with this medium.

Objects such as leaves, powders, hairs, textiles, etc., should first be examined in the dry state by incident light under a low power objective, about 16 mm., with a × 10 eyepiece. Specimens should be reduced to a suitable size and form for examination in a water or dilute alkali mount with superimposed cover-slip. The usual clearing agents may be employed, but this is seldom necessary except when using transmitted light. A brief study has been made of the appearance under ultra-violet light of leaves, whole and powdered, seeds, fibres, animal and vegetable hairs, powdered roots, cereals, flour and insects. Chase and Pratt[6] have described the colour of the general fluorescence observed under comparatively low intensities of ultra-violet light of wavelength approximately 3650 A. of 151 powdered drugs, and of their alcoholic extracts; but our investigation relates to the fluorescence, under intense ultra-violet light, exhibited by individual structures. Stanfill[7] has also given some account of the detection of contamination by rodents.

Certain constituents of vegetable and animal matter exhibit marked fluorescence, *e.g.*, aesculin, fisetin, lignin, xylin, riboflavine, lyochromes, porphyrins, vitamin A. Differential staining with fluorescent dyes such as fluorescein or acriflavine may be used when the substance under examination does not exhibit a natural fluorescence, but this subject has not been pursued by us, our attention having been given entirely to naturally fluorescent substances. A fluorescent paste has also been described[8] to render interstices in surfaces fluorescent.

As a rule no special treatment of objects to be examined by ultra-violet light is necessary. They may be examined in the dry state or mounted in fluids such as water, dilute glycerol, 1 per cent. sodium hydroxide, or 2 *N* hydrochloric acid, without clearing. The translucency of starch gelatinised by the alkaline ferricyanide mounting fluid referred to later enables ultra-violet light to penetrate readily to cellular structure in starchy powders; if this fluoresces,

its fine structure is seen much more clearly than when submitted to normal microscopy with transmitted light. The jelly holds the fragments of fibre in positions that enable the structure to be seen in perspective if a binocular microscope is used with the direct rays,

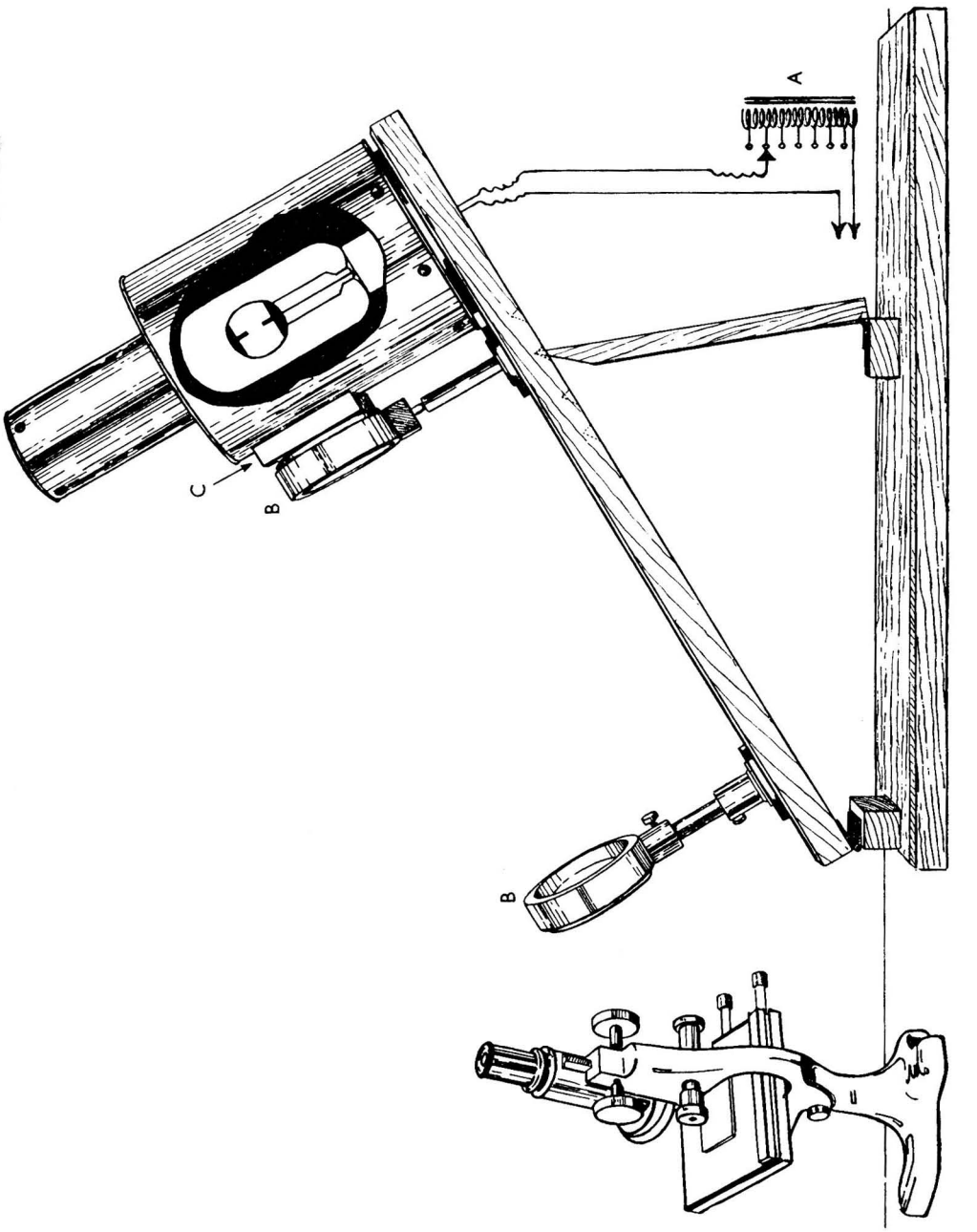

Fig. 1

and this greatly facilitates the examination of starchy powders such as wheat flour. The interior structure of leaves can only be seen by drying and powdering or by suitable dissection. Many oils, the dry endosperm of cereals, etc., fluoresce so brilliantly that the visible light emitted greatly impairs the definition of the fine structure of cellular matter. It is therefore an advantage to mount in a fluid, and sometimes to remove the oil by extraction

with a suitable solvent. Hairs of either plants or animals almost invariably fluoresce under ultra-violet light. The cell walls of most plants exhibit a green fluorescence in 1 per cent. caustic soda solution and blue violet in an acid or neutral aqueous medium. In some cases, *e.g.*, powdered rue, the aqueous mounting medium, particularly if alkaline, becomes fluorescent. On the whole, as stated by Helmholz,[9] the image is better differentiated, and the detail of structure is more readily discernable, when fluorescent substances are associated with the objects under examination, and it is our experience that for many substances differentiation and recognition is easier by the ultra-violet technique than by ordinary microscopy, and certainly with far less preliminary preparation.

The following is a short account of the fluorescence microscopy of some well-known foods and drugs, with their characteristic features.

Hairs—Most vegetable hairs fluoresce in the dry state, but it is better to examine them in an aqueous medium which may be neutral, acid or alkaline. Wheat hairs fluoresce with a blue colour when mounted in 1 per cent. sodium hydroxide, and the structure is well defined.

Rat and mouse hairs also fluoresce with a blue colour in an aqueous medium, and the structure is thrown into sharp relief. A few hairs exhibit a pale pink colour. This structure can readily be seen in contaminated flour in 1 per cent. sodium hydroxide solution, but unless contamination is gross, the method cannot replace that described for hairs and insect fragments by Kent-Jones, Amos, Elias, Bradshaw and Thackray.[10]

Vegetable structure—Many naturally occurring oils fluoresce both in the oil cells of plants and as oils. Thus the germ oils of cereals fluoresce with a violet colour, and the oil-containing cells also appear to be violet, the cell walls being green in an alkaline mounting fluid. The bast fibres of roots, stems, etc., fluoresce with a blue or blue-green colour when mounted in water or in an alkaline medium.

Insects—Mounted dry, the outer surface of insects exhibits a variety of brown and red colours, the hairs standing out in sharp relief. The compound eyes fluoresce with a blue colour. Insect fragments can be recognised in powdered cereals mounted in sodium hydroxide solution, but a slight contamination could only be established after a most exhaustive examination by this method. Mites exhibit a very pale violet fluorescence.

Detection of aneurine hydrochloride added to wheat flour in the form of a solution as a fine spray—In view of the possibility of flours being sprayed with a solution of aneurine hydrochloride for enrichment purposes, a special study of the method as applied to the detection of such flours was undertaken. Experience has shown that, while the method of Hinton[11] for the detection of added powdered aneurine hydrochloride is adequately diagnostic, sufficient light to be visible to the unaided eye is not obtainable when the vitamin is added as a solution of aneurine hydrochloride. If, however, the specimens are examined by fluorescence microscopy using our technique, the violet fluorescent spots due to thiochrome formed by the action of the oxidising medium are readily apparent, even when the addition has been made in the form of extremely fine drops produced by an atomiser. The technique is as follows: the mounting fluid consists of an aqueous solution containing 0·1 per cent. of $K_3Fe(CN)_6$ and 1 per cent. of sodium hydroxide, with the addition of a wetting agent such as "Teepol." The latter enables the microscope slide to be wetted easily, and makes the preparation of a bubble-free slide much easier. The mounting fluid is spread with a glass rod on to a microscope slide 3 inches $\times$ 1½ inches (as free as possible from flaws that fluoresce). The flour is sprinkled as evenly as possible over the wetted surface by means of a small sieve (mesh about 0·7 mm. square). Only sufficient flour is used to cover amply but to avoid thick patches, which are not completely wetted by the oxidising fluid and in the dry state fluoresce brilliantly. It is essential to avoid fluorescence due to this dry flour. A slide of similar dimensions is wetted with the same fluid and carefully placed wetted side down on to the prepared sample engaging one edge first and making complete contact slowly so as to avoid the formation of air bubbles. The slides are then inverted and examined systematically, on a travelling stage, with a 2-inch objective in combination with a $\times$ 10 eyepiece. The presence of diffused round spots of violet fluorescence which cannot be sharply focussed and are not associated with recognisable cell structure are indicative of a sprayed solution of the vitamin. Care should be taken not to mistake for thiochrome, fluorescence derived from other sources such as wheat germ oil, flaws in the glass slide, or dry material in the preparation or on the surface of the slide. A little experience is necessary before such spots can be recognised with certainty and it may be necessary to examine a number of slides, depending on the quantity added and the size of the drops, as even with flours known to have been enriched

by fluid aneurine sprays, a proportion of slides exhibiting no typical spots may be encountered. Comparison with flours of known authenticity is necessary in the event of doubt. In the samples of flour examined by us as little as 0·1 μg. of aneurine per g. of flour, added in the form of finely atomised droplets, has been detected. We prefer to use a binocular microscope and mechanical stage for the examination of these specimens, the slides being made from old photographic quarter-plates from which the gelatin has been removed. For rapid scanning, the slides may be manipulated by hand, resort being had to the screw mechanism only when a systematic survey of the whole of the material on such large slides is necessary. A moderate contamination by insect fragments or rodent hairs may be detected by this arrangement.

The various parts of the wheat grain appear as follows when mounted in water or alkaline solutions.

Wheat hairs—In water mounting the fluorescence is blue-green; the fluorescence is much stronger in 1 per cent. sodium hydroxide.

Germ—In water the fluorescence is pale blue-violet; in 1 per cent. sodium hydroxide the contents of the cell fluoresce with a stronger violet colour and the cell walls brilliant green. After de-fatting, which removes the oil, the violet colour no longer appears.

Cellular tissue—In water the fluorescence is pale blue; in 1 per cent. sodium hydroxide the aleurone cells walls are a brilliant green. Parts of the surface of the outer bran may be a pale brown in colour and show little distinctive green fluorescence.

Wholemeal—Mounted in 1 per cent. sodium hydroxide the starchy matter is invisible, but the structures enumerated above can be seen quite clearly. Occasionally a single field contains specimens of hairs (blue), internal cellular structure (cell walls brilliant green) and germ (cell walls green with violet contents), the whole exhibiting a very striking effect and giving a much clearer delineation than is possible by using ordinary microscopy. Much time is saved as no previous preparation of the specimen is necessary, the structure being as a rule more clearly defined.

The authors wish to express their thanks for gifts of samples of "enriched" flour prepared by Dr. D. W. Kent-Jones, Dr. L. George, and Dr. T. Moran and his staff of the Research Association of British Flour Millers, and also to the Government Chemist for permission to publish this paper.

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Government Laboratory
Strand, London, W.C.2

February, 1950

Gasometric Method for the Estimation of Creta Praeparata in National Flour

By J. R. FRASER and R. E. WESTON

Synopsis—A method for the routine estimation of Creta praeparata in National flour has been developed on similar lines to the Chittick gasometric method for baking powder. Apparatus for multiple determinations is described, and a mathematical formula has been evolved from which the amount of Creta praeparata in a sack of flour may be calculated directly from the volume of gas evolved and from factors which can be tabulated. These factors take account of the temperature, barometric pressure and vapour pressure of the liquid under which the estimation has been carried out.

The following method has been adopted at the Government Laboratory as rather more convenient for the routine examination of a large number of samples than other methods[1,2] previously published for this purpose. The procedure has been developed on similar lines to the Chittick gasometric method for baking powder.[3] It has proved applicable to multiple simultaneous determinations, is reasonably easy to manipulate and free from hidden sources of error, whilst the operator has the advantage of a progressive visual change to observe.

Experimental

Apparatus—

All flasks and fittings are of standard pattern and interchangeable. An approximately 400-ml. flask with ground glass fitted head is connected with a manometer burette by means of well secured pressure tubing, as shown in the diagram (Fig. 1). The reaction flasks are mounted on a shaking machine.

Procedure—

Introduce 20 g. of flour into a flask, add 50 ml. of saturated brine, insert the stopper and shake the flask gently to mix the contents thoroughly. Wash the rubber stopper and neck of flask with a further 20 ml. of brine. Then place the flask on the platform of the shaker and clip it into position.

Cautiously lower the special phial containing 20 ml. of hydrochloric acid (1 + 1) so that it stands upright in the middle of the flask. Fit the head connecting the flask with the measuring limb of the burette, and allow the apparatus to stand for a few minutes until it has reached temperature equilibrium with the surrounding atmosphere. Adjust the brine in the burette by running sufficient out of the tap provided at the U-bend so that the levels are the same in both limbs (the air within the flask is then at atmospheric pressure) and note the level (see Fig. 1), and record the temperature and barometric pressure.

Start the shaking device; this causes the acid phial to fall over and allows the acid to mix with the other contents of the flask. The carbon dioxide evolved from the reaction of the acid with any chalk present in the flour will cause a displacement of air in the head into the burette limb, the pressure in which will be apparent by the alteration of the levels in the two limbs. Periodic adjustment must be made during the course of the reaction so that it may proceed at atmospheric pressure all the time. Twenty minutes is sufficient to reach a final equilibrium. Stop the shaker, allow the contents of the apparatus to settle for 2 or 3 minutes, make the final adjustment of the levels, and record the increase in volume of gas in the system. Vigorous shaking is necessary to ensure rapid and efficient evolution of gas, as is demonstrated in Fig. 2.

This volume, adjusted for vapour pressure, temperature and barometric pressure to N.T.P., is directly proportional to the amount of chalk originally present.

The temperature of the surroundings must be kept as steady as possible throughout the determinations. There is a slight rise of about 0·5° C. between the initial and final temperatures of the liquor in the reaction flask. This difference is always checked as well as any differences between the air temperatures.

As all standards will experience the same minor fluctuations, the effect of these will have been taken into account and therefore may be ignored in the final calculation.

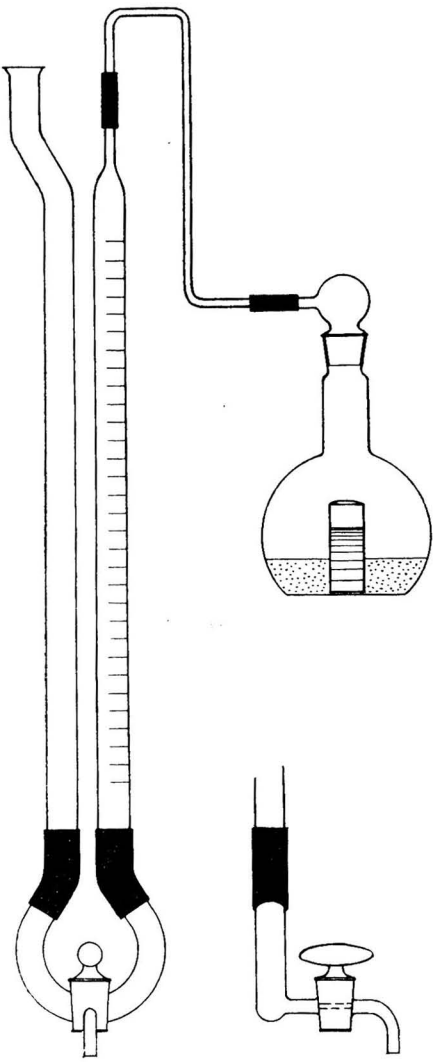

Fig. 1. Apparatus

Standards can be included in a batch of determinations so that direct comparison can be made and much calculation avoided. Alternatively, it is sufficient to make one calibration from a series of standards, and this will be within the accuracy required, provided that the necessary corrections for pressure and temperature are first applied to all data.

Calculation of results

Let V ≡ volume increase observed,
T ≡ temperature in degrees absolute,
B ≡ barometric pressure,
Aq ≡ vapour tension of liquid at temperature T,
1·976 g. ≡ weight of 1 litre of carbon dioxide at N.T.P.

It can be demonstrated that:

$$\text{Ounces of Creta praeparata per sack of 280 lb.} = \left[V \left(\frac{273}{T} \times \frac{B - Aq}{760} \times \frac{1{\cdot}976}{1000} \times \frac{100}{44} \times \frac{280}{28{\cdot}35} \times \frac{453{\cdot}6}{20} \right) \times F \right] - 0{\cdot}5$$

$$= \left[V \left(\frac{273}{T} \times \frac{B - Aq}{760} \times 1{\cdot}006 \right) \times F \right] - 0{\cdot}5$$

The factor within brackets (≡K) can be tabulated for values of B, T and Aq (Fig. 4). The vapour tension of brine is approximately 0·75 of that of water at the same temperature.

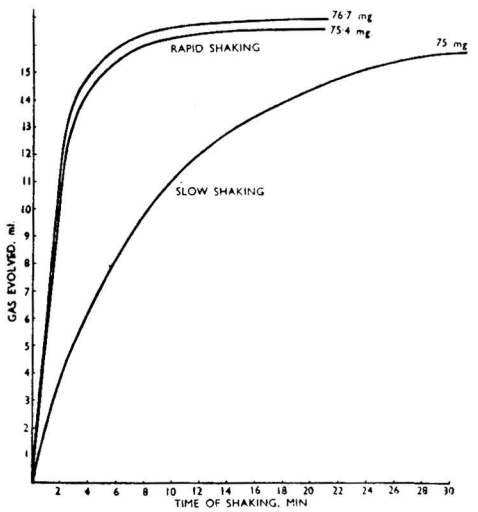

Fig. 2. Effect of Shaking

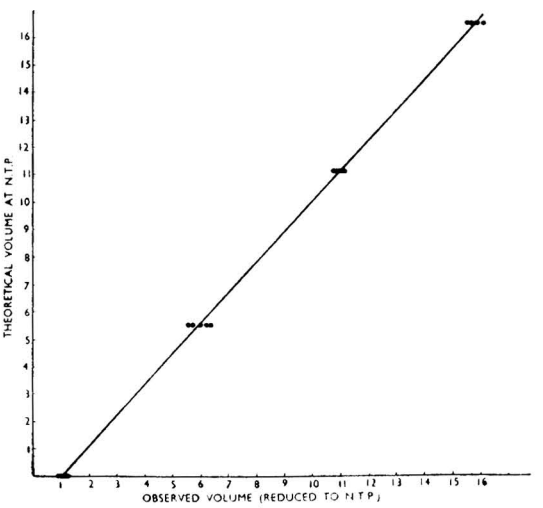

Fig. 3

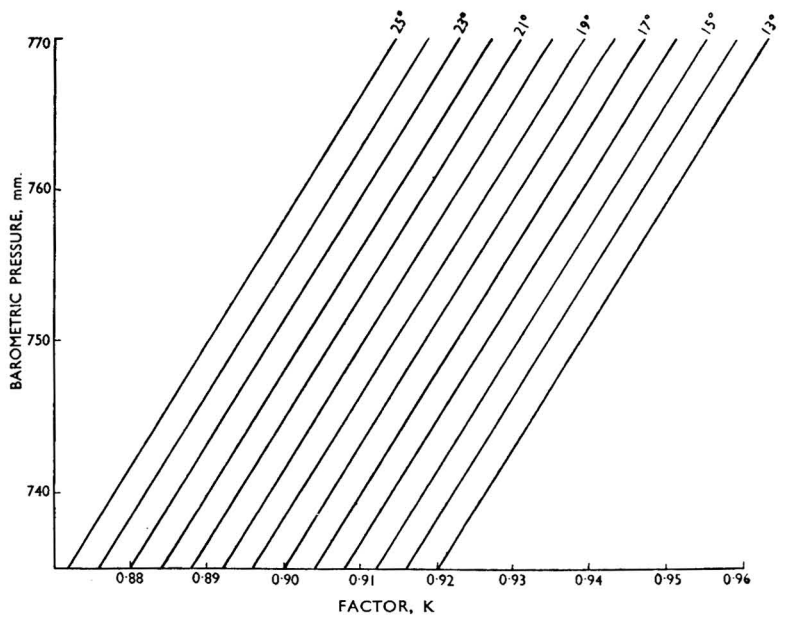

Fig. 4. Factors for values of B, T and Aq.

It has been determined experimentally for the brine - acid mixture used and found to be 0·70 of that of water.

A correction for the "blank" in Creta-free flour is necessary. This varies only slightly from an average of 1 mg. of carbon dioxide per 20 g. of flour, which may be taken as the standard deduction to be made, and this deduction is represented by the figure of 0·5 appearing as the final term in the expression above.

The factor F is dependent upon the solubility of carbon dioxide and the nature of the apparatus used and must be determined experimentally. The actual value obtained was 1·14 which was found to be fairly constant throughout the working range (17° ± 5° C.). Factors found from standard runs containing known additions of Creta praeparata (0, 25, 50 and 75 g.) are as follows—

Temperature	Barometric pressure	Experimental factor F
14	763	1·150
18	750	1·135
20	755	1·140
21	755	1·145
22	749	1·140

These results are shown graphically (Fig. 3). An approximate relation $F = 1 + \frac{TS_T}{273H}$ may be deduced, where:

$S_T \equiv$ solubility of the gas in liquor at temperature T, and

$H \equiv$ headspace of flask, *i.e.*, total volume less volume of liquor.

Some calculated factors obtained from this expression and recorded solubilities of carbon dioxide,[4,5] taking H as 300 ml. and volume of liquor as 100 ml. are:

	Calculated factor F at temperatures of		
	15° C.	20° C.	25° C.
Water	1·36	1·31	1·28
Brine, 30 per cent. w/v of NaCl ..	1·12	1·10	(1·09)
Brine, 15 per cent. w/v of NaCl ..	1·18	1·16	(1·14)
0·5 *N* hydrochloric acid	1·37	—	1·30

Hence, routine examination comprises the recording of volume of gas evolved, temperature and pressure and then making the calculation—

$$\text{Ounces per sack} = (V \times K \times F) - 0{\cdot}5,$$

where values of K and F are defined as above.

We wish to thank the Government Chemist for permission to publish this work.

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GOVERNMENT LABORATORY
LONDON, W.C.2

December, 1949

ERRATUM: April (1950) issue, p. 206. Below Fig. 3, *for* "g." *read* "μg."

The Determination of the Purity of Propylene Glycol

By G. MIDDLETON and R. E. STUCKEY

Synopsis—A description is given of a test using the critical solution temperature of propylene glycol and ether. With this test the presence of 0·1 per cent. of ethylene glycol, 0·1 per cent. of di-propylene glycol or similar amounts of water and ethyl alcohol in propylene glycol can be determined.

In spite of the large number of organic solvents that are at present available, only a few can be administered internally, while the number available for parenteral administration is extremely restricted. The addition of propylene glycol to the last group is therefore of special interest, and a high standard of purity is necessary for material required for injection purposes.

A number of specifications[1,2,3] have been published for propylene glycol for pharmaceutical purposes. These specifications include upper and lower limits for physical constants, together with tests for general impurities such as sulphates, arsenic and lead; the American National Formulary VIII has an assay using periodic acid. An examination of these specifications shows them to be, in general, deficient with regard to tests for impurities of a glycol character. Ethylene glycol is an impurity that is not likely to be found to any great extent in commercial propylene glycol, more likely impurities being di- and tri-propylene glycols; at the same time, in view of possible confusion between various glycols, a test that would limit the presence of glycols other than propylene glycol appears to be desirable.

The refractive indices of propylene glycol and ethylene glycol are practically the same, and although there is an appreciable difference in density between the two compounds, this can hardly be relied upon to show the presence of small amounts, say a few per cent., of ethylene glycol in propylene glycol. The method given by Warskowsky and Elvig[4] for the determination of ethylene glycol and propylene glycol in admixture, being based on periodate oxidation followed by estimation of the relative proportions of acetaldehyde and formaldehyde produced, is equally unsuitable for this purpose. It is, similarly, difficult to determine the presence in propylene glycol of condensed compounds, such as di-propylene glycol, which can be formed from propylene glycol by the elimination of water and the formation of an ether linkage.

A search for a chemical test does not appear to be a very promising line of attack. Direct physical measurements, such as refractive index and density, are also, as has been indicated, of little value in solving the problem. Attempts were therefore made, in experiments on mixtures of known amounts of ethylene glycol with propylene glycol, to concentrate the ethylene glycol by extraction with ether. Propylene glycol is about eight times more soluble in ether than is ethylene glycol, but preliminary trial of this method showed considerable difficulties. It did, however, indicate that a more sensitive test could probably be based on the critical solution temperature with a solvent, and ethyl ether was found to be suitable. In using such a test it is necessary to obtain a sample of pure propylene glycol, a matter of some difficulty owing to the ease with which the substance absorbs water. As a suitable criterion of purity, the constancy of the critical solution temperature of a series of successive fractions obtained by distillation in an efficient fractionating apparatus was used.

For the determination of the critical solution temperature, a method based on that of the Institute of Petroleum[5] for the determination of the aniline point was used. Owing to the necessity of guarding against errors due to the volatility of the ether and to the hygroscopic nature of the components, the test was carried out in a closed vessel and, to avoid the need for stirring, with a falling temperature. A clear mixture of propylene glycol and ether, when cooled slowly, shows at first a faint turbidity, which gradually increases as the temperature falls, so that it is not possible to state a definite point at which turbidity commences. On continuing the cooling there is, at a particular temperature, a sudden increase in turbidity, followed immediately by a "streakiness" due to convection currents and separation into two phases. This point, at which there is a marked increase in the turbidity, was found to be the most easily reproducible, and it was taken as the critical solution temperature, or what we have termed the "ether-point."

TECHNIQUE OF THE TEST—

The ether used must be anhydrous and free from alcohol; it can be prepared by distilling pure Analytical Reagent grade ether over sodium; the distillate may, if required, be kept for a short time over sodium. The ether and propylene glycol were mixed in a 10-ml. stoppered measuring cylinder, the stopper of which was specially ground with fine corundum. Propylene

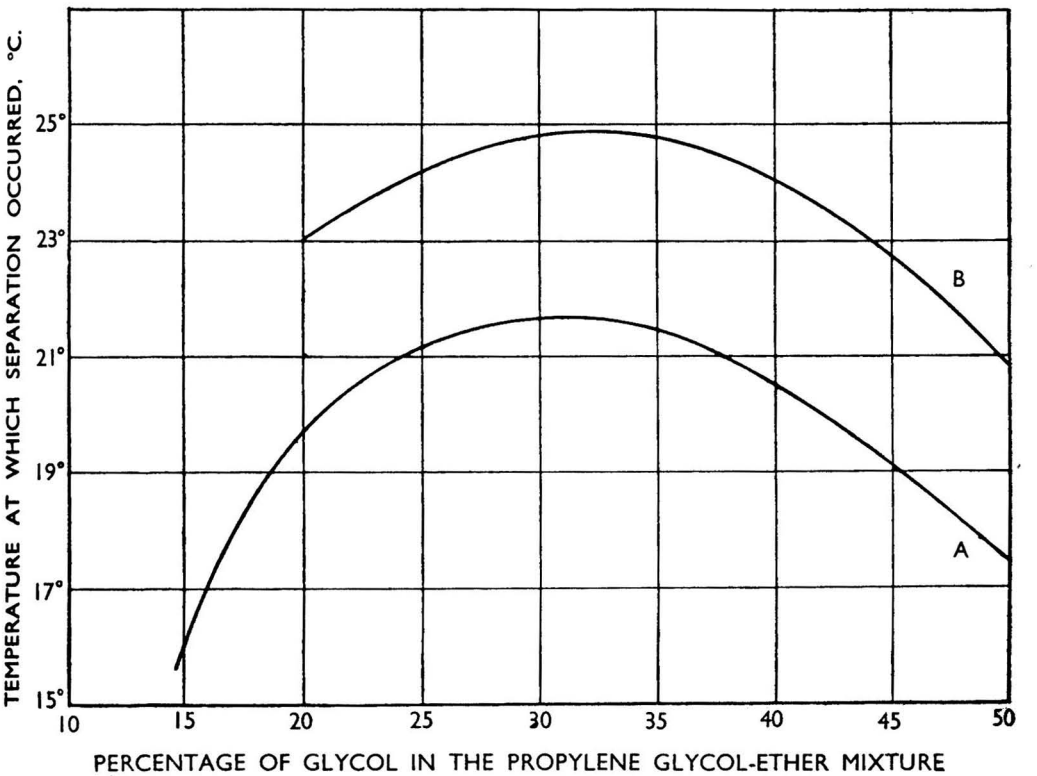

Fig. 1. Temperatures of separation of propylene glycol-ether mixtures. Curve A, pure propylene glycol and ether; curve B, propylene glycol containing 1 per cent. of water and ether.

glycol was first added quickly from a wide-bore bulb tube (an inverted pipette is satisfactory), care being taken to see that none of the glycol was allowed to come into contact with the ground portion of the neck of the cylinder. When the propylene glycol had run down in the cylinder, the volume was noted and the requisite quantity of ether was added. The ether - propylene glycol mixture was then stoppered, warmed and shaken to obtain a clear solution. The cylinder, suitably weighted, was placed in a rectangular glass trough forming a water-bath and containing a stirrer, and cold water was allowed to drip in from a tap-funnel to give the desired rate of fall of temperature. The test was carried out with top lighting, and a dead black background with a horizontal white line 0·5 cm. in width was placed so that the cylinder cut across the line. The "ether-point" was taken as that point at which the white line was indistinguishable through the cylinder. This point can be reproduced very accurately in successive experiments.

The results of experiments with pure propylene glycol and ether in different proportions are shown in Fig. 1 (curve A). It will be seen that the two liquids are completely miscible above 21·6° C., but that at lower temperatures two solutions of different concentrations are in equilibrium with each other. The summit of the curve is flattened, so that at concentrations of propylene glycol between 30 and 35 per cent. the temperature of separation is only slightly altered by variation in concentration. For this reason a concentration of propylene glycol of 33·3 per cent. (one volume of propylene glycol plus two volumes of ether), practically at the peak of the curve, was selected as a convenient standard concentration.

Purification of the propylene glycol—After preliminary work had shown the value of the ether-point in classifying samples of the glycol, a quantity of 1 litre of purified glycol was fractionated through an electrically heated column, 4 feet long, containing glass helices, with a reflux ratio head. Details of the fractionation are given in Table I. Fractions 1D to 9, showing no significant difference in ether-point, were mixed together, the resulting product being taken as pure propylene glycol of ether-point 21·62° C.

TABLE I

FRACTIONATION OF PROPYLENE GLYCOL

Fraction	Boiling-point, ° C.	Pressure of distillation, mm. of Hg.	Approx. vol. of fraction, ml.	"Ether-point" of fraction, ° C.
1A	23–26	24	2	—
1B	90–95	24	25	30
1C	to 96	22	25	21·95
1D	to 97	24	40	21·60
2	97	24	80	21·60
3	97	24	100	21·67
4	97–8	24	140	21·62
5	97	23	100	21·65
6	97	23	100	21·60
7	97	23	100	21·60
8	97	23	140	21·63
9	97	23	100	21·60
10	97	23	40	21·76
Residue	—	—	20	—

The physical constants of pure propylene glycol were determined on the sample prepared as described above and the following values were obtained—

Weight per ml. at 20° C. = 1·0374 g.
Refractive index at 20° C. = 1·4330

The effect of 1 per cent. of water added to the carefully purified propylene glycol is shown in Fig. 1, curve B, and it will be noted that this curve remains parallel to the original one.

Fig. 2 shows the alteration in the ether-point resulting from the addition of water and other substances. Within the range of concentrations examined, the graphs are practically straight lines. The alteration in ether-point produced by the presence of 0·1 per cent. of various impurities is shown in Table II.

TABLE II

EFFECT OF IMPURITIES

Impurity	Alteration of "ether-point" per 0·1 per cent., ° C.
Water	+ 0·35
Ethylene glycol	+ 0·15
Di-propylene glycol	− 0·055
Ethanol	− 0·25

APPLICATION OF THE TEST—DEHYDRATION OF THE SAMPLE—

The ether-point cannot be used directly as a measure of purity as it is greatly affected by the moisture that is usually present in samples of propylene glycol. In passing, however, it may be mentioned that, if the absence of other substances can be assumed, the ether-point can be used accurately to determine the percentage of moisture present provided that the amount is small. When 3 per cent. of water is present, the critical solution temperature approaches the boiling-point of the ether.

A method was, therefore, required for the dehydration of samples of propylene glycol. This was first attempted by the direct addition of suitable substances to the propylene glycol and to the propylene glycol - ether mixture. None of the agents tried, *viz.*, calcium oxide, barium oxide, anhydrone, silica gel, sodium sulphate, magnesium sulphate, sodium sulphite, copper sulphate and sodium metal, was of any value; either they were ineffective or they dissolved in the liquid and altered the equilibrium.

Both ethylene glycol and di-propylene glycol have a boiling-point higher than that of propylene glycol, and although the difference in boiling-point between ethylene and propylene glycols is only about 10° C., this should be sufficient to ensure that the concentration of the former would increase as part of the mixture is removed by distillation. This was confirmed in experiments in which samples of propylene glycol, to which were added small quantities

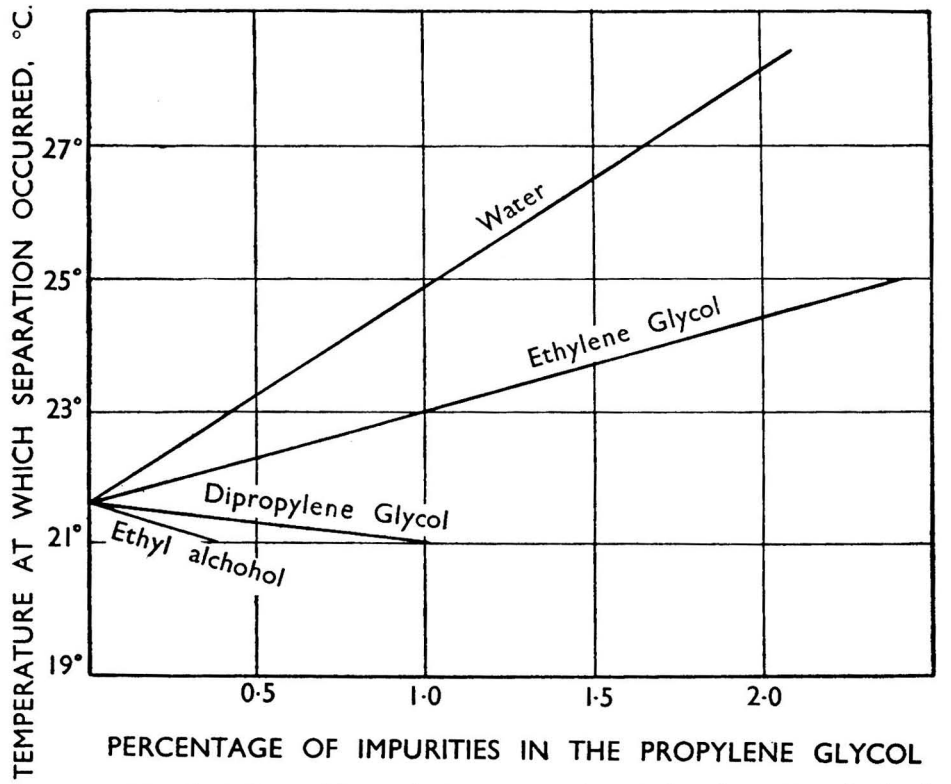

Fig. 2. The effect of impurities on the temperature of separation of propylene glycol - ether mixtures.

of the other glycols and of water, were distilled in a simple distillation apparatus. The results given in Table I showed that the water present in an ordinary commercial sample was removed in the first 5 per cent. of distillate. The results shown in Table II were obtained by distillation at atmospheric pressure in an ordinary distillation apparatus with a splash head.

TABLE III

DETECTION OF IMPURITIES IN PROPYLENE GLYCOL

Added impurity	Added water, %	Residual quantity, %	"Ether-point" of residue, ° C.	Impurity in residue, calculated from "ether-point," %
nil	1·0	90	21·6	0
Ethylene glycol				
1·0 per cent.	1·0	90	23·3	1·2
0·2 "	1·0	90	21·9	0·22
0·1 "	1·0	10	22·05	0·32
Di-propylene glycol				
0·5 per cent.	1·0	90	21·2	0·66
0·1 "	1·0	10	21·15	0·75

The results given in Table III show that it is possible by this method to detect less than 0·1 per cent. of ethylene glycol or of di-propylene glycol in propylene glycol. The percentage

of the latter was found gradually to increase in the residue during distillation, although the increase was slight. The sensitivity of the test can be increased by taking the ether-point of the residual 10 per cent. rather than of the residual 90 per cent. (*i.e.*, with only 10 per cent. removed by distillation) in which only slight concentration of the impurity had been achieved.

In view of the ease with which propylene glycol polymerises in the presence of a trace of acid or alkali,[6] it was thought advisable to check the possibility of such a reaction occurring on prolonged boiling. Pure propylene glycol was boiled under reflux for 4 hours, 10 per cent. was distilled off in order to remove any water, and the ether-point was determined on the residue. It was found to be 21·58°, which indicates at the most such a slight degree of polymerisation as to be negligible.

In order to apply the method to a sample of propylene glycol about which little is known, the glycol should be distilled and the ether-points of fractions of the distillate determined. Water will be removed in the first fractions and the trend of the ether-point of subsequent fractions and of the residue will give an indication of the purity of the glycol under test. Although the only impurities studied were ethylene glycol, di-propylene glycol, ethyl alcohol and water, the test can obviously be extended to other miscible impurities. It is possible that suitable amounts of impurities, *e.g.*, ethyl alcohol and ethylene glycol, would mutually compensate each other and would produce an ether-point of pure propylene glycol; distillation, however, followed by a determination of the ether-point of the fractions, would reveal such impurities.

The authors wish to thank the Directors of the British Drug Houses Ltd., for permission to publish this paper.

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The Determination of the Marc Content of Fresh and Dried Sugar Beets

BY THOMAS J. MITCHELL

SYNOPSIS—The marc content of the sugar beet is important on account of the volume error it introduces in the determination of the sugar in the beet. After a short reference to earlier work, some experiments on the estimation and effects of the marc in fresh and in dried cossettes produced from beets grown in Oxfordshire and Buckinghamshire are described.

The volume correction for the normal saccharimetric weight (26 g.) of fresh beets was found to be close to 1·2 ml., with a specific gravity of 1·19 for the dry marc.

In a series of 100-ml. mixtures of different weights of dried marc (1 to 8 g.) with a pure sucrose solution that itself polarised 36·9, the polarisations of the filtered mixtures showed increases that averaged 0·34 per gram of marc taken.

The marc content of dried beet cossettes appears to be higher than might be expected from calculations from the marc in the fresh beets.

CLAASSEN[1] defined beet marc as the residue remaining after complete extraction of the sugar and the readily soluble non-sugars from the beet under conditions similar to those in the factory, the extraction being effected as rapidly as possible in order to avoid the formation of soluble substances from hemicelluloses, pectin, and the like.

This marc, or insoluble cellular matter, is sometimes assumed to be present in a constant amount, 5·0 or 4·75 per cent., but this cannot be true, because the proportion of cellular matter varies considerably with climatic conditions, the age of the plant and the soil fertility.

Claassen's definition must therefore be regarded as empirical, and the marc cannot be regarded as the water-insoluble portion of the beet without further definition of how it is determined. The particle size of the beet pulp, the length and mode of treatment and in particular the quantity and temperature of the water used for extraction influence the result considerably. The temperature must be at least 60° C. to destroy protoplasm.

The proportion of marc present in the sugar beet is important on account of the volume error caused by the presence of this insoluble matter in determinations of sugar in the beet by digestion methods. The corrections generally accepted are those of Rapp and Degener[2] who assumed 4·8 per cent. as the average dry marc content of the beet, and 2·0 as the density of the marc, which gives a correction of 0·6 ml. per normal weight (26 g.).

Heintz[3] found that dried sugar beet marc would absorb water from sugar solutions, and Scheibler[4] later showed the presence of colloidal water in beet marc. He found an average of 2·5 ml. for the hydrated marc per normal weight of beet, this is much higher than the volume occupied by the dry marc. The principal corrections that have been proposed for the marc volume per normal weight of beet are shown in Table I.

TABLE I

Authority	Reference	Correction per normal weight,	Remarks
Stammer, K.	*Z. ver. deut. Zucker-Ind.*, 1882, **32**, 634	—	Original method
Rapp, G., and Degener, P.	*Ibid.*, 1882, **32**, 786	0·6	4·8% of dry marc; density 2·0
Pellet, H.	*Ibid.*, 1906, **56**, 903	0·80	
Fribourg, C.	"Analyse chimique," Paris, 1907	0·75	
Claassen, H.	*Z. ver. deut. Zucker-Ind.*, 1916, **66**, 359	—	Improved method of estimation
Sidersky, D.	"Manuel du chimiste de Sucrerie," Paris, 1909, p. 241	1·35	
Spengler, O., and Brendel, C.	*Z. ver. deut. Zucker-Ind.*, 1926, **76**, 880	2·42	3·0 to 4·5% of dry marc, Av. 3·5%; density 1·13
Staněk, V., and Vondrák, J.	*Z. Zuckerind. Cechoslov.*, 1926–27, **51**, 101, 115	1·54	In presence of lead acetate
Müller, E., and Pucherna, J.	*Ibid.*, 1929–30, **54**, 99	1·7	
Kopecký, O.	*Z. ver. deut. Zucker-Ind.*, 1931, **81**, 447	1·30	
Spengler, O., and Paar, W.	*Ibid.*, 1933, **83**, 342	0·83	In presence of lead acetate
Bachler, F. R.	*Facts about Sugar*, 1934, **29**, 191	2·31	
Osborn, S. J.	*Ind. Eng. Chem., Anal. Ed.*, 1934, **6**, 37	1·00	
Spengler, O., Paar, W., and Mück, E.	*Z. ver. deut. Zucker-Ind.*, 1937, **87**, 594	2·1	
Staněk, V., and Pavlas, P.	*Z. Zuckerind. Cechoslov.*, 1937–38, **62**, 357, 365	1·06	In presence of lead acetate
Carolan, R. J.	*Int. Sugar J.*, 1944, **46**, 179	—	Confirms work of Spengler, Paar and Mück (1937)

METHODS OF ESTIMATING MARC IN SUGAR BEET—

Stammer's method—The finely divided sample (20 g.) is digested in a beaker with 300 to 400 ml. of cold water for 30 minutes. The liquid is sucked off and digestion and filtration continued until the filtrate is free from sucrose (by α-naphthol test). The residue is treated with boiling distilled water, collected on a dry weighed filter-paper, and washed with alcohol and ether. It is dried first at low temperature and then at 100° to 110° C. to constant weight. The final residue is ignited and the ash weight deducted from the first weight. The difference, multiplied by five, gives the percentage of dry marc.

Claassen's method—The ground pulp (25 g.) is placed in a beaker marked at 400 ml. and boiling water poured in up to the mark. After 2 minutes digestion the pulp is rapidly filtered on a Buchner funnel and returned to the beaker. The extraction is repeated three times, and the marc collected on a tared filter-paper, washed with a few ml. of alcohol and dried for 6 to 8 hours at 105° to 110° C. The calculation to percentage of dry marc is made as in Stammer's method, but by multiplying by 4 instead of 5.

The amount of marc found by these methods depends primarily upon the temperature of digestion. During exhaustive hot water extraction certain normally insoluble pectins are known to undergo hydrolysis and pass into solution; other constituents may behave similarly.

Experimental

The data following were obtained in a study of sugar beets grown in Oxfordshire and Buckinghamshire and of fresh and dried cossettes prepared from them.

Estimation of the specific gravity of dried beet cossettes and of the marc from them—A composite sample of dried cossettes taken over a 3 months' drying campaign was found to contain 7·5 per cent. of water and 64·4 per cent. of sugar by polarisation. The specific gravity was determined by weighing 5 g. of the fine cossette "flour" in light petroleum of sp.gr. 0·79216 at 20° C. The average of six tests gave 1·4300 at 20° C.

Dried marc—A similar method applied to dried marc gave specific gravity 1·19012 as the average of six determinations.

The specific gravity of sucrose at 20° C. is 1·5877.

Check on specific gravity from the composition of dried beets—Assuming 64·4 per cent. of sucrose at sp.gr. 1·5877 and 7·5 per cent. of water in the dried cossettes, there would remain 28·1 per cent. of marc and soluble non-sugars which can be assumed to have a specific gravity of 1·19.

On this basis—

64·4 at 1·5877/100	=	1·022
28·1 at 1·19/100	=	0·334
7·5 at 1·00/100	=	0·075
Total		1·431

Weight per cubic foot of dried beet cossettes and of fresh beets—Loose dried cossettes vary widely in weight per cubic foot according to how the slices are packed.

	Lb. per cu. ft.	Cu. ft. per ton
Dried cossettes:		
Composite sample over 7 days ..	14·9	150·8
Average from storage bins (3-months composite sample)	19·0	120·0
Staněk and Sandera[5]	25·0	89·6
Fresh beets:		
Fresh beet cossettes	26·2	85·5
Washed beets (Claassen)	34–37	60–66

Direct estimation of marc in dried beet cossettes—The sample of dried cossettes was ground in an impact mill to a fine flour passing an 80-mesh sieve. A weighed amount was treated in a beaker with water at 50° C. using 100 ml. of water per 5 g. of sample. Digestion was continued for 5 minutes, and the pulp rapidly filtered off on a Buchner funnel. The pulp was returned to the beaker and the process repeated thrice. After the fourth digestion the marc was collected on a tared filter-paper and washed with water at 50° C. until there was no reaction with the α-naphthol test. A final washing was given with a few ml. of alcohol. The residue was dried at 105° C. for 6 hours and weighed; it was then ashed and re-weighed.

Table II

No.	Cossettes weighed, g.	Marc, %	Ash, %	Ash-free marc, %
1	3	20·83	2·07	18·76
2	3	21·20	2·43	18·77
3	4	21·30	2·43	18·87
4	4	21·48	2·40	19·08
5	4	21·73	2·60	19·13
6	2	19·60	2·60	17·00
7	20	20·61	2·50	18·11
Average		20·96	2·43	18·53

These beets were selected from a week's average delivery in early October at the beginning of a crop.

TABLE III

Method as for Table II, but using proportions of a normal weight

No.	Cossettes weighed, g.	Marc, %	Ash, %	Ash-free marc, %	Volume occupied, ml.	Correction per normal weight of fresh beets, ml.
1	6·5	23·37	2·59	20·78	1·16	1·16
2	6·5	24·54	2·71	21·83		
3	13·0	24·80	2·46	22·34	2·43	1·22
4	13·0	24·75	2·63	22·12		
5	26·0	23·38	2·55	20·83	4·67	1·17
6	26·0	24·30	2·42	21·88		

In Table III the volume occupied by the dry ash-free marc was obtained by dividing the weight of marc found with each proportion taken originally by the specific gravity of the marc (taken as 1·19). The correction per normal weight of fresh beets assumes a drying factor of 4·0 for dried cossettes, *i.e.*, 1 part of dried cossettes is equivalent to 4 parts of fresh beets.

The beets used in this test were an average sample from a week's delivery in mid-December.

Indirect estimation of marc volume error—About 50 g. of dried cossettes were continuously extracted with cold water for 9 hours until free from sugar (as shown by α-naphthol test). The resultant pulp was dried for 6 hours at 105° C. Various amounts of this dry marc were weighed into dry 100-ml. flasks which were then filled to the mark with a sucrose solution containing 96 g. per litre. Air was removed from the solutions before completing to volume. The solutions were filtered and read in a saccharimeter. The results are shown in Table IV.

TABLE IV

No.	Dry pulp taken, g.	Polarisation reading, %	Polarisation reading calculated, %	Difference per g. of marc
1	0	36·9	36·9	—
2	0·5	37·2	37·1	0·60
3	1·0	37·3	37·2	0·40
4	2·0	37·5	37·5	0·30
5	3·0	37·8	37·8	0·30
6	4·0	38·2	38·2	0·33
7	5·0	38·6	38·5	0·34
8	6·0	38·8	38·9	0·32
9	7·0	39·3	39·2	0·34
10	8·0	39·9	39·6	0·38

Their precision is limited by the accuracy possible in reading the saccharimeter. The calculated figures were obtained by using the specific gravity of 1·19 for the dried marc. The average difference per gram of marc, omitting No. 2, is 0·34.

Average figures for the marc content of fresh beets and dried cossettes derived from them are given in Table V. The fresh beets were treated by Claassen's method, and the dried cossettes by the method used for Tables II and III. An extraction temperature of 50° C. is permissible with dried cossettes because the protoplasm is no longer active.

TABLE V

AVERAGE MARC CONTENT OF BEETS

	Fresh beets	Dried cossettes
Number of samples	15	20
Average percentage of marc	4·84	22·45
Minimum " "	4·00	19·60
Maximum " "	5·40	26·70
Average water content, %	78·00	7·50

The average percentage of marc in the fresh beets, calculated to a dry basis, becomes 22·00, and calculated to 7·5 per cent. of water, becomes 20·35 per cent. The latter value is 2·1 per cent. below the percentage of marc found. Owing to the difficulty of comparing

fresh and dried samples, and to the limited accuracy of the method it is not possible to attach much importance to this difference; it may point to coagulation rendering insoluble certain constituents of the beet during the drying process.

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DEPARTMENT OF TECHNICAL CHEMISTRY
THE ROYAL TECHNICAL COLLEGE
GLASGOW

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A New Method for the Determination of Sodium in Calcined Alumina and Aluminium Hydrates

BY H. JACKSON

SYNOPSIS—A method is described for the determination of sodium in alumina and aluminium hydrates. It consists in heating the sample with hydrochloric acid in a sealed tube at 200° C. Only partial dissolution of alumina is necessary for complete solution of the sodium; complete dissolution of alumina can, however, be effected under suitable conditions. Aluminium hydrates are dissolved completely.

Sodium is determined gravimetrically, after removal of excess acid, by precipitation as the triple acetate. Separation of aluminium salts is unnecessary. The method is simple and the blank low.

THE standard method for the determination of small amounts of sodium in calcined alumina (α-alumina) by the J. Lawrence Smith method is tedious and needs a skilled analyst to carry it out. The method involves sintering with calcium carbonate and ammonium chloride, extraction of calcium and sodium chlorides with water, and separation of the calcium as oxalate before the final determination of sodium by one of the usual procedures. The main fault of the method apart from the time required, lies in the high blank obtained, which is a disadvantage when the sample has a low sodium content.

Wichers, Schlecht and Gordon[1] used a technique involving treatment with hydrochloric acid at high temperatures in sealed tubes for the dissolution of a number of refractory oxides, ceramic materials and minerals.

The method described below, p. 417, is based on this technique. Complete solution of the sample is unnecessary because the sodium appears to be present entirely on the surface of the crystal. Complete solution of α-alumina in hydrochloric acid can be readily attained, however, under suitable conditions.

In preliminary work it was observed that when α-alumina was shaken with water, an alkaline solution with a pH of about 10 was obtained. Extraction of sodium from alumina with water in a Soxhlet apparatus was incomplete, however, even after 24 hours. Extraction with diluted hydrochloric acid, 1 + 1, on the other hand, gave a solution containing all the sodium together with most of the iron and a small amount of aluminium after boiling for 24 hours. The treatment was carried out in an all-silica apparatus consisting of a flask fitted with a stirrer and reflux condenser. Sodium was determined in the filtrate, after removal of iron and aluminium with ammonia, by precipitating with zinc uranyl acetate reagent.

As this method was rather lengthy, and required a considerable expenditure on apparatus to carry it out as a batch process, experiments were carried out on the extraction at temperatures above 100° C. in sealed glass tubes.

It was found that alumina calcined at 1200° C. (α-alumina) could be completely dissolved in a 25 per cent. excess of hydrochloric acid by heating at 250° C. for 5 hours. Hence a

simple method is available whereby the minor impurities present in alumina, *e.g.*, sodium, calcium, iron, sulphur and phosphorus may readily be determined without introducing a high blank from reagents. Aluminium mono- and tri-hydrates, which are only slowly dissolved by hydrochloric acid at normal boiling-point, are also readily dissolved under pressure by heating with excess of hydrochloric acid for 1 to 2 hours at 200° C. The time required to carry out the determination was shortened by precipitating the sodium directly with zinc uranyl acetate, without separation of aluminium, which did not interfere.

Monax tubing of 4·5 mm. bore and 1·5 to 2·0 mm. wall thickness was used throughout the work since this glass was easy to seal by means of an air-gas flame and no contamination of the solution by soda from this glass was observed. Any other borosilicate glass with low soda content, such as W1* or Pyrex, is suitable, but Pyrex requires a gas-oxygen flame for sealing off.

Taking the tensile strength of glass as approximately 10,000 lb. per sq. in. and the bursting pressure (A) in lb. per sq. in. as $A = (2W/D)\ 10{,}000$, where W is the wall thickness and D the internal diameter, the calculated bursting pressure of tubing of the above dimensions is over 6000 lb. per sq. in. If this is halved for safety the bursting pressure will still be over 3000 lb. per sq. in. The pressure developed by hydrochloric acid (32 weight per cent., sp.gr. 1·16) calculated from the formula given (*loc. cit.*) is 743 lb. per sq. in. and 1635 lb. per sq. in. at 200° and 250° C. respectively so that the safety margin is high, especially when working at the lower temperature.

No accidents occurred from bursting tubes throughout this work, although well over 100 determinations have been carried out. As a precaution, however, each tube may be wrapped in asbestos paper and placed in a steel shell made from a piece of ⅜-inch gas piping of suitable length with a loose-fitting screwed cap at each end.

Experimental

Section I—The determination of sodium in calcined alumina—

The method described on p. 417 was applied to several samples of alumina from normal to finely ground samples. The sodium was also determined by the J. Lawrence Smith method.

Treatment of reaction tubes—Surface impurities were removed from the tubes by heating in a chromic - sulphuric acid mixture for half an hour, followed by boiling in nitric acid for half an hour after an intermediate rinse in distilled water. The tubes were then washed in distilled water and dried. Tubes 14 inches long (35·5 cm.) with a bore of 4·5 mm. gave a total volume of 5·65 ml. Thus a sample weight of 0·4 g. alumina with a 10 per cent. excess of hydrochloric acid, sp.gr. 1·16, over the stoichiometric amount, could be taken without having the tube more than two-thirds full.

Dissolution of alumina at 250° C.—Preliminary tests were carried out at 250° C. in a thermostatically controlled air-oven on 0·4 g. calcined alumina and 2·6 ml. hydrochloric acid (10 per cent. excess) for various times. Each tube was sealed off, after thickening the wall, with a well-rounded end and, after cooling, the contents of the tube were mixed by a vigorous shaking. The tube was then placed horizontally in the oven with the alumina distributed along its length. Even after 6 hours, complete dissolution of the alumina was not reached. Since the reaction is of the first order, increased acid should increase the rate. This was found to be so and by using a 25 per cent. excess of acid over the stoichiometric amount (total 3·0 ml.), 0·4 g. alumina was completely dissolved in 5 hours at 250° C.

After treatment, the tubes were removed from the oven and placed vertically to cool after shaking the contents out of the top of the tube. As there is no pressure increase due to the reaction, the tubes were opened by making a scratch about half an inch from the end, which was then broken off. The contents of the tube were washed out by inversion over one limb of a U-tube of 2 to 3 mm. diameter drawn down to a jet and reaching to the end of the reaction tube. The other wider limb of the U-tube was connected to a wash-bottle. Any undissolved alumina was removed directly by filtration under reduced pressure through a pulp pad contained in a sintered glass funnel of porosity 1 (see Fig. 1).

Aluminium was removed from the solution as chloride[2] and sodium determined gravimetrically in the filtrate by precipitation with zinc uranyl acetate in the usual manner. High blanks were obtained, amounting to as much as 16 mg. (weight of precipitate), compared to a weight of 120 mg. for the sample.

* A tungsten-sealing glass manufactured by the General Electric Company, Wembley.

Extraction of sodium at 200° *C.*—In an attempt to reduce the value of the blank, further experiments were carried out at a lower temperature (200° C.) and sodium determinations made on the resulting solutions, although complete solution of alumina was not attained. The results are given in Table I and show that complete solution of the alumina is unnecessary for a perfect extraction of the sodium. Moreover the blank value is reduced to a much more reasonable level (2 to 4 mg. weight of precipitate). This indicates that the attack on the glass at 200° C. and with a shorter time of extraction is much less than the attack at 250° C. Examination of the tubes after treatment failed to show any surface attack; several of the tubes were used for a number of determinations.

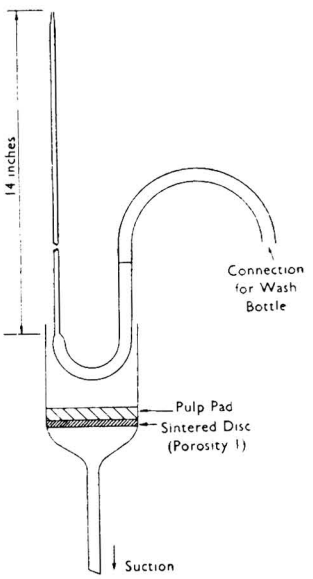

Fig. 1. U-tube for washing reaction tubes

As shown in Section II, p. 419, aluminium was found to have no effect on the precipitation of the triple sodium salt. Consequently, no attempt was made to separate it in these tests. The time saved was considerable.

Care had to be taken, however, during the final stages of evaporation. Loss by spirting was prevented by finishing the evaporation on a low temperature hot-plate, working at 120° to 130° C., controlled by a Sunvic Energy Regulator; with this low temperature there was no danger of hydrolysis of the aluminium chloride to hydroxide, and excess acid was readily removed.

TABLE I

EXTRACTION OF SODIUM (CALCULATED TO Na_2O) FROM ALUMINA WITH HYDROCHLORIC ACID (25 PER CENT. EXCESS) (SAMPLE R.406)

0·4 g. alumina, 3·0 ml. hydrochloric acid

Temperature	Time of treatment	Alumina dissolved, %	Na_2O found, %
200° C.	1 hour	12·33	0·505
"	2 "	19·95	0·507
"	4 "	31·45	0·502
250° C.	5 "	100	0·504

As a result of these experiments the sample weight was increased to 1 g. using the same amount of hydrochloric acid, thereby increasing the precision of the analysis, especially where the sodium content of the sample was low. Experiments using the larger sample weight gave results in close agreement with those obtained for a 0·4 g. sample.

Variations in time and temperature of treatment—Further experiments were carried out to determine the limits of the treatment, in which the time was reduced to 30 minutes and the temperature to 160° C. The results are summarised in Table II.

TABLE II

EFFECT OF VARIATION IN TIME AND TEMPERATURE ON THE EXTRACTION OF SODIUM FROM ALUMINA

1 g. alumina, 3·0 ml. hydrochloric acid

Sample No.	Time	Na_2O found at a temperature of 160°C., %	170°C., %	180°C., %	190°C., %	200°C., %	Na_2O (mean of four results at 200° C. for 1 hour), %
R103	1 hour	0·267	0·292	0·307	0·308	—	0·313
	30 min.				0·306	0·314	
R104	1 hour	0·181	0·190	0·202	0·210	—	0·210
	30 min.				0·205	0·210	
R391	1 hour	0·263	—	—	—	—	0·283
R392	1 „	0·326	—	—	—	—	0·348
R406	1 „	0·242	—	—	—	—	0·505

Table II shows that below 190° C. the sodium is incompletely extracted in 1 hour and that a time of 30 minutes is just sufficient at 200° C. It is however recommended that a temperature of 200° C. for 1 hour should be used to ensure complete extraction of all samples.

At temperatures above 200° C., the blank increased with an increase of temperature. Since the pressure increases rapidly above 200° C., it is not advisable to go much above this temperature in the interests of safety.

Blank determinations varied from 0·7 to 4·9 mg. with an average of just over 2 mg. (weights of precipitates), and are very small compared with the weight of precipitate from a sample.

METHOD

I. THE DETERMINATION OF SODIUM IN ALUMINA

REAGENTS—

Diluted acetic acid—(1 + 1).

Ether—dry.

Hydrochloric acid—sp.gr. 1·16 (32 per cent. by weight).

Zinc uranyl acetate reagent—

Solution A—77 g. of uranyl acetate, $UO_2(C_2H_3O_2)_2.2H_2O$, 13·3 ml. of glacial acetic acid and 410 ml. of water.

Solution B—231 g. of zinc acetate, $Zn(C_2H_3O_2)_2.3H_2O$, 6·6 ml. of glacial acetic acid and 262 ml. of water.

Heat solutions A and B to 70° C., mix, stir until clear and allow to stand for 24 hours. Filter immediately before use.

Alcoholic wash solution—Alcohol (95 per cent., saturated with sodium zinc uranyl acetate, $NaZn(UO_2)_3(C_2H_3O_2)_9.6H_2O$). Prepare by precipitating 0·1 g. of sodium chloride, dissolved in 5 ml. of water, with 50 ml. of zinc uranyl acetate reagent as described below under procedure. Filter the precipitate on a small sintered glass crucible (porosity 3) and wash thoroughly with 95 per cent. ethyl alcohol. Transfer the precipitate to a bottle containing 1 litre of ethyl alcohol, 95 per cent., shake, and allow to stand for 24 hours. Filter the solution immediately before use.

Store the zinc uranyl acetate reagent and the alcoholic wash solution at 20° C. Exposure to low temperatures during cold weather should be avoided.

PROCEDURE—

Introduce 1 g. of alumina into a dry glass tube 8 mm. external × 4 to 5 mm. internal diameter and 14 inches long of Monax, W1 or similar borosilicate, low soda, glass. (If a greater volume is required, the tube should be longer rather than wider. The contents should not occupy more than two-thirds of the volume.) Add 3·0 ml. of hydrochloric acid and seal off the tube as close to the end as possible, making sure that the end is thickened and

well-rounded and that no pinhole remains. Shake the tube vigorously to mix the contents and place it horizontally in an air-oven maintained at a temperature of 200° C. ± 5° for 1 hour, with the alumina distributed along the length of the tube. Then remove the tube, stand it vertically and allow to cool to room temperature. Cut off the tip and wash it and the contents of the tube on to a medium-tight pulp pad contained in a sintered glass funnel of porosity 1. Draw the filtrate, under suction, into a 150-ml. silica beaker, using a Witt's apparatus, and wash the pad well with water.

Evaporate the filtrate to dryness, carrying out the final stage on a hot-plate at 120° to 130° C. Add 1·0 ml. of water and 5 drops of acetic acid (1 + 1). Stir until the residue has dissolved, add a further 1 ml. of water if the aluminium chloride does not dissolve completely, and place the beaker in a thermostat at 20° C. ± 1°. Precipitate the sodium with 10 ml. of zinc uranyl acetate reagent and allow to stand for 30 minutes with occasional stirring. Filter off the precipitate through a sintered glass crucible, porosity 3, and wash with five 2-ml. portions of zinc uranyl acetate reagent followed by five 1-ml. portions of alcoholic wash solution. Finally, wash with 5 ml. of ether, dry at 100° C. for 30 minutes, cool and weigh.

Wash the crucible, under suction, with hot water followed by alcohol and ether, and again dry and weigh. The difference between the two weights is sodium zinc uranyl acetate. Carry out a blank determination on 3·0 ml. of hydrochloric acid. The factor for conversion to Na_2O is 0·02015.

A selection of results obtained by the procedure described above, together with figures obtained by the Lawrence Smith method, are shown in Table III. The standard deviation estimated from sixteen results on five samples of alumina with sodium contents from 0·2 to 0·5 per cent. as Na_2O was ±0·006 per cent.

TABLE III

DETERMINATION OF SODIUM IN ALUMINA BY EXTRACTING WITH HYDROCHLORIC ACID FOR 1 HOUR AT 200° C.

		Na_2O, %		
Sample No.	Sample weight, g.	Found	Mean	Lawrence Smith method
R103	0·4	0·300		
	0·4	0·316	0·313	0·319
	0·5	0·317		
	1·0	0·317		
R104	0·4	0·206		
	0·4	0·219	0·210	0·227
	0·5	0·206		
	1·0	0·209		
R391	1·0	0·274	—	0·265
R392	1·0	0·349	—	0·348
R406*	0·4	0·505	—	0·460

* See also Table I.

SECTION II—THE DETERMINATION OF SODIUM IN ALUMINIUM MONO- AND TRI-HYDRATES

Similar experiments to those in Section I were carried out on samples of aluminium mono- and tri-hydrates, using sample weights of 0·5 g. and 0·6 g. respectively with 3·0 ml. of hydrochloric acid (25 per cent. excess), and heating at a temperature of 200° C. Complete solution was attained after 1 to 1½ hours heating for the tri-hydrate, and 1½ to 2 hours for the mono-hydrate. It was necessary, however, to take the tubes out of the oven after half an hour and shake vigorously to disperse the cake that formed along the glass, and which otherwise was difficult to dissolve.

THE DETERMINATION OF SODIUM IN THE PRESENCE OF ALUMINIUM CHLORIDE

A series of tests was carried out on synthetic solutions containing different amounts of standard sodium solution, together with aluminium chloride solution, prepared from super purity aluminium, equivalent to that obtained from 0·6 g. of aluminium tri-hydrate. The solutions were evaporated to dryness, the final stage being carried out on a low temperature hot-plate working at 120° to 130° C. as before.

The normal treatment of the residue prior to precipitation is to dissolve it in 1 ml. of water and a few drops of acetic acid. It was found that this was insufficient in the presence of so much aluminium chloride as a viscous solution was obtained and the salt was frequently co-precipitated with the sodium triple salt, which made the solution difficult to filter. The amount of water was increased to (*a*) 2 ml. and (*b*) 3 ml., and the sodium precipitated with 20 ml. of reagent. This was wasteful of reagent, and so synthetic solutions with 2 ml. of water and only 10 ml. of reagent were tested. The results are given in Table IV, and show that satisfactory recoveries are obtained by this latter method. Filtration and washing were carried out in the normal way and the determinations completed gravimetrically.

TABLE IV

DETERMINATION OF SODIUM IN SYNTHETIC SOLUTIONS CONTAINING ALUMINIUM CHLORIDE

Aluminium chloride added equivalent to 0·6 g. of $Al(OH)_3$

Volume of solution before precipitation, ml.	Volume of reagent added, ml.	Na_2O on 0·6 g. Found, %	Na_2O on 0·6 g. Taken, %
2	20	0·134	0·133
2	20	0·270	0·267
2	10	0·134	0·133
2	10	0·269	0·267
3	20	0·134	0·133
3	20	0·268	0·267

II. THE DETERMINATION OF SODIUM IN ALUMINIUM HYDRATES

REAGENTS—

As for Method I.

PROCEDURE—

Introduce 0·6 g. of tri-hydrate or 0·5 g. of mono-hydrate into a reaction tube, add 3·0 ml. of hydrochloric acid and seal off the end of the tube. Mix the contents of the tube by vigorous shaking and place horizontally in an air-oven at 200° C. ± 5° with the hydrate spread along the length of the tube.

Allow the reaction to proceed for 1 to 2 hours until the solid has dissolved completely. This is facilitated by removing the tube from the oven after ½ to ¾ hour, and shaking vigorously to re-distribute the solid which tends to cake on the walls of the tube.

After solution of the sample, remove the tube from the oven and stand it vertically until cold.

Cut off the end of the tube and wash it and the contents of the tube into a 150-ml. silica beaker. Evaporate the solution to dryness, carry out the last stages on a hot-plate at 120° to 130° C. to effect removal of excess hydrochloric acid without decomposition of the aluminium chloride or loss by spirting.

Dissolve the residue in 2·0 ml. of water and 5 drops of acetic acid (1 + 1). Precipitate the sodium with 10 ml. of zinc uranyl acetate reagent and complete the determination as in I., p. 418.

Carry out a blank determination on 3·0 ml. of hydrochloric acid.

A series of eighteen determinations was carried out on three samples of tri-hydrate, each containing approximately 0·3 per cent. of Na_2O, by the recommended procedure. The standard deviation was ±0·006 per cent. The results are shown in Table V.

TABLE V

DETERMINATIONS OF SODIUM IN ALUMINIUM TRI-HYDRATE

$Al(OH)_3$, 0·6 g. Hydrochloric acid, 3·0 ml.
1 to 1½ hours at 200° C.

Sample No.	Na_2O found, %						Mean Na_2O, %
S4890	0·324	0·323	0·307	0·313	0·313	0·317	0·316
S6002	0·290	0·296	0·275	0·280	0·289	0·293	0·287
S6003	0·286	0·290	0·281	0·279	0·286	0·288	0·285

Treatment of residues

All solutions containing uranium salts should be collected and the uranium recovered by one of the published methods.[3,4,5]

The author wishes to thank the British Aluminium Company for permission to publish this work.

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The Determination of Small Amounts of Aluminium in Iron*

By H. G. SHORT

Synopsis—Details are given of a method for the determination of aluminium in iron in amounts down to a lower limit of 0·001 per cent., with an accuracy of ± 0·0005 per cent. Iron and other interfering elements are separated by an ether separation, followed by chloroform extraction of cupferron complexes at pH 0·5. Aluminium is determined in the resulting aqueous layer by means of "aluminon" under carefully controlled conditions.

The method as described was intended for use with high-purity iron, but it is suggested that its scope could be extended by including a second cupferron separation at pH 3·5, when aluminium alone is extracted.

In the course of some work on the preparation of high-purity iron the metal was being melted in dry hydrogen in an alumina crucible at 1600° to 1700° C., and it was desired to determine acid-soluble aluminium in the product to within ±0·0005 per cent. This was of particular interest in view of the values which have been published[1,2] for the equilibrium constant of the reaction

$$Al_2O_3 + 3Fe \rightleftharpoons 3FeO + 2Al$$

and of the difference of opinion between authorities as to the possibility of melting iron in alumina vessels without contamination.

Many papers on the colorimetric determination of small amounts of aluminium, and on the determination of aluminium in iron, have been published,[3] but none appeared to fulfil the requirements. Indeed, from the large amount of available information it was impossible to select any one colorimetric reagent or procedure as being clearly superior to the remainder.

Separation of iron and aluminium

All the colorimetric reagents for aluminium have the disadvantage that iron reacts quantitatively and must be completely separated, and, as there is no method of extracting the aluminium from the iron solution, the main problem is this removal of the large amount of iron.

Three methods, with variations, have been considered—

1. Extraction of iron as iron cupferride from aqueous solution by chloroform.
2. Ether (or amyl acetate) extraction of most of the iron as chloride, followed by separation of the last traces by method 1.
3. Mercury cathode separation of iron.

* Communication from the National Physical Laboratory.

Method 3 is attractive in that other elements, such as nickel, chromium and molybdenum, are separated from aluminium together with the iron, but it does not conveniently deal with more than 1 g. or so of iron. At the 0·001 per cent. level a 10-g. sample is desirable in order that aliquots of the final solution may be taken for trial colorimetric determinations of aluminium in samples of unknown aluminium content. In addition, the removal of iron by mercury cathode electrolysis is never so complete that aluminium can be determined at the 0·001 per cent. level without a further separation of the last traces of iron.

Method 1 also suffers from the disadvantage of limited range: more than 0·5 g. of iron requires a considerable amount of cupferron and chloroform for its removal, although, as will be shown later, removal of iron by this method is complete. Method 2 was therefore adopted.

PRELIMINARY SEPARATION—

The ether separation is carried out in the usual manner, using *iso*propyl or ethyl ether and hydrochloric acid of sp.gr. 1·15. The iron in the aqueous layer should be reduced in amount to 2 to 3 mg. and for this, two ether separations are necessary with 10 g. of iron.

Amyl acetate extraction from concentrated hydrochloric acid solution gives a much better separation in the presence of an appreciable amount of sulphuric acid, but the latter is undesirable in the subsequent colorimetric determination of aluminium, and in its absence two separations are necessary to reduce the amount of iron to the desired level even with amyl acetate extraction.

SEPARATION OF IRON AND ALUMINIUM WITH CUPFERRON—

Separation of the remainder of the iron is achieved by addition of a solution of 0·1 g. of cupferron to the iron solution, followed by extraction with chloroform until the extract is colourless. In this separation certain points require attention. After the ether separation, traces of iron are in the reduced state, and since ferrous iron is not removed by cupferron, the solution should be oxidised by warming with a few drops of nitric acid. Secondly, quantitative extraction of the iron is achieved much more readily in the presence of 2 or 3 g. of ammonium chloride.

Quantitative formation of the iron - cupferron complex is not immediate, and after addition of the cupferron the solution must be allowed to stand for 5 minutes before extraction with chloroform. In some experiments to determine the pH range over which iron was quantitatively extracted, the first extractions were made almost immediately after the addition of the cupferron. Under these conditions complete extraction of iron was obtained at pH 0·8 or higher, but in more acid solutions irregular amounts of iron up to 0·3 mg. were left in the aqueous layer (volume 50 ml.). When time was allowed for the formation of the complex, complete extraction of iron was achieved down to pH 0·2, the amount of iron remaining in the aqueous layer (volume 50 ml.) being less than 5 μg. In view of the tendency of aluminium to be extracted with the iron at pH values above 0·5, this possibility of working in more acid solutions is important.

It is perhaps not generally realised that aluminium itself is, under certain conditions,[4] precipitated by cupferron. The precipitate is soluble in chloroform. Some experiments were made to establish the pH range over which extraction of aluminium occurred. One hundred millilitres of solution containing 2 mg. of aluminium and 3 g. of ammonium chloride were extracted with chloroform, after adjustment of the acidity over a range of pH values and addition of cupferron solution. The acidity value was taken as that of the solution after extraction had been carried out, as the addition of the cupferron increased the pH value by about 0·2. The results are given in Table I.

It is apparent that unless the extraction of iron is carried out in a solution more acid than pH 0·5 there will be slight losses of aluminium, although at the 0·01 per cent. level these losses only amount to 0·00015 per cent. on a 10-g. sample at an operating pH of 0·7. Extraction of aluminium is virtually complete over the pH range 3·2 to 4·0. This was confirmed by the testing of portions of the aqueous layers, after extraction, by the "aluminon" colour test.

COLORIMETRIC DETERMINATION OF ALUMINIUM

In the present work "aluminon" (ammonium aurin tricarboxylate) has been used for the colorimetric determination of the aluminium. As with other reagents for aluminium, careful control of the pH value is the most critical factor in obtaining reproducible results and it

is, in fact, necessary to control this to ±0·05. In Fig. 1 is plotted the Spekker drum difference reading for 50 μg. of aluminium against pH value, and it will be seen that the variation with pH is linear and that an error of 0·1 in pH of the test corresponds to an error of 2·5 μg. of aluminium in 50 μg., or 5 per cent. at a working pH of 4·9.

TABLE I

EXTRACTION OF ALUMINIUM CUPFERRIDE AT VARIOUS pH VALUES

Aluminium added, mg.	pH	Aluminium extracted, mg.
2·0	0·42	0·010
2·0	0·62	0·020
2·0	0·85	0·043
2·0	1·05	0·083
2·0	1·3	0·30
2·0	1·5	0·65
2·0	2·37	1·20
2·0	3·02	1·92
2·0	3·47	1·96 (±0·04)
2·0	3·92	1·98 (±0·04)
1·0	0·70	0·016
3·0	0·68	0·120

In methods previously published the pH has been controlled by the addition of acetate buffer solutions, but the buffering power of these solutions is insufficient to give ±0·05 pH over the range of acidity which may occur in the solution to be tested. It has been found convenient to make the pH adjustment using a glass electrode combined with a small pencil-type calomel electrode and a standard pH meter. The two electrodes are mounted together on a small stand and can readily be raised or lowered. With this arrangement the addition of reagents drop by drop to adjust the pH to within 0·05 is no more difficult than by use of an indicator.

The intensity of colour produced by a given amount of aluminium is also slightly affected by the presence of ammonium salts, and the conditions of test have therefore been standardised by the addition of 1 g. of ammonium chloride before adjustment of the pH.

Procedures with "aluminon," recommending both acid and alkaline media, have been published.[5] The advantages of the latter are the weaker colour of the excess reagent in alkaline solution and the fact that the interference of tervalent chromium is much reduced. This second point, however, only applies to procedures in which the solution is heated to develop the colour. In acid solution, tervalent chromium produces a considerable colour with the reagent on heating (200 μg. of chromium ≡ 40 μg. of aluminium), but if the colour is developed by standing the solution at room temperature, comparatively large amounts of chromium (1 mg.) can be tolerated without any interference. Performance of the test in the cold has another advantage in that up to 100 μg. of aluminium may be present without flocculation of the aluminium lake occurring, whereas with heating, about 50 to 60 μg. is the maximum permissible amount of aluminium, even in the presence of glycerol.

The exact acidity chosen for the development of the colour is governed by two factors. The colour produced by a given amount of aluminium decreases with increasing pH value, but on the other hand, the reagent itself has an appreciable colour in acid solution. In the lower curve in Fig. 1 is shown the background colour due to the reagent at different pH values. It will be seen that this levels off above pH 4·9 and for this reason the range from pH 4·9 to 5·0 has been chosen for the test.

The reagent is added to the weakly acid solution and followed by ammonium acetate. Adjustment of the acidity is then carried out as described below. Under the conditions used, milligram quantities of other elements likely to be present in iron (chromium, nickel and manganese) do not interfere. Copper gives only slight interference (300 μg. of copper ≡ 8 μg. of aluminium), but in any case it is almost completely extracted by the organic solvent in the cupferron separation at pH 0·5. Titanium and vanadium, if present, are also completely removed at this stage.

In the present work the method has been applied only to iron samples of high purity, containing one additional element in appreciable amounts, but it is suggested that if the amount of other elements present is sufficient to cause interference, the aluminium could be separated by extraction of aluminium cupferride at pH 3·5, after removal of the iron.

This procedure has been tried on other metals and found to give satisfactory results. The chloroform extract of the cupferride is evaporated to dryness in a platinum dish, and the

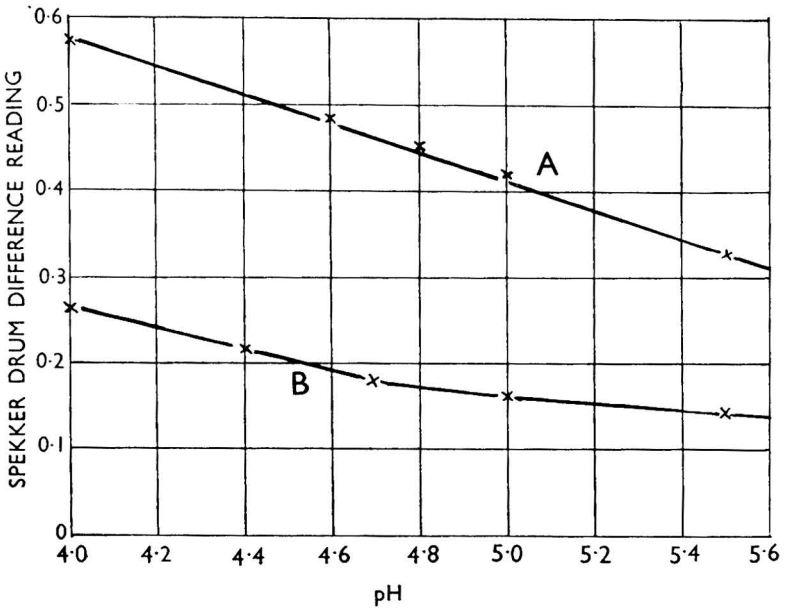

Fig. 1. Colour due to aluminium at various acidities.
Curve A, 50 μg. of aluminium in 50 ml. volume; curve B, reagent blank in 50 ml. volume.

residue ignited at 450° to 500° C. in a muffle and dissolved in a small amount of concentrated hydrochloric acid. Colorimetric aluminium determinations by the "aluminon" method are then carried out directly on aliquots of this solution.

RESULTS OF DETERMINATIONS ON HIGH-PURITY IRON SAMPLES—

Table II gives the results of tests of the whole procedure, carried out on 10-g. portions of iron with known amounts of aluminium added.

TABLE II

RECOVERY OF ADDED ALUMINIUM FROM 10 G. OF IRON

Added, %	Found, %
nil	0·0003
0·003	0·0033
0·005	0·0048
0·01	0·010
0·02	0·019
0·03	0·028

The figure of 0·0003 per cent., with no added aluminium, represents a sample of iron specially chosen for low aluminium content. The normal figure in this de-oxidised iron is of the order of 0·001 per cent. These figures relate to acid-soluble aluminium only. The material also contains small amounts of insoluble inclusions, and if the solution of the sample is filtered and aluminium determined in the insoluble matter, an additional 0·001 per cent. of aluminium is usually found.

METHOD

Fig. 2 shows the percentage transmission curve of the coloured solution produced by 50 μg. of aluminium, and also that of the reagent solution alone, measured on a Beckman spectrophotometer. From these it may be seen that a filter with maximum transmission at 5300 A. is suitable for measurement of the optical density on an absorptiometer. Ilford No. 604 filters have been used.

REAGENTS—

Ammonium acetate solution—Dissolve 115 g. of ammonium acetate in water, dilute to 300 ml. and filter before use.

Ammonium aurin tricarboxylate ("*aluminon*")—A 0·2 per cent. aqueous solution.

PROCEDURE—

Dissolve 10 g. of sample in concentrated hydrochloric acid, with a minimum of nitric acid to oxidise the iron. Evaporate to dryness, dissolve the residue in 80 ml. of hydrochloric acid and again evaporate until separation of solids begins. Add 50 ml. of hydrochloric acid (sp.gr. 1·12) and transfer to a separating funnel; wash out the beaker with small amounts

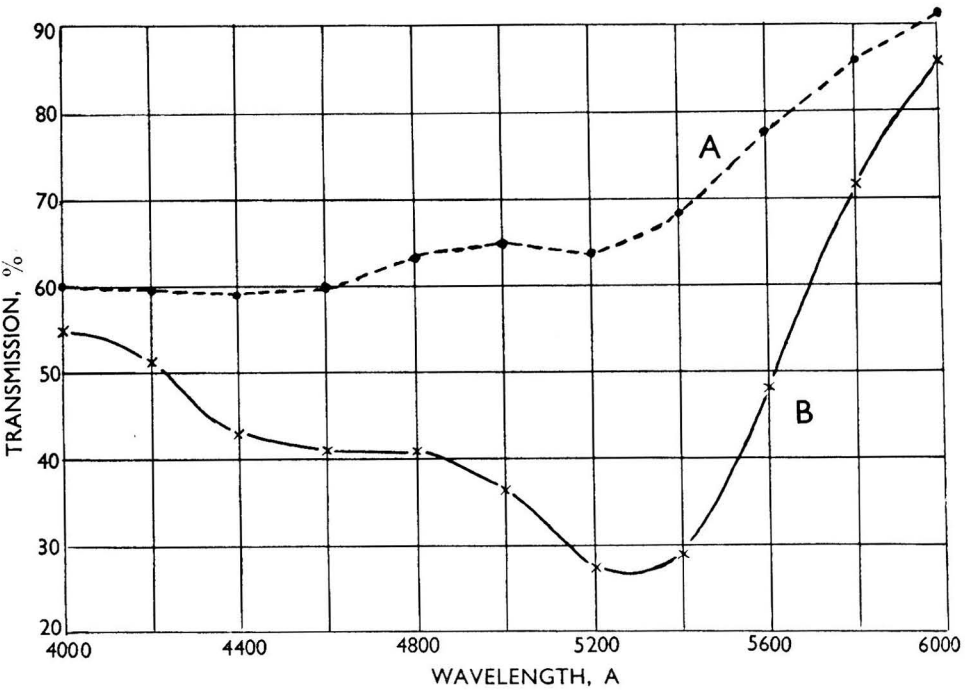

Fig. 2. Transmission curve for aluminium solution plus "aluminon."

Curve A, reagent blank in 50 ml. volume, 1-cm. layer; curve B, 50 μg. of aluminium in 50 ml. volume, 1-cm. layer.

of acid of the same strength. Carry out the extraction of ferric chloride with ethyl or *iso*propyl ether in the usual manner. Run the aqueous layer into a beaker and warm on a steam-bath to remove ether. Oxidise with a few drops of nitric acid and evaporate almost to dryness. Add a small volume of hydrochloric acid (sp.gr. 1·12) and repeat the ether separation, keeping the aqueous layer as small as possible. Warm to remove ether, add a few drops of nitric acid and again evaporate to low volume.

Dilute to 50 ml. and add 3 g. of ammonium chloride. Adjust the pH of the solution to 0·3 to 0·4, using a glass electrode - calomel electrode assembly, as described above, to measure pH. Transfer the solution to a separating funnel and add 0·1 g. of cupferron dissolved in a small amount of water. Allow the solution to stand for at least 5 minutes and then extract three times with chloroform; continue the extraction if necessary until the chloroform layer is colourless. Warm the aqueous layer for a few minutes to remove chloroform, and make the volume up to 50 ml. or 100 ml.

Take a suitable aliquot (usually 1/5 or 2/5), add 1 g. of ammonium chloride, 5 ml. of "aluminon" reagent and 5 ml. of ammonium acetate solution. Adjust the pH to within the range 4·9 to 5·0 by addition of hydrochloric acid or ammonia, using the glass electrode to measure pH. Make the volume up to 50 ml. and allow the solution to stand for 15 minutes. Measure the solution on a Spekker absorptiometer in a 1-cm. cell with Ilford No. 604 filters,

using as a comparison solution one containing the reagents but no aluminium. Read off the amount of aluminium from a graph prepared by carrying out a similar procedure on tests containing known amounts of standard aluminium solution.

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The Photometric Determination of Copper in Aluminium Alloys with Tetraethylene Pentamine

BY L. H. WILLIAMS

SYNOPSIS—For the control analysis of aluminium alloys a rapid and reasonably accurate method for determining copper is essential. A photometric method based on the complex formed with sodium diethyldithiocarbamate has been used, but this reagent is somewhat too sensitive for the purpose because the maximum concentration of copper that can be measured in a standard 4-cm. cell is but 2·5 μg. per ml. As a consequence, the conversion factor from extinction to percentage is large and, under routine conditions, the standard error for alloying quantities becomes excessive. Furthermore, in the important group of aluminium - copper - nickel alloys the nickel interferes. The ammonia reaction, on the other hand, is not sufficiently sensitive.

Tetraethylene pentamine is about three times as sensitive as ammonia and about one-fortieth as sensitive as sodium diethyldithiocarbamate.

The method described is rapid. It is free from interference and has a standard error of 0·04 per cent. for the range 1 to 4·5 per cent. of copper.

THE photometric diethyldithiocarbamate method for the determination of copper in aluminium alloys[1] is not very specific, several metals form complexes with the reagent and interfere with the determination. In particular, nickel forms a greenish-brown product, iron a golden-brown ferric citrate, and lead, tin, antimony, cadmium and chromium, if present in quantity, form white turbidities. A separation from the interfering metals is then necessary. Furthermore, the method has not proved particularly reliable in routine use, especially for the larger quantities of copper.

During an investigation of a number of amines as possible reagents for copper, tetraethylene pentamine was found to be the most sensitive of those available, its sensitivity being more than three times that of ammonia. This amine was suggested as a reagent for copper by Crumpler[2] who stated that it was subject to the same interferences as those associated with ammonia. This statement, for which no experimental evidence was offered, was not confirmed. Crumpler's tests were made by adjusting the pH of an acid copper solution to 3·5 to 4·0 with dilute sodium hydroxide solution, followed by the addition of 10 ml. of a 2 per cent. solution of the amine. Experiments using an ammoniacal citrate or tartrate solution carried out in these laboratories showed no appreciable intereference from the metals usually found in aluminium alloys.

It was therefore appreciated that a method based on the use of tetraethylene pentamine for the determination of copper in aluminium alloys might represent a considerable improvement on the existing diethyldithiocarbamate method. A full investigation was therefore undertaken.

Experimental

Choice of amine—

A standard copper solution was prepared from electrolytic copper, and aliquots representing 0·01 g. of copper were treated with ammonia until formation of the cuprammonium complex began. Five ml. of a 5 per cent. solution of the amine were added, the solutions diluted to 200 ml. and their extinctions measured on the Spekker absorptiometer in 4-cm. cells. The results are shown in Table I for the full range of available amines.

Table I

Amine	Ilford filter No. 605	606	607	608	609
Ethanolamine	0·150	0·158	0·152	0·160	0·083
Diethanolamine	0·172	0·208	0·225	0·268	0·152
Triethanolamine	0·116	0·150	0·197	0·262	0·223
Ethylene diamine	0·218	0·220	0·190	0·205	0·112
Diethylene triamine	0·232	0·308	0·350	0·398	0·310
Triethylene tetramine	0·390	0·410	0·405	0·405	0·255
Tetraethylene pentamine	0·245	0·372	0·485	0·580	0·505
Hydroxyethyl ethylene diamine	0·230	0·265	0·262	0·308	0·193
Mixed *iso*propanolamine	0·116	0·157	0·196	0·270	0·220
Tri-*iso*propanolamine	0·110	0·153	0·200	0·285	0·222
Diethylamino ethanol	0·245	0·232	0·205	0·225	0·124
Phenyl diethanolamine	0·179	0·223	0·248	0·293	0·182

These results show that tetraethylene pentamine is the most sensitive amine and that the Ilford No. 608 filter gives the highest sensitivity. A calibration graph using this amine and filter gave a straight line plot of extinction against concentration.

Properties of the cupramine complex—

Experiments showed that the complex was not influenced by temperature variations and was stable for a long period of time, at least 7 days. The effect of cellulose was studied and two filtrations through a medium texture filter paper had no effect; although repeated filtrations or the addition of accelerators and subsequent filtration resulted in slightly high extinctions.

Amount of amine required—

It was found that 0·01 g. of copper required approximately 5 ml. of a freshly prepared 2 per cent. solution of the amine for complete colour development; excess of the amine solution had no effect on the result. As there was some evidence that the amine solution deteriorated and became less reactive after several weeks' standing, 10 ml. was decided upon as being sufficient, even if old reagents were used.

The effect of some anions—

Since the amines are related to ammonia, the radicals showing interference with the cuprammonium complex might be expected to show the same effect with the cupramine

Table II

Anion	Anion added, p.p.m.	Cu found, p.p.m.
Tartaric acid	20,000	50·2
	50,000	50·9
Citric acid	10,000	50·2
	20,000	50·2
	50,000	50·6
Sulphuric acid	25,000	50·0
	50,000	50·2
	100,000	50·4
Nitric acid	20,000	49·6
	50,000	49·8
	100,000	49·6
Hydrochloric acid	50,000	49·8
	100,000	50·2

complex. Table II shows the effect of some of the anions that may be present as their ammonium salts with aluminium in ammoniacal solution. In each experiment 50 p.p.m. of copper was present in solution and the radical was added as free acid and then neutralised with ammonia.

Since the variations in these results are all within ±2 per cent. of the theoretical result the experiment showed that it was permissible to have tartrate, citrate, sulphate, nitrate or chloride present in at least the amounts shown. These amounts are far in excess of the quantities likely to be required in practice. The quantity of tartaric acid finally decided upon corresponds to 25,000 p.p.m. so that the variation is reduced to ±1 per cent.

As was to be expected, the presence of cyanide in the solution inhibits the formation of the cupramine complex.

THE EFFECT OF SOME METALS—

Similar experiments were carried out to study the effect of the commoner metals likely to be found in aluminium alloys, the results of these are shown in Table III. In each experiment the test solution contained 50 p.p.m. of copper, 10 ml. of 20 per cent. tartaric acid solution and sufficient ammonia to form the cuprammonium complex before the addition of the amine.

TABLE III

Metal	Metal added, p.p.m.	Cu found, p.p.m.
Aluminium	2000	49·8
	5000	50·2
Antimony	10	50·0
	20	49·6
Cadmium	60	49·6
Chromium	10	50·2
Iron	10	49·4
	20	49·6
Lead	2	50·0
	10	49·8
Magnesium	20	49·8
	100	49·6
Manganese	10	50·0
	20	51·3
Nickel	20	50·2
	40	49·8
	60	50·0
Silicon	20	precipitation occurred
	100	precipitation occurred
Tin	2	50·2
	10	50·2
Titanium	5	49·8
	10	49·4
Zinc	50	50·2
	240	50·6

These experiments showed that none of the elements commonly present in aluminium alloys (with the exception of silicon and possibly manganese) interfered in ammoniacal tartrate solution, even when present in the maximum amounts likely to be encountered.

In an attempt to prevent precipitation of silicic acid, experiments were made in sodium hydroxide - tartrate solution. Satisfactory results were obtained, but in the presence of manganese the extinctions were high owing to the formation of yellow colloidal hydroxide.

A number of attempts were made to prevent the formation of this hydroxide by keeping the pH of the final solution below 9 (at which pH manganous hydroxide is formed) but over the range pH 4 to 9 the results were sensitive to changes of acidity, the colour of the solution changing from violet to blue. After a number of other tests had been made, which showed no prospect of overcoming the problem of manganese interference, it was decided not to proceed further with the use of a caustic medium.

THE EFFECT OF MANGANESE IN AMMONIACAL SOLUTION—

When a sample weight of 0·2 g. was used, pure aluminium and aluminium - manganese alloys with additions of a copper solution were attacked with five pellets of sodium hydroxide and a little water, acidified with 5 ml. of nitric acid and made just ammoniacal in the presence of 10 ml. of 20 per cent. tartaric acid solution. Ten ml. of 2 per cent. tetraethylene pentamine solution were added, the assays diluted to 100 ml. and their extinctions measured. The results are shown in Table IV.

TABLE IV

Sample	Cu added, %	Cu recovered, %
Pure aluminium	1·00	1·01
	2·50	2·48
	5·00	5·00
Al - Mn alloy (1·00% Mn)	1·01	1·00
	2·51	2·49
	5·01	5·01
Al - Mn alloy (1·75% Mn)	1·17	1·15
	1·67	1·62
	5·17	5·27

These results indicate that there is no systematic error due to the presence of manganese in ammoniacal tartrate solution.

THE EFFECT OF SILICON IN AMMONIACAL SOLUTION—

Tests on a number of alloys by the method outlined in the preceding section showed that with silicon contents greater than 2 per cent., silicic acid was precipitated on the addition of the amine and with silicon contents of 1 to 2 per cent., precipitation occurred slowly and caused uncertain results. When such samples were diluted to volume and were filtered after standing to complete the precipitation of silicic acid, the results obtained were low, the error increasing with increasing silicon content. This suggested that the error was due to adsorption of the cupramine complex on the gelatinous silicic acid. When the precipitate was filtered off and washed well before diluting to volume no improvement was evident. It therefore became necessary to remove silicon by some other technique and a number of possibilities was investigated.

The usual method of hydrochloric - nitric acid attack followed by filtration through a very fine texture filter-paper is extremely slow and was not favoured as the filtrates are not always perfectly free from turbidity. The latter consideration is not so important in the diethyldithiocarbamate method in which a small aliquot of the filtrate is used for the final test, but in the present method in which no aliquot is taken a slight turbidity will cause markedly high results. Filtration through a pad on a Buchner funnel is not suitable for a large number of samples. The addition of gelatin solution to the acid mixture was found helpful, and filtration could then generally be accomplished on a filter-paper of medium texture but a general rule could not be applied; occasional samples gave turbid filtrates.

The addition of a few drops of hydrofluoric acid to a hydrochloric - nitric acid solution, dissolved all the silicon, but precipitation frequently occurred on adding the amine.

Fuming with sulphuric or perchloric acids gave some good results, but here again there were several objections to the use of either acid. Fuming with sulphuric acid resulted in a great deal of "spattering" and losses frequently occurred. To overcome this by the use of more acid was not practicable as the extra acid had then to be neutralised with ammonia, which generated enough heat to boil the solution with the accompanying danger of loss. Perchloric acid is readily fumed and neutralised, but filtration after fuming is slow and the dangers of using fuming perchloric acid in a routine process are well known.

Precipitation of silica with gelatin in several acid media was attempted without much success. Here again a strong acid concentration was required.

By using a hydrochloric - nitric acid attack, making ammoniacal after the addition of tartaric acid and then adding the amine solution, it was found that the silicon was coagulated and readily removed by filtration, generally on a Whatman No. 541 paper, invariably on a Whatman No. 40 paper. The time of filtration was shortened considerably by this method, and no loss of copper occurred. As a result, this procedure was adopted as standard for all types of alloys. It is worth recording that the same experiment carried out substituting

citric for tartaric acid resulted in only slight coagulation of the silicon, filtration not being reliable on a medium texture paper.

THE EFFECT OF NICKEL AND IRON—

In view of the serious interference from nickel and to a lesser extent from iron in the colorimetric method for determining copper as the blue cuprammonium complex and of the statement by Crumpler, a more comprehensive investigation of the effects of these metals in the cupramine process was thought desirable. Standard samples with varying iron and nickel contents and other samples with additions of standard iron and nickel solutions were analysed. Some of the results obtained are shown in Table V.

TABLE V

Sample	Fe content, %	Ni content, %	Cu by volumetric method,[3] %	Cu by amine method, %
DTD.133C	1·53	1·33	1·96	1·95, 2·00, 1·99
DTD.424	0·78	0·09	2·48	2·45
DTD.424	0·69	0·23	2·97	2·97, 2·97, 2·94
2L.24	0·62	2·14	4·37	4·36
2L.24	0·78	2·51	4·48	4·53, 4·45, 4·46
DTD.364A	0·16	0·02	3·44	3·40
DTD.364A	0·88	0·02	2·96	2·90
2L.40	0·49	0·03	3·23	3·22, 3·21, 3·23
" + 0·002 g. Ni	0·49	1·03	3·23	3·21
" + 0·004 g. Ni	0·49	2·03	3·23	3·19
" + 0·006 g. Ni	0·49	3·03	3·23	3·19
" + 0·002 g. Fe	1·49	0·03	3·23	3·21
" + 0·003 g. Fe	1·99	0·03	3·23	3·21

The results showed that satisfactory figures are obtained in the presence of iron and nickel.

METHOD

REAGENTS—

Hydrochloric - nitric acid mixture—1 vol. of hydrochloric acid, sp.gr. 1·16, and 1 vol. of nitric acid, sp.gr. 1·42.

Tartaric acid—A 20 per cent. w/w solution.

Tetraethylene pentamine—A 2 per cent. solution. Dissolve 10 ml. of the amine in 500 ml. of water, heat to between 60° and 70° C. with activated charcoal, cool and filter through a Whatman No. 40 paper.

PROCEDURE—

Attack 0·2 g. of the sample with 5 ml. of hydrochloric - nitric acid mixture in a 100-ml. squat beaker. When the attack is complete, wash down the sides of the beaker with a little water and boil for 3 to 4 minutes. Remove from the hot-plate, dilute to between 10 and 15 ml. and allow to cool somewhat. Add 10 ml. of tartaric acid solution, 10 ml. of concentrated ammonia, sp.gr. 0·880, and 10 ml. of tetraethylene pentamine solution. Mix the liquid thoroughly after each addition. Filter through a 9-cm. Whatman No. 40 filter-paper into a 100-ml. graduated flask and wash the residue six times with cold water and dilute to the mark. Mix well and measure the absorption on the Spekker absorptiometer in 4-cm. cells, with Chance No. ON13 and Ilford No. 608 filters, and the tungsten filament lamp. Deduct a blank determination on the reagents.

NOTES—

(1) For copper contents of 5 to 10 per cent. reduce the sample weight to 0·1 g.

(2) The instrument is calibrated by carrying aliquots of a standard copper solution through the above procedure.

RESULTS

The method has been in use on a routine scale for several months. The standard error has been calculated from some 200 determinations made on a series of standard samples of DTD.424, 2L.24, LAC.112A, DTD.364A and DTD.687; all the results being obtained under routine conditions. The copper contents of these standards range from 1·2 to 4·5 per cent. and the standard error is $\pm 0{\cdot}04$ per cent.

SUMMARY

1. The method is applicable to the determination of copper in all types of aluminium alloys in which it is an alloying constituent.

2. The procedure is direct; no interference is caused by any of the normal constituents.

3. The method has several advantages over the alternative photometric methods. It is much more sensitive and at the same time less subject to interference and variation from extraneous causes than that based on the cuprammonium reaction. It is less sensitive than the diethyldithiocarbamate method, which is more suited to the determination of trace quantities. This enables a larger and more representative sample to be taken and also obviates the necessity to take an aliquot of the solution. It is less subject to interference than the diethyldithiocarbamate method and the separation of silicon has been simplified.

4. The colour is stable and the precision is satisfactory for a photometric method.

5. Tetraethylene pentamine is about three times as sensitive as ammonia and about 1/40 as sensitive as sodium diethyldithiocarbamate. The method is rapid. It is free from interference and has a standard error of ±0·04 per cent. for the range 1 to 4·5 per cent. of copper.

By reducing the sample weight of 0·1 g. the upper limit of the method can be extended to 10 per cent.

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The Colorimetric Determination of *p*-Aminophenol in *p*-Methylaminophenol Sulphate (Metol)

BY C. W. BALLARD

SYNOPSIS—As published methods for the determination of *p*-aminophenol in metol either deal with large amounts, or are approximate or tedious, two colorimetric methods have been developed in an attempt to overcome these objections.

The two methods described are (*a*) diazotisation followed by coupling with resorcinol in presence of sodium carbonate, and (*b*) the reaction with *p*-dimethylaminobenzaldehyde in presence of citrate buffer. The results obtained by these two methods are compared. The latter method is simpler, more rapid and more precise, but requires photo-electric matching because the colour is yellow and the reagent blank is relatively large; the diazotisation method gives a red colour, suitable for visual comparison, and an almost colourless blank.

The difficulties encountered were (*a*) the behaviour of N-nitrosometol under the conditions of diazotisation and coupling, and (*b*) the non-specificity of *p*-dimethylaminobenzaldehyde as a reagent for primary arylamines.

PUBLISHED methods for the determination of *p*-aminophenol in metol either deal with relatively large *p*-aminophenol contents[1,2] or are approximate[3] or tedious.[4] None of these methods is colorimetric and hence in view of the success of colorimetric methods involving diazotisation and coupling or the use of *p*-dimethylaminobenzaldehyde for the determination of primary arylamines in traces[5,6,7] it was decided to attempt to establish a colorimetric method. There appeared to be some doubt whether *p*-dimethylaminobenzaldehyde reacts with secondary arylamines and therefore diazotisation and coupling, being specific for primary arylamines, was studied first.

DIAZOTISATION AND COUPLING METHOD

When metol containing *p*-aminophenol is treated with nitrous acid the diazo-oxide of *p*-diazophenol and the nitrosamine (N-nitroso-*p*-methylaminophenol) are formed. The diazo-oxide does not couple readily with secondary amines in acid or neutral solution,[8] hydrolysis to the diazotate being necessary before coupling can occur, but it couples readily with phenols in alkaline solution. The nitrosamine is coloured and must be either compensated for[5,9] or removed, as for example, by extraction from acid solution with an organic solvent.[2]

CHOICE OF PHENOL FOR COUPLING—

Nitroso-metol was found to be unstable in sodium hydroxide solution, yellow, green and purple colours being produced, but was relatively stable in sodium carbonate solution. Many different phenols were tested and it was found that resorcinol coupled best with *p*-diazophenol in sodium carbonate solution. Alkaline solutions of resorcinol rapidly became coloured purple, yellow or green according to the concentration of resorcinol and the time for which the solution stood; shielding from light or removal of oxygen from the solutions made little difference. However, it was found that if a relatively concentrated solution was allowed to pass through this series of colour changes ending with deep yellow after about 10 minutes and then diluted considerably immediately before use, a reagent blank with an extinction value as low as 0·01 for a 1-cm. cell was obtained.

DIAZOTISATION AND COUPLING OF *p*-AMINOPHENOL ALONE—

The effect of various factors on the diazotisation and coupling of *p*-aminophenol was determined and, under the conditions adopted, the coupling reaction was found to be of the first order with respect to *p*-diazophenol and was 99 per cent. complete in 10 minutes. The azo-dye produced had a $\lambda_{max.}$ of 480 mμ., and, using Ilford filter No. 602, calibration curves (extinction against concentration) were almost linear, and passed through the origin, 0·09 mg. of *p*-aminophenol giving an extinction value of 1·0 with a 1-cm. cell.

PREPARATION AND TESTING OF PURE METOL—

Metol was recrystallised nine times from dilute alcohol and from water[10] in order to remove *p*-aminophenol and other possible impurities such as *o*-aminophenol and *p*-dimethylaminophenol.[11] Nitrosation of the purified metol yielded two fractions one of which, N-nitrosometol, could be extracted from acid solution with ether[2] and the other, which could not be so extracted and was apparent by its pale orange-yellow colour. The amount of this non-extractable fraction varied with the time of nitrosation; thus when ether extraction was made immediately after adding the nitrite the nitrosometol obtained was equivalent to 100 per cent. of the original metol, but was equivalent to only 90 per cent. when 30 minutes were allowed for nitrosation. Furthermore, when pure N-nitrosometol was treated with hydrochloric acid, free nitrous acid was detectable after several minutes but not after 24 hours, when, however, extraction with ether gave a colourless solution. It seems probable that the nitrosamine undergoes a type of Fischer - Hepp transformation[23] under the influence of the dilute hydrochloric acid yielding a ring-substituted nitroso compound[12,24] which, having a basic centre, is not extracted from acid solution by ether.

Although no loss of *p*-diazophenol occurred when acid solutions were extracted with ether, it was not possible to utilise this fact because the non-extractable nitroso fraction gave with resorcinol an orange colour which would be measured with any due to *p*-aminophenol. On the other hand, in sodium carbonate solution without resorcinol this fraction gave a similar but less intense colour, hence compensation by a blank test was not possible.

The total nitrosation product from purified metol gave colours in sodium carbonate solution both alone and with resorcinol, the ratio of the intensities depending upon nitrosation time and the time after making alkaline (Table I). The development of colour in sodium carbonate alone may be due to decomposition or oxidation, nitroso compounds being particularly unstable to light,[13,26] whilst the increase in colour produced with resorcinol with increase in nitrosation time suggests that the non-extractable nitroso fraction reacts with resorcinol, N-nitrosometol reacting to a less extent (Table II) (*cf.* reaction of N-nitroso-tryparsamide with β-naphthol[5]).

From Table I it is seen that for a nitrosation time of 1 minute the colours produced in sodium carbonate solution with and without resorcinol after from 10 to 20 minutes are equal,

indicating that no azo-dye was present. Furthermore, comparison of these data with those given in Table II shows that the colour produced by the nitrosated purified metol is equal to that given by N-nitrosometol under similar conditions. Hence it was concluded that the purified metol was free from *p*-aminophenol.

TABLE I

NITROSATION OF PURIFIED METOL—COLOUR PRODUCED IN SODIUM CARBONATE SOLUTION WITH AND WITHOUT RESORCINOL

Nitrosation time, minutes			Extinction in 1-cm. cell (Ilford filter No. 602) Minutes after making alkaline 10	15	20
1	(*a*)	Sodium carbonate with resorcinol ..	0·095	0·12	0·135
	(*b*)	Sodium carbonate alone..	0·1	0·11	0·123
	(*c*)	Ratio	0·95	1·1	1·1
2	(*a*)	Sodium carbonate with resorcinol ..	0·15	0·16	0·17
	(*b*)	Sodium carbonate alone..	0·09	0·13	0·17
	(*c*)	Ratio	1·6	1·23	1·0
6	(*a*)	Sodium carbonate with resorcinol ..	0·19	0·19	0·205
	(*b*)	Sodium carbonate alone..	0·09	0·12	0·14
	(*c*)	Ratio	2·1	1·6	1·5
30	(*a*)	Sodium carbonate with resorcinol ..	0·26	0·276	0·28
	(*b*)	Sodium carbonate alone..	0·09	0·107	0·13
	(*c*)	Ratio	3·0	2·6	2·1

DIAZOTISATION AND COUPLING OF *p*-AMINOPHENOL IN PRESENCE OF METOL—

As seen from Table I, the colour arising from nitrosation of metol increased with time of nitrosation; closer examination showed that the rate of this reaction was exponential so that, at 10 minutes, 1 minute difference of time would result in a relative error of less than 1·5 per cent. in the determination of 0·8 per cent. of *p*-aminophenol in metol. Nitrosyl chloride is believed to be involved in the Fischer - Hepp transformation[27] but substitution of sulphuric

TABLE II

COLOUR PRODUCED BY N-NITROSOMETOL IN SODIUM CARBONATE SOLUTION WITH AND WITHOUT RESORCINOL

Time under influence of acid, minutes			Extinction in 1-cm. cell (Ilford filter No. 602) Minutes after making alkaline 10	15	20
0	(*a*)	Sodium carbonate with resorcinol ..	0·065	0·072	0·077
	(*b*)	Sodium carbonate alone..	0·062	0·06	0·06
	(*c*)	Ratio	1·05	1·2	1·3
1	(*a*)	Sodium carbonate with resorcinol ..	0·095	0·115	0·135
	(*b*)	Sodium carbonate alone..	0·09	0·11	0·115
	(*c*)	Ratio	1·06	1·04	1·1

acid for hydrochloric, with a view to eliminating NOCl, gave only slightly less colour in the nitrosation reaction. To ensure complete diazotisation sufficient nitrite for complete nitrosation was necessary,[5] a considerable excess being avoided and a low temperature used in order to diminish nuclear nitrosation.[24,28] Measurement of colour at exactly 10 minutes was necessary because of the continuous development of colour from the nitroso compounds (Table I). Under these conditions recovery of *p*-aminophenol added to metol was about 95 per cent., so calibration curves were prepared by an internal-standard method using purified metol. The curves were almost linear (Table III) but the slopes were not exactly

proportional to optical path length; the use of cells of sizes other than 1 cm. may be avoided by Stross's method.[25]

TABLE III

CALIBRATION DATA FOR DETERMINATION OF *p*-AMINOPHENOL IN 0·005 G. OF METOL BY DIAZOTISATION AND COUPLING

p-Aminophenol, mg. (*a*)	Extinction of 1 cm.* (*b*)	$\frac{b - 0{\cdot}245}{a}$
0·00	0·245	—
0·02	0·460	10·7
0·04	0·640	9·9
0·06	0·860	10·3
0·08	1·040	9·9

* Means of five values.

PROPOSED METHOD

REAGENTS—

Sodium nitrite solution—0·5 per cent. (freshly prepared).

Sulphamic acid solution—5 per cent.

Resorcinol solution—(This must be freshly prepared.) Dissolve 1 g. of resorcinol in 20 ml. of 2 *N* sodium carbonate solution, set aside for about 10 minutes and then dilute 1 part to 50 parts with 2 *N* sodium carbonate solution and use immediately.

PROCEDURE—

Dissolve 0·05 g. of sample in 50 ml. of *N* hydrochloric acid and dilute to 100 ml. with water. Cool 10 ml. of this solution to about 5° C., add 1 ml. of sodium nitrite solution, mix and set aside for 10 minutes at about 5° C. Add 1 ml. of sulphamic acid solution, mix by shaking and, after 5 minutes, add 10 ml. of resorcinol solution, mix and set aside at room temperature. Carry out a blank at the same time, omitting the metol, and after exactly 10 minutes measure the extinction difference of the two solutions, using a 1-cm. cell and Ilford No. 602 filter. Read the amount of *p*-aminophenol from a calibration curve.

p-DIMETHYLAMINOBENZALDEHYDE METHOD

OPTIMUM CONDITIONS—

Optimum conditions for the determination of *p*-aminophenol were determined and although suitable for *o*- and *m*-aminophenol, they differed from those for sulphathiazole and arsanilic acid,[7] a lower pH and a higher concentration of *p*-dimethylaminobenzaldehyde being required. Temperature was important, a 5° C. rise in temperature giving 2 per cent. less colour.[20] The Schiff's base 4-(4′-dimethylaminobenzalamino)phenol to which the yellow colour is attributed[14] was prepared[15] and recrystallised, but had a melting-point of 281° C. compared with 265° C. reported by Möhlau and Adams[15]; analysis was satisfactory (found: C, 75; H, 7; N, 11·5. $C_{15}H_{16}ON_2$ requires C, 75; H, 6·66; N, 11·7). The extinctions given by the Schiff's base and by the equivalent amount of *p*-aminophenol under the optimum conditions differed by less than 2 per cent.

APPLICATION TO PURIFIED METOL—

Under the optimum conditions for the determination of *p*-aminophenol, purified metol gave a distinct yellow colour which gave a spectral absorption curve (Fig. 1) with $\lambda_{max.}$ of 415 mμ. compared with 450 mμ. for the Schiff's base of *p*-aminophenol under the same conditions; a similar relationship was observed for *o*-aminophenol and a N-alkyl derivative. In neither case was there any indication of an inflexion at the $\lambda_{max.}$ for the aminophenol, in the curve for the alkyl derivative. This fact confirmed the purity of the latter and suggested that interaction occurred between it and the *p*-dimethylaminobenzaldehyde.

p-Dimethylaminobenzaldehyde gives colour reactions with a wide variety of compounds[16,17,29,30] including phenols, but there is disagreement as to whether secondary aromatic amines give a colour. According to some workers,[18,19,31] secondary amino compounds such as methylaniline give a colour reaction, whereas Werner[20] stated they do not. Schiff[32] described in 1864 a reaction product of N-ethylaniline and benzaldehyde, and more recently Veer[35] showed that di-secondary arylalkylamines react readily with benzaldehyde. Other

possible modes of reaction are the formation of a coloured salt as in the case of furfural and secondary amines,[33,34] or of a triphenylmethane derivative such as is most probably produced with phenols.[17] The conditions for the development of colour found to be optimum for *p*-aminophenol were not so for metol and it was concluded that two different reactions were involved. Thus decrease in pH had a much greater effect on the colour from metol than on

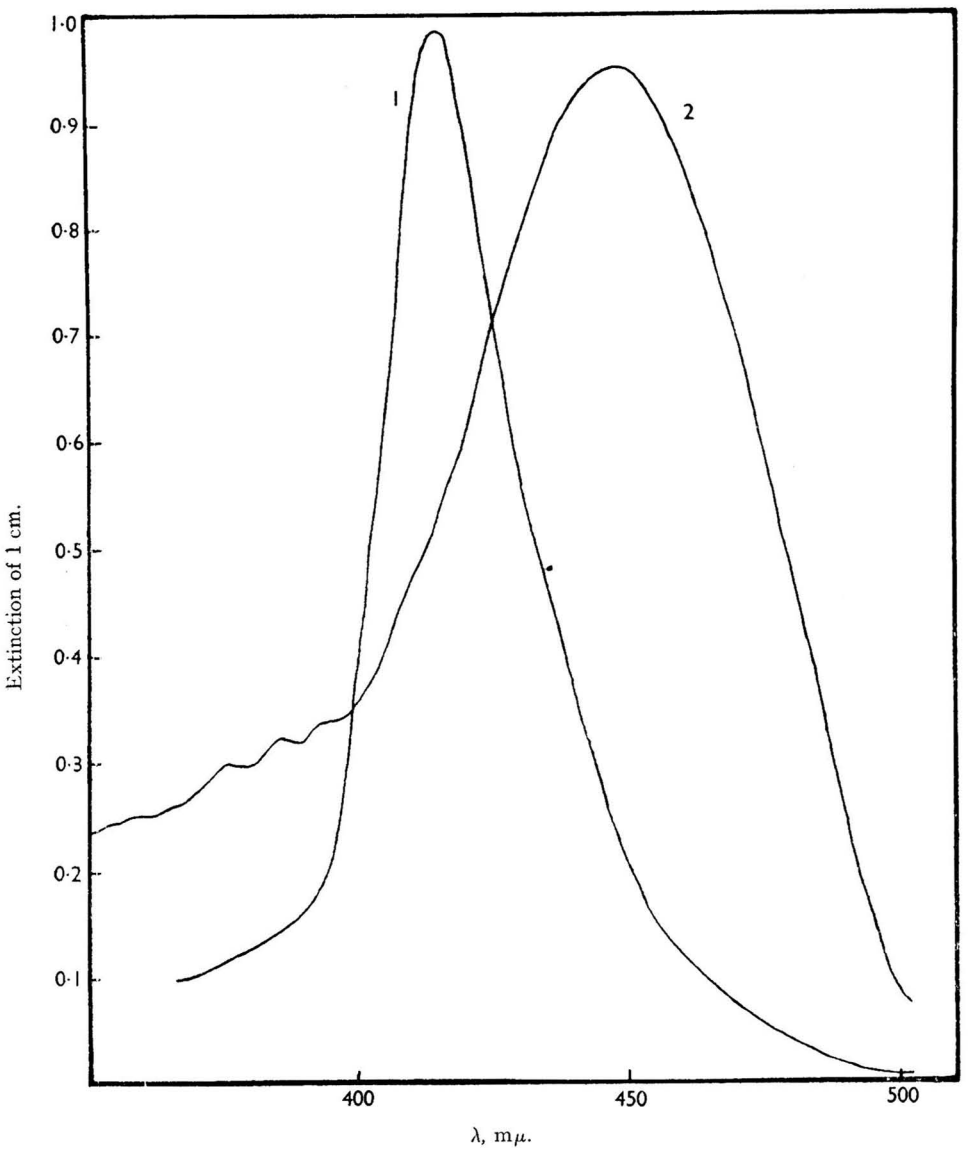

Fig. 1. Spectrophotometric absorption curves of coloured reaction products of *p*-dimethylaminobenzaldehyde with metol and with *p*-aminophenol under the conditions of the test.

Curve 1, 2·8 mg. of purified metol; curve 2, 0·06 mg. of *p*-aminophenol.

that from *p*-aminophenol. Under conditions when the ratio of colour from *p*-aminophenol to colour from metol was a minimum, the colour from metol was equivalent to 0·67 per cent. of *p*-aminophenol, but it was equivalent to only 0·28 per cent. under conditions when the ratio was a maximum. Thus there can be no doubt that metol reacts with *p*-dimethylaminobenzaldehyde; experiments are in progress to ascertain the mechanism of the reaction.

PROPOSED METHOD

For the purposes of an analytical method it was decided to use the conditions which are optimum for *p*-aminophenol, preparing calibration curves by an internal standard method. These were almost linear (Table IV) but the slopes were not proportional to the optical path lengths.

TABLE IV

CALIBRATION DATA FOR THE DETERMINATION OF *p*-AMINOPHENOL IN 0·005 G. OF METOL WITH *p*-DIMETHYLAMINOBENZALDEHYDE

p-Aminophenol, mg. (*a*)	Extinction of 1 cm.* (*b*)	$\frac{b - 0{\cdot}265}{a}$
0·00	0·265	—
0·01	0·41	14·5
0·015	0·492	15·1
0·02	0·56	14·8
0·03	0·70	14·5
0·04	0·825	14·0
0·05	0·948	13·7

* Means of three values.

It is necessary to measure the extinction after exactly 15 minutes owing to the continuous slow development of colour from the metol itself.

REAGENTS—

Citrate buffer[14]—Dissolve 39·4 g. of citric acid in 188 ml. of 2 *N* sodium hydroxide solution and dilute with water to 250 ml.

p-*Dimethylaminobenzaldehyde solution*—5 per cent. solution of reprecipitated *p*-dimethylaminobenzaldehyde[21] (or recrystallised from light petroleum, b.pt. 60° to 80° C.) in 95 per cent. alcohol.

PROCEDURE—

Dissolve 0·05 g. of sample in 50 ml. of *N* hydrochloric acid. To 3 ml. of this solution add 5 ml. of alcohol (95 per cent.), 10 ml. of *p*-dimethylaminobenzaldehyde solution and 2 ml. of buffer; mix and dilute with water to 25 ml. Set aside in a water-bath at 20° C. for about 12 minutes and re-adjust volume to 25 ml. with water. Carry out a blank determination omitting the sample and measure the extinction difference of the two solutions at exactly 15 minutes after adding the *p*-dimethylaminobenzaldehyde, using Ilford No. 601 filter and a 1-cm. cell. Read the amount of *p*-aminophenol from a calibration curve.

COMPARISON OF METHODS

From Table V it is seen that the *p*-dimethylaminobenzaldehyde method gives more concordant results than the diazotisation method and that the latter method gives results from 0·11 to 0·14 per cent. higher, irrespective of the *p*-aminophenol content. This fairly

TABLE V

COMPARISON OF RESULTS BY THE TWO METHODS

Sample	*p*-Aminophenol, per cent.				Difference
	Diazotisation and coupling	Mean	*p*-Dimethylaminobenzaldehyde	Mean	
A	0·7, 0·77	0·74	0·62, 0·60	0·61	0·13
B	0·38, 0·45	0·42	0·29, 0·27	0·28	0·14
C	1·02, 1·09, 1·0	1·04	0·93, 0·93, 0·91	0·92	0·12
D	0·28, 0·22	0·25	0·13, 0·11	0·12	0·13
E	0·67, 0·74, 0·71	0·71	0·58, 0·57, 0·58	0·58	0·13
F	0·14, 0·09, 0·15	0·13	0·04, 0·0	0·02	0·11
G	0·87, 0·91, 0·83	0·87	0·72, 0·73, 0·75	0·73	0·14
H	0·54, 0·62	0·58	0·45, 0·47	0·46	0·12
I	1·22, 1·17, 1·14	1·18	1·07, 1·1, 1·05	1·07	0·11

constant difference is attributed to the presence in the samples tested, but not in purified material used to prepare the calibration curve, of a trace of material which causes extra colour to be developed in the diazotisation method but not in the other method. It is seen, therefore, that whilst the diazotisation and coupling technique was necessary to establish the purity of the purified metol, a quantitative method based on this technique must be standardised by the *p*-dimethylaminobenzaldehyde method if true and not merely comparative results are required. The *p*-aminophenol contents found may be compared with the limit of 8 per cent. proposed by Popov[22] and that of 2·5 per cent. specified by the American Standards Association.[1]

The *p*-dimethylaminobenzaldehyde method is simpler, more rapid and more precise, but photo-electric matching is essential because the colour is yellow and the reagent blank relatively large. In the less rapid and less precise diazotisation and coupling method the reagent blank is almost colourless and the red colour produced is suitable for visual comparison.

Summary

1. Two colorimetric methods have been developed for the determination of *p*-aminophenol in metol.

2. Difficulties encountered in this work arose mainly from (*a*) the behaviour of N-nitrosometol under the conditions of diazotisation and coupling, and (*b*) the non-specificity of *p*-dimethylaminobenzaldehyde as a reagent for primary arylamines.

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February, 1950

Method for the Determination of Propamidine and M & B 1270 (Di-Bromo-Propamidine) by means of Pentacyanoammonioferrate

By HILDA TROUGHT, G. C. ASHTON and R. G. BAKER

Synopsis—A method for the determination of Propamidine and M & B 1270 by means of pentacyanoammonioferrate has been demonstrated, and its application to the estimation in blood serum has been given preliminary trials, the range of blood level so far found being between 2 and 10 mg. per 100 ml. calculated as free base. The method is capable of being carried out in an ordinarily equipped hospital laboratory.

The estimation of the aromatic diamidine type of chemotherapeutic agents has been extensively investigated in the last few years owing to the need of a method for the control of their clinical applications. Fuller[1] described a method of determination based on the reaction of aromatic diamidines with a glyoxal - bisulphite compound, using boric acid as a buffer. Jackson, Kuhl and Irvin[2] elaborated Fuller's glyoxal - bisulphite method by the addition of benzaldehyde to the aromatic diamidine, the reaction being developed in alkaline solution. Under suitable experimental conditions, fluorescent glyoxalidone derivatives are formed and can be quantitatively determined on a fluorimeter.

The method described is based on the addition of Fearon's pentacyanoammonioferrate reagent,[3] referred to in this paper as PCAF, to an aqueous solution of the aromatic diamidine; the compound chosen for the development of the method was propamidine isethionate because of its reasonable solubility in water. Under controlled experimental conditions, the addition of PCAF results in the formation of a yellow to orange coloration which varies quantitatively with the concentration of the diamidine in solution, provided the concentration is not too high. At high concentrations a coloured complex is thrown out of solution and can be filtered off and dried as an apparently homogeneous orange-coloured substance, from which the original base may be recovered quantitatively.

Fearon suggests, as a possible formula for an amidine + PCAF compound,

$$(Fe^{III}(CN)_5NH_2C(NH).R)'''$$

From a somewhat limited analysis of the propamidine + PCAF compound, it is suggested that it is formed from one molecule of PCAF + one molecule of diamidine. Further analysis would have to be made, however, before this chemical structure is definitely established.

Preparation of calibration graphs for propamidine isethionate

Reagents—

Pentacyanoammonioferrate—A 1 per cent. solution, diluted to 1 in 10 immediately before use.

Propamidine isethionate—Aqueous solutions of 1·0, 0·10 and 0·05 per cent. concentration.

Two calibration curves were prepared by adding calculated quantities of a standard 1 per cent. propamidine isethionate solution to pre-determined volumes of water contained in test tubes in order to bring the combined volumes to 5 or 3 ml. To the prepared dilution, 1 ml. of the diluted PCAF solution was then added, bringing the final volumes to 6 and 4 ml. respectively. The colour was allowed to develop for 20 to 30 minutes, after which time it underwent no further observable deepening. The readings were taken on a photo-electric colorimeter, using a blue-green filter, OB2, the solutions being placed in test tubes specially matched for use in the colorimeter.

The reagents alone gave a reading of 2 units, and this blank has been subtracted from the figures, given in Tables I and II.

TABLE I

DATA FOR CALIBRATION GRAPH FOR THE DETERMINATION OF PROPAMIDINE ISETHIONATE IN A 6-ML. TEST SOLUTION

Propamidine isethionate, mg.	2	1·5	1·0	0·5	0·2	0·15	0·10	0·05	0·025	0·012	0·006
Galvanometer reading* ..	79	60	41	26	11	8	5	3	2·5	2	1

* A reagent blank of two divisions has been subtracted.

TABLE II

DATA FOR CALIBRATION GRAPH FOR THE DETERMINATION OF PROPAMIDINE ISETHIONATE IN A 4-ML. TEST SOLUTION

Propamidine isethionate, mg. ..	1·5	1·0	0·5	0·2	0·10	0·05
Galvanometer reading*	108	71	36	15	7	4

* A reagent blank of two divisions has been subtracted.

Calibration graphs of galvanometer readings on a logarithmic scale plotted against milligrams of propamidine isethionate proved to be straight lines passing through the origin.

I. Galvanometer reading 26 ≡ 0·5 mg. of propamidine isethionate in 6·0 ml.
II. Galvanometer reading 36 ≡ 0·5 mg. of propamidine isethionate in 4·0 ml.

From Table I it can be seen that 12 μg. of propamidine isethionate, corresponding to 6 μg. of the free base, in 6·0 ml. of solution, can be determined; the lowest figure was discarded, as it was outside the limits of experimental error.

Two of the authors (G. C. A. and R. G. B.) independently checked the validity of the principle of the above experiments by preparing calibration curves for a 1·833 per cent. solution of the propamidine isethionate, which corresponds to a theoretical 1 per cent. solution of the free base, and subsequent dilutions. Using a Beckmann spectrophotometer for the observations, quantities down to 5 μg. could be detected.

The virtual agreement of these independent results is the basis for the establishment of the PCAF method of estimating propamidine. The method can be extended to the estimation of M & B 1270 and M & B 1314, for which analogous calibration curves can be prepared.

ESTIMATION OF AMIDINES IN BIOLOGICAL FLUIDS

The application of the method to the estimation of propamidine, M & B 1270 and M & B 1314 in blood serum was next investigated. A modification of Jackson's method[2] for the removal of protein by dialysed iron followed by centrifugation was used to obtain, with comparative regularity, a clear supernatant fluid. The diamidine content of this fluid was then estimated by means of the PCAF reagent. A test with a serum blank must be made at the same time and the value subtracted from the reading for the unknown amount of diamidine.

Although Fearon's reagent gives orange colours with urea, creatine, guanidines and other substances occurring in blood, at the concentrations usually present the colours produced appear relatively negligible, and remain so over a wide range of concentrations. For example, a solution of urea corresponding to 500 mg. per 100 ml. gave no significant galvanometer reading.

A series of serum recovery experiments was carried out by adding known quantities of the diamidine to measured quantities of stock serum and finding the percentages that can be quantitatively recovered.

PROCEDURE—

Place 2·5 ml. of serum in an Erlenmeyer flask and add 7·0 ml. of water. Heat over a small flame until the solution is definitely beginning to boil. Stop heating, and add 2·0 ml. of dialysed iron dropwise from a pipette, swirling the contents of the flask after the addition of each drop. Return the flask to the flame for about 1 to 2 minutes, and continue the swirling.

It can usually be seen at this stage whether the fluid is going to be clear. Transfer the contents of the flask to a centrifuge tube, and centrifuge. The supernatant fluid should be perfectly clear; if it is not, it can sometimes be cleared by putting it back into the flask and re-boiling gently, but the boiling should not be prolonged so as to alter the volume

significantly. If the solution still does not clear, it should be discarded and the procedure recommenced.

Between 5 and 6 ml. of clear supernatant fluid should be obtained in this way.

COLORIMETRIC DETERMINATION OF DIAMIDINE—

Place 2 ml. of the supernatant fluid, 1 ml. of distilled water and 1 ml. of PCAF reagent into a test tube, allow to stand of half an hour, and then read the absorption on the photo-electric colorimeter. This value is the serum blank of the stock serum. Table III gives details of serum recovery experiments carried out by this method.

TABLE III

Known quantities of diamidine added to 2·5 ml. of stock sera

PROPAMIDINE ISETHIONATE								
Added, mg.	20	10	5	3	2·5	2·0	2·0	1·0
Determined, mg.	23·0	11·5	5·2	2·64	2·3	2·07	1·9	1·2
	20·4	10·0						
DI-BROM-PROPAMIDINE								
Added, mg.	2·0	1·0	1·0	0·4	0·1	0·1		
Determined, mg.	1·90	0·96	1·12	0·52	0·07	0·12		

EXPERIMENTS *in vivo*

A series of experiments *in vivo* were carried out on patients undergoing treatment with M & B 1270 either by intravenous injection or by irrigation of drainage tubes in surgical procedures; the results of these experiments are given in Table IV.

TABLE IV

	Nov. 5	Nov. 9	Nov. 12	Nov. 22
Patient 1	9·0 mg.	5·0 mg.	6·5 mg.	2·4 mg.
	Dec. 21		Jan. 5	Jan. 10
Patient 2	10 mg.		6·8 mg.	3·7 mg.
Patient 3	13 mg.			
Patient 4	6·3 mg.			
	1st day	5th day		
Patient 5	2·8 mg.	2·8 mg.		

NOTES—

Patient 1—Daily intravenous injections of 100 mg. of M & B 1270 started about 5 days before the first specimen of blood was taken for quantitative analysis.

Patient 2—Daily irrigation of drainage tube from a subphrenic abscess by 1 g. of M & B 1270 in 20 ml. of saline.

Patients 3 *and* 4—Irrigation of drainage tube with M & B 1270.

All the percentages are calculated as free base. For each patient a serum blank must be determined, preferably before medication with the drug begins; if this is not practicable, the blank should be determined not less than 10 days after discontinuation of the drug.

Interference from the simultaneous use of the M & B "Sulpha" triad appears to be negligible at the concentrations commonly used. A minimum percentage of 240 mg. of sulphathiazole or 100 mg. of sulphamerazine or sulphadiazine is necessary before any colour is given with the PCAF reagent.

The accuracy of low level determinations of diamidine in blood serum could be increased by the use of 2·0-ml. cells in the photo-electric colorimeter, although this involves the determination of calibration curves in 2·0-ml. cells.

Occasionally anomalous results are obtained, and it is possible that some interference, at present unknown, is caused by the phenomenon of *cis* and *trans* isomerism closely associated with some of the diamidines.

The authors thank the Clinical Staff at Dudley Road Hospital for their co-operation and collaboration in allowing access to patients who were undergoing post-operative treatment

with M & B 1270; Dr. Whitelaw, Director of the laboratories; Dr. W. R. Thrower, M.R.C.P., and Dr. H. Campbell, of May & Baker Ltd., who initiated the Diamidine Research at Dudley Road Hospital, without whose helpful criticism and encouragement the work would not have reached this stage.

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Polarographic Cells with Fused-in Capillaries

BY G. S. SMITH

SYNOPSIS—Two new types of all-glass polarographic cells with included capillaries and connections to reference electrodes are described. One type is designed to be specially suitable for operation in a thermostat bath.

THE two polarographic cells, Types A and B, described in this paper were designed as units in which all the parts were to be permanently connected. Although they are quite different in design, each embodies a fused-in capillary, a connection through a liquid junction to a standard half-cell, provision for de-oxygenation of solutions by nitrogen gas in a delivery funnel fitted with a seal and not in the dropping electrode compartments, and means for washing the whole of the interior of the cell *in situ* and for withdrawing mercury drops for weighing.

The fused-in capillary was introduced to obviate the need for insertion and removal of a capillary each time a fresh solution was examined, and to protect the capillary from mechanical damage and contamination. The cells can, however, be used with capillaries inserted through small bungs or ground-glass connections by a minor modification of the design.

TYPE A

The apparatus shown in Fig. 1 depends on the use of a second mercury reservoir for filling and emptying the cell. It is simple to operate but the apparatus does not readily lend itself to insertion in a thermostat bath. The liquid junction between the polarographic cell and a calomel electrode is formed by Coates's method.[1] The mercury that passes through the capillary in a given time can be withdrawn from the bottom of the cell by a turn of the double-bored tap.

The dropping electrode vessel, A, with capillary, B, and stand tube, C, for accurate reading of the pressure of mercury at the capillary tip, has an internally-sealed nitrogen-inlet tube fitted with a tap, T_1, which can be turned so as to establish connection with nitrogen or with the atmosphere, and a double-bored tap, T_2, one outlet of which is joined with flexible tubing to a mercury reservoir, E, and the other can be used for withdrawing mercury drops for weighing.

At the top of A a side tube, G, so arranged that no dead space is left in the cell after filling, passes to the three-way tap, T_3, which is connected also to the delivery funnel, H, fitted with a stopper, preferably a ground-glass connection, carrying a bent tube and inverted funnel dipping into sodium sulphite solution (to prevent diffusion of oxygen through the seal), and to the double-bored tap, T_4, through the liquid junction tube, J. One outlet of tap T_4 is for draining off the waste liquid from the cell after use; the other outlet is connected by means of a ground-glass joint or a short rubber tube to the upturned delivery tube of the calomel electrode vessel, K, containing 3·5 *N* potassium chloride. The side arm of the stand tube, C, is connected by plastic tubing to the reservoir, F, containing pure mercury for the capillary electrode, B.

OPERATION—

By suitably turning taps T_3 and T_4, and tap T_5 of the potassium chloride reservoir of the calomel electrode, allow a little of the potassium chloride solution to flow into the junction tube, J. Then turn tap T_4 through 180° and allow the junction tube to empty. Raise the mercury reservoir, E, and turn taps T_2 and T_3 so that any electrolyte in the cell is driven into the delivery funnel, H, and then out to waste through tap T_4. Fill the funnel, H, with water, allowing some water to run to waste through T_4 before closing this tap. Lower the mercury reservoir, E, in order to fill the cell with water, and then expel the water by raising

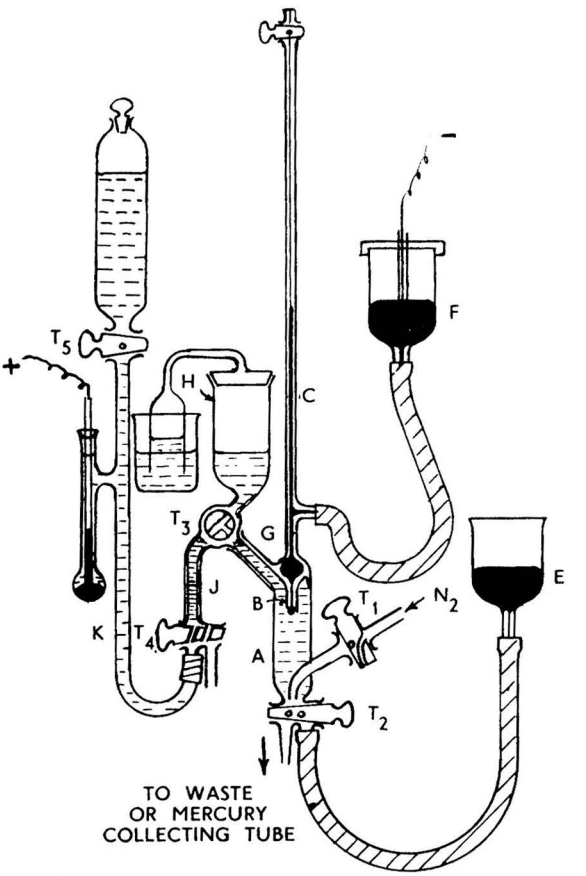

Fig. 1. Polarographic cell with fused-in capillary and mercury-operated filling.

the reservoir and allowing the water to run to waste through T_4. Wash the cell in this manner two or three times and leave it empty. Pass a stream of nitrogen through tap T_1, place the solution to be examined in the funnel H, insert the stopper and de-oxygenate the solution in the funnel with tap T_3 turned so as to make connection only between the funnel and the cell. After a suitable time, close tap T_1 and turn tap T_2 so that any mercury above it flows to waste, and a little of the electrolyte from H washes out the cell. Then turn tap T_2 through 180°, turn T_1 to connect with nitrogen, raise the mercury reservoir, E, and allow the mercury to rise and make contact with the solution in the funnel, the nitrogen in the cell being forced out through the solution. Close tap T_1, leaving some mercury in the tube, lower the reservoir and bring the solution into the cell. Close tap T_2. When it is desired subsequently to weigh a number of mercury drops, allow the mercury in the side tube below T_1 to be replaced by electrolyte by forcing some of the electrolyte up the tube and then letting the mercury run down into the cell, and then out through T_2. Turn tap T_3 so that connection is made in three

directions, fill tube J, allowing a little of the de-oxygenated electrolyte to flow to waste through tap T_4, and then turn taps T_4 and T_5 so that the 3·5 *N* potassium chloride rises a centimetre or so in tube J. Leave taps T_1 and T_2 closed, while tap T_4 establishes contact between the calomel electrode and the liquid in J, and tap T_3 establishes contact between the liquid in J and that in the funnel and in the cell. The apparatus is now ready for the recording of a polarogram.

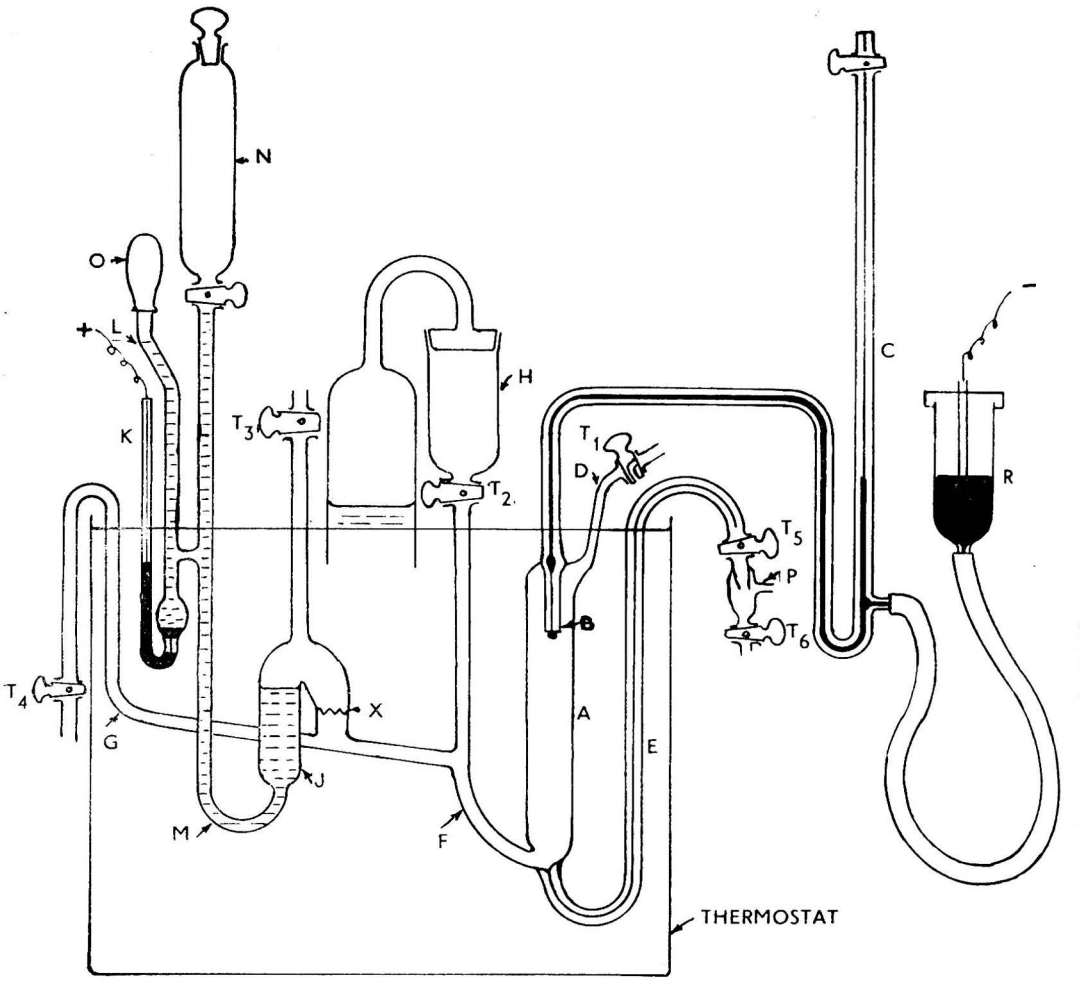

Fig. 2. Polarographic cell with fused-in capillary designed for use in a thermostat.

After use wash out the cell with water as described above. Raise the reservoir, E, so as to cover the capillary tip with mercury, and turn a tap (not shown) at the bottom of the other reservoir, F, to stop the flow of mercury through the capillary. By this means water does not get sucked into the capillary, as frequently occurs when the mercury reservoir attached to a capillary dipping into water is lowered to stop the flow of mercury.

Polarography with a mercury pool anode instead of the calomel electrode may be carried out with this apparatus if an electrical connection is made to E and tap T_2 is suitably turned.

Type B

The apparatus shown in Fig. 2 was designed to facilitate the measurement or control, or both, of all important factors in polarographic work, *e.g.*, constancy of temperature, de-oxygenation in absence of mercury, accurate establishment of potential *versus* a standard

reference electrode, freedom from contamination by substances extracted from rubber stoppers, and so on, and measurement of rate of flow of mercury under the actual conditions of an experiment, coupled with simple means for filling, emptying, and washing out the cell, and for making liquid connection with a reference electrode. The apparatus can be accommodated in a 600- or 1000-ml. beaker. The method of forming the liquid junction follows that described recently elsewhere.[2] The dropping and calomel electrodes are immersed in the bath liquid, but all the operating taps are outside.

The dropping electrode vessel, A, contains the fused-in capillary, B, connected to a stand tube, C. To it are joined the nitrogen-inlet tube, D, fitted with a tap, T_1, which can be turned so as to establish contact either with nitrogen or the atmosphere, the narrow bore tube, E, used for removal of the mercury that collects at the bottom of the cell, and the tube F which provides connection through tap T_2 to the delivery funnel, H, and also to the liquid junction tube, J, and to waste, G. The waste tube, G, terminates in a siphon tube with tap T_4 to which slight suction can be applied when necessary. The narrow bore tube, E, leads to tap T_5 with delivery into a wider tube fitted with a side tube, P, for application of suction, and a tap, T_6. The calomel electrode, K, consists of a tube, L, holding the calomel electrode proper, joined to the delivery tube, M, of a tap-funnel, N, containing 3·5 *N* potassium chloride. The delivery tube, M, is bent upwards and joined to a somewhat wider tube, J, which has a sharp downward bend near the top for connection at X by means of a fused joint, ground-glass joint, or rubber tubing to a short tube fused on to the waste tube, G. The upper part of tube J carries a tube terminating in a tap T_3. The top of tube L is closed by means of a rubber teat, O. The delivery funnel, H, is fitted with a stopper, preferably with a ground-glass joint, carrying a bent tube connected to an inverted funnel dipping into sodium sulphite solution. The side arm of the stand tube, C, is connected by plastic tubing to the reservoir, R, containing pure mercury.

OPERATION—

Allow potassium chloride solution to run into tube J from N until the level of liquid is just below the bend, as shown in Fig. 2. Remove air from A and the connecting tubes by passing nitrogen gas through tap T_1 and out through taps T_2, T_3 and T_4, and then close these taps. Place the solution to be examined in the delivery funnel, H, and de-oxygenate it by passing nitrogen gas through the empty cell A and up through tap T_2. After passage of gas for a few minutes, allow the vessel A to fill by turning tap T_1 so that the nitrogen in A is forced out through the barrel of this tap. Then turn tap T_1 again so that the cell is closed at the tap. With tap T_2 still open, cautiously open tap T_3 to allow the electrolyte to pass along tube G and flow gently into tube J on top of the more dense 3·5 *N* potassium chloride. Connection has now been established between the dropping electrode vessel and the calomel electrode, and the apparatus is ready for the taking of a polarogram.

To withdraw mercury drops for weighing, apply suction at P with tap T_5 open and tap T_6 closed. A convenient method of applying suction is by connecting P to a partially evacuated vessel. The mercury that collects above tap T_6 can be run off into a vessel such as that described previously,[3] and then washed and weighed.

After use close tap T_2, apply gentle suction at T_4, open tap T_3, flush out the junction liquid with potassium chloride solution from the reservoir N, close the reservoir tap, and finally squeeze the teat so that the level in tube J is left just below the bend. Open tap T_2 and let the electrolyte in H flow away through T_4. Open tap T_1 so that the electrolyte in A is driven through G to T_4 by the pressure of nitrogen. Then wash out the cell two or three times by placing water in H, filling and emptying A, etc., as described above. Provided that tap T_3 is kept closed, the liquid used for washing cannot come into contact with the potassium chloride solution in J. Finally de-oxygenate some water in H and allow it to flow into A and cover the orifice of the capillary, and lower the mercury reservoir until dropping stops.

With a second connection similar to that at X on tube G it would be possible to have alternative reference electrodes. Both could be left permanently in position, but only the one required for any particular purpose would be brought into operation. Thus, if polarography in a medium of concentrated calcium chloride solution were necessary, a *N* calomel electrode might be used and the liquid junction formed by flowing the lighter liquid from the calomel electrode reservoir on to the top of the calcium chloride which had been allowed to go no further than the bend on the right-hand side of tube J.

This paper is published by permission of the Ministry of Supply. The figures are reproduced by permission of the Controller of H.M. Stationery Office, Crown copyright reserved. The apparatus described as Type B forms part of the subject-matter of Patent Specification No. 14306/49, dated May 27th, 1949.

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AERONAUTICAL INSPECTION DIRECTORATE
TEST HOUSE
HAREFIELD, MIDDX.

March, 1950

Notes

IDENTIFICATION AND APPROXIMATE ESTIMATION OF REDUCING SUGARS IN THE PRESENCE OF SUCROSE

THE routine examination of samples of chocolate, sugar confectionery and similar goods normally includes the determination of sucrose by some form of double-polarisation process, and of reducing sugars by copper reduction. When it is desired to identify and estimate the individual reducing sugars present, additional work, using special methods, is usually called for. This may be tedious and time-consuming. It is the object of this note to demonstrate that, provided no more than two reducing sugars are present in the sample (invert sugar and "liquid glucose" being regarded for this purpose as single sugars), the desired information may be obtained by calculation from the "direct" and "invert" saccharimeter readings and the reducing power—*i.e.*, from the results of the routine examination.

In this laboratory, Fehlings solution is standardised against inverted sucrose and all reducing sugars are expressed, in the first instance, as invert sugar. The percentage of reducing sugars *before inversion* in any sample is referred to as the B.I. The standard procedure for the determination of sucrose in composite goods is the Clerget process, carried out on a 5 per cent. solution of the sample in a 50/55-ml. flask.

If a is the "direct" saccharimeter reading in International Sugar Degrees (° S.) in a 2 dm. tube at 20° C., and if b is the "invert" reading, uncorrected for dilution, with appropriate sign + or −, then the percentage of sucrose in the 5 per cent. solution of the sample is given by

$$(a - 1{\cdot}1b)\,\frac{26}{132{\cdot}6}$$

Since a 1 per cent. solution of sucrose produces a rotation of + 3·84° S. in a 2-dm. tube at 20° C., it follows that that portion of the "direct" reading which is due to sugars other than sucrose is equal to

$$a - \left\{(a - 1{\cdot}1b)\,\frac{26}{132{\cdot}6} \times 3{\cdot}84\right\} = \text{A (say)} = 0{\cdot}245a + 0{\cdot}83b$$

It is evident that this "rotation due to sugars other than sucrose" will be related to the B.I., and that the nature of the relationship will depend upon the actual reducing sugars present and upon their amounts. As an example, consider a mixture of sucrose, lactose and invert sugar. The specific rotation of lactose hydrate, $[\alpha]_D^{20} = +52{\cdot}5°$ and a 1 per cent. solution therefore shows a rotation in a 2-dm. tube at 20° C. of $52{\cdot}5 \times 0{\cdot}05777 = +3{\cdot}04°$ S. Its reducing power is 75 (invert sugar = 100). The specific rotation of invert sugar, $[\alpha]_D^{20} = -20°$ and a 1 per cent. solution therefore shows a rotation of $-20 \times 0{\cdot}05777 = -1{\cdot}15°$ S. If there is x per cent. of lactose hydrate and y per cent. of invert sugar in the 5 per cent. solution of the sample, then

$$3{\cdot}04x - 1{\cdot}15y = \text{A}$$

$$\text{and} \quad 0{\cdot}75x + y = \frac{1}{20}\,\text{B.I.}$$

Solving these simultaneous equations for y we obtain

$$y = 0{\cdot}039\ \text{B.I.} - 0{\cdot}1925\text{A}$$

$$\text{or} \quad \underline{20y} = \underline{0{\cdot}78\ \text{B.I.} - 3{\cdot}85\text{A}}$$

and $20y$ is the percentage of invert sugar in the sample.

In similar manner the following relationships have been derived.

When reduction is due to presence of—

A mixture of invert sugar and lactose ..	Invert sugar	= 0·78 B.I. − 3·85A	(1)
A mixture of liquid glucose and lactose	Liquid glucose (as invert sugar)	= 1·75A − 0·355 B.I.	(2)
A mixture of liquid glucose and invert sugar	Liquid glucose (as invert sugar)	= 1·2A − 0·069 B.I.	(3)
A mixture of dextrose (or cerelose) and lactose	Dextrose (as invert sugar)	= 3·69 B.I. − 18·2A	(4)
Lactose alone	Lactose (as invert sugar)	= 4·93A = B.I.	(5)
Invert sugar alone	Invert sugar	= −17·4A = B.I.	(6)
Dextrose (or cerelose) alone	Dextrose (as invert sugar)	= 6·74A = B.I.	(7)
Liquid glucose alone	Liquid glucose (as invert sugar)	= 1·29A = B.I.	(8)

To arrive at the above identities, liquid glucose has been assumed to have a specific rotation, $[\alpha]_D^{20} = 112°$ and a reducing power of 42 (invert sugar = 100). In actual fact, the specific rotation and reducing power of liquid glucose vary within fairly wide limits, and consequently the formulae for mixtures including liquid glucose are less exact than the others.

The use of the formulae is best illustrated by some examples—

Example 1. Milk Chocolate.

$a = +9·7°$
$b = -0·7°$ } Sucrose = 41·1 per cent.
B.I. = 8·9
A = $0·245a + 0·83b = 1·80°$

Theoretical B.I. if reduction wholly due to lactose (formula (5)).

= 4·93A = 8·87.

Example 2. Bakery Mixture. Reputed to consist of 50 per cent. of sucrose, 30 per cent. of fat, 20 per cent. of whey paste.

$a = +6·4°$
$b = -1·5°$ } Sucrose = 31·6 per cent.
B.I. = 23·1
A = $0·245a + 0·83b = 0·33°$

The low sucrose and high B.I. suggest partial inversion of the sucrose. Applying the lactose and invert sugar formula (formula (1)), we obtain—

Invert sugar = (0·78 × 23·1) − (3·85 × 0·33) = 16·7 per cent.
Hence, lactose (as invert sugar) = 23·1 − 16·7 = 6·4 per cent.

which results are in accordance with the reputed composition of the sample, the sucrose being partly inverted.

Example 3. Toffee.

$a = +22·2°$
$b = +7·9°$ } Sucrose = 52·9 per cent.
B.I. = 17·1
A = $0·245a + 0·83b = 12·0°$

If reduction were due wholly to liquid glucose, the theoretical B.I. should be 12·0 × 1·29 (formula (8)) = 15·5. The agreement with the B.I. found is as good as can be expected in the case of liquid glucose.

Hence, liquid glucose (as invert sugar) = 17·1 per cent.

$$\text{or liquid glucose} = 17·1 \times \frac{100}{42} = 40·7 \text{ per cent.}$$

The nomogram (see Fig. 1) facilitates the evaluation of $0{\cdot}245a + 0{\cdot}83b$. When A has been found, either by calculation or from the nomogram, the proportion of a particular sugar in a known mixture can be quickly found; if the mixture is unknown, a study of the above equations affords considerable assistance in deciding what reducing sugar or sugars are likely to be present.

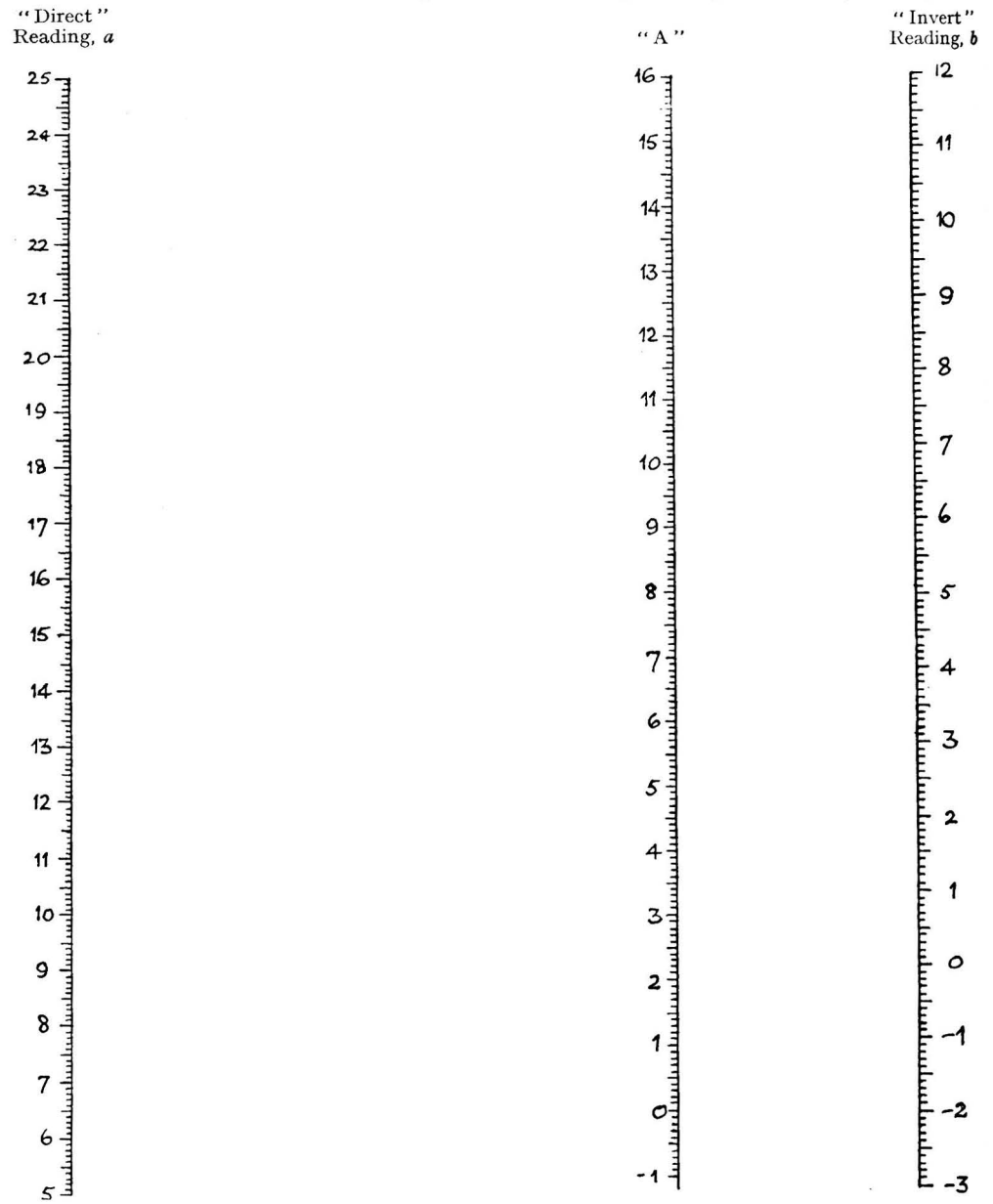

Fig. 1. Nomogram for obtaining values of A from readings a and b $A = 0{\cdot}245a + 0{\cdot}83b$.

The formulae make no pretence to extreme precision, since they neglect various factors, notably the influence of sucrose on the reduction of Fehlings solution and the effect of volume of the clarification precipitate on the Clerget readings. Nevertheless, they have found constant and practical use in this laboratory over a period of many years in the general examination of a wide variety of composite sugar goods. The estimation of reducing sugars in terms of the commercial articles "liquid glucose," "invert sugar," etc., is frequently of greater use to manufacturers

and analysts than their expression as "dextrose," "laevulose," "maltose," etc. Expressions similar to those given above can be readily calculated to suit special needs—*e.g.*, other sugars, reducing sugars expressed as dextrose, etc.

Thanks are due to the Government Chemist, Dr. G. M. Bennett, C.B., F.R.S., for permission to publish these formulae, and also to Mr. R. I. Savage, B.A., B.Sc., for the trouble he has taken in constructing the nomogram and for his assistance in drafting this note and checking the figures.

THE LABORATORY
CUSTOM HOUSE, LONDON, E.C.3

A. H. RHEINLANDER
April, 1950

COLOURS DEVELOPED IN THE PAPER CHROMATOGRAPHY OF AMINO-ACIDS

IN developing a quantitative use of the ninhydrin method of Consden, Gordon and Martin,[1] it was observed that many amino-acids gave a reddish colour after irrigation with acid media and a bluish colour after irrigation with basic media. It was thought that this red - blue colour variation could be due to keto-enol tautomerism, as shown by hydrindantoin.[2]

The blue colour, shown to be due to diketohydrindylidenediketohydrindamine,[3] was found to be readily water-soluble; but the red colour was more firmly retained by the paper fibre, being extracted slowly and incompletely even after vigorous shaking. However, elution with a mixture of 60 per cent. of 2 : 4-lutidine and 40 per cent. of water, or 20 per cent. of pyridine and 80 per cent. of water, caused a change in colour from red to blue; the resulting colour dissolved readily and in this way could be entirely washed from the cut-out spot by gentle swilling with the solvent for about 1 minute. Filtration was found to be unnecessary and a direct measurement of the absorption spectrum of the resulting blue solution could be made. The blue colour was unstable in the presence of strong alkalis and in the presence of ammonia; vigorous shaking with pyridine was liable to peptise the paper fabric, giving a translucent colloidal suspension.

It was found that glycine, asparagine, tyrosine, phenylalanine, histidine and tryptophan gave anomalous results in that they either did not yield a molar proportion of blue dye, or else gave only an orange-brown colour. In attempting (with respect to the red-blue colour change) to prevent the formation of the red colour, the papers were irrigated with phenol in the presence of a 3 per cent. solution of ammonia, dried, and then developed by spraying with ninhydrin dissolved in the lutidine mixture. Finally, the papers were dried at 50° C. for 15 minutes. The spots obtained with these six acids were then all brown or orange-brown, in contrast to the blue spots obtained under identical conditions with glutamic acid, aspartic acid, the valines, the leucines, alanine, methionine, serine, arginine, threonine, lysine and glutamine. On using irrigation with isobutyric acid in place of the phenol - ammonia system, followed by development with the lutidine solution of ninhydrin, the six anomalous acids gave a trace of blue dye mixed with the orange or brown coloured product.

As described by Ruhemann,[3] the brown colours are almost certainly due to the condensation of the aldehyde formed in the ninhydrin reaction with the unstable primary base 1 : 3-diketohydrindylamine formed simultaneously. The resulting 1 : 3-diketo-2-arylidenehydrindamines were found by Ruhemann to be orange dyes which in solution were hydrolysed to darker colours by boiling. The different colours (brown, greenish or grey) obtained with the anomalous acids,[1] are almost certainly due to the presence of different relative proportions of orange and blue dyes.

It appeared possible that aliphatic amino-acids might yield an aldehyde in the ninhydrin reaction which would condense to form a cyclic compound with the primary amine. Thus glycine was found to give a variable amount of blue dye when the chromatogram was developed with ninhydrin in butanol; formaldehyde is produced in this reaction, and, under favourable conditions, could condense with 1 : 3-diketohydrindylamine to give a hexamine derivative. In this connection asparagine gives $CONH_2.CH_2.CHO$, which could theoretically form a cyclic base; glutamine, however, giving $CONH_2.CH_2.CH_2.CHO$, develops the normal blue dye.

The behaviour of the acids listed above is in contrast to that of proline and hydroxy-proline which gave yellow colours under slightly acid conditions and red or purple colours in basic solutions; according to Grassman and Von Arnim,[4] the reaction of these acids follows a different course.

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THE BRITISH DRUG HOUSES LTD.
CITY ROAD, LONDON, N.1

R. O. ATKINSON
R. G. STUART
R. E. STUCKEY
March, 1950

THE SELENIOUS ACID TEST FOR BARBITURATES

TURFITT[1] gives the following selenious acid reaction as a preliminary test for barbiturates—

> "A trace of the test residue is mixed with an equal quantity of solid selenious acid in a small tube, 2 in. × ¼ in., 2 drops of concentrated sulphuric acid are added, and the contents of the tube gradually heated to boiling. In the presence of a barbiturate there is developed a green colour, which disappears on continued heating. If, at the green stage, the contents of the tube are poured into a white porcelain basin and a few drops of alcohol added, a bright red turbidity is obtained. Alcohol treatment at the colourless stage does not give this reaction."

It is well known that selenium or a selenide, but not oxidised selenium compounds, on gently warming with concentrated sulphuric acid gives rise to a green colour, the intensity of which varies from a light green to almost black, depending on the concentration of selenium. This green colour is due to the compound selenosulphur trioxide, $SSeO_3$. This colour, in concentrated sulphuric acid, is destroyed by further warming the solution for a few minutes. By analogy with tellurium, this is presumably due to the loss of sulphur dioxide and the formation of the basic sulphate. If the green solution is diluted, red elementary selenium is precipitated.

It is therefore probable that the reaction described as a presumptive test for barbiturates is no more than a test for substances that will reduce selenious acid in concentrated sulphuric acid, and that the majority of organic compounds would do so. Under the exact conditions prescribed, resorcinol, aspirin, salicylic acid, phenacetin, β-naphthol, benzocaine, caffeine and sucrose were tried, all of which, with the exception of caffeine, gave the reaction; this exception was no doubt due to the greater stability of caffeine in sulphuric acid.

REFERENCE

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E. B. PARKES

SOUTH-WESTERN FORENSIC SCIENCE LABORATORY
BRISTOL

April, 1950

Ministry of Food

STATUTORY INSTRUMENT*

1950—No. 794. The Soap (Licensing of Manufacturers and Rationing) Order 1950. Price 4d.

This Order, which came into force on May 21st, 1950, re-enacts the Soap (Licensing of Manufacturers and Rationing) Order, 1949, as amended, and includes in the definitions the following—

"No. 1 liquid soap" means liquid soap containing more than 12½ per cent. of fatty and resin acids.

"No. 2 liquid soap" means liquid soap containing 12½ per cent. or less of fatty and resin acids.

"No. 1 soap powder" means soap powder (other than shampoo powder) containing more than 28 per cent. but not more than 56 per cent. of anhydrous soap.

"No. 2 soap powder" means soap powder (other than shampoo powder) containing more than 6 per cent. but not more than 28 per cent. of anhydrous soap.

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

British Standards Institution

NEW SPECIFICATIONS*

B.S. 868 : 1950. Cod Oil for Sulphonation. Price 2s.
B.S. 1582 : 1950. Technical Compound Cod Oil. Price 2s. 6d.
B.S. 1647 : 1950. pH Scale. Price 2s.

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 Specification prepared by Technical Committee M/1—Units and Technical Data.
CM(M) 2812—Draft British Standard Rounding-Off Rule.
Draft Specification prepared by Technical Committee DAC/8—Parchment Paper for Dairy Products.
CM(DAC) 2916—Revised Draft B.S. for Vegetable Parchment for the Wrapping of Dairy and Other Food Products.

Reviews

PRÉCIS D'ANALYSE CHIMIQUE DES ACIERS ET DES FONTES. By M. JEAN, Pharmacien Chemist de la Marine, Rapporteur de la Commission d'Analyse Chimique des Aciers de l'Association Française de Normalisation. Publié sous les auspices de l'Institute de Recherches de la Sidérurgie et du Centre de Documentation Sidérurgique. Pp. xxii + 542. Paris: Dunod. 1949. Price 3360 fr.

If the title of this book is to do full justice to the comprehensive nature of its contents and to the manner of their presentation, the word "précis" must be interpreted in its widest sense: that is to say, it must be read as connoting a combination of summary, résumé, synopsis, abstract, epitome and compendium, and the author allowed to use it in any or all of these meanings, separately or conjointly, as and when he pleases.

The book is based on a collection of methods for the analysis of iron and steel that was made by the *Association française de Normalisation* (Afnor) for the purpose of preparing a set of standard methods for use by French manufacturers and engineers, on the lines of those that have already been published in other countries.

France, as a late comer to the publication of accepted chemical procedures for iron and steel analysis, has evidently taken full advantage of the privileged position proverbially enjoyed by the looker-on; for, in addition to previous collections of methods that were already available in France, the publications of the American Society for Testing Materials, the British Standards Institution and the "Handbuch für das Eisenhütten Laboratorium," have been freely used, as have also the World's chemical journals. Ninety-seven references to papers and abstracts in *The Analyst*, dating from 1911 to 1949, were counted.

The book contains twenty-two chapters, each of which deals with one of the following constituents or alloying elements: C, Si, Mn, S, P, Cu, Ni, Cr, Mo, W, Co, As, Ti, V, Al, N, Sn, Pb, Be, Zr, Ta, Nb and B. Each chapter opens with an account of the properties conferred on the metal by the element in question and indicates the procedure most useful for its determination in the varying quantities in which it may be found.

The methods for those elements for which a standard procedure has been prescribed are not given in working detail, but are submitted to a critical examination in which their advantages, limitations and useful range are fully discussed. For some of the lesser known methods of proved worth, the operative details are given in full and those of more recent introduction, which have not yet been subjected to extended trial, are listed in the bibliographies of the separate elements.

The book is well bound and indexed. It contains a few misspellings of English surnames, but is free from errors of other kinds.

It can be recommended as probably the widest conspectus of methods for iron and steel analysis at present in print. F. L. OKELL

* Obtainable from the British Standards Institution, Sales Department, 24, Victoria Street, London, S.W.1.

STRUCTURAL CARBOHYDRATE CHEMISTRY. By E. G. V. PERCIVAL, D.Sc., Ph.D., F.R.I.C. Pp. viii + 246. London: Frederick Muller Ltd. 1950. Price 25s. net.

In this book the author has given a very complete account of the progress made during the past twenty years in the chemistry of carbohydrates, the result of the researches of a large number of gifted workers. After reading the book one is left with the impression that up to the stage of, say, the trisaccharides, there is little left to investigate; it is, of course, quite otherwise with the polysaccharides, although much has been done.

Dr. Percival has the gift of clear exposition and shows a judicial habit of mind in weighing the evidence for and against the various theories of structure. The book is remarkably free from even trivial errors; only one statement was noted to which exception could be taken, a statement to the effect that glucose and fructose reduce equally vigorously (p. 5). Glucose has a reducing power towards Fehling's solution that is about 6 per cent. more than that of fructose, a difference which has to be taken into account by the analyst.

The book should be of great value, not only to those for whom primarily it has been written, but as a "refresher course" for chemists in general. LEWIS EYNON

THE CHEMISTRY OF ORGANIC MEDICINAL PRODUCTS. By GLENN L. JENKINS and WALTER H. HARTUNG. Third Edition. Pp. ix + 745. New York: John Wiley & Sons, Inc. London: Chapman & Hall, Ltd. 1949. Price $7.50; 60s.

The authors, who are professors of pharmaceutical chemistry at two American universities, state that the book has been written "as a textbook for students who have had basic courses in chemistry and are interested in the chemistry of medicinal and related products. Although it presents many elementary aspects of the subject so that the beginner in this field of study can readily find his way to an understanding of the more advanced topics, it is intended primarily for students in the more advanced courses in pharmaceutical, chemical, biological and medicinal science."

It is doubtful whether the attempt to combine in a single textbook information on a specialised branch of a subject with such information of an elementary nature as will make the former comprehensible is ever successful. The organic compounds used in medicine are derived from so many classes and are so varied in structure that it is difficult to see how a student can gain anything but mental confusion from an attempt to study the subject without a very thorough grounding in organic chemistry. Surely it is better for him to obtain this knowledge from a general textbook of organic chemistry and to proceed thence to a specialised branch in a book confined to the subject.

The book under review is a typical example of the results of making such an attempt. The authors have been forced by the nature of their task to attempt a chemical classification of drugs. Whereas no system of classification of drugs is really satisfactory, the system adopted here cannot be commended. For example, sterols occur in the chapter on hydroxyl derivatives, but the steroidal hormones are found under ketones. Cholanic acid, not being a hydroxy acid, is separated from the bile acids. Ethyl chaulmoograte jostles the pyrethrins, both being esters of cyclic acids. Numerous other examples could be quoted, but it would take too long to point out all the incongruities to which this rather naïve system of classification leads. The treatment is uneven; important drugs like the cardiotonics or steroidal hormones occupy a very small space, and the attention given to compounds of little or no importance medicinally is often disproportionate.

In spite of these defects, the information is accurate and up-to-date, the structural formulae are free from errors and clearly presented, but several misprints in the text have been noted.

There are useful chapters on Stereoisomerism and on Some Physico-chemical Properties of Medicinal Products. Many of the American proprietary names appearing in the text will be unfamiliar to British readers.

It is unfortunate that the amount of careful work which has evidently been done in preparing this book should be spoilt by a faulty arrangement of the information. N. EVERS

TERMS USED IN SPECTROGRAPHIC ANALYSIS. PART 1: EMISSION SPECTROGRAPHY. British Standard 1636 : Part 1 : 1950. Pp. 11. Price 2s.

This standard, issued by the B.S.I., is stated to have been prepared at the request of the Glasgow Spectrographic Discussion Group, and it formulates definitions applicable to analysis by emission spectrography. The lack of uniformity in the choice of symbols and terms, which has been obvious in published papers dealing with spectrographic analysis, makes it most desirable that there should be not only national, but international, agreement, and not only on definitions, but also on all symbols and terms used. The fundamental quantities would appear to be primarily

the concern of bodies like the International Unions of Chemistry and of Physics and societies such as the Physical and the Faraday Society. And it is to be hoped that the B.S.I. has made and will make sure that its proposals are strictly in line with fundamental scientific work.

The present pamphlet is to be welcomed as a step in the right direction. H. E. Cox

75th Anniversary of the American Chemical Society, 1951

THE American Chemical Society will celebrate its 75th Anniversary at the 1951 Fall Meeting, which will be held in New York City, September 3rd to 7th, 1951. The following week the International Congress of Pure and Applied Chemistry will meet in New York and Washington. The International Union of Pure and Applied Chemistry will hold its bi-annual meeting during this period.

The American Chemical Society's Division of Analytical Chemistry will co-ordinate its programme with these meetings, and it is hoped that many of their colleagues from other countries will participate in the Analytical Chemistry Symposia. It is planned to devote a half-day to each of the following theoretical aspects of the subject: Chemical Reaction Kinetics and Mechanisms, Physical and Chemical Equilibria, and Absorption and Emission of Radiant Energy; there will be four Symposia dealing with applied analysis.

Notice to Authors

THE Society publishes papers concerned with all aspects of analytical chemistry, inorganic and organic, including chemical, physical, biological, bacteriological and micro-methods. Papers on these and allied subjects, by members of the Society or by non-members, may be submitted for publication; they may—

(1) record proposals for new methods and the investigations on which the proposals are based;

(2) record the results of original investigations into known methods or improvements therein;

(3) record analytical results obtained by known methods where these results, by virtue of special circumstances, such as their range or the novelty of the materials examined, make a useful addition to analytical information;

(4) record the application of new apparatus and new devices in analytical technique and the interpretation of results.

The results of minor investigations or kindred matter may be accepted for publication as Notes.

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The title should be brief but descriptive.

Conciseness of expression should be aimed at; clarity is facilitated by the adoption of a logical order of presentation, with the insertion of suitable paragraph or sectional headings.

Descriptions of new methods should be supported by experimental data showing their precision and specificity.

Generally, the best order of presentation is as indicated below—

(*a*) Synopsis.

(*b*) Statement of object of investigation and, if necessary, historical introduction.

(*c*) Preliminary experimental work.

(*d*) Description of method. Working details of proposed methods are most concisely and clearly given in the imperative mood, and should normally be given in this form, *e.g.*, "Dissolve 1 g. of sample in 10 ml. of water and add . . ." Well-known procedures must not be described in detail.

(*e*) Presentation of results.

(*f*) Scope and validity of results.

(*g*) Conclusions, and if required, a short summary of the principal results.

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1. Dunn, J. T., and Bloxam, H. C. L., *J. Soc. Chem. Ind.*, 1933, **52**, 189T.
2. Allen, A. H., "Commercial Organic Analysis," Churchill, London, 1882, p. 123.

For books, the publisher, and place and date of publication should be given, followed by volume or page number, or both if required.

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