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THE ANALYST

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Some Physico-Chemical Methods in Microchemistry

Part II. Molecular Weight

BY CECIL L. WILSON

(*Read at a Joint Meeting of the Microchemistry and Physical Methods Groups of the Society held at Cambridge, on Friday, September 26th*, 1947)

MOST physico-chemical determinations can now be carried out readily on the micro or semi-micro scale. As already shown by the author, the number of methods or modifications that have been proposed for the determination of viscosity, surface tension and refractive index,[1] and of density,[2] is very great. It is clear that a critical survey of such methods would often be of value. Such a survey has been instituted by the author and his co-workers. Its primary aim is not so much the devising of new methods (although in some instances this may well be an outcome of the survey) as the provision of a valid basis for the evaluation of the various methods when used by the individual worker. Such factors as the accuracy of a method, its speed and the time taken by the operator to attain a standard accuracy and speed when various methods are carried out under comparable conditions, are all part of the information that would undoubtedly influence the choice of a method if such data were available.

One of the most important physico-chemical measurements is the determination of molecular weight. As part of the programme of investigating proposed methods for the determination of this constant, a review of the literature has been made. From 1890 up to the present, over 300 publications have dealt specifically with methods of determining molecular weight, and of this number over eighty are concerned with small-scale determinations. It is clear that, over such a range, anyone seeking a reliable method requires some guidance that would assist his choice. Even if one limits oneself to a specific mode of determination, such as the ebullioscopic, the number of methods is still very large. A considerable body of information has been amassed concerning the relative efficiency of a number of the methods, and it is hoped to make this the subject of further communications.

Broadly speaking, methods of practical importance for the determination of molecular weight can be classified as follows.

A. Determinations of properties in solutions—
   1. Ebullioscopic: elevation of the boiling-point.
   2. Cryoscopic: depression of the freezing-point.
   3. Vaporimetric: depression of vapour pressure.

B. Determinations of properties in the vapour phase—
   1. Gas density.
   2. Vapour density.

This classification is by no means a rigid one, and is merely proposed here for convenience in the discussion of the specific methods that follow. These, the classical methods for the determination of molecular weight, have all been widely adapted to the micro scale. For ordinary liquids and solids, the ebullioscopic and cryoscopic methods, as well as other methods based on osmotic pressure phenomena, have a considerable currency. For gases, the determination of density by a modification of the method of Dumas is probably the most convenient. The method of Victor Meyer, directly reduced, or modified in various ways, has been applied to a considerable extent to volatile liquids.

A.1. Ebullioscopic methods

Probably the most widely known of all micro-methods for the determination of molecular weight is that of Pregl,[3] first proposed about 1910. The ebullioscopic apparatus itself, shown in Fig. 1, is very simple. Care is taken to ensure even ebullition by addition of platinum

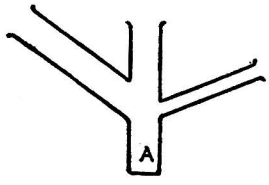

Fig. 1.
Pregl's boiling vessel.

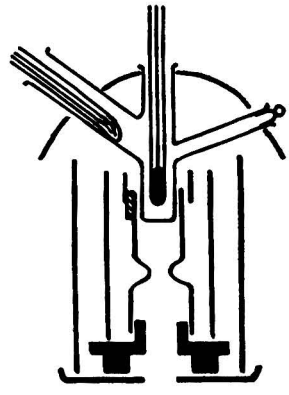

Fig. 2.
Complete Pregl apparatus.

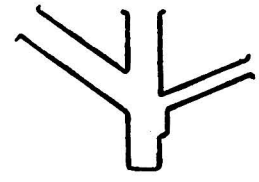

Fig. 3.
Rezek's modification.

tetrahedra to the boiling vessel, A. Much importance attaches to the choice of a heating device. An elaborate system of air-currents, produced by the use of four concentric cylinders (three of glass and one of mica) arranged around the boiling vessel as shown in Fig. 2, allows constant temperature to be maintained. Elevation of the boiling-point is measured by a Beckmann thermometer of reduced size, reading to 0·002° C. The method, as described by Pregl, is suitable for use with 7 to 10 mg. of solute, a volume of 1·5 to 4 ml. of solvent being appropriate.

The original Pregl apparatus has been modified by Rezek[4] as shown in Fig. 3. The side-arm is widened as shown, so as to allow easier insertion of the pellet of solute, avoiding condensation of the solvent on the insertion tube. It is claimed that the apparatus is easier to use than Pregl's and that the time of a determination is shortened, since the pellet can be projected directly into the solvent.

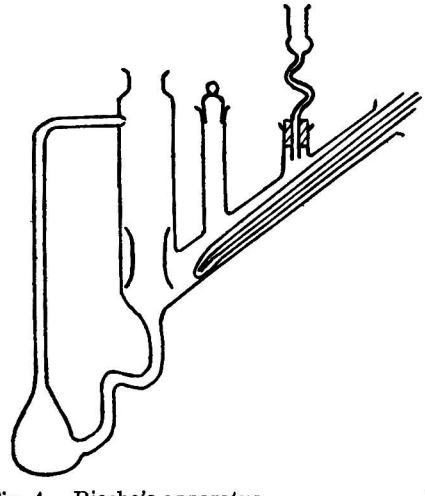

Fig. 4. Rieche's apparatus.

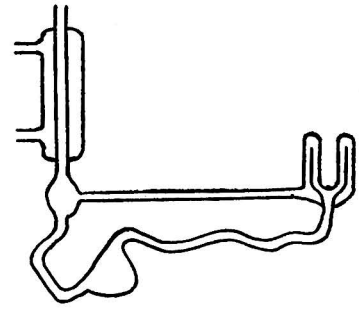

Fig. 5. Apparatus of Bobranski and Sucharda.

In the method of Rieche,[5] a similar amount of solvent is used, and between 15 and 25 mg. of solute sample. The principle is precisely as in the Pregl method, but a more complex ebullioscope, shown in Fig. 4, is used to ensure a constant flow of both boiling liquid

and vapour over the bulb of the thermometer. Again, to ensure even boiling, platinum tetrahedra are usually added to the boiling vessel. A paraffin bath is recommended for heating, instead of the burner used in the Pregl method; its temperature is maintained constant (within 0·5° C.) between 25° and 40° above the boiling-point of the solvent in the apparatus. An accuracy to within 5 per cent. is claimed for this apparatus.

The apparatus of Bobranski and Sucharda,[6] shown in Fig. 5, may more properly be regarded as semi-micro. A sample weight of 20 to 30 mg. is recommended (in the first years after the method was proposed the sample was usually weighed on an ordinary analytical balance to 0·0001 g.) and a solvent volume of 4 to 5 ml. As in the previous apparatus, the design aims at ensuring circulation of the boiling liquid, in this case on to a mercury bath which holds the thermometer bulb. A little powdered glass is sintered into the inside of the base of the boiling vessel, to promote even boiling.

Menzies and Wright[7] devised an ebullioscopic method on the macro scale which, instead of a Beckmann thermometer, used a differential thermometer[8]. In such a thermometer (Fig. 6) water is enclosed in a small evacuated bulb which forms the lower end of the thermometer. A similar bulb at the end of a piece of narrow glass tubing forms the upper bulb of the thermometer. The lower bulb is wetted by the spill from a Cottrell[8a] pump. This "pump" is simply a small funnel used to direct an intimate mixture of boiling solution and vapour on to the bulb and is a modification of that used by Cottrell and others in macro boiling point apparatus. The upper bulb and stem are wetted by the vapours which pass up and the hot condensate which drains back on two guide wires. Thus, the lower bulb is at the temperature of the boiling solution, whilst the upper bulb is at the temperature of the boiling solvent (vapour). Two different vapour pressures will cause the water in the connecting tube to rise to different heights. The height of the water in this tube above the level in the lower bulb will therefore depend, at any temperature, on the small difference in temperature between the two bulbs, and hence on the concentration of the solution. By means of a calibration table, the vapour pressure change of water equivalent to 1-mm. difference in the water levels can be converted to temperature differences.

With water in the thermometer, solvents whose boiling-points range from 33° to 101° C. may be used. Other thermometer liquids may be used for high-boiling solvents. Values obtained with the differential thermometer are not subject to changes in atmospheric pressure, irregularities in capillary bore or sticking of a mercury thread. All of these, it is claimed, are defects inherent in the Beckmann type of thermometer. Furthermore, the alteration of level in the differential thermometer of Menzies is greater than in the Beckmann thermometer, so that a more precise reading is possible.

Smith and Milner[9] have adapted Menzies' apparatus to the micro scale. Their apparatus, shown in Fig. 7, takes about 3 ml. of solvent, and from 5 to 25 mg. of solute. The flame that heats the boiling vessel is shielded from draughts and between the flask and condenser the tube is vacuum-jacketed. A short length of tungsten or platinum wire is sealed through the base to promote even boiling. Bubbles formed on this wire are directed through the Cottrell pump, so that a constant stream of liquid is pumped over the lower bulb of the thermometer. The upper portion of the thermometer is wrapped with a copper-wire spiral to ensure proper drainage of boiling-hot solvent over the upper bulb. The total height of the apparatus is about 25 cm.

The bulb of the apparatus is graduated so that the working volume of solvent may be measured quickly at its boiling-point, by removing the flame, immersing the bulb momentarily in a beaker of cold water, and reading the volume. This takes into account the fact that the working volume of solvent liquid is appreciably different, because of temperature and vaporisation, from the volume of solvent measured into the apparatus at room temperature.

The solvent is boiled until the levels in the thermometer are constant, and these are read with a lens. The weighed solute is then added and readings are made when equilibrium is reached once more. In order to prevent condensation of atmospheric moisture within the apparatus, it is recommended that water should be run through the condenser from a reservoir maintained at room temperature, at a rate of 4 ml. per minute, rather than from a service tap. An accuracy to within ± 1 per cent. is claimed for this method, which, it is stated, requires 20 to 30 minutes for a single determination.

Colson[10] has proposed an apparatus that is essentially similar in principle. Electrical heating, however, is employed, the heating coil being immersed in the boiling liquid, as shown in Fig. 8. The source of heat is therefore not so susceptible to draughts. The solvent has

a volume of 6 ml. The solute, 5 to 15 mg., is weighed in a small glass cup 0·4 cm. in diameter and 0·4 cm. deep.

The height to which the solvent rises in the condenser, and hence the volume of liquid in the boiling-vessel, can be kept constant by keeping the current constant. There are therefore no errors in the measurement of the volume of solvent, which is done simply by interrupting the current momentarily; boiling ceases and the volume is read off against the graduations. There is no need for the cooling process described in the method of Smith and Milner.

Fig. 6. Differential thermometer.

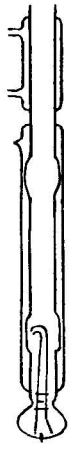

Fig. 7. Apparatus of Smith and Milner.

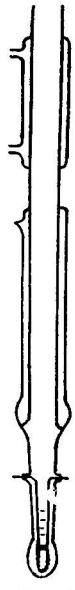

Fig. 8. Colson's apparatus.

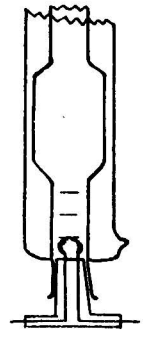

Fig. 9. Ketchum's modification.

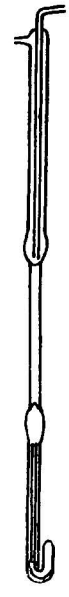

Fig. 10. Colson's differential thermometer.

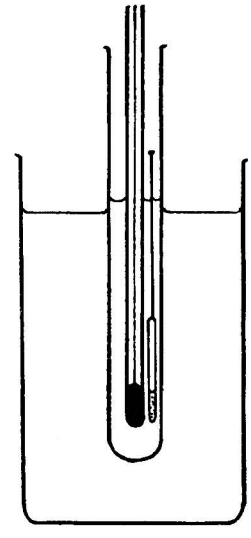

Fig. 11. Heating bath of Wilson and Harris.

The macro apparatus proposed by Hanson and Brown,[11] with a claimed accuracy to 1 per cent., resembles the previous types closely, the principle differences being a Cottrell pump fixed in position, an improved jacketing system, and a collar for the thermometer.

This apparatus has been reduced to the micro scale by two workers, Morse[12] and Hallett.[13] Such a modification requires 1 to 2 ml. of solvent and, with a determination time of 20 minutes, gives an accuracy to within ± 1 per cent.

Recently, yet another modification has been proposed by Ketchum,[14] in the form of a removable plug at the bottom of the apparatus, as shown in Fig. 9. This carries the heating coil, and permits ready access to the inside of the apparatus for cleaning, or for mending the heating coil when necessary.

Finally, the addition, proposed by Colson,[15] of a cold-finger condenser attached to the differential thermometer for more efficient control of the temperature of the upper bulb, as shown in Fig. 10, should be mentioned.

A.2. Cryoscopic methods

Cryoscopic micro-methods directly based on the classical apparatus have not been popular, since many obvious difficulties of manipulation present themselves. Iwamoto[16] devised such an apparatus to make use of the usual micro-Beckmann thermometer, and to be operable with 1·5 ml. of solvent and 7 to 30 mg. of solute. Jorg,[17] whose apparatus is designed to handle 500 mg. of solvent and 5 to 10 mg. of solute, and Salge[18] recommend the use of a thermo-element for the measurement of the depression, and Kubota and Yamane[19] have proposed the use of a silver resistance thermometer.

Undoubtedly, however, the problems confronting the chemist in cryoscopic micro-determinations have largely been solved by the introduction of Rast's well known method, using

camphor as a solvent. As ordinarily practised[20] this is, itself, essentially a semimicro-method. Simple reduction in scale immediately gives it the status of a full micro-method. This has been done by Pregl.[21] The sample weight is of the order of 0·2 to 0·3 mg., and 2 to 3 mg. of camphor are used as solvent. In comparison with K for water (18·6) and for benzene (50), K for camphor is about 400, so that a reasonably open-scale thermometer can be used, and the melting-point of the solution is determined after the fashion of an ordinary organic melting-point determination. The value of K must be redetermined for each sample of camphor, using a substance of known molecular weight. An accuracy to about 1 per cent. is attained.

Camphor is not universally applicable as a solvent in Rast's method. The usefulness of the method has been considerably extended by the introduction of other solvents of the same nature. Thus, *d*-limonene tetrabromide has been recommended[22] for general use. Wendt[23] recommends the lactam of *cis*-4-amino-hexahydrobenzoic acid for compounds containing the $-CONH_2$ group in particular, and for polar compounds in general, and describes the method of preparing this compound. Pirsch[24] has determined the melting-points and molar depressions of a range of 25 compounds, derivatives of camphane and of α-di*cyclo*pentadiene, suitable for Rast's method. Thus a solvent chemically similar to the substance under investigation can be chosen. One or two of these are especially worthy of note. Dihydroxy-α-di*cyclo*pentadiene is particularly recommended for liquids and for solids of low melting-point, whilst camphorquinone or borneol is more appropriate for solids of high melting-point. In addition, pinene dibromide and bornylamine deserve special mention. Similar compounds have been investigated by Uchida and Shimojama.[25] Keller and von Halban[26] propose tetrabromomethane.

Particular interest lies in the recommended use of *cyclo*hexanol. Chavanne and van Roelen,[27] many years ago, proposed this compound, for which they determined the cryoscopic constant, K, as 615. They noted, however, that its use was circumscribed by its instability and by the fact that it is not suitable for a wide range of compounds. According to Wilson and Heron,[28] pure *cyclo*hexanol has a melting-point of about 24·5° C., and a molar depression of 377, which is sufficiently great to permit use of an ordinary thermometer. In the method described by them, from 300 to 1 mg. of sample may be used and an amount of solvent to produce an approximately 3 per cent. solution. On the smallest scale the error is about ±5 per cent. The weighed solute, and a weighed amount of solvent, are contained in a capillary, and are frozen by cooling in ice-water. The capillary is then immersed in a boiling tube containing water at a temperature a few degrees below the expected melting-point. This, in turn, is immersed in a beaker of water a few degrees above that temperature, as shown in Fig. 11, and the capillary is observed till melting takes place. The temperature of the water in the boiling-tube is now adjusted to 1 degree below the melting-point observed, and that in the outer beaker to 1 degree above. A repeat observation of the melting-point—the point at which the last crystal just disappears—is made. Repeat determinations on a single sample should give melting-points within 0·1° C. Two separate determinations on one substance should lie within 0·2° C.

A number of related topics may be mentioned in this consideration of cryoscopic determinations. When dark-coloured solids are the subject of investigation it is difficult to get a precise determination of the melting-point or freezing-point. Aluise[29] has examined this problem and recommends, in the first place, that the freezing-point, rather than the melting-point, should be determined in every case, and secondly, that the first formation of crystals, which denotes the freezing-point, should be observed through a thin layer of liquid. This is achieved by making a capsule from tubing just narrow enough to fit into the freezing-point capillary, and weighting this by means of a small lead shot, as shown in Fig. 12. Thus the bottom of the freezing-point capillary contains only a very thin film of solution, and the first appearance of crystals is obvious. As with many other molecular weight determinations, those carried out in this fashion are claimed to be accurate to ±5 per cent.

Any determination based on the Rast method, where melting of solid in a capillary is to be observed, must depend to a considerable extent on the nature of the heating bath employed. This point will already have been made clear from the method of Heron and Wilson, and other workers have also paid attention to this point. Pirsch[30] recommends an air-bath for even heating of the capillaries. When this is heated as shown in Fig. 13 there is constant circulation of air in the direction of the arrows. Pirsch also[31] gives precise details for the filling of capillaries, and the mixing of solution and solvent to avoid segregation, as

well as a modified procedure[32] to cope with the determination of the molecular weight of liquids.

A different type of bath specially devised for molecular weight determinations has been described by Tiedcke.[33] This bath, shown in Fig. 14, is convenient for long series of determinations. It also deals with the difficulty of placing the capillaries accurately beside the thermometer. The capillary, as shown at A, is provided with an "umbrella" handle. This is hung on a small platinum hook at the end of a glass rod. The capillary is guided by a short length of glass tubing, also fixed to the end of the glass rod, to a constant position in the bath beside the thermometer. Since sulphuric acid is the bath liquid used, ground glass joints are preferable in making Tiedcke's apparatus.

Mason[34] has proposed the use of his electrically heated block, originally devised for ordinary melting-point determinations, for molecular weight purposes.

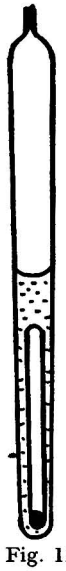

Fig. 12.
Aluise's melting-point capillary.

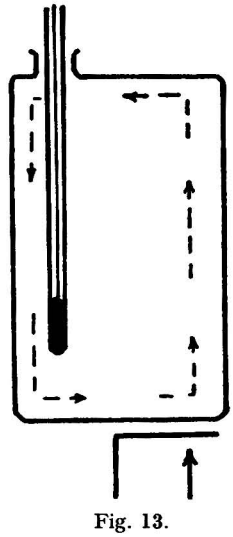

Fig. 13.
Pirsch's heating bath.

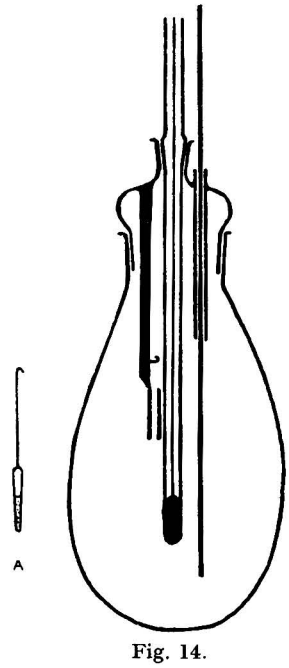

Fig. 14.
Tiedcke's heating bath and capillary, A.

Fig. 17.
Method of Schmit and Niederl.

One or two indirect determinations of the depression of the freezing-point may be included here. Reilly and Pyne[35] have proposed the determination of the cooling curve of camphor rather than the freezing-point or melting-point, in particular for substances of low solubility. Fabinyi[36] has devised an automatic apparatus depending on electrical circuits connecting three platinum wires, one coated with solvent, one coated with solution and one bare. These are dipped in a mercury-bath, and are connected to two bells which indicate the precise moment of melting of the coatings. Straub[37] recommends the determination of the time interval between the melting of the solution and that of the pure solvent, when subjected to a constant rate of warming. This condition is achieved by immersion of the capillaries in a liquid, such as alcohol, cooled well below the melting points of solvent and solution.

A.3. Vaporimetric methods

Methods for the determination of molecular weight by isothermal distillation, thus producing isotonic solutions, are quite common. They have advantages in that they may, if necessary, be carried out at low temperatures, that a wide range of solvents is available, and that the mild temperature conditions avoid risk of decomposition. The method proposed by Barger[38] was one of the earliest in this class. If two solutions, one osmotically stronger than the other, are contained in the same vessel, the concentrations of the two tend to become equal by passage of solvent from the weaker to the stronger. The volume of the more concentrated solution accordingly grows at the expense of the other.

A test solution can be compared with the unknown, and the molarity of the unknown thus determined. Standard solutions are prepared in long-necked ampoules, suitable substances being azobenzene or β-naphthol in organic solvents. A solution containing several milligrams of the unknown in 50 to 100 mg. of solvent is also prepared. Drops of both solutions are filled alternately into capillaries 1 to 2 mm. in bore, with air bubbles between the drops, as shown in Fig. 15. The capillaries are then stuck on a microscope slide, placed in a Petri dish, and covered with water. The lengths of the droplets are measured by means of a microscope fitted with an eyepiece micrometer. With water or pyridine as solvent, measurements are repeated after several days. Alcohol droplets will have come to equilibrium after several hours, and with more volatile solvents the drops may be measured in a matter of minutes. From a series of capillaries it is easy to determine which droplets show no change in size and are therefore equimolar. It is thus possible to determine the molecular concentration of the unknown solution to ±0·02 molar.

Fig. 15. Barger's osmotic capillary.

Fig. 16. Rast's osmotic capillary.

Rast[39] modified this method so that only 2 drops of solution, one of unknown and one of standard, are inserted in each capillary, as shown in Fig. 16. The motion of the air bubble between the 2 drops, rather than the size of the drops, is then followed through the microscope. Berl and Hefler[40] have used a similar method, and a larger modification of the apparatus, using about 10 mg. of solute in 1·5 to 1·7 ml. of solvent, has been proposed by Signer.[41]

All of these methods so far mentioned involve the enclosure of standard and unknown in the same tube, thus leading to contamination of the unknown so that it cannot be made use of for other purposes immediately, if at all, after the determination. Stephens,[42] in 1920, proposed that the two tubes should be enclosed in separate cups, in a single vessel, thus permitting interchange of the solvent without contamination of the unknown. The two solutions, by this method, come to equilibrium after about ninety days. Schwarz[43] and Hallström[44] applied the same principle.

Schmitt and Niederl[45] improved the method as follows: using 0·3 to 15 mg. of unknown sample in 50 to 80 cu. mm. of solution (approximately 0·5 to 2·5 per cent.) some of this solution is placed in a capillary side by side with another capillary which contains standard solution, from 0·05 to 2·0 molar, in the same solvent. The mounted tubes are inserted in a larger tube, which is evacuated and is allowed to stand for some time, microscope readings being taken at intervals. The molar concentration is determined from the behaviour of the standards, and an accuracy to within 1 per cent. is achieved in the determination. Because of the evacuation the final measurement can be made in 2 or 3 days. A suitable arrangement is shown in Fig. 17.

The principle of a method devised by Blank and Willard[46] is that of differential vapour pressure at the boiling-point. It, like the method of Schmitt and Niederl, can be carried out on a wide variety of scales, as will be obvious from the apparatus shown in Fig. 18. The bath liquid is heated to allow the solutions in the tubes to boil, and boiling is continued for 20 to 60 minutes. Each vessel is then weighed to determine the equilibrium weights, and hence the equilibrium volumes. Then from the equation

$$\frac{M_1}{M_2} = \frac{W_1 . V_2}{W_2 \ V_1}$$

where M and W are the molecular weights and the weights of each solute, and V is the volume of the solvent, the unknown molecular weight can be determined. If, in the first instance, the same weight of each solute is weighed out, the molecular weights are inversely proportional to the volumes. Typical scales for this method are 0·02 to 0·05 mg. dissolved in 2 to 5 ml. The method is rapid and the accuracy claimed is to within 2 to 5 per cent.

B.1. Gas density measurements

We turn now to methods that deal with the gas or vapour phase. Dumas' well-known method has been transformed to the small scale by Blank.[47] A small glass bulb (Fig. 19) of capacity 8 to 12 ml. is weighed on a microchemical balance, preferably with a similar bulb as counterpoise. The bulb is fitted with a tap and a ground joint. It is evacuated and filled in a thermostat with the gas under investigation, and is reweighed immediately after adjustment to atmospheric pressure by rapid opening and closing of the tap. A bulb of the size mentioned will contain about 0·01 to 0·02 g. of a gas such as carbon dioxide.

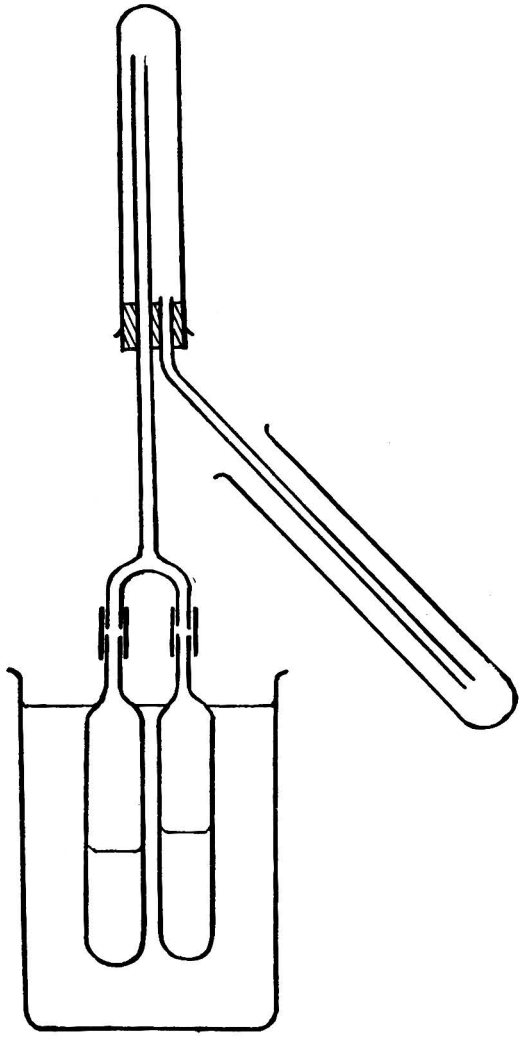

Fig. 18. Differential vapour pressure method of Blank and Willard.

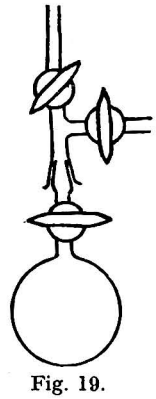

Fig. 19. Blank's gas density bulb.

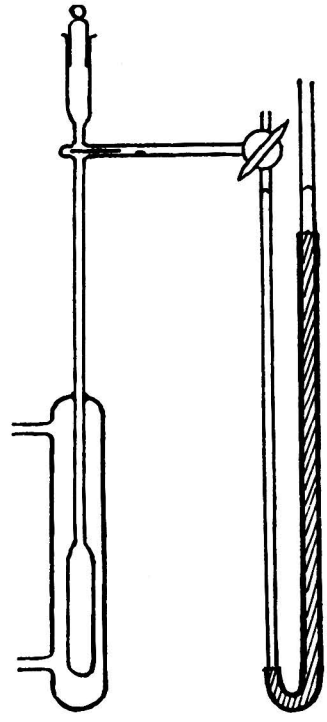

Fig. 20. Apparatus of Conklin Mote, and Sommerhalten.

The gas, contaminated by only a very small amount of residual air from the bulb, is easily removed from the bulb under mercury, using a bent capillary to insert mercury into the bulb, the gas being displaced into a waiting receiver.

B.2. Vapour density measurements

Chemists are, however, more concerned with the applications of Victor Meyer's method, which, it has been claimed by Newman and Meanes,[48] gives more reproducible results than, for example, cryoscopic methods.

Direct modifications of Victor Meyer's method have been put forward by a number of workers. The method of Mote, Conklin and Sommerhalter[49] is obviously a straightforward reduction of the classical method. It is used with samples of 10 to 30 mg. and the average error is ±4 per cent. The time required to carry out a determination is about 10 to 15 minutes. The capillary containing the liquid is held in the neck of the apparatus, shown in Fig. 20, by a magnetic valve, which consists merely of a small tube filled with iron filings or iron wire. This is drawn back by a magnet after the apparatus has come to equilibrium, to allow the sample to fall into the vaporisation chamber. The vaporisation is carried out by a steam bath. The expanding vapour drives air over into the calibrated reception vessel made of 6-mm. tubing.

Another direct modification[50] claims an accuracy to ±2 per cent. on samples of 2 to 10 mg. and a determination may be carried out in 20 minutes either at atmospheric pressure or under reduced pressure. The liquid, in a sealed capillary, is held between the side-wall of the vaporising vessel and a central rod by means of small projections (Fig. 21). At

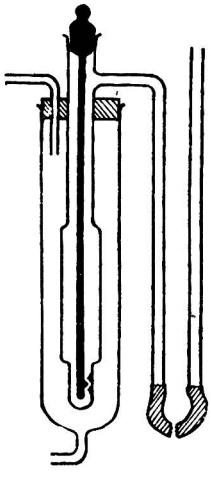

Fig. 21.
Lakshminarayan's apparatus.

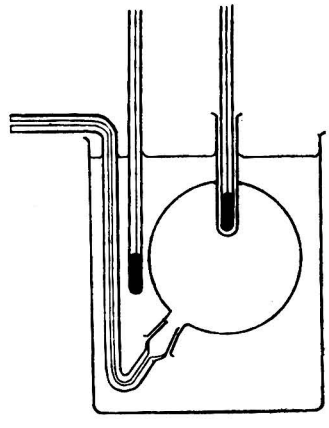

Fig. 22.
Low-boiling apparatus of Niederl.

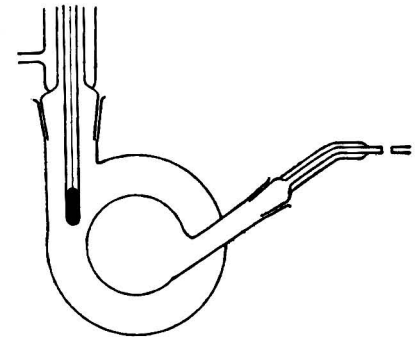

Fig. 23.
High-boiling apparatus of Niederl.

equilibrium the capillary is broken by turning the central rod. As bath liquids, water (b.p. 100° C.), *p*-cymene (180° C.), α-naphthyl methyl ether (269° C.) or benzyl benzoate (319° C.) are recommended.

P. de Ceuster[51] gives an historical review of methods deriving from the classical Meyer method. In his method the sample is weighed in a capillary, dropped into a vessel heated by aniline vapour, and the displaced air is driven into a 3-ml. burette filled with mercury, and there measured. With a 7 to 15-mg. sample the accuracy claimed is better than to ±1·5 per cent.

The volume of vapour produced, instead of being measured directly, may be measured indirectly, in terms of, for example, displaced mercury. Apparatus has been described both for low-boiling[52] and high-boiling[53] liquids In the former (Fig. 22), which may be made in different sizes to suit different scales, 1 to 20 mg. of material may be vaporised. The principle of both pieces of apparatus may be gathered from the description of a determination with the high-boiling apparatus, which is the more universally applicable of the two and is shown in Fig. 23. An outer vessel contains the bath liquid, and is fitted at the top with a condenser wide enough to allow a thermometer to be inserted. The sample is weighed in a capillary and is inserted through a ground joint into the inner vessel. This is then completely filled with mercury, and the ground joint is closed by a capillary stopper. The capillary of the stopper is likewise filled with mercury, through a capillary funnel. On vaporisation of the sample, mercury is expelled from the side-tube into a receiving vessel, and is weighed to ±0·01 g. From its weight the volume of vapour is determined. A correction is applied for the mercury expelled by expansion, this having been determined in a blank. The vaporisation bulb has a volume of 12 to 15 ml. Samples of 3 to 9 mg. are weighed to

0·005 mg. With a larger bulb, 10- to 20-mg. samples, weighed to ±0·05 mg., may be handled conveniently. The accuracy of the determination is to ±2 per cent. It is possible to carry out determinations at reduced pressure, using a side-arm on the receiver and a source of constant pressure. It is clear that the normal pressure in the bulb is greater than atmospheric.

On a much smaller scale, Blank and Willard[54] have devised a method using the microscope. A capillary with a bore of 1·5 to 3 mm. forms the main vessel. A weighed drop of liquid is confined between 2 drops of mercury, and the tube is closed off at one end by a wooden plug (Fig. 24). The capillary is heated in a horizontal vapour-bath, to volatilise the sample, and the distance between the two mercury drops is measured under the microscope, giving the volume of the vapour. The temperature and pressure are measured at the same time.

Fig. 24. Microscopic method of Blank and Willard.

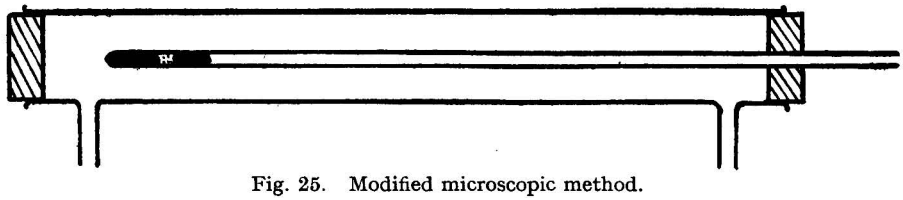

Fig. 25. Modified microscopic method.

Fig. 26. Kay's method.

It is possible to effect a variation of this[55] using a capillary closed at one end. The drops of mercury and liquid are inserted alternately by capillary pipettes. Alternatively, the tube is weighed containing a large drop of mercury at the closed end. A drop of liquid is then inserted, by means of a capillary pipette, in the centre of the mercury drop, and the whole is reweighed, giving the weight of the liquid. The appearance is then as shown in Fig. 25. The capillary is then inserted in a horizontal vapour-bath and heated as in the method of Blank and Willard.

Kay[56] uses a relative method, in which two substances, one known and one unknown, have their vapour densities compared. The specific gravities of the liquids at ordinary temperature must be known. A 1-cm. thread of mercury is inserted, about halfway along, in a metre-length of thick-walled capillary. On either side 0·5 to 1·0 cm. (about 1 to 5 mg.) of each of the liquids is inserted, as shown in Fig. 26. The tube is then sealed at each end. The lengths of the liquid threads are first measured to 0·01 cm. Then the whole is heated in a horizontal vapour-bath, considerably above the boiling-point of both liquids, and the lengths of the vapour threads are measured, in this case the measurement being only to 0·1 cm. An approximate formula that enables the vapour density of the unknown liquid to be calculated is

$$\frac{L_1 \times V.D._1}{l_1 \times s.g._1} = \frac{L_2 \times V.D._2}{l_2 \times s.g._2}$$

where L = length of vapour column, l = length of liquid column.

Instead of measuring the volume of the vapour it is sufficient to measure the pressure or the temperature required to produce a standard volume. A method devised by Blank and Willard[57] relies on this principle. A tube 12 × 0·6 cm. long, of the shape shown in Fig. 27, has a glass-rod handle attached for ease of manipulation. This may be done simply by using a rubber sleeve. The tube is filled with mercury and is inverted with the mouth dipping into a crucible of mercury. The whole is then placed in water or another suitable bath liquid. The sample, weighed in a capillary bottle, is inserted into the bottom of the tube and the bath is heated until the mercury is driven back to a mark on the neck of the tube (the mark being kept at the same level as the mercury surface in the crucible). Barometric

pressure must be corrected for the added pressure on the vapour, due to the difference in the water and mercury levels. The temperature of the bath is read. The volume of the vapour is corrected by these readings to N.T.P.

The method of Bratton and Lochte[58] is a modification in which the pressure required to produce a given volume is measured. A capillary, sealed as shown in Fig. 28, containing a weighed amount of liquid, is inserted in the top of the apparatus shown in Fig. 29, where

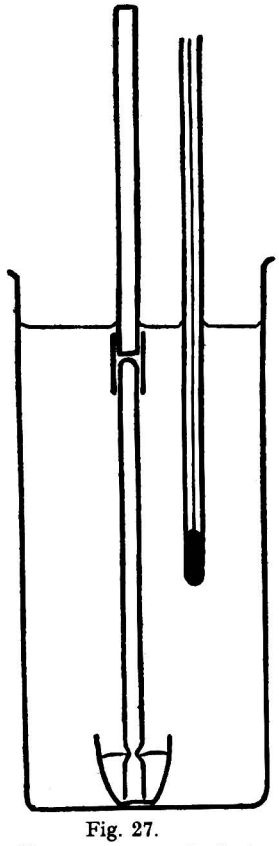

Fig. 27.
Temperature method of Blank and Willard.

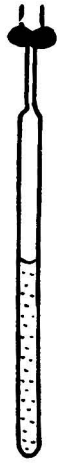

Fig. 28.
Capillary for method of Bratton and Lochte.

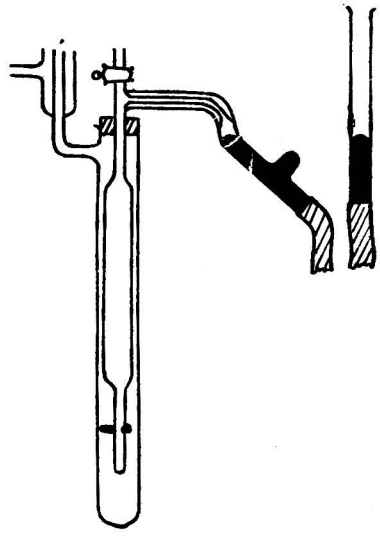

Fig. 29.
Apparatus of Bratton and Lochte.

it is held from passing through the tap by a small glass bulb fused on its end. On closing the tap the capillary is broken and falls to the bottom of the heated reservoir. As the liquid vaporises, the mercury reservoir is raised to maintain the level of the mercury in the side-arm approximately at the mark. Chance air bubbles from the mercury are trapped in a small side-arm. When equilibrium is attained the pressure is adjusted to bring the mercury precisely to the mark and is read. By calculating a constant for the apparatus the molecular weight may be calculated from the relation,

$$\text{M.W.} = \frac{\text{T.W.}}{\Delta P} \text{K}$$

where T = absolute temperature of the bath, W = weight of sample and ΔP = change in pressure. The average error of the method is less than $\pm$ 2·5 per cent. and 20 minutes suffice for a determination.

Finally, a method which really comes into the category of submicro-determinations has recently been described by Young and Taylor.[59] In their apparatus they measure the change in pressure on vaporisation of the unknown into a vacuum. Using a constant either calculated or determined empirically for the apparatus, the molecular weight is determined from this value. The time required for a determination is only 3 to 4 minutes, the sample may be as small as 2×10^{-4} ml. and the accuracy claimed for the determination is to $\pm$2 per cent.

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August, 1948

Some Physico-Chemical Methods in Microchemistry

Part II. Stabilisation of the Micro-Beckmann Thermometer in Ebullioscopic Determinations of Molecular Weight

By ROBERT J. MAGEE and CECIL L. WILSON

A review of micro-methods for the determination of molecular weight[1] has shown that the micro-Beckmann thermometer, the Menzies - Wright differential thermometer and the thermocouple are the usual instruments for measuring temperature differences, and of these the first is the most widely applied. However, whereas most of the original literature freely mentions "reading the Beckmann thermometer to 0·001 or 0·002° C.," it has been found, during work on a critical survey of reported methods, that, even with the most desirable type of boiling, fluctuations well outside these limits occur. This leaves the precise value of the elevation uncertain.

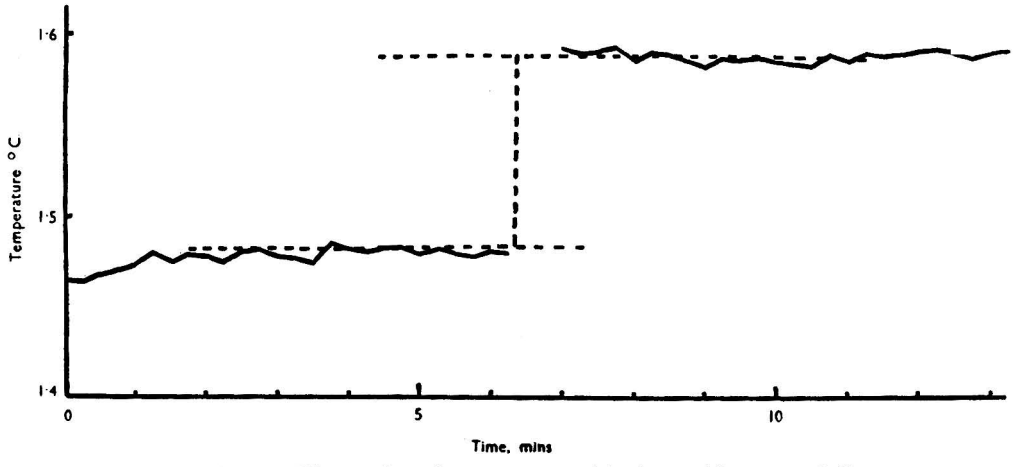

Fig. 1. Fluctuation of temperature with time, without "sock."

Using the Rieche ebulliometer in which a mixture of liquid and vapour is pumped over the bulb of the micro-Beckmann thermometer, and reading the thermometer with a cathetometer to eliminate parallax errors, a series of observations of temperature against time was made in the course of carrying out normal molecular weight determinations. In every experiment the fluctuations excluded the possibility of reading the thermometer to 0·001° C.; one typical curve plotted from the results and chosen at random from the series is shown in Fig. 1.

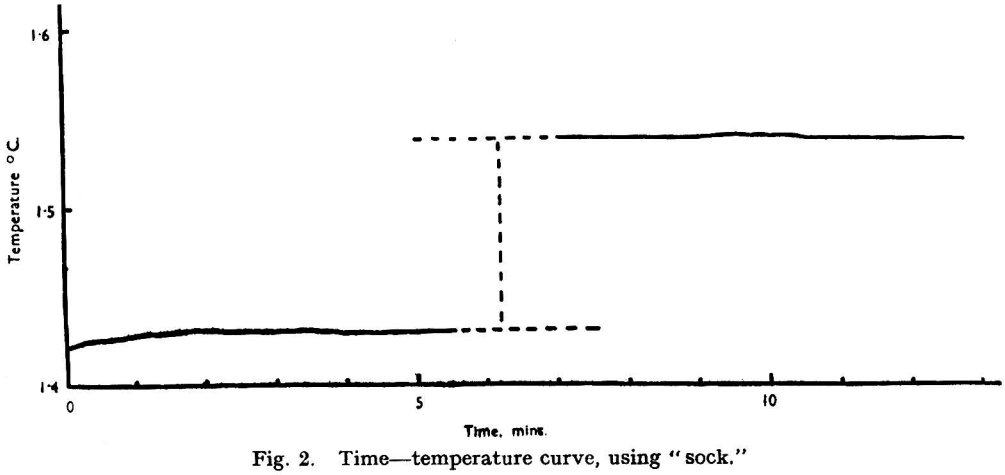

Fig. 2. Time—temperature curve, using "sock."

When the bulb of the thermometer was covered with a tightly-fitting "sock" of "butter muslin," and a similar series of determinations made, the fluctuations were considerably reduced, or, indeed, almost completely absent, and accurate reading of the thermometer was much easier.* A typical curve, again chosen at random, is shown in Fig. 2.

Because of the possibility that the steadying effect of the "sock" might be offset by an error arising from adsorption of solute on the fibres, a series of comparative experiments was performed with the Rieche apparatus, using purified *p*-nitrotoluene (m.p. 51·2° C.) as solute and benzene as solvent. Temperatures were read with a cathetometer. The volume of solvent used in each experiment was 6 ml., and in order to bring to light any errors due to adsorption the weights of solute were varied between 10 and 40 mg. The results obtained are shown in Table I.

TABLE I

	Without "sock"			With "sock"		
Experiment	Weight of solute g.	Molecular weight found	Deviation from average, *d*	Weight of solute g.	Molecular weight found	Deviation from average, *d*
1	0·0272	127·6	− 5·3	0·0265	131·0	−0·1
2	0·0248	140·7	+ 7·8	0·0277	130·0	−1·1
3	0·0251	135·9	+ 3·0	0·0229	131·7	+0·6
4	0·0267	136·9	+ 4·0	0·0282	130·9	−0·2
5	0·0272	132·6	− 0·3	0·0279	130·4	−0·7
6	0·0360	149·9	+17·0	0·0297	132·7	+1·6
7	0·0190	126·0	− 6·9	0·0164	130·0	−1·1
8	0·0350	130·4	− 2·5	0·0358	132·5	+1·4
9	0·0160	118·2	−14·7	0·0169	130·8	−0·3
10	0·0188	130·8	− 2·1	0·0189	131·0	−0·1
Average		132·9			131·1	

Examination of the results in detail gives no indication of an adsorption error, for widely differing weights of solute give values of the molecular weight that lie close together. For example, a value of 130·0 was obtained with 16·4 mg. and with 27·7 mg. of solute. In order to avoid possible contamination from one determination to the next it is suggested that instead of the "sock" being replaced by a fresh one the thermometer might be fitted with a permanent sock of fibre-glass, platinum wool or silica wool.

It can be seen from Fig. 3, where the elevations are plotted against the corresponding weights of solute, that with the "sock" in position the values obtained lie much nearer a straight line than those obtained without the "sock" which are quite erratic. Statistical analysis of the results in Table I provides an interesting set of comparisons shown in Table II.

TABLE II

	Without "sock"	With "sock"
Average deviation of a single result, $\alpha = \pm \frac{\Sigma d}{n}$	6·36	0·72
Standard deviation, $\sigma = \sqrt{\frac{\Sigma d^2}{n-1}}$ ..	8·65	0·94
Probable error of a single result, $r = \pm 0{\cdot}6745\sqrt{\frac{\Sigma d^2}{n-1}}$ ..	5·84	0·63

Using the standard deviation as probably the most useful indication, it can be seen that without the "sock" the spread of the results is almost ten times as great as with the "sock." This is expressed in other terms in Table III, which shows the probable limits within which a single result will lie.

TABLE III

	Without "sock"	With "sock"
Limits for 95 per cent. of results	± 15·43	± 1·84
Limits for 99 per cent. of results	± 22·06	± 2·43
Limits for 99·9 per cent. of results..	± 28·47	± 3·09

* Since adopting this procedure the authors have had their attention drawn to the recommendation, by Ramsay and Young,[2] concerning the use of cotton-wool or asbestos on thermometer bulbs for the accurate determination of boiling-point. The authors are not, however, familiar with any application of the principle to ebullioscopic molecular weight determinations.

A further point should be considered. The correct value for the molecular weight of *p*-nitrotoluene is 137·1. It has been pointed out,[3] and the authors' experience with the Rieche apparatus confirms this finding, that owing to the construction of the apparatus a certain proportion of the solute is always present in the vapour state, and on this account

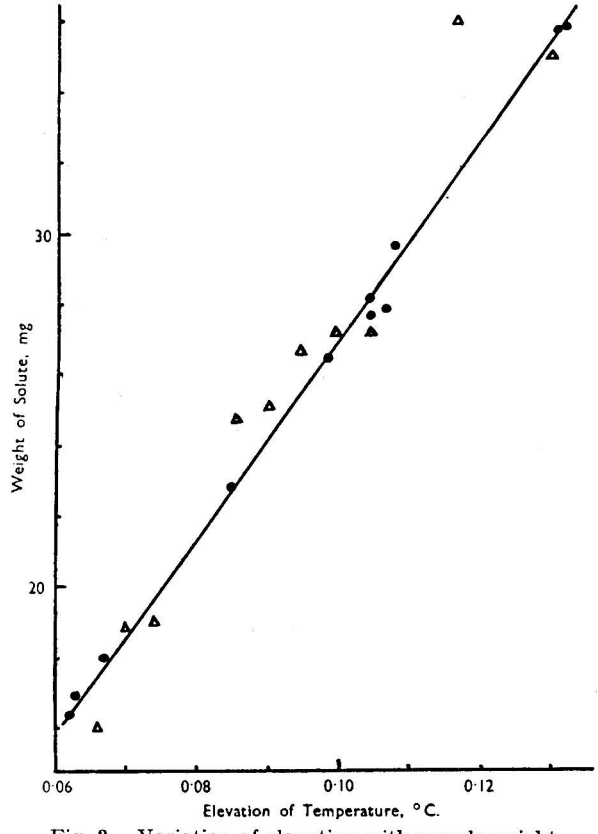

Fig. 3. Variation of elevation with sample weight
△ without "sock." ● with "sock."

results are low by about 5 per cent. In other words, the value to be expected in a satisfactory determination with Rieche's apparatus would be 130·25. Accepting the validity of this the average value obtained with the "sock" (131·1) is considerably better than that obtained without the "sock" (132·9).

Summary

The micro-Beckmann thermometer is frequently used for the ebullioscopic determination of molecular weight in an apparatus involving the pumping of solvent and vapour over the thermometer bulb. Fluctuations occur which prevent a precise estimation of the elevation of the boiling-point, but these can be restrained by fitting the bulb of the thermometer with a "sock." Statistical analysis of the results shows that the values obtained for the molecular weight show much less spread with the "sock" in position.

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August, 1948

The Microchemical Aspects of Electrolytic Conductivity

By J. T. STOCK

(*Read at the Annual General Meeting of the Microchemistry Group, January 30th,* 1948)

Since the electrical conductivity of a solution is governed by the concentrations and the mobilities of all the ions contained in it, conductivity can be used as a measure of ionic strength. For certain purposes, as in the assessment of impurities in water or in the examination of biological fluids, the actual conductivity of the sample is required. More commonly, however, changes in electrical conductivity resulting from a chemical reaction are observed merely as a means of following the progress of the reaction. Conductometric titration is the most important example of this technique.

The effect of traces of dissolved electrolytes on the conductivity of water may be used to determine small amounts of salts in steam.[1] In this and similar examples, although the concentration of the substances being estimated is small, an ample volume of sample is available. When it is not, conductivity cells of small capacity must be employed.

An example of a small cell is shown in Fig. 1; it has a capacity of about 0·5 ml. and was developed for the examination of solutions in heavy water.[2] The conductivity chamber, A, is 15 mm. long and 4 mm. in diameter, the attached capillaries, BB, which permit the introduction of the sample being of 0·8 mm. bore. Sandblasted platinum electrodes, CC, of 3 mm. diameter, are attached to platinum stems, which are sealed through the ends of the chamber and are welded to copper connecting wires, DD. For supporting in a thermostat, an ebonite clamp is used. A smaller cell of similar type has been developed by Walker.[3] It has a working volume of 0·07 ml., the conductivity chamber being horizontal and containing tiny electrodes in the form of tight coils of platinum wire.

The cell shown in Fig. 2 is of unusual design, and can be dismantled for cleaning.[4] A constriction, A, in the central, female-socketted, portion acts as the conductivity chamber, the sample being introduced by means of a micro-pipette. The male portions, BB, carrying the electrodes, CC, which are of 0·28 mm. diameter platinum wire are then inserted so that the electrodes enter the conductivity chamber as shown at (*a*). To prevent the sample being displaced in assembling the cell, a pressure-balancing tube, D, which serves also as a handle, is incorporated. Since the conductivity chamber is only 0·4 mm. in diameter and about 2 mm. long, a fraction of a cu. mm. of solution may be examined. A cell of similar capacity has been described by Bayliss and Walker.[5] It is constructed from two blocks of vulcanite, the conductivity chamber being a small recess formed between them. In the apparatus designed by Remensow,[6] the sample is placed in a small dish, into which dip the micro-electrodes. To ensure constancy of temperature, the apparatus is assembled within a vacuum-jacketted container.

Various pipette-type micro-cells have also been described. That shown in Fig. 3 was designed for conductivity measurements in heavy water systems[7] and was used subsequently for the determination of the ionisation constant of deutero-acetic acid and similar substances.[8] The cell has a capacity of 0·25 ml., bare platinum wire electrodes being used.

The construction of the cell shown in Fig. 4 is interesting. A length of 0·7 mm. bore Pyrex capillary is broken cleanly. One of the cylindrical platinum electrodes with its connecting wire attached is inserted into the end of one of the pieces of tubing, and, by use of a fine glass rod, is shaped to fit the bore. Having re-united the two pieces of tubing with the connecting wire passing through the wall, a second break is made at a suitable distance from the first. The other electrode is then inserted in a similar manner, the finished electrode assembly being as shown enlarged at (*a*). Samples of 10 cu. mm. or less may be examined in this cell.[9] Where a larger volume is available, the cell described by Sunderman, and having a capacity of 1 ml., may be used.[10]

Accurate determination of electrolytic conductivity requires the elimination of polarisation and heating effects, which increase with the current density at the electrodes. Since, in a cell designed for examining small samples, the electrode area is limited, it follows that the maximum permissible flow of current through the cell is small. Apart from suitable

temperature control of the sample and other obvious points, the cell design, suitability of current supply, and sensitivity of balance-detection are all important factors.

It was shown by Kohlrausch and also by Washburn[11] that the design of a conductivity cell depends upon the magnitude of the specific conductivity of the samples to be examined. The microchemical aspects of this have been discussed by White.[4]

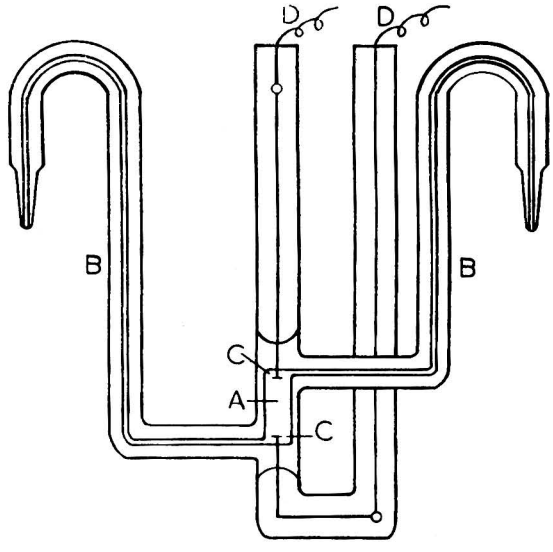

Fig. 1

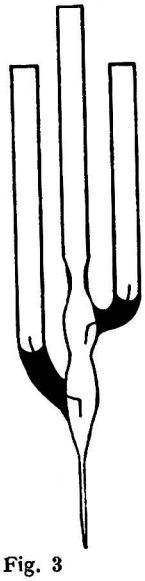

Fig. 3

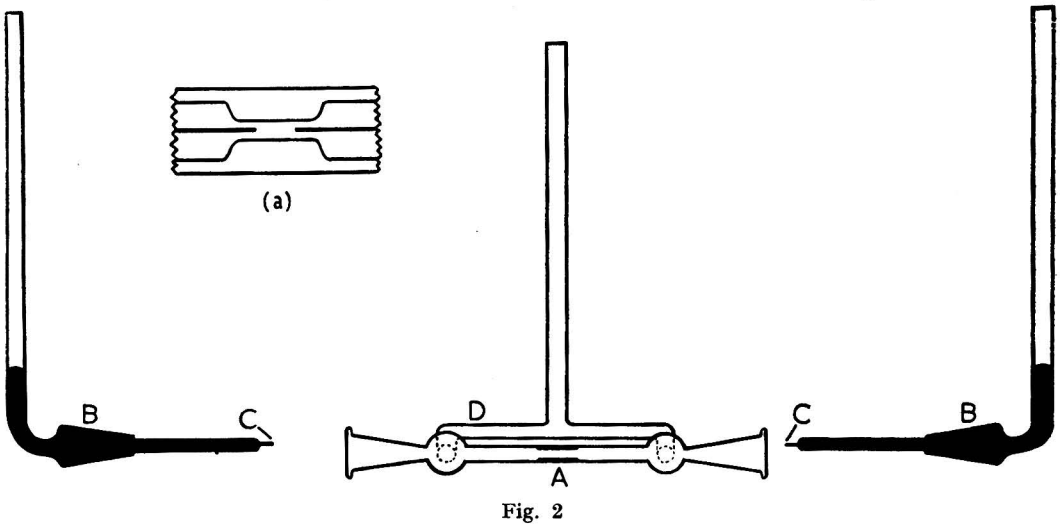

Fig. 2

To avoid electrode polarisation, an alternating current is used in the measurement. This measurement is usually made by a Wheatstone bridge method, the balance being indicated by the position of minimum sound in a telephone receiver.[12] For the best results the alternations should be sufficiently rapid, of pure sine form and such that the quantity of electricity passing in one direction is small and exactly equal to that passing in the other direction. Although simple to use, a small induction coil is not very satisfactory, as the output has a marked uni-directional component and is of poor wave form. A microphone reed vibrator, a motor-driven alternating current generator or an electronic oscillator may with advantage be used instead. Various electronic oscillators have been described.[13] A simple example is shown in Fig. 5. It is designed to operate from a 6-volt accumulator.[14]

The features desirable in a bridge system for the measurement of electrolytic conductivity have been discussed by Jones and Josephs.[15] If, as in the examination of micro-samples,

the maximum current is small, an electronic amplifier tuned to the frequency of the oscillator may be used to increase the sound.[3] Visual methods may replace the use of a telephone receiver. In these an A.C. galvanometer, a D.C. instrument equipped with a rectifier, or an electronic device such as a "magic eye" tuning indicator may be used to detect the state of balance. Visual observation suffers no interference from extraneous noise and, when conditions are not very critical enables the necessary alternating current to be drawn from the mains by way of a transformer. This arrangement is useful in conductometric titrations.

In conductometric titration the removal of an ion (*e.g.*, by precipitation) or its replacement by another of different mobility as the titrant is added, causes a progressive change in conductivity. If, for suitable samples, the conductivity of the sample is plotted against the volume of titrant added, an almost linear curve is obtained. This changes its direction in the region of the end-point and enables the latter to be recognised. Since the two arms of the titration curve are approximately linear (very nearly so if the diluting effect of the titrant is allowed for, or if the latter is much more concentrated than the sample), the plotting of a few points before and after the end-point enables it to be located as the point of intersection of the two straight lines drawn through the experimental points, as shown diagrammatically in Fig. 6.

In conductometric, as in amperometric titration,[16,17] and in contrast to potentiometric and chemical indicator methods, readings near the end-point have no special value. Rounding of the titration curve in the end-point region caused by solubility of the precipitate, hydrolysis, etc., and shown diagrammatically in Fig. 6 by chain lines, does not, therefore, prevent the location of the end-point, nor, unless excessive, greatly affect the accuracy of its location. Methods for precise determination of end-points have been discussed by Mika,[18] and by Langer and Stevenson.[19] Conductometric titration may therefore succeed where potentiometric or chemical methods fail. By contributing to the total conductivity foreign electrolytes lower the accuracy, so that conductometric titration is less generally applicable than are potentiometric or amperometric techniques. Nevertheless, by careful control of conditions (especially temperature) conductometric methods can be applied when the foreign electrolyte concentration is appreciable.[20,21,22,23]

The application of conductometric titration on the micro scale has been largely due to Jander and his co-workers, who have shown that quantities of a few micrograms of various ions may be determined with an average error of about 3 per cent. For example, hydrogen sulphide solution may be used to titrate bismuth in quantities down to 10 μg.; for copper, silver, lead, and cadmium, the lower limit is about 1 μg.[24] Using alcoholic iodine as a reagent, Jander and Harms were able to titrate a fraction of a microgram of arsenic.[25] The same workers showed that 12 μg. and upwards of fluoride could be titrated with aluminium chloride solution.[26] Chloride in amounts down to 10 μg., or, with lessened accuracy, down to 1 μg., are similarly determinable by silver nitrate titration.[27]

Jander thoroughly investigated visual methods for following the progress of the titration and developed methods which are convenient and accurate. He used either an A.C. galvanometer or a D.C. instrument fed by a commutator rectifier synchronised with the motor-driven A.C. generator to provide the bridge current. Fig. 7 shows the cell, of 3 to 4 ml. capacity, which was used in the examination of highly dilute solutions.[25] The platinum electrodes are in the form of concentric half-circles, as shown at (*a*). Titration was performed by means of a screw-operated micro-burette reading in divisions of 0·0005 ml.

For more concentrated solutions a smaller version of the Callan and Horrabin cell[28] is useful and a dipping electrode system equipped with an electronic visual conductivity bridge is available commercially.[29] A simple dipping electrode assembly for the titration of 1-ml. samples is shown in Fig. 8.[30] In constructing it, two pieces of glass tubing are placed parallel to one another and fused together for a length of about 2 cm. Without allowing them to cool, the tubes are softened all round in the region of the joint and pulled out. When cold, a 5-cm. length of the double tubing, each bore of which should be about 2 mm., is cut off and electrodes of 24 S.W.G. platinum wire are sealed in as shown. A small glass bridge joining the outer ends of the electrodes renders the assembly rigid. Electrical connections are made by copper wires dipping into beads of mercury in contact with the inner ends of the electrodes.

An interesting differential method of conductometric titration has been developed by Duval and Duval[31] and has been adapted by them to the microchemical scale.[32] The circuit arrangement is conventional, except that a second, identical, titration cell replaces the

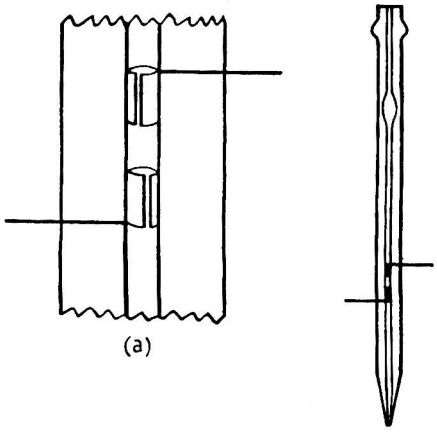

Fig. 4

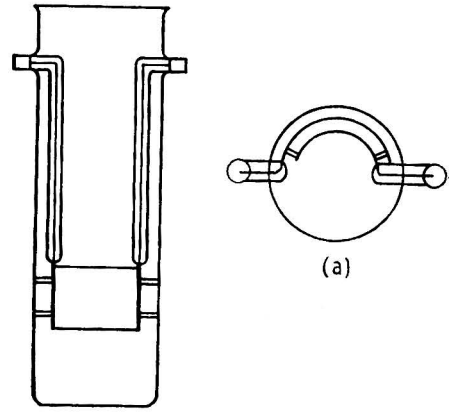

Fig. 7

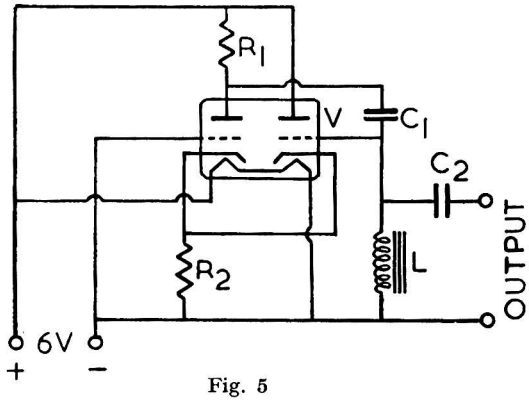

Fig. 5

R_1 0.1-megohm, 1-watt resistor
R_2 3000-ohm, 1-watt resistor
L 4-henry choke
C_1 0.1-mfd. condenser
C_2 0.01-mfd. condenser
V Double triode valve 6SN7GT

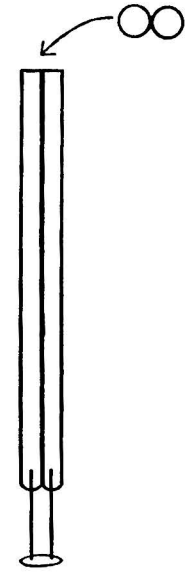

Fig. 8

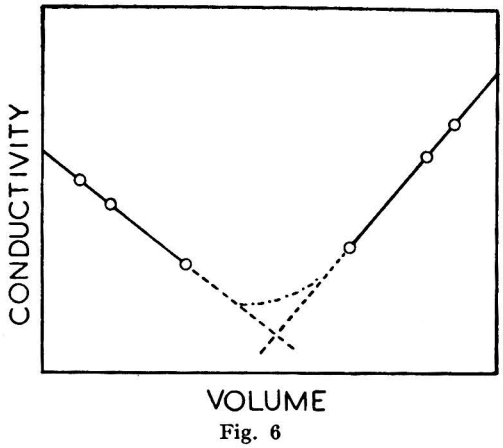

Fig. 6

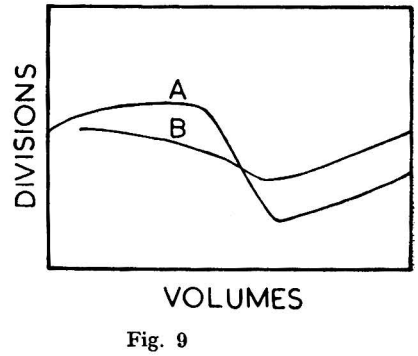

Fig. 9

resistance box. Equal volumes, 2 to 5 ml., of sample are placed in each cell and equal additions of titrant are made alternately to each cell, so that the volume added to cell A is 0·05 or 0·1 ml. in advance of that added to B. The procedure resembles the potentiometric titration of Cox.[33] After each addition, the bridge is balanced and the readings plotted against the corresponding volumes of titrant added. Two curves intersecting at the end-point, as shown diagrammatically in Fig. 9, are obtained. Quantities of a few milligrams of magnesium, calcium, zinc, iron, and sulphate were titrated with an error of between 1 and 5 per cent. The symmetry of the circuit minimises inductance and capacity effects so that a sharp audible end-point is obtained.

An ingenious and rapid method for the examination of water has been described by Polsky.[34] In this specific conductivity of the sample is measured initially and after the addition of a definite and excessive volume of a selective precipitant (*e.g.*, of silver nitrate solution in the determination of chloride). The increase in conductivity due to the addition is partially offset by a decrease due to removal of ions by precipitation, the net increase varying inversely as the quantity of substance being precipitated. After the application of a correction, based on the initial specific conductivity, to allow for the presence of ions foreign to the reaction, the result is obtainable directly and titration is unnecessary. The method was used for samples of varying hardness to determine calcium down to 15 p.p.m. and chloride and sulphate down to 5 p.p.m. of either ion.

Since the specific organic precipitants for metals have proved useful in amperometric micro-titration,[35] a publication dealing with conductometric titration involving these reagents is interesting.[36] Another development lies in the use of high-frequency oscillators.[37] As this method appears to be sensitive and does not require the insertion of electrodes into the solution, it may prove useful on the microchemical scale.

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A Note on the Estimation of Sulphur Dioxide in Fish Muscle

By J. M. SHEWAN

In some recent experiments on the use of sodium metabisulphite as a preservative in the desalting of cod, anomalous results were obtained in determining the sulphur dioxide content of the flesh, using the modified Monier-Williams method[1] of Kirkpatrick,[2] but acidifying with phosphoric acid as recommended by Jacobs.[3] For example, it was found that whereas good recoveries of sulphur dioxide were obtained from sodium metabisulphite, in pure solution or when added to salt cod which had been well desalted, very poor recoveries were obtained in the presence of fresh cod, muscle juice prepared from fresh cod, or freshly-salted cod (Table I).

TABLE I

RECOVERY OF SULPHUR DIOXIDE FROM MIXTURES OF SODIUM METABISULPHITE AND FRESH, SALTED AND WELL DE-SALTED COD FLESH

50 g. of fish flesh + 10 ml. of solution containing a known quantity of SO_2

Type of flesh	SO_2 in mg. Found	SO_2 in mg. Theoretical
Fresh cod	0	23·7
Well desalted cod (1)	16·0	,,
,, ,, ,, (2)	21·0	,,
Control (10 ml of solution)	21·9	,,
"Wet-stack" salt fish (over 1 year since salting)	20·6	32·8
,, ,, ,, ,, ,, ,, ,,	24·8	,,
,, ,, ,, (freshly salted—2 to 3 months)	2·6	,,
Control (10 ml. of solution)	30·6	,,
"Wet-stack" salt cod	10·0	16·4
Fresh cod	5·5	,,
Control (10 ml. of solution)	14·4	,,
Muscle juice (from fresh cod)	10·5	22·4
Control (10 ml. of solution)	20·8	,,

It was later shown that good recovery could be obtained with fresh brown trout and perch (freshwater fish), but that losses occurred as with cod when other marine species (haddock, sole, etc.) were used. Beef, mutton and pork were found to behave like freshwater fish. (Table II).

It thus appeared that some constituent was present in the muscle of marine fishes, but absent in that of freshwater fish, in meats and desalted cod, that reacted with the sulphite and so interfered with the determination of SO_2 by the procedure used.

TABLE II

RECOVERY OF SO_2, ADDED AS SODIUM METABISULPHITE, FROM FISH FLESH AND MEATS

50 g. of flesh + 10 ml. of solution containing a known quantity of SO_2

Type of flesh	SO_2 in mg. Found	SO_2 in mg. Theoretical	Control Solution (10 c.c.)	Blank
Fresh cod	11·5	22·9	20·8	0
,, haddock	10·9	,,	22·1	—
,, lemon sole	17·1	,,	21·0	—
,, perch	21·0	,,	21·0	—
,, trout	18·9	,,	20·0	—
,, pork	20·8	,,	20·8	—
,, mutton	20·8	,,	21.0	—
,, beef	21·0	,,	20·8	—

It is now well established that one of the chemical differences between marine and freshwater fish is the presence in the former of considerable amounts of trimethylamine oxide, the actual amount present varying with the species and possibly also with factors such as food, season, etc.[4] In freshwater fish[4,6] and meats[5] this compound is either absent or present only in traces. It has also been established that trimethylamine oxide may break down, under

certain conditions to dimethylamine and formaldehyde[7,8]. It was therefore thought conceivable that sulphite by combining with the formaldehyde might accelerate this decomposition according to the following equation,

$$NaHSO_3 + (CH_3)_3.NO \rightarrow (CH_3)_2.NH + (H{\cdot}CHO.NaHSO_3) \quad \text{or} \quad (CH_2(OH)SO_3.Na).$$

and the sulphur dioxide so bound might then fail to be estimated by the modification of the Monier-William's method used. In presence of semicarbazide, however, which might have been expected to combine similarly with any formaldehyde present, no breakdown of trimethylamine oxide, as evidenced by formation of dimethylamine, could be detected.

The flesh from a variety of sea fish treated with sulphur dioxide ($NaHSO_3$ or $Na_2S_2O_5$) has indeed shown a marked increase in the amount of dimethylamine and formaldehyde as compared with untreated controls. Freshwater fish and meats have shown no such increase (Table III).

TABLE III

DETERMINATION OF DIMETHYLAMINE AND FORMALDEHYDE IN FISH FLESH AND MEATS, TREATED WITH AND WITHOUT SO_2

50 g. of flesh + 5 ml. of a solution containing 23 mg. of SO_2. After the immediate determination of SO_2 (by the modified Monier-Williams method) the amounts of $(CH_3)_2NH$ and HCHO were determined in the suspension.

Type of flesh	Dimethylamine nitrogen per 50 g. of muscle mg.	Formaldehyde per 50 g. of muscle mg.
Haddock (untreated)	0·24	0·22
,, + $Na_2S_2O_5$	2·9	1·8
Lemon sole (untreated)	trace	trace
,, + $Na_2S_2O_5$	1·30	0·40
Codling (untreated)	trace	trace
,, + $Na_2S_2O_5$	2·7	0·34
Perch (untreated)	trace	trace
,, + $Na_2S_2O_5$	,,	,,
Beef (untreated)	,,	,,
,, (treated)	,,	,,
Pork (untreated)	,,	,,
,, (treated)	,,	,,
Mutton (untreated)	,,	,,
,, (treated)	,,	,,

It has not been possible to study the reaction in detail, but qualitative and quantitative determinations, using mixtures of pure solutions of sodium metabisulphite and trimethylamine oxide, have shown that formaldehyde and dimethylamine are formed in a short time even at room temperatures, the amounts of dimethylamine formed and of the metabisulphite disappearing being approximately that expected from the equation. (Table IV).

TABLE IV

DETERMINATION OF $(CH_3)_2NH$ AND HCHO FORMED ON MIXING PURE SOLUTIONS OF $Na_2S_2O_5$ AND TRIMETHYLAMINE OXIDE $(CH_3)_3.NO$) AND OF THE AMOUNT OF $Na_2S_2O_5$ BOUND

	Component	Theoretical* mg.	Found mg.
Experiment I	$Na_2S_2O_5$		20·0 (Bound)
	$(CH_3)_2.NH$ as N	2·95	2·50
	H.CHO	6·30	1·40
Experiment II	$Na_2S_2O_5$		11·1 (Bound)
	$(CH_3)_2.NH$ as N	1·63	1·90
	H.CHO	3·50	2·60

* The theoretical values for dimethylamine and formaldehyde have been calculated from the actual amount of metabisulphite bound according to equation

$$Na_2S_2O_5 + 2(CH_3)_3NO + H_2O \rightarrow 2(CH_3)_2NH + 2CH_2(OH)SO_3Na.$$

The figures for the sodium metabisulphite bound are taken as the amount found in the mixed solution subtracted from that originally present.

On the other hand, the amount of formaldehyde that can be estimated is much lower than that expected from the equation, presumably because formaldehyde cannot be determined accurately by Schryver's method in presence of excess of sulphur dioxide. (Table V).

TABLE V

EFFECT OF METABISULPHITE ON THE DETERMINATION OF FORMALDEHYDE BY SCHRYVER'S METHOD

Solution A = 2·14 mg. of HCHO per ml.
,, B = 13·6 mg. of $Na_2S_2O_5$ per ml.
Mixed solutions stood 18 hours at 20° C.

		HCHO mg. in 20 c.c. of original solution
Solution	(A) 20 ml. + 20 ml. H_2O	42·8 mg.
,,	(B) 20 ,, + 20 ,, ,, ...	0
,,	(A) 20 ,, + 20 ,, Solution (B)	12·8 ,,
,,	(A) 20 ,, + 5 ,, ,, ,, + 10 ml. H_2O ..	21·6 ,,
,,	(A) 10 ,, + 2 ,, ,, ,, + 10 ,, ,, ..	18·4 ,,

CONCLUSIONS—

Sulphur dioxide cannot be determined accurately in the presence of muscle from marine fish, presumably owing to the breakdown of the trimethylamine oxide to dimethylamine and formaldehyde and the formation of a formaldehyde-sulphite complex. The latter apparently *is not broken up in the modification of the Monier-Williams procedure used*, so as to permit determination of the bound sulphur dioxide.

The above note is based on work carried out as part of the programme of the Food Investigation Board, and is published by permission of the Department of Scientific and Industrial Research.

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May, 1947

A New Method for the Micro-determination of Beryllium with Particular Reference to its Determination in Biological Materials

BY W. N. ALDRIDGE* AND H. F. LIDDELL†

AT an early stage in the experimental study of the toxicology of beryllium, it became apparent that a very sensistive and specific method for the determination of beryllium in biological materials must be devised. There are few available methods for the estimation of beryllium and most of these cannot be applied to biological materials. The morin fluorimetric method[1] is of high sensitivity, but difficult to carry out in presence of calcium. The quinalizarin[2] and the curcumin[3] methods have indistinct colour changes and are difficult to apply to quantitative measurement. A more recent method[4] has been published by Fairhall; in this, iron and phosphate interfere and have to be removed and also the dye reacts with aluminium, but no steps have been taken to separate this metal from beryllium.

In the work here described we have used two dyestuffs for determining beryllium in pure solution and have been able to estimate quantities down to 0·2 μg. In digests of biological

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materials calcium, iron and phosphate are always present in comparatively large amounts, together with small amounts of aluminium. The amount of aluminium in normal tissues may be up to 10 times that of the beryllium to be estimated (Mackenzie[5]) so it is essential to have a method in which aluminium does not interfere.

Experimental

Principles of method—

After an examination of a number of mordant dyestuffs, it was found that Naphthochrome Green G (Schultz 851) and Naphthochrome Azurine 2B gave the best reaction for beryllium. Both of these dyes are of the triarylmethane mordant type. Although both react with beryllium in pure solution at *p*H 11·5 and 12·3 it has only been found possible to use Naphthochrome Green G at *p*H 12·3 for the determination in biological materials. At this *p*H a colour change from green to red is given which can be readily measured. All metals that precipitate at this *p*H value must be removed, *i.e.*, iron and also calcium in presence of phosphate. Iron is removed as Prussian blue and beryllium is precipitated from solution as phosphate by using aluminium phosphate as a collector. At *p*H 12·3 aluminium does not interfere.

Reagents—

Digesting materials—Concentrated sulphuric and nitric acids.

Potassium ferrocyanide solution containing 10 g. per 100 ml. Stored in a brown bottle.

Aluminium solution containing 1·5 mg. of Al per ml. A 2·63 per cent. solution of potassium alum.

Disodium phosphate solution, saturated.

Bromocresol green solution, 0·1 per cent.

Neutralising reagents—Approximately 10*N* sodium hydroxide, *N* sulphuric acid and 0·1 *N* sodium hydroxide.

Sodium chloride solution containing 1 g. per 100 ml.

N *Sodium hydroxide*, standardised.

Trisodium phosphate solution, 10 g. per 100 ml. in water free from carbon dioxide. Stored under soda-lime.

Dye reagent—The dye as supplied by Clayton Aniline Co. is the sodium salt. The free dye acid is prepared as follows. Dissolve 20 g. of Naphthochrome Green G in 300 ml. of boiling water. Cool to 30° C. and precipitate the dye acid with dilute hydrochloric acid until acid to Congo red. Filter and wash, and dry at 70° C. Yield 5 g. The reagent for use in the method is prepared by dissolving 0·15 g. of the dye acid in 100 ml. of alcohol containing 1 ml. of *N* hydrochloric acid.

Standard beryllium solution—Dissolve 9·833 g. of beryllium sulphate, $BeSO_4.H_2O$, in water and dilute to 500 ml. This solution contains 1 mg. of beryllium per ml. For the preparation of calibration curves dilute 5 ml. to 1 litre with distilled water, to obtain a solution containing 5 μg. of beryllium per ml. The diluted standard is stable.

Destruction of organic matter—

Dry ashing has been avoided in view of the danger of loss of beryllium due to the volatility of the chloride. We have destroyed organic matter by an ordinary sulphuric acid-nitric acid digestion with no special precautions except to avoid excessive overheating. As will be seen later, the amount of sulphuric acid that can be used is limited and should not exceed 2 ml. In order to avoid excessive conversion of ferrocyanide to ferricyanide during the subsequent removal of iron it is essential to remove all the nitric acid from the digest. This can readily be achieved by two evaporations after addition of a small amount of water.

Removal of iron—

Since iron is precipitated at the *p*H of the colour reaction and also is not separated from beryllium by the phosphate precipitation technique at *p*H 4·5 to be described later, it must be completely removed initially. Any method used must give complete removal in a highly acid medium or beryllium will be carried down with it, *e.g.*, ferric hydroxide is a very efficient collector for beryllium. Fairhall[4] removes iron by extraction with a chloroform solution of cupferron, an extremely unwieldy procedure when there are many estimations to do. Ferrocyanide does not interfere in the method, but the conversion of iron to ferrocyanide, requires an excess of cyanide which causes a rapid fading of the dye in the final colour reaction. However, iron may be completely and easily removed from highly acid solutions by precipitation

as Prussian-blue. The concentration of sulphuric acid must not rise above 7 *N* because precipitation of hydroferrocyanic acid would occur and no Prussian-blue would be formed.

SEPARATION OF BERYLLIUM—

Beryllium must be separated from calcium and the high concentration of salts present in the digest after iron removal and must be in a state in which it may be used for the colorimetric determination. As aluminium does not interfere in the method, it may be used either as hydroxide or phosphate, as a collector for beryllium. Aluminium hydroxide is an efficient collector for traces of beryllium, but in presence of phosphate calcium is also carried down. However, precipitation of 0·75 mg. of aluminium as phosphate at about *p*H 4·5 collects traces of beryllium extremely efficiently and gives a good separation from calcium and magnesium. The recoveries given in Table I illustrate this.

TABLE I

RECOVERY OF BERYLLIUM BY USING ALUMINIUM AS A COLLECTOR

0·75 mg. of aluminium was added and phosphate precipitated at *p*H 4·5

Beryllium taken, μg.	0·0	1·0	2·0	3·0	4·0	5.0
,, found, μg.	0·0	1·0	2·1	2·95	4·0	4·9

As a test of the efficiency of collection of beryllium by this procedure, 4 μg. were precipitated from solution with 0·75 mg. of aluminium as phosphate at *p*H 4·5. The resulting precipitate was dissolved in acid and the precipitation repeated. This was again repeated and the amount of beryllium recovered from the precipitate after one, two or three precipitations was determined in the usual way.

TABLE II

EFFICIENCY OF ALUMINIUM AS A COLLECTOR

4 μg. of beryllium were precipitated, with aluminium phosphate as a collector, redissolved and reprecipitated up to three times.

Number of pptns. of Be	1	2	3
Be recovered (duplicates), μg.	4·0; 4·0	3·9; 4·0	3·9; 3·9

The aluminium phosphate must be thoroughly washed to remove all substances that might alter the *p*H of the solution, or precipitate at *p*H 12, or oxidise the colouring matter (ferricyanide is the chief offender here). When water was used for washing we found that on suspending the precipitate in water the second time it sometimes passed into colloidal solution. This does not occur if the washing is carried out with 1 per cent. sodium chloride solution.

BUFFERING TO *p*H 12·3—

Solutions of *p*H 10 and above are extremely difficult to maintain unchanged. We have used a solution of trisodium phosphate stored free from carbon dioxide. However, this is hardly a buffer at all and great care must be taken to prevent substances that will alter the *p*H of the solution from reaching the colorimetric stage. A few trials with tetramethyl

TABLE III

AMOUNT OF ALKALI REQUIRED TO CORRECT FOR ALUMINIUM USED AS A COLLECTOR

0·75 mg. of aluminium was precipitated as phosphate as in the method. Various amounts of *N* NaOH were added and the whole was diluted to 5 ml. with trisodium phosphate solution and dye reagent added. The mixture was warmed for 20 min. at 30° C. and read against a blank with no aluminium, in 1-cm. cells and using Chance red filters No. 1.

N NaOH added, ml. ..	0·23	0·19	0·14	0·12	0·10	0·08	0·06
Reading against blank	+ 95	+ 80	+ 75	+ 40	+ 30	nil	− 20

\+ signifies more intense than blank
− ,, less ,, ,, ,,

ammonium hydroxide as a buffer indicated that this would be considerably better, but its cost was considered to be prohibitive. When trisodium phosphate solution is used the 0·75 mg. of aluminium alters the *p*H sufficiently to render the results inaccurate. In order to correct for this change in *p*H we have determined empirically the amount of alkali necessary to compensate. This has been done by using the dye as an indicator. As the *p*H increases

from 12 to 12·8, the intensity of the colour, as measured with a red filter, increases. The precipitate of aluminium phosphate used in the method was washed and various amounts of *N* sodium hydroxide were added. The results given in Table III show that 0·08 ml. is required for this purpose. They further indicate that the sodium hydroxide must be measured accurately *e.g.*, with a micro-burette.

THE COLOUR REACTION—

Fig. 1 gives the absorption spectrum for the unchanged dye solution and for one containing 3 μg. of beryllium. It can be seen that a much greater sensitivity is obtained by measuring a decrease in green at 650 mμ. than by measuring an increase in red at 500 mμ.

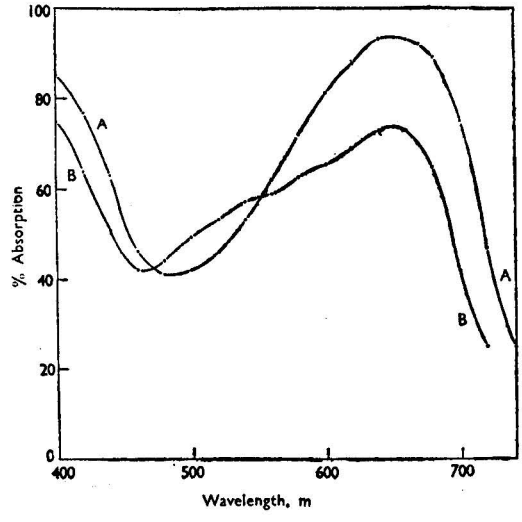

Fig. 1. Absorption spectrum of Naphthochrome Green G in 10 per cent. trisodium phosphate solution with and without beryllium. Measurements made on the Beckmann spectrophotometer with a slit width of 0·03 mm.
Curve A—Dye alone (blank).
Curve B—Dye + 3μg. of beryllium.

We have therefore measured the change in colour of the dye due to the presence of beryllium as a decrease in green, using a red filter. The instrument we have used is one designed by Wilson and Watt[6] utilising a wedge screen and giving readings on a linear scale. With Chance red filter No. 1 a straight line relationship between beryllium concentration and photo-electric reading as given in Table IV is obtained. Above 5 μg. of beryllium a straight line is no longer obtained and the sensitivity decreases rapidly. However, when it is obvious that the concentration of beryllium is too high for an accurate reading to be obtained the solution may be diluted with blank dye solution before warming to 25° C. It is recommended that a calibration curve be made frequently as a check on the reagents (especially the trisodium phosphate solution and dye reagent). It should be noted that because the colorimetry is "negative," the final volume of 5 ml. and the 0·2 ml. of dye reagent must be accurately measured; we have always used a Krogh pipette for the measurement of the dye (accuracy 0·1 per cent.).

TABLE IV

DATA FOR STANDARD BERYLLIUM CALIBRATION CURVE

Colorimetric readings with 1-cm. cells and Chance red filter No. 1.

Beryllium, μg.	0·0	1·0	2·0	3·0	4·0	5·0
Photo-electric reading ..	0·0	80	165	240	325	405

The colour development is noticeably slower at 15° than at 25° C., so some control of the temperature during colour development is essential. We have immersed the tubes in a water bath at 30° C. for 20 minutes. There is a slow colour development after this time, but little error is produced in this way.

The dye, Naphthochrome Green G (Schultz 851), as supplied by Clayton Aniline Co. is the sodium salt, soluble in water but extremely unstable and quite unsuitable as a standard reagent for the method. Other salts of the dye acid have been prepared in solution—the ammonium, the pyridine and the mono- and the tri-ethanolamine salts—but these are all unstable. The dye acid is stable but must be stored out of all contact with alkali. As stated in the list of reagents, we have used a solution in alcohol rendered approximately 0·01 *N* with respect to hydrochloric acid. This solution we have found to be stable and should keep for several months if stored in a brown bottle in the dark.

OTHER DYE REACTIONS—

We have found eight dyestuffs that will react with beryllium at *p*H 11 and above, but with most of them the colour changes are unsuitable for measurement. However, Naphthochrome Azurine 2 B will give a colour change in presence of beryllium at *p*H 11·5. This is useful only in pure solution since phosphate inhibits the reaction, but we include the calibration curve values in view of their high sensitivity. The buffer used was 10 per cent. v/v monoethanolamine containing 8 per cent. v/v of *N* hydrochloric acid and the colorimetric readings were carried out on a Coleman Junior Spectrophotometer at 500 mμ.

TABLE V

DATA FOR BERYLLIUM CALIBRATION CURVE FOR NAPHTHOCHROME AZURINE 2 B AT *p*H 11·5

Monoethanolamine buffer used and colours read on a Coleman Junior Spectrophotometer at 500 mμ.

Beryllium, μg.	..	..	..	0·0	0·4	0·8	1·2	1·6	2·0
Absorption, %	..	..	..	0·0	25	39	49	56	60

RECOVERY OF BERYLLIUM FROM BIOLOGICAL MATERIALS—

In order to check the method, various amounts of beryllium have been added to tissues and the whole estimation carried out as given in the text (*infra*). Excellent recoveries have been obtained from all tissues except those producing a large amount of calcium sulphate precipitate during the digestion. Representative results are given in Table VI.

TABLE VI

RECOVERY OF ADDED BERYLLIUM FROM TISSUES

Tissue	Beryllium added μg.	Beryllium recovered μg.	Beryllium lost μg.
Liver, 3 g.	0	0	
	4·0	4·1	
	8·0	7·9	
	12·0	12·0	
Bone, 2 g.	0	0	0
	4	1·5	2·5
	8	6·0	2·0
	12	9·6	2·4

With materials containing a large amount of calcium it is highly probable that the loss of beryllium is purely by mechanical entrainment and will thus be dependent on the quantity of calcium sulphate obtained in the digestion. That this is possible is shown by the calculation of loss of beryllium obtained with the same amount of bone, but with various amounts of added beryllium. This is hardly unexpected when it is realised that the ratio of beryllium to calcium in such circumstances is of the order of 1 to 10^5 or 10^6.

METHOD AS APPLIED TO BIOLOGICAL MATERIALS

To the weighed sample of tissue in a silica Kjeldahl flask add 2 ml. of concentrated sulphuric acid and 2 to 5 ml. of concentrated nitric acid. Heat on a sand bath and continue the digestion with addition of further quantities of nitric acid where necessary, until the digest is colourless or pale yellow and the white fumes of sulphuric acid are seen. Cool, and evaporate twice after addition of 2 ml. portions of water. We have found it convenient to finish the digestion and the evaporations over a low bunsen flame. Wash the digest into a 15-ml. graduated flask or tube and add 1 ml. of 10 per cent. potassium ferrocyanide solution. If the solution becomes pale-blue and the pigment does not coagulate add 1 drop of 5 per cent.

ferric chloride solution, dilute to 15 ml, mix, pour into a centrifuge tube and allow to stand for an hour or preferably overnight in a dark cupboard. Centrifuge for 10 minutes at 2500 revs. per min. Pipette 5 ml. of the colourless or pale-yellow solution (an intense yellow indicates that all the nitric acid has not been removed and ferrocyanide has been oxidised to ferricyanide) into a 15-ml. centrifuge tube, add 0·5 ml. of 2·63 per cent. potash alum solution (0·75 mg. of Al.), 1 ml. of saturated disodium phosphate solution and 3 drops of bromocresol green indicator. Add 10 *N* sodium hydroxide drop by drop, mixing with a flat-ended glass rod until the solution is alkaline (indicator blue). Bring back to yellow with *N* sulphuric acid and finally bring to a green colour with 0·1 *N* sodium hydroxide. Mix and allow to stand at room temperature for 30 minutes. Centrifuge 2500 revs. per min. for 5 minutes, carefully decant the supernatant fluid and drain on a filter paper. Add about 0·5 ml. of 1 per cent. sodium chloride solution and suspend the precipitate by tapping the tube. Add a further 5 ml. of sodium chloride solution and centrifuge at 2500 revs. per min. for 3 minutes. Pour off the supernatant liquid and repeat the washing. The washing must be carried out very thoroughly and the precipitate must be suspended in the solution and not superficially washed on the bottom of the centrifuge tube. Pour off the last washing liquor and drain. To the precipitate add 0·08 ml. of standardised *N* sodium hydroxide from a micro-burette. Transfer and rinse the tube with trisodium phosphate solution into a 5-ml. graduated flask or tube. Dilute to 5 ml. and add 0·2 ml. of the dye reagent with an accurate pipette (Krogh syringe pipette recommended), mix and allow to stand at 30° C. for 20 minutes. Prepare a blank with 5 ml. of trisodium phosphate solution and 0·2 ml. of dye reagent and treat in the same way as the unknown. Read on a photo-electric instrument, using 1-cm. cells and Chance red filters No. 1. As a decrease in green is being measured, the blank is brought to zero first on a null point instrument and the unknown then measured. Usually when carrying out estimations on unknowns we have run a blank through as a check from the removal-of-iron stage.

Discussion

In the method described above there are two main factors that narrow the possible conditions for the estimation of beryllium. First, beryllium phosphate is insoluble down to at least *p*H 3 and secondly, aluminium is not rendered completely non-interfering until above *p*H 11. Further, we have found that the extremely sensitive reaction of Naphthochrome Azurine 2 B in pure solution at approximately *p*H 11·5 is completely inhibited by the presence of traces of phosphate. Also, if the *p*H is increased above 12·3 the colour change decreases in amount until at *p*H 13 there is hardly any reaction. The degree of specificity attained is therefore not a function of the dye itself, but rather of the conditions chosen for the reaction.

The effect of aluminium in lowering the *p*H has been prevented by the addition of a known amount of standard alkali. This procedure completely disregards the aluminium normally present in tissues. However, a decrease of 20 per cent. in the amount of *N* sodium hydroxide added produces an error of 20 in the colorimetric reading (equivalent to about 0·2 μg. of beryllium). Therefore the method will allow an increase of aluminium in the aliquot analysed (a third of the total) of 20 per cent. (about 150 μg.). Taking the results obtained for normal rat livers by Mackenzie (1931)[5] (this organ contains the highest concentration, of the order of 17 μg. per gram of wet tissue) it can easily be calculated up to 25 g. of liver may be digested without producing any marked effect on the method due to aluminium initially present. We have never obtained a positive reading for normal tissues.

Finally, although we have carried out many satisfactory estimations of beryllium by this method, we would like to point out its disadvantages:—

(*a*) The colorimetry, being the reverse of the normal procedure, *i.e.*, a decrease of colour is being measured, places the greatest inaccuracies on the determination of the smallest amounts of beryllium.

(*b*) The procedure is long and complicated. Further, it requires considerable practice and technical skill to produce consistently reliable results.

(*c*) The reaction is carried out at a *p*H at which it is almost impossible to buffer effectively.

(*d*) We have not been able to produce good recoveries of added beryllium from bone or any organ that produces a large precipitate of calcium sulphate during the digestion.

Summary

(1) A new method for the determination of small amounts (down to 1 μg.) of beryllium is described. (2) New methods for the separation of beryllium from interfering substances

have been worked out. (3) Details of the determination of beryllium in biological materials are given. (4) The method is specific for beryllium among the elements normally present in biological materials. (5) Good recoveries have been obtained for known amounts of beryllium added to normal tissues except bone or any other material containing large amounts of calcium.

We wish to thank the Clayton Aniline Co. for the gift of a large quantity of Naphthochrome Green G and one of us (H. F. L.) thanks the Chief Scientist of the Ministry of Supply for permission to publish this work.

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April, 1948

The Simultaneous Determination of Nickel and Zinc in Secondary Aluminium Alloys by Means of the Polarograph

BY B. A. SCOTT

THE determination of small quantities of zinc in secondary aluminium alloys by the usual methods is a somewhat tedious process, and it was felt that the polarographic technique might give some improvement in speed and convenience, particularly if a direct method not involving chemical separations could be devised.

The first experiments were directed towards the possibility of carrying out the polarographic analysis in a strongly alkaline medium, but tests soon showed that there were serious irregularities, due probably to variable adsorption of zinc by the precipitated hydroxides. Previous removal of copper, etc., by treatment with hydrogen sulphide in acid solution gave no improvement, and it was concluded that the interference was probably due to the precipitated MnO(OH). As manganese could not be easily removed, this method was given up.

Experiments were next carried out to determine the possibility of using a slightly acid solution for the polarogram. The chief difficulty here was the control of the *p*H at such a value that the hydrogen wave would not interfere with the results and at the same time there would be no risk of precipitation of aluminium hydroxide. With secondary metal containing nickel, there was also the additional drawback of the coincidence of the nickel and zinc waves under these conditions, unless a suitable complex-forming substance was present. Citric acid was first tried in order to prevent precipitation of alumina at the optimum *p*H for zinc determination (*p*H 6·0, bromocresol purple indicator), copper being first removed as sulphide in strongly acid solution, as its wave precedes that of zinc. Even on samples free from nickel, however, erratic results were obtained and nickel, when present, interfered with the zinc wave. As it is usual to prevent this interference by addition of ammonium oxalate, and as oxalic acid is also capable of forming a loose complex with aluminium salts, this acid was next tried instead of citric acid, the *p*H being adjusted to 6·0, as before, with ammonia. Although with this modification some samples gave fairly good results for zinc, a certain amount of hydrolysis was difficult to avoid, and in a number of instances low and erratic results were obtained, probably owing to adsorption. Numerous modifications of this method were tried, but these poor results were always in evidence.

In consequence of the repeated failure of all these methods, a search was made through the literature for other possible methods. The most promising features were shown by the work of Stout *et al.*[1] on the simultaneous determination of nickel and zinc in plant ash, after a preliminary dithizone extraction, using a medium containing potassium thiocyanate and ammonium acetate at *p*H 4·6. It seemed likely that this procedure could be applied to aluminium alloys, without the necessity for dithizone extraction, for the following reasons. (1) Hydrolysis of aluminium salts would not take place at the relatively low *p*H. (2) Copper could be precipitated as cuprous thiocyanate after a preliminary reduction which would also remove the ferric iron wave. (3) Other elements might form stable complexes with excess thiocyanate.

The method was tried in the form given below, hydroxylamine being selected for the reduction as it is not itself reduced at a mercury cathode.

Of the metal to be analysed 0·5 g. was dissolved in 10 ml. of *aqua regia* and when the action was complete the solution was boiled down almost to crystallisation, to remove excess of acid. Three grams of sodium acetate and 2 ml. of 10 per cent. hydroxylamine hydrochloride solution were added to the diluted solution, which was warmed for a few minutes to complete the reduction of copper and iron. The copper was then precipitated by addition of 3 ml. of 25 per cent. potassium thiocyanate solution and the mixture was cooled without filtration. Five ml. of 0·1 per cent. gelatine solution were added and the solution was diluted to 50 ml. and polarographed between — 0·4 v. and — 1·2 v. after removal of dissolved oxygen.

This procedure gave very encouraging results with synthetic standards containing nickel and zinc only, both waves being well separated (Ni — 0·6 v. and Zn — 0·9 v.), but with actual samples the waves showed a number of unsatisfactory features. The first of these took the form of occasional maxima and irregularities in shape of the zinc wave, and was finally traced to the use of gelatin, for a slight increase in concentration of this substance was found to have a very strong suppressive action on the waves without affecting the maxima. However, after a number of tests with other maximum suppressors, this drawback was finally overcome by the addition of 10 ml. of a freshly-made solution containing 0·1 per cent. of sucrose and 30 per cent, of potassium chloride ("regulating solution") to the mixture, instead of the gelatin solution. A point of interest was that neither sucrose nor potassium chloride alone gave entirely satisfactory results, although together they improved the shape of the waves considerably. Sucrose probably suppresses the water wave and any maxima, but the action of the potassium chloride is not clear.

The second, and less important irregularity was a small kink on the upper plateau of the nickel wave. This was not a true nickel maximum, and was unaffected by the usual maximum suppressors. However, further tests showed that the height of the kink varied inversely with the *p*H, and that the wave was completely suppressed at a *p*H of 4·4, obtained by titrating the solution to the change point of bromocresol green with saturated sodium acetate before addition of the thiocyanate. With this technique, the addition of an excess of acetate does little harm and polarographic waves were of almost ideal shape. As shown in the table the relationship between wave height and concentration was linear and the two waves were independent.

COMPARISON OF STANDARDS

Standard	Wave height, mm.	
	Nickel	Zinc
0·40% of Ni; 0·40% of Zn	30	33
0·40% of Ni alone	30	—
0·40% of Zn alone	—	34
0·20% of Ni; 0·20% of Zn	15	17

It was therefore possible, by these means, to determine both elements from one polarogram after a simple direct attack. The final method is given below.

METHOD

Attack 0·5 g. of the metal with 10 ml. of *aqua regia*, and when the attack is complete boil down the mixture nearly to crystallisation to remove excess of acid. Then add 2 ml. of 10 per cent. hydroxylamine hydrochloride solution and allow the beaker to stand for 2 minutes on the edge of the hot plate. Add 1 or 2 drops of bromocresol green indicator solution, and run in saturated sodium acetate solution until the green colour is produced. Then add 3 ml. of 25 per cent. potassium thiocyanate solution and, after cooling the mixture add 10 ml. of a freshly-made solution containing 0·1 per cent. of sucrose and 30 per cent. of potassium chloride and dilute the mixture to 50 ml. Polarograph between — 0·4 v. and — 1·2 v. after removal of dissolved oxygen, The nickel wave will be at about — 0·6 v. and the zinc wave at about — 0·9 v. The wave heights so obtained are compared with those found by carrying out the same procedure on 0·5 g. of aluminium or aluminium alloy, free from nickel and zinc, plus 2 ml. of standard nickel and zinc solution containing 6·75 g. of nickel ammonium sulphate and 1 g. of zinc per litre.

The table below shows some typical results for a range of secondary alloys. As can be seen, none of the common elements interfere and the results appear to be free from systematic error for quantities of nickel or zinc up to about 0·5 per cent.

SIMULTANEOUS POLAROGRAPHIC NICKEL AND ZINC

Sample	Alloy	Nickel %		Zinc %	
		Polarographic	Grav.	Polarographic	Grav.
1	BA35	0·03; 0·03; 0·04	not sought	0·18; 0·19; 0·18	0·19
2	,,	nil	nil	0·23; 0·25; 0·24	0·25
3	,,	nil	nil	0·05; 0·07	0·05
4	,,	0·10; 0·10	0·09	nil	nil
5	5L3	0·04; 0·04; 0·03	not sought	0·12; 0·12; 0·12	0·10
6	,,	0·09; 0·12; 0·12	0·11	0·12; 0·14; 0·13	0·11
7	,,	0·21; 0·18; 0·18	0·21	0·18; 0·18; 0·19	0·21
8	,,	0·37; 0·37	0·36	0·38; 0·36	0·38
9	,,	0·40; 0·42; 0·38	0·42	0·38; 0·37; 0·34	0·37
10	,,	0·13; 0·13; 0·12	0·11	0·24; 0·26; 0·26	0·23
11	DTD424	0·20; 0·19	0·20	0·12; 0·11	0·08
12	,,	0·31; 0·29	0·32	0·18; 0·18	0·18
13	DTD310B	nil	nil	0·43; 0·42	0·46
14	,,	nil	nil	0·05; 0·06	0·04
15	,,	nil	nil	0·10; 0·10	0·09

The results of ten replicate analyses of sample 8 indicated that the standard deviation of a single determination of either nickel or zinc was ± 0·02 per cent. A batch of eight analyses may be taken through the complete process by one operator in two hours. This represents a considerable saving in time over the standard methods for zinc and nickel. The accuracy is ample for most purposes.

The author is indebted to the British Aluminium Company Limited for permission to publish this work which formed part of a thesis accepted by the University of London for the degree of Doctor of Philosophy.

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RESEARCH LABORATORIES of
THE BRITISH ALUMINIUM CO. LTD.
CHALFONT PARK
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Notes

A CASE OF FATAL POISONING BY SODIUM NITROPRUSSIDE

As fatal poisoning by sodium nitroprusside appears to be extremely rare and the writer can find no record of the analytical procedures in the few cases reported, it may be of some value to record a case recently investigated in this laboratory. The victim, a male nurse, had some motive for suicide, and there was sufficient evidence of his behaviour and conversation to justify a verdict at the inquest of "suicide whilst in a depressed condition of mind." His body was found in the open air some 17 hours after he had last been seen alive, and medical opinion at the time of the discovery was that he had been dead at least 10 hours. On the ground nearby there were some "reddish crystals," subsequently identified as sodium nitroprusside.

The following details of the post-mortem findings are quoted from the pathologist's report: "The lower lip has a somewhat bleached and wrinkled appearance. Near the middle of the upper and lower lip there is a small abraded area. For practically the whole of its length down to the cardiac end the mucosa of the oesophagus has a congested and swollen appearance as if it had been acted on by an alkali. Almost all of the surface epithelium has gone. The cardiac end of the stomach shows congestion, mucosal swelling and numerous small submucosal haemorrhages. There is some light brownish fluid in the stomach which smells faintly of almonds. There is nothing abnormal at the pyloric end of the stomach or in the rest of the alimentary tract. There are no lesions of the tongue or within the mouth. Double dentures are present. There is moderate oedema of the glottis. . . . The findings show that death has been caused through the drinking of some form of irritant poison, probably a cyanide."

In spite of the use of the word "drinking" in this report, the finding of the crystals and some of the post-mortem appearances would seem to suggest that solid sodium nitroprusside had been taken. The stomach contents, total volume 78 ml., were brownish in colour, and, when the jar containing them was opened, smelt distinctly but not strongly of hydrogen cyanide. They were faintly acid, and gave a positive reaction for nitroprusside by the sodium sulphide test. The analytical procedures, which were inevitably tentative, were as follows.

(1) A portion was centrifuged, and nitroprusside determined colorimetrically in the clear liquor by means of sodium sulphide. This gave a figure of 0·055 g. of sodium nitroprusside in the whole stomach contents. In this connection two points worthy of note emerged: (*a*) the nitroprusside - sulphide colour is very fugitive, so the determination must be carried out as quickly as possible; (*b*) sodium nitroprusside is very unstable in alkaline solution, so the liquid under examination must be kept slightly acid until the actual

development of the colour. Separation by dialysis would probably have been preferable to centrifuging, but no suitable membrane was immediately available and time was important.

(2) Another portion was made distinctly acid with dilute sulphuric acid and steam-distilled. The hydrogen cyanide in the distillate was, after conversion to thiocyanate, estimated by the colour developed with ferric chloride, and gave a figure of 0·008 g. of free hydrogen cyanide in the whole stomach contents.

(3) The residue from (2) was made alkaline with sodium hydroxide and boiled under reflux for 2 hours to convert any unchanged nitroprusside to ferrocyanide.[1] This was then precipitated as benzidine ferrocyanide,[2] and ignited to ferric oxide, which was weighed. Calculation of the weight back to $Na_2Fe(NO)(CN)_5 \cdot 2H_2O$ gave 0·16 g. of sodium nitroprusside in the whole stomach contents. (This did not prove a very suitable method for the determination of small quantities of ferrocyanide, as the precipitate of benzidine ferrocyanide was almost too bulky to filter on an 11-cm. paper and yet yielded only 0·0020 g. of Fe_2O_3 on ignition). The discrepancy between the nitroprusside figure obtained in this way and that given by direct colorimetric estimation is probably due partly to decomposition of the nitroprusside in the stomach and partly to the inadequacy of the sodium sulphide - nitroprusside colour test for quantitative work.

There was nothing about the stomach contents to suggest the presence of any other irritant poison. As the free hydrogen cyanide found was equivalent to 0·018 g. of sodium nitroprusside, the equivalent total of sodium nitroprusside for the whole stomach contents is approximately 0·17 to 0·18 g.

As cyanides are known to be excreted in the urine in the form of thiocyanates,[3] the contents of the bladder were tested for thiocyanate. A positive reaction was obtained, and colorimetric estimation with ferric chloride indicated 0·0011 g. of sodium thiocyanate in the total urine (72 ml.) The total nitroprusside taken must therefore have exceeded, by an unknown but probably considerable amount, that found in the stomach.

The writer could find only three reports of previous cases (all suicides) of poisoning by sodium nitroprusside[4,5]. (A fourth reference, to an Italian journal, proved inaccessible). Fowweather[4] also states that he could find no record of any previous case. It may be convenient to summarise some of the facts of this case and of the previous three cases as reported:—

	This case	Fowweather[4]	L-B. & N. (1)[5]	L-B. & N. (2)[5]
Time, ingestion to death	< 7 hr.	> 2½ hr.	> 2½ hr.	?
Smell of HCN in stomach	+	+	not definitely identifiable	?
HCN found in stomach	0·008 g.	positive reaction	< 0·02 g.	0·2–0·25 g*
Na nitroprusside found in stomach	0·16 g.	positive reaction	ca. 0·4 g.	26 g.*

* Total, stomach contents and viscera.

In the second case described by Lazarus-Barlow and Norman arsenic was also taken, but these authors believe that death occurred quickly and that the arsenic played no part. In the case described by Fowweather a bottle containing an aqueous solution of sodium nitroprusside was found by the body.

In view of the simplicity of the test with sodium sulphide, it would seem to be worth while to make it as a matter of routine in all cases of cyanide poisoning in which there is any possibility of sodium nitroprusside having been the causative agent.

The writer is indebted to Dr. A. D. Fraser, Honorary Patholgist to the Bristol Royal Infirmary, for permission to quote from his post-mortem report, to Messrs. B. Lilliman, B.Sc. and E. J. Fishlock for their assistance with the experimental work, and to Mr. E. B. Parkes, M.Sc., F.R.I.C., Director of this Laboratory, for permission to publish this note.

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April, 1948

A CHROMATOGRAPHIC METHOD FOR THE IDENTIFICATION AND ESTIMATION OF MINERAL JELLY IN PROPELLANT EXPLOSIVES

Although mineral jelly is an ingredient of many types of propellant powders, both British and foreign, its estimation, except in the simplest compositions, is often difficult. In the more complex compositions it has, in fact, been common practice to derive the mineral jelly content by difference. It is of interest, therefore, to record a rapid and simple method, depending on the wide differences between the chromatographic

adsorption characteristics of mineral jelly and those of other ether-soluble propellant ingredients, which provides a reliable identity test for mineral jelly as well as a reasonably accurate estimation of its amount.

The method is as follows. Extract 5 g. of the ground or sliced propellant with ethyl ether in the usual way, and remove the ether *in vacuo*. Extract the ether-soluble residue with four successive quantities of light petroleum (b.p. 40° to 50° C.), boiling each portion in contact with the residue by immersing the extraction flask in water at 60° C. and then decanting into a second vessel. Reduce the volume of the combined extracts to about 25 ml. and pour on to a chromatographic column. Wash the unadsorbed mineral jelly through the column with light petroleum, using a volume of solvent such that all the other ingredients present remain adsorbed. Recover the mineral jelly in the percolate by evaporation, dry and weigh. Multiply the weight by an appropriate factor (see below) to correct for the proportion of mineral jelly remaining adsorbed.

This determination can be completed within 2 hours once the ether extract of the propellant has been obtained.

A suitable adsorbent is the B.D.H. "Aluminium oxide for chromatographic adsorption for preparatory work." This should be washed with light petroleum and activated by heating for 3 hours at 250° C. With a column of 17 mm. internal diameter containing 10 g. of this adsorbent, the optimum volume of light petroleum required to wash the jelly through the column without eluting any of the other ingredients was found to be 150 ml. (plus the 25 ml. used for transfer).

Experiments with various samples of mineral jelly revealed that uncracked jellies contained 80 to 90 per cent. and cracked jellies (such as have been commonly employed in propellant manufacture) 75 to 85 per cent. of unadsorbed matter. Recoveries for any given sample were reproducible within an over-all range of 3 per cent. The unadsorbed fractions recovered from all samples were exactly alike, and consisted of perfectly colourless, smooth grease of characteristic appearance and consistency. In contrast to the original samples they were only very slightly fluorescent in ultra-violet light, the fluorescent components having been adsorbed by the column. On visual inspection these fractions could be distinguished immediately from fractions obtained in the same way from mineral and rosin oils, mineral and other waxes, bituminous products and similar materials containing unsaponifiable matter. The latter fractions were either clear colourless oils or hard white waxes. It should here be mentioned that the unsaponifiable matter in various edible oils has been subjected to chromatographic treatment by a number of earlier workers,[1,2,3] and more recently the use of aluminium oxide columns for the study of ageing of turbine oils,[4] the separation and determination of hydrocarbons (such as mineral oil) present in fatty materials,[5] and the determination of unsaponifiable matter in oils and fats[6] has been reported.

As has already been mentioned, the total mineral jelly content is obtained from the weight of unadsorbed jelly by the use of a factor. Examination of a large number of propellants of different ages indicated a factor of 1·3 for freshly manufactured propellants rising to 1·4 for those over 5 years old. The following two examples will illustrate the degree of accuracy to be expected.

(i) A sample of Cordite WM of recent manufacture, containing nominally 3·6 per cent. of mineral jelly, yielded 2·73 per cent. of unadsorbed jelly, which becomes 3·55 per cent. on correction.

(ii) Of six aged propellant samples of known mineral jelly content, the largest error occurred with one containing 5·1 per cent. of jelly, for which a corrected figure of 4·8 per cent. was obtained.

The author wishes to thank the Admiralty for permission to publish this note.

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October, 1947

NOTE ON THE B.P. SULPHURIC ACID TEST FOR LIQUID PARAFFIN

Some time ago, several consignments of medicinal Liquid Paraffin examined gave a deep brown colour when tested by the B.P. Sulphuric Acid Test, using 97 per cent. w/w Acid. This strength of acid has been used for several years in this laboratory and most genuine pre-war samples of Liquid Paraffin gave only faint colorations. It was therefore decided to prepare acids of various strengths and to subject different qualities of Liquid Paraffin to the B.P. test, using the carefully assayed acids. The results of these tests indicate that an exactly standardised acid is essential, particularly if a paraffin sample happens to be a border-line case.[1]

In view of the fact that some samples of Liquid Paraffin develop a yellow colour and paraffin-like taste on keeping, it was thought that those Liquid Paraffins that give a deep brown colour with 97 per cent. acid, yet nevertheless just pass the B.P. test with 96 per cent. acid, would probably deteriorate on storing.

Samples of Liquid Paraffin were, therefore stored under various conditions, for example in white and amber bottles, in direct sunlight, away from direct sunlight, and in the dark. All these samples of Liquid Paraffin were tested by the B.P. test, using an exactly standardised 96 per cent. w/w Sulphuric Acid. These experiments showed that:

(*a*) Liquid Paraffin stored in white glass bottles in direct sunlight deteriorates rapidly. Under these conditions it develops a yellow colour or white opalescence and acquires a paraffin-like taste and odour. Amber glass affords complete protection from sunlight.

(*b*) In diffused light, Liquid Paraffin that originally conformed to the B.P. test with 96 per cent. acid but gave a deep colour with 97 per cent. acid, deteriorates within about 12 months.

(*c*) In darkness the deterioration follows the same trend, albeit much more slowly.

In view of the fact that it has been suggested that the deterioration of Liquid Paraffin is due to the production of unsaturated substances, an attempt was made to determine the Iodine Values. The method used was that suggested by Brindle[2] for the determination of Iodine Values of Soft Paraffins.

These iodine values were determined over a period of many months but proved to be too small to make the test sufficiently significant.

SUMMARY

The strength of the sulphuric acid is critical in the B.P. test for Liquid Paraffin.

97 per cent. acid gives a more satisfactory indication of the completeness of the refining of Liquid Paraffin than 96 per cent. acid and serves to show if it can be safely kept under shop conditions for a reasonable length of time.

Thanks are due to Stotherts Limited for permission to publish this note.

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THE LABORATORY
STOTHERTS LTD.
ATHERTON, MANCHESTER

S. DRUCE
May 20th, 1947

NEW REACTION BETWEEN IRON AND DITHIZONE

DITHIZONE has been found to react with either ferrous or ferric iron to produce an intensely yellow compound, which is believed not to have been described hitherto.

Dithizone in carbon tetrachloride, chloroform or toluene solution reacts slowly with iron salts in aqueous solution at *p*H about 9, and in several minutes the organic solvent becomes yellow and free from dithizone. The conversion is slight or nil outside a relatively narrow *p*H range, but takes place to an appreciable extent if the *p*H value is caused to pass rapidly through that range during an interval in the shaking. The reaction is not quantitative with respect to iron, and a considerable excess of iron is necessary to convert the dithizone completely. Cyanide, citrate and phosphate have no effect on the reaction.

The new compound has properties quite unlike those of known dithizonates and is therefore assumed not to be a dithizonate. It is soluble, with some loss of colour but otherwise unchanged, in acids; hot concentrated oxidising acids are necessary to decompose it, and do so only by destruction of the organic radical. Sulphur dioxide and hydrogen peroxide are without effect. Boiling 10 per cent. sodium hydroxide solution, which produces some sodium dithizonate as well as tarry matter from known dithizonates, fails to do so from the yellow iron compound, but the iron is liberated in the ferrous state by this means. The inference, that this is a ferrous compound whether prepared from ferrous or ferric iron, is supported by the fact that a slightly smaller yield is obtained from a given amount of dithizone when ferric iron is used, the difference presumably being accounted for by oxidative side-reactions. The iron content of the compound is about 15 per cent. Further work on its constitution is outside the scope and purpose of these laboratories.

When a mixture of dithizonates with the yellow compound is destroyed by wet oxidation, the resulting solution does not contain enough iron to interfere in subsequent dithizone extractions, but high results may be obtained in Monier-Williams's method for lead if the sulphate precipitation is omitted.[1] The dithizone extract from a material containing much iron may contain enough of the yellow compound to invalidate a direct absorptiometric determination of a small quantity of lead, zinc, bismuth, etc., without intermediate processing. To minimise this interference, the *p*H range 8 to 10 should be avoided after the dithizone has been added. If necessary the following separation may be carried out. From the dithizonate solution (A) remove the yellow compound by two extractions with diluted hydrochloric acid (1 in 3), and wash the combined acid extracts (B) free from dithizone, adding the washings to the solution (A). Make the extract (B) alkaline with ammonia, and extract the yellow compound from it with two portions of carbon tetrachloride. Use solution (A) and then a little fresh dithizone to extract the metals from (B).

It is generally assumed (*e.g.*, Sandell[2]) that the brown solution of impurities obtained from dithizone consists mainly of diphenylthiocarbodiazone, but it has been found that much of the colour is due to this

yellow ferrous compound together with red dithizonates. Sandell[2] also states that ferric iron oxidises dithizone to the diazone, but it is suggested that the chief product is the compound here described.

Thanks are due to Messrs. Kearley & Tonge Ltd., for permission to publish this note, and to their Chief Chemist, Mr. D. J. Patient, for suggestions.

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KEARLEY & TONGE LTD.
SOUTHALL, MIDDLESEX

E. C. DAWSON
May, 1948

Ministry of Food

STATUTORY INSTRUMENT*

1948—No. 2460. The Edible Gelatin Order, 1948. Price 1d.

This Order, which came into operation on November 22nd, 1948, replaces the Edible Gelatin (Control) Order, 1947 (S.R. & O., No. 161 of 1947; see ANALYST, 1947, **72**, 66). *It continues the prohibition of the use of gelatin other than edible gelatin in the manufacture of food, but does not continue the restriction of the use of edible gelatin to particular named foods.*

It defines edible gelatin in the same way as S.R. & O., No. 161 of 1947.

* Italics signify changed wording.

ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

Food and Drugs

Determination of Sugar in Bread. R. M. Sandstedt and J. C. Fleming (*J. Assoc. Off. Agric. Chem.*, 1947, **30**, 550–552)—The tentative method given in "*Methods of Analysis of the A.O.A.C.*," 1945, involves extraction of sugars with boiling 50 per cent. alcohol and subsequent clarification with lead acetate. This procedure is not satisfactory with products containing protein soluble in 50 per cent. alcohol, and the proteins of bakery products are not rendered insoluble by the baking procedure. Since extraction with water and subsequent clarification with acid tungstate solution followed by determination of sugars by the ferricyanide method has proved a satisfactory method for the determination of sugars in flour (*op. cit.*, 20·28, 20·29, 20·30), its suitability for use with bread was investigated, and the method was applied to the determination of known amounts of maltose and sucrose that had been added to bread crumb.

Bread crumb of low sugar content, baked from dough that had been largely depleted of sugar by fermentation, was dried *in vacuo* at 70° C. and was then ground in a burr mill. Samples equivalent to 5·2 g. of fresh crumb (≡ 3·22 g. dry weight) were placed in 100-ml. Erlenmeyer flasks, aliquots of solutions of maltose or sucrose or both were added and the samples were re-dried. This procedure gave materials containing known amounts of added sugar and simulating a series of breads varying widely in content of reducing and non-reducing sugar. The reagents used for the determination of sugars were those specified for the determination of sugars in flour (*op. cit.*).

Fifty ml. of the acid buffer solution were added to the 3·22 g. of prepared dry bread crumb, and the crumb was dispersed by shaking the flask. Two ml. of the sodium tungstate solution were added and the suspension was thoroughly mixed. The mixture was filtered and 5-ml. aliquots of the filtrate were used for the determination of reducing and non-reducing sugars as directed for flour. The results showed the method to be satisfactory, but, since the data were obtained with bread crumb containing no milk, it is recommended that similar experiments should be made with bread containing 6 to 12 per cent. of dry milk solids (lactose being included with maltose and dextrose as reducing sugars), and also with bakery products containing eggs. A. O. JONES

Determination of Dextrose and Laevulose in Cane [Sugar] Products containing Unfermentable Reducing Substances. F. W. Zerban and C. Erb (*J. Assoc. Off. Agric. Chem.*, 1947, **30**, 585–590)—In some methods devised for the determination of dextrose and laevulose in cane sugar products it is tacitly assumed that the two sugars are the only reducing substances present in solution after clarification with lead acetate. Cane sugar products, however, especially molasses, contain varying amounts of reducing substances that differ from dextrose and laevulose in not being fermented by bakers' yeast. An attempt has now been made to correct the dextrose and laevulose found in cane molasses by means of methods at present used for determining unfermentable reducing substances. In a mixture of two pure reducing sugars each can be determined by the combination of any two reduction methods provided that the reducing ratios of the sugars are different enough to afford a trustworthy basis for calculation, and similarly a mixture of three pure reducing sugars can be analysed by combining three reducing methods that fulfil the same condition. Zerban and Sattler (*Ind. Eng. Chem., Anal. Ed.*, 1938, **10**, 669) have shown that in a mixture of dextrose, laevulose, and maltose, each can be determined satisfactorily by combining the method of Jackson and Matthews (*J. Res. Nat*

Bur. Stand., 1932, **8**, 403) for the selective determination of laevulose, the Zerban - Sattler modification of the Steinhoff copper acetate method (*Ind. Eng. Chem., Anal. Ed.*, 1938, **10**, 669) for the determination of monosaccharides in presence of reducing disaccharides, and Steinhoff's method for the determination of total reducing sugars by means of Fehling's solution (*Z. Spiritusind.*, 1933, **56**, 64). When only dextrose and laevulose are present, each can be determined by combining any two of these methods. When maltose is absent the three equations, by combination of which the single sugars are calculated, are (1) $R_1 = 0{\cdot}0806G + F$; (2) $R_2 = aG + F$; and (3) $R_3 = G + bF$. When maltose is present the third equation becomes $R_3 = G + bF + cM$. R_1 (mg.) is the apparent laevulose found by the Jackson and Matthews method; R_2 (mg.) is the content of dextrose and laevulose, expressed as laevulose, found by the Steinhoff copper acetate method; R_3 (mg.) is the total reducing sugar content expressed as dextrose found by the Steinhoff method with Fehling's solution; G, F, and M are mg. of dextrose, laevulose, and maltose, respectively; a is the reducing ratio of dextrose to laevulose, and b and c are the reducing ratios of laevulose and maltose respectively to dextrose. From (1) and (2), $G = (R_2 - R_1)/(a - 0{\cdot}0806)$ and $F = R_2 - aG$; from (2) and (3), $G = (R_3 - bR_1)/(1 - ab)$ and $F = R_2 - aG$; from (1) and (3), $G = (R_3 - bR_1)/(1 - 0{\cdot}0806b)$ and $F = R_1 - 0{\cdot}0806G$. If (1), (2), and (3) are combined, dextrose, laevulose, and any maltose or its equivalent are calculated as described by Zerban and Sattler (*loc. cit.*).

These methods of determination and calculation were applied to a Puerto Rican blackstrap syrup and to a refiner's syrup before and after fermentation with Fleischmann's bakers' yeast. Although the unfermentable portion does not contain dextrose or laevulose, it does reduce not only Fehling's solution but also Jackson and Matthew's reagent as well as the Steinhoff copper acetate reagent.

The total reducing sugars determined directly by the Steinhoff method with Fehling's solution (R_3) and expressed as invert sugar were found to be 19·73 per cent. before fermentation and 5·65 per cent. after fermentation. The sum of the dextrose and laevulose calculated from the results of the combination of any two methods must agree with these figures within narrow limits, because the reducing ratio of laevulose to invert sugar is about 0·96 and that of dextrose to invert sugar is about 1·04. The sum of dextrose and laevulose found by combining R_1 and R_2 was always considerably lower than the invert sugar figures given, and the results are obviously erroneous. The values found for the sum of dextrose and laevulose by combining R_2 and R_3 were near the invert sugar values, but in three out of the total four instances the laevulose figure was negative and the percentage ratio of dextrose to total reducing sugars exceeded 100, which is impossible. This combination, therefore, also gives erroneous results.

The combination of R_1 and R_3 gave values for total reducing sugars agreeing closely with those for the invert sugar found directly, and, in addition, both the dextrose and the laevulose figures were always positive. When R_1, R_2, and R_3 were combined and the "maltose" found, but not actually present, was converted into dextrose, the dextrose and laevulose results agreed closely with those obtained by combining R_1 and R_3 only. This makes it probable that these values are correct. This conclusion has been confirmed by an independent method devised by Browne (Browne and Zerban, "*Physical and Chemical Methods of Sugar Analysis*," 3rd Edition, 1941, p. 991), in which the dextrose and laevulose are calculated from the direct polarisation, invert polarisation, and reducing power. This method applied to the blackstrap syrup gave 8·32 per cent. of dextrose, 11·52 per cent. of laevulose, and 19·84 per cent. of total reducing sugars, in good agreement with the values found by combining R_1 and R_3. The percentage ratio of dextrose to total reducing sugars (41·9) also agreed with that found by combining R_1 and R_3 (41·6). Correction for the unfermentable reducing substance for the same combination did not appreciably alter the percentage ratios found in the original products.

It may be concluded that the actual dextrose and laevulose present in cane products can be found by determining the reducing power with the Jackson and Matthews' reagent and with Fehling's solution, as described, before and after fermentation, calculating the percentages of each sugar, and deducting the values found from those found before fermentation. There are, however, several sources of error in the method. A small error is introduced by the omission of a correction for the reducing power of the sucrose contained in the products. Reducing substances not present in the original substance but formed by fermentation with yeast, introduce another error, the magnitude of which is being investigated.

It is evident that the Steinhoff copper acetate reagent, which is being used successfully for the determination of dextrose in starch conversion products, cannot be used for differentiating between monosaccharides and higher saccharides in mixed corn and cane products when the cane products contain unfermentable reducing substances. The problem of the analysis of such products has not yet been satisfactorily solved. A. O. JONES

Oxidase Activity in Potato Tubers. I. o-Phenylenediamine as a Colorimetric Reagent. J. S. Wallerstein, R. T. Alba, and M. G. Hale (*Biochim. et Biophys. Acta*, 1947, **1**, 175–183)—The ability of certain plant extracts to react with phenolic substances with the aid of molecular oxygen and eventually form the dark pigment melanin has been variously attributed to the action of a single enzyme (tyrosinase) or of multiple enzymes (monophenolase, polyphenolase). With potato, the reaction is apparently due to the action of a single enzyme, *viz.*, tyrosinase, upon free tyrosine. The first step in the enzymic oxidation is presumably the introduction of a second hydroxyl group in the *ortho* position to the one already present in the monophenol. When the enzyme and substrate are brought into contact

by rupture of the tissues of the potato, oxidation of tyrosine is initiated and a grey discoloration occurs on the cut surface. In the blanching of potatoes before dehydration the tyrosinase is inactivated by treatment with boiling water or with steam or sulphite. Excessive blanching impairs the quality of the dehydrated product, so that precise control of the blanching procedure is necessary.

To test for completeness of blanching, various methods have been used for determining residual enzymic activity, involving the application of indicators to the cut surface, *e.g.*, guaiacol. benzidene, or gum guaiac for peroxidase, and catechol and *p*-cresol for oxidase activity. The spontaneous colour formation of macerated potato tissue has also been measured photometrically under standard conditions.

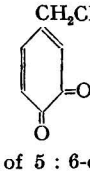
In the present work the capacity for colour formation of a potato sample is determined by trapping one of the intermediate products of the melanin reaction and forming with it a stable complex determinable colorimetrically. When raw potato tissue is mechanically dispersed in a solution of *o*-phenylenediamine in presence of oxygen, there is formed a highly coloured compound that is water-soluble and, unlike the colour formed spontaneously, can be extracted with organic solvents. Such a reaction is to be expected as a result of the condensation of the *o*-quinones formed in the course of the melanin reaction with *o*-phenylenediamine at the favourably low *p*H (6·0) of the juice. No coloration forms with completely blanched potatoes and the depth of colour with any given sample is an inverse function of the extent of blanching. In presence of sufficient *o*-phenylenediamine the greying or blackening that forms with untreated juice does not occur, but one of the intermediate products is fixed by the *o*-phenylenediamine to form an orange-red compound, which is provisionally termed "tyrophenazine" on the assumption that it owes its origin to the phenazine type of condensation. Stabilisation of this colour can be effected by extraction with ethyl acetate or preferably by addition of acetone, which precipitates colloids and assists filtration. The colour of the acetone solution is then measured photo-electrically.

Procedure—Peel the potato and cut it under water into approximately 1-cm. cubes. When a number of determinations are to be made with a given batch, wash the cubes in running water for an hour to remove all exposed matter from the cut cells. Mix 50 g. intimately with 10 ml. of 1 per cent. *o*-phenylenediamine hydrochloride solution and 240 ml. of water in a Waring blendor. Blend for 2 min. and strain the liquid through a cotton-wool plug into a 500-ml. flask, keeping the temperature at 25° to 30° C. After 10 min. from the beginning of blending, pipette 10 ml. of the strained liquid into 40 ml. of acetone, which stops the enzyme reaction. Filter the liquid through Whatman No. 5 paper into a colorimeter tube, and measure the colour in a Klett Summerson photo-electric colorimeter fitted with a blue filter (No. 42) against 80 per cent. aqueous acetone as control liquid. The colour of the acetone extract is stable for at least 4 hr. and virtually stable even after 24 hr. The method is reproducible within a variation of about 5 per cent. from the mean.

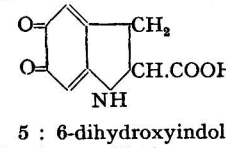
When a series of maceration extracts are pipetted into acetone 3, 5, 10, 15, 20, 25, and 30 min. after addition of the *o*-phenylenediamine and the colour measurement is plotted against the time of reaction, it is seen that, although the depth of colour varies with different samples, the curves follow parallel courses. Similar experiments in which the time of blanching is varied, the time of reaction with *o*-phenylenediamine being maintained at 15 min., show that, despite a variation of about 250 per cent. in the colour formation with different samples, the production of tyrophenazine is completely inhibited by blanching for 60 sec. and that the depth of colour is inversely related to the time of blanching. Maximum colour formation occurs at about 40° C., but the values obtained at 25° to 30° C., the range suggested for the test, are not much lower. Maximum colour formation occurs also at about *p*H 5·0, *i.e.*, near the *p*H of the blended mixture, which is more acid than the potato juice (*p*H 5·9).

Purified potato phenolase, prepared by the method of Kubowitz (*Biochem. Z.*, 1937, **292**, 221), showing an activity of 66 catecholase units per ml. when tested by Dawson's method (Miller and Dawson, *J. Amer. Chem. Soc.*, 1941, **63**, 3375; 1944, **66**, 514), failed to cause an appreciable colour when added to an *o*-phenylenediamine solution. Addition of a maceration of fully blanched potato to the system resulted in colour formation, indicating that the formation of tyrophenazine is not due to direct oxidation of the *o*-phenylenediamine.

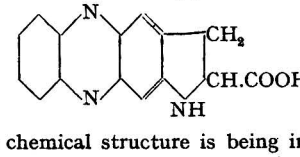
The reaction leading from tyrosine to melanin has been pictured by Raper (*Ergebn. Enzymforsch.*, 1932, **1**, 270) as a conversion of tyrosine through the quinone of 3 : 4-dihydroxyphenylalanine



to the quinone of 5 : 6-dihydroxydihydroindole-2-carboxylic acid



This yields 5 : 6-dihydroxyindole, which by successive reduction, oxidation, and condensation yields melanin. Of the intermediate products the two *o*-quinones formulated appear to be the most likely to react with *o*-phenylenediamine to yield a phenazine of the type



Its exact chemical structure is being investigated.

A. O. Jones

Oxidase Activity in Potato Tubers. II. o-Phenylenediamine as Fluorimetric Reagent. J. A. Wallerstein, R. T. Alba, and M. G. Hale (*Biochim. et Biophys. Acta*, 1947, **1**, 184–189)—Solutions of "tyrophenazine" (see preceding abstract) in 80 per cent. acetone show a marked green fluorescence when exposed to filtered radiation from a mercury high-pressure lamp (360 mμ.). When an aqueous solution is extracted with *n*-butyl alcohol the extract maintains the intensity of its fluorescence for at least half an hour. Addition of 1 part of ethyl acetate to 2 parts of *n*-butanol does not affect the stability of the fluorescence and improves the ease of separation from the aqueous phase, but larger proportions of ethyl acetate cause progressive fading. Under these conditions the degree of fluorescence is closely related to the colour formation described in the preceding abstract, and the depth of colour is practically identical whether measured in 80 per cent. acetone or in the butanol and ethyl acetate extract.

Procedure—Macerate 50 g. of potato with *o*-phenylenediamine as described in the preceding abstract, strain the blended mixture through cotton-wool, and allow it to stand for a total time of 15 min. at room temperature (25° to 30° C.) from the beginning of blending. Pipette 5 ml. of the solution into a separating funnel containing 40 ml. of water and 50 ml. of a mixture of 2 parts of *n*-butanol and 1 part of ethyl acetate. Shake the mixture vigorously for 30 sec., allow it to separate, remove the aqueous layer, and filter the yellow organic solvent layer through Whatman No. 5 paper. Mix 1 part of the filtrate with 24 parts of the extracting solvent before measuring the fluorescence. This concentration corresponds to about 1 to 1·5 μg. per ml. of *o*-phenylenediamine. Measure the fluorescence of the diluted solution against that of a solution of fluorescein (1 in 4×10^6) in 0·01 *N* sodium hydroxide with a Pfaltz and Bauer fluoro-photometer at 440 mμ., with the galvanometer adjusted to a scale reading of 100 for the standard fluorescein solution.

In blanching experiments 1-cm. cubes of potato were dropped into a colander in sufficient boiling water to maintain boiling temperature despite the addition of the sample, and the material was withdrawn after a measured number of seconds. For steam blanching the cubes were placed on a cheese-cloth holder in a desiccator through which steam circulated freely from below the cloth. Determinations of *p*H were made with a Beckmann *p*H meter and colorimetric determinations with a Klett Summerson photo-electric colorimeter with blue filter No. 42. The results are reproducible within about 6 per cent. of the mean.

The curve of fluorescence plotted against time of reaction runs practically parallel to the curve of colour formation and tends to become asymptotic to the time axis after 15 min. A curve showing the relation between fluorescence and blanching time is again parallel to the corresponding colour formation curve, colour formation and fluorescence being inversely related to the blanching time and both being abolished by a 60-sec. blanching period. With steam blanching a 2·5 to 3-min. period is required to complete enzymic inactivation. By adding varying amounts of dilute phosphoric acid and sodium hydroxide to the mixture of potato and *o*-phenylenediamine at the beginning of blending the effect of *p*H was studied, the *p*H of the filtrate being determined. Both colour formation and fluorescence are progressively diminished by addition of acid or alkali, and inactivation is complete at *p*H 3·7 and at *p*H 7·9. The degree of fluorescence is a function of the temperature, reaches its maximum at about 40° C., and diminishes progressively as the temperature is raised or lowered beyond this point. The temperature at which the experiments were conducted (25° to 30° C.) differs but slightly from this optimum temperature. The close parallelism between fluorescence and colour formation suggests that a single coloured compound or type of compound is involved, which becomes fluorescent under the appropriate conditions. The high sensitivity of the fluorometric method when applied to investigation of the oxidase activity of potatoes makes it of special value when only traces of such activity are likely to be present, as, for example, in blanched or dehydrated materials.

A. O. JONES

Colorimetric Determination of Homatropine Methylbromide. F. J. Bandelin (*J. Amer. Pharm. Assoc., Sci. Ed.*, 1948, **37**, 10–12)—The method described is based on the quantitative precipitation of the insoluble reineckate by ammonium reineckate. The coloured precipitate is separated, dissolved in acetone, and determined spectrophotometrically.

Procedure—Transfer 1, 2, 3, 4, and 5 ml., respectively, of a standard solution of homatropine methylbromide containing 2 mg. of homatropine methylbromide per ml., into each of 5 test tubes and adjust the volume in each tube to approximately 10 ml. with water. To each tube add 1 ml. of 20 per cent. sulphuric acid, and also 2 ml. of ammonium reineckate solution prepared by stirring mechanically 2 g. of ammonium reineckate with 100 ml. of water for 10 min., filtering through a hardened filter paper, storing in a refrigerator, and using within 48 hr. after its preparation. Set the tubes aside for 1 hr., filter through small sintered-glass crucibles of medium porosity, and wash each residue twice with 2 ml. of water. Apply suction to the filters until all excess of moisture has been removed. With the aid of gentle suction, pass four successive 2-ml. quantities of acetone through each of the crucibles, collect the resulting coloured solutions, and dilute each to 10 ml. with acetone. Determine in a spectrophotometer the percentage transmittance of the acetone solutions at a wavelength of 525 mμ., and plot the values obtained on semilogarithmic paper.

Procedure for determination in tablets—Grind about 20 tablets to a fine powder, weigh an aliquot equivalent to 5 mg. of homatropine methylbromide and extract with 5 ml. of water by allowing to stand in a test tube with occasional shaking for 30 min. Filter, wash with five successive quantities of 1 ml. of water and add 1 ml. of 20 per cent. sulphuric acid and 2 ml. of ammonium reineckate solution to

the combined filtrates. Allow to stand for 1 hr., filter, wash and dry the residue, dissolve the residue in acetone, and determine the percentage transmittance of the coloured acetone solution as previously described.

Comparison of results obtained by this method with those obtained by the Kjeldahl method indicate fair agreement of the mean values found; there is less deviation from the mean with the proposed method, which was accurate to within 5 per cent. A. H. A. ABBOTT

Determination of the Acetyl Value of Fats and Oils. K. Helrich and W. Rieman, 3rd. (*Anal. Chem.*, 1947, **19**, 691)—The method is a modification of that of Roberts and Schuette (*Ind. Eng. Chem., Anal. Ed.*, 1932, **4**, 257).

Procedure—Prepare reaction tubes by drawing out a 30-cm. length of 12-mm. glass tubing until the middle is constricted to about 6 mm., and then cutting the tubing into two halves and sealing the large ends.

Weigh an empty tube, add 1 to 1·2 g. of acetic anhydride from a pipette, and re-weigh. Similarly, add 2·8 to 3·2 g. of the sample and weigh again. Seal the tube, shake it, and then heat it for 1 hr. in a vertical position in an oven at 180° ± 5° C. Cool, break the tube, and pour the contents into a 500-ml. Erlenmeyer flask containing 50 ml. of water. Rinse the tube first with hot water and then repeatedly with cold water until the volume in the flask is about 200 ml. Bring the mixture just to the boiling-point beneath a reflux condenser, cool for 5 min., and then rinse the condenser. Cool, and titrate with 0·5 *N* sodium hydroxide to phenolphthalein indicator.

Make a blank experiment to standardise the acetic anhydride. The procedure is as described except that the sample is omitted and the tubes are not heated, because decomposition occurs at 180° C. if the anhydride is heated alone, whereas it apparently does not occur if fat or oil is present. If the acetic anhydride is stored in a dark, glass-stoppered bottle, standardisation is necessary only once a month. If the sample contains free fatty acid, a correction must be applied. To determine this, add 3·0 ± 0·5 g. of the sample to 200 ml. of water in a 500-ml. flask and titrate as already described. A. O. JONES

Determination of Diethylbromoacetylurea in Admixture with Diethylbarbituric Acid and Potassium Bromide. J. A. C. van Pinxteren and M. A. G. Smeets (*Pharm. Weekblad*, 1948, **83**, 322–326)—In this mixture, separation of the two organic components by means of alkali was unsatisfactory, some diethylbromoacetylurea being decomposed by the alkali. A buffer solution can, however, be used.

Procedure—Dissolve 0·25 g. of the mixture in 20 ml. of buffer solution of *p*H 11 (2·7 ml. of 0·1 *M* borax + 97·3 ml. of 0·1 *N* sodium hydroxide). Shake out the solution successively with 40, 20, and 10 ml. of ether, dry the ether solution with sodium sulphate, evaporate to dryness, and dry the residue at 50° C. to constant weight. The method can be used for bromural, which is also decomposed by alkali. G. MIDDLETON

Biochemical

Colour Reactions of Native and Denatured Proteins. F. Haurowitz and S. Tekman (*Biochim. et Biophys. Acta*, 1947, **1**, 484–486)—One of the few chemical reactions by which native and denatured proteins can be distinguished is the nitroprusside test. Denatured ovalbumin gives an intense pink colour with nitroprusside, whereas no colour is formed in solutions of native ovalbumin (A. Heffter, *Chem. Ztg.*, 1907, **11**, 822; Arnold, *Z. physiol. Chem.*, 1911, **70**, 300; ANALYST, 1911, **36**, 235; Anson, *Adv. Protein Chem.*, 1945, **2**, 361). Two explanations of this are possible, *viz.*, either the SH-group found in the denatured protein is present also in the native protein but is inaccessible to the reagent until changes in the peptide chains have occurred during denaturing, or these SH-groups are formed by chemical changes, such as the cleavage of dithio-, or thioester-, or of other sulphur-containing groups. On the first assumption the constitution of the protein molecule in the native and denatured state is the same but for the changes in the peptide chains, and it would be expected that the intensity of other colour reactions also would increase during denaturation. The proteins examined were ovalbumin from hens' eggs, beef serum albumin, and beef serum globulin (both prepared by fractional precipitation with ammonium sulphate) and lactoglobulin from cows' milk. Denaturation was effected by mixing 2 ml. (24 mg.) of the aqueous protein solution with 3 ml. of a buffer solution at *p*H 9 and placing the mixture in boiling water for 10 to 30 min. With the biuret test, the Sakaguchi test for arginine (Thomas *et al.*, *J. Biol. Chem.*, 1939, **129**, 263), and the Voisinet test for tryptophan (Fürth and Lieben, *Biochem. Z.*, 1920, **124**, 109), no differences were observed between the colours given by native and by denatured proteins.

For the diazo reaction 10 ml. (24 mg.) of the protein solutions were mixed with 3·6 ml. of the soda solution and 1·5 ml. of the diazo solution of Koessler and Hanke (*J. Biol. Chem.*, 1919, **39**, 497). The red colour was measured with the Pulfrich step photometer with colour filter S53. After denaturing, the protein solutions gave more intense reactions than the corresponding native protein solutions. The increase was greater with ovalbumin than with the other proteins, and 1·5 ml. of an 8·4 per cent. solution of this protein was therefore mixed with 4·5 ml. of a 60 per cent. solution of urea, and the mixture was maintained at room temperature for 24 hr., after which the urea was removed by dialysis. The solution of the protein thus denatured (12 mg. in 5 ml.) was mixed with 1·8 ml. of the soda solution and 0·75 ml. of the diazo solution (*supra*) and the colour was measured as already described. An increase of the extinction coefficient with denaturing was again found.

For the phenol reaction of Folin-Wu-Ciocalteu (Heidelberger and MacPherson, *Science*, 1943, **97**,

405) solutions containing 10 mg. of the protein in 25 ml. of 0·0008 *N* sodium hydroxide were used. Two-ml. portions of these solutions were mixed with 6 ml. of a saturated solution of sodium carbonate and 1 ml. of 1 per cent. copper sulphate solution. One set of test tubes was cooled immediately in an ice-bath while a duplicate set was immersed for 5 min. in boiling water. To both solutions 1 ml. of the diluted (1 : 3) phenol reagent (*loc. cit.*) was added and after 30 min. the blue colour was measured in the step photometer with colour filter S72 and 10-mm. vessels. With each protein, an increase of the extinction coefficient occurred after denaturing. Similar results with trypsinogen and ovalbumin have been reported previously by Herriot (*J. Gen. Physiol.*, 1935, **19**, 283; 1938, **21**, 501).

Increase of colour formation with these reagents after denaturing cannot be ascribed to partial hydrolysis of the protein because the same increase occurs when the denaturing is effected by urea at room temperature. Apparently in the native protein not only the SH-groups but also phenolic OH-groups and probably other groups are inaccessible to the chemical reagents, owing to steric hindrance exerted by the tightly bound peptide chains. After denaturing, these groups become accessible by unfolding of the peptide chains.

The failure of the biuret test, the arginine test, and the tryptophan test to distinguish between native and denatured proteins is due to the denaturing of the proteins by the alkali present in the first two tests and the strong acid present in the last. A. O. JONES

Microbiological Determination of Threonine in Proteins and Foods. M. J. Horn, D. B. Jones, and A. E. Blum (*J. Biol. Chem.*, 1947, **169**, 739–743)—Various methods described for the determination of threonine with *Lactobacillus arabinosus*, *Leuconostoc mesenteroides*, and *Streptococcus faecalis* have been tested. A satisfactory blank could not be obtained with *L. arabinosus*, and *L. meserentoides* gave a sigmoidal standard curve in spite of modifications of the method. *S. faecalis* gave a satisfactory curve using the following method.

Method—The medium of Horn *et al.* (*Ibid.*, 1946, **166**, 321; ANALYST, Abst., 1947, **72**, 211) was used with the pyridoxine replaced by 400 μg. of pyridoxamine per litre. The procedures followed for the culture, inoculum, and preparation of samples were identical with those described for the assay of lysine by Horn *et al.* (*Ibid.*, 1947, **169**, 71; ANALYST, Abst., 1948, **73**, 286). As only one-half of threonine racemate is active, solutions ranging in concentration from 5 to 100 μg. per ml. of the optically active isomer were prepared by weighing the DL-compound. Titration values were not altered by the addition to the medium of 1·2 mg. of any of 19 amino acids.

Recovery of threonine added in different proportions to hydrolysates of casein, ox muscle, soya bean flour, and whole meal yellow corn gave results well within the experimental error of the assay.

Tables comparing the threonine contents of thirty-one proteins and foods, as determined by this method, with the values obtained by other workers using microbiological and chemical methods show that the results agree well. J. S. HARRISON

Microbiological Determination of Valine in Proteins and Foods. M. J. Horn, D. B. Jones, and A. E. Blum (*J. Biol. Chem.*, 1947, **170**, 719–723)—Satisfactory standard curves were developed with both *Lactobacillus arabinosus* 17–5 and *Streptococcus faecalis* 9790 (American Type Culture Collection), but the latter was used for the assay.

Method—The basal medium was that previously described (*Ibid.*, 1946, **166**, 321; ANALYST, Abst., 1947, **72**, 211) with the exception that the pyridoxine was replaced by 400 μg. of pyridoxamine. The procedure for cultures, inoculum, and preparation of samples has been previously described (*Ibid.*, 1946, **166**, 321; 1947, **169**, 739). The valine standard solutions used for the standard curve were made from the synthetic DL-racemate using twice the quantity that would be required with the L-isomer, as the racemate has exactly one-half the activity of the L-isomer.

The titration values on the standard curve are not altered by addition to the medium of 1·2 mg. of any of the other nineteen amino acids. Recovery of valine added to hydrolysates of natural materials gave results well within the experimental error for the assay. The valine contents of thirty-one proteins and foods determined by this method are given, and the results agree closely with those obtained on the same materials by other microbiological methods. J. S. HARRISON

Comparison of a Modified Folin Photometric Procedure and the Ninhydrin Manometric Method for the Determination of Amino Acid Nitrogen in Plasma. F. P. Chinard and D. D. Van Slyke (*J. Biol. Chem.*, 1947, **169**, 571–581)—The photometric and ninhydrin manometric methods of analysis for the amino acid nitrogen content of plasma are compared.

Method—Photometric—The procedure was that described by Frame *et al.* (*Ibid.*, 1943, **149**, 255; ANALYST, Abst., 1943, **68**, 378) with the modifications of Russell (*Ibid.*, 1944, **156**, 467; ANALYST, Abst., 1945, **70**, 221). The 1 : 2-naphthoquinone-4-sodium sulphonate was purified by Folin's method (*Ibid.*, 1922, **51**, 377). A standard curve for glutamic acid was used for the individual amino acids and for plasma determinations, instead of mixed glycine and glutamic acid, at three levels, usually 4, 8, and 12 μg. of glutamic acid nitrogen per 15 ml. of final coloured solution. Measurements were made with the Coleman junior spectrophotometer with the wavelength scale set at 475 mμ., and a few determinations on the Beckman quartz spectrophotometer to check the position of the absorption maxima observed in the Coleman instrument with individual amino acids. Tungstic acid filtrates were used.

Ninhydrin manometric analysis—The procedure described by Hamilton and Van Slyke (*Ibid.*, 1943, **150**, 231) as modified by the same authors (*Ibid.*, 1946, **164**, 249; ANALYST, Abst., 1946, **71**, 588)

was used. Results were corrected for urea nitrogen for plasmas with normal urea nitrogen. With abnormally high urea nitrogens the carboxyl tubes were incubated for 3 hr. at 60° C. with 200 mg. of ninhydrin. The photometric determinations with the Beckman instrument were more precise than those with the Coleman, but the reproducibility of the standard curve with the latter was found to be adequate in the range used. Mean optical densities in cylindrical cuvettes of 16-mm. bore for 4·0, 8·0, and 12·0 μg. of glutamic acid α-amino nitrogen were 0·110 ± 0·006, 0·212 ± 0·004, and 0·317 ± 0·006, respectively.

Analyses were carried out on solutions of twenty-five amino acids. Purity was checked by the manometric ninhydrin method. The colours were developed, as described by Russell, from duplicate portions of each amino acid solution diluted to 5 ml., and the final coloured solutions were brought to a volume of 15 ml. Readings were made from 10 to 20 min. after the final dilutions. Calculations were made from standard glutamic acid curves. All densities were measured at $\lambda = 475$ mμ. Duplicate readings on solutions with the same concentrations usually agreed within 2 or 3 per cent., but different concentrations differed by more than 5 per cent. in the amount of theoretical amino nitrogen indicated. The order of the normal rate of fading was 1 to 2 per cent. 10 min. after dilution, but proline and hydroxyproline gave gross deviations from the theoretical values; the colours developed by these two amino acids faded much more rapidly than the others, the values being as low as 59 per cent. 10 min. after the final dilution and 25 per cent. after 30 min. Not all the amino acids obeyed Beer's law at the wavelength of 475 mμ. The compounds formed by naphthoquinone with the different amino acids had different peaks.

Results—Comparison of results obtained in plasma analyses with the ninhydrin manometric and the photometric procedures showed that approximately 20 per cent. of the photometric values were within ± 5 per cent. of the ninhydrin values, but 54 per cent. deviated by more than ± 15 per cent., whilst some deviations exceeded 400 per cent. Maximum deviations were −37 and + 491 per cent. The gross plus deviations of the photometric results were observed in analyses of uremic plasmas, which gave high results by the nitrous acid method. The high photometric results in these cases may be due to the presence of amino nitrogen other than α-amino acid nitrogen.

The average recovery of added amino acid nitrogen by the ninhydrin procedure was 97 to 99 per cent. in terms of ninhydrin nitrogen and 100 to 116 per cent. for the photometric procedure in terms of photometric nitrogen. There were differences of 19 to 48 per cent. between the ninhydrin and photometric nitrogen determined on the same filtrates.

Unsuccessful attempts, including the use of reducing agents other than thiosulphate, and the use of different quinone reagents, were made to improve the photometric procedure.

J. S. HARRISON

Micro-Determination of Non-Protein Nitrogen in Serum, Plasma, or Blood. F. Rappaport and F. Eichhorn (*Lancet*, 1947, **253**, 171–172)—The method estimates urea, ammonia, and amino compounds, but not indole compounds, with nitrogen in the ring. Results agree well with the Kjeldahl method. The de-proteinised sample is treated with alkaline hypobromite - borate solution and the excess of hypobromite is determined iodimetrically.

REAGENTS—*Deproteinisation fluid*—Dissolve 44·8 ml. of 10 per cent. sodium tungstate solution, 2 g. of sodium citrate, and 6·4 g. of sodium sulphate in about 800 ml. of distilled water. Add 44·8 ml. of *N* sulphuric acid and 2 g. of cadmium sulphate. Dilute to 1 litre. "*Hypobromite solution*"—(*a*) Dissolve 84·5 g. of boric acid and 15·6 g. of sodium hydroxide in about 500 ml. of water, boil for 30 min., cool, and dilute to 1 litre. (*b*) Saturated sodium fluoride solution. (*c*) 27 per cent. sodium hydroxide solution. Mix 5 parts of (*a*), 3 parts of (*b*), and 1 of (*c*). This mixture keeps well. *Bromine solution*—(*a*) Dissolve 20 g. of potassium bromide and 8 g. of bromine in water and dilute to 1 litre, or (*b*) dissolve 32 g. of potassium bromide and 2·8 g. of potassium bromate in water, add 100 ml. of *N* sulphuric acid, and after 30 min. dilute to 1 litre. Immediately before use, add 1 part of the bromine solution to 9 parts of the "hypobromite solution."

PROCEDURE—To 5 ml. of de-proteinisation fluid add 0·1 ml. of serum, plasma, or blood, using a capillary pipette. Wash the pipette by repeatedly sucking up and expelling the fluid. Centrifuge or allow to stand for 5 min., and filter. To 4 ml. of the clear liquid add 5 ml. of the hypobromite reagent mixture. Mix, allow to stand 1 or 2 min., add a few crystals of potassium iodide and 2 or 3 ml. of 18 per cent. hydrochloric acid. Titrate with 0·005 *N* sodium thiosulphate adding a few drops of 0·25 per cent. starch solution at the end-point. Carry out a blank on 4 ml. of de-proteinisation fluid and 5 ml. of hypobromite reagent mixture. From the difference between blank and experiment calculate the non-protein nitrogen in the sample. One nitrogen atom requires 3 bromine atoms. Interference by glucose is prevented by the boric acid in the reagent, and the cadmium sulphate prevents interference by sulphur compounds of the mercaptan type.

G. R. PRIMAVESI

Factors Affecting Folic Acid Determination. E. P. Daniel and O. L. Kline (*J. Biol. Chem.*, 1947, **170**, 739–746)—The stability of folic acid under the conditions of extraction, sterilisation, and storage involved in microbiological assay procedures has been studied. The determinations were made by a microbiological method using *Lactobacillus casei* as the test organism. Crystalline folic acid, tomato juice serum, and dehydrated liver extract powder were used as the sources of folic acid.

No loss of folic acid was observed during the sterilisation process or during 3 months storage of stock solutions of reference standards at 40° C. Destruction during autoclaving for 30 min. at

*p*H 3·0 was marked and became progressively greater with lowered *p*H.

To extract folic acid from test materials, a preliminary hydrolysis, followed by enzyme treatment, was frequently necessary, but data as to the best method of quantitative extraction are lacking.

Methods of removing folic acid from natural sources such as liver extract and tomato juice were examined with a view to preparing supplements suitable for addition to the basal medium. The use of heat in acid solution, irradiation, or treatment with sulphite was unsatisfactory for the purpose. Of a number of adsorbing agents studied, activated charcoal was the most efficient for tomato juice at a *p*H below 3·0. For aqueous solutions of folic acid, visual comparison of intensity of fluorescence after adsorption treatment was used to evaluate the adsorbing agents studied. The use of fluorescence may prove of value in the determination of folic acid. J. S. HARRISON

Tryptophan Content of Normal Human Urine. C. P. Berg and W. G. Rohse (*J. Biol. Chem.*, 1947, **170**, 725–729)—Assays with *Lactobacillus arabinosus* have raised doubt as to the accuracy of the Albanese and Frankston colorimetric method (*Ibid.*, 1945, **157**, 59) for the determination of tryptophan. Samples of urine were prepared by the preliminary steps suggested by Albanese and Frankston, involving percolation through activated Permutit, extraction of the acidified filtrate with ether, precipitation with mercuric sulphate, and dissolution of the precipitate in trichloroacetic - hydrochloric acid reagent. The Albanese - Frankston and the Shaw - McFarlane methods (*Canad. J. Res., Sect. B*, 1938, **16**, 361 and *J. Biol. Chem.*, 1940, **132**, 387) were applied to the test solutions.

Recovery of tryptophan added to the solution was almost complete as measured by the Shaw - McFarlane technique, whereas by the Albanese - Frankston method much of the added tryptophan was unaccounted for. On the other hand, the latter method indicated the presence of much more tryptophan in the original urine. The discrepancies obtained by the Albanese - Frankston method are attributed chiefly to errors inherent in the colorimetric technique.

Judged by variations in colour development with concentration and by the capacity to measure accurately additions of tryptophan to urine, the Shaw - McFarlane method is the more trustworthy and accurate of the two methods. In 24-hr. urine specimens, estimated by the Albanese - Frankston procedure to contain 137 to 240 mg. of tryptophan, only 20 to 42 mg. were measured by the Shaw - McFarlane procedure. The latter values are not markedly greater than the results of microbiological assays reported in the literature.

J. S. HARRISON

Comparison of Three Thiochrome Methods for Urinary Thiamine [Aneurine] by a Simplified Base-Exchange Procedure. E. Papageorge and M. V. Lamar (*Arch. Biochem.*, 1947 **14**, 315–324)—The presence of interfering materials in urine invalidates assays by direct oxidation to thiochrome and necessitates a preliminary base-exchange operation for the isolation of aneurine, and modifications of the oxidation and final extraction steps to remove or correct for non-thiochrome material that affects the oxidation step. In the method described, adsorption, elution, oxidation, and extraction are all carried out successively in a glass-stoppered tube with a conical base. This simplified technique was applied to three modifications of the Hennessy method suggested by Najjar and Ketron (*J. Biol. Chem.*, 1944, **152**, 579; ANALYST, Abst., 1944, **69**, 352), Urban and Goldman (*Ibid.*, 1944, **152**, 329; ANALYST, Abst., 1944, **69**, 280), and Mickelsen *et al.* (*Ibid.*, 1945, **160**, 361).

METHOD—*Reagent—Activated Permutit*—Add 700 ml. of a 25 per cent. potassium chloride - 5 per cent. acetic acid solution to 100 g. of Permutit, boil the mixture for 1 hr. with mechanical stirring, discard the supernatant liquid, and repeat the potassium chloride - acetic acid treatment. Wash the Permutit with boiling water until it is chloride-free, dry at 100° C., and store dry. The activation process should be carried out once a month.

Preservation of urine—Urine will retain its aneurine content for at least a month if 2 ml. of glacial acetic acid are added to 100 ml. of urine before storing in the refrigerator.

Base-exchange—Place 300 mg. of activated Permutit into each of three conical centrifuge tubes with ground-glass stoppers and of at least 30-ml. capacity. Add 2 to 5 ml. of urine to each tube according to the aneurine content; the amount of aneurine in each aliquot should not exceed 1·2 μg. Dilute to 10 ml., stopper, and shake for 3 min., remove the stopper, and wash the Permutit into the tube. If necessary, add 1 or 2 drops of caprylic alcohol to prevent foaming, allow to settle, decant and discard the supernatant liquid, add 10 ml. of water, and repeat the operation. Add to each tube 5 ml. of a 25 per cent. solution of potassium chloride in 0·1 *N* hydrochloric acid, stopper, and shake for 3 min. Oxidation to thiochrome and extraction with *iso*butanol are carried out in the presence of Permutit according to any of the following three procedures.

Oxidation and extraction—1. *Modification of the Najjar - Ketron procedure*—Add 15 ml. of *iso*butanol to all three tubes, then 3 ml. of 15 per cent. sodium hydroxide solution to the blank, and 3 ml. of alkaline potassium ferricyanide reagent (made by mixing 1 ml. of 2 per cent. potassium ferricyanide solution with 29 ml. of 15 per cent. sodium hydroxide solution) to each of the other two tubes, stopper, shake for exactly 1·5 min., allow to stand for 10 min., transfer the liquid to separating funnels without removing any Permutit, and remove and discard the aqueous layer. Add about 2 g. of anhydrous sodium sulphate that has been freed from fluorescent material by heating at 800° to 1000° F. in a muffle furnace for several hours, stopper, and shake until clear. Decant the liquid into small brown bottles without transferring any of the sodium sulphate, stopper, and store in the

refrigerator until the fluorescence is determined. The thiochrome solution will keep for at least 24 hr. in this way.

2. *Modification of the Urban - Goldman procedure*—(Benzenesulphonyl chloride blank.) Treat the two tubes for oxidation to thiochrome as above. To the blank add 3 ml. of 15 per cent. sodium hydroxide solution and 1 drop of benzenesulphonyl chloride, shake for 3 min., add 0·1 ml. of a 2 per cent. ferricyanide solution, allow to stand for 1 min., add 15 ml. of *iso*butanol, and shake for 1·5 min. Proceed as above for the separation of layers, clearing of the *iso*butanol solution, etc.

3. *Modification of the Mickelsen, Condiff, and Keys procedure*—(Adjustment of *p*H before extraction of *iso*butanol.) Add 3 ml. of 15 per cent. sodium hydroxide solution to the blank and 3 ml. of alkaline ferricyanide solution to each of the other tubes, stopper, and shake for exactly 1·5 min., adjust to *p*H 8·0 to 10·0 by addition of 0·45 to 0·55 ml. of a mixture of equal volumes of concentrated hydrochloric acid and 85 per cent. phosphoric acid. The end-point is indicated by the formation of a white precipitate. The exact amount of acid mixture necessary to make the *p*H adjustment is determined by checking by *p*H meter every time a new batch of acid potassium chloride, 15 per cent. sodium hydroxide solution, or acid mixture is prepared. Immediately the *p*H is adjusted cool the tubes in running water, add 15 ml. of *iso*butanol to each tube, shake for 1·5 min., and proceed as is described above.

Thiochrome standards and standard blank—These are prepared, without preliminary adsorption and elution, for each series of fluorimetric readings. Measure 1 ml. of dilute aneurine standard solution containing 1·00 μg. per ml. (prepared weekly by diluting with 0·05 *N* hydrochloric acid a stock solution containing 0·1 mg. of aneurine per ml. in 0·05 *N* hydrochloric acid) into each of three small separating funnels with glass stoppers, or into similar reaction vessels, to each add 4 ml. of acid potassium chloride solution and 15 ml. of *iso*butanol. To the blank add 3 ml. of sodium hydroxide solution, and to each of the other two 3 ml. of alkaline ferricyanide solution, stopper, shake immediately for exactly 1·5 min., allow to stand for 10 min., and remove the aqueous layer. Add about 2 g. of anhydrous sodium sulphate to each *iso*butanol extract, stopper, shake to clear, pour into small brown bottles, and store in the refrigerator until the fluorimetric determinations are made.

Fluorimetric assay—In these experiments, a model B Pfaltz and Bauer instrument was used, the diaphragm was set at 40 so that the standard 1 μg. of aneurine gave a deflection of 50 to 60. Quinine sulphate standards were not used. A standard thiochrome block was employed to check the light intensity during the fluorimetric readings.

Calculations—The amount of aneurine in micrograms in the test sample is calculated as follows. The symbols represent galvanometer readings. U = unknown; S = standard; A = alkali blank; B = standard blank; and $BSCl$ = benzenesulphonyl chloride blank.

Najjar - Ketron

$$[(U - B) - 0{\cdot}2(A - B)]/(S - B)$$

Benzenesulphonyl chloride blank

$$(U - BSCl)/(S - B)$$

*p*H Adjustment

$$(U - A)/(S - B)$$

Results—Comparative values are given for urinary aneurine using the simplified methods described and the corresponding base-exchange methods, and a table of recovery values for added aneurine is also given. Agreement between the three methods was generally good. Slightly lower values were obtained by the *p*H adjustment technique. Recovery tests showed no significant variation between any of the three methods. The use of benzenesulphonyl chloride for the specific determination of aneurine offers the most convenient method for the determination of non-thiochrome fluorescent material in the *iso*butanol extract. J. S. HARRISON

Improved Method for the Assay of Vitamin B_6 with *Streptococcus faecalis*. J. C. Rabinowitz and E. E. Snell (*J. Biol. Chem.*, 1947, **169**, 631–642)—*Streptococcus faecalis* R requires vitamin B_6 for growth under defined conditions, but pyridoxal and pyridoxamine are highly active, whilst pyridoxine is essentially inactive. Because the application of the assay led to certain inconsistencies, a detailed investigation of the method was carried out.

Experimental—Test organism and culture medium—*S. faecalis* R (American Type Culture Collection No. 8043) was carried by monthly transfer in yeast-dextrose agar, with incubation at 30° C. Originally the medium and technique of Snell and Rannefeld (*Ibid.*, 1945, **157**, 475) were employed. 0·1 μg. per tube of synthetic folic acid was used in place of folic acid concentrate and the other vitamins were added to twice the levels originally recommended. Adenine sulphate, guanine hydrochloride, and uracil were omitted. Because of the lability of pyridoxal on autoclaving, all B_6-containing supplements were sterilised separately and added aseptically to the medium with enough water to bring the total volume to 5 ml. The double strength basal medium was autoclaved separately and 5 ml. were added aseptically to each tube.

Inoculum—The inoculum was prepared from a 20- to 24-hr. culture of *S. faecalis* grown in 10 ml. of a sodium citrate medium that was supplemented by 100 μg. of pyridoxal hydrochloride. The cells were centrifuged, washed in 10 ml. of sterile saline, again centrifuged, and re-suspended in 10 ml. of sterile saline. Each tube was inoculated with 1 drop of this suspension. Higher inocula gave sigmoidal growth response curves with pyridoxal.

Effect of citrate—Citrate was used in the medium because replacement of the sodium acetate in the original medium by 2 per cent. of sodium citrate greatly increased growth. The growth tended to be erratic, but not more so than that obtained with the original medium.

Effect of autoclaving—In some experiments the addition of a mixture of synthetic amino acids to the basal medium markedly stimulated growth.

Further study showed that the unautoclaved medium did not support growth, but that the addition of reducing agents such as ascorbic acid, sodium thioglycolate, cysteine or alkali-treated glucose permitted extensive growth. Also, medium autoclaved for more than 6 min. at 15 lb. pressure did not support maximum growth, owing to the destruction of cystine and cysteine. This effect was overcome by supplementing the autoclaved medium with cystine that had been sterilised separately, or by adding an excess of cystine before autoclaving. Modifications based on these findings largely eliminated the erratic and variable results obtained with the earlier method.

In applying the method to natural materials, the time of incubation affected the results. Certain materials contained substances that stimulated growth in the early stages, and satisfactory results were only obtained after 20 to 24 hr. incubation.

Recommended procedure—Using enough water to give a volume of 5 ml. when the supplements are added, autoclave for 10 min. at 15 lb. pressure in 18 × 150 mm. Pyrex test tubes that are covered with aluminium caps. Cool the tubes and add the supplements to the tubes aseptically. Autoclave sufficient double strength basal medium for the assay in one flask for 10 min. at 15 lb. pressure and, when cool, add an autoclaved solution of cystine (3 mg. per ml. dissolved in hot water with the aid of a few drops of concentrated hydrochloric acid) to supply 1 mg. per 5 ml. Add 5 ml. of basal medium aseptically to each tube, inoculate with 1 drop of heavy inoculum, and incubate at 30° C. for 22 to 24 hr. At the end of this period, heat in flowing steam for 10 min., cool, and determine the turbidities in an Evelyn colorimeter with the 600-mμ. filter.

Preparation of samples—Prepare samples as described by Rabinowitz and Snell (*Anal. Chem.*, **1947, 19**, 277). The vitamin B_6 contents determined by the *S. faecalis* assay are always smaller than those measured by *Saccharomyces carlsbergensis*. The differences can be explained in terms of their pyridoxine, pyridoxamine, and pyridoxal contents alone.

Four different strains of *S. faecalis* were tested and all gave identical values for the vitamin B_6 content of yeast extract. The improved medium may be used without modification in the assay of nicotinic acid, folic acid, and pantothenic acid with *S. faecalis* as test organism. It can also be used for the assay of biotin if the casein is treated with charcoal at *p*H 2·0 to 3·5. J. S. HARRISON

Naturally Occurring Penicillins: Assay Method for Penicillin G. T. C. Grenfell, J. A. Means, and E. V. Brown (*J. Biol. Chem.*, 1947, **170**, 527–535)—1. *Total penicillin*—The method proposed for determining total penicillin by measuring optical rotation is based on the observations that (*a*) the specific rotations of aqueous solutions of the sodium salts of the different penicillins are approximately identical, and (*b*) the decomposition products of penicillin possess only slight optical activity. For commercial penicillin, 800 units or more per mg., good agreement is shown between this polariscopic method and the iodimetric method.

Procedure—Dissolve the weighed sample in 10 ml. of freshly boiled and cooled distilled water at 22° to 24° C. Determine the specific rotation. Repeat, using pure penicillin G. The fraction of total penicillin in the sample is then the ratio of the specific rotations. The specific rotation of pure sodium penicillin G is +298° at 25° C.

2. *Penicillin G*—The method depends on the fact that the ultra-violet absorption spectrum of sodium penicillin G in aqueous solution shows strong bands at 258 and 263 mμ. characteristic of the benzyl group. Other penicillins not containing an aromatic side-chain possess only weak non-characteristic absorption in this region of the spectrum. Penicillin X has a very strong absorption, and this method is not recommended for samples containing more than 1 per cent. of penicillin X. Curves of the ultra-violet absorption spectra of penicillins F, G, X, and K are presented. At 280 mμ., the pure penicillins have a negligible absorption, whereas decomposition products of penicillin produce an increased absorption. By subtracting the optical density at 280 mμ. from that at 263 mμ. it is possible to estimate only the active penicillin G.

Procedure—(*a*) For highly pure penicillin. Dissolve the sample in freshly boiled and cooled distilled water, and determine the concentration of total penicillin by the polariscopic method above. Dilute to a concentration of 1·8 mg. of total sodium penicillin per ml. Place the solution in a 1-cm. quartz cell and, using a Beckman spectrophotometer, determine the optical densities at 263 and 280 mμ. The optical density at 280 mμ. should be less than 0·1. From a calibration curve of optical density difference plotted against percentage of penicillin G prepared similarly using known mixtures of pure penicillin F (or K) and penicillin G, the G content of the sample as a percentage of the total penicillin is read.

Procedure—(*b*) For samples showing an optical density greater than 0·1 at 280 mμ. Dissolve 180 mg. of sample in 10 ml. of water and assay polariscopically. To 5 ml. of the solution, add 1·8 g. of pure ammonium sulphate and 1 drop of 3 per cent. aqueous ammonia solution. Cool slowly to −5° C. with stirring and hold at this temperature for 1 hr. Filter through a pre-chilled, semi-micro Buchner funnel, and wash with 1 to 3 ml. of ice-cold, 40 per cent. ammonium sulphate solution. Dissolve the precipitate in 5 ml. of water and assay this solution and the mother-liquor polariscopically. Dilute the ammonium salt solution to a concentration equivalent to 1·8 mg. of total sodium penicillin per ml., and estimate according to procedure (*a*). For material of 1400 units (or more) per mg., the recovery in the purification process is better than 90 per cent., and no change in the ratio of the different penicillins occurs.

Procedure—(*c*) For crude penicillin (about 1000 units per mg.). Assay 5 ml. of solution as in procedure (*b*). Repeat with another 5 ml. of solution, but increase the amount of ammonium

sulphate used to 2·1 g. These two procedures will give different yields from the purification process and different values for the penicillin G content. By plotting the percentage of penicillin G against the percentage yield, and extrapolating linearly to 100 per cent. yield, the true penicillin G content of the original material is calculated. An accuracy to within ± 3 per cent. is claimed. G. H. TWIGG

Microbiological Method for the Determination of Manganese. O. G. Bentley, E. E. Snell, and P. H. Phillips (*J. Biol. Chem.*, 1947, **170**, 343–350)—*Lactobacillus arabinosus* 17–5 is grown in a semi-synthetic medium that has previously been made deficient in manganese by pre-absorption with the test organism. *L. casei* was used in preliminary work, but did not respond so favourably to manganese addition as did *L. arabinosus*.

Method—Stock culture and inocula—Stock cultures are carried by semi-monthly transfer in the medium of Nyman and Gortner (*Ibid.*, 1946, **163**, 277; ANALYST, Abst., 1946, **71**, 390). Stab cultures are incubated for 48 hr. at 37° C. and stored in the refrigerator. To prepare the inoculum make a transfer from a stab culture to a tube containing 10 ml. of the same sterile medium without agar, incubate for 24 hr. at 37° C., centrifuge, pour off the supernatant liquid, add 10 ml. of sterile saline, and re-suspend the cells. Use 1 drop of this suspension to inoculate each tube.

Medium—For the assay, the medium of Roberts and Snell (*Ibid.*, 1946, **163**, 499; ANALYST, Abst., 1946, **71**, 440), with manganese omitted, is used with minor modifications. Ingredients of the highest purity contain sufficient manganese to permit maximum growth. This difficulty is overcome by growing the test organism for 24 hr. at 37° C. in the double strength medium after sterilisation at 15 lb. pressure for 5 min. After cooling, centrifuge, adjust the *p*H to 6·8 to 7·0 with aqueous ammonia solution, and add additional allotments of uracil, guanine, adenine sulphate, *p*-aminobenzoic acid, and the vitamins.

Assay procedure—In each tube dilute 5 ml. of the pre-treated medium to 10 ml. by adding water, sample, or water plus sample. Use three or more levels in duplicate. Sterilise at 15 lb. pressure for 15 min., cool, inoculate, and incubate at 37° C. for 72 hr. Measure the response by electrometric titration of the acid produced. A calomel half-cell - quinhydrone system with a sensitive galvanometer is convenient for this purpose. Duplicate tubes should agree within 0·3 ml. of alkali.

Standard curve—The standard solution of manganese sulphate contains 0·5 μg. per ml. of manganese. Standard levels per tube range from 0·05 to 0·5 μg.; the preferred range is 0·1 to 0·3 μg. Manganese added as potassium permanganate gives identical curves. In assaying an unknown sample at least three levels are used; these should agree within 10 per cent. of the average value.

Preparation of sample—Either acid extraction or ashing methods can be used to obtain the manganese in a suitable form for the assay. Ashing is preferred. Carefully and slowly heat the dried, finely ground sample in a platinum dish over a burner until foaming has ceased, add 0·3 g. of sodium carbonate per g. of sample to plant materials to make silicates more soluble. Place in an electric oven at 550° to 650° C. for 3 to 5 hr. If any carbon remains, moisten the residue with nitric acid and heat over a burner for less than 1 min. Dissolve the ash by adding 1 to 2 ml. of concentrated hydrochloric acid with 5 to 10 ml. of water and warming, transfer quantitatively to an Erlenmeyer flask, and dilute to the required volume with water. Neutralise with aqueous ammonia solution before the assay. The quantity of ammonium chloride produced should be less than 175 mg. per assay tube; more will cause some inhibition of growth of the test organism.

As an alternative method of extraction, autoclave the sample with 75 ml. of *N* hydrochloric acid for 12 hr. at 15 lb. pressure. Filter the solution, neutralise with aqueous ammonia solution, and dilute to volume.

Results—Results on alfalfa hay, liver, blood, and similar materials are consistent, duplication of results is satisfactory, and recoveries of added manganese are quantitative within the experimental error of ±10 per cent. The acid extraction procedure gives considerably higher values than the ashing method on liver samples, but the values obtained by the two methods agree well when alfalfa is assayed. Fatty acids and lecithin do not interfere with the assay. Results on ashed samples agree closely with those obtained by the chemical periodate method. Values for the manganese content of several plant and animal materials are given. J. S. HARRISON

Micro-colorimetric Determination of Sodium in Human Biological Fluids. A. A. Albanese and M. Lein (*J. Lab. Clin. Med.*, 1948, **33**, 246–250)—The method described is based on the measurement of the yellow colour of the triple salt, uranyl zinc sodium acetate, obtained when the micro-quantities of sodium in 0·2 ml. of urine, cerebrospinal fluid, and various blood fractions react with uranyl zinc acetate. The experimental error of the method is approximately ± 5 per cent.

Preparation of reagent—Dissolve 10 g. of uranyl acetate in 50 ml. of boiling water containing 2 ml. of glacial acetic acid. Dissolve separately 30 g. of zinc acetate in 50 ml. of boiling water containing 1 ml. of glacial acetic acid. Mix the solutions and heat the mixture just to boiling. Allow to stand overnight, filter, and mix the filtrate with an equal volume of 95 per cent. alcohol. Store the solution at 4° C. for 48 hr. and filter again for use. The reagent is stable at room temperature.

Procedure for urine and spinal fluid—Transfer 0·2 ml. of urine to a 15-ml. centrifuge tube and add 1 ml. of reagent. Maintain at 4° C. for 1 hr. and then centrifuge at 3000 r.p.m. for 10 min. Discard the supernatant liquid and carefully drain the tube by inversion. Re-suspend the yellow precipitate in 2 ml. of 95 per cent. alcohol, wash by centrifuging, and again drain by inversion. Dissolve the precipitate in 5 ml. of water, transfer

to colorimeter tubes, and determine the intensity of the yellow colour in a photo-electric colorimeter. Conduct a parallel determination on a 0·2-ml. aliquot of a standard solution containing 2 mg. of sodium per ml., prepared by dissolving 0·5084 g. of sodium chloride, dried at 100° C., in 100 ml. of water. The sodium content of the urine is obtained from the formula: mg. of sodium per 100 ml. of sample = 200 × reading of sample/reading of standard.

Procedure for whole blood, serum, or plasma—Add 0·2 ml. of the sample to 0·6 ml. of 20 per cent. trichloroacetic acid solution and centrifuge. Transfer 0·4 ml. of the supernatant liquid (equivalent to 0·1 ml. of the sample) to another centrifuge tube, add 1 ml. of reagent, and complete the determination as described for urine.

The accompanying table shows the results of analyses of biological fluids obtained from human subjects by this method. They are in the range of values reported by others.

SODIUM CONTENT OF VARIOUS HUMAN BIOLOGICAL FLUIDS

Subject	Sample	Sodium content mg. per 100 ml.
Adult, male	Serum	316
Adult, female	Plasma	307
Adult, male	Whole blood	224
Infant, female	Serum	325
Infant, male	Cerebrospinal fluid	285
Infant, female	" "	312
Infant, female	" "	294
		g. per day
Premature infant, male	Urine	0·129
Infant, male	Urine	0·314
Infant, male	Urine	0·550
Adult, male	Urine	3·75
Adult, female	Urine	2·37

A. H. A. ABBOTT

Some Mineral Requirements of the Lactic Acid Bacteria. R. A. MacLeod and E. E. Snell (*J. Biol. Chem.*, 1947, **170**, 351–365)—The study of the mineral requirements of lactic acid bacteria used in microbiological assays is made difficult by the fact that the complex medium required often contains, as contaminants, sufficient essential mineral elements to permit limited or extensive growth even though none of the mineral is added. The presence of large amounts of organic materials renders ineffective many of the procedures used in other investigations for the removal of traces of inorganic ions. By the technique used in this study, $Mn^{\cdot\cdot}$, $Mg^{\cdot\cdot}$, $Fe^{\cdot\cdot}$, $K^{\cdot}$, and PO_4''' are removed by specific lactic acid bacteria. The inhibiting action of citrate due to the formation of non-ionic complexes with certain metallic ions is also investigated.

$Mn^{\cdot\cdot}$ is essential for the growth of *Leuconostoc mesenteroides*, all lactobacilli, and probably *Streptococcus faecalis*. Potassium ion is required in comparatively large amounts by all the lactic acid bacteria investigated. Magnesium stimulates growth, but is not necessarily essential. The amount of magnesium required is less than that required by yeast, but the amounts of magnesium and potassium commonly added to assay media are marginal for maximum growth in 24 hr. The remainder of the metals tested appear to be present in adequate amounts. Most of the organisms require about 3 mg. of PO_4''' per 10 ml. of medium. Citrate inhibition of growth can be prevented by increasing the amounts of manganese and magnesium present in the medium. For some organisms, manganese alone prevents the inhibitory action of citrate. The amount of manganese required is, however, always decreased by addition of magnesium and also by addition of other bivalent metallic ions, such as calcium, that form complex ions with citrate. J. S. HARRISON

Reaction of Certain Carotenoids with Antimony Trichloride. R. M. Johnson and C. A. Baumann (*J. Biol. Chem.*, 1947, **169**, 83–90)—Carotenoids, which occur in certain natural materials, produce with antimony trichloride a blue colour that interferes with the evaluation of vitamin A. The colour due to vitamin A develops in 2 to 5 sec., whereas that from the carotenoids forms more slowly. The present study deals with variations in the intensity and rate of formation of the blue colour when antimony trichloride reacts with derivatives of carotene.

Procedure—Solutions of carotene from various sources, both fresh and after oxidation or isomerisation, were analysed for both carotene and apparent vitamin A by standard colorimetric procedures. The pigment was dissolved in 10 ml. of re-distilled chloroform and the intensity of light was measured in an Evelyn photo-electric colorimeter with a 440-mμ. filter. Galvanometer readings were converted into micrograms of carotene by multiplying the L value by 3·35. Blue values were determined by heating the solutions with antimony trichloride by the method of Dann and Evelyn (*Biochem. J.*, 1938, **32**, 1008), and a drop of acetic anhydride was added to each tube to prevent turbidity. Readings were taken with a 620-mμ. filter at intervals of 5 to 10 sec. and the apparent vitamin A content was calculated from the maximum intensity of the blue colour attained, by multiplying the L value by 1·33. Thus, the apparent vitamin A content for any carotenoid represented the correction factor that would have to be applied to the total blue colour in a determination of vitamin A in mixtures.

Experimental—On treatment with antimony trichloride, pure carotene developed a maximum colour within 5 to 10 sec. and faded rapidly. The apparent vitamin A value was 5·6 μg. per 100 μg. of pure carotene. Carotene fractions extracted from carrot roots developed a blue colour at a rate similar to that of carotene. Extracts from all other crude materials formed blue colours that reached maximum intensity in 15 to 120 sec. with apparent vitamin A equivalents ranging from 7·6 to 52·4 μg. per 100 μg. of carotene. Fractions from crude materials were prepared in the following way. The material was extracted repeatedly with a mixture of ethanol and Skellysolve B (b.p. 64° to

68° C.), and saponified with alcoholic potassium hydroxide, the pigment was transferred to light petroleum (Skellysolve A, b.p. 34° to 37° C. or Skellysolve B), and the xanthophylls were removed with 85 per cent. ethanol. The light petroleum was evaporated under vacuum without heating and the pigment was taken up in chloroform. Partially oxidised carotene developed with antimony trichloride blue colours that increased in intensity for as long as 15 min. after the addition of the reagent with grossly abnormal blue to yellow ratios. The interfering agents could be separated from the carotene by extraction with 80 per cent. methanol or by chromatography. Various isomers such as neo-β-carotene U, β-carotene, neo-β-carotene B, neo-β-carotene E, and several unidentified minor bands that were separated by chromatography were tested. Most stereoisomers of β-carotene reacted with antimony trichloride somewhat more slowly than the natural all-*trans*-isomer and the blue to yellow ratio was also increased slightly.

Discussion—The wide variation in the blue and yellow ratios observed on carotene fractions from natural materials raises the question whether it is ever advisable to use such correction factors in the determination of vitamin A in crude mixtures. If vitamin A and pure carotene only were present a satisfactory correction would be possible, but in crude materials oxidation has often taken place before the sample is submitted for analysis, and, therefore, any attempt to determine the vitamin A becomes one of measuring the vitamin in the presence of several interfering agents. These agents include carotene itself, the effect of which is constant and reproducible, and oxidation products that represent a variable component capable of exerting a much greater effect on the antimony trichloride reaction, both qualitatively and quantitatively, than carotene itself. In addition, other carotenoids could exert variable effects on the reaction. Accordingly, it is almost imperative that these compounds be separated by chromatography if trustworthy analyses for vitamin A are to be obtained.

Thompson *et al.* (*Ind. Eng. Chem., Anal. Ed.*, 1946, **18**, 113; ANALYST, Abst., 1946, **71**, 355) have established conditions under which vitamin A from dehydrated egg is distributed on calcium hydroxide between and including the zones due to β-carotene and cryptoxanthine. Under these conditions, the more harmful oxidation products of carotene would be retained at the top of the absorption column.

A table of the characteristics of the blue colour formed with antimony trichloride and the carotene fractions of certain plant materials is given.

J. S. HARRISON

Organic

Determination of Ethyl and Other Alkyl Sulphates. N. Q. Trinh and M. Sequin (*Compt. rend.*, 1948, **226**, 334–335)—The usual method of determining alkyl sulphates by neutralisation with alkali is slow and inconvenient, and its accuracy is no better than that of the determination by calcination (± 3 per cent.). The method used by Nicloux (*Bull. Soc. Chim.*, 1906, [iii], **35**, 331) for the determination of alcohol in chloroform has been adapted.

Procedure—Into a test tube introduce 5 ml. of the solution of alkyl sulphate (equivalent to an alcohol concentration of about 2 ml. per litre), add 5 ml. of concentrated sulphuric acid followed by about 1 ml. of a solution of potassium dichromate containing 19·0 g. per litre. Warm gently until the solution just boils and continue adding the dichromate, warming after each addition, until the blue-green colour becomes yellowish. If *V* ml. of dichromate are used, each millilitre of solution contains the equivalent of 0·01 *V* ml. of absolute alcohol.

It is claimed that the results are within 1 per cent. of the correct values, and that the assay can be completed in 30 min. J. ALLEN

Determination of Chloroacetic Acid. J. Frankiel and P. Rombau (*Chimie Analyt.*, 1948, **30**, 60)—Chloroacetic acid is determined by making use of the following reaction

$$ClCH_2COONa + HS.CH_2COONa + NaOH \rightarrow S(CH_2COONa)_2 + NaCl + H_2O$$

The reaction is carried out with an excess of a standard solution of thioglycollic acid and the excess is titrated with standard iodine

$$2HS.CH_2COOH + I_2 \rightarrow (.S.CH_2COOH)_2 + 2HI$$

Although the first reaction takes place in the presence of strong alkali there is no error due to formation of glycollic acid. The method is particularly useful when chloroacetates are to be determined in mixtures containing chlorides.

Procedure—Dissolve 0·5 g. of anhydrous sodium carbonate in 20 ml. of 0·1 *N* thioglycollic acid and add 5 ml. of 40 per cent. w/v sodium hydroxide solution. To this mixture add the neutralised solution of chloroacetate containing 0·001 to 0·0015 g.-mol. in 20 ml. Boil for 1 min., cool, acidify with 5 ml. of hydrochloric acid, s.g. 1·18, and titrate with 0·1 *N* iodine. W. C. JOHNSON

Determination of Anti-oxidants in Gasoline. L. R. Williams and B. R. Strickland (*Anal. Chem.*, 1947, **19**, 633–634)—Anti-oxidant compounds are added to gasoline to inhibit the formation of gum and the precipitation of lead, and to maintain stability. Alkyl-substituted *p*-aminophenol and *p*-phenylenediamine derivatives are most commonly used, and since these may be lost by contact of the gasoline with acid, alkali or water it is often necessary to determine the amount present. The method for this purpose depends upon extraction of the aminophenol or phenylenediamine type of inhibitor with hydrochloric acid, neutralisation of the extract with sodium carbonate in presence of phosphotungstic acid, and measurement of the blue colour thus formed, which is proportional to the concentration of inhibitor. To prepare the reagent of Folin and Denis (*J. Biol. Chem.*, 1912, **12**, 239) boil under refluxing conditions a mixture

of 100 g. of sodium tungstate, 750 ml. of water, and 80 ml. of 85 per cent. phosphoric acid for 2 hr., cool, filter, if necessary, and dilute to 1 litre. The components of the reagent must be free from nitrates.

Procedure—Extract 100 ml. of the gasoline (less if the concentration of inhibitor is above 1 lb. per 5000 gall.) with 20 ml. of 5 per cent. hydrochloric acid by shaking vigorously in a separating funnel for 3 min., separate the acid layer, and extract again with 10 ml. of the acid, shaking for 1 min. Rinse the sides of the funnel with about 10 ml. of water after each extraction, and add this to the acid extract. Decolorise the acid extract with 20-ml. portions of dye solvent, if necessary, until all colour has been removed. The dye solvent is a mixture of equal parts of toluene and alkylate or *iso*octane, free from inhibitor. Ether may also be used. If the inhibitors present are predominantly of the aminophenol type, add 5 ml. of the Folin and Denis reagent, and with the aid of a *p*H meter adjust the *p*H to 7 by an 18 per cent. solution of sodium carbonate with constant stirring. If the inhibitor is a phenylenediamine derivative, adjust the *p*H to 7 before adding the reagent, then add more sodium carbonate solution to raise the *p*H to 8. Dilute the solution to 100 ml. and measure the light absorption or transmittance immediately in a suitable colorimeter, *e.g.*, a photo-electric colorimeter with a red filter at about 650 mμ. where absorption by the blue solution is in a constant and maximum range. If the solution is turbid, centrifuge before measuring the colour. Read the concentration of inhibitor directly from a standard curve prepared for each inhibitor by applying the procedure to known amounts of the particular inhibitor dissolved in benzene. Express the results to the nearest 0·01 lb. per 5000 gall. of gasoline. Inhibitors and their solutions used for making standard curves must be pure and, since they are very reactive, must be protected from light and air. Large amounts should be transferred to small ampoules or bottles, sealed with an atmosphere of nitrogen and stored in the dark. The use of an electric *p*H meter and a photo-electric colorimeter are recommended for convenience and accuracy, but the *p*H may be regulated with *p*H paper, and the colour compared visually against that obtained from samples of known inhibitor content. The *p*H should be controlled carefully, however, since both the depth and the stability of the colour are markedly affected by changes in *p*H.

The exact reaction that occurs between the inhibitor and the phosphotungstic acid is not known, but the colour is produced only in alkaline solution and apparently involves reduction of tungstic oxides. Sodium carbonate is used for neutralisation because potassium and ammonium carbonates form precipitates with the reagent (Folin and Denis, *loc. cit.*).

Many organic and inorganic compounds respond to the reagent, *e.g.*, hydroquinone, catechol pyrogallol, α-naphthol (but not β-naphthol), ferrous chloride, and sodium thiosulphate. Phenol, cresol, thymol, aniline, and salicylic acid do not react. Alkyl phenol inhibitors and the usual additives in commercial gasoline (*e.g.*, dye and tetra-ethyl lead) do not interfere with the test. Aged gasoline containing oxidised inhibitors may respond abnormally to the test, causing poor reproducibility of the results.

If the pure inhibitors or their concentrated solutions come into contact with the skin, they should be washed off immediately with alcohol.

A. O. JONES

Composition of Rosin Size (Aluminium Resinates) Precipitates. Analyses of Standard Size Precipitates. D. Price (*Anal. Chem.*, 1948, **20**, 444–449)—Unlike other solvents, light petroleum (m.p. about 40° to 60° C.) does not change the composition of fresh rosin size precipitate extraction residues, even when used in presence of an excess of water; fresh rosin size precipitates contain little or no oxidised resin acids that are insoluble in this solvent. When analyses of size precipitates were attempted by the standard T.A.P.P.I. method, T408 m–44 (see also, ANALYST, 1944, **69**, 160), a clear solution in acidified alcohol was obtained, but the residue on evaporation of this solution could not be extracted completely with light petroleum in a reasonable time. Instead of an analysis for total resin therefore, the carbon/aluminium ratios of the precipitates were determined by Fieser's semimicro-method ("*Experiments in Organic Chemistry*," 1935, p. 350). In making the ash determination the high ignition temperature of 1200° C. is necessary in order to decompose any aluminium sulphate into alumina. The benzene-insoluble constituents were determined by extracting a 2-g. sample with 50 ml. of warm, anhydrous benzene, filtering through a weighed Selas crucible, drying the residue at 100° C., and reweighing. Results are tabulated for sizes containing from 0 to 75 per cent. of free rosin, standard size precipitates being made by adding 25 ml. of 3 per cent. size solution to 1 litre of distilled water, and then adding sufficient aluminium sulphate to produce a *p*H of 4·5. A substance having the composition of aluminium diresinate was isolated from size precipitates and sized papers by the methods described above. The reaction

$$Al''' + 3\ Res' + H_2O \rightarrow Al.Res_2OH + H.Res,$$

and the free rosin contents of the original sizes tested, can account for the compositions of the size precipitates found.

J. GRANT

Rapid Differentiation of Papers by Means of Ultra-Violet Spark Spectra. A. Berton (*Chimie Analyt.*, 1948, **30**, 124–126)—A high-frequency, condensed (5000 volts) spark is passed between two vertical, pure graphite electrodes of square cross-section (5 × 5 sq. mm.), 5 mm. apart. A strip of the paper sample is fastened to a mechanically rotated drum in such a way that its edge travels horizontally between the electrodes, at about 1 cm. per sec., and is about 1 mm. above the lower of them. The resulting spectrum is examined with a Féry ultra-violet spectrograph, in a direction perpendicular to the plane face of the drum, using a slit width of 0·02 mm., and a 10-sec. exposure. The spectrographs so obtained cover

the range 2297 A. to 4267 A., and are assessed by comparison with spectrographs of standard lines and also with those produced without insertion of the paper. Spectrographs from twelve different types of papers are illustrated; they show how qualitative differentiation is possible in terms of the nature and amounts of the metallic constituents present. Of particular diagnostic value are calcium, magnesium, barium and titanium (from various types of loadings), aluminium (from sizing), and iron (usually present as an impurity). Manganese, copper and lead are found in some papers, but in much smaller quantities. The method is reproducible and rapid, and is unaffected by the moisture content of the paper. However, the results are influenced by the thickness and formation (texture) of the paper, as these factors affect the intensities of the characteristic lines observed. Thus, with papers having a close texture, the effect of thickness is apparent only in sheets more than 0·1 mm. thick; for those of loose texture (*e.g.*, filter paper) the corresponding limit is about 0·25 mm. J. GRANT

Polarographic Determination of Furfuraldehyde. N. I. Malyugina and I. A. Korshunov (*J. Anal. Chem. Russ.*, 1947, **2**, 341–344)—Reduction of furfuraldehyde occurs in acid, neutral, and alkaline media giving a single wave in acid and alkaline solutions and two waves in solutions of *p*H 4·0 to 7·0. Within the latter range, with increasing acidity the first wave gets smaller and the second larger, but the total wave-height remains unchanged. With $pH < 7$, the reduction potential is 0·25 to 0·30 v. more positive than that of hydrogen, and under these conditions formaldehyde does not interfere.

Procedure—With synthetic resins, extract the furfuraldehyde by means of ethyl alcohol and dilute to a suitable volume with alcohol. In a polarographic cell place 5 ml. of a mixture of equal volumes of 0·1 *N* acetic acid and 0·1 *N* sodium hydroxide, and then 0·5 ml. of the furfuraldehyde solution. Pass hydrogen gas for 15 to 20 min., and record the polarographic curve starting at 1·0 v. Obtain the content by means of a calibration curve for standard solutions similarly treated.

Results are accurate to within 3 parts in 100.

G. S. SMITH

Polarographic Determination of Naphthalenes in Petroleum Fractions. R. A. Burdett and B. E. Gordon (*Anal. Chem.*, 1947, **19**, 843–846)—The naphthalene compounds found in petroleum fractions can be determined polarographically by means of their reduction waves at −2·5 v. *versus* the saturated calomel electrode.

Procedure—Weigh not more than 0·32 g. of kerosene or 0·25 g. of light gas oil into a 10-ml. volumetric flask by means of a Lunge pipette and dilute to 10 ml. with a base solution which is 0·1 *M* with respect to tetra-*n*-butylammonium iodide and contains 85 per cent. by volume of freshly fractionated dioxan. Transfer the solution to a polarographic cell, remove the dissolved oxygen by passing nitrogen gas through the solution for 15 min., and record a polarogram from −1·8 v. to −2·3 v. *versus* the mercury pool. The naphthalene content of the solution can be calculated from the height of the step by reference to a calibration curve.

The cell and dropping mercury electrode should be well rinsed with acetone between runs. To prevent contamination of the dropping mercury electrode, lower it into the solution only 2 min. before recording a polarogram and remove it at the end of the run. When a capillary gives erratic results, immerse it in nitric acid for a few minutes with the mercury flowing. J. G. WALLER

The Polarographic Properties of Sulphur. Analytical Applications, Reaction with Vulcanisation-Accelerators. G. Proske (*Z. angew. Chem.*, 1947, **59**, 121–122)—The polarographic reduction wave of sulphur can be used for the determination of sulphur in vulcanised rubber or in any other material where the sulphur is not chemically bound. Aqueous acetone or aqueous pyridine is used as solvent, pyridine being preferable, since oxygen can be removed more readily.

Procedure for vulcanised rubber—Heat 0·5 to 2·0 g. of rubber under refluxing conditions with 50 ml. of pyridine for 30 min., and filter the solution into a 100-ml. flask. Extract the residue with a further 40 ml. of pyridine for 30 min., filter the liquid into the same flask and dilute the combined extracts to 100 ml. with water. Dilute an aliquot portion of this solution to 20 ml., and then add 6 ml. of the base electrolyte consisting of 12·5 g. of glacial acetic acid, 27·2 g. of sodium acetate, and 2·0 g. of tylose dissolved in 600 ml. of water. After removing dissolved oxygen, record a polarogram from 0 v. to −1·0 v. *versus* the mercury anode.

Any accelerators present in the rubber have very little effect on the sulphur step, since the sulphur concentration is so much greater than that of the accelerators. J. G. WALLER

Measurement of Internal Double Bonds in Polymers by Perbenzoic Acid Addition. A. Saffer and B. L. Johnson (*Ind. Eng. Chem.*, 1948, **40**, 538–541)—Internal double bonds are those that result from 1 : 4 polymerisation of a butadiene and are distinguished from the double bonds of vinyl groups that result from 1 : 2 addition occurring during the polymerisation. Perbenzoic acid adds more rapidly to internal double bonds than to vinyl groups and an extrapolation of the reaction rate enables the former to be separated from the total unsaturation. The method was tested with mixtures of oleic acid and 10-undecylenic acid and results accurate to 1 per cent. are recorded. Interference due to side-chain oxidation is discussed and is, in the present method, minimised by use of a low temperature and a small excess only of the peracid.

Procedure—Using Braun's method (*Organic Syntheses*, 1933, **13**, 86), prepare a normal solution of the perbenzoic acid in chloroform, dilute to 0·56 to 0·57 *N* with chloroform, and store in a refrigerator. The storage life under these conditions is at least 2 months. Extract small pieces of the polymer, made soluble by milling if necessary, with

acetone. Dissolve in 50 ml. of chloroform an amount of the sample to give 11·1 ± 0·1 mg.-equivalents of double bonds. Add 25 ml. of the 0·57 *N* peracid solution and dilute to 100 ml. in a standard flask. Measure all volumes and maintain the reaction flask at 6° C. Determine the unreacted perbenzoic acid by pipetting 10-ml. aliquot portions of the reaction solution into a 500-ml. Erlenmeyer flask containing 20 ml. of 10 per cent. potassium iodide solution and 25 ml. of 0·4 *N* acetic acid. Titrate the liberated iodine with 0·05 *N* sodium thiosulphate. The unreacted perbenzoic acid is determined at convenient intervals extending over 24 hr. Plot the apparent percentage of double bonds reacted as ordinate against time as abscissa. The graph will be found to become linear after all the internal double bonds have reacted (about 10 hr. for GR–S, the butadiene - styrene copolymer) and this linear portion, extrapolated to zero time, gives the amount of internal double bonds as the intercept on the ordinate.

Results obtained—The results show that natural rubber has at least 95 per cent. internal double bonds, but that synthetic rubbers based on butadiene have from 58 per cent. (for sodium polymerised poly*iso*prene) to 89 per cent. (for emulsion polymerised polydimethylbutadiene) internal double bonds. W. C. WAKE

Inorganic

Thermal Behaviour of Analytical Precipitates. V. Determination of Magnesium. VI. Determination of Beryllium. VII. Determination of Lithium. T. Duval and C. Duval (*Analyt. Chim. Acta*, 1948, **2**, 45–52, 53–56, 57–59)—A study has been made of the various forms in which magnesium, beryllium and lithium are generally weighed (*cf.* ANALYST, 1948, **73**, 522).

Magnesium—The hydroxide loses water at all temperatures, stable magnesium oxide being formed above 800° C. Brucite becomes constant in weight at 815° C., but above 947° C. there is a slight increase in weight owing to oxidation of traces of ferrous oxide present.

The sulphate monohydrate is stable between 140° and 170° C., and the anhydrous sulphate from 320° C. to, at least, 880° C.

The fluoride is precipitated as a mixture of the neutral and acid salts, but can be weighed as magnesium fluoride after heating above 411° C.

Magnesium ammonium phosphate hexahydrate is stable below 40° C., and the pyrophosphate above 477° C. Heating the latter to 900° C. is unnecessary.

The analogous hydrated arsenate compound is unstable on heating, and weighing the compound containing 1·5 molecules of water between 82° and 93° C., the monohydrate obtained at 100° C., or the anhydrous form at 149° C., as recommended in the literature, are all unsuitable. The stable pyroarsenate is obtained above 415° C. An arsenical odour can be detected at 750° C., but up to 885° C., loss in weight is insignificant.

The double carbonate with ammonia is of uncertain composition, but can be ignited to magnesium oxide above 420° C.

The oxalate dihydrate is stable below 176° C. and the anhydrous form between 233° and 397° C. Magnesium oxide is obtained on heating between 480° and 1013° C.

The 8-hydroxyquinoline complex can be weighed as the dihydrate after heating below 90° C., and is anhydrous at 155° C., but decomposition to magnesium oxide begins at this temperature and is not complete until 950° C. The existence of a tetrahydrate is doubtful.

Beryllium—The hydroxide is completely converted to beryllium oxide at 951° C.

The crystalline tetrahydrated sulphate loses water to form the dihydrate, which is stable between 88° and 92° C. The anhydrous sulphate is stable between 346° and 679° C. Beryllium oxide is not formed below 1031° C. The moist sulphate containing an excess of sulphuric acid begins to decompose before it is dry, pure beryllium oxide not being obtained even at 1050° C.

Beryllium ammonium phosphate hexahydrate begins to decompose at 30° C. and the pyrophosphate is obtained at 640° C.; this is the best form in which to weigh beryllium.

Lithium—On drying the precipitated chloride, a stable hemihydrate is obtained between 80° and 96° C. The anhydrous chloride is stable between 175° and 606° C. Sublimation occurs above this range.

The sulphate exists as a stable monohydrate between 50° and 72° C., and is anhydrous between 160° and 877° C.

The phosphate is stable as lithium orthophosphate above 450° C.

An aluminate, $2Li_2O.5Al_2O_3$, is obtained anhydrous above 471° C. M. E. DALZIEL

Systematic Analysis of Cations by the Polarographic Method. I. Conditions for Determining Elements of the Copper and Iron Analytical Sub-groups. M. A. Portnov and A. A. Kozlova (*J. Anal. Chem. Russ.*, 1947, **2**, 345–352)—A study of the effect of various factors on the reduction potentials and polarographic wave-heights of copper, bismuth, cadmium, lead, iron, chromium, and aluminium shows that (1) the half-wave potential does not depend on the concentration of the ion being determined provided that the concentration is not more than about 0·01 *N*, (2) the half-wave potential depends on the nature and concentration of the supporting electrolyte, and it may change its value by some tenths of a volt, (3) the wave-height also depends on the nature and concentration of the supporting electrolyte, and (4) the presence of bivalent tin in the determination of lead in alkaline medium shifts the reduction potential of the latter element by values of the order of 600 millivolts.

Copper—In ammoniacal medium with concentrations of copper from 0·00003 to 0·007 *N* the reduction potentials lay between −0·40 and −0·43 v., and the half-wave potentials between −0·46 and −0·51 v., and thus may be considered constant. In ammoniacal media containing potassium chloride, barium chloride, calcium chloride, ammonium sulphate, or ammonium nitrate, and the same

copper concentration, 0·00330 *N*, the reduction potentials, half-wave potentials, and wave-heights were approximately the same in the different solutions.

Bismuth—With ammoniacal tartrate media, 0·00363 *N* in bismuth, and 0·1 *N* in ammonium chloride, potassium chloride, sodium chloride, barium chloride, ammonium nitrate, or ammonium sulphate, the reduction potentials and half-wave potentials, and the wave-heights, were nearly the same in all the solutions. A variation of the ammonium nitrate concentration had a pronounced effect; thus with 8·0, 3·6, 0·36, and 0·10 *N* ammonium nitrate the reduction potentials were, respectively, −0·54, −0·55, −0·58, and −0·61 v., and the half-wave potentials −0·66, −0·73, −0·75, and −0·78 v., whilst the wave-heights were 31, 35, 39, and 40 mm.

Lead—Determinations of lead in acidic, alkaline, and ammoniacal tartrate media gave consistently a linear relation between wave-height and lead concentration. In alkaline solutions, arsenic, antimony, chromium, and aluminium had practically no effect on the determination of lead. The presence of tin in 0·0020 *N* concentration changed the reduction and half-wave potentials of a 0·0020 *N* lead solution from −0·73 to −0·044 v., and from −0·80 to −0·11 v., respectively.

Cadmium—In ammoniacal medium the reduction potential of cadmium was not affected by a change in its concentration. With 0·0013 *N* cadmium solutions, variations in the ammonia concentration, 0·46, 4·6, and 6·4 *N*, effected changes in the reduction potential, −0·71, −0·82, and −0·90 v., the half-wave potential, −0·76, −0·88, and −0·95 v., and the wave-height, 26·2, 25·5, and 23·2 mm., respectively. With constant ammonia concentration, the presence of ammonium chloride, sodium chloride, potassium chloride, calcium chloride, or barium chloride, in 0·1 *N* concentration had no effect on the reduction and half-wave potentials, and the wave-height. Variation of the concentration of ammonium nitrate did not affect the reduction potential, but the wave-height was somewhat reduced with increasing concentration. In acid solution, the half-wave potential was −0·58 v. for all cadmium concentrations up to 0·005 *N*, but above this it became more positive, *viz.*, −0·51 v. at 0·02 *N*.

Iron—Ammonium sulphate in different concentrations had no effect on the reduction potential and wave-height, but when sodium potassium tartrate was used non-reproducible waves were obtained at the lower concentrations of this salt, less than 5 per cent. With increasing concentration the half-wave potential was nearly constant, but the wave-height became slightly less.

Aluminium—With calcium chloride as supporting electrolyte, variations in its concentration had little effect on the reduction and half-wave potentials of aluminium, but a considerable effect on the wave-height. Thus with 0·0050 *N* aluminium and varying calcium chloride concentrations, 3·46, 2·74, 1·82, 0·91, and 0·18 *N*, the wave-heights were 11·5, 16·8, 17·0, 20·3, and 30·5 mm., respectively. The 3·46 *N* calcium chloride gave reduction and half-wave potentials of −1·66 and −1·75 v., respectively, but the corresponding values in the other cases were all about −1·50 and −1·60 v., respectively.

Chromium—The reduction and half-wave potentials of the two chromium waves obtained in calcium chloride solution were not affected by changes in the chromium content. The potentials of both waves became more negative as the calcium chloride concentration was increased. With other salts as supporting electrolytes in similar concentration, the half-wave potential of the first wave, about −1·0 v., was the same as with calcium chloride, but that of the second varied between −1·50 and −1·55 v. as against −1·58 v. with 0·1 *N* calcium chloride, at a chromium concentration of 0·0046 *N*. Chromium should be determined in 0·1 to 0·2 *N* calcium chloride at a *p*H of 3·9 to 5. Either of the two waves may be used.

G. S. SMITH

Polarographic Determination of Zinc in Metallic Cadmium after Preliminary Electrodeposition of Cadmium from Hydrochloric Acid Solution. P. N. Kovalenko (*J. Anal. Chem. Russ.*, 1947, **2**, 334–340)—The method previously described (*Ibid.*, p. 85; ANALYST, 1948, **73**, 173) is modified to permit the determination of smaller amounts, 0·004 to 0·1 per cent., of zinc in cadmium.

Procedure—Treat 10·0 g. of cadmium metal in a conical flask with 60 ml. of diluted hydrochloric acid (1 + 1) and, gradually, with 0·5 ml. of concentrated nitric acid. Heat to 100° C., cool slightly, and add more nitric acid. Repeat the additions of nitric acid as necessary, but avoid more than 2·5 ml. in all. After dissolution of the metal transfer the solution to a beaker of 400 to 500 ml. capacity, and cool the contents to 50° to 60° C., washing down the sides but keeping the volume between 80 and 100 ml. Place in the solution a double spiral of aluminium wire of diameter 0·22 cm., prepared by bending one end of a 4-m. length into a spiral of diameter 5 cm., and the other into a spiral of diameter 1·5 cm. with a middle untwisted length of 10 to 12 cm., and then inserting the smaller spiral into the larger. Cover the beaker and cool in cold water when the reaction starts to be violent, possibly introducing some cold water into the beaker itself. When the reaction has moderated bring the solution to the boiling-point. Cool and re-heat, repeating these operations until the solution is light in colour.

Remove the spirals, wash down the cover and the sides of the beaker with cold water, add 3 drops of 0·1 per cent. tropaeolin OO solution and then, dropwise, either concentrated aqueous ammonia solution or diluted hydrochloric acid solution (1 + 1) to give an orange-red colour. Heat to boiling and electrolyse, using aluminium electrodes with an external E.M.F. of 0·5 to 0·6 v. Zinc tends to deposit at higher voltages. After 30 min., remove and wash the electrodes, transfer the solution to a 200-ml. graduated flask, and take two aliquot portions of 50 ml. in 100-ml. graduated flasks. To each portion add 10 ml. of 40 per cent. citric acid solution and 15 to 20 ml. of 25 per cent. aqueous ammonia solution, and to one add in

addition a grams of zinc in the form of a standard zinc chloride solution. Obtain the polarogram of each solution, after treatment with 3 drops of 1 per cent. glue solution and 0·5 g. sodium sulphite per 20 ml.

Calculate the zinc content from the formula, percentage of zinc $= 400\,ah/(H - h)g$, where H is the wave-height for the solution containing added zinc, h is the wave-height without added zinc, g is the sample weight, and a is the number of grams of added zinc in 100 ml. of the solution tested.

With zinc contents less than 0·05 per cent. the method of double addition is preferable. To one of the aliquots add a grams of zinc, and to the other $2a$ grams of zinc. Calculate from the formula, percentage of zinc $= 400\,a(2h - H)/(H - h)g$.

G. S. SMITH

Determination of Oxidised Zinc in Ores and Concentrator Products. C. W. Barker and R. S. Young (*J. Soc. Chem. Ind.*, 1948, **67**, 61)—Oxidised zinc minerals are extracted satisfactorily from a sulphide mixture by treatment with dilute sulphuric acid saturated with sulphur dioxide. The procedure can be applied to ores and concentrator products, but the zinc ferrite in the calcines from a flash roaster was not completely soluble.

Procedure—Weigh 0·5 to 1·0 g. of a 200-mesh sample into a 300-ml. flask fitted with a Bunsen valve. Add 50 ml. of 2 per cent. v/v sulphuric acid saturated with sulphur dioxide. Stopper the flask and keep it at 30° to 40° C. for 1 hr. swirling every 10 min. Filter, wash thoroughly with hot water, add 5 ml. of diluted sulphuric acid (1 : 1) to the filtrate, and evaporate to small volume. Determine the zinc by any standard procedure allowing for possible interfering elements. This figure gives the oxidised zinc. Lead, iron, vanadium, manganese, copper, etc., do not interfere with the extraction. With very refractory silicate minerals, a few drops of hydrofluoric acid may safely be added to the leaching solution. C. F. HERBERT

Detection of Aluminium by Means of Aluminon. C. J. van Nieuwenburg and G. Uitenbroek (*Analyt. Chim. Acta*, 1948, **2**, 88–91)—The reagent is not ideally selective, but the interference of the chromium, indium, gallium, and titanium compounds and the pink colour of the reagent may be eliminated by treatment with sulphurous acid, the aluminium complex being stable. Beryllium, scandium, zirconium, and small amounts of ferric iron form similar compounds, which are decomposed when the solution is acidified, any reagent precipitated being dissolved by the addition of methanol or ethanol. Large amounts of ferric iron must be removed by treatment with potassium hydroxide or better, by extraction of the thiocyanate complex with benzyl alcohol and carbon tetrachloride.

Method—Reagent—Dissolve 100 mg. of aluminon in 10 ml. of water in a 100-ml. graduated flask and add 7·5 g. of ammonium acetate dissolved in 10 ml. of water neutralised to litmus by means of aqueous ammonia solution. Shake the solution and add, while still shaking, 5 ml. of a cold, saturated sulphurous acid solution. Neutralise the solution to litmus with ammonia and dilute to volume. The faint yellow-brown solution can be stored for some weeks.

Procedure—Shake 3 ml. of the test solution and 1 ml. of 30 per cent. potassium thiocyanate solution with 2 ml. of a mixture (3 : 1) of benzyl alcohol and carbon tetrachloride until the red colour is removed.

Add 3 drops of the reagent to 1 ml. of the nearly neutral test solution. Heat to about 70° C. and cool. Add 2 ml. of ethanol and shake the solution, then add 1 ml. of 2 N hydrochloric and shake again. If the solution remains pink, aluminium is present.

Under these conditions the sensitivity of the test is reduced to 1 in 5×10^5. Large amounts of phosphate, oxalate, and fluoride also reduce the sensitivity. M. E. DALZIEL

Acidimetric Determination of Aluminium. A. H. Bushey (*Anal. Chem.*, 1948, **20**, 169–172)—In titrating alkaline aluminate solutions with acid, a sharp end-point is obtained when neutralisation of the free alkali is complete, although the actual pH varies with concentration; this end-point is the starting-point for the acidimetric determination of aluminium and is found potentiometrically. In neutral solution, aluminate is determined by adding hydrochloric acid in slight excess over that required to form aluminium chloride, precipitating the aluminium as potassium cryolite, and titrating the slight excess of acid with standard alkali.

Procedure—Add an alkaline sample containing about 0·2 g. of aluminium to sufficient 10 per cent. barium chloride solution, contained in a 250-ml. beaker and diluted to 175 ml., to precipitate all the carbonate present. If only a faint turbidity results, dilute the solution with 50 ml. of water and proceed with the titration. Otherwise, filter through a small Buchner funnel, and wash the precipitate with 100 ml. of water in small portions.

Transfer the filtrate and washings to a 600-ml. beaker and place on the titrating-assembly with the glass electrodes and a mechanical stirrer dipping into the solution; connect to the pH meter. Start the stirrer and titrate with N hydrochloric acid to the free sodium hydroxide end-point (pH about 11), the point at which the rate of change of pH per 0·2 ml. of added acid is found by trial to be a maximum. Continue adding the standard acid rapidly until all but traces of the precipitate dissolve and the solution contains a slight excess of hydrochloric acid over that required to form aluminium chloride. Add several drops of 50 per cent. potassium fluoride solution (neutralised to phenolphthalein and stored in a paraffin-lined bottle) to dissolve the last traces of precipitate and 30 ml. of the fluoride solution to precipitate the potassium aluminofluoride. Add 4 drops of phenolphthalein solution, and titrate back the excess of acid with N sodium hydroxide (pH 8·3) to a pink colour lasting 15 sec.

From the total acid titre subtract the back-titration and the "free alkali titration," the

remainder being equivalent to the aluminium present. The reactions involved are:—

$NaOH + HCl \rightarrow NaCl + H_2O$;
$NaAlO_2 + 4HCl \rightarrow AlCl_3 + NaCl + 2H_2O$;
$AlCl_3 + 6KF \rightarrow K_3AlF_6 + 3KCl$; and
$(K_3AlF_6) + HCl$ (excess) $+ NaOH \rightarrow (K_3AlF_6) + NaCl + H_2O$

With 0·02 g. of aluminium, use 0·1 *N* solutions for the titrations and 10 ml. of potassium fluoride solution. Acid aluminium solutions should be treated dropwise with saturated sodium hydroxide solution until 1 or 2 drops are present in excess of the volume necessary just to dissolve the precipitate.

Interfering substances—The titration covers the *p*H range 11 to 8·3 and substances consuming acid in that range interfere. Hence, ammonia must be removed by distillation from the alkaline solution and carbonate must be precipitated, as small quantities are converted to bicarbonate and larger quantities are decomposed and carbon dioxide is evolved. The precipitated barium carbonate can be separately titrated for a carbonate determination. In moderate quantities, fluoride has no effect, and phosphate and acetate only minor effects. Iron and silica interfere, the former forming a stable fluoride complex and the latter forming silicofluoride, which is decomposed by acid under the experimental conditions employed.

The method is rapid for materials without interfering ions, but can only be used for metal analysis after appropriate separations.

M. E. DALZIEL

Semi-quantitative Determination of Gold. P. E. Wenger, D. Monnier, and Y. Rusconi (*Helv. Chim. Acta*, 1947, **30**, 1636–1638)—Gold in cyanide baths is separated from other ions by precipitation as the metal, which is determined, after dissolution in *aqua regia*, by treatment with mercurous chloride to which the finely-divided precipitate of gold imparts a violet colour, visible at a dilution of 0·00018 g. of gold in 100 ml. of solution, which may be weakly or strongly acid.

Method—Treat 10 ml. of the cyanide bath in a glass vessel with 3 ml. of concentrated hydrochloric acid and some granulated zinc. Heat gently until the zinc is dissolved, decant the solution, and wash the precipitated gold several times by decantation with water. Attack the gold with the minimum quantity of *aqua regia* (1 to 2 ml.), transfer the solution to a 100-ml. volumetric flask, and dilute to volume.

Prepare dilutions in 10 vessels according to Wenger, Monnier and Piguet, (*Ibid.*, 1946, **29**, 1698; ANALYST, 1947, **72**, 116; Wenger, Monnier, and Rusconi, *Analyt. Chim. Acta*, 1947, **1**, 190; ANALYST, 1948, **73**, 521), each containing 10 drops of liquid, ranging from 1 of the solution and 9 of water to 10 of the solution. [ABSTRACTOR'S NOTE: Mercurous chloride is then added, presumably.] If all give a positive reaction, dilute the solution tenfold and repeat the test. Dilute progressively until the limit of dilution for the reaction is reached, and calculate from the sensitivity and the dilution the concentration of gold in the original bath.

If the concentration of the bath is known approximately, an estimate of the necessary dilution may be made for the original solution. M. E. DALZIEL

Simple Method for Determining Iodine in Presence of Chlorides and Bromides. J. C. de Jong and J. B. Lenstra (*Pharm. Weekblad*, 1948, **83**, 161–169)—PROCEDURE—*Inorganic compounds*—Mix 10 ml. of the solution (containing, *e.g.*, 0·083 g. of potassium iodide) with 10 ml. of *N* potassium permanganate and 10 ml. of 4 *N* sulphuric acid. Add, drop by drop, 10 per cent. sodium nitrite solution until the liquid is clear and colourless, then 10 ml. of 30 per cent. urea solution to react with the excess of nitrite, and 2 g. of powdered tartaric acid to reduce any periodate. After 10 min., rinse down the walls of the container, add 5 ml. of *N* potassium iodide, and titrate with 0·1 *N* sodium thiosulphate.

Iodine in thyroid gland—Dissolve 0·2 g. of the powdered gland in 5 ml. of 4 *N* sodium hydroxide, add 50 ml. of 4 per cent. potassium permanganate, 25 ml. of 4 *N* sulphuric acid, and a few glass beads. After boiling the solution for 5 min., cool the mixture, and continue, as described above, by adding sodium nitrite. G. MIDDLETON

Determination of Nitrates by the Method of Cotte and Kahane. P. Karsten and C. A. J. Grabé (*Chem. Weekblad*, 1948, **44**, 237–238)—In Cotte and Kahane's method (*Bull. Soc. Chim.*, 1946, 542), the nitrates are reduced to ammonia by ferrous hydroxide in alkaline solution, silver sulphate being used as catalyst. The method is superior to those of Devarda and others, as the reaction is quicker and there is no evolution of gas. The speed of reaction is dependent on the alkali concentration, which should be, in the final volume, 20 per cent. of sodium hydroxide; a 20 per cent. excess of ferrous sulphate is also desirable. There should be added, per milligram-molecule of nitrate, 2·7 g. of crystalline ferrous sulphate dissolved in a minimum amount of warm water. The catalyst is added as 20 ml. of 0·5 per cent. silver sulphate solution. Distillation in steam is preferred in order to avoid formation of a deposit in the flask. Other heavy metals can be used as catalysts, but extra ferrous sulphate must be added to allow for that used in reducing the metallic ion to metal. Sulphides can be removed by adding a suspension of silver oxide, followed by the alkali, and warming for 10 min. at 90° C. before adding the ferrous sulphate and silver sulphate. G. MIDDLETON

Acidimetric Determination of Phosphoric Acid and Phosphates. G. Brunisholz (*Helv. Chim. Acta*, 1947, **30**, 2028–2035)—Neutralisation of phosphoric acid with sodium hydroxide gives primary phosphate when bromocresol green is used as indicator, and secondary phosphate when thymolphthalein is used, but as the dissociation constant of the tertiary phosphate is only $1·3 \times 10^{-12}$ it cannot be determined by direct titration. Hydrolysis effects must be eliminated by precipitation of the insoluble tertiary phosphate either with barium chloride, calcium chloride, or silver nitrate. The last is best as it forms no basic salt and yields

a pure tertiary phosphate precipitate. The titration for total acidity in a solution containing phosphoric acid is then represented by the equation

$$H_3PO_4 + 3AgNO_3 + 3NaOH = Ag_3PO_4 + 3NaNO_3 + 3H_2O.$$

For phosphate determination in a mixture of acids or salts, the *p*H value is adjusted to correspond to that of a solution of diacid phosphate, and the solution is titrated according to the equation

$$H_2PO_4' + 3Ag^{\cdot} + 2OH' = Ag_3PO_4 + 2H_2O$$

Moerk and Hugues (*Amer. J. Pharm.*, 1922, **94**, 650), Sanfourche and Focet (*Bull. Soc. Chim.*, [4], 1933, **53**, 963), and Gerber and Miles (*Ind. Eng. Chem., Anal. Ed.*, 1938, **10**, 519) use these reactions with methyl red as indicator. Simmich (*Z. angew. Chem.*, 1935, **48**, 566) used bromothymol blue.

The effect of silver concentration, and of light, and the choice of indicator have been studied, and a modified method has been evolved.

PROCEDURE—*Total acidity*—Add to the aqueous solution or suspension, for each 10 ml. of final volume, 0·5 to 1·0 ml. of *N* silver nitrate in excess of that required to precipitate the phosphate, chloride, and bromide, so that after the titration the concentration is between 0·05 and 0·1 *N* with respect to silver. Add 1 drop of a 0·1 per cent. solution of chlorophenol red in 20 per cent. ethanol for each 5 ml. of solution and titrate with carbonate-free 0·1 *N* sodium hydroxide with vigorous shaking. Near the end-point the precipitate coagulates and when, on shaking, the supernatant liquid changes from yellow to violet, the equivalence point is reached.

Disregard a light mauve coloration forming slowly in the solution just before the end-point. The titration must be conducted dropwise and with constant shaking to avoid precipitation of silver oxide.

Phosphate determination—Add 1 drop of a 0·1 per cent. solution of bromocresol green in 20 per cent. ethanol for every 10 ml. of solution and adjust the *p*H with 0·1 *N* sodium hydroxide or hydrochloric acid to match a solution of potassium dihydrogen phosphate containing the same indicator. Then proceed as for total acidity. The presence of bromocresol green has no effect on the end-point as it is completely adsorbed on the precipitate, but the green-tinged solid makes the colour change slightly less distinct.

Alternatively, diacid phosphate may be determined by difference, by titrating two aliquots, one for total acidity, and the other with bromocresol green in the absence of silver.

Alkaline earth, chloride, and sulphate ions do not interfere, but ammonium, aluminium, and ferric ions must not be present. The illumination must be weak and diffuse. M. E. DALZIEL

Quantitative Determination of Permanganate and Manganate Ions when Present Together. M. Shchigol and N. Burchinskaya (*J. Anal. Chem. Russ.*, 1947, **2**, 359–363)—The rate of the reaction

$$4KMnO_4 + 4KOH = 4K_2MnO_4 + 2H_2O + O_2$$

depends on the temperature and the concentration of alkali. In 12 *N* potassium hydroxide, 0·1 *N* potassium permanganate is decomposed immediately even in the cold. In 2 *N* alkali, potassium permanganate is unaffected at room temperature, in the light as well as in the dark, but in presence of iodide quantitative reduction to manganate occurs:

$$6MnO_4' + I' + 6OH' \rightarrow 6MnO_4'' + IO_3' + 3H_2O.$$

Titration by thiosulphate of the iodate formed gives the permanganate content. Addition of a ferrous salt to the solution serves to eliminate the manganate ions:

$$MnO_4'' + 2Fe^{\cdot\cdot} + 8OH' \rightarrow 2Fe(OH)_3 + MnO_2 + 4H_2O$$

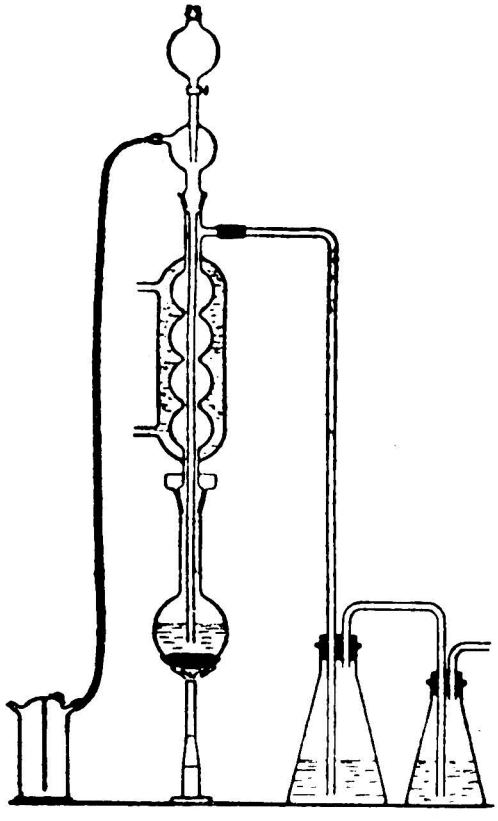
Procedure—To 10 ml. of approximately 0·1 *N* permanganate and manganate mixture in a 100-ml. graduated flask add 25 ml. of 2 *N* potassium hydroxide, followed by 20 ml. of 0·5 *N* potassium iodide, and shake. Add dropwise from a burette, with shaking, 0·1 *N* ferrous ammonium sulphate until a clear solution is obtained over the precipitated manganese dioxide and ferric hydroxide. Dilute to the mark, mix, filter, transfer 20 ml. of the filtrate to a stoppered flask, and add to it 10 ml. of 8 *N* sulphuric acid, and 20 ml. of water, and leave in the dark for 10 min. Titrate the liberated iodine with thiosulphate. Carry out a blank with 5 ml. of 2 *N* potassium hydroxide, 5 ml. of 0·5 *N* potassium iodide, 10 ml. of water, and 10 ml. of 8 *N* sulphuric acid under the same conditions. Calculate the result to permanganate. To determine the total permanganate and manganate, add to 10 ml. of the sample 20 ml. of 0·5 *N* potassium iodide, and 10 ml. of 8 *N* sulphuric acid, and, after keeping in the dark for 10 min., titrate the liberated iodine with thiosulphate. Deduct from the result the amount corresponding to the permanganate as obtained in the first titration, and calculate to manganate.

The interaction of permanganate and iodide in 2 *N* potassium hydroxide gives iodate and manganate only and not manganese dioxide as stated by various authors.

Results on synthetic mixtures show very close agreement with the amounts taken. G. S. SMITH

Determination of Pyrites Sulphur in Presence of Sulphates. E. A. Ostroumov and B. N. Ivanov-Emin (*J. Anal. Chem. Russ.*, 1947, **2**, 314–322)—For determining sulphur combined in the form of pyrites, Bartsch (*Chem.-Ztg.*, 1919, **43**, 33) heated the material, in presence of much mercury and little tin, with hydrobromic acid in an atmosphere of carbon dioxide, and absorbed the liberated hydrogen sulphide in cadmium acetate solution. This method has been found faulty in some respects. Traces of mercury tend to deposit in the vertical condenser above the reaction flask and form mercuric sulphide away from the sphere of reaction. This trouble could be prevented by lengthening the neck of the flask and by having alcohol present to improve the washing-down of the condenser by the condensate. Also, any soluble sulphates present, including

calcium sulphate, were reduced to hydrogen sulphide; in this case it was found necessary to add barium bromide and a small quantity of water to give insoluble, non-reacting barium sulphate. Free sulphur reacted, but it was removable by a preliminary extraction with carbon disulphide. Interference by ferrous sulphide could be avoided by preliminary treatment with hydrobromic acid under conditions that left the pyrites unattacked.



Apparatus—The reaction flask (see Fig.) is a 160-ml. round-bottomed flask with a neck 120 mm. long, carrying a water-sealed, ground-glass joint to a vertical water condenser near the top of which is a side-tube. A special delivery tube with ground-glass joint passes right down the condenser into the reaction flask and allows the passage of carbon dioxide into the flask and, by means of a sealed-in tap-funnel, the addition of reagents. The outlet tube is connected to two flasks in series containing cadmium acetate. Carbon dioxide is delivered under water pressure from large glass bottles, filled from a cylinder, and is carefully purified by successive passage through wash bottles containing titanous sulphate in sulphuric acid solution, copper sulphate solution, and cotton wool.

Procedure—Place in the flask 20 ml. of mercury and, normally, 3 ml. of water and 1 ml. of a solution of 20 g. of barium bromide in 40 ml. of water. For the analysis of gypsum, use 1 ml. of water and 3 ml. of the barium bromide solution. Take 0·2 to 0·3 g. of sample of content about 1·5 per cent. of pyritic sulphur, 0·1 g. for contents up to 10 per cent., 0·05 g. for contents above 10 per cent., and 0·5 g. for gypsum. Place the sample in a tin-foil beaker, 14 mm. in diameter and 18 mm. high, of approximate weight 0·2 g., moisten it with 1 ml. of the barium bromide solution, mix by means of a glass rod, 42 mm. long and 2 mm. in diameter, and then carefully introduce the beaker, still containing the glass rod, into the flask by means of forceps. The glass rod upsets the contents of the beaker. Connect the flask with the condenser without shaking or inclining the flask, seal the joint with water, and connect the inlet tube for carbon dioxide and the receivers. The first receiver contains 50 ml. of a solution containing 25 g. of cadmium acetate and 250 ml. of glacial acetic acid per litre, and 100 ml. of water, and the second 30 ml. of the cadmium acetate solution and 50 ml. of water. Remove air from the apparatus by passing carbon dioxide for 15 to 20 min., then reduce the flow of gas, run down the funnel a mixture of 50 ml. of hydrobromic acid (sp.gr. 1·49) and 1 to 1·5 ml. of ethyl alcohol, and with a gas-flow of about 30 bubbles per min., heat the flask gently for about 1 hr. with a small luminous flame under an asbestos mat. Then heat more strongly for 4 hr., without boiling the liquid or causing movement of the mercury, but increasing the rate of flow of carbon dioxide to 50 bubbles per min. Finally, reduce the rate of flow somewhat, boil the liquid for 10 min., remove the burner, and continue the passage of carbon dioxide for 20 min. Disconnect the receivers, add the contents of the second, if any cadmium sulphide is present therein, to the first receiver, detaching any precipitate in the tube by means of filter paper, and then treat the shaken liquid dropwise with 15 to 20 ml. of a solution prepared by adding 120 ml. of concentrated sulphuric acid to a solution of 120 g. of copper sulphate pentahydrate in 800 ml. of water. Heat gently to form and coagulate copper sulphide, cool, filter, and wash the precipitate with cold, boiled-out water until the washings give no reaction with sodium sulphide solution. Ignite the precipitate with the filter. The weight of copper oxide multiplied by 0·40304 gives the weight of pyritic sulphur in the sample.

Procedure for materials containing ferrous sulphide—Treat the sample with a small amount of diluted hydrobromic acid (1 + 2) mixed with 1 ml. of ethyl alcohol in a similar apparatus under an atmosphere of carbon dioxide, heating gently for 20 min., and then boiling for 5 min. Collect the hydrogen sulphide from the easily decomposable sulphides, *e.g.*, ferrous sulphide, in cadmium acetate solution. Pour the contents of the reaction flask, which is preferably a conical flask in this case, through an asbestos-lined Gooch crucible, and wash with water saturated with carbon dioxide. Transfer the asbestos pad and insoluble matter to a tin-foil beaker, and proceed as described above.

With pyrites containing 50·5 per cent. of sulphur, mixtures of pyrites with gypsum, coal seams, shale, and various coals containing 0·08 to 16·6 per cent. of pyritic sulphur, together with sulphates,

the errors calculated on the sample weights never exceeded 0·02 per cent.

The hydrobromic acid can be recovered by distillation. Fresh mercury is used ach time; it can be reconditioned by treating the amount obtained after ten experiments with hydrobromic acid at the boiling-point, washing with water, and drying with filter paper. Barium bromide can be prepared as follows. Mix 200 g. of powdered barium carbonate with 100 ml. of water, add dropwise with stirring 250 ml. of hydrobromic acid (sp.gr. 1·48), heat gently, add a small excess of baiium carbonate, filter, add 450 to 500 ml. of hydrobromic acid to the filtrate, and leave cold for several days. Collect the crystals and dry them at 120° to 130° C.

G. S. SMITH

Physical Methods, Apparatus, etc.

Crystallographic Data. Armour Research Foundation of Illinois Institute of Technology (*Anal. Chem.*, 1948, **20**, 385–386)—Continuing the proposed scheme (*Ind. Eng. Chem., Anal. Ed.*, 1948, **20**, 274; *cf.* ANALYST, 1948, **73**, 579), adipic acid and *trans*-azobenzene are dealt with under the headings previously proposed. M. E. DALZIEL

Technique for Testing the Accuracy of Analytical Data. W. J. Youden (*Anal. Chem., Anal. Ed.*, 1947, **19**, 946–950)—In developing a new quantitative analytical procedure usually a number of samples are taken and determinations made; *e.g.*, in determining the atomic weight of iodine, samples of silver are converted into silver iodide and a series of paired values is obtained. The ratios of silver to iodine can be calculated and the average taken. However, the pairs may also be used to plot a graph that should be a straight line through the origin. A non-zero intercept should correspond to a blank determination. The slope is the change in iodine corresponding to one unit of silver. The estimate of the ratio of iodine to silver obtained from the slope is independent of any constant errors in the chemical analysis, and disagreement between the ratio as usually computed and as computed from the slope is a warning to the analyst.

The standard least squares method for calculating the slope and intercept of a line allows estimates of their standard errors to be made and therefore of whether the intercept may be regarded as zero. The procedure of calculating the ratios y/x for each pair of points and then taking their average assumes that the intercept is zero and gives each ratio equal weight regardless of the sample size. The procedure is correct when the analytical error increases proportionately with the size of the sample. Where the error is constant in absolute magnitude, however, it gives a less efficient estimate than the standard least squares solution.

The data of Oesting and Kaufman (*Ibid.*, 1945, **17**, 245) on a comparison of a proposed rapid control method for fat in meat with the A.O.A.C. method is discussed. The least squares line is $y = 1{\cdot}05 + 0{\cdot}9663x$ and the tests of significance show that the intercept differs significantly from zero and that the slope differs significantly from unity.

In a further example the data of Hazel and Eglof (*Ibid.*, 1946, **18**, 759) on the comparison of two methods for the determination of calcium in presence of large amounts of magnesium are examined with the least squares technique. Both methods were tested on standard mixtures, and both lines could be considered to pass through the origin. The residual standard deviation about the line was smaller for the new method, indicating it to be the more accurate. However, a further series of replicate samples showed the new method's standard deviation to be larger, and this discrepancy calls for further investigation.

Other examples relate to a determination of the atomic weights of iodine, in which an analysis of variance shows suspicious variability in the results according to the sources of silver and iodine, and to a determination of the voltage across antimony and hydrogen electrodes. Here the assumption that the voltage is independent of pH is shown to be false by fitting a parabola, and a result for pH 8·0, which had been rejected as anomalous, is shown to be perfectly consistent with the remainder of the data. K. A. BROWNLEE

Industrial Control of Size Grading. E. Sharratt (*Trans. Brit. Ceram. Soc.*, 1948, **47**, 22–37)—The sources of error inherent in the methods available for the size grading of powders in the range 2 to 150 μ. are briefly discussed, and experimental data are given to show the comparison between the various methods, which include sieving analysis for coarse particles, the pipette, turbidimetric, and microscopic methods for particles in the sub-sieve range, and the turbidimetric and air-permeability methods for total specific surface. Good agreement for a number of fine powders was obtained between all three methods of size analysis in the sub-sieve range, and between the two methods for total specific surface. It is concluded that a sieving test on two or three sieves down to 200 B.S.S. to control the coarse material, and a specific surface measurement by the Murex "Spekker" method (Sharratt *et al.*, *J. Soc. Chem. Ind.*, 1945, **64**, 73) to control the fine material, are most suitable for routine use, particularly where a rapid comparison of powders of similar type is required.

B. A. SCOTT

Determination of Powder Density, using the Rees - Hugill Flask. A. H. B. Cross (*Trans. Brit. Ceram. Soc.*, 1948, **47**, 38–52)—The determination of the density of powders by means of the Rees - Hugill flask, a modified burette method (Cross, *Ibid.*, 1947, **46**, 105), and the specific gravity bottle, has been investigated. Differences between the results obtained by the two methods using the Rees - Hugill flask were traced to calibration errors, and discrepancies between triplicate results to temperature fluctuations, a change of 1° C. producing a change of 0·016 in measured density. When suitable corrections were applied, the final result was within ±0·003 of the specific gravity bottle result in 23 out of 25 cases. The burette method is slightly more accurate than the direct method. B. In SCOTT

Reviews

COLORIMETRIC METHODS OF ANAYLSIS. By FOSTER DEE SNELL, Ph.D. and CORNELIA T. SNELL, Ph.D. Vol. I. Pp. xxi + 239. New York: D. Van Nostrand Company Inc. London: Macmillan & Co., Ltd. 1948. Price 25s. net.

Most analysts will be familiar with the second edition of this important book in two large volumes published ten years ago. The work has now been extended into three volumes and the first of these presents an expansion of that part in the earlier Vol. I devoted to a discussion of theoretical principles, the instruments employed in colorimetric analysis and the colorimetric determination of hydrogen ion concentration.

It is questionable if the large amount of space devoted to the description of instruments is justified, since most people contemplating the purchase of a colorimeter would consult the catalogues of laboratory furnishers. In any event the authors of this book do not appear to have taken sufficient trouble to recast the original material, for whilst detailed accounts, fortified with illustrations and schematic diagrams of the various Duboscq-type instruments are included, the descriptions of the more modern photo-electric filter photometers and particularly the spectro-photometers are relatively inadequate. For example, the account of the Beckman photo-electric quartz spectrophotometer lacks a diagram, essential for its description, and the brief letter-press is merely illustrated by a meaningless and extremely badly reproduced photograph showing an external view of the instrument.

The section on the Colorimetric Determination of Hydrogen Ion Concentration now occupies 89, compared with 58 pages in the second edition, and the amplification includes many useful numerical data. However, in the opinion of the present reviewer the most valuable portions of this volume are the summaries on Turbidimetry and Nephelometry and, best of all, the chapter on Filters, in which the essential optical properties of a large number of series issued by various manufacturers are presented.

It is explained in the Preface that Vol. II will be devoted to the colorimetric determination of inorganic substances and that Vol. III will deal with organic applications. The authors further state that many of the older references given in the second edition have been deleted, which is surely an unfortunate decision since, while the space thereby saved is negligible, the historical foundations upon which all this work has been built are lost. Thus, there would have been real satisfaction in knowing that Sir William Crookes and his collaborators proposed the measurement of the colour of London water in terms of artificial standards in the year 1881.

N. L. ALLPORT

THE RARE-EARTH ELEMENTS AND THEIR COMPOUNDS. By DON M. YOST, HORACE RUSSELL, JUN. AND CLIFFORD S. GARNER. Pp. ix + 92. New York: John Wiley & Sons, Inc. London: Chapman & Hall, Ltd. 1947. Price $2·50.

With the discovery of isotopes of the rare earth elements among the products of the fission of the uranium atom, a new interest in these elements has been aroused. New methods for their separation have been devised. Dr. Marsh at Oxford has made notable advances in chemical methods of separation and in America ion-exchange columns have been used with some success.

Anyone wishing to gain a full knowledge of the intricacies of the rare earths will obtain only partial satisfaction from a study of the book under review. It certainly will serve as a useful introduction, however, particularly to those who are interested rather in the physics than the chemistry of the rare earths. The book is divided into only six chapters dealing with, respectively, electronic structures and oxidation states of the rare earth elements, paramagnetic properties of rare-earth compounds, absorption spectra of rare-earth compounds, evidence for the existence of element 61, separation of the rare earths and chemical and physical properties of the rare earths. Appendixes contain information on nuclear properties of the rare earth elements and general physical constants and include a table of the periodic system.

Although the physical aspects are dealt with very fully and well, the chemical chapters are all too brief and give no more than a survey of the more important chemical properties of the metals and their compounds. The complex problems associated with the separation of the individual earths from their naturally occurring mixtures are dealt with only superficially. A table rather akin to that used in the teaching of qualitative analysis, while being a most useful reference, hardly indicates the years of work required, the difficulties to be overcome or the patience and skill needed to prepare every rare earth in a state of purity.

Inaccuracies and omissions are few in number but occur mainly in the chemical chapters. Marsh's dimethylphosphate method (not "phosphate" method, as cited on p. 41) for the separation of the yttria groups elements is erroneously explained on p. 47; the whole point of the method is that these compounds are very soluble in ice-cold water, but much less soluble in water at 50° C. so that on warming the cold solution the compounds crystallise out. The sodium amalgam method of Marsh for producing high purity samarium is not mentioned.

In spite of its shortcomings, however, the book must be recommended, for it will impart to those reading it a desire to know more of this most complex and difficult, yet intriguing, branch of inorganic chemistry.

F. M. LEVER

PHYSICAL METHODS GROUP

A MEETING of the Physical Methods Group will be held in the Chemistry Lecture Theatre, Imperial College, Imperial Institute Road, South Kensington, London S.W.7, at 6 p.m. on Tuesday, January 25th, 1949.

The subject of the meeting will be:

"Rheological Methods of Control"

and the following papers will be read:

At 6.5 p.m. "Industrial applications of rheology," by G. W. Scott Blair, D.Sc., Ph.D., F.R.I.C.

At 6.35 p.m. "The application of rheological methods in the milling and baking industries," by A. J. Amos, B.Sc., Ph.D., F.R.I.C.

At 6.55 p.m. "The use of rheological tests in the pharmaceutical and cosmetic industries," by R. H. Marriott, D.Sc., F.R.I.C.

At 7.15 p.m. "Rheological methods and their uses in the paint industry," by P. S. Williams, B.Sc., A.R.C.S.

At 7.35 p.m. an open discussion will follow.

NUTRITION PANEL OF THE FOOD GROUP OF THE SOCIETY OF CHEMICAL INDUSTRY

WE have been asked to make known to our members that the Nutrition Panel of the Food Group of the Society of Chemical Industry is inaugurating a series of meetings for discussing problems affecting "The Nation's Manufactured Foods." The first of these meetings will be held on Wednesday, January 5th, 1949, at Gas Industry House, 1, Grosvenor Place, London S.W.1, at 6.30 p.m.

The subject will be:

"The Sausage as Food."

The following papers will be read:

"The history of sausages," by Mr. H. P. Blunt, of the Ministry of Food.

"The composition of fresh sausages," by Mr. Osman Jones.

"The composition of processed sausages," by Mr. M. G. Read.

The Chairman of the Panel, Dr. Magnus Pyke, will review these papers in a contribution—"Sausages and Nutrition."

A general discussion will follow and in order that it may be directed along constructive lines the Hon. Secretary of the Panel would like those who contemplate contributing to it to send him their names and give some indication of the points on which they wish to speak.

At this, as at subsequent meetings, visitors will be very welcome.

The Hon. Secretary of the Nutrition Panel is Mr. L. C. Dutton, F.R.I.C., c/o Virol, Limited, Hanger Lane, Ealing, London W.5.

HIS MAJESTY'S COLONIAL CHEMICAL SERVICE

There are vacancies for Chemists in the Joint Department of Chemistry, Federation of Malaya and Singapore. Qualifications are an Honours Degree in Chemistry from a British University with the Fellowship or Associateship of the Royal Institute of Chemistry. Candidates should have had in addition at least 2 years' specialised experience in some line (Food, Drugs, Water Bacteriology, Pharmacy, Public Health, etc.) relevant to the work in Malaya or research experience indicated by, e.g., a Ph.D. qualification. Successful candidates will be required to pass an examination in colloquial Malay within 18 months of their engagement. Preference will be given to unmarried candidates of from 25 to 27 years of age. Appointments are on probation for 3 years for permanent and pensionable service. *Salary scale* is—$430 a month rising by annual increments of $30 a month to $670 and then $720 a month rising by annual increments of $30 to $900 a month. There is, in addition, *Expatriation Pay* at the following rates:—

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(*b*) Graduates (Hons. B.Sc.) to work in various sections of the above department.

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