THE ANALYST

PROCEEDINGS OF THE SOCIETY OF PUBLIC ANALYSTS AND OTHER ANALYTICAL CHEMISTS

AN Ordinary Meeting of the Society was held at 6 p.m. on Wednesday, May 7th, at The Chemical Society's Rooms, Burlington House, London, W.1, with the President, Mr. Lewis Eynon, in the chair. The following paper was presented and discussed: "A Micro-chromatographic Method for the Detection and Approximate Determination of the different Penicillins in a Mixture," by R. R. Goodall, Ph.D., and A. A. Levi, Ph.D., A.R.I.C. The paper was preceded by a brief account of the chemical nature of the Penicillins by F. A. Robinson, M.Sc., LL.B., F.R.I.C.

NEW MEMBERS

Professor Marti Fernando Burriel, D.Chem. (Madrid and Brussels); Albert Cecil Case, B.Sc. (Lond.), A.R.I.C.; Daniel Corkery, M.Sc.; William Francis Jack Cuthbertson, B.Sc., Ph.D. (Lond.), A.R.I.C.; Alfred Davidsohn; Eric Coning Dawson, B.Sc. (Lond.), M.Sc. (Dunelm), A.R.I.C.; Francis Cyril James Poulton.

DEATHS

WE deeply regret to record the deaths of

Sir Frederick Gowland Hopkins, Honorary Member.
Robert Ellison.

The Fundamental Laws of Polarography

BY J. HEYROVSKÝ

(*Lecture delivered at a Meeting of the Society on October 31st, 1946*)

THE foundations of the polarographic method are closely connected both theoretically and experimentally with fundamental researches on the significance of electrode potential and polarisation. In the seventies, G. Lippmann introduced the cell consisting of a mercury meniscus in a capillary, as one electrode, and a large mercury pool as the other. The capillary was connected by means of rubber tubing to a mercury reservoir, the height of which could regulate the position of the meniscus in the capillary. The rôle of the large mercury electrode was to ensure that, owing to its small current density, its potential would remain constant, so that the total external E.M.F. applied on the electrodes of this cell would produce changes of potential at the small electrode only. This provided a convenient system comprising a polarisable electrode (the capillary one) and an unpolarisable electrode (the bottom one). From Lippmann's studies of electrocapillarity carried out with this cell Helmholtz was able to work out his theory of electrode potential being the potential of a double-layer condenser, of which one layer lies on the metal and the oppositely charged layer is in the surrounding solution. In 1903, B. Kučera developed Lippmann's method further by raising the mercury reservoir so that mercury would drop slowly out of the capillary. He has shown that the drop-weight method is more convenient for the determination of the interfacial tension (γ) of polarised mercury than Lippmann's method of observations on the meniscus, since the drop-weight, w, is given by $w = 2\pi r\gamma$, r being the radius of the mouth of the capillary. However, w is equal to $m.t$, m being the rate of outflow of mercury from the capillary and t the drop-time; hence, at a constant applied E.M.F., $w = m.t = 2\pi r\gamma = k$ is a constant. Since, however, m is proportional to the height, h, of the mercury reservoir, t must be inversely proportional to h.

Kučera obtained with his method a number of electrocapillary parabolas, some of which, however, showed an anomalous maximum. In 1918 he suggested that the present writer should investigate the cause of such anomalies; in the course of these investigations it became evident that a much better insight into the processes occurring at the dropping electrode

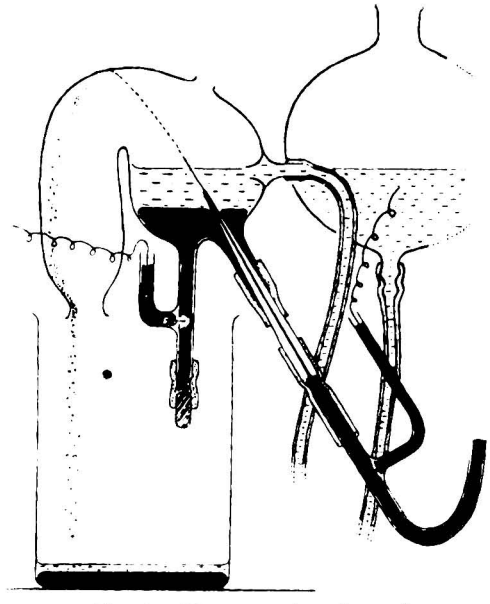

Fig. 3. The streaming electrode.

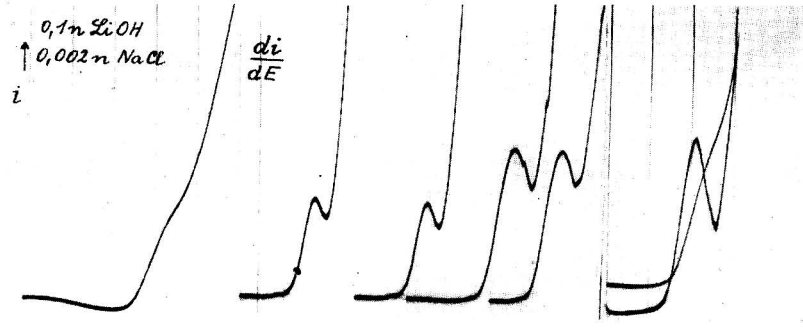

Fig. 7. Left: the current - voltage curve; middle: its derivative curve; right: the current - voltage curve and its derivative.

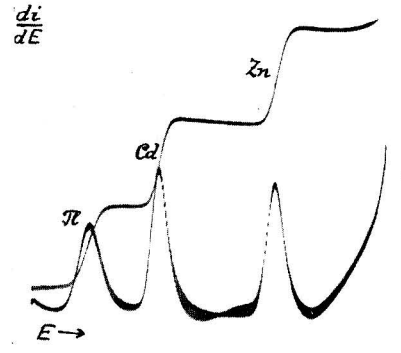

Fig. 8. The current - voltage curve of a solution containing 10^{-3} N, Tl', Cd", and Zn" and its derivative.

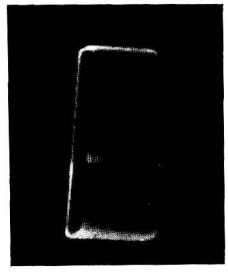

Fig. 10. Oscillographic "spectral line."

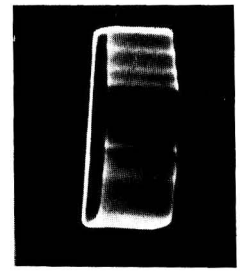

Fig. 11. Oscillographic "spectrum" due to 10^{-3}N Tl', Cd", Zn", Fe" and Mn" in ammoniacal solution.

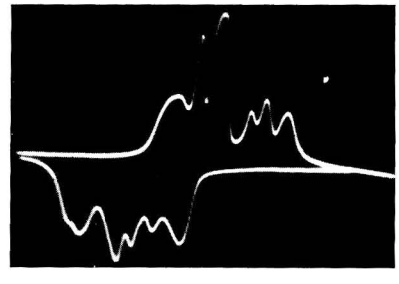

Fig 12. The derivative of the potential - time curve.

could be obtained by measuring the currents which pass through the cell at different voltages. In this respect the dropping mercury electrode was found to be especially suited to show a variety of exactly reproducible phenomena, as it offers the following advantages: (1) it develops always a fresh, regularly renewed surface of mercury and of the adjacent electrolyte solution, so that no time effects due to accumulating products of electrolysis are possible; (2) the large overvoltage on pure mercury prevents hydrogen deposition, so that even alkali metals may be deposited without decomposition of water taking place; (3) since the extent of electrolysis is small, changes in the concentration of the depolariser in solution are negligible, and consequently the measurements may be repeated with the same small amount of solution many times with identical results; (4) owing to the perfectly regular dropping, all processes repeat themselves at each fresh mercury drop with a high degree of exactitude, so that the current is a well-defined function of the dropping-electrode potential.

From the regularity of conditions under which the surface of each drop is developed, Ilkovič, and, later, Rideal and MacGillavry, were able to deduce that the mean current, i_E, due to electrolysis of a depolariser present at a concentration C in a concentrated solution of an indifferent electrolyte, is determined by the equation:

$$i_E = 0{\cdot}627\ \nu.F.D^{1/2}m^{2/3}t^{1/6}(C-C_0),$$

where ν is the electrovalency, F Faraday's constant, D the diffusion coefficient and C_0 the concentration of the depolariser at the electrode surface, all quantities being expressed in absolute units; i_E reaches its limit when $C_0 = 0$, at which condition the depolariser is completely exhausted at the electrode surface. This current is then called "diffusion current"; it gives the "height of the polarographic wave" and is proportional to the concentration C of the depolariser in the body of the solution, so that it serves as a measure of its concentration. From the relationships given above between m, t and h it follows that the "diffusion current" is proportional to $\sqrt{h}$, *i.e.*, it varies as the square root of the height of the mercury reservoir.

The relationship between the current, i, and the potential, π, of the polarised mercury electrode* has been deduced by Ilkovič and the present writer from the formula of Peters $\left(\pi = -\frac{RT}{\nu F}\log\frac{[\text{red.}]_0}{[\text{ox.}]_0}.k\right)$ as

$$\pi = -\frac{RT}{\nu F}\log\frac{i}{i_d-i}\sqrt{\frac{D}{D'}}.k,$$

where D and D′ denote the diffusion coefficients of the depolariser in its oxidised and reduced states respectively, the electrolytic process being ox. $+ \nu\ominus \rightarrow$ red., *e.g.*, $Pb^{\cdot\cdot} + 2\ominus \rightarrow Pb$. The curve, giving this dependence of the current, i, on the potential, π, *i.e.*, the current - voltage curve has the shape of curve 1 in Fig. 1. It has an inflexion for $i = i_d/2$, at which point the concentration of the depolariser at the electrode surface is half exhausted ($C_0 = C/2$) and also the concentration of the lead amalgam reaches half of its final value. The potential, $\pi_{\frac{1}{2}}$, at which this happens is a constant independent of the concentration of the depolariser, C, and of the properties of the capillary (m, t). This is the so-called "half-wave potential" characterising the quality of the depolariser.

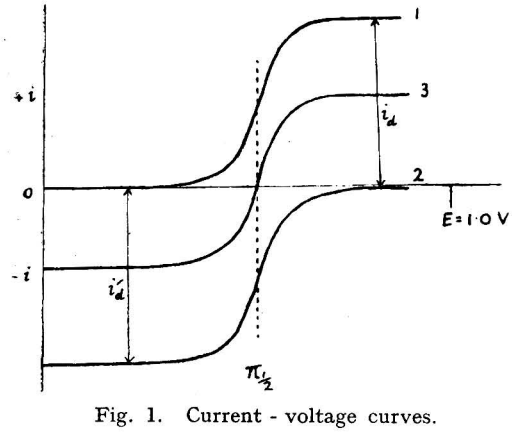

Fig. 1. Current - voltage curves.

If we use instead of pure mercury in the dropping electrode a dilute, say, 0·001 per cent. amalgam of lead and polarise it in a pure solution of an indifferent electrolyte cathodically, starting with a large voltage, say, E = 1·0 v., the current is practically zero (Fig. 1, curve 2). On decreasing the voltage, *i.e.*, imparting to the dropping electrode more positive potentials, we start anodic dissolution of lead, *viz.*, $Pb \rightarrow Pb^{\cdot\cdot} + 2\ominus$. At a potential at which half of the lead content from the surface dissolves, the "half-wave potential" of lead is reached and

* π depends on the voltage V (*i.e.*, external applied E.M.F.) as $\pi = -V + i.\rho$, where ρ denotes the resistance in the circuit. Since both i and ρ are small, π is practically identical with V.

when all the lead from the surface of the amalgamated drop is dissolved, an anodic "diffusion current," i'_d, ensues. We thus obtain a curve of the same shape as when using pure mercury dropping into a lead salt in the solution. If the lead amalgam drops into a solution containing a lead salt, there is a cathodic diffusion current, i_d, as well as an anodic diffusion current, i'_d, the first indicating the concentration of lead in the solution, the second that in the mercury. In fact, in polarography we always deal with an electrode equilibrium of the depolariser in its oxidised (ox.) and reduced (red.) states, *i.e.*, with redox potentials. The current - voltage curve for cases in which both the oxidised and the reduced forms are present is represented by the equation

$$\pi = -\frac{RT}{\nu.F} \log \frac{i-i'_d}{i_d-i} \sqrt{\frac{D}{D'}} . k$$

This formula is mostly applied to solutions of redox indicators and other systems containing in the solutions red. and ox. components, *e.g.*, $Fe^{\cdot\cdot} - Fe^{\cdot\cdot\cdot}$, $Cr^{\cdot\cdot} - Cr^{\cdot\cdot\cdot}$, $Sn^{\cdot\cdot} - Sn^{\cdot\cdot\cdot\cdot}$, quinone - hydroquinone, lactoflavin - leuko-base, etc. The study of current - voltage curves gives us, indeed, the same information as the Michaelis "potentiometric titration curves." In these, increasing amounts of a reducing agent, say, $Cr^{\cdot\cdot}$, are added to the depolariser, *e.g.*, $Fe^{\cdot\cdot\cdot}$, whereby the ratio of ox. to red. is changed and the corresponding π is noted (Fig. 2*a*). In polarography the reduction of ox. to red. is effected by the current of electrons, which reduce the depolariser at the interphase, and the corresponding π is shown on the abscissa (Fig. 2*b*). To change diagram *a* into *b* one has first to turn *a* round through 90° and then turn its paper upside down to cover exactly the curve *b*. From this it is evident that all deductions that are derived from Michaelis curves also follow from the polarographic waves, notably as regards the formation of semiquinones and dimers. Moreover, in polarography one of the components suffices, the second being formed at the electrode; this is of special value if one of the forms is unstable. Also the range of negative potentials at which the redox systems may be studied is considerably extended (to −2·0 v. from the 1 *N* KCl calomel zero).

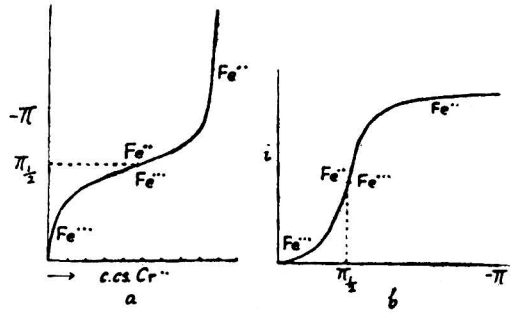

Fig. 2. (*a*) Potentiometric titration curve.
(*b*) Polarographic current - voltage curve.

When examining solutions containing more than one depolariser, we obtain the corresponding number of waves, which allow simultaneous qualitative and quantitative determination of the depolarisers. If the half-wave potentials are near to each other, the waves coalesce so that the determination is uncertain; however, an addition, to the electrolyte, of a compound that makes complexes of different stability with the various depolarisers helps to resolve such coincidences into separated waves. Another difficulty arises when we have to determine accurately small quantities of a baser component in excess of nobler ones, *e.g.*, traces of Zn or Cd in Cu. Then the noblest component ($Cu^{\cdot\cdot}$) forms such a large wave at a positive potential that waves of the minor components, reducible at more negative potentials, are hardly distinguishable at the given sensitivity. This difficulty might be removed by applying a differential method described recently by Semerano and by Kanewsky, who use two solutions in separate vessels with exactly identical capillaries in each; the difference of currents flowing through the two capillaries is registered against the voltage. One of the vessels contains the sample to be analysed, the other only the indifferent electrolyte used in the first solution. Next, the noblest (most electro-positive) component is added to the indifferent electrolyte in such an amount as to make its concentration equal to that in the vessel with the sample, so that the two diffusion currents balance each other. On increasing the voltage the diffusion currents of the baser components may be registered with a large sensitivity. The presence of air does not interfere, since the oxygen waves exactly balance each other. This differential method assumes equal drop-times and synchronous dropping for both electrodes, which is very difficult to maintain. Therefore the present author recommends here the use of two streaming electrodes instead of the dropping ones. One type of the streaming electrode is shown in Fig. 3. It consists of a thick-walled capillary with an inside diameter of 1 to 2 mm., drawn out at the tip to an internal diameter of about 0·1 mm.

It is essential to keep the level of the solution in the vessel constant, so as to have always the same length of the electrode given by the jet of mercury. Two such electrodes, each surrounded by a solution in the same indifferent electrolyte, coupled according to the differential scheme, can be used to show slight differences between samples of similar composition. In Figs. 4 and 5 curves obtained with two streaming electrodes are shown, the solutions being exposed open to the air; the sensitivity of the galvanometer was decreased 1:30. The method is very sensitive, but the consumption of mercury is large, amounting to 600 to 1000 g. per hour (under a pressure of about 50 cm. of Hg). One streaming electrode alone is not suitable for analytical purposes, since the charging (or "capacity," *i.e.*, "condenser") current is about 100 times larger than that of the dropping electrode.

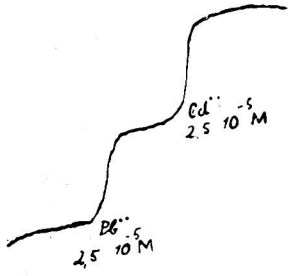

Fig. 4. Comparison of solutions: one solution pure N HCl, the other containing Pb" and Cd" in a concentration $2{\cdot}5.10^{-5}M$.

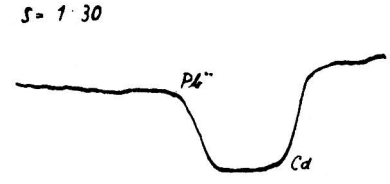

Fig. 5. One solution contains Pb", the other Cd" ions in a concentration $2{\cdot}5.10^{-5}M$, both in N HCl.

Another method, which enables small quantities of baser constituents to be determined in presence of excess of nobler ones, and which at the same time gives new information on the shape of current - voltage curves, consists in obtaining the "derivative" curve, $\frac{di}{d\pi} - \pi$. Here again a double electrode is used (Fig. 6), but dipping into one solution only. Since synchronous dropping is difficult to attain, the drop-time is reduced (0·8 to 1 sec.) without synchronising the drops. Thus the curves in Figs. 7 and 8 were obtained. The equation of the current - voltage curves gives for the differential quotient at the half-wave potential $\frac{di}{d\pi} = -\frac{\nu.F}{4RT} \cdot i_d = 10\nu \cdot i_d$, as a maximum. The advantage of this method for analytical applications is due to the ease with which the half-wave potential and the height of the maximum, indicating the quantity required, are determined.

The precision with which a component may be determined polarographically reaches in favourable case ±1 per cent. of its absolute amount. Where greater exactness is required "polarometric titrations" may be used. In these the diffusion current of the component is followed at a certain constant voltage as it is diminished during precipitation with a suitable agent. The end-point of the titration is shown by the minimum value of the diffusion current; this minimum is best found graphically from a hand-drawn diagram, two measurements before and two after the end-point being necessary. By this method the precision is increased to 0·1 per cent. The component or the precipitating agent has to act as a depolariser. It is best when both are polarographically active, because in this case a very sharp minimum of the diffusion current is obtained (*e.g.*, nickel with dimethylglyoxime). The school of I. M. Kolthoff has worked out many analytical applications of these polarometric (called also "amperometric") titrations. An interesting case is encountered in the polarometric titration of thorium salts with fluorides, neither of which is a depolariser, yet the presence of Th^{++++} ions provokes a wave of nitrate ions added for that purpose. As soon as all thorium ions are precipitated the nitrate wave disappears.

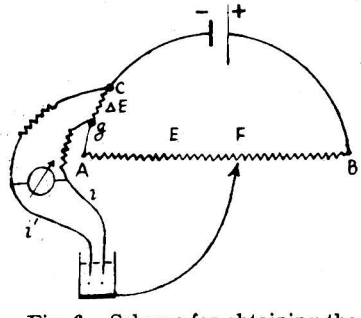

Fig. 6. Scheme for obtaining the derivative curve.

In the last few years a new development of polarographic research has been started through the use of potential - time curves, obtained on the fluorescent screen of the cathode ray oscillograph.[2]

The voltage of the ordinary alternating current supply (of 50 cycles per sec.) acts on the dropping mercury electrode and the changes of its potential are recorded on the oscilloscope. The curve shown with pure indifferent electrolyte has the shape *a* (Fig. 9), and a trace of depolariser changes it into the shape *b* (Fig. 9) with a time-lag at the depolarisation potential.

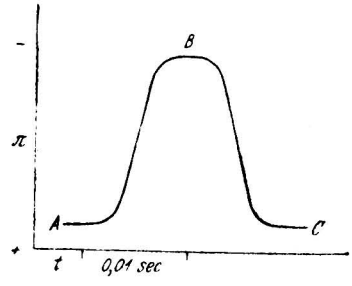

Fig. 9 (*a*).

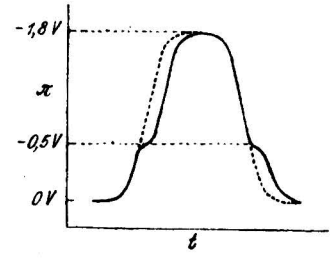

Fig. 9 (*b*).

Oscillographic potential - time curves.

Increased frequency of the time base (to about 100,000 per sec.) changes curve *b* into Fig. 10, where the depolariser is characterised by a line at a certain potential. If more depolarisers are present, the corresponding number of lines appears as an "oscillographic spectrum" (Fig. 11). The quantity of the depolariser is deduced from the "derivative" curve (representing $\frac{dV}{dt} - t$) through the depth of the cut-in, shown on the curve in Fig. 12. To obtain a steady oscillogram the streaming mercury electrode has to be substituted for the dropping electrode.

References

1. Heyrovský, J., "The Differential Method with the Streaming Electrode" (in Czech), *Chem. Listy*, 1946, **40**, 222–224.
2. Heyrovský, J., and Forejt, J., "Oscillographische Polarographie," *Z. physik. Chem.*, 1943, **193**, 77–96.

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Charles University
Prague

Discussion

The President, Dr. G. W. Monier-Williams, expressed the cordial thanks of the Society to Professor Heyrovský for enabling them to hear this authoritative account of the fundamental principles of polarography from one who was not only the originator but also the leading exponent of the subject. The Society was grateful also to the British Council, to whom they owed this opportunity of welcoming Professor Heyrovský as their guest lecturer.

Dr. J. E. Page said it was a great privilege for him to open the discussion on Professor Heyrovský's lecture. Few branches of analysis could have owed so much to one man as polarography owed to Professor Heyrovský. A high percentage of the 1400 papers published on polarography had come from Professor Heyrovský's laboratory and the bulk of the remainder had been directly inspired by work initiated at Prague. He (the speaker) was particularly interested in Professor Heyrovský's recent work with the cathode ray oscillograph and would like to know if he had used this technique to study (i) oxidation - reduction systems such as benzoquinone - hydroquinone and (ii) catalytic steps of the type formed by cysteine and cystine in ammoniacal cobalt buffer solutions.

Mr. J. Haslam said it was a privilege to have had the opportunity of listening to the lecture of Professor Heyrovský, which was of fundamental value to the analyst. More and more they saw the fundamental nature of the work, which made possible the determination of reducible substances by clean and neat methods, and they saw also the selective nature of the polarographic method, as in the determination of nitrobenzene in aniline, for which the chemical method of reduction with titanous solution only gave a figure for total reducible matter calculated as nitrobenzene. He thought that in the future when they had more information about the correct ground solutions, the polarographic method would be of great value in that difficult problem for all organic chemists—the rapid quantitative determination of small amounts of various metals present in the sulphuric acid digestion products of organic materials.

Mr. F. STEGHART, speaking of the Ilkovič equation, said that experiments by Maas and Kolthoff seemed to confirm its validity, but some experiments in the laboratory with which he was associated threw some doubt on it. In these experiments there was included in the polarographic circuit a high-speed high-precision amplifier designed in such a way that no interference with the current took place. The output from the amplifier was connected to two instruments, one representing the ordinary highly damped galvanometer and the other a high-speed high-precision recorder. Whilst the damped recorder, which for all practical purposes gave an exact replica of the ordinary galvanometer, confirmed the tests by Maas and Kolthoff, the high-speed recorder contradicted them. The curve obtained was practically never in agreement with one-sixth power of the time, but varied usually between two-thirds and one-third power. As the difference between such curves and the curve of one-sixth power is comparatively small and would only be shown by a highly accurate recorder, it appeared rather likely that the previous tests and those carried out by Ilkovič to obtain confirmation of his theory were obtained only because of the inadequacy of the instruments used at the time. The results of the experiments described above do not affect the practical usability of the polarograph in any way, but they make it appear very likely that the Ilkovič equation describes only part of the effect and that other factors, *e.g.*, the turbulence near the surface of the drop, may play a part. Furthermore, it appeared from micro-photographic experiments that the increase of the mass of the drop is not a linear function of the time, and if this is taken into account it increases the discrepancy between the actual record and the one-sixth power law. From all these tests it appeared that further investigation into the basic theory of the polarograph would be of great interest and might lead to further improvement in the usability and accuracy of the instrument.

Professor HEYROVSKÝ, in reply to Dr. Page's question concerning the oscillographic investigations with oxidation - reduction systems such as benzoquinone - hydroquinone, said that this particular case—as well as that of quinhydrone—shows perfect oscillographic reversibility and so does the system cystine - cysteine. Such red-ox electrode reactions must, therefore, come to the thermodynamically defined state of equilibrium with an extremely high rapidity. The catalytic reactions with cysteine were not yet investigated, but the reaction of blood-proteins in ammoniacal cobalt buffer solutions were found to show characteristic time-lags in the oscillographic potential - time curve, again proving the high rate of this catalysed reaction.

To Mr. Haslam's remark on the possibility of the determination of traces of metals in organic materials he expressed the opinion that such determinations, *e.g.*, of iron, copper, bismuth, antimony, lead, thallium, cadmium, zinc, and so on, are possible without difficulties.

In reply to Mr. Steghart, Professor Heyrovský remarks that the validity of the one-sixth power law of Ilkovič has been firmly established experimentally by measurements with a torsion thread galvanometer of 0·01 sec. period of swing (*Collection of Czechoslovak Chemical Communications*, **8**, 1936, p. 31), and also by the fact that the diffusion current, i_d, varies as the square root of the height of the reservoir, h, as was pointed out at the beginning of this paper (*Collection*, **6**, 1934, p. 510). Thus the one-sixth power holds equally for momentary currents and for the mean current. If a one-third power law held, then i_d would be proportional to the cube root of h (for i_d would equal $k.m^{2/3}.t^{1/3} = k'.h^{2/3}.h^{-1/3}$); and if a two-thirds power law held, then i_d would be independent of h (for i_d would equal $k.m^{2/3}.t^{2/3} = k'.h^{2/3}.h^{-2/3}$). Experimentally the two latter cases are found only when a kinetic reaction takes place at the interphase (see *e.g.*, Wiesner, *Collection*, **12**, 1947, p.64), or when motion of the electrolyte occurs (as with maxima, when $i = k.t^{1/3}$). Turbulence was never observed in the outflow of mercury, and as the rate of outflow is constant and given by the height of the mercury reservoir, the mass of the drop must be a linear function of time. There is a small capillary back-pressure at the beginning of the formation of each drop, amounting to a few per cent. of h and disappearing as the drop grows; this small effect, however, cannot account for the discrepancy referred to by Mr. Steghart.

Ilkovič's law was derived on the assumption that each particle of the depolariser, say a cation, is deposited as soon as it touches the electrode. However, oscillographic investigations carried out by the present author (see *e.g.*, Faraday Soc. Discussion on Electrode Reactions, April, 1947), show that the transfer of two or more electrons, *e.g.*, in the deposition of $Cu^{\cdot\cdot}$ or $In^{\cdot\cdot\cdot}$ ions, involves consecutive (dismutation) reactions; in such instances—encountered in sulphate or nitrite solutions—the electro-deposition is retarded and the assumption of Ilkovič does not hold; consequently, also the one-sixth power relationship changes towards the one-third power. But such cases are anomalous. As soon as excess of chloride is added, the law of Ilkovič holds, just as it holds in the electro-deposition of any univalent cations ($Tl^{\cdot}$, $Na^{\cdot}$, $K^{\cdot}$) in any solution.

The Determination of Acetyl Groups by Trans-Esterification

By R. G. STUART

The method of assay of acetyl groups by catalysed trans-esterification to ethyl acetate was investigated by Matchett and Levine.[1] They used absolute alcohol as a solvent, concentrated hydrochloric acid as a catalyst, fractionation through a column packed with glass helices, and intermittent small-volume take-offs into a receiver charged with excess of alcoholic alkali. The take-off device resembled that shown at E in Figure 1. The efficiency of the method depended on the difference in boiling-points between the ternary mixture, water, alcohol and ester, and the binary, alcohol and water. O-Acetyl compounds were given about two hours fractionation, from 2 ml. of acid in 50 ml. of alcohol, with a 2-ml. take-off every 15 min. N-Acetyl compounds, reacting more slowly, received four hours treatment, with 3 ml. of catalyst and similar take-offs, at half-hour intervals.

In these laboratories, the first attempt to use this method was made on N-acetyl-phenetidine, which is used as an "analytical standard" for acetyl groups. For quantitative recovery, it was found necessary to distil a large volume of alcohol (more than 100 ml.) and in doing so to refill the still-pot several times. The subsequent titrations had an unduly large possible error, and the time of distillation exceeded 8 hours.

To reduce inaccuracy and running costs, an apparatus was designed (Fig. 1) to allow the redistillation of the alcohol from the receiver into the still-head at the same rate as that from the still-pot into the receiver. It was then possible to examine rates of recovery of ethyl acetate under standardised conditions, the volume in the two flasks remaining constant. At this stage it was found that introduction of a few per cent. of water or of methyl alcohol caused no significant change in rates of recovery, but anhydrous ethanol was retained as a reagent because of greater ease of purification.

Apparatus (Fig. 1)

The still-pot A (100-ml. round-bottomed pyrex flask, B24 neck, with lugs) is attached by springs to the fractionating column C, which has an effective length of 90 cm., an effective bore of 14 mm., a vacuum jacket (pressure about 1 micron Hg), a working hold-up of 7 ml., a static hold-up of 3 ml., a through-put, just before flooding, of about 20 ml. per minute, and a filling of 4 mm. of Fenske single-turn glass helices.[2,3]

The still-head includes a total reflux condenser D into the U-tube E. This U-tube has a variable constant-ratio take-off through tap F into a visual drip-feed into condenser R, which cools the take-off and refluxes the reagent alcoholic potash in flask B (a replica of flask A). The tube between condenser R and flask B is connected by a 10-mm. bore vertical lagged air tube to a small reservoir P, above tap G, which controls a visual drip-feed into the top of condenser D. Condenser H is a total reflux into reservoir P, and condenser K cools the pressure outlet to flask A and the fractionating column.

The two outlets to the whole system, the tops of condensers H and K, are capped by soda-lime tubes L, attached *via* B10 joints.

For strength, the four condensers are jointed by 10-mm. bore water-carrying tubes, the whole still-head assembly being of glass blown in one piece, with its centre of gravity near enough to that of the fractionating column to allow it to be rested in and supported by the B34 joint which tops the column. The column is clamped at two symmetrical points to the bench framework, and the stillhead is lightly clamped round the jacket of the air-tube at X.

Flasks A and B are heated by small electric heaters, each of about 500 watts, and the upper halves of the glass joints are lightly greased with petroleum jelly.

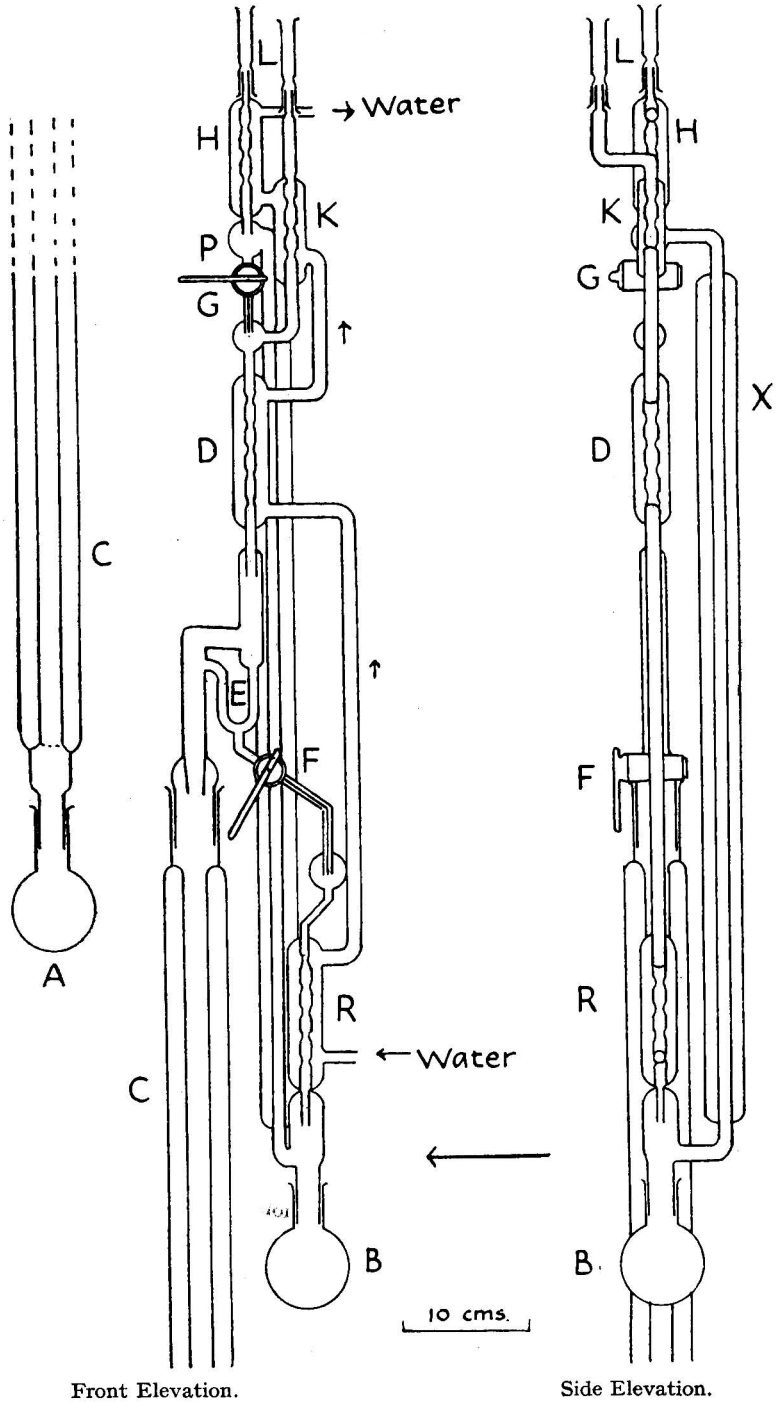
Method of operation

Macro analysis

Reagents—

(1) *Anhydrous ethanol 99·5 per cent.*—This is freed from aldehyde by distillation in vacuum from 1 per cent. of sodium metal and subsequent fractionation through column C (Fig. 1), using 250-ml. flasks and charging the still-pot with 200 ml. The first 50 ml. contain all the remaining aldehyde bodies, and the bulked first fractions are re-fractionated, separating

the first 50 ml. The bulked main fractions, 140 ml. each, are stored in rubber-stoppered amber bottles.



Front Elevation. Side Elevation.

Fig. 1.

(2) *Potassium hydroxide, 0·8 per cent. solution in the purified alcohol*—This solution, which is about 0·11 *N*, is colourless and remains so indefinitely when exposed to daylight. It is stored in a rubber-stoppered white glass bottle, allowed to stand two days for carbonate

to precipitate and settle, and then pipetted as required from the supernatant clear layer. The temperature correction is about 0·1 per cent. per degree, and the titre changes very slowly, a new standardisation being required monthly.

(3) *Hydrochloric acid, concentrated AnalaR*—35 per cent. of HCl in water.

PROCEDURE—

Charge flask A with 45 ml. of the alcohol, 3 ml. of concentrated hydrochloric acid, a few carborundum chips, and enough of the sample under test to yield 0·043 to 0·086 g. of CH_3CO, *i.e.*, requiring 10 to 20 ml. of 0·1 *N* potassium hydroxide. Charge flask B with 20 ml. of 0·1 *N* potassium hydroxide and 15 ml. of alcohol.

Boil the contents of both A and B until U-tube E and reservoir P are filled with alcohol, and adjust both drip-feeds to about one drop per second. The relative drop sizes of the two feeds have been previously found by experiment to have the ratio 6 : 5, and the rates can be adjusted sufficiently accurately by eye to keep the volumes in the flasks constant, that is, about 35 ml. in each.

Distil from both flasks for one hour from the initial time of boiling of flask A, turn off the taps, and titrate the contents of flask B with 0·1 *N* sulphuric acid, using phenolphthalein as indicator. Titrate a "blank" on the 0·1 *N* potassium hydroxide alone. Deduct 0·15 ml. from the net reading to allow for carbon dioxide in the alcohol (0·1 ml.), and for alkali absorbed by the glass of flask B (0·05 ml.). Carborundum chips are not used in flask B because they are susceptible to attack by alkali. The carbon dioxide in the system becomes concentrated in flask B, and the correction is found from a blank titration on a portion of the alcohol, which does not change in this respect on storage.

It is unnecessary to boil flask B for longer than one hour, because, when the rate of reaction has been found from the first hour's run, the time required to reach a quantitative yield is read off graphically (see Fig. 3 and p. 238); flask B is charged with excess of 0·1 *N* potassium hydroxide, plus alcohol to make up to 35 ml., and distillation from it is resumed for an hour after flask A has been boiling for an hour less than the total time.

For the final back-titration the correction is 0·05 ml. of 0·1 *N* potassium hydroxide.

It is unnecessary to clean the apparatus internally, and it is mounted semi-permanently. No hydrochloric acid remains in the column after draining, and if necessary alcohol can be removed from the column by aspirating a stream of air downwards through it after removal of the soda-lime tubes. (This is sometimes advisable, to remove stale alcohol before an assay.)

With known O-acetyl compounds, one distillation, over a time of two hours, is sufficient.

RESULTS—

To check the efficiency of fractionation, ethyl acetate itself was distilled in the apparatus, with the quantities of reagents as specified, 92 per cent. was recovered in 30 minutes, and 99·5 per cent. in the first hour. From sodium acetate the equivalent of 99·8 per cent. was recovered, as ethyl acetate, in one hour.

For many O-acetyl compounds and N-acetyl compounds curves were drawn of the rates of acetyl recovery (Fig. 2). For all O-acetyl bodies, 99·9 per cent. was recovered in the first two hours; but for N-acetyl compounds the rate varied considerably with the molecular structure, although it was remarkably constant for each compound.

Compound	*% Recovered*
(1) Ethyl acetate	30 min., 92·6; 1 hr., 99·5.
(2) Acetylsalicylic acid	1 hr., 98·0; 2 hr., 99·9.
(3) Acetanilide	1 hr., 59·5; 2 hr., 82·9; 3 hr., 93·1; 7 hr., 99·8.
(4) N-Acetylphenetidine	1 hr., 40·5; 2 hr., 65·0; 5 hr., 93·2; 12 hr., 99·9.
(5) Toluene-azo-diacetylaminotoluene, 2:1:1:4:3	1 hr., 79·2; 3·5 hr., 97·8; 12 hr., 100·2.
(6) N-Methylacetanilide	1 hr., 10·4; 4 hr., 33·2; 8 hr., 59·1.
(7) N-Ethylacetanilide	2 hr., 9·6; 9 hr., 33·3.
(8) Diacetyl-*p*-phenylene-diamine	1 hr., 70·6; 2 hr., 91·4; 3 hr., 97·5; 5 hr., 99·8.
(9) Triacetoxybenzene, 1:2:3	2 hr., 100·0.
(10) Triacetoxybenzene, 1:2:4	2 hr., 100·1.

Other O-acetyl compounds tested included acetic esters of higher alcohols, phenols and sugars; without exception, all the ethyl acetate was recovered in two hours. The same was

true with crystalline vinyl acetate, with 98 per cent. of the theoretical amount in the unpolymerised body; further distillation gave no yield.

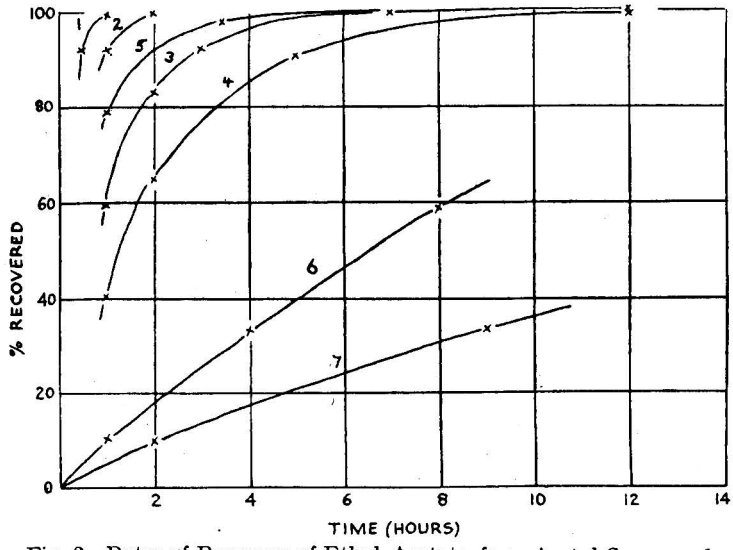


Fig. 2. Rates of Recovery of Ethyl Acetate from Acetyl Compounds.

(1) Ethyl acetate
(2) Acetylsalicylic acid
(3) Acetanilide
(4) N-Acetylphenetidine
(5) 2:1:1:4:3-Toluene-azo-diacetylaminotoluene
(6) N-Methyl-acetanilide
(7) N-Ethylacetanilide

MATHEMATICAL INTERPRETATION—

Every recovery curve obtained so far, including the fractionation curve for ethyl acetate, has been asymptotic and exponential in form; that is, the rate of recovery depends on the concentration of unchanged acetyl body, and the reactions follow the unimolecular law. From this it follows that the shape of each curve should be invariable under constant conditions, and *independent of the weight of acetyl body*. Application of the method to the micro scale, with the same apparatus, showed this to be true.

Exponential form—If the fraction of the original sample that remains unaltered in the still-pot after unit time is a, then after x units of time the fraction, y, remaining will be a^x. Hence, $\log y = x \log a$, and, as for each compound a is a constant, $\log y$ is proportional to x.

Hence a curve in which $\log y$ is plotted against time should be a straight line. The typical constant for each compound is the fraction remaining after unit time.

In practice, the percentage *recovered* is plotted against time, using a log scale, inverted, to measure percentages as ordinates, and a linear scale to measure times as abscissae. The first log cycle is calibrated up to 90 per cent., the second up to 99 per cent., and the third up to 99·9 per cent. (see Fig. 3).

One point in the curve is plotted, and a straight line, drawn through it and the origin, will cut the 99·9 per cent. line at the time required to reach that figure in the trans-esterification. (For convenience, a sheet of graph paper is mounted on a stiff backing, with a cord attached at the zero point.) This procedure is of course only necessary with N-acetyl derivatives.

ANALYSIS OF "UNKNOWN" SAMPLES—

Method—Take burette reading after two equal units of time.

Let a = titration after unit time
b = total titration after 2 units of time.

From these two values the titration, T, corresponding to the completion of the reaction, can be calculated from the formula $T = a^2/(2a - b)$, which is easily derivable from the relations given above.

The percentage recovery in the first unit of time is $\frac{100a}{T} = \left(2 - \frac{b}{a}\right)100$.

From this percentage, the time required to reach, say, 99·9 per cent. is read off on the graph, and the distillation completed.

These methods have been used in the analysis of research intermediates, and the rate of reaction can be used to suggest the location of the acetyl group, although the possibility of more than one type being present must be borne in mind.

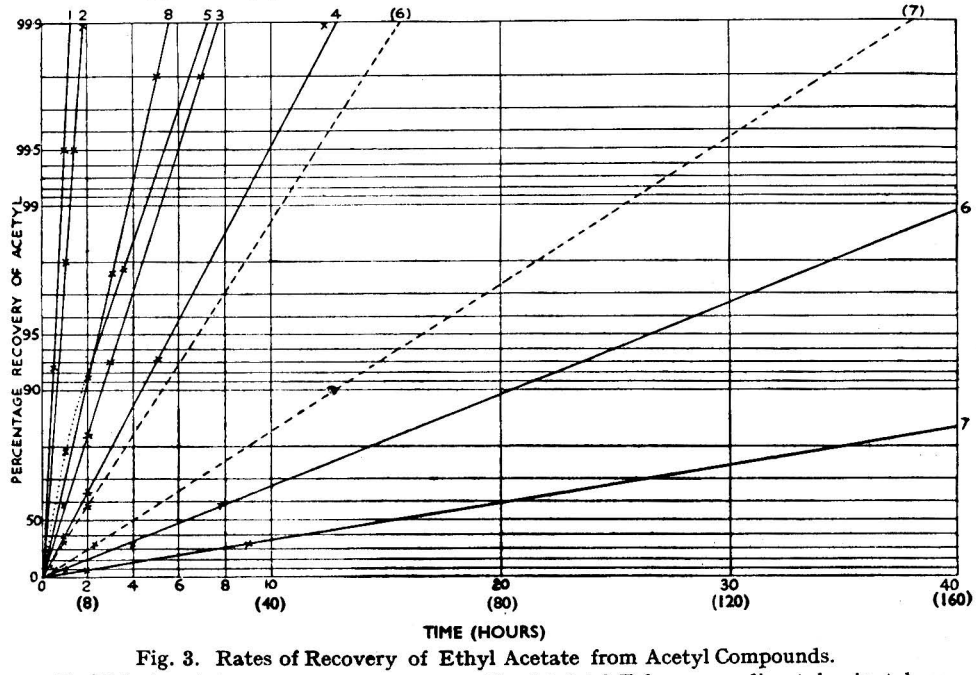


Fig. 3. Rates of Recovery of Ethyl Acetate from Acetyl Compounds.

(1) Ethyl acetate
(2) Acetylsalicylic acid
(3) Acetanilide
(4) N-Acetylphenetidine
(5) 2:1:1:4:3-Toluene-azo-diacetylaminotoluene
(6) N-Methylacetanilide
(7) N-Ethylacetanilide
(8) Diacetyl-*p*-phenylenediamine

N.B.—Dotted lines (6) and (7) indicate a reduced time-scale for 6 and 7; the corresponding time figures are given in parenthesis at the bottom of the graph.

REVERSIBILITY OF REACTION (on cooling)—

The necessity of sometimes allowing the still-pot to stand overnight, and to continue its heating next day, made advisable a check on the effect of this. Using acetanilide, refluxing two hours, standing overnight and then distilling one hour, 93·5 per cent. was recovered. From the exponential curve for this compound (measured in one day, without cooling) the theoretical recovery is 93 per cent. in 3 hours. It follows that the reversibility of the reaction under these conditions is not significant and that probably a small amount of trans-esterification occurs during cooling and re-warming. The rapid removal of ethyl acetate from the reaction chamber into the U-tube will reduce the reverse reaction, if any occurs. O-Acetyl compounds were not considered.

EFFECT OF CONCENTRATION OF CATALYST—

Following are results obtained with acetanilide and acetylsalicylic acid when different amounts of hydrochloric acid were used.

(1) Acetanilide: 1 hour's distillation and trans-esterification.

1·5 ml. of hydrochloric acid;	45 ml. of alcohol;	acetyl recovery	38·5	per cent.
3·0 ,, ,, ,, ,, ;	45 ,, ,, ,, ;	,, ,,	59·5	,, ,,
6·0 ,, ,, ,, ,, ;	45 ,, ,, ,, ;	,, ,,	75·0	,, ,,

(2) Acetyl-salicylic acid: 1 hour.

1·5 ml. of hydrochloric acid;	45 ml. of alcohol;	acetyl recovery	96·8	per cent.
3·0 ,, ,, ,, ,, ;	45 ,, ,, ,, ;	,, ,,	99·0	,, ,,
3·0 ,, ,, ,, ,, ;	45 ,, ,, ,, ;	,, ,,	97·8	,, ,,
6·0 ,, ,, ,, ,, ;	45 ,, ,, ,, ;	,, ,,	98·8	,, ,,

From these figures it appears that an O-acetyl trans-esterification is not significantly affected by alteration of the concentration of hydrochloric acid, which appears to act as a true catalyst. With N-acetyl compounds, the rate plotted against the concentration of acid appears to follow a curve similar in shape to the trans-esterification curves. The application of this to the speeding of reactions is limited by the capacity of the column to retain hydrochloric acid vapour under the working conditions. When 3 ml. of concentrated hydrochloric acid were used, the quantity of chloride in the distillate was not significant, even when, on one occasion, flask A was accidentally allowed to run dry; but with 6 ml. of the acid its rate of distillation was equivalent to 0·15 ml. of 0·1 *N* per hour. The column could be lengthened to increase retention.

For convenience, the volume of hydrochloric acid used in all determinations was kept at 3 ml.

Micro analysis

Method—

The distillation rates are left unchanged, but the total volume of alcohol used is reduced from 80 ml. to 60 ml. In flask A are 35 ml. of alcohol and 3 ml. of concentrated hydrochloric acid, and in flask B, 20 ml. of 0·01 *N* alcoholic potassium hydroxide plus 5 ml. of alcohol. Enough sample is weighed to give a net titration of less than 10 ml., the back titration being carried out with 0·01 *N* sulphuric acid, and neutralised phenolphthalein as indicator. As before the only significant errors are due to carbon dioxide in the alcohol (usually $\equiv$ 0·4 ml. of 0·01 *N* per 20 ml.), and to the effect of boiling 0·01 *N* alkali in a glass flask (usually $\equiv$ 0·2 ml. of 0·01 *N* per 20 ml. per hour). In practice, only a blank titration on 20 ml. of alcohol is necessary. There is less margin of error for variations in rate of distillation and re-distillation; but the rate of trans-esterification of N-acetyl-compounds is increased about 10 per cent. The relatively larger excess of 0·01 *N* potassium hydroxide in flask B is to ensure that hydrolysis of the distilled ethyl acetate will be rapid enough to prevent its remaining in circulation.

Results—

With known compounds the curves obtained were, as expected, similar to those obtained on the macro scale, with an approximately 10 per cent. increase in the rates of reaction of N-acetyl compounds. The method has been applied in the analysis of a number of research intermediates, both for percentages and for identification.

Discussion

A trans-esterification under these conditions is essentially a hydrolysis of the acetyl compound, followed by an esterification of the acetic acid. This esterification is in all cases at a uniform rate, not significantly affected by the concentration of catalyst. The wide differences in the rates of recovery depend solely on the rates of initial hydrolysis. This rate is fast and uniform throughout a wide range of O-acetyl compounds, and is of the same order as the rate of esterification, because the two together give a rate of recovery not far below that for ethyl acetate. (The exponential form of the straight distillation is due to the mechanism of fractionation, including the slight washing-out effect of the distillate from flask B crossing the inflowing distillate from flask A).

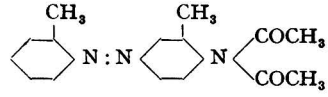
It follows that for O-acetyl compounds the reaction at the boiling-point is almost instantaneous and ionic and depends on the hydrogen ion concentration, which itself has a maximum not affected by a large excess of strong acid.

With N-acetyl compounds, the rate of hydrolysis depends on the concentration of hydrogen chloride, in accordance with Dawson's "Dual Theory of Acid Catalysts," which ascribes the catalytic effect to the concentration of HCl molecules, in addition to that of H and Cl ions. This leaves a wide field of investigation into other causes of variation, namely, the shape of the molecule and the effect of substitution, especially of the spare H atom of the acetylamino group. A recent experiment points to the molecular shape as the prime influence; because N-acetyl-diphenylamine reacts more rapidly than methyl acetanilide, and this more rapidly than the ethyl derivative.

The difficulty of dislodging the strongly held N-acetyl by collision with hydrochloric acid molecules or ions is increased by a protective effect of substituted groups and by the subsequent decrease in polarity, which probably more than counterbalances the decreased strength of the bond holding the acetyl group. Once the dislodging has occurred, the acetyl group will be esterified, the chances of the necessary collision being very high, for there are initially,

with 0·1 *N* solution, about twenty hydrochloric acid molecules to every acetyl. Also the freed amino group will be much more likely to attach a hydrochloric acid molecule than a free acetyl group. With the large excess of hydrochloric acid molecules, the collision rate will depend on the concentration of acetyl compound only; hence the first-order reaction, characterised by an exponential curve.

An interesting case is that of 2:1:1:4:3-toluene-azo-diacetylaminotoluene—



This compound behaves as would a mixture of an O-acetyl compound yielding 100 per cent. of the theoretical amount of acetyl in two hours, and an N-acetyl compound yielding 58 per cent. in one hour. A mixture of this kind would give 79 per cent. of the total acetyl in one hour, 96 per cent. in three hours, and 99·8 per cent. in six hours, figures which agree with the curve obtained. It would be expected that one group would be released, or expelled, at the same rate, approximately, as an O-acetyl group, and that the remaining group would be more strongly held. The derived figure of 58 per cent. per hour for the mono-acetyl derivative is similar to that for acetanilide, faster than that for acetyl-phenetidine (40·5 per cent.) and slower than that for diacetyl-*p*-phenylenediamine (70·6 per cent.).

Every trans-esterification of an N-acetyl compound so far investigated has followed a fixed unalterable rate with remarkable accuracy, so much so that any variation from the theoretical curve showed an error in the technique, and thus helped in the improvement of design and method.

SUMMARY—

Determination of acetyl groups led to the investigation of the rate of trans-esterification of acetyl compounds to ethyl acetate, with hydrochloric acid as catalyst, and to the design of an apparatus which improved the accuracy of the method. All trans-esterifications followed exponential curves, the shape of the curve depending on the molecular structure of the acetyl compound. The time required to reach sufficiently quantitative yields was arrived at graphically. O-acetyl compounds yielded 100 per cent. after two hours. N-acetyl compounds gave rates depending on the substitution of the N hydrogen atom, on the substituent groups in other parts of the molecule and on the concentration of hydrochloric acid.

My thanks are due to Mr. I. J. Fine for the glass-blowing construction, to Mr. T. Tusting Cocking for his advice and encouragement in the experiments described and to the Directors of The British Drug Houses, Ltd., for their permission to publish the results.

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GRAHAM STREET, CITY ROAD
LONDON, N.1

March, 1947

The Determination of Micro-Quantities of some Derivatives of Phenarsazine

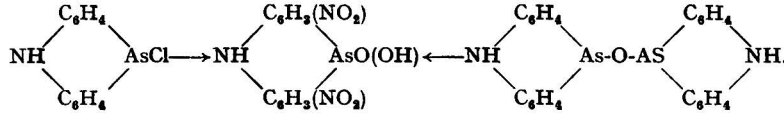
BY H. BARNES

THE usual method for the determination of derivatives of phenarsazine involves wet oxidation of the compound followed by determination of the contained arsenic (Sartori[6]). When only small quantities of the arsenical are available either the Gutzeit method or the determination of the arsenic (after reduction and volatilisation as trichloride or as arsine) by the ammonium molybdate reagent, could no doubt be used. Delga[1] has described a colour reaction of phenarsazine chloride; a yellow colour is obtained when aqueous solutions of this substance, in concentrations of 8 mg. or more per litre, are treated with a reagent consisting of silver nitrate in glacial acetic acid.

This paper describes a simple, sensitive, although not specific method for determining some phenarsazine derivatives. Up to the present it has been principally used with pure

solutions of the arsenicals in organic solvents (see, however, p. 243), but it has proved of considerable value in toxicity work. It depends upon the action of concentrated nitric acid on the arsenical and the subsequent addition of sodium hydroxide, whereupon an intensely red coloured solution is obtained.

The nitro derivatives of 10-chloro-5 : 10-dihydrophenarsazine and the corresponding phenarsazinic acids yield red salts with sodium hydroxide when the nitro groups are in the 2 and 8 positions (Gibson and Johnson,[2] Raiziss and Gavron,[5] Wieland and Rheinheimer[8]). Further, Schmidt[7] has shown that hot nitric acid oxidises and nitrates 10:10′-oxy-5:10-dihydrophenarsazine. It is suggested, therefore, that under the conditions used in the method given below, both oxidation and nitration take place with the production of the 2:8-dinitro derivative of the phenarsazinic acid, which on addition of sodium hydroxide yields the intensely red coloured quinonoid aci-salt.



METHOD

Reagents—

Nitric acid (A.R.), concentrated.

Aqueous acetone, 50 per cent. v/v solution.

Sodium hydroxide, (A.R.), 10 per cent. aqueous solution.

Sodium potassium tartrate (A.R.), 10 per cent. aqueous solution.

Procedure—

Transfer the test solution to an evaporating dish and remove the solvent on a hot water bath. Add 2 ml. of nitric acid and evaporate just to dryness on a vigorously boiling water-bath. After cooling, dissolve the residue in 10 ml. of aqueous acetone with the aid of a rubber-tipped glass rod and add 1 ml. of the tartrate solution. Add 0·4 ml. of sodium hydroxide solution with stirring. A red colour is produced on the addition of the alkali. Transfer the solution to the absorptiometer cell and measure the transmittance. Calibration curves may be obtained by the use of benzene solutions of phenarsazine derivatives. A blank should be run on the reagents and solvents used.

Measurement of transmittance—

For the determination of transmittances the Spekker photo-electric absorptiometer has been employed throughout, with a 1-cm. cell and green filters (Ilford 604). The instrument is set at 0·400 against distilled water. It has been the practice first to set the instrument and take a reading of the unknown; the instrument is then reset and, with the shutter closed, the drum is moved into the previous position of balance. The unknown is then placed in position and the final adjustment made rapidly.

DISCUSSION

1. The method has been applied to phenarsazine chloride (5-chloro-5:10-dihydrophenarsazine), phenarsazine oxide (10:10′-oxy-5:10-dihydrophenarsazine) and 5-ethyl-5:10-dihydrophenarsazine. Preliminary tests have indicated that the method could be applied to other derivatives of phenarsazine and to compounds with a similar structure.

2. The maximum absorption is obtained with use of green filters (Ilford 604). With a 1-cm. cell the calibration curves are linear over the range 5 to 25 μg. for all the above substances.

3. The limit of the method as given is of the order of 2 μg. of arsenical. It should be possible to determine smaller quantities by reducing the volume of the final solution, but this would necessitate the use of a smaller cell than the standard 1 cm. Spekker cell (approximate volume 10 ml.) and slight changes in the technique may then be found necessary.

4. The spread of the calibration curve indicates that differences of 1 μg. correspond approximately to a drum difference reading of 0·010 (*i.e.*, one drum division).

5. The tartrate solution prevents the precipitation, when the solution is made alkaline, of any small quantities of impurities carried over from the reagents.

6. Care should be taken to bring all the material into solution when the aqueous acetone and the alkali are added; the upper portions of the dish should be carefully rubbed down since the substances show a tendency to "creep."

7. The transmittances should be determined within a short time; the red colour fades, particularly if the solution is left in the evaporating dish. In a covered cell no significant change takes place within 10 minutes.

8. Table I gives a series of results obtained by the method.

TABLE I

AMOUNTS IN MICROGRAMS

Phenarsazine chloride		5-Ethyl derivative		Phenarsazine oxide	
Present	Found	Present	Found	Present	Found
3·4	4·0	16·4	17·2	16·8	16·8
5·0	5·8	—	—	—	—
4·4	4·0	25·2	25·6	15·6	16·2
6·2	5·4	29·6	30·4	20·0	19·4
11·8	11·4	25·6	25·6	23·2	22·6
12·2	12·2	20·0	19·0	26·4	25·2
12·8	13·4	13·2	12·0	17·6	16·8
14·0	13·6	10·4	10·6	28·4	27·2
14·8	14·8	—	—	15·6	15·0
15·0	14·8	—	—	—	—
15·2	15·0	—	—	—	—
15·2	15·4	—	—	—	—
15·2	15·2	—	—	—	—
25·0	24·0	—	—	—	—
28·0	27·6	—	—	—	—

THE DETERMINATION OF PHENARSAZINE CHLORIDE IN AQUEOUS SOLUTION

Derivatives of phenarsazine have been used in experimental anti-fouling compositions[3] and assessments of raft exposures have indicated their effectiveness. In recent work on the behaviour of anti-fouling compositions containing cuprous oxide as the poisonous pigment, a so-called leaching technique has been developed (Harris,[3] Ketchum *et alia*[4]). Painted panels (3″ × 1″ microscope slides, 12·9 sq.cm. painted area) are exposed in the sea and at regular intervals the rate of loss of cuprous oxide (leaching rate) is measured by a standardised laboratory method. These tests of leaching rate have proved to be of great value in interpreting the behaviour of anti-fouling compositions and the technique has been adapted to investigate the rate of release of phenarsazine derivatives from compositions containing them, with or without cuprous oxide. In a four-hours leaching test, using 60 ml. of sea water, a "normal" composition will lose up to 30 μg. of arsenical. A relatively simple method was required, so that large numbers of test panels could be examined, and an application of the method outlined above appeared possible. Preliminary investigations indicated that under the prescribed conditions, in a slightly alkaline medium, hydrolysis of small amounts of phenarsazine chloride was virtually complete and the following method has been used for sea water solutions of this substance; in view of the rather specialised application only a brief description is given. The method must be regarded as of a preliminary nature, since the work has been interrupted before a more complete investigation was possible.*

The sea water extract from the leaching test (60 ml.) is transferred to a 250 ml. separating funnel and shaken for three minutes with an equal volume of chloroform; after the two layers have separated the chloroform layer is run off into a small distilling flask and the major portion of the solvent removed on the water bath. The remaining solution (about 3 ml.) is transferred to an evaporating dish, the flask is rinsed twice with small amounts of chloroform and the washings are added to the bulk. The solvent is then completely removed on a hot water bath and the residue treated exactly as in the direct procedure already described. (Under certain circumstances it may be necessary to centrifuge the solution after development of the red colour). A calibration curve is constructed using sea water solutions of phenarsazine chloride which have been standing for some hours. In order to obtain satisfactory results it is essential to adhere to standardised conditions. Blanks are run on extracts from panels painted with a composition in which the arsenical is replaced by an inert pigment.

* A progressive hydrolysis on shaking a benzene solution of phenarsazinechloride with alkaline solutions has been demonstrated by analysis of the benzene layer after varying periods. This hydrolysis is suppressed on replacing the slightly alkaline medium by concentrated hydrochloric acid. Preliminary work indicates that as with most substituted derivatives of arsenic trichloride, phenarsazine chloride is comparatively readily hydrolysed—the hydrolysis being difficult to detect or determine owing to the extreme insolubility of the product in water.

The distribution of the hydrolysis product is in favour of the water and this, together with the more involved procedure, renders the results less accurate than those of the direct method. Nevertheless, several hundred determinations by the method have indicated that it is capable of yielding useful information on the behaviour of phenarsazine chloride in anti-fouling paints.

The author is indebted to the Marine Corrosion Sub-Committee of the Iron and Steel Institute for permission to publish this work and to Professor J. E. Harris for his interest in it.

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THE MARINE STATION
MILLPORT, SCOTLAND

February, 1947

The Separation of Crystals and "Gums" on the Micro or Semimicro Scale

BY A. L. BACHARACH

(*Communicated to the Annual General Meeting of the Microchemistry Group on January 31st*, 1947)

IT often happens during work on the isolation of individual compounds from complex materials of biological origin—and also, doubtless, during preparative and synthetic operations—that a rather intractable mixture is obtained, consisting of a relatively small quantity of obviously crystalline nature embedded in a more or less viscous fluid. Recrystallisation of such mixtures, with a view to isolating the crystalline constituent, is at best fraught with appreciable losses; at worst, it results in the whole of the mixture being taken up in the solvent and ultimate recovery, by evaporation, of the fluid material holding the crystals no longer in suspension but in solution. It is a common experience that such crystalline material is more soluble in a solution of the accompanying "impurities" than in the solvent used; this accounts for the fact that purification by recrystallisation tends to become progressively easier as the fluid material is proportionately reduced. In this business it is the first step that counts (and may lose) most.

For this first step there are also available chromatographic methods. Elegant and effective though these so often are, for a particular problem under consideration they involve a search for the right adsorbent and eluent, as well as several manipulative stages. What the chemist wants in the circumstances is a simple method that is so rapid as to reduce to a minimum waste of time in examining what may ultimately turn out to be crystalline material of no interest. (In work connected with the extraction of "active principles" from vegetable drugs—plants, leaves, roots—it is surprising how often the first crop of crystalline matter was found to be ammonium oxalate!)

It therefore seems that a description might with advantage be given of a procedure first shown to me many years ago by the late Dr. Frank Tutin and now modified in two directions. On various occasions during the past twenty years the procedure has been described or demonstrated to colleagues working in the laboratory and the matter has generally been treated with indifference or scepticism, but recently Dr. C. H. Gray (private communication) has used the technique for recovering crystalline stercobilin hydrochloride from faeces. By this means he has on occasion been able to improve the yield of pure material by as much as 30 per cent.

Although the mechanism of the procedure was never explained by Tutin—a man, in any event, not over-much given to theorising—the experiments described below make it clear that it is merely an application of those phenomena of diffusion so brilliantly exploited by Conway for quantitative micro-analysis. In essence the device involves separating "gum" from crystals on porous plate, using a solvent and yet never wetting the mixture with the solvent.

In the original technique a fragment of unglazed plate or tile—from 1 to 4 or 6 sq.cm. in area, and of any outline, but preferably as flat as possible,—was selected from the pieces of plate produced by dropping one on a hard surface. A quantity of the mixed crystals and gum is spread uniformly on the fragment, in a layer a few mm. thick, and the fragment is laid ("butter-side up") on a smooth surface, such as a glazed tile or a sheet of glass. An ordinary glass funnel is inverted over the fragment, with the stem upwards and open to the air. Solvent from a wash-bottle is then blown round the outside of the funnel where this makes contact with the smooth tile or glass, so as to give a sealing ring of solvent (Fig. 1). When this has evaporated beyond a certain point, the ring will be broken and must be replenished with more solvent. It will be found that after a comparatively short time, depending on the solvent chosen, the solubility of the gum, the viscosity of the mixture, the relative amounts of gum and crystals and so on, the gum has penetrated into the porous plate, leaving the crystals more or less "high and dry" on the surface and ready for further manipulative treatment. Ordinary recrystallisation, for example, can now be applied, especially on the micro scale, with every prospect of preventing precious material from eluding the investigator altogether. Furthermore, after the crystals have been scraped off the porous plate, the gummy material—often the more interesting constituent for further chemical study—can be recovered by simply extracting it from the porous plate in a Soxhlet or other suitable apparatus.

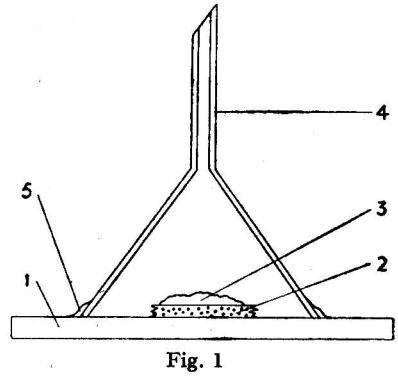

Fig. 1

1. Smooth tile. 2. Porous plate.
3. Mixture under treatment.
4. Glass funnel. 5. Solvent "seal."

The chief objections to this procedure as formerly practised, and possibly as originally devised, by Tutin are two: first, waste of solvent, because of continuous evaporation; secondly, and more important, the need for frequent renewal of the solvent and thus for continuous attention, so that ordinarily the process cannot be allowed to continue without aid overnight. As clearance may be achieved in two or three working days, say, 24 hours, continuous action overnight should save one or two days.

It seemed reasonable to assume that similar results might be achieved on a micro-scale by use of the Conway diffusion unit. The only material available for putting this to practical test was some commercial "carotene paste," consisting of a mass of small carotene crystals embedded in a greenish semi-fluid matrix. The fluid portion could pass through a porous plate without the presence of any solvent, but it took many days to do so, and the residual crystals were by no means cleanly separated. If, however, the tile fragment and the attached carotene paste were put in the inner chamber of a Conway unit, the outer chamber being charged with a little acetone and the whole unit covered with the usual ground glass square plate, almost complete separation was observed in 24 hours or less, without any attention for replenishing the solvent. The Conway unit is, of course, designed to secure maximum diffusion with minimum loss by evaporation, and that is exactly what is wanted for separations of this kind.

For larger scale work the method is still applicable by using an arrangement of two glass cylindrical flat-bottomed evaporating dishes, one inside the other, with an annular space between, to give what is in fact a macro Conway unit that can be taken apart. Using the same carotene paste, I found that ether was much more rapid and effective in "forcing" the gum into the plate than was acetone, although evaporation losses with this set-up may be relatively as well as absolutely rather greater than with the Conway micro unit. Nevertheless, three or four such arrangements of two dishes, with a suitable solvent and pieces of tile of about 10 sq.cm., would probably allow a gram or more of carotene to be separated overnight from the paste used.

The process, like recrystallisation and chromatography, can be carried out again on partly purified material, but it is so effective in cleaning up even the most unpromising mixtures that the investigator who uses it will generally be able to proceed at once to those final stages of purification necessary before any quantitative analyses or physico-chemical tests can be properly undertaken.

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The Identification of Phenol from Synthetic and Natural Sources

By E. J. BOORMAN, C. G. DAUBNEY, and A. E. MARTIN

A number of specimens of phenol, derived from three sources, namely (1) alkali fusion of benzenesulphonic acid, (2) high temperature hydrolysis of chlorobenzene, and (3) fractionation of coal tar, have been examined in order to find whether these three types of phenol could be distinguished analytically with certainty, it being assumed that the material may be so discoloured as to prevent the use of the simple coloration test by passing of air.

Each class of sample was always found to contain a small but identifiable quantity of characteristic impurity which was absent from samples of different origin. These impurities were, for class (1), *o*- and *p*-hydroxydiphenyls; for class (2), diphenyl ether; for class (3), naphthalene. The *p*-hydroxydiphenyl was usually isolated and identified by analysis (Found: C, 84·4; H, 5·7. Calc.: C, 84·7; H, 5·9 per cent.) and by the melting-point, 164° C., not depressed by admixture with an authentic specimen; but in general the identification was made by observing the infra-red spectrum of a solution of the residue in carbon tetrachloride.

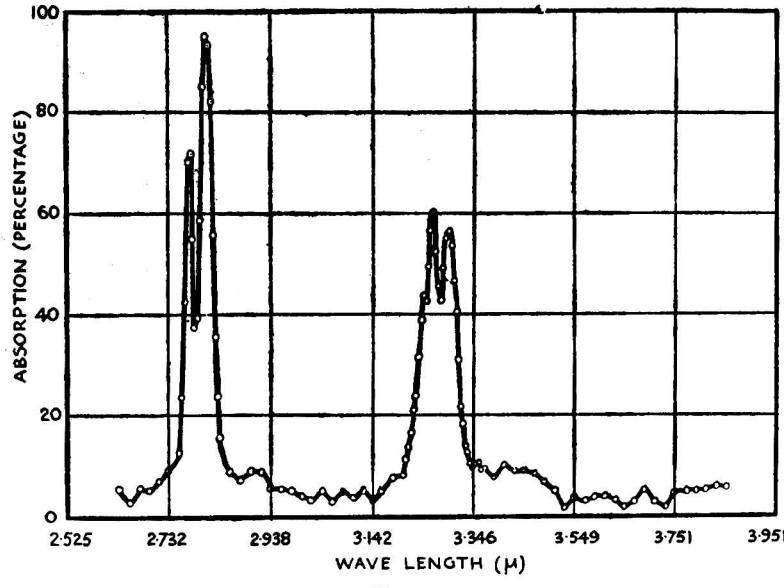

Fig. 1.

Procedure (A)—Distil the sample at 1 to 1·5 mm. pressure (bath at 58° to 60° C.) until distillation ceases. Take up the residue from 1 kilo in ether, filter, evaporate and heat the residue at 100° C. in a current of air to remove traces of phenol. The yield of residue depends on the quality of the original phenol.

Materials of class (1) yield from 0·3 g. to 10 g. of residue, consisting largely of *o*- and *p*-hydroxydiphenyls in the ratio of approximately 4 to 10 parts of *o*- to 1 part of *p*-isomeride. Materials of class (2) or (3) leave not more than a trace of residue. Separate tests showed that added *p*-hydroxydiphenyl in amounts down to 0·02 per cent. can be recovered by this procedure.

Procedure (B)—Dissolve 500 g. of the sample in 800 ml. of sodium hydroxide solution (30 per cent. w/v) and distil in a current of steam until 200 ml. have been collected. Add 80 ml. of the alkali to this, and repeat the steam distillation, collecting 100 ml. and allowing the condenser to become warm towards the end of the operation. Extract the final distillate with 20 ml. of carbon tetrachloride and examine the infra-red spectrum of the solution.

Materials of class (2) show the presence of a trace up to 13 mg. of diphenyl ether from 0·5 kilo of material. Experiments show that added *o*-hydroxydiphenyl does not appear in

this distillate. Materials of class (3) yield from an identifiable trace to 10 mg. of impurity from 200 to 500 g. of sample and infra-red examination reveals the presence of naphthalene.

INFRA-RED EXAMINATION—A grating spectrometer was used (compare Fox and Martin[1]), the solution, in an absorption cell 1 cm. or 5 cm. long, being compared with pure carbon tetrachloride in a second similar cell. For identification and estimation of the hydroxydiphenyls the OH band at 2·77 μ was used for the *p*-isomeride, and for the *o*-isomeride a double band having the weaker limb at the same point. The C–H bands of phenol and *p*-hydroxydiphenyl at 3·283 μ. and 3·295 μ. respectively are distinguishable from each other and also from the related bands for *m*- and *o*-hydroxydiphenyls. Bands in the region of 3·3 to 3·5 μ. are not useful, being obscured by absorption due to oily impurities.

Fig. 1 shows a curve for the residue from a phenol of class (1) with the split-off band characteristic of *o*-hydroxydiphenyl (80 per cent. of *ortho*, 20 per cent. of *para*).

Fig. 2 shows the diphenyl ether bands at 3·256, 3·288 and (weaker) 3·298 μ. in residues from phenols of class (2).

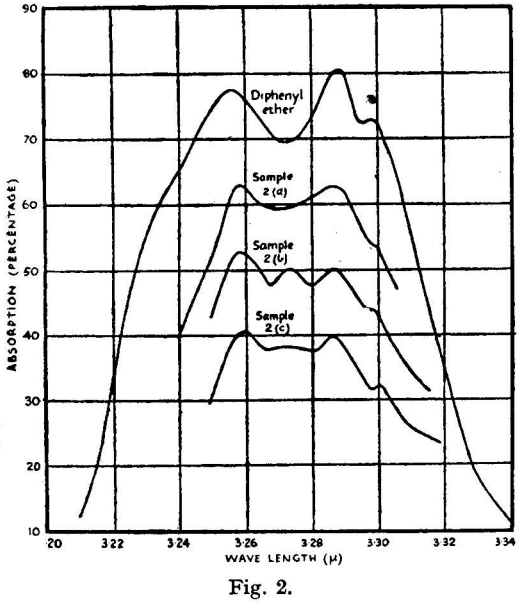

Fig. 2.

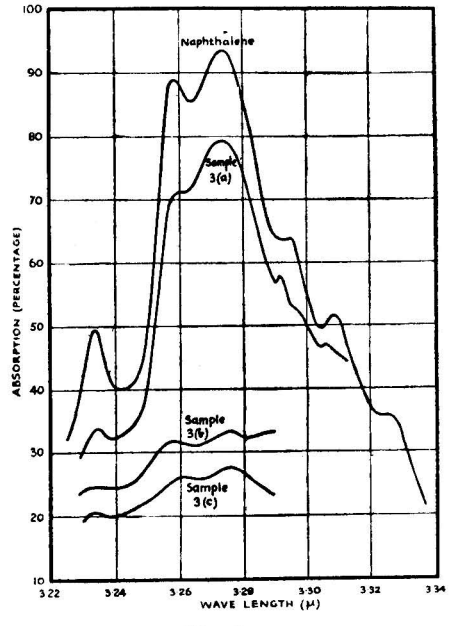

Fig. 3.

Fig. 3 shows the identification of naphthalene in residues from phenols of class (3) the main peaks being at 3·234, 3·259 and 3·276 μ.

The authors are indebted to the Government Chemist for permission to publish this work.

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Reductometric Determination of the Sulphoxide and Amine Oxide Groups

By ERICA GLYNN

(*Read at the Meeting of the Society on February 5th*, 1947)

From the analytical point of view the presence of the sulphoxide or amine oxide groups has hitherto been determined indirectly from the figures for the ultimate analysis of the substance under investigation. A direct method is clearly desirable for the sake of accuracy and in order to distinguish, for example, a dioxide from the isomeric sulphide sulphone.

The problem is similar to that of the nitro and azo groups, and similar methods should, therefore, be applicable. For these groups reduction with an excess of stannous chloride[1] or titanous chloride[2] is commonly used and the excess titrated back with standard iodine or ferric alum. Stannous chloride and ferric alum have been adopted in the present work, for iodine is unsuitable in presence of organic sulphides.

Choice of an indicator—The end-point in presence of thiocyanate was found not to be very sharp and an oxidation-reduction indicator was, therefore, adopted. The oxidation-reduction potential concerned lies between 0·55 and 0·22 volt.[3] Nile Blue, Lauth's Violet and Brilliant Cresyl Blue were tried and rejected owing to the dependence of the colour on acid concentration. Potassium indigo trisulphonate[4] was finally chosen (value of $\pi_0 = 0{\cdot}332$), as the passage of the final blue to a deep blue with a reddish tinge, as seen in artificial light, was sharp and easily recognisable. (This characteristic reddish tinge is not observed in absence of artificial light. It is sufficient to place an electric lamp directly behind the flask during the titration.)

Method

Reagents—Water and acid used for the preparation of all reagents should be boiled free from air and saturated with carbon dioxide.

Standard iodine solution—Approximately 0·1 *N*, standardised against potassium iodate in the usual way.

Standard stannous chloride solution—Approximately 0·2 *N*. Dissolve 54 g. of stannous chloride in 150 ml. of concentrated hydrochloric acid and 1850 ml. of water. Preserve under carbon dioxide and syphon direct into the burette. Standardise by titration against iodine under carbon dioxide with starch as an indicator.

Ferric alum solution—Approximately 0·1 *N*. Dissolve 100 g. of ferric ammonium alum in 400 ml. of 5 *N* sulphuric acid and 1600 ml. water. Filter after it has stood overnight and keep at least a week before use. Standardise this solution by adding an excess of stannous chloride and titrating with iodine. The oxygen equivalent of 1 ml. of 0·1 *N* ferric alum is 0·0008 g.

Procedure—(1) In titrating the stannous chloride with the ferric alum solution take 20 ml. of diluted hydrochloric acid (1+1), with carbon dioxide bubbling, add 10 ml. of stannous chloride from the burette, 5 to 10 drops of 1 per cent. potassium indigo trisulphonate solution and 10 ml. of water and titrate in the cold with ferric alum solution, shaking thoroughly after each addition in the last 0·5 ml. and allowing 5 to 10 seconds for any change of colour, until one further drop causes no further deepening in the final reddish blue. The result agreed with the iodimetric titration within 0·1 per cent.

(2) In titrating a sulphoxide, introduce 20 ml. of an air-free solution in water or alcohol of the substance, containing approximately 0·5 g. of reducible oxygen per litre, into a 250-ml. conical flask on a hot plate, passing carbon dioxide throughout the operation, and add 10 ml. of stannous chloride from the burette. Now add 10 ml. of hot concentrated hydrochloric acid and boil gently for 45 minutes, adding a further 25 ml. of hot water and 5 ml. of concentrated acid after 15 minutes. Finally add 20 ml. of concentrated hydrochloric acid, 10 ml. of water and 5 to 10 drops of 1 per cent. potassium indigo trisulphonate solution and titrate as above with ferric alum. Carry out a blank titration with 20 ml. of air-free water in place of the solution of the substance, heating as before.

Correction of the blank titration—The blank titration is essential, as some oxidation of stannous chloride by atmospheric oxygen is inevitable. It is safe to assume that the reduction of the sulphoxide is largely completed in a very short time and the actual amount of stannous

chloride subsequently liable to oxidation is the excess then left in solution. Using the simple blank estimation, the results were in fact found to be low (92 to 98 per cent. of the calculated figure).

Ingold and Smith,[5] in titrating the nitro group with titanous chloride, adopt the device of adding, in their blank experiment, only the amount of reducing solution found to be in excess. The method used here is to make a correction of the blank titration based on the same principle. It has the advantage that the blank determination need not then be repeated for each titration.

If a constant volume, n ml., of stannous chloride solution is always used and titrations are in ml. of standard ferric alum solution, then if titration (cold) without substance is v_c, and titration with heating for a definite period without substance is v_h, whilst titration with heating for the same period with substance is v_s, the apparent titre for the substance (blank uncorrected) is $v_h - v_s$. The true value should be the apparent titre $(v_c - v_s)$ less the blank correction $\times(1 -$ fraction of stannous chloride used up in the reduction of the substance). That is to say, the true titre X is given by

$$X = (v_c - v_s) - \left[(v_c - v_h)(1 - \frac{X}{v_c})\right],$$

which gives

$$X = \frac{v_c(v_h - v_s)}{v_h}.$$

As the total hot blank $v_c - v_h$ approaches zero, so the true value of X approaches $v_h - v_s$. The application of this device is shown in Table I with dithian dioxide as the substance analysed.

TABLE I

19·965 ML. OF A SOLUTION OF 2·6964 G. (0·01772 G.-MOL.) OF 1:4-DITHIAN DIOXIDE IN 1 LITRE, TITRATED WITH 0·09977 N FERRIC ALUM

v_c ml.	v_h ml.	v_s ml.	X ml. (calcd. 14·18)	Peroxidic O% found (calcd. 21·02)
25·76	25·21	11·30	14·21	21·06
25·76	24·33	10·92	14·20	21·04
25·76	24·33	11·01	14·10	20·91
25·76	24·33	10·87	14·25	21·13
21·32	19·63	6·53	14·21	21·06

Results with a number of sulphoxides or sulphones are shown in Table II.

TABLE II

Substance	Total O% (Calcd.)	Peroxidic oxygen	
		Found %	Calcd. %
α-Dithian dioxide	21·02	21·04	21·02
Dithian monoxide	11·74	11·77	11·74
Dithian trioxide	28·54	9·41	9·51
Dibenzyl sulphoxide	6·95	6·87	6·95
Dibenzyl sulphone	12·99	0	0
α-Phenylene-1:3-dimethyl disulphoxide ..	15·82	15·82	15·82
α-2:5-Dimethyl-thiolbenzoic acid dioxide ..	25·98	12·95	12·99

Amine oxides—The determination was carried out as for sulphoxides. Amine oxides are often most conveniently isolated as picrates and for the analysis of these salts the picric acid must first be removed.

To the solution of an amine oxide picrate in alcohol, add alcoholic potassium hydroxide, warm and allow to stand overnight. Filter into a 100-ml. flask, wash the precipitate with absolute alcohol, make up to 100 ml. with water and take aliquot portions for analysis.

The results for two amine oxides are given below:

Substance	Total oxygen Calcd. %	Peroxidic oxygen	
		Found %	Calcd. %
Dimethylaniline oxide picrate	34·9	4·37	4·28
Diphenylpiperazine α-dioxide octahydrate ..	38·7	7·76	7·73

SUMMARY

A method is described for the determination of sulphoxide and amine oxide groups by quantitative reduction with excess of stannous chloride and back titration with ferric alum, using potassium indigo trisulphonate as an indicator. Results are given for some sulphoxides, sulphoxide sulphones, amine oxide and amine oxide picrate; the average error was less than 1 per cent.

The author's thanks are due to Dr. G. M. Bennett for suggesting this investigation.

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Determination of Sulphate in Sodium Dichromate

BY A. W. WYLIE

FOR the determination of sulphate in the presence of sexivalent chromium compounds, it is usually recommended that chromium be reduced to the tervalent state before precipitation of sulphate with barium chloride. Various combinations of acids and reducing agents have been proposed. Haring and Barrows[1] used hydrochloric acid and ethyl alcohol. Willard and Schneidewind[2] recommended hydrochloric acid and either ethyl or methyl alcohol, hydroxylamine hydrochloride or hydrogen peroxide. Acetic acid and formaldehyde were suggested by Nikitina and Babaeva[3] and by Rabovskii and Raskatova.[4] Macchia[5] advocated the use of acetic acid and hydroxylamine.

The method proposed by Haring and Barrows for the determination of sulphate in chromic acid yields low results unless acetic acid is added before precipitation of barium sulphate. The addition of acetic acid was proposed by Willard and Schneidewind, after Weinland[6] had shown that acetic acid displaces non-precipitable sulphate groups from complex chromium ions and thus brings about almost complete precipitation of sulphate by barium chloride. Most subsequent experimenters have added acetic acid to the solution, whether hydrochloric acid was present or not. In the course of experiments to find whether small amounts of sulphate could be determined in sodium dichromate with the same accuracy as in chromic acid, it has been found (1) that the method of Willard and Schneidewind is equally applicable to sodium dichromate; (2) that increasing proportions of hydrochloric acid increase the quantity of barium sulphate precipitated in the absence of acetic acid; (3) that sulphate may be satisfactorily determined in sodium dichromate without the addition of acetic acid provided 2·7 times the theoretical requirement of hydrochloric acid is used, and the solution is allowed to stand for 15 hours. It has also been found that the presence of large amounts of acetic acid in hydrochloric acid solutions of chromic chloride may cause high results, although it is more usual to obtain results which are slightly low.

To determine approximately 1 per cent. (dry basis) of sodium sulphate in sodium dichromate the procedure of Willard and Schneidewind for the determination of sulphate in chromic acid may be varied as follows:

A 1 to 2 g. sample should be taken and the procedure described below followed; the volume of hydrochloric acid (sp. gr. 1·178) added should be 1·3 times the theoretical requirement and the volume of glacial acetic acid used should be equal to the volume of hydrochloric acid. The precipitation volume should be 500 ml. The time of standing should be 15 hours for the best results (error approx. −0·5 per cent.) although fairly satisfactory results may be obtained by doubling the volume of acetic acid and allowing the solution to stand 1 hour (error approx. −1·5 per cent.). To determine sodium sulphate in sodium dichromate without the addition of acetic acid, a 1 to 2 g. sample should be used and 2·7 times the theoretical requirement of concentrated hydrochloric acid must be added. If the procedure is then carried out as before, the time of standing being 15 hours, the accuracy of the results is equal to that obtained in the presence of acetic acid.

Experimental

AnalaR sodium sulphate and twice recrystallised sodium dichromate were used in all experiments. Solutions of these reagents were standardised, the former by determination of sulphate as barium sulphate and the latter by titration with ferrous ammonium sulphate, using barium diphenylamine sulphonate as indicator. Mixed aliquots of these solutions were acidified with hydrochloric acid (sp.gr. 1·178), or hydrochloric acid and glacial acetic acid, and 50 ml. of ethyl alcohol were added. If the weight of sodium dichromate exceeded 1 g., 70 ml. of alcohol was used. Chromium was completely reduced by heating this solution, of total volume 200 ml., for 30 minutes at 90° to 95° C. The solution was then diluted to 500 ml., heated to 90° C. and treated with 20 ml. of 10 per cent. barium chloride solution added dropwise from a burette. During precipitation, and for periods of standing up to 4½ hours thereafter, the solutions were maintained at 90° C.; for standing periods of 15 hours or more, the solutions were kept at 90° C. for 1 hour only and then cooled to room temperature. The precipitated sulphate was filtered on a No. 42 Whatman paper, thoroughly washed with hot water and ignited at 800°C. Blank tests showed no sulphate in any of the reagents employed. The ignited precipitates were white or only slightly discoloured when cold but usually showed a faint yellowish green colour when hot. Each determination was made in duplicate and the mean values are recorded in Tables I to III. When the error in the mean weight of sodium sulphate found did not exceed ±5 per cent. the maximum variation of duplicates from their mean was ±0·2 mg. and the average variation 0·08 mg. When the error in the mean weight of sodium sulphate exceeded ±5 per cent., agreement between duplicates was less satisfactory. An average value, however, is recorded for purposes of comparison.

Effect of hydrochloric and acetic acids on the precipitation of barium sulphate in presence of chromium salts

Table I

In each experiment 5·00 g. of $Na_2Cr_2O_7$ and 0·0546 g. of Na_2SO_4 were present in a final volume of 500 ml.

Expt.	Hydrochloric acid* ml.	Acetic acid ml.	Standing period hrs.	Na_2SO_4 found g.	Error mg.	Error per cent.
1	12·5	10	15	0·0540	−0·6	−1·1
2	14·0	10	15	0·0540	−0·6	−1·1
3	15·0	10	15	0·0541	−0·5	−0·92
4	16·0	10	15	0·0544	−0·2	−0·37
5	16·0	10	2	0·0517	−2·9	−5·3
6	13·0	25	15	0·0556	+1·0	+1·8
7	13·0	50	15	0·0553	+0·7	+1·3
8	13·0	75	15	0·0554	+0·8	+1·5
9	12·5	nil	15	0·0247	−29·9	−54·8
10	25·0	nil	15	0·0510	−3·6	−6·6
11	35·0	nil	15	0·0540	−0·6	−1·1

* Volume of hydrochloric acid theoretically required to convert $Na_2Cr_2O_7$ to NaCl and $CrCl_3$, 13·0 ml.

Experiments 1 to 4, Table I, lend no support to the contention of Willard and Schneidewind that decreasing concentrations of hydrochloric acid cause an increase in the quantity of barium sulphate precipitated from solutions of chromium salts in presence of hydrochloric and acetic acids, and suggest that the contrary may be true. Experiment 5 emphasises the necessity of allowing solutions of the concentration used to stand for longer than 2 hours. Experiments 6 to 8 show that high results may be obtained when larger amounts of acetic acid are added. Experiments 9 to 11 indicate that a fairly reliable result may be obtained in absence of acetic acid if the quantity of hydrochloric acid added is 2·7 times the theoretical requirement, excess hydrochloric acid up to this total producing a marked increase in the quantity of barium sulphate precipitated. This optimum quantity of hydrochloric acid may be compared with that recommended by Haring and Barrows for the determination of sulphate in chromic acid, *viz.*, approximately 1·5 times the theoretical requirement.

Experiments 1 and 3 (Table II) reveal no increase in the quantity of barium sulphate precipitated in presence of acetic acid when the concentration of hydrochloric acid is lowered. The smaller concentration of chromium in solution appears to convert a positive error of the order of 1 to 2 per cent. which might have been expected when the volume of acetic acid is twice that of hydrochloric acid (Table I, Experiment 6) to a negative error of the same order (Table II, Experiment 1). Experiments 5, 7 and 9 confirm the results of Experiments 9 to 11,

Table I. It is also shown (Experiments 6 and 8) that prolonged standing greatly increases the quantity of barium sulphate precipitated in absence of acetic acid, particularly at lower

TABLE II

In each experiment 2·0 g. of $Na_2Cr_2O_7$ and 0·0219 g. of Na_2SO_4 were present in a final volume of 500 ml.

Expt.	Hydrochloric acid* ml.	Acetic acid ml.	Standing period hrs.	Na_2SO_4 found g.	Error mg.	Error per cent.
1	5·2	10	15	0·0216	−0·3	−1·4
2	5·2	10	1	0·0216	−0·3	−1·4
3	7·0	10	15	0·0218	−0·1	−0·46
4	7·0	10	4·5	0·0217	−0·2	−0·91
5	7	—	15	0·0092	−12·7	−57·9
6	7	—	64	0·0198	−2·1	−9·6
7	8	—	15	0·0152	−7·7	−35·2
8	8	—	40	0·0212	−0·7	−3·2
9	14	—	15	0·0217	−0·2	−0·9
10	14	—	2	0·0199	−2·0	−9·1

* Theoretical requirement of hydrochloric acid 5·2 ml.

concentrations of hydrochloirc acid. It seems reasonable to assume that almost all sulphate would be precipitated under these conditions if sufficient time were allowed. In presence of acetic acid the time factor is of less importance.

TABLE III

In each experiment 1·0 g. of $Na_2Cr_2O_7$ and 0·0219 g. of Na_2SO_4 were present in a final volume of 500 ml.

Expt.	Hydrochloric acid* ml.	Acetic acid ml.	Standing period hrs.	Na_2SO_4 found g.	Error mg.	Error per cent.
1	3	10	4·5	0·0218	−0·1	−0·46
2	3	10	15	0·0221	+0·2	+0·91
3	7	10	4	0·0217	−0·2	−0·91
4	3	—	15	0·0103	−11·6	−53·0
5	5	—	15	0·0164	−5·5	−25·1
6	7	—	15	0·0218	−0·1	−0·46
7	7	—	2	0·0192	−2·7	−12·3

* Theoretical requirement of hydrochloric acid 2·6 ml.

The results in Table III confirm previous findings. The high result in Experiment 2 is attributed to the relatively large volume of acetic acid present. No obvious explanation is apparent for the high results obtained in the presence of larger quantities of acetic acid but such results could be caused by occlusion of greater quantities of chromium or barium salts in the precipitates.

The assistance of Miss J. Mather in carrying out the experimental work involved in this investigation is gratefully acknowledged.

SUMMARY

A method for the determination of small quantities of sodium sulphate (1 per cent.) in sodium dichromate is described. An amount of hydrochloric acid equal to 2·7 times the theoretical requirement is added and the dichromate reduced to a mixture of chromic chloride and sodium chloride by heating with excess of ethyl alcohol. Sodium sulphate in the resulting solution is then precipitated as barium sulphate and the solution allowed to stand for 15 hours before filtration. The results, on the average, are 1 per cent. low.

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5. Macchia, O., *Industria chimica*, 1930, **5**, 1346; *Chem. Abst.*, 1931, **25**, 1760.
6. Weinland, R., quoted by Willard and Schneidewind, Reference 2.

DIVISION OF INDUSTRIAL CHEMISTRY
COUNCIL FOR SCIENTIFIC AND INDUSTRIAL RESEARCH
MELBOURNE, AUSTRALIA

November, 1946

Notes

THE CHROMIUM TRIOXIDE TEST FOR COCAINE HYDROCHLORIDE

THIS identity test for cocaine hydrochloride is given in both the British Pharmacopoeia (1932) and in the United States Pharmacopoeia (20th Ed.). The former requires the use of a 1 per cent. solution of cocaine hydrochloride and a 3 per cent. solution of chromium trioxide; while the latter requires the use of a 2 per cent. solution of cocaine hydrochloride and a 5 per cent. solution of chromium trioxide. In neither case is mention made of the temperature at which the test is to be carried out, nor has any modification of the details of the test appeared in any addendum to the British Pharmacopoeia published since 1932. In the test as given in the British Pharmacopoeia it is stated that a precipitate, which dissolves on shaking, is formed on addition of one or two drops of 3 per cent. solution of chromium trioxide to 1 ml. of 1 per cent. solution of cocaine hydrochloride. Further addition of the chromium trioxide solution is stated to produce a permanent precipitate.

We have found that the test, carried out according to the instructions of the B.P., gives a positive result only if the temperature of the solutions is below 35° C., and then only if considerable care is taken to avoid undue mixing of the drop of chromium trioxide solution with the solution of cocaine hydrochloride. At 35° a definite, but slight, precipitate, which dissolved on shaking, was obtained with the second drop of chromium trioxide solution; but at 36° twelve drops were required to give a precipitate and at higher temperatures still more. At 32° a definite precipitate was obtained with one drop of chromium trioxide solution and a permanent precipitate with ten drops. As the temperature was still further reduced the amount of precipitate obtained with one drop became more substantial. At 25° a permanent precipitate was obtained with the sixth drop.

With a 1 per cent. solution of cocaine hydrochloride and a 5 per cent. solution of chromium trioxide a precipitate, soluble on shaking, was obtained with one drop at all temperatures up to 48°; but at 50° eight drops were required to give a precipitate soluble on shaking, whilst twelve drops gave a permanent precipitate. Using a 2 per cent. solution of cocaine hydrochloride and a 5 per cent. solution of chromium trioxide a precipitate was obtained with one drop at all temperatures up to 56°.

For carrying out these tests the solution of cocaine hydrochloride was contained in a short test-tube (3 × $\frac{5}{8}$ inch) and the drop of chromium trioxide solution was allowed to fall on the side of the tube just above the surface of the solution and to run down into the solution.

As laboratory temperatures in the tropics are frequently in the region of 36 to 40° C. or higher, the procedure of the British Pharmacopoeia for carrying out this test is inadequate, as an unsatisfactory positive, or a negative, result is obtained at temperatures above 32°. We consider, therefore, that the details of the test should be modified, either in the direction of conforming to the conditions of concentration of the United States Pharmacopoeia, or by stating that the temperature of the solutions shall not exceed 20° C.

We are indebted to the Director, Sudan Medical Service, for permission to publish this note.

WELLCOME CHEMICAL LABORATORIES
SUDAN MEDICAL SERVICE, KHARTOUM

A. J. HENRY
RIAD MANSOUR

February 22nd, 1947

Official Appointments

PUBLIC ANALYST APPOINTMENTS

NOTIFICATION of the following appointments of Public Analysts has been received from the Ministry of Health since the last record in THE ANALYST (1947, **72**, 152).

Public Analysts	*Appointments*
BRANSON, V. C. (Deputy) ..	County of Essex and Borough of Hove.
CHALMERS, F. G. D.	Urban District of Solihull.
DEDICOAT, H. (Deputy)	County Borough of Leeds.
LOVETT, T. W. (Deputy) ..	County Borough of Oldham.
WHITTLE, E. G.	County Borough of Bristol.

OFFICIAL AGRICULTURAL ANALYST APPOINTMENTS

NOTIFICATION of the following appointments of Official Agricultural Analysts has been received from the Ministry of Agriculture and Fisheries since the last record in THE ANALYST (1947, **72**, 153).

Official Agricultural Analysts	*Appointments*
DEDICOAT, H. (Deputy)	County Borough of Leeds.
TAYLOR, W. W.	Administrative County of the Parts of Lindsey, Lincolnshire.

STANDARD METHODS FOR TESTING PETROLEUM AND ITS PRODUCTS*

THE eighth edition of the Standard Methods includes new methods for the determination of metals in unused lubricating oils, the examination of the tendency of grease to corrode copper, the determination of the resistance of turbine oils to oxidation, the flash-point of liquid asphaltic bitumen and a modification of the Schwartz method of determining tetraethyl lead in motor fuel.

Fifteen of the previous methods have been amended. The amendments include that of the procedure for determining kinematic viscosity in absolute units, for which the use of a 60 per cent. sucrose solution as a primary standard has been discontinued. Minor amendments have been made to methods for determining knock-rating of aviation fuels and of motor fuels. The bromination method for tetraethyl lead in motor fuel has been withdrawn.

STANDARDISED SUBSTANCES

FOR SPECTROGRAPHY, CHEMICAL ANALYSIS AND RESEARCH

WE have received from Messrs. Johnson, Matthey & Co., Ltd., their Publication No. 1760, which deals with Standardised Substances for Spectrography, Chemical Analysis and Research. One section of this catalogue contains details of the Spectrographically Standardised Substances formerly supplied by Messrs. Adam Hilger, Ltd.

The number of elements listed is 68 although some are available only in the form of oxides or salts. The materials are believed to be the purest obtainable from various research and industrial laboratories throughout the world, whilst many are specially prepared in the suppliers' own laboratories. Some of the problems associated with the control of purity of these substances have already been described in this Journal (D. M. Smith, ANALYST, 1946, **71**, 368). In the catalogue an indication is given of the origin of the material, the form in which it is available and, in some instances, the purity attained.

British Standards Institution

A FEW copies of the following draft Specifications, issued for comment only, are available to interested members of the Society and may be obtained on application to the Secretary, J. H. Lane, 7-8, Idol Lane, London, E.C.3.

Draft Specification prepared by Sub-Committee C/8/1A—Volumetric Mouldblown and Lampblown Glassware.

CH(C)9938—Draft for Interchangeable Conical Ground Glass Joints (Third Revision of B.S. 572: 1934).

Draft Specifications prepared by Technical Committee OSC/21—Marine Animal and Fish Oils.

CJ(OSC)167—Draft for Vitamin D in Oil for Poultry Feeding Purposes (Types 200 and 400).
CJ(OSC)168—Draft for Vitamins A & D in Oil for Poultry and Other Animal Feeding Purposes.
CH(OSC)107—Draft for Whale Oil (Grades 1, 2, 3 and 4) (Revision of B.S.856).

Draft Specifications prepared by Technical Committee FCC/4—Solvents.

CJ(FCC)507—Draft for Trichlorethylene (Revision of B.S.580).

Draft Specifications prepared by Technical Committee OSC/24—British National Committee on Soaps and Fats of the International Commission for the Study of Fats.

CJ(OSC)516—Draft for Iron in Oils and Fats.
CJ(OSC)517—Draft for Soap in Oils.
CJ(OSC)518—Draft for Peroxide Value of Oils and Fats.
CJ(OSC)519—Draft for Specific Gravity and Apparent Density.
CJ(OSC)520—Draft for Solvent.

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* Eighth Edition. Pp. 576, with 152 diagrams. Published by The Institute of Petroleum, 26, Portland Place, London, W.1. 1947. Price 17s. 6d.

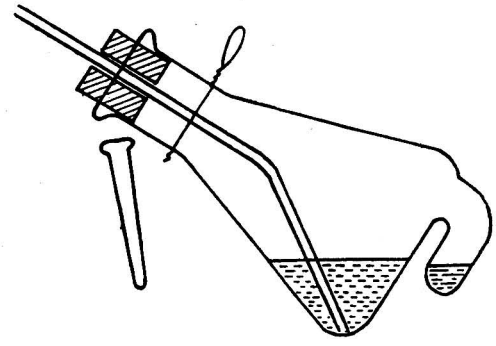
ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

Food and Drugs

Determination of Peroxide Values of Edible Fats and Oils. The Iodimetric Method. C. H. Lea (*J. Soc. Chem. Ind.*, 1946, **65**, 286–291)—It has been shown (Lea, *Ibid.*, 1945, **64**, 106; ANALYST 1945, **70**, 308) that peroxide values determined by the Chapman and McFarlane procedure (*Canadian J. Res.*, 1943, **21**B, 133), in which the fat is heated at 50° C. with ferrous ammonium sulphate and ammonium thiocyanate in 96 per cent. acetone solution and the ferric thiocyanate produced is measured photometrically, are reduced by about 75 per cent. when the reagents are de-aerated before use and the reaction is carried out in an inert atmosphere. Apparently, further oxidation of the fat (or reagent) occurs in presence of free oxygen to a degree roughly proportional to the amount of peroxide already present, probably as the result of a chain reaction initiated by the peroxide. The method used by Bolland *et al.* (*Trans. Inst. Rubber Ind.*, 1941, **17**, 29) depending upon the same principle under different conditions is affected similarly, and attention was therefore directed towards this possible source of error in the iodimetric method, the numerous variations of which have in common the reaction of the fat with potassium iodide in glacial acetic acid and chloroform solution and the subsequent titration of the liberated iodine with sodium thiosulphate solution.

In spite of the replacement of air in the reaction vessel by inert gas in some recent modifications of this method, the iodimetric procedure has been considered somewhat empirical, different procedures giving different results, which vary also with the amount of sample taken. This latter effect has been attributed to re-absorption of part of the liberated iodine by the fat, and attempts to minimise it include progressive reduction of the size of sample taken as the peroxide value increases, so that the titration is kept below 10 ml. of 0·002 *N* sodium thiosulphate (Lampitt and Sylvester, *Biochem. J.*, 1936, **30**, 2237), and estimation of the residual potassium iodide instead of the liberated iodine (Gangl and Rumpel, *Z. Unters. Lebensm.*, 1934, **68**, 533; Dastur and Lea, ANALYST, 1941, **66**, 90). It is now shown that de-aeration of the reagent is necessary before it is brought into contact with the fat if further oxidation during the determination is to be avoided. This can be effected by means of the following apparatus.

Fuse a small piece of Pyrex tube of diameter 13 mm. into the base of a 100-ml. conical flask so as to form a side tube of about 3 ml. capacity as shown in the figure. For the introduction of inert gas fit a bent tube passing loosely through a hole in a rubber stopper from which it can be subsequently withdrawn and replaced by the tightly-fitting tapered glass plug. Fix the flask in a notched cork ring so that it can be supported either vertically or in an inclined position inside a can (slotted to accommodate the neck of the flask), which protects the reaction mixture from daylight during de-aeration. The side tube allows the fat or the potassium iodide (or both) to be kept apart from the solvent during de-aeration and solid fats can be melted without heating the reagent.



Investigation of the conditions of the reaction led to the following recommended procedure.

Cold method—Weigh about 1 g. of the oil or fat (less if the peroxide value is expected to exceed 100) into the side tube and support the flask in an inclined position. Introduce into the flask 20 ml. of a mixture of glacial acetic acid and chloroform (3 : 2 by volume) and 1·2 ml. of freshly prepared, saturated potassium iodide solution, and de-aerate the solvent immediately by passing in oxygen-free nitrogen or carbon dioxide for 15 min. Withdraw the gas delivery tube while the gas is still flowing and close the orifice in the stopper by means of the glass plug. Restore the flask to an upright position thereby flooding the sample with solvent, swirl the mixture immediately, and set the flask aside in a dark place for 1 hr. Add 30 to 50 ml. of water and titrate the mixture with 0·002 to 0·1 *N* sodium thiosulphate with vigorous shaking, adding starch indicator as the end-point is approached. Solid fat should be melted by application of a small flame to the side tube immediately before flooding the tube with solvent.

Hot method—The procedure is as for the cold method as far as the completion of de-aeration. The gas delivery tube is then withdrawn, the glass plug is inserted loosely, and the flask is heated in

boiling water until chloroform vapour condenses on the plug, which is then forced home. The flask is immediately placed in a bath at 77° C. for 2 min., and cooled under the tap, and the contents are diluted and titrated as in the cold method.

Since it has hitherto been believed that the most probable sources of error in the iodimetric procedure have been incomplete reaction of the more stable peroxides with the reagent, possible decomposition of very labile peroxides in side reactions, and loss of part of the liberated iodine by re-absorption, the tendency has been to consider the highest values obtained as most nearly correct. The observation that in the iodimetric method, as in the ferrous salt method, further oxidation occurs during the determination indicates that the values given by both methods must frequently have been in error. The ratio between the results of the "aerobic" and "anaerobic" methods is smaller for the iodimetric method (1 to 1·5) than for the photometric ferrous salt method (about 4). This may be due to the difference in the concentration of fat used in the two methods and perhaps to the presence of iron in the latter method. The highest peroxide value obtained is therefore likely to be most nearly correct only when free oxygen is adequately excluded during the determination. Although results obtained by the recommended procedures cannot be regarded as absolutely correct, a number of the more obvious errors of the earlier procedures have been eliminated. A. O. J.

Study of Rancidity of Olive Oils. S. A. Kaloyereas (*J. Amer. Oil Chem. Soc.*, 1947, **24**, 39)—The Wheeler test for peroxides proved relatively the most reliable for assessing rancidity, agreeing more closely with organoleptic tests than did other tests considered. The Issoglio test was of no value for the purpose, but can be of use in differentiating natural olive oils from refined ones. This test (Kerr, R. H., *Ind. Eng. Chem.*, 1918, **10**, 471; Bolton, E. R., *Oils, Fats, and Fatty Foods*, 1928, p. 319) gives values usually below 6 for sweet refined olive oils, and of 12 or over for natural oils.

Rapid Qualitative and Quantitative Determination of Barbiturates from Post-mortem Specimens. P. Valov (*Ind. Eng. Chem., Anal. Ed.*, 1946, **18**, 456)—The prepared material is extracted with aqueous sodium hydroxide solution, the proteins in the extract are coagulated by addition of sodium tungstate and sulphuric acid and are removed by filtration, and the barbiturates are extracted from the filtrate with ether. The yield is complete and the barbiturates are obtained in a crystalline form suitable for qualitative tests and quantitative determination.

Method—Shake 60 ml. of blood or minced viscera with 410 ml. of water and 10 ml. of 10 per cent. sodium hydroxide solution in a litre flask for 5 min. Add 60 ml. of 10 per cent. sodium tungstate solution followed by 60 ml. of 0·67 *N* sulphuric acid, added slowly with continuous shaking, and finally acidify the liquid to Universal Indicator paper with 18 *N* sulphuric acid. Filter the mixture and collect 450 ml. of extract, add an equal volume of ether and shake for 5 min. Separate the ether layer, remove the solvent by distillation, and weigh the residue, deducting the result of a blank determination. Calculate the amount (mg.) of barbiturate in 100 g. of the original material. To determine the blank correction add 250 mg. of phenobarbitone to 60 g. of liver and extract the mixture in the manner described. The average amount of residue found is 253 mg. Extract 60 g. of the liver in the same way. The average amount of residue found is 3 mg. This established average amount may be deducted in the quantitative determination of relatively large amounts of barbiturate; with small amounts, a colorimetric determination of barbiturate in the residue is advisable. The Rotondaro method (*J. Assoc. Off. Agric. Chem.*, 1940, **23**, 777; ANALYST, 1941, **66**, 70) may be used for final purification of the residue, but this is not always necessary.

Extraction with sodium hydroxide separates the barbiturates effectively from fats and oils, the coagulation of proteins yields a clear solution substantially purified, and the relatively large volume of ether used prevents formation of emulsions and promotes rapid and complete extraction. The residue is usually crystalline and in a suitable form for sublimation for melting-point determination. Secanol residues and, sometimes, nembutal residues are non-crystalline, but the latter become crystalline after evaporation of added water. In practice, the following residual amounts of ingested barbiturates are encountered; for secanol, up to 3 mg. per 100 g. of liver; for nembutal, up to 16 mg. and for phenobarbitone, up to 39 mg. It is only very rarely that barbiturate is accompanied by other substances extractable by ether from an acid medium (*e.g.*, salicylic acid, sedormid, or acetophenetidine), but an abnormal weight exceeding 40 mg. of ingested barbiturate per 100 g. of tissue could not be overlooked and would indicate interference of this type. This makes the method practically specific for commonly used barbiturates. A. O. J.

Method of Estimating Penicillin and other Bacteriostatics. J. Fielding (*Brit. Med. J.*, 1947, i, 136–137)—The method depends on the fermentation of glucose in a serum medium, with acid production and subsequent clotting of the serum as an indicator of growth.

Procedure—Prepare a medium of the following composition: sterile horse serum, 1 part; water, 3 parts; glucose, to give a 1 per cent. solution; and Andrade's indicator to give a 1 per cent. solution.

Transfer to sterile containers and sterilise in a steamer for 30 min. Inoculate the medium with 10 cu.mm. per ml. of medium of a 24-hour old broth culture of the Oxford strain of staphylococcus. Put several 25-cu.mm. portions of the seeded medium in a row on the paraffin-waxed surface of a glass slide and mix the first portion with an equal volume of the penicillin-containing sample. Make serial dilutions along the row by transferring 25 cu.mm. the mixture to the next portion, and so on, leaving the last volume unmixed as a control. Take up each of the mixtures and the control into capillary tubes (2 to 3 cm. long and 1 mm. bore), seal the ends in a flame, and incubate the tubes overnight. Where full growth of the organism has occurred the column of fluid in the capillary tube shows opaque clotted serum, coloured uniformly pink by the indicator; where no growth has occurred, the fluid retains its original transparency. The end-point is generally sharp, one capillary-tube dilution containing clear fluid and the next completely clotted pink serum.

The method was successfully used for the estimation of penicillin in blood serum, cerebrospinal fluid, and urine. F. A. R.

Biochemical

Modification of the Fluorimetric Method of Estimating Riboflavine in Biological Materials. E. C. Slater and D. B. Morell (*Biochem. J.*, 1946, **40**, 644–652)—The method is a modification of that of Najjar (*J. Biol. Chem.*, 1941, **141**, 355), but differs from those previously described by including a test for specificity.

Procedure—Carry out the entire operation in weak artificial light and, if solutions have to be kept, store them in darkness. Into three 50-ml., glass-stoppered measuring cylinders, A, B, and C, pipette 1 ml. of acetic acid. (It is preferable to prepare all solutions in duplicate, and then six cylinders are employed.) Into cylinders A and B, put v ml. of the extract, containing not more than 1·2 μg. of riboflavine, followed by 1 ml. of water in cylinder A, and 1 ml. of riboflavine solution (containing 1·2 μg. of riboflavine per ml.) in cylinder B; put $(v+1)$ ml. of water in cylinder C. To each cylinder add 1 ml. of 6 per cent. potassium permanganate solution, mix, and leave for exactly 1 min. Add 1 ml. of 3 per cent. hydrogen peroxide, agitate gently to expel gases, and add 3 g. of anhydrous sodium sulphate followed by 25 ml. of *n*-butanol - pyridine (92 vols. : 8 vols.). Immerse the cylinders in water at 45° to 60° C. for a few minutes to raise the temperature to at least 40° C., and then remove them from the bath and shake vigorously to dislodge the cake of sodium sulphate at the bottom. Cool to room temperature, again shake vigorously for 2 min., and transfer the contents to a 50-ml. centrifuge tube. Centrifuge at 2,000 r.p.m. for 4 min., and pipette 20 ml. of the clear, upper layer into thin-walled, soda-glass test tubes containing 3 ml. of ethanol and stoppered with corks covered with tinfoil. Mix, and transfer 20 ml. to the cuvette of the fluorimeter and read the fluorescence, keeping the solution in the light-beam for exactly 30 sec. Return the solution in the cuvette to the test tube when readings have been taken on all three solutions, expose the test tubes to sunlight or to artificial light of uniform intensity for a sufficient time to destroy between 80 and 95 per cent. of the riboflavine, and measure the fluorescence again. Solution C should not change by more than 1 scale reading after exposure to sunlight.

To calculate the results, subtract all the readings after the exposure to sunlight from the corresponding readings before exposure, and from the values so obtained for cylinders A and B subtract the value obtained for the blank solution in cylinder C. Then, if A and B represent the corrected values for the reduction in fluorescence on exposure to sunlight of the solutions in cylinders A and B respectively, $B-A$ is equivalent to the reduction in fluorescence of the added riboflavine, *i.e.*, 1·2 μg., and the riboflavine content of the extract is equal to $\frac{A \times 1{\cdot}2}{B-A}$ μg. The accuracy of the method is about 10 per cent. and the limit of sensitivity about 0·01 μg. F. A. R.

Fluorimetric Estimation of Riboflavine in Urine. D. B. Morell and E. C. Slater (*Biochem. J.*, 1946, **40**, 652–657)—Seven different methods of estimating riboflavine in rat and human urine were compared. Methods using a hyposulphite ($Na_2S_2O_4$) blank, even without adsorption on Florisil, gave high results because they included "apparent riboflavine." Urine contains not only "apparent riboflavine," but also precursors that are changed into "apparent riboflavine" by sodium hyposulphite - stannous chloride reduction, by potassium permanganate oxidation, or during the process of adsorption on Florisil. The methods of Najjar (*J. Biol. Chem.*, 1941, **141**, 355) were not specific, giving high results for urines of low riboflavine content, and low values for urines of high riboflavine content. No completely satisfactory method for overcoming these difficulties was discovered. F. A. R.

Determination of Carbon Monoxide in Blood and of Total and Active Haemoglobin by Carbon Monoxide Capacity. D. D. van Slyke, A. Hiller, J. R. Weisiger, and W. O. Cruz (*J. Biol. Chem.*, 1946, **166**, 121–148)—The procedure for estimating total haemoglobin comprises: (1) reducing the ferrihaemoglobin to ferrohaemoglobin with sodium hyposulphite ($Na_2S_2O_4$); (2) saturating the haemoglobin with carbon monoxide; (3) extracting the uncombined carbon monoxide and the nitrogen from the solution; (4) adding ferricyanide to

liberate carbon monoxide from carboxyhaemoglobin; and (5) extracting and measuring the carbon monoxide thus liberated. For active haemoglobin the procedure is the same as for total haemoglobin except that step (1) is omitted, whilst for estimating the carbon monoxide content of blood the procedure is the same except that step (2) is omitted, and pressure readings are taken at 0·5-ml. volume even when the sample is 2 ml. The following procedure is that used for total haemoglobin with 1-ml. samples, and indication is given of the steps that are omitted when active haemoglobin or carbon monoxide contents are determined. For samples of 2·0, 0·5, 0·2, or 0·1 ml. the procedure is the same as for the 1-ml. sample except that different amounts of reagent are used, as indicated in Table I, and that, when 2-ml. samples are used for total or active haemoglobin the pressure readings are made with the carbon monoxide at 2·0 ml. rather than 0·5 ml. volume.

TABLE I

Vol. of blood sample taken, ml. ..	2	1	0·5	0·1–0·2
Capryl alcohol used, drops . ..	3	2	1	1
Vol. of saponin - borax solution used, ml. ..	4	3	1·5	1·0
$Na_2S_2O_4$ used, mg.*	35 ± 5	35 ± 5	18 ± 2	7 ± 1
Vol. of CO gas in chamber to saturate Hb, ml.† ..	10	10	10	10
Vol. of ferricyanide - acetate solution used, ml. ..	1·5	1·5	1·0	0·5
Vol. of *N* NaOH: Measured into cup, ml. ..	3·0	3·0	2·0	2·0
Admitted into chamber, ml.	1·5	1·5	1·0	0·5
Vol. of gas when p_1 and p_0 are read, ml.	2·0	0·5	0·5	0·5

* To determine *active* Hb, omit the $Na_2S_2O_4$. In all other details, the procedure is the same as for total Hb.

† To determine CO *content* of blood as drawn, omit the saturation with 10 ml. of CO. Make pressure readings with the gas at 0·5 ml. volume, even when the sample is 2 ml. Other details, including addition of $Na_2S_2O_4$, are the same as for total Hb.

Method: Step 1—Put 2 drops of capryl alcohol into the cup of the chamber of the Van Slyke - Neill manometric apparatus and draw down most of it into the chamber, leaving enough above the stop-cock to fill the capillary between it and the cup. Pipette 3 ml. of saponin - borax solution (dissolve 1 g. of saponin and 3 g. of borax in 100 ml. of water and add 0·1 ml. of capryl alcohol as a preservative) and draw about 0·5 ml. into the chamber. Draw the blood sample into a 1-ml. pipette calibrated for delivery and fitted at the tip with a rubber ring. Put the tip of the pipette containing the sample through the solution in the cup so that the rubber ring presses on the bottom of the cup and then deliver the blood at a slow, even rate into the chamber, taking about 2 min., until a small bubble of air follows the blood into the capillary beneath. Close the stop-cock and dislodge the bubble at the top of the capillary by means of a fine wire dipped in capryl alcohol. Run a little of the saponin - borax solution into the chamber to wash the blood out of the capillary. Introduce from a calibrated glass spoon 35 ± 5 mg. of powdered sodium hyposulphite into the cup, dissolve in the saponin - borax solution by stirring for a few seconds, and immediately draw the solution into the chamber. Put about 0·5 ml. of mercury into the cup to provide a seal for the tube from which the carbon monoxide gas is next delivered.

Step 2 (omitted in estimating the carbon monoxide content of blood)—Transfer 10 ml. of carbon monoxide from a Hempel pipette into the chamber, lower the mercury meniscus to the 50-ml. mark and alternately admit carbon monoxide at the top of the chamber and mercury at the bottom until the pressure indicated by the manometer has risen to 150 mm. Run the 0·5 ml. of mercury from the cup into the chamber, leaving sufficient to fill the capillary above the stop-cock, lower the mercury in the chamber, to the 50-ml. mark, and shake the chamber for 1·5 to 2 min., but not longer. Avoid exposure of the chamber to direct sunlight during shaking. Eject the excess of carbon monoxide from the chamber. This treatment results in laking of the blood cells by the saponin, reduction of the oxygen originally present in the blood by the hyposulphite, reduction of ferrihaemoglobin to ferrohaemoglobin, and combination of the latter with carbon monoxide to form carboxyhaemoglobin. In addition, the greater part of the nitrogen is extracted from the solution.

Step 3—Seal the stop-cock with mercury, lower the mercury into the chamber to the 50-ml. mark, and shake the evacuated chamber for 2 min. at a rate of 300 to 400 oscillations per minute. Protect the solution from light, eject the extracted gas, and again seal the stop-cock with mercury. The only gases now left in solution are carbon monoxide, in the form of carboxyhaemoglobin, and carbon dioxide.

Step 4—Lower the mercury in the chamber sufficiently to bring the surface of the aqueous solution down into the broad portion of the chamber, and into the cup above the chamber put 0·5 ml. of mercury and 1 or 2 ml. of water. Now introduce into the chamber 1·5 ml. of ferricyanide - acetate reagent (freshly prepared each day by mixing 5 ml. of an acetate buffer solution containing 75 g. of sodium acetate trihydrate in 100 ml. of water, to which are added 15 ml. of glacial acetic acid, with 15 ml. of 32 per cent. potassium ferri-cyanide

solution) by means of the pipette with the rubber ring attachment previously described. Seal the stop-cock with mercury from the cup and rinse the cup with water to remove any ferricyanide left in it. Lower the mercury in the chamber to the 50-ml. mark and shake the chamber for 3 mins. to extract the carbon monoxide plus the carbon dioxide liberated from the blood and reagents by the acid in the ferricyanide solution.

Step 5—Admit mercury from the levelling bulb to the chamber until the gas space is decreased to 3·5 to 4 ml. Put 3 ml. of air-free *N* sodium hydroxide into the cup and at once run 1·5 ml. into the chamber. Admit a few droplets of mercury and then after about 1 min. raise the meniscus to the 0·5-ml. mark and record the reading, p_1, on the manometer and the temperature of the water-jacket. Eject the gas from the chamber, lower the meniscus of the solution below the 0·5-ml. mark, and then raise to the mark and record the reading, p_0.

Blank Analysis—Carry out a blank analysis in precisely the same way, substituting water for the blood. The value of p_1-p_0 thus found is the correction *c*. With 1-ml. samples of blood this value should not exceed 1·5 mm. in estimating the total haemoglobin or 3·5 mm. in estimating active haemoglobin, provided the reagents have been kept free from air.

Washing the chamber after analysis—In washing the chamber after analyses, it is important to remove all particles of methaemoglobin precipitate from the walls of the chamber. After recording the reading, p_0, and without releasing the vacuum, admit about 10 ml. of water into the chamber, mix with the blood solution, and eject the mixture from the chamber. Fill the cup with *N* sodium hydroxide, dissolve in this about 70 mg. of sodium hyposulphite, and admit the solution to the chamber followed by about 20 ml. of water. Lower the mercury below the 50-ml. mark and shake with the motor until all particles adhering to the wall are dissolved. Eject the solution from the chamber, wash once with water, and then admit 20 ml. of water and 2 ml. of 2 *N* sulphuric acid. Shake with the mercury surface in the broad part of the chamber somewhat above the 50-ml. mark. Eject the acid solution and wash twice with 20-ml. portions of water.

Calculation—If P_{CO} be the pressure exerted by the carbon monoxide, then $P_{CO}=p_1-p_0-c$. The result in volumes per cent., or mg.-mols. per litre, or grams of haemoglobin per 100 ml. of blood, is calculated by multiplying P_{CO} by the proper factors given in Tables II or III [in which *S* = final liquid volume and *a* = final gas volume]. The amount of inactive haemoglobin is obtained by subtracting the active haemoglobin (without sodium hyposulphite) from the total haemoglobin (with sodium hyposulphite).

TABLE II

FACTORS BY WHICH MM. OF P_{CO} ARE MULTIPLIED TO GIVE BLOOD CO CONTENT

	Volumes per cent. of CO in blood					Mg.-mols. of CO per litre of blood				
Temperature °C.	Sample =2 ml. *S*=7·5 ml. *a*=2·0 ml. *i*=1·00	Sample =2 ml. *S*=7·5 ml. *a*=0·5 ml. *i*=1·00	Sample =1 ml. *S*=5·5 ml. *a*=0·5 ml. *i*=1·00	Sample =0·5 ml. *S*=3 ml. *a*=0·5 ml. *i*=1·00	Sample =0·2 ml. *S*=1·7 ml. *a*=0·5 ml. *i*=1·00	Sample =2 ml. *S*=7·5 ml. *a*=2·0 ml. *i*=1·00	Sample =2 ml. *S*=7·5 ml. *a*=0·5 ml. *i*=1·00	Sample =1 ml. *S*=5·5 ml. *a*=0·5 ml. *i*=1·00	Sample =0·5 ml. *S*=3 ml. *a*=0·5 ml. *i*=1·00	Sample =0·2 ml. *S*=1·7 ml. *a*=0·5 ml. *i*=1·00
15	0·1248	0·03120	0·06240	0·1246	0·3113	0·05569	0·01392	0·02784	0·05558	0·1389
16	43	08	16	41	02	50	87	74	38	84
17	39	0·03097	0·06194	37	0·3091	30	82	64	19	79
18	35	86	72	32	80	10	77	54	0·05499	74
19	30	75	50	28	69	0·05490	72	44	80	69
20	26	63	26	24	57	71	67	34	60	64
21	22	53	06	19	47	51	62	24	40	59
22	18	42	0·06084	15	36	32	57	14	20	54
23	13	31	62	10	25	12	53	05	00	50
24	09	26	41	06	14	0·05392	48	0·02695	0·05381	45
25	04	10	19	02	04	73	43	85	62	40
26	01	00	0·05999	0·1198	0·2993	54	38	76	42	35
27	0·1196	0·02989	78	94	83	36	34	67	24	31
28	92	79	57	89	73	18	29	58	05	26
29	88	68	36	85	62	0·05299	25	49	0·05287	22
30	83	58	15	81	51	79	20	39	68	17
31	79	48	0·05895	77	41	62	15	20	50	13
32	75	37	74	73	31	44	11	21	33	08
33	71	27	54	69	21	26	06	12	15	04
34	67	17	33	65	11	08	02	03	0·05198	0·1299

For samples of 0·1 ml., use 10 times the factors for samples of 1·0 ml.

TABLE III

FACTORS BY WHICH MM. OF P_{CO} ARE MULTIPLIED TO GIVE G. OF HAEMOGLOBIN PER 100 ML. OF BLOOD

Temperature °C.	Sample = 2 ml. S = 7·5 ml. a = 2·0 ml. i = 1·00	Sample = 2 ml. S = 7·5 ml. a = 0·5 ml. i = 1·00	Sample = 1 ml. S = 5·5 ml. a = 0·5 ml. i = 1·00	Sample = 0·5 ml. S = 3 ml. a = 0·5 ml. i = 1·00	Sample = 0·2 ml. S = 1·7 ml. a = 0·5 ml. i = 1·00
15	0·09147	0·02288	0·04575	0·09129	0·2281
16	14	79	58	0·09097	73
17	0·09082	70	40	64	65
18	50	62	23	32	57
19	18	53	06	00	49
20	0·08986	45	0·04489	0·08968	40
21	54	37	73	36	33
22	20	29	57	02	25
23	0·08888	21	42	0·08870	17
24	56	13	26	38	09
25	24	06	11	06	01
26	0·08792	0·02198	0·04396	0·08776	0·2193
27	62	90	80	46	85
28	32	83	65	15	78
29	02	75	50	0·08684	70
30	0·08671	68	35	54	63
31	42	60	19	24	55
32	14	52	04	0·08594	48
33	0·08584	45	0·04290	66	40
34	54	38	75	37	33

For samples of 0·1 ml., use 10 times the factor for samples of 1·0 ml.

F. A. R.

Spectrophotometric Determination of Tyrosine and Tryptophan in Proteins. T. W. Goodwin and R. A. Morton (*Biochem. J.*, 1946, **40**, 628–632)—The ultra-violet absorption spectra of tyrosine and tryptophan in 0·1 *N* sodium hydroxide intersect at 257·15 and 294·4 mμ (ϵ 2748 and 2375, respectively). By measuring the intensity of absorption at 294·4 and 280 mμ by means of a photo-electric spectrophotometer, mixtures of tyrosine and tryptophan can be analysed with considerable accuracy provided the molar ratios are not greater than 20:1 either way.

The observed intensity of absorption for a 1-cm. cell is given by the expression $yA + (x - y)\,B$, where x equals the total g.-mol. per litre in solution, y equals the g.-mol. of tyrosine per litre, and A and B are the values of ϵ for tyrosine and tryptophan, respectively, at any wavelength other than a point of intersection. In examining the spectra of protein solutions, irrelevant absorptions at 294·4 and 280 mμ have to be allowed for. The simplest procedure is to extrapolate linearly from 370 and 340 mμ to those wavelengths. Analyses, by this method, of mixtures of tyrosine and tryptophan of known composition, gave recoveries of the two amino acids ranging from 91 to 106 per cent. when the 20:1 ratio was not exceeded. F. A. R.

Colorimetric Determination of Pyridoxine, Pyridoxal, and Pyridoxamine. A. A. Ormsby, A. Fisher, and F. Schlenk (*Arch. Biochem.*, 1947, **12**, 79–81)—Pyridoxal, pyridoxamine, and pyridoxine give a bright yellow colour, an orange to pink colour, and an orange colour, respectively, with a solution of diazotised sulphanilic acid. Maximum intensity is reached one minute after addition of the reagent, but the stability of the colours obtained is very limited. The method can be used to estimate the amount of any one of the three substances in a biological preparation, provided the other two substances are absent. The reagent is prepared according to the method of Bina, Thomas, and Brown (*J. Biol. Chem.*, 1943, **148**, 111; ANALYST, 1943, **68**, 256). F. A. R.

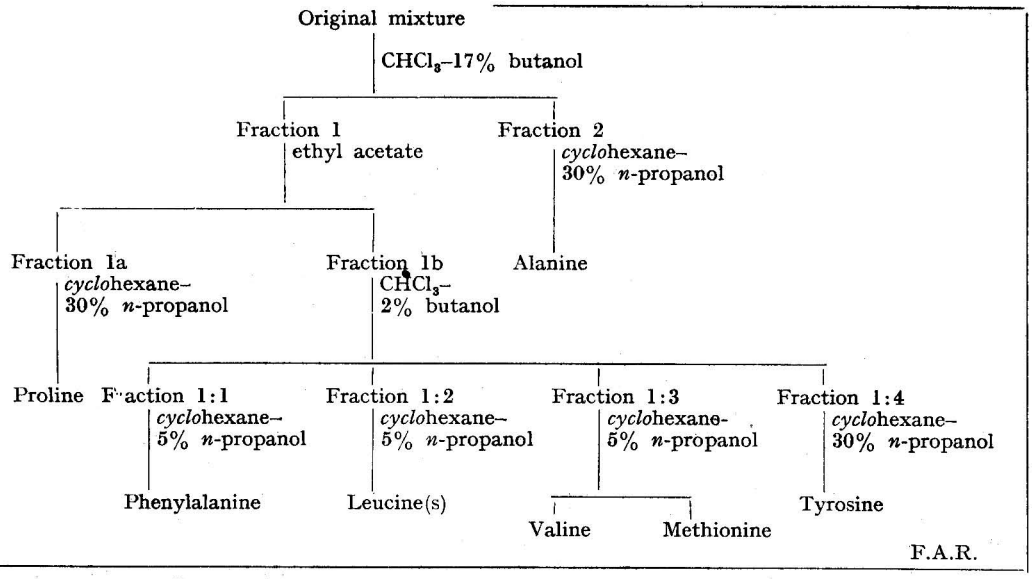
Application of Partition Chromatography to the Estimation of the Monoamino Acids in Proteins. G. R. Tristram (*Biochem. J.*, 1946, **40**, 721–733)—Difficulties were encountered in the application of the original method of Gordon, Martin and Synge (*Ibid.*, 1943, **37**, 79), in which separation of acetamino acids was obtained by eluting with various solvents from a column of silica gel in presence of coloured indicators, and a modified procedure was therefore developed; this proved to be satisfactory for the amino acid systems studied. It was shown that the adsorption of acetamino acids on iron-free silica gel is dependent on the water content of the silica.

Procedure—Hydrolyse the protein by dissolution in 5 volumes of concentrated hydrochloric acid at 37° C., heating under reflux in boiling water until

frothing ceases, then diluting with water so that the acid concentration is 5·5 *N*, and finally heating under reflux for 24 hours. Remove the excess acid by repeated evaporation under reduced pressure from a water-bath at 45° C.

Adjust the sample to a volume of 2 to 3 ml., acetylate the amino acids by the method of Gordon *et al.* (*loc. cit.*), and extract the acetamino acids from the acetylated mixture by the chromatographic procedure of Gordon *et al.* (*Ibid.*, 1943, **37**, 313), using Alphamine Red-R as indicator. It was found that small amounts of sulphuric acid sufficient to cause esterification and decomposition were being extracted by the eluting solvent. This was overcome by adding 5 ml. of water to the receiver at the beginning of the elution and, when elution was complete, testing a 1-ml. sample for sulphate. If sulphate was present, the aqueous layer was removed in a separating funnel and washed several times with the eluting solvent, all the organic solvent being filtered through a No. 2 Whatman filter paper. It was also found essential to acidify the acetylated mixture to *p*H 1·0 to ensure complete extraction of the acetamino acids. The complete removal of ethanol, butanol, and acetic acid is also extremely important, and is ensured by removing the ethanol under reduced pressure and drying the residue overnight in a vacuum desiccator at 0·005 mm. over anhydrous calcium chloride and potassium hydroxide pellets.

The scheme for fractionation recommended by Gordon *et al.* was found to give low recoveries of proline, so the following procedure was adopted:



F.A.R.

Determination and Isolation of the Organic Acids in Fruit. F. A. Isherwood (*Biochem. J.*, 1946, **40**, 688–695)—A method was developed for the quantitative separation of many of the acids commonly found in fruit. This can readily be modified to allow the constituent acids to be isolated in a relatively pure state and identified. The acids are extracted from an acidified extract of fruit by means of a 1+1 mixture of *n*-butanol and chloro-

Organic

Quantitative Analysis of Hydrocarbon Mixtures by means of Raman Spectra. E. J. Rosenbaum, C. C. Martin, and J. L. Lauer (*Ind. Eng. Chem., Anal. Ed.*, 1946, **18**, 731–733)—A method is described for the analysis of mixtures containing up to four or five components, without the use of an internal standard, giving an average deviation of less than 2 per cent. The assumption is made that for constant illumination of the sample (usually 15 ml.), the intensity of light scattered by a hydrocarbon in a mixture is directly proportional to the concentration of that hydrocarbon. Special mercury arc lamps giving little continuous radiation were used to reduce background scattering.

Calibration mixtures, each containing the same series of pure components in different proportions, were prepared, and the corrected optical density of each suitable Raman line of each component in a mixture was measured. From the optical density, the effective intensity of each line was calculated, and, using an empirical averaging process, relations between the effective intensity and the concentration were obtained which enabled the analysis of a mixture of these components of unknown composition to be carried out.

The method is illustrated by results obtained in analysing synthetic mixtures of *o*-, *m*-, and *p*-xylene, and ethylbenzene; of *o*-, *m*-, and *p*-ethyltoluene, mesitylene, and pseudocumene; and of the trimethylpentanes. D. A. P.

form, and a concentrated solution of the acids is then prepared in a 1+1 mixture of *tertiary* amyl alcohol and chloroform. The mixture of acids thus obtained is separated into its components by means of a modified partition chromatogram.

Procedure. Extraction of acids—Grind the frozen fruit at −20° C. to a flour and mix 50 g. with 20 ml. of 0·2 *N* sulphuric acid. Allow the mixture to thaw and, if necessary, adjust the *p*H to 2·0 ± 0·2. Centrifuge and decant the supernatant liquid. Add 10 g. of the resulting extract, which should contain organic acids equivalent to 10 to 20 ml. of 0·1 *N* sodium hydroxide, to 12 g. of specially prepared silica gel (see below) and incorporate the liquid into the gel so that a superficially dry powder is obtained. Add about 50 ml. of a 1+1 mixture of *n*-butanol and chloroform, and transfer the mixture to a glass tube, 20 cm. long and 5·2 cm. in diameter. Collect the filtrate, allow the column to run dry, and then fill up the tube with fresh solvent. Collect 350 to 400 ml. of the filtrate, and then a further 50 ml. in a separate receiver; titrate this with 0·1 *N* sodium hydroxide to *p*H 9·4. Not more than 0·2 ml. should be required if extraction has been complete. Titrate the main bulk of the filtrate with 0·1 *N* sodium hydroxide to *p*H 9·4 and leave for half an hour. Separate the lower solvent layer and wash twice with 10 to 15 ml. of water. Concentrate the combined aqueous layers under reduced pressure to about 2 ml. in a water-bath at 35° C., transfer quantitatively to a small beaker, and dry *in vacuo* over phosphorus pentoxide. Add sufficient 2 *N* sulphuric acid to liberate the organic acids, and mix the liquid, which should have a volume of 0·7 to 1·0 ml., with 1·0 g. of silica gel. Add about 10 ml. of a 1+1 mixture of *tertiary* amyl alcohol and chloroform, transfer the mixture quantitatively to a glass tube, 25 cm. long and 1·3 cm. in diameter, and collect the filtrate. Thereafter the procedure is the same as that described above for extraction with the butanol - chloroform mixture. Finally, rinse the beaker with 5 ml. of solvent and transfer this to the tube when the top of the column is dry. Add fresh solvent to the column until 30 ml. of filtrate have been collected. Check the completeness of the extraction by collecting a further 5 ml. and titrating with 0·1 *N* sodium hydroxide to *p*H 8·4; less than 0·1 ml. should be required.

Separation and analysis of a mixture of acids—To 3·0 ml. of 0·5 *N* sulphuric acid add 3·0 g. of silica gel and mix thoroughly. Add 20 ml. of a 35:65 mixture of *n*-butanol and chloroform, pour the suspension into a glass tube (1·3 cm. intern. diam.) and allow the column to pack down. Add to the filtrate at the rate of 0·1 ml. per min. a 0·0004 *M* solution of thymol blue [0·186 g./litre]. Put 2 ml. of a solution of the acids in a 1 + 1 mixture of *tertiary* amyl alcohol and chloroform on to the top of the column and develop the column with a 35 per cent. *n*-butanol - chloroform mixture at the rate of 1 ml. per min., carefully observing the colour of the filtrate. The volume of indicator should be 1/8th to 1/10th the volume of the solvent. The mixture of solvent and indicator is blue when no organic acid is present, changing to green and then to yellow, and finally to pink when each organic acid begins to wash out of the column. Change the receiver at the point when the colour is still greenish-blue and again when the colour reverts to blue. Record the volume of solvent and indicator in the receiver containing each fraction and titrate to *p*H 8·4 with 0·01 *N* barium hydroxide, following the addition of 20 ml. of carbon-dioxide-free water. With a mixture of acids, of which the composition is unknown, a 35 per cent. (v/v) mixture of *n*-butanol and chloroform should be used as the developing solvent, but if, as sometimes happens, an acid fraction comes through the column before the oxalic and malic acid fractions, the chromatographic separation should be repeated and the column developed first with 5 per cent. *n*-butanol - chloroform, if acetic and formic acids have to be separated, and then, after about 100 ml. of solvent have passed through the column, with 10 per cent., with 20 per cent., and eventually with 35 per cent. *n*-butanol - chloroform.

Preparation of silica gel—The method used by Gordon *et al.* (*Ibid.*, 1943, **37**, 79) was found to be unsuitable, and the following method is recommended. Dilute water glass (6 lb.) with twice its volume of warm water, filter twice through hardened filter paper to remove suspended matter, and then add a little methyl orange followed by 10 *N* hydrochloric acid with vigorous stirring. When the mixture is acid, add a further 200 to 300 ml. of hydrochloric acid, leave for several hours, and filter through a hardened filter paper. Suspend the silica in 5 litres of 10 *N* hydrochloric acid, leave overnight, filter, and wash with 10 litres of 5 *N* hydrochloric acid to remove all but traces of aluminium and iron. Wash with about 20 litres of water or until free from chloride, then with 10 litres of absolute alcohol, and finally with 5 litres of dry ether. Leave the dry silica gel for 2 weeks and then suspend it in 7 litres of 10 *N* hydrochloric acid. Leave overnight, filter, and wash with 10 litres of 5 *N* hydrochloric acid, and then with water, ethanol, and ether as before. Store in a closed vessel.

The acids commonly occurring in fruit are washed out of the column in the following order: acetic, fumaric, glutaric, formic, succinic, *trans*-aconitic, malonic, oxalic, tricarballylic, glycollic, malic, citric, and tartaric. The acids were washed off the column most readily by means of the 35 per cent. solvent mixture and least readily by the 5 per cent. mixture. The recovery of individual acids from a mixture of six acids was almost quantitative.

F. A. R.

Identification of Fructose. C. Neuberg and I. Mandl (*Arch. Biochem.*, 1946, **11**, 451–456)—Fructose can be identified in quantities less than 0·00225 g., and can be differentiated from glucose, by the formation of the methylphenylosazone. The reaction can be carried out in presence of glucose, saccharose, and sorbose, and can be used as a micro-reaction. The derivative forms at temperatures as low as 4° C., separates as characteristic crystals (photomicrographs are given), and melts (decomp.) at 158° to 160° C.

Method. Preparation of the methylphenylosazone—To a solution of 4·1 g. of sodium acetate trihydrate in 10 ml. of warm water were added 5·13 g. of methylphenylhydrazine sulphate (from Eastman Kodak), followed by 2 ml. of glacial acetic acid. After cooling to 20 to 25° C., a solution of 1·8 g. of fructose in 2 ml. of water was added. The liquid solidified to a crystalline mass after standing for 1 hour at ordinary temperature. Crystallisation sets in 25 min. after inoculation. Recrystallisation from ethyl acetate, or aqueous methyl Cellosolve, or dioxan, yields long needles. The reaction mixtures are well buffered and have a *p*H of 4·55 to 4·58, at which *p*H saccharose is stable and does not react with the methylphenylhydrazine. In presence of sorbose, resinous masses sometimes separate; this can be remedied by addition of small quantities of alcohol.

For the micro-test, place a small drop of the reaction mixture, by means of a capillary, on a microscope slide with a single concavity, and scratch with a glass thread, evaporation being prevented by means of a cover-glass. In presence of fructose, yellow needles form at room temperature in 40 min. or even less. The yields in the macro-technique are 64–80 per cent. of the theoretical.

The methylphenylhydrazine sulphate can usually be used directly; if the aqueous solution is yellow, recrystallisation from ethyl alcohol and ether may be necessary, but stirring with a mixture of 4 parts of ether and 1 part of absolute alcohol is often sufficient to extract the yellow impurity.

E. M. P.

Micro-Kjeldahl Determination of Nitrogen in Gramicidin and Tryptophan. Comparison of the Gunning-Arnold-Dyer and Friedrich Methods. L. M. White and G. E. Secor (*Ind. Eng. Chem., Anal. Ed.*, 1946, **18**, 457–458)—Hotchkiss and Dubos (*J. Biol. Chem.*, 1941, **141**, 155) reported that the Dumas combustion method is unreliable when applied to the determination of nitrogen in gramicidin, and that consistent results are obtainable with the Kjeldahl method only when the sample is treated with hydriodic acid by the Friedrich method (Pregl and Roth, "Quantitative Organic Micro-analysis," translated by E. B. Daw, 3rd Ed., p. 89, Philadelphia, Blakiston Co., 1937) before digestion. Similar difficulties are encountered in the determination of the nitrogen in tryptophan. Tryptophan constitutes up to 40 per cent. of the gramicidin molecule, and the failure to obtain quantitative yields of nitrogen from these substances may be due to the presence of half the nitrogen of tryptophan in the indole ring. Experience with the Gunning-Arnold-Dyer method as described by Clark (*J. Assoc. Off. Agric. Chem.*, 1941, **24**, 641) suggested that the nitrogen in tryptophan could be completely recovered by this method without the tedious pre-treatment with hydriodic acid.

Procedure—Digest a 5- to 7-mg. sample (weighed by difference) in a 30-ml. Kjeldahl flask containing a glass bead with 1·5 ml. of conc. sulphuric acid, 500 mg. of potassium sulphate, and 40 mg. of mercuric oxide until the liquid becomes clear, and for a further 30 min. Cool, add 1 drop of ethyl alcohol, and heat again until the liquid is colourless. The digests must be boiled vigorously during the later stages of the digestion, otherwise low results may be obtained. Distil the liquid according to the method of Clark (*loc. cit.*), but receive the ammonia in 5 ml. of 2 per cent. by weight boric acid solution containing 1 per cent. by volume of the mixed indicator of Ma and Zuazaga (*Ind. Eng. Chem., Anal. Ed.*, 1942, **14**, 280). Titrate the absorbed ammonia with 0·01 *N* hydrochloric acid in a final volume of 25±2 ml. The acid should be standardised by determination of the nitrogen in acetanilide (Nat. Bur. Standards, standard sample 141) or cystine (standard sample 143). Make blank determinations daily.

The authors' modification of the Friedrich method differs from that described by Clark (*loc. cit.*) in the same particulars as does their modification of the Gunning-Arnold-Dyer method, and, in addition, they use red phosphorus with the hydriodic acid as prescribed by Pregl and Roth (*op. cit.*).

The percentages of nitrogen found by the Gunning-Arnold-Dyer method in a number of samples of tryptophan and gramicidin and their derivatives showed no significant difference from those obtained by the Friedrich method. Nitrogen in the carboline ring might be expected to be resistant to oxidation, but analysis of 3: 4: 5: 6-tetrahydro-4-carboline-5-carboxylic acid gave a nitrogen recovery of 99·5 per cent. by the method described. The complete recovery of nitrogen from the indole ring of tryptophan is in accord with the findings of Clark (*loc. cit.*) and other investigators, who demonstrated the applicability of mercury as a catalyst to similar refractory, nitrogen-containing rings, *e.g.*, pyridine, quinoline, purine, pyrimidine, thiazole, and pyrrole. The authors, working with solid samples, have been unable to confirm the results of Miller and Houghton (*J. Biol. Chem.*, 1945, **159**, 373; ANALYST, 1945, **70**, 479), who recovered only 92·8 per cent. of the nitrogen in tryptophan

solutions after a 2-hr. digestion with mercuric oxide. The authors found 92·7 per cent. recovery after a total digestion of 35 min., 97 per cent. after 55 min., 99·4 per cent. after 85 min., and 100 per cent. after 115 min. The method described is applicable to the gramicidin-formaldehyde reaction product. A. O. J.

Determination of Silicon in Relatively Non-volatile Organosilicon Compounds. H. Gilman, R. N. Clark, R. E. Wiley, and H. Diehl (*J. Amer. Chem. Soc.*, 1946, **68**, 2728)—Of the availa to methods for the conversion of such compoundsble silicon dioxide, heating with 60 per cent. perchloric acid has been found both rapid and accurate, and preferable to heating with 72 per cent. acid as it reduces carbonisation and produces a smoother reaction. With easily oxidised compounds, a mixture of nitric and perchloric acids can be used.

Procedure (A)—Treat 0·2 g. of the compound in a platinum crucible with 5 ml. of 60 per cent. perchloric acid, and evaporate off the acid by heating on an air-bath over a Meker burner; then ignite to constant weight. Conversion to silica is complete, and the reaction is smooth; a complete determination takes about 3 hr. Condensation of fumes on wood must be avoided, as the impregnated wood then burns vigorously and may explode when heated.

Procedure (B)—Treat the sample as in (A), but use a mixture of 2 ml. of concentrated nitric acid with 5 ml. of 60 per cent. perchloric acid. Heat cautiously as there is a tendency to frothing; after the preliminary evaporation, rinse down the ring of foam near the crucible rim with 3 ml. of perchloric acid, and evaporate again to dryness.

Results—On four samples analysed by method A, a maximum deviation of 1 part in 103 is recorded, results being reproducible to 1 in 130. By method (B) the reproducibility is within 1 in 133, and the accuracy on one sample is approximately 1 in 300. M. E. D.

Determination of the Pyrethrin Content of Dilute Preparations of Pyrethrum Flowers in Oil. G. T. Bray and K. A. Lord (*J. Soc. Chem. Ind.*, 1946, **65**, 382–384)—The authors have investigated the effects of the following factors on the determination of the pyrethrin content of preparations containing less than 0·2 per cent. of total pyrethrins by the Wilcoxon-Holaday method as modified by Martin and Brightwell (*Ibid.*, p. 379): time of saponification; concentration of alkali and time of saponification; degree of dilution of the solution; removal of free acids; and replacement of alcohol by ethylene glycol monoethyl ether as a solvent for sodium hydroxide in the hydrolysis of the pyrethrins. They conclude that (1) for preparations containing 0·2 per cent. (w/v) of total pyrethrins or over, two hours' saponification with *N* alcoholic sodium hydroxide is adequate. (2) For preparations containing less than 0·2 per cent. of total pyrethrins: (*a*) the time of saponification should be 4 hours with *N* alcoholic sodium hydroxide, or 2 hours with 2 *N* alkali, the latter being preferable, (*b*) there is usually no need to remove free acids before saponification, and (*c*) should it be desired to remove the free acids, no solvent should be added when shaking with barium hydroxide, and the aqueous solutions should later be washed with light petroleum. (3) Ethylene glycol monoethyl ether is a satisfactory solvent for use with dilute pyrethrum preparations, and permits a reduction in the time required for saponification. E. M. P.

Quantitative Analysis of Isomeric Cresols and Cresol-Phenol Mixtures by Ultra-violet Absorption Spectra of Vapours. W. W. Robertson, N. Ginsburg, and F. A. Matsen (*Ind. Eng. Chem., Anal. Ed.*, 1946, **18**, 746–750)—Working curves for cresol mixtures were prepared from microphotometer tracings of ultra-violet absorption spectrograms of the vapours of the three pure cresols and a number of synthetic mixtures. The strongest bands in the spectra of the *o*- and *p*-isomers with wavelengths 2744 and 2830A, respectively, are relatively independent; the concentration of either *o*- or *p*-cresol in a mixture can thus be obtained from the density, corrected for background, of the centre of the corresponding band. As the strongest band of *m*-cresol, at 2779A, has a low extinction coefficient and is overlapped by an *ortho* band of almost equal intensity, it is not possible to determine the *meta* concentration by the same direct method. The difference in density between bands of *o*- and *m*-cresols in a single mixture as a function of the meta concentration is used instead.

Owing to the very strong absorption of the *o*- and *p*-bands used for the analysis, samples containing more than 50 per cent. of *ortho*, or 70 per cent. of *para*-cresol cannot be determined directly. The relative concentrations of these isomers can, however, be lowered by addition of a known amount of one of the isomers as a diluent. The mean deviation between the composition of the synthetic cresol mixtures and the composition of these samples as obtained from the working curves is less than 2 per cent.

Working curves for phenol - cresol mixtures based on the phenol band 2637A were prepared as indicated above, and gave a mean deviation of less than 3 per cent. for the phenol content of synthetic mixtures. Samples containing more than 40 per cent. of phenol cannot be analysed directly, but the dilution method already mentioned can be applied.

Using the phenol band 2750A, low concentrations of phenol, probably down to 0·3 per cent., can be determined with a reasonable degree of accuracy. D. A. P.

Inorganic

Photometric Determination of Bismuth in Copper. A. I. Kokorin and I. G. Dermanova (*Zavod. Lab.*, 1946, **12**, 59–63)—Optimum conditions for the determination of bismuth by means of the yellow colour given by ammonium thiocyanate are worked out. As applied to the analysis of copper, it is superior to the potassium iodide method, which requires more separations.

Under similar conditions, the colour intensity is proportional to the bismuth concentration. With increasing amounts of diluted sulphuric acid (1+10) in a total volume of 10 ml. containing 0·06 mg. of bismuth and 0·5 g. of ammonium thiocyanate, the intensity increases with amounts up to 3 ml. of the acid, and then remains constant up to 8 ml.; in 3·5 *N* acid, decomposition occurs. With increasing amounts of ammonium thiocyanate (50 per cent. solution) in 10 ml. containing 3 ml. of diluted sulphuric acid (1+10) and 0·06 mg. of bismuth, the intensity increases with amounts up to 0·9 ml., and then remains constant up to 4 ml. Hydrochloric acid and other chlorides reduce the intensity; 1 ml. of diluted hydrochloric acid (1+1) completely decolorises 10 ml. of solution containing 0·06 mg. of bismuth and 0·5 g. of thiocyanate, but 0·1 ml. has no appreciable effect. Tin introduced as chloride reduces the intensity only because of the chloride present. Iron interferes unless reduced to the ferrous state; for reduction, stannous chloride may be used if comparison is made with standards containing the same amount of reagent. Thus, with 5 mg. of iron, 3 to 4 drops of a 20 per cent. solution of $SnCl_2,2H_2O$ in diluted hydrochloric acid (1+1) may be used satisfactorily. Antimony gives no colour, and when less than 0·5 mg. in amount, has no effect on the bismuth colour. Lead gives lead sulphate, which must be filtered off. Separation from copper may be effected by double precipitation with aqueous ammonia in the presence of aluminium salts. The error in the determination of bismuth in the range 0·0005 to 0·003 per cent. with a 5-g. sample does not exceed about 1 in 10.

Procedure—Dissolve 5 g. of copper in 60 ml. of diluted nitric acid (1+1), boil to remove oxides of nitrogen, dilute with water to about 200 ml., cool, introduce 2 ml. of 10 per cent. aluminium nitrate solution, and, avoiding a large excess, add 25 per cent. aqueous ammonia solution (about 28 ml. will be required) until all the copper is in the form of the ammonia complex. Leave cold for 15 to 20 min., filter through a filter funnel (diameter of pores, 100–110 μ), wash with cold water containing 1·5 to 2 ml. of concentrated aqueous ammonia in 100 ml., dissolve the precipitate in 4·5 ml. of hot diluted sulphuric acid (1+2), wash with hot water, and precipitate as before. Again dissolve in diluted sulphuric acid (4·5 ml. of 1+2 acid), but collect the filtrate in a 15-ml. graduated flask (or burette). If necessary, refilter. Cool the solution, the volume of which with the washings should be about 13 ml., add 2 ml. of 50 per cent. ammonium thiocyanate solution, and four drops of stannous chloride solution in hydrochloric acid (1+1), close the flask, and shake to decolorise the ferric thiocyanate complex. Measure the intensity of colour by means of a compensation photo-colorimeter, and determine the bismuth concentration from calibration curves.

G. S. S.

Quantitative Method for Estimation of the Arsenoso Group. C. K. Banks and J. A. Sultzaberger (*J. Amer. Chem. Soc.*, 1947, **69**, 1–4)—Arsenoso compounds prepared by the usual synthetic methods are frequently of dubious homogeneity, being contaminated with the corresponding arsonic acid, the de-arsenated organic residue, arsenic trioxide, or mixtures of these substances. The determination of total arsenic by wet combustion will detect gross contamination, but considerable amounts of impurities may be present without changing the total arsenic content by an amount greatly different from the tolerance limits usually prescribed for such analytical procedures. Examination of the literature showed that reduction reactions are not quantitative, but that it might be possible to develop a quantitative oxidation method. Apparently, the general applicability of iodine titration of arsenoso groups has not been thoroughly investigated.

Theoretically, an arsenoso compound or an arsonous acid should react with iodine according to the equation

$$R\text{-}As(OH)_2 + I_2 + H_2O \rightleftharpoons R\text{-}AsO_3H_2 + 2HI.$$

Of the common impurities, only arsenic trioxide might be expected to react with iodine. Also, iodination of the organic nucleus of the arsonic acid or de-arsenated organic residue might interfere with the stoicheiometric reaction. Although arsines and arseno compounds will also react with iodine, the first cannot exist in presence of arsenoso compounds, and the second has never been known to occur as an impurity, although the presence of small amounts of arsenoso compounds in arseno compounds is frequent.

Highly purified samples of 3-amino-4-hydroxybenzenearsonous acid and 4-[2′:4′-diamino-6′-triazinylamino]-benzenearsonous acid hydrate were used to test the stoicheiometric behaviour of the arsenoso-iodine reaction, and these, in acidified aqueous solution, were titrated with standard iodine solution. The reaction was found to be both stoicheiometric and of adequate precision. The effect of interfering substances was also investigated. From previous unpublished work it was known that, in the presence of an excess of iodide

ion, most benzenearsonic acids do not reduce above *p*H 1·1, and none of those examined reduced above *p*H 2. On the other hand, arsenic acid showed evidence of reduction at all *p*H values below 4·8. The reaction of iodine with varying amounts of arsonous acid, arsonic acid, and arsenic trioxide between *p*H 2 and 6·8 was therefore investigated. It was found that the reaction between the arsonous acid and iodine remained stoicheiometric between these *p*H values, and that the presence of the arsonic acid had no effect on the titration. Arsenic trioxide interfered above *p*H 5 when it constituted more than 4 per cent. of the arsonous acid, but between *p*H 2 and 3 the reaction was within the analytical limits with as much as 8 per cent. of arsenic trioxide present. With 11 per cent. of arsenic trioxide, the end-point of the reaction was not definite, and the iodine consumption was not stoicheiometric for the arsonous acid, and did not approach the value required for the combined organic and inorganic tervalent arsenic. The amount of arsenic trioxide that failed to interfere with the stoicheiometric properties of the arsonous acid and iodine reaction appeared to be a function of the amount of arsonous acid present.

To determine why small amounts of arsenic trioxide failed to influence the titration appreciably, the effect of each constituent present in the reaction was investigated. Variation of *p*H alone, although influencing the arsenic trioxide and iodine reaction, did not explain the exclusion of inorganic arsenic from the titration. Similarly, the presence of the amount of iodide ion equivalent to that produced during the titration of about 0·1 g. of arsenoso compound failed to influence the arsenite reaction. The amount of *p*-arsanilic acid that would be produced under such conditions did significantly alter the reaction, and a combination of *p*-arsanilic acid and iodide ion sufficed to reduce the iodine titre of 1·5 mg. of arsenic trioxide from 0·570 to 0·008 ml. Similarly, the titre for 12 mg. of arsenic trioxide was reduced from 4·560 to 0·024 ml. Since the titration of 0·1 g. of most arsenoso compounds or arsonous acid involves about 20 ml. of such an iodine solution, 0·008 ml. would be insignificant and 0·024 ml. barely noticeable. This phenomenon was noted with over twenty benzenearsonic acids besides *p*-arsanilic acid.

A number of arsenoso compounds and arsonous acids were titrated under what appeared to be optimum conditions, and the values found all compared favourably with those obtained by combustion methods and with those required by theory. The following method was found satisfactory for the titration of arsenoso compounds and arsonous acids.

Procedure—Dissolve the sample (0·15 to 0·20 g.) in 40 ml. of water and add, if necessary for complete solution, a few drops of hydrochloric acid. When the sample is not soluble under these conditions, dissolve it in 15 ml. of propylene glycol by warming gently, and dilute the solution with water. Adjust the *p*H to about 3·5 and titrate with 0·05 *N* iodine, using starch as indicator.

It is found advisable in practice to determine both the tervalent organic and total arsenic values. As an arbitrary standard, if both values are within ±0·1 per cent. of theory and each other, the compound is considered to be pure within the limits of the analysis. If theory and the two assays agree within 0·3 per cent., the compound is generally considered sufficiently pure for pharmacological purposes (over 99 per cent.). Occasionally, samples are obtained for which the two assays agree to within ±0·1 per cent., but do not agree with the theoretical value either for the arsonous acid or the arsenoso compound. Although this may be due to the presence of non-arsenical organic or inorganic compounds, it is frequently caused by incomplete dehydration of the forms $RAsO_3H_4$ or $RAsO_2H_2$.

The iodine solution should be standardised against Bureau of Standards arsenic trioxide and acidified standard sodium thiosulphate solution. Iodine solutions may acquire a small amount of iodate on standing, and although this is not important in assay procedures where the iodine solution is standardised under the conditions of the assay, it is a source of appreciable error in this determination. In acid solution the iodate is available, but it does not react under the conditions of the standardisation against arsenic trioxide. Iodine solutions showing detectable amounts of iodate should not be used to titrate arsonous acids. The inhibiting effect of *p*-arsanilic acid on titration of the arsenite ion with iodine is being investigated. A. O. J.

Rapid Method of Determining Sulphate Impurities in Cryolite. J. V. Karjakin and V. I. Muraschova (*Zavod. Lab.*, 1946, **12**, 284–286) —Sulphate-contamination of manufactured cryolite is apparently an adsorption effect. Double precipitation of the hydrochloric acid solution with aqueous ammonia and solid sodium chloride gives almost complete desorption, and sulphate may then be determined by boiling the solution with barium chromate suspension in hydrochloric acid, neutralising with aqueous ammonia, and titrating the chromate in solution equivalent to the barium sulphate precipitated (Andrews, *Amer. Chem. J.*, 1889, **11**, 567; Komarowsky, *Chem.-Ztg.*, 1907, **31**, 498). The method is claimed to be more rapid than any previously described (it takes from 50 min. to 1 hour) and to have a maximum relative error of ±10 per cent. The results compare well with those obtained by Schrenk and Ode's method (*Ind. Eng. Chem., Anal. Ed.*, 1929, **1**, 206) based on the removal of fluorine by evaporation with hydrochloric and boric acids.

Procedure—Dissolve 1 g. of cryolite in 25 ml. of diluted hydrochloric acid (1+2) by heating in a 100-ml. conical flask under cover. Make just alkaline to methyl red with diluted aqueous ammonia (1+1), add 3 g. of dry sodium chloride, heat to boiling, allow to stand for a short time, and filter through a 9-cm. paper, washing flask and filter once with sodium chloride solution (1·5 per cent.). Return the filter paper and precipitate to the original flask, break up the paper, add 20 ml. of diluted hydrochloric acid (1+2), and heat to dissolve the cryolite and to pulp the paper. Repeat the precipitation with aqueous ammonia and sodium chloride, filter, wash three times with the sodium chloride solution, combine the filtrates from the first and second precipitations, make just acid to methyl red with hydrochloric acid, heat to boiling, and add 2 ml. of barium chromate suspension (obtained by mixing hot solutions of 19·44 g. of potassium chromate in 1 litre of water and 24·44 g. of $BaCl_2.2H_2O$ in 1 litre of water) and sufficient diluted hydrochloric acid (1+2) to ensure solution of the excess barium chromate. Boil the mixture, containing insoluble barium sulphate, for 10 to 15 min., cool under the tap, make just alkaline with aqueous ammonia (yellow to methyl red), transfer the solution to a 100-ml. measuring flask, and make up to volume. Mix, filter through a dry paper, and pipette 25 ml. of the filtrate into a 100-ml. flask already containing a mixture of 10 ml. of potassium iodide (10 per cent.) and 10 ml. of diluted hydrochloric acid (1+1) that has been allowed to stand for 5 min., and has been decolorised, if necessary, by means of a drop or two of thiosulphate. Leave for 5 min. in the dark, and titrate the iodine liberated, using starch as indicator, with 0·1 *N* thiosulphate, the titre of which has been established on cryolite containing a known percentage of sulphate.

G. S. S.

Gas Analysis

Traces of Oxygen in Hydrogen or Nitrogen. H. A. Liebhafsky and E. H. Winslow (*J. Amer. Chem. Soc.*, 1946, **68**, 2734–2735)—By the method of Winslow and Liebhafsky (*Ind. Eng. Chem., Anal. Ed.*, 1946, **18**, 565), which is sensitive to 0·0001 per cent. of oxygen by volume, the efficiency of hot copper in removing oxygen from gas samples has been tested. Passage of hydrogen over copper turnings at 600° C. in a fused-silica tube removes all the oxygen, whilst 0·0017 per cent. by volume remains if a temperature of 230° C. is used.

The removal of oxygen from nitrogen is facilitated by adding hydrogen, the end-product then being water and not copper oxides. At a temperature of about 400° C., introduction of 25 per cent. of hydrogen results in a purified gas retaining 0·0009 per cent. of oxygen, while with only 3 per cent. of hydrogen the purification is to 0·0002 per cent. of oxygen.

"Oxygen-free" gases are contaminated by oxygen in storage. Thus, hydrogen was found to pick up 0·0026 per cent. of oxygen on passing through 10 ft. of rubber tubing, and nitrogen 0·0035 per cent. of oxygen in a "sealed" steel system. M. E. D.

Microchemical

Micro-determination of Pentoses by the Bisulphite Method. V. Heines (*Arch. Biochem.*, 1946, **11**, 531–535)—The micro-determination of *d*-xylose and *d*-ribose by conversion to furfural has been studied by two methods, one involving distillation of the furfural into a sodium bisulphite solution, and the other depending on the titration of the furfural without preliminary distillation. The first method can be used for the determination of 10 to 30 mg. of pentose, and the second for 500 μg. to 5 mg.

Reagents—Dissolve 0·950 g. of anhydrous sodium metabisulphite in water to form 1 litre of 0·02 *N* solution. Standardise 0·01 *N* iodine against 0·01 *N* thiosulphate, using starch as indicator; prepare the iodine solution fresh before each series of experiments, and check it frequently. Prepare aniline acetate test paper from freshly distilled aniline, free from furfural.

Pentose determination with distillation of furfural—Pipette 3 ml. of pentose solution containing 10 to 30 mg. of the sugar into a test tube and add 10 ml. of 85 per cent. phosphoric acid solution. Heat in an electrically controlled asbestos mantle to 125° C., and then connect to a steam generator and steam-distil into an ice-cooled receiver. Allow the temperature of the mantle to reach 175° C., but not higher, and continue the distillation until the distillate fails to show a red colour with aniline acetate paper. Dilute to a definite volume, usually 200 ml., and treat aliquot portions with an excess of 0·02 *N* bisulphite to form the furfural-bisulphite addition compound. Oxidise the excess of bisulphite with 0·01 *N* iodine, using starch as indicator. After the first end-point, discharge the blue colour with a few ml. of saturated sodium bicarbonate solution, and titrate the freed bisulphite to the reappearance of the blue colour. If a blank determination is run, a check may be made on the first end-point, under exactly the same conditions of temperature, *p*H, and concentration.

Pentose determination without distillation of the furfural—Place 1 ml. of a solution containing from 500 μg. to 5 mg. of pentose in a flask connected to a small reflux condenser, and add 2 ml. of 85 per cent. phosphoric acid. Maintain the mantle at about 130° C. for 10 min., allow to cool, and dilute to 100 ml. Take aliquot portions, chill, and neutralise to methyl orange. Treat with an excess of 0·01 *N* bisulphite, and allow to stand for about 2 hours at

room temperature to ensure complete formation of the furfural-bisulphite compound. Titrate with 0·005 *N* iodine in a burette graduated in 0·05-ml. divisions. Record the first end-point.

Calculation—A sample of 15 mg. of *d*-xylose converted to furfural by heating with 85 per cent. phosphoric acid was steam-distilled into 25 ml. of 0·02 *N* sodium metabisulphite solution. On titrating with 0·0096 *N* iodine, the second end-point gave 10·40 ml. of iodine used to react with the freed bisulphite: then, ml. of iodine used × normality of iodine × 75 = mg. of pentose. Hence, 10·40 ml. of iodine × 0·0096 *N* iodine × 75 = 7·47 mg. of *d*-xylose. Using the factor 2, the recovery in milligrams equals 14·94, representing a 99·6 per cent. recovery of pentose.

Data showing that determinations, without distillation of the furfural, gave an average of 95 per cent. recovery of sugar from samples containing 500 μg. to 5 mg. of *d*-xylose or d-ribose per millilitre are recorded. E. M. P.

Physical Methods, Apparatus, etc.

Polarographic Analysis of Brass Plating Baths and Brass Coatings. D. A. Viachirev (*Zavod. Lab.*, 1946, **12**, 276–278)—Since only 0·5 ml. of the electrolyte is required, complete decomposition of the cyanides may be effected in a few minutes, and by using a small Kjeldahl flask with a 25-ml. mark on the neck, the decomposition flask may be used as a measuring flask. *Procedure*—Decompose 0·5 ml. of the cyanide electrolyte, in a small Kjeldahl flask having a narrowed neck with a 25-ml. mark, by heating with a mixture of concentrated sulphuric and nitric acids. The flask is placed in an inclined position on a sand-bath. After destruction of the cyanide complexes (about 5 to 10 min.), cool in running water, add 2 to 5 ml. of water, and carefully neutralise with aqueous ammonia, bringing copper and zinc into solution and precipitating iron. Add 1 ml. of freshly-prepared gelatin solution, cool, and make up to the mark with 25 per cent. aqueous ammonia solution. Leave to stand and decant, or filter directly into a polarographic cell, and take the copper and zinc waves. Calculate the contents of copper and zinc from calibration curves.

For coatings, make the sample the anode and a platinum spiral the cathode in a mixture of 30 ml. of 25 per cent. aqueous ammonia solution and 2 ml. of saturated ammonium nitrate solution, electrolyse for 10 to 15 min. with a current density of 2 to 3 amp. per sq.dm., remove the electrodes, re-insert the platinum spiral, and stir to dissolve the deposit. Take polarograms for copper and zinc. Alternatively, dissolve the coating on the sample, without electrolysis, as described by Tyler and Brown (*Ind. Eng. Chem., Anal. Ed.*, 1943, **15**, 521).

The accuracy is 2 to 3 parts in 100 with the electrolyte and 4 to 5 parts in 100 with the coating. G. S. S.

Polarographic Determination of Lead in Lead Slags. S. J. Fainberg and E. M. Tal (*Zavod. Lab.* 1946, **12**, 265–268)—*Procedure*—To 0·1 g. of slag in a 50-ml. conical flask, add 0·1 g. of ammonium or sodium fluoride and about 1 ml. of water, carefully mix, add 5 ml. of concentrated hydrochloric acid, heat gently to effect complete decomposition, and evaporate nearly to dryness. Cool, add 10 ml. of diluted hydrochloric acid (1+1), boil to dissolve the salts, cool somewhat, and pour into a 25-ml. measuring flask, rinsing with a few ml. of diluted hydrochloric acid (1+1). Reduce ferric iron by heating the solution to complete decolorisation with about 1 g. of reduced iron (1 to 2 min.) or a 2 to 3-cm. iron nail (20 to 25 min.), cool in running water, dilute to the mark with diluted hydrochloric acid (1+1) adding 5 to 6 drops of 0·25 per cent. glue solution, and take a polarogram for lead. Passage of hydrogen is unnecessary. A complete determination takes only 20 to 25 min. To standardise, place in three separate measuring flasks (25-ml. capacity) 2, 3, and 5 mg. of lead as lead nitrate solution, add 30 mg. of iron as ferric chloride, evaporate to dryness, add 10 ml. of diluted hydrochloric acid (1+1), and then reduce with iron and proceed as above. The method was tested on samples containing 1·5 to 2·3 per cent. of lead. Errors do not exceed about 1 in 30. Tungsten, thallium, tin, and arsenic, which would interfere, are not encountered in lead slags. Bismuth and copper in small amounts have no effect. G. S. S.

Polarographic Analysis of Nitrite and of Nitrite - Nitrate Mixtures. B. Keilin and J. W. Otvos (*J. Amer. Chem. Soc.*, 1946, **68**, 2665–2668)—In the presence of uranyl ions and dilute hydrochloric acid, nitrite and nitrate ions are both reduced at approximately −0·9 v. (*versus* the Saturated Calomel Electrode). The diffusion current is proportional to the nitrite concentration when the ratio of uranyl ion to nitrite is greater than unity. Under these conditions, the reduction of nitrite involves three electrons, indicating a reduction to nitrogen. Analysis of the wave shows that the reduction is irreversible. A solution can be analysed for both nitrate and nitrite ions in two polarographic experiments. The diffusion current due to the two constituents in the original solution is first measured. The nitrite in a second sample is oxidised to nitrate by hydrogen peroxide in acid solution, the excess of peroxide destroyed catalytically by manganese dioxide in alkaline solution, and the diffusion current measured.

Procedure—Nitrite—Prepare two stock solutions that are 0·2 *M* with respect to potassium chloride and 0·02 *M* with respect to hydrochloric acid, the

first being 4×10^{-4} *M*, and the second 1×10^{-4} *M* with respect to uranyl acetate. Measure into a 50-ml. flask a volume of nitrite solution, such that the final nitrite concentration lies between 5×10^{-5} and 5×10^{-3} *M*, and add the 25 ml. of either the first stock solution for nitrite concentrations above 1×10^{-4} *M*, or the second stock solution for lower concentrations. Dilute to 50 ml., transfer an aliquot portion to a polarographic cell, pass nitrogen gas to remove dissolved oxygen, and measure the diffusion current at a potential of −1·2 v. against the Saturated Calomel Electrode. Carry out a blank determination on the stock solution, and from the corrected diffusion current, calculate the nitrite concentration by reference to a calibration curve.

Nitrite and Nitrate—Divide the solution to be analysed into two equal portions. Examine the first as above, and obtain the diffusion current due to nitrite and nitrate. Neutralise the second portion with 2 *N* hydrochloric acid, add an excess of 5 drops, and 1 ml. of 30 per cent. hydrogen peroxide. Allow to stand at room temperature for 30 min., add 8 drops of 2 *N* sodium hydroxide and a small quantity of manganese dioxide. After the evolution of gas has ceased, decant the solution quantitatively to a 50-ml. flask, add 3 drops of 2 *N* hydrochloric acid, and 25 ml. of the appropriate uranyl acetate solution, dilute to 50 ml., and measure the diffusion current for an aliquot portion. The nitrite and nitrate concentration can be calculated from a standard curve, or from equations (1) and (2):

$$C_1 = \frac{i'_d - i_d}{6{\cdot}35\, m^{2/3} t^{1/6}} \quad .. \quad .. \quad (1)$$

$$C_2 = \frac{i'_d}{13{\cdot}8\, m^{2/3} t^{1/6}} - C_1 \quad .. \quad (2)$$

where C_1 and C_2 are the concentrations of nitrite and nitrate, respectively; i_d and i'_d are the corrected diffusion currents for the original and the oxidised solutions, respectively; m is the weight of mercury flowing in mg. per sec., and t is the drop-time in seconds.

Interferences are the same as those described in the method for determining nitrate (ANALYST, 1945, **70**, 101). Large amounts of sulphate ions also reduce the diffusion current. J. G. W.

Electron Diffraction and Electron Microscopic Study of Oxide Films formed on Metals and Alloys at Moderate Temperatures. R. T. Phelps, E. A. Gulbransen, and J. W. Hickman (*Ind. Eng. Chem., Anal. Ed.*, 1946, **18**, 391–400)—The physical and chemical structure of oxide films formed under known conditions on chromium, cobalt, copper, iron, molybdenum, nickel, aluminium, niobium, and tungsten have been studied by reflection and transmission methods of electron diffraction, and by electron microscopy. Electron diffraction reflection patterns of the polished metal specimen are taken in a camera furnace (E. A. Gulbransen, *J. Applied Phys.*, 1945, **16**, 718–724) before and after oxidation, and after cooling *in vacuo*. The specimen is then cut in half and photomicrographs × 100 and × 1000 are taken of one piece, and a thin film is formed on the surface of the other from a 0·01 per cent. solution of nitrocellulose in amyl acetate. The film is cut into 1 mm. square sections with a needle point, and the remainder of the specimen is painted with a protective coating. The film is removed electrolytically or chemically, washed, mounted, and examined by electron microscopy and transmission diffraction.

The electrolytic cell used for the removal of the films is modified from that of Evans and Stockdale (*J. Chem. Soc.*, 1929, 2651). A U-shaped cell fitted with three side arms is divided by a fritted-glass filter. The electrolyte is boiled saturated potassium chloride solution, and the atmosphere above the cell is pure hydrogen. The oxidised metal is the anode and the cathode is the same metal, or platinum. A current source of 110 v. D.C. is controlled by a potentiometer-rheostat, and a second rheostat in series with the electrodes. A current of 10 m.amp. is increased gradually to 60 m.amp. if the film is not loosened within an hour.

The experimental values for lattice spacings are compared with X-ray data, and are used to calculate the lattice parameter for cubic-type oxides. Electron reflection yields parameters 0·7 per cent. higher than accepted X-ray values, and the transmission method gives values that average 0·2 per cent. low. The electron and light micrographs show that the films consist of oxide crystals 100 to 2500 A. in size; they are about 10^{-3} to 10^{-5} of the linear dimension of the metal crystal grain. The crystal size is found to be a function of time and temperature of oxidation.

The oxide films are largely non-uniform, as they consist of thicker and thinner sections; this indicates a multi-layer film of oxide crystals. Nucleation thus occurs in contact not only with the initial thin oxide layer but also with other oxide crystals. Overlapping of one crystal on another often occurs. The oxides formed on tungsten, niobium, and chromium give the smallest average size, whilst the largest crystals occur during oxidation of copper, iron, molybdenum, nickel, and cobalt. The more protective metals thus appear to have the more uniform films.

Electron micrographs, light micrographs, and electron diffraction patterns for the nine metals investigated are reproduced in the paper. G. A. B.

Determination of Tetraethyl Lead in Gasoline by X-Ray Absorption. M. V. Sullivan and H. Friedman (*Ind. Eng. Chem., Anal. Ed.*, 1946, **18**, 304–306)—The X-ray absorption technique

described by Aborn and Brown (*Ibid.*, 1929, **1**, 26–27) is limited in sensitivity by the use of an ionisation chamber to detect the radiation. This paper gives details of the use of a Geiger counter as detector, and describes how this modified method can be used to detect, for example, 0·005 ml. of tetraethyl lead per gallon of gasoline. The accuracy is comparable with that obtained by chemical methods, yet this method takes only about 5 min. to carry out and is potentially semi-automatic.

Lead has a high absorption coefficient for nearly all X-ray wavelengths and therefore no attempt was made to select a characteristic absorption edge. Using an absorption cell length of 15 to 25 cm., the optimum conditions were obtained with an X-ray tube (molybdenum target and line focus) operated at 17 kv. The Geiger counter used (see Friedman, *Electronics*, 1945, **18**, 132–7), had a quantum efficiency of 80 per cent. for the radiation employed, and was connected to an amplifier capable of passing 25,000 random counts per sec. coupled to a scale of 32, the output being registered by a mechanical impulse counter.

Development of this method for the detection of metals in general by making use of absorption edges may open up new fields for this method of analysis in fuel and oil research, in the study of "break in" and "break down," and in the selective observation of rates of wear of different metallic sections such as shafts and bearings. E. G. S.

Preparation of Standard Powders for Reference in Particle-size Measurement. E. L. Gooden and R. L. Updike, jun. (*Ind. Eng. Chem., Anal. Ed.*, 1946, **18**, 802)—Two economic procedures for preparing standard powders are given. (A) Glass beads used for coating projection screens contain a considerable percentage of material, easily separated by sieves, that is close to 100 microns in diameter. Non-spherical particles are removed from this material by passing it through a slightly oversize sieve with little or no agitation. (B) A grade of glass wool with a cross-sectional diameter about 8 μ. is cut into short lengths with shears or a print trimmer and ground in a hammer mill. Final calibration of the powder is done with a microscope. The acicular powder has a surface mean particle diameter about 11 μ., and the spherical powder about 110 μ.

G. A. B.

Qualitative Method for Detecting Surface-Active Agents. L. F. Hoyt (*J. Amer. Oil Chem. Soc.*, 1947, **24**, 54)—The method is based on the solubilisation in aqueous solution of certain oil soluble dyes, particularly Brilliant Oil Blue BMA.

Preparation of sample—(1) Dry materials—Grind to a fine powder; digest 10 g. with 50 ml. of hot, 50 per cent. alcohol and filter through paper with suction. Evaporate the residue to dryness; digest this residue with 3 ml. of distilled water by warming in a steam-bath, and filter the water extract through a wet filter in a 1-inch funnel. (2) Liquids or pastes—Extract 10 g. with three successive portions of benzene, chloroform, or trichloroethylene; combine the extracts, filter, and evaporate to dryness. Treat the residue with 3 ml. of water as in (1).

Procedure—Add 0·02 g. of a finely ground mixture of 2 parts of National Brilliant Oil Blue BMA with 98 parts of sodium chloride, prepared by ball-milling, to the water extract from (1) or (2). Shake and allow to stand for an hour or more. Compare with a "blank" of 0·02 g. of the dye reagent in 2 ml. of water. Half a milligram, often less, of the organic component of a surface active agent will produce a blue solution, the blank test showing only a faint red-purple tinge.

Oil Red O or Oil Yellow AB may be used as dyes in the test, but are not universally applicable. With the blue dye, 48 agents gave positive results. Alframine DCA and a cationic agent, Retravon, gave negative results. Soap solubilises all three dyes and must be eliminated by acidifying, filtering, and neutralising the filtrate before the test is made.

Reviews

CURRENT WATERWORKS PRACTICE: A PRACTICAL TREATISE ON THE PROVISION OF WATER SUPPLIES FOR URBAN AND RURAL COMMUNITIES. By W. H. MAXWELL, A.M.Inst.C.E. Pp. viii + 254. London: B. T. Batsford, Ltd. 1946. Price 18s. net.

During the past ten or fifteen years, no more pressing subject to the sanitarian than that of water supply has occupied public attention and, despite the impact of war with all its pre-occupations and disturbing effects, the passing of the Rural Water Supplies and Sewerage Act, 1944, and the Water Act, 1945, bears testimony to the will of Parliament to deal with a truly national problem. These enactments have focussed attention on the implications of hydrology in its widest interpretation and it is more than ever realised—with the ever-increasing and co-ordinated knowledge and application of the principles underlying conservation, storage, distribution and sterilisation of potable water—how ultimate success depends upon the co-operation of the engineer, the practical geologist, the chemist and the biologist.

The appearance, therefore, of a limited treatise, embodying a condensed survey of the many mechanical, structural and technical branches of the subject—from initial hydro-geological considerations of the proposed supply to its final conditioning for consumption—

written by a reputable civil engineer of many years' *practical* experience in waterworks' design and equipment, is timely and should appeal to the chemist entrusted with joint responsibilities governing the provision and scientific control of pure and wholesome drinking water. At the same time, he must not expect to find herein much beyond what may help him towards a clearer appreciation and more complete understanding of the work of his engineering and other colleagues.

About the substance, however, of what has been included in a wide review of modern practice, there is evidence of a careful discrimination and selection, although one gets the impression that the author, in his desire to incorporate a digest of all that is germane to the subject, has, in certain respects, not allowed himself sufficient elbow room. This is not to be interpreted as a call for any drastic expurgation of the text but rather the desirability of a re-editing, in some future revision, of certain of the contents which, in other respects, are concise and eminently readable.

Naturally, the mechanical and constructional aspects of the reservoir site, plant, and auxiliaries and so forth, receive major attention and, generally speaking, the book is well abreast of the latest engineering developments and makes a useful contribution to waterworks' literature and should thus prove of service to practising water engineers and others engaged in the industry, being clearly outlined and ably written. The value of the text, in this section of the review, is much enhanced by inclusion of many helpful references to existing undertakings which illustrate good practice. In this relation, for example, the use of concrete is fully discussed and much up-to-date information is summarised. The subject of distribution, however, is but briefly dealt with, and it would appear more could appropriately have been included, particularly about the design of such systems. Nor can one resist the impression that more prominence might well have been given to the conservation of water from other than underground sources.

Methods of treatment and purification—including all-too-brief reference to such recent developments as "break-point" chlorination, "Calgon" stabilisation, ion-exchange systems, anti-corrosion measures and so forth—in which the chemist is more intimately concerned, are confined almost entirely to two chapters (XI and XIII). The second of these is, in effect, an abbreviated *olla podrida*, ranging from a timely caution on engineering structures *vis-a-vis* modern methods of destruction to flocculation of fine suspensions by supersonic vibrations! Incidentally, their significance in such a compendium would have been materially enriched by an adequate extension of footnote references to current literature, enabling the reader to follow up the theme in more detail. It is in no spirit of carping criticism to suggest that the various phases which are within the ambit of the chemist should have been accorded a more orderly presentation *in one chapter or section* and the ritual of purification principles adopted, from source to consumer's tap—pre-treatment, storage, flocculation, sedimentation, filtration, correction, and so on—assembled, however much condensed, in logical sequence.

These minor defects notwithstanding, there is so much sound and finely illustrated material set out that, as a book of reference, it can be commended to the chemist who is primarily concerned with water supply, as an intelligible and useful volume to retain in his library.

S. ERNEST MELLING

THE NATION'S FOOD: A SURVEY OF SCIENTIFIC DATA. Edited for the Society of Chemical Industry by A. L. BACHARACH, M.A., F.R.I.C., and T. RENDLE, F.R.I.C. Pp. 349. London: Society of Chemical Industry. 1946. Price 18s. net.

At the outbreak of war the Nutrition Panel of the Society of Chemical Industry decided to concentrate its activities on those best calculated to be of the greatest utility under the conditions prevailing. This policy led to a series of meetings, held between the years 1940 and 1943, at which papers were contributed on "the nation's food." Each paper covered a separate and specialised field of knowledge and was written by an authority on the subject under review. The authors numbered rather more than thirty, and included amongst the better known were Lampitt, Bacharach, Kent-Jones, Kon, Mamie Oliver and Harriette Chick. This series of papers, edited by Bacharach and Rendle, have been brought together to constitute the book under review.

The foods dealt with included eggs, potatoes, vegetables, cereals, meat, fish and milk. The first article of diet considered was the egg, and the egg, as a food, for instance, was studied from three angles, namely, the biological, chemical, and nutritional. In addition, two chapters were devoted to the preservation of, and the effect of cooking upon, eggs. It may be readily

appreciated that, from the chemist's and dietician's point of view, the information about the egg is almost exhaustive. In other chapters, other foods are dealt with in a similarly thorough manner.

Some of the chapters provide material of more than ordinary interest and should make a special appeal to a wide variety of readers. These include, for example, the one that gives an account of the biology of the sea fisheries.

An endeavour is generally made by a reviewer to indicate the class of reader to whom a volume should be of particular use. In dealing with this book a difficulty arises because it contains so much information that it should appeal to anyone who takes a scientific interest in food. Not only does it present a survey of knowledge concerning certain foods but it gives an invaluable collection of analytical results. The student, too, has in this volume information respecting the chemical, physical, and physiological properties of many foods that it would be difficult, or even impossible, for him to obtain elsewhere.

The only regret, perhaps, is that more subjects have not been dealt with, but the Editors express the hope that other foods will be considered in the near future, and presumably therefore, a further volume will be published. May this be soon! F. W. F. ARNAUD