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Volume 71 | See Note on p. 95 | February, 1946

No. 839, Pages 49—96

THE ANALYST

The Journal of The Society of Public Analysts and other Analytical Chemists

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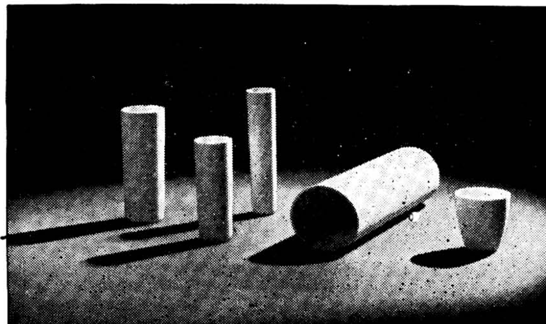

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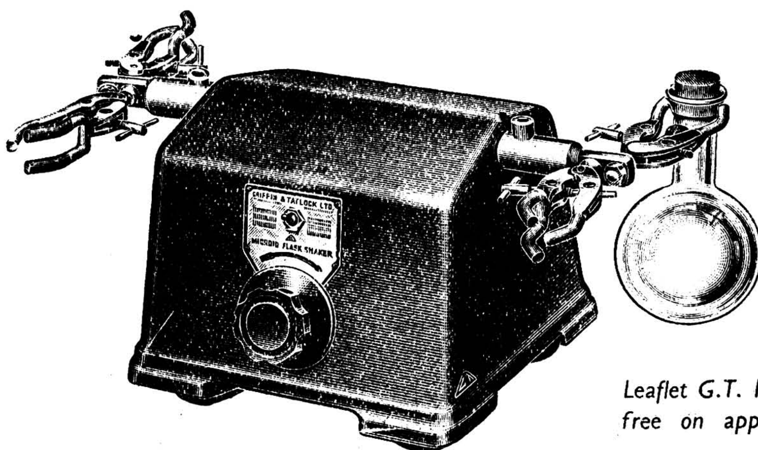

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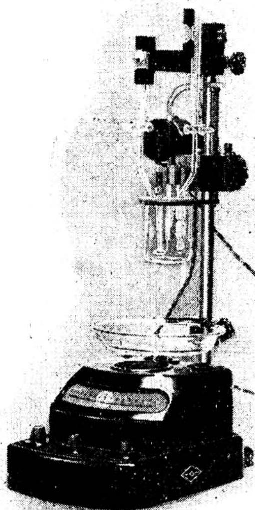

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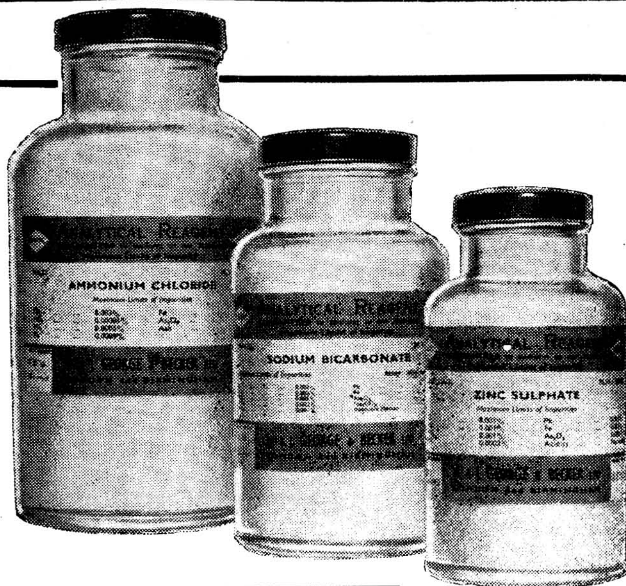

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DEATH

We record with regret the death of Alfred Lucas, who had been a member since 1899.

Symposium on Polarography

The following four Papers on Polarography were read and discussed at the Joint Meeting of the Physical Methods Group and the North of England Section, at Manchester, on October 20th, 1945. The Discussion follows the last of the four, on p. 64.

General Principles of Polarographic Analysis

By W. CULE DAVIES

Introduction—It is just 20 years since Heyrovsky began his experiments with the dropping mercury electrode, which led to the invention of the polarograph, a machine for recording current-voltage curves, by Heyrovsky and Shikata.[1] Applications of the new instrument in analysis very rapidly followed and the general principles of the method of polarography were soon understood. Throughout the whole period Heyrovsky and his pupils have been leading workers in this field. A little before the war, interest quickened in the U.S.A., and important analytical applications have appeared there, notably from Kolthoff and his pupils. During the war many analysts in our own country have investigated applications of the polarograph, and without doubt it can be said that the method has established itself alongside the other important physical methods of analysis.

A continuously increasing potential is applied to a cell which has a pool of mercury as anode and mercury dropping from a capillary as a cathode (about 1 drop per 3 secs.) and which contains the substance being analysed dissolved in a solution containing excess of an electrolyte (ground solution). The current flowing is recorded photographically or by a pen-recorder. The voltage across the cell is varied slowly and uniformly from 0 to 2 or 4 volts by means of a rotating potentiometer and the current flowing is measured by a mirror galvanometer or the like, the sensitivity of which can be adjusted by a shunt box (Fig. 1).

The type of current - voltage curve obtained is shown in Fig. 2. When the voltage reaches the characteristic value (reduction or deposition potential) associated with any electro-reducible substance present there is a rapid increase in current which finally reaches a more or less constant value (saturation current) dependent on the concentration of substance being reduced.

Qualitative polarographic analysis depends on the determination of reduction potential, and has had as yet few applications. As an example of its possible use I might mention the detection of minute quantities of impurity in pure materials (aldehydes and peroxides in ether).

Quantitative polarographic analysis depends on the measurement of wave height.

Measurement of Wave Height—Generally, polarographic workers have used highly damped long-period galvanometers in order to limit the current oscillations which are caused by the "building up" and falling of each mercury drop. The waves then obtained appear as in Fig. 2. There are many ways of measuring a wave, and the analyst should choose a convenient one for his type of work and strictly keep to it. The method described by the

British Aluminium Co. Ltd.,[2] is a neat one. It makes a correction for the residual current and can be applied equally well when, as is most usual, the top and bottom of the wave are not parallel.

It is possible with a high speed undamped polarographic pen-recorder to record true current values throughout the building up of each drop of mercury, and one manufacturer recommends this method of recording and of measuring, for example, the maximum current before and after the wave. I would recommend users of such instruments to try the method; it certainly gives a truer measurement than that obtained by taking, as is usual, the average of highly damped oscillations.

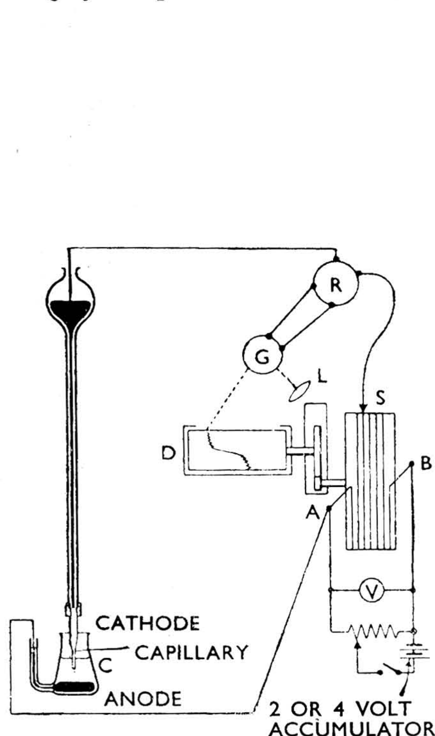

Fig. 1.

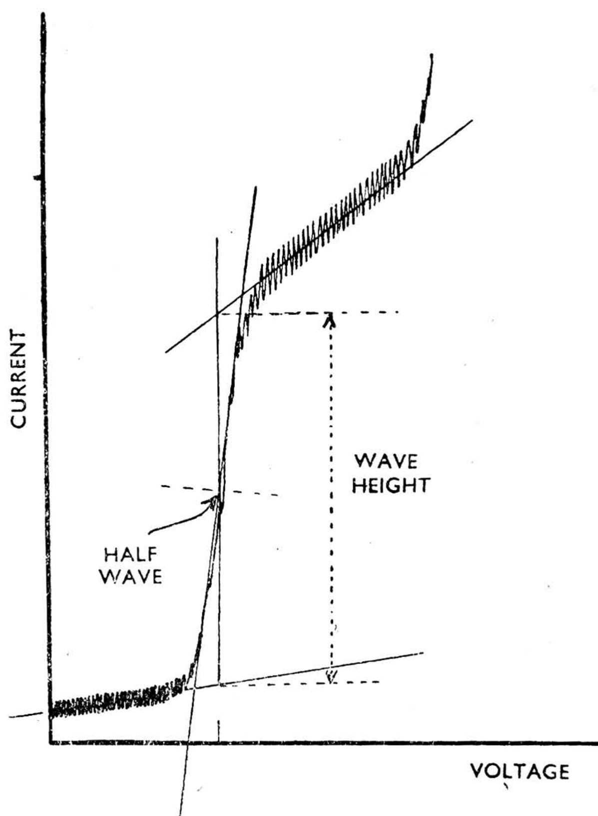

Fig. 2.

The Ilkovic equation is a relation which has been developed mathematically in several ways to calculate the saturation current, i, from the known characteristics of the dropping electrode being used and the concentration of the substance being reduced—

$$i = 605nD^{\frac{1}{2}}Cm^{\frac{2}{3}}t^{\frac{1}{6}}$$

where n = Faradays per mole of electrode reaction, D = diffusion coefficient of substance being reduced, C = concentration, m = rate of flow of mercury, and t = drop time; i is proportional to C.

Assumptions, some of questionable value, are made in deriving the equation, and it has been found by one investigator that an undamped recording obtained in the way just described did not entirely support the above values of certain of the indices. Doubt must arise from the fact that i in the equation is the so-called average current measured by using a slow damped galvanometer.

In the past it has been very difficult to compare results in published works because writers have often neglected to report the characteristics of their dropping mercury electrode (m and t) and also to give the current units of their diffusion currents. It is urged that serious polarographers should attend to this important matter. It is very desirable that the

doubt attending the indices of the Ilkovic equation should be cleared up as soon as possible, but the experimental data now asked for should nevertheless always be given.

POLAROGRAPHS—Polarographs are of 3 main types, differing solely in the method of recording the current - voltage curves.

(1) Manual recording. In this, for successive steps in voltage applied to the electrolytic cell, current is measured, and the results are plotted on squared paper. Clearly the method is slow, but the set up is cheap. Investigation of a new process of analysis is often best done with a hand-operated polarograph, and where purse strings are tight it might be possible to convince a management of the value and economy of an automatic polarograph in a particular case by the use of a home-made manual instrument. For amperometric titrations the manual instrument is excellent in any event. A sensitive micro-ammeter is the best type of current measuring instrument, with a 2000 mf. condenser across its terminals as a means of damping the oscillations.

(2) Automatic polarograph with photographic recording of current - voltage curves. In this the potentiometer is motor driven and the current passing is recorded photographically by means of a spot of light reflected from a mirror galvanometer. The first polarograph was of this type.

(3) Automatic polarograph with pen-recording of current - voltage curves.

I have worked for several years with both a pen- and a photo-recorder, and for convenience and speed there is little doubt that in every way the pen-recorder is the best type of instrument so far produced. One particular advantage, of course, is that the actual current is recorded.

Interesting experiments have been made with cathode ray tube recorders, but so far no commercial instrument of this type has been developed.

GENERAL PRECAUTIONS—For reliable work (except in amperometric titrations) it is essential to control the temperature of the cells and whenever low concentrations of material are being determined all the usual precautions of micro-analysis must be strictly adhered to. Needless to say the mercury should be pure; an apparatus for vacuum distillation of mercury is desirable.

INVESTIGATION OF A NEW METHOD—In developing a method of determination for a new single substance or for a small group of substances a considerable expenditure of time and research may have to be undertaken. Consideration of the basic principles of polarography will act as a guide; for example, if the deposition potentials of two cations are similar, no separation into two separate waves will occur, and devices such as the addition of a reagent which forms a complex with one of the cations only will have to be tried, in order to obtain a wider voltage separation. The choice of the best ground solution is largely empirical, but the success of a new polarographic determination depends essentially on this choice. For instance, suppose the problem of determining ferric iron, $titanium^{IV}$ and aluminium in admixture is presented. First, various solutions of the separate elements will be tried to find out which give well-developed waves and which are likely to give separations in the mixture of elements. Then various binary mixtures in various ground solutions would be tried, and so on. It may, of course, be found impossible to get all three waves fully developed simultaneously on a single polarogram. In this particular case iron and titanium together (and in presence of aluminium) give a nicely separated pair of waves of good shape in a mixture of normal and acid oxalate solutions, but no separate aluminium wave appears because of the simultaneous discharge of hydrogen from the acid solution. Thus, the investigation is one calling for much patience and must be done by an expert. Chemical separation should be a last resort and if necessary at all may finally spoil the use of the polarograph for that particular case. On the other hand it may be that by addition of the proper reagent to precipitate an interfering element (filtration being often unnecessary) the desired determination can be made advantageously by the polarograph. The element to be determined, however, should never be precipitated, collected and redissolved for its polarographic determination in solution. It is far better, if such a separation must be done, to dry and weigh the precipitate.

Once the simplest polarographic method has been discovered, however, a relatively unskilled analyst can be trained to use the method as a routine test.

CONCLUSION—No doubt enthusiasts have often over-rated the value of polarography, with the effect of bringing it to some extent into disrepute. There does seem to be an impression amongst those who are relatively unacquainted with the technique that polarographic

methods have little advantage over conventional methods. The sooner it is realised that polarography has limitations the better; the sooner its scope is fully defined, the more useful it will be. We must try to educate those who feel that the polarograph is the automatic analyst for every cation, anion, etc., in any substance, and those who in order to use the polarograph in every possible instance take every route in their endeavours. The polarograph has its rightful and useful place in the laboratory and it should be used only when it is properly called for. Without doubt in certain routine tests it is supreme—I know of analytical laboratories where the polarograph does a single, simple determination, day in day out, with great advantage and economy, for example, lead in special steels or sulphate in aluminium hydroxide, saving time and labour over other methods. At International Alloys Ltd.[3] it has been shown that zinc can be determined in 60 samples of aluminium alloy by one operator in 8 hours with reliability and economy of reagents, glassware and bench space. An inexperienced operator may readily be trained. The cost of equipment is soon covered by saving in reagents, filter papers and glassware. No other methods were so favourable in this instance.

The polarograph, however, offers little advantage over colorimetric, volumetric and other methods if complicated chemical separations and such like have to be carried out before it can be used at all.

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2. The British Aluminium Co. Ltd., "*Spectrochemical and Polarographic Analysis of Aluminium and its Alloys*," 1943.
3. Stross, W., and Osborn, G. H., *Light Metals*, July, 1944.

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Biochemical Applications of Polarographic Analysis

BY JAMES E. PAGE

MY remarks should be prefaced by a few words of warning. There has been a tendency amongst some enthusiasts to be uncritical of polarographic methods and to recommend a polarographic procedure whether or not some other more satisfactory method exists. Some of the polarographic analyses suggested in the literature do not compare at all favourably with methods depending on the more orthodox analytical techniques. Whenever a new analytical problem is being examined a polarographic approach should always be considered, but before the method is adopted for routine use the merits of the procedure must be compared with those of methods based on other analytical techniques. There is no doubt that for many analyses the polarograph can offer a method that has considerable advantages over all others.

In this short survey of the more important biochemical applications of polarographic analysis, I shall for the most part refer to analyses of which I have had practical experience and in several instances I shall add a few words of personal criticism.

The simplest biochemical application of the polarograph is to the estimation of traces of metals such as lead,[1] arsenic,[2] vanadium,[3] zinc,[4] cadmium[5] and magnesium,[6] and is a direct extension of the methods used in metallurgy. Unfortunately the procedures are not always quite so simple when applied to biological material. Blood and urine containing traces of lead, arsenic and vanadium require lengthy pre-treatments to remove reducible organic matter before an extract is obtained suitable for polarographic analysis. These particular pre-treatments are probably as complicated as those required for any other method of estimating these metals in blood and urine, and it is doubtful whether anything is to be gained by using a polarographic procedure.

On the other hand the polarograph has undoubted advantages for the estimation of antimony in blood and urine[7] and has proved to be of value for studying the metabolism of antimony compounds used in the therapy of bilharziasis and kala-azar. It is difficult by ordinary chemical methods to distinguish between ter- and quinque-valent antimony in biological material. However, tervalent antimony in normal hydrochloric acid solution (*cf.* Fig. 1) forms a good polarographic step with a half-wave potential versus the saturated calomel electrode at −0·15 v., whereas quinquevalent antimony does not; consequently the

tervalent form can readily be determined by polarography in presence of the quinquevalent. The quinquevalent antimony can be determined after reduction with sodium sulphite. The procedure proved to be surprisingly rapid as well as accurate, so that it was possible to make a large number of measurements which would have been impracticable by the earlier methods. Samples of blood needed relatively little pre-treatment. Urine could be examined directly. Since the half-wave potential of tervalent antimony in acid solution is relatively low, at −0·15 v., the characteristic step for tervalent antimony appears before those due to the other reducible substances in urine, and the steps formed by them would completely mask those produced by small quantities of anything less readily reducible than tervalent antimony.

The polarographic estimation of oxygen[8] has attracted considerable attention. Oxygen dissolved in electrolyte solutions (*cf.* Fig. 2) is reduced at the dropping-mercury electrode and yields two distinct steps. The first oxygen step is due to the reduction of oxygen to hydrogen peroxide, and the second to the reduction of hydrogen peroxide to either water or hydroxyl

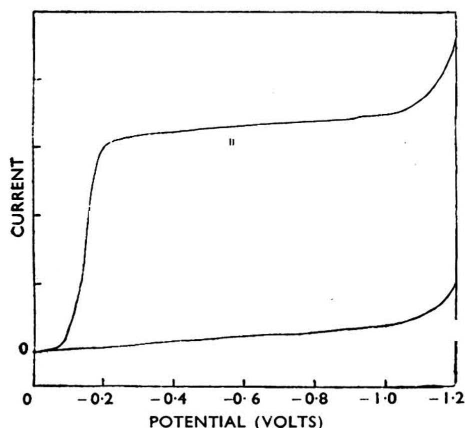


FIG. 1.

Current - voltage curves for antimony in normal hydrochloric acid solution.
I. 0·0001 *M* sodium quinquevalent antimony gluconate.
II. 0·0001 *M* sodium tervalent antimony gluconate.

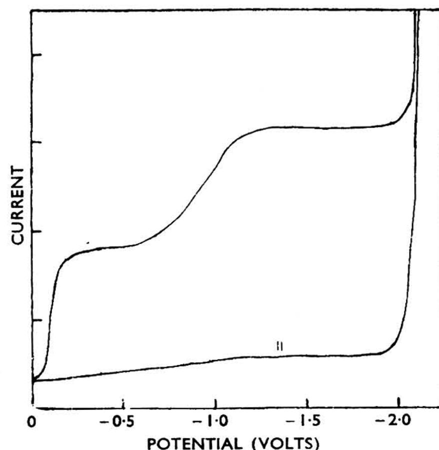


FIG. 2.

Current - voltage curves for oxygen.
I. 0·05 *N* potassium chloride solution saturated with air and containing a trace of methyl red.
II. The same solution after removal of air by nitrogen.

ion. The second oxygen step coincides with that obtained for the electrolysis of an air-free solution of hydrogen peroxide. These steps have been used for measuring the oxygen content of body fluids,[9] and for studying the respiration rates of micro-organisms[10] such as green algae (*Chlorella pyrenoidosa*), yeast, blood cells and animal tissues. The method is quite sensitive and compares favourably with the classical manometric methods, especially when the oxygen uptake is very small.

The polarograph is of considerable theoretical importance for the study of oxidation - reduction systems. An ideal example of a reversible reduction at the dropping-mercury electrode is given by quinhydrone and its components.[11] In a well-buffered solution (*cf.* Fig. 3) the step due to reduction of benzoquinone has the same characteristic half-wave potential as the step due to oxidation of hydroquinone. If quinhydrone is examined, one half of the curve represents an oxidation of hydroquinone at the mercury anode and the other half represents a reduction of benzoquinone at the mercury cathode. An oxidation - reduction system is thermo-dynamically reversible if identical polarographic half-wave potentials are obtained for the reduction of the oxidant and oxidation of the reductant. As would be expected, values for the oxidation - reduction potentials[12,13] of such systems determined polarographically are in good agreement with those obtained by potentiometric methods. The polarographic method of measuring oxidation - reduction potentials has several distinct advantages over the potentiometric methods; unfortunately the accuracy is inferior.

The electrolytic examination of most organic substances of biological interest involves reactions not thermodynamically reversible, for reduction products are formed that cannot

be oxidised back to the starting material at the same electrode potential. The reductions of aldehydes, ketones, unsaturated acids and nitro-compounds belong to this group.

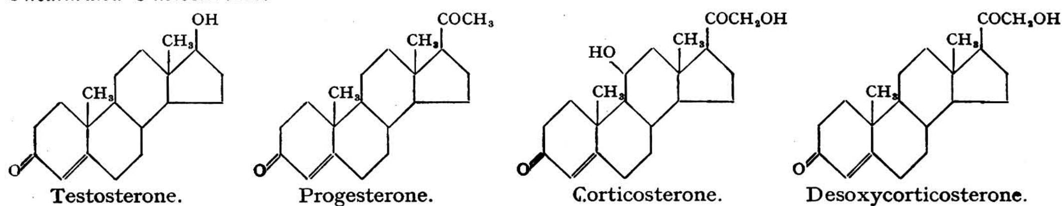
The polarographic behaviour of the various vitamins containing either reducible or oxidisable groupings has been studied extensively. Aneurine (vitamin B_1),[14] riboflavine,[14] nicotinic acid,[14] pantothenic acid,[14] ascorbic acid (vitamin C),[15] α-tocopherol (vitamin E)[16] and vitamin K[17,18] can be determined in pure solution; however, in most instances further work is required to devise methods for their estimation in presence of other reducible substances.

Lingane and Davis[14] found that riboflavine, aneurine, and nicotinic acid (*cf.* Fig. 4) can be determined together in aqueous solution, but the method cannot be used for the direct determination of aneurine and nicotinic acid in natural products, as other reducible materials present mask the steps formed by them. Owing to the relatively low reduction potential of riboflavine, it is possible to determine it in purified liver extracts. The mechanism of the riboflavine step has been investigated by Brdička and Knoblock[19] and that of the nicotinic acid step by Tompkins and Schmidt.[20] Recently Wollenberger [21] has pointed out that aneurine under certain conditions of *p*H forms a catalytic step about 4000 times higher than that given by aneurine reduced in the usual way. This catalytic step promises to be of value for the detection of very small quantities of aneurine, but it is unlikely to be used as the basis of a method for its estimation. The effect of other biological substances on this catalytic step has still to be investigated.

The estimation of ascorbic acid (*cf.* Fig. 5) is of some interest, as it is an example of a polarographic oxidation. The *p*H of the buffer solution used as the supporting medium must be within the range 3·4 to 5·3. If the *p*H value is too low, the anodic step of ascorbic acid is shifted towards such high positive potentials that the limiting current cannot develop. At high *p*H values the vitamin becomes unstable. A number of investigators have claimed reliable results for the estimation of vitamin C in fruits and vegetables by this method but I was unable to obtain satisfactory results until I tried a simple device which has recently been described by Werthessen and Baker[22] in a paper on the polarographic estimation of keto-steroids. The potential range of the instrument is reduced, so that the complete range of the recording drum corresponds to a range of 0 to 1·0 v. instead of 0 to 3·0 v., thus separating steps that are close together and facilitating the measurement of their height. The steps shown in Fig. 5 were obtained by this method.

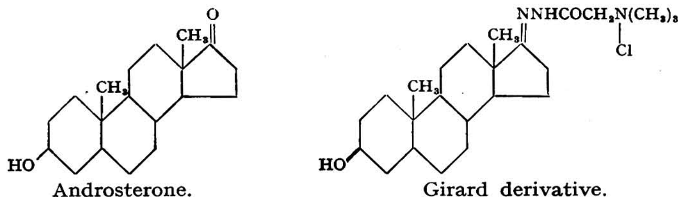
α-Tocopherol[23] cannot be determined quantitatively in concentrations of less than 10^{-3} *M* in solutions containing small amounts of sesame oil, fish oil, or cholesterol.

Unsaturated 3-*ketosteroids.*



Testosterone. Progesterone. Corticosterone. Desoxycorticosterone.

17-*Ketosteroids.*



Androsterone. Girard derivative.

The polarograph can be used for the determination of certain steroid hormones.[24] 4:5-Unsaturated-3-ketosteroids (*e.g.*, testosterone, progesterone, corticosterone, and desoxy-corticosterone) are reducible, but the saturated 17-ketosteroids (*e.g.*, androsterone) are not. However, Fieser and his collaborators at Harvard[25] found that the saturated 17-ketosteroids condensed with an excess of Girard's reagent T (trimethylacethydrazide ammonium chloride) to yield derivatives with an electro-reducible molecule. Girard derivatives of the

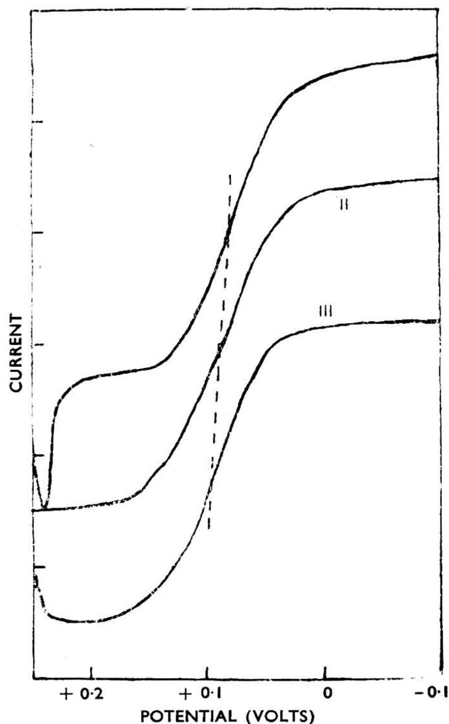


FIG. 3.

Current - voltage curves for hydroquinone-quinone system.
I. Benzoquinone buffered at pH 7·00.
II. Quinhydrone „ „ „ „
III. Hydroquinone „ „ „

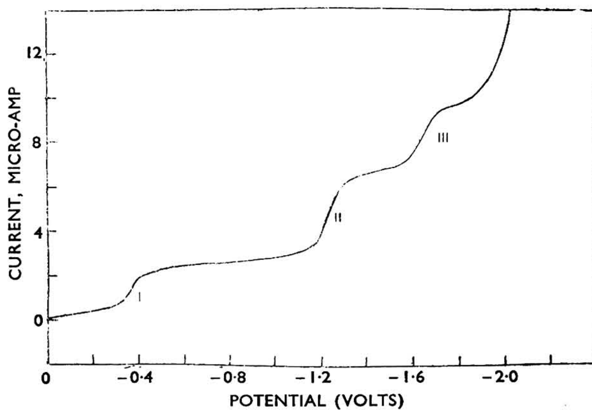


FIG. 4.

Current - voltage curves for a mixture of 3×10^{-4} M riboflavine (step I), 4×10^{-4} M aneurine (step II), and 4×10^{-4} M sodium nicotinate (step III) in 0·1 N potassium chloride solution.

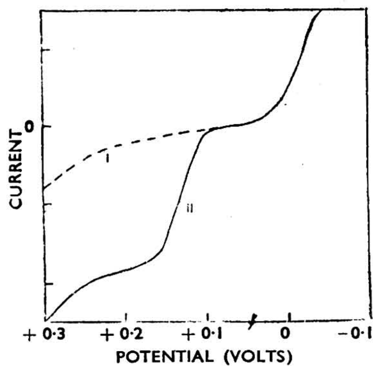


FIG. 5.

Current - voltage curves for ascorbic acid.
I. 0·05 M phthalate buffer solution(pH 4·0).
II. 0·0002 M ascorbic acid in 0·05 M phthalate buffer solution.

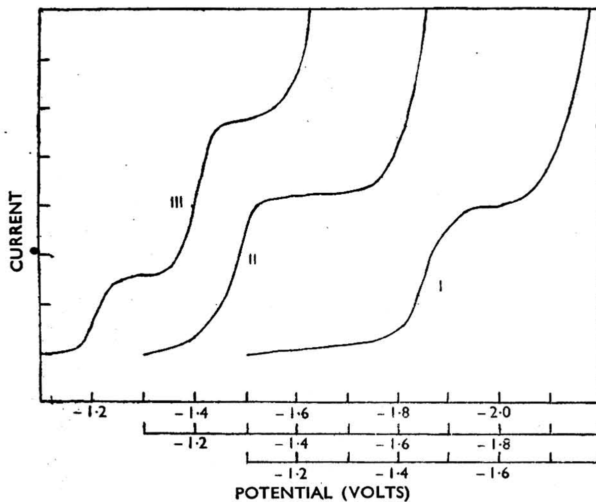


FIG. 6.

Current - voltage curves for Girard derivatives of keto-steroids.
I. 0·00035 M androsterone.
II. 0·00035 M testosterone.
III. 0·00018 M testosterone and 0·00035 M androsterone.

4:5-unsaturated ketosteroids also form well defined steps, which can be used for their determination in urine extracts. Some typical curves for the Girard derivatives of androsterone and testosterone are shown in Fig. 6. The biological assay of corticosterone in urine is extremely laborious and would justify a full investigation of the polarographic method. The polarographic method is of no value for mixtures of ketosteroids, as the individual steps are too close together. Nevertheless a separation can sometimes be achieved by adopting

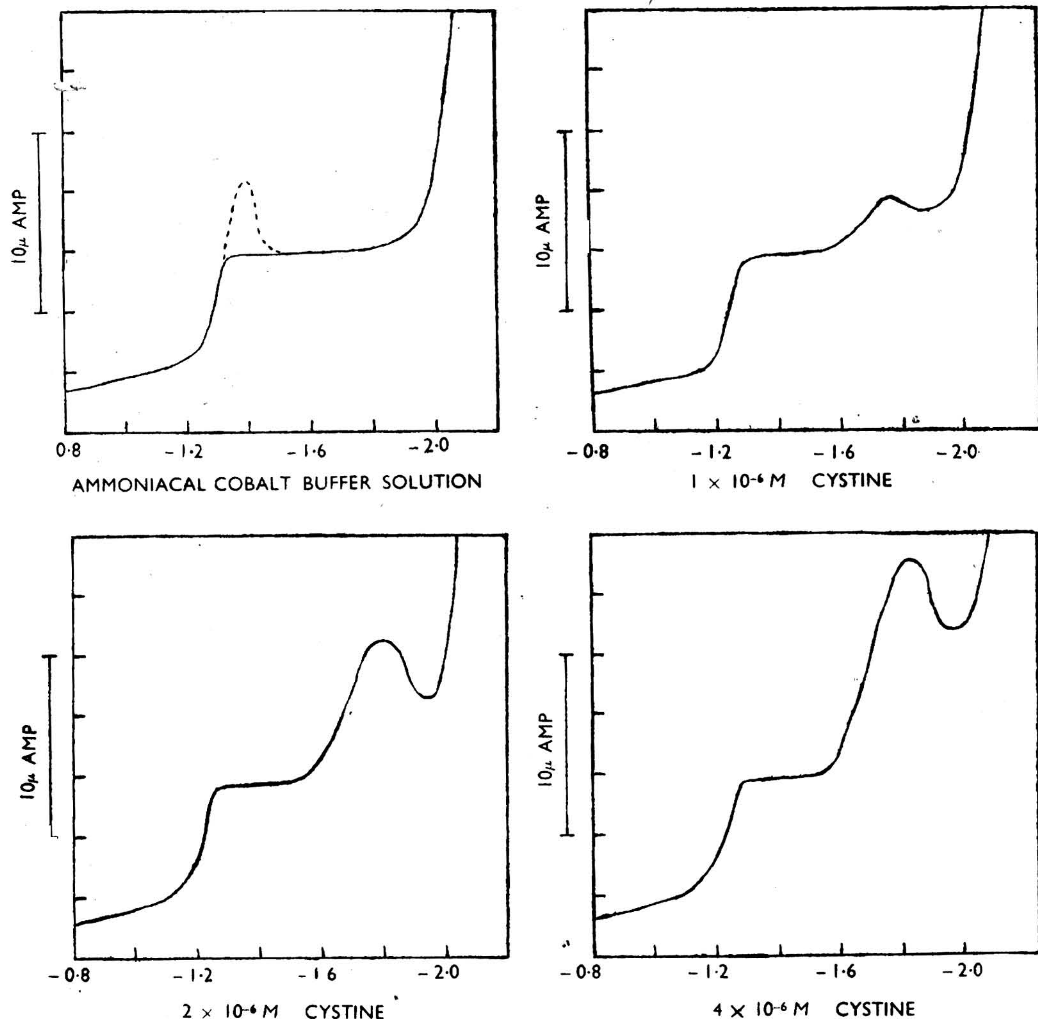


FIG. 7.

Current-voltage curves for cystine in an ammoniacal cobalt buffer solution containing 0·1% of gelatin.

Werthessen and Baker's suggestion[22] for magnifying the potential scale of the polarograph. Polarographically inert non-ketonic sterols (such as cholesterol)[26] can be determined polarographically after oxidising to the corresponding ketones with aluminium *t*-butoxide, and then condensing with Girard's reagent.

In addition to reversible and irreversible direct reductions, catalytic reductions may also occur at the dropping-mercury electrode. Catalytic reductions have been observed for a number of inorganic substances such as metals of the platinum group and perrhenates, as well as for organic substances such as quinine,[27] strychnine[28] and aneurine.[21] The phenomenon is due to the ability of certain ions that deposit at more negative potentials than hydrogen

to increase greatly the hydrogen over-potential. The resulting catalytic steps are frequently many hundreds of times higher than the corresponding normal reduction steps. Brdička[29] observed that if sulphur-containing proteins were reduced in buffered cobalt or nickel solution, a large double step formed. Cystine and cysteine behaved in the same way, but only gave a single large catalytic step (*cf.* Fig. 7). The height of the catalytic step formed by cystine is twice that given by the same molar quantity of cysteine, but is 500 times as great as that of the step corresponding to the reduction of cystine to cysteine. Since the catalytic steps are not obtained in absence of cobalt and nickel, Brdička concluded that the hydrogen evolution from the sulphhydryl groups is catalysed by the metals.

The catalytic steps formed by cysteine and cystine in ammoniacal cobalt buffer solution can be used for the determination of very small amounts of these compounds. The optimum concentration of cystine (*cf.* Fig. 8) for these determinations is between $1{\cdot}0 \times 10^{-6}$ and 5×10^{-6} molar, *i.e.*, between 0·025 and 0·125 mg. per 100 ml. of solution. At high concentrations the height of the catalytic step becomes independent of further increases in the concentration of cystine. Brdička was able to determine the cystine in as little as 0·5 mg. of human hair.

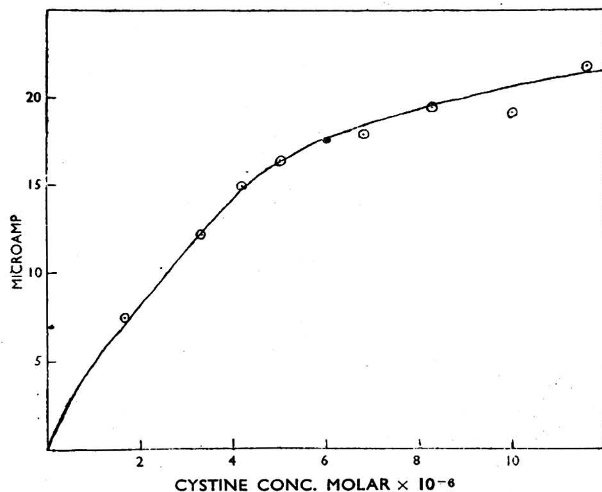


FIG. 8.

Relation between the height of the catalytic cystine step and the concentration of cystine in an ammoniacal cobalt buffer solution.

The catalytic steps formed by sulphur-containing proteins have attracted a good deal of attention in the medical world.[30] When blood proteins from different individuals were examined it was noticed that the protein double step formed by the serum from cancer patients was much smaller than that formed by the serum of normal patients. This unexpected behaviour appeared to offer possibilities for the clinical diagnosis of cancer. The effect was investigated at a large number of research institutions in Central Europe and America, but it is now generally agreed that despite many improvements of technique (i) a positive test is not obtained in all cases of cancer or sarcoma and (ii) a positive test is not specific for carcinoma or sarcoma, as it is often got from patients suffering from pneumonia and arthritis.

Many of the biochemical applications of the polarograph are of strictly limited scope but they are all of some importance in their own particular field and they all serve to indicate the versatility of the polarograph and the general usefulness of polarography.

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GREENFORD, MIDDLESEX

Inorganic Applications of Polarographic Analysis, with special reference to Aluminium, Magnesium and Zinc

BY A. S. NICKELSON

1. INTRODUCTION—A general acceleration in production in the chemical and metallurgical industries has taken place during the past ten years, and it has become increasingly evident that the older classical chemical methods of analysis require revision. The need for this is particularly necessary for plant control or inspection. Physico-chemical technique has been invaluable in the development of the newer methods. The versatility of the quartz spectrograph is well known. By its use practically the whole range of elements can be detected qualitatively and many can be determined quantitatively. Indeed, the instrument is now almost indispensable in most laboratories. Likewise other physico-chemical methods are beginning to become important. The polarographic technique in particular has many advantages where speed and accuracy are a necessity, and for some years now we have found polarographic methods invaluable.

Although theoretically any material, organic or inorganic, reducible at the dropping mercury electrode can be determined quantitatively, it is often necessary to subject the material to a series of chemical operations before a polarographic determination can be made. We maintain that thereby much of the elegance of the technique is lost and whilst chemical separations may be essential for some determinations our aim, primarily, has been to use or devise methods involving none.

Some of the analyses we carry out by the polarograph are given below.

Determination	*Material*
Lead	Lead restricted paints, etc.
Lead	Phosphor bronze
Lead and copper	Steel
Copper	Fabrics, etc.
Potassium	Various compounds
Nitrates	Inorganic salts
Bromates and iodates	Inorganic salts
Iron, copper, lead, tin and cadmium	Zinc and zinc alloys for diecasting
Iron, copper, lead and zinc	Aluminium and magnesium

In the brief account of some of the methods we use for zinc and aluminium, it will be noted that all the metals determined have reduction potentials below that of hydrogen, *i.e.*, from —0·0 v. to —1·4 v., enabling the analyses to be carried out in acid solution. Some metals have coincident reduction potentials, *e.g.*, iron and copper at zero applied potential, lead and tin at —0·4 v., but their determination has nevertheless been effected without chemical separation.

2. ANALYSIS OF ZINC AND ZINC BASE ALLOYS—Much of the work carried out on these metals was in conjunction with the British Standards Institution. Their recommended methods have been published.[1] The experimental work leading up to the final methods has also been published.[2]

Zinc is dissolved in hydrochloric acid and a part of the solution is adjusted to a definite acidity with the aid of methyl violet and polarographed. Well defined steps for lead and

cadmium are obtained at —0·46 v. and —0·63 v. respectively. Tin in this "neutralised" solution does not give a step. Potassium bromide is added to another part of the original solution and a step is then obtained for lead and tin at —0·47 v. The step for tin is obtained by difference. Percentage contents of the metals are read off from calibration curves obtained by polarographing a series of synthetic standards.

Copper may be determined from the "neutral" solution to which gelatin has been added as a maximum suppressor. The second copper (cuprous) step is measured.

After addition of sodium citrate to a solution of zinc in nitric acid and careful adjustment of concentration and acidity, steps for copper, iron, lead and cadmium are obtained on polarographing. In this medium the iron citrate complex is less readily reduced and the iron step occurs at —0·31 v. instead of at zero potential as in chloride solution.

Magnesium may be determined after chemical separation, by measurement of the diminution in height of the first step of 8-hydroxyquinoline in a buffered solution, when magnesium oxine is precipitated from it. There are some advantages in this procedure over the usual gravimetric and colorimetric determination.

Aluminium may be determined by amperometric titration.

3. ANALYSIS OF ALUMINIUM—In the following method, steps occur at

Applied potential	Zero	—0·25 v.	—0·45 v.	—1·1 v.
	$Fe^{\cdot\cdot\cdot} \rightarrow Fe^{\cdot\cdot}$	$Cu^{\cdot} \rightarrow Cu$	$Pb^{\cdot\cdot} \rightarrow Pb$	$Zn^{\cdot\cdot} \rightarrow Zn$
	$Cu^{\cdot\cdot} \rightarrow Cu^{\cdot}$			

Two factors cause difficulties in the analysis—

(a) coalescence of the ferric and cupric steps, and

(b) the ease with which aluminium in solution hydrolyses.

There is no doubt that these are serious difficulties and it is of interest to note that published methods generally rely upon elimination of the aluminium and some separation of the constituent metals prior to polarographic analysis. While these procedures may be necessary for particular purposes, it is considered that their use considerably reduces the special value of the polarographic technique.

The method we employ is based on the use of a formate buffer and no chemical separations are made. Aluminium is dissolved in hydrochloric acid with addition of a little chlorate to complete solution. A maximum suppressor (0·1 per cent. solution of proteose peptone) is then added. The solution is adjusted to *p*H 3·7 by addition of sodium formate and formate buffer and polarographed, with an input to the potentiometer of 2 v. to give a reasonable interval between the first combined step and the second copper step. The combined copper and iron step is measured from a zero current line obtained when the cell circuit is broken. In practice it is found that the ratio of the cupric to the cuprous step is about 0·9, so that the height of the iron step is the height of the combined step less 0·9 times the height of the cuprous step. The heights of the lead and zinc steps are measured in the normal way.

Iron and copper in amounts up to 1%, lead up to 0·2% and zinc up to 2% have been readily determined.

4. ANALYSIS OF MAGNESIUM—Similar methods to those for aluminium can be used. Separate iron and copper steps may also be obtained in nitric acid solution in presence of citric acid, but the adjustment of acidity is far more critical than it is in the method for zinc.

Papers in connection with the determination of magnesium and aluminium and with those methods outlined in 3 and 4 are being prepared.

5. CONCLUSION—This short account of our use of the polarograph is intended to show how the instrument can be applied for the analysis of a few non-ferrous metals and it can only touch on the fringe of the polarograph's potentialities.

It is essential, however, to appreciate that a method which has proved to be satisfactory for a particular alloy may not necessarily be suitable for other alloys of different compositions without careful checking. In other words, each problem must be treated as an individual one. Failure to realise this will inevitably lead to inaccurate results and discredit to a technique unsurpassed for some analyses, *e.g.*, to cite only one instance, the accurate determination of traces of cadmium (0·0005% and upwards) in zinc and zinc alloys.

It follows therefore that the control of the development and use of the technique should be in the hands of a sound experimentalist with a good knowledge of physical chemistry if the instrument is not to suffer the fate of relegation to the "museum" corner of the laboratory.

Results obtained by the polarograph have compared more than favourably with chemical methods, both in accuracy and speed, with the added advantage that the work can be carried out by comparatively inexperienced staff.

Even so, many problems remain to be solved. The difficulty due to "drop wave" encountered in measuring a small step following a very large step is well known to polarographers, *e.g.* in the determination of traces of lead and tin in zinc alloys when copper is present in amounts over 0·01%, and a method to deal with cases like this would be invaluable.

Polarographic recording is generally carried out by means of a drum camera or pen recorder, and there is very little published information as yet about the use of the cathode ray oscillograph. It is therefore of some interest to state that a colleague, Mr. J. E. B. Randles, has carried out an investigation with the oscillograph as a recording instrument. Results indicate that it can be successfully used in routine operation and also for problems in connection with the fundamental principles of polarography.

This paper is published with the permission of the Chief Scientific Officer, Ministry of Supply.

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Applications of Polarographic Analysis to the Examination of High Purity Selenium, Nickel and Cobalt Compounds

BY R. H. JONES

SELENIUM DIOXIDE

SELENIUM dioxide used in the manufacture of selenium rectifiers is required to conform to exceptionally high standards of purity. Gravimetric analytical methods which are satisfactory for the examination of technical grade selenium and crude selenium dioxide will not detect the traces of tellurium, lead, copper, nickel and cobalt which are present in the pure selenium dioxide.

An approximation of the order of quantities of these impurities present was obtained by analysing the residue remaining after re-subliming several kilos of the product at 340° C. Some analytical results are tabulated in Table I.

TABLE I
IMPURITIES IN SELENIUM DIOXIDE

Sample weight ..	Crude, 50 g. %	Pure 1940, 7 kg. pts./million	Pure 1945, 500 g. (Polarographic method) pts./million
Te	0·031	0·8	0·1
Pb	0·011	0·4	0·3
Cu	0·002	0·6	0·2
Ni	0·003	4·3	0·3
Co	0·001	1·2	0·4
Fe	0·033	2·8	0·9

Consideration was given to the application of polarographic methods to this analytical problem.

The polarographic behaviour of selenious acid and selenites was studied by Schwaer and Suchy,[1] who reported that the reduction of the acid proceeded in three steps with half wave potentials of —0·12 v., —0·4 v. and —0·55 v. versus the saturated calomel electrode. In ammoniacal ammonium chloride solution, however, the selenite ion reduced at —1·5 v. and in strongly alkaline solution it was not reduced.

An alkaline ground solution would appear to be the ideal condition for the determination of impurities in selenium dioxide, but these are so low in amount and A.R. reagent blanks are relatively too large to permit this method to be used directly for examination of the pure product.

An analytical scheme which included preliminary concentration, chemical separation of impurities and subsequent polarographic determination was studied, using a manual polarograph to investigate each procedure.

CONCENTRATION OF IMPURITIES—A few experiments with known additions of impurities to the pure selenium dioxide established that dry sublimation of the selenium dioxide gave low results, but this was overcome by volatilising the selenium dioxide from concentrated sulphuric acid solution.

CHEMICAL SEPARATION—In analysing the concentrated dry residue it was first necessary to separate the tellurium from the other impurities present, which could then be examined in two polarographic groups, *viz.*, for copper and lead in hydrochloric acid and for nickel and cobalt in pyridine solution. The residue remaining after volatilising the selenium dioxide was dissolved in 25% hydrochloric acid and filtered from siliceous matter, and tellurium was precipitated by reduction with sulphur dioxide in boiling solution.

For the complete precipitation of traces of tellurium it was found necessary to add a few mg. of hydrazine hydrochloride and a similar small quantity of pure selenium dioxide to act as a collector of the tellurium, and to continue bubbling sulphur dioxide until the solution was cold.

After settling overnight the precipitate of tellurium and selenium was filtered on a paper pad, treated with concentrated nitric and sulphuric acid mixture and evaporated to dryness to decompose organic matter, volatilise the added selenium dioxide and convert the tellurium to the dioxide. Low temperature ignition of the tellurium - selenium precipitate results in complete loss of traces of tellurium.

The filtrate from the tellurium separation was evaporated to dryness and the residue, containing the base metal impurities, was dissolved in 10 ml. of 0·25 *N* hydrochloric acid and examined by the following polarographic methods.

POLAROGRAPHIC DETERMINATION—*Copper and Lead*—To 5 ml. of the 0·25 *N* hydrochloric acid solution add a few mg. of hydroxylamine hydrochloride, warm to reduce iron, cool and dilute to 10 ml. with 0·2 *N* potassium chloride containing 0·02% of gelatin. Transfer to the polarograph cell, remove dissolved oxygen and electrolyse. Record galvanometer deflections between 0·1 and 0·4 v. for copper, $Cu^{\cdot} \rightarrow Cu$, and between 0·4 and 0·6 v. for lead, $Pb^{\cdot\cdot} \rightarrow Pb$.

Nickel and Cobalt—To the remaining 5 ml. of solution add 5 ml. of 10% pyridine solution containing 0·02% of gelatin and electrolyse after the usual preliminaries, recording galvanometer deflections between 0·7 and 1·0 v. for nickel, and between 1·0 and 1·3 v. for cobalt. It is unnecessary to remove the traces of iron and lead hydroxides precipitated on addition of the pyridine solution. Copper may be determined in the pyridine ground solution if desired, and the result agrees with that obtained in chloride solution if the appropriate standard is used.

Tellurium—Dissolve the tellurium dioxide in 5 ml. of 0·2 *N* hydrochloric acid and add 5 ml. of 0·8 *N* ammonium hydroxide. Transfer to the polarograph cell, remove oxygen and electrolyse. The half wave reduction potential of tellurite ion occurs at about 0·7 v. applied voltage. Interference from possible trace of extraneous nickel contamination may be overcome by adding 0·1 ml. of 2 *N* potassium cyanide. In this case, record galvanometer deflections between 0·1 v. and 0·6 v. with mercury pool as reference electrode.

STANDARDISATION—The quantitative recovery of small amounts of tellurium from 25% hydrochloric acid solution by the chemical procedures described is shown by the following results which have been used to construct the standard calibration chart.

TABLE II

Te taken mg.	Galvanometer sensitivity	Wave height cm.
0·05	1	4·0
0·1	1	8·3
0·2	½	8·1
0·3	⅓	8·4

In no case was tellurium detected in the filtrate from the sulphur dioxide reduction or in the siliceous residue.

Conventional methods were used for standardising the polarographic technique and measuring wave heights over suitable ranges of concentration for the other elements determined.

Generally, the plateaux were so well defined that two point recordings were satisfactory, and the results confirmed the proportional relation between wave height and concentration in the ground solutions employed.

It is essential to use only A.R. reagents and acid-rinsed beakers and to run reagent blanks through the analytical procedures to correct the analyses.

As a result of process and plant improvements, including the installation of air filtration plant, the pure selenium dioxide now produced contains less than 3 parts/million of total base metal impurities excluding silica.

Nickel and Cobalt Salts

Standard methods for the determination of copper and lead as impurities in nickel and cobalt salts are based on hydrogen sulphide separation, the copper being determined electrolytically or colorimetrically after separation of the lead as lead sulphate.

Polarographic analysis provides an alternative method which is much more rapid and permits the determination of these impurities without chemical separations.

The order of the copper and lead impurities in the range of nickel and cobalt salts produced in the Refinery varies between 0·002 and 0·02% of Cu, and between $<0{\cdot}001$ and 0·03% of Pb. Within these limits a solution of the salt in *N* hydrochloric acid was found to give satisfactory well-separated steps for copper and lead.

Method—Weigh 20 g. of the crystals, dissolve in *N* hydrochloric acid and dilute with this acid to 100 ml. To 10 ml. of this solution add a few mg. of hydroxylamine hydrochloride and warm. Cool, add one drop of 1% gelatin solution and correct the volume to 10 ml. Transfer to a polarograph cell, remove dissolved oxygen by inert gas, and electrolyse between 0 and 0·7 v. applied voltage. The copper wave $Cu^{\cdot} \rightarrow Cu$ occurs between 0·1 and 0·3 v. and the lead wave $Pb^{\cdot\cdot} \rightarrow Pb$ between 0·4 and 0·6 v. Record wave heights and read off percentage impurity from standard graphs.

This general procedure has been applied to the determination of copper and lead impurities in:

Nickel chloride, sulphate and formate (10% solution) and nickel ammonium sulphate.
Cobalt acetate, chloride and sulphate.

Nickel and cobalt hydroxides and carbonates are dissolved in hydrochloric acid and a 5% solution of these compounds is suitable for direct polarographic analysis.

Calibration—Standard solutions of copper and lead containing 0·1 g. of the metal per litre were used in the calibration experiments. Measured volumes of these solutions, added from a semi-micro burette, were evaporated to dryness and dissolved in 10 ml. of 20% nickel or cobalt sulphate solution in *N* hydrochloric acid. Wave heights for copper and lead were recorded and plotted against percentage of the respective metal impurity.

Similar experiments with nickel sulphate prepared from carbonyl nickel powder which is free from copper and lead gave a straight line graph passing through the origin, and parallel to the former graph.

The percentages of copper and lead in the samples of nickel and cobalt sulphate used as ground solution blanks were obtained by extrapolation.

In Tables III and IV are tabulated typical results, from which the calibration graphs were constructed.

Table III

Cobalt Sulphate 20% Solution

Instrument settings: Sensitivity = ½; Counter current = 4

Copper				Lead			
added		Wave height	Wave ht. for Cu added	added		Wave height	Wave ht. for Pb added
mg.	≡%	cm.	cm.	mg.	≡%	cm.	cm.
—	—	1·5	—	—	—	5·1	—
0·02	0·001	2·1	0·6	0·04	0·002	5·8	0·7
0·04	0·002	2·9	1·4	0·10	0·005	7·2	2·1
0·08	0·004	4·2	2·7	0·16	0·008	8·1	3·0
0·10	0·005	4·9	3·4	0·20	0·010	9·0	3·9
0·16	0·008	6·8	5·3	0·30	0·015	11·1	6·0

In routine analysis it is advisable to run a standard with each batch of samples, and the instrument settings, *e.g.*, counter current, used in the calibration must be adhered to in the analysis.

NICKEL IN COBALT—The determination of traces of nickel impurity in cobalt salts was for many years a difficult analytical problem. Methods depending on the precipitation of nickel with dimethylglyoxime required the cobalt to be in the oxidised condition and probably the best separation is obtained when cobalt is present as the stable cobalticyanide and nickel is precipitated from the cyanide solution with solid dimethylglyoxime and formaldehyde. Reprecipitation is necessary and the method, although reliable, is tedious.

A rapid polarographic method was developed by Lingane and Kerlinger[2] in 1941 for the determination of traces of nickel in cobalt metal and its salts, in a pyridine ground solution. The method of standard additions was used and the results obtained were accurate to within ±3% of the amount of nickel present.

TABLE IV

ADDITIONS OF LEAD TO LEAD-FREE NICKEL SOLUTIONS

Instrument settings: Sensitivity = 1; Counter current = 8

Pb in 10 ml. nickel solution mg	Wave height cm.
0·02	0·8
0·04	1·5
0·08	3·0
0·10	3·8
0·15	6·0
0·16	6·3
0·20	7·9
0·28	11·2
0·32	12·8

TABLE V

COMPARATIVE RESULTS

	Polarographic		Gravimetric	
	% Cu	% Pb	% Cu	% Pb
Nickel sulphate	0·002	0·0005	0·002	<0·001
Nickel formate	0·009	0·020	0·009	0·020
Cobalt sulphate (*a*)	0·0015	0·008	0·0015	0·009
(*b*)	—	0·0085	—	0·0085
(*c*)	0·0022	0·012	0·0023	0·011
Cobalt acetate	0·003	0·018	0·003	0·019

The greatest accuracy of which the polarograph is capable without elaborate precautions is probably in the determination of ratios. In this application variables such as capillary constant, drop rate and temperature, are eliminated, and the results are accurate to within 1%.

In conclusion I will refer briefly to one use of this method. The value of the ratio nickel to copper in mattes during volatilisation by the carbonyl process is a measure of the extent of nickel extraction, and rapid information of this is an advantage in process control. A polarographic method was developed to provide this information accurately in less than half an hour, compared with several hours required for gravimetric determinations. Some results are shown in Table VI.

TABLE VI

Gravimetric analysis					Polarographic analysis	
Ni %	Co %	Cu %	Ni : Cu	Ni : Co	Ni : Cu	Ni : Co
51·08	3·88	17·92	2·85	13·2	2·88	—
45·40	4·70	20·40	2·22	9·7	2·25	—
32·10	6·85	31·90	1·01	4·7	1·02	4·6
10·54	7·38	34·76	0·30	1·4	0·31	1·4

Polarographic methods have earned a well-deserved place among the more established physical methods and the increase in research on them during recent years is a good augury for future applications.

I wish to acknowledge the help of Mr. W. G. Bolton and Miss R. Samel in the experimental work, and to thank the Mond Nickel Co. Ltd. for permission to publish this paper.

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DISCUSSION ON THE PRECEDING FOUR PAPERS

The CHAIRMAN, Mr. R. C. CHIRNSIDE, said that all the readers of the papers had emphasised that the polarograph should be regarded as a complementary tool and not as something to be used ill-advisedly in replacing conventional methods. Another factor which had to be considered was the effect it must have on the type of person employed to do analysis; this effect might well be to raise the status of the analyst, because highly trained chemists with a considerable knowledge of physics would be needed to show how to apply the polarograph to any particular problem. The method could subsequently be used by relatively unskilled operators. Mr. Jones had mentioned that tellurium and selenium were being separated by volatilisation. At what temperature did that take place? Also, was there any possibility of accurately determining nickel and cobalt by the aid of the polarograph when they occurred together in considerable proportions?

Mr. JONES replied that one difficulty in the accurate determination of nickel and cobalt was concerned with amounts present. If small traces of nickel were present with considerable excess of cobalt, instead of good wave separation there would be a very nasty curve. Lingane had given the accuracy in determining nickel in cobalt as within about 2–3%. This error would be considerable if the amount of nickel present were, say, 40%, but on 0·001–0·02% or even more it would not matter. Regarding the volatilisation of tellurium on ignition, very small traces volatilised at about 400° C.

Mr. J. T. MINSTER asked if there was a satisfactory method of determining, say, 0·5% of cobalt in about 99·5% of nickel.

Mr. JONES replied that there was, by titration with potassium ferricyanide in ammoniacal solution.

Mr. G. W. C. MILNER remarked that a hydrochloric acid medium was being used by several of the speakers for the determination of copper and lead. Had the possible interference of antimony and bismuth been considered, for their half wave potentials were very close to that of copper in hydrochloric acid solution?

Both Mr. NICKELSON and Mr. JONES replied that the substances for which they had developed their methods did not contain bismuth or antimony.

Mr. N. STRAFFORD asked what were the smallest quantities of ions determinable by the polarograph in the normal manner, *i.e.*, without the device of a catalytic reaction as described by Dr. Page. From the figures given by the speakers it seemed that the amounts determined were of the order of milligrams. But many colorimetric methods would determine micrograms.

Mr. NICKELSON said it was possible to determine 0·002% of lead, tin and cadmium in zinc alloys, but what that would be in micrograms would depend on the amount of sample taken.

Dr. DAVIES considered that with the main materials the polarographic method was as sensitive as the colorimetric, provided proper precautions were taken. The difficulty of working with micro-quantities was the exceedingly high concentration of the ground solution compared with that of the trace elements to be determined. Analytical reagents often contained a large number of trace impurities far in excess of the small traces the analyst was trying to determine.

Dr. W. STROSS asked for fuller information about the technique of investigating waves extending from positive to negative potentials and, at the same time, reaching below the galvanometer zero. He also said that he had tried, in the laboratory of International Alloys, Ltd., the method mentioned by Mr. Nickelson for copper, lead and zinc in aluminium alloys. He had found 0·25 ml. of a 2% solution of gum ghatti (kept sterile by addition of a crystal of thymol) per 10 ml. of final solution a very satisfactory maximum suppressor for this method, but he had not been very satisfied with the determinations of iron and copper—iron in particular. The "factor" mentioned by Mr. Nickelson varied from day to day, the calibration gave correct answers only over a limited range of concentrations and the results for iron tended to be low.

Dr. DAVIES explained that most of the commercial instruments had a device incorporated in them which enabled them to pass through zero, or a positive applied voltage to a negative one. He could not speak for the Cambridge instrument as he had never used it, but the Tinsley instrument had such a device. In one paper read in Czechoslovakia a very simple device of two resistances added across the potentiometer was described. This enabled the operator to pass from a positive, through zero, to a negative voltage continuously.

Dr. PAGE confirmed that both the Cambridge and the Tinsley instruments were fitted with a device to enable the applied potential to pass from a positive value through zero to a negative value. The technique for reducing the potential range of the polarograph from one of 0 to 3·0 v. to one of 0 to 1·0 v. had been described by Page and Waller (this Vol., p. 65).

Mr. NICKELSON, in reply to Dr. Stross, said that if he could send one of his staff to Woolwich, the question of the varying "factor" could be investigated. No difficulty had been encountered in the determination of iron and copper in amounts from 0·2% to 2% in any ratio, provided conditions were rigidly adhered to.

Mr. J. C. GAGE asked Dr. Page if complex bases like the alkaloids in acid solution were discharged at the cathode in the same way as metal ions. If so, how was it possible to tell whether a wave thus obtained really represented a discharge or was due to a reduction of the molecule or was a catalytic hydrogen wave?

Dr. PAGE said he was not familiar with the reduction of alkaloids, though he was aware that both strychnine and quinine gave catalytic waves. If the height of the step formed was much greater than that estimated from a knowledge of the number of electrons involved in the reduction the step could be assumed to be catalytic. The height of the catalytic step for aneurine was 4000 times greater than that of the normal reduction step for aneurine.

Mr. F. A. PAINE asked what was the method of measuring catalytic wave heights, since they all showed pronounced maxima and it is known that after a maximum has occurred the current does not necessarily fall to the value of the limiting diffusion current.

Dr. PAGE said that he was afraid the measurement of all catalytic waves had to be somewhat arbitrary. For cystine, he measured the vertical distance between the previous horizontal plateau and the minimum occurring after the catalytic maximum.

Mr. H. F. BAMFORD asked if wet ashing was a suitable method of preparing organic materials for the polarographic estimation of trace metals.

Dr. PAGE said it was.

Mr. B. S. COOPER raised a general point regarding the reliability of the polarograph. Was there any danger of slight defects arising which might pass unnoticed by the semi-skilled routine operators using the instrument but might vitiate the results and remain unsuspected?

Dr. DAVIES considered that the normal practice was for the polarograph to be re-standardised at least once a day. He had found that if an electrical defect occurred it soon became evident in some unexpected irregularity of the curve. It was not difficult to discover that an instrument was becoming faulty.

Mr. N. A. HURT asked if Dr. Davies could suggest the most satisfactory methods of varying the position of the zero on the applied potential drum of a polarograph, to give a wider choice of positive or negative applied potential when working, for example, with platinum micro-electrodes. He also asked if Dr. Page had obtained results similar to those obtained with the thermodynamically reversible oxidation - reduction system quinone - hydroquinone with more complex quinone structures. From these results had it been possible to evolve a satisfactory method for the determination of synthetic vitamin K substitutes that compared favourably with the colorimetric methods? Had Dr. Page any experience of the polarography of methionine, after removal of the methyl group and, if so, did the results closely correspond with the cysteine - cystine system?

Dr. PAGE said, in reply to Mr. Hurt, that many substituted quinones gave satisfactory steps (*cf. J. Chem. Soc.*, 1943, 133). He had not compared the accuracy of the polarographic method for 2-methyl-1 : 4-naphthaquinone with that of the colorimetric method. He had been unable to devise a simple polarographic method for the estimation of methionine owing to the difficulty of removing the methyl group. Homocysteine in an ammoniacal cobalt buffer solution gave a catalytic step similar to that given by cysteine.

Mr. J. HASLAM suggested that there was some danger in the use of a calibration curve, even if associated with a thermostat. There did not appear to be absolute certainty that the conditions were the same for the dropping electrode when a test was made and when the curve was prepared. He preferred to put the standard on as an individual test.

Mr. NICKELSON said the simplest way was to determine the capillary constant each day, by measuring the step obtained from a standard cadmium sulphate solution. Over a period of several months no large differences in the capillary constant were obtained.

Mr. JONES said he had had a capillary in use for almost twelve months and it had not varied considerably over that length of time. They always ran a standard.

Polarographic Studies: IV. A Note on the Determination of Ascorbic Acid

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

A POLAROGRAPHIC method for the estimation of ascorbic acid in fruits and vegetables has been used by a number of investigators.[1,2,3,4,5,6] The technique employed differs from that generally used in that it entails oxidation at a dropping mercury anode rather than reduction at a dropping mercury cathode.

The method is fully described in a recent paper by Gillam.[6] We have tried to use a Cambridge polarograph for this estimation, but experienced considerable difficulty in measuring the anodic step height. However, we have since found that the method can be improved considerably by modifying the technique as recently described by Werthessen and Baker[7] in a paper on the polarographic estimation of ketosteroids.

These authors increased the potential scale of their polarograph by decreasing the range from one of 0 to $-3{\cdot}0$ v. to one of 0 to $-2{\cdot}0$ v. The recording system and automatically changing potentiometer were allowed to rotate at their normal speed. Werthessen and Baker found that under these conditions definition of the ketosteroid steps was much improved, and they were able to effect satisfactory separation of the steps for the 20-ketosteroids from those for the 17-ketosteroids.

The polarographic steps for the reduction of the ketosteroids occur at about —1·5 v.; therefore in an examination of these substances the voltage range must extend to —2·0 v. On the other hand, during the oxidation of ascorbic acid it is only necessary to consider potentials up to +0·3 v.; in a buffer solution of *p*H 4·6 the vitamin is oxidized at +0·10 v. (versus the saturated calomel electrode).[6]

We accordingly changed the potential range of our Cambridge polarograph so that the complete range was from 0 to —1·0 v. instead of from 0 to —3·0 v. This was achieved by connecting a 2 v. accumulator to the polarograph in place of the usual 6 v. battery and adjusting the applied potential to one-third of its normal value. Thus the reading of a voltmeter connected across the cell terminals of the polarograph was one-third of that indicated on the potentiometer scale of the polarograph. The horizontal voltage scales of polarograms obtained in this way have been magnified threefold. By reversing the connections to the polarograph cell, we were able to use the indicated range of 0 to —0·9 v. (now equivalent to 0 to +0·3 v.) for oxidations. The scale cannot be magnified more than threefold unless an external potentiometer is employed to reduce the voltage applied by the 2 v. accumulator. We found that a three-fold magnification gave satisfactory results for ascorbic acid.

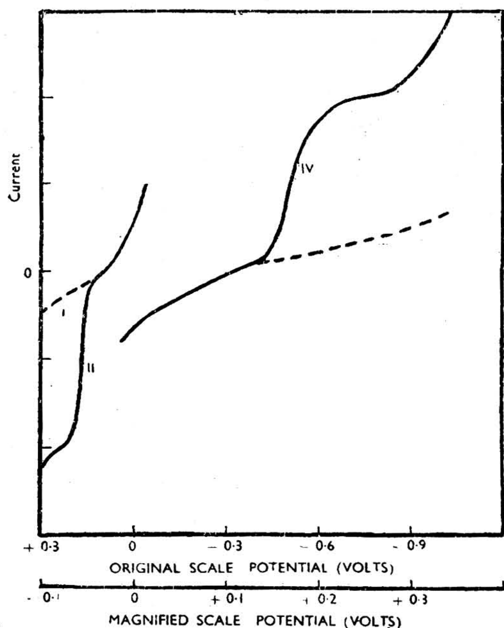

Fig. 1.

I. 0·05 *M* Phthalate buffer solution (original scale).
II. 0·0002 *M* Ascorbic Acid " "
III. 0·05 *M* Phthalate buffer solution (magnified scale).
IV. 0·0002 *M* Ascorbic Acid " "

Some typical curves for ascorbic acid in a 0·05 *M* potassium hydrogen phthalate buffer solution (*p*H 4·0) are shown in Fig. 1. The curves obtained by magnifying the scale have a much better definition and their step heights are the easier to measure.

It is thought that this modification of technique is likely to be of value for other polarographic analyses in which it is necessary to measure the height of steps that are very close together. The technique should also facilitate the accurate measurement of half-wave potentials.

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GLAXO LABORATORIES, LTD.
GREENFORD, MIDDLESEX

November, 1945

Colour Reactions of Stilboestrol

BY T. TUSTING COCKING

IN a recent contribution[1] two new colour reactions for stilboestrol were described, and it was stated that, while these could be used for approximate quantitative estimations, great accuracy could not be claimed owing to a variety of causes. Further experimental work has resulted in improved technique for both the green and violet reactions and has brought to light a number of points regarding both tests that appear worth recording.

VIOLET COLOUR REACTION—With regard to the method whereby a purple or violet colour is produced by the action of water on a solution of brominated stilboestrol in glacial acetic acid, improvements in technique have resulted in increasing the accuracy of the test sufficiently to justify its use for quantitative colorimetric measurements, provided a satisfactory method can be evolved for separating stilboestrol in a pure state from its preparations.

As already stated, the coloured colloidal precipitate is readily soluble in all kinds of organic solvents, but the coloured solutions so formed rapidly fade and are useless for quantitative work. The colloidal solution itself varies in colour from purple to violet according to the dispersion of the colloidal particles and the colour fades slowly as the particles coalesce and precipitate. It has been found, however, that the use of a solution of gelatin instead of water produces a perfectly transparent solution, the colour of which is relatively stable and suitable for comparisons in a colorimeter of the Duboscq type. It has been found that the *maximum* colour is only produced when an enormous excess of bromine at a high concentration is used, the most suitable being 5% v/v in glacial acetic acid, and a proportion of about 100 atoms of bromine per molecule of stilboestrol. Less bromine or more dilute solutions yield colours which are weaker, variable and red instead of violet. Thus, 1 millilitre of stilboestrol, fully brominated and diluted to 100 ml. with gelatin solution, gave a colour in a 1 cm. cell of 14 red + 2·6 blue Lovibond, whereas the colour of the same amount less brominated was only about 10 red and no blue. In order to avoid loss of bromine the heating is best carried out in a small sealed tube (ampoule).

A standard solution of brominated stilboestrol may be prepared by heating in a boiling water-bath for 10 minutes, in a 50 ml. sealed ampoule, 100 mg. of stilboestrol with 20 ml. of a 5 per cent. v/v solution of bromine in glacial acetic acid and, after cooling, diluting to 100 ml. with glacial acetic acid. The resulting solution, 1 ml. of which ≡ 1 mg. of stilboestrol, keeps indefinitely in a stoppered bottle. In order to prepare the colour standard, 1 ml. of this solution is mixed with 2 ml. of absolute alcohol and then sufficient of a clear 0·4% aqueous solution of gelatin to produce 100 ml. With this solution, a setting of the colorimeter at about 25 mm. gives the most accurate results.

For quantitative determinations it is essential that the stilboestrol should be first separated from its preparations in a *pure* state. Mere traces of "fatty" matter prevent the formation of the colloidal solution and, although the colouring matter is produced on the addition of water, any "fatty" impurity also separates and carries down with it the bulk of the colour, leaving the aqueous portion turbid and weakly coloured. Ampoules of stilboestrol invariably have an oily base; tablets usually contain a lubricant such as magnesium stearate and, up to the present, I have been unable to separate the stilboestrol in a sufficiently pure state for quantitative determinations.

GREEN COLOUR REACTION—In the earlier report[1] it was stated that the full development of the green colour necessitated the addition of a definite proportion of bromine, approximately 4 equivalents. With less bromine the colour was weak and with very little more no colour developed. The test therefore was only useful as an identity test if carried out more or less quantitatively. If, however, a slight excess of bromine is added to the acetic acid solution of stilboestrol and allowed to react at ordinary temperature for about 20 seconds, and the excess is then removed by addition of a little phenol, the resulting liquid on heating in a water-bath for 2 minutes develops the full green colour. The same colour develops in the cold but only after long standing, 12 to 18 hours. Thus, for an identity test, it is only necessary to dissolve about 1 mg. in a few ml. of glacial acetic acid, add 0·2 ml. of 1% v/v bromine solution in glacial acetic acid, allow to stand for about 20 seconds, then add 1 or 2 drops of a 50% phenol solution in glacial acetic acid and, as soon as the excess of bromine has been removed, heat in a water-bath for 2 minutes.

The change of the green colour to blue, grey, straw and finally reddish brown on addition of sucrose and further heating also takes place in the cold, but requires about 48 hours. Of all sugars tried, sucrose, laevulose, sorbose and raffinose alone give this reaction. It would thus appear to be due to the laevulose portion of the molecule, which all these sugars possess, and the reaction may be useful in identifying a small quantity of a sugar.

The green colour reaction is not suitable for quantitative work, as not only the depth of the colour but also the shade varies with the concentration. 0·2 mg. of stilboestrol in a volume of 5 ml. develops a yellow colour with only a faint tinge of green; 0·4, 0·6, 0·8 and 1 mg. develop disproportionately deeper colours becoming less yellow and more green as the concentration increases. Thus, the 1 mg. colour is slightly deeper and less yellow than

the 0·6 mg. colour superimposed on the 0·8 mg. colour. A similar difference occurs with the further colours produced by the addition of sucrose.

The above two colour reactions are also given by dienoestrol.

The work entailed by the above has been carried out in the laboratories of The British Drug Houses, Ltd., to the Directors of which I am indebted for permission to publish these results.

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THE BRITISH DRUG HOUSES LTD.
LONDON, N.1

6th November, 1945

A Method for the Determination of Selenium in Steels, with Notes on the Determination of Sulphur

BY B. S. EVANS*

SELENIUM

AN attempt to apply the method worked out for the determination of selenium in copper[1] to its determination in steels was not very successful, low results being obtained. It would seem that the carbon compounds present in steel are fairly active in precipitating selenium and that the recovery of such selenium without loss by volatilisation is a matter of some difficulty.

The following experiments illustrate this point.

Quantities of 5 g. of electrolytic iron were taken and various amounts of selenium were added. Each was dissolved without heating in 100 ml. of diluted hydrochloric acid (1+1) containing 10 ml. of bromine. After solution 2 ml. of 20% cupric chloride solution were added, the liquid was warmed to about 50° C. and a solution of sodium hypophosphite in diluted hydrochloric acid (1+1) was added a little at a time until the iron was completely reduced and the selenium began to precipitate. Excess of the hypophosphite solution was then added and the beaker was allowed to stand on the steam bath until the selenium was filterable. From this point the precipitate was treated in exactly the same manner as the hypophosphite precipitate obtained in the method published for determination of selenium in copper. Results were as follows:

Iron taken g.	Selenium added g.	Titration $N/100$ $Na_2S_2O_3$ ml.	Selenium found g.	% of Se Added	% of Se Found
5·0	0·0050	23·87	0·0047	0·100	0·094
5·0	0·0040	19·72	0·0039	0·080	0·078
5·0	0·0030	14·16	0·0028	0·060	0·056
5·0	0·0020	9·03	0·0018	0·040	0·036
5·0	0·0010	4·87	0·00096	0·020	0·019

While these results are reasonably accurate, the same method tried with standard steels to which selenium had been added gave invariably low results, and the error was very roughly proportional to the amount of carbon in the steel.

	Steel taken g.	Carbon %	Selenium added g.	Titration $N/100$ $Na_2S_2O_3$ ml.	Selenium found g.	% of Se Added	% of Se Found
"V" ..	5·0	0·5	0·0050	22·00	0·0044	0·100	0·088
"T" ..	5·0	0·3	0·0050	22·80	0·0045	0·100	0·090
"R" ..	5·0	0·79	0·0050	21·08	0·0042	0·100	0·084

A subsequent attempt to recover the selenium precipitated with the carbon in the "R" steel experiment by treatment of the precipitated carbon with nitric acid brought the amount up to 0·0045 g.

Trials were then made by dissolving the steels in 100 ml. of diluted hydrochloric acid (1+1) to which had been added 10 g. of potassium chlorate. Results were better, though

* Communication from the Armament Research Dept., late Research Dept., Woolwich.

still low ("R" steel 0·0048, 0·0046), but, the method being unpleasant to work and some of the carbon being left still undissolved, it was abandoned.

METHOD—The method finally adopted was as follows:

(*a*) *For steels that will dissolve under the treatment*—Dissolve 5 g. of sample by warming in 100 ml. of diluted sulphuric acid (1+3) + 50 ml. of water containing 10 g. of potassium permanganate. After solution, boil for five minutes and then dissolve the precipitated manganese dioxide by addition of oxalic acid a little at a time. Pour the liquid into a flask, rinsing in with diluted hydrochloric acid (1+1); add 100 ml. of strong hydrochloric acid and 2 ml. of 20% cupric chloride solution; warm to about 50° C., add sodium hypophosphite a little at a time until the iron is reduced and then an additional 4–5 g. Boil under a reflux condenser for 15 minutes. Filter immediately while hot through a pulp filter and wash as rapidly as possible with a boiling solution of 2 g. of sodium hypophosphite in 100 ml. of diluted hydrochloric acid (1+1), adding each quantity of wash liquid directly the preceding one has run through; change the receiver and continue the washing (about 4 times) with cold 5% ammonium chloride solution. Test the filtrate and first washings for completeness of precipitation by heating to boiling (it should remain bright). Dissolve the precipitated selenium through into a flask by treatment on the pulp with a solution of bromine in dilute hydrochloric acid (5 ml. of saturated solution of bromine in conc. hydrochloric acid + 10 ml. of water), if necessary break up the precipitate with a jet of 5% hydrochloric acid and pass the filtrate through again. Wash the filter with 5% hydrochloric acid; this should result in a total filtrate of 100–150 ml. coloured deep orange with bromine and of acidity slightly over 5% HCl; the filter, which is discarded, should be white. Remove bromine from the solution by bubbling through it a rapid stream of air until its colour is a pale lemon yellow. Add 20 ml. of 4% potassium iodide solution, shake well and allow to stand for 1–1½ hour with occasional shaking; filter off the (scarlet) precipitate of selenium on a tight pulp filter and wash

(1) with 100 ml. of a solution of 5% hydrochloric acid and 5% ammonium chloride,
(2) twice with 5% ammonium chloride solution (to remove acid),
(3) five or 6 times with 5% ammonium nitrate solution (to remove chlorides).

Suck the filter dry by momentarily attaching the stem of the funnel to a filter pump, blow it out into a beaker and wipe the funnel with a piece of moist filter paper, which is then added to the beaker; run 10 ml. of 10% potassium cyanide solution over the walls of the funnel into the beaker and rinse with hot water. Add sufficient water to allow of the paper pulp being broken up with a glass rod, stir-in 0–4 g. of sodium nitrite and place on the steam bath. After solution of the precipitate run in 25 ml. of diluted nitric acid (sp.gr. 1·2), stir thoroughly and heat to boiling. Filter into an Erlenmeyer flask (about 600 ml.) wash the pulp thoroughly with hot water and discard it. Evaporate the solution to about 40 ml., cool and roughly measure it in a graduated tube. Place 1 g. of urea in the Erlenmeyer flask, pour the solution back into that flask and rinse in the measuring tube with cold water to bring the total volume to about 100 ml. Add 2 ml. of 10% solution of potassium cobalticyanide, shake, leave for about 1 minute, add 10 ml. of 4% potassium iodide solution and 5 ml. of chloroform and shake again, thoroughly. Leave for 5 minutes with repeated shaking and titrate the liberated iodine with $N/100$ thiosulphate, with vigorous shaking towards the end and using starch for the final end-point.

$$1 \text{ ml. of } N/100\ Na_2S_2O_3 \equiv 0{\cdot}0001975 \text{ g. Se.}$$

(*b*) *For steels which will not dissolve by method* (*a*)—"18 : 18" steels and certain others will not dissolve in the acid liquid used in method (*a*). For such steels the apparatus illustrated in THE ANALYST, 1929, **64**, 286, may be used.*

Place the sample in the flask, insert the stopper and put a beaker under the end of the leading tube so that the tube passes to the bottom of the beaker. Run into the beaker about 100 ml. of 5% sulphuric acid coloured dark purple with permanganate and add 100 ml. of diluted sulphuric acid (1+3) to the flask through the tapped funnel; close the tap and heat the flask until the attack is finished, the escaping gases bubbling through the permangate solution in the beaker. Open the tap and heat the liquid in the flask to boiling; add through the funnel saturated permanganate solution a little at a time (the tap being left open) until a permanent precipitate of manganese dioxide is produced. Boil for five minutes, remove the source of heat, close the tap and allow the solution in the beaker to suck back into the

* A flask with a ground-in stopper carrying a leading tube and a tapped funnel.

flask. Rinse the tube and beaker by allowing successive small quantities of diluted hydrochloric acid (1+1) to suck back similarly into the flask. Take 100 ml. of hydrochloric acid (conc.) and use a small portion of it to rinse-in the funnel; remove the funnel, pour the liquid in the flask into an ordinary flask, rinse in with the remainer of the hydrochloric acid and add 2 ml. of 20% cupric chloride solution. From this point on treat the solution in exactly the same manner as that of method (*a*) after the addition of cupric chloride.

Results on standard steels with selenium additions were as follows:

Method	Steel taken g.	Carbon %	Selenium added g.	Titration $N/100$ $Na_2S_2O_3$ ml.	Selenium found g.	% of Se Added	% of Se Found
(*a*)	"V" 5·0	0·55	0·00500	24·70	0·00487	0·100	0·097
(*a*)	"R" 5·0	0·79	0·00500	24·70	0·00487	0·100	0·097
(*b*)	"T" 5·0	0·3(ap.)	0·00500	24·70	0·00487	0·100	0·097
(*b*)	"18:8" 5·0	—	0·00500	24·55	0·00485	0·100	0·097

These results seem to be fair, though still showing a slightly low tendency.

When the selenium precipitated by the hypophosphite had been dissolved in bromine-hydrochloric acid a trace of brownish precipitate remained on the washed filter. This, presumably, was still unattacked carbon compound and probably accounted for the low tendency. The practice therefore has been adopted of reserving this filter and using it for filtering off the selenium obtained by iodide precipitation, thus ensuring that this carbonaceous matter receives additional attack by cyanide and by nitric acid. No figures can be shown in support of this modification.

Sulphur

Many experiments were conducted to find out whether the ordinary gravimetric process for sulphur required modification in presence of selenium. These experiments, though not very conclusive, showed that if errors occur they are so small as to lie within the bounds of general experimental error, and that therefore the general method can be used with confidence and without modification.

One fact that emerged was that in steels containing appreciable amounts of carbon a part, or even the whole, of the selenium is precipitated and is filtered off with the silicon. The amount of this precipitation seems to be proportional to the carbon content.

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November, 1945

The Precipitation of Tin by Tannin

By H. HOLNESS and W. R. SCHOELLER

This paper continues and expands the subject of the two earlier ones on certain novel analytical applications of tannin. The first paper dealt with the tannin precipitation of zirconium,[1] the second with that of titanium,[2] from acid chloride soln. We have now completed a study of the action of the reagent upon stannic chloride and oxalate solns., with the result that we may claim to have enriched the analytical chemistry of tin by the introduction of a more tractable precipitate than those hitherto employed for its gravimetric determination.

Only one reference to the tin - tannin complex has been found in the literature. In 1929, Moser and List[3] published a scheme, involving tannin precipitation from acetate solution, for the quantitative separation of beryllium from other metals. It is not our object here to question the value of this scheme, which has been criticised by other workers.[4,5,6,7] We are concerned merely with the procedure for the separation of beryllium from tin. Moser and List find that both stannic sulphide, and stannic acid precipitated by hydrolysis, strongly adsorb beryllium, hence they recommend precipitation of the tin by tannin from acetate solution. From their brief directions it appears that double precipitation is necessary for more than 0·2 g. of SnO_2, the precipitate first produced requiring hot strong hydrochloric acid for its solution. We ascribed the necessity for the unattractive double treatment to the

choice of the unsuitable acetate medium, and decided to investigate the tannin precipitation of tin from chloride and from oxalate solns. as likely to lead to a cleaner separation.

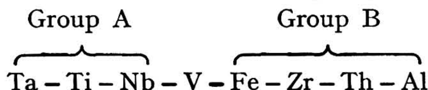
A: CHLORIDE SOLUTIONS—Stannic chloride solns. are quantitatively precipitated by tannin in presence of free hydrochloric acid at and below 0·05 *N* concentration. In solutions containing ammonium chloride the white ppt. is coarsely flocculent, settles quickly, does not adhere to the glass and is easily filtered off and washed, especially when mixed with filter pulp. In fact it is in all respects similar to the zirconium complex, the comparative insolubility and ready flocculation of which we have related to the pronounced hydrolysis of zirconium chloride solutions.[2] The strong tendency of stannic chloride solutions to undergo hydrolytic decomposition is a familiar fact, in accordance with which we found that, above 0·35 *N* acidity, tin is precipitated by tannin to a greater extent than zirconium; hence the serial order of precipitability appears to be Sn–Zr–Ti above, and Zr–Sn–Ti below, 0·35 *N* acidity. The figures expressing the percentage precipitation of the 3 metals as a function of the acidity, in chloride and in oxalate solution, will be published in graph form in due course.

The similarity in the action of tannin upon zirconium and stannic chloride solutions justified the inference—which was borne out by experiment—that tin is readily separable from aluminium and ferric chlorides, and also from vanadyl chloride at a slightly higher acidity (Expts. 5–7). In the separation of tin from iron, it was found once more that the ferric salt affects the colour of the tannin complex,[1] which is obtained as a dark-brown ppt., yielding, however, pure white stannic oxide upon ignition (Expt. 4). In all the separations here described the tin was recovered in one fraction and by a single precipitation, by the use of the technique forecast in our titanium paper.[2] Instead of filtering off the major fraction and completing the precipitation in the filtrate, we added the reagent to the acid solution, and then gradually treated it with *N* ammonia until the whole of the tin was precipitated. This mode of working proved reliable and more expeditious, and we expect it to be of general applicability in this class of work.

The separation of tin from the bivalent metals copper, lead, and beryllium gave no trouble (Expts. 8–12). Our interest in the tin - beryllium separation as such is rather remote, but we wanted to prove that it is unnecessary to resort to Moser and List's procedure in acetate solution (*supra*). The separation of tin from copper and lead points to promising simplifications in the treatment of the hydrogen sulphide group (as *e.g.*, in Lambie and Schoeller's gravimetric tin assay[8]). Antimony is precipitated by tannin from chloride and from oxalate solution, and we are at present studying the best means for overcoming its interference. Arsenic is not precipitated by tannin, since its hydroxy compounds do not form colloidal suspensions.

As the field of the analytical application of tannin is becoming more extensively surveyed, all our work and our correct prediction of the behaviour of several elements towards the reagent support the "flocculation theory," according to which the precipitation is due to reciprocal flocculation of a disperse hydroxide or basic salt by the negative tannin sol.[2] It is interesting in this connection to note that, in acidified sulphate solution, zirconium and tin react quite differently towards tannin; the former is not affected whilst the latter is precipitated even at 4 *N* acidity. This can be explained by the instability of stannic sulphate, which is hydrolysed to hydrated stannic oxide, whilst zirconium sulphate yields a soluble hydrolysis product, complex zirconylsulphuric acid.[9]

B. OXALATE SOLUTIONS—Separations of paramount importance in earth-acid analysis can be effected by tannin precipitation from oxalate solution. Schoeller and his co-workers, who devised the methods,[10] observed a serial order of precipitability



in oxalate solutions[11] free from tin, previously removed by other means.[12] An awkward separation problem was thus avoided, for we now find that tin is a member of Group A and takes its place side by side with tantalum in the above precipitation series. Hence it is co-precipitated completely with tantalum (Expt. 14), whilst a common oxalate solution of tin and niobium yields a slightly niobiferous precipitate (Expt. 15). It is obvious that mixed pentoxides intended for the separation of tantalum from niobium must be free from tin.

In accordance with the position of tin at the head of Group A, its separation from all elements, including and following vanadium in the above tannin precipitation series, as well

as from copper and beryllium, is accurate (Expts. 16–22); in all these tests the tin was recovered in one fraction by a single precipitation.

The above tests proved that oxalate solutions are to be preferred to chloride solutions for the separation of tin from Group B. Precipitation intervals in oxalate solutions appear to be wider and more sharply defined than those in other media. We ascribe this to the formation of relatively stable oxalate complexes of the more basic elements and the comparative instability of those of Group A, on the reasonable assumption that the order of precipitability by tannin is the measure of such instability. As a matter of fact, the separation of tin in oxalate solution is cleaner; whilst the tin complex produced in chloride solutions settles readily enough, the coarsely flocculent precipitate formed in oxalate solution is even superior in this respect and leaves a perfectly clear supernatant liquor. In the separation of tin from vanadium, control of the acidity is more critical in chloride than in oxalate solution (Expts. 5–7); the tin precipitate obtained in oxalate solutions containing ferric iron is pure white, not brown as in solutions of ferric chloride (*supra*); and tin is separable from zirconium, which is not the case in chloride solution (see A). The separation of beryllium from tin is more elegant than Moser and List's procedure in acetate solution.

Experimental

C. Gravimetric Determination of Tin—Two solutions of doubly recrystallised ammonium chlorostannate in N hydrochloric acid were standardised by precipitation with hydrogen sulphide and ignition of the washed precipitate to SnO_2; 25-ml. portions gave 0·0509 and 0·0512 g. respectively. In all our tests the tin precipitates were washed with 2% ammonium nitrate soln. until free from chloride, to prevent loss by volatilisation on ignition. The tannin precipitation was done by addition of 5 g. of ammonium chloride and a freshly made solution of 1 g. of tannin to the hot liquid, nearly complete neutralisation with N ammonia, dilution to 200 ml. and short boiling. The precipitate was collected, washed with ammonium nitrate solution and ignited wet in a tared porcelain crucible. Found, 0·0510 and 0·0511 g. respectively.

D. Separations in Chloride Solution—Zirconium is necessarily co-precipitated with tin from chloride soln.; nor did we attempt the separation of tin from titanium. Antimony and tin are not quantitatively separable (Expt. 13).

In Expts. 3–11 the following procedure was used: 25-ml. portions of tin solution, 25 ml. of saturated ammonium chloride solution, and 50 ml. of chloride solution of the metal to be separated, were transferred to 600 ml. beakers. Sufficient hydrochloric acid was added to produce 0·25 normality in a bulk of 200 ml., and water to make up 185 ml. The boiling solutions were treated with 1 g. of tannin in 15 ml. of hot water, most of the tin being precipitated. N Ammonia was then added to the boiling solution during agitation, until the acidity was reduced to the normality given in the Table ("final acidity") in a total bulk of about 250 ml. The precipitates were left to settle for some hours, collected, washed with 2% ammonium nitrate solution to eliminate chloride and ignited wet to SnO_2. The amount of metal added is given in the Table in terms of oxide.

Expt.	SnO_2 taken g.	Added g.		Final acidity	Tannin ppt. g.	SnO_2 error g.
3	0·0510	Al_2O_3	0·378	0·023 N	0·0507	−0·0003
4	,,	Fe_2O_3	0·250	0·028 N	0·0512	+0·0002
5	,,	V_2O_4	0·142	0·022 N	0·0541	+0·0031
6	,,		,,	0·044 N	0·0523	+0·0013
7	,,		,,	0·068 N	0·0512	+0·0002
8	,,	CuO	0·500	0·022 N	0·0511	+0·0001
9	,,	PbO	0·106	0·022 N	0·0511	+0·0001
10	,,	BeO	0·0456	0·028 N	0·0505	−0·0005
11	,,	,,	0·713	0·022 N	0·0513	+0·0003
12	0·2040	,,	1·426	0·025 N	0·2044	+0·0004
13	0·0510	Sb_2O_3	0·100	0·055 N	0·0648	+0·0138

Expts. 5–7 prove that the separation of tin from vanadium requires a higher final acidity than the other separations. It is also advisable to wash the rim of the filter containing the tannin precipitate with a solution of ammonium chloride in 0·1 N hydrochloric acid before applying the ammonium nitrate wash, to prevent precipitation of the blue vanadium complex. Contamination with vanadium produces a yellow discoloration of the ignited stannic oxide.

In Expt. 12 large amounts of beryllium and tin were successfully separated in one operation. A solution of 4 g. of tannin was added to the hot 0·3 N acid mixed solution

(bulk 700 ml.) which was then treated with 195 ml. of *N* ammonia. In Expt. 13 the positive error is actually higher than shown in the Table, a little antimony having been lost during the ignition of the tannin precipitate.

E. SEPARATIONS IN OXALATE SOLUTION—

Expt. 14. *Tin and tantalum*—0·1153 g. of Ta_2O_5 was fused with bisulphate, the melt was dissolved in ammonium oxalate solution and 25 ml. of tin solution (≡ 0·0511 g. of SnO_2) were added. Fractionation with tannin gave three yellow fractions which were collected and SnO_2 was determined in each.[12]

Fraction	Total weight	SnO_2 in fraction	Ta_2O_5 in fraction (by difference)
	g.	g.	g.
P^1	0·0670	0·0251	0·0419
P^{1a}	0·0573	0·0224	0·0349
P^{1b}	0·0305	0·0035	0·0270
		0·0510	0·1038

The test shows that tin tends to be precipitated more readily than tantalum.

Expt. 15. *Tin and niobium*—0·1037 g. of Nb_2O_5 was taken and the procedure of Expt. 14 was followed:

Fraction	Colour	Total weight	SnO_2 in fraction	Nb_2O_5 in fraction (by difference)
		g.	g.	g.
P^1	Pale rose	0·0535	0·0494	0·0041
P^{1a}	Orange	0·0032	Mixed ppt.	
P^{1b}	Red	Voluminous Nb ppt.		

There is thus marked differentiation, P^1 consisting chiefly of the tin complex.

Expts. 16–22. *Separation of tin from Group B, etc.*—The procedure was the same as that used under *D*, except that 3–6 g. of ammonium oxalate crystals were added to the solutions prior to precipitation with tannin. An excess of ammonium oxalate over 3 g. was required in some tests (see Table) to obtain a clear solution. In all the experiments 0·0511 g. of SnO_2 was taken.

Expt.	Added		Ammonium oxalate added	Final acidity	Tannin ppt.	SnO_2 error
		g.	g.		g.	g.
16	V_2O_4	0·284	3	0·08 *N*	0·0510	−0·0001
17	Fe_2O_3	0·250	4	0·08 *N*	0·0511	0·0000
18	ZrO_2	0·0536	3	0·08 *N*	0·0511	0·0000
19	ThO_2	0·0520	4	0·08 *N*	0·0510	−0·0001
20	Al_2O_3	0·378	5	0·08 *N*	0·0509	−0·0002
21	BeO	0·0456	3	0·08 *N*	0·0513	+0·0002
22	CuO	0·500	6	0·08 *N*	0·0512	+0·0001

F. ANALYTICAL APPLICATION—The proposed gravimetric determination of tin based on tannin precipitation is not advanced as an alternative method for the assay of high-grade tin ores and alloys. In that class of work the volumetric iodine process is indispensable. On the other hand we feel confident that the tannin method will prove a valuable adjunct for the detection and determination of tin occurring as a minor constituent or impurity in low-grade and complex ores, alloys, slags, and other metallurgical products. We are engaged in investigating the use of tannin in this direction, and have so far obtained encouraging results with bronze, and lead-base alloys. It is hoped that this work will be reported in a further communication.

SUMMARY—Tin (stannic) is quantitatively precipitated by tannin from chloride solution at 0·05 *N* acidity; the white ppt. settles quickly and is easy to filter and wash. Tin can thus be separated from iron, vanadium, aluminium, beryllium, copper, and lead (not from zirconium, titanium or antimony), and determined gravimetrically as SnO_2 after ignition of the tannin precipitate washed free from chloride. In oxalate solution the tin precipitate settles remarkably well, and clean separations are effected in one operation from elements of tannin group B including zirconium. Tin is shown to be a member of group A, being precipitated at the same acidity as tantalum.

We desire to thank the Governors, Principal and Head of the Science Department of the South-West Essex Technical College for permission to carry out this investigation.

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WALTHAMSTOW, LONDON, E.17

September, 1945

The Determination of Magnesium in Nickel Alloys

BY J. T. MINSTER

THE separation of magnesium from nickel in alloys containing up to 18 per cent. of magnesium by means of electrolysis with a mercury cathode has been described by Chirnside, Dauncey and Proffitt[1] and the same authors, with C. F. Pritchard, have extended the method to determine the magnesium volumetrically after precipitation with 8-hydroxyquinoline,[2] as was suggested in the discussion on the paper mentioned above.[1] The present paper is a record of the application of these methods to the routine analysis of fairly large numbers of nickel alloy samples. Chirnside *et al.* devised electrodes and vessels which could be readily fabricated from normal laboratory equipment, avoiding the necessity for specialised apparatus. Such an arrangement has the additional advantage of flexibility, so that it can meet the various requirements necessary for the analysis of a wide range of materials.

The method, when tentatively tested in this laboratory, was found generally satisfactory, but for its adaption to routine analyses it was decided that the number of similar samples regularly analysed warranted the installation of a piece of apparatus for this specific purpose. It was confirmed that no mechanical stirring is required, but some loss by spraying occurred even when the beaker was covered as well as possible with a watch glass.

A self-contained unit to electrolyse up to six samples simultaneously was therefore devised. The only external connection is a standard 3 pin plug that connects to the 110 volt D.C. mains, which source of supply is available in this laboratory. Safety from shock to operators is secured by gate breaks which automatically disconnect the electrode circuit from the main supply on opening a door covering the electrolysis vessels.

The cylindrical electrolysis vessel which has been developed to suit the particular requirements of this case is shown at Fig. 1, and needs little additional description. The aqueous layer is removed by means of the side tube and tap, which has been found more convenient than syphoning. Other types of analyses, in which different quantities of electrolyte and mercury are employed, would need modified vessels. The necessary alterations to dimensions can easily be planned whilst retaining the essential features of the cell; allowance must be made in the design of the cabinet to accommodate the largest cell it is proposed to use and to provide support for smaller ones if a range of sizes is to be employed. The electrode system, Fig. 2, consists of two tubes, 6 mm. in diameter and ½ in. apart, fused through a deeply concave watch-glass. The tubes are of such length that when the watch-glass lid rests on the top of the vessel the end of the longer tube almost touches the bottom. Platinum electrodes are fused through the lower ends of the glass tubes, contact with the flexible leads being made with mercury. The shape and size of the electrodes were not the subject of any investigation; they were made of platinum strip which happened to be available.

During electrolysis the electrode vessels stand on a shelf (Figs. 3 and 4) and are supported in a vertical position by passing through holes in a higher shelf. In the fronts of these holes are slots to allow the passage of the side tubes carrying the taps, and the lower shelf has shallow depressions into which the bottoms of the vessels fit. The electrode system rests

with the watch-glass lid on the top of the vessel, thus eliminating losses due to spray, and also rendering unnecessary the use of any other support for the electrodes. Flexes from the electrodes terminate in red and black "Clix" plugs, which are pushed into corresponding coloured sockets, the black, negative, plug being attached to the electrode dipping into the mercury. A door, hinged at the top, covers the upper part of the electrolysis vessels, plugs, etc., upwards from the shelf with slotted holes; gate breaks connected in the circuit ensure that all the sockets are dead when this door is opened, so that there is no chance of operators receiving a shock. Behind the electrolysis vessels, between the two shelves, is a sheet of opal glass which is illuminated from the rear by two electric lamps. This form of illumination enables the colour of the solutions being electrolysed to be easily discerned; and, since the lamps are not switched off by the gate break contacts on opening the door, but are directly wired to the main supply entering the cabinet, the lamps also act as pilot lights to prevent the current being left switched on inadvertently.

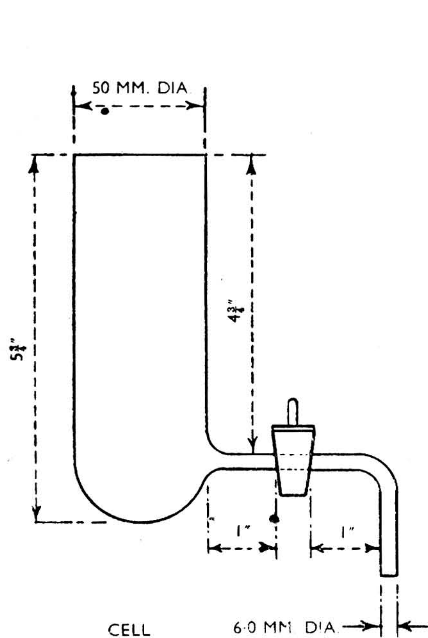

Fig. 1.
Electrolysis vessel.

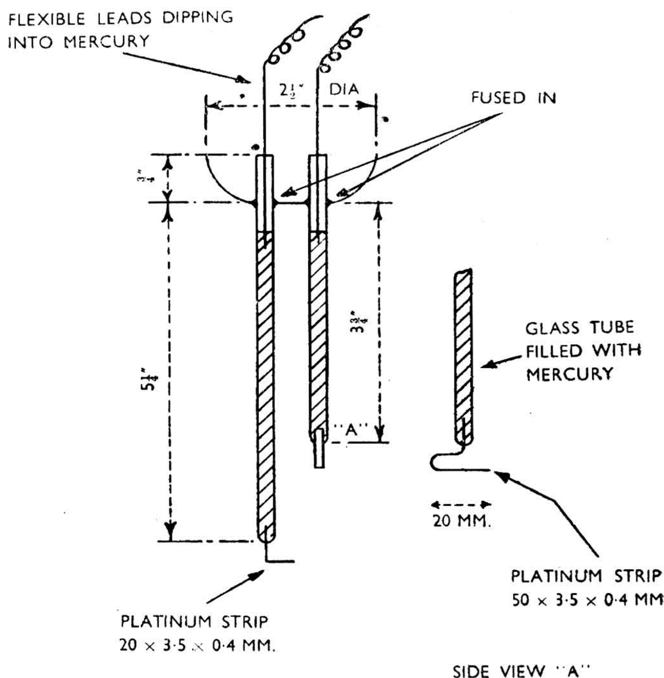

Fig. 2.
Electrodes.

The arrangement of meters, switches, etc., will be readily understood by considering the wiring diagram, Fig. 5. Since they are in series, the current is the same through each of the six cells. To enable the current to be maintained when less than six cells are in use, or when some have been emptied but others still require the current to be passed until they are also emptied in their turn, provision has been made for the inclusion of resistances in the circuit. These resistances, wired in parallel with the cells, are shown at R. When the current is switched off from any cell its parallel resistance is automatically brought into the circuit and so the current is maintained in the other vessels. Slight variations in current may occur as the result of such an operation, since the resistances have average values of those of the liquids being electrolysed, but such changes are of little significance and are easily rectified by adjusting the variable resistance. The voltage across any pair of electrodes can be measured by means of the voltmeter and selector switch.

During preliminary work the heat generated by the variable resistance was found to be excessive for installation in a cabinet. By choosing the right type of resistance and fitting a perforated metal back to the cabinet this overheating has been eliminated; so, although it becomes fairly hot, no dangerous temperature develops even after many hours' running.

To prepare the solution for electrolysis the alloy is dissolved in nitric acid and nitrates are then destroyed by evaporation with sulphuric acid. Bumping and spitting during this process were found to be excessive when carried out by straightforward heating in a beaker, even on a sandbath, so a procedure has been adopted in which the evaporation takes place in a long necked flask and a current of air is continuously blown through the liquid. This has entirely eliminated losses due to erratic boiling. The solution is neutralised and then made slightly acid for electrolysis. If insufficient acid is present a little precipitation may occur during the early stages of the electrolysis, the solution clearing as nickel is removed from solution. Accompanying this low acidity is a slightly increased resistance, indicated by the voltage across the electrodes being a little higher than that across normal cells in series with the hazy one.

The following are the practical details:

Dissolve 3 g. of alloy in 50 ml. of diluted nitric acid (1+1) in a 150 ml. Kjeldahl flask. Add 5 ml. of sulphuric acid (conc.), support in a slanting position on a retort stand in a fume cupboard and pass a fairly rapid current of air through the liquid by means of a glass tube.

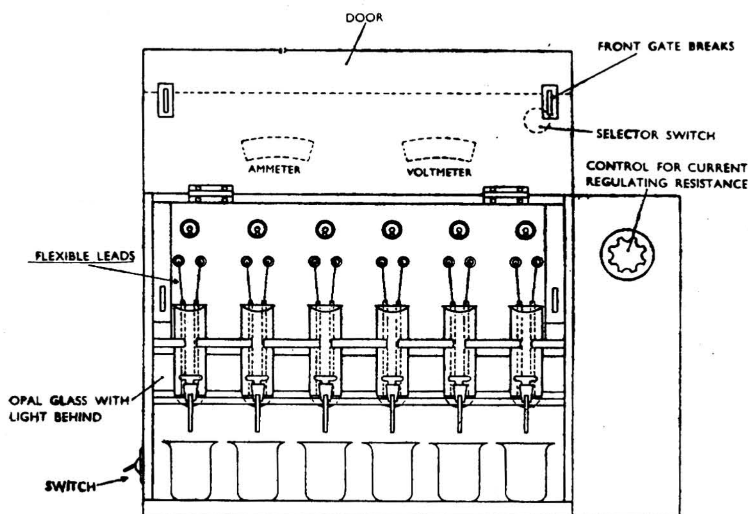

FIG. 3.

View of apparatus with the door in its open position. During electrolysis the door must be closed. The dotted lines indicate the components which are mounted on the top panel but which are now concealed by the door.

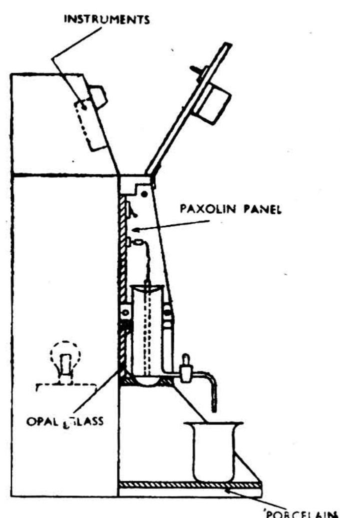

FIG. 4.

Part sectional side view. Back wiring not shown.

Heat with a small naked flame and evaporate to white fuming. Allow to cool, add 100 ml. of distilled water and boil, maintaining the air current, until all solid has dissolved. Rinse and remove the air tube and add freshly prepared caustic soda solution (1 part of NaOH to 4 parts of water) until a faint permanent precipitate appears. Add diluted sulphuric acid (1+3) until the precipitate just dissolves and 3 ml. in excess. Pour clean mercury into an electrolysis vessel up to the bottom of the outlet tube. Transfer the nickel solution to the vessel and make up the volume to about 200 ml. Insert the electrodes, connect up so that the mercury is negative, and pass a current of 3·5 amps. until the solution is colourless. Alternatively, a current of 0·5–1 amp. may be passed overnight. Continue the electrolysis for 15 minutes after complete decolorisation and run off the aqueous layer into a 500 ml. beaker before switching off the current. Immediately each cell is emptied change its switch to the "off" position. Reclose the door of the electrolysis cabinet during any delay so that the current is maintained through the cells that have not yet been emptied. Remove the electrodes and rinse the mercury three times with about 10 ml. of distilled water each time.

Precipitation of the magnesium with 8-hydroxyquinoline and subsequent filtration and titration with decinormal potassium bromate follow established procedure.

SUMMARY—A permanently installed piece of apparatus for separating magnesium from nickel by electrolysis with a mercury cathode is described. The magnesium is subsequently determined volumetrically by means of 8-hydroxyquinoline.

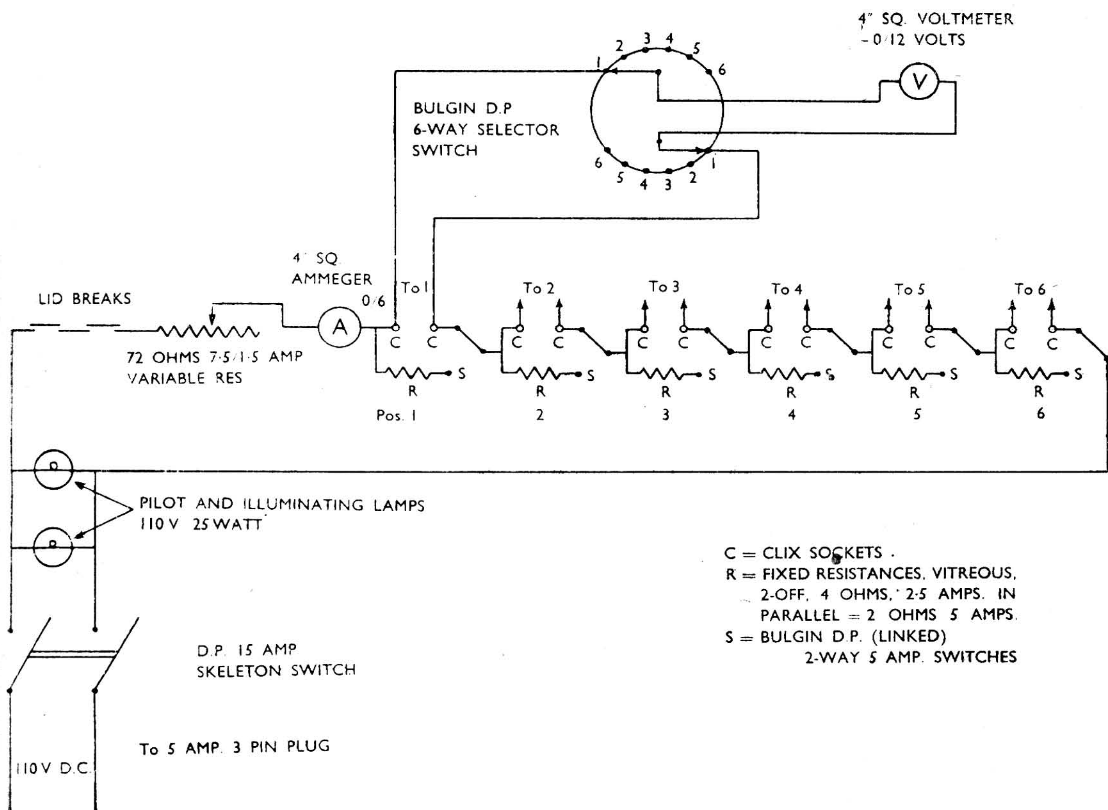

FIG. 5.

I must thank the electrical engineering staff at this factory for their co-operation in designing and constructing the electrolysis cabinet, Messrs. Chirnside and Dauncey for advice in the presentation of the paper, and the Management of this factory, in whose laboratory the work was carried out, for permission to publish the paper.

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THE GENERAL ELECTRIC CO. LTD.
CAPE MILL, SHAW, OLDHAM, LANCS

July, 1945

Notes

THE COLORIMETRIC DETERMINATION OF *p*-HYDROXYBENZOIC ACID AND ITS ESTERS

THE use of Millon's reagent to determine *p*-hydroxybenzoic acid and its esters colorimetrically has been described by Edwards, Nanji and Hassan[1] and later by Sabalitschka[2] and by Diemar, Riffart and Schmelk.[3] The last two papers cited state that the development of colour depends upon temperature and time, but Edwards, Nanji and Hassan merely direct exactly 2 minutes immersion in a boiling water-bath. Following this method, consistent colour formation could not be obtained and the method, therefore, was investigated.

A neutral solution of the ammonium salt of the acid was prepared in accordance with Edwards's method, and diluted to known concentration not exceeding the equivalent of 2·0 mg. of *para*-acid in 20 ml.

More consistent and intense colour formation was obtained by heating the solution just to boiling point, adding the reagent and immersing immediately for two minutes in the boiling water-bath than by the original procedure.

By varying the time of immersion in the boiling water-bath it was found that the colour intensity becomes constant in approximately five minutes, as can be seen from the following table, whereas at two minutes the colour intensity is developing rapidly with time.

Immersion time in mins.	0·5	1	1·5	2	3	4	5	7·5	10
1·0 mg. in 20 ml. Red Units	—	0·2	0·4	0·8	1·3	1·6	2·1	2·1	2·1
1·7 mg. in 20 ml. Red Units	—	0·4	0·8	1·4	2·6	3·4	3·6	3·6	3·6

Variation in the time between dilution and comparison in the Tintometer did not affect the colour intensity, but since in solutions containing more than about 1·5 mg. in 20 ml. a slight opalescence appeared as the solution cooled, rendering colour matching difficult, the readings should be determined within a few minutes after dilution.

Amounts of Millon's reagent between 2 and 3 ml. produced no variation in colour intensity, but uantities less than 2 ml. failed to produce the maximum colour.

The following method, therefore, was adopted.

Pipette 20 ml. of a dilution of the neutral ammonium salt containing not more than 2·0 mgm. of *p*-hydroxybenzoic acid into a boiling tube, heat just to boiling point, add 2 ml. of Millon's reagent and immerse in a boiling water-bath for not less than 5 minutes. Then remove and immediately dilute with 25 ml. of distilled water, make up to 50 ml., mix thoroughly and, in less than 5 minutes, match the colour in the Lovibond tintometer, using a 1 cm. cell, and record Red Units.

A standard curve constructed in accordance with this method, with known amounts of pure *para*-acid plotted against Red Units, was found to be linear and to pass through the origin. With a 1 cm. cell and using C.I.E. standard illuminant B, 1 Red Unit was found to be equivalent to 1·84 mg. of *p*-hydroxybenzoic acid.

Thanks are due to Dr. E. F. Hersant for advice and to the Directors of May & Baker, Ltd., for permission to publish these results.

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MAY & BAKER, LTD.,
ANALYTICAL CONTROL DIVISION
DAGENHAM, ESSEX

H. W. JOHNSON

November, 1945

Fruit and Vegetable Preservation Research Station, Campden

ANNUAL REPORT, 1944

DURING the past few years the scientific activities of the Research Station have been directed largely to the investigation of short-term practical problems connected with preservation of food under war-time conditions. The canning industry is making full use of the advice and assistance of the Station as is shown by the increased number of problems submitted for investigation.

FRUIT GUMMING OF VICTORIA PLUMS—The progress report by W. B. Adam and D. Dickinson shows that frequency of gumming is positively correlated with the rainfall occurring during July and August, *i.e.*, during the later stages of ripening. Watering tests to confirm this were unsuccessful, probably owing to the difficulty of reproducing natural conditions. There is evidence that gumming is associated with turgidity of the fruit cells, which is dependent upon the humidity of the air. Previous observations that boric acid injected into the branches or sprayed on the leaves inhibits gumming have been supported by a significant correlation between gumming and boron content of the leaves of one lot of plum trees. Experiments on the effect of artificial fertilisers on the availability and uptake of boron have hitherto given negative results but are being continued.

VITAMIN C CONTENT OF CANNED POTATOES AND CANNED PEAS (W. B. Adam)—Potatoes have been canned on a large scale during the war, and to the majority of canners this was a new operation. The general quality and nutritive value of the product has often lacked uniformity, especially in the ascorbic acid content. Canned peas also have varied considerably. In potatoes the most important factors are those connected with the raw tubers, *e.g.*, storage period, variety and soil. A number of investigators have reported a heavy fall in the ascorbic acid content of potatoes lifted after September and stored in clamps, the final content being as low as one-third of the initial content. Losses in the canning operations, apart from blanching losses and the distribution of ascorbic acid between the liquid and solid material, are not high. Blanching losses may attain 30 to 40% and the final content of the canned product is generally about 30 to 40% that of the raw tubers. The ascorbic acid content shows a progressive fall with length of storage of the raw tubers. The full range of ascorbic acid contents recorded for commercial packs was 2 to 14 mg. per 100 g. The early season pack constitutes a fairly rich source of vitamin C. No detectable change in the vitamin C content of the canned product was found after 6 months' storage.

Canned Fresh Peas—Raw peas vary greatly in ascorbic acid content, maturity being an important factor and variety having some influence. The effects of soil and manurial treatment have not yet been fully determined. Fresh peas for canning have an average ascorbic acid content of about 30 to 35 mg. per 100 g., and the average for canned peas is about 9 to 10 mg. per 100 g., the average retention being thus about 30%. The average losses are about 40 to 50% during blanching and about 10% during storage, and the ascorbic acid remaining in the product is distributed between the solid and liquid portions in the ratio of about 5 to 3. The observed copper content of commercial packs did not exceed 5·7 p.p.m. and most samples were within the range of 1·7 to 3·2 p.p.m. The higher figures for copper content were not associated with low ascorbic acid values. Statistical analyses of results showed that maturity and final

vacuum in the can were both significantly correlated with ascorbic acid content, the former negatively and the latter positively. The average ascorbic acid content of commercial cans of green peas was about 9·2 mg. per 100 g. and the recorded range 2 to 13 mg. per 100 g.

INTERNAL CORROSION OF CANS (D. Dickinson)—In the 1943 report the author described two accelerated tests by which the corrodibility of a steel base plate might be estimated. Experiments have been made to test the validity of the methods when applied to de-tinned plate for diagnosis in any instance of abnormally rapid occurrence of hydrogen swells. The question arose as to whether the pattern left on the exposed steel after stripping it with Clarke's solution had any influence on the subsequent corrosion of the steel in the rapid hydrochloric acid test, and, if so, whether it was permissible to remove the pattern by abrasion. The conclusion was reached that the pattern has little effect and that removal of the pattern with emery paper is not desirable because the act of abrasion increases the rate of corrosion of the steel in hydrochloric acid. A simple method for the comparison of the corrosivities of fruit juices and syrups was described in the previous report. Data are now available for a more satisfactory treatment of the results. The liquid from bottled fruits was sterilised and steel strips of standard size were allowed to corrode first in boiling hydrochloric acid according to the standard procedure and then in the extracts for 72 hr. at 25° C. under anaerobic and sterile conditions to prevent fermentation. The weight of iron dissolved from the strip by hydrochloric acid was plotted (abscissa) against the weight dissolved by the fruit extract (ordinate). Except with Pershore plums the curves were practically straight lines, the general equation being $L_J = S(L_A - 100) + K$, where L_J and L_A are the weights lost by the strip in fruit extract and in hydrochloric acid respectively. S is the slope of the mean line and K is the intercept of this line on the ordinate corresponding with $L_A = 100$, this ordinate being chosen for reference to avoid extrapolation. In previous work a corrosivity index $100L_J/L_A$ was used. It is now apparent that K is a more satisfactory index, although the difference between the two indices is not numerically great.

CORROSION AND HYDROGEN SWELLS IN CANNED VEGETABLES (W. B. Adam)—For most vegetable products stored in this country the risks of internal corrosion are not great, but with the large quantities sent to tropical countries during the war the storage life was much reduced and there has been evidence of hydrogen swells in some vegetable packs. The worst pack was undoubtedly beetroot with which some cans had formed swells after 2½ years' storage and a high proportion after 3 to 3½ years' storage. Stringless and runner beans had also formed hydrogen swells somewhat rapidly especially in cans 3 to 4 years old. There was less trouble from carrots except when in lacquered cans, and the packs generally survived 4 years' storage. Not a single instance was recorded among canned fresh peas, although this was the product most thoroughly sampled. The proportion of old stock was smaller for this popular product; nevertheless, occasional samples of four years' storage were examined without a single instance of internal corrosion. Sampling of spinach, turnips, potatoes, and vegetable macedoine was on a smaller scale, but a high proportion of the samples consisted of old stock and none had hydrogen swells. The relatively slow rate of corrosion of fresh peas in lacquered cans, as indicated by observations on commercial stock, was confirmed by experimental storage tests. Loss of vacuum was measured by the spherometer method (*v.* 1943 Report, ANALYST, 1944, **69**, 309). There is some evidence that hydrogen swells occur more rapidly in smaller cans than in the medium size cans. The rate of formation of hydrogen in lacquered cans is much more rapid than in plain cans, the differences being much greater than those noted with canned fruits. There is little, if any, dissolution of iron in beans in tomato sauce packed in plain cans, and this is generally true also of canned carrots and potatoes. The dissolution of tin in beans in tomato sauce and carrots in plain cans is severe, and there may be some stripping of the metal by oxidising agents present in the contents.

DETERMINATION OF TIN IN CANNED FOODS (D. Dickinson)—The need for a more rapid method of determining tin in canned foods led to the modification of an existing method (*v.* ANALYST, 1946, **71**, 41).

ABSTRACTS OF PAPERS PUBLISHED IN OTHER JOURNALS

Food and Drugs

Determination and Detection of Dichloroacetic Acid in Food Products. C. F. Bruening (*J. Assoc. Off. Agr. Chem.*, 1945, **28**, 620–627)—The quantitative method involves isolation of the dichloroacetic acid by ether extraction in a continuous extractor, subsequent conversion of the organically combined chlorine to ionised chloride by ignition with sodium carbonate or by hydrolysis with alkali and finally determination of the chlorine by Volhard's method.

Place the aqueous soln. containing not more than 200 mg. of dichloroacetic acid in the outer part of a continuous extractor, add 10 ml. of dil. sulphuric acid (50% w/w), insert the inner tube and add water until the level of the liquid is about 1 cm. below the side arm of the extractor. Attach a suitable flask containing 100 to 150 ml. of ether to the side arm and extract for 3 hr. To the extract add 2 g. of sodium carbonate in 20 ml. of water, remove the ether on the steam bath and evaporate the aqueous residue to dryness in a platinum dish. Dry the residue at 100° C. for 30 min. and finally ignite at 500°–550° C. for 15 min. Dissolve the ignited material in water, add 15 ml. of nitric acid and an excess of $N/10$ silver nitrate, remove the ppt. of silver chloride and carbonised matter by filtration, washing the filter with water, and to the combined filtrate and washings add 1–3 ml. of sat. ferric alum soln. Titrate the excess of silver nitrate with $N/10$ ammonium thiocyanate soln. (1 ml. ≡ 0·006447 g. of $CHCl_2COOH$).

Alternatively, treat the extract with 5 ml. of 3 N alcoholic potassium hydroxide and remove ether on the steam bath. Add a further 25 ml. of the potassium hydroxide soln. and boil gently under a reflux condenser for 1 hr. Add 75–100 ml. of water, 15 ml. of nitric acid, an excess of $N/10$ silver nitrate and filter and treat the filtrate as before. The alternative method is preferable when ether-soluble substances other than dichloroacetic acid are absent. These form dark coloured solns. in which the end-point is obscure, and in their presence the method first described is preferable. Since accumulation of chlorine atoms on the same carbon atom of simple aliphatic compounds increases their resistance to hydrolysis,

dichloroacetic acid is more difficult to hydrolyse than monochloroacetic acid, and it was found that alcoholic potassium hydroxide of at least 3 *N* concn. and a refluxing time of 1 hr. are necessary for complete hydrolysis.

Dichloroacetic acid can be readily identified by the m.p. of its *p*-toluide derivative, which is 153° C. To prepare this, extract enough sample in one or more continuous extractors to provide 100 mg. of the acid in ethereal soln. Extract the combined ethereal extracts with small amounts of *N*/10 sodium hydroxide until the total aqueous extract is alkaline to litmus. Add *N*/10 sulphuric acid until the extract is slightly acid, evaporate to dryness on a steam bath and dry the residue at 100° C. for 30 min. Boil the dried salt in a test tube or small flask under a reflux condenser with 2 ml. or more of thionyl chloride for ½ hr. To the cooled liquid add 1–2 g. of *p*-toluidine in 50 ml. of benzene and heat on the steam bath for 2 min. Extract the product with two 30-ml. portions of water and then similarly with 5% hydrochloric acid, 5% sodium hydroxide soln. and finally water. Filter the benzene soln. and evaporate the filtrate to dryness on the steam bath. Crystallise the residue from small amounts of 50% methyl alcohol and dry at 100° C. A. O. J.

Persistence of Monochloroacetic Acid in Wine. J. B. Wilson (*J. Assoc. Off. Agr. Chem.*, 1945, **28**, 627–633)—As with fruit juices and carbonated beverages (*J. Assoc. Off. Agr. Chem.*, 1944, **27**, 195; ANALYST, 1944, **69**, 216), it has been claimed that monochloroacetic acid hydrolyses in wine in a short time, and experiments were made to determine to what extent the alleged hydrolysis takes place. Samples of Muscatel wine were prepared containing 30, 50 and 100 mg. of monochloroacetic acid per 100 ml. and these were bottled and stored in the laboratory. A similar series of samples was prepared with Burgundy wine. The monochloroacetic acid content of each sample was determined on the first day of storage and after lapse of various periods up to 192 days by the following method. Distil 100 ml. of wine, collecting at least 2 ml. of distillate for each ml. of alcohol present in the sample. Add to the distillate enough 10 *N* sodium hydroxide to make the mixture 1 *N* and digest on the steam bath for 2 hr. Determine chloride ion as described in the second method described (*loc. cit.*), beginning "add 50 ml. of water, 15–20 ml. of nitric acid and 1 ml. of ferric indicator . . .". Transfer the residue in the distillation flask to a continuous extractor and determine monochloroacetic acid as described in the first method (*loc. cit.*), beginning "Dilute if necessary to 150 ml., *etc.*" The sum of the quantities found in the two determinations is the amount in the sample. The results showed that there is no essential change in the monochloroacetic acid content of the wine even after six months' storage in the laboratory. The investigation was then continued on the semi-commercial scale and was applied to samples taken from commercial plant at the various stages of wine manufacture. It was found that monochloroacetic acid does not hydrolyse when added to wine at any stage during the process of manufacture and remains unchanged even after two years' storage. Further expts. showed that the addition of 0·05% or less of monochloroacetic acid did not prevent secondary fermentation of wine containing less than 12% by volume of alcohol when fermentable sugars were present, and that addition of 0·01% did not prevent secondary fermentation of wine containing more than 12% of alcohol by vol. when fermentable sugars were present. A. O. J.

Use of Buffers in the Determination of Colour by means of Titanium Trichloride. IV. O. L. Evenson (*J. Assoc. Off. Agr. Chem.*, 1945, **28**, 633–636)—In the first of the three previous papers (*Ind. Eng. Chem.*, 1928, **20**, 860; ANALYST, 1928, **53**, 602) as well as in a paper on the electrometric titration of food colours (*Ind. Eng. Chem.*, *Anal. Ed.*, 1931, **4**, 151) it was shown that the pure dye in F D & C Red No. 2 (the tri-sodium salt of 1-(4-sulpho-1-naphthylazo)-2-naphthol-3 : 6-disulphonic acid; also known as amaranth) could not be correctly evaluated when sodium bitartrate was used as a buffer. Other failures with sodium bitartrate were noted. The same discrepancies were noted with F D & C Red No. 1 (the di-sodium salt of 1-pseudo-cumylazo-2-naphthol-3:6-disulphonic acid; also known as Ponceau 3R). It was shown further that correct results could be obtained for these two colours if sodium citrate was used as buffer, and that for all other water-soluble F D & C colours the tartrates were satisfactory buffers. The object of the present investigation was to obtain further information on the efficacy of tartrate buffers and on its correlation with *p*H, and F D & C Red Nos. 1 and 2 were used for this purpose. The titanium trichloride soln. was approx. 0·1 *N* as reducing agent and 1·45 *N* with respect to the hydrochloric acid present. Its acidity was determined as follows. Add a known excess of *N* sodium hydroxide to 10 ml. of the soln. and allow the reaction to proceed until the black titanous hydroxide changes to the white titanic compound. Determine the excess of sodium hydroxide by titration with standard hydrochloric acid using phenolphthalein as indicator. The *p*H determinations were made with a Beckman *p*H meter thus. Dissolve the buffer salt in 130 ml. of water and titrate the hot soln. slowly with the titanium trichloride soln. with mechanical stirring in an atmosphere of carbon dioxide. Determine the *p*H of the solns. before and after titration at about 25° C. after eliminating the carbon dioxide by boiling. The results showed that sodium bitartrate always gives low results. With sodium tartrate the results are satisfactory only if enough of the buffer salt is used to keep the *p*H at 4 or above after the titration. This value cannot be obtained with sodium bitartrate. The amount of sodium tartrate necessary will depend upon the acidity of the titanium trichloride soln. and the quantity used for reduction, this in turn varying with the weight of dye being reduced. With 0·25 g. of F D & C Red No. 1 and 0·3–0·4 g. of F D & C Red No. 2 (90% and 86% of pure dye respectively) and titanium trichloride of the strength already mentioned, 15 g. of sodium tartrate is enough to keep the *p*H at about 4 and give correct results. Sodium tartrate may therefore be used under these conditions for all the water-soluble F D & C colours and all mixtures of these. It seems probable also that it may be used for most other certified colours since it gives a final *p*H intermediate between those given by sodium citrate and sodium bitartrate. Sodium citrate gives equally correct results although the final *p*H of the titrated soln. varies from 5·5 to 2·5. A. O. J.

Estimation of F D & C Yellow Nos. 3 and 4 in Cottonseed and Other Vegetable Oils. S. H. Newberger (*J. Assoc. Off. Agr. Chem.*, 1945, **28**, 636–639)—Among the colours submitted for

certification under the Food, Drugs and Cosmetic Act are solns. of F D & C Yellow Nos. 3 and 4 in oils such as cottonseed, peanut, soya, maize and castor oils, the pure dye content of these solns. varying from 2 to 8%. The solns. can readily be analysed spectrophotometrically, but there is no satisfactory chemical method. The oils are not soluble in the usual solvents, and the end points in the titanium trichloride method are obscured by their yellow colour. A method devised for the separation of the dyes from the oils gave accurate and reproducible results with cottonseed oil. To a sample weighing not more than 3 g. and containing from 0·05 to 0·10 g. of dye add 25 ml. of alcohol and 1 g. of potassium hydroxide and boil the mixture vigorously under reflux for ½ hr. Treat the cooled liquid with 80 ml. of water and extract with four 50-ml. portions of ether. Wash the combined ethereal extracts gently with two 25-ml. portions of water and vigorously with a third portion. Rinse the washed extract into a titration flask with ether, remove the ether on the steam bath, dissolve the residue in 100 ml. of alcohol, add 120 ml. of hot water containing 15 g. of sodium hydrogen tartrate and heat the soln. to boiling. Add 1 ml. of aqueous F D & C Green No. 2 indicator soln. (1 ml. ≡ 0·2 ml. of 0·1 *N* $TiCl_3$) and titrate the hot soln. with 0·1 *N* titanium trichloride in an atmosphere of carbon dioxide. The end-point is a change from blue to a pale reddish orange. Subtract the indicator blank and calculate the pure dye content (1 ml. of 0·1 *N* $TiCl_3$ ≡ 0·00618 g. of F D & C Yellow No. 3 or 0·00653 g. of F D & C Yellow No. 4). The method was applied to solns. of known amounts of the dyes in cottonseed oil with satisfactory results and with an accuracy within 1%. The procedure was then applied to colour samples submitted for certification and containing soya, peanut, castor and maize oils; results agreed satisfactorily with those obtained spectrophotometrically. When the soln. contains both dyes, it is necessary to know the ratio in which they are present to calculate the theoretical titration value. If, however, a 1 : 1 ratio is assumed (1 ml. of 0·1 *N* $TiCl_3$ ≡ 0·00635 g. of the dye mixture) the error will not be excessive. Spectrophotometrically the dyes have very similar absorption curves and cannot therefore be individually determined in solns. containing both. However, their extinction values at 437 mμ for equal concns. are the same, and this makes it possible to analyse accurately the total dye content irrespective of the ratio in which the two components are present.

A. O. J.

Determination of Salt in Butter by a Mercurimetric Method. W. S. Arbuckle (*J. Assoc. Off. Agr. Chem.*, 1945, **28**, 676)—The method is an adaptation of that of Schales and Schales (*J. Biol. Chem.*, 1941, **140**, 879)—To prepare 0·1711 *N* mercuric nitrate soln. dissolve 29·31 g. of pure mercuric nitrate in a few hundred ml. of water containing exactly 40 ml. of 2 *N* nitric acid and dilute the soln. to 1 litre with water. Standardise the mercuric nitrate soln. against 0·1 *N* sodium chloride soln. To prepare the indicator dissolve 100 mg. of *s*-diphenylcarbazone in 100 ml. of neutral alcohol and store the soln. in a dark place, preferably in a refrigerator. From 10 g. of butter prepare 250 ml. of washings as described in the Official Method for determination of salt in butter ("*Methods of Analysis*," A.O.A.C., 1940, 299) and titrate a 25-ml. portion slowly with standard mercuric nitrate soln. in presence of 0·6 ml. of the indicator soln. The clear and colourless soln. becomes pale violet on addition of 1 drop (0·05 ml.) of the mercuric nitrate soln. at the end-point and an intense violet-blue on addition of 1 drop in excess. The vol. of mercuric nitrate soln. used in the titration (ml.) indicates the % of salt in the sample. To secure a sharp end-point the mercuric nitrate soln. should contain the exact amount of nitric acid specified and the indicator should be stored not longer than one month.

A. O. J.

Method for Differentiating Egg Lecithin and Soya Bean Lecithin in Macaroni and Noodle Products. J. J. Winston and B. R. Jacobs (*J. Assoc. Off. Agr. Chem.*, 1945, **28**, 607–615)—Lecithin derived from soya bean exhibits a strong blue fluorescence in ultra-violet light, whereas lecithin from flour and egg products shows this property only very feebly. This difference was investigated, the apparatus used being a Pfaltz and Bauer fluorophotometer, Model B, with a 370 mμ ultra-violet filter and a double 460 mμ filter placed between the sample cell and the photo-cell. These filters are those used in the thiochrome method. The following method was developed. Set the fluorophotometer to give a deflection of 50 on the galvanometer scale with a soln. containing 1 μg. of quinine sulphate per ml. in 0·1 *N* sulphuric acid. Soak 5 g. of such samples as flour, semolina, macaroni and noodles with 7 ml. of 95% alcohol and 0·5 g. of anhydrous sodium sulphate in a wide stoppered bottle. Add 93 ml. of naphtha, shake well and allow to stand overnight in the dark. Pipette the clear liquid into the photometer cell and measure the fluorescence, correcting the result by means of a blank determination. For yolks and whole eggs treat a suitable amount weighed on an aluminium strip with 7 ml. of acetone and break up the egg with a glass rod. Add 0·5 g. of anhydrous sodium sulphate, soak for 30 min., add 93 ml. of naphtha and proceed as before. Expts. with soya lecithin containing 60% of phospholipids and 40% of vegetable oil (thereby resembling commercial products) showed that up to a concn. of 212 mg. per 100 ml. the relation between the concn. and the measured fluorescence obeyed the Lambert-Beer law. Inasmuch as egg noodles cannot be expected to contain more than 3% of lecithin, owing to its shortening effect, this concn.-fluorescence relationship is satisfactory for the determination of added soya lecithin in noodle and macaroni products. Samples (5 g.) of flour, farina and semolina when extracted for 24 hr. with the alcohol-naphtha solvent (7 : 93) exhibited only a small degree of fluorescence, *viz.* 0·5–1·0 scale division. Similar expts. were made with egg products consisting of frozen egg yolk and whole eggs. The fluorescence of the yolks ranged from 1·0 to 3·0 scale divisions, the amounts extracted being from 601·6 mg. to 853·6 mg. The concn., however, had no significant effect on the fluorescence. Whole eggs showed less fluorescence with a max. deflection of 1·5 scale divisions, whereas 18·8 mg. of soya lecithin gave a deflection of 4 divisions. By treating soya lecithin with acetone to extract vegetable oils and the residue with ether to extract lecithin, it was shown that the phospholipid fraction is responsible for the fluorescence. Macaroni products were manufactured under supervision and varying amounts of soya lecithin were incorporated. Samples (5 g.) of the finished product were finely ground and extracted with the alcohol-naphtha solvent and the fluorescence was measured. The results showed that as little as 0·5% of soya lecithin will serve to give an abnormal degree of fluorescence

and that in general the relation between the galvanometer deflection and the concn. of soya lecithin is linear. Similar results were obtained with a large number of macaroni products from different factories, the deflection obtained being not more than 1 division, whereas the inclusion of 0·5% of soya lecithin caused a deflection of 2·5 scale divisions. Egg noodles containing 5·5% of egg solids (as yolk) and varying amounts of soya lecithin were examined similarly. The extract from the finely ground product (5 g.) gave a deflection of 1·0 divisions in the absence of soya lecithin, whereas the presence of 0·5% of soya lecithin caused a deflection of 4·5 divisions and the deflection with varying amounts was practically proportional to the amount of soya lecithin in the sample. With 209 samples of egg noodles containing 5·5% of egg solids a statistical analysis indicated that the max. fluorescence was 2·5, the min. fluorescence 1·0, the average 1·6 and the standard deviation 0·45 scale divisions. Expts. with macaroni products showed that the inclusion of 15% of soya flour, although causing a slight increase in fluorescence, does not interfere with the detection of soya lecithin. A. O. J.

Development of a Phosphatase Test Applicable to Cheddar Cheese. G. P. Sanders and O. S. Sager (*J. Assoc. Off. Agr. Chem.*, 1945, **28**, 656–675)—Difficulties were encountered in determining whether or not cheese can be tested by the phosphatase test to detect under-pasteurisation. The buffer capacity of cheese is greater than that of milk, samples differ greatly in composition, and titratable acidity increases with age so that the buffer solns. used with milk do not always produce the optimal *p*H conditions for the phosphatase test with cheese. It was necessary to find a reagent that would, in one operation and in all samples, establish optimal *p*H conditions and remove interfering proteins and salts. A soln. containing barium hydroxide and barium borate was found to have this property.

Mix 8 g. of pure barium hydroxide, $Ba(OH)_2$, $8H_2O$, with 8 g. of boric acid and enough water to produce 1 litre, warm and stir the mixture until no more solid will dissolve and filter. The *p*H of the filtrate is 10·2 and that of a mixture of 10 ml. of it with 0·5 g. of cheese is about 9·6. For adjusting the *p*H in preparing standard phenol solns. and in extracting free phenol from the disodium phenyl phosphate used as substrate, prepare a buffer soln. with a *p*H of about 9·8 by mixing 13 g. of barium hydroxide with 6·5 g. of boric acid and treating the mixture as above. A mixture of 9 ml. of this buffer soln. with 1 ml. of milk has a *p*H of about 9·6 and is suitable for tests with milk. To prepare BQC soln. dissolve 20 mg. of pure 2,6-dibromoquinonechloroimine in 5 ml. of methyl alcohol and store the soln. in a dark coloured bottle in a refrigerator. A fresh portion of this soln. should be prepared every few days. To prepare the substrate dissolve 1 g. of disodium phenyl phosphate in 8 ml. of water, adjust the *p*H by adding 1 ml. of the buffer soln., developing the colour due to any free phenol that may be present by adding 2 drops of BQC soln., incubating for 30 min. at 37°–38° C. and extracting the colour with 5 ml. of *n*-butyl alcohol containing 0·5 ml. of 0·1 *N* sodium hydroxide per litre and repeating the extraction until the alcoholic layer is free from colour. Store this stock soln. in a refrigerator and re-extract it daily before use. Prepare fresh substrate soln. daily by adding 1 ml. of the stock soln. to 100 ml. of buffer soln. To prepare standard phenol solns. treat 1 g. of warmed phenol with 900 ml. of water, add 4 ml. of *N* sodium hydroxide and 3 ml. of chloroform and dilute to 1 litre. Dilute 1 ml. of this stock soln. to 1 litre to produce a standard soln. containing 1 µg. of phenol (1 unit) per ml. and prepare solns. containing 2, 5, 10, 20 and 40 units per ml. by appropriate dilutions of the stock soln. From these measure appropriate quantities into a series of tubes graduated at 5, 10 and 15 ml. to provide a suitable range of phenol standards containing from 0·5 to 40 units. Add water to the 5 ml. graduation, 2·5 ml. of buffer to each standard containing from 0 to 25 µg. of phenol or 3 ml. of buffer to each standard containing more than 25 µg. of phenol, and then water to the 10-ml. graduation. Treat these comparison solns. in the same manner as the sample.

Break up 0·5 g. of cheese sample in a suitable tube by means of a glass rod, add 1 ml. of buffer substrate (*supra*), complete the breaking up, add 9 ml. more of buffer substrate, mix thoroughly and incubate the stoppered tube at 37°–38° C., for 1 hr. in a water-bath. Transfer the tube in a boiling water-bath until proteins are seen to separate and bubbles begin to appear, then remove the source of heat and allow the tube to remain in the hot water for 5 min. Cool the tube in running water and filter the contents into a tube graduated at 5, 10, and 15 ml. Draw off the filtrate to the 5 ml. graduation, add water to the 10 ml. graduation, add 6 drops (0·12 ml.) of BQC soln., mix and incubate at 37°–38° C. for 30 min. At this point the blue colour in tests with phosphatase values of 10 or more units can be easily detected in the liquid. For samples with lower values and for quantitative results add *n*-butyl alcohol to the 15 ml. graduation and invert the tube slowly several times. Compare the colour in the alcohol layer with a set of standards prepared similarly or evaluate the colour in a photometer. Since 0·5 g. of cheese is used and the amount of filtrate collected is equal to half of the amount of buffer substrate used, the result is recorded as units of colour (µg. of phenol) per 0·25 g. of cheese. It is highly desirable to make a blank test daily, using 0·5 g. of cheese, breaking up and boiling it in the tube before adding the buffer substrate. The optimal *p*H for activity of the enzyme was found to be 9·6, and a range of 9·4–9·8 is satisfactory.

Cheese was made from pasteurised milk to which varying amounts of raw milk had been added and the phosphatase values of the raw milk, the pasteurised milk, the mixed milk, the cheese and the whey were determined. The results showed that as little as 0·1% of raw milk added to pasteurised milk could be detected in the resulting cheese and also that the enzymic activity of the milk was concentrated in the cheese at the expense of the whey.

A. O. J.

Determination of Sulphonamides in Tablets. H. S. Conway (*J. Amer. Pharm. Assoc.*, 1945, **34**, 236–237)—The diazotisation procedure of the U.S.P. XII for the determination of sulphonamides is criticised on the grounds that it requires a fairly narrow temperature range for accurate results and that acylated sulphonamides must be hydrolysed to liberate the aromatic amino group. In the proposed method these inconveniences are overcome, while no external indicator is needed and the end-point is sharp and stable.

Method—To an accurately weighed sample of the powdered tablets, expected to contain 0·25–0·35 g. of sulphanilamide, sulphathiazole, sulphadiazine or

sulphamerazine, 0·35–0·45 g. of sulphaguanidine or 1·0–1·4 g. of succinylsulphathiazole, contained in a glass-stoppered flask, add 20 ml. of 2% sodium hydroxide and shake vigorously to dissolve the sulphonamide. Add a measured volume of 0·2 *N* bromate - bromide solution so that a few ml. are in excess, followed by 80 ml. of glacial acetic acid and 5 ml. of conc. hydrochloric acid; immediately stopper the flask and shake vigorously for 30 seconds to redissolve any precipitated sulphonamide. Allow to stand for a further 90 seconds and then titrate with 0·1 *N* arsenious oxide solution until the yellow bromine colour is discharged. In analyses of sulphanilamide and sulphathiazole the sodium hydroxide may be omitted, in which event the acetic acid may be added first, the bromate-bromide being then added with constant swirling to avoid any local excess of aqueous bromine. Finally the hydrochloric acid is added and the excess bromine titrated with arsenious oxide. Each ml. of 0·2 *N* bromate - bromide is equivalent to 0·008610 g. of sulphanilamide, 0·008511 g. of sulphathiazole, 0·008343 g. of sulphadiazine, 0·008810 g. of sulphamerazine, 0·01161 g. of sulphaguanidine monohydrate or 0·03734 g. of succinylsulphathiazole monohydrate. It is claimed that tablet excipients are not affected and results are cited which indicate that the procedure is equivalent in accuracy to the diazotisation method of the U.S.P. XII. J. A.

Gravimetric Determination of Aluminium in Pharmaceutical Preparations. F. J. Bandelin (*J. Amer. Pharm. Assoc.*, 1945, **34**, 232–234)—Willard and Tang (*J. Amer. Chem. Soc.*, 1937, **59**, 1190; *Ind. Eng. Chem. Anal. Ed.*, 1937, **9**, 357) have demonstrated that aluminium may be quantitatively precipitated as the basic salt of an organic anion such as oxalate, succinate, benzoate or phthalate in presence of urea and then gives a dense granular precipitate that is easily filtered and does not adsorb other materials to the same extent as aluminium hydroxide. *Method*—Dilute a solution of the sample expected to contain the equivalent of about 0·2 g. of Al_2O_3 and contained in a 800-ml. beaker to about 200 ml. with distilled water, add 10% ammonium hydroxide until a slight turbidity forms, followed by 10% hydrochloric acid, dropwise, to redissolve the precipitate and then add two or three drops of the acid in excess. Add 5 g. of succinic acid previously dissolved in 100 ml. of water, 10 g. of ammonium chloride and 4 g. of urea. Dilute to 400 ml. and boil the solution gently until it becomes turbid and then for a further period of 90 min. Turbidity occurs at a *p*H at which methyl orange changes colour, usually about twenty to thirty min. after boiling begins. Allow the mixture to cool to room temperature, filter through a tared Gooch crucible and wash the beaker and precipitate with 6 10-ml. portions of 1% succinic acid solution previously neutralised to methyl red with ammonium hydroxide. Ignite the crucible and contents at 1000° for about 1 hr., cool in a desiccator and weight as Al_2O_3. Results cited indicate that the proposed method gives more accurate and uniform results than does the hydroxide procedure, while the maximum *p*H obtainable is 4·6, thus eliminating the possibility of errors arising from excess alkalinity. J. A.

A Chemical Assay for Ergonovine [Ergometrine] in Ergot compared with the Biologic Assay. D. C. Grove and B. J. Vos, Jr. (*J. Amer. Pharm. Assoc.*, 1945, **34**, 256–259)—It is considered that the results given by all the recently published methods for the chemical determination of ergonovine (ergometrine) in ergot are high owing to contamination of the ergometrine fraction with impurities that give a colour with *p*-dimethylaminobenzaldehyde reagent. Except by Powell, Reagan, Stevens and Swanson (*J. Amer. Pharm. Assoc.*, 1941, **30**, 255), no attempt has been made to show any correlation between the colorimetric assay and the biological activity of the ergometrine fraction, and since it is considered that if a chemical assay is to be adopted the results obtained should demonstrably agree with those of the biological method, the following procedure has been devised.

Method—Weigh 10 g. of non-defatted, powdered ergot into a 100-ml. beaker, add 4 ml. of lead subacetate solution (about 22·5% Pb) (Allport and Porter, *Quart. J. Pharm.*, 1938, **11**, 96), mix thoroughly, transfer completely to an extraction thimble and extract with 100 ml. of ether for four hours on the steam bath. Transfer the ether extract to a 125-ml. separator, wash the flask several times with small portions of ether and then with two 5-ml. portions of 1% tartaric acid solution, adding each washing to the separator. Shake the separator, draw off the separated tartaric acid solution and extract with six successive 5-ml. portions of 1% tartaric acid solution. Emulsification may be eliminated by adding 1 ml. of alcohol after the third shaking and a further 1 ml. after the fifth shaking. Combine the acid extracts in a long necked 200-ml. round bottomed flask and remove the dissolved ether at room temperature *in vacuo*. Transfer the solution to a 50-ml. volumetric flask with small portions of water and dilute to the mark. Dilute a 5-ml. aliquot to 50 ml. and reserve this for the colorimetric determination of the total alkaloids; it represents the alkaloids from 1 g. of ergot. Rinse the 5 ml. pipette with about 2 ml. of water and add this to the 45 ml. of tartaric acid solution. Add to this sufficient 10% ammonia solution to precipitate the water-insoluble alkaloids and to render the solution alkaline to litmus and dilute to 50 ml. Filter, transfer 45 ml. to a 125-ml. separator, add 0·5 g. of powdered tartaric acid, 15 g. of sodium chloride and 50 ml. of ether, previously washed with water to remove alcohol, and shake thoroughly. Withdraw the aqueous layer to a second separator, wash the ether with two 5-ml. portions of saturated sodium chloride solution containing 1% of tartaric acid and discard the ether. To the mixed tartaric acid extract and washings in the second separator add sufficient ammonia to render alkaline to litmus and extract with four 50-ml. portions of washed ether. Filter the combined ether extracts through cotton wool and evaporate on the steam bath to 20–25 ml. in a beaker. Transfer to a 125-ml. separator, marked for a volume of 35 ml., with small portions of ether and dilute with more solvent to 35 ml. Wash the beaker with 10, 10 and 5 ml. of ammonia water (1 drop of 10% ammonia solution in 200 ml. of water) and add the washings to the separator. Shake the mixture fairly vigorously for 2 minutes (this time should be used for all subsequent shakings), withdraw the separated aqueous layer into a second separator containing 35 ml. of U.S.P. ether and shake. Withdraw the aqueous layer from this second extraction to a third separator containing 35 ml. of U.S.P. ether and shake, and finally run the aqueous layer into a 250-ml. separator. Repeat the whole extraction procedure with six successive 10-ml. portions of ammonia water. Add 25–30 g.

of sodium chloride to the combined aqueous extracts, shake with 10 ml. of chloroform and filter the solvent layer through cotton wool into a 100-ml. beaker. Extract with five further 10-ml. portions of chloroform, evaporate the combined filtered extracts just to dryness on the steam bath, dissolve the residue in 1% tartaric acid solution and dilute to a convenient volume depending upon the amount of ergometrine expected to be present. This solution is used for the colorimetric and the biological assays. The amount of ergometrine in this solution is that derived from 8·1 g. of ergot.

The colorimetric determinations were carried out using 3 ml. of the alkaloidal solution and 6 ml. of the *p*-dimethylaminobenzaldehyde reagent of Allport and Cocking (*Quart. J. Pharm.*, 1932, **5**, 341) and the colours compared photoelectrically at 560 mμ. The biological determination was performed on the isolated rabbit uterus as described by Vos (*J. Amer. Pharm. Assoc.*, 1943, **32**, 138). Any ergometrinine which may be present is eliminated by the three 35-ml. ether washes (Grove, *ibid.*, 1941, **30**, 260) while the purpose of the transference to chloroform is to give a dry residue of the alkaloid free from traces of alcohol and ammonia which may otherwise affect the biological assay; if only the colorimetric assay is required, this stage may be omitted. Recovery experiments indicated that an average of 91% of added ergometrine acid maleate was obtained. The relationship between the results of the colorimetric and of the biological assays has been studied and in all cases the biological figure is lower than that obtained chemically. The ratio between them is fairly constant (0·80 to 0·92, av. 0·85) and the experimental results indicate that the difference between results by the two procedures increases with the length of time elapsing between the grinding of the drug and the assay. Thus in four assays performed within a month of grinding the drug none of the ratios fell below 0·85, while in four done seven to nine months after grinding, all the ratios were less than this figure. It is suggested that if the biological assay were considered to indicate more correctly the actual ergometrine content of the drug, a factor such as 0·85 might be used to convert the results of the chemical assay to their approximate biological equivalents, although it is considered that, in view of the effect of storage in the ground state on the ratio between the results by the two methods, it may be advisable to study the effects of deterioration of ergot on the results by the chemical assay before discarding the biological method. J. A.

The Assay of Alkaloidal Galenicals, using the Chromatograph. Part I. Preparations of Belladonna, Stramonium and Nux Vomica. G. W. Brownlee (*Quart. J. Pharm.*, 1945, **18**, 163–171)—The use of chromatographic methods in the assay of preparations of belladonna, stramonium and nux vomica has been examined and it is shown that the analytical results obtained compare favourably with those by official methods. The apparatus employed consists of a glass tube 60 cm. long and with an internal diameter of 1·3 cm. One end of this is closed by a rubber bung carrying a tube 8 mm. in diameter and 8 cm. long which passes through one hole of a two-holed bung attached to a flask of convenient size and marked at 30 and 40 ml.; the other hole carries a tube which may be attached to a vacuum pump. The adsorbent used is alumina and the tube is charged with 50 g. of this material supported on a plug of cotton wool and introduced in quantities of about 5 to 8 g., each portion being evenly pressed down before the next is added. The total length of the alumina column is about 37 cm. A piece of filter paper cut to size and moistened with the particular galenical menstruum used is placed on top of the column. For preparations of nux vomica and soft extracts generally, a plug of cotton wool, suitably moistened, has been found more convenient.

Belladonna and Stramonium—Transfer to the top of the column a known weight of the galenical, or, in the case of soft extracts, a known weight dissolved in a suitable menstruum, attach the apparatus to a vacuum pump and apply gentle suction. When 1 to 2 ml. of liquid remains on top of the column wash the sides of the tube with 2 ml. of 70% alcohol, continue the addition of the alcohol and finally fill up the tube. Allowing the suction to proceed, collect 30 to 35 ml. of liquid and transfer to a separator with the aid of 10 ml. of 0·1 *N* sulphuric acid and 20 ml. of water. Wash the liquid in the separator with two 10 ml. portions of chloroform, wash each with the same 10 ml. of 0·1 *N* sulphuric acid contained in a second separator, and reject the chloroform layers. Transfer the acid washings to the first separator, make alkaline with ammonia and extract the alkaloids completely with successive portions of chloroform. Remove the solvent, dry the residue to constant weight at 100° C., dissolve in 20 ml. of 0·02 *N* sulphuric acid and titrate the excess with 0·02 *N* sodium hydroxide using methyl red as indicator.

Preparations of Nux Vomica—Carry out the process as described above, collecting 35 to 40 ml. of liquid from the chromatograph. Transfer the liquid to a separator with 10 ml. of *N* sulphuric acid and 20 ml. of water, wash with two 10-ml. quantities of chloroform, wash each chloroform extract with two separate 10-ml. quantities of 0·1 *N* sulphuric acid and reject the chloroform. Mix the acid liquids, make distinctly alkaline with dilute ammonia solution and extract with successive portions of chloroform until complete extraction of the alkaloids is effected. Remove the solvent, add 5 ml. of 95% alcohol, evaporate to dryness and continue the assay as described in the B.P. 1932 beginning "Dissolve the residue in 15 ml. of 3% w/v sulphuric acid." Webster and Pursel (*Amer. J. Pharm.*, 1907, **79**, 1) have shown that direct treatment of extracts of nux vomica with nitric and sulphuric acids does not affect the normal nitration and oxidation of the brucine, and a method of assay has been devised applying this principle. *Method*—To 10 ml. of liquid extract in a small beaker add 5 ml. of 9% w/v sulphuric acid and 2 ml. of nitric acid. Leave for 30 min. at 15 to 20° C., then make the liquid slightly alkaline to litmus with 40% sodium hydroxide, acidify with 33% acetic acid and add 0·5 to 1 ml. in excess. Chromatograph this liquid as before, using 70% alcohol containing 1 ml. of 33% acetic acid per 100 ml. as eluant. Collect 40 to 45 ml. of liquid, transfer to a separator with 20 ml. of water and make alkaline with sodium hydroxide solution. Extract with 20-ml. quantities of chloroform until complete extraction of the alkaloids is effected, combine the solvent solutions and extract with 20 ml. of *N* sulphuric acid, followed by successive portions of 0·2 *N* sulphuric acid containing 10 ml. of 90% alcohol per 100 ml. Wash the combined acid layers with two 10-ml. portions of chloroform, washing each separate chloroform layer with the same 10 ml. of 0·1 *N* sulphuric acid. Reject the chloroform, mix the acid liquids, make alkaline

with sodium hydroxide and extract with successive portions of chloroform until extraction is complete, washing each chloroform layer with the same 10 ml. of water. Remove the solvent, add 5 ml. of alcohol, evaporate to dryness, dry the residue for 30 minutes at 100° C., dissolve in 10 ml. of 0·1 *N* sulphuric acid and titrate the excess with 0·1 *N* sodium hydroxide with methyl red as indicator. The result should be multiplied by 1·02 to correct for loss of strychnine in the process. Other preparations of nux vomica are treated as follows: *Tincture*—Evaporate 100 ml. to dryness, dissolve the residue in 8·5 ml. of 50% alcohol, add 5 ml. of 9% w/v sulphuric acid and 2 ml. of nitric acid and continue as above. *Dry Extract*—Extract 3 g. with 50% alcohol as in the official method, evaporate to dryness, dissolve the residue in 8·5 ml. of 50% alcohol, add 5 ml. of 9% w/v sulphuric acid and 2 ml. of nitric acid and continue as above. *Soft Extract*—Dissolve 2 g. in 8 ml. of 50% alcohol, add 5 ml. of 9% w/v sulphuric acid and 2 ml. of nitric acid and continue as above.

The chromatograms of the various preparations are discussed and the similarity in those of drugs from various sources is demonstrated. The chromatogram of English belladonna leaf is more or less in agreement with that shown by Merz and Franck (*Arch. Pharm.*, 1937, **82**, 345). It is also pointed out that the complexity of the nitration products of brucine is indicated in the chromatogram derived from the reaction mixture. J. A.

The Assay of Alkaloidal Galenicals using the Chromatograph. Part II. Liquid Extract of Ergot B.P. and Extract of Ergot B.P.C. G. W. Brownlee (*Quart. J. Pharm.*, 1945, **18**, 172–174)—*Liquid Extract of Ergot*—Prepare a chromatograph column using 25 to 30 g. of alumina (see previous abstract) and place on it 5 ml. of the liquid extract. Apply gentle suction and, when about 1 ml. remains, wash the sides of the tube with 2 ml. of a mixture of equal volumes of acetone and water. When only 1 ml. of this remains continue the elution with AnalaR acetone and collect 35 to 40 ml. of liquid. Transfer to a separator, washing the flask with anaesthetic ether until 120 ml. in all have been used. Extract the mixture with 10, 5, 5, 5 and 5 ml. portions of 1% tartaric acid solution. Remove the solvents from the mixed acid extracts under reduced pressure and continue the assay as described in the B.P. 1932 (Sixth Addendum), commencing with the words ". . . dilute to 50 ml. with water. . . ." *Extract of Ergot B.P.C.*—Dissolve 0·5 g., accurately weighed, in 3 ml. of a mixture of equal parts of 90% alcohol and water containing 1% of tartaric acid. Transfer the solution to the chromatograph and rinse the vessel with small quantities of the solvent. Continue the assay as described above. It is stated that the tartaric acid extracts are comparable in colour with those obtained by the method of Hampshire and Page (*Quart. J. Pharm.*, 1938, **11**, 57) and are less highly coloured than those from the Allport and Jones procedure (*Quart. J. Pharm.*, 1941, **14**, 106). The results obtained agreed well with those by the Hampshire and Page method and the process has the advantage of eliminating the six-hour extraction, the process of chromatography being completed in 30 minutes under a moderate vacuum. The assay in its present form is unsatisfactory for liquid extract of ergot B.P. 1914, owing in part to the large amount of extractive matter and in part to the low alkaloidal content. The chromatographs of the preparations are described and a distinction is demonstrated between those of extract of ergot B.P. 1914 and B.P. 1932 extract. J. A.

Erratum.—January issue, p. 42, col. 2, lines 9–8 from the bottom of the page, *for* "2-aminopyridine" *read* "2-aminopyrimidine."

Biochemical

New Reagent for the Determination of Sugars. M. Somogyi (*J. Biol. Chem.*, 1945, **160**, 61–68)—It is frequently necessary to use different modifications of the alkaline copper tartrate reagent for different purposes, and it would be an advantage to have a reagent of general applicability. One has now been devised which is claimed to possess several advantages. It is sufficiently alkaline to permit the determination of maltose and other slowly reacting sugars; it contains no potassium iodide, and yet is stable, showing no auto-reduction at room temperature, even in sunlight; moreover its high sulphate content and the absence of iodide make it unlikely that the cuprous oxide will be reoxidised. The reagent can be used for colorimetric work and its range is particularly extensive; it can be used to determine as little as 0·01 mg. or as much as 3·0 mg. of glucose. The main change from existing reagents is the substitution of a phosphate buffer for the carbonate - bicarbonate buffer mixture hitherto used. The composition of the reagent is as follows: 28 g. of Na_2HPO_4 (anhyd.), 100 ml. of *N* sodium hydroxide, 40 g. of Rochelle salt, 8 g. of $CuSO_45H_2O$, and 180 g. of Na_2SO_4 (anhyd.) per litre. It is prepared as follows: Dissolve the phosphate and tartrate in about 700 ml. of water, add the sodium hydroxide and then, with stirring, 80 ml. of 10% copper sulphate soln. Finally, add the sodium sulphate and when it is dissolved dilute to 1 litre and leave for 1 to 2 days. Decant the clear supernatant liquid and filter the remainder.

Colorimetric procedure—Mix 2 ml. of the reagent and 2 ml. of the sugar soln. in a test-tube, cover the mouth of the tube with a glass marble and immerse in boiling water for 10 mins. Cool, add Nelson's arsenomolybdate reagent (*J. Biol. Chem.*, 1944, **153**, 375; ANALYST, 1944, **69**, 313), dilute to a definite volume, and evaluate the colour of the soln.

Iodimetric procedure—To a convenient quantity of the reagent add 5, 10 or 20 ml. of *N* potassium iodate per litre according as to whether the glucose concentration is expected to be about 0·5, 1 or 2–3 mg. per 5 ml. respectively. Mix 5 ml. of the reagent with 5 ml. of the sugar soln., and heat in a boiling water-bath for a period of time varying with the nature of the sugar, *e.g.*, with glucose heat for 10 mins. and with maltose 20 mins. Cool and add 0·5, 1·0 or 2·0 ml. of 2·5% potassium iodide solution, according as 5, 10 or 25 ml. of iodate per litre are present in the reagent. Add rapidly 1·5 ml. of approx. 2 *N* sulphuric acid, and titrate with 0·005 *N* sodium thiosulphate soln. With glucose or maltose the amount of copper reduced is directly proportional to the amount of the respective sugar between 0·05 and 3·0 mg. One ml. of 0·005 *N* thiosulphate is equivalent to 0·135 mg. of glucose or to 0·26 mg. of maltose.

F. A. R.

Determination of Blood Sugar. M. Somogyi (*J. Biol. Chem.*, 1945, **160**, 69–73)—The new copper reagent (*cf.* preceding abstract) was used for the

estimation of sugar in blood filtrates prepared by addition of zinc sulphate and barium hydroxide. Take 1 vol. of blood in a measured amount of water (this may vary from 5 to 75 vols.), add 2 vols. of 0·3 *N* barium hydroxide, and 2 vols. of 5% $ZnSO_4.7H_2O$ soln. Shake vigorously and filter. To a convenient vol. of reagent add 8 g. of potassium iodide and 25 ml. of *N* potassium iodate per litre. Mix 2 ml. of blood filtrate with 2 ml. of the reagent in a test-tube, and heat in a boiling water-bath for 12 mins. Cool, add 1 ml. of approx. 2 *N* sulphuric acid and titrate with thiosulphate as previously described. For accurate results it is better to add the potassium iodide separately after the heating.

Alternatively, the glucose may be estimated colorimetrically as follows: Mix 2 ml. of the filtrate with 2 ml. of the copper reagent (containing neither iodate nor iodide), heat for 10 mins. and, after cooling, add Nelson's reagent, and evaluate the colour in a colorimeter. F. A. R.

Determination of Glucose in Blood. O. Schales and S. S. Schales (*Arch. Biochem.*, 1945, **8**, 285–292)—A simple and accurate routine method of estimating glucose in blood has been devised based on the photoelectric determination of the disappearance of ferricyanide by reaction with glucose.

Into test tubes graduated at 25 ml., pipette 1 ml. of Folin - Wu filtrate and add exactly 10 ml. of alkali ferricyanide soln. (dissolve 2·5–3·0 g. of potassium ferricyanide, 140 g. of K_2HPO_4 and 42 g. of K_3PO_4 in 1 litre of water and dilute the resulting stock soln. 5-fold before use). Prepare a blank with 1 ml. of water and 10 ml. of the reagent. Immerse the tubes in boiling water for 15 min. and in cold water for a further 3 min. Dilute to the mark and evaluate the colours of the solns. in a sensitive photoelectric colorimeter against distilled water, using a filter transmitting maximally at 420 mμ. The difference between the extinction values of the blank and unknown was found to be proportional to the glucose concentration. Prepare a calibration curve using 10 standard solns. containing 50 to 500 mg. of glucose per 100 ml., and use this for calculating the glucose contents of the unknowns. Known amounts of glucose added to a Folin - Wu filtrate were recovered with a maximum error of 2·7%.

The removal of non-fermentable substances, which would otherwise reduce the reagent, leading to high values, is effected by treatment with cadmium hydroxide (Fujita and Iwatake, *Biochem. Z.*, 1931, **242**, 43), which also precipitates proteins. Where elimination of such interfering substances is necessary, substitute 1 ml. of a cadmium hydroxide filtrate for the Folin - Wu filtrate. The presence of small amounts of cadmium does not interfere with the method. F. A. R.

Specific Micro-method for the Colorimetric Estimation of Glycine in Blood and Urine. B. Alexander, G. Landwehr and A. M. Seligman (*J. Biol. Chem.*, 1945, **160**, 51–59)—The basis of the method is the conversion of glycine by ninhydrin into formaldehyde which is then measured by allowing it to react with chromotropic acid (1 : 8-dihydroxynaphthalene-3 : 6-disulphonic acid).

Put 5·0 ml. of a protein-free filtrate of blood, prepared by addition of sulphuric acid and sodium tungstate soln., or 5 ml. of urine diluted 50-fold, into the flask of an all-glass still containing 2 ml. of phosphate buffer, *p*H 5·5 (add 3·5 g. of K_3PO_4 to 100 ml. of 20% KH_2PO_4 soln.), 1 ml. of 1% ninhydrin soln. and a glass bead. Attach the flask to the condenser and distil the contents rapidly into a test-tube calibrated at 10·0 ml. Collect 7 ml. of distillate, cool the flask to room temperature, add 2 ml. of water, and continue the distillation to dryness. The total distillation time should not exceed 15 min. Heat the neck of the still gently to distil the drops that have condensed there, remove the receiver, and make up the volume to 10·0 ml. Pipette 5·0 ml. into an Evelyn colorimeter tube, cool in an ice-bath and add 4·0 ml. of conc. sulphuric acid. Allow the mixture to warm to room temperature and add 0·1 ml. of 5% chromotropic acid soln. Cork the tube lightly, immerse in a boiling water-bath for 30 mins., cool, and evaluate the colour in an Evelyn or Klett photoelectric colorimeter with a No. 565 or 540 filter. With urine it is necessary to run a blank on another 5·0-ml. aliquot of the diluted sample, adding 1 ml. of water in place of the ninhydrin soln.

As little as 0·2 μg. of formaldehyde, equivalent to 0·5 μg. of glycine, can be estimated in this way. The only substance likely to interfere is furfural, which is volatile in steam and gives a pink colour with the reagent, though only in high concentrations. The average deviation in duplicate estimations of glycine was 4%, and the recovery of glycine added to blood or urine varied from 86 to 105%. F. A. R.

Multiple Nature of Vitamin B_6. Critique of Methods for the Determination of the Complex and its Components. D. Melnick, M. Hochberg, H. W. Himes and B. L. Oser (*J. Biol. Chem.*, 1945, **160**, 1–15)—Pyridoxine, pyridoxal and pyridoxamine all possess vitamin B_6 activity, but their relative potencies towards different organisms vary greatly. The earlier chemical and microbiological methods were published before this was realised, and the available methods have now been reinvestigated to ascertain their reliability for measuring pyridoxine itself, as well as other compounds with similar biological activity.

The ultraviolet absorption curves of pyridoxine, pyridoxal and pyridoxamine differ from one another, and at *p*H 2·1 each has a single absorption maximum at 288, 286 and 292 mμ respectively. At *p*H 6·75 the three compounds show maxima at 254 and 325, 251 and 316; and 250 and 325 mμ respectively. By measuring the absorption at 325 mμ of solutions of *p*H 6·7 it is possible to obtain an approx. estimate of the total of the three compounds, despite variations in their relative concentrations, since at this wave length all three compounds absorb to the same extent; the value of $E^{1\%}_{1 cm.}$ is 440.

The method of Hochberg *et al* (*J. Biol. Chem.*, 1944, **155**, 109; ANALYST, 1945, **70**, 95) can be used to determine pyridoxine in presence of pyridoxal and pyridoxamine. The published procedure is followed except that the ammonia soln. in the buffer is reduced from 160 to 40 ml. per litre, and the reaction time increased from 1 to 15 mins. Pyridoxal and pyridoxamine react with the chloroimide reagent to yield a blue pigment but the reactions, unlike that with pyridoxine, are not inhibited by borate, and the difference in the photometric density in absence and presence of borate is therefore due solely to the pyridoxine.

Microbiological assays with *Saccharomyces carlsbergensis* (Atkin *et al.*, *Ind. Eng. Chem.*, *Anal. Ed.*, 1943, **15**, 141) estimate the total of the three compounds, assays with *Lactobacillus helveticus* (Snell and Rannefeld, *J. Biol. Chem.*, 1945, **157**,

475) estimate pyridoxal but not pyridoxine or pyridoxamine, whilst *Streptococcus faecalis* (Snell, *J. Biol. Chem.*, 1945, **157**, 491) estimates pyridoxamine and pyridoxal but not pyridoxine. By using these three organisms it is possible to demonstrate the presence of pyridoxal or pyridoxamine, but unfortunately it is not possible to estimate the amounts of each factor in substances such as yeast, which appear to contain in addition a labile factor possessing the same vitamin B_6 activity for the rat as for *Saccharomyces carlsbergensis*. Where only pyridoxine is present the chemical method of assay gives the best results, but where additional substances with vitamin B_6 activity are present the microbiological method with *Saccharomyces carlsbergensis* is more satisfactory; its only disadvantage is the relatively high error (15%). F. A. R.

Bioassay of Lysine by use of a Mutant of *Neurospora*. A. H. Doermann (*J. Biol. Chem.*, 1945, **160**, 95–103)—In this method a mutant of *Neurospora crassa* (strain 4545), which fails to grow in absence of lysine, is used. The basal medium has the following composition: ammonium tartrate 5·0 g., ammonium nitrate 1 g., monopotassium phosphate 1·0 g., magnesium sulphate ($7H_2O$) 0·5 g., sodium chloride 0·1 g., calcium chloride (anhydrous) 0·1 g., sucrose 20·0 g., biotin 5·0 × 10^{-6} g., boron 0·01 g., molybdenum 0·02 mg., iron 0·20 mg., copper 0·10 mg., manganese 0·02 mg., zinc 2·00 mg., phosphate buffer, 0·5 *M*, *p*H 5·5, 100 ml., asparagine 1·0 g., glutamic acid 1·0 g., *l*(+)-lysine (calculated as free base) 5·84 mg., water to bring vol. to 1 litre.

Maintain stock cultures on the basal medium plus agar 2%, casein hydrolysate 0·05%, yeast extract 0·5%, malt extract 0·5%, and *l*(+)-lysine 0·01%. The cultures are ready for preparing the conidial suspension used for inoculating the test solns. after 5–6 days' incubation. Put 25 ml. of basal medium (without lysine) into each of a series of 125 ml. conical flasks and add amounts of *l*(+)-lysine ranging from 0 to 0·6 mg. (calculated as free base).

Hydrolyse the material by any method that does not racemise amino-acids, and remove arginine, which is inhibitory for strain 4545, either by precipitating as the silver salt, described by Kossel and Kutscher (*Z. physiol. Chem.*, 1900–01, **31**, 165) or, more simply, by hydrolysis with arginase. Prepare the enzyme as follows: Grind a quantity of liver and shake vigorously with an equal weight of water for 10 mins. Heat the container at 62–65° C. with constant stirring, and maintain the contents at about 58° C. for 5 mins. Cool, filter, adjust the filtrate to *p*H 7·0, and evaporate to dryness. The dry preparation keeps for about 9 months. Put 0·5 g. of the hydrolysate into a 50-ml. volumetric flask, and add 25 mg. of the arginase preparation and 20 ml. of 0·25 *M* pyrophosphate buffer. Adjust the *p*H to 8·5–9·0 with *N* sodium hydroxide and dilute to approx. 48 ml. Layer the soln. with toluene, incubate at 35° C. overnight and then adjust the *p*H to 5·5–6·5 with 12 *N* sulphuric acid and dilute to 50 ml. Immerse in a boiling water-bath for 15 mins. and filter.

Add different amounts of the hydrolysate to another set of conical flasks each containing 25 ml. of lysine-less basal medium, plug all the flasks with cotton wool, autoclave at 15 lb. pressure for 10–15 mins. and then inoculate with 1 or 2 drops of a conidial suspension of *Neurospora*. Incubate for 7 days at 25° C. and then remove the mycelium with a needle or forceps, press as dry as possible on filter paper, heat in an oven at 75 to 90° C. for 3 hrs. and weigh. Plot the weights of mycelia against the corresponding amounts of sample and standard and calculate the results from the standard curve in the usual way.

Duplicate values on the same material agreed within 4%, whilst the recovery of lysine added to protein hydrolysates ranged from 97 to 109%. F. A. R.

Chemical Determination of Tocopherols in Animal Fats. J. R. Chipault, W. O. Lundberg and G. O. Burr (*Arch. Biochem.*, 1945, **8**, 321–335)—A sensitive and specific method of estimating tocopherol in animal fat was devised. This was based on Furter and Mayer's procedure (*Helv. Chim. Acta*, 1939, **22**, 240) as modified by Andrews and Binnington. The fat is extracted and saponified and the unsaponifiable matter oxidised with nitric acid; the oxidation products are chromatographed on alumina and the red orthoquinones separated and measured by reaction with leuco-methylene blue.

Saponify 8·9 g. of the fat with 17 ml. of air-free alkali soln. (dissolve 400 g. of KOH pellets in 2 litres of absolute methanol, passing a current of purified nitrogen through the soln., and filter through a sintered glass funnel whilst maintaining an atmosphere of nitrogen) and 13 ml. of air-free methanol. Dilute with air-free water and extract the unsaponifiable matter with ether, maintaining an atmosphere of nitrogen throughout all the operations. Wash the extract, dry over sodium sulphate and evaporate to dryness. Add 2 ml. of benzene and remove sterols and carotenoids by adsorption on a column (5 × 100 mm.) of Florisil. Wash the column with three 2-ml. portions of benzene and evaporate the benzene soln. in a current of nitrogen. Add 10 ml. of absolute *n*-butanol to the residue and then 2 ml. of conc. nitric acid. Immerse in boiling water for 2 min. (± 5 sec.) and then cool to room temperature as rapidly as possible.

Transfer the soln. with the aid of three 5-ml. portions of absolute ethyl alcohol to a 250-ml. separating funnel containing 150 ml. of water. Add 25 ml. of light petroleum, shake, allow to separate, and discard the lower layer. Wash the petroleum with two 100-ml. portions of water, dry over sodium sulphate and evaporate the petroleum soln. in a stream of nitrogen. Dissolve the residue in 2 ml. of light petroleum, and draw the soln. through a column (3 × 75 mm.) of Merck's Brockmann alumina containing 12% of moisture. (The moisture content of the alumina is very important, and may be adjusted to the correct value by exposing the dried alumina to the atmosphere above 33·2% sulphuric acid.) Rinse the flask and column with another 2 ml. of light petroleum and then wash with benzene until the red zone of orthoquinone has almost reached the bottom of the column. Wash once with light petroleum and dry by suction. Elute the portion of the column containing the red zone with a small amount of peroxide-free ether and transfer to a spectrophotometer tube after suitable dilution, if necessary. When relatively large amounts of tocopherols are present, the analysis can be carried out by colorimetric estimation of the orthoquinone in alcohol solution, using an Evelyn colorimeter with a 490 mμ filter.

With small amounts of tocopherol, however, the following procedure is necessary. Dissolve methylene blue in 95% alcohol to give a few ml. of a concentrated solution, filter, and dilute with a mixture of 80 parts by volume of 95% ethyl alcohol, 15 parts of water, and 5 parts of glacial acetic acid, giving a

stock solution with an optical density of 2·0 to 2·5 per cm. of cell thickness at 650 mμ. Put 15 ml. of the stock soln. into a separating funnel containing a few g. of granulated zinc, and pass a gentle stream of nitrogen up through the stem of the funnel. Put the ethereal eluate containing the orthoquinone into a side-arm sealed on to a spectrophotometer tube and remove the solvent by evaporation in a gentle stream of nitrogen. To the colorimeter tube attach a separating funnel in which is a sintered glass plate; on to this put a pinch of zinc dust and evacuate the entire apparatus to a pressure of 10 to 20 mm. Now close the tap of the separating funnel and fill it with nitrogen. Run the reduced methylene blue soln. into the funnel and again evacuate to 20 mm. or less. When reduction is complete allow 10 ml. of the reduced methylene blue soln. to flow into the colorimeter tube and then close the tap of the funnel. Mix the leuco-methylene blue soln. with the quinone soln. by tipping the colorimeter tube and measure the transmission at 650 mμ. The results are calculated from a reference curve prepared by treating a sample of fat of known tocopherol content by the same procedure. Within the limits of experimental error, α- and γ-tocopherol give the same standard curves.

For details of the apparatus used, the original paper should be consulted. F. A. R.

Simplified Hydrogenation Technique for the Estimation of Blood Plasma Tocopherols. M. L. Quaife and R. Biehler (*J. Biol. Chem.*, 1945, **159**, 663–665)—The method of Quaife and Harris (*id.*, 1944, **156**, 499; Abst., ANALYST, 1945, **70**, 183) employs catalytic hydrogenation

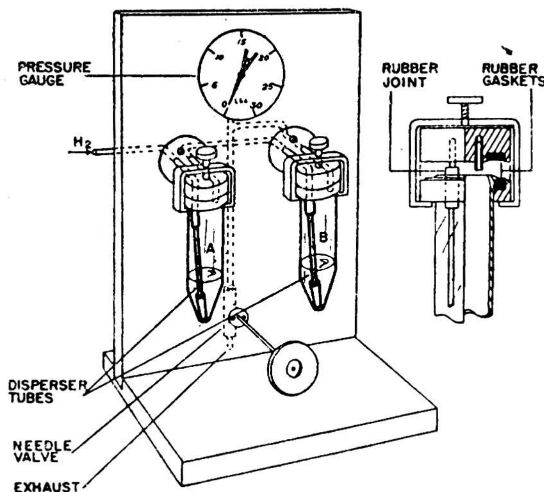


Fig. 1.

for the removal of interfering vitamin A and carotenoids. As originally described this method is lengthy and requires equipment not generally available. A hydrogenator is now described which reduces the time for a single estimation of vitamin E in plasma to about 30 mins. The apparatus is shown in Fig. 1. Hydrogen is bubbled through the alcohol in tube A by means of the microporous disperser tube and the gas, saturated with alcohol vapour, is then bubbled through the sample in tube B, the exhaust gas passing out through a pressure gauge and needle-valve.

Evaporate 10 ml. of the light petroleum extract of plasma to dryness in a 50-ml. conical centrifuge tube and dissolve the residue in 10 ml. of ethanol. Cool, add the catalyst and clamp the tube into position. Pass a rapid, smooth flow of gas through the soln. by adjusting the needle-valve and after 1 min. stop the flow of gas, adjust to atmospheric pressure, remove the tube and centrifuge. The remainder of the analysis is carried out as described previously. Solutions of pure α-tocopherol in ethanol gave recoveries of 97·8%. F. A. R.

Indophenol - Xylene Extraction Method for Ascorbic Acid and Modifications for Interfering Substances. W. B. Robinson and E. Stotz (*J. Biol. Chem.*, 1945, **160**, 217–225)—*Procedure A, Basic method*—Put 1 to 5 ml. of the tissue extract (prepared with fresh 2% metaphosphoric acid in *N* sulphuric acid) containing 0·02 to 0·15 mg. of ascorbic acid into a test-tube and add an equal vol. of acetate buffer *p*H 4·0 (mix 1 litre of 50% hydrated sodium acetate soln. and 1 litre of glacial acetic acid), followed immediately by 2 ml. of a 0·0125% soln. of 2 : 6-dichloro-benzenone-indophenol and 10 ml. of redistilled xylene. Shake vigorously for 6 to 10 secs. to extract the excess dye. In a second tube extract 2 ml. of dye soln. with 10 ml. of xylene, and in a third tube treat a soln. containing 0·10 mg. of ascorbic acid with 2 ml. of dye soln. and 10 ml. of xylene in precisely the same way. Centrifuge the three tubes, withdraw the xylene layers, and evaluate the colours in a photoelectric colorimeter with a filter transmitting maximally at 500 mμ. Use xylene as the reference substance. Since ascorbic acid is unstable at *p*H 4·0 the mixing and extraction should be carried out as quickly as possible.

If in a preliminary test xylene removes colour from the acetate-buffered tissue extract an additional operation must be carried out. After the first reading add 2 drops of hydroquinone soln. (prepared by mixing equal vols. of acetone and a saturated soln. of hydroquinone in acetone) to the xylene extract to bleach the orange colour due to the excess dye. After 30 secs., take a second reading to measure the blank due to the tissue extract.

Procedure B, Peroxide modification—To eliminate the reducing action of sulphite or reduced iron or tin, add a vol. of 3% hydrogen peroxide equal to that of the acetate buffer extract just prior to the addition of the dye. Then continue the procedure as described above.

Procedure C, Formaldehyde modification—To correct for the action of reductones, destroy the reducing action of ascorbic acid by interaction with formaldehyde at *p*H 3·6 to 3·8 for 10 mins. For this purpose put 2 ml. of tissue extract into each of two tubes and add to the second tube 2 ml. of acetate buffer and 1 ml. of 40% formaldehyde. After 10 mins. put 2 ml. of acetate buffer and 1 ml. of water into the first tube and then add 2 ml. of dye soln. to each tube and proceed as described above. The difference between the amounts of dye reduced in the two tubes represents the true ascorbic acid. In procedures A and B, the amount of ascorbic acid is equal to

$$0{\cdot}1\ \text{mg.} \times \frac{E^{1\%}_{1\,\text{cm.}}\ \text{for dye} - E^{1\%}_{1\,\text{cm.}}\ \text{for sample}}{E^{1\%}_{1\,\text{cm.}}\ \text{for dye} - E^{1\%}_{1\,\text{cm.}}\ \text{for standard}}$$

When hydroquinone is employed in the basic method, $E^{1\%}_{1\,\text{cm}}$ for sample in this expression is replaced by ($E^{1\%}_{1\,\text{cm.}}$ for sample − $E^{1\%}_{1\,\text{cm.}}$ for sample

bleached with hydroquinone). In procedure C the amount of ascorbic acid is equal to

$$0{\cdot}1 \text{ mg.} \times \frac{E^{1\%}_{1\,cm.} \text{ for tube 2} - E^{1\%}_{1\,cm.} \text{ for tube 1}}{E^{1\%}_{1\,cm.} \text{ for dye} - E^{1\%}_{1\,cm.} \text{ for standard.}}$$

F. A. R.

Organic

Separation and Identification of the Volatile Saturated Fatty Acids (C_1 to C_4). L. L. Rawsey and W. I. Patterson (*J. Assoc. Off. Agr. Chem.*, **1945, 28,** 644–656)—The method is an adaptation of the "partition chromatography" of Martin and Synge (*Biochem. J.*, 1941, **35,** 1358; ANALYST, 1942, **67,** 210) and, within the limits of confirmatory tests, complete separation of the lower fatty acids, *viz.*, formic, acetic, propionic and butyric, is achieved, even when one or more may constitute only a small fraction of the total acids. The method described is limited to these four acids. The first three, having no isomers, are obtained pure, but butyric and isobutyric acids are not separated on the column. For material high in protein distil 50 g. of comminuted sample with 150 ml. of water and enough diluted sulphuric acid (1 + 1) to make the liquid acid to Congo red paper. Collect 300 ml. of distillate, neutralise it with $N/10$ sodium hydroxide, evaporate to less than 5 ml. and complete the evaporation in a test tube with the aid of a current of air. To the cold residue add a few glass beads and enough dil. sulphuric acid to liberate the acids, avoiding a large excess and wetting all the solid salt by rotating the beads in the tube. Add enough anhydrous sodium sulphate to make the contents of the tube semi-solid, add 1 ml. of 1% butanol in chloroform and again ensure thorough wetting of the material. Transfer the chloroform by means of an eye-dropper pipette to the surface of the silicic acid column (*infra*), avoiding any loosening of the surface. Rinse the tube twice more with 1 ml. of the butanol - chloroform mixture, shaking the tube vigorously during the final rinsing. For material of high carbohydrate content an extraction method is suitable, and material containing no insoluble solids may be extracted directly. For samples containing insoluble solids comminute 250 g. mixed with an equal wt. of water in a Waring blendor. Centrifuge and acidify 200 ml. of the supernatant liquid with dil. sulphuric acid to Congo red paper and extract with ether in a continuous extractor for 2 hr. Wash the ethereal extract with 30 ml. of water containing enough sodium hydroxide to neutralise the acids, transfer the aq. layer to a distillation flask, add some glass beads, acidify the liquid to Congo red and distil rapidly until the residue measures 5 ml. or less. Neutralise the distillate and follow the procedure already described.

To prepare a small column for amounts of acid up to 2 ml. of $N/10$ use a tube of diam. 11–13 mm. and length 30 cm. with the constricted end drawn out to 5 or 6 mm. diam. To 5 g. of commercial silicic acid add 1 ml. of the R–NH_4 indicator (50 mg. of 3:6-disulpho-β-naphthalene-azo-N-phenyl-α-naphthylamine in 25 ml. of water) or bromocresol green indicator (100 mg. of dye in 25 ml. of water and 1·5 ml. of $N/10$ ammonia) and enough N ammonia to change the dye to its alkaline form. Add some water (the optimum amount must be determined for each batch of silicic acid) mix well in a mortar and add enough of the 1% butanol in chloroform solvent to make a paste and then 20–30 ml. until the mixture pours readily. Pour the suspension slowly into the sloped tube, the lower end of which is closed with cottonwool, and apply pressure by means of compressed air to the upper end of the tube so that the solvent is forced through the column drop by drop. When the column becomes so viscous that it will no longer pour, it is ready for use, but care must be taken that the column does not dry out below the surface. After draining the third butanol - chloroform extract obtained in the preparation of the acids for separation (*supra*) into the gel, fill the tube above the gel with solvent and apply pressure. As the solvent percolates through the column it carries the acids with it, the higher members of the series moving the faster. The column changes from its alkaline to its acid colour in the section containing acid. A mixture of formic, acetic, propionic and butyric acids first forms one blue band (R–NH_4 indicator) at the top. Gradually this band separates into 4 distinct bands representing each of the acids in the order given, reading from the top. Collect each band as it moves through the column. After the band tentatively identified as butyric acid (by comparison with a mixture of the four acids passing through another column) has moved through, change the washing solvent to 10% butanol in chloroform to increase the rate of movement of the propionic and acetic acid bands. Formic acid is not readily removed by this solvent. After separation of the other acids withdraw the column from the tube, place the top portion containing the formic acid in a small flask, add a little water and excess of alkali, stir the mixture, separate the chloroform and filter the aq. layer. Wash the gel with two small portions of water and aerate the filtrate to remove dissolved chloroform. Distil the acidified filtrate almost to dryness and titrate the distillate with $N/10$ or $N/100$ alkali. Large columns for amounts of acid ranging from 2 to 6 ml. $N/10$ should be made with a tube of diam. 22–25 mm. and length 15–20 cm., and the column should be supported upon two perforated porcelain or glass plates enclosing a pad of cottonwool. For microscopical identification of the acids the sodium salts must be practically free from soluble impurities such as excess of alkali or indicator. Add 10–25 ml. of water to the percolates of each band and titrate the acids with standard alkali to phenolphthalein, correcting the titration by means of a blank determination. Separate each of the titrated percolates from the solvent and remove chloroform by aeration. Distil each fraction, including the formic acid fraction, with dil. sulphuric acid almost to dryness as described for formic acid. To each distillate add 90% of its titre of silica-free sodium hydroxide without indicator, evaporate the neutralised liquids to dryness on the steam bath and add enough water to give the concn. required for the microscopical tests.

Formic acid is identified by adding a drop of the soln. ($N/10$) to fragments of cerous nitrate on a microscope slide, allowing the mixture to dry and comparing the crystals with those of cerous formate prepared in the same way. As a confirmatory test reduce 5% mercuric chloride solution with the acidified soln. on the steam bath. To identify acetic acid add a drop of the soln. ($N/10$) to fragments of mercurous nitrate, allowing initially only the edge of the drop to make contact with the solid. Compare the preparation microscopically with mercurous acetate prepared in the same way. Propionic acid is identified in the same manner. Examination of the mercurous salts of butyric and isobutyric acids fails to identify each individually, the crystals being similar for either salt or the

mixture. Make the remainder of the soln. up to 5 or 10 ml. with water, evaporate half of the soln. to dryness and heat the residue with 5 ml. of conc. sulphuric acid at 200° C. for 1 hr. Dilute the cold product to 60 ml. and distil, collecting 50 ml. of distillate. Titrate the distillate, acidify with N sulphuric acid re-distil, and add 90% of its titre of sodium hydroxide to the distillate and evaporate to dryness. Prepare and examine the mercurous salt, which should now be that of *n*-butyric acid. To the remaining half of the soln. add 0·5 ml. of dil. sulphuric acid, 5 ml. of 4% potassium permanganate soln. a few mg. of silver sulphate, some glass beads and enough water to make 20 ml. Boil gently under reflux for 10 min., cool, rinse down the condenser and distil, collecting 5 to 10 ml. of distillate under 10 ml. of water. Dilute with water to 25 ml. To an 8 ml. aliquot in a 25 ml. stoppered cylinder add 8 ml. of 40% sodium hydroxide soln. and 1 ml. of a soln. of 1·6 ml. of salicylaldehyde in 25 ml. of abs. alcohol, heat in a water-bath at 55° C. for 30 min. and compare the red colour with that developed by the same amount of *n*-butyric acid oxidised and otherwise treated in the same manner. When the colour, which is due to reaction between acetone (formed by oxidation of isobutyric acid) and salicylaldehyde, exceeds that given by *n*-butyric acid, isobutyric acid is present. The comparison expt. with *n*-butyric acid is necessary because this acid yields a small amount of acetone by oxidation, probably owing to presence of traces of isobutyric acid.

The separation of the four acids fails in presence of sulphur dioxide, which therefore must be removed from the sample. For a laboratory method of preparing silica gel for the columns the original paper should be consulted. A. O. J.

Inorganic

Determination of Sodium and Potassium in Silicates. G. G. Marvin and L. B. Woolaver (*Ind. Eng. Chem., Anal. Ed.*, 1945, **17**, 554–556)—A method based on the decomposition of the sample with hydrofluoric and perchloric acids is claimed to retain most of the advantages of the Berzelius and J. Lawrence Smith methods and to eliminate their difficulties. The procedure described yields the pure mixed alkali chlorides; for the separation of the alkalies the method of G. F. Smith (*J. Amer. Chem. Soc.*, 1925, **47**, 762) is suggested. Good results have been obtained with synthetic solns. containing sodium, potassium, calcium, iron, aluminium, magnesium, titanium, manganese, lead, barium and arsenic, and on Bureau of Standards samples of potash and soda feldspars and lead, barium, high boron, opal and soda-lime glasses. Phosphorus and sulphate must be absent.

Weigh 1 g. of sample into a flat-bottom platinum dish and add 15 ml. of 60–70% perchloric acid and 10 ml. of 47% hydrofluoric acid. Heat very cautiously on a hot plate until the vol. is substantially reduced. Place under a heating unit suspended over a hot plate and heat until copious fumes of perchloric acid are evolved. When nearly dry, remove, add 5 ml. of perchloric acid and again evaporate almost to dryness. Wash into a 250 ml. tall-form beaker and evaporate to dryness. Cover and place in a muffle furnace at 550° C. for 30 min. Alkali and calcium perchlorates are converted to chlorides, iron, aluminium and magnesium perchlorates to oxides. Cool, crush the residue and extract it with two successive portions of 50 ml. of hot water each containing 5 ml. of ammonia soln. (sp.gr. 0·90) and follow with two 10 ml. portions. Filter, heat the filtrates nearly to boiling, add 10 ml. of 0·5 N ammonium oxalate, leave for 1 hour and filter. Heat the filtrate to 60° C., add 5 drops of a 5% soln. of 8-hydroxyquinoline in diluted acetic acid (1+10) and leave for 1 hour. Filter, acidify the filtrate with hydrochloric acid and evaporate to dryness. (The two pptns. might be combined, but this has not been tried.) Treat the residue with 25 ml. of conc. hydrochloric acid and 5 ml. of conc. nitric acid and boil vigorously. Add a further 25 ml. of hydrochloric acid, boil and finally evaporate to dryness. Heat the beaker cautiously over a flame to remove any remaining ammonium salts or organic matter. Dissolve the residue in water, filter into a weighed 100 ml. beaker, add a few ml. of conc. hydrochloric acid and evaporate to dryness. Heat cautiously, cool and re-weigh. L. A. D.

Rapid Determination of Alkalies in Portland Cement. J. J. S. Cornes (*New Zealand Journal of Science and Technology*, 1945, **26**, 239–242)—As alkalies have an important influence on the deterioration of concrete (Stanton, *Proc. Amer. Soc. Civil Eng.*, 1940, **66**, 1781) rapid methods for their accurate determination in cement are desirable. The full procedure described takes 10 to 12 hours and comprises dissolving the samples in acid, removing silica and then iron and alumina, finishing by the gravimetric determination of sodium as magnesium uranyl acetate on one portion and the volumetric determination of potassium as cobaltinitrite on another. In a less accurate modification taking about 8 hours the silica, iron and alumina are removed in one operation.

Method—(*a*) *Preparation of sample* (*full method*)—Weigh each separate 1 g. portion into a 100 ml. basin, preferably of platinum, stir in 10–15 ml. of cold water and then add 5 ml. of conc. hydrochloric acid. Evaporate to dryness on a water-bath and heat for 30 min. at 105–110° C. Dissolve in 20 ml. of hot diluted hydrochloric acid (1+3), filter and wash the ppt. thoroughly with small amounts of hot water. Return the filtrate to the cleaned basin and again evaporate to dryness and heat for 30 min. at 105–110° C. (*b*) *Sodium*—To one dried residue add 20 ml. of hot water and then gradually stir in 1 g. of alkali-free calcium carbonate. Boil, add 2 ml. of barium chloride soln. (5%), boil for 1–2 min. and filter into a 150 ml. Pyrex basin. Wash the ppt. until the washings contain only a trace of chloride. Add 2 drops of conc. hydrochloric acid to the filtrate and evaporate to dryness. Determine sodium gravimetrically by Kahane's method (C. S. Piper, "*Soil and Plant Analysis*," Univ. of Adelaide, 1942, pp. 161, 176; Review, ANALYST, 1942, **68**, 230). (*c*) *Potassium*—To a second residue add 20 ml. of hot water and 1 g. of calcium or sodium carbonate (free from potassium), boil and filter and wash as for sodium. Evaporate the filtrate to dryness, dissolve the residue in 3–4 ml. of water and determine potassium by Piper's method (*J. Soc. Chem. Ind.*, 1934, **53**, 392T), titrating with ceric sulphate rather than potassium permanganate (Cornes, ANALYST, 1944, **69**, 239–240). Comparison with results obtained by the Lawrence Smith method shows that the sodium values agree, while the potassium values are usually about 0·02% higher by the new method. (*d*) *Shortened method*—Dissolve the samples as under (*a*) above, evaporate the solution and dry the residue. Omit the further preparatory steps and treat the residues as under (*b*) and (*c*), silica, iron and alumina being removed

in one filtration. Attention is drawn to the looseness of the term "total alkali" as applied to cement, and it is suggested that the alkali should be expresse d as total equivalent soda in research work on interaction between cement and aggregate. L. A. D.

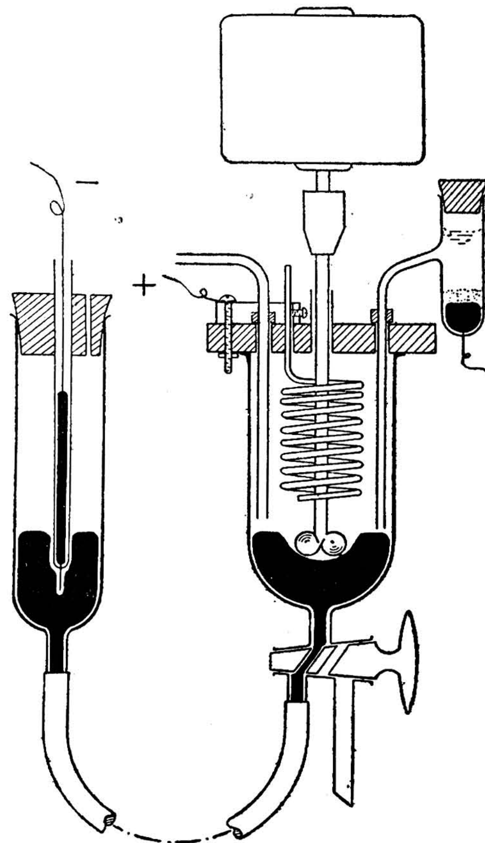
Physical Methods, Apparatus, etc.

The Polarography of Uranium. I. Reduction in Moderately Acid Solutions. W. E. Harris and I. M. Kolthoff (*J. Amer. Chem. Soc.*, 1945, **67**, 1484–1490)—In solns. which are 0·01 to 0·2 M with respect to hydrochloric acid, uranyl chloride gives two waves. These have half wave potentials of −0·18 (slightly affected by acidity) and −0·92 v. respectively (all potentials are versus the saturated calomel electrode); they correspond with the reductions U^{6+} to U^{5+} and U^{5+} to U^{3+}, respectively. For the determination of uranium the diffusion current of the first wave is measured. Though the total diffusion current obtained at −1·2 v. is three times greater, more substances may interfere at this potential and measurement is difficult owing to the proximity of the hydrogen wave. Uranous sulphate gives a single wave having the same half wave potential as the second of the uranyl waves. Up to at least +0·4 v. uranous sulphate gives no anodic wave.

Method.—Measure a suitable volume of unknown uranium soln. (pH 2 to 3; in solns. too acid or too basic, the diffusion current is not proportional to the concentration). Add 10 ml. of stock soln. (0·5 M in potassium chloride and 0·05 M in hydrochloric acid) and about 0·05 ml. of 0·1% thymol soln. (caffeine is alternative and probably preferable to thymol). Dilute to 50 ml., so that the final uranium concentration is between 1×10^{-4} and 5×10^{-3} M. Transfer to a polarographic cell, remove dissolved air by bubbling with nitrogen, and measure the apparent diffusion current at −0·5 v. Subtract the residual current found for a soln. containing no uranium. The corrected diffusion current is proportional to the concentration of uranium; the proportionality-constant is found by using a known uranium soln. Interference by ferric iron may be prevented by adding excess (up to 2 M final concentration) of hydroxylamine hydrochloride and warming to 50° C. for 10 min. Since numerous organic compounds change both diffusion current and half wave potential, ashing should be carried out if the sample contains organic matter. Phosphate interferes and must be removed.

Determination of very small amounts of uranium—Uranyl or uranous uranium catalyses the reduction of nitrate (Kolthoff, Harris and Matsuyama, *J. Amer. Chem. Soc.*, 1944, **66**, 1782; Abst., ANALYST, **1945, 70,** 101); this effect permits the determination of traces (10^{-6} to 3×10^{-5} M) of uranium. The waves obtained under the following conditions have no region of constant diffusion current, but are about 100 times greater than those obtained by the previous method. Measure a suitable vol. of unknown uranium soln. and add 10 ml. of a stock soln. which is 0·005 M in potassium nitrate, 0·5 M in potassium chloride, and 0·05 M in hydrochloric acid. Dilute to 50 ml. and measure the apparent diffusion current at −1·2 v. after removing dissolved air. Subtract the residual current; the corrected diffusion current is *not* strictly proportional to the uranium concentration, hence a calibration curve should be constructed from a series of known uranium solns. Interferences are, in general, the same as in the polarographic determination of nitrate (Kolthoff, Harris and Matsuyama, *loc. cit.*). J. T. S.

Coulometric Analysis. J. J. Lingane (*J. Amer. Chem. Soc.*, 1945, **67**, 1916–1922)—The quantity of electricity necessary for the quantitative reaction (*e.g.*, reduction) of the substance to be determined is measured. A stirred mercury cathode, the potential of which is controlled at 0·1 to 0·2 v. more negative than the polarographic half wave potential of the substance to be determined, is used.



Polarography is thus a convenient pilot technique for establishing the optimum conditions for a given analysis. Throughout the analysis the constant cathode potential is maintained either manually or by an automatic controller (Lingane, *Ind. Eng. Chem., Anal. Ed.*, 1945, **17**, 332). The electrolysis cell, which holds about 100 ml. of soln., has a cathode area of about 30 sq. cm. The anode is a closely-wound helix, 4 cm. long and 3 cm. diam., of No. 10 gauge silver wire (total surface about 80 sq. cm.). A propeller-stirrer is immersed in the mercury - solution interface (very efficient stirring is important), while a second pair of blades stirs the soln. The cathode potential is measured by a saturated calomel electrode connected by a 4-mm. bridge tube filled with 3 to 4% agar gel saturated with potassium chloride. When the cathode is stirred it just touches the tip of the bridge tube. Throughout the determination nitrogen enters through the inlet tube and escapes around the

stirrer shaft. To measure the quantity of electricity a hydrogen - oxygen coulometer with bright platinum electrodes about 1·7 sq. cm. in area is connected in series with the cell. The gas evolved is measured by the vol. of confining soln. displaced into an adjoining burette. The electrolysis tube of the coulometer is about 40 cm. long and permits about 100 ml. of gas (corresponding to about 500 coulombs) to be collected. The coulometer is filled with 0·5 *M* potassium sulphate, which must be completely free from reducible impurities, especially those capable of cyclic oxidation and reduction (*e.g.*, iron salts). As shown by Lehfeldt (*Phil. Mag.*, 1908, **15**, (6), 614), sodium and potassium hydroxide solns. give results which are 1 to 2% low. Immediately before use the confining soln. must be saturated with gas by passing a current of 50 to 100 ma. for at least 5 min.

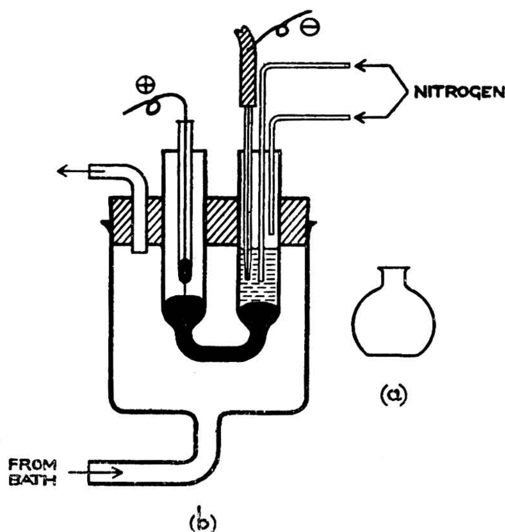
Procedure—Place in the cell 50 to 90 ml. of a suitable supporting electrolyte soln. After passing in nitrogen for about 5 min., introduce the cathode mercury by opening the stopcock and raising the reservoir. Apply an e.m.f. such that the potential of the cathode is 0·3 to 0·4 v. more negative than that used in the determination and leave until the current becomes negligible (this removes traces of reducible impurities; ordinarily, the current decreases to about 1 ma. after about 10 min.). Without disconnecting, reduce the cathode potential to the desired value and read the coulometer burette. Add to the cell 10 to 50 ml. of the test soln., readjust the cathode potential and allow the electrolysis to proceed until the current has fallen to 1 ma. or less. The time required is usually 40 to 60 min. Read the coulometer burette and correct the vol. of gas to S.T.P., allowing for the vapour pressure of the confining soln. Calculate the result by using an appropriate factor. Using a supporting soln. which was 0·4 *M* in sodium tartrate, 0·1 *M* in sodium hydrogen tartrate and 0·1 to 0·3 *M* in sodium chloride with a cathode potential of −0·24 ± 0·02 v. (all potentials are versus the saturated calomel electrode), quantities of copper from 6·2 to 73·7 mg. were determined with an aver. error of ±0·3 mg. (1 mg. of copper ≡ 0·5285 ml. of gas at S.T.P.). With a cathode potential of −0·35 ± 0·02 v. and the same supporting soln., 13·2 to 105·3 mg. of bismuth were determined with an aver. error of ±0·7 mg. (1 mg. of bismuth ≡ 0·2411 ml. of gas). In a 0·5 *M* potassium chloride supporting soln. with a cathode potential of −0·50 ± 0·02 v., 41·3 to 207·2 mg. of lead were determined with an aver. error of ±0·9 mg. (1 mg. of lead ≡ 0·1622 ml. of gas). Addition of small or large amounts of cadmium did not affect the accuracy; hence the sharp separation and successive determination of metals whose reduction potentials differ by only 0·20 v. are relatively easy. The time required is practically independent of the concentration of the metal being determined. Theoretically, an infinite time is required; this is the chief limitation to the accuracy. However, the electrolysis is more than 99% complete when the current has fallen to 1% of its initial value; an equation is given from which the extent of reduction after any given time may be computed. For determining reduction states, *e.g.*, of organic compounds, coulometric technique is reliable. Thus, in a supporting soln. which is 0·4 *M* in potassium chloride and 0·1 *M* in hydrochloric acid, picric acid requires 17 electrons per mol., whereas complete reduction of the 3 nitro-groups to amino-groups would require 18 electrons. Probably a hydrazine derivative is formed.

J. T. S.

Determination of Copper in Plant Materials. **J. F. Reed and R. W. Cummings** (*Ind. Eng. Chem., Anal. Ed.*, 1941, **13**, 124–127)—By precipitating interfering elements by addition of a slight excess of ammonia, copper may be determined polarographically in presence of all constituents normally present in plant ash. The limits of the method are from 0·2 to 0·0002% on a 1 g. sample. *Procedure*—Weigh 0·5 to 2·0 g. of the sample into a 30-ml. micro-Kjeldahl flask. Add 5 ml. of conc. nitric acid, and heat until brown fumes are evolved. Add 1 ml. of conc. sulphuric acid and heat until charring begins and all nitric acid is eliminated. Add 1 to 2 ml. of 60% perchloric acid and heat until colourless or pale yellow, excess perchloric acid being driven off. Dilute to 15 to 20 ml., heat to boiling, and add a slight excess of ammonia. Boil for 1 min., filter, wash with faintly ammoniacal water and evaporate the filtrate to dryness. Take up the residue in 9 ml. of a soln. made by mixing equal vols. of 0·5 *M* sodium hydroxide and 0·5 *M* citric acid soln. and add 1 ml. of a 0·05% soln. of acid fuchsin, $C_{20}H_{17}N_3(SO_2ONa)_2$. Transfer to a polarographic cell, remove dissolved oxygen by bubbling nitrogen through the soln., polarograph and measure the height of the copper wave (the half wave potential is about −0·15 v. versus S.C.E.). Deduce the copper concn. by comparing with the results obtained by adding known amounts of copper to portions of the acid citrate soln. A blank should also be performed.

J. T. S.

Determination of Copper in Copper Proteins. **S. R. Ames and C. E. Dawson** (*Ind. Eng. Chem., Anal. Ed.*, 1945, **17**, 249–253)—By treatment with citric acid soln. copper may be quantitatively extracted from copper proteins. It is not adsorbed to the residual protein when the *p*H is subsequently raised to 4 by addition of sodium hydroxide.



Hence the supporting soln. recommended by Reed and Cummings (*Ind. Eng. Chem., Anal. Ed.*, 1941, **13**, 124; see preceding abstract) may be used for the determination of copper in copper proteins without the need for wet-ashing. *Procedure*—Use copper-free redistilled water throughout. Dialyse the

protein soln. against repeated changes of water for 50 to 100 hr. Transfer a portion containing 1 to 100 μg. of copper to a tared 5-ml. Pyrex bulb (*a*) and evaporate to constant "dry weight" at 110° C. Add a measured vol. (usually 0·5 ml.) of 0·5 *M* citric acid, insert the neck of the bulb into a gum rubber sleeve attached to a motor set at 60° to the horizontal, and rotate at 60 to 120 r.p.m. for about 30 min. (longer if the dry protein exceeds 3 mg.). Add a vol. of 0·5 *M* sodium hydroxide equal to that of the citric acid soln. and mix by rotation for 10 to 15 min. If a large amount of ppt. appears filter through a sintered glass funnel. Transfer an aliquot (usually 0·5 ml.) to the special polarographic cell (*b*), add a measured vol. (about 10% of that of the aliquot) of 0·05% acid fuchsin soln. and about 0·01 ml. of heptyl alcohol. Maintain at 25° C., remove dissolved oxygen by bubbling wet nitrogen through the soln. and measure the limiting current (the half wave potential is −0·31 v. versus the mercury pool, or −0·18 v. versus the S.C.E. A manual apparatus, the Fisher Electropode, was used.) Subtract the residual current of the supporting soln. and calculate the result from the current-concn. relationship obtained by using known amounts of copper. The limits of the method are from 1 to 100 μg. of copper per ml. of supporting soln., and the average deviation ±3%. Though ferric iron interferes, no interference occurred in an expt. in which haemoglobin was added to the supporting soln. This indicates that no free iron is liberated at *p*H 4. J. T. S.

Polarographic Determination of Iron and Zinc in Phosphate Coatings. J. Knanishu and T. Rice (*Ind. Eng. Chem., Anal. Ed.*, 1945, **17**, 444–446)—The simultaneous determination of iron and zinc in commercial phosphate coatings on ferrous articles may be carried out upon a few mg. of coating scraped from the surface. By removing the coating from a unit area different coatings may be compared. *Procedure*—Carefully scrape the coating from the unoiled surface. Dissolve 4 mg. in 20 ml. of 0·75 *M* oxalic acid, add 2 drops of methyl red and neutralise by dropwise addition of conc. ammonia. Add 2 drops of ammonia in excess and dilute to 50 ml. Transfer a 5-ml. aliquot to a polarographic cell and add 1 drop of 0·05% glue soln. Remove dissolved oxygen by bubbling nitrogen through the soln. for 7 min., and polarograph over the range 0 to −1·6 v. (A Leeds and Northrup Electro-Chemograph, operated at max. sensitivity was used. The cell had a mercury pool anode and the capillary a drop-time of 6 sec. The temp. was 77° F.) Measure the heights of the iron and zinc waves, the half wave potentials of which are about −0·3 and about −1·4 v. respectively. Read off the concns. of the two metals from (linear) calibration curves prepared by adding known amounts of standard iron and zinc solns. respectively to 20-ml. portions of oxalic acid soln. and proceeding as above. The method was applied to the examination of machine gun links. No comparison of the results with those of other methods has yet been made. J. T. S.

Reviews

COOKING AND NUTRITIVE VALUE. By A. BARBARA CALLOW. Pp. 156. London: Oxford University Press. 1945. Price 7s. 6d.

Investigations into the nutritional value of foodstuffs have been given a special impetus during the last few years, and a mass of valuable information has been accumulated. The main difficulty now lies in the transmission of this and earlier-acquired knowledge to those who so urgently need it—the housewife, the dietitian, the canteen manager, the doctor and the food manufacturer. Mrs. Callow has done much to meet this difficulty in her latest book. The most outstanding findings of workers engaged in the field of nutrition are presented in an easy style, but a high degree of accuracy and thoroughness has been maintained throughout. Some errors have been found which will undoubtedly be corrected in subsequent editions.

After a preliminary discussion on the nutritive value of foods, there are separate chapters on meat, poultry and game; fruits and nuts; milk, butter and cheese; eggs and cereals. In each instance the composition of the untreated food is dealt with and then follows a description of the effect of various methods of preparation on the nutritive value. The title of the book is somewhat misleading, as many processing operations such as canning, dehydration and freezing are considered in addition to the more familiar household cooking.

The analyst will find in the 21 tables a useful collection of data for rapid reference, although no details of analytical methods are given. The literature list deals only with the more fundamental publications in the field and is obviously not intended to be more than an introduction to the various subjects discussed. The print and general set-up are of the high standard to be expected from the Oxford University Press. The price is moderate for so comprehensive a publication, and it is recommended that this book should be owned by all those interested in nutrition. M. OLLIVER

MANUAL OF NUTRITION, 1945. Ministry of Food Scientific Adviser's Division. Pp. iv+64. London: H.M. Stationery Office. 1945. Price 1s.

Dr. Magnus Pyke is known to analysts for many publications, in this journal and in others, connected with the estimation of vitamins in foods. When he left his position in

industry at the beginning of the war to take up a post under Sir Jack Drummond at the Ministry of Food, he clearly found it necessary to widen his knowledge of nutrition in general and to leave behind him the technical minutiae of titrations with dichlorophenolindophenol, fluorimetry of thiochrome and riboflavine, and chromatography of carotenoids. His appearance from time to time at scientific meetings has shown him to be a perspicacious student of food patterns in various parts of the country and a racy recorder of the varied and frequently entertaining experiences that have fallen to his lot as an itinerant investigator for the Ministry.

It is clear that in the course of his work Dr. Pyke has been convinced of the need for writing some kind of general instruction on nutritional principles that might make the large-scale caterer and the individual housewife better able to conserve the nutritive value of the foods they handle. He has therefore written this little book which has been published, with the blessing of the Ministry of Food, at a very modest price. It is just about the best and completest statement of the main and most easily grasped facts of modern nutritional knowledge that has so far come my way. It can be read with profit by everybody concerned in any way with nutrition—the student of domestic science, the Professor of Physiology, the *maître d'hôtel*, the Public Analyst, and even the other analytical chemist! This manual is characterised throughout by that extreme clarity of presentation associated in the minds of many with Dr. Pyke in person. Apart from being thoroughly readable, the manual contains a mass of facts that one is delighted to have collected together so compactly between two covers.

A. L. BACHARACH

STUDIES ON THE NATURE OF THE BROMATE EFFECT. By HOLGER JØRGENSEN. Pp. 435. Copenhagen: Einar Munksgaard; London: Humphrey Milford, Oxford University Press. Price, Dan. Cr. 40.

We fear that many readers will not gather from the title of this well printed and comparatively large volume that the book only deals with the explanation of why the addition of minute amounts of certain chemicals to flour may bring about considerable improvement in baking strength. In other words, it deals exhaustively—and some may consider unnecessarily exhaustively—with one small aspect of the chemistry of the baking of wheaten bread. It is true that potassium bromate is one of the principal chemicals used as an "improver,"—as such chemicals are termed in the flour milling industry,—and, when added to flour at the rate of one part in 100,000, enhances the baking value, but, if the theory of the action which is so strenuously argued in the book is correct, then the theory is one of more general application and should also account for the improving action of other improvers, such as nitrogen trichloride. For these reasons we feel that the title of the book is not well chosen, although it is admitted that most of the experiments in this restricted field of work have dealt with bromate.

The reason why traces of bromate, persulphate, ascorbic acid, chlorine, chlorine dioxide, nitrogen trichloride and other chemicals act as improvers has not been understood until comparatively recently, and it is now generally agreed that the theory first put forward by Jørgensen in 1935 offers a reasonable explanation of this curious but important phenomenon. The theory advanced by the Ministry of Health Departmental Committee on Improvers in 1927, that improvement was related to change in *p*H, is no longer acceptable and the simple theory that beneficial colloidal changes in the protein take place owing to coagulation no longer holds. The present theory is that such substances act by partial suppression of the proteinase activity of the flour, either directly or by inactivation of substances such as glutathione which activate the proteinases. Jørgensen's theory, based on numerous experiments, seized the imagination of cereal chemists and his point of view received confirmation by Balls and Hale, who independently came to a similar conclusion a year later. Although other workers such as Elion supported this theory, criticism came from a number of workers such as Freilich and Frey, Read and Haas in America and Bungenberg de Jong and Ford and Maiden in Europe. The books deals in detail with some twenty-one papers on the subject, and it must be granted deals with the subject in a painstaking and convincing way. Although we would not be prepared to agree with everything the author says or to subscribe to the statement that the Jørgensen theory is correct in every detail, yet it must be stated that he has made out an excellent case and dealt with his critics in a masterly manner. It can now scarcely be doubted that his theory is substantially correct even if it may not always account for all the known phenomena.

The translation is good, the subject matter is clear and the book well printed. Yet it must be emphasised that the book is of a very restricted character and indeed shows considerable signs of "padding" to occupy, as it does, 435 pages. It is not surprising, therefore, to find that it is in fact a translation from the Danish of Dr. Jørgensen's doctorial thesis entitled "Studier over Bromatvirkningens Natur," the translation into English and German being financed by the Research Foundation of Dansk Gaerings-Industri Ltd., in whose laboratories the work was carried out. Always bearing in mind the general criticism as to the restricted nature of the book, it can be recommended to those cereal chemists who wish to have at their disposal a very full and able account of this branch of cereal chemistry.

D. W. Kent-Jones

BIOLOGIGAL METHODS GROUP

The Annual General Meeting of the Group will be held in the Rooms of The Chemical Society, Burlington House, London, W.1, on Monday, February 25th, 1946, at 6 p.m.

It will be followed, at 6.30, by an Ordinary Meeting of the Group at which the following papers will be read.

"The Transformation of Metameters, with special reference to Vitamin D Assays," by N. T. Gridgeman, B.Sc.

"Some Remarks on the Statistical Background of Bio-Assays," by E. C. Fieller, M.A.

The Annual General Meeting will be confined to members of the Group but guests will be welcomed at the Ordinary Meeting.

ADVICE TO AUTHORS

THE Council has approved the following notice by the Publication Committee, which is here given in condensed form.

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

(1) record proposals for new methods and the investigations on which the proposals are based;
(2) record the results of original investigations into known methods or improvements therein;
(3) record analytical results obtained by known methods where these results, by virtue of special circumstances, such as their range or the novelty of the materials examined, make a useful addition to analytical information;
(4) record the application of new apparatus and new devices in analytical technique and the interpretation of results.

Communications.—Papers (which should be sent to the Editor) will normally be submitted to at least one referee, on whose advice the Publication Committee will be guided as to the acceptance or rejection of any communication. Papers or Notes accepted by the Publication Committee may not be published elsewhere except by permission of the Committee.

Abstracts.—The MS. should be accompanied by a brief abstract of about 100 to 150 words indicating the scope and results of the investigation.

Proofs.—Proofs should be carefully checked and returned within 48 hours. Two galley proofs* will normally be sent out, one of which should be retained by the Author.

Reprints.—Ten Reprints are supplied gratis to the Author. Additional reprints may be obtained at cost if the Author orders them directly from the printers, W. Heffer & Sons Ltd., 104, Hills Road, Cambridge, at the time of publication. Details are sent to Authors with the proofs.

Notes on the writing of papers for THE ANALYST

Manuscript.—Papers and Notes should preferably be typewritten.

The title should be descriptive and should set out clearly the scope of the paper. Degrees are now omitted after the names of Authors in the headings of papers.

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

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

(*a*) General, including historical, introduction.
(*b*) Statement of object of investigation.
(*c*) Description of methods used. Working details of methods are usually most concisely and clearly given in the imperative mood, and should be given in this form, at least while economy of paper is pressing, *e.g.*, "Dissolve 1 g. in 10 ml. of water and add . . .". Well-known procedures must not be described in detail.
(*d*) Presentation of results.
(*e*) Discussion of results.
(*f*) Conclusions.

ollowed by a short summary (100 to 250 words) of the whole paper: items (*e*) and (*f*) can often be combined.

Illustrations, diagrams, etc.—The cost of setting up tabular matter is high and columns should therefore be as few as possible. Column headings should be brief or replaced by a number or letter to be used in combination with an explanatory footnote to the table.

Sketches or diagrams should be on white Bristol board, not larger than foolscap size, in Indian ink. Lettering should be in light pencil.

Tables or graphs may be used, but normally not both for the same set of results. Graphs should have the co-ordinate lines clearly drawn in black ink.

References.—References should be numbered serially in the text and collected in that order under "REFERENCES" at the end of the paper. They should be given in the following form:

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

Notes on the Presentation of Papers before Meetings of the Society are appended to the "ADVICE," copies of which may be obtained on application to the Secretary, 7/8, Idol Lane, London, E.C.3

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* During the paper shortage two copies of the MS. will not be insisted on, nor will two galley proofs be sent.

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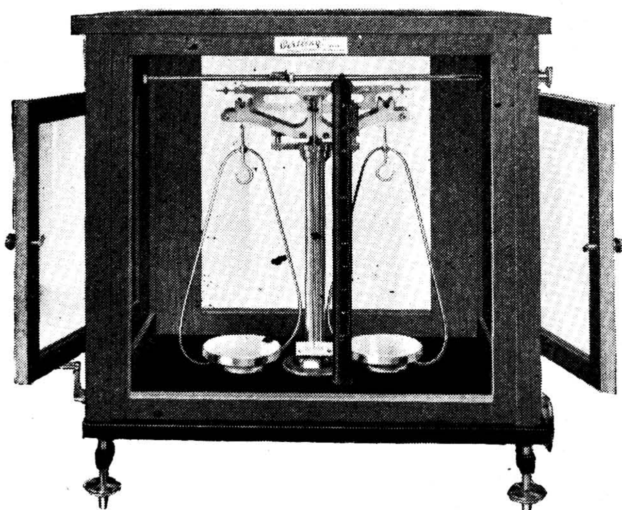

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Printed for the Society of Public Analysts and other Analytical Chemists by W. Heffer & Sons Ltd., Hills Road, Cambridge. Communications to be addressed to the Editor, J. H. Lane, 7-8, Idol Lane, London, E.C.3.