THE ANALYST

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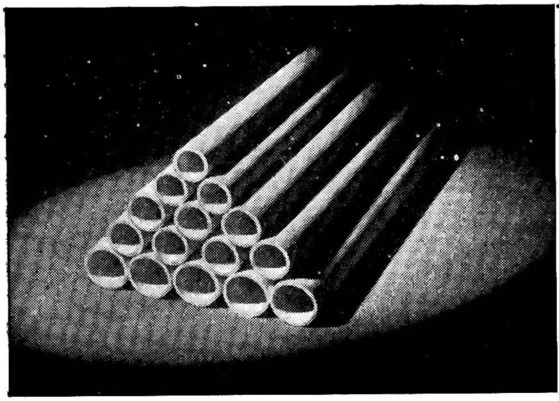

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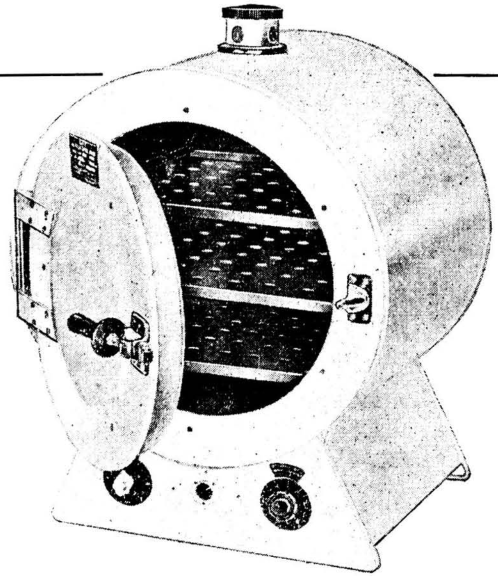

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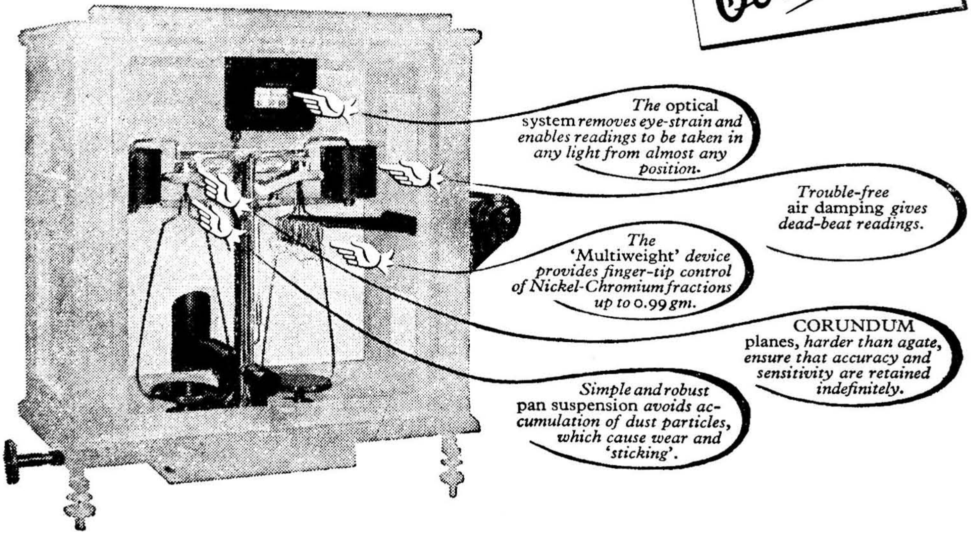

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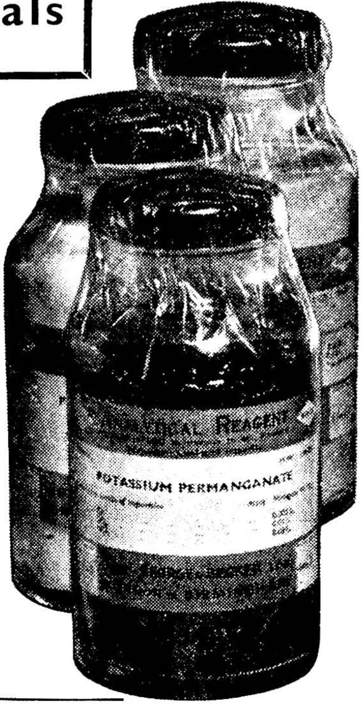

JUNE, 1950 Vol. 75, No. 891

THE ANALYST

PROCEEDINGS OF THE SOCIETY OF PUBLIC ANALYSTS AND OTHER ANALYTICAL CHEMISTS

AN Ordinary Meeting of the Society was held at 7 p.m. on Wednesday, April 5th, 1950, in the Meeting Room of the Chemical Society, Burlington House, Piccadilly, London, W.1. The chair was taken by the President, Mr. George Taylor, O.B.E., F.R.I.C.

The meeting was devoted to "Bromine" and the following papers were presented and discussed: "The Determination of Bromine in Brine," by J. Haslam, M.Sc., F.R.I.C., and G. Moses, A.M.C.T., F.R.I.C.; "The Bromine Content of the Cheshire Salt Deposit and of Some Borehole and Other Brines," by E. C. Allberry, B.A., J. Haslam, M.Sc., F.R.I.C., and G. Moses, A.M.C.T., F.R.I.C.; "Survey of the In-shore Waters round the Coasts of Great Britain, with particular reference to the Bromine Content," by R. O. Gibson, D.Sc., F.R.I.C., and J. Haslam, M.Sc., F.R.I.C.; "The Analysis of Bromine and Compounds containing Bromine," by J. Haslam, M.Sc., F.R.I.C.

PHYSICAL METHODS GROUP

THE twenty-fourth Ordinary Meeting of the Group was held at 6 p.m. on Tuesday, January 3rd, 1950, in the Chemistry Lecture Theatre of Imperial College, London, S.W.7. Mr. B. S. Cooper, the Chairman of the Group, occupied the chair and about sixty members and visitors were present.

The following papers on Spectroscopy were presented and discussed: "The Determination of Strontium in Sea-Water, a Combination of Flame Photometry and Radiochemistry," by A. A. Smales, B.Sc., A.R.I.C.; "A Photo-electric Spectrophotometer for the Visible and Ultra-Violet Regions," by R. A. C. Isbell, A.Inst.P.; "The Application of the Uvispek Spectrophotometer to Biochemical Problems," by D. C. M. Adamson, F.R.I.C.

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Mercury Drop Control: Application to Derivative and Differential Polarography

BY L. AIREY AND A. A. SMALES

SYNOPSIS—Controlled disengagement of a mercury drop may be achieved either by the abrupt application of an electrical pulse to reduce the interfacial tension, or by a small lateral movement of the capillary, effectively "shearing" the drop from the mercury thread. The fundamentals of the two techniques are examined and it is concluded that the mechanical method possesses marked advantages.

The modification of a standard Cambridge polarograph to permit operation either normally or as a bridge circuit for multiple electrode work, as required, is described and illustrated.

The performance of the bridge circuit as used for both derivative and differential working is examined theoretically and practically. Illustrative examples of the utility of the two techniques are given. Possible future developments are briefly discussed and some observations on the Ilkovic equation are included.

DURING the past few years several investigators[1,2,3,4,5] have published details of bridge circuits incorporating two dropping-mercury electrodes. The general applicability of these methods, however, has been somewhat restricted by difficulties in the synchronisation of the drop rates. Heyrovsky[1] attempted to eliminate the objection by the use of streaming-jet mercury electrodes; these are fairly satisfactory in theory, but in practice are cumbersome. Rapidly dropping electrodes (approximately 1 sec.) have also been employed by the same author, with some success.

Lingane,[6] in his review of polarographic development, comments that some means of synchronising the drop rates in these circuits would be a useful improvement in technique. Muller[7] has observed that "knock off drops" have been made, but at present are in the category of laboratory toys. Sevcik[5] reports the use of electrical pulsing as a means of drop synchronisation in his work on cathode ray polarography.

This paper considers the problem of mercury drop control from three angles, *viz.* (*a*) fundamental principles governing the method, (*b*) the application of the technique to standard polarographic equipment, and (*c*) applications based upon non-standard apparatus.

FUNDAMENTALS OF DROP CONTROL

Two general methods may be envisaged for inducing the disengagement of a pendant mercury drop from a capillary tube. Either the surface tension may be abruptly reduced to a value which renders the drop unstable, or the shape of the drop may be changed to such an extent that a condition of instability is attained. The primary process of severance from the capillary thread is the result of surface tension forces: gravitational action removes the drop from the vicinity of the capillary tube. The reduction in surface tension required by the first method is obtained by a change of the electrical potential difference between the mercury and the solution: the distortion of the drop in the second method by a movement of the capillary tube. The two techniques are conveniently designated "electrostatic" and "electromechanical" respectively, and will be considered separately under those headings.

ELECTROSTATIC CONTROL—

The variation of the interfacial tension between a mercury drop and a salt solution theoretically provides a means of producing instability under any conditions. In practice, however, the extent to which the potential difference between the drop and the solution may be changed is determined largely by the presence and nature of the ions in solution. The mechanism of the process can be understood by reference to Fig. 1, which depicts the equivalent circuit of the cell assembly. If we assume an electrolyte of dilute hydrochloric acid, the passage of an electron current from B to A will charge C_2; R_3 is practically infinite. The potential of C_2 will build up to a value of about 1 volt, and then reduction of hydrogen ions will commence; R_3 now has an appreciable finite value, which will vary somewhat as a diffusion gradient is produced. To obtain any further potential change across C_2, the impressed current must be much increased because the shunting action of R_3 is disproportionately increased with an increase of applied potential.

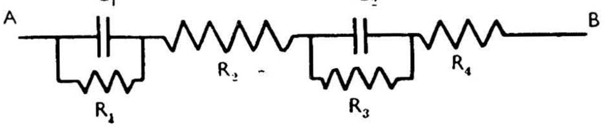


Fig. 1. Simple equivalent circuit of polarographic cell

C_1	Anode double layer capacitance
C_2	Mercury drop double layer capacitance
R_1	Anode leakage resistance
R_2	Cell electrolyte resistance
R_3	Mercury drop leakage resistance
R_4	Capillary thread resistance

The presence of such a large steady current in a cell would be highly objectionable, as it causes changes in local concentration, hydrogen evolution and heating effects, particularly in R_4. A practicable compromise is to discharge a condenser through the cell, giving a momentarily large current. A value of 0·1 microfarad at a potential of up to 250 volts has been found suitable when using a mercury pool anode. If R_2 is increased by the use of an agar bridge, greater voltages would be needed, with attendant heating effects.

Fig. 2 shows a series of instantaneous photomicrographs of a freely falling mercury drop. The salient fact is that during the last stages of its growth a drop assumes a pear shape, rupture occurs at the rapidly constricting neck and, after a few oscillations, a spherical shape is recovered within a few milliseconds. It is inferred that the elongated shape is an indispensable condition for detachment (assuming that reduction of interfacial tension nearly to zero is impossible). Hence, although a small abrupt change of potential during the last 10 per cent. or so of the life of a drop may be sufficient to detach it, the problem of detaching the drop is much more difficult during the early stages of growth. Not only must the change of interfacial tension be greater (*i.e.*, a greater change of potential difference) but the reduction must be maintained for a longer time—sufficient, in fact, to allow the nearly spherical drop to extend to the required pear shape. In the presence of large concentrations of hydrogen ion, *e.g.*, in normal acids, it is frequently impossible to effect this without the simultaneous evolution of large amounts of hydrogen and disruptive heating of the capillary thread.

In the practical utilisation of this method, it would be very desirable to be able to maintain a constant drop time of about 3 seconds over the range 0 to −2 volts. If a time of free fall of 3 seconds at −2 volts is assumed, the drop rate at −0·6 volts would be about 6 seconds, and in acid solutions it would be quite impossible to disengage the drop after only 3 seconds of life. This applies with rather more force to the synchronisation of two *approximately* equal drops.

Figs. 5 (*a*) and (*b*) respectively show the Ilkovic curves obtained at a freely falling drop and at a drop synchronised electrostatically to an external timing unit. The curves were

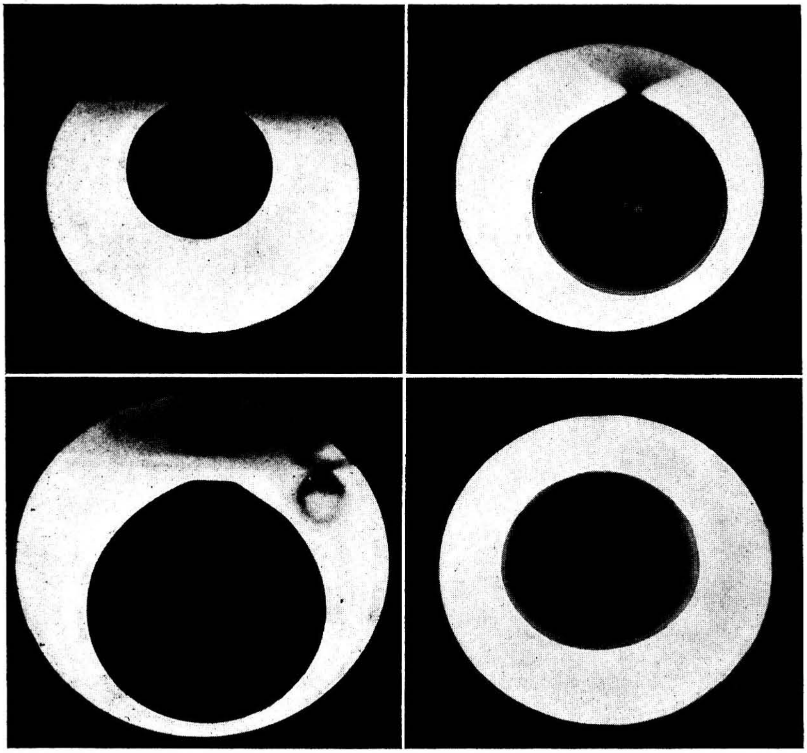


Fig. 2. Instantaneous photomicrographs of a freely falling mercury drop

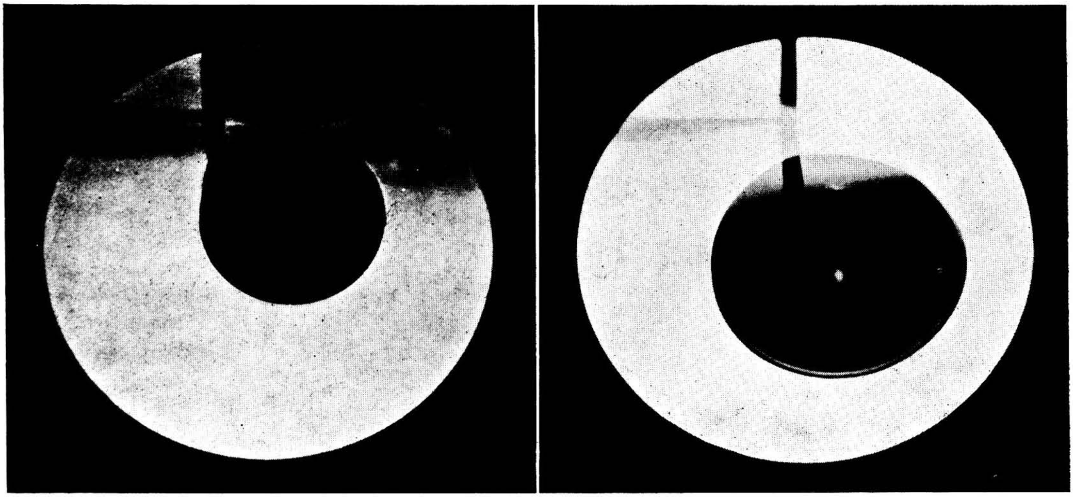


Fig. 4. Instantaneous photomicrographs of a mechanically disengaged drop

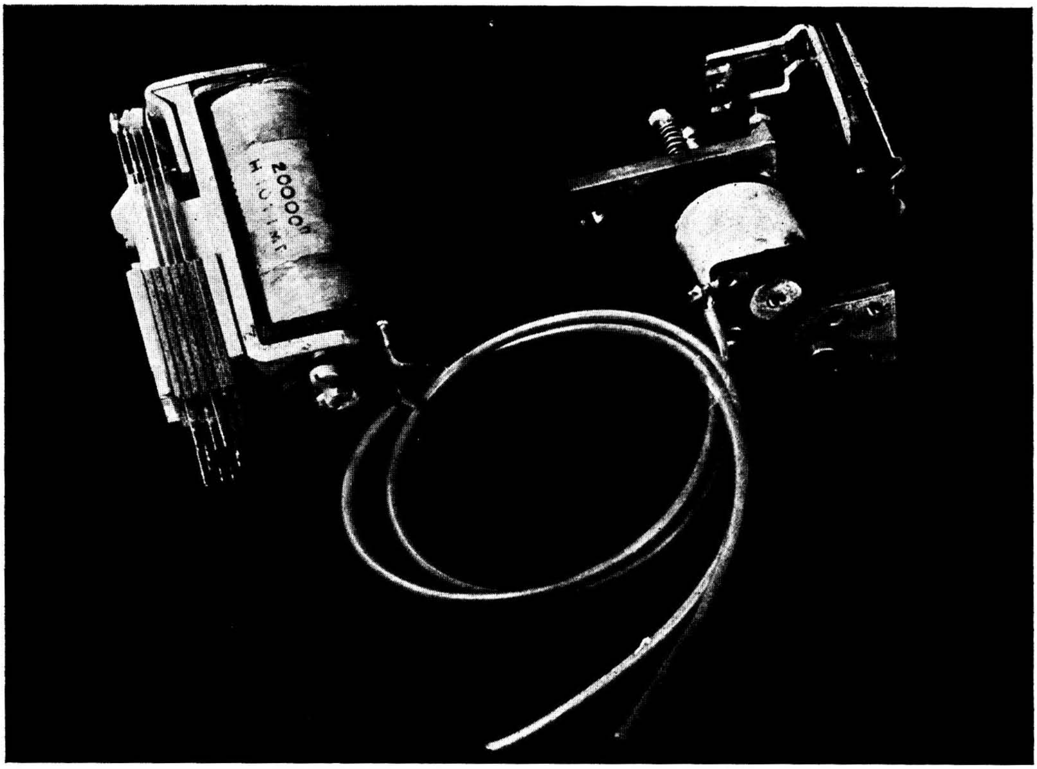


Fig. 3 (*a*). Pulsator unit

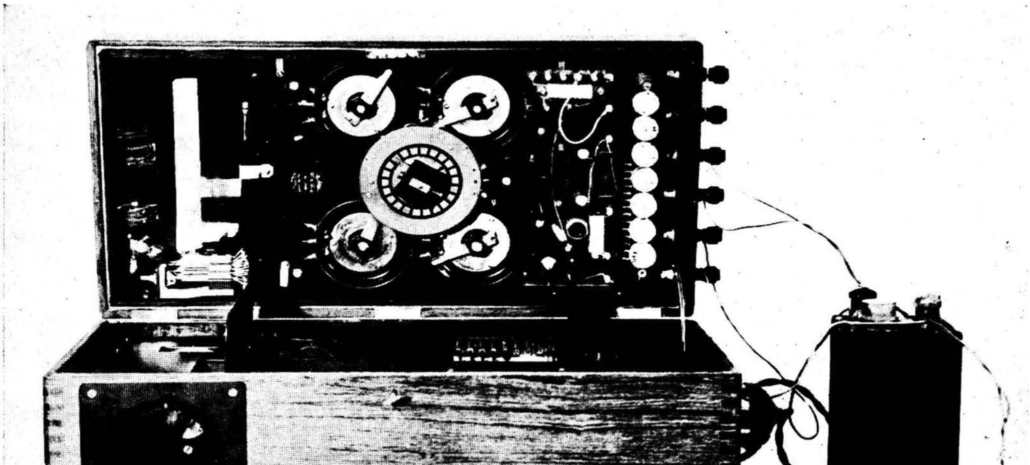


Fig. 7 (*b*). Modified Cambridge polarograph described on a cathode ray tube, using a modified version of the equipment designed by Randles,[8] and photographed in the usual way. It can be seen that some distortion of the curve is produced by the electrostatic control. The cause is rather uncertain, but is probably associated with convection stirring, which will be described later. In spite of this modification of shape, the reproducibility is good (the diagram shows a single curve only).

In conclusion, it should be noted that the galvanometer in the circuit must be paralysed and protected from the current pulses. The most convenient method is to short-circuit the galvanometer with a relay operated slightly in advance of the one that injects the pulse. Some care is necessary in the disposition of the apparatus with respect to stray A.C. magnetic fields, or mechanical rectification of the induced alternating current may occur, and give rise to random fluctuations on the galvanometer. For cathode ray polarographic circuits, protection is unnecessary.

ELECTROMECHANICAL CONTROL—

Two possible motions of the capillary tube may be envisaged, *viz.*, (*a*) vertical axial displacement, effectively "stretching" the drop, and (*b*) lateral movement, "shearing" the drop from the capillary thread. The primary requirement is that any such motion shall cause the minimum of stirring in the solution, and since effective disengagement is only produced by a rapid movement of the capillary tip, the shearing action is the only practicable method. Fig. 3 shows the apparatus by which this may be achieved. The upper end of the capillary tube (or tubes) is clamped rigidly to the armature of a small electromagnetic relay divested of its contacts. The relay is held open by adjustable springs, and a screw stop provides means for adjusting the travel of the armature. A small piece of thin sheet rubber, about 1/32-inch thick, is inserted into the gap to form a resilient buffer, and the adjusting screw is tightened until the rubber is lightly gripped. If the rubber buffer is omitted, it will be found that the working gap is only of the order of a few thousandths of an inch and, although initially the action may be quite satisfactory, such a small gap soon becomes wedged open by particles of almost impalpable dust. The electromagnet is "pulse" energised by the discharge of a 4-microfarad condenser. By tightening the adjusting screw of the relay armature, a condition is attained in which each discharge produces a very rapid lateral movement of the capillary tip of about 0·2 mm., followed by a highly damped oscillatory recovery. Under these conditions, which are not critical, mercury drops may be cleanly detached at any stage of their growth and under any conditions of polarisation or current flow.

Fig. 4 shows two typical instantaneous photomicrographs of the detachment of mercury drops at different stages in their lives. The rapidity of the process is apparent from the absence of any trace of the succeeding drop. The freedom from any marked distortion of the falling drop suggests that there is little turbulence produced by the movement.

Fig. 3 (*a*) is a photograph of a pulsator, as it is convenient to name the unit, together with the Post Office type relay from which it was made. The real value of the latter as a starting-point lay in the facts that the yoke is made of high permeability iron showing low residual magnetism and that the "knife edge" method of supporting the armature is ideal for this purpose, permitting easy removal whenever desired. Other parts of the relay are discarded. The type of coil used is not very important. Mainly for reasons of compactness, the 100-ohm latch-relay coil from a Siemens uniselector switch (standard Post Office equipment) was used.

Included in Fig. 3 (*b*) is a simple control circuit. The only important requirement is that the discharge of the condenser through the pulsator coil shall be abrupt and unhindered by such features as poor contact or "bouncing" of the relay. The latter is very detrimental to the regularity of the galvanometer oscillations. The authors use as a timing control a small geared-down Klaxon split-phase (constant speed) motor to which has been fitted a pair of contacts and a cam. Closing of the contacts operates the relay which discharges a 4-microfarad condenser through the pulsator coil. Very little power is consumed in operating the contactor and the type of motive power for this is not of great importance. Electronic control of the relay by means of a multivibrator circuit has been successfully used and possesses the advantage of easy variation of drop time.

Figs. 5 (*d*) to (*f*) show the Ilkovic curves delineated as described above, using electromechanical control with variable timing. The curves were obtained with the optimum setting of the pulsator and by comparison with Fig. 5 (*c*), the curve for a freely falling drop, it will be seen that the stirring effect is quite negligible, and that the four curves are all congruent within the common portions. Figs. 5 (*g*) and (*h*) illustrate the effect of progressively increasing the movement of the capillary tip to 1 to 2 mm. Figs. 5 (*i*) and (*j*) are curves obtained from a thallium solution in the absence of maximum suppressor, with and without drop control respectively. Three curves at corresponding potentials are shown in each figure. The experiments were carried out to ascertain whether the initial slight stirring during drop disengagement modified in any way the stirring present during maximum production. The latter stirring is always very irregular, and the photographs from which the figures were prepared suggest that there is no marked effect.

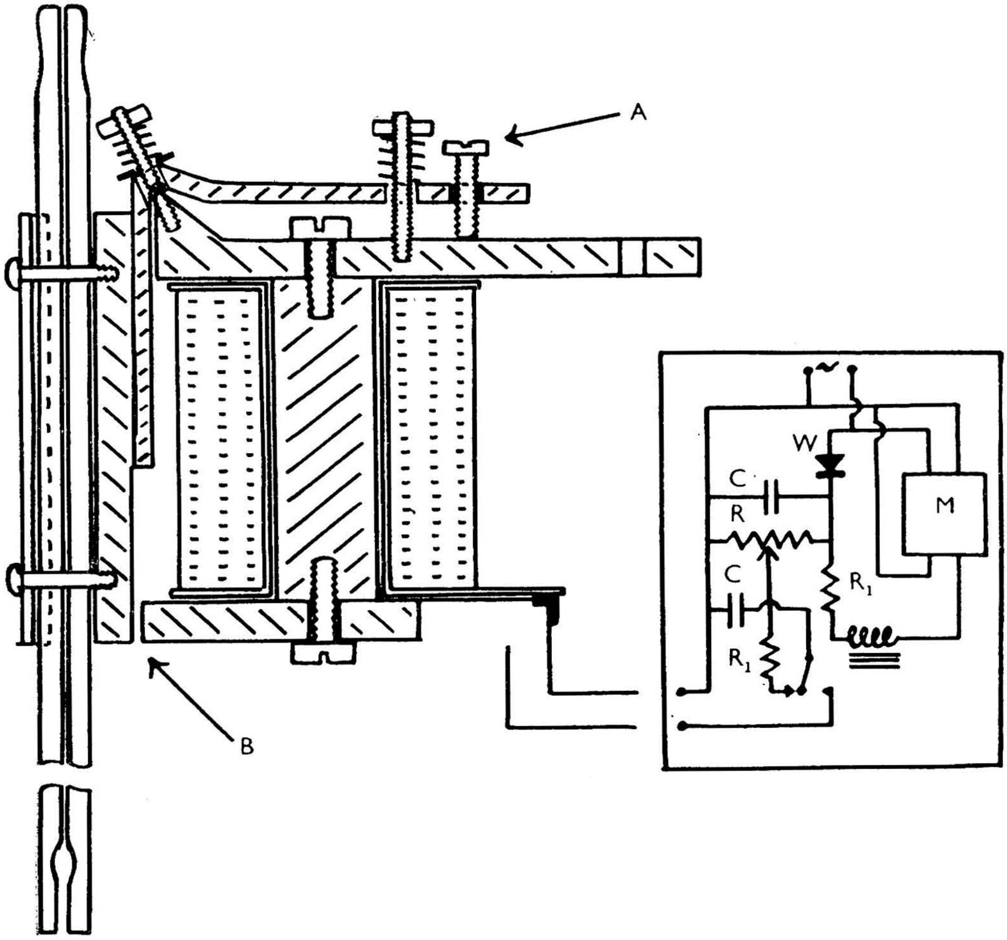


Fig. 3 (*b*). Pulsator (section) and control circuit

A	Adjusting screw
B	Armature - pole face gap. Sheet rubber insertion
M	Motor driven contactor
C	Paper condenser, 4 μF., 600 v. wkg.
R	Potentiometer 50,000 ohms w/w
R$_1$	20,000 ohms
W	Westinghouse rectifier, H 100
RELAY.	P.O. type 10,000-ohm coil, tungsten contacts

Fig. 6 shows some typical curves obtained on a Cambridge polarograph using a drop controlled as described. The regularity of the drop wave is quite satisfactory, and under conditions of constant rate of mercury flow, the variation of wave height with drop time is clearly seen. Reference will be made to this and similar experiments when the Ilkovic

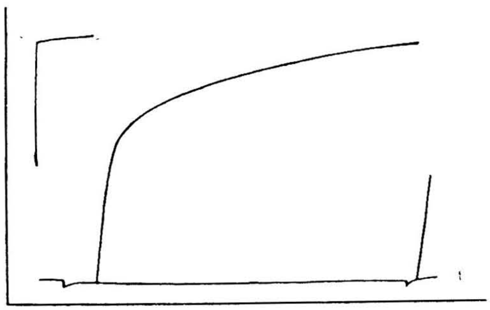


Fig. 5 (*a*). Free fall

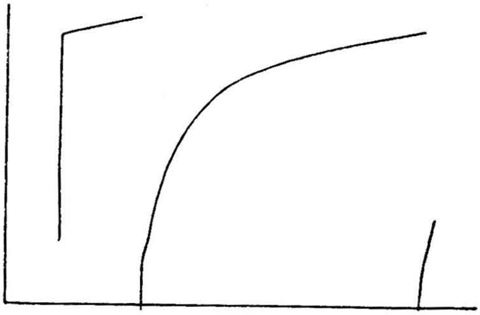


Fig. 5 (*b*). Electrostatic control

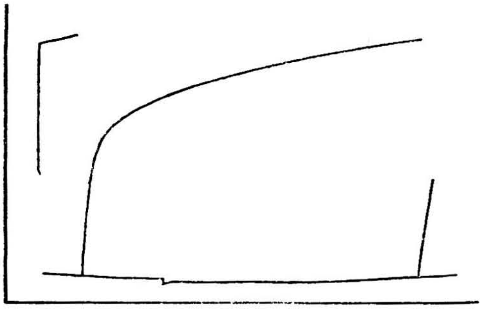


Fig. 5 (*c*). Free fall, $t = 4$ sec.

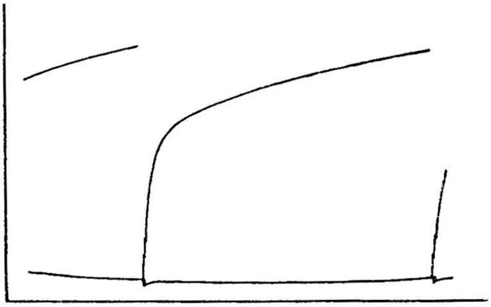


Fig. 5 (*d*). Electromechanical control, $t = 3{\cdot}5$ sec.

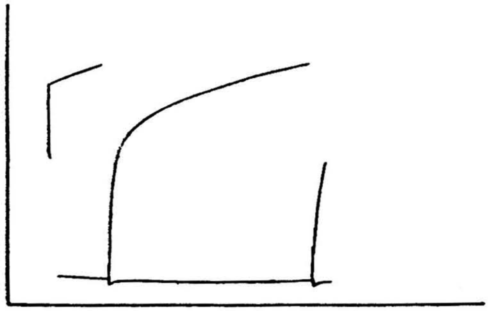


Fig. 5 (*e*). Electromechanical control, $t = 2{\cdot}5$ sec.

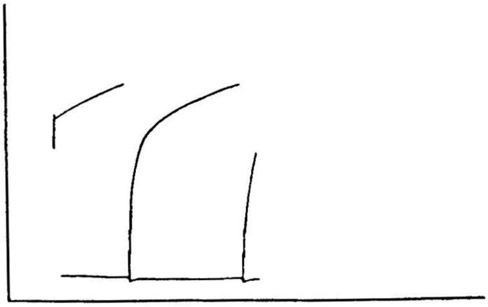


Fig. 5 (*f*). Electromechanical control, $t = 1{\cdot}5$ sec.

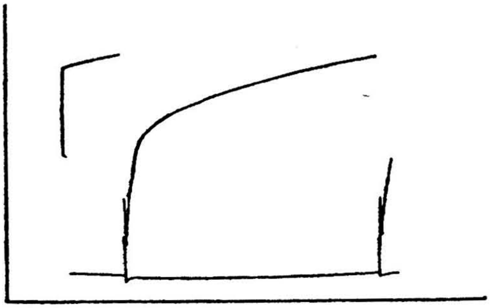


Fig. 5 (*g*). Electromechanical control. Increased amplitude of capillary movement

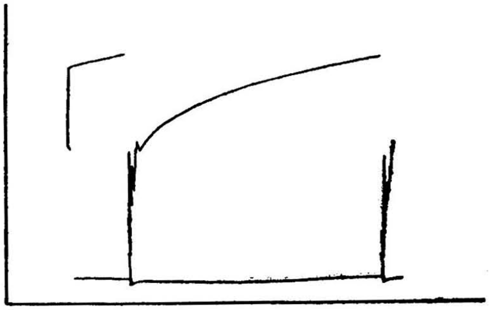


Fig. 5 (*h*). Electromechanical control. Increased amplitude of capillary movement equation is discussed. At the optimum setting of the pulsator, relatively large variations in the electrical energy in the condenser (± 50 per cent.) are practically without effect on the wave; an increase in capillary movement results in a slight increase in the step height and in extreme cases to some "raggedness" of the wave top, as might be anticipated from the Ilkovic curve shown in Fig. 5 (*h*). It is estimated that an increase in capillary-tip displacement of 10 times produces approximately a 2 per cent. increase in wave height. Control to within 0·5 per cent. should be maintained with ease.

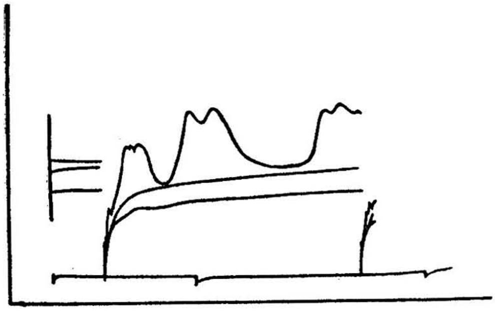


Fig. 5 (*i*). Electromechanical control. Three curves, (*a*) preceding maximum, (*b*) at maximum and (*c*) following maximum

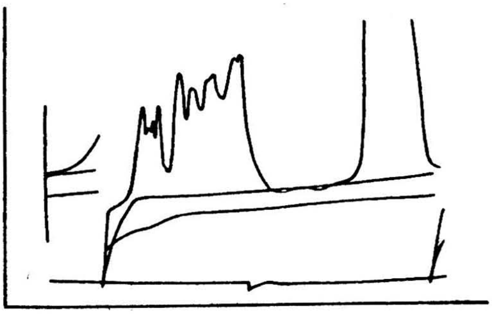


Fig. 5 (*j*). Free fall. Three curves, (*a*) preceding maximum, (*b*) at maximum and (*c*) following maximum

The magnetic field of the pulsator will induce an oscillatory current in the electrodes and associated wiring. It is desirable that this should decay to zero before the drop is detached, or else partial mechanical rectification may occur, leading to the possibility of erratic fluctuations of the galvanometer. Precise data are not available, but this factor will probably set an upper limit to the speed of action (*i.e.*, time between injection of pulse and disengagement of drop) of a pulsator, and it should be considered in any future designs.

Applications involving standard polarographic equipment

It will be appreciated from the above that of the two methods of drop control, the electromechanical is much the more practicable. A limited success has been obtained in the use of the electrostatic method on the cathode ray polarograph, but continuation of this work is not envisaged.

A single controlled capillary, used in conjunction with a conventional type of polarograph, may have limited advantages in so far as the product $m^{2/3}t^{1/6}$ is then independent of the applied potential. Furthermore, provided that a suitable capillary is chosen, the variation of t with capillary-active substances—*e.g.*, proteins—may be obviated. The real value of the control scheme however, lies in the ease with which two capillaries may be accurately synchronised, and following from this, it is profitable by slight modifications and additions to extend the versatility of commercial polarographs.

The circuit of the Cambridge polarograph is suitable for modification to bridge working. Fig. 7 (*a*) shows the electrical modifications made; Fig. 7 (*b*) shows the spatial arrangement of the additional components within the instrument. Apart from a pulsator, a controller, and a suitable stand for holding two capillaries, a large 2500-ohm potentiometer (standard electronic component) and a 2-volt accumulator are required. A change from normal working to bridge conditions is effected simply by the operation of the 6-circuit switch (standard Post Office equipment). On the latter setting, change from derivative to differential working is effected simply by moving the wander lead on the delay voltage potentiometer to the zero position. To compensate for variations in the e.m.f. of the 2-volt accumulator, a small variable resistance is included in this latter circuit. A 1-volt tap is taken from this network via a press-button switch to the wiping contact of the main drum potentiometer. With this set at 1 volt, any lack of equality in the two potentials will cause, on operation of the push-button, a deflection of the instrument galvanometer. Adjustments are made to the 50-ohm variable resistor in the delay voltage circuit until balance is attained. A precision of 1 per cent. is realisable, but the ultimate reference standard is the current milliameter on the instrument. Alternatively, for work of very high accuracy, the circuit could be standardised by the use of a Weston cell and an additional galvanometer.

No information on the circuits of other types of polarographs is accessible, but it is considered that modifications such as the ones described should be quite practicable. For convenience and neatness, the circuit diagram of the Cambridge instrument is not identical with the wiring layout.

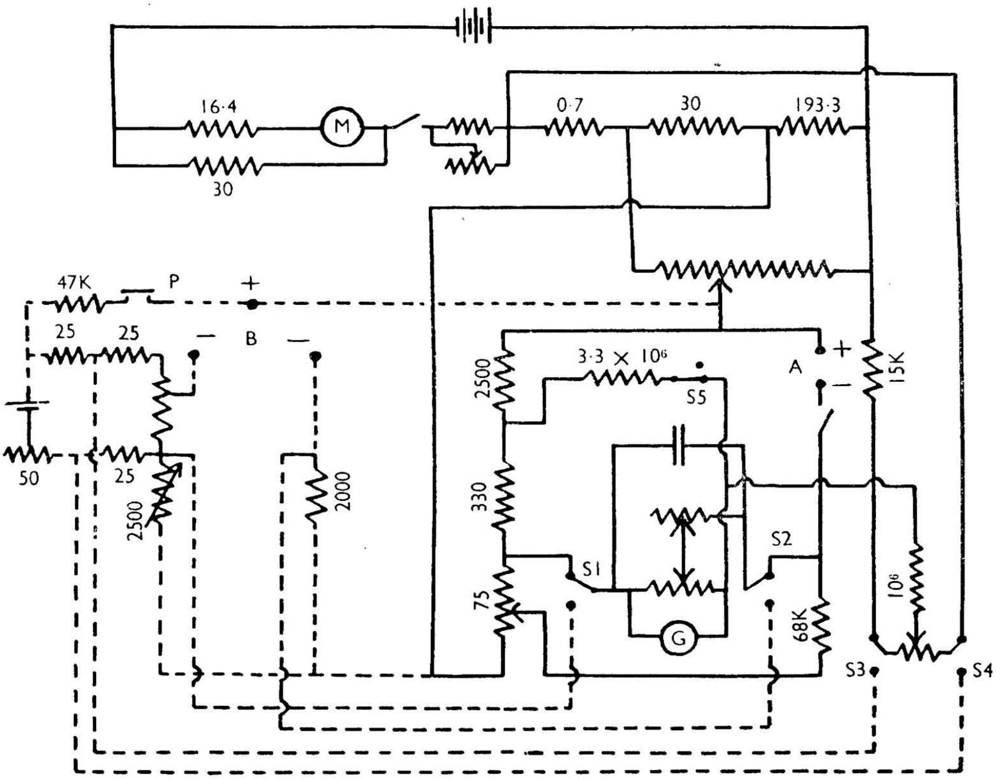


Fig. 7 (*a*). Circuit diagram of modified polarograph

Additional circuits shown dotted. Switches S1, S2, S3, S4 and S5 ganged on one frame. P = standardising press switch

A Polarographic cell position—normal working

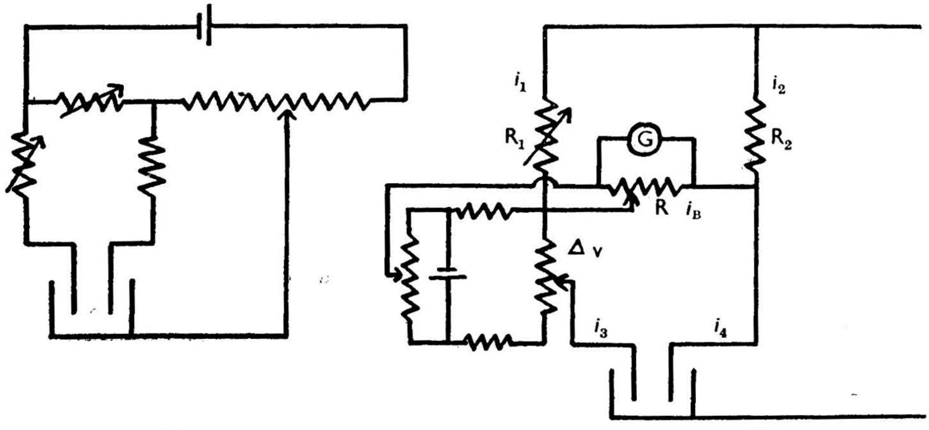
B Polarographic cell positions—derivative and differential working

DERIVATIVE POLAROGRAPHY—

Following Heyrovsky, derivative polarography is defined as polarographic work involving the automatic production and delineation, with respect to the applied voltage, of curves representing the slopes of normal waves. In contrast, differential polarography involves the elimination or minimisation of unwanted waves or residual currents by automatic subtraction.

The simple circuit described by Heyrovsky,[1] while adequate to illustrate the principle of the method, is unsuitable for general analytical purposes. Reference to Fig. 8 (*a*) will show that there is a steady current through the galvanometer even when connections are not made to the electrodes. The actual difference in potential between the two dropping electrodes is thus equal to the IR drop across the galvanometer resistance. Since it is very desirable to include the galvanometer in an Ayrton shunt circuit, and the net resistance of this varies with the setting, the true voltage difference, which we may designate as Δv, becomes a function of the shunt setting. To this may be added the facts that there is a permanent deflection of the galvanometer, also variable with the shunt position, and that other than the torsion head there is no means of adjusting the zero.

In the circuit of Fig. 8 (*b*) these inconveniences are all avoided, although an additional source of e.m.f. is unfortunately necessary. The tapped resistance provides values of Δv from 0 to 50 mv. in nominal increments of 10 mv.



(*a*) (*b*)

Fig. 8. (*a*) Heyrovsky derivative circuit, and (*b*) modification

Making the assumptions of identical electrodes and equality of bridge arms, a simple theoretical analysis of the circuit performance may be made.

Differentiating the fundamental equation[12] we have—

$$E = E_{\frac{1}{2}} - \frac{QT}{nF} \log \frac{i}{i_D - i}$$

where—

E = applied potential
$E_{\frac{1}{2}}$ = half-wave potential
n = number of electrons in reduction
i = current flowing
i_D = limiting current
Q = gas constant

and, re-arranging, at the half-wave potential, *i.e.*, where $i = \frac{1}{2}i_D$, we have—

$$\frac{di}{dE} = - \frac{nF}{4QT} i_D$$

Let Δv be the applied delay voltage and let δv be the voltage drop across the galvanometer network in consequence of the current flowing. Approximating the central portion of a polarogram to a straight line, the following equations hold when δv is a maximum

$$i_3 = \frac{i_D}{2}\left\{1 - \frac{nF}{2QT} \frac{(\Delta v - \delta v)}{2}\right\} \quad \ldots \quad \ldots \quad \ldots \quad \ldots \quad (1)$$

$$i_4 = \frac{i_D}{2}\left\{1 + \frac{nF}{2QT} \frac{(\Delta v - \delta v)}{2}\right\} \quad \ldots \quad \ldots \quad \ldots \quad \ldots \quad (2)$$

Applying the Kirchoff laws to the network, substituting and simplifying, we have—

$$i_B = \frac{i_D}{2} \frac{\frac{nF}{4QT}.\Delta v.2R_1}{(2R_1 + R) + \frac{nF}{4QT} \cdot \frac{i_D}{2} \cdot 2R_1R} \quad \ldots \quad \ldots \quad \ldots \quad (3)$$

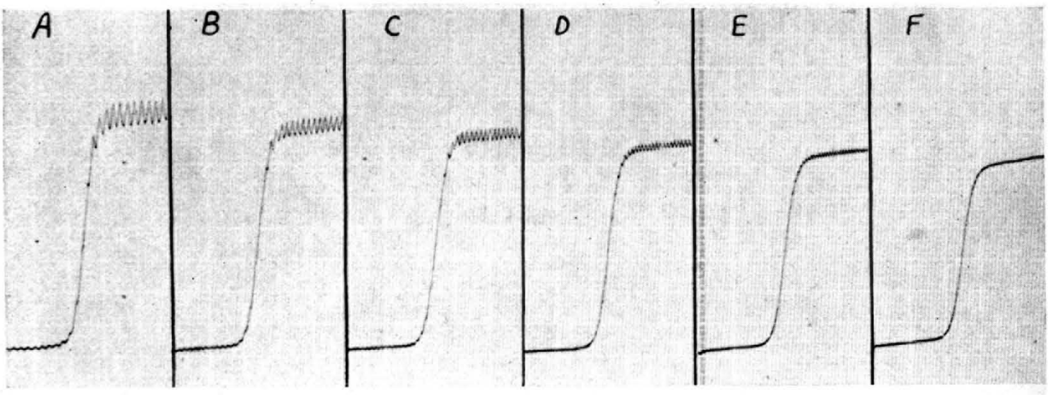


Fig. 6. Normal polarograms, electromechanical drop control, of 0·001 *M* cadmium in solution containing 0·5 *M* potassium chloride + 0·01 per cent. of gelatin. S = 1/50

Curve	A	B	C	D	E	F
t, sec.	3·90	3·05	2·50	1·93	1·50	1·00

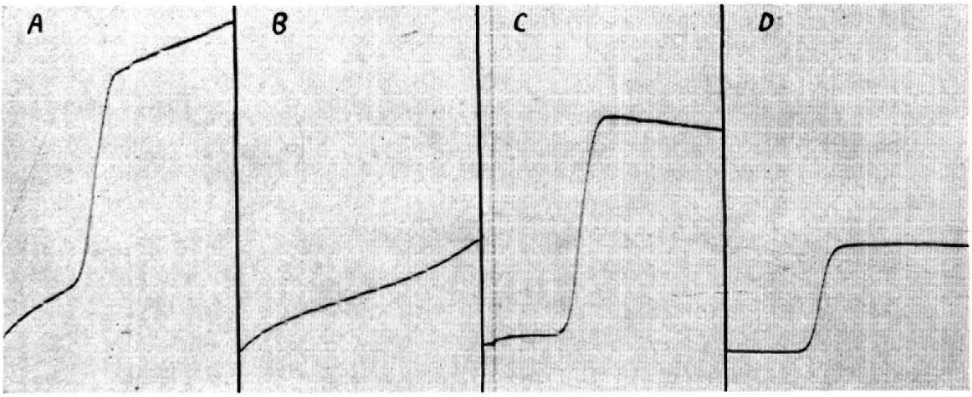


Fig. 13. Normal and differential polarograms of 8 × 10^{-5} *M* lead in solution containing 0·25 *M* potassium nitrate + 0·01 per cent. of gelatin. S = $\frac{2}{3}$

Curve A, simple polarogram; curve B, residual current; curve C, applied counter current; curve D, differential polarogram

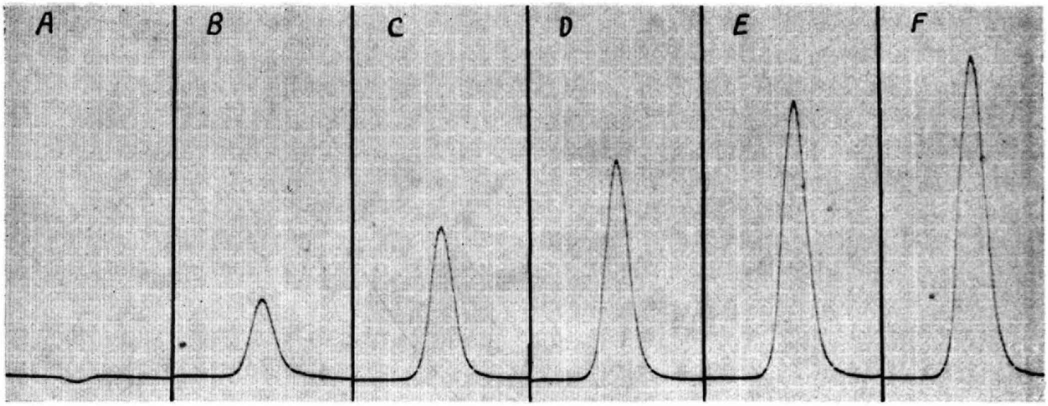


Fig. 9 (*a*)

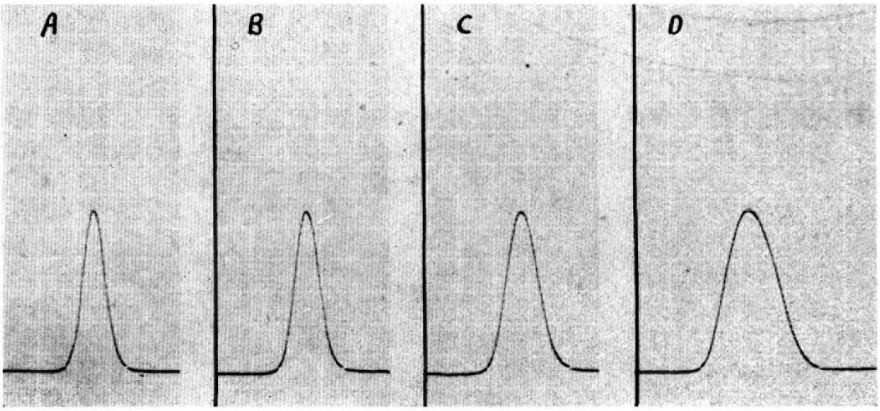


Fig. 9 (*b*)

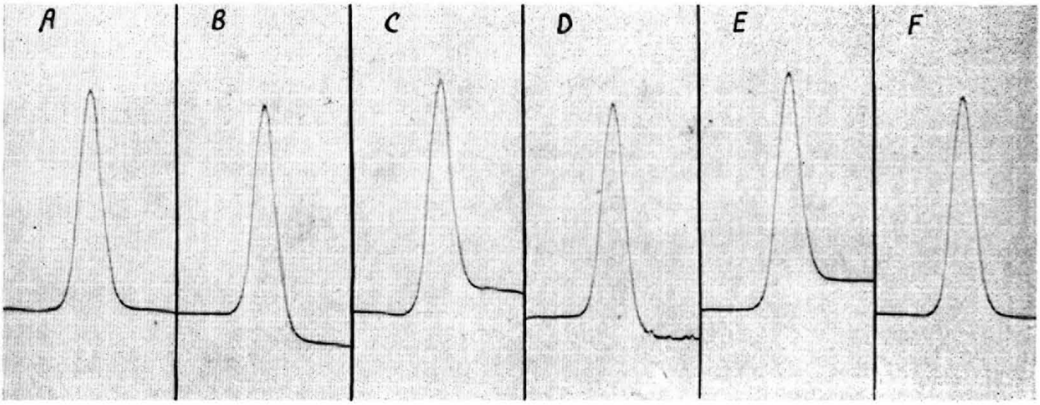


Fig. 9 (*c*)

Derivative polarograms of 0·001 *M* cadmium in solution containing 0·5 *M* potassium chloride + 0·01 per cent. of gelatin. S = 1/10. For details see opposite page

The significant inferences to be drawn from this equation are as follows—

(*a*) If other factors are constant, $i_B \propto \Delta v$.

Ignoring the second term in the denominator (it is shown below that it may be neglected) and if other factors are constant,

(*b*) $$i_B \propto n.$$

(*c*) Similarly, $i_B \propto \dfrac{2R_1}{2R_1 + R}$.

Since R is variable with shunt setting, the total sensitivity will thus be compounded of a shunt sensitivity and a network sensitivity.

(*d*) If other factors are constant, i_B is not strictly proportional to i_D.

If (*d*) is considered in more detail it will be seen that the magnitude of the error depends upon the product $i_D \times R$. Approximate proportionality exists between i_B, the maximum bridge current, and i_D, the cell diffusion current, but a graphical plot of $i_D \times R$ against i_B would show discontinuities because it is necessary to reduce the sensitivity (and thus the value of R) whenever full-scale deflection of the galvanometer is attained. The magnitude of the error may be calculated in the following manner. For a two-electron reduction, the ratio of i_B to i_D is, with the circuit constants shown, approximately 0·2. At any given setting of the Ayrton shunt, the bridge current giving full-scale deflection, together with the total resistance R, may be measured or calculated. The corresponding value of i_D is then obtained from the ratio above and, hence, for full-scale deflection conditions the magnitude of the error factor,

$$\frac{\dfrac{nF}{4QT}\,\dfrac{i_D}{2}\,2R_1R}{2R_1 + R},$$

may be computed.

Fig. 9. Derivative polarograms of 0·001 *M* cadmium in solution containing 0·5 *M* potassium chloride + 0·01 per cent. of gelatin. S = 1/10. (Facing this page)

(*a*) Effect of variation of Δv

Curve	A	B	C	D	E	F
Δv, mv. ..	0	9	18·2	27·2	36·4	46·3

(*b*) Effect of resistance in anode lead (Δv = 18·2 mv.)

Curve	A	B	C	D
Resistance, ohms	0	1000	5000	15,000

(*c*) Effect of bridge asymmetry (Δv = 18·2 mv.)

Curve	A	B	C	D	E	F
Conditions	$R_1 = R_2$	$R_1 < R_2$	$R_1 > R_2$	$R_1 = R_2$		$R_1 = R_2$ $i_{D_1} = i_{D_2}$ $R_1 i_{D_1} = R_2 i_{D_2}$
	$i_{D_1} = i_{D_2}$			$i_{D_1} > i_{D_2}$	$i_{D_1} < i_{D_2}$	

Proceeding on these lines, it has been calculated that for the *particular galvanometer and circuit constants employed*, the error factor is −0·7 per cent. at a shunt setting of 1, rising to −3·5 per cent. at a sensitivity of 1/1000. As an example, if a comparison were being made at S = 1/5 (error factor = 3·0 per cent.) between two solutions which should give deflections of 100 (full scale) and 50 respectively, the actual values obtained would be 97 and 49·25, as the error factor for the second peak is only 1·5 per cent. The observed ratio would be 1·97 instead of 2·00, an error of only 1·5 per cent. A similar error occurs for other ratios. Furthermore, since $i_D \propto i_B/n$, the error factor, which is proportional to $i_D \times n$, will be proportional to i_B and independent of n.

The validity of relation (*a*) is shown by the results in Table I. The departure from proportionality at greater values of Δv than 25 mv. is due to the non-linearity of the polarogram over such extended ranges. The derivative curves are presented in Fig. 9 (*a*) as typical examples of the performance of the apparatus.

The effect of bridge setting on the over-all sensitivity is demonstrated by the results of Table II. The constancy of the results in the last column is satisfactory. Verification of (*b*)

TABLE I

VERIFICATION OF THE RELATION $i_B \propto \Delta v$

Data from experiments similar to Fig. 9 (*a*)

Δv, mv.	Peak height, h	$h/\Delta v$
0	0	—
9	15·2	1·69
18·2	30·7	1·69
27·2	44·0	1·62
36·4	55·1	1·51
46·3	64·2	1·39

has not been attempted and, for the particular instrument employed, the error in (*d*) is not of sufficient practical importance to justify the extreme accuracy which would be necessary to verify its existence by experiment.

TABLE II

RELATION BETWEEN GALVANOMETER DEFLECTION AND SHUNT SENSITIVITY FOR BRIDGE CIRCUIT

Derivative polarograms for solution of 0·001 *M* cadmium in 0·5 *M* potassium chloride; $\Delta v = 18{\cdot}2$ mv., $R_1 = 2000$ ohms

Shunt sensitivity, S	Peak height, h	R	$\frac{2R_1}{2R_1 + R}$	$\frac{h}{S}$	$\frac{h}{S} \times \frac{2R_1 + R}{2R_1}$
1/5	61·5	336	1·084	308	334
1/7	45·2	253	1·064	316	336
1/10	32·2	185	1·047	322	337
1/15	21·7	127	1·032	326	336
1/20	16·3	96	1·024	326	333
1/30	11·0	65	1·018	330	335

The over-all validity of equation (3) is demonstrated by the results of Table III. It may be noted that the simple equation derived by Heyrovsky[1] does not adequately represent the behaviour of a derivative bridge circuit.

TABLE III

VERIFICATION OF EQUATION (3)

Solution of 0·001 *M* cadmium in 0·5 *M* potassium chloride; $t = 3{\cdot}05$ sec.

i_D, μ amps.	i_B, μ amps.	S	R, ohms	2R$_1$, ohms	T	Δv, mv.	$\frac{i_B}{i_D}$ obs.	$\frac{i_B}{i_D}$ theoret.
6·98	1·09	1/10	185	4000	21° C.	18·2	0·16	0·18

The postulated condition of identical electrodes is very rarely attained in practice. If the two diffusion currents are i_{D_1} and i_{D_2} it may be shown that, providing

$$R_1 i_{D_1} = R_2 i_{D_2},$$

equation (3) is still valid and may be re-written as—

$$i_B = \frac{i_{D_2}}{2} \frac{\frac{nF}{4QT}\,2R_2}{(R_1 + R_2 + R) + \frac{i_{D_2}}{2}\,\frac{nF}{4QT}\,2R_2R} \quad \cdots \quad \cdots \quad \cdots \quad (4)$$

i_{D_2} is preferred to i_{D_1}, since R_2 is a fixed resistance).

Note, however, that $R_1 + R_2 + R$ replaces $2R_1 + R$ in the denominator, which implies a changed bridge sensitivity.

The effect of extraneous resistance in the circuit is dependent upon its position. If, for example, the resistance is common to the two cells, as in an agar bridge, the peak is merely widened. Fig. 9 (*b*) illustrates this. The instrument measures di/dV, where V is the potential difference across the mercury surface, but delineates it with respect to E, the total applied potential. If, however, the position of the resistance is such as to produce bridge asymmetry, as for example, a very high resistance capillary, the unbalanced voltage drop either assists or opposes Δv, with a corresponding change of peak height. Since i_B is proportional to Δv, the magnitude of such errors may be estimated by expressing the potential difference across the extraneous resistance as a percentage of Δv. Ideally, both capillaries should have zero resistance; the usual value of about 50 ohms produces but negligible errors except at high currents.

THE TECHNIQUE OF DERIVATIVE POLAROGRAPHY—

Since, as is mentioned above, identical rates of mercury flow in the two capillaries would be improbable, the primary requirement is that the condition expressed by the equation $R_1 i_{D_1} = R_2 i_{D_2}$ (see above) be established. Fig. 9 (*c*) shows a number of derivative polarograms obtained under conditions when this equation was not satisfied, together with one designed to prove its validity. In the latter case the recovery of the original shape will be observed. It will be seen that the curves obtained may, to a first approximation, be regarded as compounded of a derivative curve and a positive or negative differential polarogram (dotted curve). For small deviations from exact balance, the peak height measured from the fore-foot of the curve will be the algebraic sum of a derivative peak proportional to the concentration of reducible ion and half of a differential wave, also proportional to the ionic concentration. Hence, except for the verification of equation (4), balance to within 5 to 10 per cent. is adequate. Necessarily, any such setting must be maintained constant for a series of experiments.

The method of balancing the bridge assumes a primary polarographic curve of ideal shape. The applied cell potential is adjusted to 0·3 to 0·4 volt more negative than the half-wave potential, *i.e.*, both electrodes are operating under conditions of limiting diffusion, and the variable resistance arm of the bridge is adjusted until there is no galvanometer deflection. In general, this must be done as a separate initial procedure, using some ion such as cadmium or thallium, as the derivative method is only of value when dealing with waves which are but poorly defined.

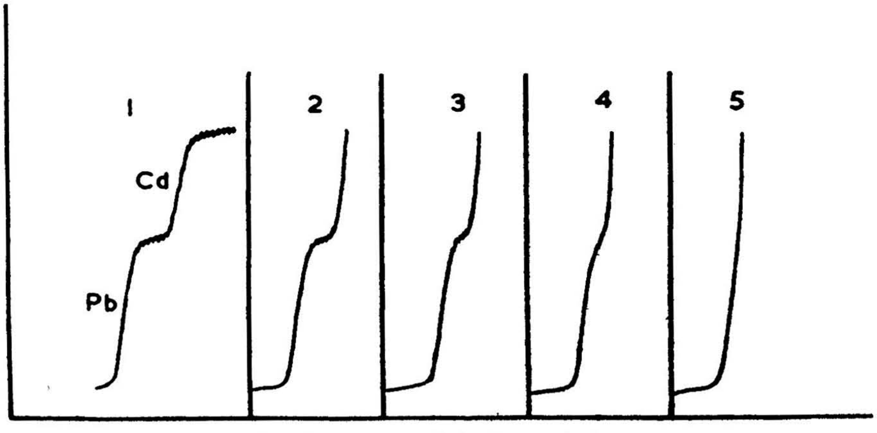


Fig. 10 (*a*). Normal polarograms of lead + cadmium in 0·25 *M* potassium nitrate + 0·01 per cent. gelatin solution. S = 1/10. 1·3 × 10^{-4} *M* lead
Cadmium, (1) 10^{-4} *M*, (2) 10^{-3} *M*, (3) 10^{-2} *M*, (4) 10^{-1} *M* and (5) 1 *M*

In other respects, the operation of the polarograph is similar to normal working. The slow recording speed must be employed, particularly when working at S = 1 owing to the sharpness of the peak. Additional damping may be secured by the use of the appropriate control, but a reduction in bridge sensitivity is simultaneously effected. Provided that the bridge resistances are all known and approximately constant, it is apparently (see Table II) satisfactory to calculate the over-all sensitivity and make a correction table for the Ayrton shunt. Alternatively, calibration at each setting is feasible, although time-consuming.

Illustrative examples of the possibilities of derivative methods are provided by Figs. 10 and 11. Fig. 10 (*a*) shows the ordinary polarograms of solutions of lead and cadmium in which the ratio of lead to cadmium was varied from approximately 1/1 to 1/10^4. Figs. 10 (*b*) and (*c*) are the corresponding derivative polarograms, the experiment having been designed to ascertain the point at which the simple proportionality between peak height and concentration is vitiated by interference from the cadmium peak. It will be seen that significant interference, due to a constant contribution from the fore-foot of the cadmium peak, only appears in the last example. Even under these conditions, an approximate correction for the interference could be obtained by making a blank measurement on a lead-free cadmium solution of equal concentration, *i.e.*, by subtracting the height of the cadmium peak fore-foot at the lead half-wave potential.

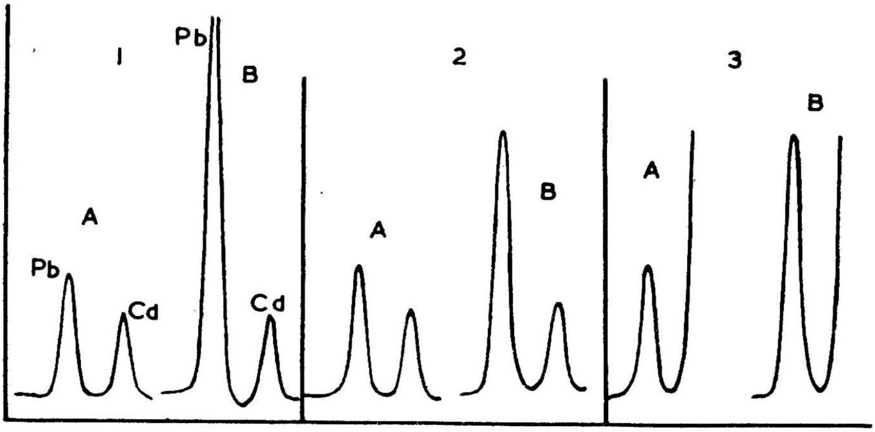


Fig. 10 (*b*). Derivative polarograms of lead + cadmium in 0·25 M potassium nitrate + 0·01 per cent. gelatin solution. $\Delta v = 18{\cdot}2$ mv., $S = \frac{2}{3}$

1	2	3
Cd = 10^{-4} M	Cd = 10^{-4} M	Cd = 10^{-3} M
(A) Pb = $1{\cdot}3 \times 10^{-4}$ M	(A) Pb = $1{\cdot}3 \times 10^{-4}$ M	(A) Pb = $1{\cdot}3 \times 10^{-4}$ M
(B) Pb = $3{\cdot}9 \times 10^{-4}$ M	(B) Pb = $2{\cdot}6 \times 10^{-4}$ M	(B) Pb = $2{\cdot}6 \times 10^{-4}$ M
	$h_a/h_b = 2{\cdot}00$	$h_a/h_b = 2{\cdot}00$

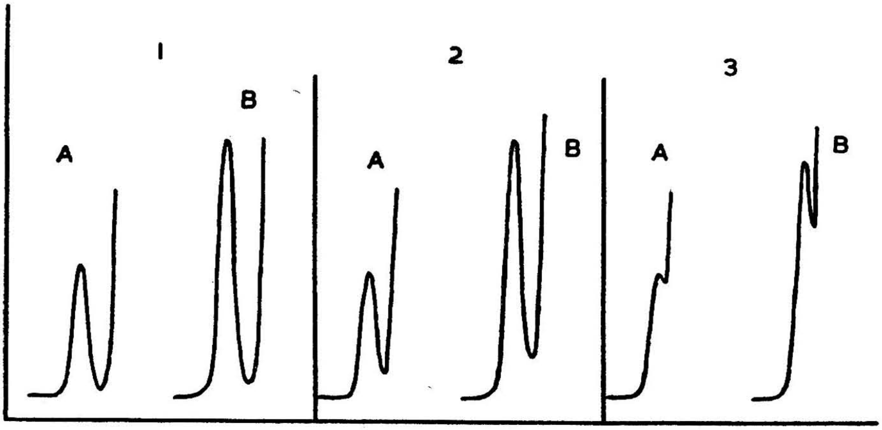


Fig. 10 (*c*). Derivative polarograms of lead + cadmium in 0·25 M potassium nitrate + 0·01 per cent. gelatin solution. $\Delta v = 18{\cdot}2$ mv., $S = \frac{2}{3}$

1	2	3
Cd = 10^{-2} M	Cd = 10^{-1} M	Cd = 1 M
(A) Pb = $1{\cdot}3 \times 10^{-4}$ M	(A) Pb = $1{\cdot}3 \times 10^{-4}$ M	(A) Pb = $1{\cdot}3 \times 10^{-4}$ M
(B) Pb = $2{\cdot}6 \times 10^{-4}$ M	(B) Pb = $2{\cdot}6 \times 10^{-4}$ M	(B) Pb = $2{\cdot}6 \times 10^{-4}$ M
$h_a/h_b = 1{\cdot}99$	$h_a/h_b = 2{\cdot}02$	$h_a/h_b = 1{\cdot}88$

Figs. 11 (*a*) and (*b*) respectively show the normal and derivative polarograms of lead in admixture with increasing amounts of thallium. The half-wave potentials of the two waves differ by 0·08 volt.

No consideration has been given to the use of the derivative curve for qualitative purposes. Corrections for delay voltage, net bridge resistance, cell resistance and other factors might be of importance.

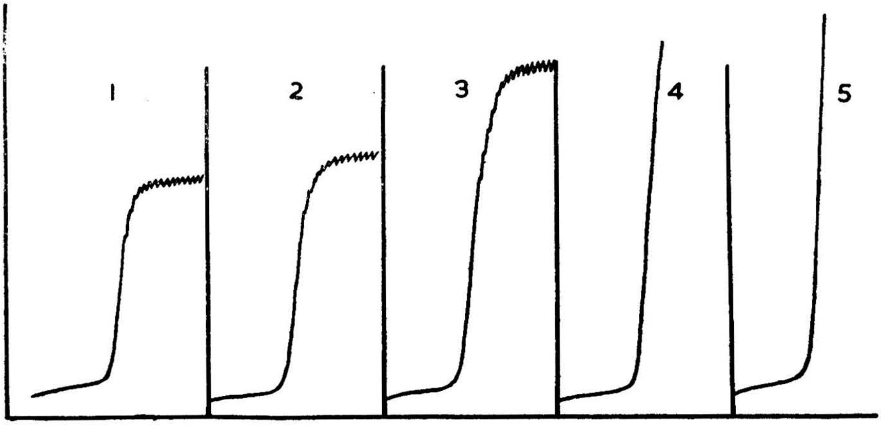


Fig. 11 (*a*). Normal polarograms of lead + thallium in 0·25 *M* potassium nitrate + 0·01 per cent. gelatin. S = 1/15. 2·7 × 10^{-4} *M* lead
Thallium, (1) nil, (2) 0·5 × 10^{-4} *M*, (3) 2 × 10^{-4} *M*, (4) 5 × 10^{-4} *M* and (5) 20 × 10^{-4} *M*

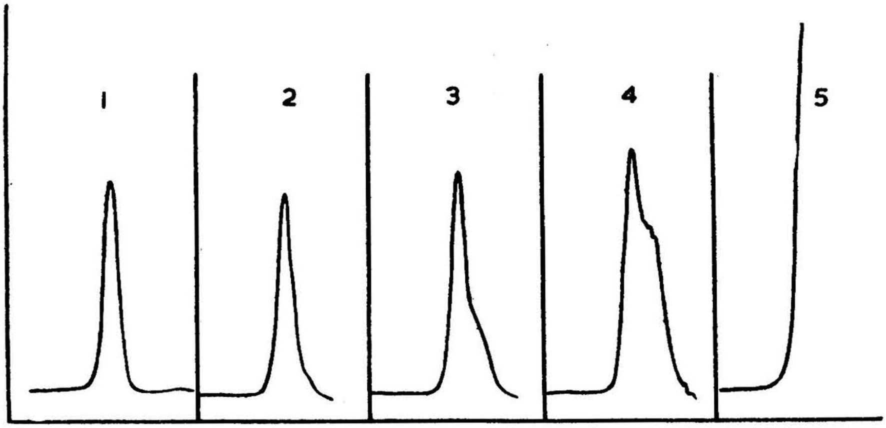


Fig. 11 (*b*). Derivative polarograms of lead + thallium in 0·25 *M* potassium nitrate + 0·01 per cent. gelatin. Δv = 18·2 mv., S = ½. 2·7 × 10^{-4} *M* lead
Thallium, (1) nil, (2) 0·5 × 10^{-4} *M*, (3) 20 × 10^{-4} *M*, (4) 5 × 10^{-4} *M* and (5) 20 × 10^{-4} *M*

DIFFERENTIAL POLAROGRAPHY—

With the omission, for simplicity, of the resistances r_1 and r_2, the circuit of Semerano and Riccoboni[2] has been adopted (Fig. 12). A simple theoretical analysis is possible on the following considerations. Assuming a symmetrical bridge with identical electrodes, let a limiting current of i_D flow in one cell and a current of $i_D + i'_D$ in the other. It is required to measure i'_D and eliminate i_D. If attention is focussed on the horizontal portions of the waves, the relation between cell current and voltage is expressed by $di/dE = 0$. With this condition, a simple application of the Kirchoff laws gives

$$i_B = i'_D \frac{R_2}{2R_2 + R} \quad \dots \quad \dots \quad \dots \quad \dots \quad \dots \quad (5)$$

The over-all sensitivity of the bridge plus galvanometer circuit can, therefore, never exceed 50 per cent. of the sensitivity of the galvanometer alone at a comparable setting. The validity of the equation is shown by the results given in Table IV. Inequality in the electrodes requires, as before, that $R_1 i_{D_1} = R_2 i_{D_2}$, and the equation assumes the form

$$i_B = i'_D \frac{R_2}{R_1 + R_2 + R} \qquad (6)$$

TABLE IV

VERIFICATION OF EQUATION (5)

Solution of 0·001 *M* thallium in 0·25 *M* potassium nitrate; S = 1/15, t = 3·05 sec.

i_D, μ amps.	i_B, μ amps.	R_1, ohms	R, ohms	$\frac{i_B}{i_D}$	$\frac{R_1}{2R_1 + R}$
2·64	1·29	2000	127	0·487	0·484

THE TECHNIQUE OF DIFFERENTIAL POLAROGRAPHY—

As for derivative work, the balancing of the bridge is the most important requirement. The purpose of the differential method is to remove unwanted currents, whether residual or otherwise. If it is known that after such removal an ideally or nearly ideally shaped polarogram should be obtained, the procedure is merely to adjust the variable resistance bridge arm until such a shape is obtained. If, however, as in an irreversible electrode process, a distorted wave is expected, it is desirable to balance the bridge initially with *e.g.*, a cadmium or thallium solution.

As two electrodes dropping into two solutions of possibly different concentrations are now concerned, the number of variables is increased. For theoretically perfect compensation, the requirement that $R_1 i_1 = R_2 i_2$ must be satisfied for both the reduction and condenser currents. Ideally, therefore, the bridge should be balanced with the same cadmium or thallium solution in both cells, the unknown solution should be placed in cell 2 and the concentration of a solution containing the ion giving the unwanted wave should be adjusted until balance is again attained. Practically, this would be tedious, and no noticeable error results if the final accurate balancing is done by the variable bridge resistance.

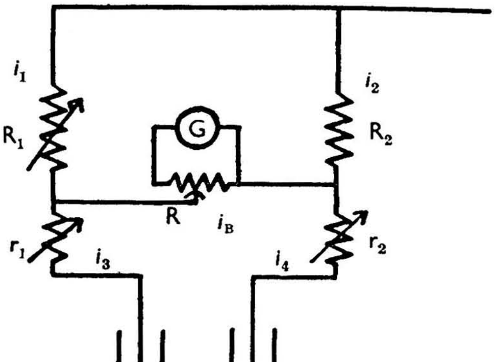


Fig. 12

Differential circuit of Semerano and Raccoboni

The relatively high temperature coefficient of the diffusion current (2 per cent. per ° C.) necessitates temperature equality, if not temperature constancy, in the two cells, and a thermostat is almost indispensable. As yet, insufficient evidence has been accumulated to decide whether the two electrodes must be operated on one pulsator or whether two pulsators, each carrying one capillary and operated simultaneously, are satisfactory. The latter scheme is advantageous in that the standard Cambridge thermostat and dropping electrode stand are immediately adaptable. In the case of the derivative method, the separation of the two electrodes should be a minimum and the question does not arise.

Perfect compensation of two waves *at all voltages* requires, apart from the conditions already discussed, that they have identical *apparent* half-wave potentials and shapes. Any differences in these quantities, caused by the inequality of calomel half-cells or cell resistances, will be observed by the galvanometer both in magnitude and sign, giving rise to minor derivative effects superimposed on the differential. The resistances r_1 and r_2 (Fig. 12) are designed to minimise such phenomena. However, within the ratio limit of 10/1 discussed below, the effects are small and the two compensating resistances may be discarded.

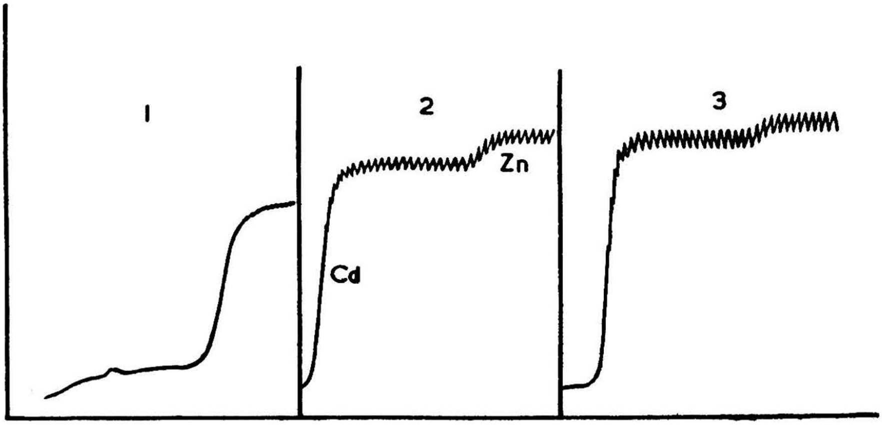


Fig. 14 (*a*). Normal polarograms of zinc + cadmium in 0·5 M ammonium chloride + 0·5 M ammonium hydroxide + 0·01 per cent. gelatin solution. 8×10^{-5} M zinc

(1) Cadmium, nil; S = 1/5. (2) Cadmium, 8×10^{-4} M; S = 1/30. (3) Cadmium, 2×10^{-3} M; S = 1/20

The utility of the differential technique is shown by Figs. 13 and 14. As shown by the former example, nearly perfect elimination of the residual current over the whole working voltage range may be achieved. This is an attribute not possessed by the counter-current method of Ilkovic and Semerano, and more than compensates for the 50 per cent. loss in sensitivity.

Fig. 14 illustrates the minimisation of a large cadmium wave preceding a zinc wave. The polarogram of Fig. 14 (*b*, 3) is anomalous. Fig. 14 (*c*) presents three consecutive repeats carried out several hours later (a somewhat smaller mercury flow rate, in consequence of a

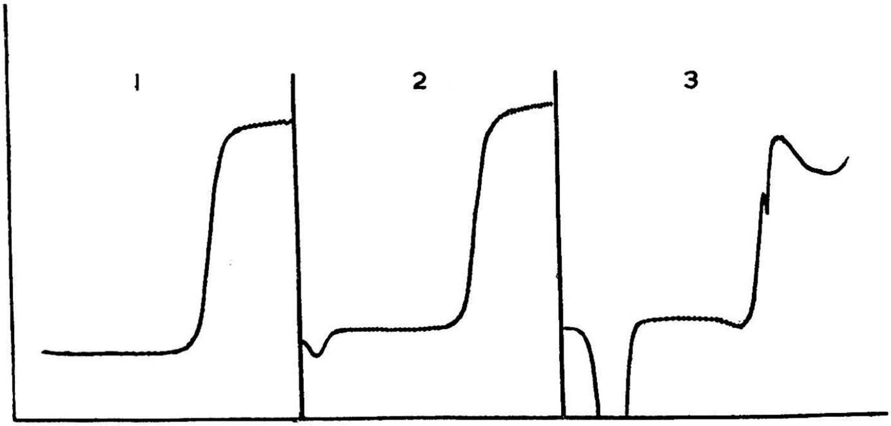


Fig. 14 (*b*). Differential polarograms of zinc + cadmium in 0·5 M ammonium chloride + 0·5 M ammonium hydroxide + 0·01 per cent. gelatin solution. S = $\frac{2}{3}$. 8×10^{-5} M zinc

Cadmium, (1) nil, (2) 8×10^{-4} M and (3) 2×10^{-3} M

reduced head, was employed). The regularity of the galvanometer oscillations in each case is good: from the known cadmium to zinc ratio of 25/1, it will be seen that the average reduction current associated with each drop is constant to well within 0·1 per cent. It must be emphasised, however, that such regularity can only be obtained if the pulsator receives electrical excitations of constant energy and duration. As was briefly noted earlier, the phenomenon known as "relay bouncing" is particularly objectionable. On the first closure of the contacts, the condenser is partially discharged and the drop is probably detached. The second closure completes the discharge, causing a further shock to the capillary, which results in a slight irregular stirring and corresponding variations in the reduction current. Normally, such effects would be almost indiscernible, but the differential method magnifies them to such an extent that they may produce a "raggedness" of the wave which almost obscures it.

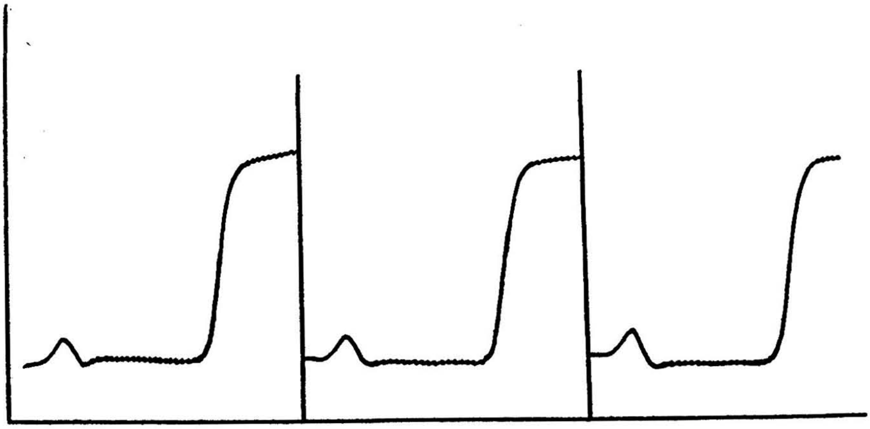


Fig. 14 (*c*). Differential polarograms of zinc + cadmium in 0·5 *M* ammonium chloride + 0·5 *M* ammonium hydroxide + 0·01 per cent. gelatin solution. S = $\frac{2}{3}$. 8×10^{-5} *M* zinc + 2×10^{-3} *M* cadmium

With reasonably careful working it is possible to carry out estimations on solutions in which the ratio of the unwanted to the desired wave is 100/1. By comparison, the simple compensation procedure of Lingane and Kerlinger[9] is applicable in favourable cases up to a limit of 50/1. However, it is considered that above a ratio of 10/1 the use of automatic controlled potential electrolysis at a mercury cathode is a much more effective method of dealing with large preceding waves. The special merit of the differential method is that, unlike that of Lingane and Kerlinger, it is possible to compensate waves of any shape, such as those frequently obtained in the reduction of organic compounds.

APPLICATIONS INVOLVING NON-STANDARD EQUIPMENT

MULTI-TIP ELECTRODES—

McGilvery, Hawkins and Thode[10] have described multi-tip electrodes as a means of increasing sensitivity. Simultaneously, there occurs a proportionate increase in the condenser current, but it is claimed that the natural asynchronism of dropping rates materially reduces what would otherwise be an enhanced drop wave. Bricker and Furman[11] have criticised this latter statement, claiming that an irregular wave-top results. A five-tip electrode has been made (unfortunately of a very rapid drop rate) and the drops have been shown to be capable of synchronisation by means of a pulsator. Work is in abeyance owing to the difficulty of obtaining a suitable size of capillary tubing, but there appears to be no reason why this type of electrode should not prove very profitable if the residual current is eliminated by means of a differential circuit.

COMBINATIONS OF DERIVATIVE AND DIFFERENTIAL METHODS—

The practicability of the separation of two waves by derivative methods depends upon the difference of half-wave potentials and the ratio of the concentrations. Instances may arise in which a separation is feasible when the two substances are present in equal amounts, but not when present in a ratio of, for example, 1/10. Theoretically it should be possible to set up two derivative bridge circuits, one operating on the second reducible substance only. By means of a galvanometer having independent differential windings, automatic subtraction of the two derivative curves should be feasible. Numerous other combinations might be advanced; in particular it should be possible to gain the desired results with only three synchronised mercury drops, but as yet no satisfactory circuits have been designed.

CATHODE RAY POLAROGRAPHY—

The apparatus devised by Randles[8] has been elaborated to permit differential operation. As an example of the performance, one part of zinc in 10^4 parts of cadmium may be detected with certainty. The work is as yet in a preliminary stage and will be reported fully in a subsequent paper.

MISCELLANEOUS OBSERVATIONS

The Ilkovic curves of Fig. 5 show that, with proper adjustment of operation, the pulsator has no directly discernable effect on the diffusion current. It thus becomes possible to separate the variables m and t in the Ilkovic equation. The results of Table V show that the relation $i \propto m^{2/3}$ is followed to within less than ±2 per cent. over a 2 to 1 range in flow rates.

TABLE V

VERIFICATION OF RELATION $i_D \propto m^{2/3}$

Solution of cadmium in 0·5 M potassium chloride; $t = 3{\cdot}05$ sec.

h	m, mg./sec.	$\frac{h}{m^{2/3}}$
54·0	1·41	42·9
49·5	1·278	42·0
44·0	1·056	42·4
36·7	0·831	41·5
32·9	0·706	41·5
		42·1 + 1·9%
		− 1·4%

Table VI shows results verifying the relation $i \propto t^{1/6}$ to within approximately ±2·5 per cent. However, the Ilkovic curves of Fig. 5 do not exactly fit a one-sixth power law, the slope of the initial portions being too low. This could be qualitatively explained by the postulate that during the life of a drop there is some small accumulation (by convection and, possibly, mercury vortex action) of the depleted solution from the diffusion layer around the neck of the mercury drop. This would not be entirely removed by the falling drop,

TABLE VI

VERIFICATION OF RELATION $i_D \propto t^{1/6}$

Data from experiments similar to Fig. 6

	h	t, sec.	$\frac{h}{t^{1/6}}$
Series 1	43·3	3·9	34·6
	41·1	3·05	34·1
	39·7	2·50	34·0
	37·8	1·93	34·0
	35·4	1·50	33·1
	33·4	1·00	33·4
			mean 33·9 + 2·1%
			− 2·4%
Series 2	43·0	3·9	34·3
	42·6	3·66	34·3
	40·7	3·05	33·8
	37·5	2·15	33·0
	37·0	1·58	34·3
	32·0	0·75	33·5
			mean 33·9 + 1·2%
			− 2·7%

and the new drop would therefore grow through a portion of solution of concentration somewhat lower than that in the bulk of the electrolyte. With increase of drop size the diffusion conditions would become those required by the Ilkovic equation. Some substantiation of this hypothesis is provided by results obtained on the cathode ray polarograph. With this instrument a polarogram is observed during the life of a drop; one such polarogram may be observed or the process may be repetitive, depending on the particular drop frequency employed. In several cases examined, single polarograms appear to be a few per cent. larger than those obtained when there has been a preceding wave. It would be of interest to examine a reduction process in which the product of the reaction remains in solution and is more dense than the bulk of the electrolyte.

A small distortion of the Ilkovic equation such as is considered would not markedly affect the one-sixth power law as determined by an integration of the curve, *i.e.*, a mean current measurement by a galvanometer. There is a suggestion of a drift in the results of Table VI, but the order of accuracy is insufficient for unequivocal deductions.

DESIGN OF CAPILLARIES—

Composite capillary tubes consisting of a 10-cm. length of 0·05-mm. bore tubing plus a 1-cm. tip of 0·1-mm. bore, are employed with the pulsators. The free-drop time may be as great as 15 seconds, but the drop time is easily controlled to the convenient value of 3 seconds. The relatively large bore of the tip makes such capillaries much less liable to clogging, which for protein-like substances may be a decided advantage.

ADDENDUM

Since the submission of this work for publication, a paper by Lévêque[13] has appeared. Mention is made of experiments on electromechanical drop-control, using a somewhat similar scheme to that described, and failure to devise a useful technique was reported. From an examination of the circuit diagram it would appear that the lack of success was probably due to the stirring effects arising from the cessation of the magnet-energising current after the relatively long time of energisation of the driving magnet.

Acknowledgment is made to the Director, Atomic Energy Research Establishment, Ministry of Supply, for permission to publish this work.

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October, 1949

The Determination of Egg in Salad Cream

Part I

By C. G. DAUBNEY and G. E. W. SEXTON

SYNOPSIS—A method is proposed for determining the amount of egg yolk solids and dry egg solids in salad cream. It is based on a colorimetric determination of the choline derived from egg lecithin by means of acid hydrolysis and conversion of the choline into its pink reineckate, which is soluble in acetone.

The choline content of dried whole egg, sugar-dried egg, and dried egg yolk has been determined and shown to be constant in any one type of product.

Mustard, a common ingredient of salad cream, causes a complication on account of the choline derived from the sinalbin of white mustard. The method described determines total choline and is applicable only when the nature and amount of any mustard present is known. Satisfactory results were attained for salad creams of known composition.

Work is in progress to devise a means of determining egg and mustard separately.

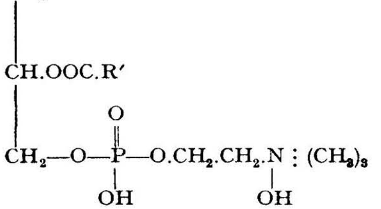
THE determination of the egg content of certain foodstuffs is often required for the purpose of quality control and for checking their compliance with Orders issued by the Ministry of Food.

The first of these Orders is the Food Standards (Salad Cream and Mayonnaise) Order, 1945 (S.R. & O., 1945, No. 1177), which lays down that in the case of salad cream, mayonnaise and salad dressing, "the product shall contain . . . not less than 1·35 per cent. by weight of egg yolk solids." The second is the Flour Confectionery (Control and Maximum Prices) (Amendment) Order, 1950 (S.I., 1950, No. 87), by which a certain maximum price may be charged for flour confectionery, which includes cakes, puddings, pastry, etc., provided "the combined fat, sugar, and dry egg solids content (or the combined content of any two of these ingredients) . . . is 45 per cent. or more." The third is the Food Standards (Preserves) (Amendment) Order, 1949 (S.I., 1949, No. 1893), by which fruit curd shall contain not less than 1 per cent. of dried whole egg (or its equivalent as sugar-dried whole egg, liquid or frozen whole egg or shell egg). It will be noticed that in one Order it is "egg yolk solids" that is mentioned and in another "dry egg solids." None of the Orders specifies a method for the determination of the egg content.

It was in consequence of these Government Regulations that the experiments described below on egg and salad cream were carried out; work is now in progress on flour confectionery and fruit curd, the result of which it is hoped to publish shortly.

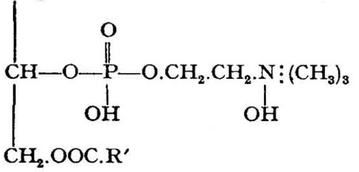
So far as the analyst is concerned, the term "egg" generally implies that of the hen. In the main, the white of egg is composed of protein and water, whilst the yolk contains, in addition to protein and water, some 30 per cent. of fatty matter and 1·5 per cent. of cholesterol. Of this fatty matter about one-third is phosphatide, chiefly lecithin.

The term phosphatide (or phospholipin) refers to a group of substances in which one or more fatty acids and a nitrogenous base are combined with a third constituent, glycerophosphoric acid. This group can be further subdivided into (*a*) the lecithins and (*b*) the cephalins. The compounds in each sub-group conform to the same type of structure but differ in the nature and position of the fatty acid radicals contained in the molecule. The lecithins are represented by the formulae—



$$\begin{array}{l} CH_2.OOC.R \\ | \\ CH.OOC.R' \\ | \\ CH_2{-}O{-}\underset{\underset{OH}{|}}{\overset{\overset{O}{\|}}{P}}{-}O.CH_2.CH_2.\underset{\underset{OH}{|}}{N}:(CH_3)_3 \end{array}$$

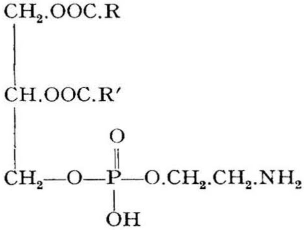
α-form



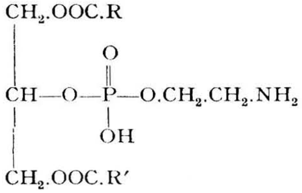
$$\begin{array}{l} CH_2.OOC.R \\ | \\ CH{-}O{-}\underset{\underset{OH}{|}}{\overset{\overset{O}{\|}}{P}}{-}O.CH_2.CH_2.\underset{\underset{OH}{|}}{N}:(CH_3)_3 \\ | \\ CH_2.OOC.R' \end{array}$$

β-form

The cephalins are identical in structure,* except that they have β-amino ethanol (cholamine) in place of choline—



α-form



β-form

These two groups of phosphatides occur in animal tissues in varying proportions, and in egg the β-form of lecithin is said to predominate.

The phosphatides are soluble in most organic solvents except acetone, whilst cephalin is insoluble in alcohol unless water or other lipoid bodies are present. In considering the analytical chemistry of this class of substance it must be borne in mind that lecithin and cephalin appear to undergo oxidation and hydrolysis more readily than the fats. Lecithin is hydrolysed on boiling with dilute acid into glycerophosphoric acid, fatty acids and the base choline. Similar decomposition is brought about by the enzyme lecithinase which occurs in animal tissue.[1,2] Cephalin decomposes similarily to give cholamine.

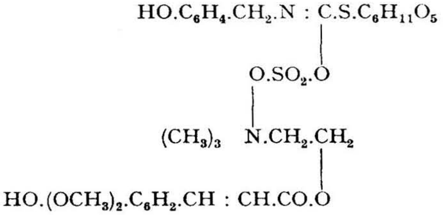
The methods proposed from time to time for the determination of egg in various foodstuffs have usually depended on a determination of either the phosphatide or the cholesterol content.[3] In deciding on a method which was likely to be applicable to salad cream, one based on the determination of the phosphatide fraction of egg seemed to be the most promising, either by way of organically-bound phosphorus, as a measure of the total phosphatides, or by way of choline as a measure of lecithin.[4]

Methods[5,6,7,8,9] based on the determination of organically-bound phosphorus require the complete extraction of the phosphatide with suitable solvents and the subsequent determination of phosphorus in the extract. Such methods are, however, liable to give low results owing to the susceptibility of lecithin to decomposition by enzymes or acids, with the consequent splitting off of the phosphoric acid radicle as mentioned above.[10] Further, the physical nature of salad cream renders solvent extraction methods unsuitable.

Methods based on the determination of choline after hydrolysis of the lecithin, on the other hand, present certain advantages. The products of hydrolysis will include any choline previously split off by acid or enzyme action and the experimental technique is readily applicable to salad cream.

A disadvantage is that under the conditions of hydrolysis to be described, choline is also derived from white mustard (*Sinapis alba*) which is often an ingredient of salad cream.

White mustard contains the glucoside sinalbin, which was shown by Gadamer[11] to be—



This is made up of the non-volatile mustard oil, *p*-hydroxybenzyl *iso*thiocyanate, linked to glucose and the acid sulphate of the choline ester of sinapinic acid.

Black mustard (*Sinapis nigra*), on the other hand, contains the glucoside sinigrin, which is less complex, and contains no choline.

An evaluation of mustard has been described by Viehoever and Nelson[12] and by Terry and Corran,[13] both of whom have based their method on a determination of the liberated sulphate after enzymatic decomposition.

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* This formula has recently been disputed; see Hutt, H. H., Malkin, T., Poole, A. G., and Watt, P. R., *Nature*, 1950, **165**, 314.

The non-volatile mustard oil has been studied by several workers, but it does not lend itself readily to determination.

The other important ingredient of salad cream which might give rise to choline is the vegetable oil.[14] At the present time there are available to manufacturers various oils all of which, in the crude state, contain small but significant quantities of choline. Examination of the refined oils, as used, has shown that they yield no choline when treated by the method given below.

It will be seen, therefore, that any method for the determination of egg in salad cream that depends on the choline content of the egg must also be capable of modification when white mustard is present. The method to be described determines total choline and is only applicable when the nature and amount of any mustard ingredient is known. Experiments are proceeding at the present time with the object of determining the choline from egg lecithin or from sinalbin separately.

Choline may be determined in a variety of ways, which include colorimetric,[15,16,17,18] gravimetric,[19,20] volumetric[21] and micro-biological methods.[22] For the present purpose, a colorimetric method was found to be suitable, in which the choline is precipitated by ammonium reineckate, $[Cr(NH_3)_2(SCN)_4]NH_4$, the choline reineckate filtered off, dissolved in acetone and the (pink) colour of the solution compared with standards similarly prepared. The use of artificial standards of methyl red in a buffer solution has been suggested.

EXPERIMENTAL

Choline has been determined in the various forms of liquid and dried egg at present available and in salad creams containing known amounts of egg. Where mustard is present the choline content of this ingredient has been determined in the same way, using 0·5 g. of the powder, and due correction made.

METHOD—

Weigh 0·5 g. of dried egg, 2·0 g. of frozen whole egg or 10 g. of salad cream into a 150-ml. flask containing a few glass beads and add 20 ml. of diluted hydrochloric acid (5 + 3). Warm the mixture on the steam-bath for 5 minutes to reduce subsequent frothing and then boil under a reflux condenser for 1 hour, in apparatus fitted with ground-glass joints. Wash down the condenser with 10 ml. of hot water and transfer the contents of the flask, when cool, to a separator. Extract the aqueous liquid by shaking with 40 ml. of methylated ether in order to remove the bulk of the fatty matter. Allow to separate and run the aqueous layer through a filter into a small conical flask. Wash the ether layer twice with 20-ml. portions of water and run the washings through the same filter. Boil the combined filtrate and washings to expel the ether and reduce the volume. After cooling, add the calculated volume of potassium hydroxide solution to neutralise the acid present, followed by 20 ml. of cold, saturated aqueous baryta solution. Add 1 drop of 1 per cent. alcoholic thymol-phthalein solution followed by glacial acetic acid added dropwise till the blue colour just disappears. Cool the liquid by standing it in a refrigerator for about 1 hour and filter by gentle suction through a filter funnel fitted with a sintered glass plate (No. 3 porosity) or a hardened filter-paper. Wash the filter with water and, to the clear combined filtrate and washings, add 6·0 ml. of 2 per cent. ammonium reineckate in methyl alcohol. Leave overnight in a refrigerator. Collect the pink precipitate of choline reineckate on a sintered glass filter funnel (No. 3 porosity) with gentle suction, wash twice with 10-ml. portions of ice-cold water and finally three times with 2·5-ml. portions of *n*-propyl alcohol. When free from solvent, dissolve the precipitate in acetone, collect the solution in a graduated flask and make up to 50 ml. with acetone. Transfer to a Nessler tube and match the colour against standards prepared as follows. Dissolve sufficient choline chloride in water to give a solution containing 2 per cent. of choline and standardise against 0·1 *N* silver nitrate.[23] From this prepare a dilute solution containing 1 mg. of choline per ml. Add appropriate volumes of this second solution to 150-ml. flasks, dilute to 50 ml. with water, add 6 ml. of 2 per cent. ammonium reineckate in methyl alcohol and proceed as above. In this way prepare the standards that may be necessary containing between 1 and 12 mg. of choline in 1 mg. steps. Fractional standards may be prepared therefrom by dilution with acetone. The colours should be matched within 30 minutes of dissolving the reineckate in acetone as some batches of the solvent have been found to cause a marked deterioration in the pink colour on standing. The reineckate precipitates can however be left on their separate filters after washing with the *n*-propyl alcohol and then dissolved when ready for matching.

RESULTS

Egg—Various types of egg products were examined by the above method. Half a gram was taken for each determination.

TABLE I

Product	Choline, mg. per g.	Product	Choline, mg. per g.
Dried whole egg:		Dried egg yolk:	
Canadian	16·0	Chinese	22·0
Canadian	14·0	Chinese	22·0
Swedish (S quality)	15·0	Foreign origin	22·0
Swedish (D quality)	14·0	Foreign origin	24·0
Foreign origin	17·0	Foreign origin	20·0
Sample A	16·0	Sample E	21·0
„ B	15·0	Frozen whole egg:	
„ C	16·0	U.S.A. (sample F)	4·5
Sugar-dried egg:		Egg albumen:	
Canadian (grade A)	10·0	Foreign origin	nil
Canadian	10·0		
Sample D	12·0		

NOTE—Samples A–F supplied by manufacturers of salad creams as typical of egg ingredient used.

Salad cream—Various commercial products. Ten grams of sample were taken for each determination.

TABLE II

		Mustard			Egg	
Sample	Egg component	Present, %	mg. of choline/g.	Choline in sample, mg./g.	Added, %	Found,* %
1	Dried whole egg, 16 mg. of choline/g.		—	0·18; 0·19	1·42	1·1; 1·2
2			—	0·51; 0·55	3·25	3·2; 3·4
3			—	0·90	5·62	5·6
4			—	1·20	7·64	7·5†
5		1·5	4·4	0·55	3·1	3·0
6	**	5·3	7·8	1·07	3·6	4·1
7	Dried egg yolk, 22 mg. of choline/g.	3·0	8·0	0·65	2·2	1·9
8		3·0	8·0	0·65	2·2	1·9
9		3·0	8·0	0·70	1·87	2·1
10		3·0	8·0	0·65	1·87	1·9
11	21 mg. of choline/g. ..	5·3	7·8	0·88	2·3	2·2
12	**	5·3	7·8	1·27	3·9	4·0
13		5·3	7·8	0·90	2·3	2·3
14	Frozen whole egg, 4·5 mg. of choline/g.**	3·0	8·0	0·6	10·5	8·0
15	**	3·0	8·0	0·65	10·5	9·1
16		5·5	9·0	0·90	9·6	9·1
17		5·5	9·0	0·75	6·2	5·7
18	Sugar-dried egg, 12 mg. of choline/g.	1·0 (approx.)	5·6	0·70	6·0	5·4

NOTE—Samples 1–4 specially prepared by B.F.M.I.R.A.

* Corrected for mustard content. ** Presumed choline content of egg ingredient.
† 7·75% found by B.F.M.I.R.A. using the same method.

DISCUSSION

As described, the choline is determined by matching colours in Nessler tubes. It is possible to use a Spekker absorptiometer, in which method the graph of the optical density of the solution plotted against choline is a straight line passing through the point of origin (private communication from C. L. Hinton).

The method has been applied to a number of samples of dried and liquid egg from different sources including bulk shipments and manufacturers' supplies. The results given in Table I show that the choline content of any one type of product is virtually constant.

For the purpose of calculation the following values may be taken—

Dried whole egg	.. ..	16 mg. of choline per g.
Dried egg yolk	.. ..	22 ,,
Frozen whole egg	.. ..	4·5 ,,
Sugar-dried egg	.. ..	11 ,,

These results are in conformity with the known relationship in chemical composition between the various products.

In applying the method to the determination of egg in salad cream, products prepared on a laboratory scale as well as proprietary brands have been examined.

The method can be applied directly to preparations that do not contain mustard, but in the presence of mustard it is necessary to know the nature and proportion of this ingredient in order to apply a correction. A sample of white mustard yields some 9 mg. of choline per gram when hydrolysed in the manner described, but most commercial flours were found to contain a lower proportion on account of blending. Work is in hand to devise a method for determining egg and mustard separately. Table II sets out the results obtained and satisfactory agreement is shown between the declared egg used and the amount found. It must be borne in mind that the majority of samples were commercial products and in many of them no sample of the actual egg ingredient used was available. In determining the choline, matching was carried out to the nearest 0·5 mg. standard.

The authors wish to thank the Government Chemist for permission to publish this paper and Miss B. Askew for assistance with much of the experimental work. Their thanks are also due to the British Food Manufacturing Industries Research Association, and to Messrs. Crosse and Blackwell, Heinz, Ocean Preserving Co., Rayner and Sutton, for supplies of salad cream.

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A Modified Rapid Technique for the Separation and Determination of Penicillin Types by Partition Chromatography on Paper

By G. A. GLISTER and A. GRAINGER

SYNOPSIS—A modified rapid-development technique for the micro-chromatographic assay of penicillin types is presented. Problems arising out of the quantitative interpretation are discussed, and it is indicated how, by using developed mixtures of pure penicillins as standards, the results are rendered more truly representative.

THE method of Goodall and Levi,[1] using the principle of Consden and Martin,[2] for the separation and estimation of penicillin types is well known and requires no introduction. It is recognised as the method giving the most comprehensive picture of the constituents in a mixture of penicillins. The recently published methods of Winsten and Spark[3] and Kluener[4] describe rapid development at room temperature. In view of the advantages offered by this in routine practice, our procedure was modified and, by using a simplified developing apparatus and narrow paper strips, the basic principle of filter-paper impregnated with phosphate buffer as support for the stationary aqueous phase and diethyl ether as the mobile phase being retained, development time was cut from 24 hours at 4° C. to 4 hours at room temperature.

Quantitative interpretation of chromatographic assays of this type has been the subject of much criticism and investigation, the issue being confused by the varying shapes of the inhibition zones that are produced and by the use of undeveloped standards prepared from only one entity. Maximum zone widths, lengths and areas have all been considered as measures of activity, and various corrective factors and formulae have been evolved, but so far none has proved satisfactory.

The hydroxamic acid method of Baker, Dobson and Martin[5] offers a useful alternative to the microbiological finish, but has the disadvantage that it is not so readily applicable to samples of low penicillin content, such as broth culture filtrates and the more dilute extracts therefrom. The quantitative interpretation of the microbiological chromatographic technique has therefore remained only comparatively accurate for any one technique.

EXPERIMENTAL

RAPID DEVELOPMENT—

Experimental work finally led to the use of micro-strips of Whatman No. 1 filter-paper (30 cm. × 1 cm.) impregnated with potassium phosphate, pH 6·2, and a decreased dose of penicillin of 5 units per strip instead of the original 30 units. Development of these strips with water-saturated diethyl ether as mobile phase for 3 to 4 hours at room temperature, and using a glass jar (14 inches × 8 inches diameter) and an evaporating basin as developing chamber and trough respectively, produced chromatograms which were reproducible and comparable with those produced by other methods, especially by that of Goodall and Levi in which strips twice the size are used and developed at 4° C. for 24 hours.

QUANTITATIVE INTERPRETATION—

Goodall and Levi, using standard dilutions of penicillin G, found that the ratio of the biological assay "slopes" from the inhibition zones of developed to those from undeveloped, or stationary, spots was approximately 1·2. They therefore introduced this factor as a correction to be applied to plate slopes determined by using undeveloped standards.

In our early experimental work we confirmed the factor over the range 30 to 0·3 units of penicillin G per spot (a concentration ratio of 100/1), but found that it varied considerably outside these limits.

Developed standards were then introduced as indicated below.

The grade of filter-paper and the pH of the phosphate buffer used to impregnate the paper, as well as the concentration of penicillin per strip and vapour conditions within the developing chamber, are all known to be factors affecting chromatographic separation and hence the shape of the final inhibition zones of the penicillins. Observation and measurement of numerous series of typical inhibition zones obtained from the paper chromatograms indicate that in descending order of development—

p-Hydroxybenzyl penicillin (X) ..	Produces a circular zone.
Benzyl penicillin (G)	All produce elliptical zones of similar shape, *e.g.*, the ratios of major to minor axis are constant.
Δ^2-Pentenyl penicillin (F)	
n-Amyl penicillin (dihydro F) ..	
n-Heptyl penicillin (K)	Produces an elliptical zone which is elongated out of all proportion to the others.

From consideration of the zone shapes it may be seen that if penicillin G standards (developed under identical conditions as the test penicillins) are used and the maximum zone width is taken as the criterion against log units then, as there is a slight decrease in zone width for an increase in zone length, there results an over-estimation of penicillin X and an under-estimation of penicillin K. Therefore a standard should be strictly only applicable to penicillins producing zones of identical shape. Fortunately for practical purposes, penicillin X is rarely present in commercial penicillin in quantities exceeding 0·5 per cent. (of activity) and may be neglected. Penicillin K, however, has to be considered. One or two closely associated unknown antibiotics are often present in small amounts and, as they are almost inseparable from penicillin K, they may contribute slightly to the zone length, although in practice the effect is negligible. While working with mixtures of pure penicillins G and K, a procedure was evolved by which this under-estimation of K could be overcome.

An equal number of units of penicillins G and K were mixed together in the same solution, and a series of dilutions, such as those shown in Table I, was prepared, each containing the number of units of G and K per ml. stated. Each sample was chromatographed in replicates of six strips and plated out as usual on assay plates, using *B. subtilis* as test organism. Maximum widths of the inhibition zones obtained were measured and graphs plotted against log units. The results showed that in each case the graph of maximum zone width plotted against log units was linear, but that the slope for developed K was less than that for developed G, as shown in Fig. 1.

TABLE I

A DEVELOPED "STANDARD CURVE"

Standard solution, containing penicillins G and K in equal amounts, *subtilis* units per ml.	Maximum zone width	
	G zone, mm.	K zone, mm.
10,000	37	35
7000	36	34
4000	34	33
2000	33	31
1000	31	30
700	28	27
400	27	26
200	25	24
100	22	20
70	20	20
40	18	19
20	16	18

The graph clearly demonstrates how penicillin K may be under-estimated by using a G slope for zone width of K. In mixtures where the proportion of K is very small, the reverse holds, an over-estimation being obtained below the intersection.

EXPERIMENTAL CONCLUSIONS—

From the experimental findings it was concluded that the inclusion of penicillin K with G as developed standard would greatly improve the accuracy of the quantitative procedure, G, F and dihydro F being calculated as usual with the G standards, and K being calculated separately with a standard of similar type. Mixtures containing appreciable amounts of penicillin X should be accompanied by standards which include X.

MODIFIED METHOD

APPARATUS AND REAGENTS—

Developing chamber and trough—A wide-necked cylindrical glass jar, 14 inches high and 8 inches in diam. fitted with a gas-tight lid, is suitable. The trough consists of a 3-inch porcelain evaporating basin resting on the top of an 11 to 12-inch cylinder which stands vertically in the centre of the jar. The cylinder is bandaged with absorbent lint.

Buffered paper strips—Sheets of Whatman No. 1 filter-paper are soaked in 20 per cent. w/v potassium phosphate buffer solution, pH 6·2, blotted between blotting paper, and air-dried. The sheets are then cut by means of a razor and a steel straight-edge into strips 30 cm. × 1 cm. in size.

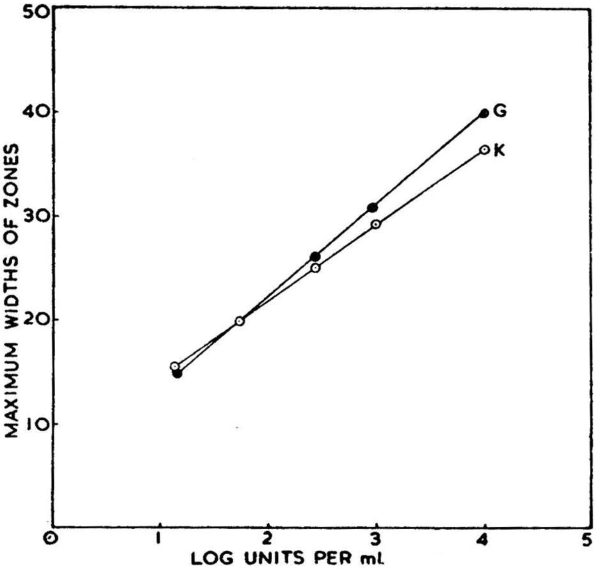

Fig. 1. Difference of slope for G and K

Micro-pipettes—These are made from 1-mm. bore heavy-walled glass tubing drawn out to give a long capillary tip. A calibration mark is made on the capillary at a point from which delivery of water on to No. 1 filter-paper produces a 2 to 3-mm. diameter spot. This volume is approximately 2 μl. These pipettes give relative or comparative measurements required by the technique. Accuracy must be maintained in the primary preparations of the standard and test solutions.

Assay plates—These are prepared from sheets of plate glass 19 inches × 14 inches. Half-inch wide strips may be cemented to form walls by means of cements such as Reuter's dichromate gelatin mixture.

Standard solutions—As the diameter - log units relationship is linear over a wide range, it is only necessary to prepare two standard solutions.

(*a*) *High standard*—containing 5000 *B. subtilis* units each of sodium penicillin G and K per ml.

(*b*) *Low standard*—containing 50 *B. subtilis* units of sodium penicillin G and K per ml.

The solutions are made in 1 per cent. phosphate buffer, pH 6·5, and usually the low standard is prepared from the high one by dilution.

Nutrient agar for assay plates—The medium contains: 10 g. of Peptone, 3 g. of Lab. Lemco, 2 g. of sodium chloride, 20 g. of agar and 1 litre of distilled water.

Dissolve the ingredients by boiling, and adjust the pH to 7·5. While still molten, dispense the medium into 410-ml. lots and sterilise in an autoclave.

Developing solvent—Anaesthetic diethyl ether saturated with distilled water at room temperature.

PROCEDURE—

Take the required number of buffered strips. For ordinary work nine are used, three for each standard and three for the test. Fold each strip 2 inches from one end and make a pencil-mark a half-inch from the fold in the centre of the strip on the long end of the fold. This serves as a point for the spot. The identification mark of the sample may be marked on the strip in pencil. By means of the same micro-pipette, spot identical volumes of the standards on to their respective strips. In the case of the test sample one should aim at spotting a similar amount of penicillin. With concentrated samples this may be achieved by preliminary dilution with 1 per cent. phosphate buffer to approximately 5000 *B. subtilis* units per ml.

When the test sample is of low activity, *e.g.*, as with culture filtrates, it is necessary to superimpose several spots, drying between applications, in order to keep a small concentrated spot. Thus the required units per spot may be obtained. Solutions in organic solvents may be applied direct in the same manner.

When the spots are dry, hang the strips in the developing chamber which should be previously prepared by soaking the cylinder bandage with water and placing a 1-inch layer of diethyl ether in the bottom of the jar. Place the short ends of the strips in the basin, with the long ends hanging down. Then fill the basin to within half an inch of the top with the wet ether and place a small glass block in the basin to hold the strips firmly. Cover the jar with the lid, and allow development to proceed for 3½ to 4 hours.

In the meantime assay plates should be poured with agar seeded with *B. subtilis* spores and stored in a cold chamber. When development is complete, remove the strips and lay them on the surface of the *B. subtilis*-seeded agar. Allow diffusion to proceed for half an hour and then incubate the plates at 27° C. overnight. Measure the maximum zone widths with a transparent plastic rule graduated in millimetres and calculate the averages.

CALCULATION (PENICILLIN X PRACTICALLY ABSENT)—

$$\frac{\text{Mean max. zone width high G standard} - \text{Mean max. zone width low G standard}}{\text{log conc. ratio } (e.g., 100/1)} \equiv \text{G slope}$$

Similarly for the K slope.

The mean maximum zone widths of G, F and dihydro F of the test divided by the G slope give values the antilogs of which are proportional to the activities (in *B. subtilis* units) of these types.

Similarly, the mean maximum width of the K zone of the test divided by the K slope gives a value whose antilog is proportional to its activity.

The individual proportionate activities expressed as percentage of the sum of these activities. ≡ percentage activity (*B. subtilis* units) for each type.

The percentage activity (*S. aureus* units) may be obtained by using this organism in place of *B. subtilis* in the technique. Alternatively, a series of standards could form a "developed standard curve" and a graph be plotted. Test readings could be made on the graph direct.

Typical results are shown in Tables II to IV.

TABLE II

ANALYSIS OF ARTIFICIALLY PREPARED MIXTURES OF PENICILLINS

Results expressed as activity (*B. subtilis* units), per cent.

Known penicillin K content, %	Found Goodall and Levi method, %	 Modified method, %
5	3·3	5·1
10	6·8	9·8
20	10·8	19·6
50	32·0	51·4
67	40·2	64·8

TABLE III

COMPARISON OF RESULTS FROM CULTURE FILTRATES

Goodall and Levi method				Modified method			
X	G %	F_1 and F_2 (dihydro) %	K %	X	G %	F_1 and F_2 (dihydro) %	K %
trace	94	3	3	trace	87	5	8
trace	97	1	2	trace	95	1	4
trace	92	4	4	trace	88	5	7
trace	80	6	14	trace	75	7	18

TABLE IV

REPLICATE ANALYSES OF THE SAME SAMPLE

G	F_1 and F_2 (dihydro)	K
92·4	3·1	4·5
93·8	2·2	4·0
91·0	2·7	6·3

We wish to thank the Directors of the Distillers Company (Biochemicals) Limited for their kind permission to publish this paper.

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Critical Factors in the Resorcinol Reaction for the Determination of Fructose

BY D. J. S. GRAY

SYNOPSIS—A method is described for the colorimetric determination of fructose in fermentation media by the use of the colour reaction between fructose, resorcinol and hydrochloric acid.

The effect of purity of the reagents has been studied, and it has been found that the intensity of the colour produced depends not only on the purity of the reagents, but also on the time of heating and the temperature of reaction.

As the fermentation media under examination contained 0·5 per cent. of ammonium nitrate, the effect of nitrate ion was also studied, and it was found that the intensity of the colour was increased by the presence of nitrate ion up to 0·25 per cent., beyond which value the intensity of the colour lessened. The ammonium ion alone had no effect.

Interference by glucose or kojic and gluconic acids is negligible. The fructose contents of three samples of fermentation media have been checked by the method of Jackson and Matthews, and gave comparable results.

THE colour reaction between fructose, resorcinol and hydrochloric acid was first described by Seliwanoff.[6] The reaction is not specific for fructose but is also given by other sugars that have a keto-group. Roe[5] has adapted the reaction to the colorimetric estimation of fructose in blood and urine and this method has been modified by Cole. This latter modification has been described by Bacon and Bell,[1] but, as yet, Cole has not published his work.

The method depends on the conversion of fructose to 5-hydroxymethylfurfural by strong hydrochloric acid, and the subsequent reaction of this substance with resorcinol to produce a compound which has a cherry-red colour. Glucose will give the reaction only if submitted to some preliminary treatment.[7] The stages of the conversion of fructose to 5-hydroxymethylfurfural have been discussed by Haworth and Jones.[3]

This reaction has been applied to the determination of fructose in fermentation media, where it was necessary to carry out analyses quickly and on small samples. As a result of this, certain critical conditions in the application of the method have been observed.

METHOD

The method outlined below is a modification of Roe's procedure.

REAGENTS—

Alcoholic resorcinol solution (0·1 *per cent.*)—0·5 g. resorcinol dissolved in 500 ml. of pure 95 per cent. alcohol.

Hydrochloric acid solution (*sp.gr.* 1·17)—The acid must be free from iron and give no blue colour when 10 ml. are mixed with a solution of starch and pure potassium iodide.

Standard fructose solution—A stock standard solution may be prepared by dissolving 0·5 g. of fructose in 500 ml. of distilled water saturated with benzoic acid. (Benzoic acid is added as a preservative.) Working standards may be obtained by diluting this solution.

PROCEDURE—

Measure 2 ml. of a solution containing 0 to 60 mg. of fructose per 100 ml. into a clean, dry test tube by means of a pipette. Add 2 ml. of alcoholic resorcinol solution and 6 ml. of hydrochloric acid. Mix the contents of the tube and suspend in a water-bath maintained at a constant temperature of 80° C. (±0·2° C.) for exactly 8 minutes. Remove from the bath and cool rapidly in running water. Allow the tube to stand for 10 minutes and then transfer the contents to an absorptiometer cell and measure the absorption immediately.

In the work described, a Spekker absorptiometer was used with Chance's glass filters, OB2, and 4-cm. cells. The red solution has a distinct absorption band at approximately 486 $m\mu$.[2]

NOTES ON THE METHOD

Efficiency of the method—One hundred ml. of a solution containing 437 mg. of fructose and 401 mg. of glucose were prepared. This solution was analysed by the method described and found to contain 438 mg. fructose.

Purity of alcohol—It is important that the reagents used for the reaction are of the highest purity. Errors may be introduced if the alcohol used to prepare the resorcinol solution contains aldehydes. A blank determination should give practically the same transmittance as pure water.

Purity of hydrochloric acid—The commercially available "reagent" hydrochloric acid proved also to be a source of error. The method was standardised with one batch of hydrochloric acid and reproducible results were obtained, but when another batch of acid was used the colours obtained with similar concentrations of fructose were less intense. A sample of pure hydrochloric acid was prepared and it was noted that the addition of hypochlorite to the reaction solution caused a reduction of colour intensity. It is probable that free chlorine was present in the batch of acid that gave low results.

Effect of iron—The presence of as little as 2 parts of ferric iron per million in the hydrochloric acid caused a marked increase in the intensity of the colour produced (Fig. 1). It is interesting to note that Cole,[1] in his modification of the method, recommends addition of ferric iron to the solution being analysed, presumably to intensify the colour produced by the reaction. In the presence of iron an orange-red colour is obtained, whereas when iron is absent this colour ranges from pink to cherry-red.

Concentration of hydrochloric acid—The concentration of hydrochloric acid in the solution must be kept constant when carrying out a series of analyses, as any variation may affect the results. The specific gravity of the acid used should be as close to 1·17 as possible. The intensity of the colour obtained increases with increasing acid concentration and the sensitivity of the method may be increased by using acid of higher specific gravity (Fig. 2).

Heating time and reaction temperature—The heating time (Fig. 3) and temperature of reaction are also variable factors and affect the intensity of the colour obtained. Roe recommended that the sample analysed be heated for 8 minutes at a temperature of 80° C. and used 30 per cent. hydrochloric acid. In the method described above the only alteration in procedure has been to increase the acid concentration to about 33·5 per cent. This has the advantage of increasing the sensitivity of the method and an absorption curve is obtained which is more nearly a straight line than that obtained with the lower acid concentration.

Effect of nitrate ion—The fermentation media under examination contained 0·5 per cent. of ammonium nitrate. Investigations showed that the presence of 0·01 per cent. of ammonium nitrate in the 2 ml. portion of the solution used for analysis caused a marked increase in the intensity of the colour produced by the reaction (Fig. 4). This intensification of colour is caused by the presence of nitrate and no increase is observed when ammonium alone is in the solution. When the ammonium nitrate concentration was increased to 0·25 per cent. the colour intensity was practically the same. However, a lessening of the colour intensity

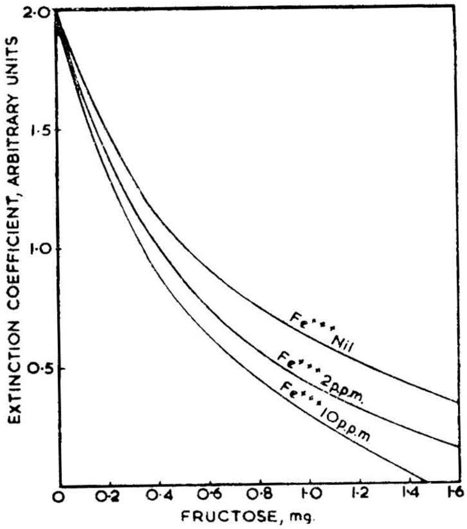

Fig. 1. Effect of iron. Heating time 5 min.

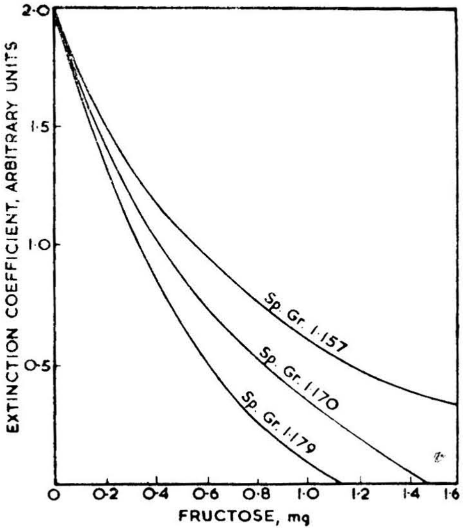

Fig. 2. Effect of specific gravity of hydrochloric acid used

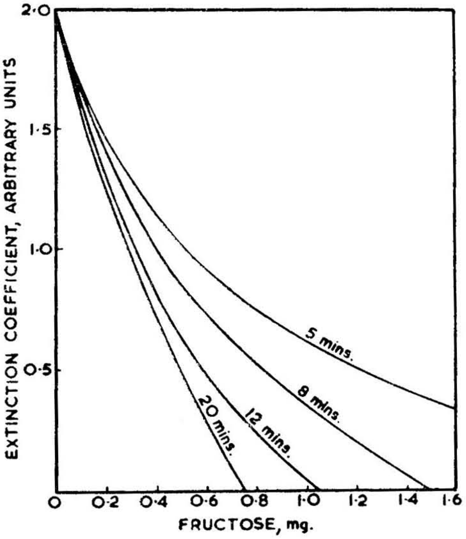

Fig. 3. Effect of heating time

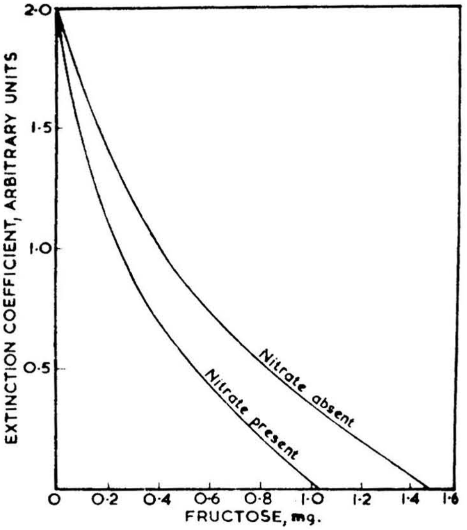

Fig. 4. Effect of nitrate ion

was noted when the ammonium nitrate concentration was increased beyond this limit. In view of the concordance of the absorption curves for fructose in the presence of concentrations of ammonium nitrate which vary between 0·01 and 0·25 per cent. in the 2-ml. portion analysed, it is possible to estimate the fructose content of fermentation media, which contain nitrate, by the preparation of a standard curve from a series of fructose solutions to which ammonium nitrate has been added. The reaction colour obtained in the presence of nitrate is similar to the colour observed when iron is present.

The analyses given below are of three samples of fermentation media. The fructose content has been determined by the method of Jackson and Matthews[4] and by the resorcinol method. The results were read from a standard absorption curve prepared from a series of fructose solutions containing ammonium nitrate.

Sample	Fructose, %	
	Jackson and Matthews	Resorcinol method
A	1·6	1·5
B	0·28	0·27
C	0·44	0·44

Interference by other substances—Glucose does not interfere appreciably with the reaction. A solution containing 600 mg. of glucose per 100 ml. was prepared. When submitted to the analytical procedure it gave a colour corresponding to 7·5 mg. of fructose per 100 ml. The presence of kojic and gluconic acids in the solution does not appear to affect the reaction.

It will be apparent that the resorcinol reaction for fructose can be used for the determination of fructose under a variety of conditions. By variation of the experimental conditions the method can be given a greater or a lesser sensitivity. The method is simple, accurate and rapid.

The author's thanks for permission to publish this work are due to the South African Council for Scientific and Industrial Research.

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ERRATUM: May (1950) issue, p. 251. Paper by Sutton and Markland. The date of reading should be *November 26th*, 1949.

The Quantitative Estimation of 1-Methyl-5-Amino-Acridine

By J. R. A. ANDERSON and M. LEDERER

SYNOPSIS—By double decomposition between aqueous solutions of 1-methyl-5-amino-acridine and picric acid, a yellow insoluble precipitate, $C_{20}H_{15}N_5O_7$, is formed quantitatively. The compound is only sparingly soluble in many common organic solvents, and melts at 274° C.

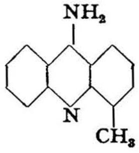
Aqueous solutions of 1-methyl-5-amino-acridine and picrolonic acid react quantitatively to give a yellow precipitate, $C_{24}H_{20}N_6O_5$, which is only sparingly soluble in water and in many common organic solvents. It melts at 296° to 298° C. with decomposition.

Both picric and picrolonic acids may be used as reagents for the gravimetric estimation of 1-methyl-5-amino-acridine and its salts, and are quantitative even in the presence of glucose or sodium chloride.

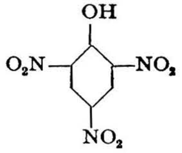
THE chemotherapeutic properties of 1-methyl-5-amino-acridine were discussed by Albert, and his co-workers.[1] A method for its synthesis was reported by Albert and Gledhill,[2] and the pharmaceutical properties of its hydrochloride were discussed by Falk and Lederer.[3] Pedley[4] has described a method of assay of the closely related 5-amino-acridine which utilises the insolubility of the dichromate, and Hall and Powell[5] utilised the insolubility of the ferrocyanides of euflavine, acriflavine and proflavine for their determination. Bolliger[6] has estimated proflavine, etc. by precipitation of the picrate at 0° C. and back-titration of the excess picric acid with methylene blue.

DISCUSSION

In this paper two methods for the quantitative estimation of 1-methyl-5-amino-acridine, in its salts and in solution, are described. The methods described by Pedley[4] and Hall and Powell[5] for the estimation of 5-amino-acridine hydrochloride were considered unsatisfactory for 1-methyl-5-amino-acridine and its salts. In the first method the dichromate of 1-methyl-5-amino-acridine is too soluble, and in the second[5] the procedure is rather tedious. The volumetric method recommended by Bolliger[6] is not readily applied to dilute solutions. It was found, however, that the picrate of 1-methyl-5-amino-acridine precipitates quantitatively under controlled conditions and can be weighed after drying at 105° C. A guide to the identification of the picrate is provided by the melting-point (which is accompanied by slight decomposition). The compound formed has the composition $C_{20}H_{15}N_5O_7$. Picrolonic acid (1-*p*-nitrophenyl-3-methyl-4-nitro-5-pyrazolone) has been estimated by Bolliger[7,8] by titration with standard solution of methylene blue in a manner similar to that employed for the estimation of picric acid.[6] Schiedwitz[9] has described its determination by converting it to its acridine salt, which has a low solubility and a high molecular weight. It was found that 1-methyl-5-amino-acridine hydrochloride reacted with picrolonic acid to yield a yellow insoluble precipitate; hence it was decided to investigate the use of picrolonic acid as a reagent for the quantitative determination of 1-methyl-5-amino-acridine and its salts. In the case of 1-methyl-5-amino-acridine hydrochloride monohydrate, the precipitate obtained may be weighed after drying at 130° C., and the melting-point, 296° to 298° C. (accompanied by slight decomposition), may be determined. The compound has the composition $C_{24}H_{20}N_6O_5$. The reactions of 1-methyl-5-amino-acridine with picric acid and with picrolonic acid are presumably molecular.

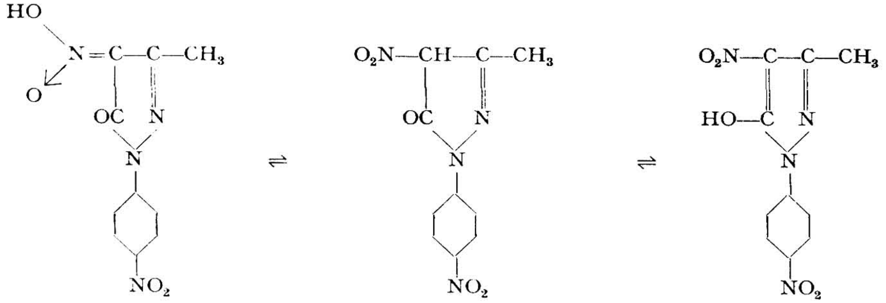


1-Methyl-5-amino-acridine



Picric acid

According to Welcher,[10] three possible tautomeric structures have been assigned to picrolonic acid, which may be shown as follows—



(I) (II) (III)

Picrolonic acid

Structures (I) and (III) account for the acid properties of the picrolonic acid molecule, although structure (I), containing the acid-form of the nitro group, seems the more probable.

PICRIC ACID METHOD

Dissolve a sample containing 0·1 g. of 1-methyl-5-amino-acridine or its salts in 100 ml. of water. If the insoluble free base is to be analysed, it will be found to dissolve freely in a buffer solution consisting of 260 g. of sodium acetate and 280 g. of 30 per cent. acetic acid per litre. Add 5 ml. of the acetate buffer and heat the solution until nearly boiling. Then add 15 ml. of a saturated solution of picric acid in water (100 per cent. excess) with constant stirring. Then leave the mixture at 0° C. for 3 hours. Transfer the precipitate to a sintered porcelain crucible and wash with ice-cold distilled water (approximately 50 ml.). Bring the crucible to constant weight at 105° C. and weigh as 1-methyl-5-amino-acridine monopicrate, a yellow crystalline powder, melting-point 274° C. (uncorrected under slight decomposition).

EXPERIMENTAL—

A sample of pure 1-methyl-5-amino-acridine was prepared, and it gave an equivalent weight of 208·2 by addition of excess of 0·1 *N* hydrochloric acid and back-titration with 0·1 *N* sodium hydroxide, corresponding to 99·9 per cent. of the base. This sample was used to determine the conditions for a suitable analysis, and the results are shown in Table I, using the method described above. A sample of the hydrochloride was then prepared (moisture content 12·90 per cent.), which gave on analysis 73·9 per cent. of 1-methyl-5-amino-acridine by the picric acid method, thus showing that this sample was the dihydrate of the hydrochloride. Glucose and sodium chloride were added to the solution for analysis and neither substance affected the results to any extent. The loss due to excessive washing of the precipitate was also investigated, and it was found that 100 ml. of water, on being poured over the precipitate, dissolved 1·8 mg., corresponding to about 0·5 per cent. of the total. The picrate was soluble in pyridine, somewhat so in alcohols, acetone and ethyl acetate, but only slightly soluble in most other common organic solvents. Details of results are given in Table I.

TABLE I

RECOVERY OF 1-METHYL-5-AMINO-ACRIDINE BY PICRIC ACID METHOD

Weight taken, g.	**Volume of solution, ml.**	Volume of saturated picric acid added, ml.	Temperature for complete precipitation, ° C.	Time of standing	**Recovery, %**
0·4000	100	50	*ca.* 15	A few minutes	99·4
0·1000	100	15	*ca.* 15	A few minutes	98·4
0·1000	100	15	*ca.* 15	18 hours	99·35
0·1000	200	15	0	1 hour	99·0
0·1000	100	15	0	18 hours	100·5
0·1000	100	15	0	3 hours	99·0

PICROLONIC ACID METHOD

Dissolve 0·1 g. of 1-methyl-5-amino-acridine hydrochloride monohydrate in 50 ml. distilled water, boil the solution and add 40 ml. of 0·01 *N* picrolonic acid, prepared as described by Dworzak and Reich-Rohrwig,[11] from a burette, dropwise and with constant stirring to the boiling solution. Then run in a further 40 ml. of 0·01 *N* picrolonic acid, giving 100 per cent. excess of precipitant. Allow the mixture to stand for at least 1 hour, and then cool to about 15° to 20° C., and transfer to a sintered crucible and wash with cold distilled water (approximately 60 ml.). Bring the crucible to constant weight at 130° C. and weigh as 1-methyl-5-amino-acridine mono-picrolonate, a yellowish crystalline powder.

EXPERIMENTAL—

Samples of pure 1-methyl-5-amino-acridine hydrochloride monohydrate weighing 0·1 g. (6·80 per cent. water of hydration; melting-point of free base, 196° to 197° C. (corrected)) were treated with 0·01 *N* picrolonic acid by the method described above. A 100 per cent. excess of picrolonic acid was found to be sufficient for complete precipitation. The precipitates formed were dried over sulphuric acid in a vacuum desiccator, an average yield of 100·3 per cent. of the monohydrated salt being obtained. Subsequent drying of a number of precipitates for about 2 hours at 130° C. gave the anhydrous salt, the yield of this being 99·7 to 100·1 per cent. of the theoretical yield. The addition of glucose or of sodium chloride to the solution being analysed had no serious effect on the results obtained. The anhydrous 1-methyl-5-amino-acridine mono-picrolonate showed only slight solubility in water and in many common organic solvents. It was soluble in pyridine and somewhat so in alcohols, acetone and ethyl acetate. It was found to have a melting-point of 296° to 298° C. (accompanied by decomposition). The analytical data is summarised in Table II.

TABLE II

RECOVERY OF 1-METHYL-5-AMINO-ACRIDINE HYDROCHLORIDE MONOHYDRATE BY PICROLONIC ACID METHOD

Weight taken, g.	Volume of solution, ml.	0·01 *N* picrolonic acid added, ml.	Temperature for complete precipitation, ° C.	Time of standing	Weight of glucose added, g.	Weight of sodium chloride added, g.	Recovery, %
0·1000	50	120	16	18 hours	—	—	99·7
0·1000	50	120	16	3 hours	—	—	99·7
0·1000	50	80	18	3 hours	—	—	100·1
0·1000	50	80	18	1 hour	—	—	99·7
0·1000	100	80	20	1 hour	5	—	99·1
0·1000	100	80	18	2 hours	—	0·9	100·1
0·1000	100	80	19	1·5 hours	1	0·9	99·9
0·1000	100	80	16	6 days	—	0·9	99·8

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August, 1949

The Photometric Determination of Phosphorus in Low-Alloy Steels

By A. BACON

SYNOPSIS—Although methods utilising the molybdate reaction and stannous chloride or sodium sulphite as the reducing agent have been described by previous workers, concordant results were not obtained when attempts were made to apply the technique as described. It was thought that incomplete reduction was the reason for this failure, and this paper describes the effect of residual ferric iron on the reduction of phosphomolybdate, both by stannous chloride and by ferrous sulphate.

The production of ferrous sulphate by the reduction of ferric iron with sulphurous acid is studied and some details of the fundamental reagent concentrations are also given.

From the results of these experiments a method has been devised which, if carefully controlled, gives results reproducible within ±0·001 per cent. of phosphorus. The values obtained by this method are slightly lower than those obtained by a referee gravimetric method, and calibration using steels analysed by the referee method is recommended.

A DIRECT method for the determination of phosphorus in steel has been described,[1] in which the phosphorus is converted to phosphomolybdate, followed by reduction to molybdenum blue by means of stannous chloride. Attempts to apply the method proved unsatisfactory and some of the fundamental conditions were therefore studied in order to evolve a satisfactory procedure.

Preliminary experiments were conducted in order to determine the effect of variations in the molybdate concentration and in acidity on the production of the yellow phosphomolybdate. These effects were studied on the Spekker absorptiometer, measurements being made in the violet waveband where the yellow phosphomolybdate exerts maximum absorption. As high concentrations of ammonium molybdate resulted in the formation of a yellow precipitate, presumably ammonium phosphomolybdate, the curves shown in Fig. 1 were produced using sodium molybdate. These results show that the optimum acidity, and also the acidity range over which maximum colour develops, increase with the sodium molybdate concentration. Readings showed the absorption of the solutions to be constant over a period of 30 minutes.

Substitution of ammonium molybdate at a concentration of 5 g. per litre for the same concentration of sodium molybdate had little effect on the readings obtained, and the absorption was almost constant over a period of 30 minutes. At a concentration of 10 g. of ammonium molybdate per litre, however, precipitation occurred within 10 minutes over an acidity range of 0·2 to 0·3 N sulphuric acid. The poor sensitivity and the resulting high concentrations of ferric salts, which absorb strongly in the violet waveband, prohibit the application of this method to the analysis of steels.

STANNOUS CHLORIDE REDUCTION

Previous work[2] on the determination of silicon by the formation of a silicomolybdate and subsequent reduction with stannous chloride has shown that the background colour is dependent on the concentrations of the three reagents, sulphuric acid, sodium (or ammonium) molybdate and stannous chloride. The effect of stannous chloride is shown in Fig. 2, where it can be seen that there is a critical acidity below which excessive reduction of the molybdic acid occurs. This critical acidity increases with the molybdate and stannous chloride concentrations, and although a stannous chloride concentration of 0·001 N was chosen for further experiments, acidities lower than 0·5 N sulphuric acid could not be studied when using a concentration of 5 g. of ammonium molybdate per litre. By reference to Fig. 1 it can be seen that the region of maximum formation of phosphomolybdate, as indicated by the absorption in the violet waveband, cannot be studied using stannous chloride. When the formation of silicomolybdate was studied, the solution was strongly acidified after silicomolybdate formation and before addition of the stannous chloride. It was not possible to do this with phosphomolybdate because decomposition occurred immediately.

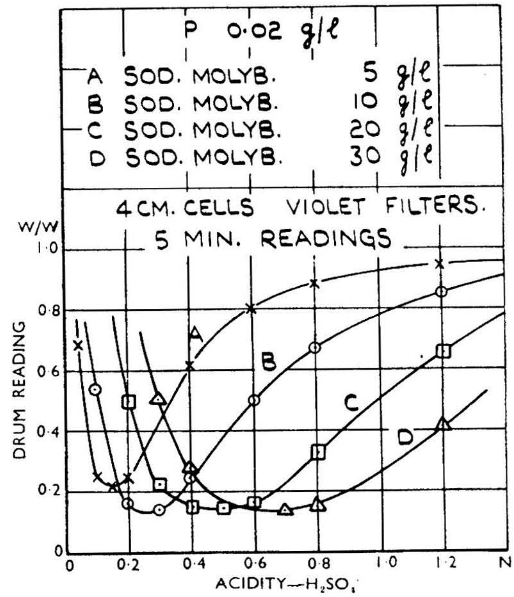

Fig. 1. Formation of yellow phosphomolybdate. The effect of molybdate concentrations on the acidity range

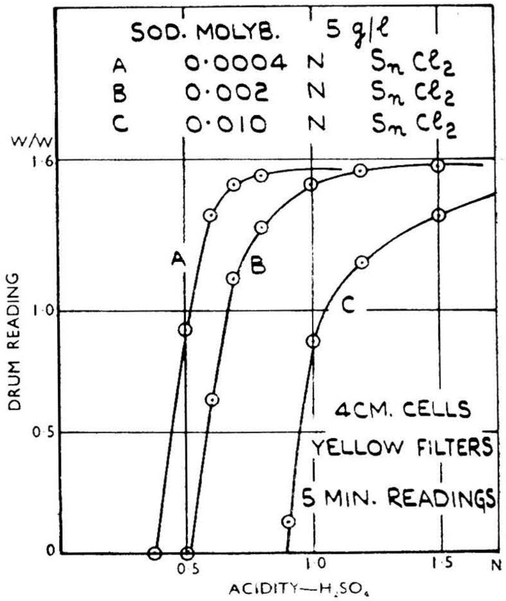

Fig. 2. Effect of stannous chloride on the background colour

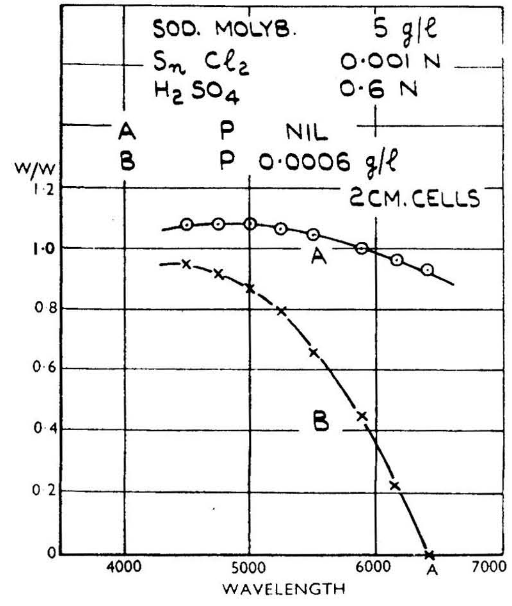

Fig. 3. Transmission curve for molybdenum blue

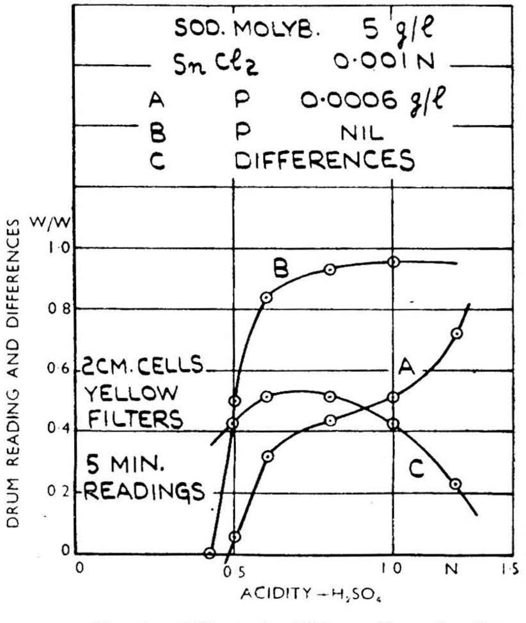

Fig. 4. Effect of acidity on the reduction by stannous chloride

ABSORPTION BY THE MOLYBDENUM-BLUE COMPOUND—

Although the maximum absorption by the reduced phosphomolybdate occurs in the red waveband, it was found more convenient to use yellow filters in conjunction with the mercury-vapour light source. The transmission curve shown in Fig. 3 illustrates the relative sensitivity in the yellow and red wavebands, and it should be noted that the background also shows maximum absorption in the red waveband. The transmission graph was obtained by plotting the Spekker readings against the wavelengths of maximum transmission of the colour filters, a tungsten-filament lamp being used as the source of illumination.

THE EFFECT OF ACIDITY ON THE REDUCTION BY STANNOUS CHLORIDE—

The effect of acidity on the production of the molybdenum-blue colour was studied in a series of experiments using concentrations of 5 g. of sodium molybdate per litre, 0·001 *N* stannous chloride, 0·0006 g. of phosphorus per litre, and various acidities in the range 0·5 to 1·2 *N* sulphuric acid. The molybdate was added to the acid solution containing phosphorus and then the stannous chloride was added with agitation of the solution. The results are shown in Fig. 4 (curve A), together with those for a similar series without the phosphorus (curve B), and the difference between the absorptions is shown in curve C. It can be seen that below 0·6 *N* sulphuric acid excessive reduction of molybdic acid occurs and that the maximum absorption attributable to the phosphorus is attained at approximately 0·7 *N* sulphuric acid.

THE EFFECT OF CONCENTRATION OF MOLYBDATE ON THE ACIDITY RANGE—

On increasing the strength of the sodium molybdate to 10 g. per litre, excessive reduction of molybdic acid occurred at acidities below 0·75 *N* sulphuric acid, and the optimum acidity was increased to 0·9 *N* sulphuric acid. Substitution of ammonium molybdate, 5 g. per litre, for the sodium salt did not result in any material change in the position of the curves shown in Fig. 4.

THE STABILITY OF THE MOLYBDENUM-BLUE COMPOUND—

Readings taken after the solutions had stood for 30 minutes showed that the background colour was stable at acidities greater than 0·6 *N* sulphuric acid. The solutions containing phosphorus showed a loss in absorption equivalent to 0·05 in drum reading over the acidity range of 0·7 to 1·0 *N*, while at 1·2 *N* sulphuric acid an increase in absorption occurred equivalent to 0·12 in drum reading.

THE EFFECT OF THE STANNOUS CHLORIDE CONCENTRATION—

In order to study the effect of the stannous chloride, a series of experiments was conducted using concentrations of 5 g. of ammonium molybdate per litre, 1·0 *N* sulphuric acid, 0·0006 g. of phosphorus per litre, and various concentrations of stannous chloride in the range 0·0002 to 0·010 *N*. The series was then repeated without the phosphorus. The results are shown in Fig. 5.

It can be seen that maximum sensitivity occurs at 0·0005 *N* stannous chloride. Below 0·0002 *N*, reduction of the phosphomolybdate is incomplete, and increasing the strength of the stannous chloride above 0·0005 *N* tends to reduce the sensitivity (curve C), because the background absorption increases more rapidly than that due to phosphorus. It was noted that decreasing the stannous chloride below 0·001 *N* did not result in any decrease in the background absorption. This residual colour is due to small amounts of phosphorus present as impurities in the reagents used. On the assumption that all of the impurity is in the sulphuric acid, the residual colour is equivalent to approximately 2 p.p.m. (w/v) of phosphorus in the concentrated acid.

A series of experiments similar to those used to construct Fig. 5 was conducted at an acidity of 0·8 *N* sulphuric acid instead of 1·0 *N*. The results obtained at this acidity are shown in Fig. 6, and by comparing the two figures it can be seen that the sensitivity at 0·001 *N* stannous chloride is practically unchanged. However, the background colour is much more sensitive to variations of the stannous chloride concentration and the sensitivity, as shown by the differences in the drum readings, increases with the stannous chloride concentration.

THE EFFECT OF FERRIC SULPHATE ON THE STANNOUS CHLORIDE REDUCTION—

The effect of ferric sulphate on the reduction of the phosphomolybdate by stannous chloride was studied in a series of experiments using concentrations of 5 g. of ammonium

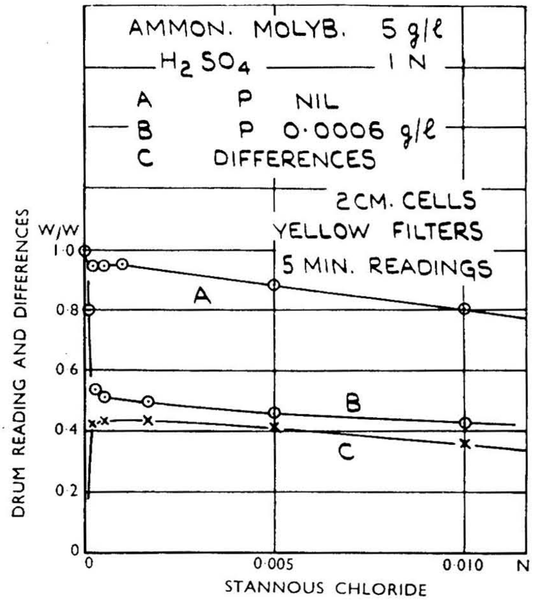

Fig. 5. Effect of stannous chloride on the sensitivity
(Sulphuric acid, 1·0 *N*)

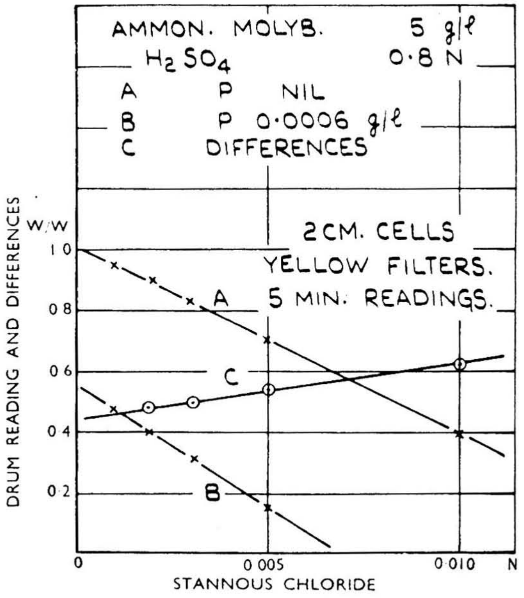

Fig. 6. Effect of stannous chloride on the sensitivity
(Sulphuric acid, 0·8 *N*)

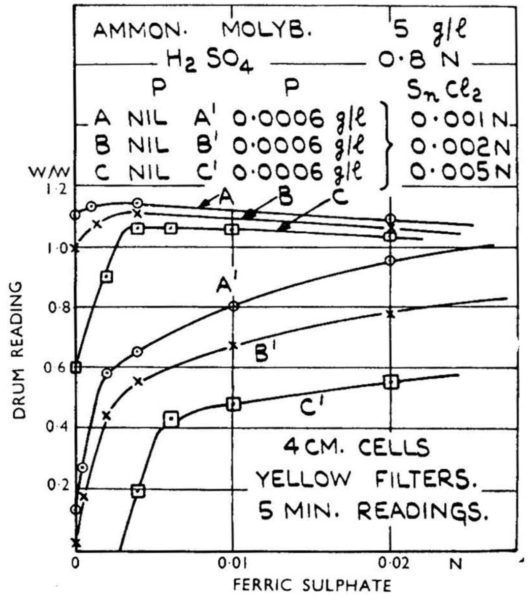

Fig. 7. Effect of ferric sulphate on the reduction of phosphomolybdate by stannous chloride

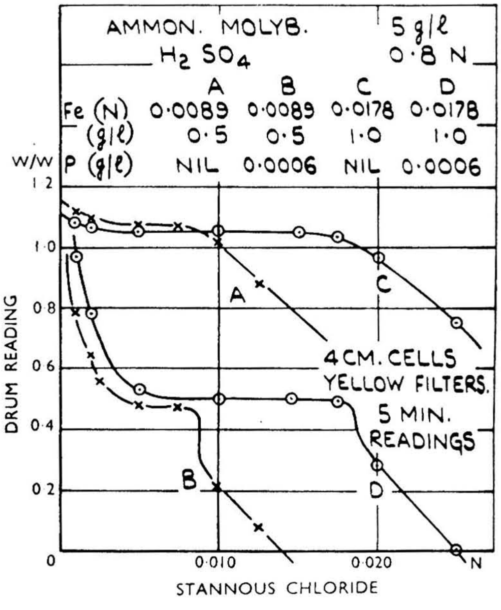

Fig. 8. Effect of stannous chloride on the sensitivity in the presence of ferric sulphate

molybdate per litre, 0·8 *N* sulphuric acid, 0·001 *N* stannous chloride and 0·0006 g. of phosphorus per litre and the iron content was varied over the range 0 to 0·02 *N* by means of a ferric sulphate solution. A second series was conducted without the phosphorus. The results given in Fig. 7, curves A and A′, show that the background colour and the colour associated with the phosphorus are seriously affected by quite low concentrations of ferric sulphate.

Repeating the experiments at two further concentrations of stannous chloride, 0·002 and 0·005 *N*, yielded curves B, B′ and C, C′, from which it is evident that the sudden change in slope of the curves occurs when the iron concentration is approximately equal to that of the stannous chloride. At a concentration of 0·02 *N* ferric iron, increasing the stannous chloride over the range 0·001 to 0·005 *N* had little effect on the background colour. Appreciable changes occur, however, in the solutions containing phosphorus, and a series of experiments was conducted to determine the stannous chloride concentration necessary to ensure maximum absorption when the solution contained approximately 0·010 and 0·020 *N* ferric sulphate. The results are given in Fig. 8 and show that as the concentration of the stannous chloride increases, the absorption of the solutions containing phosphorus increases, then remains constant for a period, and then suddenly increases, this sudden increase occurring when the stannous chloride concentration exceeds that of the ferric iron and coinciding with a similar rapid increase in absorption by those solutions not containing phosphorus. The values plotted in Fig. 8 are the average of a large number of determinations, for the readings obtained were not reproducible. Such factors as speed of addition of the reducing agent, temperature of the solution, agitation, and preparation of stannous chloride were all found to affect the partition of the stannous chloride between the ferric salt and the phosphomolybdate; the graphs showing the effect of ferric iron must, therefore, be interpreted generally.

THE STABILITY OF THE MOLYBDENUM-BLUE COMPOUND IN THE PRESENCE OF FERRIC SULPHATE—

A typical time-curve for a solution containing a concentration of stannous chloride less than that of the ferric iron is shown in Fig. 9. During the first few minutes, rapid formation of molybdenum blue occurs, but as reduction of the ferric iron proceeds, with a subsequent reduction in the stannous chloride concentration, the formation of molybdenum blue slows down and finally, when the whole of the stannous chloride has been removed by the ferric iron, a sudden drop in absorption occurs, most probably because the residual ferric salt attacks the molybdenum-blue compound. After attaining the new level of absorption, the solution fades slowly. The time-curve shown in Fig. 10 is for a solution containing a stannous chloride concentration larger than that required to reduce completely the ferric iron present. In the early stages, a considerable concentration of stannous chloride is present, and this results in the formation of deep and erratic background colours. The development of colour due to phosphorus progresses to a maximum in concordance with removal of ferric iron, with excess of stannous chloride always present. The results obtained from over 300 tests, covering a wide range of solution conditions, were not sufficiently reproducible to offer any hope of a satisfactory method of determining phosphorus in the presence of ferric salts.

FERROUS SULPHATE REDUCTION

Preliminary tests showed that the phosphomolybdate could be reduced by ferrous sulphate; the sensitivity, however, is considerably less than that attained when using stannous chloride. As with stannous chloride, the background colour was found to be dependent on the concentrations of the three reagents sulphuric acid, ammonium (or sodium) molybdate and ferrous sulphate. This is shown in Fig. 11, and it can be seen that there is a critical acidity below which excessive reduction of the molybdic acid occurs. With ferrous sulphate this acidity is less critically dependent on the concentrations of reducing agent and molybdate than with stannous chloride, and comparatively high concentrations of ferrous sulphate produce only slight background colours. The acidity below which excessive reduction of molybdic acid occurs even in the presence of 0·4 *N* ferrous sulphate is in the region of 0·3 to 0·4 *N* sulphuric acid. Substitution of ferrous sulphate for stannous chloride does not result in any material change in the transmission curve of the molybdenum-blue colour.

THE EFFECT OF ACIDITY ON THE REDUCTION BY FERROUS SULPHATE—

The effect of acidity on the production of the molybdenum-blue colour was studied in a series of experiments using concentrations of 5 g. of sodium molybdate per litre, 0·004 g.

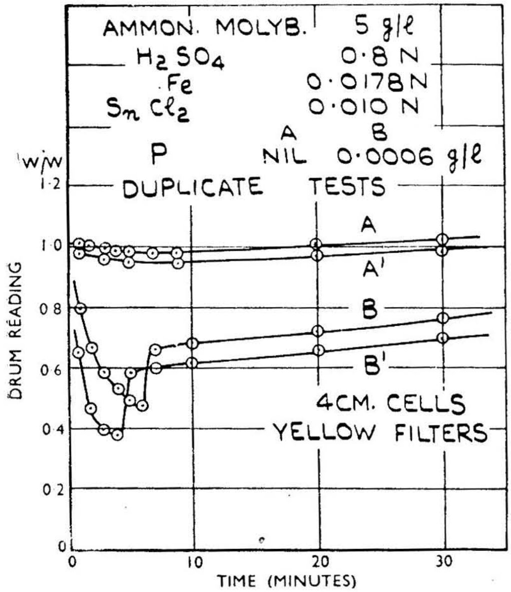

Fig. 9. Time - absorption curve. Stannous chloride less than ferric iron concentration

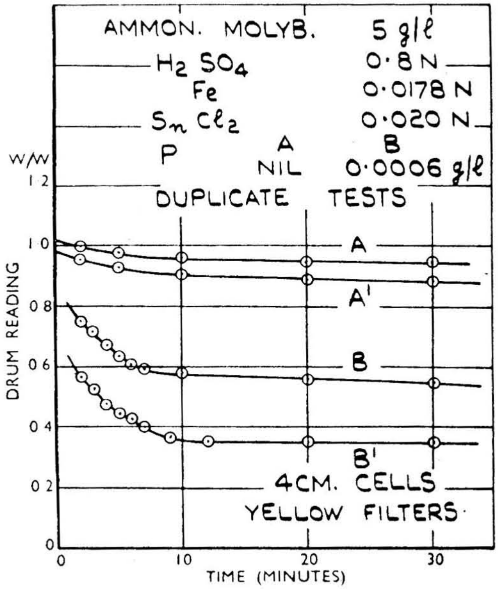

Fig. 10. Time - absorption curve. Stannous chloride greater than ferric iron concentration

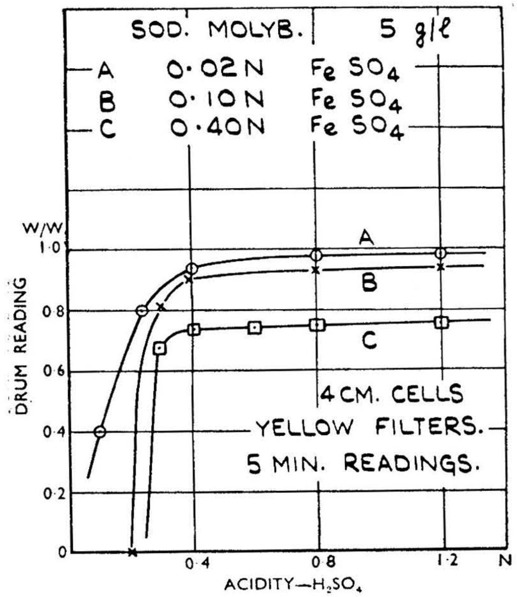

Fig. 11. Effect of ferrous sulphate on the background colour

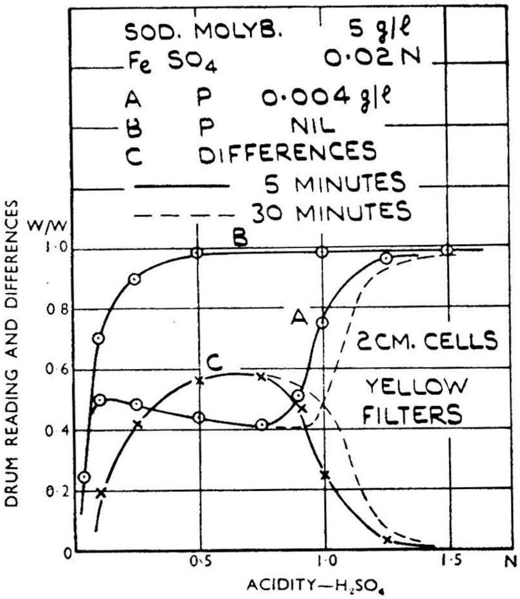

Fig. 12. Effect of acidity on the reduction by ferrous sulphate

of phosphorus per litre, 0·02 *N* ferrous sulphate and various acidities in the range 0·1 to 1·5 *N* sulphuric acid. The results are shown in Fig. 12, curve A, together with those for a similar series conducted without phosphorus, curve B. The difference in absorption between A and B is shown in curve C. From these curves it can be seen that, under these conditions, maximum sensitivity is attained at 0·6 *N* sulphuric acid, and that the absorption by those solutions containing phosphorus decreases rapidly at acidities above 0·75 *N* sulphuric acid. At acidities greater than 0·3 *N* sulphuric acid, the background absorption is slight.

THE EFFECT OF CONCENTRATION OF MOLYBDATE ON THE ACIDITY RANGE—

Substitution of ammonium molybdate, 5 g. per litre, for the sodium salt had little effect on the values obtained, the curves being similar in shape but with a slight displacement towards the higher acidities.

Increasing the concentration of the sodium molybdate to 10 g. per litre resulted in excessive blanks occurring at acidities below 0·5 *N* sulphuric acid. The difference curve was similar in shape and magnitude, but the peak occurred in the region of 0·8 to 1·0 *N* sulphuric acid.

THE STABILITY OF THE MOLYBDENUM-BLUE COMPOUND—

Readings taken after 30 minutes showed the background colour to be stable at acidities greater than 0·25 *N* sulphuric acid. The solutions containing phosphorus were stable up to 0·75 *N* sulphuric acid. Above this value the solutions showed an appreciable increase in absorption during a period of 30 minutes.

THE EFFECT OF THE FERROUS SULPHATE CONCENTRATION—

It can be seen from Fig. 13 that the background colour is proportional to the concentration of ferrous sulphate. It can also be seen that a stage is reached where any further addition of ferrous sulphate does not produce an increase in absorption by the solution containing phosphorus. At a concentration of 0·004 g. of phosphorus per litre, maximum sensitivity, as shown by curve C, is attained at a ferrous sulphate concentration of 0·05 *N*. A further series of experiments showed that 0·016 g. of phosphorus per litre required a concentration of 0·15 *N* ferrous sulphate to attain maximum sensitivity. It was also deduced from these experiments that when sufficient ferrous sulphate was present to ensure maximum sensitivity, the absorption produced was proportional to the phosphorus content. It is evident from these results that the values shown in Fig. 12 were obtained at a ferrous sulphate concentration that was insufficient to ensure maximum colour from the phosphorus. When these experiments were repeated at a concentration of 0·1 *N* ferrous sulphate, the shape and position of the curves were not affected by the values obtained. The maximum sensitivity, as indicated by the peak of the difference curve, was slightly increased to a difference of 0·62 in drum reading.

THE EFFECT OF FERRIC SULPHATE ON THE FERROUS SULPHATE REDUCTION—

In order to study the effect of ferric sulphate on the reduction of the phosphomolybdate by ferrous sulphate, a series of experiments was conducted using concentrations of 5 g. of ammonium molybdate per litre, 0·8 *N* sulphuric acid, 0·02 *N* ferrous sulphate and 0·004 g. of phosphorus per litre. The ferric iron content was varied over the range 0 to 0·012 *N* by means of a ferric sulphate solution. The series was repeated omitting the phosphorus. Three further series were conducted at ferrous sulphate concentrations of 0·10, 0·20 and 0·4 *N*, and the results are shown in Fig. 14. A study of this figure shows that the absorption due to phosphorus is considerably reduced by the introduction of ferric sulphate, and that the effect is minimised by increasing the concentration of the ferrous sulphate. The background colour is unaffected by the ferric salt.

From Fig. 14 conditions can be derived such that a range of 0 to 0·5 per cent. of phosphorus in steel can be directly determined in the presence of the ferric iron; but for a more limited phosphorus range, employing a larger weight of sample, the ferric iron must be reduced prior to coloration.

THE STABILITY OF THE MOLYBDENUM-BLUE COMPOUND IN THE PRESENCE OF FERRIC SULPHATE—

Readings taken over a period of 40 minutes during the determination of the values used in the construction of the curves shown in Fig. 14 showed that the background colour

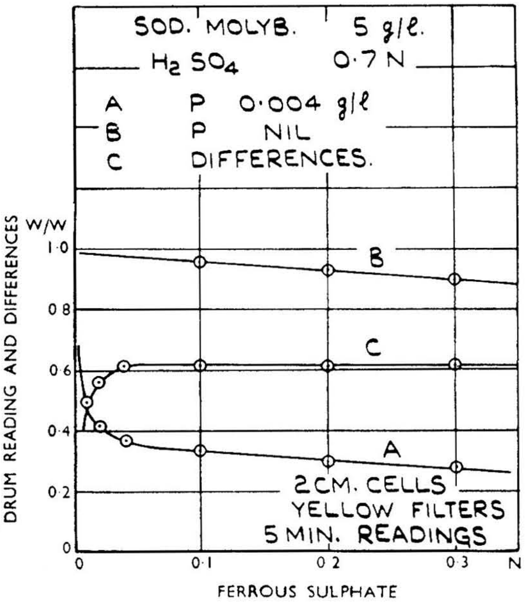

Fig. 13. The effect of ferrous sulphate on the sensitivity

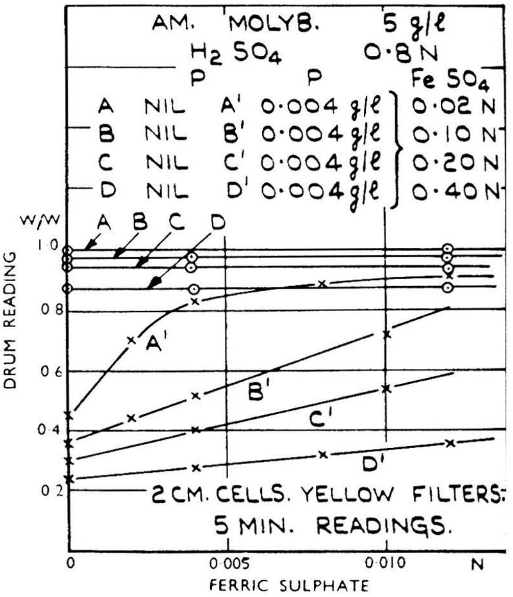

Fig. 14. The effect of ferric sulphate on the reduction of phosphomolybdate by ferrous sulphate

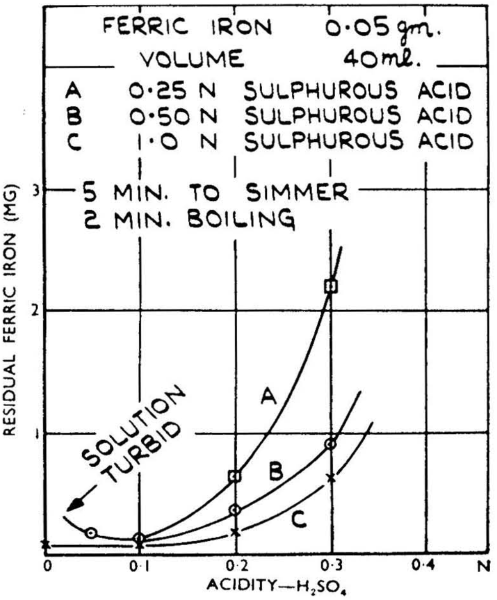

Fig. 15. Reduction of 0·05 g. of ferric iron by sulphurous acid

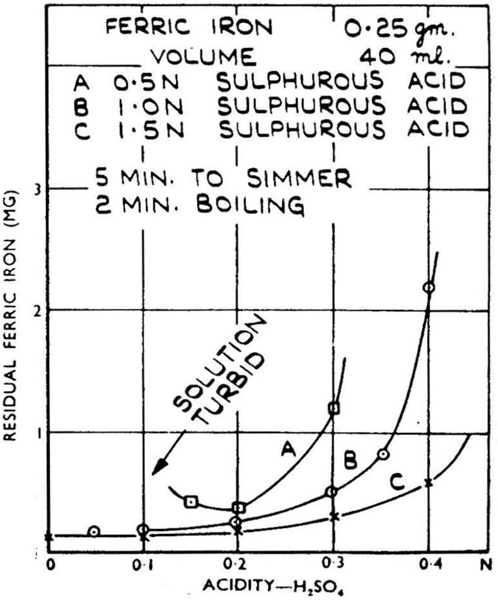

Fig. 16. Reduction of 0·25 g. of ferric iron by sulphurous acid

was stable. The solutions containing phosphorus, however, deepened slightly to a maximum absorption at 20 minutes, both in the presence and in the absence of iron. This is not unexpected, for it can be seen from Fig. 12 that the acidity at 0·8 *N* sulphuric acid is slightly greater than the optimum.

REDUCTION OF THE FERRIC IRON BY SULPHUROUS ACID

For the experiments on the reduction of ferric sulphate by sulphurous acid, a solution of ferric sulphate was prepared and the iron content determined by reduction with stannous chloride and then titrating with standard potassium dichromate using sodium diphenylamine sulphonate as internal indicator. The acidity of the ferric sulphate solution was determined by adding an excess of standard alkali and titrating back with standard acid using phenolphthalein as indicator. The sulphuric acid generated during the reduction was taken into account during the construction of the graphs. Thus for zero acidity, before adding the sulphurous acid, an alkali addition was made which neutralised the free sulphuric acid, the acid associated with the ferric sulphate and the sulphuric acid generated during reduction.

Solutions of sodium sulphite prepared from various samples of the salt were so variable and weak that recourse was had to a saturated solution of sulphur dioxide in water as the reducing agent. The solution was standardised against potassium permanganate, and the strength so found was regularly between 2·0 and 2·5 *N*. Preliminary tests showed that immersion in boiling water for at least 30 minutes was necessary for the completion of the reduction by the sulphur dioxide. Heating rapidly to boiling-point, however, resulted in the sulphur dioxide being evolved too quickly. Experiments showed that by heating slowly so that 5 minutes elapsed before the appearance of gas bubbles, efficient reduction by the sulphurous acid occurred. The introduction of a short period, about 2 minutes, of boiling resulted in a slightly better reduction, presumably because of a rise in pH as the sulphur dioxide was evolved. Tests showed that increasing the heating period and a variation of 1 minute in the boiling period had little effect on the residual ferric iron. All the experiments on the reduction by sulphurous acid, described below, were therefore conducted by using gentle heating for 5 minutes followed by boiling for 2 minutes. The residual ferric iron was determined by a thiocyanate absorptiometric method.

APPLICATION TO THE STANNOUS CHLORIDE METHOD—

It can be calculated from Fig. 6 that, using a sample weight of 0·05 g. and a volume of 50 ml. for coloration, the range covered by the use of 4-cm. cells and yellow filters would be from 0 to 0·06 per cent. of phosphorus in the absence of ferric iron. By reference to Fig. 7 it can be deduced that the introduction of 0·0001 g. of ferric iron results in a change in absorption of 1 per cent. The prior reduction of the ferric salt by sulphurous acid should, therefore, ensure a difference of less than ± 0·0001 g. between the ferric iron in the test solution and the ferric iron content of the solutions used to determine the calibration points, if an accuracy of ± 1 per cent. is to be attained. Experiments, using 0·05 g. of ferric iron, were conducted, therefore, to determine the optimum conditions for the reduction by sulphurous acid. The results are given in Fig. 15 and show that maximum reduction is obtained over an acidity range of 0 to 0·1 *N*. Over this range, as reduction occurs, the acid is associated with the iron as ferrous sulphate. At higher acidities, the free acid causes a rapid fall in pH which prevents reduction of the ferric salt by sulphurous acid. At low concentrations of sulphurous acid, and in the presence of only small amounts of free acid, the precipitated ferric hydrate fails to re-dissolve.

APPLICATION TO THE FERROUS SULPHATE METHOD—

It can be calculated from Fig. 13 that, using a 0·25-g. sample and a volume of 50 ml. for coloration, the range of phosphorus contents that can be determined by the use of 4-cm. cells and yellow filters would be 0 to 0·065 per cent. of phosphorus in the absence of ferric iron. By reference to Fig. 14, it may be deduced that the introduction of 0·0007 g. of ferric iron results in a change in absorption of 1 per cent. The prior reduction of the ferric salt by sulphurous acid should therefore ensure a difference of less than ± 0·0007 g. between the ferric iron in the test solution and the ferric iron contents of the solutions used in the determination of the calibration points, if an accuracy of ± 1 per cent. is to be attained. Experiments, using 0·25 g. of ferric iron, were, therefore, conducted to determine the optimum conditions for the reduction by sulphurous acid. The results are given in Fig. 16 and are similar to those obtained using 0·05 g. of ferric iron. At the lower sulphurous acid concentration of 0·5 *N*, the ferric hydrate fails to re-dissolve at acidities of less than 0·15 *N* sulphuric acid. Maximum reduction is attained over an acidity range of 0 to 0·2 *N* sulphuric acid; the increase in the acidity range is caused by the higher concentration of ferrous sulphate.

CHOICE OF REDUCING AGENT—

The values in Figs. 15 and 16 were obtained by using pure ferric sulphate, and further experiments were carried out to determine the residual ferric iron after a sulphurous acid reduction on solutions prepared from standard steels. For these experiments, the steels were dissolved in dilute nitric acid, which was subsequently removed by fuming with sulphuric acid. Suitable conditions were deduced from Figs. 15 and 16 to ensure maximum reduction by the sulphurous acid, and the results are shown in Table I.

TABLE I

SULPHUROUS ACID REDUCTION USING STANDARD STEELS

Sample	Residual ferric iron after treatment of 0·05-g. sample, g.	Residual ferric iron after treatment of 0·25-g. sample. g.
Electrolytically pure iron ..	0·00007	0·00012
B.C.S. 218	0·000145	0·000385
B.C.S. 225	0·000145	0·00030

Although the residual ferric iron is greater for the B.C.S. samples than for the electrolytically pure samples, the difference is sufficiently small to permit the use of either reducing agent in the development of the phosphorus colour. It has already been shown that the stannous chloride method is more sensitive to changes in the concentrations of the reducing agent and ferric iron, and changes in acidity; these changes appear to be some of the reasons why such poor reproduction was attained during attempts to evolve a method based on the stannous chloride reduction after a preliminary reduction of the ferric iron by sulphurous acid. The background colours were difficult to reproduce and apparently depended on the order of addition of the reagents, the speed at which the reducing agent was added and the temperature of the solution. The results obtained were unsatisfactory, and therefore attention has been confined to the use of ferrous sulphate in the development of a method for determining phosphorus in steel.

APPLICATION OF THE FERROUS SULPHATE METHOD TO THE DETERMINATION OF PHOSPHORUS IN STEEL

FUMING WITH SULPHURIC ACID TO REMOVE NITRIC ACID—

Several 0·25-g. samples of pure iron were dissolved in dilute nitric acid, 2·5 ml. of 10 *N* sulphuric acid were added, and the solutions were fumed for various times and subsequently reduced by sulphurous acid after neutralising with 6 ml. of 4 *N* caustic soda. The residual ferric iron was determined by thiocyanate, and the results, given in Table II, indicate that a fuming period of 5 minutes is sufficient.

TABLE II

THE EFFECT OF THE FUMING PERIOD ON THE AMOUNT OF FERRIC IRON REMAINING AFTER REDUCTION BY SULPHUROUS ACID

Samples of pure iron weighing 0·25 g.

Fuming period, minutes ..	0·5	1	2	5	20
Residual ferric iron, g.	0·00050	0·00036	0·00042	0·00020	0·00025

A similar series of experiments was conducted to determine the quantity of sulphuric acid lost during fuming in a covered beaker for 5 minutes. The samples used were 0·25 g. of pure iron, and 25 ml. of *N* sulphuric acid were added. After fuming, the sulphuric acid that remained was determined by titration, and 20·4, 22·0, 19·5 and 19·5 ml. were found. These results show that an average loss of 5 ml. of *N* sulphuric acid occurs. That phosphorus was not lost during this fuming period was proved by adding phosphorus both before and after fuming; the readings obtained were identical.

FINAL ACIDITY FOR PRODUCTION OF MAXIMUM SENSITIVITY—

After reduction, the addition of 3 ml. of 10 *N* sulphuric acid in conjunction with that introduced by the ferrous sulphate reagent ensures an acidity of 0·7 *N* in a final bulk of 50 ml., and this can be seen from Fig. 12 to give the maximum sensitivity when buffered by 5 g. of ammonium molybdate per litre.

STABILITY TESTS TO ESTABLISH THE STANDING PERIOD—

Readings taken at intervals over a period of 1 hour showed that maximum colour was only just developed in 5 minutes, and as the absorption remained constant for a further 55 minutes, a standing period of 10 minutes was chosen.

METHOD FOR THE DETERMINATION OF PHOSPHORUS IN STEEL

SOLUTIONS—

Ferrous sulphate solution—Make a 14 per cent. solution by dissolving 14 g. of pure ferrous sulphate in approximately 80 ml. of water containing 5 ml. of 10 *N* sulphuric acid, and then dilute to 100 ml.

Reference solution—Dissolve 1·28 g. of pure ferrous sulphate in 5 ml. of dilute nitric acid, sp.gr. 1·2, add 1·6 ml. of 10 *N* sulphuric acid and treat according to the procedure given below.

PROCEDURE—

Dissolve 0·25 g. of steel in 5 ml. of dilute nitric acid, sp.gr. 1·2. Add 2·5 ml. of 10 *N* sulphuric acid and a few drops of hydrochloric acid to prevent spurting; boil down and fume for 5 minutes. A 100-ml. beaker is recommended and the cover should not be removed during fuming. Cool, add 10 ml. of water and digest on a hot-plate to a clear solution. Cool and add 6 ml. of 4 *N* sodium hydroxide, agitating the solution. Add 25 ml. of water saturated with sulphur dioxide (the solution should be between 2·0 and 2·5 *N*). Heat slowly on the hot-plate, allowing at least 5 minutes to elapse before the evolution of gas bubbles, and then boil for 2 minutes. Add 3 ml. of 10 *N* sulphuric acid and boil down to 30 ml. Cool, add 5 ml. of 5 per cent. ammonium molybdate solution while agitating the solution, followed by 10 ml. of the 14 per cent. solution of ferrous sulphate. Transfer to a 50-ml. graduated flask, dilute to the mark, and allow the solution to stand for 10 minutes. The temperature of the solution should be within ± 2° C. of the calibration temperature. Read the absorption on the Spekker using 4-cm. cells, yellow filters and a setting water - water of 1·0. Derive the differences with respect to the reference solution and convert them to the phosphorus content by reference to the calibration curve.

CALIBRATION—

Phosphorus solution—Dissolve 0·4260 g. of di-ammonium hydrogen phosphate, $(NH_4)_2HPO_4$, in water and dilute to exactly 1 litre.

$$1 \text{ ml.} \equiv 0{\cdot}0001 \text{ g. of phosphorus.}$$

Dissolve several 0·25-g. samples of pure iron in 5 ml. of dilute nitric acid and add incremental amounts of phosphorus as shown below—

Phosphorus solution, ml.	0	0·5	1·0	1·5
Phosphorus, per cent.	0	0·02	0·04	0·06

Proceed as described in the method, derive differences with respect to the zero calibration-point and construct a calibration graph by plotting these differences against the phosphorus added.

RELATING THE DIFFERENCES OBTAINED BY THE METHOD TO THE CALIBRATION SYSTEM—

The readings obtained on a calibration series using pure iron are given in Table III, and differences are shown derived with respect to (*a*) the water reading of the cell and (*b*) the reading of the zero calibrating solution.

TABLE III

CALIBRATION SERIES USING PURE IRON

Cell reading for water only = 1·00

Phosphorus, %	Drum reading		(a) Differences with respect to readings of cell containing water		(b) Differences with respect to readings of zero phosphorus solution	
0	0·80	0·805	0·20	0·195	0	0
0·02	0·475	0·475	0·525	0·525	0·325	0·330
0·04	0·16	0·155	0·84	0·845	0·64	0·65
0·06	0·05*	0·045*	1·15	1·155	0·95	0·96

* Reading obtained using W/W = 1·20

Construction of a calibration graph from the first series (*a*) of differences results in a calibration curve which intersects the ordinate at an absorption value equivalent to 0·20 difference in drum reading. This absorption is produced by

(*a*) the background absorption,
(*b*) the phosphorus in the pure iron,
(*c*) the phosphorus in the reagents,

and any change in these factors results in a different calibration curve. In the case of a steel sample, when differences are derived with respect to the water reading of the cell, the absorption is due to

(*a*) the background absorption,
(*b*) the phosphorus in the reagents,
(*c*) the inherent colour of the solution,
(*d*) the phosphorus content of the steel.

It can be seen that the phosphorus content derived from the calibration graph would be in error if the background absorption or the phosphorus in the reagents was different from that used in calibration. It would also be in error to the extent of the phosphorus content of the pure iron and the absorption due to the inherent colour of the solution (alloying elements). The system is similar in principle to that employing direct readings and no advantage is gained by deriving differences with respect to the water reading of the cell.

Construction of a calibration graph from the second series (*b*) of differences results in a calibration curve which passes through the origin. The position of this curve is not affected by the background absorption, the phosphorus content of the pure iron or the phosphorus content of the reagents, provided they are constant for the calibration series. In the case of the steel samples, however, differences must be derived such that the value obtained for the absorption is due only to the phosphorus content of the steel. This can only be attained if the reference solution compensates for all absorptions other than that due to the phosphorus content of the steel. Where this is not possible, corrections must be applied. Any reagent which is introduced into the reference solution but not into the sample solution must be free from phosphorus, or the equivalent phosphorus content must be determined and a correction applied.

DETERMINATION OF THE SUBSIDIARY ABSORPTIONS

PHOSPHORUS INTRODUCED WITH THE IRON—

A calibration graph was prepared by substituting the corresponding quantity of ferrous sulphate for the pure iron used when constructing Table III. Allowance was made for the acid radicle introduced, and the values obtained are given in Table IV.

The slope of the calibration curve is the same in both instances, but the difference in absorption between the corresponding calibration points is 0·07 drum difference, equivalent to 0·004 per cent. of phosphorus. This corresponds to the difference in phosphorus content of the two sources of the iron.

In order to determine the phosphorus content of the ferrous sulphate, 28 g. were dissolved in water, 5 mg. of ferric iron and 2 drops of ammonia were added and the solution was boiled and filtered. The precipitate was redissolved and a phosphorus determination conducted as in the method for steel. A control was conducted simultaneously and the difference in absorption read on the calibration graph as the equivalent phosphorus percentage. This value was 0·0105 per cent. of phosphorus, and thus the equivalent phosphorus percentage introduced into the solution by 1·28 g. of ferrous sulphate is of the order of 0·0005 per cent. of phosphorus. As previously stated, when the calibration curve is constructed from the differences derived with respect to the zero phosphorus solution, the position of the curve is unaffected whether pure iron or ferrous sulphate is used. It is preferable, however, to use ferrous sulphate to produce the reference solution in order to minimise the correction which must be applied. It must be emphasised that, in the ferrous sulphate method, the base metal after reduction becomes a reagent and affects the background colour. It cannot be omitted from the reference solution because it accounts for approximately half the concentration of reducing agent.

TABLE IV

CALIBRATION SERIES USING FERROUS SULPHATE

Cell reading for water only = 1·00

Phosphorus, %	Drum reading		(*a*) Differences with respect to readings of cell containing water		(*b*) Differences with respect to readings of zero phosphorus solution	
0	0·87	0·87	0·13	0·13	0	0
0·02	0·55	0·555	0·45	0·445	0·32	0·315
0·04	0·225	0·230	0·775	0·77	0·645	0·64
0·06	0·12*	0·125*	1·08	1·075	0·95	0·945

* Reading obtained using W/W = 1·20.

THE REFERENCE SOLUTION—

Experiments were conducted to determine whether the phosphorus colour could be completely inhibited by strong acid, but with samples containing 0·04 per cent. of phosphorus absorptions attributable to the phosphorus were found even at acidities as high as 2 *N* sulphuric acid. A change in absorption occurs when the solution corresponding to the zero point of the calibration curve is strongly acidified. It can be calculated that some of this change is due to the suppression of the phosphorus colour, but the remainder is due to change in background colour. If, therefore, the sample is strongly acidified and used as a reference solution, no compensation is made for the phosphorus in the reagents, for the change in background colour and for the uninhibited phosphorus colour at the higher phosphorus contents. By deriving differences with respect to a solution prepared under the same conditions as the sample but using 1·28 g. of ferrous sulphate to introduce the iron, compensation is made for the background colour and the phosphorus content of the reagents. Allowance should be made, however, for the small amount of phosphorus introduced into the reference solution by the addition of 1·28 g. of ferrous sulphate; absorption due to this is not present in the sample solution. Correction must also be applied for any absorption due to colours inherent in the sample solution and not present in the reference solution.

CORRECTION FOR ALLOYING ELEMENTS—

For every 1 per cent. of chromium, 0·0045 per cent. of phosphorus should be deducted from the phosphorus value found by reference to the calibration graph.

This correction was derived by adding chromium as a dichromate solution both in the presence and absence of phosphorus prior to reducing with sulphurous acid. Nickel gives an interference absorption equivalent to approximately 0·0002 per cent. of phosphorus for each 1 per cent. of nickel.

ARSENIC INTERFERENCE—

An interfering absorption was found when arsenic was added just prior to the final coloration. The colour forms slowly and many hours are required for the colour to develop fully. Interference by arsenic does not occur, however, after reduction by sulphurous acid.[3] This is shown in Table V.

Table V

Influence of arsenic

Calibration solution made with pure iron and 0·040 per cent. of phosphorus
Arsenic added prior to solution in nitric acid

Arsenic added, %	Time, minutes 5	10	15	20	30
0	0·175	0·160	0·160	0·160	0·160
0·2	0·165	0·155	0·155	0·160	0·160
0·4	0·165	0·160	0·160	0·160	0·160
0·6	0·175	0·155	0·155	0·155	0·155

Effect of temperature—

Table VI shows that variation in temperature has little effect on the final absorption produced after the solution has stood for some time but that it affects the speed of formation of the colour.

Table VI

The effect of temperature

Calibration solution made with pure iron and 0·40 per cent. of phosphorus

Temperature, ° C.	Time, minutes 1	2	3	4	5	6	7	8	9	10	15	20
18·0	0·60	0·40	0·29	0·23	0·20	0·17	0·16	0·145	0·145	0·145	0·145	0·145
21·5	0·35	0·27	0·21	0·18	0·16	—	0·155	—	0·155	0·155	0·155	0·155
25·0	0·18	0·16	0·16	—	0·16	—	—	0·16	—	0·16	0·16	0·16

Values obtained for the phosphorus content of British chemical standard steels

Phosphorus determinations were conducted, using the absorptiometric method as described, on the following standard steels—

B.C.S. 218	0·15 per cent. carbon steel ..	0·045 per cent. phosphorus
B.C.S. 189	Ni - Cr - Mo steel B	0·024 per cent. phosphorus
B.C.S. 159	Carbon steel F	0·049 per cent. phosphorus
B.C.S. 225	Ni - Cr - Mo steel	0·021 per cent. phosphorus

The values given above are the averages of the results submitted by several independent analysts after careful investigation. A study of the literature issued by the Bureau of Analysed Samples Ltd. shows that, with B.C.S. 218, all chemists used the gravimetric method issued by the British Standards Institution.[4] The values obtained by the different analysts were within the range 0·044 to 0·047 per cent. of phosphorus, the average being 0·045 per cent. With B.C.S. 189, various methods were used, the figures submitted ranging between 0·021 and 0·026 per cent. of phosphorus, and with B.C.S. 159, all chemists titrated the phosphomolybdate with standard alkali and acid, the values obtained ranging between 0·046 and 0·051 per cent. of phosphorus. With B.C.S. 225, most chemists used the British Standards Institution method,[4] but four of the analysts finished volumetrically, the values submitted ranging between 0·020 and 0·023 per cent. of phosphorus.

Results obtained by the absorptiometric method—

The values obtained by the method described in this paper are given in Table VII, and are 0·002 to 0·005 per cent. lower than the standard values. An explanation of this discrepancy has not yet been found. During these analyses a control with a synthetic solution containing 0·040 per cent. of phosphorus was repeatedly conducted, and the value found was always in the range 0·039 to 0·041 per cent. of phosphorus. The di-ammonium hydrogen phosphate solution used for the calibration was checked against another solution prepared by using the theoretical amount of sodium ammonium hydrogen phosphate, and the values obtained were identical.

The discrepancy in values can be overcome by preparing the calibration graph using steel samples of which the phosphorus contents have been determined by the B.S.I. method.

TABLE VII

PERCENTAGE OF PHOSPHORUS FOUND IN B.C.S. SAMPLES BY THE ABSORPTIOMETRIC METHOD

	B.C.S. 218	B.C.S. 189	B.C.S. 159	B.C.S. 225
	0·0425	0·0190	0·0465	0·0200
	0·0420	0·0200	0·0450	0·0185
	0·0420	0·0195	0·0460	0·0190
	0·0410	0·0190	0·0460	0·0180
	0·0420			
	0·0410	0·0185		
	0·0420			
	0·0420			
	0·0425			
	0·0430			
Average	0·0420	0·0192	0·0460	0·0190

SUMMARY AND CONCLUSIONS—

The stannous chloride method is unsatisfactory for the photometric determination of phosphorus in steel, and a suitable procedure has been developed using ferrous sulphate as reducing agent. The method is suitable for routine batch analysis and, if carefully controlled, the reproducibility is within ±0·001 per cent. of phosphorus. The values obtained are slightly lower than those obtained by a reference gravimetric method unless steels analysed by this method are used to construct the calibration graph.

Acknowledgment is made to the Chief Scientist, Ministry of Supply, for permission to publish this paper. Reproduced with the permission of the Controller, His Majesty's Stationery Office.

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4. British Standard 1121, part 1, 1943.

METALLURGY DEPARTMENT
ROYAL AIRCRAFT ESTABLISHMENT
FARNBOROUGH, HANTS.

September, 1949

Notes

THE DETERMINATION OF OXYGEN IN CHROMIUM METAL*

HITHERTO the only reliable method for the determination of total oxygen in electrolytic chromium has been considered to be the vacuum fusion method, in which the sample is heated *in vacuo* with a solution of carbon in molten iron and the evolved gases are subsequently analysed. It has been shown that the simpler method suggested by Adcock[1] can also give useful results. The sample is annealed in a good vacuum for 2 hours at 800° C. and, after cooling, dissolved in dilute (10 per cent. v/v 1·18 sp.gr.) hydrochloric acid. The oxygen in the insoluble chromic oxide represents the total oxygen in the sample.

It should be noted that a sample dissolved as deposited, *i.e.*, without annealing, may give very little insoluble oxide in spite of a considerable oxygen content. Low results are also obtained if acid stronger than 10 per cent. v/v is used, presumably owing to the solubility of the chromic oxide.

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* Communication from the National Physical Laboratory.

Results on a few samples by the two methods are given below—

Sample	Oxygen by vacuum fusion %	Oxygen calculated from insoluble Cr_2O_3: 10% v/v HCl %	Oxygen calculated from insoluble Cr_2O_3: 5% v/v HCl %
1. Electrolytic, small nodules	0·48	0·59	0·61
2. 2nd sample do.	0·57	0·58	0·59
3. 3rd sample do.	0·55	0·60	0·58
4. Electrolytic powder (−100 mesh)	0·28	0·28	0·31
5. 2nd sample do.	0·22	0·23	0·22

REFERENCE

1. Adcock, F., *J. Iron and Steel Inst.*, 1927, **115**, 369.

H. G. SHORT
December, 1949

THE ESTIMATION OF HORSE-FAT IN ADMIXTURE WITH OTHER FATS

THE method of Paschke[1] for the detection of horse-fat in admixture with lard, beef-fat and mutton-fat has been investigated and has been extended to include a number of other fats and oils. The method depends upon the presence in horse-fat of 1 to 2 per cent. of linolenic acid, and its relative scarcity in other animal fats. The linolenic acid is determined as its hexabromide using the method given below which is essentially that described by Paschke.

PROCEDURE

Heat 10 g. of the fat on a water-bath with 100 ml. of 0·5 *N* alcoholic potash for half an hour under a reflux condenser. Connect to a distillation apparatus and distil until 80 ml. of distillate (containing most of the alcohol) have been collected.

Dilute the hot residue with distilled water and wash into a 1000-ml. separating funnel, using a total amount of 250 ml. of water. Add 15 ml. of 5 *N* sulphuric acid, 250 ml. of saturated sodium chloride solution and 50 ml. of ether. Shake vigorously for 1 minute. Allow to stand for 10 minutes and then discard the aqueous layer. Wash the ethereal extract (containing the fatty acids), with three 15-ml. washings of saturated sodium chloride solution, and filter it through a filter-paper.

Place 5 ml. of the ethereal filtrate into a test tube by means of a pipette, and cool to −15° C. in an ice - salt mixture. Cool a second test tube containing 5 ml. of pure ether and 0·45 ml. of bromine to the same temperature. Add the cooled ethereal solution of bromine in portions of about 1 ml. to the ethereal fatty acid solution; the temperature must not be allowed to rise above 0° C. Keep in the ice - salt mixture for 10 minutes and then keep at 5° to 10° C. for a further 15 to 18 hours.

Allow the solution to stand at room temperature for half an hour, in order that any separated free fatty acids may be redissolved, then filter through a porcelain Gooch crucible having a porous base. Transfer any residue remaining in the test tube to the Gooch and wash the sides of the Gooch with total of 10 ml. of ether cooled to −10° C. The contents of the crucible should remain just covered with liquid until the washing is complete. In order to remove all the fatty acids from the insides of the Gooch it was found necessary to remove the crucible from the adaptor and to pour small portions of the 10 ml. of ether down the sides.

Carefully wipe the outside of the crucible, dry at 100° C. for 1 hour, cool to room temperature, and then wash with a further 5 ml. of ether (at room temperature) to remove any remaining free fatty acids. The hexabromide is less soluble in ether when dry than when freshly precipitated. Dry for a further hour at 100° C., cool and weigh.

APPLICATION TO PURE FATS

The method described above was used to examine a number of pure fats. All the samples of horse-fat were obtained direct from a horse-slaughterer (each from a different horse), and these and a number of the other fats were rendered down in the laboratory. Table I gives the results expressed as milligrams of bromide yielded by 1 gram of fat. The numbers in brackets represent the number of samples examined in each case.

The most interesting feature of these results is the high figure of 191 mg. per g. obtained for horse-fat, compared with the figure of 41·2 mg. per g. reported by Paschke. No explanation has been found for this. The only difference between the original method and the method described

TABLE I

Sample	Insoluble bromide found, mg./g. Average	Limits
Horse-fat	191 (10)	177 to 213
Beef-fat	1·5 (3)	1·0 to 1·8
Mutton-fat	5·6 (3)	5·0 to 6·2
Pig-fat	3·2 (5)	1·0 to 4·8
Butter-fat	3·1 (3)	2·6 to 3·5
Neatsfoot oil	nil (1)	
Cod-liver oil	450 (2)	420 to 480
Arachis oil	3·0 (1)	
Almond oil	nil (1)	
Peach-kernel oil	nil (1)	
Sesame oil	nil (1)	
Olive oil	0·5 (1)	
Linseed oil	538 (2)	500 to 575

above is that in the latter slightly more ether is used to wash the hexabromide. If anything, this would have been expected to produce slightly lower results than those obtained by Paschke.

No proof was given in Paschke's paper that the bromide compound was actually the hexabromide of linolenic acid. Arachidonic acid, which has been reported in small quantities in some animal fats,[2] gives an octabromide under the same conditions, as do also the highly unsaturated acids of marine animal oils. The two bromides may, however, be distinguished by their melting-points; the hexabromide of linolenic acid melts at 181° C., whereas the octabromide of arachidonic acid does not melt but blackens above a temperature of about 220° C. With the 10 samples of horse-fat examined, all the bromides melted within the range 180° to 183° C., whereas the bromides obtained from the two samples of cod-liver oil did not melt but blackened at 220° to 230° C.

APPLICATION TO MIXTURES

A number of mixtures of horse-fat with animal and vegetable fats were examined with the results shown in Table II. The composition of the mixtures was unknown to the analyst at the time of the examination.

TABLE II

Mixture				
Horse-fat, %	Other fats present	Weight of hexabromide, mg./g.	Correction allowed for other fats present, mg.	Horse-fat found, %
12·4	pig, mutton	24·0	4·0	10·5
10·0	beef, mutton	21·0	3·0	9·4
5·0	beef	7·0	1·5	2·9
10·0	beef, mutton, pig	20·6	4·0	8·7
7·5	beef, arachis	14·0	2·0	6·3
30·0	beef, arachis	57·0	2·0	28·8
10·0	neatsfoot, almond	16·2	nil	8·5
25·0	peach-kernel	39·0	nil	20·4
64·8	neatsfoot, almond	126·0	nil	66·0

The results show that the presence of 10 per cent. or more of horse-fat in a mixture can be detected with certainty and the amount estimated with a fair degree of accuracy. Indeed, if the examination of a greater number of samples of beef, mutton and pig fats confirms the limits obtained above for these samples, it would appear possible to detect the presence of as little as 5·0 per cent. of horse-fat in admixture with them.

The results given in Table I indicate that of the fats examined only linseed and cod-liver oils would interfere with the estimation of horse-fat. Neither of these is considered to be a likely adulterant, but the latter can be ruled out if the melting-point of the bromide is determined. Two unknown mixtures (not shown in Table II) were in fact examined and the hexabromides were found not to melt at 181° C. but to char at about 220° C., and it was reported that no opinion could be given as to the presence or absence of horse-fat in these based on the test as above reported. It was subsequently learnt that cod-liver oil was present in each of these mixtures.

SUMMARY

An investigation has been made into the method of Paschke for the determination of horse-fat in admixture with other fats. The method depends upon the presence in horse-fat of 1 to 2 per

cent. of linolenic acid and its relative scarcity in most other fats. The linolenic acid is determined as its hexabromide, the results being expressed as mg. of hexabromide yielded by 1 g. of fat.

Pure horse-fat has been found to give an average of 191 mg. of hexabromide per g., with limits of 177 to 213 mg. per g.; this is a much higher figure than that reported by Paschke (41·2 mg.).

The method has been used to determine the amount of horse-fat in admixture with other animal fats and with vegetable oils, and has given satisfactory results with mixtures containing as little as 5 to 10 per cent. of horse-fat. Although only one sample of neatsfoot oil has been examined the method would appear to be of use in distinguishing this oil from horse-fat.

I wish to express my thanks to Mr. E. T. Illing, B.Sc., F.R.I.C., for preparing the unknown mixtures, and for his advice throughout this work.

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COUNTY ANALYST'S LABORATORY
COUNTY HALL
TAUNTON

R. A. DALLEY
January, 1950

A NEW QUALITATIVE REACTION FOR YOHIMBINE

THE important group of alkaloids containing the indole nucleus ranges in complexity from simple substances like hypaphorin, the methylbetaine of tryptophan, to the complicated structures of yohimbine and strychnine. Some of these alkaloids have as parent substance the amino acid, tryptophan; in others a carbazole nucleus is present. Consequently these alkaloids have some analytical reactions in common. The best method to use for their detection varies according to the substance present and is influenced by constitutional differences. We find, for example, that with sugars, carbazole does not react, but methyl indole[1] produces deeply coloured compounds almost instantly. A broader application, as is well known, makes use of dimethylaminobenzaldehyde, which gives coloured compounds with a series of indole derivatives in presence of mineral acids; these, according to Voisènet,[2] can be transformed to blue compounds in the presence of weak oxidants like hydrogen peroxide or nitrous acid. We found that yohimbine also gives this very sensitive reaction.

When aldehydes under certain conditions react with indole derivatives, a red intermediate is formed which changes to a blue pigment in presence of a weak oxidant. The constitution of the coloured compound is not known, but it can be assumed that its formation is the result of multiple condensation reactions between the aldehyde and the indole compound which lead to more complicated ring structures.[3]

Amongst the alkaloids that undergo the same type of reaction as tryptophan are ergotamine, ergonovine[4] and yohimbine, which latter is in its constitution related to harman and carbazole, but contains one more nitrogen atom in its nucleus. Physostigmine does not react, as position five of the indole nucleus is occupied by a urethane group.

PROCEDURE

Dissolve a small amount of the alkaloid in about 1 ml. of concentrated hydrochloric acid. Add 4 drops of a 2 per cent. solution of *p*-dimethylaminobenzaldehyde in concentrated hydrochloric acid and warm. Add 2 drops of a 0·05 per cent. solution of sodium nitrite to the colourless test liquid. In the presence of yohimbine, a deep violet-blue ring appears after a short time. On shaking gently the whole solution becomes deep blue. One milligram of yohimbine is sufficient to give a distinct reaction. Tablets containing yohimbine can be treated in the same way, with the same result.

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LAWRENCE S. MALOWAN
DEPARTMENT OF BIOCHEMISTRY
UNIVERSITY OF PANAMA

First submitted, *August*, 1948
Amended, *April*, 1950

Ministry of Food

STATUTORY INSTRUMENTS*

1950.—No. 554. The Milk (Great Britain) (General Licence No. 2) Order, 1950. Price 1d.

This Order, which came into operation on the 9th of April, 1950, is a general authority, during the period April 9th to July 1st, 1950, inclusive, for the supply of milk to any manufacturer for use by him in the preparation or manufacture of food.

— No. 555. The Use of Milk (Suspension of Restriction) Order, 1950. Price 1d.

For the period April 9th to July 1st, 1950, inclusive, this Order suspends the operation of the Use of Milk (Restriction) Order, 1945 (S.R. & O., 1945, No. 304), and permits the use of milk, milk powder or condensed milk in the preparation or manufacture of biscuits, bread, buns, pastries, cakes, rolls, scones and other similar articles, ice-cream, soft cheese and curd cheese, sweetmeats (including sugar confectionery and chocolate) and synthetic cream.

— No. 589. The Food Standards (Fish Cakes) Order, 1950. Price 1d.

This Order, which came into operation on the 15th of April, 1950, and which should be read with the Food Standards (General Provisions) Order, 1944, as amended by S.R. & O., 1944, Nos. 42 and 654, maintains the standard for fish cakes hitherto prescribed in the Fish Cakes (Maximum Prices) Order, 1943, viz., that not less than 35 per cent. by weight of the fish cake shall consist of fish.

Proceedings for infringement may be brought in England and Northern Ireland by a Food and Drugs Authority without the consent of the Minister.

— No. 596. The Soft Drinks (Amendment) Order, 1950. Price 2d.

1. The Soft Drinks Order, 1947, as amended by S.R. & O., 1947, No. 2756, S.I., 1948, No. 1291, and 1949, No. 1378, shall be further amended by substituting for the Schedule thereto the Schedule to this Order.
2. The Soft Drinks (Amendment) Order, 1949, as amended by S.I., 1949, No. 1378, is hereby revoked, but without prejudice to any proceedings in respect of any contravention of the Soft Drinks Order, 1947, as thereby amended.

This Order, which came into operation on the 19th of April, 1950, prescribes specifications for the ingredients of all soft drinks containing citrus fruit juice and barley. These specifications are the same as those that previously applied to "lemon barley" only.

THE SCHEDULE

(*To be substituted for the Schedule to the Soft Drinks Order,* 1947)

PROVISIONS AS TO INGREDIENTS

NOTE—These provisions do not apply to unsweetened soft drinks, other than soda water, or to fruit juice (as defined in Article 1).

PART I

Soft drinks suitable for consumption without dilution

Description of soft drink	Minimum quantity of fruit juice (expressed in terms of unconcentrated juice of natural strength) per 10 gallons	Minimum quantity of added sugar per 10 gallons	Maximum quantity of saccharin per 10 gallons
Non-alcoholic cider and non-alcoholic perry	120 fluid oz.	18 oz.	82 grains
Any citrus fruit juice and barley drink	48 fluid oz.	18 oz.	82 grains
Lime juice and soda	48 fluid oz.	18 oz.	82 grains
Any other soft drink containing fruit juice	80 fluid oz.	18 oz.	82 grains
Non-alcoholic wine	—	7½ lb.	No maximum
Indian or quinine tonic-water		18 oz.	82 grains
	Not less than ½ grain of quinine (calculated as quinine sulphate B.P.) per pint		

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

Soda-water	Not less than 5 grains of sodium bicarbonate per pint		
Any other soft drink (except those mentioned in Part II of this Schedule)	—	18 oz.	82 grains

PART II

Soft drinks intended for consumption after dilution

Any citrus fruit juice and barley drink ..	1½ gallons	7½ lb.	⅞ oz.
Any other squash, crush, cordial or concentrate containing citrus fruit juice ..	2½ gallons	7½ lb.	⅞ oz.
Any squash, crush, cordial or concentrate containing any other fruit juice	1 gallon	7½ lb.	⅞ oz.
Any other soft drink (except those mentioned in Part I of this Schedule)	—	7½ lb.	⅞ oz.

British Standards Institution

NEW SPECIFICATION†

B.S. 572 : 1950. Interchangeable Conical Ground Glass Joints.

DRAFT SPECIFICATIONS

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

Draft Specifications prepared by Technical Committee LBC/1—Volumetric, Mouldblown and Lampblown Glassware.

CM(LBC) 361—Draft for Automatic Pipettes.
CM(LBC) 371—Draft Revision of B.S. 700: Graduated Pipettes and Straight Pipettes.

Draft Specifications prepared by Technical Committee LBC/3—Glassware for Pharmaceutical Purposes.

CM(LBC) 382—Draft for Pharmacists' Graduated Pipettes.
CM(LBC) 383—Draft for Pharmacists' Conical Measures.

Crystallographic Data

ARMOUR RESEARCH FOUNDATION OF ILLINOIS INSTITUTE OF TECHNOLOGY

THE Illinois Institute of Technology, 3300, South Federal Street, Chicago, 16, Illinois, U.S.A., announce that the National Registry of Rare Crystallographic Data has been established by the Armour Research Foundation of the Institute. The Registry is a public service, available to scientists throughout the world. Dr. Walter C. McCrone, supervisor of the analytical section of the Foundation's Chemistry and Chemical Engineering Department, is in charge.

Scientists are invited to submit data on new compounds which they identify. Acknowledgment of persons and laboratories who contribute this information will be included in the files. Due recognition in proportion to the extent of the contribution will be made, varying from senior authorship to acknowledgment within published papers. The current series of monthly reports on new information in the Registry, appearing in *Analytical Chemistry*, is an example of the intended use of such data.

Persons and organisations may obtain information from the Registry on request by describing an unknown compound crystallographically. If it is listed in the file, the information will be sent to the enquirer, identifying the compound for him.

The system of conventions and nomenclature set up after the 1947 New York meeting of the American Chemical Society is used by the Registry. This system was described in *Analytical Chemistry*, 1948, **20**, 274 and abstracted in *The Analyst*, 1948, **73**, 579.

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† Obtainable from the British Standards Institution, Publications Department, 28, Victoria Street, London, S.W.1.

A VACANCY exists in the Central Research and Development Department of the Distillers Company Limited, Great Burgh, Epsom, Surrey, for a Biochemist for work on antibiotic fermentations. Applicants, aged under 27 years, should possess an Honours degree or equivalent, and preferably previous biochemical or analytical experience. Apply to The Controller of Research and Development.

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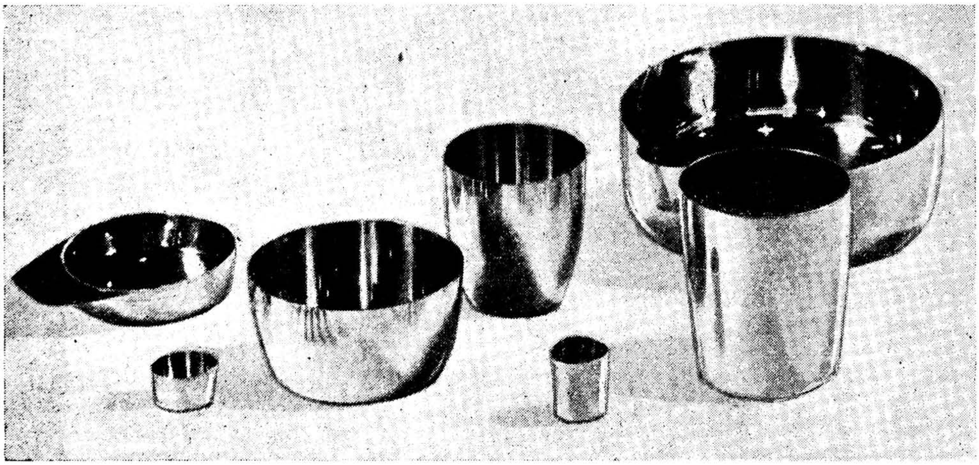

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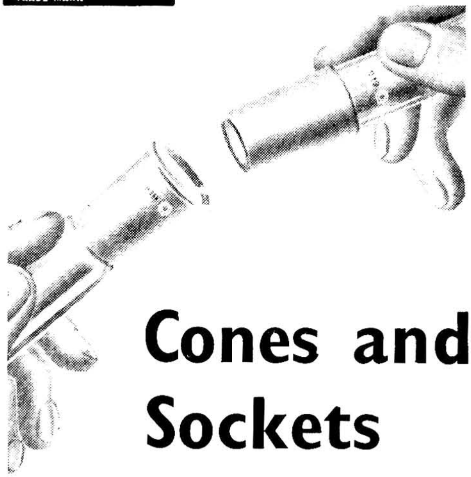

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