NEW APPARATUS

A MANUAL POLAROGRAPH

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The accuracy of recording polarographs is usually of the order of ± 1 per cent. for full scale deflection; at intermediate points on the scale the error may be considerably greater, and is apt to increase with the age of the instrument. In the course of a polarographic investigation, it was desired to measure diffusion currents with an accuracy of ± 0·1 per cent., and a potentiometric method of determination of current and voltage was used for this purpose. The determination of current-voltage curves by manual means is admittedly tedious; it should be borne in mind, however, that in routine analysis, measurements are frequently made at a fixed polarising voltage, and the plotting of a complete polarogram is unnecessary. In applications in which precision, high current sensitivity, and permanence of calibration are of primary importance, a potentiometric manual instrument offers advantages over the costly automatic recorders.

GENERAL DESCRIPTION

The potentiometric principle has been applied to polarographic measurements by Lingane[1] and by Dell and Gentry[2]. A potentiometric instrument which, however, entails the use of an auxiliary reference electrode in addition to the normal anode and dropping mercury cathode, has also been described recently by Adams, Reilley, and Furman[3]. In the circuit arrangement used by Lingane[1], the diffusion current I produced by applying a voltage V to a series combination of the polarographic cell and a standard resistor R is determined by measuring potentiometrically the potential drop IR across the resistor. The method has the drawback that the cell voltage V–IR is not identical with the applied voltage V, and must be determined by connecting a potentiometer across the electrodes.

Dell and Gentry[2] overcome this disadvantage by connecting the potentiometer in series-opposition to the potential difference across the resistor, and a similar device is used in the instrument now described. The modified circuit is shown in a simplified form in Figure 1, in which P_1 and P_2 are series-connected potentiometers supplied with power from a common source C. The potentiometer P_2 is adjusted to provide any desired polarising voltage V_2, and the resulting diffusion current I is measured by adjusting the potentiometer P_1 to voltage V_1 such that a null point detector connected across AB is undeflected. The voltage drop IR across R is thus counterbalanced by the equal potential drop across P_1, and the cell voltage is accordingly equal to the applied polarising voltage V_2.

In view of the oscillatory nature of the polarographic current, it is desirable that the null point detector should be free from mechanical inertia. Dell and Gentry[2] apply the unbalanced E.M.F., interrupted at a frequency of 50 c/s. by means of a high-speed relay, to the amplifier of a cathode ray oscillograph, and use the impulses generated by the fall of the mercury drops, after amplification, to control the time base of the oscillograph. In the instrument now described, a considerable economy is effected, without sacrificing the advantages of high sensitivity and freedom from mechanical inertia, by using as the null point detector the inexpensive EM34 cathode ray tuning indicator. Indicators of this type have been used in polarography by Boeke and van Suchtelen[4]; in the circuit devised by these authors, however, an alternating voltage is superimposed upon the polarographic cell, and the method cannot be used in producing polarograms of the conventional form.

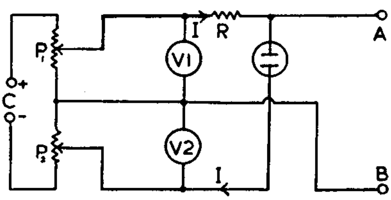

FIG. 1

In order that an unbalanced E.M.F. of ± 1 mV may be readily detected, an amplifier having a gain of at least 2500 is required. A single valve (ME1400) having semi-electrometer characteristics is used for this purpose. The operating conditions are such that the stage gain, and the grid current, are approximately 400 and 10^{-12}A. respectively. In contrast to the normal behaviour of thermionic valves, the cathode current of the EM34 decreases as the negative voltage applied to its control grid is decreased; in consequence, if a feedback resistor connected in series with the cathode is included in the grid return lead, the application of the unbalanced E.M.F. sets up a potential difference across the resistor which reinforces the applied voltage, and positive feedback is obtained. Advantage is taken of this property to enhance the sensitivity of the amplifier and, in practice, no difficulty is experienced in securing the desired gain.

The polarising voltage and the corresponding peak polarographic current are indicated, with an accuracy of ± 1 per cent., by the deflections of a multi-range meter bearing 125 subdivisions. By connecting an external direct current potentiometer to terminals provided for this purpose, the accuracy in the measurement of current and voltage may be increased to ± 0·1 per cent. In view of the growing importance of derivative polarography, a derivative circuit is included, and provision is also made for the use of the instrument in *p*H measurements with the aid of the glass electrode.

Constructional Details

The practical circuit diagram is given in Figure 2, and the specification of the components is as follows:—

Component	Specification	Component	Specification	Component	Specification	Component	Specification
V_1	ME1400	R_4	100 M Ω ± 5 per cent.	$R_{10}R_{11}$	3·3 M Ω ± 5 per cent.	R_{16}	9 K Ω ± 0·1 per cent.
V_2	EM34	R_5	1 M Ω ± 0·1 per cent.	R_{12}	50 K Ω 1W.	$R_{17}R_{18}$	0·2 Ω ± 1 per cent.
R_1	20 Ω ± 1 per cent.	R_6	10 K Ω ± 0·1 per cent.	R_{13}	2 K Ω 20W.	C_1C_2	0·02 μF.
R_2	15 Ω ± 1 per cent.	R_7R_9	1 M Ω ± 1 per cent.	R_{14}	440 Ω 5 W.	$S_1S_2S_3$	12-way 3-pole switch
R_3	25 Ω ± 1 per cent.	R_8	20 M Ω ± 5 per cent.	R_{15}	19 K Ω ± 0·1 per cent.	S_4S_5	D.P.D.T. switches

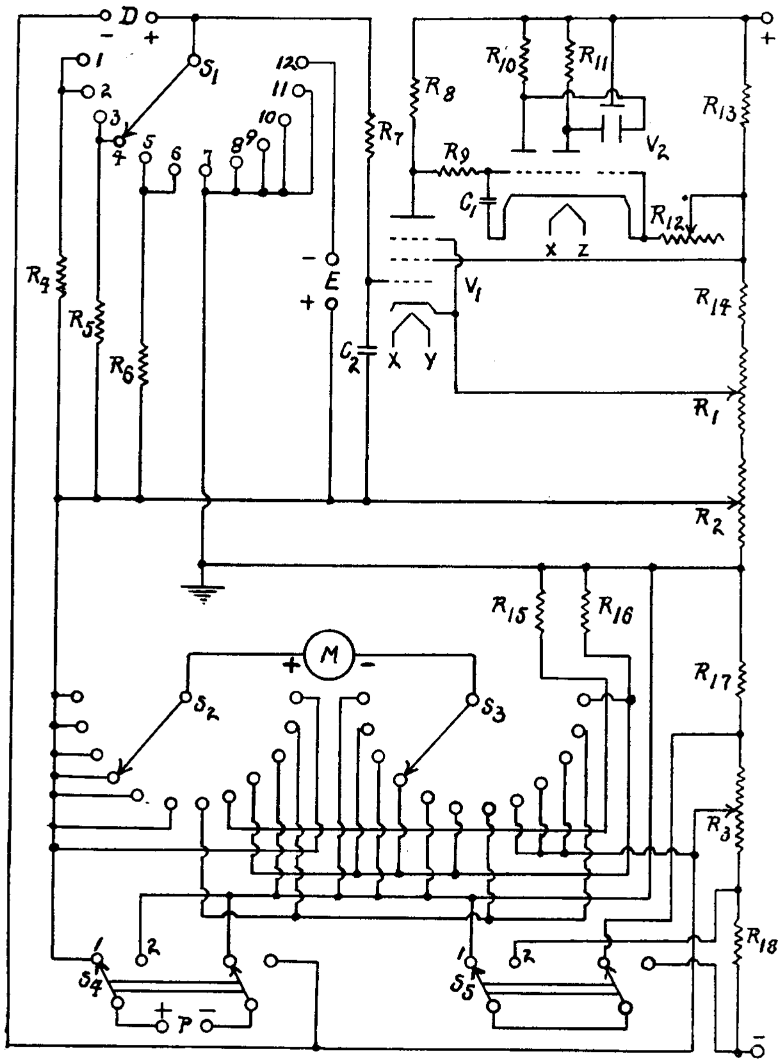

FIG. 2

Terminals for connection to the polarographic cell D, external potentiometer P, and glass electrode E are also required. The potential dividers $R_1R_2R_3$ are of the helical type, and the meter M is a 10,000 Ω/V. millivoltmeter requiring 125 mV. for full scale deflection. The mains unit (not shown in the diagram) delivers a direct current supply of 250 V. 100 mA. to the potential divider $R_{13}R_{14}R_1R_2R_{17}R_3R_{18}$, together with alternating current supplies of 4·5 and 6·3 V. to the heaters of the ME1400 and EM34 respectively; a constant voltage transformer is used to stabilise the power supply. In order to eliminate ripple voltage, filters are included in the grid circuits. The time constants of the filters, each of which consists of a 1 MΩ resistor and a 0·02 μF. mica-dielectric capacitor, are such that their effects on the waveform of the oscillatory polarographic current are negligible. The feedback resistor R_{12} is adjusted until the application of 1 mV. to the input circuit of the amplifier causes the shadow to open or close fully.

6 current ranges and 3 voltage ranges, controlled by the ganged range switch $S_1S_2S_3$, are provided:—

Switch position	Current for F.S.D.	Current per division	Switch position	Voltage for F.S.D.	Voltage per division
1	0·00125 µA.	10·0 µµA.	8	2·50 V.	20 mV.
2	0·0125 µA.	100·0 µµA.	9	1·25 V.	10 mV.
3	0·125 µA.	0·001 µA.	10	125·00 mV.	1 mV.
4	1·25 µA.	0·01 µA.	—	—	—
5	12·5 µA.	0·1 µA.	—	—	—
6	125 µA.	1 µA.	—	—	—

Measurement of Polarographic Currents

In the measurement of diffusion currents, the range switch $S_1S_2S_3$ is thrown to the appropriate voltage range and, by means of the control R_3, the polarising voltage is adjusted to the desired value, as indicated by the deflection of the meter. With the range switch in position 7 or 11, the control R_1 is adjusted until the tuning indicator displays a suitable shadow angle. Finally, the range switch is thrown to the appropriate current range, and the control R_2 is adjusted until, at the peak of each oscillation, a rapid increase in shadow angle is observed; the reading of the meter then gives the peak value of the polarographic current. When an accuracy of ± 0·1 per cent. is desired, the determination is made by connecting an external potentiometer to the terminals provided for this purpose, and throwing the switch S_4 to position 1 or 2 for the measurement of current or voltage. On ranges 1 and 2, the polarographic current flows through a resistor of 100 MΩ; as it is difficult to adjust a resistance of this ohmic value with an accuracy of ± 0·1 per cent., a correction factor is used on these ranges.

Derivative Polarography

Occasionally, in addition to the normal polarogram obtained by plotting the current I against the polarising voltage V, the derivative $\Delta I/\Delta V$ of the current-voltage curve is required. For this purpose, after measuring the value of I corresponding to a given value of V, the switch S_5, which normally rests in the position shown in Figure 2, is thrown to its alternative position, thus increasing V by a small increment ΔV equal to the potential difference (about 20 mV.) across R_{17}; the change ΔI in the current is noted and, by plotting values of ΔI against corresponding values of V, the derivative polarogram is obtained. The method is tedious, but has the advantage of yielding an accurate derivative. As Lingane and Williams[5] have shown, the derivative circuit usually incorporated in recording polarographs, viz., that devised by Leveque and Roth[6], suffers from the drawbacks that the height of the maximum in the derivative polarogram is not accurately proportional to the concentration, and that the voltage at which this maximum occurs differs appreciably from the half-wave potential.

*p*H MEASUREMENTS

The instrument has characteristics which render it suitable for use in *p*H measurements with the aid of the glass electrode; these characteristics include low grid current, together with voltage sensitivity of a higher order than that of the amplifiers usually incorporated in potentiometric *p*H meters. The use of a null point detector free from mechanical inertia and from risk of damage due to overload also has obvious advantages over the sluggish and fragile galvanometers normally employed. The electrodes are connected to the terminals E (Fig. 2) provided for this purpose and, with the range switch in position 12, the control R_2 is adjusted until a shadow angle of about 45° is observed; the E.M.F. of the electrode system is then indicated by the deflection of the meter. Alternatively, the instrument may be used as a null point detector in conjunction with an external potentiometer calibrated in *p*H units.

THEORY

For the purpose of predicting the optimum circuit conditions, the EM34 cathode ray tuning indicator may be regarded as electrically equivalent to a triode having an internal anode resistance r_a of about 0·25 MΩ and an amplification factor μ of approximately 20. The voltage appearing at the grid of the indicator is mE, where E is the voltage applied to the input stage, $m = \mu' R'_a/(r'_a + R'_a)$ is the stage gain, μ' and r'_a represent the amplification factor and internal anode resistance of the ME1400, and R'_a is the external anode resistance of the input stage. Under the operating conditions ($V_a = V_{g2} = 45$ V., $I_a = 10$ μA.) the characteristics of the ME1400 are $\mu' = 1200$ and $r'_a = 40$ MΩ; thus, using an anode load R'_a of 20 MΩ, a stage gain of approximately 400 is obtained.

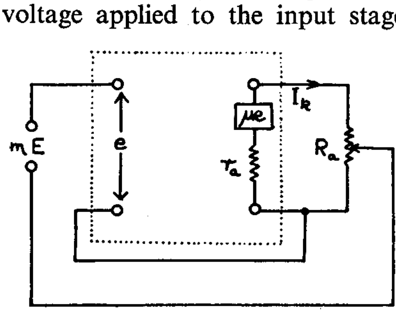

FIG. 3

Figure 3 is the equivalent electrical circuit of the output stage; I_k is the cathode current of the tuning indicator, R_a represents the feedback resistor (corresponding to R_{12} in Figure 2), and $e = mE + \beta I_k R_a$ is the voltage appearing at the grid of the indicator, β being the fraction of the output voltage $I_k R_a$ which is fed back to the grid of the EM34. Evidently

$$I_k = \mu e/(R_a + r_a) = mE/\left(\frac{r_a + R_a}{\mu} - \beta R_a\right) \quad \ldots \quad \ldots \quad \ldots \quad (1)$$

The voltage sensitivity of the amplifier is conveniently expressed in terms of $\Delta\theta/\Delta E$, the change in shadow angle, in angular degrees, produced by unit change in applied voltage. As the shadow angle is proportional to the cathode current, the value of $k = \Delta\theta/\Delta I_k$ being approximately 300°/mA., equation (1) may be written

$$\frac{\Delta\theta}{\Delta E} = \frac{\Delta\theta}{\Delta I_k} \cdot \frac{\Delta I_k}{\Delta E} = km/\left(\frac{r_a + R_a}{\mu} - \beta R_a\right) \quad \ldots \quad \ldots \quad (2)$$

In the absence of positive feedback ($R_a = O$) equation (2) reduces to

$$\Delta\theta/\Delta E = k\mu m/r_a \quad \ldots \quad \ldots \quad \ldots \quad \ldots \quad \ldots \quad \ldots \quad \ldots \quad (3)$$

By substituting $\beta = 1$, together with the numerical values of k, m, r_a, and μ given above in equations (2) and (3), it is readily calculated that the voltage sensitivity $\Delta\theta/\Delta E$ of the instrument is approximately 10°/mV. in the absence of positive feedback, and is increased tenfold (109°/mV.) when $R_a = 12$ KΩ. By careful adjustment of the feedback resistor, the voltage sensitivity may be further increased; as equation (2) indicates, however, a practical limit is reached when $R_a = r_a/(\mu - 1)$; the sensitivity is then theoretically infinitely great and, in practice, uncontrollable oscillation at a low frequency commences.

The current sensitivity $\Delta\theta/\Delta I$ of the instrument, i.e. the change in shadow angle produced by unit change in the polarographic current I flowing through the resistor R, is

$$\Delta\theta/\Delta I = R. \quad \Delta\theta/\Delta E = k\mu mR/r_a \quad \ldots \quad \ldots \quad \ldots \quad \ldots \quad (4$$

in the absence of positive feedback, or

$$\Delta\theta/\Delta I = R. \quad \Delta\theta/\Delta E = kmR/\left(\frac{r_a + R_a}{\mu} - \beta R_a\right) \quad \ldots \quad \ldots \quad (5)$$

when a feedback resistor R_a is included in the circuit. The values of R employed on current ranges 1 and 2, 3 and 4, and 5 and 6 are 100 MΩ, 1 MΩ and 10 KΩ respectively; the approximate current sensitivities of the instrument on these ranges are accordingly 1°/$\mu\mu$A., 0·01°/$\mu\mu$A., and 100°/μA., respectively, in the absence of feedback, or 10·9, 0·109 and 0·001°/$\mu\mu$A. in the presence of a feedback resistor of 12 KΩ.

SUMMARY

1. A description is given of a potentiometric manual polarograph in which a cathode ray tuning indicator is used as a null point detector.

2. The polarising voltage and the corresponding peak polarographic current are indicated, with an accuracy of $\pm$ 1 per cent., by the deflections of a voltmeter, the ranges being as follows: current, 0·00125 to 125 μA., first indication 10 $\mu\mu$A.; voltage, 125 mV. to 2·5 V., first indication 1 mV. By using the instrument in conjunction with a direct current potentiometer, the accuracy may be increased to $\pm$ 0·1 per cent.

3. A circuit for obtaining derivative polarograms is included, and provision is also made for the use of the instrument in *p*H measurements with the aid of the glass electrode.

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(ABSTRACTS *continued from p. 273*).

BACTERIOLOGY AND CLINICAL TESTS

Isoniazid Resistant Variants of Tubercle Bacilli, Pathogenicity of. G. Middlebrook and M. L. Cohn. (*Science*, 1953, **118**, 297.) Strains of bovine (Vallée) and human (H37Rv) tubercle bacilli resistant to 10 μg./ml. of isoniazid on oleic acid-albumin solid medium were subcultured through passages in Tween-albumin medium containing 10 μg./ml. of isoniazid and then tested for pathogenicity by injection into guinea-pigs. The isoniazid-resistant Vallée strain showed striking loss of pathogenicity, the animals dying after 33 and 43 days respectively with only minimal evidence of active tuberculosis. The animals injected with the isoniazid-resistant strain of H37Rv were still alive after 60 days. 11 strains resistant to 1 μg./ml. of isoniazid, isolated from patients treated with the drug, were subcultured once or twice in Tween-albumin medium without isoniazid and injected intravenously into guinea-pigs. Organisms isolated from animals dying in less than 60 days or sacrificed at 60 days were tested for resistance to isoniazid. 3 strains, all from animals surviving for 60 days, were completely resistant to 10 μg./ml. of isoniazid and the animals showed no signs of tuberculosis. 7 strains were resistant to 1 μg./ml. but partially or completely sensitive to 10 μg./ml.; all the animals yielding these strains died within the 60-day period. 1 strain was sensitive to 1 μg./ml.; the two animals giving this strain died at 17 and 27 days respectively. Resistance of human type tubercle bacilli to 10 μg./ml. of isoniazid may therefore be accompanied by marked loss of pathogenicity for normal guinea-pigs. In similar tests on 21 strains isolated from the sputum of patients treated with isoniazid for at least 2 months, 4 strains were found to be pathogenic while the remaining 17 caused little or no tuberculosis to develop. 8 of the non-pathogenic strains failed to grow on lactic acid-albumin medium but 5 grew on American Trudeau Society egg medium. The 3 which grew on neither came from patients whose sputum concentrates contained enormous numbers of acid-fast rods, probably derived from a multiplying population in a lung cavity. The observation supports the suggestion that strains resistant to isoniazid have growth requirements differing from those of the parent sensitive strains. Possibly necrotic tissue contains a growth substance which is unavailable in normal tissue, the substance being present in moderate but not always sufficient amount in egg medium or in much smaller quantities in lactic acid-albumin medium. H. T. B.