POLAROGRAPHY OF ORGANIC COMPOUNDS

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During the last ten years polarography has attracted the attention of many organic chemists. The technique has been employed in the qualitative detection and quantitative analysis of a wide range of organic substances and has found a place in the study of keto–enol tautomerism, oxidation–reduction equilibria, hydrogen-bonding, and electrochemical oxidation and reduction mechanisms.

Polarography was devised in 1922 by Professor J. Heyrovský ; [1, 2] most of the papers on the subject published before 1938 either emanated from Heyrovský's laboratory in the Charles University at Prague or represent the work of chemists from other countries who had been trained by him. These early papers established the fundamental principles of polarography, which are now universally accepted. The general principles are described by I. M. Kolthoff and J. J. Lingane [3] and by J. Heyrovský [4] in monographs published in 1941. More recent monographs by O. H. Müller [5] and by M. von Stackelberg [6] provide, respectively, an introduction to the practical aspects of polarography and a review of polarographic literature to the end of 1949. In recent review articles, O. H. Müller [7] has set forth the theoretical principles to be observed in applying polarography to organic compounds and J. E. Page [8] has surveyed the applications of polarography to pharmaceutical analysis. A bibliography [9] listing over 3000 polarographic papers published between 1922 and 1950 has been printed as a part of the Proceedings of the First International Polarographic Congress, which was held at Prague on the occasion of Professor Heyrovský's 60th birthday.

General principles of polarography

Polarography is an electrochemical method of analysis and incorporates features of both potentiometry and electrolysis. The sample to be examined is dissolved in an aqueous solution containing an excess of a " base " or " supporting " electrolyte, and the solution is placed in a special electrolytic cell, which has as anode a relatively large pool of mercury, and as cathode mercury dropping from a glass capillary at a steady rate of one

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[1] *Chem. Listy*, 1922, **16**, 256 ; *Phil. Mag.*, 1923, **45**, 303.

[2] J. Heyrovský and M. Shikata, *Rec. Trav. chim.*, 1925, **44**, 496.

[3] " Polarography ", Interscience Publ., New York, 1941 ; see also Vol. I, 2nd Edn., 1952.

[4] " Polarographie ", Springer-Verlag, Vienna, 1941.

[5] " The Polarographic Method of Analysis ", Chemical Education Publ. Co., Easton, Pa., 2nd Edn., 1951.

[6] " Polarographische Arbeitsmethoden ", Walter de Gruyter, Berlin, 1950.

[7] In A. Weissberger's " Physical Methods of Organic Chemistry ", Vol. I, Part II, Interscience Publ., New York, 2nd Edn., 1949, p. 1785.

[8] *J. Pharm. Pharmacol.*, 1952, **4**, 1.

[9] Proc. 1st Intern. Polarographic Congr., Prague, 1951, Vol. II.

drop every 2—4 seconds. A gradually increasing voltage is applied to the cell and the corresponding current measured on a galvanometer. If the current is plotted against the applied voltage, a characteristic S-shaped current–voltage curve, a polarogram, is obtained. From this curve it is possible to determine both the nature and the concentration of reducible substances in the sample. If the dropping-mercury electrode is made the anode of the cell, similar information on the oxidisable substances in the sample may be obtained. Polarograms may be plotted manually or recorded automatically either on photographic paper or with ink on a paper chart. Photographic recording was employed in Heyrovský and Shikata's original instrument,[2] but ink-recording is now generally favoured.[10]

The layout for a simple photographic recording polarograph is illustrated in Fig. 1. A constant voltage is supplied from the battery, *B*, to

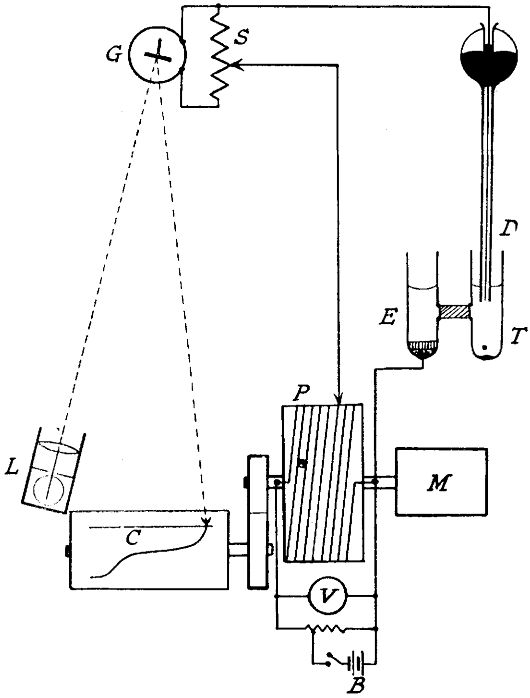

FIG. 1

Diagram of simple photographic recording polarograph.

B, Battery. *V*, Voltmeter.
P, Rotary potentiometer.
M, Motor.
C, Camera slit.
G, Reflecting galvanometer.
S, Galvanometer shunt.
L, Galvanometer lamp.
D, Dropping-mercury electrode.
E, Calomel half-cell.
T, Test solution.

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[10] J. T. Stock in R. F. Milton and W. A. Waters's " Methods of Quantitative Microanalysis ", Ed. Arnold & Co., London, 1949, p. 434 ; J. J. Lingane, *Analyt. Chem.*, 1951, **23,** 86.

the rotary potentiometer, *P*. When the potentiometer is rotated by the motor, *M*, a sliding contact moves along the potentiometer wire so that the voltage applied to the electrolytic cell increases gradually from zero to about — 2·6 v. At the same time the photographic paper is rotated past a slit in the camera, *C* ; the light beam from the mirror galvanometer, *G*, is focused on to the camera slit, *C*, so that any deflection of the galvanometer mirror will be recorded on the photographic paper. Mercury from the glass capillary drops into the test solution, which is connected through a liquid junction to a saturated calomel half-cell. The latter is included so that the applied potential can be measured directly against the saturated calomel electrode.

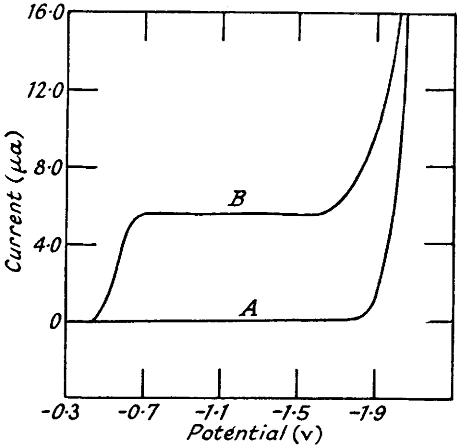

FIG. 2

Current–voltage curves for air-free 10% *ethanolic sodium phosphate buffer solutions containing* 0·005% *of gelatin.*

A, Base solution alone (residual current).

B, $5{\cdot}0 \times 10^{-4}$M-*Nitrobenzene in base solution.*

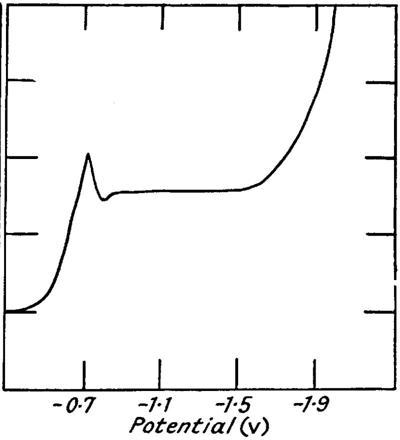

FIG. 3

Current–voltage curve for $5{\cdot}0 \times 10^{-4}$M-*nitrobenzene in air-free* 10% *ethanolic sodium phosphate buffer solution.*

A typical polarogram for $5{\cdot}0 \times 10^{-4}$M-nitrobenzene in an air-free 10% ethanolic sodium phosphate buffer solution (pH 7·0) containing 0·005 % of gelatin is shown in Fig. 2. Under standard conditions the diffusion current (*i.e.*, the step or wave-height) is proportional to the concentration of electro-reducible substance present in the solution. This relationship forms the basis of quantitative polarography. The half-wave potential, which was the name given by J. Heyrovský and D. Ilkovič [11] to the value of the potential of the dropping-mercury electrode, measured against a standard reference electrode, at the point on the current–voltage curve at which the current is one-half of its limiting value, is a special property of the reducible substance under examination ; it is independent of the concentration of the

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[11] *Coll. Czech. Chem. Comm.*, 1935, **7**, 198.

substance and of the characteristics of the dropping-mercury electrode. The saturated calomel electrode is generally accepted as the standard reference electrode, and all half-wave potentials mentioned in this Review will be referred to it.

Polarographic Current.—When an organic substance is electrolysed at a dropping-mercury electrode, six different types of current may contribute to the final polarogram. These are condenser or residual, migration, diffusion, adsorption, catalytic, and kinetic currents. Each of these currents will be discussed briefly but no attempt will be made to give a detailed theoretical exposition.

(1) **Condenser or residual current.** When a supporting electrolyte is polarographed in the absence of reducible material, a small condenser or residual current is recorded ; the current rises almost linearly with applied voltage and does not show a polarographic step. If the supporting electrolyte contains any impurities, they may give rise to small steps, which will be superimposed on the condenser current. The residual current for the sodium phosphate buffer solution is shown in Fig. 2. In quantitative measurements the residual current must always be subtracted from the total observed current.

(2) **Migration current.** The migration current is produced by the movement of charged particles in an electric field ; it is eliminated by adding to the solution a supporting electrolyte (*e.g.*, a sodium phosphate buffer mixture) at a concentration so large that its ions will carry practically all the current. In order to produce a good diffusion current, the added electrolyte should be about 25 times as concentrated as the reducible substance.

(3) **Diffusion current.** The diffusion current is formed by the diffusion of charged particles in the gradient produced by the reduction of material at the dropping-mercury electrode. Its magnitude is governed by D. Ilkovič's equation,[12] $i_{\mathrm{d}} = 607nD^{\frac{1}{2}}cm^{\frac{2}{3}}t^{\frac{1}{6}}$, in which i_{d} is the diffusion current in μa., n is the number of electrons involved in the reduction of one molecule of reducible substance, D is the diffusion coefficient of the reducible substance in sq. cm. per sec., c is its concentration in millimoles per l., m is the weight of mercury (in mg.) flowing out of the capillary per sec., and t is the drop time in sec. The equation was derived independently by D. MacGillavry and E. K. Rideal [13] and has been shown to be of general application. Recent work, however, has shown that the ratio $i_{\mathrm{d}}/m^{\frac{2}{3}}t^{\frac{1}{6}}$ varies slightly, owing to neglect, during the derivation of the equation, of the fact that the diffusion is towards a curved surface. For absolute values, Ilkovič's equation should be multiplied by the factor $(1 + xD^{\frac{1}{2}}t^{\frac{1}{6}})/m^{\frac{1}{3}}$. J. J. Lingane and B. A. Loveridge [14] propose a value of 39 for the factor x, but H. Strehlow and M. von Stackelberg [15] suggest 17 (cf. however, L. Meites and T. Meites [16]). It follows from Ilkovič's equation that results obtained with different capillaries may be compared, provided that the product,

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[12] *Coll. Czech. Chem. Comm.*, 1934, **6**, 498.

[13] *Rec. Trav. chim.*, 1937, **56**, 1013.

[14] *J. Amer. Chem. Soc.*, 1950, **72**, 438.

[15] *Z. Elektrochem.*, 1950, **54**, 51.

[16] *J. Amer. Chem. Soc.*, 1950, **72**, 4843.

$m^{\frac{2}{3}}t^{\frac{1}{6}}$, is known. Data for m and t should therefore be included in all polarographic reports.

(4) **Adsorption current.** The adsorption current is responsible for the formation of characteristic maxima on current–voltage curves. If nitrobenzene is examined in a gelatin-free base solution, a polarogram of the type shown in Fig. 3 is obtained. The current rises sharply, but, instead of developing into a normal diffusion current, increases abnormally until a critical value is reached and then rapidly decreases to a limiting value corresponding with the normal diffusion current. Maxima of this type are readily suppressed by adding to the solution small quantities of a surface-active substance. Gelatin and methyl-red at a concentration of 0·001—0·1% are frequently used (see Fig. 2). Larger amounts of surface-active substances suppress and alter the shape of the polarographic step. L. Holleck and H. J. Exner [17] have demonstrated the suppressant action of large amounts of gelatin, tylose, and agar on polarograms formed by *p*-nitroaniline, *m*-nitrobenzoic acid, and *m*- and *p*-nitrobenzaldehyde.

(5) **Catalytic current.** When an organic substance is reduced at the dropping-mercury electrode, reactions frequently occur at the interface; the rates of these reactions in many instances control the strength of the resulting current. Such currents may produce either a large maximum on the current–voltage curve or an abnormally large apparent diffusion current. The former is termed a catalytic and the latter a kinetic current.

Catalytic waves are formed under certain conditions by ions that deposit at more negative potentials than hydrogen and are able to increase greatly the hydrogen over-potential. These waves may be distinguished from ordinary reduction steps by observing the effect of pH on their height. Catalytic waves show big changes in height for small changes in pH and frequently attain a height many hundreds of times greater than that of the corresponding reduction step; they cannot be suppressed by the methods used for ordinary maxima.

The phenomenon was discovered by J. Heyrovský and J. Babička [18] while studying the polarographic behaviour of albumin in an ammoniacal buffer solution. R. Brdička [19] subsequently found that cystine and cysteine dissolved in an ammoniacal buffer solution containing bivalent (but not tervalent) cobalt or nickel gave a characteristic catalytic wave (see Fig. 4). The wave appeared at a more negative potential than corresponded to the reduction of cystine to cysteine and was thus due to the reduced form, cysteine. The height of the catalytic wave due to cystine was twice that formed by the same molar quantity of cysteine, but 500 times as great as that of the step associated with the normal reduction of cystine to cysteine.

Brdička attributed the catalytic cysteine wave to the formation of a cobalt–cysteine complex, in which there is a co-ordinate link between the cobalt atom and the thiol group. This link activates the bond between the sulphur and the hydrogen atom in the thiol group and facilitates the deposition of hydrogen at the dropping-mercury electrode. Müller,[7] how-

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[17] Proc. 1st Intern. Polarographic Congr., Prague, 1951, Vol. I, p. 97.

[18] *Coll. Czech. Chem. Comm.*, 1930, **2**, 370.

[19] *Ibid.*, 1933, **5**, 112, 148, 238.

ever, has pointed out that, since the cobalt ions are discharged before the catalytic wave potential is reached, the electrode becomes a dropping-cobalt-amalgam electrode, which by itself produces a catalytic reaction with thiol groups. J. Klumpar [20] has recently shown that the reciprocal of the catalytic wave-height is proportional to the reciprocal of the cystine concentration (if the cobalt concentration is constant) and to the reciprocal of the cobalt concentration (if the cystine concentration is constant).

In addition to thiol-containing compounds, many other substances, such as pyridine, nicotinic acid,[21] most alkaloids,[22] aneurin,[23] and cyanocobalamin (vitamin B_{12}),[24] form catalytic waves.

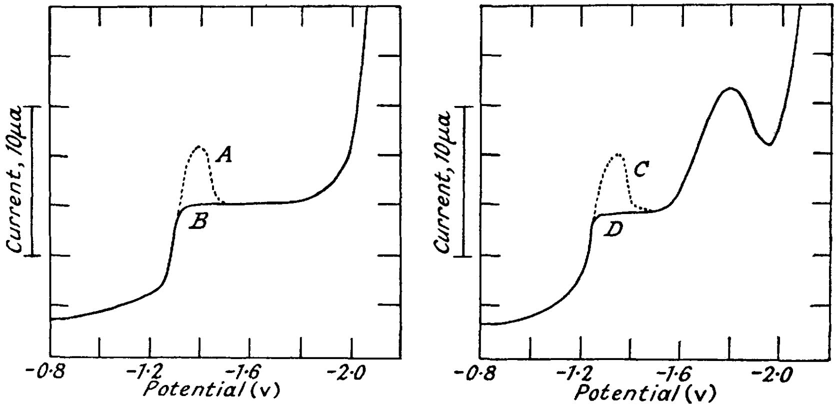

FIG. 4

Polarograms showing the cystine catalytic wave.
Ammoniacal cobalt buffer solution.

A, Alone.
B, Containing 0·01% *of gelatin.*
C, Containing 0·00005% *of cystine.*
D, Containing 0·00005% *of cystine and* 0·01% *of gelatin.*

(6) **Kinetic current.** The kinetic current is controlled by the rate of the reaction taking place at the dropping-mercury electrode. Such currents are observed during the reduction of certain carboxylic acids and their anions and of keto–enol tautomeric substances : the electrolytic removal of material from the interface unbalances the equilibrium, which is restored at a measurable rate.

K. Wiesner [25] has shown that the kinetic current is a direct function of mercury drop-size and is independent of the velocity of mercury flow

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[20] *Coll. Czech. Chem. Comm.*, 1948, **13**, 11.

[21] P. C. Tompkins and C. L. A. Schmidt, *J. Biol. Chem.*, 1942, **143**, 643 ; E. Knobloch, *Coll. Czech. Chem. Comm.*, 1947, **12**, 407.

[22] H. F. W. Kirkpatrick, *Quart. J. Pharm.*, 1945, **18**, 245, 338 ; 1946, **19**, 8, 127, 526 ; 1947, **20**, 87 ; F. Šantavý, *Coll. Czech. Chem. Comm.*, 1949, **14**, 145, 377.

[23] A. Wollenberger, *Science*, 1945, **101**, 386.

[24] K. H. Fantes, J. E. Page, L. F. J. Parker, and E. L. Smith, *Proc. Roy. Soc.*, 1949, *B*, **136**, 592.

[25] *Z. Elektrochem.*, 1943, **49**, 164.

from a given capillary. R. Brdička and K. Wiesner [26] have further demonstrated that the kinetic current is proportional to the rate constant of the reaction and to the volume of the interface in which the reaction takes place. A more rigorous mathematical treatment of the phenomenon has been provided by J. Koutecký and R. Brdička [27] and by P. Delahay.[28]

Additional examples of the six types of current will be given later in this Review.

Half-wave Potential.—The concept of half-wave potential was not introduced by Heyrovský and Ilkovič [11] until 1935 and so in the earlier polarographic papers arbitrary " reduction " potentials were reported instead of half-wave potentials. Different empirical methods for assigning reduction potentials were used by J. Heyrovský,[29] G. Semerano,[30] and M. Shikata.[31] When referring to early polarographic literature, it is important to ascertain the method used for measuring reduction potentials and to ensure that reduction potentials are not confused with half-wave potentials. Reduction potentials are less satisfactory than half-wave potentials in that they vary with the concentration of reducible substance, with galvanometer sensitivity, and with mercury drop-time and flow-rate.

P. Zuman [32] has recently provided a useful reference list of the half-wave potentials of several hundred organic substances.

Practical polarography

General Effects.—(1) **Solvent.** Since the substance to be examined must be in true solution, organic substances that are insufficiently soluble in water are usually dissolved in a mixture of water and a miscible organic solvent ; the supporting electrolyte must also dissolve in the solvent mixture. The monohydric and polyhydric alcohols and dioxan are suitable organic solvents.

A small amount of polarographic work on organic substances has been conducted in non-aqueous media, such as methanol, ethanol, formamide, glycerol,[33] Cellosolves (ethylene glycol monoalkyl ethers),[34] glacial acetic acid,[35] and concentrated sulphuric acid.[36] The resultant steps are lower and usually more drawn out than those for the same substance in water. A mixture of benzene and methanol has been used for oil-soluble substances, such as oxidised fat and petroleum peroxides.[37] A few inorganic

[26] *Coll. Czech. Chem. Comm.*, 1947, **12**, 138.

[27] *Ibid.*, p. 337.

[28] *J. Amer. Chem. Soc.*, 1951, **73**, 4944 ; 1952, **74**, 3506.

[29] *Rec. Trav. chim.*, 1925, **44**, 488.

[30] *Gazzetta*, 1932, **62**, 518.

[31] *Mem. Coll. Agric. Kyoto*, 1927, **4**, 1.

[32] *Coll. Czech. Chem. Comm.*, 1950, **15**, 1107.

[33] A. M. Zanko and F. A. Manusova, *J. Gen. Chem., U.S.S.R.*, 1940, **10**, 1171.

[34] T. D. Parks and K. A. Hansen, *Analyt. Chem.*, 1950, **22**, 1268.

[35] G. B. Bachman and M. J. Astle, *J. Amer. Chem. Soc.*, 1942, **64**, 1303, 2177; E. Hála, *Chem. Obzor*, 1948, **23**, 145.

[36] J. C. James, *Trans. Faraday Soc.*, 1951, **47**, 1240.

[37] W. R. Lewis, F. W. Quackenbush, and T. De Vries, *Analyt. Chem.*, 1949, **21**, 762.

substances have been examined in ethylene glycol,[38] liquid ammonia,[39] and fused salts.[40]

All the ingredients used in the preparation of a polarographic base solution must first be tested to ensure that they do not contain impurities that would be reduced at a more positive potential than the substance under examination. Ethanol, *iso*propyl alcohol, and dioxan must be free from aldehydes and peroxides.

Addition of an organic solvent to an aqueous solution affects not only the pH value of the solution but also the diffusion current and half-wave potential of the reducible substance. It can be deduced from Ilkovič's equation that diffusion currents should be smaller in non-aqueous than in aqueous solvents. Thus ethanol depresses the diffusion current of nitrobenzene in solutions buffered at pH 4·0 and 8·0 ; [41] the diffusion current of *p*-nitroaniline decreases with increase of ethanol content up to 10M-ethanol and then increases slightly.[42] Half-wave potentials are usually more negative in organic solvents than in aqueous solution. Thus the addition of 30% of ethanol to a nitrobenzene solution buffered at pH 4·0 changes the half-wave potential of the nitrobenzene from — 0·37 to — 0·48 v.[41] Ethanol has a similar effect on the reduction potential of *p*-nitroaniline.[42] When working with mixtures of water and an organic solvent, it should be remembered that the potential of the reference electrode and of the liquid junction may be different from that observed in aqueous media and that the cell resistance may be considerably greater. These factors must be considered during the calculation of the half-wave potential and a correction applied for the potential drop between the electrodes.

(2) **Base electrolyte.** Special care is needed in selecting the base electrolyte to be used in the study of organic substances. Since pH value has a big effect on the half-wave potential of aldehydes, ketones, quinones, unsaturated and halogeno-acids and nitro-, nitroso-, and azo-compounds, polarographic solutions should be buffered. In unbuffered solutions pH changes due to depletion of hydrogen ions at the electrode surface frequently lead to the formation of either drawn-out or double steps. Theoretical interpretation of many published polarograms is prevented because unbuffered solutions were employed.

If alkali or alkaline-earth metal salts are used as base electrolytes at pH values higher than 8·0, the potential range of the dropping-mercury electrode is limited to about — 1·8 v. By using tetramethyl- and tetrabutyl-ammonium salts the range may be extended to — 2·6 v [43, 44] and

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[38] C. H. R. Gentry, *Nature*, 1946, **157**, 479.

[39] H. A. Laitinen and C. J. Nyman, *J. Amer. Chem. Soc.*, 1948, **70**, 2241, 3002 ; H. A. Laitinen and C. E. Shoemaker, *ibid.*, 1950, **72**, 663, 4975.

[40] N. H. Nachtrieb and M. Steinberg, *ibid.*, 1948, **70**, 2613 ; 1950, **72**, 3558.

[41] J. E. Page, J. W. Smith, and J. G. Waller, *J. Phys. Colloid Chem.*, 1949, **53**, 545.

[42] O. D. Shreve and E. C. Markham, *J. Amer. Chem. Soc.*, 1949, **71**, 2993.

[43] J. Pech, *Coll. Czech. Chem. Comm.*, 1934, **6**, 126.

[44] P. van Rysselberghe and J. M. McGee, *J. Amer. Chem. Soc.*, 1945, **67**, 1039.

— 2·8 v [45] respectively. In this way, M. von Stackelberg and W. Stracke [46] were able to reduce acetone and other substances that were previously considered not to be reducible at the dropping-mercury electrode.

The half-wave potentials of certain organic substances depend on the concentration and valencies of the electrolytes present in the solution. Thus at alkaline pH values, barium and calcium ions have a greater effect than potassium ions on the half-wave potential of diacetyl.[47] K. G. Stone [48] believes that buffer composition is important whenever the buffer anion forms a relatively stable compound with either the starting material or one of its reduction products; he urges that in future work the effect of different buffer systems as well as changes in pH value should be studied. This feature has been re-emphasised by the recent work of P. J. Elving, J. C. Komyathy, R. E. van Atta, C.-S. Tang, and I. Rosenthal [49] on the polarographic reduction of α-bromo-*n*-butyric acid over the pH range 1—12, using various buffer systems with ionic strengths ranging from 0·1 to 3M. They found that the half-wave potential depended on the nature and concentration of the buffer mixture and on the pH value and ionic strength of the solution. For every substance there is an ionic-strength region in which half-wave potential changes are at a minimum; for α-bromo-*n*-butyric acid this region is between 1·0 and 1·5M.

(3) **Temperature.** Temperature changes have a slight effect on half-wave potential and a greater effect on diffusion current. The temperature coefficient for half-wave potential is less than one mv. per degree and is usually negative; that for diffusion current is about 1·5% per degree and is usually positive. To restrict diffusion-current variations within ± 1·0%, the polarograph cell should therefore be mounted in a thermostat, of which the temperature does not vary by more than ± 0·5°.

(4) **Oxygen.** Since oxygen is reduced at the dropping-mercury electrode and the resulting steps interfere with the current–voltage curves for most organic substances, it is usually essential to remove dissolved oxygen from solutions before polarographic examination. This is done either by bubbling oxygen-free nitrogen or hydrogen through the solution or, if the solution is neutral or alkaline, adding about 1·0% (w/v) of sodium sulphite.

Special Polarographic Techniques.—Recently several new polarographic techniques have been introduced. So far they have been mainly used in the study of inorganic systems, but there is no doubt that they will be of great value in the study of organic compounds.

(1) **Platinum micro-electrodes.** For certain measurements the dropping-mercury electrode may be replaced by a platinum micro-electrode; it may be either stationary,[50] rotating,[51] or vibrating.[52] Platinum electrodes can

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[45] H. A. Laitinen and S. Wawzonek, *J. Amer. Chem. Soc.*, 1942, **64**, 1765.

[46] *Z. Elektrochem.*, 1949, **53**, 118.

[47] A. Winkel and G. Proske, *Ber.*, 1938, **71**, 1785.

[48] *J. Electrochem. Soc.*, 1950, **97**, 63.

[49] *Analyt. Chem.*, 1951, **23**, 1218.

[50] D. B. Julian and W. R. Ruby, *J. Amer. Chem. Soc.*, 1950, **72**, 4719.

[51] C. J. O. R. Morris, *Analyst*, 1947, **72**, 298.

[52] E. R. Roberts and J. S. Meek, *ibid.*, 1952, **77**, 43.

be used for measurements in the positive-potential range, in which mercury would be oxidised. Thus a platinum electrode was used in a study of the anodic oxidation of quinol, catechol, ascorbic acid,[53] and several substituted *p*-phenylenediamines,[50] and for the determination of alkyl peroxides.[52] So far their greatest use has been in the amperometric titration of organic substances.

(2) **Differential polarography.** In differential polarography, which was devised by G. Semerano and L. Riccoboni,[54] two dropping-mercury electrodes with the same characteristics are introduced into separate solutions that differ only in the quantity of the unknown substance that they contain. Both dropping-mercury cathodes are supplied from the same mercury reservoir, but the two anodes are connected together through a suitably shunted galvanometer and also with the anodic end of the potentiometer unit. Thus any residual current due to the supporting electrolyte is balanced out and only the differential current produced by the unknown substance is recorded. The technique is useful when high sensitivity is required and for studying substances reduced at the high potentials at which alkali metals are deposited.

(3) **Derivative polarography.** Derivative polarography can be used to determine half-wave potentials and wave heights.[5] In this method, instead of the current, i, the current increment for a small change in potential, $\mathrm{d}i/\mathrm{d}E$, is plotted against the potential, E. The resulting curves (see Fig. 5) have sharp peaks at the half-wave potential and the height of the peak is proportional to the concentration of reducible substance. Steps that are too close together to be separated by ordinary polarography can sometimes be resolved by derivative polarography. Several methods involving the use of either one or two dropping-mercury electrodes to obtain derivative curves automatically have been described by J. Heyrovský.[55]

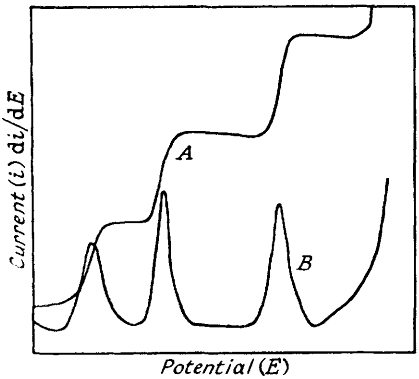

FIG. 5

A normal current voltage curve (A) for a solution containing thallium, cadmium, and zinc ions and the corresponding di/dE*–voltage curve (B).*

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[53] E. M. Skobets and N. N. Atamanenko, *Zavod. Lab.*, 1949, **15**, 1291.

[54] *Gazzetta*, 1942, **72**, 297.

[55] *Analyst*, 1947, **72**, 229.

(4) **Oscillographic polarography.** Cathode-ray oscillographs have been used to obtain a current–voltage curve for a single mercury drop, the drop time being synchronised with sweep frequency,[56] and to obtain potential–time curves at high frequencies.[57] By means of derivative potential (dE/dt)–potential (E) curves, J. Heyrovský [58] was able to distinguish between *o*-, *m*-, and *p*-nitrophenol in the presence of nitrobenzene (see Fig. 6) and between nicotinic, picolinic, and *iso*nicotinic acid ; in these curves, the half-wave potentials are indicated by minima. Oscillographic and alternating-current polarography has recently been reviewed by H. A. Laitinen.[59]

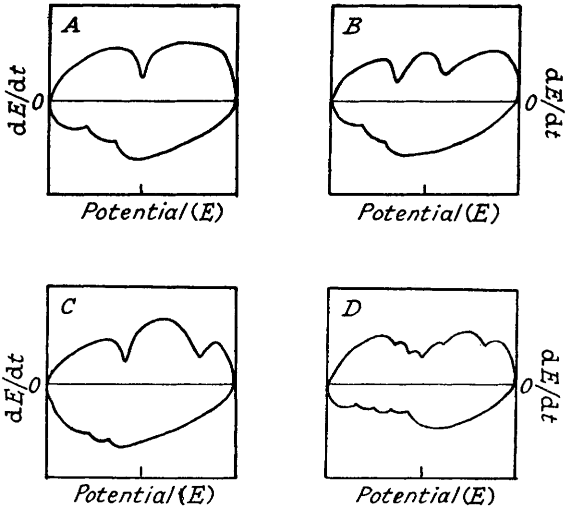

FIG. 6

Differential potential–potential oscillograms for 0·001M-o-*nitrophenol* (*A*), m-*nitrophenol* (*B*), p-*nitrophenol* (*C*), *and their mixture* (*D*) *in* 1·0N-*potassium hydroxide.*

These newer techniques have already provided important information on the rate of electrode processes and on the reversibility of certain reactions, but they need further development before they can be accepted for routine studies on organic substances.

Analytical Applications of Polarography.—(1) Scope of polarographic analysis. Theoretically every substance that is either electro-reducible or electro-oxidisable within the voltage range of the dropping-mercury electrode (*i.e.*, between + 0·6 and − 2·8 v) can be determined polarographically. Simple aliphatic olefins are not reducible at the dropping-mercury electrode ; if, however, the unsaturation is conjugated with an aryl, carbonyl, or

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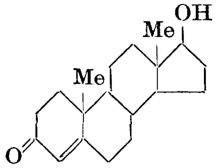
[56] J. E. B. Randles, *Trans. Faraday Soc.*, 1948, **44**, 322, 327 ; F. C. Snowden and H. T. Page, *Analyt. Chem.*, 1950, **22**, 969 ; 1952, **24**, 1152.

[57] J. Heyrovský and J. Forejt, *Z. physikal. Chem.*, 1943, **193**, 77.

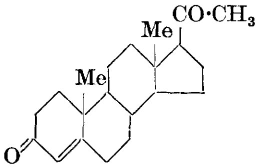
[58] *Analyt. Chim. Acta*, 1948, **2**, 533.

[59] *Ann. Review Phys. Chem.*, 1950, **1**, 309.

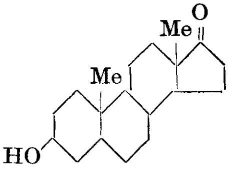
ethylene group, reduction becomes possible. Most keto-, aldehydo-, nitro-, and halogen groups are reducible. Organic substances that are not polarographically reducible can sometimes be converted into reducible derivatives. Thus although 4 : 5-unsaturated 3-keto-steroids, such as testosterone (I), progesterone (II), corticosterone, and deoxycorticosterone, are reducible at the dropping-mercury electrode,[60] saturated 17-keto-steroids, such as androsterone (III), are not. However, when the latter are condensed with excess of Girard T reagent (trimethylammonium acetohydrazide chloride), an electro-reducible derivative (IV) is obtained.[61] Certain non-ketonic steroids, such as cholesterol, can be determined after oxidation with aluminium *tert.*-butoxide to the corresponding ketone and subsequent condensation with Girard T reagent.[62] The technique has been adopted for the determination of 17-keto-steroids in urine.[61, 63]



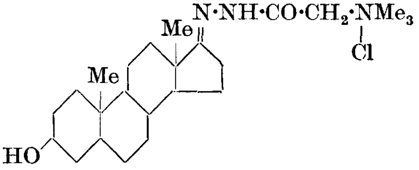
(I)



(II)



(III)



(IV)

(2) Examination of mixtures. If several electro-reducible substances are present together in the same solution, they can all be determined if their half-wave potentials are more than 0·2 v apart. Steps that are less than 0·2 v apart can sometimes be separated sufficiently for measurement by changing the pH value of the base solution, converting the reactants into complexes from which they are deposited at different potentials, or using derivative polarography.[58] If these methods are unsuccessful, the mixture must be chemically separated into suitable fractions and the individual fractions submitted for polarographic analysis.

Partition polarography should be of value for determining members of a homologous series. The technique was first used by B. A. Gosman[64] to deter-

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[60] J. Eisenbrand and H. Picher, *Z. physiol. Chem.*, 1939, **260**, 83.

[61] J. K. Wolfe, E. B. Hershberg, and L. F. Fieser, *J. Biol. Chem.*, 1940, **136**, 653; V. Prelog and O. Häfliger, *Helv. Chim. Acta*, 1949, **32**, 2088.

[62] E. B. Hershberg, J. K. Wolfe, and L. F. Fieser, *ibid.*, 1941, **140**, 215.

[63] J. Barnett, A. A. Henly, and C. J. O. R. Morris, *Biochem. J.*, 1946, **40**, 445; J. Barnett and C. J. O. R. Morris, *ibid.*, p. 450; J. Barnett, A. A. Henly, C. J. O. R. Morris, and F. L. Warren, *ibid.*, p. 778; W. R. Butt, A. A. Henly, and C. J. O. R. Morris, *ibid.*, 1948, **42**, 447.

[64] *Coll. Czech. Chem. Comm.*, 1935, **7**, 467.

mine the acetaldehyde content of ether. The sample was extracted twice with a lithium hydroxide solution, and both extracts were polarographed. By comparing the heights of the steps in the two polarograms he was able to calculate the original concentration of acetaldehyde. V. I. Gnyubkin, A. A. Dobrinskaya, and M. B. Neiman [65] were able to calculate the amount of acetaldehyde, propaldehyde, and *n*-butaldehyde in an aqueous solution, by measuring the rate of decrease in step height when the solution was deoxygenated with hydrogen. Recently B. E. Gordon and L. C. Jones [66] have described a benzene-extraction technique for the partition-polarographic determination of these aldehydes.

(3) **Analytical applications of maxima suppression.** The suppressive action of certain organic substances on the maximum formed by dissolved oxygen in 0·001N-potassium chloride provides a sensitive method for their determination. The technique has been used for the estimation of soaps, gelatin, dyes,[67] diethylstilbœstrol,[68] and cholesterol,[69] and for the measurement of surface-active impurities in refined sugar.[70]

(4) **Amperometric titrations.** Amperometric or polarometric titration, as the technique was originally called, has many applications in organic chemistry.[71] Precipitation, oxidation–reduction, and neutralisation reactions involving electro-active substances may be studied; such substances may be titrated or used as titrating agents. The sample dissolved in a suitable supporting solution is placed in a cell provided with either a dropping-mercury or a platinum electrode. The potential applied to the cell is maintained at a constant value, which is greater than the half-wave potential of the electro-active reagent; the diffusion current is observed on a galvanometer throughout the titration. The diffusion current is then plotted against the volume of reagent added, and the end-point is deduced from a change in the slope of the curve. Amperometric titrations need relatively simple equipment and frequently yield more accurate results than can be obtained by ordinary polarography.

The technique has been used to determine a wide range of organic substances, including organic peroxides,[72] vinyl acetate,[73] styrene,[74] ascorbic acid,[75] α-tocopherol,[76] thiols,[77] alkyl disulphides in the absence and pres-

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[65] *Acta Physicochim. U.R.S.S.*, 1939, **11**, 701.

[66] *Analyt. Chem.*, 1950, **22**, 981.

[67] M. von Stackelberg and H. Schütz, *Kolloid Z.*, 1943, **105**, 20.

[68] L. E. Bingenheimer and J. E. Christian, *J. Amer. Pharm. Assoc.*, 1949, **38**, 117.

[69] E. Talafant, *Coll. Czech. Chem. Comm.*, 1950, **15**, 232.

[70] I. Vavruch, *Analyt. Chem.*, 1950, **22**, 930.

[71] I. M. Kolthoff, *Analyt. Chim. Acta*, 1948, **2**, 606; H. A. Laitinen, *Analyt. Chem.*, 1949, **21**, 66; 1952, **24**, 46.

[72] I. M. Kolthoff and A. I. Medalia, *ibid.*, 1951, **23**, 595.

[73] O. Samuelson, *Svensk Kem. Tidskr.*, 1945, **57**, 27.

[74] I. M. Kolthoff and F. A. Bovey, *Analyt. Chem.*, 1947, **19**, 498.

[75] D. M. Coulson, W. R. Crowell, and S. L. Friess, *ibid.*, 1950, **22**, 525.

[76] L. I. Smith, I. M. Kolthoff, and L. J. Spillane, *J. Amer. Chem. Soc.*, 1942, **64**, 646.

[77] I. M. Kolthoff and W. E. Harris, *Ind. Eng. Chem. Anal.*, 1946, **18**, 161; *Analyt. Chem.*, 1949, **21**, 963; N. Strafford, F. R. Cropper, and A. Hamer, *Analyst*, 1950, **75**, 55.

ence of thiols,[78] thiodiglycol,[79] thiol groups in amino-acids and proteins,[80] cysteine and cystine,[81] *p*-aminosalicylic [82] and picrolonic acid,[83] nicotine,[84] nornicotine,[85] diamidines,[86] dimethylglyoxime,[87] and dye-couplers such as pyrazolones and naphthols.[88]

(5) **Merits of polarographic analysis.** Under normal conditions the precision of polarographic analysis is about ± 2% in the concentration range 10^{-2}—10^{-4}M, and ± 5% at 10^{-4}—10^{-5}M. By taking special precautions a higher precision can sometimes be obtained. However, when the small quantity of material (about 2 ml. of a 10^{-2}—10^{-5}M-solution) needed for an analysis is considered, the precision is seen to compare favourably with that of other microanalytical techniques. Since the total current passing during a determination is small, the test solution is practically unchanged and the measurement may be repeated several times on the same solution. If necessary the sample may be recovered for further studies.

Applications of Polarography in Reaction Kinetics.—Use of polarographic analysis in the field of reaction kinetics has been reviewed by G. Semerano.[89] The applicability of the method depends on the direct or induced polarographic activity of one or more of the organic substances taking part in the reaction. The technique has been employed in kinetic measurements during tautomeric changes (*e.g.*, conversion of nitroparaffins into the sodium salt of the *aci*-form [90]), decomposition reactions (*e.g.*, of methyl 4 : 5-diketopent-2-enoate 5-oxime $HO{\cdot}N{:}CH{\cdot}CO{\cdot}CH{:}CH{\cdot}CO_2Me$ [91]), addition reactions (*e.g.*, between glycine and iodoacetic acid [92]), oxidation–reduction reactions (*e.g.*, enzymic oxidation of tyrosine [93]), hydrolytic reactions (*e.g.*, hydrolysis of nicotinamide in hydrochloric acid solution [94]), dehydrochlorination reactions [*e.g.*, elimination of hydrogen chloride from D.D.T., $(p\text{-}C_6H_4Cl)_2CH{\cdot}CCl_3$ [95]], autoxidation reactions (*e.g.*, of fats [37]), polymerisation reactions (*e.g.*, of

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[78] I. M. Kolthoff, D. R. May, P. Morgan, H. A. Laitinen, and A. S. O'Brien, *Ind. Eng. Chem. Anal.*, 1946, **18**, 442.

[79] J. W. Sease, C. Niemann, and E. H. Swift, *Analyt. Chem.*, 1947, **19**, 197.

[80] R. Benesch and R. E. Benesch, *Arch. Biochem.*, 1948, **19**, 35 ; S. Rosenberg, J. C. Perrone, and P. L. Kirk, *Analyt. Chem.*, 1950, **22**, 1186 ; N. Weissman, E. B. Schoenbach, and E. B. Armistead, *J. Biol. Chem.*, 1950, **187**, 153.

[81] I. M. Kolthoff and W. Stricks, *J. Amer. Chem. Soc.*, 1950, **72**, 1952 ; *Analyt. Chem.*, 1951, **23**, 763.

[82] A. Liberti, *Atti R. Accad. Lincei*, 1950, **8**, 608.

[83] G. Cohn and I. M. Kolthoff, *J. Biol. Chem.*, 1943, **148**, 711.

[84] G. De Angelis, *Ric. sci.*, 1951, **21**, 62.

[85] C. O. Willits and C. Ricciuti, *Analyt. Chem.*, 1951, **23**, 1712.

[86] J. B. Conn, *ibid.*, 1948, **20**, 585.

[87] A. G. Stromberg and A. I. Zelyanskaya, *J. Analyt. Chem., U.S.S.R.*, 1949, **4**, 286.

[88] R. M. Elofson and P. A. Mecherly, *Analyt. Chem.*, 1949, **21**, 565.

[89] Proc. 1st Intern. Polarographic Congr., Prague, 1951, Vol. I, p. 300.

[90] E. W. Miller, A. P. Arnold, and M. J. Astle, *J. Amer. Chem. Soc.*, 1948, **70**, 3971.

[91] E. D. Hartnell and C. E. Bricker, *ibid.*, p. 3385.

[92] R. Brdička, *J. Gen. Physiol.*, 1936, **19**, 843, 899.

[93] K. Wiesner, *Biochem. Z.*, 1943, **314**, 214.

[94] H. H. G. Jellinek and A. Gordon, *J. Phys. Colloid Chem.*, 1949, **53**, 996.

[95] H. Keller, M. Hochweber, and H. von Halban, *Helv. Chim. Acta*, 1946, **29**, 761.

styrene [96]), diazotisation reactions,[97] and various types of electrode reactions (*e.g.*, electrolytic dissociation equilibrium of weak acids [98]). These and other applications of polarographic analysis to reaction kinetics will be discussed in later sections of this Review.

Polarographic Oxidation and Reduction Mechanisms.—A reaction mechanism can usually be assigned to the polarographic oxidation or reduction of an organic substance if the final product is known. Sufficient of the product for identification can sometimes be isolated by continuing the electrolysis at a quiet mercury electrode. Nevertheless it cannot be assumed that the product thus formed will be the same as that at a dropping-mercury electrode. When isolation of the product is not feasible, the number of electrons involved in the reduction should be determined by either coulometric measurements, calculation from Ilkovič's equation, or comparison with the polarographic behaviour of similar compounds. Whenever a mechanism is assigned, the chemical properties of the compound, the variation of the half-wave potential with pH value, and the slope of the polarographic step should be considered.

Coulometric measurement provides the most accurate method for determining the number of electrons involved in a reduction. It is of particular value for reductions, such as that of picric acid, in which a large number of electrons is involved. J. J. Lingane [99] has developed a method in which the current consumed during the quantitative electrolysis at a large stirred mercury cathode, whose potential is controlled by a potentiostat [100] at a value previously selected from a polarographic measurement, is integrated and compared with the number of moles of reducible material originally present. The method was satisfactory for the study of picric acid, but it cannot always be assumed that the reduction of an organic substance at a large stirred mercury cathode proceeds in the same way as at a dropping-mercury electrode. S. Bogan, L. Meites, E. Peters, and J. M. Sturtevant [101] have therefore designed a millicoulometer that can be used to integrate the current flowing through a polarograph cell under conditions such that a big decrease in the concentration of reducible material takes place. Recently G. A. Gilbert and E. K. Rideal [102] have described a micropolarograph cell that they used to determine the number of electrons involved in the electro-reduction of an azo-sulphonate dye.

In order to use Ilkovič's equation, the concentration, diffusion current, and diffusion coefficient of the reducible compound must be known. Ionic-diffusion coefficients may be calculated from ionic-conductance data, and those of uncharged substances of high molecular weight from the molecular weights. The diffusion coefficient of an organic substance can be deter-

[96] F. A. Bovey and I. M. Kolthoff, *J. Amer. Chem. Soc.*, 1947, **69**, 2143.

[97] R. M. Elofson, R. L. Edsberg, and P. A. Mecherly, *J. Electrochem. Soc.*, 1950, **97**, 166.

[98] R. Brdička, *Coll. Czech. Chem. Comm.*, 1947, **12**, 212.

[99] *J. Amer. Chem. Soc.*, 1945, **67**, 1916.

[100] J. J. Lingane, *Ind. Eng. Chem. Anal.*, 1944, **16**, 147.

[101] *J. Amer. Chem. Soc.*, 1951, **73**, 1584.

[102] *Trans. Faraday Soc.*, 1951, **47**, 396.

mined by J. H. Northrup and M. L. Anson's method.[103] The substance is dissolved in the medium in which the diffusion coefficient is required and the rate at which it diffuses through a sintered glass membrane is measured (cf. Stone [48]).

Reversible oxidation-reduction systems

Polarography provides an important method for distinguishing between reversible and irreversible organic reductions. In a reversible oxidation–reduction system, the half-wave potential for the oxidation of the reductant is identical with that for the reduction of the oxidant and is equal to the potentiometrically determined normal potential of the system. Thus O. H. Müller and J. P. Baumberger [104] found that in buffered solutions the half-wave potential for the reduction of benzoquinone is the same as that for the oxidation of quinol (see Fig. 7). If quinhydrone is examined, half of the curve represents oxidation of quinol at the mercury anode and the other half represents reduction of benzoquinone at the mercury cathode.

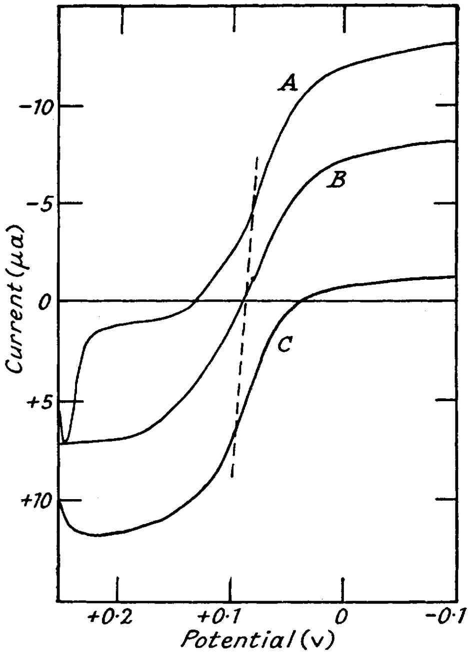

FIG. 7

Polarograms for quinol–benzoquinone system.
A, Benzoquinone buffered at pH 7·0.
B, Quinhydrone buffered at pH 7·0.
C, Quinol buffered at pH 7·0.

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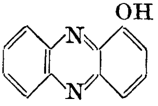
[103] *J. Gen. Physiol.*, 1929, **12**, 543; A. R. Gordon, *Ann. N.Y. Acad. Sci.*, 1945, **46**, 285.

[104] *Trans. Electrochem. Soc.*, 1937, **71**, 181.

The half-wave potential of a reversible system is a linear function of pH, the slope of the curve being − 0·0591 v per pH unit. Oscillographic techniques, in which both oxidation and reduction processes at the dropping-mercury electrode are presented simultaneously, promise to be useful in evaluating reversibility, but it must be remembered that oscillographically observed reversibility may differ from that detected by polarography and potentiometry.


(V)

Many dyes belonging to reversible oxidation–reduction systems form steps that under suitable conditions split into one or more steps. Usually a small anomalous step precedes the normal reduction step. For 1-hydroxyphenazine (V) a single wave with a more positive half-wave potential than the normal one appears at concentrations of up to about 10^{-4}M.[105] At higher concentrations, the anomalous wave ceases to increase in height and a second wave with the correct half-wave potential appears and continues to grow with increase of concentration (see Fig. 8). The anomalous wave cannot be attributed to a secondary reaction of the reduction product, for the wave is obtained for both oxidations and reductions. The sum of the two wave-heights is always proportional to the concentration of the dye. However, big changes in the height of the anomalous waves are produced when the drop-time of the electrode is changed. Anomalous waves are not formed by benzoquinone

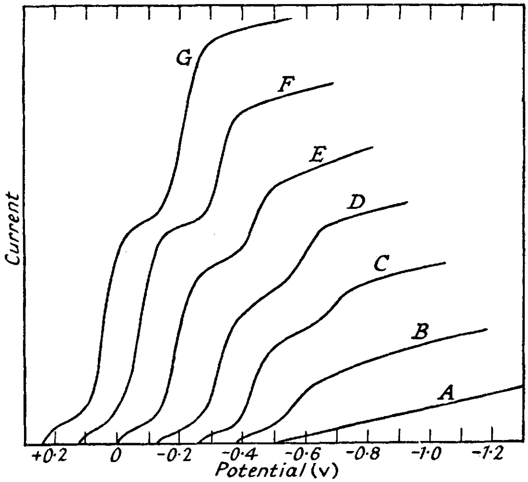


FIG. 8

Polarograms for 0 (*A*), 0·5 (*B*), 1·3 (*C*), 2·1 (*D*), 2·4 (*E*), 3·1 (*F*), *and* 3·7 (*G*) × 10^{-4}M-1-*hydroxyphenazine in* 0·1N-*sulphuric acid. The potential scale for curve G is marked on the abscissa. The other curves have been shifted progressively to the right by* 0·120 v.

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[105] O. H. Müller, *J. Biol. Chem.*, 1942, **145**, 425; *Trans. Electrochem. Soc.*, 1945, **87**, 441.

or duroquinone but are exhibited by methylene-blue,[106] riboflavin,[107] and pyocyanine,[108] and by a few irreversible systems, such as colchicine [109] and a styrene–oxygen polymer.[96]

Müller [105] believes that the anomalous wave is due to an unusually high "activity" of the dye in dilute solutions, but Brdička [109] attributes the phenomenon to adsorption of the reduced dye on the mercury drop. He considers that the energy liberated during the adsorption helps reduction in the same way that electrodeposition of metals on mercury is aided by the energy of amalgam formation. If the oxidised form of the oxidation–reduction system is adsorbed (*e.g.*, phenosafranine), a small wave appears at a potential more negative than the main one. When both forms are adsorbed equally, the polarographic steps are unaffected. The anomalous or, as it is now usually called, the adsorption wave is independent of concentration above a certain limit and is directly proportional to the height of the mercury reservoir. The wave-height decreases with increase in temperature; [109] the waves are readily suppressed by eosin and other substances that are strongly adsorbed but are not reduced polarographically. The wave suppressors also shift the reversible steps to more negative potentials.[110] Substances of high molecular weight have a greater suppressant action than those of low molecular weight.[111]

H. Eyring, L. Marker, and T.-C. Kwoh [112] have developed from the theory of absolute reaction rates a general equation explaining reversible and some types of irreversible organic waves.

Examples of Reversible Systems.—The observations of Müller and Baumberger [104] on the benzoquinone system have been extended to other reversible *p*-quinone systems, including substituted benzoquinones and toluquinones,[113] substituted naphthaquinones (including vitamin K),[114] quinones from α-tocopherol,[115] and substituted anthraquinones.[116] All these systems have been shown to be polarographically reversible. The methylenequinone system that occurs in citrinin (VI),[117] fuchsones (VII),[118] and the coloured form

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[106] R. Brdička, *Z. Elektrochem.*, 1942, **48,** 278.

[107] R. Brdička and E. Knobloch, *ibid.*, 1941, **47,** 721; R. Brdička, *ibid.*, 1942, **48,** 686.

[108] C. Cattaneo and G. Sartori, *Gazzetta*, 1942, **72,** 38; M. Voříšková, *Coll. Czech. Chem. Comm.*, 1947, **12,** 607.

[109] R. Brdička, *ibid.*, p. 522.

[110] K. Wiesner, *ibid.*, p. 594.

[111] B. Keilin, *J. Amer. Chem. Soc.*, 1948, **70,** 1984.

[112] *J. Phys. Colloid Chem.*, 1949, **53,** 1453.

[113] L. I. Smith, I. M. Kolthoff, S. Wawzonek, and P. M. Ruoff, *J. Amer. Chem. Soc.*, 1941, **63,** 1018; J. E. Page and F. A. Robinson, *J.*, 1943, 133.

[114] E. B. Hershberg, J. K. Wolfe, and L. F. Fieser, *J. Amer. Chem. Soc.*, 1940, **62,** 3516; G. Sartori and C. Cattaneo, *Gazzetta*, 1941, **71,** 713; J. E. Page and F. A. Robinson, *Brit. J. Exp. Path.*, 1943, **24,** 89; F. Poupě, *Coll. Czech. Chem. Comm.*, 1947, **12,** 225; H. Onrust and B. Wöstmann, *Rec. Trav. chim.*, 1950, **69,** 1207.

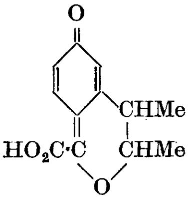
[115] L. I. Smith, L. J. Spillane, and I. M. Kolthoff, *J. Amer. Chem. Soc.*, 1942, **64,** 644.

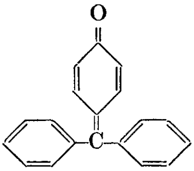
[116] N. H. Furman and K. G. Stone, *ibid.*, 1948, **70,** 3055, 3062; L. A. Wiles, *J.*, 1952, 1358; R. Gill and H. I. Stonehill, *J.*, 1952, 1845, 1857.

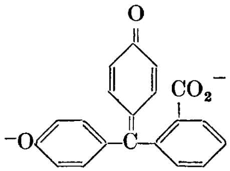
[117] H. W. Hirschy and P. M. Ruoff, *J. Amer. Chem. Soc.*, 1942, **64,** 1490.

[118] A. G. Stromberg and L. M. Reĭnus, *J. Gen. Chem., U.S.S.R.*, 1946, **16,** 1431.

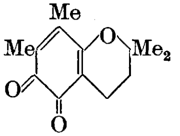
of phenolphthalein (VIII) [119] is irreversible, in spite of the fact that these compounds are structurally similar to *p*-quinones. Thus the half-wave potential of the red form of phenolphthalein does not shift with change in pH.

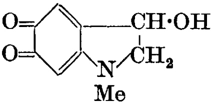

(VI)


(VII)


(VIII)

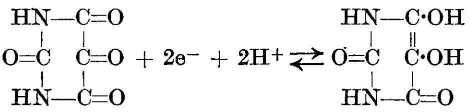
Among the *o*-quinones examined polarographically have been 1 : 2-benzoquinone, 4-methyl-1 : 2-benzoquinone, 1 : 2-naphthaquinone,[120] 2 : 2 : 7 : 8-tetramethylchroman-5 : 6-quinone (IX),[115] adrenochrome (X), which is obtained on enzymic oxidation of adrenaline,[121] and the corresponding

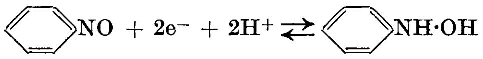
(IX)



(X)

o-quinone from enzymic oxidation of tyrosine.[93] Although these systems have been shown to be reversible, *o*-quinones are generally much more difficult to study than are *p*-quinones, being less stable, so that side-reactions are more likely to take place.[120]

Several other oxidation–reduction systems, such as alloxan (XI)–dialuric acid (XII) [122] and nitrosobenzene (XIII)–*N*-phenylhydroxylamine (XIV),[123]

(XI)

(XII)

(XIII)

(XIV)

have been shown to be polarographically reversible. Alloxan forms two reduction steps, the first of which corresponds with the oxidation step formed by dialuric acid ; the second is a catalytic wave produced by the —NH·CO·NH·CO— grouping and is similar to the catalytic wave formed by alloxanic acid.

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[119] I. M. Kolthoff and D. J. Lehmicke, *J. Amer. Chem. Soc.*, 1948, **70,** 1879.

[120] J. Doskočil, *Coll. Czech. Chem. Comm.*, 1950, **15,** 599, 780.

[121] K. Wiesner, *Biochem. Z.*, 1942, **313,** 48 ; G. B. Koelle and J. S. Friedenwald, *Arch. Biochem.*, 1951, **32,** 370.

[122] G. Sartori and A. Liberti, *Ric. sci.*, 1946, **16,** 313.

[123] J. W. Smith and J. G. Waller, *Trans. Faraday Soc.*, 1950, **46,** 290.

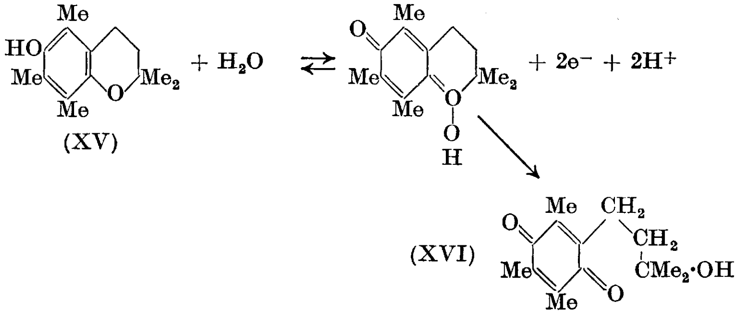
Irreversible oxidation-reduction systems

The polarographic oxidations and reductions of most organic substances are irreversible. Irreversible reactions fall into two general classes :

(1) Reactions with a reversible first stage. There is a direct transfer of electrons between the electrode and the organic compound, but either the product reacts further or the final equilibrium is reached slowly.

(2) Reactions with an irreversible first stage. Hydrogen ions are reduced to atomic or molecular hydrogen, which then reacts, either directly or after activation at the electrode, irreversibly with the organic compound.

The first type of mechanism gives rise to polarograms similar to those formed by reversible systems. The steps have a symmetrical S-shape, and their half-wave potentials vary linearly with pH. Oxidation of 6-hydroxychromans and 5-hydroxycoumarans provides examples of a reaction in which the products are unstable.[113] The final oxidation products are quinones that can be reversibly reduced to the corresponding quinols but not to the original hydroxychromans and hydroxycoumarans. Thus 6-hydroxy-2 : 2 : 5 : 7 : 8-pentamethylchroman (XV) is probably converted into a quinone (XVI) in the following way :



The behaviour of quinhydrone in a carbonate buffer solution may be considered as an example of a reaction in which the final equilibrium is reached slowly ; it gives separate anodic and cathodic steps, from which is inferred the formation of an acid that cannot react fast enough to maintain a constant pH during the electrode reaction.[7]

J. Pearson [124] has postulated that the primary electrode reaction for the irreversible reduction of a nitro-group, which involves four or more electrons and hydrogen ions, consists of the reversible deposition of hydrogen ions at the cathode ; this reaction determines the potential of the cathode and actually produces the observed current. Since the hydrogen atoms do not combine to form hydrogen molecules, they remain on the electrode surface in the atomic state. The speed of the reaction depends on the rate of diffusion of organic molecules to the cathode ; fresh hydrogen ions can only be deposited when some of the hydrogen atoms have reacted with the reducible material. The current is thus a function of the rate at which reducible

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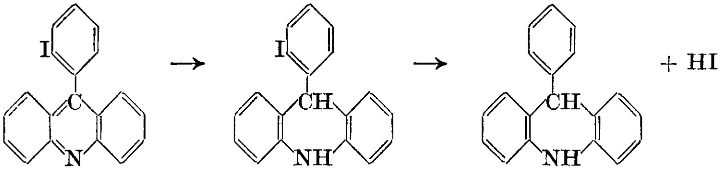
[124] *Trans. Faraday Soc.*, 1948, **44,** 683.

material diffuses to the electrode ; since the pH of the cathode surface layer is kept constant by the buffering action of the supporting electrolyte, the current is independent of the diffusion rate of the hydrogen ions.

Many organic substances undergo stepwise irreversible reactions at the dropping-mercury electrode. Thus 5-*o*-iodophenylacridine (XVII) in a mixture of 0·1N-potassium hydroxide and 0·5M-potassium acetate in 90% ethanol gives two reduction steps, at − 1·32 and − 1·62 v. The first step corresponds with reduction to dihydro-5-*o*-iodophenylacridine (XVIII), and the second with elimination of iodine to yield dihydro-5-phenylacridine (XIX). By controlling the potential of the mercury cathode, J. J. Lingane, C. G. Swain, and M. Fields [125] were able to prepare either product electrolytically in a high state of purity and in almost quantitative yield.



(XVII) (XVIII) (XIX)

P. Delahay and J. E. Strassner [126] (cf. Eyring, Marker, and Kwoh [112]) have derived an equation for an irreversible polarographic wave by assuming that the current at any point on the wave is controlled by the rate of the electrode process and by the diffusion rate of the substance reacting at the electrode.

Examples of irreversible systems

Unsaturated Hydrocarbons.—Isolated ethylene and acetylene bonds are not reduced at the dropping-mercury electrode, but double bonds either conjugated with carbonyl groups (*e.g.*, crotonaldehyde [127]) or present in heterocyclic rings (*e.g.*, quinoline [127]) are reducible. Laitinen and Wawzonek [45] first showed that phenyl-substituted olefin and acetylene compounds are reducible. Nine compounds were examined in a 3 : 1 mixture of dioxan and water that was 0·175M with respect to tetrabutylammonium iodide. Each of the hydrocarbons showed a single well-defined step, the half-wave potential of which was independent of hydrocarbon concentration and was unaffected by changes in pH. Increased substitution in the hydrocarbon molecule led to easier reduction of the ethylene and acetylene linkages. The conjugated diene, 1 : 4-diphenylbuta-1 : 3-diene, gave a diffusion current that corresponded with a two-electron reduction, thus indicating 1 : 4-addition of hydrogen to the conjugated system. The steps formed by phenylacetylene and diphenylacetylene indicated complete reduction of the acetylene linkage.

From a similar study of 17 aromatic polynuclear hydrocarbons,

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[125] *J. Amer. Chem. Soc.*, 1943, **65**, 1348.

[126] *Ibid.*, 1951, **73**, 5219.

[127] H. Adkins and F. W. Cox, *ibid.*, 1938, **60**, 1151.

S. Wawzonek and H. A. Laitinen [128] concluded that aromatic compounds possessing a high degree of resonance, such as benzene, are not reduced, but that aromatic polynuclear hydrocarbons with a decreased degree of resonance (*e.g.*, naphthalene, acenaphthylene, indene, fluorene, 3 : 4-benzopyrene, 3-methylcholanthrene, 1 : 2-benzanthracene, and 1 : 2-5 : 6-dibenzanthracene) are. The half-wave potentials were independent of changes in pH and concentration but characteristic of certain structures. The authors suggest that polarography might be used to determine double-bond arrangement in the various rings.

The reduction of ethylene and acetylene bonds probably proceeds in three stages : [45]

$$\begin{aligned} &(1)\quad R + e^- \rightleftarrows R^- \\ &(2)\quad R^- + e^- \longrightarrow R^{2-} \\ &(3)\quad R^{2-} + 2H_2O \longrightarrow RH_2 + 2OH^- \end{aligned}$$

The first stage is considered to be reversible and to fix the half-wave potential. The second and third stages are both irreversible ; if they were reversible, the half-wave potential would be dependent on pH.

On polarographic reduction, certain olefins, such as 1 : 1-diphenylethylene, dimerise in the same way as they do on treatment with sodium :

$$2CPh_2{:}CH_2 + 2Na \longrightarrow (Na{\cdot}CPh_2{\cdot}CH_2{\cdot})_2$$

Two mechanisms had been proposed to account for the reaction with sodium but it had not been possible to decide between them. However, S. Wawzonek and J. W. Fan [129] determined the number of electrons involved in the polarographic reduction of one olefin molecule. They deduced that most unsaturated compounds dimerise by adding two electrons, but that cinnamylidenefluorene and fluoranthene follow a free-radical mechanism ; di-9-fluorenylidene was exceptional in that it behaves as a diradical. A. Maccoll [130] has interpreted these observations in terms of the molecular orbital concept.

F. Goulden and F. L. Warren [131] extended the polarographic study of unsaturated compounds to 37 stilbene and stilbœstrol derivatives. All the half-wave potentials fell within a relatively narrow range ; in the stilbene series, substitution in one or more aromatic nuclei shifted the half-wave potentials to slightly more negative values. Alkyl or aryl monosubstitution on the α-carbon atom of a stilbene gave derivatives that were reduced at approximately the same or slightly higher potentials than stilbene itself. With the exception of αβ-diethylstilbene $(CPhEt{:})_2$, all αβ-dialkyl-substituted stilbenes (*e.g.*, αβ-diethylstilbœstrol) were not reduced ; the *cis*-, but not the *trans*-, form of αβ-diethylstilbene was reducible. F. Wessely and J. Wratil [132] showed that *cis*- and *trans*-stilbene may also be distinguished polarographically. When dissolved in 80% aqueous ethanol, *cis*-stilbene is reduced at – 2·18 v, and the *trans*-compound at – 1·13 v. Owing to their high negative half-wave potentials, stilbene derivatives cannot be identified polarographically in biological materials.

[128] *J. Amer. Chem. Soc.*, 1942, **64,** 2365.

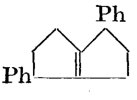
[129] *Ibid.*, 1946, **68,** 2541.

[130] *Nature,* 1949, **163,** 178.

[131] *Biochem. J.*, 1948, **42,** 420.

[132] *Mikrochem. Mikrochim. Acta,* 1947, **33,** 248.

von Stackelberg and Stracke[46] studied twelve additional unsaturated hydrocarbons, using as base solution, 0·05M-tetramethylammonium bromide in either 75% aqueous dioxan or 90% aqueous butanol. They confirmed that only ethylene and acetylene linkages forming part of a conjugated system are reducible and that the half-wave potentials of unsaturated non-ionic compounds are independent of changes in pH value. However the half-wave potentials of unsaturated acids (*e.g.*, maleic and fumaric) shift linearly with pH.[133]



(XX)

Polarography has been used to determine styrene in the styrene fraction of crude benzene and in the presence of polystyrene,[134] to determine naphthalene in petroleum fractions,[135] and to confirm the position of the double bond in 2 : 6-diphenyl*bicyclo*[3 : 3 : 0]-oct-1(5)-ene (XX).[136] *cyclo*-Octatetraene in tetramethylammonium salt solutions gives a two-electron step that is independent of pH. Since the step is poorly defined in other salt solutions, R. M. Elofson[137] has proposed the following unusual mechanism :

$$C_8H_8 + 2e^- + 2Me_4N^+ \longrightarrow (Me_4N^+)_2C_8H_8{}^{2-}$$

The double bonds in many heterocyclic nitrogen compounds are reducible, unless the substances give catalytic waves. Pyridine and nicotinic acid[21] yield catalytic waves, but the acridines being weaker bases are reduced to the corresponding dihydroacridines.[125, 138] Quinaldinic acid, 8-hydroxyquinoline, and quinoline-8-carboxylic acid yield three polarographic waves ; the first is probably catalytic, the second an adsorption wave, and the third a diffusion wave corresponding to the reduction of the quinoline molecule.[139] The mechanism of the reduction of these and other quinoline derivatives has been discussed by D. J. Casimer and L. E. Lyons.[140] Other heterocyclic compounds that have been examined include aneurin,[141] adenosine, adenine, adenylic acid,[142] folic acid and related pterins,[143] and many alkaloids.[22]

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[133] E. Vopička, *Coll. Czech. Chem. Comm.*, 1936, **8,** 349 ; B. Warshowsky, P. J. Elving, and J. Mandel, *Analyt. Chem.*, 1947, **19,** 161 ; P. J. Elving and C. Teitelbaum, *J. Amer. Chem. Soc.*, 1949, **71,** 3916.

[134] A. G. Pozdeyeva and A. G. Stromberg, *J. Analyt. Chem., U.S.S.R.*, 1950, **5,** 101 ; *J. Gen. Chem., U.S.S.R.*, 1950, **20,** 54.

[135] R. A. Burdett and B. E. Gordon, *Analyt. Chem.*, 1947, **19,** 843 ; D. P. Thornton, *Petrol. Process.*, 1948, **3,** 673.

[136] S. Wawzonek, *J. Amer. Chem. Soc.*, 1943, **65,** 839.

[137] *Analyt. Chem.*, 1949, **21,** 917.

[138] B. Breyer, G. S. Buchanan, and H. Duewell, *J.*, 1944, 360 ; D. Ll. Hammick and S. F. Mason, *J.*, 1950, 345 ; R. C. Kaye, *J. Pharm. Pharmacol.*, 1950, **2,** 902 ; R. C. Kaye and H. I. Stonehill, *J.*, 1951, 27, 2638.

[139] J. T. Stock, *J.*, 1944, 427 ; 1949, 586, 763.

[140] *J.*, 1950, 783.

[141] J. J. Lingane and O. L. Davis, *J. Biol. Chem.*, 1941, **137,** 567 ; G. Sartori and C. Cattaneo, *Gazzetta*, 1944, **74,** 166.

[142] J. C. Heath, *Nature*, 1946, **158,** 23.

[143] E. L. Rickes, N. R. Trenner, J. B. Conn, and J. C. Keresztesy, *J. Amer. Chem. Soc.*, 1947, **69,** 2751 ; W. J. Mader and H. A. Frediani, *Analyt. Chem.*, 1948, **20,** 1199 ; W. Allen, R. L. Pasternak, and W. Seaman, *J. Amer. Chem. Soc.*, 1952, **74,** 3264.

Organic Peroxides.—The steps formed by organic peroxides are usually flat and drawn out ; those of methyl and ethyl hydroperoxide in dilute acid medium start at − 0·25 v and hardly show a region of constant diffusion current. Their half-wave potentials are apparently independent of pH.[144] The reduction of an alkyl peroxide usually proceeds normally but, if its concentration in hydrochloric acid solution is high, decomposition may occur with formation of mercurous chloride, which is deposited on the mercury anode and interferes with the reduction ; [145] this difficulty can be overcome by replacing the dropping-mercury electrode with a vibrating platinum electrode.[52]

F. Reimers [146] has used polarography to determine peroxides and aldehydes in ether. The sample was mixed with a solution of tetramethylammonium hydroxide in ethanol and polarographed before and after removal of oxygen with ether-saturated nitrogen. The first polarogram gave information on the aldehyde content of the sample, and the second on the peroxide content. Lewis, Quackenbush, and De Vries [37] recommend an ethanol–benzene mixture containing lithium chloride as base solution for the determination of benzoyl peroxide, methyl linoleate peroxide, and ethyl, *iso*propyl, and *n*-butyl ether peroxides.

P. A. Giguère and D. Lamontagne [147] studied the reduction of benzoyl peroxide and cumene hydroperoxide and used the method to follow the disappearance of these catalysts in polymerisation reactions. The polarographic behaviour of *tert.*-butyl peroxide has been examined by Bovey and Kolthoff,[96] who used the technique to show that a styrene–oxygen polymer had a peroxide structure.

Aldehydes.—Most aldehydes and ketones are reduced at the dropping-mercury electrode ; in general, aldehydes are reduced more easily than ketones. Much of the earlier work on aliphatic aldehydes [127, 148] does not contain information on half-wave potential constancy, the mechanism of the reduction, or the relation between diffusion current and concentration ; recent work has been devoted to elucidation of the reduction mechanisms of individual aldehydes.

Formaldehyde undergoes a two-electron reduction according to the following mechanism : [148]

$$\mathrm{H{\cdot}CHO} + 2e^- + 2\mathrm{H}^+ \longrightarrow \mathrm{CH_3{\cdot}OH}$$

Formaldehyde differs from other aldehydes in that it readily forms hydrates ; since only free formaldehyde is reducible, its diffusion current is controlled by the speed of dehydration [149] and hence by factors, such as temperature, pH, solvent,[150] and buffer composition,[149] that influence the equilibrium. This mechanism has been confirmed by measurements on oscillographic potential–time curves and by observing that the diffusion current is inde-

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[144] V. Shtern and S. Pollyak, *Acta Physicochim. U.R.S.S.*, 1939, **11**, 797.

[145] M. B. Neĭman and M. I. Gerber, *J. Analyt. Chem., U.S.S.R.*, 1946, **1**, 211.

[146] *Quart. J. Pharm.*, 1946, **19**, 473.

[147] *Canad. J. Chem.*, 1951, **29**, 54.

[148] F. G. Jahoda, *Coll. Czech. Chem. Comm.*, 1935, **7**, 415.

[149] R. Bieber and G. Trümpler, *Helv. Chim. Acta*, 1947, **30**, 706 ; K. Veselý and R. Brdička, *Coll. Czech. Chem. Comm.*, 1947, **12**, 313.

[150] R. Bieber and G. Trümpler, *Helv. Chim. Acta*, 1947, **30**, 971, 1109, 1286, 1534.

pendent of the height of the mercury column above the cathode. The diffusion current is only proportional to concentration if the solution is buffered and contains a suitable maximum suppressor.[150] The heat of hydration of formaldehyde (13,000 cal. per mole) has been calculated from its temperature–diffusion current curve.[151]

The polarographic steps formed by acetaldehyde and propaldehyde are less dependent on external factors than the steps formed by formaldehyde, since the first two are less hydrated.[152] Acetaldehyde is largely reduced to ethanol but some dimethylglycol may be formed in the following way : [153]

$$2CH_3{\cdot}CHO + 2e^- + 2H^+ \longrightarrow (CH_3{\cdot}\dot{C}H{\cdot}OH)_2$$

These steps have been used to determine formaldehyde in pharmaceutical preparations [154] and in the presence of acraldehyde and other aldehydes,[155] to study the reaction of formaldehyde with urea, acetamide, and benzamide,[156] and to estimate acetaldehyde in complex liquid mixtures and in distillation fractions obtained during the conversion of ethanol into butadiene.[157] The technique has been used to determine indirectly compounds that yield either formaldehyde or acetaldehyde on oxidation with periodic acid. Thus serine has been estimated in protein hydrolysates,[158] glycerol in fermentation residues,[159] diethylene and dipropylene glycol in mixtures with ethylene and propylene glycol,[160] and ethylene and propylene chlorohydrin in the presence of each other.[161]

Glycollic aldehyde, aldoses (*e.g.*, glucose, rhamnose, arabinose, mannose, galactose, and lyxose) and disaccharides (*e.g.*, maltose, lactose, and sucrose) behave similarly to formaldehyde in that the steps formed by all of them are sensitive to temperature and pH changes. Glycollic aldehyde is normally present in equilibrium with its hydrate,[152] whereas the aldoses exist as cyclic hemiacetals. S. M. Cantor and Q. P. Peniston [162] thought that the polarographic steps were dependent on the appearance of the free *aldehydo*-form of the sugars and used the technique to measure the concentration of free aldehyde formed under various conditions. K. Wiesner [163]

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[151] M. B. Neĭman and M. I. Gerber, *J. Analyt. Chem., U.S.S.R.*, 1947, **2**, 135.

[152] R. Bieber and G. Trümpler, *Helv. Chim. Acta*, 1947, **30**, 2000 ; 1948, **31**, 5 ; W. Dirscherl and H.-U. Bergmeyer, *Chem. Ber.*, 1949, **82**, 291.

[153] I. Smoleř, *Coll. Czech. Chem. Comm.*, 1930, **2**, 699 ; G. Semerano and B. Polacsek, *Gazzetta*, 1938, **68**, 292.

[154] R. Portillo and G. Varella, *Anal. Fís. Quím.*, 1944, **40**, 794 ; R. Portillo and G. V. Mosquera, *Farm. nueva, Madrid*, 1945, **10**, 659.

[155] G. C. Whitnack and R. W. Moshier, *Ind. Eng. Chem. Anal.*, 1944, **16**, 496 ; A. S. Bogorad and S. N. Aleksandrov, *J. Analyt. Chem., U.S.S.R.*, 1951, **6**, 276.

[156] G. A. Crowe and C. C. Lynch, *J. Amer. Chem. Soc.*, 1948, **70**, 3795 ; 1949, **17**, 3731 ; 1950, **72**, 3622.

[157] P. J. Elving and E. Rutner, *Ind. Eng. Chem. Anal.*, 1946, **18**, 176.

[158] M. J. Boyd and K. Bambach, *ibid.*, 1943, **15**, 314.

[159] P. J. Elving, B. Warshowsky, E. Shoemaker, and J. Margolit, *Analyt. Chem.*, 1948, **20**, 25.

[160] B. Warshowsky and P. J. Elving, *Ind. Eng. Chem. Anal.*, 1946, **18**, 253 ; C. V. Francis, *Analyt. Chem.*, 1949, **21**, 1238.

[161] W. A. Cannon, *ibid.*, 1950, **22**, 928.

[162] *J. Amer. Chem. Soc.*, 1940, **62**, 2113.

[163] *Coll. Czech. Chem. Comm.*, 1947, **12**, 64.

has, however, since shown that the reaction is kinetic in nature and that the height of the polarographic step depends on the velocity of liberation of free aldehyde at the dropping-mercury electrode.

Unsaturated aldehydes, in which the aldehyde group is conjugated with one or more double bonds, yield two reduction steps. The first step formed by acraldehyde [164] and crotonaldehyde [127] corresponds to a bimolecular 1 : 4-reduction and the second to reduction of the aldehyde group. With higher unsaturated aliphatic aldehydes, such as 2 : 4-hexadienal, S. Wawzonek [165] believes that 1 : 4-addition of hydrogen occurs instead of the 1 : 2-addition postulated by M. Fields and E. R. Blout.[166] A. Kirrmann and P. Federlin [167] have examined a few aliphatic chloro- and bromo-aldehydes.

Aromatic aldehydes are reduced at lower potentials than the corresponding aliphatic aldehydes. Below pH 2 benzaldehyde yields a single one-electron step ; above pH 6 a single two-electron step appears. At intermediate pH values two steps are formed ; their total height corresponds with that of the two-electron step formed at higher pH values.[168] Thus at low pH values benzaldehyde is reduced to hydrobenzoin and at higher values to benzyl alcohol :

$$2\text{Ph·CHO} + 2e^- + 2\text{H}^+ \longrightarrow (\text{·CHPh·OH})_2$$
$$(\text{·CHPh·OH})_2 + 2e^- + 2\text{H}^+ \longrightarrow 2\text{CH}_2\text{Ph·OH}$$

R. Pasternak [169] has confirmed this mechanism by a controlled electrolytic reduction.

The reduction of substituted benzaldehydes has been studied extensively.[127, 170] J. W. Baker, W. C. Davies, and M. L. Hemming,[171] who were interested in the effects of substituents on the " reduction " potentials of aromatic aldehydes, explained their observations in terms of the inductive and tautomeric electron-release effects.

Furfuraldehyde is irreversibly reduced to the corresponding alcohol, two steps being formed.[172] Polarography has been used for estimation of furfuraldehyde in alcoholic extracts of resins [173] and in the products formed during the deterioration of apricots.[174]

Ketones.—Aliphatic and cyclic ketones are not reduced in ammonium or lithium chloride solution, but yield polarographic steps when examined in a tetra-alkylammonium salt solution.[46, 175] The hydrazone, phenyl-

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[164] R. W. Moshier, *Ind. Eng. Chem. Anal.*, 1943, **15,** 107.

[165] *Analyt. Chem.*, 1949, **21,** 61.

[166] *J. Amer. Chem. Soc.*, 1948, **70,** 930 ; see also D. M. Coulson and W. R. Crowell, *ibid.*, 1952, **74,** 1290, 1294.

[167] *Compt. rend.*, 1950, **230,** 1066 ; P. Federlin, *ibid.*, 1951, **232,** 60.

[168] G. Semerano and G. de Ponte, *Gazzetta*, 1932, **62,** 991 ; M. Tokuoka, *Coll. Czech. Chem. Comm.*, 1935, **7,** 392 ; I. A. Korshunov and L. N. Sazanova, *J. Phys. Chem., U.S.S.R.*, 1949, **23,** 202.

[169] *Helv. Chim. Acta*, 1948, **31,** 753.

[170] G. Semerano and A. Chisini, *Gazzetta*, 1933, **63,** 802 ; A. Winkel and G. Proske, *Ber.*, 1936, **69,** 1917.

[171] *J.*, 1940, 692.

[172] I. A. Korshunov and S. A. Ermoldeva, *J. Gen. Chem., U.S.S.R.*, 1947, **17,** 181.

[173] N. I. Malyugina and I. A. Korshunov, *J. Analyt. Chem., U.S.S.R.*, 1947, **2,** 341.

[174] G. MacKinney and O. Temmer, *J. Amer. Chem. Soc.*, 1948, **70,** 3586.

[175] M. B. Neiman and Z. V. Markina, *Zavod. Lab.*, 1947, **13,** 1174.

hydrazone,[176] and Girard T [61] derivatives of most ketones are reducible. Acetone in a concentrated ammoniacal buffer solution yields a step at − 1·6 v, which P. Zuman [177] has attributed to reduction of the imino-group in the acetone–ammonia complex. Similar steps are formed by primary amines and amino-acids in the presence of other carbonyl compounds. Introduction of halogen increases the reducibility of acetone; iodine has a greater effect than bromine, and bromine than chlorine.[178]

Ketoses, such as fructose and sorbose, give well-defined two-electron steps in 0·02N-lithium chloride and hydroxide. The technique has been used for estimation of fructose, in the presence of sucrose and glucose, in materials such as honey and urine.[179]

Acetophenone, substituted acetophenones,[180] and diacetylresorcinols [181] yield two one-electron steps; the first corresponds to the formation of a pinacol, and the second to the formation of a secondary alcohol. Pasternak [169] has confirmed by controlled electrolytic reduction that benzophenone, which also gives two one-electron steps,[178, 182] yields benzpinacol and diphenylmethanol. The mechanism of the reduction has been discussed in greater detail by R. A. Day and J. J. Kirkland [183] and by H. J. Gardner.[184] α-Diketones such as diacetyl and benzil are reduced to acetoin and benzoin, respectively.[185]

Aliphatic and aromatic ketones, in which the carbonyl group is conjugated with either a double bond or a second carbonyl group,[127, 186] are reduced at the dropping-mercury electrode. In αβ-unsaturated ketones the ethylene group is reduced before the carbonyl group. R. Pasternak and H. von Halban [187] have studied in some detail the reduction of benzylideneacetophenone and H. Keller, R. Pasternak, and H. von Halban [188] the reduction of *cis*- and *trans*-dibenzoylethylene; the *trans*-isomer is reduced more readily than the *cis*-isomer. Dibenzoylmethane [187] and terpene β-keto-aldehydes [189] behave like αβ-unsaturated carbonyl compounds and are probably reduced *via* the enolic form. Polarography has been used for determination of methyl vinyl ketone in mixtures with but-3-en-2-ol

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[176] J. M. Lupton and C. C. Lynch, *J. Amer. Chem. Soc.*, 1944, **66**, 697.

[177] *Nature*, 1950, **165**, 485; *Coll. Czech. Chem. Comm.*, 1950, **15**, 839.

[178] A. Winkle and G. Proske, *Ber.*, 1936, **69**, 693.

[179] J. Heyrovsky and I. Smoleř, *Coll. Czech. Chem. Comm.*, 1932, **4**, 521; K. T. Williams, E. A. McComb, and E. F. Potter, *Analyt. Chem.*, 1950, **22**, 1031.

[180] W. C. Davies and D. P. Evans, *J.*, 1939, 546; F. W. Cox and H. Adkins, *J. Amer. Chem. Soc.*, 1939, **61**, 3364; S. Wawzonek and H. A. Laitinen, *ibid.*, 1941, **63**, 2341; R. Portillo and G. Varela, *Anal. Fís. Quím.*, 1947, **43**, 850; N. A. Valyashko and Y. S. Rozum, *J. Gen. Chem.*, *U.S.S.R.*, 1948, **18**, 710; I. A. Korshunov, A. S. Kirillova, and Z. B. Kuznetsova, *J. Phys. Chem.*, *U.S.S.R.*, 1950, **24**, 551.

[181] G. Scaramelli, *Boll. sci., Bologna,* 1941, **2**, 122.

[182] M. Ashworth, *Coll. Czech. Chem. Comm.*, 1948, **13**, 229.

[183] *J. Amer. Chem. Soc.*, 1950, **72**, 2766.

[184] *Chem. and Ind.*, 1951, 819.

[185] R. Pasternak, *Helv. Chim. Acta*, 1947, **30**, 1984.

[186] I. Tachi, *Mem. Coll. Agric. Kyoto*, 1938, **42**, 27.

[187] *Helv. Chim. Acta*, 1946, **29**, 190.

[188] *Ibid.*, p. 512.

[189] G. Scaramelli, *Boll. sci., Bologna,* 1941, **2**, 129.

and ethyl methyl ketone, diacetyl in mixtures with methyl vinyl ketone and ethyl methyl ketone,[190] and steroids containing a carbonyl group conjugated with a double bond.[60] The Girard T technique has been widely used for the estimation of 17-keto-steroids in urine.[63]

Carboxylic Acids.—Carboxylic acids containing a carbonyl group, such as pyruvic and phenylglyoxylic acids, give either one or two steps, depending on the pH of the solution and the dissociation constant of the acid ; the diffusion currents are independent of the height of the mercury reservoir. The total height of the two steps remains constant but the ratio of the heights varies with pH. Thus below pH 4·0, pyruvic acid in buffered solution gives a single step. At pH between 4 and 11 a second step appears ; the first step disappears above pH 11. O. H. Müller and J. P. Baumberger [191] attributed this behaviour to the keto–enol tautomerism of pyruvic acid and suggested that the first step was due to the keto-form and the second to the enol form of the acid. Brdička [98] has, however, since shown that the more positive step is formed by the undissociated acid and the second step by the anion. Brdička's interpretation is supported by the fact that phenylglyoxylic acid, which cannot enolise, also forms two steps in buffered solution and that the esters of pyruvic and phenylglyoxylic acids only give one step so long as they are not hydrolysed. If the ratio of the heights of the two pyruvic acid steps is plotted against pH a curve resembling a dissociation curve is obtained. Koutecký and Brdička [27] showed that the p*K* value obtained from this curve was several pH units higher than the true p*K* of the acid and that the shift was due to the rapid recombination of acid ions with protons at the dropping-mercury electrode. E. G. Clair and K. Wiesner [192] have calculated the velocity constant of the recombination reaction for several substituted pyruvic acids.

I. A. Korshunov, Z. B. Kuznetsova, and M. K. Shchennikova [193] have examined the polarographic behaviour of a wide range of carboxylic acids ; the reduction steps were due to the discharge of the hydrogen ions. Mesoxalic acid is not reduced, but its hydrazone and oxime yield a two-electron step.[194] S. Wawzonek and his colleagues [195] have used polarography to determine the structure of the esters and amides derived from γ-keto- and γ-aldehydo-acids, such as *o*-benzoylbenzoic and β-*p*-bromobenzoylcrotonic acid.

Acids containing a carboxyl group conjugated with a double bond are reducible. The reduction is sensitive to *cis–trans* structural changes, so that maleic can be determined in the presence of fumaric acid.[133] Fumaric can be determined in the presence of aconitic and citraconic acids ; [196] aspartic

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[190] E. I. Fulmer, J. J. Kolfenbach and L. A. Underkofler, *Ind. Eng. Chem. Anal.*, 1944, **16**, 469.

[191] *J. Amer. Chem. Soc.*, 1939, **61**, 590.

[192] *Nature*, 1950, **165**, 202.

[193] *J. Phys. Chem., U.S.S.R.*, 1949, **23**, 1292; *J. Analyt. Chem., U.S.S.R.*, 1951, **6**, 96.

[194] G. Sartori and A. Gaudiano, *Gazzetta*, 1948, **78**, 77.

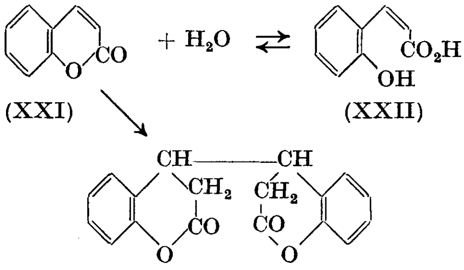
[195] S. Wawzonek, H. A. Laitinen, and S. J. Kwiatkowski, *J. Amer. Chem. Soc.*, 1944, **66**, 827, 830 ; S. Wawzonek, R. C. Reck, W. W. Vaught, and J. W. Fan, *ibid.*, 1945, **67**, 1300 ; S. Wawzonek and J. H. Fossum, *J. Electrochem. Soc.*, 1949, **96**, 234.

[196] G. Semerano, *Mikrochem.*, 1938, **24**, 10 ; I. D. S. Rao, *J. Univ. Bombay*, 1941, **10**, 56.

acid in acid-hydrolysed proteins may be estimated after conversion into a mixture of fumaric and maleic acids.[197]

Polarography has been used for determination of αβ-unsaturated esters in the presence of βγ-unsaturated isomers,[198] unchanged maleic anhydride in styrene–maleic anhydride and vinyl acetate–maleic anhydride polymers,[199] and maleic in succinic acid,[200] and for a study of the formation of the oxime and hydrazone of mesoxalic acid,[194] the polymerisation of methyl methacrylate in the presence of peroxides,[201] the depolymerisation of aconitic acid,[196] and the decomposition of methyl 4 : 5-diketopent-2-enoate 5-oxime.[91]

Lactones are reduced at the dropping-mercury electrode. H. Matheson, H. S. Isbell, and E. R. Smith [202] used polarography to follow the formation of lactones from polyhydroxy-carboxylic acids. A. J. Harle and L. E. Lyons [203] demonstrated that above pH 11·2 coumarin (XXI) exists in the lactone form and below pH 6·8 as coumaric acid (XXII); at intermediate pH values both forms are present. Reduction leads to the *meso*- and racemic forms of tetrahydrodi-4-coumarinyl (XXIII):



(XXI) (XXII)

(XXIII)

αβ- but not βγ-angelica lactones are reduced polarographically; both lactones absorb oxygen in the basic media with formation of peroxides that are irreversibly reduced in the same way. E. V. Kirkland, C. A. Reynolds, and C. A. VanderWerf [204] deduced that both lactones form the same peroxide and that the βγ-isomer yields the peroxide more readily than the αβ-isomer.

The ene-diol system, such as is present in ascorbic acid, forms an anodic step [205] (see Fig. 9). Since the position of the ascorbic acid step shifts with

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[197] B. Warshowsky and M. W. Rice, *Analyt. Chem.*, 1948, **20**, 341.

[198] A. C. Cope and E. M. Hardy, *J. Amer. Chem. Soc.*, 1940, **62**, 3319.

[199] G. C. Whitnack, *Analyt. Chem.*, 1948, **20**, 658.

[200] L. Silverman, *Chem. Analyst*, 1947, **36**, 57.

[201] M. B. Neĭman and M. A. Shubenko, *Zavod. Lab.*, 1948, **14**, 394.

[202] *J. Res. Nat. Bur. Stand.*, 1942, **28**, 95.

[203] *J.*, 1950, 1575.

[204] *J. Amer. Chem. Soc.*, 1950, **72**, 1764.

[205] E. Kodíček and K. Wenig, *Nature*, 1938, **142**, 35; K. Schwarz, *Z. anal. Chem.*, 1939, **115**, 161; C. Cattaneo and G. Sartori, *Gazzetta*, 1942, **72**, 351; W. S. Gillam, *Ind. Eng. Chem. Anal.*, 1945, **17**, 217; J. E. Page and J. G. Waller, *Analyst*, 1946, **71**, 65; D. R. Perrin and D. D. Perrin, *N.Z. J. Sci. Tech.*, 1946, **28**, *A*, 266; Z. Vavřín, *Coll. Czech. Chem. Comm.*, 1949, **14**, 367.

change in pH, a strongly buffered solution is recommended for analytical work. If the pH of the solution is too low, the anodic step moves to such a high positive potential that the diffusion current cannot develop ; at high pH's the vitamin is unstable. Polarography has been used for estimation of ascorbic acid in fruit, vegetables, milk, and pharmaceutical preparations.[205] Reductone (hydroxymethyleneglycollic aldehyde) and coumarindiol also yield anodic steps.[206]

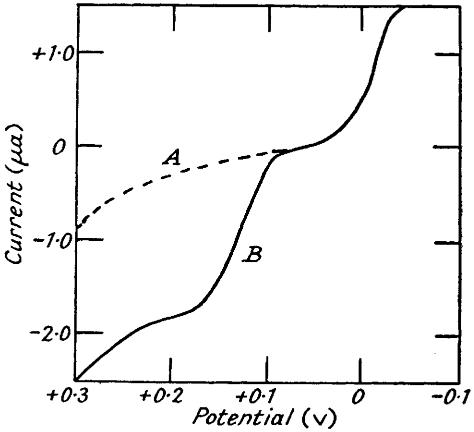

FIG. 9

Polarograms for ascorbic acid.
A, 0·05M-*Phthalate buffer solution* (pH 4·0).
B, 0·0002M-*Ascorbic acid in* 0·05M-*phthalate buffer solution.*

Halogen Compounds.—Until recently few halogen-containing organic compounds had been examined polarographically. Winkel and Proske's [178] early work had shown that iodo-organic compounds are reduced more readily than bromo-, and bromo- than chloro- compounds. Thus monoiodo-, monobromo-, and monochloro-acetone in 0·1N-ammonium chloride give steps with " reduction " potentials of — 0·14, — 0·30, and — 1·13 v, respectively.

In 1936, Brdička [92] observed that iodoacetic acid in 0·08N-sodium carbonate gave a characteristic polarogram with a step whose height was proportional to concentration. He attributed the step to the elimination of iodine, according to the equation :

$$CH_2I{\cdot}CO_2H + 2e^- + 2H^+ \longrightarrow CH_3{\cdot}CO_2H + HI$$

Brdička used the step analytically, to investigate the kinetics of the reaction between glycine and iodoacetic acid and of the hydroxylation of iodoacetic acid to glycollic acid. Lingane, Swain, and Fields [125] confirmed that the iodine atom in 5-*o*-iodophenylacridine on polarographic reduction is eliminated in a two-electron step.

Pasternak and von Halban [187] made the first attempt to understand the mechanism of the polarographic reduction of halogenated organic com-

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[206] R. Brdička and P. Zuman, *Coll. Czech. Chem. Comm.*, 1950, **15**, 766.

pounds. They found that the half-wave potential of ω-bromoacetophenone did not move to more positive values when the buffer medium was changed from alkaline to acid, thus suggesting that the mechanism is a direct electron addition and not a hydrogenation. In a subsequent paper, however, on the reduction of chloro-compounds, such as 1 : 1 : 1-trichloro-2 : 2-diphenylethane and triphenylmethyl chloride, it was suggested [95] that only halogen atoms bound to the aliphatic part of the molecule are reducible at the dropping-mercury electrode.

von Stackelberg and Stracke [46] in an extensive study of halogenated hydrocarbons showed that methyl iodide, methylene iodide, and iodoform are reduced more readily than the corresponding bromine compounds, which in turn are reduced more readily than the chlorine compounds (cf. M. B. Neĭman, A. V. Ryabov, and E. M. Sheyanova [207]). Methyl iodide is reduced to methane, ethyl bromide to ethane, α-bromonaphthalene to naphthalene, allyl bromide to propylene, di-iodoacetylene to acetylene, 2 : 3-dibromobutane to butene, and 1 : 2-dibromoethane to a mixture containing 80% of ethylene and 20% of ethane. The half-wave potentials for all the steps are independent of pH.[46] The polarographic behaviour of chloroform and carbon tetrachloride has been studied in greater detail by I. M. Kolthoff, T. S. Lee, D. Stočesová, and E. P. Parry.[208] Carbon tetrachloride gives two two-electron steps ; the first corresponds to the formation of chloroform and the second to methylene chloride.

Recent work on mono-, di-, and tri-chloroacetic,[46, 209] monoiodoacetic, mono-, di-, and tri-bromoacetic,[210, 211] and α-bromopropionic acid [211] has confirmed that reduction involves fission of the carbon–halogen bond and formation of a saturated acid. Reduction of *meso*-αα′-dibromosuccinic acid, however, yields fumaric acid.[212] This behaviour is in harmony with von Stackelberg and Stracke's observation on the reduction of 1 : 2-dibromoethane and 2 : 3-dibromobutane and may take place in other compounds containing two adjacent halogen atoms whose removal will lead to a stable double or triple bond.

E. Gergely and T. Iredale [213] confirmed that the half-wave potentials for the reduction of iodinated hydrocarbons are independent of pH, but found that those for the reduction of iodoacetic acid, *o*-, *m*-, and *p*-iodobenzoic acid, *o*-, *m*-, and *p*-iodophenol, and *o*- and *p*-iodoaniline do change. Thus the half-wave potential of iodoacetic acid shifts from – 0·17 to – 0·68 v when the pH is increased from 1·12 to 12·5.

Observations on the polarographic behaviour of 3 : 5-di-iodotyrosine

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[207] *Compt. rend. Acad. Sci., U.R.S.S.*, 1949, **68**, 1065.

[208] *Analyt. Chem.*, 1950, **22**, 521.

[209] P. J. Elving and C.-S. Tang, *J. Amer. Chem. Soc.*, 1950, **72**, 3244 ; *Analyt. Chem.*, 1951, **23**, 341.

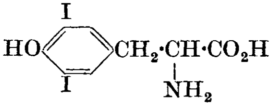
[210] P. J. Elving, I. Rosenthal, and M. K. Kramer, *J. Amer. Chem. Soc.*, 1951, **73**, 1717.

[211] E. Saito, *Bull. Soc. chim.*, 1948, 404 ; 1950, 1185 ; 1951, 957.

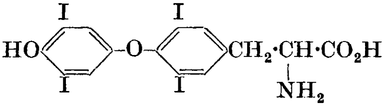
[212] I. Rosenthal and P. J. Elving, *J. Amer. Chem. Soc.*, 1951, **73**, 1880.

[213] *J.*, 1951, 13, 3052 ; see also P. J. Elving and C. L. Hilton, *J. Amer. Chem. Soc.*, 1952, **74**, 3368.

(XXIV), thyroxine (XXV), and other iodinated aromatic acids indicate that their half-wave potentials are dependent on pH.[214, 215] Thyroxine in 40% ethanolic 0·5N-sodium carbonate containing 1·0% of tetramethylammonium bromide gives three steps, the first step having a half-wave potential of — 1·24 v. In the same medium di-iodotyrosine gives a double step with a half-wave potential of — 1·72 v, thus permitting thyroxine to be determined in the presence of di-iodotyrosine (see Fig. 10). The technique has been extended to the measurement of thyroxine in iodinated proteins [215, 216] and to estimation of the chemical purity of thyroxine labelled with radioactive iodine.[217]



(XXIV)



(XXV)

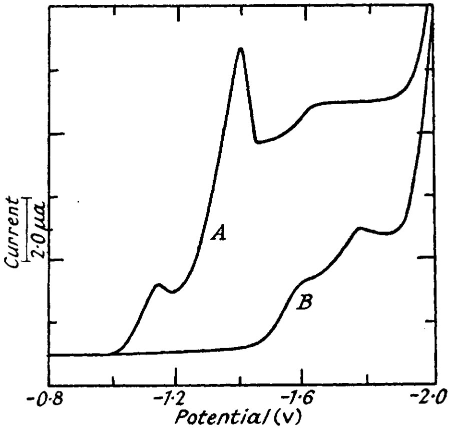


FIG. 10

Polarograms for 6·0 × 10^{-4}M-*thyroxine* (*A*) *and* 6·0 × 10^{-4}M-3 : 5-*di-iodotyrosine* (*B*) *in* 20% iso*propanolic solution containing* 1·0% *of tetramethylammonium bromide and* 2·65% *of sodium carbonate.*

A study [215, 218] of 26 compounds structurally related to thyroxine showed that acids containing one, two, or four iodine atoms give one, two, or three reduction steps, respectively. Each step, except the second step formed by the tetraiodo-compounds (*e.g.*, thyroxine), corresponds with the elimination of one iodine atom, *i.e.*, a two-electron change. The second thyroxine

[214] G. K. Simpson and D. Traill, *Biochem. J.*, 1946, **40,** 116.

[215] E. T. Borrows, B. A. Hems, and J. E. Page, *J.*, 1949, *S* 204.

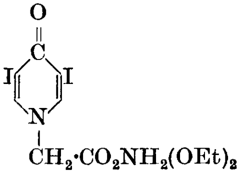
[216] G. K. Simpson, A. G. Johnston, and D. Traill, *Biochem. J.*, 1947, **41,** 181.

[217] J. C. Clayton, A. A. Free, J. E. Page, G. F. Somers, and E. A. Woollett, *ibid.*, 1950, **46,** 598.

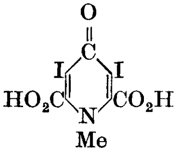
[218] J. E. Page, Proc. 1st Intern. Polarographic Congr., Prague, 1951, Vol. I, p. 193.

step is associated with the elimination of two iodine atoms, *i.e.*, a four-electron change. Substitution in the 4-hydroxyl group of 4-hydroxy-3 : 5-di-iodobenzoic acid and di-iodotyrosine (XXIV) facilitates reduction of the acids. Acetylation of the amino-group of di-iodotyrosine and thyroxine (XXV) does not affect their reduction potentials.

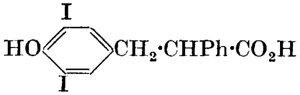
S. Wawzonek, S. C. Wang, and P. Lyons [219] have shown that thyroxine analogues of cinnamic acid yield steps, which can be associated with both elimination of the iodine atoms and reduction of the $\alpha\beta$-double bond. The iodine steps occur at a much lower potential than those for the double bond.



(XXVI)



(XXVII)



(XXVIII)

As might be expected, the iodine-containing contrast agents, diodone (XXVI), iodoxyl (XXVII), and pheniodol (XXVIII),[218] used in *X*-ray examination of various body organs, yield characteristic polarograms which can serve for identification and determination of these compounds in simple solutions. A. A. Free, J. E. Page, and E. A. Woollett [220] have used polarography in estimating the chemical purity of pheniodol labelled with radioactive iodine.

Other halogen-containing compounds that have been examined polarographically include chloramine-T,[221] iodofuchsone,[118] and the 1 : 2 : 3 : 4 : 5 : 6-hexachloro*cyclo*hexanes.[222] The γ-isomer (the insecticide Gammexane) is the only isomer of hexachloro*cyclo*hexane that is polarographically reducible, which is unusual ; in this instance the reducibility is attributed to the polar structure of the γ-isomer.

Nitro-compounds.—The polarographic behaviour of nitro-compounds has attracted considerable attention. The nitro-group is a powerful electron-seeking group ; it is therefore to be expected that its ability to capture an electron and hence its reducibility at the dropping-mercury electrode will be affected by the nature and position of other electron-donating or electron-seeking substituents in the molecule.

Nitrobenzene was the first organic substance to be studied polarographically. M. Shikata [223] measured its " reduction " potential at various pH values and subsequently, with the aid of his collaborators, studied the

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[219] *J. Org. Chem.*, 1950, **15**, 593.

[220] *Biochem. J.*, 1951, **48**, 490.

[221] K. Heller and E. N. Jenkins, *Nature*, 1946, **158**, 706.

[222] G. B. Ingram and H. K. Southern, *ibid.*, 1948, **161**, 437 ; G. Dragt, *Analyt. Chem.*, 1948, **20**, 737 ; D. Monnier, L. Roesgen, and R. Monnier, *Analyt. Chim. Acta*, 1950, **4**, 309 ; H. Hasselbach and K. Schwabe, *Z. anal. Chem.*, 1951, **132**, 94 ; K. Schwabe and H. Frind, *Z. physikal. Chem.*, 1951, **196**, 342 ; M. Nakazima, Y. Katumura, and T. Okubo, Proc. 1st Intern. Polarographic Congr., Prague, 1951, Vol. I, p. 173.

[223] *Trans. Faraday Soc.*, 1925, **21**, 42.

nitrophenols,[224] dinitrobenzenes, dinitrophenols,[225] and nitroanilines.[226] Unfortunately they did not appreciate the significance of half-wave potentials and, although they attempted to calculate the number of electrons involved in a reduction, their values are open to criticism. Winkel and Proske [47] later investigated the effect of pH on the " reduction " potential of nitrobenzene.

Since the earlier work does not contain information on the nature of the electrode reactions, and as arbitrary " reduction " potentials were quoted rather than half-wave potentials, the investigation has been repeated and additional nitro-compounds have been examined, mainly by M. J. Astle and his colleagues in America and by J. Pearson and by J. E. Page and his colleagues in this country. The substances studied include the mono-, di-, and tri-nitrobenzenes,[41, 124, 227, 228, 229] -nitrotoluenes,[124, 228, 229] -nitrophenols,[41, 230] and -nitroresorcinols,[231] *p*-nitroaniline,[42] the mono-nitrocresols,[232] -nitroanisoles, and -nitrobenzoic acids,[41, 229] and the methyl nitrobenzoates.[41] A mechanism suggested by Pearson [124] for the reduction of the nitro-group has been discussed earlier in this review.

Below pH 4, nitrobenzene gives a four- and a two-electron step, representing reduction to the hydroxylamine and aniline respectively. The second step is not well-defined and disappears above pH 4. Addition of alcohol, however, shifts the steps to more negative potentials and makes the second step more distinct. The nitrotoluenes, nitroanisoles and methyl nitrobenzoates differ from nitrobenzene in the pH value at which the second step disappears. The dinitrobenzenes and dinitrotoluenes show a twelve-electron reduction in acid and a ten-electron (or less) reduction in alkaline solution. Trinitrobenzene and trinitrotoluene undergo a greater than twelve-electron reduction in acid and a twelve-electron reduction in alkaline solution. Thus mono-, di-, and tri-nitrobenzene and -nitrotoluene in neutral and alkaline solution are reduced to the corresponding hydroxylamine, but at acid pH the reduction proceeds stepwise through the hydroxylamine to the amine. At pH's between 3 and 6 a mixture of the hydroxylamine and the amine is formed. The half-wave potentials vary linearly with pH, but the slope of the curve changes in the pH range (*i.e.*, about pH 4) in which the reduction process changes.

o-, *m*-, and *p*-Nitrophenol resemble nitrobenzene in forming two steps in acid solution, but the *o*- and *p*-isomers differ from nitrobenzene in that

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[224] M. Shikata and M. Watanabe, *J. Agric. Chem. Soc. Japan*, 1928, **4**, 924.

[225] M. Shikata and N. Hozaki, *Mem. Coll. Agric. Kyoto*, 1931, **17** 1, 21.

[226] M. Shikata and E. Taguchi, *ibid.*, 1934, **29**, 1.

[227] I. A. Korshunov and A. S. Kirillova, *J. Gen. Chem.*, *U.S.S.R.*, 1948, **18**, 785; I. M. Kolthoff and F. A. Bovey, *J. Amer. Chem. Soc.*, 1948, **70**, 791.

[228] M. Fields, C. Valle, and M. Kane, *ibid.*, 1949, **71**, 421.

[229] S. F. Dennis, A. S. Powell, and M. J. Astle, *ibid.*, p. 1484; D. Stočesová, *Coll. Czech. Chem. Comm.*, 1949, **14**, 615.

[230] M. J. Astle and W. V. McConnell, *J. Amer. Chem. Soc.*, 1943, **65**, 35; J. Pearson, *Trans. Faraday Soc.*, 1948, **44**, 692.

[231] M. J. Astle and S. P. Stephenson, *J. Amer. Chem. Soc.*, 1943, **65**, 2399; J. Pearson, *Trans. Faraday Soc.*, 1949, **45**, 199.

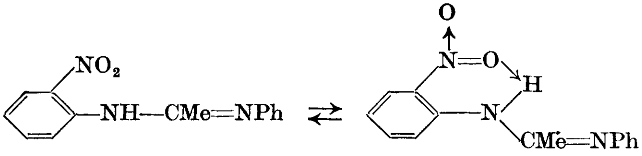
[232] M. J. Astle and W. P. Cropper, *J. Amer. Chem. Soc.*, 1943, **65**, 2395.

the four-electron step increases to a six-electron step with increase in pH. The pH–half-wave potential relation is a straight line without the inflection at pH 4 that occurs with the nitrobenzenes and nitrotoluenes. The anionic or quinonoid form present in alkaline solution is reduced directly to the amine. Reduction of the *p*-isomer in alkaline solution proceeds through the nitroso-state. 2 : 4- and 2 : 6-Dinitrophenol resemble the dinitrobenzenes, but picric acid shows a 17-electron reduction and probably is reduced to bis-3 : 5-diamino-4-hydroxyphenylhydrazine, which rearranges in acid to give a benzidine (cf. Lingane [99]).

Polarographic reduction of 2-nitro-, 2 : 4-dinitro-, and 2 : 6-dinitroresorcinol and styphnic acid is simpler than that of the nitrobenzenes and nitrophenols; the appropriate amine is formed at all pH's. Styphnic acid behaves like picric acid in giving a 17-electron step.[231]

The *o*-, *m*-, and *p*-nitro-derivatives of anisole, benzoic acid, and methyl benzoate form characteristic polarograms. The nitrobenzoic acids yield two reduction steps in both acid and alkaline solution. Their half-wave potentials do not change with concentration, but vary linearly with pH over a limited range.[41]

Dennis, Powell, and Astle [229] observed that the substituents, nitro, carboxyl, chloro, methyl, make the reduction of nitrobenzene increasingly difficult, in that order. Nitro and carboxyl in the *ortho*- and the *para*-position show a greater effect than in the *meta*-position. Two methyl substituents, as in 1 : 4-dimethyl-2-nitrobenzene and 1 : 2-dimethyl-3-nitrobenzene, have little effect on the ease of reduction of the nitro-group, but methyl groups substituted in a way that inhibits sterically resonance of the nitro-group, as in nitromesitylene and nitrodurene, make the nitro-group more difficult to reduce.[228]



(XXIX) (XXX)

M. E. Runner, M. L. Kilpatrick, and E. C. Wagner [233] have shown that the *o*-nitro-isomer of *N*-nitrophenyl-*N*′-phenylacetamidine (XXIX) is reduced more readily than the *m*- or the *p*-isomer, indicating that internal hydrogen bonding (cf. XXX) takes place.

The well-defined polarographic steps associated with nitro-compounds have found many analytical applications. These include the determination of nitrobenzene in aniline [234] and blood,[235] 1 : 3 : 5-trinitro- and *m*-dinitro-

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[233] *J. Amer. Chem. Soc.*, 1947, **69**, 1406.

[234] J. V. A. Novák, *Coll. Czech. Chem. Comm.*, 1939, **11**, 573; J. Haslam and L. H. Cross, *J. Soc. Chem. Ind.*, 1944, **63**, 94; I. A. Korshunov, A. V. Ryabov, L. N. Sazanova, and A. S. Kirillova, *Zavod. Lab.*, 1948, **14**, 519.

[235] J. Teisinger, *Mikrochem. Mikrochim. Acta*, 1938, **25**, 151.

benzene in the presence of each other,[236] 1-nitronaphthalene in 1-naphthylamine [237] and mono- and di-nitroxylenes during the commercial hydrogenation of nitroxylene to xylidine.[238] Benzene may be determined after nitration to dinitrobenzene; if toluene and xylene are present, the nitration product must be oxidised and the acids removed with alkali before polarography.[239] Because of the nitro-group present in each, the antibiotic, chloramphenicol $p\text{-NO}_2\cdot\text{C}_6\text{H}_4\cdot\text{CH(OH)}\cdot\text{CH(CH}_2\cdot\text{OH)}\cdot\text{NH}\cdot\text{CO}\cdot\text{CHCl}_2$,[240] and the insecticide, parathion $p\text{-NO}_2\cdot\text{C}_6\text{H}_4\cdot\text{O}\cdot\text{PS(OEt)}_2$,[241] may be determined polarographically. Tyrosine, phenylalanine, and albumin are determined after nitration with nitric acid.[242]

As would be expected, nitroso-compounds, such as 1-nitroso-2-naphthol, *p*-nitrosophenylhydroxylamine,[243] and nitrosobenzene,[123] are reduced at the dropping-mercury electrode. This provides the basis of a method for estimating substances of pharmaceutical interest, such as œstrone,[244] stilbœstrol,[245] and morphine,[246] which are easily converted into nitroso-derivatives.

Nitroparaffins in strongly acid solution yield one polarographic step, which has been attributed to formation of the alkylhydroxylamine; in weakly acid solution a second step that has been attributed to the alkylamine appears.[247] According to P. E. Stewart and W. A. Bonner,[248] however, in well-buffered solutions only one four-electron step appears at all pH's. Since the *aci*-form of a nitroparaffin is not reduced, Miller, Arnold, and Astle [90] were able to use polarography to study the kinetics of the conversion of nitroparaffins into their *aci*-forms in the pH range 6—11. Reduction of the hydroxynitrobutanes and their esters and ethers has been discussed by W. J. Seagers and P. J. Elving.[249]

H. N. Wilson and W. Hutchinson [250] have determined the nitromethane content of air by absorbing the nitromethane in 0·2N-sulphuric acid and measuring the concentration polarographically. Dimethylamine in an aqueous solution containing ammonia and mono- and tri-methylamines may

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[236] K. Cruse, *Angew. Chem.*, 1948, **60**, *A*, 66; K. Cruse and R. Haul, *Z. Elektrochem.*, 1949, **53**, 115.

[237] Y. I. Vainshtein, *Zavod. Lab.*, 1948, **14**, 517.

[238] C. H. Hale, *Analyt. Chem.*, 1951, **23**, 572.

[239] S. Škramovský and J. Teisinger, *Chim. et Ind.*, 1948, **59**, 48; A. S. Landry, *Analyt. Chem.*, 1949, **21**, 674.

[240] G. B. Hess, *ibid.*, 1950, **22**, 649.

[241] C. V. Bowen and F. I. Edwards, *ibid.*, p. 706.

[242] D. Monnier and Y. Rusconi, *Helv. Chim. Acta*, 1951, **34**, 1297; Y. Rusconi, D. Monnier, and P. E. Wenger, *ibid.*, p. 1943.

[243] I. M. Kolthoff and A. Langer, *J. Amer. Chem. Soc.*, 1940, **62**, 3172; I. M. Kolthoff and A. Liberti, *ibid.*, 1948, **70**, 1885.

[244] C. Heusghem, *Bull. Soc. Chim. biol.*, 1949, **31**, 1114.

[245] O. Gry, *Dansk Tidsskr. Farm.*, 1949, **23**, 139.

[246] H. B. Rasmussen, C. Hahn, and K. Ilver, *ibid.*, 1945, **19**, 41; H. B. Rasmussen and O. Lanng, *ibid.*, 1948, **22**, 201.

[247] T. De Vries and R. W. Ivett, *Ind. Eng. Chem. Anal.*, 1941, **13**, 339; F. Petrü, *Coll. Czech. Chem. Comm.*, 1947, **12**, 620.

[248] *Analyt. Chem.*, 1950, **22**, 793.

[249] *J. Amer. Chem. Soc.*, 1950, **72**, 3241, 5183; 1951, **73**, 947.

[250] *Analyst*, 1947, **72**, 432.

be determined after conversion into its nitrosamine, which is steam-distilled, after which the acidified distillate is polarographed.[251]

Miscellaneous Nitrogen-containing Compounds.—Azobenzene and its derivatives in acid, neutral, and alkaline solution are irreversibly reduced at the dropping-mercury electrode to the corresponding hydrazo-compound; steps are formed that are suitable for analytical purposes.[252] W. Stricks and I. M. Kolthoff [253] used the step associated with the reduction of the azo-group in methyl-orange as the basis of a novel method for measuring the molecular weight of serum albumin. Small amounts of gelatin (about 0·01%) decrease by 10% the diffusion current of methyl-orange dissolved in a phosphate buffer solution; larger amounts (up to 1%) of gelatin have no further effect. On the other hand, concentrations of up to 0·5% of serum albumin continue to suppress the methyl-orange diffusion current. Stricks and Kolthoff attributed the suppression to combination between the serum albumin and methyl-orange and deduced that at a concentration level of 0·5% of serum albumin, all the methyl-orange in solution had combined with protein. The diffusion coefficient of the complex was calculated from Ilkovič's equation and substituted in the Stokes–Einstein equation to give a value of 66,000 for the molecular weight of serum albumin. This figure is in good agreement with values of 68,000—70,000 obtained by other methods.

E. R. Atkinson, H. H. Warren, P. I. Abell, and R. E. Wing [254] showed that diazo-compounds prepared from aniline and the three isomeric amino-benzoic acids yield two polarographic steps, the half-wave potentials of which vary with pH. The first step is attributed to the slow irreversible reduction of the diazonium ion, $R·N_2^+$, and the second to the rapid irreversible reduction of the azoic acid, R·N:N·OH. At all pH's one electron is involved in the reduction of one diazonium group. Elofson, Edsberg, and Mecherly [97] used polarography to measure the diazotisation rates of aromatic amines; the method is the only one available for studying the rapid formation of an insoluble azo-compound. Hantzch's *anti*-diazosulphonates are reduced at a more negative potential than the corresponding *syn*-diazosulphonates; this is in harmony with the greater stability of the *anti*-isomers.[255]

N-Dialkyl-*p*-aminoanilines, such as 2-acetamido-4-amino-*N*-diethyl-aniline, undergo oxidation at a platinum micro-electrode.[256] Their half-wave potentials become more positive when electron-donating groups are substituted either on the tertiary nitrogen atom or in the *ortho*-position to

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[251] A. A. Smales and H. N. Wilson, *J. Soc. Chem. Ind.*, 1948, **67**, 210; F. L. English, *Analyt. Chem.*, 1951, **23**, 344.

[252] M. Shikata and I. Tachi, *Mem. Coll. Agric. Kyoto*, 1937, **40**, 1; I. Tachi, *ibid.*, p. 11; A. Volpi, *Gazzetta*, 1947, **77**, 473; P. J. Hillson and P. P. Birnbaum, *Trans. Faraday Soc.*, 1952, **48**, 478.

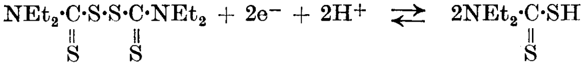
[253] *J. Amer. Chem. Soc.*, 49, **71**, 1519. [254] *Ibid.*, 1950, **72**, 915.

[255] H. C. Freeman and W. P. Georgans, *Chem. and Ind.*, 1951, 148.

[256] R. L. Bent, J. C. Dessloch, F. C. Duennebier, D. W. Fassett, D. B. Glass, T. H. James, D. B. Julian, W. R. Ruby, J. M. Snell, J. H. Sterner, J. R. Thirtle, P. W. Vittum, and A. Weissberger, *J. Amer. Chem. Soc.*, 1951, **73**, 3100.

the primary amino-group in the benzene ring ; the reverse holds for electron-seeking groups. Substituted *p*-phenylenediamines are also oxidised at a platinum micro-electrode.[50]

Sulphur-containing Compounds.—The disulphide linkage is the only sulphur bond that is polarographically reducible ; the thiol group is oxidisable. I. M. Kolthoff and C. Barnum [257] have demonstrated that, although cysteine forms an anodic and cystine a cathodic step, the cysteine–cystine system is not reversible. E. C. Gregg and W. P. Tyler [258] have examined the reversibility of the bisdiethylthiocarbamyl disulphide–diethyldithiocarbamate system :



$$\mathrm{NEt_2{\cdot}\underset{\overset{\|}{S}}{C}{\cdot}S{\cdot}S{\cdot}\underset{\overset{\|}{S}}{C}{\cdot}NEt_2 + 2e^- + 2H^+ \rightleftarrows 2NEt_2{\cdot}\underset{\overset{\|}{S}}{C}{\cdot}SH}$$

The two-step reduction of the disulphide and the two-step oxidation of the carbamate are produced by adsorption at the mercury–solution interface. G. Sartori and A. Liberti [259] have shown that the anodic step formed by mercaptobenzothiazole is associated with the formation of a mercurous derivative at the dropping-mercury electrode and not with oxidation to disulphide. Thioglycollic acid also yields an anodic step.[260] K. G. Stone [261] found that benzothiomorpholide, but neither dibenzyl sulphoxide nor benzomorpholide, is reducible.

Thiol groups substituted on the α-carbon atom of a carboxylic acid (*e.g.*, mercaptoacetic [19] and $\alpha\alpha'$-dimercaptoadipic acid [262]) or the β-carbon atom of an amino-acid [*e.g.*, cysteine, cysteylglycine,[19] homocystine,[263] and penicillamine ($\beta\beta$-dimethylcysteine) [264]], dissolved in an ammoniacal cobalt buffer solution, yield a catalytic wave at about — 1·80 v (see Fig. 4). Sulphur-containing amino-acids, in which the thiol group has an alkyl substituent (*e.g.*, methionine), and mercapto-alcohols (*e.g.*, 2 : 3-dimercaptopropanol [262]), do not form catalytic waves. The nature of the catalytic waves formed by cystine has been discussed above. The waves have been used to determine cystine in a wide range of biological materials,[19, 265] to estimate penicillin,[264] and to study the cysteine-inactivation of antibacterial substances [266] and the relation of the thiol and disulphide forms of aneurin.[267] The most important application of the catalytic waves has been in the serological study of cancer [268] and the clinical examination of

[257] *J. Amer. Chem. Soc.*, 1940, **62**, 3061 ; 1941, **63**, 520. [258] *Ibid.*, 1950, **72**, 4561.

[259] *J. Electrochem. Soc.*, 1950, **97**, 20.

[260] A. Liberti and E. Cervone, *Ann. Chim. appl.*, 1951, **41**, 95.

[261] *J. Amer. Chem. Soc.*, 1947, **69**, 1832.

[262] J. B. Fraser, L. N. Owen, and G. Shaw, *Biochem. J.*, 1947, **41**, 328.

[263] A. Stern and E. F. Beach, *Proc. Soc. Exp. Biol. N.Y.*, 1940, **43**, 104.

[264] J. E. Page, *Analyst*, 1948, **73**, 214 ; H. T. Clarke, J. R. Johnson, and R. Robinson, "The Chemistry of Penicillin", Oxford Univ. Press, 1949, p. 1028.

[265] G. Reed, *J. Biol. Chem.*, 1942, **142**, 61.

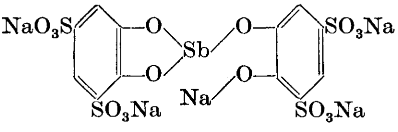
[266] J. E. Page and J. G. Waller, *Nature*, 1946, **157**, 838.

[267] E. Hamamoto and I. Yamanouchi, Proc. 1st Intern. Polarographic Congr., Prague, 1951, Vol. I, p. 77 ; I. Tachi and S. Koide, *op. cit.*, p. 469.

[268] R. Brdička, *Nature*, 1937, **139**, 330, 1020 ; *Research*, 1947, **1**, 25 ; C. Carruthers and V. Suntzeff, Proc. 1st Intern. Polarographic Congr., Prague, 1951, **1**, 11 ; A. M. Robinson, *op. cit.*, p. 213.

blood sera; for the latter purpose, O. H. Müller and J. S. Davis [269] introduced the term, " protein index ", which is a measure of the thiol-content of the sera.

Metal-containing Compounds.—Most metal-containing organic compounds are reduced at the dropping-mercury electrode. In acid solution, organic compounds containing tervalent antimony [*e.g.* tartar emetic and stibophen (XXXI)] break down, and a step corresponding to the reduction of the metal in its ionic state is obtained; in alkaline solution the step corresponds to reduction of the anionic complex.[270] Mercury-containing compounds, such as phenylmercuric acetate, in acid solution, form steps caused by reduction of the mercury-containing cation; compounds, such as thiomersalate, *o*-$CO_2Na{\cdot}C_6H_4{\cdot}S{\cdot}HgEt$, form steps owing to reduction of the anion.[271] The cobalt-containing complex, cyanocobalamin (vitamin B_{12}), yields both a reduction step [272] and a catalytic wave.[24]


(XXXI)

Polarography has been used to study the oxidation and reduction of the complex ions formed by metal atoms and organic acids and bases. The systems that have been examined include ferric and ferrous complexes with oxalic, citric, tartaric, and sulphosalicylic acid,[273] copper complexes with tartaric, citric, oxalic,[274] nitrilotriacetic, ethylenediaminetetra-acetic,[275] and aspartic acid, glycine, alanine,[276] cysteine,[277] ethylenediamine, propylenediamine, diethylenetriamine,[278] and pyridine,[279] lead complexes with tartaric and oxalic acid,[280] titanium [281] and vanadium [282] complexes with oxalic acid, and cadmium, zinc,[283] and uranium [284] complexes with citric acid. A. J. P.

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[269] *J. Biol. Chem.*, 1945, **159,** 667; *Arch. Biochem.*, 1947, **15,** 39; *Amer. J. Med. Sci.*, 1950, **220,** 298.

[270] J. E. Page and F. A. Robinson, *J. Soc. Chem. Ind.*, 1942, **61,** 93; L. G. Goodwin and J. E. Page, *Biochem. J.*, 1943, **37,** 198.

[271] J. E. Page and J. G. Waller, *Analyst*, 1949, **74,** 292; R. Benesch and R. E. Benesch, *J. Amer. Chem. Soc.*, 1951, **73,** 3391; *J. Phys. Chem.*, 1952, **56,** 648.

[272] H. Diehl, R. R. Sealock, and J. I. Morrison, *Iowa State Coll. J. Sci.*, 1950, **24,** 433; *Experientia*, 1951, **7,** 60.

[273] J. J. Lingane, *J. Amer. Chem. Soc.*, 1946, **68,** 2448; M. Bobtelsky and J. Jordan, *ibid.*, 1947, **69,** 2286; C. V. Banks and J. H. Patterson, *ibid.*, 1951, **73,** 3062.

[274] L. Meites, *ibid.*, 1949, **71,** 3269; 1950, **72,** 180, 184.

[275] W. C. Davies and W. Furness, Proc. 1st Intern. Polarographic Congr., Prague, 1951, Vol. I, p. 28.

[276] R. M. Keefer, *J. Amer. Chem. Soc.*, 1946, **68,** 2329; N. C. Li and E. Doody, *ibid.*, 1950, **72,** 1891.

[277] W. Stricks and I. M. Kolthoff, *ibid.*, 1951, **73,** 1723, 1728.

[278] H. A. Laitinen, E. I. Onstott, J. C. Bailar, and S. Swann, *ibid.*, 1949, **71,** 1550.

[279] I. A. Korshunov and N. I. Malyugina, *J. Gen. Chem., U.S.S.R.*, 1950, **20,** 402.

[280] T. Meites and L. Meites, *J. Amer. Chem. Soc.*, 1951, **73,** 1161.

[281] R. L. Pecsok, *ibid.*, p. 1304.

[282] J. J. Lingane and L. Meites, *ibid.*, 1947, **69,** 1021.

[283] L. Meites, *J. Amer. Chem. Soc.*, 1951, **73,** 3727.

[284] W. F. Neuman, J. R. Havill, and I. Feldman, *ibid.*, p. 3593.

Martin and R. Mittelmann [285] used the step formed by amino-acid complexes with copper as the basis of a method for making quantitative the paper-chromatographic analysis of amino-acids.

Recently G. Costa [286] has described in some detail the polarographic behaviour of the alkyl halides of mercury, lead, thallium, and tin. Tetraethyl-lead decomposes in anhydrous Cellosolve that is 1·0N with respect to hydrogen chloride; lead ions are obtained, which yield a polarographic step. K. A. Hansen, T. D. Parks, and L. Lykken [287] used the method for the estimation of tetraethyl-lead in petroleum products.

The metal content of complexes that do not form reducible ions in solution can be determined after the organic matter has been destroyed. A polarographic method for determining vanadium in sodium vanadyl tartrate has been described by J. E. Page and F. A. Robinson [288] and for cobalt in cyanocobalamin (vitamin B_{12}) by F. Ender and H. Steeg.[289]

Conclusion

For full discussion of the applications of polarography to organic chemistry the references cited should be consulted. An attempt has been made in this Review to indicate the directions in which polarographic analysis is likely to be of most value. It should be emphasised that the use of the polarograph in organic chemistry is largely complementary to that of other modern physical techniques, such as infra-red and ultra-violet absorption spectroscopy. Whenever a new problem is being examined, a polarographic approach should be considered, but, before a method is finally adopted, its merits must be compared with those of methods based on other physical techniques. For many problems in organic chemistry the polarograph can offer advantages over all other techniques.

[285] *Biochem. J.*, 1948, **43,** 353.

[286] *Ann. Chim. appl.*, 1948, **38,** 655; 1950, **40,** 541, 559; 1951, **41,** 207; *Gazzetta*, 1950, **80,** 42.

[287] *Analyt. Chem.*, 1950, **22,** 1232.

[288] *Analyst*, 1943, **68,** 269.

[289] *Biochem. Z.*, 1951, **321,** 426.