CHAPTER **16**

Polarography

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16.1 INTRODUCTION

Polarography is the best known of several electroanalytical techniques involving controlled electrolysis and referred to as "voltammetry." In potentiometry (voltammetry at zero current) the potentials of electrochemical cells are normally measured when no appreciable current is flowing, while in voltammetry a finite current is allowed to flow through the electrochemical cell causing electrolysis. The extent of electrolysis may vary from complete electrolysis in electrogravimetry and coulometry, to that involving only a minute fraction of the analyte (the electroactive substance) at the surface of the microelectrode in polarography and amperometry.

Polarography originated with Professor Heyrovský[1] at Prague University in 1922. The term "polarography" is usually restricted to that voltammetric method in which the analyte, dissolved in a suitable medium, is placed in an electrolysis cell where the electrolysis is controlled by a variable known potential applied to the dropping mercury electrode (DME). The latter is polarized relative to a nonpolarizable electrode, usually the saturated calomel reference electrode (SCE). The electrolysis current resulting from a controlled increase in the potential of the DME, in an unstirred system, may be represented as a current-voltage curve called a "polarogram." This technique is used in the qualitative and quantitative analysis of many inorganic and organic electroactive substances.

If a polarizable solid microelectrode, such as a platinum wire, is employed in place of the DME and the solution is stirred under reproducible conditions the process is called "solid electrode voltammetry" and the resulting current-voltage curve is referred to as a "voltammogram." When the concentration of the electroactive substance(s) is altered by the addition of a titrant to either of the forementioned processes, the method is known as an "amperometric titration."

This chapter is confined mainly to conventional dc polarography which is still by far the most versatile of the voltammetric methods. The student is referred to recent references[2-5] and the literature for extensions and modifications of the polarographic method.

16.2 THEORETICAL CONSIDERATIONS

A. FACTORS WHICH INFLUENCE CURRENT FLOW DURING CONTROLLED ELECTROLYSIS

In electrolysis, certain constituents of a solution can be made to undergo a change in oxidative state, under appropriate conditions, by a flow of current at the electrode-solution interface. For example, at the cathode (where reduction occurs):

$$Ox + ne^- \rightarrow Red \tag{16.1}$$

at the anode (where oxidation occurs):

$$2H_2O \rightarrow O_2 + 2H^+ + 2e^- \tag{16.2}$$

where Ox is the electroreducible substance and Red is the product. In this instance, the cathode is a polarizable working electrode, e.g., DME, while the anode is the nonpolarizable electrode, e.g., SCE with a large-surface salt bridge. The working microelectrode becomes "polarized" when an external source of potential is impressed across the electrolysis cell. The electrons supplied to the cathode, under these conditions, are transferred to the electroreducible substance Ox at the electrode surface. Oxidation occurs at the larger anode surface, where electrons are received from the solution. The amount of current flowing through the anode is equal to that through the cathode, but due to the larger surface area of the anode the current density is much lower and polarization does not occur at this electrode.

The current-potential (i-E) relationships are simplified in polarography (and somewhat similarly in solid microelectrode voltammetry) by the following conditions: (a) the potential of one electrode, the reference electrode, is made independent of current flow by employing a large-surface electrode of constant potential (e.g., SCE), while the microelectrode (e.g., DME) becomes polarized under the same current flow; (b) the ohmic drop iR across the cell is kept to a low value, perhaps a few millivolts. Under these conditions the actual applied potential (voltage) is equal to the potential of the large-surfaced SCE less the potential of the polarized DME. In other words, the voltage applied across the electrolysis cell equals the potential of the polarized electrode if the unpolarized electrode of constant potential is considered the reference. This is what is implied when the potential of the microelectrode (DME) is reported as E_{appl} (vs. SCE). Since the potential of the SCE is +0.2444 V vs. the normal hydrogen electrode (NHE) at 25°C, the potential of the DME can also be related to the NHE (0.000 V) if this is desired.

Current-potential relationships in polarography are readily studied by means of apparatus schematically represented by Fig. 16.1.

An external potential (E_{appl}) is applied across the electrolysis cell by

means of a potentiometer and the resulting current is read by means of a sensitive galvanometer or microammeter (G).

Electrolysis of any species involves at least three factors which control the flow of current: mass transfer, electron transfer, and removal of the product.[6,7] An appreciation of the control of these factors will assist in obtaining accurate analytical results.
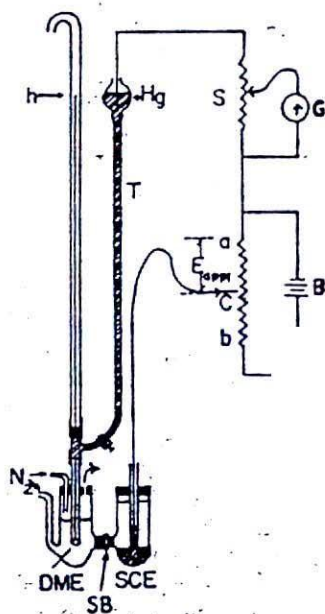

FIGURE 16.1: Simple polarographic apparatus and circuit. The H-type electrolysis cell includes a dropping mercury electrode (DME) and saturated calomel electrode (SCE) with a large-surfaced salt bridge (SB). The height (h) of the mercury column is controlled by the mercury (H_g) in a reservoir. A battery (B) or other source of constant voltage is accurately divided by a sliding contact (C) along a slide wire (a-b). A shunt (S) is used to change the sensitivity of a sensitive galvanometer (G) reading. T is a flexible plastic tubing.

1. Mass Transfer

Three basic mechanisms are involved in the mass transfer of an analyte (e.g., Ox) from the bulk of the solution to the polarized electrode surface, where it can be reduced. These mechanisms are migration, convection, and diffusion.

a. Migration. This means of transport involves the potential gradient produced in the solution whereby the electroactive species (e.g., Ox) is attracted toward the polarized microelectrode DME, while other species

having an electrical charge of like sign are repelled by this electrode. For example, a negatively charged cathode (DME) attracts cations while repelling anions. This means of mass transport is undesirable in polarography. The migration of the electroactive substance of interest is minimized by the addition, to the same solution, of a large excess of an "inert" (nonelectrolyzed) substance called the "supporting electrolyte" (SE). This SE conducts almost the entire current through the cell, but does not interfere with the reaction of interest at the microelectrode. For example, if the solution in the electrolysis cell is 0.1 M in KCl and only 0.001 M in the electroactive species, then the latter will conduct only about 1% of the current, everything else being equal. As the chloride ions migrate toward the anode, the potassium ions migrate toward the cathode. Since the potassium cations are not reduced at the selected applied potential on the cathode, they merely form a layer of positively charged particles around the microelectrode, where they decrease the attraction of this electrode for the reducible species, thereby reducing this means of mass transport to a negligible value.

b. Convection. This means of transport is operative when the solution is agitated. This agitation may be caused by density or temperature differences in various parts of the electrolysis cell or it may be produced by some form of mechanical stirring. Agitation brings more electroactive substances to the electrode surface and produces an increase in the current flow.

c. Diffusion. This transport mechanism is a result of a concentration gradient involving the electroactive species. When an appropriate potential is applied to the microelectrode (e.g., DME), the concentration of the reducible species at the electrode surface is rapidly reduced and more of this material will diffuse from the bulk of the solution, where the concentration C is higher, toward the electrode surface where the concentration C_0 is nearly depleted. Thus a concentration gradient $C - C_0$ exists for the electroactive species and "concentration polarization" (or "concentration over potential") occurs at this electrode. The electroactive species is frequently referred to as the "depolarizer" or "depolarizing substance." This concentration gradient causes the depolarizer (ion or molecule) to move through a diffusion layer d surrounding the polarized microelectrode. Agitation of the solution by means of a constant-rate stirrer causes the thickness of d to decrease and the current to increase until the latter reaches a steady limiting value.

Diffusion is a very slow means of mass transport relative to stirring and the limiting current value is therefore correspondingly lower in unstirred systems. Each depolarizer substance has a characteristic diffusion rate which is expressed as the diffusion coefficient D in units of square centimeters per second.

In view of the several factors which affect the current flow during electrolysis in the type of cell discussed here, the limiting current i_L is related to these

factors as well as the surface area A of the microelectrode by the following expression

$$i_L = \frac{nFDA}{d}(C - C_0) \tag{16.3}$$

where n is the number of electrons per molecule of the depolarizer and F is the faraday.

A constant limiting current i_L value can only be achieved in unstirred solution (the usual condition in polarography) or in solution stirred at a constant rate (as in voltammetry employing a polarized rotating or vibrating platinum microelectrode). In the latter instance, the limiting current values are less reproducible than when the DME is used. If there is a sufficient excess of a supporting electrolyte in the cell along with the depolarizer substance and if measures are taken to eliminate all agitation of the solution, then the limiting current becomes solely a diffusion current.

2. Electron Transfer Process

The electron transfer process occurs on the electrode surface between the electrode and the electroactive species. If this species is in equilibrium with other species, then the equilibrium must shift to produce more of the active species as it is reduced at the electrode during the electron transfer process. This equilibrium shift to produce more of the depolarizer species occurs either at the electrode surface or in the diffusion layer d, where it gives rise to a "kinetic current."

If this equilibrium shift is slower than that for mass transfer, then the observed current flow is dependent on this rate of conversion. Thus, polarography has been employed in electrochemical kinetic studies. Once the active species is at the electrode surface, the actual electron transfer process begins, providing the applied potential exceeds the decomposition potential. The current increases with increasing applied potential since the mass transport rate (MTR) is adequate to replace the species as it is depleted at the electrode surface. At this point it is the electron transfer rate (ETR) which limits the current flow. At a higher applied potential the ETR surpasses the MTR and the current reaches a limiting value controlled by the MTR. The greater the MTR of a species, the greater its limiting current value.[6]

The slope of the i vs. E_{appl} curve reflects the ETR, the greater the ETR, the steeper the slope. However, the i_L value ultimately depends upon the MTR, regardless of the slope of the i-E_{appl} curve.[6]

The electron transfer process is reversible only while the product is at the electrode surface. The ratio of the forward and reverse ETR depends on the electrode potential. When no external potential is applied to the electrode, the electrode adopts the equilibrium potential. Under this condition the two electron transfer rates differ only in their directions. When the forward

and reverse ETR are much greater than the MTR and the rate of conversion of a species to the "active" form, the electrode process is said to be "reversible." However, when the forward and reverse ETR are about the same or less than the two just mentioned rate processes, the electrode process is "irreversible." This irreversibility increases in degree, the slower these electron transfer rates become relative to these other processes.[6,7]

3. Removal of the Product

Since product accumulation at the electrode surface may influence nearly all the just mentioned processes, the rate of product removal could become the overall rate determining process in the electrode reaction.

The importance of the proper choice of experimental conditions can be seen when attempting to study the relationship between samples and corresponding i vs. E_{appl} curves.

B.   CURRENT-VOLTAGE RELATIONSHIPS

The apparatus which is frequently used in conventional dc polarography is represented by Fig. 16.1. The electrodes consist of a polarizable microelectrode, usually the dropping mercury electrode (DME), and a large-surfaced nonpolarizable reference electrode such as the saturated calomel electrode (SCE). When an external potential is applied to the DME, it is forced-to assume a potential other than that representing the equilibrium potential of the bulk of the solution. A small electrolysis current is now produced, providing some soluble electroreducible substance is present the electrolysis cell, and the DME becomes polarized. A similar situation exists when the DME becomes an anode, except that oxidation may occur at the electrode surface. Only a minute amount of the electroactive substance is removed from solution since a very small electrolysis current is employed in the polarographic method. In fact, several i-E_{appl} curves can be obtained using the same solution without detectable differences. The method is applicable to both inorganic and organic materials within an optimum concentration range of 10^{-2} to 10^{-5} M and can be performed on volumes as small as 0.5 ml or less in appropriate microcells.

1. The Nernst Equation

The potential E of the microelectrode at equilibrium is defined by the Nernst equation

$$E = E^{o'} + \frac{2.303RT}{nF} \log \frac{[Ox]}{[Red]}$$ (16.4)

where $E^{o'}$ is the formal electrode potential, with reference to the normal hydrogen electrode $E = E^{o'}$, when $[Ox] = [Red]$. The molar concentrations of the two soluble species involved in the electrode reaction are

represented by [Ox] and [Red], n equals the number of electrons required in the conversion of Ox to Red, and F is a faraday. Molar concentrations of each soluble species can be used in place of activities normally found in the Nernst equation, without appreciable error in dilute solutions. The relationship between these terms is shown by

$$a_{Ox} = [Ox]f \qquad (16.5)$$

where the activity coefficient f approaches unity in solutions which are approximately 10^{-3} M or less. For this reason, and because accurate values of f are not often known, the molar concentrations are used to facilitate calculations.

This form of the Nernst equation can be employed to define the potential of the microelectrode in polarography if the molar concentrations of the Ox and Red species represent those only at this electrode surface. The electrode reaction must also be reversible and the electrode in equilibrium with the solution immediately surrounding it. This latter condition is approximated closely if the ETR is very large relative to other electrolysis processes such as the diffusion rate of active species, and if both the Ox and Red forms of the species are soluble in the surrounding solution.[8]

2. Overpotential

To obtain an electrode reaction it is frequently necessary to apply a potential to the DME which is different, e.g., more negative relative to the SCE, than that which would be calculated by means of the Nernst equation under equilibrium conditions for a reversible electrode reaction. This difference in the DME potential is referred to as the "overpotential" of the electroactive substance on that electrode. The hydrogen-overpotential on the DME is fortunately quite large, therefore a considerable negative E_{appl} (vs. SCE) is required before hydrogen gas is evolved at the DME due to the reduction of the hydrogen ions in aqueous solution. This permits many substances to be reduced at the DME at E_{appl} values which are less negative than those required for the discharge of hydrogen gas.

3. Current-Voltage Curves

A typical polarogram (i-E_{appl} curve) is shown in Fig. 16.2. In this generalized discussion, the sample might be any soluble electroreducible substance (Ox) of appropriate concentration (10^{-2} to 10^{-5} M) in a large excess of a nonreducible electrolyte, e.g., KCl, 0.1 to 1 M. This supporting electrolyte should be about 50 times, or more, the concentration of the depolarizer in order to effectively eliminate the migration current of the latter.

a. Typical Polarogram (Fig. 16.2, curve a). As the DME is made to assume an increasingly negative potential (relative to SCE) an E_{appl} is reached where current begins to flow due to the reduction of the electroactive species

Ox in solution. This potential is referred to as the "decomposition potential" (E_d). As the applied potential is increased further, the current rises, controlled by the diffusion of Ox (or by the ETR in the case of irreversible electrode reactions) and eventually reaches a limiting current (i_L) plateau. Finally the current rises steeply due to the reduction of some SE component which may be water or other substance if its decomposition potential is lower.
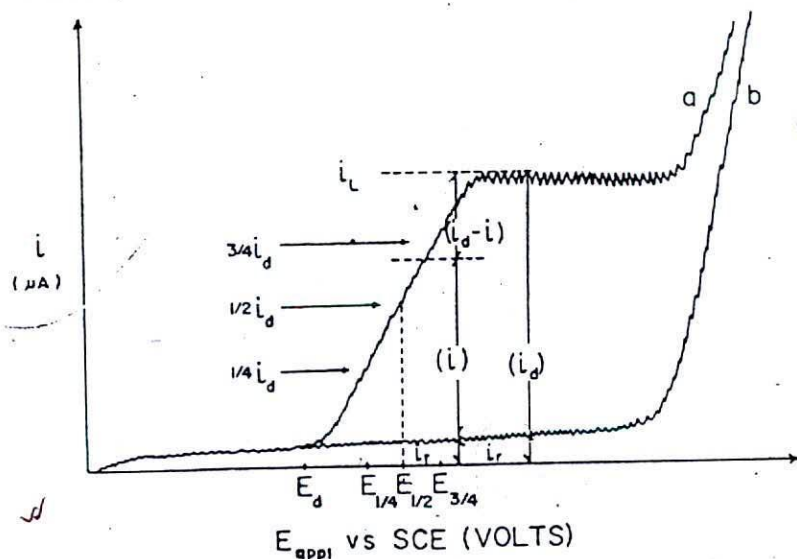

FIGURE 16.2: Typical polarogram. Curve a is the i vs. E_{appl} curve of an electroactive substance. Curve b is that for the supporting electrolyte, etc., less the electroactive substance. The symbols are identified as follows: i (μA) is the current reading; E_{appl} is the applied potential; E_d is the decomposition potential; $E_{1/4}$, $E_{1/2}$, and $E_{3/4}$, are the potentials corresponding to $\frac{1}{4}$, $\frac{1}{2}$, and $\frac{3}{4}$ the diffusion current (i_d), respectively; i_r is the residual current; i is the current reading anywhere on the curve corrected for i_r; i_d is the diffusion current ($i_L - i_r$) and i_L is the limiting current.

b. Residual Current (Fig. 16.2, Curve b). This is a i-E_{appl} curve of the supporting electrolyte alone. As the potential of the DME becomes increasingly negative the residual current i_r rises very gradually from a value near 0. Eventually a point is reached where an abrupt rise in the current flow occurs due to the reason given for curve a. The saw-toothed curves are the result of current oscillations caused by the gradual increasing electrode surface area [Eq. (16.3)] followed by the detachment of the mercury drop from the capillary tip.

c. Components of the Polarographic Wave. While it is the diffusion current i_d of the electroactive substance which is the most important and

characteristic portion of the i-E_{appl} curve, other contributions to the limiting current i_L may alter the overall curve and must be understood to be controlled.

1. Limiting current (i_L). This is the total current read from a current meter or recording. It includes the residual current i_r and diffusion current i_d, as well as the migration current of the reducible substance if this has not been adequately suppressed.

2. Residual current (i_r). This portion of the total current is the result of contributions from (a) a minute faradaic current i_f caused by the reduction of trace impurities and (b) a much larger condenser current i_c caused by the charging of the Helmholtz double-layer capacitance at the mercury-solution interface. This "condenser" is formed by positively charged ions of the supporting electrolyte (e.g., K^+) forming a layer around the negatively charged mercury drop, which in turn are surrounded by a layer of negative ions (e.g., Cl^-) to form the double layer.

3. Migration current (i_m). If the DME is a negatively charged cathode, then cations of an electroreducible sample will migrate (due to electrostatic attraction) to this electrode where they are reduced upon arrival. This undesirable contribution to the cathodic current is referred to as a migration current i_m. The effect of the i_m can be essentially eliminated by the addition of a large excess of "inert" electrolyte which conducts nearly the total current across the electrolysis cell but which does not interfere with the electrode reaction. Under these conditions the contribution of the migration current to the cathodic current is negligibly small and can be ignored.

4. Diffusion current (i_d). This is the net current which results from the subtraction of the residual and migration currents from the limiting current

$$i_d = i_L - (i_r + i_m) \tag{16.6}$$

Since the i_m can be eliminated as explained earlier, the Eq. (16.6) reduces to

$$\boxed{i_d = i_L - i_r} \tag{16.7}$$

This subtraction is normally done graphically, as illustrated in Fig. 16.2. When the diffusion current i_d is determined as just described, it is entirely dependent upon the rate of diffusion of the electroactive species from the bulk of the solution to the electrode surface.

C. FACTORS GOVERNING THE DIFFUSION CURRENT

I. Ilkovic Equation

A variety of factors govern the diffusion current at the DME for any electroactive species in a supporting electrolyte. A theoretical equation

was derived by Ilkovic (1934) for the diffusion current under controlled conditions

$$i_d = knD^{1/2}Cm^{2/3}t^{1/6} \qquad (16.8)$$

where i_d is the diffusion current (μA) during the life t of the mercury drop.

The constant k includes several physical and numerical constants, including the density of mercury, which were used in the derivation of the equation; $k = 706$ for maximum i_d, and $k = 607$ for average i_d measurements. The upper limit of the oscillations recorded by a fast recorder (<1 sec full-scale deflection) along the i_L plateau in Fig. 16.2 is used when measuring $(i_d)_{max}$; the average of these oscillations is employed when measuring $(i_d)_{aver}$ on a damped recorder. Also the $(i_d)_{aver}$ is the value actually measured on a galvanometer which does not respond quickly enough to follow the actual current oscillations. The $(i_d)_{max} = \frac{7}{6}(i_d)_{aver}$. There are several ways of defining n, such as: (1) the number of electrons per ion or molecule of the electroactive species or (2) the number of electron equivalents required per mole of the electroactive species involved in the electrode reaction. D is the diffusion coefficient of the electroactive species in square centimeters per second. C is the concentration of the electroactive substance, in millimoles per liter (mM/liter). m is the rate of mercury flow from DME capillary in milligrams per second. t is the drop life, in seconds; the value of t varies with the potential applied to the DME, because the interfacial tension (γ) at the mercury drop surface greatly influences the value of t and γ depends on E_{appl}. Therefore t is measured either at the E_{appl} where the i_d is to be measured or at the $E_{1/2}$ of the electroactive species.

In very precise work, modified forms of the Ilkovic equation may be employed which allow for the curvature of the mercury drops, since diffusion is spherical rather than linear, as originally supposed by Ilkovic. The Ilkovic equation is valid as long as t is longer than 2.5 sec and if a constant temperature is maintained in the approximate range of 15 to 40°C. If t is too short, the falling drops produce a stirring effect which decreases the diffusion layer d and increases the observed current.

The linear relationship between i_d and C can be seen if all of the factors on the right-hand side of the Ilkovic equation, except C, are held constant and represented by k:

$$i_d = kC \qquad (16.9)$$

The Ilkovic equation may be arranged to give

$$knD^{1/2} = \frac{i_d}{Cm^{2/3}t^{1/6}} \qquad (16.10)$$

The characteristics which are independent of the electrodes and instrument are grouped together on the left and are collectively referred to as the "diffusion current constant" (I_d). This value is frequently recorded in the literature and is reproducible within $\pm 5\%$, under specified conditions.

The term $m^{2/3}t^{1/6}$ is called the "capillary characteristic." When m and t have been experimentally determined, comparisons can be made between capillaries of varying length and bore diameter, as well as with the same capillary under different head pressure of mercury or at various applied potentials.

The factors which affect the i_d include those which produce a change in any term in the Ilkovic equation and these, as well as others, must be controlled when using the polarographic method.

a. Measurement of the Diffusion Current (i_d). This term varies with the sixth root of t. However, most galvanometers have a time period which is not short enough to accurately follow the current produced at each mercury drop.

The galvanometer oscillations observed correspond closely to the average of the true diffusion current and correspond to the $(i_d)_{aver}$ in the Ilkovic equation rather than the maximal or minimal reading. An exact diffusion current i_d of an electroactive substance can only be determined when the exact residual current is known and subtracted from the limiting current value. The most accurate measurement of i_r can be made by means of a separate polarogram of the supporting electrolyte, such as that shown in Fig. 16.2, curve b. In actual practice, an adequate approximation of the i_r can be obtained by extrapolating the portion of the sample polarogram, which preceeds the decomposition potential, and measuring the distance between this extrapolated line and the limiting current to obtain the i_d at a specified point. This method is illustrated in a later section.

b. Height of the Mercury Column (h). Both the mercury flow rate m and the drop life t are dependent on the dimensions of the capillary of the DME and on the height of the mercury column above the tip of this electrode. An increase in the height of the mercury column h produces no increase in drop size (a function of bore size), but rather increases the number of drops formed per unit of time. The relationship between m and h has been shown to be

$$m = kh_{corr} \qquad (16.11)$$

where k is a constant and h_{corr} is the height of the mercury column (in centimeters) above the DME tip corrected for the back pressure due to the interfacial tension between the drop and solution.

It can be shown that for aqueous solutions the back pressure (h_{back}) is given by the equation

$$h_{back} = \frac{3.1}{m^{1/3}t^{1/3}} \text{ cm mercury} \qquad (16.12)$$

and $h_{corr} = h - h_{back}$, where h is the uncorrected height of the mercury column in centimeters.

The value of t varies inversely with the height of the mercury column, as shown by the equation

$$t = \frac{k'}{h_{corr}^2} \tag{16.13}$$

If Eqs. (16.11) and (16.13) are substituted into the Ilkovic equation (Eq, 16.8), and providing all other factors which could affect i_d remain constant, then it can be shown that i_d varies with the square root of the corrected height of the mercury column above the tip of the DME

$$i_d \propto h_{corr}^{1/2} \tag{16.14}$$

This equation provides a means of ascertaining whether the current is actually diffusion controlled.
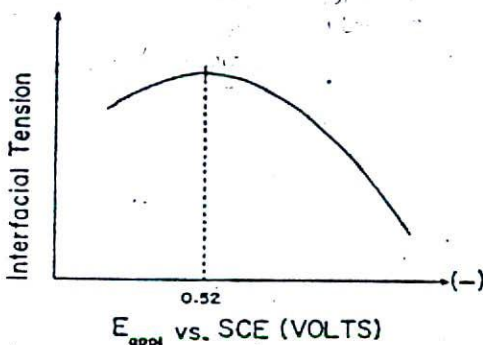

FIGURE 16.3: Electrocapillary curve for mercury.

c. The Applied Potential (E_{appl}). The drop life t is affected by the applied potential E_{appl} on the DME. Actually the value of t is affected by the mercury drop-solution interfacial tension γ, which in turn is affected by the applied potential as indicated in Fig. 16.3.

As the negative applied potential ($-E_{appl}$) is increased on the mercury drop, the drop-solution interfacial tension γ, passes through a maximum at approximately -0.52 V (relative to SCE) and then decreases rapidly. In practice, since the product $m^{2/3}t^{1/6}$ is only influenced by the sixth root of t, it is considered almost constant, as it varies less than 0.5% over the applied potential range of 0 to -1.0 V. However, at more negative potentials the product $m^{2/3}t^{1/6}$ shows a more rapid decline in value, which must be taken into account.

When diffusion currents are to be compared with those calculated by means of the Ilkovic equation, it is essential that t and i_d be measured at the same potential.

d. **Temperature Effect.** The effect of temperature on the diffusion current is quite pronounced. Although a temperature term does not appear in the Ilkovic equation, a temperature change affects every term except n. The diffusion coefficient D is very sensitive to variations in temperature because of its influence on viscosity, mobilities of ions, etc. These changes produce an increase in the i_d of 1 to 2%/deg rise in temperature in the vicinity of 25°C. To eliminate this error in i_d measurement it is essential to control the temperature of the electrolysis cell to within ±0.25 or ±0.5°C to hold errors within 1%.

e. **Viscosity of Solution.** The factor which is affected most by the viscosity of the solution is the diffusion coefficient D.

Changes in the supporting electrolyte do not appreciably change the viscosity unless they are large, i.e., in excess of ±10%. However, a change in the amount of a maximum suppressor, such as gelatin, may produce a marked change in the solution viscosity. A high concentration of a maximum suppressor is to be avoided for this reason.

The diffusion coefficient D of an electroactive species is also affected by complex formation with any component of the supporting electrolyte, or by other means.

D. KINETIC CURRENT

A kinetic current is a complex-limiting current which may occur when one or both of the oxidative states of the electroactive substance is in a dissociation equilibrium or involved in a chemical reaction with other substances. Under such circumstances the electroactive form of the species is depleted at the DME surface by a rapid electron transfer process and must be replenished by a relatively slower nonelectrode reaction such as dissociation or a shift in a chemical equilibrium. The magnitude of the kinetic current flow is proportional to the rate constant of the slower chemical reaction.

Kinetic waves are more common in the polarographic analysis of organic compounds such as formaldehyde, carboxylic acids, etc.

E. CATALYTIC CURRENT

A catalytic current is an increase in the current which may be brought about by an unstable electrolysis product, which may suddenly revert to the original electroactive species. For example, catalytic current waves have been observed during the reduction of cupric ions to cuprous ions, where part of the latter is spontaneously oxidized to cupric ions while another part is simultaneously reduced to copper.[8] Well-defined catalytic hydrogen waves have been observed for a number of alkaloids in certain supporting electrolytes.[9]

F. MAXIMA AND SUPPRESSORS

Frequently a pronounced increase in current above the normal limiting value is observed on a polarographic curve. This is often caused by an increase in mass transport of the depolarizer (electroactive substance) to the electrode surface brought about by a streaming effect in the solution. These maxima are reproducible and are subdivided into maxima of the first and second kind. Maxima of the first kind, which appear on the rising portion of the wave, as shown in Fig. 16.4, are rather narrow and frequently occur
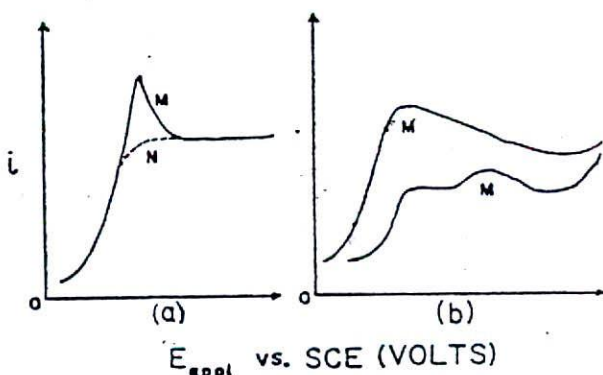

FIGURE 16.4: (a) Maximum of the first kind (M). The dotted line (N) indicates the normal wave. (b) Maxima of the second kind (M).
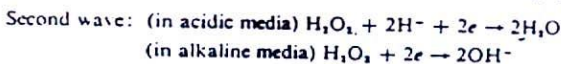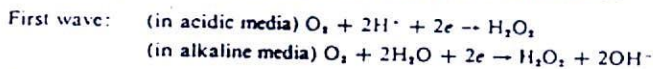
in dilute solutions. Those of the second kind occur in more concentrated solutions, at higher mercury flow rates; only in the limiting current range, are rounded, and do not fall as abruptly to the limiting-current plateau as do the maxima of the first kind. Heyrovský and Kůta discuss both kinds of maxima in detail.[2]

The occurrence of maxima is undesirable because of the interference with diffusion current measurements. Maxima of both the first and second kinds can be eliminated by adding a suitable maxima suppressor. The suppressor substance is usually some nonreducible high-molecular-weight organic surface-active material such as gelatin, nonionic detergents such at Triton X-100, as well as cationic and anionic types, methyl cellulose, agar, alcohols, and certain dyes such as methyl red, and basic or acid fuchin, etc. Gelatin is one of the most widely used substances, but solutions must be prepared freshly. The final concentrations of these maxima suppressors in solution range between 0.001 to 0.01%. The optimum amount of substance required for complete suppression of a maximum is proportional to the concentration of the depolarizer, varies from substance to substance, and is influenced by the nature of the supporting electrolyte. The optimum concentration of

the suppressor substance is found by trial and error. Care must be taken to avoid higher concentrations as several terms in the Ilkovic equation are affected by the resultant increase in viscosity and a lower diffusion current results.

G. OXYGEN WAVES

(If air (oxygen) is not removed from the solution prior to analysis, two waves will result from the reduction of oxygen and its reduction product, hydrogen peroxide, as the potential of the DME is made increasingly negative. Although the half-wave potentials are pH dependent, one may occur about -0.10 V and another about -0.90 V (vs. the SCE). The reactions which produce these reduction waves are as follows:

First wave: (in acidic media) $O_2 + 2H^+ + 2e \rightarrow H_2O_2$
              (in alkaline media) $O_2 + 2H_2O + 2e \rightarrow H_2O_2 + 2OH^-$

Second wave: (in acidic media) $H_2O_2 + 2H^+ + 2e \rightarrow 2H_2O$
              (in alkaline media) $H_2O_2 + 2e \rightarrow 2OH^-$

The oxygen (air) must be removed from the solution or these pronounced oxygen waves will be superimposed on the desired polarogram and interfere with current measurements. This air is usually removed by bubbling some inert gas such as oxygen-free nitrogen through the solution prior to analysis and allowing a gentle flow of nitrogen to layer above the solution surface to prevent oxygen reabsorption during analysis) If a gas dispersion tube is used, the deaeration time can be reduced considerably. However, if an ordinary small-bore glass tube is used for this purpose, about 10–15 min are normally required to remove the dissolved oxygen. A purified grade of commercial tank nitrogen (99.9+ %) is frequently adequate for this purpose. If the last trace of oxygen must be removed, the nitrogen should either be passed through a heated tube of copper turnings or a gas washer containing some suitable reducing agent.

H. SUPPORTING ELECTROLYTE

The supporting electrolyte is added to carry most of the current between the electrodes, to reduce the migration current of the electroactive substance. This condition must be satisfied if the diffusion current i_d of the depolarizer is to be directly proportional to the concentration of this electroactive substance. The concentration of the supporting electrolyte should be at least 50-fold greater than that of the depolarizer for this purpose. The choice of the supporting electrolyte can be a most important factor since it frequently interacts with, and affects the $E_{1/2}$ value of the depolarizer, and may even determine whether any wave will result or not. A mixture of two electroreducible substances may have closely spaced $E_{1/2}$ values in one supporting electrolyte (SE) and yield unresolved waves. Another SE can

often be found which will form a complex with one of them, producing a shift in its $E_{1/2}$ value and thereby allowing the waves to be resolved adequately for both substances to be determined simultaneously in the mixture. The reader is referred to texts on polarography for extensive lists of supporting electrolytes.[2,5,10]

As the applied potential across the electrolysis cell is increased, a point is eventually reached where the water (solvent) will be electrolyzed. This point determines the upper voltage limit of the working electrode in that media, for once the electrolysis of the solvent begins, other electrode reactions will be masked. The solvent is seldom electrolyzed at the reference electrode. When the microelectrode (DME) functions as a cathode the hydrogen ions of water are reduced and hydrogen gas begins to be evolved about -1.8 V (vs. SCE) in neutral solution. Under more alkaline conditions this electrolysis is shifted to slightly more negative potentials. If a working electrode, other than the DME, becomes sufficiently anodic, the water is oxidized and oxygen is evolved at this electrode surface. The positive voltage-range limit for the DME is determined by the potential at which mercury begins to be oxidized to mercuric ions, while the negative limit of the DME potential is that at which some component of the SE becomes discharged. Certain quaternary salts and hydroxides serve as supporting electrolytes and permit the use of applied potentials more negative than -2.0 V (vs. SCE). These SE materials increase the hydrogen overpotential and allow polarographic reduction studies on alkali and alkaline earth-metal cations. Salts of the latter substances may be employed as supporting electrolytes for other more readily reduced substances in aqueous solutions at the DME over a range of $+0.4$ to about -1.9 V (vs. SCE).

Organic substances are frequently dissolved in nonaqueous supporting electrolytes.[5]

All substances to be used in supporting electrolytes should be polarographed under high sensitivity to check for possible interfering waves due to impurities.

IX MIXTURES OF ELECTROACTIVE SUBSTANCES

Frequently mixtures of electroactive substances can be analyzed provided the half-wave potentials ($E_{1,2}$) of the component substances are adequately spaced so that there is no interference between adjacent waves. If the half-wave potentials are not, or cannot be made sufficiently different by complexing the depolarizer or changing the solvent, then physical, chemical, or electrochemical methods of separating the interfering components are mandatory prior to polarography.

The wave of the most readily reduced component can easily be observed at a high sensitivity, but the second wave may go off scale. When this occurs, the analyst is faced with a choice. If the second component is present in

higher concentration, the sensitivity may be decreased sufficiently to keep the second wave on scale. However, if the second wave is produced by a minor component, greater sensitivity is required for its wave. Many instruments are equipped with a device which permits a compensating current to be passed through the galvanometer, in opposition to the reduction (or oxidation) current, which either reduces the height of the preceding wave or eliminates it. With the first wave electrically cancelled the second wave can be developed with the same or a higher sensitivity to permit an accurate measurement of the minor component.

The relative wave heights (and indirectly concentration), the number of electrons n involved in each reversible reduction, as well as the degree of reversibility of each reduction all affect the resolution of successive reduction waves for mixtures of reducible constituents.

Usually the wave for a two-electron reduction is steeper and occurs over a shorter voltage span than for a single-electron reduction. A greater degree of irreversibility of the electrode reaction also causes a spread in the wave over a wider voltage range. It should be noted that the $E_{1/2}$ values of two electro-reducible species showing irreversible waves would have to differ to a greater extent than those of adjacent reversible waves in order to obtain comparable resolution.

J. EQUATIONS FOR REVERSIBLE POLAROGRAPHIC WAVES

If the oxidized state only of a depolarizer [e.g., Cd(II), Fe(III), reducible organic molecule, etc.] is in solution, it will accept electrons from the DME (cathode) which are supplied from the SCE (nonpolarizable anode) via the external circuit.

The equation for the polarographic reduction (cathodic) wave can be arrived at from the following considerations:

Reduction at the DME surface is represented by Eq. (16.1), repeated here:

$$Ox + ne^- \rightarrow Red$$
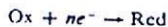
where Ox and Red are the oxidized and reduced forms of the electroactive species. During electrolysis at a potential corresponding to the limiting-current plateau, the concentration of the electroreducible substance at the microelectrode surface, $[Ox]_0$, is negligibly small compared to that in the bulk of the solution $[Ox]$. At some lower applied potential, corresponding to the rising current on an i vs. E_{appl} plot, the $[Ox]$ is not negligible in the layer surrounding the DME and, for a reversible reaction in which both Ox and Red are soluble, the Nernst equation can be applied. The potential of the DME under these conditions and at 25°C is

$$E_{DME} = E^{o'} + \frac{0.0591}{n} \log \frac{[Ox]_0}{[Red]_0} \tag{16.15}$$

where $E^{o'}$ is the formal reduction potential of the redox couple in the electrode reaction [Eq. (16.1)] under experimental conditions. As discussed earlier [Eq. (16.4)] molar concentrations can be employed in this equation instead of activities, providing dilute solutions are used. When this is done $E^{o'}$ replaces the standard potential E^{o}. The subscript $_0$ denotes the negligible concentration at the electrode surface. It can be seen from Eq. (16.15) that the potential on the DME determines the ratio Ox/Red.

The average current supplied by diffusion of the reducible depolarizer to the DME surface is

$$i = K([Ox] - [Ox]_0)D_{Ox}^{1/2} \tag{16.16}$$

where K is a constant which also includes terms n, m, and t of the Ilkovic equation [see Eq. (16.8)]. Equation (16.16) is similar to Eq. (16.3). The Ilkovic equation may now be written in the form of

$$i_d = 607nm^{2/3}t^{1/6}D_{Ox}^{1/2}([Ox] - [Ox]_0) \tag{16.17}$$

where the current reaches its limiting value and is solely dependent on the gradient existing between the concentration of depolarizer in the bulk of the solution [Ox] and that at the electrode surface $[Ox]_0$. Since $[Ox]_0 = 0$, i.e., it is negligible since Ox is reduced as rapidly as it arrives at the electrode surface, Eq. (16.17) reduces to

$$i_d = K[Ox]D_{Ox}^{1/2} \tag{16.18}$$

By solving for $[Ox]_0$ in Eq. (16.16) and combining with Eq. (16.18)

$$[Ox]_0 = \frac{i_d - i}{KD_{Ox}^{1/2}} \tag{16.19}$$

The reduced form (Red) of the depolarizer formed at the DME may diffuse away or form amalgams and diffuse into the mercury drop. The current depends not only on the rate at which Ox arrives at the microelectrode [see Eq. (16.18)] but also on the rate at which the reduced form, Red, diffuses from this electrode surface:

$$i = K([Red]_0 - [Red])D_{Red}^{1/2} \tag{16.20}$$

and since only the oxidized form of the depolarizer is present in the bulk of the solution, [Red] = 0, and

$$i = K[Red]_0 D_{Red}^{1/2} \tag{16.21}$$

By solving Eq. (16.21) for $[Red]_0$ and substituting this value, as well as Eq. (16.19) in the Nernst [Eq. (16.15)], the potential of a redox system is given by

$$E_{DME} = E^{o'} + \frac{0.0591}{n} \log \frac{i_d - i}{i} - \frac{0.0591}{n} \log \left(\frac{D_{Ox}}{D_{Red}}\right)^{1/2} \tag{16.22}$$

Since the diffusion coefficients D for the Ox and Red forms of many depolarizer substances are very nearly equal and appear in this equation only as the square root, the factor $(D_{Ox}/D_{Red})^{1/2}$ can be let equal unity and Eq. (16.22) reduces to the following at 25°C:

$$E_{DME} = E^{o'} + \frac{0.0591}{n} \log \frac{i_d - i}{i} \qquad (16.23)$$

This is the simplest expression for the shape of a reversible polarographic reduction wave, i.e., a cathodic wave.

The potential on the microelectrode (DME) corresponding to the current value i at one-half the diffusion current i_d is called the "half-wave potential" ($E_{1/2}$). A mathematical expression for the $E_{1/2}$ is obtained from Eq. (16.22) by introducing $i = i_d/2$:

$$E_{1/2} = E^{o'} - \frac{0.0591}{n} \log \left(\frac{D_{Ox}}{D_{Red}}\right)^{1/2} \qquad (16.24)$$

or

$$E_{1/2} = E^{o'} \qquad (16.25)$$

can be obtained from Eq. (16.23).

The half-wave potential ($E_{1/2}$) value is characteristic of a given depolarizer in a specified medium, but is independent of the depolarizer concentration, of the capillary characteristics, and of the galvanometer sensitivity.[2] The $E_{1/2}$ value obtained in a specified supporting electrolyte is frequently used to identify a depolarizer.

It is frequently so that the diffusion coefficients of the oxidized and reduced states D_{Ox} and D_{Red} of a redox couple are approximately equal and in such instances the $E_{1/2}$ corresponds closely to the value of the formal potential $E^{o'}$. If either the Ox or Red form of the depolarizer substance is complexed, their rates of diffusion will differ. By measuring the ratio of anodic to cathodic diffusion currents in solutions where $[Ox] = [Red]$, the ratio $D_{Ox}^{1/2}/D_{Red}^{1/2}$ can be found. Equation (16.24) shows that for a reversible electrode reaction the $E_{1/2}$ is related to the formal reduction potential $E^{o'}$ of the redox couple. However, the $E_{1/2}$ value may differ from the $E^{o'}$ somewhat depending on the difference in the diffusion coefficients of the Ox and Red forms.

If a polarographic system consisting of a perfectly polarizable DME and nonpolarizable SCE is employed, $E_{DME} = E_{appl}$. At an applied potential corresponding to the $E_{1/2}$ value, where $i = i_d/2$ by definition, $E_{1/2} = E^{o'}$ due to the disappearance of the log term in Eq. (16.23), thus, at 25°C,

$$E_{appl} = E_{1/2} + \frac{0.0591}{n} \log \frac{i_d - i}{i} \qquad (16.26)$$

This equation with slight rearrangement is a form of $y = mx + b$, the equation for a straight line. Therefore the reversibility of an oxidation-reduction system at the DME surface can be checked by plotting $\log (i_d - i)/i$ vs. E_{appl} (as abscissa). For reversible redox systems, a straight line results with

slope $\doteq 0.0591/n$ V and abscissa intercept equal to $E_{1/2}$, where the log term of Eq. (16.26) becomes 0. The value of n can be determined by the expression $n = 0.0591/\text{slope}$, where the slope is 0.0591, 0.0296, and 0.0197 V, for a 1-, 2-, or 3-electron transfer in the electrode process, respectively. These values apply only at 25°C.

It is unfortunate that a linear relationship between log $(i_d - i)/i$ vs. E_{appl} is not absolute proof of reversibility.[5] This method cannot be applied if the product of the electrode reaction is not soluble in the mercury drop as an amalgam, or soluble in the solution of supporting electrolyte employed.

The preceding discussion dealt only with the cathodic wave. For discussions and equations of anodic waves and cathodic-anodic combined waves the reader is directed to Ref. 2.

K.   REVERSIBLE AND IRREVERSIBLE ELECTRODE REACTIONS

In reversible redox systems (Ox $+ ne^- \rightleftharpoons$ Red) where the equilibrium is rapidly established by fast electron-transfer rates (ETR) and when the current is controlled solely by diffusion of the depolarizer, the electrode potential is equal, or very nearly equal, to that value calculated by means of the Nernst equation [Eq. (16.4)]. It has been shown [Eq. (16.25) and Fig. 16.5, curve a] that for reversible reactions the $E^{o'}$ very nearly coincides with the $E_{1/2}$ for each form of the redox couple.

A reversible electrode process is indicated when the $E_{1/2}$ values of the anodic (oxidation) and cathodic (reduction) waves coincide. This situation occurs with the Fe(III)/Fe(II) redox couple. When both oxidation states of the depolarizer substance [e.g., Fe(III)/Fe(II)] are present in solution, a composite anodic-cathodic wave may be obtained (Fig. 16.6, curve a) the steep slope of which is defined by the Nernst equation.

If the electrode reaction is irreversible, as it generally is for organic molecules and some inorganic substances, the Nernst equation has no application. It should be noted also that irreversible reactions occur when either the Ox or Red form is insoluble in either the supporting medium or the mercury drop. Some reversible electrode processes may become "irreversible" with a shortened mercury drop life t or under other circumstances where the time required for establishing equilibrium conditions at the solution-drop interface is inadequate.[2]

A smooth polarographic wave may result from a mixture of oxidized and reduced forms of a substance, even though the electrode processes are irreversible under the conditions used. However, in this situation the slope of the combined anodic-cathodic wave is much less steep and does not conform to theory. Figure 16.5 shows generalized forms of individual and combined anodic and cathodic waves for both reversible and irreversible systems; a qualitative difference is readily seen between these curves. In reversible processes the $E_{1/2}$ values of both anodic and cathodic waves are equal and very near the value of $E^{o'}$ in the Nernst equation. However, the $E_{1/2}$ values

of the anodic and cathodic waves in irreversible systems are separated and neither is equal to the $E^{o'}$. The greater the degree of irreversibility, the greater the separation of the $E_{1/2}$ values of the oxidized and reduced forms. Irreversibility in an electrode reaction (slow ETR) yields an $E_{1/2}$ value more negative than $E^{o'}$ for a reduction (more positive for an oxidation).
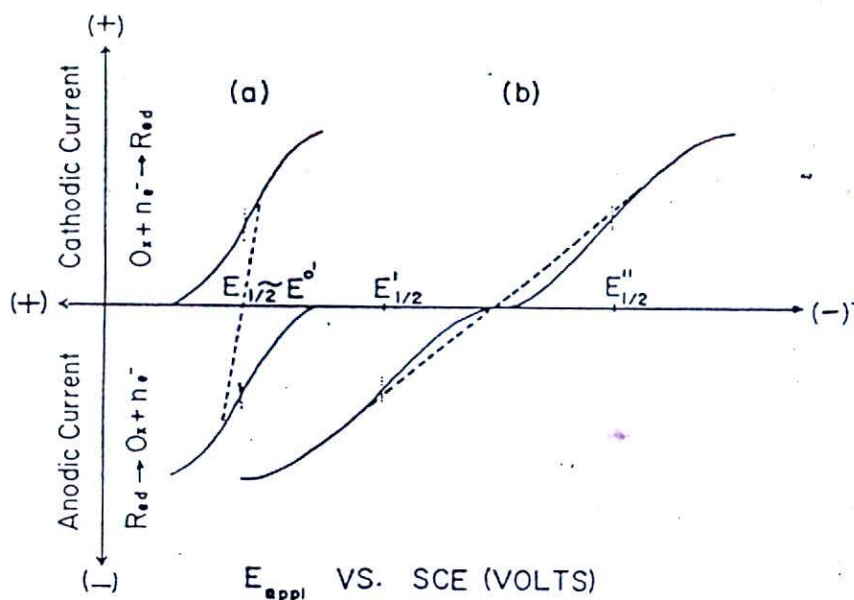

FIGURE 16.5: Anodic and cathodic i-E_{appl} curves for a reversible (a) and an irreversible (b) redox couple. The dotted lines represent the combined waves of a mixture of equal concentrations of Ox and Red forms in both instances, while the solid lines represent the waves for the Ox and Red forms of each couple separately. $E^{o'}$ is the formal redox potential and $E_{1/2}$, $E'_{1/2}$, and $E''_{1/2}$ are half-wave potentials.

If we now let $E_{1/2}$ in Fig. 16.5, curve a, represent the calculated theoretical value and $E_{1/2}$ on curve b represent the actual observed value for a specific substance under identical conditions, then the difference between these values correspond to the "overvoltage" (ov) of that substance (depolarizer) on that electrode. The magnitude of this overvoltage is indicative of the degree of irreversibility of the reaction occurring at the microelectrode (e.g., DME) surface.

Irreversible waves are also defined by the Ilkovic equation [Eq. (16.8)] and the $E_{1/2}$ is still an identifying characteristic of the depolarizer, even though it occurs at a position which differs from that which would result if the reaction were reversible.

Another method used for distinguishing between reversibility and irreversibility of electrode processes involves the calculation of the difference between $E_{3/4}$ and $E_{1/4}$, where these terms are defined in a manner similar to that for $E_{1/2}$. For ideal cathodic $(i - E)$ waves, a plot of i/i_d vs. $(E_{DME} - E^{o\prime})$ is made and the value $(E_{3/4} - E_{1/4})$ measured. For reversible reactions $(E_{3/4} - E_{1/4}) = 0.0564/n$ V (at 25°C), and for irreversible reactions this value is greater. Mathematical expressions are available for the $E_{1/2}$ of a

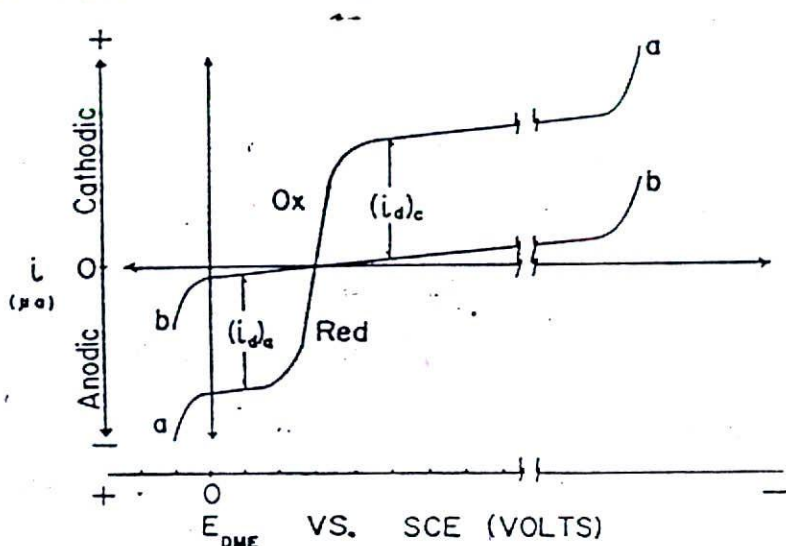FIGURE 16.6: Polarographic waves of (a) a reversible oxidation-reduction couple, where [Ox] = [Red], and (b) the supporting electrolyte alone. The symbols $(i_d)_a$ and $(i_d)_c$ represent the anodic and cathodic diffusion currents, respectively.

totally irreversible wave (very slow ETR) and for the relationship between this value and drop time. For adequate resolution in quantitative analysis, the difference between the $E_{1/2}$ values of two successive polarographic waves should be three or more times the sum of their respective $E_{3/4} - E_{1/4}$ values. A greater difference is required if the wave heights are very different.[5] The reader is referred to the references, particularly Refs. 2 and 5, for a detailed discussion involving differentiating between reversible and irreversible electrode processes.

L.   CONVENTIONS EMPLOYED IN PLOTTING $i - E_{appl}$ CURVES

The conventional method of plotting $i - E_{appl}$ curves is shown in Fig. 16.6. The potential applied to the microelectrode relative to a nonpolarizable reference electrode (e.g., SCE) is shown on the horizontal axis. This applied

potential value (E_{DME}) becomes increasingly negative to the right of $E_{DME} = 0$, the potential of the SCE, and increasingly positive to the left. The current is represented on the vertical axis and, by convention, cathodic currents due to reduction are recorded as being positive and appears above the zero current line. The anodic currents due to oxidation are considered negative and appear below the zero current line.

Curve a (Fig. 16.6) is the polarogram of a solution containing equimolar concentrations of the soluble oxidized (Ox) and reduced (Red) states of an oxidation-reduction system in a deaerated solution containing a supporting electrolyte (SE) such as 0.1 M KCl. Curve b is the polarogram of the same deaerated SE and maximum suppressor but no depolarizer substance. At the extreme left, the anodic current increase is due to oxidation of the mercury of the drop ($2Hg \rightarrow Hg(II) + 2e^-$). This occurs at a lower (+) potential than the oxidation of water to oxygen ($2H_2O \rightarrow O_2 + 4H^- + 4e^-$). The negative current to the right of the mercury dissolution wave up to the zero current line represents the anodic oxidation of the Red form of the depolarizer substance. The intercept of this mixed wave with the zero current line represents the E_{DME}, which is very nearly equal to the formal potential ($E^{\circ\prime}$) in the Nernst equation for reversible systems (fast electron-transfer rates) when [Ox] = [Red], assuming little or no change in the diffusion coefficients D of the two states of oxidation. Also in truly reversible redox systems the value of $E^{\circ\prime} \sim E_{1/2}$ of both the Ox and the Red forms. The (+) cathodic current in curve a (Fig. 16.6) represents the electroreduction of the oxidized form of the substance. At the extreme right of curve a, the rapid rise in the cathodic current at an applied potential of about -1.8 to -2.0 V, is a result of the reduction of hydrogen ions in the water or to the discharge of some cation of the SE. Curve b is the polarogram of the SE alone, the increased anodic and cathodic currents are due to the same causes discussed in curve a. Between these two extremes of rapid current increase, the DME is perfectly polarized and only a small residual current flows, which is shown here running close to the horizontal zero current line.

The cathodic diffusion (i_d)$_c$ will closely approximate the anodic diffusion current (i_d)$_a$, when [Ox] = [Red] in the mixture.

16.3   BASIC INSTRUMENTATION, APPARATUS, AND PRINCIPLES

A.   CIRCUITRY

The essential circuitry is shown schematically in Fig. 16.1. This diagram represents the basic requirements for a conventional manual dc polarograph.

The potential of the dropping mercury electrode (DME) can be made increasingly negative (or positive) relative to the constant known potential
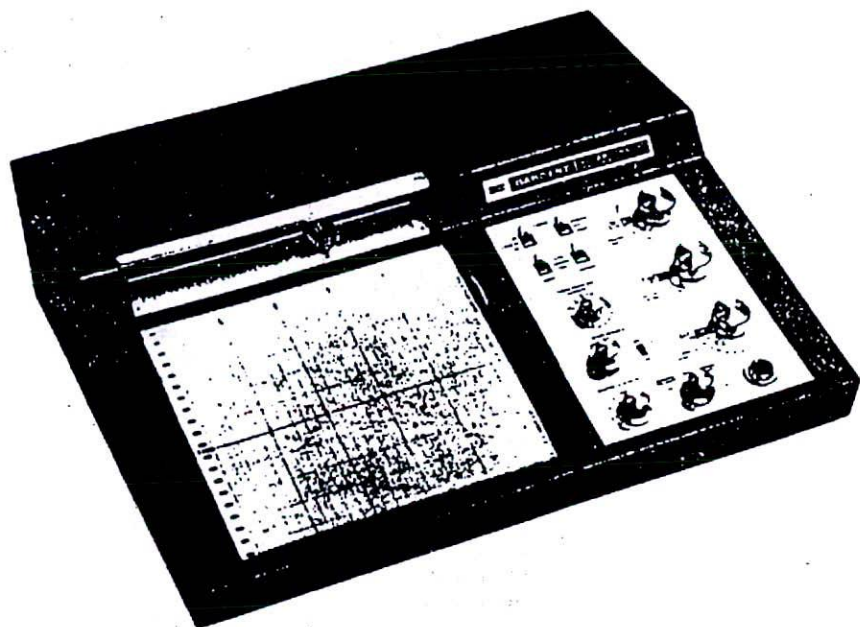
FIGURE 16.7: Sargent recording polarograph, model XVI. Courtesy E. H. Sargent and Co.

3. Electrolysis Cells

Polarographic electrolysis cells vary widely in design and capacity. The more common are: (a) a small open beaker-type of cell which may be used when a mercury pool (nonpolarized) electrode is to be used (Fig. 16.8a) and (b) the H-type cell in which the electrolysis compartment containing the DME is connected to an external reference electrode (SCE). These two compartments are electrically connected via a short agar gel plug saturated with KCl and supported in a wide diameter tube closed at one end with a sintered-glass disk (Fig. 16.8b)

A wide variety of electrolysis cells are commercially available from E. H. Sargent & Co. and other sources. These cells are designed for polarographic analysis of solutions having a total volume range from less than 1 ml up to several ml. Many different designs are possible as a literature survey will readily show.

4. Electrodes

a. Polarizable Microelectrodes. Actually the term "polarography" infers that the polarizable microelectrode is the dropping mercury electrode (DME).
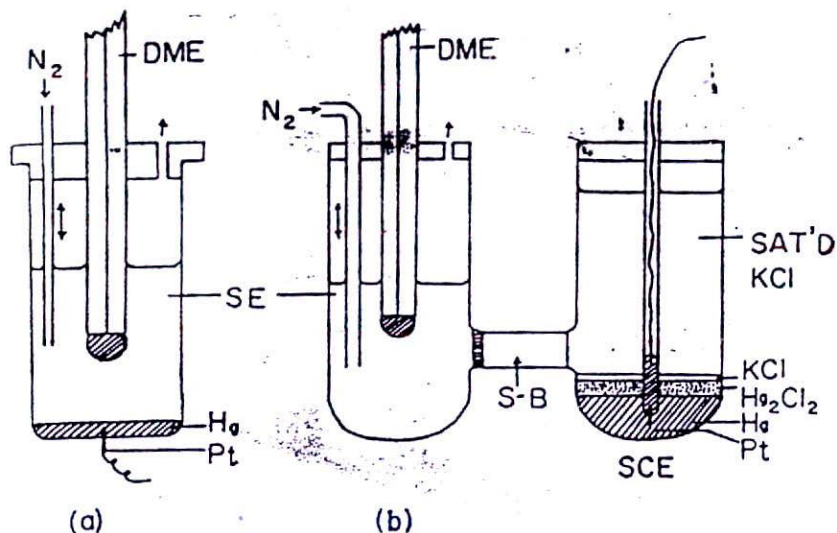
FIGURE 16.8: (a) Beaker-type cell with mercury pool and dropping mercury electrodes. (b) H-type cell with saturated calomel electrode (SCE) and DME.

This is still the most widely used electrode, however numerous other mercury electrodes have been employed such as the hanging mercury drop, streaming mercury, inverted mercury, and horizontal mercury electrode, as well as many others. Microelectrodes such as the rotating and vibrating platinum electrodes are used for very closely related methods of voltammetry, especially in amperometric titrations.

1. *Dropping Mercury Electrode (DME).* The DME consists of a short length of glass capillary tubing (0.03–0.05 mm id) which is attached to a length of large-bore glass tubing used to support a mercury column. The constant height of this mercury column is maintained by a mercury reservoir bulb which is connected to the mercury column and capillary as shown in Fig. 16.1. Electrical contact with the DME may be made via the mercury reservoir, or by means of a platinum wire fused into the glass wall of the mercury column support or elsewhere. The mercury drops, formed under a constant head pressure of mercury, leave the capillary tip at a constant rate. A repetitive process is involved in which each mercury drop gradually grows into a microsphere until it reaches a critical weight and detaches itself.

The advantages of the DME relative to other microelectrodes are:

(a) The continually renewed smooth surface of the drop prevents the accumulation of electrode reaction products which would adversely affect the potential at the surface.

(b) The reproducible surface area of the drop can be readily calculated at any time during the drop life.

(c) The high overvoltage (overpotential) of hydrogen on this mercury electrode makes possible the electroreduction of many species at negative potentials unattainable on solid microelectrodes. The DME can be used up to -1.6 V (vs. SCE) in acidic solution and up to -2.6 V in basic solutions or in nonaqueous solutions.

(d) The mercury drop forms amalgams (solid solutions) with many metals, which results in a lower reduction potential for the substance.

(e) Several polarograms can be run on the same solution without appreciable change since a very small amount of the analyte is changed by the minute current flow through this electrode.

(f) The diffusion current reaches a steady value rapidly and is reproducible.

The DME has certain disadvantages such as:

(a) Mercury has a very limited potential range as an anode, since mercury is oxidized at an applied potential of about $+0.4$ V (vs. SCE).

(b) The surface area of this electrode is always changing giving rise to current oscillations.

(c) The applied potential affects the interfacial tension at the mercury drop-solution interface thus affects the mercury drop size. The latter is also influenced by chemical agents which affect this interfacial tension.

2. *Solid Microelectrodes.* The rotating or vibrating platinum microelectrodes are among the most useful in voltammetry. Platinum has a much lower hydrogen overpotential than mercury, and thus has a very limited potential range as a cathode. However, platinum can be used at positive applied potentials up to about $+1.1$ V (vs. SCE).

When not rotated or vibrated, these solid electrodes have a number of disadvantages such as:

(a) Require several minutes to reach a steady current value, then it declines slowly.

(b) After a voltammogram is obtained, the curve is not retraced when the E_{appl} is gradually reversed to zero. The $i - E_{appl}$ curve shape also varies with the rate of the applied potential increase.

(c) Temperature changes produce greater changes in the diffusion current obtained with this electrode than for the DME.

By rotating or vibrating the platinum-wire electrode the current is greatly enhanced over that obtained with a DME and assumes a steady value immediately. Since the rate of stirring affects the current flow, constant rate rotating (or vibrating) platinum electrodes are mandatory. These electrodes are widely used for amperometric titrations, but their lack of reproducibility precludes single measurements of current.

b. Reference Electrodes. Two reference electrodes which are widely employed in polarography will be discussed here.

1. *Mercury Pool Electrode* (Hg-*pool*). The mercury pool electrode consists of a pool of mercury in the bottom of the electrolysis cell (Fig. 16.8a). Its relatively large surface area prevents its polarization by the small current flow through it due to the potential impressed across the cell. While the exact potential of this electrode is unknown, it remains quite constant. The mercury pool electrode may be employed for routine work in which the exact value of the potential applied to the DME is not important.

2. *Saturated Calomel Electrode* (*SCE*). For accurate work or research the potential of the DME must be known, therefore an isolated reference electrode is connected to the electrolysis cell by means of a relatively large-surface "salt bridge." The reference electrode which has been almost universally adopted is the SCE (Fig. 16.8b).

3. *Auxiliary Electrodes.* In addition to the polarized microelectrode (e.g., DME) and reference electrode (e.g., SCE), a third electrode which is frequently a platinum wire (or mercury pool) electrode may be used. This additional electrode is called an "auxiliary electrode." It is not polarized and reduces the resistance to the flow of current in poorly conducting supporting media. Since the potential on the DME must be proportional to the applied potential in many instances, either one or both of the SCE and auxiliary electrodes are required depending on the conditions used. When an applied potential is impressed across an electrolysis cell a potential drop occurs at three sites, the cathode (usually DME), the anode (usually SCE), and in the solution between these two electrodes, thus

$$\text{emf}_{\text{cell}} = (E_{\text{SCE}} - E_{\text{DME}}) + iR \qquad (16.27)$$

where iR is the potential drop (ohmic drop) between the electrodes. It can be seen that when the exact value of the E_{DME} must be known, the other values in Eq. (16.27) must be known or measurable. Three different situations are possible:

(a) Only a large surface (nonpolarizable) SCE is required when the electrolysis current is small (under 1 μA) and the supporting electrolyte has a low resistance and the ohmic drop iR between the electrodes is negligibly small (under 1 mV) or known.

(b) Only a nonpolarizable auxiliary electrode (e.g., mercury pool) of reasonably constant but unknown potential is required when the iR drop is small (under 10 mV), is known, or where the DME potential does not have to be accurately known.

(c) When an accurate E_{DME} is required, both the SCE and auxiliary electrodes are required when the SE has a high specific resistance, (e.g.,

nonaqueous media). In the latter situation it is also possible to measure the voltage between a closely spaced DME and SCE salt bridge, with a sensitive voltameter, such as a pH meter.[8]

16.4   GENERAL POLAROGRAPHIC ANALYSIS

Substances which can be reduced or oxidized at the DME to produce an $i\text{-}E_{appl}$ wave can usually be polarographically analyzed. If more than one electroactive substance (including impurities) is present, the successive $E_{1/2}$ values of each substance must differ by 150 mV or more to obtain resolution of the waves. Many inorganic and organic substances yield well defined cathodic waves when the DME is employed. Quantitative analysis of such substances is normally performed within a concentration range of 10^{-2}–10^{-5} M. The lower concentration limit is usually governed by the magnitude of the residual current. Solubility factors at the DME surface may produce false or unpredictable currents at concentrations greater than 10^{-2} M.

When a new supporting electrolyte is employed, it should be checked polarographically at a high sensitivity to preclude the possibility of waves arising from impurities.

A.   TECHNIQUES FOR MEASURING DIFFUSION CURRENTS AND HALF-WAVE POTENTIALS

Many polarographic waves are not as ideally shaped as that depicted in Fig. 16.2, therefore several arbitrary procedures have been devised for measuring diffusion currents (i_d) and half-wave potentials ($E_{1/2}$) from a polarogram. These procedures differ in the manner of estimating the residual current.

1.   Accurate Measurement of Residual Current

The residual current i_r is most accurately determined separately from the polarogram of a blank solution which is identical to the test solution except for the deletion of the depolarizer. This value is then subtracted from the limiting current plateau value i_l for the test solution measured at the same potential to obtain i_d.

2.   Approximate Measurement of Residual Current

A close approximation of the i_r can be made and the i_d and $E_{1/2}$ values determined with sufficient reproducibility by employing one of the graphical procedures which follow.

a. For ill-Defined Waves. In the measurement of the diffusion current of depolarizer substance 1 (Fig. 16.9), i_r is approximated by extrapolating the residual current to give line a. Since the limiting current is impossible to measure accurately for species 1, a line b is drawn parallel to line a and the $(i_d)_1$ measured as indicated. Since the residual current for species 2 includes the $(i_d)_1$ and is not parallel to the limiting current $(i_L)_{1+2}$, line d is drawn parallel to the horizontal axis from the point of intersection of line b and the
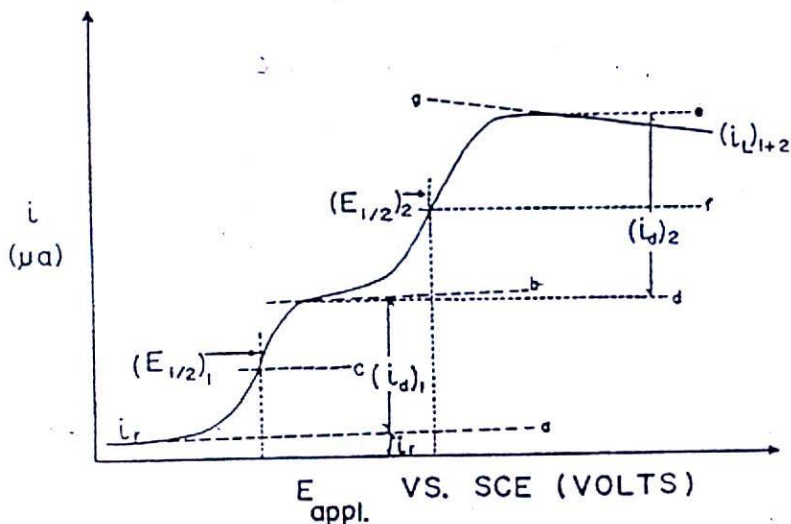

FIGURE 16.9: Double wave polarogram. See the text for the explanation of the measurement of the diffusion currents i_d and half-wave potential $E_{1/2}$.

curve. Extrapolation of the upper limiting current gives line g. Line e is drawn parallel to line d from the point of intersection of line g and the curve. The diffusion current $(i_d)_2$ for species 2 is measured as indicated.

The $E_{1/2}$ values of species 1 and 2 are determined from the point of intersection of the curve with lines c and f, respectively. These lines are drawn from points equal to $i_d/2$ for each species and parallel to lines a and d, respectively.

b. For Well-Defined Waves. The i_d and $E_{1/2}$ values for well-defined waves, where the i_r and i_L portions of the curve are parallel, are easily measured. The procedure is much simpler as shown in Fig. 16.2, since the i_r value only is approximated by an extrapolation of this portion of the curve.

Reference books on polarography should be consulted for additional procedures.

B. QUALITATIVE POLAROGRAPHIC ANALYSIS

Although the half-wave potential ($E_{1/2}$) is a characteristic of an electro-active species under specified and reproducible conditions, especially with respect to the electrolysis medium, polarography finds only limited application to qualitative analysis. This statement is particularly true in the presence of mixtures of electroactive species where successive waves are not well resolved. To effect resolution the supporting medium usually has to be altered. The new $E_{1/2}$ value for a species is applicable only to that altered medium. The $E_{1/2}$ values of many electroactive species in a large variety of solvents or supporting electrolytes are to be found in reference books on polarography.[2,5,10]

It is frequently possible to identify relatively pure electroactive species by comparing the $E_{1/2}$ values of the unknown with suspected known species in a variety of supporting electrolytes (or solvent media), since the latter frequently affects the electron transfer rates involved in the electrode reactions.

C. QUANTITATIVE POLAROGRAPHIC METHODS

These methods are divided into two categories: (1) absolute method and (2) comparative methods.

The following methods presuppose that appropriate consideration has been given to the selection of the concentration range (approx. 10^{-2} to 10^{-5} M) to be studied, the appropriate solvent or supporting media, and any preanalysis depolarizer separation which may be necessary to give the desired selectivity to the quantitative method.

1. Absolute Method

Since the absolute method is based on the direct application of the Ilkovic equation, all the terms in that equation must be known or measured before the concentration can be related to the observed diffusion current. This method is not used to any extent in practical analysis for obvious reasons. These terms would have to be measured or calculated to verify this equation experimentally. The reader is referred to one of the books on polarography for further details on this method.

2. A Modified Absolute Method

This method employs a modified form of the Ilkovic equation [Eq. (16.8)]:

$$C = i_d/I_d m^{2/3} t^{1/6} \qquad (16.28)$$

The diffusion current constant I_d is independent of any specific capillary, but is dependent upon the nature and concentration of the supporting electrolyte and the temperature which must be controlled. A calibrated

galvanometer is essential for the measurement of absolute current. This method therefore suffers from tedious calibrations and is not as accurate as the relative methods which follow.

3. Comparative Methods

These relative methods can be subdivided according to the particular techniques employed. In all instances, however, a pure standard of the same species as the sample depolarizer is used for calibration purposes or relative measurements. Identical conditions are employed for the sample and standard to calculate the unknown concentration of the sample.

a. Direct Comparison Method. A series of standard solutions are prepared which are identical to sample solutions in all aspects except that of concentration. The actual analysis can be performed in one of two ways:

1. *Calibration Curve.* This method is convenient for routine analysis on large numbers of samples. The standard solutions can be polarographed one at a time or if regular waves are obtained the limiting current only may be determined for each concentration at a fixed applied potential corresponding to the limiting current plateau in the polarogram of one of the standard solutions. The residual current should be determined at the same potential from a polarogram of the blank or approximated from the polarogram of a standard solution by the extrapolation procedure. The measured diffusion currents i_d of the standard solutions are used to construct a calibration curve, i.e., a plot of i_d vs. concentration. The diffusion current value of each sample solution, measured under essentially identical conditions, is referred to the calibration curve and the concentration read. Should the concentration range be large enough to produce nonlinearity in the calibration curve, semilog graph paper may be used to retain the same precision in the concentration reading over the entire plot. For more limited concentration ranges the i_d vs. concentration plot should be linear, passing through the origin. Calibration curves should not be assumed to be valid from day to day, but must be verified for each series of analysis.

2. *Alternate Direct Comparison Method.* The wave heights of a consecutively polarographed standard and sample solutions (identical conditions) which are about equal in concentration may be compared. The simplified form of the Ilkovic equation [Eq. (16.9)], i.e., $i_d = KC$, may be used here. This equation applies equally to both sample and standard and the constant K is identical in both instances under identical conditions except for concentration. Therefore the following expression can be employed for the calculation of the sample concentration:

$$C_{\text{sample}} = \frac{(i_d)_{\text{sample}}}{(i_d)_{\text{std}}} (C_{\text{std}}) \qquad (16.29)$$

This procedure is convenient for occasional analysis on very few samples as it eliminates the necessity of constructing a calibration curve. Greatest accuracy is obtained when the concentrations of standard and sample closely approximate one another, especially for a slightly nonlinear relationship between wave height and concentration. No special knowledge of the capillary characteristics is necessary in this method, but they as well as the temperature must remain constant throughout the comparison.

b. Standard Addition Method. The polarogram of an accurately known volume of solution A containing the sample is recorded. A known weight of the standard substance (a pure form of sample substance) is added to an identical sample solution B and the polarogram recorded under the same conditions.

The weight of the substance being determined (W_{sample}) in the sample solution A may be calculated from this equation

$$W_{sample} = \frac{i_{sample} W_{std}}{i_{(sample+std)} - i_{sample}} \tag{16.30}$$

where W_{std} is the weight of standard added to the second solution B of the sample, $i_{(sample+std)}$ is wave height of solution B, and i_{sample} is the wave height of solution A.

This version of the standard addition method is less complex than an alternate version in which accurate volumes of a standard solution are added to known volumes of the sample. More complex calculations are required because of the dilution of the sample by the standard solution and vice versa. The equation for this alternate method of standard addition is

$$C_x = \frac{C_s + i_x v_s}{V_x(i_s - i_x) + i_s v_s} \tag{16.31}$$

where C_x, i_x, and V_x are the values for concentration, wave height, and volume of the sample, respectively, and C_s, i_s, and v_s are the corresponding values for the standard solution.

In both versions of the standard addition method it is assumed that all other factors which might affect the instrument reading remain constant during the consecutive measurements and also that there is a linear relationship between wave height and concentration. Maximum accuracy is obtained when the wave height caused by the standard addition approximately doubles that of the sample alone. If a semiautomatic analytical balance is available, the first-mentioned version of this would be just about as convenient as the latter and does not involve as much calculation. This method requires an expenditure of slightly more time than the "calibration curve" method discussed earlier, but is generally considered to be more accurate. One reason for this is that it is not possible to compensate exactly for the possible

effect produced by extraneous substances in the sample solution on the wave height when using the direct comparison method.

c. The Pilot Ion (Internal Standard) Method. The basis of this method depends upon the knowledge that two electroactive species of equal concentration give rise to relative wave heights, in a given supporting electrolyte, which are constant and independent of capillary characteristics m and t. Also for any electroactive substance the diffusion current constant I_d, which is the ratio of $i_d/Cm^{2/3}t^{1/6}$ [Eq. (16.28)], remains constant for a given capillary employed under a constant column height of mercury and in a specified supporting electrolyte system.

Once this ratio has been established for each of a series of ions using one capillary under specified conditions, all that is required to establish the diffusion current constants for the same substances in a second capillary is the calculation of this $i_d/Cm^{2/3}t^{1/6}$ ratio for one of these substances (i.e., a pilot ion) in the second capillary using the same specified experimental conditions. Since the i_d values of any of the ions (x) change in the same ratio as those for the pilot ion on changing capillaries, these values can be calculated as follows:

$$\frac{(I_d)_{\text{pilot.1}}}{(I_d)_{\text{pilot.2}}} = \frac{(I_d)_{x.1}}{(I_d)_{x.2}}$$

on rearrangement this gives

$$(I_d)_{x.2} = [(I_d)_{\text{pilot.2}}/(I_d)_{\text{pilot.1}}](I_d)_{x.1} \tag{16.32}$$

where I_d is the diffusion current constant $i_d/Cm^{2/3}t^{1/6}$ for the pilot ion and ion x in capillaries 1 and 2 as indicated. Equation (16.32) enables a comparison of data obtained with one capillary with that obtained with another, providing the I_d value of a pilot ion has been calculated using both capillaries. This application of the pilot ion obviates the need to redetermine the I_d, for all the ions of interest on a second capillary if the first becomes plugged or broken. It is only necessary to keep a standard stock solution of the pilot ion in each supporting electrolyte which is likely to be used.

1. *Quantitative Analysis Employing the Pilot Ion Method*

(a) Using an equation. Since the relative diffusion current constants I_d of ions in specific supporting electrolytes are independent of the capillary used, Eq. (16.28) applies to both the pilot ion p and sample depolarizer ion x to be measured. Thus, the following expressions can be written:

$$C_x = (i_d)_x/(I_d)_x m^{2/3} t^{1/6} \tag{16.33}$$

$$C_p = (i_d)_p/(I_d)_p m^{2/3} t^{1/6} \tag{16.34}$$

Under identical conditions, including a common capillary, Eq. (16.33) can be divided by Eq. (16.34), then rearranged to give

$$C_x = [(i_d)_x/(i_d)_p][(I_d)_p/(I_d)_x]C_p \tag{16.35}$$

where C_x and C_p are the concentrations of the sample ion x and pilot ion p, respectively; i_d and I_d are the diffusion currents and diffusion current constants for the sample ion and pilot ion as indicated by the subscripts.

The ratio $(I_d)_p/(I_d)_x$ is called the "internal standard ratio" (or "pilot ion ratio") and is independent of capillary characteristics. This ratio can be determined in advance using the same capillary or it may be calculated from literature values of I_d for these substances if reported for the same concentration of the supporting electrolyte system in which the $(i_d)_x$ and $(i_d)_p$ are to be determined. The latter two values.are determined in the same solution and are measured from the resulting two-step polarogram.
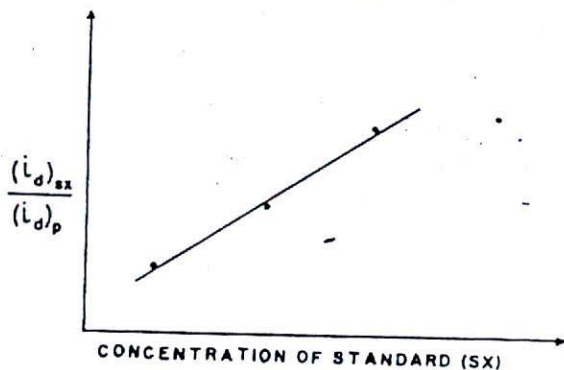

FIGURE 16.10: Calibration curve used in the pilot ion method. The subscripts sx and p represent the standard solutions of the test substance and pilot ion, respectively.

(b) Alternate Pilot Ion Method Using a Calibration Curve. A series of standard solutions sx are prepared for the depolarizer x, each containing a constant known volume of pilot ion solution. Sample solutions of unknown concentration depolarizer x are prepared to contain the same amount of the pilot ion p. The selected pilot ion must have a $E_{1/2}$ value which is considerably different from that of the depolarizer x or any other substance which may be present in solution. A polarogram is recorded and the $(i_d)_x$ and $(i_d)_p$ are determined at potentials corresponding to the limiting currents of their respective waves. This may be repeated for each standard sx and sample x solution if a recording polarograph is used. If a manual instrument is used the diffusion current values are measured only at appropriate fixed applied potentials selected from the polarogram of the mixture.

A calibration curve is then constructed by plotting the ratio $(i_d)_{sx}/(i_d)_p$ vs. C_s as shown in Fig. 16.10. The sample solutions containing unknown concentrations of the substance x in the standard solutions and identical quantities of pilot ion are then measured in the same way. The ratio of $(i_d)_x/(i_d)_p$ is referred to the calibration curve and the concentration of the sample is read in the usual manner.

This procedure compensates for small variations in temperature and capillary characteristics over extended periods of time. The method is somewhat limited by the number of possible pilot ion substances available since they must produce well-defined waves which are well resolved from the others produced in the mixed solution. Cadmium ion has frequently been used for this purpose.

The diffusion current constant (I_d) for any electroactive species can be calculated from a rearranged Eq. (16.28). To calculate m and t, we must determine the average drop time t in seconds and the mass of mercury m flowing in milligrams per second by timing the collection of 10 drops of mercury under the actual operating conditions of E_{appl}, in a supporting electrolyte system identical in nature and concentration to that for which the value of I_d is required. The 10 mercury drops can be collected in a small glass receptacle, washed with water, then acetone, dried, and weighed. This data enables the calculation of the value of m. The reader may refer to Ref. 7 for details.

D. NONAQUEOUS SOLVENTS IN POLAROGRAPHY

Many water-insoluble substances can be polarographed in nonaqueous solvents, solvent mixtures, or in melts. The current state of polarography in nonaqueous systems is rather empirical and descriptive in nature. Frequently depolarizers which yield ill-defined polarograms in aqueous media yield well-defined waves in nonaqueous media. The i_d, $E_{1/2}$, and wave shape for a depolarizer substance usually differ in the two types of media. Since traces of water often influence the nature of the depolarizer, care is necessary to exclude water when employing anhydrous solvents. Reference electrodes in nonaqueous media behave differently than in aqueous media depending upon the particular reference electrode and supporting electrolyte present in the nonaqueous medium. Frequently a mercury pool may be employed as a constant "reference" electrode, or an aqueous reference electrode (e.g., SCE), which often contains a high concentration of an indifferent electrolyte, may be employed. Since the choice of a suitable nonaqueous solvent and reference electrode depends on numerous considerations, the reader is referred to recent references on polarography.[2,3]

Solvents which have been employed include methanol, ethanol, alcohol-water mixtures, alcohol-benzene mixtures, acetic acid or anhydrous acetic acid containing ammonium acetate, sulfuric acid, liquid ammonia, acetonitrile, ethylenediamine, and many other organic substances. Furthermore, polarography has been performed in melts consisting of mixtures of inorganic salts at elevated temperatures. For details and references see the recent book by Heyrovský and Kůta[2] as well as review papers listed under References.

E.   ORGANIC POLAROGRAPHY

A large number of organic-compounds can be studied and assayed by polarographic methods. Usually the electrode reactions involve hydrogen ions, and for this reason a constant concentration of these ions is provided by means of the supporting electrolyte medium, which may be strongly buffered, acidic or basic, aqueous or nonaqueous. The general electrode reaction may be represented by

$$Org + mH^- + ne^- \rightarrow Org \cdot Hm$$
(16.36)
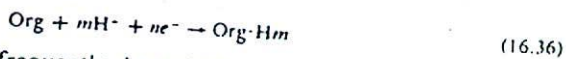
where the value of n is frequently dependent upon the pH of the medium. One or more steps may be involved in the complete reduction of a functional group and in most instances the reaction is irreversible.

While the list of functional groups in Table 16.1 is not an exhaustive list, it does serve to indicate the variety of compounds which have been studied polarographically: acids with carbonyl or conjugated double bonds; aliphatic, aromatic, and substituted aldehydes; aliphatic, aromatic substituted and unsaturated ketones, including quinones, di- and tri-ketones; certain esters; many nitrogen-containing groups such as: nitro, nitroso, azo, azoxy, hydroxylamines, amine oxides, diazonium salts, certain heterocyclic compounds such as alkaloids and others; many sulfur-containing groups such as thiols, sulfur groups conjugated with unsaturated groups; most peroxides, including hydrogen peroxide; many halogen containing compounds; and others too numerous to mention.

16.5   LABORATORY EXPERIMENTS

A.   APPARATUS

Manual and/or recording polarographs.

Polarographic electrolysis cell—such as an H-type cell with an attached SCE (if the DME potential is desired), or a beaker-type cell (if a mercury pool electrode is to be used).

Large tray to prevent the loss of poisonous and volatile mercury, (clean up spills immediately).

Dropping mercury electrode (DME) assembly containing doubly-distilled and filtered mercury.

Reference electrode—a mercury pool, and/or a nonpolarizable SCE, as required. See Fig. 16.8.

Stop watch—for determining the drop life t.

Deoxygenation apparatus—consisting of oxygen-free nitrogen gas (tank and regulator). A gas washing or scrubbing device containing a suitable solution (such as alkaline pyrogallol or chromous chloride) through which the nitrogen can be passed if it contains any oxygen.

TABLE 16.1: Organic Polarography

Substance	Concentration	Medium	$E_{1/2}$ or E_{scan} volts vs. SCE	References
Chlortetracycline HCl, and/or oxytetracycline HCl	0.1 mg/ml	0.2 M phosphate buffer, pH 8.2 ± 0.1	E: −0.8 → (−)	·
Tetracycline HCl	0.05–0.15 mg/ml	Sorensen's phosphate buffer, pH 6.2	$E_{1/2}$: −1.50 vs. Hg-pool	·
Chloramphenicol, and dosage forms	1 mg/ml	5 ml isopropanol + 25 ml 0.2 M potassium biphthalate solution + 0.2 ml 0.2 N NaOH + 0.2 ml 0.1% methylene blue, and water to make 100 ml of solution. Mix well.	E: −0.1 → −0.9 i_d at −0.78	·
Chloramphenicol	2 mg/ml	20 mg in 10 ml phosphate buffer containing 10% EtOH	E: 0 → (−1.5)	·
Streptomycin sulfate	100–400 μg/ml	20-ml aliquot of sample + 2 ml 1 M NaOH	$E_{1/2}$: −1.29 (vs. Hg-pool)	·
Cortisone and/or Prednisone		50% MeOH containing 0.1 M acetate buffer	E: −0.5 → −1.6 i_t at −0.9 i_t cortisone (−1.47) i_t prednisone (−1.31)	·
Ascorbic acid, and in dosage forms	25–250 μg/ml	7.5 ml 2 N HOAc + 37.5 ml 2 N NaOAc + 0.5 ml formaldehyde + water to make 50 ml. Saturated with sodium oxalate, filter, pH ca. 5.5	$E_{1/2}$: +0.07 E: −0.3 → (+)	·
Folic acid and tablets	250 μg/ml	Aqueous solution containing 15% NH₄Cl + 10% Me₄NOH	E: −0.9 → (−)	·
Menadione (vitamin K_3) and dosage forms	5–15 μg/ml	Light petroleum-2 M (NH₄Cl/NH₄OH) buffer, (2:3), pH 9–9.5	$E_{1/2}$: −0.35	·
Menadione	10–100 μg/ml	Benzene-MeOH-conc. NH₄OH (10:10:1) containing 0.005 M methylene blue	$E_{1/2}$: −0.4 (vs. Hg-pool) E: 0 → −1.0	·
Menadione-sodium bisulfite	10–30 μg/ml	0.1 M KCl containing 5 g sodium sulfite and 50 mg agar per liter	$E_{1/2}$: −1.1	·

Nicotinamide, also in mixtures after chromatographic separation	3-10 μg/ml	Aqueous solution of 1% Me₄NOH	$E_{1/2}$: -1.7
Pyridoxal HCl or pyridoxal-5-phosphoric acid ester (and in tablets)	100 μg/ml	5 ml 0.3% gelatin + 1% LiCl to make 100 ml	E: -0.9 → (-)
Riboflavin and dosage forms	20-50 μg/ml	0.1 M Sorensen phosphate buffer (pH 7.5)	$E_{1/2}$: -0.47
Riboflavin and dosage forms	20-30 μg/ml	Britton-Robinson buffer (pH 2.8) containing 0.08 M of each component, plus 0.4 M KCl	E: 0 → (-1.0) (vs. Hg-pool)
Thiamine HCl	50-200 μg/ml	10% Na₂CO₃-0.1 M KCl (1:9), pH ~ 11.5	$E_{1/2}$: -0.42
Chlorpromazine and dosage forms	1-8 μg/ml	0.5 N HCl	E: -0.7 → (+)
Chorpromazine HCl			E: -0.5 → (-)
Phenothiazine HCl salts	35-480 mg/liter	1 N H₂SO₄ (with R.P.E.)	$E_{1/2}$: +0.6
Diiodohydroxyquin	1.09-1.76 mM	9 N H₂SO₄ (with R.P.E.)	$E_{1/2}$: +0.37 and +0.95
Iodochlorhydroxyquin	0.08-2.5 mM	In water	E: 0 → (-)
		2-ethoxyethanol containing 0.05 M Et₄NBr	E: 0 → -2
		0.024 M KOAc + 0.013 M HOAc in 90% EtOH containing 0.06 M LiCl (pH 7.0)	E: 0 → -2
Isonicolinic acid hydrazide (and dosage forms)	0.0005 M	10-ml sample (50 mg/250 ml) + 10 ml universal buffer	E: 0 → 1.0
Thimerosal	0.01-0.1 mg/ml	0.1 to 1.0 mg in conc. HCl + 1 ml 0.1% gelatine + water to make 10 ml	E: 0 → -1.0 (first wave)
Thimerosal	20-50 ppm	10 ml of aqueous solution containing 20-50 ppm + 2 ml 0.1 M KMnO₄ + 1 ml of 0.1% gelatin	$E_{1/2}$: -0.75 (first wave)
Phenolphthalein (and in emulsions)	0.15 mg/ml	30 mg in 95% EtOH to make 50 ml. Take 25 ml of this solution and 55 ml of 25% HCl solution and 1 ml aqueous 0.1% basic fuchsine + water to make 100 ml	E: 0 → (-)

TABLE 16.1 (continued)

Substance	Concentration	Medium	$E_{1/2}$ or E_{max} volts vs. SCE	References
Barbiturates (5,5-dialkylbarbituric acids)	0.0001 0.00001 M	In 0.05 M borax solution	Using DME anodic wave of mercury salt formation	
Thiobarbiturates (e.g., pentothal)	$7 \times 10^{-5} M$ (or less)	In 0.1 N NaOH	Anodic wave of Hg salt formation	
Strychnine		0.01-0.5 mg + 1 ml HNO$_3$ + 2 ml water in closed test tube in boiling water bath (20 min) Cool, + 8 ml 2.5 M KOH; gives 2,4-dinitro-strychnine as product	2 waves at −0.64 and −0.79	

[a] P. Kabasakalian and J. H. McGlotten, in Handbook of Analytical Chemistry, 1st ed.; L. Meites (ed.), McGraw-Hill, New York 1963, Sec. 5.

[b] J. Dean and B. E. Riedel, Canad. Pharm. J., Sci. Sect., 96, 109 (1963).

[c] A. L. Summa, J. Pharm. Sci., 54, 442 (1965).

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[e] P. Kabasakalian, S. DeLeonce, and J. McGlotten, Anal. Chem., 28, 1669 (1956).

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[i] G. S. Porter, J. Pharm. Pharmacol. (Suppl.) 16, 24T (1964).

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[k] N. Chuen and B. E. Riedel, Canad. Pharm. J., Sci. Sect., 94, 51 (1961).

[l] W. Brown and L. Krupski, J. Pharm. Sci., 50, 49 (1961).

[m] J. D. Neuss, W. J. Seagers, and W. J. Mader, J. Am. Pharm. Assoc., Sci. Ed., 41, 672 (1952).

[n] J. L. Page and J. G. Waller, Analyst, 74, 292 (1949).

[o] J. Buser and J. R. Garnet, J. Pharm. Sci., 53, 1266 (1964).

A constant temperature bath and control (25°C ± 0.1°).

Maxima suppressor solution—such as 0.2% solution of Triton X-100 (Rohm & Haass Co.), or freshly prepared 0.2% gelatin solution (dissolve in warm water).

Volumetric glassware such as volumetric flasks, pipettes, etc.

B. CARE OF DROPPING MERCURY ELECTRODE

Every precaution must be taken to prevent the capillary bore from becoming plugged. This obstruction could be caused by a dust particle in the mercury, oxidation products of mercury, salt crystals, etc. Since these capillaries are extremely difficult or impossible to clean, the following precautions must be followed:

1. Protect the mercury in the reservoir from dust with a filter of glass wool. If the DME apparatus is being assembled for the first time, clean and dry all plastic or rubber tubing well to eliminate dust particles.

2. Make certain that the mercury is dropping from the capillary before the capillary is immersed in any solution. This precaution excludes contaminants from the capillary bore, where they could cause an erratic mercury flow.

3. Never stop the mercury flow while the capillary is in solution. When through, lower the electrolysis cell to remove the DME from the solution. While the mercury is still dropping, rinse the tip with distilled water, blot dry with filter paper, and place a dry test tube under the tip. Stop the mercury flow by lowering the reservoir, by a stopcock or clamp, etc, whichever is appropriate for the DME assembly used. This procedure is recommended over the immersion of the capillary tip in distilled water.

4. Occasional cleaning of the end of the capillary bore is required. This is done by immersing the tip in 50% nitric acid while the mercury is flowing, then rinsing and drying as directed under 3.

5. The DME assembly must be vibration free and the capillary vertical to avoid an erratic drop time t. A level or plumb line may be used to check the latter adjustment.

C. GENERAL POLAROGRAPHIC PROCEDURE

This general procedure should be followed in all polarographic measurements, unless otherwise directed in specific exercises:

1. If a mercury pool electrode is employed, add sufficient mercury to cover the bottom of the electrolysis cell to a depth of 1/8 inch.

2. If a SCE (or other reference electrode) is used, maintain the level of the saturated KCl (or other electrolyte) solution above the solution level in the cell.

3. Add sufficient test solution (consisting of supporting electrolyte, maximum suppressor and depolarizer) to a level above the large "salt bridge," but not too close to the top of the cell.

4. *Caution:* Read Section 16.5B on the care of the DME. Start the mercury flow, then raise the electrolysis cell to immerse the DME well below the solution surface. Adjust the mercury column to produce a drop time of 3 or 4 sec. Ideally this is done at the applied potential at which the diffusion current is to be measured.

5. Deoxygenate the test solution with oxygen-free nitrogen gas for 15 min (about 5 min if a sintered-glass gas-dispersion tube is used). Never measure the polarographic current while N_2 is bubbling through the solution. Why?

6. Consult the operating instructions for the instrument to be used. Standardize (or calibrate) the potentiometer and adjust the galvanometer index (or recorder pen) to zero.

7. Connect the electrodes to the appropriate terminals. The polarity of the DME is (−) negative for cathodic reductions. Adjust the instrument to the lowest sensitivity initially, unless the correct setting is known. The appropriate sensitivity is that which produces a large deflection but prevents the galvanometer index from going off scale. This setting may be determined by gradually increasing the applied potential over the range of interest while adjusting the sensitivity switch (shunt) to keep the index on scale at the point of greatest deflection.

D. MANUAL OPERATION

With the preceding general procedure in mind, select the applied potential span to be used. Set the applied potential to zero and note the galvanometer reading. A slight negative reading can be ignored, it may only be the end of an anodic wave of some substance in solution. The applied potential is increased in steps of 0.05–0.1 V except during a wave where 0.01- or 0.02-V steps are used. Record the applied potential and resulting current or galvanometer readings. It is often easier to record the maximum throw of the index although the average is more desirable. This manual potential scan is usually performed over the 0- to −2-V range, or until some component of the supporting electrolyte is discharged about −1.7 to −1.9 V (vs. SCE). Use the highest sensitivity that will permit the galvanometer index to remain on scale at all times.

Plot the polarogram graphically according to convention (Fig. 16.6). Measure the diffusion current as directed earlier (Fig. 16.2).

Use of Compensator (or Bias) Control

The reader should familiarize himself with this control (if available), which may be required to "cancel" a large diffusion current of a substance

which is reduced just prior to the depolarizer of interest. This enables the desired wave to be developed at a higher sensitivity. Turn this control off when finished so that it does not interfere with a subsequent analysis.

E. OPERATION OF RECORDING INSTRUMENT

Read the preceding general polarographic procedure, then consult the operating instructions for the instrument to be used. Adjust the recorder to zero, select the appropriate potential range to be scanned, the scan rate, and sensitivity, if known. Otherwise, scan rapidly, adjusting the sensitivity as required to obtain nearly a full-scale deflection (fsd) at the potential of maximum current flow. The "scan" switch usually activates a synchronous motor, which increases the applied potential in a linear manner and simultaneously starts the chart drive motor. The chart drive axis corresponds to the applied potential while current is measured on the other axis. If the scan is not automatically stopped, switch off the drive motors at about -2.0 V, or at the end of the selected voltage range.

Experiment 16.1

Familiarization with the instrument, plotting methods, maximum suppressor, role of supporting electrolyte, and the measure of various currents and potentials.

Apparatus. Carefully read over this experiment (or the assigned portions) and compile a list of the apparatus required. All apparatus must be clean. To save time obtain all the apparatus before starting.

Graph paper ($8\frac{1}{2}$ in. × 11 in.) with millimeter (or 20 lines/in.) ruling will be required.

Note: It would be desirable to have a recording polarographic instrument if all parts of this experiment are to be attempted.

Solutions (provided):

Maximum suppressor solutions: (1) 50 ml of a fresh 0.2% gelatin solution (dissolved in warm water, cooled, and diluted to volume), and/or (2) 0.2% Triton X-100 Solution.

Stock Solutions of :

$PbCl_2$ [or $Pb(NO_3)_2$] 0.02 M in distilled water

$CdCl_2$ (or $CdSO_4$)   0.02 M in distilled water

$ZnCl_2$ (or $ZnSO_4$)   0.02 M in distilled water

Supporting electrolyte (1.0 M KCl).

Mixed supporting electrolyte (1 M Sodium Potassium tartrate + 0.2 M NaOH).

Using appropriate volumetric glassware and the stock solutions provided, prepare and label the following solutions:

Maximum suppressor—0.2% gelatin solution or 0.2% Triton X-100 (if not provided).

Solution 1.   100 ml of 0.1 M KCl (do *not* add any maximum suppressor to this solution).

Solution 2.   100 ml of 0.001 M Cd(II) in aqueous solution containing 2.0 ml 0.2% gelatin.

Solution 3.   Prepare this solution so that 100 ml will contain 2.0 ml of the 0.2% gelatin, and be 0.001 M in Cd(II) and 0.1 M in KCl.

Solution 4.   100 ml is to contain 2.0 ml of 0.2% gelatin and be 0.1 M in KCl [no Cd(II) present].

Part A. To Demonstrate the Need for Deoxygenation and a Maximum Suppressor

Procedure. Transfer 20 ml (or sufficient to cover the electrodes) of solution 1 to the electrolysis compartment of the H-cell. Obtain a polarogram over the range 0 to −1.9 V (vs. SCE), by the *general polarographic procedure*, omitting the deoxygenation. Plot this curve as the data is obtained and in accord with the plotting conventions described earlier. Note the location of any waves present and their respective half-wave potentials $E_{1/2}$. Two maxima may appear in the polarogram. If this does not occur, it may be necessary to bubble air into solution 1 to enhance the oxygen waves. Repeat the polarogram in the regions of the observed maxima after adding 4 drops of the selected maximum suppressor. Plot this polarogram on the same sheet. If the maxima persist, repeat again after the addition of 4 more drops of the maximum suppressor. Repeat this process until the maxima are just eliminated. Record the number of drops or volume (pipette) of the maximum suppressor required as well as the initial volume of solution added to the vessel.

Now deoxygenate the solution for 5 min and obtain another polarogram over the same voltage range. If the waves are still evident, deoxygenate for a further 5 min and check for the oxygen wave(s). Repeat until the last oxygen wave disappears, then obtain a complete polarogram on the graph along with those obtained previously.

Part B. To Study the Role of the Supporting Electrolyte in Minimizing the Migration Current of the Depolarizer

Procedure. Transfer 20 ml (or appropriate known volume) of solution 2 to the electrolysis vessel, deoxygenate, and obtain a polarogram using the *general polarographic procedure*. Record the measured limiting current (i_L) value for this solution. Repeat using solution 3, and again with solution 4.

Plot (or record) these polarograms (or partial polarograms) on the same paper used for solution 2. Determine the diffusion current (i_d) for the Cd(II) ion concentration present (refer to Fig. 16.2). Calculate the approximate migration current (i_m) of the Cd(II) ions from the results obtained, using the expression $i_L = i_d + i_m$. Measure and compare the magnitude of the currents i_r, i_L, i_d, and i_m (refer to Fig. 16.2) in these plots and explain the causes of each. Why is it necessary to remove the oxygen when calculating the i_d for the Cd(II) ions in solution ?

Note: Comparable concentrations of Pb(II) or Zn(II) can be used instead of Cd(II) in this experiment.

Part C. Effect of the (1) Concentration of the Depolarizer and (2) Nature of the Supporting Electrolyte on the Half-Wave Potential

1. *Effect of Depolarizer Concentration.* Prepare the following solutions in 100 ml volumetric flasks:

Solution a.   20 ml of 0.02 M Pb(II), plus 2 ml of gelatin (0.2%), plus 20 ml of 1 M KCl, plus water to volume.

Solutions b–f. By substituting the following corresponding volumes of 0.02 M Pb(II) for that in solution a: (b) 15 ml, (c) 10 ml, (d) 5 ml, (e) unknown volume (supplied by instructor), (f) 0 ml (blank). Using the *general polarographic procedure*, obtain complete polarograms for solutions a and c only over an applied potential range of 0 to -1.0 V (vs. SCE). Select a potential on the limiting current plateau of the fully developed wave of solution a. Measure the current of each of the remaining solutions and the "blank" at this selected applied potential. Record all data in your notebook and determine the following:

(1) The diffusion current i_d for each solution, i.e., $i_d = (i_L - i_r)$, where i_r is the current for solution f at the same potential;

(2) The $E_{1/2}$ values for Pb(II) ion from the complete polarograms obtained with solutions a and c.

Plot i_d vs. concentration of Pb(II) for all solutions on graph paper. What relationship exists between i_d and concentration in this plot? Express this by means of a simple equation. What is the numerical value of the constant in this equation? What is the concentration of the unknown solution e? What is this quantitative polarographic method called? What effect does Cd(II) concentration have of the value of $E_{1/2}$ determined graphically? How does this $E_{1/2}$ value compare with those reported by others? See Refs. 2, 5, and 11, or others for tables of $E_{1/2}$ values in a variety of media.

2. *Effect of the Nature of the Supporting Electrolyte.* Prepare the following solution in a 100-ml volumetric flask: 20 ml of 0.02 M Pb(II), plus 2 ml of 0.2% gelatin, plus 50 ml of the mixed tartrate-NaOH supporting electrolyte (stock solution), and water to volume.

Procedure. Deoxygenate and obtain a polarogram of this solution, determine the i_d and $E_{1/2}$ for Pb(II). In this case use the approximate or extrapolation method to estimate the residual current i_r. Compare the $E_{1/2}$ value obtained with that determined for solution a in (1). Comment on the manner of reporting an $E_{1/2}$ value for any electroactive species (depolarizer).

Note: Read Experiment 16.2 before discarding solutions c and d used in part C of Experiment 16.1.

Experiment 16.2.   Quantitative Polarographic Analysis

Part A. Standard Addition Method

Prepare the following solutions in 100-ml volumetric flasks:

Solution a.   Ten (10.0) ml of "unknown Pb(II) Solution" [ca. 0.02 *M* Pb(II)], plus 2 ml of a 0.2% gelatin solution, plus 20 ml 1 *M* KCl, and water to volume.

Solution b.   Repeat solution a, except, add also an accurate volume of standard Pb(II) solution which produces a doubling of the diffusion current observed in the case of the unknown in solution a.

Note: The addition of the 0.02 *M* Pb(II) standard is made prior to diluting with water to the 100-ml mark.

Obtain polarograms according to the general procedure and calculate the concentration of the "unknown" solution from Eq. (16.30). Would it be appropriate to use Eq. (16.31) in this situation?

Note: If time is short the standard-addition method can be demonstrated with very little additional effort if Experiment 16.1, part C, has been completed and solutions c and d kept. If we let solution d represent the "unknown" solution and solution c represent the "unknown" solution to which an accurate weight of standard Pb(II) has been added to "approximately" double the wave height. Calculate the concentration of the "unknown" solution d using Eq. (16.30). How does the value compare with the actual concentration of the "unknown" solution d (known in this case)?

Part B. Pilot Ion (Internal Standard) Method

Prepare the following solutions in 100-ml volumetric flasks:

Solution a.   5.0 ml of standard 0.02 *M* Pb(II) solution, plus 5.0 ml of 0.02 *M* Cd(II), plus 2 ml of 0.2% gelatin solution, plus 10 ml of 1 *M* KCl, and water to volume.

Solution b.   Repeat the preparation of solution a, except substitute an

accurately measured volume of "unknown" Pb(II) solution for the standard Pb(II) solution.

Note: The unknown could be the same as that used in Experiment 16.1, part C1 or that in Experiment 16.2A, and a comparison of two methods of quantitative measurement made.

Obtain polarograms for the solutions a and b according to the *general polarographic procedure*. Scan the potential range 0 to -1.0 V (vs. SCE). Measure the diffusion currents i_d for both waves and calculate the concentration of the unknown using Eq. (16.35). The I_d values for Pb(II) and Cd(II) ions in 0.1 M KCl are 3.80 and 3.51, respectively.[11]

Experiment 16.3.   Conventions Used for Plotting Cathodic and Anodic Current-Voltage Waves of a Reversible Redox Couple

The following aqueous solutions are provided: 1 M sodium citrate, 0.02 M ferric ammonium sulfate.

Prepare the following aqueous solutions *freshly* each day:
   100 ml of 0.2% gelatin
   100 ml of 0.02 M ferrous ammonium sulfate [in deoxygenated water to retain the Fe(II) state]

Procedure. Transfer 50 ml of the 1 M sodium citrate (stock solution) to a 150-ml beaker, add 2.0 ml of the gelatin solution, then deoxygenate with oxygen-free nitrogen for 15 min. To this oxygen-free solution add 5 ml of each of the two iron solutions and adjust the pH by dropwise addition of 12 M HCl to 5.5 $\pm$ 0.1. Pour this solution into the H-type cell until it is about two-thirds full, deoxygenate for about 5 min, then protect the solution surface with a gentle stream of nitrogen. Obtain a polarogram according to the *general polarographic procedure* over an applied potential range of 0.1 to -0.5 V (vs. SCE). The anodic part of the curve is obtained by switching the galvanometer connections, or more conveniently by setting the galvanometer index to "zero" at midscale. The $+0.20$ to 0.00 V potentials can be applied by switching the leads to the DME and SCE or on some instruments by flipping a polarity switch on the instrument (often marked "DME + and $-$") to ($+$) side. Consult the manual supplied with a specific instrument for the exact operating procedure.

Plot i vs. E_{appl} using the plotting conventions illustrated in Fig. 16.6.

How could you determine which species is responsible for the anodic, or cathodic, currents? If time permits, carry out your plan using the solutions available. If this is done compare the $E_{1/2}$ potentials of each form of this redox couple and comment on their agreement, or lack of it.

See Chapter XIII of the book by Heyrovský and Kůta[2] for other examples and discussion of mixed currents.

Experiment 16.4.   Organic Polarography

Part A. Quantitative Analysis of Riboflavin[12]

Riboflavin has a half-wave potential $(E_{1\,2})$ of -0.47 V (vs. SCE) in Sörensen's phosphate buffer (pH 7.5) when the dropping mercury electrode (DME) is employed. The dc polarographic method is particularly useful for the assay of this vitamin in pharmaceutical preparations. The method is quite specific and other vitamins, vehicles, or excipients rarely cause interference with the riboflavin wave. The direct-comparison method, especially that involving a calibration curve, is widely used in the polarographic analysis of this vitamin.

Materials. USP reference standard riboflavin (or very pure riboflavin) sodium salicylate USP XVII.

Sörensen phosphate buffer (0.5 M, pH 7.5). This supporting electrolyte is prepared as follows: dissolve 62.5 g of $Na_2HPO_4 \cdot 2H_2O$ and 10.2 g of KH_2PO_4 in sufficient distilled (or deionized) water to make 500 ml of solution.

Preparation of Standard Curve

Weigh accurately about 25 mg of the reference standard riboflavin plus 2.5 g of pure sodium salicylate and transfer these to a 250-ml volumetric flask. Dissolve these materials in approximately 200 ml of water, then add water to the mark and protect from light. Pipette 10, 15, 20, and 25 ml of this standard riboflavin solution into four 50-ml volumetric flasks, followed by 10 ml of the buffer solution, add water to the mark, stopper, and mix well. Obtain a polarogram for each of the standard solutions according to the *general polarographic procedure,* preferably with a recording polarograph. Measure the diffusion current i_d in each instance and plot i_d vs. concentration of riboflavin (micrograms per milliliter) to obtain a calibration curve.

Preparation of the Sample. Grind a single tablet containing riboflavin in a mortar. Quantitatively transfer to a volumetric flask of appropriate capacity sufficient of the sample, sodium salicylate and buffer to make the final assay solution 1% in salicylate, 0.1 M in phosphate buffer and contain between 20–50 μg of B_2/ml. The final assay solution is analyzed under the same conditions employed for the standard solutions. Determine the diffusion current for the sample and refer it to the calibration curve to ascertain the concentration of the sample solution in μg B_2/ml. Calculate the riboflavin content in milligrams per tablet and report the result as per cent of the label claim. This polarographic method can be used to assay riboflavin in multiple vitamin capsules or tablets containing vitamins A, B_1, B_2, B_{12}, C, D, E, nicotinamide, calcium pantothenate, several mineral salts, etc., without apparent interference. For greater detail and procedure modification consult Ref. 12.

Other polarographic analysis procedures, involving direct comparison

and/or. standard addition methods, for riboflavin are given by: W. J. Seagers, *J. Am. Pharm. Assoc., Sci. Ed.*, **42**, 317 (1953); and A. J. Zimmer and C. L. Huyck, *ibid.*, **44**, 344 (1955).

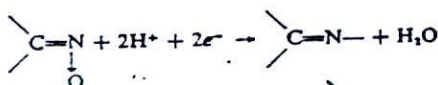**Part B. Quantitative Assay of Chlordiazepoxide[13]**

Apparatus. Recording (or manual) polarograph
H-type cell with SCE and DME assembly

Materials. Chlordiazepoxide ("Librium"), 7-chloro-2-methyl amino-5-phenyl-3H-1,4-benzodiazepine 4-oxide
Triton X-100 (Rohm and Haas Co.) 0.5% solution
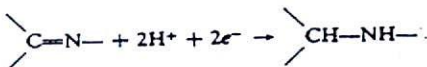Anti-foam B (Dow-Corning) 0.5% solution
1 M hydrochloric acid (SE)

Introduction. In a 1 M HCl medium this drug is reduced at the DME producing a well-defined two-step polarogram with $E_{1/2}$ values of -0.36 V and -0.67 V (vs. SCE).

A linear relationship exists between i_d and concentration from about 3 to 135 $\mu g/ml$. The cathodic reduction is represented by the following electrode reactions:

First wave:

$$\diagup \! C{=}N \! \diagdown + 2H^+ + 2e^- \rightarrow \diagdown C{=}N{-} + H_2O$$
$$\qquad\quad | \atop O$$

Second wave:

$$\diagdown C{=}N{-} + 2H^+ + 2e^- \rightarrow \diagdown CH{-}NH{-}$$

The second wave ($E_{1/2} = -0.67$ V vs. SCE) is used for diffusion current measurement since it is somewhat better defined than the first.

The original work[13] was performed in a 3-ml small volume H-type polarographic cell at a sensitivity coefficient of 0.003 $\mu A/mm$ for the estimation of this drug in biological fluids. Consult the reference for the extraction details, if required.

Procedure. Accurately weigh about 10 mg of pure chlordiazepoxide (reference standard) and quantitatively transfer this to a 100-ml volumetric flask. Add 1 M HCl to volume and mix well. From this concentrated standard solution, prepare four or five standard solutions ranging in concentration from 10 to 100 $\mu g/ml$, and containing about 10 drops of 0.5% Triton X-100 solution, 5 drops of Anti-foam B, and sufficient 1 M HCl to produce the desired volume. Transfer to the polarographic cell sufficient standard solution to cover the electrodes to a depth of about ½ in. Deoxygenate and obtain a partial polarogram over the applied potential range of -0.3 to -1.0 V (vs. SCE) employing the *general polarographic procedure.* Repeat with each standard solution. Extrapolate the slightly inclined limiting current of each wave and measure the i_d of the second wave, i.e., the vertical

distance between these two extrapolated lines at the $E_{1/2}$ value for the second wave.[14]

Plot the i_d values (second wave) vs. concentration of chlordiazepoxide (μg/ml) to obtain a calibration curve.

Prepare the sample in the same manner, estimating the concentration so that it lies within the range of the calibration curve. Measure the i_d in the same manner and refer this value to the calibration curve for the concentration in μg/ml. Calculate the amount of chlordiazepoxide in the unknown provided.

If time permits calculate the total current for the two-step reduction, corrected for the residual current at the same potential and construct a standard curve. Compare the result for the unknown calculated by this means with that obtained in the part B experiment. Does one method have any advantage over the other? Comment.

Part C. Polarographic Assays of Official Substances

Polarographic analysis is employed in the assay of acetazolamide tablets USP, sodium acetazolamide USP, sterile sodium acetazolamide USP; dichlorophenamide tablets USP; hydrochlorothiazide tablets USP; methazolamide tablets USP; nitrofurantoin oral suspension USP; nitrofurantoin tablets USP[15], and dienestrol tablets, NF[16].

Consult also the following reference for the polarographic analysis of certain of the just listed drugs and chlorothiazide. A. F. Summa, *J. Pharm. Sci.*, **51**, 474 (1962).

Experiment 16.5

Additional experiments can be selected from Table 16.1.

PROBLEMS

P16.1. Outline a procedure for the polarographic analysis of a trace of As(III) ion in the presence of a much larger concentration of Zn(II) ion. The $E_{1/2}$ values in a NH_3NH_4Cl medium are -1.46 V and -1.35 V (vs. SCE), for As(III) and Zn(II), respectively.

P16.2. It is known that $m \propto h_{corr}$ and $t \propto 1/h_{corr}$, where h_{corr} is the height in cm of the mercury column above the tip of the DME. If a Pb(II) ion solution gave a $i_d = 4.05$ μA for a drop time $t = 3.60$ sec, and $m = 2.11$ mg/sec, what is the value of the i_d when the mercury height is changed to give a drop time $t = 4.00$ sec?

P16.3. A 500-mg sample of a preparation containing riboflavin was dissolved in 100 ml of water. A 10-ml aliquot of this solution was placed in each of two flasks. To one flask was added 10 ml of buffer, to the other 10 ml of a standard solution (containing 4 mg of USP reference standard riboflavin in buffer). Both were analyzed by the general polarographic method. The diffusion current of the sample solution alone was 25 (galvanometer reading), the other solution gave an i_d reading of 45. Calculate the per cent concentration of B_2 in the solid sample.

P16.4. Compare the relative quantitative polarographic methods described in the text in each of the following situations:

a. Routine analysis of relatively pure samples.

b. Routine analysis of a substance in a complex pharmaceutical preparation of unknown composition.

P16.5. List as many factors as possible which may contribute to a limiting current. Which is the desirable factor? Discuss means of eliminating the other factor.

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