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REVIEW ARTICLE

Applications of Modern Electroanalytical Techniques to Pharmaceutical Chemistry

RALPH N. ADAMS

Keyphrases ☐ Electroanalytical technique applications ☐ Potentiometry *versus* voltammetry ☐ Voltammetry, single sweep peak ☐ Electrodes, response characteristics ☐ Stationary, electrodes—quiet solution ☐ Chronoamperometry ☐ Forced convection, electrodes ☐ Rotated disk, electrodes

Present day pharmaceutical and medicinal chemists employ a wide array of experimental techniques. The complexity of the problems encountered with biochemically important systems and the success of the methods utilized are indeed impressive. One is almost hesitant to suggest that a technique which really consists of trickling electrons in or out the end of a wire could contribute anything significant to this difficult area. It is true that modern electrochemical practice has grown to a high level of sophistication. The instrumentation compares with the best, or, perhaps, the most complex available. But the inherent simplicity lightly referred to above offers certain advantages. With their simplicity, electrochemical techniques frequently offer an incisive and revealing approach to the properties of molecules. Chemists are interested in the properties of molecules, no matter what their brand names.

Although there has been no general adoption of modern electrochemistry into the pharmaceutical and medicinal fields, two monographs dealing with applications have been available for some time. The first, by Brezina and Zuman (1) of the Czechoslovakian polarographic school, deals almost exclusively with analysis *via* the dropping mercury electrode. The book by Purdy (2) is an excellent introduction to a wide variety of electroanalytical methods.

The present article attempts to introduce the basic principles of voltammetry and to show how it is used with a variety of electrode systems. Detailed theory is not given—the approach is primarily experimental. Hopefully, the examples chosen will illustrate both the potentialities and shortcomings of electrochemistry applied to (*a*) oxidation-reduction systems of general biological significance; (*b*) physical properties and interactions of drug systems; and (*c*) practical analysis of pharmaceutical compounds. Rarely is the electrochemistry self-sufficient and a variety of spectroscopic and other physicochemical techniques which are part of modern electroanalytical practice are included.

FUNDAMENTALS OF VOLTAMMETRY

Voltammetry, the measurement of current-voltage curves, constitutes the single most important measurement in electrochemical methods. One requires a basic

feel for these measurements and their broad range of applications. An intuitive and experimental approach is given herein. For a more detailed development of the theory, particularly applied to the study of organic electrochemical processes, the reader is referred to a monograph which has recently appeared (3).

Voltammetry can be introduced by comparing it to classical potentiometry. The latter has been applied widely to biochemical systems and is, of course, familiar to all chemists. It will be interesting to see that classical potentiometry is, in reality, a rather special case of the less familiar but more general voltammetry.

Comparison of Potentiometry and Voltammetry—When measuring the oxidation-reduction (redox) potential of a hypothethical solution which was 10^{-3} *M* in Fe(III), 10^{-3} *M* in Fe(II), and perhaps 1 *M* in sulfuric acid, a supposedly inert electrode, like a platinum (Pt) wire, and a reference half-cell, typically the saturated calomel electrode (SCE) would be used. These electrodes would be immersed in the above test solution (well stirred) and now it only remains to choose a potential measuring apparatus. Here the "generation gap" must be remembered for most of the younger crowd would suggest "attach the electrodes to a pH meter on the millivolt range and read the potential"—and rightly so, for the pH meter is convenient and accurate. But many old-timers will recall the use of a so-called student potentiometer. Figure 1 shows one form of such a circuit which has by no means outlived its usefulness.

In Fig. 1, the top portion of the apparatus comprised of the battery, *B*, and the variable resistance, *R*, is enclosed in broken lines because it is a very important and fundamental circuit in electrochemistry. It is properly called a voltage divider but commonly is known as a potentiometer. (Actually, the variable resistor, when used in the configuration of Fig. 1, is itself called a potentiometer. In radio and electronic parlance the terminology is often shortened to pot. There is no known relationship between this and the more pharmacologically important "pot.") For electrochemical applications, typical values of *B* and *R* are 3–6 v. and 10–50 ohms, respectively.

The purpose of the voltage divider is to supply a source of continuously adjustable voltage between points *O–X* (Fig. 1) by varying the position of the adjustable tap on *R*. (*R* can be a simple slidewire resistor, a wire-wound radio pot, or one of the many forms of linear potentiometers).[1] The variable voltage, which can be read on voltmeter, *V*, is applied to the electrochemical cell (Pt–SCE).

If the voltage divider is adjusted by momentarily closing the switch (tapping key, *K*) until the galvanometer, *G*, shows no current deflection, then the voltage as read on *V* must be equal and opposite to the unknown voltage of the Pt–SCE electrode system, E_x. (In the "true" student potentiometer the variable voltage was ordinarily calibrated *via* a Weston standard cell. Increments along *R* represented some regularly increasing and precise fraction of the total voltage applied. Usually, instead of a voltmeter reading, the standardized position of *R* was used.)

[1] Helipot.

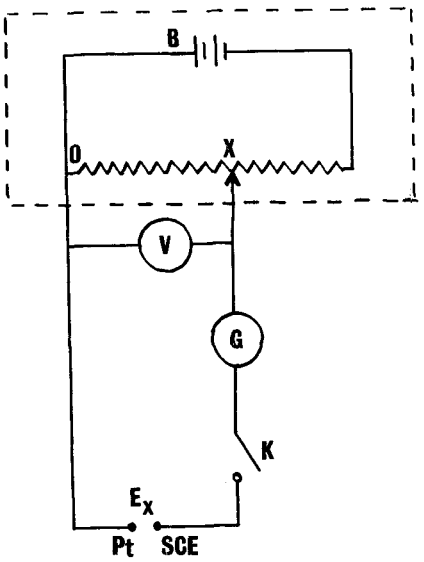


Figure 1—*Simple circuit for potentiometry and voltammetry; see text for explanation of symbols.*

The measurement above is made at essentially zero current drain from the electrodes, provided the switch is not closed for too long a time at off-balance conditions. This type of null-point measurement is called classical potentiometry. In the present case, the potential of the Pt electrode is observed to be +0.44 v. *versus* SCE. This is $E^{0\prime}$, the formal redox potential of the system Fe(III)/Fe(II) in 1 *M* sulfuric acid. (The convention in this article is the analytical or European convention in which reduction potentials are used. Thus, for the processes Ce(IV) + $e \rightleftharpoons$ Ce(III) and Zn(II) + $2e \rightleftharpoons$ Zn^0, the reduction potentials are +1.44 and −0.76 v. *versus* the hydrogen electrode, respectively. This is the convention used most frequently in biochemical studies. It is also invariably used in voltammetry. The SCE is almost the universal reference electrode in voltammetry and potentials are rarely referenced back to the hydrogen electrode. Unless otherwise noted, all potentials in this article will be in volts *versus* SCE. On the reduction scale, the potential of the SCE *versus* hydrogen is +0.246 v.) For a more thorough discussion of potential conventions, formal potentials, *etc.*, the reader is referred to *References* 3 and 4.

The Nernst equation expresses the relationship as measured above between the potential of the Pt electrode and the bulk concentrations of Fe(III) and Fe(II) in solution as:

$$E_{Pt} = E^{0\prime} + 0.059 \log \frac{[\text{Fe(III)}]}{[\text{Fe(II)}]} \qquad \text{(Eq. 1)}$$

where $E^{0\prime}$ = +0.44 v. *versus* SCE. (This is for the 1 *M* sulfuric acid medium. In general, formal potentials vary with the overall solution composition but are useful constants for any fixed set of solution environment.) Whenever the bulk concentration ratio of [Fe(III)]/[Fe(II)] is changed, Eq. 1 demonstrates E_{Pt} must vary. This is the basis of classical potentiometric titrations, where the bulk concentrations are changed by interaction with either an oxidative or reductive titrant.

There is another way in which the concentrations can be varied—by direct "electron titration" of the interface

region close to the Pt surface. In this case the bulk concentrations do not vary, only those concentrations at or very close to the electrode surface are altered and a slightly modified Nernst equation holds:

$$E_{Pt} = E^{0\prime} + 0.059 \log \frac{[Fe(III)]_{x=0}}{[Fe(II)]_{x=0}} \qquad \text{(Eq. 2)}$$

where $x = 0$ indicates the concentrations at the electrode surface and x is the distance from the electrode usually expressed in centimeters. This process is voltammetry and can be carried out with exactly the same equipment as in Fig. 1.

Now consider what happens if one simply locks the tapping key closed. If the voltage divider is set exactly at +0.44 v., no current will flow since this is exactly the condition of the potentiometric zero current measurement. If, however, the applied voltage is made less positive than +0.44 v., considerable current will flow. (In fact, the current may be too large for a galvanometer and we may wish to substitute a microammeter or even a milliammeter. This is really the only modification we need make in the circuit to change from a classical potentiometric to a voltammetric measurement.)

The reason for the current flow is clear. If one assumes that E_{Pt} is now held less positive than $E^{0\prime}$ (+0.44 v.), then it is clear that Eq. 2 cannot be satisfied unless:

$$\frac{[Fe(III)]_{x=0}}{[Fe(II)]_{x=0}} < 1$$

The only possible way this can happen (in the absence of an externally added reductant) is for electrons to be transferred to the Fe(III) species. This constitutes a current flow through the ammeter circuit and corresponds to a cathodic reduction of Fe(III) species. Note it is not necessary to alter the bulk concentration—only the concentration ratio at the electrode surface, *i.e.*, at $x = 0$, needs be changed to satisfy the value of E_{Pt} which is applied *via* the voltage divider.

In an exactly analogous fashion, if E_{Pt} is set at values more positive than +0.44 v., current will again flow—this time in the opposite direction. Now it is necessary that the ratio:

$$\frac{[Fe(III)]_{x=0}}{[Fe(II)]_{x=0}} > 1$$

to satisfy Eq. 2, and Fe(II) is oxidized at the interface corresponding to an anodic oxidation. Voltages more positive than the $E^{0\prime}$ are spoken of as anodic and the current flow is designated anodic or oxidation current. Similarly, cathodic voltages are less positive (more negative) than $E^{0\prime}$ and the corresponding current is cathodic or reduction current. [Note that it is not necessarily the sign of the applied voltage which determines the direction of current flow, but rather its value with respect to $E^{0\prime}$ of the redox system. For example, Br_2 can undergo reduction at fairly high positive voltages (about +0.7 v.), and many reductants are oxidizable at quite negative (with respect to zero) voltages. Throughout the discussion above the author has used the expression voltage—whereas in voltammetry one most frequently sees the term potential. It is actually the potential of the electrodes which is of interest. The real potential differs from the applied voltage by the amount of iR drop in the voltage divider, reference electrode-solution resistance network. The iR drop is a necessary evil of the finite current flow. With low resistance (R) solutions and circuits, the iR drop can be made small and the terms can be used interchangeably. In any event, one can always correct (either *via* instrumentation or arithmetic) applied voltage to potential. From here on the author will assume such corrections and refer to current-potential relationships.]

If plotted, this current-potential data would look like Fig. 2A. The three points with the smooth curve indicated can be reasonably connected. If one continues to move more cathodic from $E^{0\prime}$, the current could either (*a*) increase indefinitely or (*b*) reach some limiting value. A little reflection will indicate the second possibility is most likely.

If, for instance, the applied potential is several tenths of a volt less positive than $E^{0\prime}$, then:

$$\frac{[Fe(III)]_{x=0}}{[Fe(II)]_{x=0}} <<< 1$$

Thus, it is evident the concentration of Fe(III) at the electrode must decrease to a value negligibly small with respect to that in the bulk of the solution, or:

$$[Fe(III)]_{x=0} <<< [Fe(III)]_{x=\infty}$$

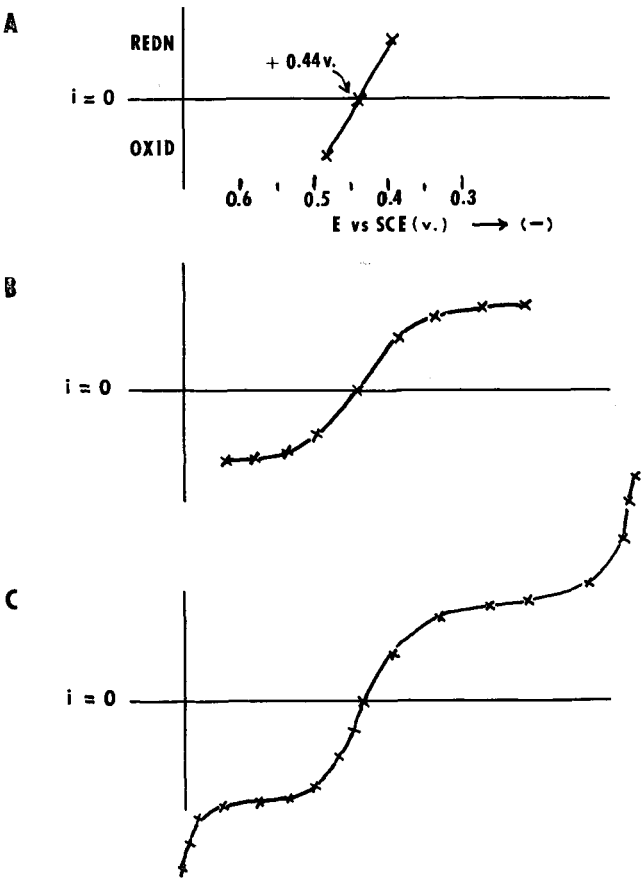


Figure 2—*Current-potential curves for ferric-ferrous system;* A, B, C: *see text.*

Thus,

$$[Fe(III)]_{x=0} \rightarrow 0$$

If, under these conditions, the Fe(III) at the electrode surface is approaching zero, to sustain continual and increasing current flow fresh Fe(III) must be transported from the bulk of the solution by some mass transfer process, be it diffusion, electrical migration, stirring, or whatever. Ordinarily this mass transfer becomes current limiting—in fact, in most voltammetric applications we arrange the mass transfer conditions so it does limit the current. Hence, both the anodic and cathodic currents can be expected to show a leveling off as indicated in Fig. 2B. Since the limiting current is due to mass transfer from the bulk of the solution (the concentration of Fe(III) or Fe(II) at $x = 0$ is essentially zero in this potential region) we can reasonably expect that these limiting currents will be proportional to the bulk concentrations of Fe(III) and Fe(II). The exact quantitative relationships for the limiting currents will depend on the electrode area, type of mass transfer involved, and other factors to be examined later.

If the applied potential is extended even more, it is possible to increase the current beyond that obtained by the ferric-ferrous system. This, of course, must occur by reducing or oxidizing additional species in the solution. The next most easily reducible and oxidizable species undergo electron transfer, which in 1 *M* sulfuric acid would be:

$$2H^+ + 2e \rightleftharpoons H_2 \text{ at more cathodic } E_{app.}$$

$$2H_2O \rightleftharpoons O_2 + 4H^+ + 4e \text{ at more anodic } E_{app.}$$

Since this represents electrolysis of the solvent or "background" solution, present in high concentrations, these currents are large and represent the limits (or cut-off) of the available potential range. The total range of potential depends on the solvent and supporting or background electrolyte. This complete current-potential curve looks like that of Fig. 2C.

The plot of Fig. 2C is called a current-potential curve, polarogram, polarographic wave, or voltammogram. In this case, it represents a mixed anodic-cathodic polarogram. If only Fe(III) or Fe(II) had been present, only the cathodic reduction or anodic oxidation waves, respectively, would have been obtained.

In summary, the essential difference between a classical potentiometric and a voltammetric measurement is that in the former method great care is taken to exclude current flow at the electrodes. Just the opposite is true in the voltammetric technique where the current is the essential response of the system. Notice Eq. 2 is applicable to both processes—it is essentially concentrations at electrode surfaces which are meaningful. In the potentiometric case, with zero current flow, electrode surface concentrations are identical with those in the bulk of solution and the "classical" Nernst expression of Eq. 1 is interchangeable.

If this is the only important difference in the two methods, one might justifiably ask, "who needs voltammetry?" There are a variety of advantages which it is hoped will become apparent in later sections. At this point merely consider the following problem. Suppose we wished to determine the $E^{0\prime}$ of the redox couple *p*-phenylenediamine (PPD) and its conjugate oxidant *p*-phenylenediimine (PDI), in pH 4 buffer solution. Even the most elegant experimentalist will find it impossible to prepare a stable solution of PDI—it undergoes rapid hydrolysis and coupling reactions and classical potentiometric measurements are not feasible. Even oxidative potentiometric titration of PPD and evaluation of the $E^{0\prime}$ from the 50% titrated point is unreliable. Some form of discontinuous titration, using extrapolation of potential to zero time of reagent addition, is required to offset the decomposition reactions of PDI. Such systems eluded the best potentiometric techniques of the late W. M. Clark *et al.* whose work on biological redox systems is monumental and should be required reading for any young upstart in electrochemistry (5).

With fast voltammetric techniques, the effects of chemical reactions coupled to the electron transfer (followup reactions) are qualitatively and quantitatively reflected in the current-potential curves. Often the chemical reactions can be by-passed by high speed measurements. This is rarely desirable for they are of utmost interest. With proper selection of voltammetric conditions, the coupled chemical reactions can be "tuned in" for precise characterization and rate measurements. Thus, the criticism of classical potentiometry in studying organic redox systems is only that it by-passes a wealth of information.

So far only one type of voltammetric procedure has been discussed. In the next section the most important voltammetric responses as a function of the experimental variables will be examined to see how each can be applied to the study of organic systems.

Response Characteristics of Electrodes—Assuming that one is exercising control over the applied potential, there are a variety of electrochemical responses which can be obtained. The diversity depends on the fact that the output response is controlled by: (*a*) nature of the mass transfer process—quiet or stirred solution, type, and nature of the working electrode (the working electrode is the one at which the electrode reaction of interest occurs); (*b*) type of applied potential signal ($E_{app.}$). $E_{app.}$ may be constant, linearly varied with time, or have any other convenient and useful time dependence.

The three main modes of mass transport encountered are migration, convection, and diffusion.

Mass transfer by migration results from the influence of the electrical field at the electrode on charged (ionic) electroactive species. It is of no interest whatsoever in voltammetry. It can be rendered negligible by using an excess of background or supporting electrolyte. A supporting (background) electrolyte is simply one which is not electroactive in the region of interest. This was the purpose of the 1 *M* sulfuric acid in the author's earlier example. Supporting electrolytes typically include salts, acids, bases, and buffer solutions. If the background electrolyte is 10–100-fold greater than the electroactive species migration effects can be neglected. From here on proper supporting electrolytes are assumed to be present and migration plays no part in the voltammetry.

Two types of convective mass transport can be distinguished. The first, due to natural convection, arises from thermal or mechanical disturbances, as well as density

gradients in the solution. It is nonreproducible. Frequently, measurements are restricted to times <60 sec. which limits density and vibration disturbances.

On the other hand, forced convection, which really means stirring, can be made very reproducible and is an important mass transfer mode in voltammetry. The most reproducible convective mass transport is achieved with rotated electrodes of special design known as rotated disk electrodes (RDE).

Diffusion is the most widely studied mass transport process and many voltammetric experiments are designed so it is the only one operative. Diffusion occurs as a result of the concentration difference developed between the electrode surface and the bulk of solution as soon as electrolysis is initiated. It is the predominant form of mass transport in quiet solutions.

It is inituitively easy to appreciate that the overall rate of convective mass transport normally exceeds that of the diffusion counterpart. Therefore different responses can be expected if rapidly varying signals are applied to the two processes. In general, the convective transport will usually "keep up" with the applied signal and the response will be a steady state or time invarient current. On the other hand, currents and current-potential responses are normally time dependent in quiet solutions. This survey of voltammetric methods will begin with a process of the latter type, since it is fundamental to an understanding of all the other methods.

STATIONARY ELECTRODES IN QUIET SOLUTION

Chronoamperometry—In the chronoamperometric (also called potentiostatic) measurement, one suddenly applies a constant potential to a working electrode in quiet solution and observes the current as a function of time. The experiment obviously can be carried out with the apparatus of Fig. 1. However, since we will wish to record current-time curves, the apparatus will be upgraded to a design which will fit all later experiments. Figure 3 shows such an instrument.

The basic voltage divider is still present, but now it is arranged so the variable tap can be driven or scanned by a synchronous motor, *SM*. One can thus hold a constant potential or provide any suitable variation with time. The current is now measured as the *iR* drop across a standard resistance, *Rs*, and fed to the pen axis of an *X*-time recorder. The scan rate of the voltage divider can be synchronized with recorder chart speed, or, in other applications, provide a signal for the *Y* axis of an *X-Y* recorder. Figure 3 is now a recording polarograph of limited versatility. (The simple voltage divider polarograph will not suffice for high speed voltammetry and precision applications. In all modern instruments, it is replaced by an operational amplifier potentiostat. The potentiostat does the same thing as the circuit of Fig. 3—only better. This type of instrumentation is discussed briefly later.)

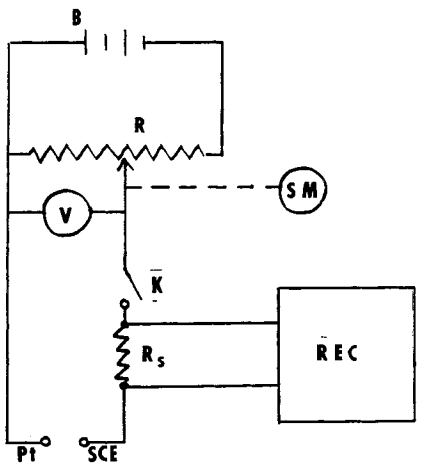


Figure 3—*Simple recording polarograph; see text for explanation of symbols.*

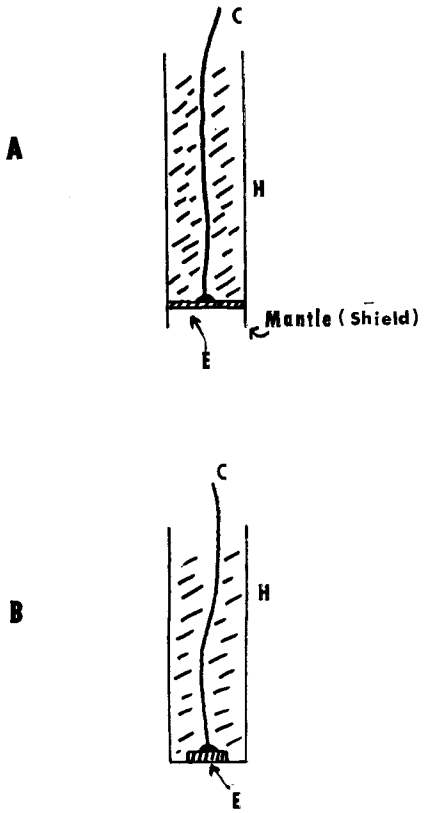


Figure 4—*Stationary electrode designs:* A, *precise linear diffusion electrode;* B, *practical linear diffusion electrode:* C, *electrical connector wire;* H, *electrode holder, usually glass, Teflon,* etc.; E, *electrode surface, Pt, carbon,* etc.

For the potentiostatic experiment, let one assume a quiet solution containing only 10^{-3} *M* Fe(II) in 1 *M* sulfuric acid. The working electrode is now chosen to provide linear diffusion as the only mode of mass transport. True adherence to linear diffusion conditions requires an electrode with a shielding mantle (which prevents edge diffusion) as shown in Fig. 4A. It is difficult to construct and use. Unshielded electrodes like those of Fig. 4B are used on a practical basis for most forms of stationary electrode applications. They provide rather close correlation with linear diffusion theory. Details of practical electrode construction can be found in *References* 3 and 6.

If one applies a potential several tenths of a volt anodic to the $E^{0\prime}$ at $t = 0$ and follows the current-time curve for about 45 sec. (longer times will allow natural

convection to interfere) it can be verified that the current will decay with time according to the well-known Cottrell equation:

$$i = \frac{nFAD^{1/2}C}{\pi^{1/2}t^{1/2}} \qquad \text{(Eq. 3)}$$

In Eq. 3, n = number of electrons, here unity for the Fe(II) oxidation; F is the Faraday; A, the electrode area; D, the diffusion coefficient of Fe(II); and C, the bulk concentration. The other symbols have their usual significance. For a rigorous derivation of Eq. 3, see *Reference* 6; for an experimental interpretation, *Reference* 3.

The instantaneous current at any time is proportional to the bulk concentration and could be used for analysis. However, the transient response makes it an inconvenient analytical tool. The chronoamperometric measurement is a precise and convenient means of calculating the diffusion coefficient of an electroactive species, provided the number of electrons, n, is known. (This is not a trivial piece of data for organic compounds and often limits D value measurements.) For molecules of biological interest, this is a relatively simple means of obtaining the diffusion coefficient. For high concentrations of supporting electrolyte, the electrochemical values can be shown to be in reasonable agreement with those obtained by tracer and capillary diffusion measurements (3).

Eq. 3 is really the most fundamental of all relationships in voltammetry where diffusional control is operative. If the electrode is spherical, or cylindrical, rather than planar, extra terms are added to Eq. 3 to account for the additional mass transport from the other diffusion paths. In many instances, these more complex expressions can be reduced to Eq. 3. The Ilkovic equation for the instantaneous current at a dropping mercury electrode is obtained by merely substituting for the area of the expanding mercury sphere in terms of experimental quantities like m, the mass of mercury falling in mg./sec. and t, the drop time.

Chronoamperometry, when it is applied to a simple electron transfer like the oxidation of Fe(II), is really pretty dull. However, the picture is very exciting when chemical follow-up reactions are present. Consider the following oxidative electrode reaction:

$$A \rightleftharpoons B + n_1e \text{ at potential } E_1$$

$$B + Z \xrightarrow{k} C$$

$$C \rightleftharpoons D + n_2e \text{ at potential } E_2$$

This is commonly called an ECE reaction because it consists of an electron transfer—a chemical interaction—and another electron transfer. The initial product B interacts with some component of the solution or solvent itself, Z, to produce the intermediate C. It very frequently happens that C is more easily oxidized than the starting material, A ($E_2 < E_1$). Hence, at the applied potential E_1, it is immediately further oxidized to D in a n_2 electron transfer.

Such a process is typical of the oxidation of a hydroquinone (A). The resulting quinone (B) may undergo a rapid 1,4-addition with a nucleophile (Z) to give a substituted hydroquinone (C). If the nucleophile is electron donating, C will be more easily oxidized than A. These processes in biochemically important quinones have been discussed by Pullman and Pullman (7). The reactant Z can be electron withdrawing or other factors can lead to species C being slightly more difficult to oxidize than A, but this increase is rarely greater than 100–200 mv. in a variety of systems examined to date. The ECE process is very widespread in organic electro-oxidations and there are many counterparts in reduction processes (3).

Now, with an applied potential of E_2 (or E_1, if sufficient), analyze the current-time (i-t) behavior for the ECE process. First, consider the i-t curve if only the first oxidation were to take place. This is the situation if k for the intervening chemical reaction is very small. The current will decay inversely with $t^{1/2}$ according to Eq. 3

$$i = \frac{n_1FAD^{1/2}C}{\pi^{1/2}t^{1/2}}$$

or:

$$\frac{i}{FAD^{1/2}C} = \frac{n_1}{\pi^{1/2}t^{1/2}} \qquad \text{(Eq. 3}a\text{)}$$

The corresponding equation in which both electron transfers occur was given by Alberts and Shain (8) as:

$$\frac{i}{FAD^{1/2}C} = \frac{n_1 + n_2(1 - e^{-kt})}{\pi^{1/2}t^{1/2}} \qquad \text{(Eq. 4)}$$

If k is very small, Eq. 4 reduces to 3a, *i.e.*, the process corresponds only to the simple oxidation of A if no chemical step takes place. This is also the case if k is finite, but t is very short, since for short times, A is essentially the only oxidizable component present. However, for long times, (t_∞), Eq. 4 becomes:

$$\frac{i}{FAD^{1/2}C} = \frac{n_1 + n_2}{\pi^{1/2}t^{1/2}} \qquad \text{(Eq. 5)}$$

corresponding to the direct oxidation of A to D. At intermediate t's, the current varies between the two limits and the switch-over point is determined by the value of the chemical rate constant, k.

In order to facilitate the determination of k, Alberts and Shain divided Eq. 4 by Eq. 5 to obtain:

$$\frac{it^{1/2}}{it_\infty^{1/2}} = 1 - \frac{n_2}{n_1 + n_2}e^{-kt} \qquad \text{(Eq. 6)}$$

Since very often $n_1 = n_2$ for ECE processes, this reduces to the remarkably simple relation:

$$\frac{it^{1/2}}{it_\infty^{1/2}} = 1 - 0.5e^{-kt} \qquad \text{(Eq. 7)}$$

A dimensionless working curve can be constructed from Eq. 7 by merely assigning values to kt and plotting against calculated ratios of $it^{1/2}/it_\infty^{1/2}$. Experimentally, one evaluates this latter ratio and for each such measurement obtains a value of k. Alternately, several values of kt can be plotted *versus* t and k evaluated from the slope of the resulting linear function. The value of $it_\infty^{1/2}$ is determined under conditions where kt is large. However, if k is small, this requires long times where natural convection disturbs the proper measurement of current-time curves. Hence, an alternate (and usually preferable) method is to determine an $it_\infty^{1/2}$ value in a

medium where the chemical reaction cannot occur, *i.e.*, $k = 0$. Then, if $n_1 = n_2$, $it^{1/2}$ is simply twice this value. This assumes that the diffusion coefficients are not appreciably altered by the change in medium (usually an alteration of pH).

A wide variety of analyses are now available for ECE processes, including first- and second-order chemical steps, dimerizations, *etc.* (8–12). Evaluation of the data has been greatly simplified by the digital simulation techniques of Feldberg, which are now summarized (13). There are several so-called "nuances" to the ECE reaction not discussed above. If the intermediate, C, is more easily oxidized than starting A, then it is clear the chemical oxidation: $B + C \rightleftharpoons D + A$ can occur along with electro-oxidation of C. This type of reaction has been called the ECC process and, when taken into account, slightly modifies the working curves. The net electrochemical effect is still the same—in the limit $(n_1 + n_2)$ electrons are transferred. Several ECE systems of pharmaceutical interest have been studied by the chronoamperometric method and these are discussed later under applications.

Single Sweep Peak Voltammetry—With quiet solutions, an experiment more common than the chronoamperometry of the previous section is the application of a linear potential sweep to the working electrode. In fact, the technique is often called linear potential sweep chronoamperometry. It also has been termed oscillographic polarography. The more descriptive name of peak voltammetry seems most appropriate.

The conditions of the experiment are the same as before. Shielded electrodes give strict adherence to theory, but the practical variety work very well. Potential sweep rates vary from 1 or 2 to several hundred volts per minute. The most common range is 2–50 volts per minute. With sweep rates less than about 20 volts per minute the current-potential curve can be fed to a pen-and-ink recorder. Higher rates require oscillographic presentation. The present status of the theory and practice of peak voltammetry is largely due to the studies of Nicholson and Shain (14).

The output response is a peak shaped current-potential curve called a peak polarogram or peak voltammogram. This is an example of electrode response in quiet solution depending on the rate of application of the applied potential. The current naturally increases with the applied potential sweep and would reach a steady state limiting value (as in either the cathodic or anodic position of Fig. 1B) if convective mass transport constantly replenished the supply of electroactive species. With only diffusion operative, the current instead reaches a maximum and then decays with the characteristic $t^{1/2}$ dependence of Eq. 3—hence, the peak behavior.

A typical example, and one of interest in pharmaceutical work, is that for the reduction of the important antibiotic, chloramphenicol (Chloromycetin) shown in Fig. 5. The maximum or peak current is given by:

$$i_p = kn^{3/2}AD^{1/2}V^{1/2}C \qquad \text{(Eq. 8)}$$

Here, V is the potential sweep rate in volts per second and the other symbols have their previous significance. The constant, k, is called the Randles-Sevcik constant

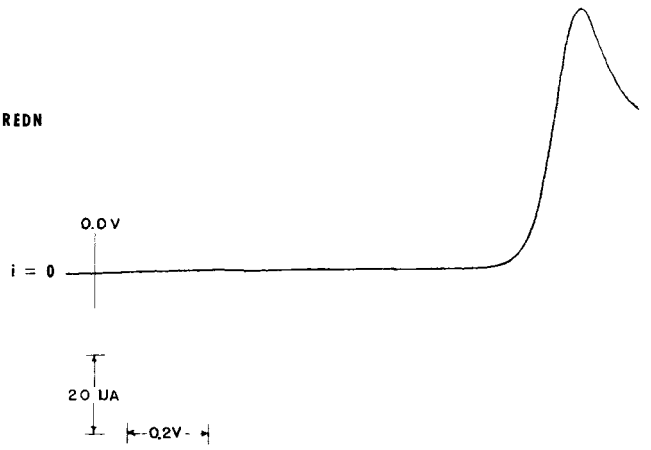


Figure 5—*Peak polarogram for reduction of chloramphenicol.*

after Randles and Sevcik, who independently first practiced this work as oscillographic polarography (3, 6). The peak current of Eq. 8 is normally expressed in amperes. Two useful expressions in terms of microamperes (μamp.) are:

$$i_{p(\mu\text{amp.})} = 269 \times 10^6 n^{3/2}AD^{1/2}V^{1/2}C \qquad \text{(Eq. 8}a\text{)}$$

where C is in moles/l. or M

or:

$$i_{p(\mu\text{amp.})} = 269 \times 10^3 n^{3/2}AD^{1/2}V^{1/2}C \qquad \text{(Eq. 8}b\text{)}$$

where C is in mmoles/l. or mM.

The value of the Randles-Sevcik constant has now been established by the rigorous calculations of Nicholson and Shain as 269 (14).

The curve may be characterized by the peak potential, E_p, or the half-peak potential, $E_{p/2}$. The latter is simply defined as the potential where $i = i_p/2$. It has no special thermodynamic significance, but is easily related to the more conventional $E_{1/2}$ and $E^{0\prime}$ values (3, 6). The peak polarogram of a reversible redox system is narrow and occupies a potential interval of about 0.12 v. for a 1-electron system. The increment between E_p and $E_{p/2}$ for a reversible process is given by:

$$|E_p - E_{p/2}| = \frac{0.057}{n} \text{ volts} \qquad \text{(Eq. 9)}$$

Equation 8 predicts that the ratio $i_p/V^{1/2}$ should be constant and independent of V for a simple electron transfer with no coupled chemical reactions. If an ECE process occurs, as the sweep rate decreases, more time is available for the homogeneous chemical reaction and the second electron transfer, hence, $i_p/V^{1/2}$ will increase. This is indeed a valuable diagnostic criterion for ECE reactions. Quantitative relationships of peak voltammetry are very useful in elucidating the nature of electrode processes (3).

One of the most valuable applications of peak voltammetry is in analysis. The sensitivity is excellent. Equations 8*a* and 8*b* show that a 1 mM solution of an electroactive species with $n = 1$ and an average $D = 4 \times 10^{-6}$ cm.^{2}/sec. will produce about 150 μamp. of current at a 1 cm.^{2} electrode using sweep rates of about 6 v. min. Most electrodes used to date have averaged about 0.2 cm.^{2} in area and peak currents of about 40–50 μamp. for a 1 mM

solution are common. This may be compared with the dropping mercury electrode where a 1 mM solution of a 1-electron reduction produces about 4–6 μamp. Both electrode areas and sweep rates can be increased considerably to give higher sensitivity ($i_p \propto A, V^{1/2}$) although a practical limit on sweep rates is set by charging current (6).

In general, any compound which can be reduced by conventional dropping mercury polarography works well in peak voltammetry. The hanging mercury drop electrode and a variety of mercury plated surfaces are used. It should also be noted that a great many reductions in nonaqueous media (acetonitrile, *N,N*-dimethylformamide) occur easily at platinum or gold electrodes. Platinum and various carbon electrodes are used for anodic oxidations. A thorough survey of the electrodes and methodology of peak voltammetry can be found in the comprehensive biannual reviews by Hume (15). A complete and continuing coverage of the voltammetry of organic compounds, including pharmaceutical materials, is contained in similar reviews started by Wawzonek and continued by Pietrzyk (16–18).

A modification of peak voltammetry known as inverse or stripping voltammetry is particularly suited to trace metal analysis in biological materials. Here a controlled potential electrolysis concentrates the metal in a hanging drop, mercury film, or other electrode surface. Then, the potential is swept anodic to give an oxidative "stripping" peak. The sensitivity has been estimated at 10^{-11} M, but higher practical limits (as in most trace determinations) are usually set by impurities in reagents, *etc.* The latest review by Hume summarizes some of the applications of stripping voltammetry (15).

Cyclic Voltammetry—The single, most fruitful, electrochemical approach to studying chemical reactions coupled with electron transfers is the technique of cyclic voltammetry (CV). In CV one employs a stationary working electrode in quiet solution and applies a repetitive, triangular (isosceles) wave potential sweep between it and an auxiliary or reference electrode. The sweep interval can include the whole potential range of interest, *i.e.*, for an aqueous media from about +1.2 to −0.2 v. *versus* SCE, or be limited to shorter intervals of interest. The sweep rates which are compatible with pen and ink recording vary between about 1–20 v./min. Much higher rates are widely used in oscillographic recording. One records the anodic and cathodic currents as a function of the applied triangular potential sweep, usually on an *X-Y* recorder (with at least 1-sec. pen response).

CV is really peak voltammetry with rapid and reproducible reversals of the potential sweep. Since the solution is quiet and the time interval between sweep reversals is short, the products generated in a reduction are available near the electrode surface for reoxidation as the triangular wave reverses. In a simple, rapid electron transfer with no chemical complications, one obtains anodic and cathodic peak polarograms in which the ratio of anodic to cathodic peak currents, $i_{p,a}/i_{p,c}$ is unity. The potentials of the two peaks are separated by a small increment:

$$|E_{p,a} - E_{p,c}| = \frac{0.057}{n} \text{ volts} \qquad \text{(Eq. 10)}$$

This behavior is seen in Fig. 6A for a reversible system. With less reversible electron transfer, the peaks are more rounded and the separation is greater, Fig. 6B. With marked irreversibility, the individual peaks may be reduced in size and widely separated as in Fig. 6C.

(The ambiguous terms reversible, quasi-reversible, irreversible, *etc.*, have clouded the minds of several generations of readers. If the electrochemical establishment had a shred of social conscience, it would ban their usage. The alternatives are actually not so simple. The quantitative measure of reversibility is embodied in the heterogeneous rate constant for the electron transfer process. This rate constant, given a variety of symbols like k_s, $k_{s,h}$, k_{el} or k°, and expressed in centimeters per second, can be measured and quoted for many systems. However, k_s, *per se*, is not an index of observable reversibility. Rather, the latter depends on the magnitude of k_s compared to the average mass transfer rate, V_{mt}, of the measuring technique.

Lady Godiva riding Man-O'-War would have been just a blonde blur. The same lady astride her slow pacing steed indeed must have been "something else." So it is with reversibility—if k_s is much faster than V_{mt} of the measuring technique, it is said that the system appears reversible. Only the slower V_{mt} can limit the current and the system obeys what we expect for the Nernst equation. Such behavior is also frequently termed Nernstian. If k_s and V_{mt} are competitive, the voltammetry appears partially or quasi-reversible. If k_s is quite small with respect to V_{mt}, the former is limiting and marked irreversibility is observed. Hence, observable reversibility depends on the speed of the measuring technique. With a fast enough electrochemical technique, it is theoretically possible to make any redox system appear irreversible, *i.e.*, k_s can be measured. In CV, the apparent reversibility will vary with sweep rate. Measurements of k_s constitute an important area of electrochemical investigation (3, 6). For the present purposes, we need only appreciate that the general shapes and peak separations

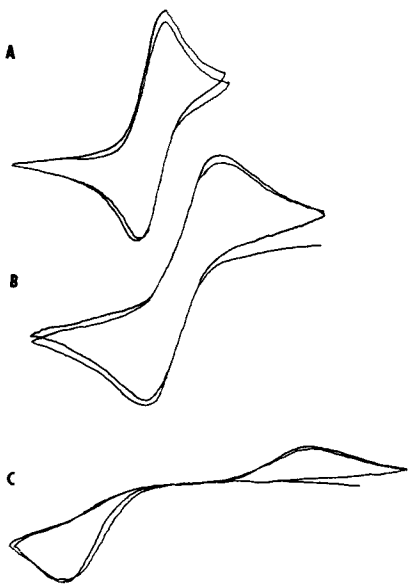


Figure 6—*Cyclic polarograms of various redox systems;* A, *fast electron transfer rate (reversible);* B, *intermediate electron transfer rate (quasi-reversible);* C, *slow electron transfer rate (irreversible).*

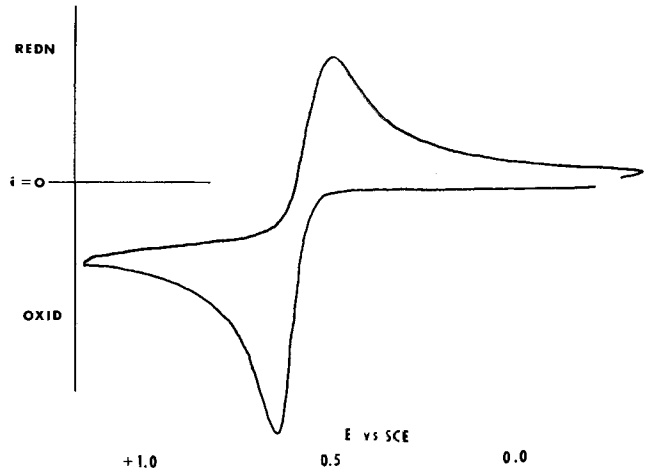
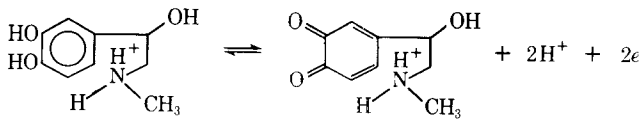


Figure 7—*Oxidation of adrenaline in strong acid about 1 m*M *adrenaline in 1* M *sulfuric acid.*

of redox couples will vary with the systems and the sweep rate. This behavior is, in a way, a nuisance. However, it is discernible and not to be confused with changes which occur as a result of chemical reactions coupled to the electron transfer.)

Although the cyclic polarograms of Fig. 6 may look quite different, they have one factor in common. They are essentially the same no matter how many cycles are examined. There is always a slight decrease in peak heights during the first few cycles. This is due to slight adjustments of the concentration profiles near the electrode surface to steady state conditions. But no major changes in shape or number of peaks occur. An approximate steady state is reached after some 5–10 cycles. Usually only the first and the next two or three cycles are of interest.

The situation is very different when a chemical follow-up reaction is present. This is strikingly shown in Figs. 7–9 for the oxidation of adrenaline (epinephrine). Figure 7 represents the oxidation of about 1 m*M* adrenaline in strong acid (1 *M* sulfuric or perchloric acid). Only one cycle is shown, but, other than minor changes, subsequent cycles are the same. The CV obviously represents a partially reversible electron transfer. It can be shown to correspond to the 2-electron oxidation of the protonated adrenaline to its open chain quinone form:



In 0.3 *M* acid, although the adrenaline/adrenaline-quinone system is considerably more irreversible, as seen in Fig. 8, the picture is still essentially the same. Note there is no major difference between the first and second forward sweeps marked 1*F* and 2*F* in Fig. 8. A forward sweep is one in the same direction as the initial potential sweep. The only real difference between Figs. 7 and 8 is that the electron transfer rate of the catechol/quinone system has become considerably slower. For qualitative comparison, the reversibility lies somewhere between the systems represented in Figs. 6B and C. (The reversibility

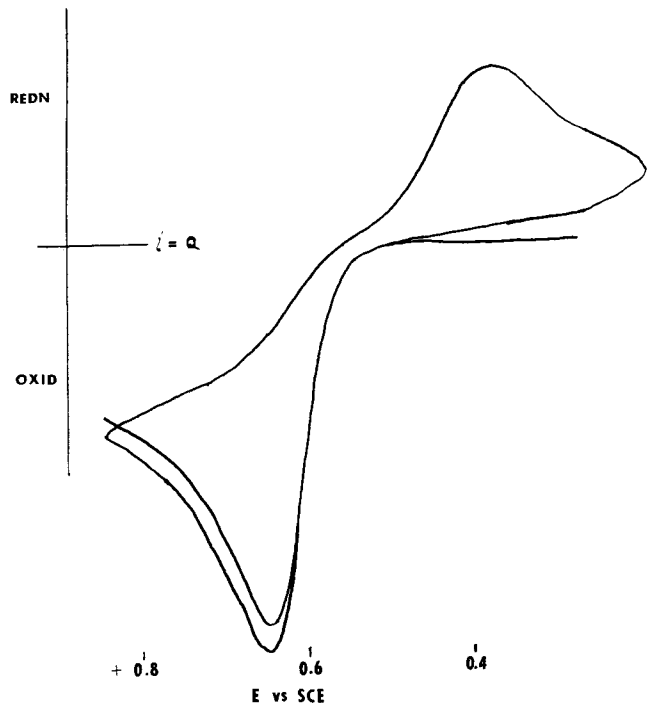


Figure 8—*Oxidation of adrenaline in 0.3* M *acid solution about 1 m*M *adrenaline in 0.3* M *sulfuric acid.*

of most hydroquinone/quinone and catechol type systems decreases with decreasing acidity).

At pH 3 the picture seen in Fig. 9 is significantly different. Starting with the first forward sweep, 1*F*, adrenaline is initially oxidized (Peak *A*) with an E_p at about +0.6 v. On the reverse, cathodic sweep some adrenaline-quinone is still seen at Peak *B*. An even greater peak separation between *A* and *B* exists at this lower acidity. Now, however, there is an additional reduction peak at *C*. Further, on the second forward sweep, 2*F*, the pattern is entirely different. A new oxidation peak, *D*, not present on the first sweep, is now observed. The semi-reversible redox system represented by Peaks *C* and *D* can be identified with the couple leucoadrenochrome/adrenochrome by comparison with the CV of an authen-

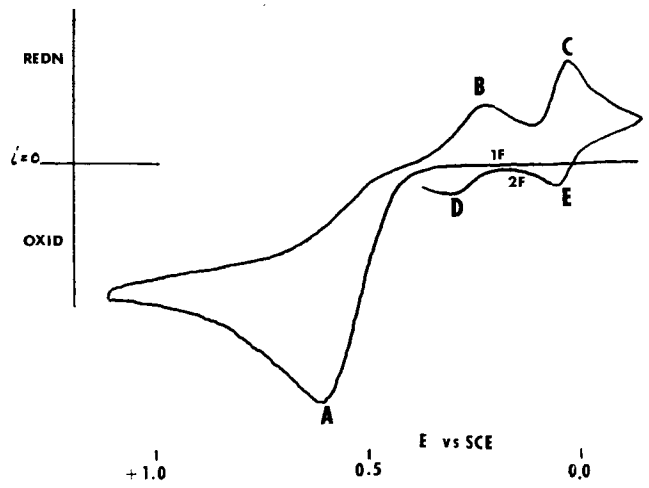
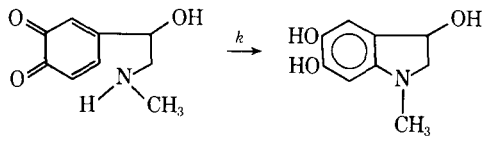
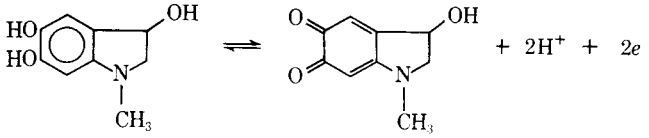


Figure 9—*Oxidation of adrenaline in pH 3 buffer solution about 1 m*M *adrenaline in Britton and Robinson, pH 3 buffer.*

tic sample of adrenochrome. As the pH is lowered, sufficient concentration of the unprotonated adrenalinequinone develops to allow the intramolecular 1,4 (Michael) addition to occur as:



Because the cyclized product, leucoadrenochrome, is more easily oxidized than adrenaline itself, it is rapidly oxidized further to adrenochrome:



Comparison of Figs. 7–9 shows immediately how powerful a tool CV is in identifying follow-up chemical reactions. Whenever there is a distinct change between the first and subsequent cycles, a chemical follow-up reaction has occurred. Often a tremendous amount of information about the overall electrode reaction can be obtained in a very short time by such simple, qualitative CV experiments. If follow-up reactions do occur, frequently the new products and intermediates can be identified *via* comparison of the CV with known, authentic samples. This is rarely satisfactory proof and every physicochemical technique which can be brought to bear is used for positive identification. Such techniques include electron spin resonance, UV, visible, and fluorescence spectroscopy, as well as chemical identifications. Although precise measurements of the rates of the coupled chemical reactions can be made *via* quantitative CV, it is often more advantageous to use chronoamperometry or other techniques, once the basic pattern has been established.

In the adrenaline case, the reaction is a clear case of the ECE process discussed previously. (Actually, due to the ease of oxidation of leucoadrenochrome, it is also chemically oxidized by the initially formed open-chain adrenalinequinone—the ECC mechanism mentioned. Precise evaluation of the cyclization rates by chronoamperometry verifies this, but the reaction is written here as an ECE.) Finally, Peak *E* in Fig. 9 can be identified as belonging to the oxidation of 5,6-dihydroxy-*N*-methylindole formed by a subsequent slow dehydration of leucoadrenochrome. Its presence is not pertinent to this discussion. The cyclization rates of various catecholamines is discussed later.

A complete description of the instrumentation and experimental details of CV has been given and a variety of applications to electron transfers with coupled chemical reactions are summarized (3). Authoritative theoretical treatments of CV, including the measurement of rate constants for coupled chemical reactions are available (19–22). Used alone, CV is by far the most rapid means of obtaining a generalized picture of an overall electrode transfer process. With the theoretical interpretations of Nicholson and Shain at hand, no electrochemical group can afford to be without CV capabilities. It is also pertinent that it is perhaps the easiest of the electrochemical techniques for investigators new to the area—because the output information is so intuitively satisfying. Consider the application to adrenaline oxidation. One can study this reaction by classical potentiometry and, from rates of change of potential with time, deduce the cyclization rates. Indeed, Ball and Chen (23) in 1933 produced elegant data on this reaction by a potentiometric flow technique. With CV, each and every intermediate of the coupled electron transfer and cyclization process appears on the chart paper or oscilloscope screen for detailed investigation. CV is far more sensitive and precise than classical potentiometry for measuring the occurrence and rates of coupled chemical reactions. Only relatively slow chemical events have been discussed herein. With fast CV very rapid chemical processes can be investigated as desired.

There are a number of other electroanalytical techniques applied to quiet solutions which are advantageous for certain situations. Many are specialized adaptations of methods covered here. There is, in addition, a whole segment of methods in which current, rather than potential, is controlled (chronopotentiometric or galvanostatic techniques). For a thorough treatment of these the reader is referred to several monographs (2,3,6) and the reviews on "relaxation methods" by Reinmuth (24), and "electroanalysis and coulometric methods" by Bard (25). In addition, amperometric methods, while usually carried out in stirred solution, embody the principles of voltammetry discussed in the previous sections. The extensive reviews by Stock (26) on "amperometric titrations" should be consulted for pharmaceutical applications.

ELECTRODES WITH FORCED CONVECTION

Rotated electrodes, or those set in stirred or flowing solutions, were among the first used. Approximate treatments of their response have been available for a long time. Rigorous equations are of recent origin. Their development is summarized in the English translation of Levich's monograph, "Physicochemical Hydrodynamics" (27).

In forced convection both diffusion and convection are operative. Unless fast sweep rates are used, the current-potential response is independent of the sweep rate. The current is a steady-state limiting current, i_L, as was shown in Fig. 2B. The transient response following initiation of the applied potential, which is important in some studies, is not considered here.

The derivation of convective voltammetry equations requires consideration of the solution hydrodynamics. Precise descriptions of the hydrodynamic flow at several stationary electrodes are available. Stirred solutions are not so easy to characterize accurately. Ordinary (*e.g.*, magnetic) stirring is not a particularly efficient means of providing high rates of convective mass transport.

The limiting current equations of most convective electrodes will be functions of the general form:

$$i_L = f(C, D^{2/3}, \nu^{-1/6}, L, U^{1/2}, etc.) \qquad \text{(Eq. 11)}$$

where C is the bulk concentration of electroactive species. (Migration is considered absent by virtue of excess supporting electrolyte.) Note the $D^{2/3}$ dependence in contrast to the $D^{1/2}$ of linear diffusion. A typical hydrodynamic property of the solution, the kinematic viscosity, ν, usually appears to the indicated power. (In ordinary aqueous solutions, kinematic viscosity is about 0.01 cm.^{2}/sec.). L represents a characteristic length or geometry of the electrode. The solution velocity, U, ordinarily has the one-half power dependence. It may appear disguised in another factor in many i_L equations. Other hydrodynamic and geometric parameters pertain to specific situations.

Rotated Disk Electrodes—The rotated disk electrode (RDE) is by far the most successful and practical of the convective mass transport electrodes. A theoretical RDE consists of a thin circle of metal (or other electrically conducting surface), large enough in diameter that its edges can be neglected. It is rotated at constant angular velocity about an axis or shaft perpendicular to the center of the disk. Practical RDE's often are of the form shown in Fig. 10, where the disk is surrounded by a glass or plastic shroud. Figure 10 also shows the approximate fluid flow patterns as the electrode rotates. Liquid close to the disk surface acquires a rotational motion and is swept away from the disk center with a radial and tangential velocity. Liquid which thus moves horizontally away from the disk must be replenished by an upward, axial flow. In addition to this basic convective mass transport pattern, one imposes the conditions of electrochemical reaction *via* the usual applied potential. RDE surfaces can be made of platinum, gold, mercury-coated metals, carbon, and other surfaces. Precision devices for varying rotation rates (2–80 r.p.s. are common, but many RDE's operate at much higher rates) and maintaining speed control to ± 0.5 to 1% are readily available. For a full description of practical RDE's, see *References* 3 and 28.

The current-potential curve at the RDE is typical of that shown in Figs. 2B and C. With high rotation rates, V_{mt} can reach the level where irreversible polarograms are obtained. Such effects often distort the *i-E* curves at high rotation rates. They are used to advantage to measure k_s. However, these measurements are beyond the interest of the present review which concentrates on the limiting current behavior of the RDE.

The i_L at the RDE is given by Levich (27) as:

$$i_L = 0.62\, n\, FACD^{2/3}\nu^{-1/6}\omega^{1/2} \qquad \text{(Eq. 12)}$$

where i_L = current in milliamps; A = electrode area in cm.^{2}; ν = kinematic viscosity in cm.^{2}/sec.; C = bulk concentration in moles/l. or M.; ω = angular velocity of disk.

The angular velocity of the disk, $\omega = 2\pi N$, where N = r.p.s. The kinematic viscosity is the ratio of viscosity to density. Note that i_L is given in milliamperes. The sensitivity of the RDE is excellent for low level analyses. As a typical example, an ordinary RDE with $A = 0.2$ cm.^{2}, operated at 10 r.p.s. (a relatively small and slowly rotating electrode) gives approximately 200 μamp. of current for a 1 mM solution (1-electron process). Compare that with the 3–4 μamp. of the dropping mercury electrode!

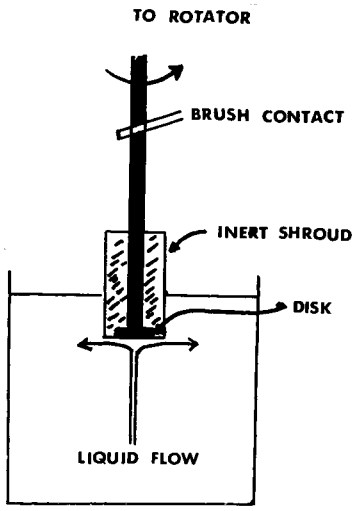


Figure 10—*Typical rotated disk electrode.*

Furthermore, i_L's at the RDE can be measured routinely with a precision of $\pm 1\%$ and, in many cases, in agreement with the theory of the Levich equation to ± 1–2%. It is a remarkably successful electrode, relatively easy to construct and simple to use.

The most important use to date of the RDE is in the study of the mechanism of electron transfers and particularly ECE processes. For this purpose one studies the variation of i_L with ω, contrary to the analytical applications which operate at any convenient, fixed rotation speed. The application of the RDE to ECE processes is intuitively simple and satisfying. Recall the ECE reaction described previously under *Chronoamperometry* where the product of the intermediate chemical reaction, C, was more easily oxidized than starting material, A. If one assumes the chemical step has a moderate rate and a potential corresponding to the i_L plateau region is applied, it may be possible to rotate the disk so rapidly that all of C will be spun away from the electrode surface too rapidly for it to undergo the second oxidation step (n_2). Hence, i_L will correspond to only n_1 electrons. On the other hand, at slow rotation rates, most or all of C is at the disk surface long enough to give rise to currents corresponding to $(n_1 + n_2)$ electrons. Thus a plot of i_L *versus* $\omega^{1/2}$ should be greater than that for a n_1 process at slow speeds and tend toward n_1 behavior at high speeds. How much of the total variation is seen depends, of course, on the relative magnitudes of the chemical rate constant and the rotation rate. Precise measurements of dimerization rates in the range 10^2–10^4 l. mole^{-1} sec.$^{-1}$ have been measured with the RDE (29, 30). Further details of this technique have been reviewed (3).

Electrodes in Flowing Solution—A number of investigations have yielded rigorous hydrodynamic i_L equations for electrodes in flowing solution. Jordan *et al.* developed the conical microelectrode, shaped much like a pencil point. This electrode was used at very high solution flow rates to measure the kinetics of electron transfer processes (31, 32). Blaedel *et al.* developed tubular platinum electrodes (TPE) in which a seam-

less joint between the electrode and the inner surface of a glass tube provides laminar flow control (33, 34). Recently a series of catalytic reactions were studied at the TPE with excellent results (35). Oesterling and Olson (36) developed simple mercury-coated TPE's and studied their chronoamperometric behavior. The TPE is ideally suited to flow stream monitoring and possible use for *in vivo* applications. Further details of the use of flowing solution electrodes have been summarized (3, 27).

Coulometric Methods—Coulometry, both at constant potential and with constant current (coulometric titrations) is usually carried out in stirred solution and should be considered in this section. These techniques are capable of excellent sensitivity and accuracy in analysis. Purdy's monograph gives a thorough coverage of these methods (2).

Instrumentation—As mentioned earlier, the simple voltage divider polarograph applied to a two electrode system will not suffice for fast and precise voltammetric measurements. It is necessary, for such work, to accurately control the real potential at the working electrode to within a few millivolts. This is done with a potentiostat, which, as its name implies, holds the potential static (constant) as desired. For dynamic operations, the potentiostat is controlled by a suitable sweep circuit—and it accurately and rapidly maintains any desired potential as a function of time. A three-electrode system is used—working and auxiliary electrode and a reference electrode. The entire polarograph is usually constructed from operational amplifiers and these circuits are commonplace in electrochemical measurements. Detailed discussions of operational amplifier polarographs are to be found in the literature (15, 25) and in a review of a special symposium on such instrumentation (37).[2]

POTENTIALITIES AND SHORTCOMINGS OF ELECTROANALYTICAL TECHNIQUES

Having examined the basic techniques of modern electrochemical practice, the purpose of this section is to review briefly some of the applications that have been made and to evaluate critically the potentialities of electrochemistry applied to compounds of pharmaceutical systems. No attempt is made to cover exhaustively the existing literature. Rather, examples are chosen to identify certain interesting and important areas.

Analytical Applications—There is no real shortage of applications of electroanalytical techniques to compounds of pharmaceutical interest. For instance, a brief perusal of the 1968 review on "Organic Polarography" (18) reveals at least fifty publications dealing with pharmaceuticals. This does not include studies of closely related interest. While many of these investigations use conventional dropping mercury electrode polarography, there appears to be an increasing number employing fast sweep techniques. It is particularly this area which certainly deserves further attention. As has been pointed out in the previous sections, both single sweep peak voltammetry and RDE methods possess distinct advantages over the dropping electrode in sensitivity, usually not at the expense of accuracy. Further, increasing attention should be paid to the use of other than mercury electrodes, *i.e.*, for anodic oxidation work. As investigators become more acquainted with the anodic possibilities, this area should prove fruitful. There are a large number of drug systems with amino, phenolic, and mercapto functions—all potentially oxidizable. The previous reluctance to use anodic voltammetry stems from a distrust of solid electrodes. There is no disputing that the constantly renewable dropping mercury electrode is difficult to surpass in surface qualities. Nevertheless, expertise in handling solid electrode surface conditions is now well established. Although platinum electrodes are troublesome in aqueous media due to surface oxidation, they behave very well, with minimum cleaning, in nonaqueous solvents for both reductions and oxidations.

The use of nonaqueous solvents is particularly advantageous—aiding, in many cases, both solubility and electrochemical characteristics. Recent studies by Woodson and Smith (50) of the a.c. and d.c. polarography and CV of some 15 pharmaceutically important compounds in acetonitrile (including reserpine, steroids, barbiturates, *etc.*) showed excellent response in almost all cases.

The carbon paste electrode, with its very low residual current, is excellent for oxidative work in aqueous media. Renewing carbon paste electrodes, or cleaning metal surfaces after each run or several runs is not that difficult, once the reasons for it are clear. It seems a small effort, especially when it opens up an entire new area of electrochemical operations on the oxidation side.

Illustrative of the kind of anodic analytical work that can be done is the data of Fig. 11. Investigations of a variety of neurotransmitters and hallucinogens have been in progress in this laboratory for some time. Mescaline was merely picked out at random from a series of these hallucinogens to illustrate the potentialities of peak voltammetry for its analysis. Figure 11 clearly shows i_p is lineally proportional to concentration over a reasonable range. These data were taken in one-hour's work and no attempt was made to look for optimum conditions or to improve the straightforward measurements (38).

One recent innovation in electrochemical instrumentation is the use of digital readout for peak and limiting currents. This is particularly useful for routine analyses. Commercial instrumentation with digital readout is already available.

A serious shortcoming of electrochemical techniques in analysis is the lack of selectivity. Not by any stretch of the imagination can electrochemistry compete with, say, spectroscopy in selectivity. The effect of substituents on $E_{1/2}$'s, *etc.*, simply is not great enough to offer the differentiation of spectroscopic methods. Switching from more classical to modern techniques is of little value in most cases. However, the derivative techniques of Perone, Mueller *et al.* applied to peak voltammetry do help in this respect and, in addition, enhance sensitivity (39–41).

[2] Commercially available instruments of this type include those manufactured by Heath, Bendix, Beckman Instruments, and Chemtrix, among others.

Oxidation-Reduction Studies of Pharmaceutical Interest—This is the area in which the fast, dynamic electrochemical techniques really show promise—and should replace classical potentiometry and polarography. Many applications have already appeared in the literature and the review mentioned previously indicates quite a few (42).

Chronoamperometry, CV, and RDE techniques, with their special relevance to the measurement of ECE processes, are essentially new and distinct ways of eliciting fundamental information on the electron transfer characteristics of organic systems. In addition, they can be used to study quantitatively the interactions of either an oxidized or reduced state with its environment, information of fundamental interest about any molecule. A few examples will illustrate the potentialities.

How rapidly would you like to look at the interactions of a drug system with its environment? While you may need moderately fancy electronics (and know how to use them), if you follow Perone, you can apply CV as rapidly as 2000 v./sec. and study processes in the 100-μsec. time scale. Perone has coupled such techniques to flash photolysis (43). At slightly "slower" sweep rates of 100–500 v./sec. Perone and Kretlow (44) showed they could handle with confidence the fast (about 10^3 sec.$^{-1}$), irreversible hydration reactions coupled to the oxidation of ascorbic acid.

Using simple potentiostatic techniques and CV, as described earlier, one can oxidize any of the catecholamines and study their rapid cyclization to indole-like structures. Such studies may be criticized as having little relation to *in vivo* behavior of this important class of biogenic amines—since, for instance, adrenochrome formation is not observed in the body. However, the relative cyclization rates of adrenaline and noradrenaline (adrenaline is about 140 times faster than noradrenaline under comparable conditions) is perfectly consistent with the relative amounts of short-lived open-chain quinone involved in interactions with DPNH, *etc.* (45, 46). Using similar techniques one can oxidize catechols, producing the biologically important (but unstable) *o*-quinone structure, and study the rate of interaction with a variety of nucleophiles. Precise measurements of the rate of 1,4 additions of a series of amines and amino acids to electrolytically generated *o*-benzoquinone and 4-methyl-*o*-benzoquinone have been made (47). Davis and Orgeron (49) employed CV to determine ligand substitution rates in iron porphyrin complexes. Many more examples can be cited.

If one wishes to employ simple i_L measurements at the RDE for dimerization rates, treatments for rates up to about 10^3–10^4 l. mole^{-1} sec.$^{-1}$ are available (32). The most recent issue of *Analytical Chemistry* describes the determination of dimerization rates from a simple cyclic polarogram (48).

The remarks of this section are enthusiastic and optimistic and are clearly designed to be so. But they also are not intended to suggest electrochemistry as a pot of gold for the pharmaceutical chemist. However, one of the main interests in pharmaceutical chemistry is the interaction of drug systems with their environment. Modern electroanalytical techniques offer some exciting possibilities of studying these interactions and should, where applicable, be considered. There is no need to get too involved in electrochemical theory or instrumentation—the state of the art is well-developed and available in usable form.

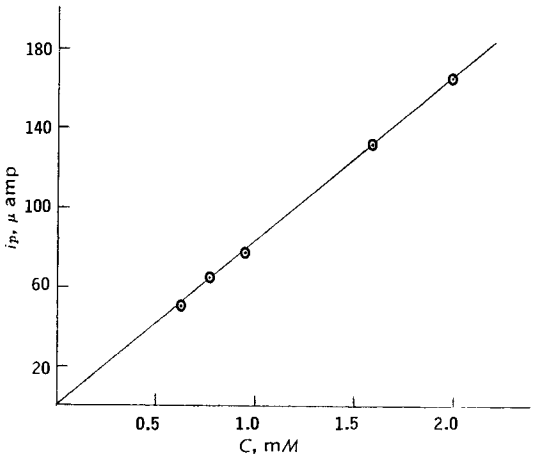


Figure 11—*Calibration curve for determination of mescaline by anodic peak voltammetry; in about 0.5* M *sodium sulfate-acetic acid solution, pH about 3, scan rate 4 v./min., carbon paste electrode.*

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RESEARCH ARTICLES

pH-Partition Behavior of Tetracyclines

JOHN L. COLAIZZI and PAUL R. KLINK*

Abstract □ The apparent partition coefficients between *n*-octyl alcohol and aqueous buffers (ranging from pH 2.1 to 8.5) were determined for several tetracyclines. Using the previously suggested microscopic dissociation constants for tetracycline, the relative amounts of each microscopic ionic form of tetracycline theoretically present at each pH were calculated. The zwitterionic form, −0+ (tricarbonyl methane system ionized, phenolic diketone moiety unionized, dimethylammonium cation postively charged), which was present in highest concentration in the pH range from 4 to 7, appeared to be the most lipid soluble form, its reduced polarity possibly resulting from an intramolecular type of ion-pair formation. Possible relationships between the biological activity of the various tetracycline analogs and their pH-octanol solubility profiles have been discussed.

Keyphrases □ Tetracyclines—pH-partition behavior □ Apparent partition coefficients—tetracyclines between *n*-octanol, aqueous buffers □ Biological activity relationship, tetracyclines—pH-octanol solubility profiles □ UV spectrophotometry—analysis

The work of Pindell *et al.* (1) has demonstrated that tetracycline is fairly rapidly absorbed from the duodenum of the dog and that some absorption can also occur from the stomach when gastric emptying is delayed. However, only a surprisingly small amount (3.1%) of the total administered dose was actually absorbed in 1.5 hr. The amounts of tetracycline absorbed were directly proportional to the dose over a tenfold range. These factors have suggested that tetracycline absorption is a passive diffusion phenomenon.

Structural modifications have been shown to alter the gastrointestinal absorption of the tetracyclines. For example, one recent study showed that the extent of minocycline absorption from the gastrointestinal tract of dogs was two to three times greater than with tetracycline (2). Doxycycline has been shown to produce nearly identical initial plasma levels as demethylchlortetracycline upon oral administration to fasting humans, even though the dose of the latter was three times as great (3). Structural modifications can likewise influence the renal clearance of tetracyclines. Although they are bound to protein to nearly the same extent, doxycycline has been shown to have a 12% of creatinine clearance in man while demethylchlortetracycline was shown to have a 27% of creatinine clearance in the same study (3). The general structural formula for the tetracycline analogs is shown as Fig. 1, and the structures of the analogs discussed in this paper are listed in Table I and explained in terms of the general structure in Fig. 1.

The tetracyclines are ionized throughout the physiological pH range, existing in cationic form at more acidic pH values, in anionic form at more alkaline pH values,