ELECTROANALYTICAL TECHNIQUES

1. Introduction

Electroanalysis employs electrochemical cells, the most common of which are batteries, consisting of two electrodes; an anode and a cathode. The principles that govern batteries and electroanalytically useful cells are the same. Electroanalytical cells typically have at least two electrodes: a working electrode and a reference electrode. The working electrode may serve as either an anode or a cathode, depending on the applied voltage, or as a source or sink for ion exchange, as in the case of ion-selective electrodes. The reference electrode invariably serves as a source or sink for ions at its interface with the solution, and as a source or sink for electrons at its interface with an external circuit even though it may not actually carry any current. The solution, or electrolyte in batteries, has a very high ionic strength in order to carry enormous amounts of current. In electroanalytical cells, the solution is generally the sample and must typically contain supporting electrolyte (electrochemically inert salts) that supports much lower current densities. Supporting electrolytes may also be used to define ionic strength for potentiometric measurements.

Cells useful for electroanalysis typically consist of two or more electrodes dipping into the solution to be analyzed. Sample solutions can range from water to blood, but can also include virtually all organic solvents. Analyte concentrations are usually in the picomolar to millimolar range. Numerous choices exist for working electrodes, ranging from electron exchangers to ion exchangers, including metals of many kinds (Pt, Au, Ag, and Hg), semiconductors (Si, Ge, and TiO_2) and plastics, ion exchangers, and sequesterers in poly(vinyl chloride) (PVC). Choices for reference electrodes are more limited. The problems involving reference electrodes are often more profound, and availability of the right reference electrode (1) may ultimately dictate the feasibility of an assay. They are inherently unstable and may drift, leak, become foul or plugged, and frequently need to be replaced.

Electrochemical measurements may be either active or passive, depending on whether or not a signal, typically a current or a voltage, must be actively applied to the sample in order to evoke an analytically useful response. Electro-

analytical techniques have also been divided into two broad categories, static and dynamic, depending on whether or not current flows in the external circuit (2). In the static case, the system is assumed to be at equilibrium. The term dynamic indicates that the system has been disturbed and is not at equilibrium when the measurement is made. These definitions are often inappropriate because active measurements can be made that hardly disturb the system at all and passive measurements can be made on systems that are far from equilibrium. The terms static and dynamic also imply some sort of artificial time constraints on the measurement. Furthermore, active and passive are terms that nonelectrochemists seem to understand more readily than static and dynamic.

2. Active Techniques

Active techniques are classified by the method of collection and display of data. If a voltage is applied to the cell and the resultant current measured and displayed as a function of time, the technique is called chronoamperometry (3). "Chrono" is a combining form from the Greek *chronos*, meaning "time". If a current is applied to the cell and the resultant voltage is measured and displayed as a function of time, the technique is called chronopotentiometry (4). Similarly, if current is measured and displayed as a function of applied potential, the technique is called voltammetry (5). Subcategorizing depends on such things as the geometry and size of the working electrode, its physical treatment, usually its rotation, and the functionality of the applied waveform. Therefore there is rotating disk voltammetry (4), cyclic voltammetry (5), pulse voltammetry (2), etc. Even postcollection treatment of the data may result in a renaming of the technique. Integration of the current, either during analogue data acquisition or digitally from computer stored currents, results in chronocoulometry (3). The functionality of the time response of the charge, measured in coulombs, provides the concentration of the electroactive species. If the electrode is small, eg, 0.01 cm^2 or so, the number of moles of the sample electrolyzed is insignificant relative to its total number of moles. Extremely large (>1 cm^2) electrodes may be used to completely electrolyze the solution, in which case the technique may be termed coulometry (6).

The question of what happens when an electrical signal is applied to an electrochemical cell needs to be answered with respect to the three components of the cell: the working electrode, the reference electrode, and the sample itself.

2.1. The Working Electrode. If the applied signal is a voltage and the intent is to measure a flow of current, the outcome depends on the sign and magnitude of the applied voltage and the chemistries of the solution and the electrode. If the electrode is a metal that appears high in the electromotive series of metals, eg, copper, mercury, or silver, and the voltage is positive with respect to the solution, then the metal may dissolve. On the other hand, mercury is an extremely useful metal for electroanalysis because at negative potentials metal ions in solution, such as Cu^{+}, Cu^{2+}, Cd^{2+}, Fe^{2+}, Fe^{+3}, Mn^{2+}, and Ni^{2+} may be reduced to the corresponding metals, which then dissolve in the mercury. If the dropping mercury electrode (DME) is used as the working electrode to replace the amalgam, and the current that flows during the reduction is monitored as a function of applied potential, then the technique is a form of

voltammetry called polarography (7). If only one drop is used, ie, a hanging mercury drop electrode (HME), then the metal ions of interest may be reduced at a suitably negative potential for a lengthy period (min) of time and then the potential may be linearly scanned in a positive direction to reoxidize the amalgam. This results in a series of current peaks appearing at different voltages on the voltammogram, each corresponding to a different species of metal ion in the solution. The maximum current of each peak is then indicative of the concentration of the corresponding ion. This technique is called anodic stripping voltammetry (8). Anodic means that the electrochemical process occurring at the surface of the electrode is oxidation. If reduction occurs, the electrode process is cathodic.

Polarography and related techniques are not confined to analysis of metal ions. Organic redox reactions can also be used for polarographic electroanalysis (9,10). The use of mercury has the advantage of high reproducibility. Each drop presents a new electrode surface to the solution, and anything that may have happened to the previous drop, such as the adsorption of product and concomitant fouling of the droplet surface, becomes inconsequential. The disadvantage of mercury usage lies in the relative ease with which mercury can be oxidized. Mercury has a small anodic limit, ie, the maximum positive voltage that can be applied without causing oxidation (dissolution), and other electrodes having more positive anodic limits are often more appropriate for use in organic solvents such as acetonitrile, dimethyl sulfoxide, methylene chloride, etc. More noble metals such as gold and especially platinum are useful for these purposes. The anodic limit in these cases is more often defined by oxidation of the solvent, not the electrode.

Charging Current. In most cases, application of a voltage to an electrode is intended to produce an analytically useful current that depends solely on the concentration of the analyte. Unfortunately, current flows even in the complete absence of the analyte. Thus, the current may have nothing to do with the electroactive species in the sample. This is charging current, and it must be circumvented or otherwise compensated.

The applied voltage injects charge (electrons) onto the electrode that must be balanced by charge (ions) in solution. The ions, present in the solution as supporting electrolyte, cannot approach the surface of the electrode any closer than their radii permit. This plane of closest approach, also called the inner-Helmholtz plane (IHP), defines one plate of a capacitor (Fig. 1). The electrode defines the other plate and solvent molecules adsorbed to the surface (most of the electrode's surface is covered by adsorbed solvent molecules) provide the dielectric material for the capacitor, which is called the compact double-layer capacitance. An OHP, defined by the line of centers of solvated ions, may also contribute to the compact double-layer capacitance. There is also a diffuse double-layer capacitance, which generally becomes important only at low ionic strength. This latter capacitance results from nonelectroneutral concentrations of anions and cations in solution that respond to the charge on the electrode, one being attracted and the other repelled. Additionally, there are diffusive forces. The higher concentration ion tries to diffuse from the electrode region back out into the solution; the lower concentration ion tries to diffuse in the opposite direction. The equilibrium between electrostatic and diffusive forces establishes the diffuse double-layer and associated capacitance. At the ionic strengths necessary for most electroanalyses,

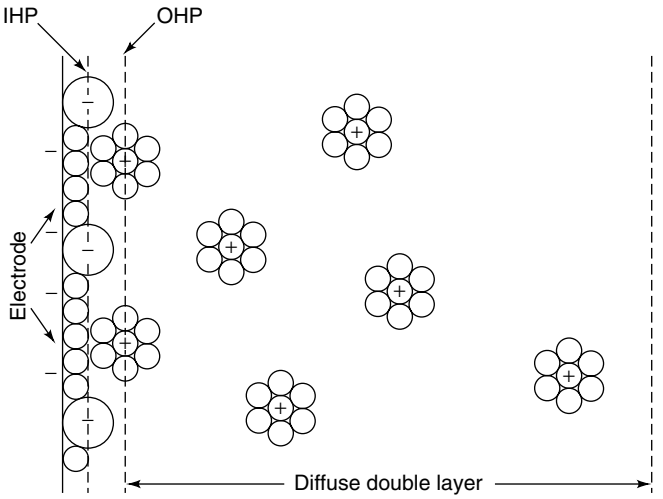


Fig. 1. Schematic representation of the electrochemical or diffuse double layer showing the IHP and outer-Helmholtz planes (OHP) and the electrode. The uncharged circles represent solvent molecules.

the electrostatic force overwhelms the diffusive forces. Thus the total double-layer capacitance, which is the sum of the two contributions, added together as capacitors in series, equals the compact double-layer capacitance.

From an electroanalytical point of view, the double-layer capacitance is a nuisance resulting in the charging current, which has no analytical value. The charging current must decay to zero, or be otherwise accounted for, before the analytically useful Faradaic current that results from electron exchange with electroactive species in the sample can be measured and used for calibration. Because the double layer is charged by ions that must move through the solution, the time constant for charging is the product of its capacitance (a few pF) and the resistance of the solution (typically 100 Ω). Charging can therefore be very fast. This consideration is important because there is a relationship between the speed with which a current can be measured and the precision and detection limit of the assay. Various pulse and square-wave techniques, eg, pulse voltammetry and square-wave voltammetry (11), are used to increase the rate of charging and therefore the precision, accuracy, and/or speed of the assay.

Faradaic Current. The double layer is a leaky capacitor because Faradaic current flows around it. This leaky nature can be represented by a voltage-dependent resistance placed in parallel with it and called the charge-transfer resistance. Basically, the electrochemical reaction at the electrode surface consists of four thermodynamically defined states, two each on either side of a transition state. These are (12): (*1*) oxidized species beyond the diffuse double layer and n electrons in the electrode; (*2*) oxidized species within the OPH and n electrons in the electrode, on one side of the transition state; (*3*) reduced species within the OHP; and (*4*) reduced species beyond the diffuse double layer, on the other.

The thermodynamics coupled with the Arrhenius activation theory suggest that the transition state surmounts a voltage-dependent activation energy barrier. The shape of this barrier is described by a parameter, α, that can have values between 0 and 1. If $\alpha = -0.5$ the barrier is symmetrical, if <0.5 it is skewed toward the reduced species, and if >0.5 it is skewed toward the oxidized species (13). The height of the barrier ultimately dictates the rate of charge transfer, and the value of the heterogeneous charge-transfer rate constant, k_s^0. These two parameters, α and k_s^0, may be used (at least empirically) to describe the charge-transfer process and the accompanying flow of current. If k_s^0 is large, the barrier is small and surface concentrations of oxidized and reduced species closely obey the Nernst equation, even though large currents may be flowing and the Nernst equation rigorously applies only to equilibrium conditions.

Smaller values of k_s^0 necessitate the application of voltages greater than those calculated from the Nernst equation to obtain a corresponding set of surface concentrations of electroactive species. These voltages are called overpotentials and indicate chemically related difficulties with the electrolysis. In other words, electron exchange between the electrode and the electroactive species is impeded by the chemistry of the process itself.

Surface concentration ratios of oxidized to reduced species relate directly to the analytically important current densities. In some cases, this problem can be solved by simply applying voltages large enough to accommodate the overpotentials. In other cases, they are too large to be accommodated, or larger voltages may result in spurious currents, breakdown of the solvent, or dissolution of the electrode. Even in the best of cases, overpotentials compromise the already inherently limited resolution of electroanalysis. Electrochemical reactions having small values of k_s^0 are said to be irreversible in a thermodynamic sense—the electron-transfer process is so inefficient that the Nernst equation is not obeyed during a flow of current. Processes that are irreversible on one time scale, however, can become reversible on a longer time scale because more time is allowed for the reaction to occur.

2.2. The Reference Electrode. The response of a reference electrode to an applied voltage depends critically on the magnitude of the applied voltage and the resultant current density, the duration of the application, and the nature of the reference electrode itself. Reference electrodes typically consist of a metal contacting one of its halide salts and a saturated potassium halide solution (of this same halide), separated from the sample solution by a salt bridge containing a high (3 *M* or more) concentration of an equitransferent salt such as KNO_3. The Ag–AgCl double junction reference electrode illustrated in Figure 2 is such an electrode. It is called a double-junction electrode because there are two *frits* separating two electrolytic solutions. Another popular electrode is the saturated calomel electrode (SCE) in which the silver is replaced by mercury, which is contacted by platinum for connection to the external circuit, and the silver chloride is replaced by a mixture of mercurous and mercuric chlorides, ie, calomel. The standard electrochemical reference electrode is the standard or normal hydrogen electrode (NHE), consisting of a platinum electrode contacted by a stream of hydrogen gas at 101.3 kPa (1 atm) and an aqueous solution of hydrochloric acid at unit activity.

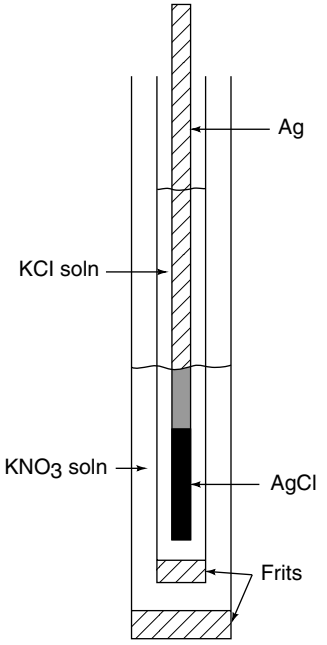


Fig. 2. Schematic of a silver–silver chloride reference electrode.

There are many variations of reference electrodes (1). For example the frits may be replaced by fibers, free-flowing capillaries, or Luggin probes (2,13), which are narrow tubes, often several centimeters long, through which the salt bridge solution flows. Although the junction must be free flowing, to provide a stable reference potential, the flow rate should be made as small as possible to avoid depletion of the internal filling solutions and/or contamination of the sample. Obviously, the reference electrode eventually contaminates a freestanding sample solution and depletes itself of some of its ingredients. This inherent instability precludes many process control applications for which long-term stability is essential, but instability is not so great a problem for most laboratory applications.

Reference electrodes can cause trouble even in the absence of applied potentials and the effects of applied voltages and resultant currents are much less subtle. The Ag–AgC1 electrode (Fig. 2) is a case in point. A positive voltage that results in a prolonged flow of current can eventually convert all of the submerged silver into silver chloride or deplete the concentration of KC1. A negative voltage eventually dissolves the silver chloride coating, which is generally produced by anodization of the silver wire in a chloride solution, and precipitates KC1 from its saturated solution. Saturated KC1 solutions are not always used, in which case the chloride concentration of this solution may rise noticeably. In any event, the change in composition of the reference electrode results in a change in its reference potential. The instability can be trivial when current densities are low and the current flows for only brief periods of time, or severe when prolonged electrolyses are involved.

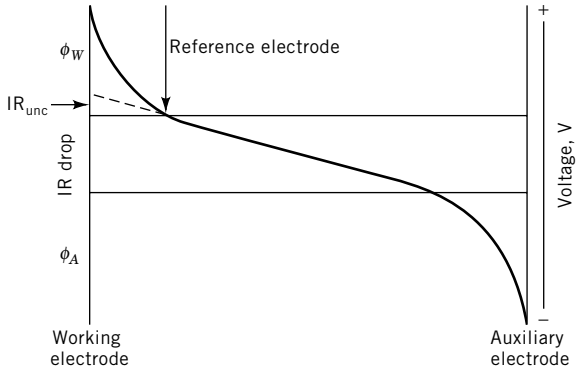


Fig. 3. Voltage distributions and the three-electrode system. Terms are defined in the text.

One solution to reference electrode instability is the introduction of a third or auxiliary electrode, intended to carry whatever current is required to keep the potential difference between the working and reference electrodes at a specified value. Virtually all potentiostats (instruments designed specifically for electrochemistry) have this three-electrode configuration. Its use is illustrated in Figure 3.

Whereas the terms "voltage" and "potential" have been used almost interchangeably, voltage is the number of volts applied between the auxiliary and working electrodes of the electrochemical cell to maintain a flow of current. Potential, ϕ, is the number of volts (usually millivolts) that drop between any two regions of the cell. The parameter ϕ_ω is the potential difference between the working and reference electrode, which is placed as close to the working electrode as possible in order to minimize the uncompensated IR drop (IR_{unc}) that results from current flow, I, through the solution resistance, R. The IR drop and the potential drop at the auxiliary electrode are unimportant except for the practical consideration that the potentiostat must be able to apply the correct voltage to the cell to maintain the correct potential at the working electrode.

The three-electrode system serves two important purposes. Because the reference electrode carries no current, but merely measures a potential relative to the working electrode, its stability is not unduly influenced by the electrolysis. Furthermore, because it is placed close to the working electrode the measured potential difference is more nearly representative of the true potential difference between the working electrode and the sample solution: the significant quantity in electroanalysis.

The resolution of electroanalysis is inherently limited. The entire potential range spanned, from anodic to cathodic limit, cannot be >6 V and even this range can only be covered using a platinum electrode and noxious nonaqueous solvents like liquid ammonia and sulfur dioxide. "Diamond Electrodes" are being introduced that may extend this range a bit more, but not so much as to alter conclusions (14). Examination of the Nernst equation reveals that a $\pm$0.1-V swing about the E° of a redox couple results in a 50:1–1:50 swing in the ratio of

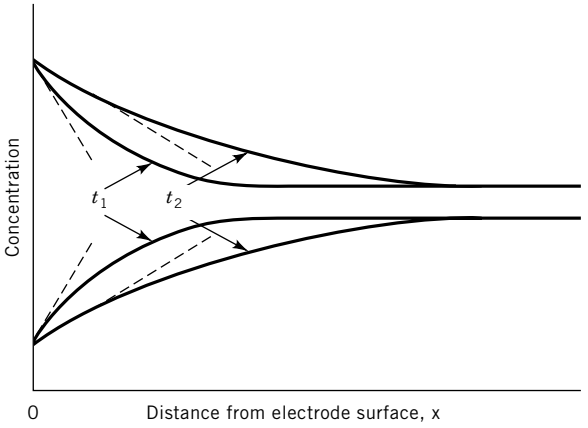


Fig. 4. Concentration profiles during electrolysis of unstirred solutions, where t_1, and t_2 represent two different times.

the oxidized to reduced species activities. The total resolution >6 V is therefore $\sim 6/0.2 = 30$ redox couples or 1 part in 30 resolution. In aqueous solutions the span is usually only ~2 V, so the resolution is 1 part in 10. Viewed in this context a millivolt is a fairly large quantity, and the precise control of applied potentials, within a few millivolts, may be critical to the success of an electroanalysis. This findings is especially true if large electrolyses are involved because high current densities result in IR drops that move the applied voltage (Fig. 4) far away from the desired potential difference, ϕ_ω. The proper selection and use of the reference electrode, and the importance of the three-electrode system, should therefore not be underestimated.

Whereas electrochemistry has poor resolution, virtually every other analytical technique except chromatography, which has lower sensitivity in the form of column or paper chromatography, suffers from lack of resolution when quantifying the analysis of one component in a mixture. The resolution of spectroscopic techniques is also limited and resolving more than two or three components in a complex solution is difficult unless the wavelength range is enormous. Chemometrics has found considerable application in this area (15). Atomic absorption spectroscopy, which can resolve a multiplicity of elements, is an exception. The development and use of hyphenated techniques in which the higher resolution of chromatography is married to the sensitivity of another technique, is intended to obtain separation, identification, and quantification of the analyte. Such an electroanalytical system is liquid chromatography with electrochemical detection (lcec)(2). The lcec detection is extremely versatile, taking advantage of the resolution of chromatography and the high precision of electroanalysis.

2.3. The Solution. The responses of working and reference electrodes to applied voltages are important because they are indicative of what goes on in the solution, or at the solution/electrode interface. The distinction between bulk (solution) and interfacial events is basically the distinction between chemical kinetics and charge-transfer kinetics.

Coulometry. If it can be assumed that kinetic nuances in the solution are unimportant and that destruction of the sample is not a problem, then the simplest technique may be to apply a potential to a large working electrode (surface area of several cm^2) and wait until the current decays to zero. The potential should be sufficiently removed from the E° of the analyte, ie, ~200 mV, that the analyte is completely electrolyzed, but electrolysis of an interferent is avoided. The integral under the current versus time curve is a charge equal to $nFCV$, where n is the number of electrons needed to electrolyze the molecule, C is the concentration of the analyte, V is the volume of the solution, and F is the Faraday constant.

Coulometry (6) is not usually the technique employed. Even in the absence of kinetics, the several minutes or hours required for the electrolysis seems excessive and destruction of the sample is not a desirable result. Furthermore, Coulometric precision can be exceptionally poor at lower concentrations, and currents almost never decay to zero because of the presence of trace contaminants. One has to decide when zero current has been obtained.

Chronoamperometry. In the more general case, a voltage is applied at time zero, the current is ignored for a few microseconds while the charging current decays, and then measured for perhaps several seconds while only that small part of the total concentration adjacent to the surface of a microworking electrode is electrolyzed. For this approach, the diameter of the working electrode is typically only a millimeter or so. The currents that flow are only in the microampere range but can nevertheless be easily detected and resolved. According to the Cottrell equation (eq. 1):

$$i = \frac{nFCD^{1/2}}{(\pi t)^{1/2}} \tag{1}$$

plots of current density, i, versus the inverse of the square root of time, t, should be linear with a slope proportional to the concentration of the analyte, C. D is the diffusion coefficient of the analyte. Equation 1 is a solution to Fick's second law of diffusion, having appropriate semi-infinite boundary conditions (2,3,5,13). This technique is chronoamperometry (3).

The chronoamperometric technique illustrates the principle that analytically useful current responses depend critically on the efficiency of analyte mass transport within the solution. The analyte mass transport in turn depends on the efficiency with which an applied voltage can maintain the surface concentrations of oxidized and reduced species at values specified by the Nernst equation. It is generally the case in chronoamperometry that the bulk concentration of one of the species is zero whereas the surface concentration of the other species is forced to zero by the applied potential, but this is not always so.

The situation illustrated in Figure 4 allows both species to coexist. Either of the two sets of curves can be considered the oxidized species; the other is the reduced species, depending on whether oxidation or reduction is occurring at the surface. Assume that the upper curve is the reduced species and the lower curve is its oxidized form. An applied voltage has maintained fixed surface concentrations for some period of time including t_1 and t_2. The concentration profile

of the oxidized species decreases at the electrode surface (0 distance) as it is being reduced. Electrolysis therefore results in an increase in the concentration of reduced species at the surface. The concentration profiles approach bulk values far from the surface of the electrode because electrolysis for short times at small electrodes cannot significantly affect the concentrations of species in large volumes of solution.

In this context, the relative terms far, short, small, and large can be defined as follows. Fick's second law of diffusion dictates that the distance, δ, that a species having a diffusion coefficient, D, may diffuse within a period of time, t, is given by (13):

$$\delta = (2Dt)^{1/2} \tag{2}$$

Aqueous diffusion coefficients are usually on the order of 5×10^{-6} cm^2/s. A second is typically a long time to an electrochemist, so $\delta \approx 30$ μm. The definition of "far" is then 30 μm. "Short" is less than a second, perhaps a few milliseconds, but microseconds are not uncommon. Small, referring to the diameter of the electrode is about 1 mm for microelectrodes, or perhaps only a few micrometers for ultramicroelectrodes (16), that have opened a whole new field of electroanalysis known as "Psychoanalytical Electrochemistry." A 200-μm carbon working electrode has been inserted into the brain of a rat and used to monitor dopamine as the rat self-administers cocaine (17). The oxidation of dopamine and the reduction of dopamine-*o*-quinone are observed as the potential is scanned back and forth between -0.4 to $+1.0$ V every 100 ms at 300 V/s.

Small electrodes allow faster measurements and are therefore very popular with electrochemists. Large solution volumes are typically a few tens of milliliters, but electroanalysis can be performed in drops as small as 10 μL or less because 30 μm times 1 mm^2 equals only 3% of a 10-μL drop. Preferred analyte concentration ranges are anywhere from subpicomolar to maybe 10 mM, depending on the technique employed.

The concentration gradients at the surface, represented by the dashed lines in Figure 4, are proportional to the current densities measured at the applied voltage

$$i = nFD_{\mathrm{o}} \frac{\partial C_{\mathrm{o}}}{\partial x}\bigg|0 = -nFD_{\mathrm{r}} \frac{\partial C_{\mathrm{r}}}{\partial x}\bigg|_0 \tag{3}$$

where C_0, D_0, and C_r, D_r represent the concentration and diffusion coefficients of the oxidized and reduced species, respectively. These gradients change with the square root of time as diffusion progresses within the time interval that includes t_1 and t_2. This phenomenon affords an enormous benefit at short times, because the gradients are initially huge. In principle, they are infinite at $t = 0$ when the voltage is first applied, resulting in infinite current flow according to equation 1. In practice, the current is limited by solution resistance and contains the charging current contribution discussed previously. One must then wait the millisecond or less needed to ensure the appropriateness of equation 1 before processing the data. The fact that both profiles are involved, even if the bulk concentration

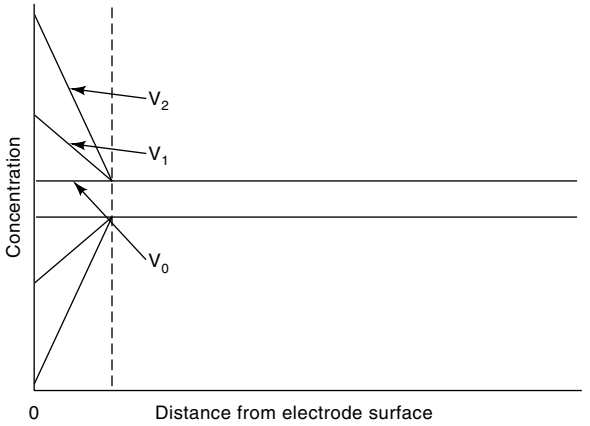


Fig. 5. Concentration profiles at a rotating electrode or in a stirred solution at applied potentials V_0, V_1, and V_2 where the dotted line represents the Nernst diffusion layer.

of the product is zero, leads to the responses seen in cyclic voltammetry (2,5) and the various kinds of pulse voltammetries (2,11). The currents driven by pulsed voltages are higher and thus more analytically useful.

Voltammetry. Diffusional effects, as embodied in equation 1, can be avoided by simply stirring the solution or rotating the electrode, eg, using the rotating disk electrode (RDE) at high rpm (4,8). The resultant concentration profiles then appear as shown in Figure 5. A time-independent Nernst diffusion layer having a thickness dictated by the laws of hydrodynamics is established. For the RDE,

$$\delta = 1.61 D^{1/3} \omega^{-1/2} \nu^{1/6} \tag{4}$$

where ω is the radial frequency of rotation and ν is the kinematic viscosity. If $\nu = 0.01$ cm^2/s, $\omega = 1000$ s^{-1}, and $D = 5 \times 10^{-6}$ cm^2/s, then $\delta = 5$ μm, corresponding to the diffusion layer thickness calculated from equation 2 with $t \cong 30$ ms. However, a steady-state current is measured because stirring maintains static concentration profiles, which may be assumed to be linear within the Nernst diffusion layer. The steady-state currents are therefore directly proportional to the concentration difference between the surface and the bulk, and depend solely on applied potential (ϕ_ω) (see Fig. 3). The resultant voltammogram is given by

$$E = E_{1/2} + \frac{RT}{nF} \ln \frac{(i_{l,c} - i)}{(i - i_{l,a})} \tag{5}$$

where

$$E_{1/2} = E^{0\prime} - \frac{RT}{nF} \ln \left(\frac{m_o}{m_r} \right) \tag{6}$$

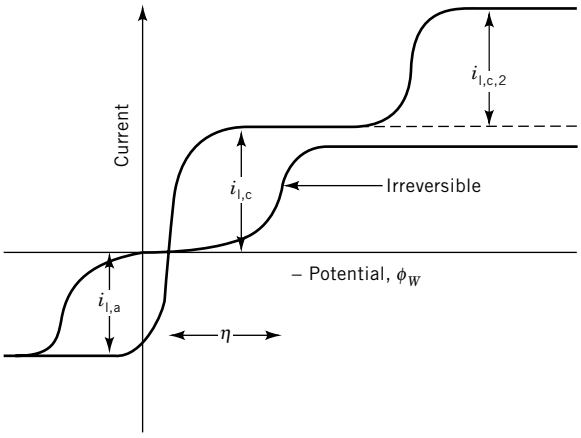


Fig. 6. Voltammogram showing both the reversible and irreversible regions. Terms are defined in the text.

cathodic and anodic current limits are given by $i_{l,c}$ and $i_{l,a}$, respectively; R is the ideal gas constant, T is the absolute temperature, and $E^{\circ\prime}$ is the formal potential of the redox reaction, ie, the E° uncorrected for activity coefficients; m_0 and m_r are mass-transfer coefficients (13) for the oxidized and reduced species, respectively, and equal to D/δ. δ depends on the mass-transport properties of the particular technique under consideration (eqs. 2 and 4). For the convective mode of mass transport considered here, eg, $(m_o/m_r) = (D_o/D_r)^{2/3}$, but for the diffusional modes used in polarography and cyclic voltammetry $(m_o/m_r) = (D_0/D_r)^{1/2}$.

Equation 5 is illustrated schematically in Figure 6. Two additional features to further demonstrate the essential characteristics of this technique are also included. First, there is a second redox couple having a limiting cathodic current of $i_{l,c,2}$. Second, an irreversible response is included to illustrate the overpotential, η, discussed previously. The parameter $E_{1/2}$ is the potential corresponding to the average of $i_{l,c}$ and $i_{l,a}$ (see eq. 6) and the potential corresponding to zero current flow is the equilibrium potential. Potential increases negatively (cathodically) to the right in the diagram, so cathodic (reductive) currents are positive. Figure 6 also applies to polarograms, measured at the DME, except that the dropping mercury modulates the current, resulting in a characteristic pattern (7). The drops of mercury essentially stir the solution. Polarography is also closely related to chronoamperometry at an expanding plane (the mercury drop) for a linearly varying voltage.

The difference between $i_{1,c}$ and $i_{1,a}$ in Figure 6 is a measure of the total concentration of the first redox couple. The parameter $i_{l,c,2}$ is a measure of the total concentration of the second redox couple. The equilibrium potential provides the two ratios of oxidized to reduced species according to the Nernst equation. From all this information the concentrations of both oxidized and reduced species can be determined. In this case the E° values of the two species are so far apart that their voltammograms are well resolved. The second redox couple must be wholly in its oxidized form whereas the first must be in a ratio equal to the ratio of

the absolute values of its limiting currents, corrected by the mass-transfer coefficients.

Two types of problem are illustrated by the curves corresponding to the irreversible response. The obvious problem is that the overpotential of one redox couple may smear into the voltammogram of another, complicating the analysis by compromising resolution. A less obvious problem has to do with the possibility of associated homogeneous (solution) kinetics. Assume that one wishes to use the first voltammogram to determine the concentration of the first redox couple, but the species providing the irreversible voltammogram is present in the sample. A likely choice of potential would be just before the cathodic limiting current, $i_{l,c}$, because the irreversible component is not reduced there at the surface of the electrode. Unfortunately, this results in a catalytic current or what electrochemists call an EC′ reaction. Just because the reduction of a species may be irreversible at an electrode surface does not mean that its reduction does not occur in solution. In the present case, the oxidized species that can be reduced electrochemically at the electrode surface may reduce the irreversible species in a following chemical reaction and thus be regenerated for further reduction at the electrode.

Examples of such irreversible species (13) include hydroxylamine, hydroxide, and perchlorate. The electrochemistries of dichromate and thiosulfate are also irreversible. The presence of any of these agents may compromise an analysis by generating currents in excess of the analytically useful values. This problem is avoided if the chemical reaction is slow enough, or if the electrode can be rotated fast enough so that the reaction does not occur within the Nernst diffusion layer and therefore does not influence the current.

Not all electroanalytical difficulties are confined to the measurement of currents. The presence of metal complexing agents has a profound effect on the selection of electrolysis potentials. Ligands decrease the activity of at least one of the redox species. Consider the polarographic reduction of a metal ion, M^{+n} at a DME in the presence of a complexing ligand, L, assuming reversibility, so the Nernst equation is obeyed. The parameters M and L obey the equilibrium,

$$\mathrm{M} + p\mathrm{L} \rightleftharpoons \mathrm{ML}_p$$

with an instability constant, K. Equation 5 is obeyed but the half-wave potential, given by equation 6, becomes (13)

$$E^C_{1/2} = E^M_{1/2} + \frac{RT}{nF}\ln K - p\,\frac{RT}{nF}\ln \mathrm{L} + \frac{RT}{nF}\ln\left(\frac{m_\mathrm{M}}{m_C}\right) \qquad (7)$$

where $E^C_{1/2}$ and $E^M_{1/2}$ are the half-wave potentials of the complex and free metal, respectively, and m_M and m_C are the corresponding mass-transfer coefficients. The results of equation 7, which can be used under favorable circumstances to determine p and K, may be a blessing or a curse. It is a curse if the intention is to measure the metal-ion concentration because the presence of the ligand shifts the half-wave potential cathodically and the complex is harder to reduce.

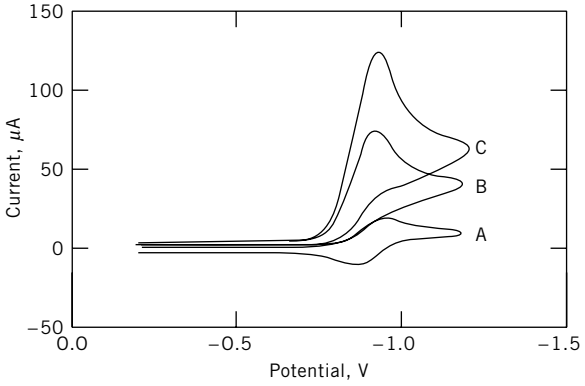


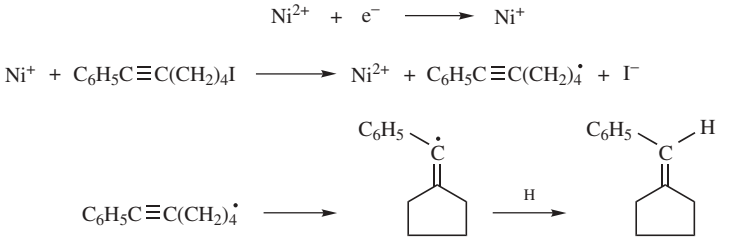
Fig. 7. Cyclic voltammograms for the reduction of 1.0 m*M* [2,2′-ethylene-bis(nitrilomethylidyne)diphenolato]nickel(II) in dimethylformamide (dmf) at a glassy carbon electrode, in A, the absence, and B and C the presence of 2.0 and 5.0 m*M* 6-iodo-1-phenyl-1-hexyne, respectively (18).

On the other hand, the addition of a specific ligand to the sample solution may remove interferents by shifting the half-wave potentials beyond that required for the analyte.

Voltammograms such as those shown in Figure 6 can be considered static because a steady-state current is measured at any given voltage. Cyclic voltammetry, on the other hand, is a dynamic technique because currents depend not only on applied voltages but also on the way the voltages are applied (sweep rates). Voltages are applied linearly as a function of time in one direction and then reversed or cycled back in the other direction. The result is a response like curve A in Figure 7, for the reduction of a Ni(II) complex (18). As the potential is swept negatively from −0.2 V, referenced against a saturated cadmium amalgam reference electrode, at 100 mV/s, the current initially rises because the electroactive species is reduced. The current decays, in accordance with equation 1, because the potential is so negative that the surface concentration of the species is held at zero and the current becomes diffusion controlled. The potential is reversed at −1.2 V and swept in the opposite direction, resulting in the reoxidation of the reduced form of the species that remains near the surface of the electrode. The solution is not stirred or disturbed in any way.

In the presence of 6-iodo-1-phenyl-1-hexyne, the current increases in the cathodic (negative potential going) direction because the hexyne catalytically regenerates the nickel(II) complex. The absence of the nickel(I) complex precludes an anodic wave upon reversal of the sweep direction; there is nothing to oxidize. If the catalytic process were slow enough it would be possible to recover the anodic wave by increasing the sweep rate to a value so fast that the reduced species [the nickel(I) complex] would be reoxidized before it could react with the hexyne. A quantitative treatment of the data, collected at several sweep rates, could then be used to calculate the rate constant for the catalytic reaction at the electrode surface. Such rate constants may be substantially different from

those measured in the bulk of the solution. The chemical and electrochemical reactions involved are



It is important to understand the fundamental electrochemistries of analytes before attempting electroanalysis. The usual approach is to perform electroanalyses so quickly that kinetic events do not have a chance to occur before charge transfer (electrolysis) has provided a response that is unequivocally related to the concentration of the analyte. Pulse techniques figure prominently into this principle. See Ref. 11 for a highly useful approach to this problem.

Active electrochemical techniques are not confined to pulse and linear sweep waveforms, which are considered large amplitude methods. A-C voltammetry, considered a small amplitude technique because an alternating voltage <10 mV is applied to actively couple through the double-layer capacitance, can also be used (19). An excellent source of additional information concerning active electroanalytical techniques can be found in Ref. 20–22. Reference 22, although directed toward clinical chemistry and medicine, also contains an excellent review of electroanalytical techniques.

3. Passive Techniques

Perhaps the most precise, reliable, accurate, convenient, selective, inexpensive, and commercially successful electroanalytical techniques are the passive techniques, which include only potentiometry and the use of ion-selective electrodes, coated wire electrodes, and ion-selective field effect transistors (ISFETs) either directly or in potentiometric titrations. Whereas these techniques receive only cursory or no treatment in electrochemistry textbooks, the subject is regularly reviewed and treated (23–26). Reference (26) is especially recommended for novices in the field. Additionally, there is a journal, *Ion-Selective Electrode Reviews*, devoted solely to the use of ion-selective electrodes.

According to the definition, a passive technique is one for which no applied signal is required to measure a response that is analytically useful. Only the potential (the equilibrium potential) corresponding to zero current is measured. Because no current flows, the auxiliary electrode is no longer needed. The two-electrode system, where the working electrode may or not be an ion-selective electrode, suffices.

The equilibrium potential may be forced to change by the addition of a titrant, as in potentiometric titrations employing nonspecific working electrodes. But equilibrium potential can also be analytically useful in its own right if the

response of the electrode is highly selective to the analyte. Chemically modified electrodes (27) are being researched that may provide this high selectivity, eg, for use in active techniques, such as the glucose sensor (28,29). However, ion-selective electrodes, of which the pH glass electrode is probably the most common example, dominate applications involving high selectivity even though their response mechanisms have nothing to do with oxidation and reduction. Potentiometry employing ion-selective electrodes without the addition of titrants is termed direct potentiometry.

3.1. Direct Potentiometry and Ion-Selective Electrodes. The Ag–AgCl reference electrode illustrated in Figure 2 may be taken as representative of reference electrodes in general. The difference in potential between this electrode and a working electrode forces a current flow that eventually discharges the cell like a battery if the two are shorted together. In practice, however, little or no current is allowed to flow during a potentiometric measurement because of the essentially infinite input impedance of the pH or millivolt meter used to measure the potential difference between them. It is convenient, therefore, to think of the current that would flow as a virtual current. The question then becomes one of what might carry virtual current. Electrons would obviously carry current through the external circuit. In the case of the Ag–AgC1 electrode an electron that enters the reference electrode would reduce AgCl to free Ag^0 and release Cl^- into the adjacent (reference) solution. Conversely, an electron removed from the reference electrode would result in the oxidation of Ag^0 to Ag^+, which would precipitate Cl^- from the reference solution as AgCl. In either case, Cl^- carries the current across the electrode/reference–solution interface.

Current flow through the frits is supported by ions. Both cations and anions support the virtual current by flowing in opposite directions, and the transference number of a particular ion is defined as the fraction of the total current it carries. The sum of all transference numbers is necessarily unity. If the fraction of the virtual current carried by the cations equals the fraction carried by the anions, then the solution is said to be equitransferent.

It is important that a junction of equitransferent ions be present between the sample and reference solutions in order to minimize junction potentials, which result when ions of opposite sign are diffusing in the same direction. The free (very slowly) flowing junctions (30) at the frits in Figure 6 can generate junction potentials if the anions and cations have unequal mobilities. The faster ion tends to get ahead of the slower one, causing the generation of a potential that slows down the fast one and speeds up the slow one until the two move with equal velocities, thus ensuring electroneutrality. Ions are equitransferent if the mobilities are equal. Thus junction potentials are minimized by equitransferent ions. "Minimized" generally means variation between samples of <0.25 mV, because a 0.25-mV error in potential measurement corresponds to a 1% error in predicted concentration (activity). This value can be derived by taking the derivative of the Nernst equation and allowing 100 da/a = % error, where a is the activity of the potential determining ion.

The construction of an ion-selective electrode, shown schematically in Figure 8, is similar to that of the reference electrode shown in Figure 2. Basically, the equitransferent frit has been replaced by an ion-selective membrane and the KNO_3 solution has been eliminated. This membrane is permselective if ions of

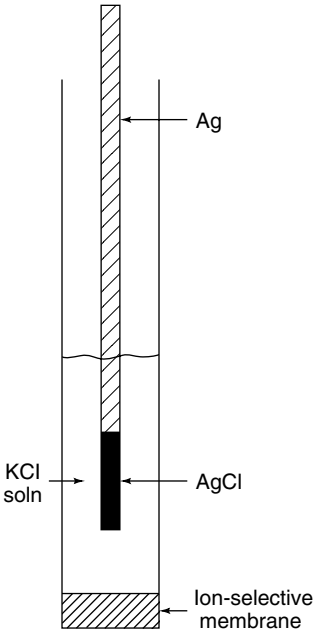


Fig. 8. Schematic of an ion-selective electrode.

only one sign can carry the virtual current. If only one particular ion can carry the virtual current, then the electrode is said to be specific to that ion. Virtually all electrodes are selective, not specific, meaning that a number of ions having the same signs are sensed, but not with equal facility. Selectivity coefficients determine the degree to which ion-selective electrodes may generate potentials in response to the presence of different ions. Simple thermodynamic arguments reveal that these potentials are Nernstian such that there are 59/z mV per decade change in activity at room temperature if only one ion of charge z is being sensed. If several ions are sensed, it is customary to apply the Nicholskii equation,

$$E = E^{0\prime} + \frac{RT}{F} \ln \left[a_i + \sum_j K_{i,j} a_j \right] \tag{8}$$

where a_i is the activity of the analyte ion, $K_{i,j}$ is the selectivity coefficient of the ith ion in the presence of the jth interferent, and a_j is the activity of the jth interferent (24). Umezawa (31) has tabulated selectivity coefficients, however, Bakker has done some revolutionary work that extends the application of ion-selective electrodes to measurements in mixed-ion solutions using equations that are very different from equation 8. His group has also demonstrated the use of dynamic techniques that extend measurements to detection limits that are substantially below a picomolar. These techniques involve counterflow of ions from the sample back into the inner-filling solution. In addition, they have

demonstrated the advantages of applying voltages to ion-selective electrodes, essentially using them as active rather than passive devices. Their work is summarized in Ref. 32.

Although the theory of ion-selective electrode responses has been presented in terms of transference, the selectivities of these electrodes have more to do with ion-exchange constants than with the mobilities of ions within their interiors (24,25). For example, protons do not carry virtual current through pH-selective glass membranes. Basically, an electrode is more selective to one ion than to another because the membrane can accommodate the presence of the one ion better than the other. In fact, Bakker (32) relies heavily upon this principle in his derivation of a "Phase Boundary Potential Model" that essentially neglects transport of ions through the membrane proper. He treats only the potential difference at the electrode/solution interface. In any event, the selectivities of ion-selective membranes may be attributed to cavities that have just the right size, and perhaps shape, to accommodate a given ion. These cavities are completely filled with specific (analyte) ions where the charges are compensated by sites of opposite sign that are confined within the membrane and, ideally, cannot enter the sample solution. These sites provide the membrane with permselectivity by electrostatically repelling ions of like sign. A potential difference is generated at one surface of the membrane when an ion is extracted from its interior into the sample solution, thus generating a charge separation across the double-layer capacitance and a corresponding potential. Samples more concentrated in the analyte tend to extract fewer analyte ions from the membrane, so the charge separation is less and the potential difference is lower. If the membrane is cation selective, its surface becomes less negative, and therefore more positive as the concentration of cations is increased in the sample solution. Large selectivity coefficients imply severely interfering ions that can extract analyte ions from the membrane by ion exchange, thus compromising the charge separation and rendering the interfacial potential difference less analytically useful.

Of interest is the manner in which cavities of the appropriate size are introduced into ion-selective membranes. These membranes typically consist of highly plasticized PVC. Plasticizers are organic solvents such as phthalates, sebacates, trimellitates, and organic phosphates of various kinds, and cavities may simply be the excluded volumes maintained by these solvent molecules themselves. More often, however, neutral carrier molecules (24) are added to the membrane. These molecules are shaped like donuts with holes that have the same sizes as the ions of interest, eg, valinomycin [2001-95-8], $C_{54}H_{90}N_6O_{18}$, and nonactin [6833-84-7], $C_{40}H_{64}O_{12}$, or have wrap-around structures like methyl monensin that have just the right length to legate selectively with the analyte. Although efforts are under way to bind these molecules to membranes, thus removing the neutral carrier feature, it has been pointed out repeatedly that the molecule must be able to physically carry the analyte ion between the two contacting solutions, ie, the sample and refs. 24,33,34. Recent results suggest that carrier molecules, also known as "ionophores", may be bound to the polymer and still transport ions, however, and there is some evidence to suggest that this notion of binding is not so far fetched after all (35–38). This would be good news because leaching of the ionophore out of the membrane upon prolonged use is a common failure mechanism that can be avoided by anchoring it in place.

Ion-selective electrodes are available for the electroanalysis of most small anions, (eg, halides, sulfide, carbonate, nitrate), and cations, (eg, lithium, sodium, potassium, hydrogen, magnesium, calcium), but with varying degrees of selectivity. The most successful uses of these electrodes involve process monitoring, (eg, for pH), where precision beyond the unstable reference electrode's ability to deliver is not generally required, and for clinical applications, eg, sodium, potassium, chloride, and carbonate in blood, urine, and serum. A recent development (39) is the demonstration that ion-selective field effect transistors, specifically the "DuraFET", manufactured by Honeywell, is an extraordinarily stable alternative to the glass electrode for pH measurements. Applications where the breakage of glass might be problematical, such as the food industry, and where high temperatures are used for processing should benefit greatly from this development. ISFETs are essentially hybrid electrodes in which the input field effect transistor of the measurement device is incorporated directly into the sensor. Reference 40 is a good reference for learning more about ISFETs.

Ion-selective electrodes can also become sensors for gases such as carbon dioxide, ammonia, and hydrogen sulfide by isolating the gas in buffered solutions protected from the sample atmosphere by gas-permeable membranes. Typically, pH glass electrodes are used, but electrodes selective to carbonate or sulfide may be more selective.

Electrodes may also be rendered selective to more complex analytes using enzyme or other overcoats. The enzyme converts the analyte into a detectable ion or gas. Glucose and blood urea nitrogen sensors can be made in this way.

3.2. Potentiometric Titrations. If one wishes to analyze electroactive analytes that are not ions or for which ion-selective electrodes are not available, two problems arise. First, the working electrodes, such as silver, platinum, mercury, etc, are not selective. Second, metallic electrodes may exhibit mixed potentials that can arise from a variety of causes. For example, silver may exchange electrons with redox couples in solution, sense Ag^+ via electron exchange with the external circuit, or tarnish to produce pH-sensitive oxide sites or Ag_2S sites that are sensitive to sulfide and halide. On the other hand, anodization of the silver electrode produces the very stable Ag–AgCl electrode, which responds selectively to Cl^-, albeit with interference by sulfide and the other halides, for use not only in reference electrodes, but also for direct potentiometry.

In a titration, the analytical utility of the measured potential lies not in its value, which may drift or be otherwise unstable, but in the magnitude of the change of its value near an endpoint. In a redox titration, the potential changes from something close to the E° of the analyte to something close to the E° of the titrant. This works fine provided the electrochemistries of both analyte and titrant are reversible. The technique may fail, however, if the electrode responds slowly to concentration changes because of irreversibility.

The Karl Fischer jack on the back of most pH meters, used to monitor Karl Fischer titrations, supplies a constant regulated current to the cell, which can consist of two identical (platinum) working electrodes. The voltammograms shown in Figure 9 illustrate the essential features of this technique. The initial potential difference, ΔE°, is small because both redox forms of the sample coexist to depolarize the electrodes. The sample corresponds to the wave on the

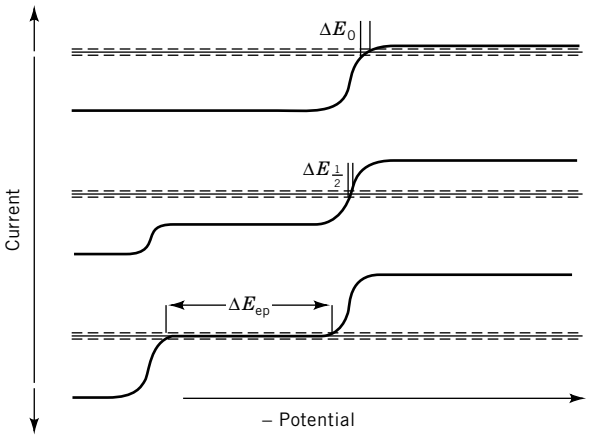


Fig. 9. Voltammograms demonstrating a potentiometric titration using dual-polarized electrodes, where the dashed lines indicate the anodic and equal-but-opposite cathodic currents that must be carried by the two opposing electrodes during the titration. Terms are defined in the text.

right-hand (cathodic) side of each figure and is therefore easily oxidized. The titrant is represented by the wave on the left-hand (anodic) side and is therefore easily reduced. Halfway to the end point the potential difference, $\Delta E_{1/2}$, remains small, but at the end point the potential difference, ΔE_{ep} is large, nearly equal to the standard potential difference, ΔE°, between the sample and titrant redox couples. The shape of the titration curve depends on the irreversibilities of the analyte and titrant, but the large potential difference at the end point is analytically useful nevertheless. An example is the oxidation of Fe^{2+} by Ce^{4+}, found in Ref. 41.

4. Static and Dynamic Measurements

The definition herein of static electroanalytical measurements implies a time independent response, regardless of whether or not that response is generated by an applied signal. If an applied signal is needed, the method is active; if not, it is passive. These terms should not be confused with other definitions (1), where static more or less equates to passive and dynamic to active. This difference can be illustrated by several examples of dynamic measurements that can be either active or passive. First there is the batch injection analysis (BLA) technique (42). Basically, an electrode is placed upside down in a beaker of supporting electrolyte so that small aliquots of the sample can be injected onto its surface. A signal, measured during the residency of the sample, rapidly decays as the sample dissipates into the supporting electrolyte, which is stirred. Peak responses correlate well to sample concentrations. The term dynamic measurements is used for this technique. Both modified and unmodified carbon-paste electrodes were used amperometrically to demonstrate what are called active measurements herein. A silver–silver chloride ion-selective electrode is used to illustrate a passive approach.

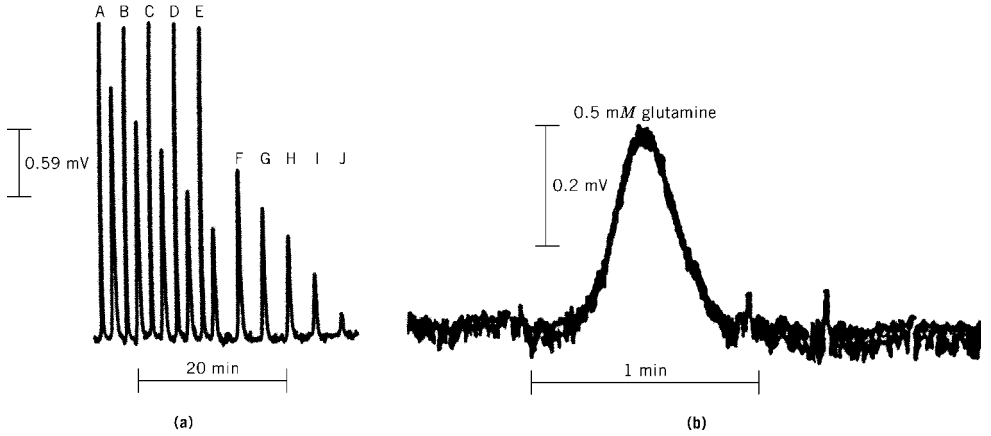


Fig. 10. Response of an ammonium ion-selective electrode that was made from nonactin dissolved in a PVC membrane mounted in a Phillips electrode body (ISE-561, Glasblaserie Möller, Zurich) referenced against an SCE to a split-stream FIA system. (**a**) FLA response to injected standards containing both glutamine and ammonium chloride: lines A–E contain both glutamine and ammonium chloride; F–G contain only glutamine. (**b**) Strip-chart recording illustrating signal to noise (S/N) for a 0.5 m*M* glutamine standard (29).

BIA is closely related to flow injection analysis (FIA), where the sample is injected into a flowing stream of supporting electrolyte that carries it past the stationary working electrode. A transient response is again generated, regardless of whether or not the method is active or passive. Thus FIA is another example of a dynamic technique (43). Figure 10 illustrates results from this technique (29). Errors from ionic interferences can be eliminated in enzyme-based, flow-injection biosensing by the intentional addition of interferences to the sample. In this case the enzyme, glutaminase, was used to generate NH_4^+ from glutamine (the analyte) in a "split-stream" FIA system. Two peaks are generated for each injection because the two streams provide sample to the detector at different times. One stream provides only the excess NH_4^+. The other passes through an enzyme reactor and therefore provides NH_4^+ generated by the glutamine as well. The presence of the interferent in relatively high concentration not only swamps out the effects of other interferences, but also linearizes the Nernst equation. The peaks in Figure 10**a** are only a few millivolts high. The peak corresponding to 0.5 m*M* glutamine (Fig 10**b**) has an amplitude of only ~0.4 mV. Such small amplitudes may be cause for concern because the junction potential generated by the reference electrode can itself be several tenths of a millivolt.

The duration of the response results primarily from the rate of elution of the sample, and not on any inherent limitation in the response time of the electrode. This is a characteristic of ion-selective electrodes, but amperometric responses depend not only on the duration of elution but also on flow rate because of the hydrodynamic effects discussed previously.

Another dynamic measurement is the lcec technique, which can be thought of, simplistically, as FIA using a chromatographic column positioned between the sample injection port and the detector. Bioanalytical systems (BAS) of West

Lafayette, Indiana, specializes in instrumentation for lcec. Their catalogs come with extensive bibliographies covering a variety of applications.

5. Economic Aspects

Companies that sell and service electroanalytical instrumentation are few in number and small in size, or they are parts of much larger companies. A quick internet survey lists Bioanalytical Systems, Brinkman, Princeton Applied Research, Radiometer, and Solartron Analytical among the companies that supply electrochemical instrumentation, but this list is by no means exhaustive.

Electroanalytical chemistry does not yet generate the kind of revenue that battery technology and electrowinning (see METALLURGY, EXTRACTIVE) do. Electroanalysis, even at the most sensitive limits, can be performed using simple instruments that cost between $100 and $1000. However, assays generally require highly skilled technicians and are thus correspondingly expensive even if the equipment used is not, and electrochemical instrumentation, fully supported with warranties, can cost $10,000 or more. The future of electroanalytical chemistry probably lies in two areas: processes control and biosensors. If it is ever to be a competitive industry, rather than a support technique, the latter of these opportunities will most probably be the greater source of future revenue. In the area of consumer products, amperometric glucose sensors hold high potential. Industrially, process monitors for the manufacture of consumer chemicals are under development. However, replacement of defective reference electrodes, which in a laboratory environment may be trivial, may be prohibitively difficult *in vivo* or in an industrial process environment. In any event, the reference electrode contributes heavily to the economics of electroanalytical chemistry.

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