

**Electroanalytical
Methods in Biochemistry
William C. Purdy**

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***Dedicated to
Robert Bruce Purdy
December 3, 1954, to
October 15, 1958***

PREFACE

Several years ago the author became associated with the Division of Biochemistry of a major research institution. As an analytical chemist, he found it rather strange to be connected with a group of scientists dealing with purely biochemical problems, and he was initially skeptical of the potential value of his services. However, it soon became apparent that his training as an analytical chemist could be of significant value to biochemical research. Aside from acquiring a fresh viewpoint toward certain problems, the author soon learned that many of the techniques developed by the modern electroanalytical chemist were not being used in biochemical research. Yet these techniques, if properly employed by the biochemist, could greatly extend biochemical research

potential and, in many cases, would permit more rapid, more precise, and more versatile experiments to be performed.

There are many fields in biochemistry where the application of modern electroanalytical techniques could prove valuable. Many of the clinical procedures still involve the development of a colored solution followed by measurement with a colorimeter or spectrophotometer. Although these procedures are satisfactory in many cases, a large number of interferences are known for each of these methods, and in many cases these interferences can completely block an analysis or lead an investigator to draw erroneous conclusions. For example, the widely accepted method for the determination of serum and urinary lead is based upon the reaction of lead with dithizone. Unfortunately, a number of cations react with dithizone under the same conditions as does lead, and several of these cations produce a color indistinguishable from that of lead. In fact, the author had experience with a case where the clinical laboratory stated that a patient was suffering from acute lead intoxication, whereas the heavy-metal poisoning was due, in reality, to cadmium. Had the polarographic technique been employed for this investigation, no such erroneous conclusion would have been possible. In addition, the polarographic technique offers greater specificity for the determination of lead than does the dithizone method.

Electroanalytical techniques have proved useful, and are widely utilized, in the determination of pH. However, not much use has been made of other potentiometric measurements. A field which has been virtually unexplored by the biochemist in this country is polarography. To be sure, the biochemist does perform amperometric titrations and other voltammetric measurements such as oxygen electrode studies. The great body of polarography, however, has been practically neglected. Polarography offers the possibility of trace-metal studies in a wide variety of media, and a means of determining quickly the oxidation potential of a large number of organic compounds. In addition, proper interpretation of polarographic data can often be of great help in the elucidation of enzyme mechanisms. Also, complex studies can be carried out relatively easily by polarographic techniques, much more easily than has ever been possible by the standard spectrophotometric or titration methods.

Coulometry and coulometric titrations have greatly extended the sensitivity of detection and determination of trace metals and of important anions. In addition, coulometric techniques can be so auto-

mated that the technician error for a particular determination can be virtually eliminated. Commercial instruments which carry out specific analyses are now available. The utility of these instruments could be greatly extended by someone familiar with the theory behind their operation.

Most of the books dealing with the subject of electroanalytical chemistry or instrumental analysis have been written by analytical or physical chemists. The emphasis, therefore, has been placed upon the physical aspects of the measurement, and sufficient discussion of the potential biochemical aspects of the technique is sparse or wholly absent. There is one major monograph dealing with the field of polarography in biochemistry, medicine, and pharmacy. This excellent book, written by two Czechoslovak chemists, contains a vast number of methods for the determination of reducible and oxidizable substances in body tissue, plants, etc. The book, however, does not contain the theory of polarography. The biochemist who desires a fuller understanding of the measurements involved must resort to one of the fine books dealing exclusively with polarography. All too often, the explanations found in these books are presented in a highly mathematical manner, which can be discouraging to one with a casual interest in one subject.

It is the purpose of this monograph, therefore, to bridge the gap between the polarographic and electrochemical texts on the one hand and the Czechoslovak monograph on the other. To accomplish this the author has drawn heavily upon a lecture series he presented to biochemists and physicians on the topic of electroanalytical chemistry.

The author acknowledges with many thanks the inspiration and help provided by the Division of Biochemistry of the Walter Reed Army Institute of Research and the particular contributions of its Director, Lt. Col. Edward C. Knoblock.

William C. Purdy

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chapter
one **INTRODUCTION**

1-1. ELECTRICAL UNITS

Ohm's law is the fundamental law describing the flow of direct current through a conductor.

$$E = iR \quad (1-1)$$

In this equation, E is the potential difference between the ends of the conductor, R is the resistance, and i is the current. The units associated with each of these quantities are volts, ohms, and amperes, respectively. Two of these units are defined by statute; the third (volt) is derived from the application of Ohm's law.

The *ampere* is the unit of intensity of electric current, and is defined as the unvarying current which, when passed through a standard solution of silver nitrate in water, deposits silver at a rate of 0.001118 g/sec.

The *ohm* is the resistance offered at 0°C by a column of mercury 106.300 cm in length, of a constant cross-sectional area, and weighing 14.4521 g.

The *volt* is the electric potential required to make a current of one ampere flow through a resistance of one ohm.

The *conductance* is a property of an electrical circuit which determines the rate at which electrical energy is converted into heat when a given potential is applied across the electrodes. Conductance is the reciprocal of electrical resistance, and its unit, the ohm^{-1} , is the reciprocal of the ohm.

The *coulomb* Q is the absolute unit for the quantity of electricity. It is defined as the quantity of electricity which must pass through a circuit to deposit 0.001118 g of silver from a solution of silver nitrate. One coulomb corresponds to the constant current of one ampere flowing for one second. One equivalent of chemical change corresponds to 96,493 coulombs, and this quantity of electricity is called the *faraday*.

The *dielectric constant* ϵ is a constant in the equation for Coulomb's law for the force between two charges separated by a dielectric or non-conductor of electricity,

$$\epsilon f \delta^2 = Q_1 Q_2 \quad (1-2)$$

where f is the force between charges Q_1 and Q_2 , separated by the distance δ .

The *inductance* L , in henrys, is a property of a conductor relating the strength of the current to the magnetic field. The henry (10^9 cm of inductance) is that inductance in which an induced electromotive force of one volt is produced when the inducing current is changed at the rate of one ampere per second. Whenever a current is passed through a conductor, there is an accompanying magnetic field around the conductor. The shape and the dimensions of the conductor are important variables; a straight wire has very small inductance, whereas a coil of wire has a high inductance.

In a-c circuits, the inductance acts to retard the alternating current by withdrawing energy for storage in the magnetic field and imposes a phase shift in the a-c wave. The inductance thus behaves in a similar manner to the resistance, which dissipates energy in the form of heat. As with resistance, it is convenient to measure the retarding effect in ohms, and this effect is called the *reactance* X of the circuit.

The *capacitance* C , in farads, is the total supplied charge Q that can be stored on a condenser at a potential difference V . The capacitance is therefore defined as

$$C = \frac{Q}{V} \quad (1-3)$$

A capacitance of one farad requires one coulomb of electricity to raise the potential by one volt. In a d-c circuit, the capacitance blocks the flow of current. For a-c circuits, the capacitance will retard the flow of current and will introduce a phase differential between the voltage across the capacitor and the current in the leads to the capacitor.

In an a-c circuit, the direction of the electron current will reverse periodically. This current may be represented by a sine wave, with electric and magnetic vectors representing the force field. These vectors are perpendicular to the direction of travel and also to one another. The interval of time between the passage of successive maxima of either of the vectors is called the period τ of the disturbance. The frequency ν is the number of such periods per second. The inductance, capacitance, and frequency can be related by the equation

$$\nu = \frac{1}{2\pi\sqrt{LC}} \quad (1-4)$$

1-2. ELECTRICAL METHODS

For every one of the electrical quantities mentioned above, there is an analytical method based upon the exact measurement of that quantity.

Conductometric methods are based upon the measurement of the conductivity (reciprocal of resistance) of a solution, or on the measurement of the change of conductivity of a solution upon the addition of a titrant.

High-frequency methods are carried out in a circuit of an oscillator operating in the megacycle frequency range. This circuit consists of a capacitor, inductance, and resistance in parallel. Any changes in the nature of the solution placed within the inductance or capacitance will result in a change in the frequency. These changes depend upon both the conductivity of the solution and its dielectric constant.

Potentiometric methods consist either of the measurement of the electromotive force (emf) of a galvanic cell or of the change of potential of one of the electrodes (the indicator electrode) during the course of a titration. The first of these measurements is commonly employed in the determination of the pH of a solution; the second measurement is called a potentiometric titration.

Voltammetric methods have become increasingly important in the past decade. These methods are based on the current-voltage curves which arise when solutions of an oxidizable or reducible substance are electrolyzed at a solid microelectrode or a dropping mercury electrode. The S-shaped curves obtained can be employed both for qualitative and quantitative determination of the electroactive substances. The inflection point of the curve, the half-wave potential, is characteristic of the substance undergoing electrode reaction, and the height of the curve, the limiting current, is directly proportional to the concentration of the electroactive species in solution.

Titration may be carried out in a cell consisting of a macro electrode and a dropping mercury or solid micro electrode. If the titrant or the substance being titrated is electroactive and the change in limiting current is plotted as a function of added titrant, the determination is called an *amperometric titration*. By careful control of the potential applied to the micro indicator electrode, amperometric titrations can be made specific for certain substances.

Coulometric methods involve the measurement of the quantity of

electricity associated with a quantitative electrode reaction. These methods are of two types, constant-potential and constant-current coulometry.

In the first type, electrolysis of the solution to be analyzed is carried out in a cell containing a "working electrode." The potential applied to the working electrode is carefully controlled so that the desired electrode reaction proceeds with 100 percent current efficiency. Upon completion of the electrode reaction, the current falls to zero. The quantity of electricity consumed is measured on a coulometer in series with the electrolysis cell; the quantity of substance reacted is computed from Faraday's law. This method is particularly specific since the potential of the working electrode is carefully controlled. In principle, this method is applicable to any reducible or oxidizable substance, inorganic or organic.

Coulometric titrations employ the application to the electrolysis cell of a carefully controlled constant current. The constant current generates the titrant in the titration vessel, and the end point of the reaction is indicated by a color indicator or some electrometric technique. The quantity of electricity passed, and hence the number of equivalents of titrant generated, is simply determined by the product of the known current and the generation time. This method offers particular advantage, for it permits titrations with reagents which are difficult to store and to standardize. The sole criterion for a coulometric titration is that the electrode reaction take place with 100 percent current efficiency. Since it is possible to measure very small quantities of electricity with ease, precise and accurate micro analyses can be accomplished in ordinary volumes of solutions.

Several other electroanalytical methods have recently been developed. *Chronopotentiometry* involves the measurement of potential as a function of time at a micro electrode on which is impressed a small constant current. Most of the analytical information obtainable from a chronopotentiogram can be more easily obtained by polarographic techniques. However, chronopotentiometry is very useful in the study of the kinetics of fast electrode reactions.

Fast-sweep polarography is a technique which increases by a factor of 100 the sensitivity of conventional polarography. The current at a hanging mercury drop electrode is measured as the applied potential is varied at rates approaching 50 millivolts/sec. The curves obtained have characteristic peaks; the peak potential identifies the electroactive

substance, and the peak current is proportional to the concentration of that substance in solution.

1-3. ELECTROANALYTICAL BIBLIOGRAPHY

For the reader who would like to gain a fuller knowledge of the theoretical principles underlying the various methods to be discussed, the following general bibliography should prove useful.

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