ELECTROCHEMISTRY OF BIOLOGICAL MOLECULES

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Preface

Nitrogen heterocyclic molecules are found extensively in biological systems, and have been chosen by nature to be involved in many, if not most, of the fundamental reactions of living organisms. Most of the nitrogen heterocyclic molecules found in nature contain extensive delocalized or mobile (π) electron systems. A result of this property is that these compounds are generally quite good electron donors and/or acceptors. This in turn allows very many biologically important nitrogen heterocyclic molecules to be studied by various electrochemical techniques, that is, their electrochemical oxidation and/or reduction and related chemical processes may be studied. The purpose of this book is to present a fairly complete summary of the electrochemistry of the more important groups of nitrogen heterocyclic molecules including purines and pyrimidines and their nucleosides and nucleotides, polynucleotides and nucleic acids, pteridines, flavins, pyrroles, porphyrins, and pyridines.

The treatment of the material tends to be, on occasion, somewhat encyclopedic. However, this is necessary to present a reasonably complete summary of the available information. Although this work will be of greatest use to electrochemists working on nitrogen heterocyclic and related systems, it is hoped that biochemists and biologists will also find the information summarized to be of use. Because of the involvement of these molecules in biological electron transfer processes, it is likely that many of the electrochemical reaction routes and intermediates will be similar to the biological electron-transfer processes.

I would like to acknowledge the extensive assistance provided by the Faculty Research Committee of the University of Oklahoma in the preparation of this book. I wish to express my appreciation to Dr. Bruce Laube, Dr. Jean P. Pinson, and Dr. David L. McAllister for reading many sections of the original

manuscript, and particularly to Dr. C. LeRoy Blank who very carefully read the final manuscript and made many valuable suggestions. However, any errors in the book, of course, must be attributed to me. I would also like to express my deep appreciation to Professor Philip J. Elving, who first gave me the opportunity to study the electrochemistry of nitrogen heterocycles. It will be obvious from this book that Professor Elving, probably more than anyone else, was responsible for opening up the modern era of electrochemistry of purines and pyrimidines, and he continues to be a major contributor to the field. I am especially grateful to my wife, June Diane Dryhurst, for her constant support and encouragement throughout the years.

Glenn Dryhurst

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Electrochemistry and Biological Processes

Studies of the electron-transfer reactions of atoms, ions, or molecules by polarographic or voltammetric techniques* can provide an extraordinary amount of information about such reactions. Thus, a characteristic potential at which the reaction occurs can be measured. It is generally referred to as the half-wave potential in DC polarography and some related techniques, or as the half-peak potential or peak potential in voltammetric techniques at stationary electrodes. These potentials characterize the electrode process to some degree and in certain instances may have direct thermodynamic significance.

By use of modern electrochemical techniques it is possible to decide precisely how many electrons are involved in the electron-transfer reaction at a particular potential. It is also possible to detect very unstable intermediates or products produced after the transfer of electrons has occurred. For example, many electrode (and biological) reactions proceed by one-electron-transfer reactions to give a free radical, a radical cation, or a radical anion species. There are examples in which the radicals so produced are extremely unstable such that electron spin resonance spectroscopy, for example, is generally incapable of detecting them, whereas they are detectable by electrochemical techniques such as fast sweep cyclic voltammetry.

It is also quite easy to examine electron-transfer reactions electrochemically under an extraordinarily large number of conditions in which most other chemical or biological studies often cannot be performed satisfactorily. Thus, it is not unusual to study an electrode reaction over a very wide pH range, in the

* Throughout this book polarography refers to electrochemical processes studied at the dropping mercury electrode. Voltammetry refers to electrochemical reactions occurring at any other type of solid or liquid electrode.

presence of quite different buffer systems, to employ a variety of temperatures and solvents, and to study the reaction in the presence and almost total absence of protons. Often, as a result of such studies, rather subtle changes in mechanism occur, or intermediates or unstable products can be observed more clearly. This potentially allows a much more detailed reaction mechanism for the electron-transfer process and related reactions to be deduced.

When a dropping mercury electrode or other microelectrodes are used, only extremely small amounts of electroactive material are involved in the electron-transfer reaction, and only minute amounts of products are formed. However, it is possible, and indeed quite common, to scale up the polarographic or voltammetric experiments several hundreds or thousands of times so that appreciable amounts of products can be isolated and identified. In relation to this it is now becoming appreciated that electrochemical methods can be utilized for some rather unique synthetic applications.

Some electrochemical techniques, in particular pulse polarography, have an extraordinary sensitivity so that they can be occasionally utilized for determining electroactive material from the 10^{-2} to 10^{-8} M concentration level. There are few techniques that can rival this range of utility or lower level of analytical detection. Indeed, although pulse polarography has not been widely employed for the solution analysis of organic compounds, it is, in fact, a most attractive analytical technique.

Thus, in essence, modern electrochemical techniques can be employed to study the electron-transfer reaction mechanisms and products of atoms, ions, and molecules, and certain techniques can provide valuable synthetic or analytical tools. The pertinent question to be asked is, "What can electrochemical studies tell one about biological electron-transfer and related processes?" First of all, there is a definite set of similarities between electrochemical and biological (e.g., enzymatic) reactions:

1. Electrochemical and biological electron-transfer, or oxidation–reduction, reactions both involve essentially heterogeneous electron-transfer processes. Electrochemically, this process occurs at the electrode–solution interface; biologically, it occurs at an enzyme–solution interface.
2. Both electrochemical and biological reactions can take place at similar pH and in the presence of similar ionic strengths of inert electrolyte.
3. Both types of processes can occur effectively under nonaqueous conditions.
4. Both types of reactions normally occur at very similar temperatures.
5. Both at an electrode and at the active site of an enzyme it is likely that the substrate molecule has to be oriented in a rather specific fashion before the electron transfer can occur.

These statements are not meant to imply that the unique selectivity often associated with an enzyme can in any way be duplicated by an electrode. On the

other hand, an enzyme cannot cause a thermodynamically impossible reaction to proceed; all of the laws and principles of chemistry are still applicable to an enzyme-catalyzed reaction. There is, however, sufficient superficial similarity between the electrochemical and biological reactions, which is not duplicated in other chemical systems, to warrant extensive study of the electrochemistry of biologically important molecules. Such studies should yield an enormous amount of evidence regarding the mechanisms of biological electron-transfer processes.

Normally, when one considers electron-transfer processes in biological systems, one tends to think of the electron-transport or respiratory chain, where an organic substrate is oxidized and usually oxygen is ultimately reduced. However, this reaction does not proceed by direct interaction of the substrate and oxygen but via a series of enzyme-catalyzed electron-transfer reactions, first between the substrate and, for example, a pyridine nucleotide, then through a large number of other transfers, until ultimately a reduced cytochrome is oxidized by oxygen and the oxygen is correspondingly reduced. There are, however, many more electron-transfer or oxidation–reduction type of reactions. Typical of these are those involved in catabolism of many biologically important organic compounds. A group of compounds of this type consists of the purines, in which the enzymatic and electrochemical oxidations are very similar. The electrochemical studies, however, reveal a great deal more information about the fine detail of the reaction than is available from enzymatic studies.

It is a reasonable contention, therefore, that biological and electrochemical redox processes are sufficiently similar that extensive studies of the electrochemistry of biologically important molecules should shed considerable light on the fundamentals of the biological reaction mechanisms.

Having made these somewhat sweeping and highly optimistic statements it is only proper to point out that not a great deal of electrochemical research has been directed at really understanding the electron-transfer reactions of biologically important molecules, particularly in relation to biological processes. A very large number of biologically important molecules have been studied, generally polarographically, but more often than not these studies unfortunately boil down to a tabulation of half-wave potentials, measurement of the limiting current at a variety of pH values, an approximate guess at the number of electrons involved in the reaction, and postulation of a rather superficial mechanism. It is all too often found that products have not been isolated, identified, or subjected to structure elucidation. It is still common for only a single electrochemical technique to be utilized. Since the early to mid-1960s, however, there has been a marked upsurge in the development of the theory of modern electrochemical techniques and reactions. In addition, the advent of the operational amplifier has led to some spectacular advances in electrochemical instrumentation. The results of these two areas of advancement have led to the rather unusual situation that both theory and instrument design are considerably

ahead of their experimental application, particularly to biologically important molecules.

In this book a fairly complete summary of the state of knowledge regarding the electrochemistry of a number of families of *N*-heterocyclic compounds is presented. Not all of the members of these families are normally found in biological systems. However, in order to understand the complete picture of the electrochemistry of the biologically important members of a family of compounds, a review of the pertinent literature regarding all the members of the family has been presented.

There are two unifying features that relate nearly all of the compounds discussed. The first, of course, is that they all contain heterocyclic nitrogen atoms. The second is that the compounds are characterized by molecular systems with mobile electrons. Nitrogen heterocycles containing delocalized or mobile (π) electrons have been chosen by nature to perform or to be involved in many, if not most, of the fundamental reactions of living organisms. For example, the most important constituents of nucleic acids are the purines and pyrimidines. The information stored in the sequences of the purines and pyrimidines in the nucleic acids directs protein synthesis and transmits genetic information. The energy-rich compounds, such as adenosine triphosphate, contain purines and are vital reactants in intermediary metabolism. Although there are hundreds of enzymes, most of these can exert their catalytic effects only in the presence of a suitable coenzyme. There are only a few coenzymes and most of these are conjugated *N*-heterocycles such as the pyridine nucleotides (NAD^+ and $NADP^+$), the flavin nucleotides (FAD and FMN), and the porphyrins, which are the heme prosthetic groups of the cytochromes. The latter are principally redox coenzymes. Folic acid, pyridoxal, and thiamine are part of the vitamin B complex, yet all contain conjugated *N*-heterocyclic groups. Apart from their involvement in folic acid, the pteridines are widely dispersed in nature, yet present considerable mystery as to their exact biological function. They are now suspected of being involved in reactions of sight and in electron transport in the photosynthetic process.

Most of the compounds just mentioned are characterized as being theoretically and experimentally quite good electron donors and/or acceptors. There are, therefore, valid reasons for electrochemically studying the electron-transfer reactions of these compounds and the consequences of such transfers.

It might also be pointed out that a very large number of drugs capable of acting on living cells are, at least in part, conjugated *N*-heterocyclic compounds.

The nitrogen heterocycles which are biologically important and which will be discussed subsequently are synthesized biologically at considerable expense. Since "nature does not indulge in luxuries"[1] there are necessarily deep and fundamental reasons why these compounds are used biologically and why they can so readily accept and/or donate electrons. It is therefore reasonable to

expect that electrochemical studies, in conjunction with all of the other experimental and theoretical tools of chemistry, should be able to contribute significantly to the ultimate understanding of many biological electron-transfer reactions.

The material presented in subsequent chapters deals primarily with electron-transfer reactions of biologically important and related compounds. This does not imply, however, that the sole application of electrochemistry to biology is the study of such reactions. Thus, electrochemists are studying modes of ion transport, membrane and surface phenomena, effects of potential and current on tissue healing and regeneration, and many other problems. Other authoritative texts and literature reports deal with these studies.

REFERENCE

1. A. Szent-Györgi, "Introduction to Submolecular Biology." Academic Press, New York, 1960.

2

Theory and Instrumentation

I. INTRODUCTION

No attempt will be made in this chapter to give an extensive account of the theory and instrumentation of electrochemistry. Rather, a summary of the usual working equations, their significance and utility, and electrochemical jargon will be presented. In addition, the principles and circuits of some, but by no means all, electrochemical instrumentation will be discussed.

II. POTENTIOMETRY

Most electrochemical techniques of the type to be discussed can, to a greater or lesser extent, be regarded as derived from potentiometry. Accordingly, it is worth reviewing this technique, even though very little mention is made of it in subsequent chapters.

If a platinum wire electrode is immersed in a solution containing two components of a redox couple (Eq. 1), a potential develops across the

$$\mathrm{Ox} + ne \rightleftharpoons \mathrm{Red} \qquad (1)$$

electrode–solution interface. Unfortunately, it is not possible to measure such a single-electrode potential directly. For this reason, the potential of the platinum electrode must be measured against a second electrode having a constant potential, i.e., a reference electrode. Potentials determined potentiometrically are normally expressed relative to the normal hydrogen electrode (NHE), which again does not have a known absolute potential, but by convention is assigned a

potential of 0.0000 V. In order to measure the voltage between the reference electrode and the indicating or platinum electrode, a salt bridge is placed between the two electrode solutions to maintain electrolytic contact, and an apparatus of the type shown in Fig. 2-1 is employed. The DC voltage supply and the electrochemical cell are connected so that their potentials are in opposition. The sliding contact C is moved along the slidewire AB until upon momentarily closing the tapping key the galvanometer (G) shows no deflection. This implies that the voltage across AC is equal to and of the same polarity as the voltage across the cell and no current flows through the galvanometer. The voltage across AC and hence of the cell is then read from the voltmeter (V). Under these conditions the potential E across the cell is given by the Nernst equation (Eq. 2).

$$E = E^0 - \frac{RT}{nF} \ln \frac{A_{\text{Red}}}{A_{\text{Ox}}} \tag{2}$$

In this expression E^0 is the standard potential for the reaction shown in Eq. 1 versus the same reference electrode, which, in other words, means the value of E when the activity of Ox and Red are equal (i.e., $A_{\text{Red}} = A_{\text{Ox}}$); R is the ideal gas

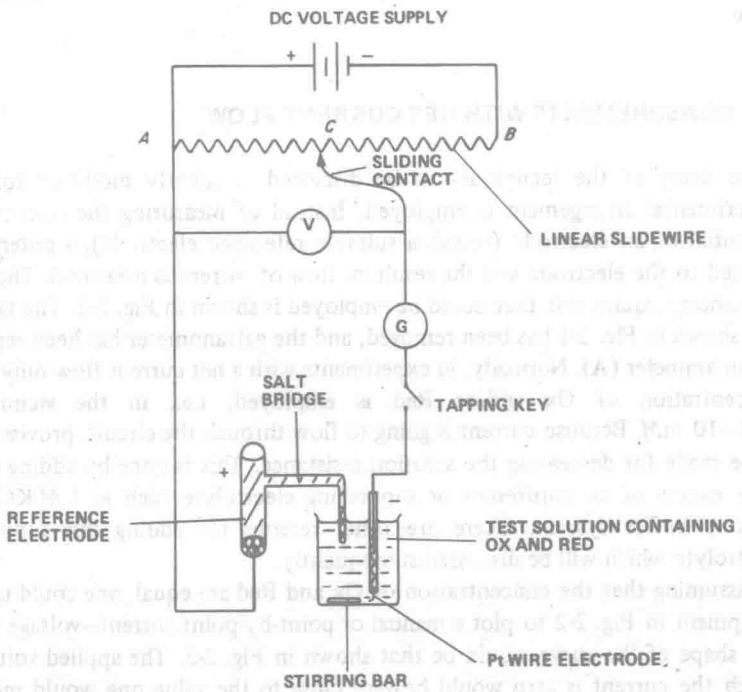

FIG. 2-1. Schematic diagram of simple apparatus for potentiometry. *A,B,C*: Linear slidewire; V: voltmeter; G: galvanometer.

constant (8.312 international joules), T is the absolute temperature, F is the faraday (96,500 C), and n is the number of electrons involved in the reaction as shown in Eq. 1.

The potentials determined potentiometrically for a redox system are of direct thermodynamic significance. Unfortunately, only a rather limited number of redox systems can be examined potentiometrically. In fact, only those reactions that exhibit electrochemical reversibility can be studied by direct potentiometry of the type described above. In a practical sense, electrochemical reversibility implies that both the oxidized and reduced forms of the redox couple are stable, and that the reaction can be carried out rapidly in either direction so that the Nernst equation is applicable. One can often find redox couples referred to as "sluggish," which implies that the reactions are not rapid and take a long time to reach equilibrium under potentiometric conditions.

The great difference between the potentiometrically determined electrode potential and that for the majority of techniques to be discussed subsequently is that in potentiometry the potential is measured when the voltage from the slidewire across AC is exactly equal to and of the same polarity as the voltage across the electrochemical cell so that the measurement is taken at zero current flow.

III. MEASUREMENTS WITH NET CURRENT FLOW

In many of the techniques to be discussed, a slightly modified form of experimental arrangement is employed. Instead of measuring the zero current potential of an electrode (versus a suitable reference electrode), a potential is applied to the electrode and the resultant flow of current is measured. The most elementary equipment that could be employed is shown in Fig. 2-2. The tapping key shown in Fig. 2-1 has been removed, and the galvanometer has been replaced by an ammeter (A). Normally, in experiments with a net current flow only a low concentration of Ox and/or Red is employed, i.e., in the vicinity of 0.01–10 mM. Because current is going to flow through the circuit, provision has to be made for decreasing the solution resistance. This is done by adding a very large excess of an indifferent or supporting electrolyte such as 1 M KCl or a suitable buffer system. There are other reasons for adding this supporting electrolyte which will be discussed subsequently.

Assuming that the concentration of Ox and Red are equal, one could use the equipment in Fig. 2-2 to plot a manual or point-by-point current–voltage curve. The shape of the curve would be that shown in Fig. 2-3. The applied voltage at which the current is zero would be very close to the value one would measure potentiometrically using the apparatus shown in Fig. 2-1, and since the concentrations of Ox and Red are equal, it would be close to the formal

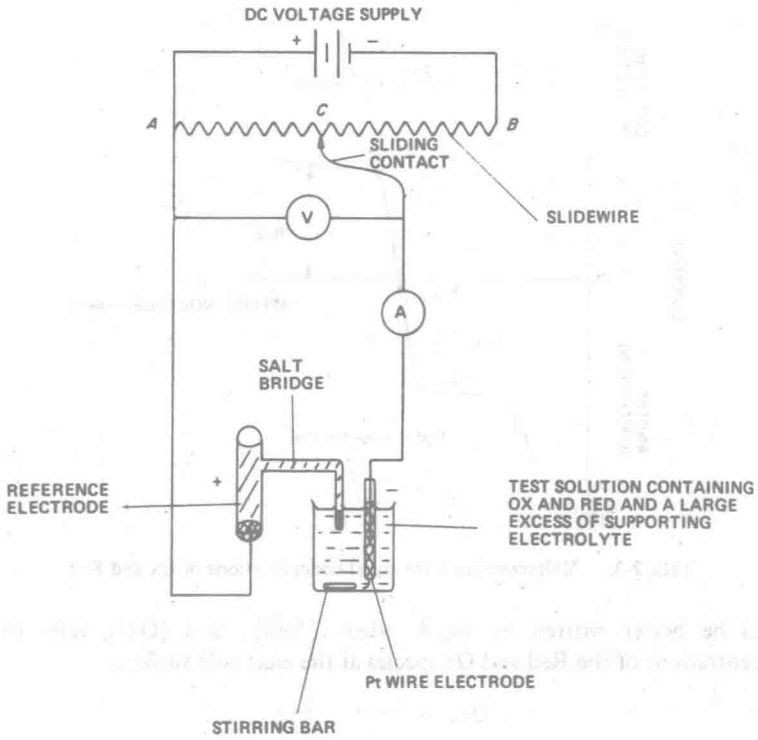

FIG. 2-2. Schematic diagram for measurement of a voltammetric current–voltage curve. *A,B,C*: Linear slidewire; V: voltmeter; A: ammeter.

potential ($E^{0\prime}$) of the system. As the applied voltage is made more positive or more negative than the zero current potential, the current clearly increases up to a limiting value, not surprisingly called the anodic $(i_L)_a$ or cathodic $(i_L)_c$ limiting current plateau. The reason for this increase in current is quite simple. If, for example, the applied voltage is made more negative than the zero current potential, then the Nernst relationship demands that the concentration of Ox decreases or, alternatively, that the concentration of Red increases. The only conceivable way in which this can happen is by electrons flowing down the platinum electrode, called the *working electrode* in voltammetry, across the electrode–solution interface and reducing Ox to Red according to Eq. 3. Hence, momentarily the concentration of Ox decreases to the desired level and the Nernst equation is obeyed. It is not necessary that the total solution undergo this concentration change, merely the solution in the immediate vicinity of the electrode surface. Accordingly, under these conditions the Nernst equation