Two-Dimensional Electrophoresis and Immunological Techniques

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Preface

This text is a summary of basic principles and techniques and is dedicated to all those students who have been told by their mentors, "Go forth and do two-dimensional gels and have the results on my desk tomorrow." No attempt has been made in this text to provide exhaustive lists of references related to basic principles or techniques or to list every company or supplier involved in this area of research. Nevertheless, it is hoped that sufficient information is given to help a new investigator or student appreciate the complexities but develop sufficient expertise to carry out these techniques successfully. The discussions are designed to instill in basic science and clinical investigators of all levels of expertise an appreciation of the power of combining a variety of techniques as well as to provide basic insight into the theories, complexities, and problems frequently encountered with electrophoretic and immunochemical methods.

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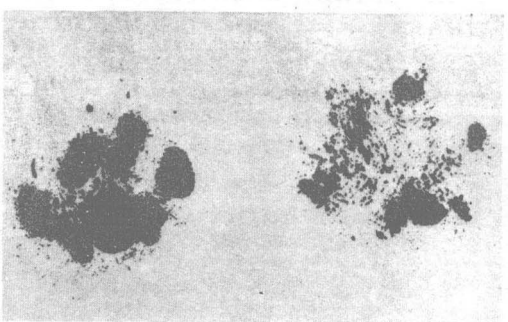

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Chapter 1

Basic Theories and Principles of Electrophoresis

I. INTRODUCTION

The optimal use of any technology requires an understanding of the basic theories and principles that are the foundation for that technology. This knowledge helps the investigator to better appreciate the strengths and weaknesses of the technology and is critical in the final interpretation of experimental results.

reaction will take place at each of the platinum electrodes. Usually, hydrogen (H_2) is evolved or some metal is deposited at the electrode (termed the *cathode*), which is connected to the *negative* pole of the battery. During electrolysis, a nonmetal (e.g., O_2) is liberated at the *anode*, which is at the *positive* pole of the battery. The usual reactions that occur in an electrophoresis chamber are as follows:

1. *Cathode reactions* (where reduction or the gain of electrons occurs):

$$2e^- + 2H_2O \rightarrow 2OH^- + H_2$$

$$HA + OH^- \rightarrow A^- + H_2O$$

2. *Anode reactions* (where oxidation or the loss of electrons occurs)

$$H_2O \rightarrow 2H^+ \rightarrow \tfrac{1}{2}O_2 + 2e$$

$$H^+ + A^- \rightarrow HA$$

In order to maintain the electric current, it is necessary to have a complete circuit (a closed-loop system in which the electric charge can return to its starting point). If this complete circuit has an electrolytic conductor, a chemical reaction must occur at the electrodes. An example

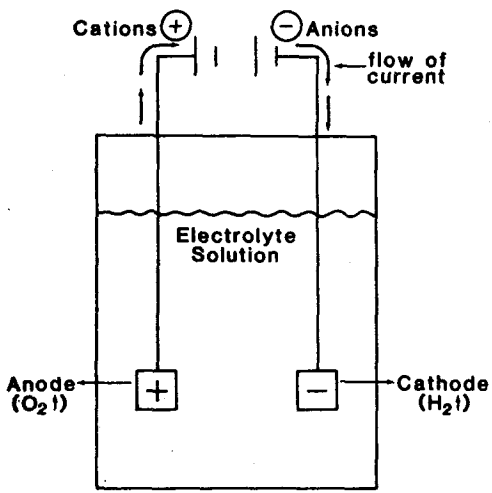

Figure 1.1. Diagram of an electrolytic cell. (Modified from Murphy and Rousseau, 1969.)

of an electrolysis unit (i.e., electrolytic cell) is shown in Figure 1.1.

As illustrated in Figure 1.1, the ions (+ and −) are free to move in an electrolytic cell. If a battery is attached to this cell, it will generate an electric field that pushes electrons through the wires in the directions given by the arrows. The rate at which electricity moves in a circuit is measured in *amperes* (A). A current of 1 A corresponds to the transfer of electricity at a rate of 1 coulomb/second. The term *coulomb* is the measurement for the quantity of electricity required to deposit 1.118×10^{-3} g of silver from a solution of silver nitrate.

Faraday further determined that the mass of any substance formed by the passage of a given amount of electricity is directly proportional to the equivalent weight of that substance. The liberation of one *equivalent weight* of any element during electrolysis requires 1 Faraday (F), which is 96.493 coulombs. A Faraday is also the charge on a mole of electrons. Because every electron has an identical negative charge, neutralization of each positive or negative charge during electrolysis requires the gain or loss of one electron. The number of electrons (Faradays) that will neutralize the total charge on 1 mole of singly charged atoms will therefore neutralize ½ mole of doubly charged atoms. For example

$$Ag^+ + e^- \rightarrow Ag^\circ, \qquad \text{equivalent weight} = \text{atomic weight}$$

$$Cu^{2+} - 2e^- \rightarrow Cu^\circ, \qquad \text{equivalent weight} = \text{atomic weight/2}$$

$$O_2^- \rightarrow \tfrac{1}{2}O_2 + 2e^-, \qquad \text{equivalent weight} = \text{atomic weight/2}$$

In electrolysis reactions, the equivalent weight of an element is therefore, equal to its atomic weight divided by the number of electrons it gains or loses (from Murphy and Rousseau, 1969).

When an electrolyte is dissolved in water, its molecules dissociate into oppositely charged fragments (*ions*). The conduction of electricity occurs when electrodes connected to a battery are placed in an electrolyte solution and the positive ions (cations) migrate to the negatively charged cathode, where each cation will pick up one or more electrons. Meantime, the negatively charged ions (anions) will lose their negative charge by transferring electrons to the positively charged anode. Electrons are therefore "pumped" out of solution at the anode through the external circuit and back into solution at the cathode; thus the current continues to flow as long as positive and negative ions are present in the solution. The voltage of this reaction is a measure of the electromotive force of this system (Murphy and Rousseau, 1969).

III. MOVEMENT OF MOLECULES IN AN ELECTRICAL FIELD

When any molecule is placed in an electric field, a *force* is exerted on it, which depends on both the *strength* of the electrical field as well as the *charge* of that molecule (see discussions by Cooper, 1977; Freifelder, 1976). The mathematical equation used to express this phenomenon is

$$F = (E/d)\,(q)$$

where F is the force, E is the potential difference between electrodes (electrical field), d is the distance between electrodes, E/d is the field strength, and q is the net charge of molecule.

If the molecule or particle with the charge q is placed in the electrical field E, it will move at a constant velocity v, which is determined by the balance between the electric force Eq and the viscous drag fv. The equation that represents this phenomenon is

$$Eq = fv$$

where Eq is the electric force, f is the frictional coefficient of the molecule (a function of the physical parameters of that molecule), v is the charge velocity, and fv is viscous drag.

Using this equation, it is possible to determine the characteristic *mobility* u of a defined particle, which is its velocity for a given external electrical field:

$$\text{Mobility} = u = v/E = q/f$$

The term *electrophoresis* refers to the transport of particles through a solvent by an electric field. If a charged molecule is placed in a vacuum, it will accelerate until it finally collides with the electrode. This does not occur, however, if the molecule is in solution or in a matrix. In these instances, the force of the electrical field is opposed by the friction that occurs between the accelerating molecule and the solution. The degree of the drag on this molecule is therefore dependent on the *size* and *shape* of the molecule as well as the *viscosity* of the medium through which it moves. If electrophoresis is carried out in a solution such as sucrose, the extent of this drag can be calculated using the Stokes equation:

$$F = 6\pi r\eta v$$