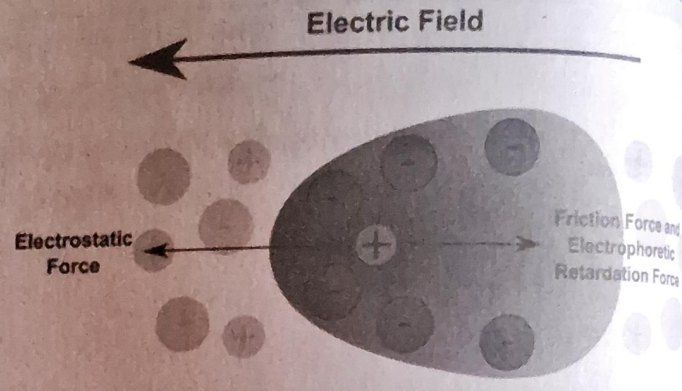
Gel Electrophoresis

10.1 INTRODUCTION

Electrophoresis is the movement of particles dispersed in a fluid, under the influence o electric field which has uniform spatial distribution. The electrophoresis is principally ba on electrokinetic phenomenon which was observed for the first time by Ferdinand Frederic Reuss in 1807. It is the basis for a number of analytical techniques used in chemistry for separating molecules by size, charge, or binding affinity. Electrophoresis of positively charged particles (cations) is known as cataphoresis, while electrophoresis of negatively charged particles (anions) is known as anaphoresis. Electrophoresis is a technique used in laborator in order to separate macromolecules based on size. The technique applies a negative cha so proteins move towards a positive charge. In electrophoresis molecules are separated the basis of their charge and molecular weight. Infact, the charge is responsible for creat a pull and molecular weight creates a drag across the matrix that creates differences in t mobility of molecules. In broad terms, electrophoresis may be grouped into two sub typ (a) Zone electrophoresis and (b) Moving Boundary electrophoresis.



Moving-boundary electrophoresis or free-boundary electrophoresis is electrophoresis a free solu- tion. It was developed by Arne Tiselius in 1930.This is based on the separati of molecules by virtue of the difference in motion of charged particles through a station liquid under the influence of an electric field. Its variants are capillary electrophores Isotachophoresis, immunoelectrophoresis etc. The capillary electrophoresis is routinely used automated DNA sequencing machines.

Zone electrophoresis, on the other hand is an electrophoretic technique in which compone are separated into zones or bands in a buffer, and stabilized in solid, porous, or any other supp medium–eg, filter paper, agar gel, or polyacrylamide gel. Although capillary electrophore was first developed as Moving boundary type electrophoresis but later modifications lead the development of its variants called capillary zone electrophoresis (CZE) (also known as F solution CE), and capillary gel electro- phoresis (CGE), Isoelectric focusing can also be do using capillary electrophoresis and is known as capillary isoelectric focusing (CIEF)

Gel electrophoresis is the most widely used type of zone electrophoresis. It is based on t principle of difference in the electrophoretic mobility of different molecules on the basis change and their molecular weight or infact shape of the molecule. Apart from the laborato

technique for the separation of biomolecules this techniques was also used for a diagnostic purpose for long time in recent past. As the gels used in the gel electrophoresis are quite delicate and fragile, further analysis of biomolecules is usually not possible on gels, hence the molecules, after their separation are either transferred to stable material such as nylon or nitrocellulose membranes (called blotting), or pieces of the gel containing biomolecules are cut and biomolecules are purified using the chemicals that can dissolve gels.

There are several criteria to categorise electrophoretic techniques in general, such as on the basis of separation principle, type of matrix, distribution of electric field etc. Following figure illustrates the outline of various electrophoretic methods. This review is limited to the description of gel electrophoresis, other variants have been discussed elsewhere.

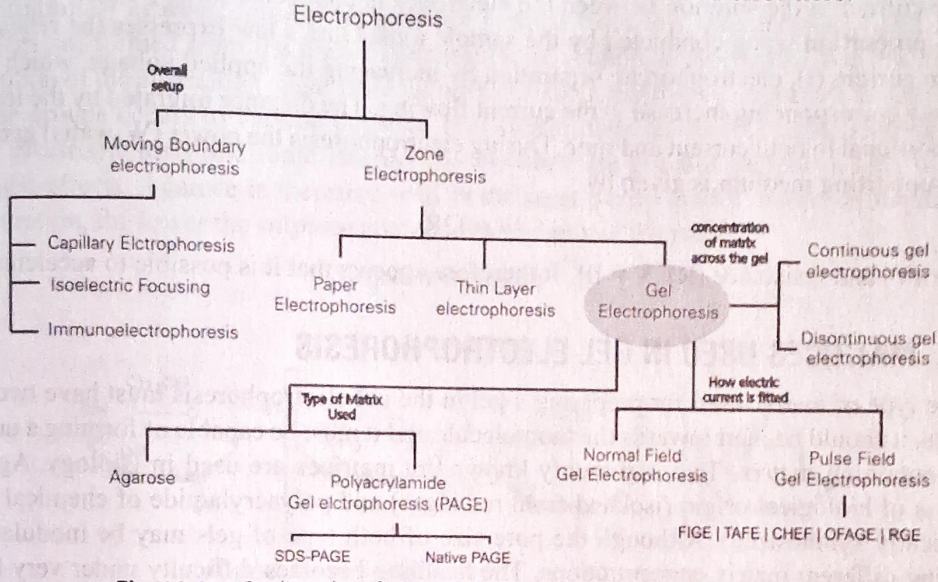


Fig. 10.1. *Outline classification of various electrophoresis techniques.*

10.2 PRINCIPLE INVOLVED IN ELECTROPHORESIS

The migration of molecules is influenced by the applied electric field, the rigid, mazelike matrix of the gel support; and the size, shape, charge, and chemical composition of the molecules to be separated. When a potential difference (voltage) is applied across the electrodes, it generates a potential gradient, (E) which is the applied voltage; (V) divided by the distance, (d) between the electrodes. When this potential gradient E is applied, the force on a molecule bearing a charge of q coulombs is F = Eq newtons. It is this force that drives a charged molecule towards an electrode. However, there is also a frictional resistance that retards the movement of this charged molecule. This frictional force is a measure of the hydrodynamic size of the molecule, the shape of the molecule, the pore size of the medium in which electrophoresis is taking place and the viscosity of the buffer. The velocity, (v) of a charged molecule in an electric field is therefore given by the equation.

$$V = Eq/f$$

where, f = frictional coefficient

During electrophoresis we commonly use the term electrophoretic mobility (p) , which is the ratio of the velocity of the ion to field strength (v/E). When a potential difference is applied, therefore, molecules with different overall charges begin to separate owing to their different

electrophoretic mobilities. Even molecules with similar charges will begin to separate if the have different molecular sizes, since they will experience different frictional forces. Some forms of electrophoresis rely almost totally on the different charges on molecules to effect separation, whilst other methods exploit differences in molecular size and therefore encourage frictional effects to bring about separation.

Electrophoresis is an incomplete form of electrolysis because the components are separated according to their electrophoretic mobility and the electric field is removed before the molecules in the sample reach the electrodes. The separated samples are then located by staining with an appropriate dye or by autoradiography.

The current in the solution between the electrodes is conducted mainly by the buffer ions, a small proportion being conducted by the sample ions. Ohm's law expresses the relationship between current (i), electrophoretic separation by increasing the applied voltage, which would result in a corresponding increase in the current flowing. The distance migrated by the ions will be proportional to both current and time. During electrophoresis the power (W, watts) generated in the supporting medium is given by

$$W = I2R$$