



## 21

# ELECTROPHORESIS

## 21.1 INTRODUCTION

The movement of particles under spatially uniform electric field in a fluid is called **electrophoresis**. In 1807, Ferdinand Frederic Reuss observed clay particles dispersed in water to migrate on applying constant electric field for the first time. It is caused by a charged interface present between the particle surface and the surrounding fluid. The rate of migration of particle depends on the strength of the field, on the net charge size and shape of the molecules and also on the ionic strength, viscosity and temperature of medium in which the molecules are moving. As an analytical tool, electrophoresis is simple, rapid and highly sensitive. It is used analytically to study the properties of a single charged species and as a separation technique. It provides the basis for a number of analytical techniques used for separating molecules by size, charge, or binding affinity, example- for the separation of deoxyribonucleic acid (DNA), ribonucleic acid (RNA), or protein molecules using an electric field applied to a gel matrix. Gel matrix used mainly is polyacrylamide and agarose. DNA Gel electrophoresis is usually performed for analytical purposes, often after amplification of DNA via PCR, but may be used as a preparative technique prior to use of other methods such as mass spectrometry, RFLP, PCR, cloning, DNA sequencing, or Southern blotting for further characterization.



## OBJECTIVES

After reading this lesson, you will be able to:

- define the electrophoresis
- describe the principle and important types of electrophoretic methods
- explain the principle and components of a electrophoresis
- explain various uses of electrophoresis

## 21.2 PRINCIPLE



Notes

The surface adsorbed sample strongly affects suspended particles by applying electric surface charge, on which an external electric field exerts an electrostatic coulomb force. According to the double layer theory, all surface charges in fluids are screened by a diffuse layer of ions, which has the same absolute charge but opposite sign with respect to that of the surface charge. The electric field also exerts a force on the ions in the diffuse layer which has direction opposite to that acting on the surface charge. This force is not actually applied to the particle, but to the ions in the diffuse layer located at some distance from the particle surface, and part of it is transferred all the way to the particle surface through viscous stress. This part of the force is also called electrophoretic retardation force. When the electric field is applied and the charged particle to be analyzed is at steady movement through the diffuse layer, the total resulting force is zero:

$$F_{\text{toto}} = 0 = F_{\text{el}} + F_{\text{f}} + F_{\text{ret}}$$

Considering the drag force on the moving particles due to the viscosity of the dispersant, in the case of low turbulence and moderate electric charge strength  $E$ , the velocity of a dispersed particle  $v$  is simply proportional to the applied field, which leaves the electrophoretic mobility  $\mu_e$  defined as:

$$\mu_e = \frac{v}{E}$$

The most known and widely used theory of electrophoresis was developed in 1903 by Smoluchowsky

$$\mu_e = \frac{\epsilon_r \epsilon_0 \zeta}{\eta},$$

where  $\epsilon_r$  is the dielectric constant of the dispersion,  $\epsilon_0$  is the permittivity of free space ( $C^2 N^{-1} m^{-2}$ ),  $\eta$  is dynamic viscosity of the dispersion medium (Pa s), and  $\zeta$  is zeta potential (i.e., the electrokinetic potential of the slipping plane in the double layer).

The Smoluchowski theory is very powerful because it works for dispersed particles of any shape at any concentration. Unfortunately, it has limitations on its validity. It follows, for instance, from the fact that it does not include Debye length  $\kappa^{-1}$ . However, Debye length must be important for electrophoresis, as follows immediately from the Figure on the right. Increasing thickness of the double layer (DL) leads to removing point of retardation force further from the particle surface. The thicker DL, the smaller retardation force must be.

Detailed theoretical analysis proved that the Smoluchowski theory is valid only for sufficiently thin DL, when particle radius  $a$  is much greater than the Debye length :

$$a\kappa \gg 1$$

This model of “thin Double Layer” offers tremendous simplifications not only for electrophoresis theory but for many other electrokinetic theories. This model is valid for most aqueous systems because the Debye length is only a few nanometers there. It breaks only for nano-colloids in solution with ionic strength close to water.

The Smoluchowski theory also neglects contribution of surface conductivity. This is expressed in modern theory as condition of small Dukhin number.

$$Du \ll 1$$

In the effort of expanding the range of validity of electrophoretic theories, the opposite asymptotic case was considered, when Debye length is larger than particle radius:

$$a\kappa < 1$$

Under this condition of a “thick Double Layer”, Huckel predicted the following relation for electrophoretic mobility:

$$\mu_e = \frac{2\varepsilon_r \varepsilon_0 \zeta}{3\eta}$$

This model can be useful for some nanoparticles and non-polar fluids, where Debye length is much larger than in the usual cases. There are several analytical theories that incorporate surface conductivity and eliminate the restriction of a small Dukhin number pioneered by Overbeek and Booth. Modern, rigorous theories valid for any zeta potential and often any  $a\kappa$  stem mostly from Dukhin-Semenikhin theory. In the **thin Double Layer** limit, these theories confirm the numerical solution to the problem provided by O’Brien and White.



### INTEXT QUESTIONS 21.1

1. Define electrophoresis.
2. The gel matrix widely used in electrophoresis is mainly ..... and .....
3. The widely used theory of electrophoresis was developed in ..... by .....
4. The part of the force that is transferred to the particle surface through viscous stress is called .....



Notes

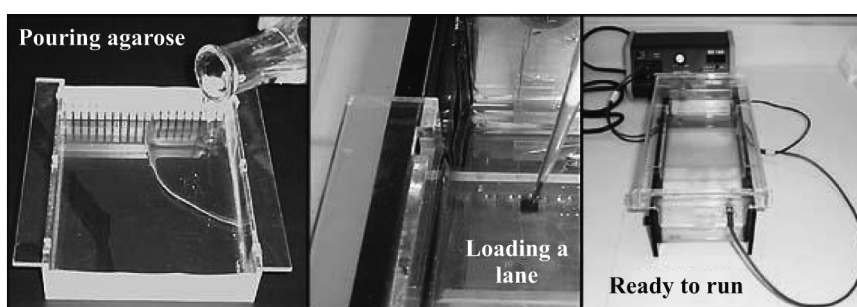
### 21.3 PREPARING AND RUNNING STANDARD AGAROSE GEL



Notes

The equipment and supplies necessary for conducting agarose gel electrophoresis are relatively simple (Fig 21.1) and include:

1. An electrophoresis chamber and power supply
2. Gel casting trays, which are available in a variety of sizes and composed of UV-transparent plastic. The open ends of the trays are closed with tape while the Gel is being cast, then removed prior to electrophoresis.
3. Sample combs, around which molten agarose is poured to form sample wells in the gel.
4. Electrophoresis buffer, usually Tris-acetate-EDTA (TAE) or Tris-borate-EDTA (TBE).
5. Loading buffer, which contains something dense (e.g. glycerol) to allow the sample to “fall” into the sample wells, and one or two tracking dyes, which migrate in the gel and allow visual monitoring of how far the electrophoresis has proceeded.
6. Ethidium bromide, a fluorescent dye used for staining nucleic acids. *NOTE: Ethidium bromide is a known mutagen and should be handled as a hazardous chemical - wear gloves while handling.*
7. Transilluminator (an ultraviolet light box), which is used to visualize Ethidium bromide-stained DNA in gels. *NOTE: always wear protective eyewear when observing DNA on a transilluminator to prevent damage to the eyes from UV light.*



**Fig. 21:** Gel Electrophoresis unit

To pour a gel, agarose powder is mixed with electrophoresis buffer to the desired concentration, and then heated in a microwave oven until completely melted. Most commonly, Ethidium bromide is added to the gel (final concentration 0.5 µg/ml) at this point to facilitate visualization of DNA after electrophoresis. After

cooling the solution to about 6°C, it is poured into a casting tray containing a sample comb and allowed to solidify at room temperature.

After the gel has solidified, the comb is removed, using care not to rip the bottom of the wells. The gel, still in its plastic tray, is inserted horizontally into the electrophoresis chamber and just covered with buffer. Samples containing DNA mixed with loading buffer are then pipeted into the sample wells, the lid and power leads are placed on the apparatus, and a current is applied. You can confirm that current is flowing by observing bubbles coming off the electrodes. DNA will migrate towards the positive electrode, which is usually colored red.

The distance DNA has migrated in the gel can be judged by visually monitoring migration of the tracking dyes. Bromophenol blue and xylene cyanol dyes migrate through agarose gel at roughly the same rate as double-stranded DNA fragments of 300 and 4000 bp, respectively.

When adequate migration has occurred, DNA fragments are visualized by staining with Ethidium bromide. This fluorescent dye intercalates between bases of DNA and RNA. It is often incorporated into the gel so that staining occurs during electrophoresis, but the gel can also be stained after electrophoresis by soaking in a dilute solution of Ethidium bromide. To visualize DNA or RNA, the gel is placed on a ultraviolet transilluminator.

Fragments of linear DNA migrate through agarose gel with a mobility that is inversely proportional to the  $\log_{10}$  of their molecular weight. In other words, if you plot the distance from the well that DNA fragments have migrated against the  $\log_{10}$  of either their molecular weights or number of base pairs, a roughly straight line will appear.

### 21.3.1 Requirements

Electrophoretic unit, conical flask, measuring cylinder, power pack, micropipette, micro tips (1X) TAE buffer, Gel loading dye, EtBr, Agarose.

### 21.3.2 Steps

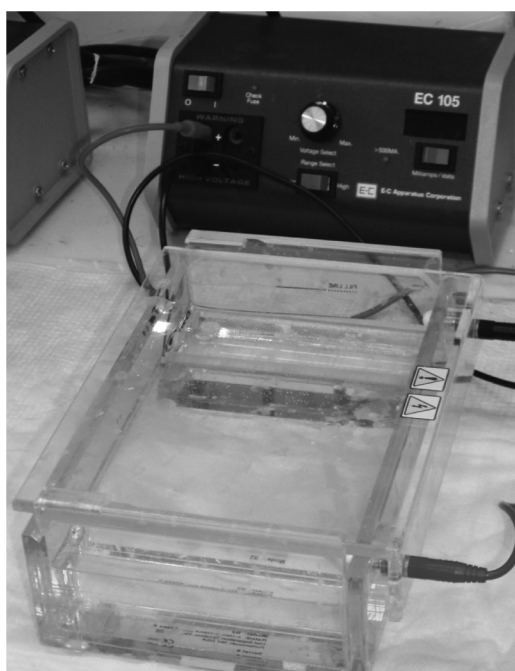
1. For preparing 0.8% agarose gel, 0.14g of Agarose was dissolved in 20ml of TAE (1X).
2. Mixture was boiled till a clear solution was obtained.
3. Left at room temperature till suspension reaches 40-45°C.
4. Then 2 $\mu$ l of 1% EtBr was added.
5. Seal the casting tray properly and placed the combs at appropriate place.

**Notes**



**Notes**

6. Pour the gel and leave at room temperature for 45-50 minutes to solidify the gel.
7. Fill the buffer tank with TAE (1X) so that the gel was dipped.
8. 5  $\mu$ l of the sample was loaded into the well by mixing with 1 $\mu$ l of 6X loading dye containing Bromophenol blue.
9. Switch on the power supply at the rate of 5V/cm (Fig. 21.2)
10. When the electrophoretic front reaches bottom of the gel power supply was switched off. The gel was placed over transilluminator and observed under UV light.



**Fig. 21.2:** Gel Electrophoresis tank with power unit



**INTEXT QUESTIONS 21.2**

1. The fluorescent dye used in the gel electrophoresis is .....
2. Give the function of a transilluminator.
3. Which two dyes migrate through the gel at same rate to help in visualizing the sample that is loaded in the gel?  
..... and .....
4. The electrophoresis buffer used is mostly .....or .....

## 21.4 TYPES OF ELECTROPHORESIS

### 21.4.1 Affinity electrophoresis

The methods include the mobility shift electrophoresis, charge shift electrophoresis and affinity capillary electrophoresis. The methods are based on changes in the electrophoretic pattern of molecules (mainly macromolecules) through biospecific interaction or complex formation. The interaction or binding of a molecule, charged or uncharged, will normally change the electrophoretic properties of a molecule. Membrane proteins may be identified by a shift in mobility induced by a charged detergent. Nucleic acids or nucleic acid fragments may be characterized by their affinity to other molecules. The methods has been used for estimation of binding constants, as for instance in lectin affinity electrophoresis or characterization of molecules with specific features like glycan content or ligand binding. For enzymes and other ligand-binding proteins, one dimensional electrophoresis similar to counter electrophoresis or to “rocket immunoelectrophoresis”, affinity electrophoresis may be used as an alternative quantification of the protein. Some of the methods are similar to affinity chromatography by use of immobilized ligands.

### 21.4.2 Capillary electrophoresis

Capillary electrophoresis (CE), can be used to separate ionic species by their charge and frictional forces and hydrodynamic radius. In traditional electrophoresis, electrically charged analytes move in a conductive liquid medium under the influence of an electric field (Fig. 21.3). Introduced in the 1960s, the technique of capillary electrophoresis (CE) was designed to separate species based on their size to charge ratio in the interior of a small capillary filled with an electrolyte.

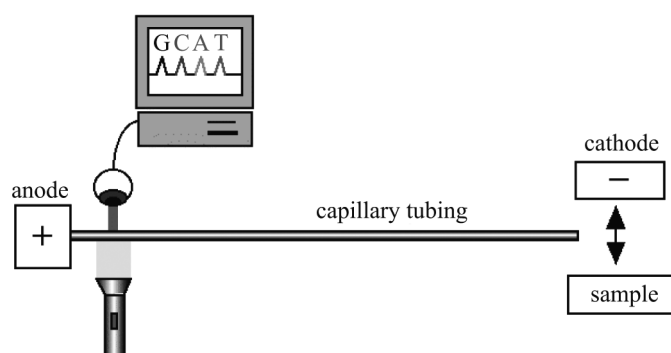


Fig. 21.3: Capillary Electrophoresis



Notes



## Notes

### 21.4.3 Immuno electrophoresis

Immuno electrophoresis is a general name for a number of biochemical methods for separation and characterization of proteins based on electrophoresis and reaction with antibodies. All variants of immuno electrophoresis require immunoglobulins, also known as antibodies reacting with the proteins to be separated or characterized.

1. **Rocket immuno electrophoresis** is one-dimensional quantitative immuno electrophoresis
2. **Fused rocket immuno electrophoresis** is a modification of one-dimensional quantitative immuno electrophoresis used for detailed measurement of proteins in fractions from protein separation experiments.
3. **Affinity immuno electrophoresis** is based on changes in the electrophoretic pattern of proteins through specific interaction or complex formation with other macromolecules or ligands.

### 21.4.4 Pulsed field gel electrophoresis

Pulsed field gel electrophoresis is a technique used for the separation of large deoxyribonucleic acid (DNA) molecules by applying to a gel matrix an electric field that periodically changes direction.

While in general small fragments can find their way through the gel matrix more easily than large DNA fragments, a threshold length exists above 30–50 kb where all large fragments will run at the same rate, and appear in a gel as a single large diffuse band. However, with periodic changing of field direction, the various lengths of DNA react to the change at differing rates. That is, larger pieces of DNA will be slower to realign their charge when field direction is changed, while smaller pieces will be quicker. Over the course of time with the consistent changing of directions, each band will begin to separate more and more even at very large lengths. Thus separation of very large DNA pieces using PFGE is made possible.

### 21.4.5 SDS-PAGE

One of the most common means of analyzing proteins by electrophoresis is by using **Sodium Dodecyl Sulfate - Polyacrylamide Gel Electrophoresis**. SDS is a detergent which denatures proteins by binding to the hydrophobic regions and essentially coating the linear protein sequence with a set of SDS molecules. The SDS is negatively charged and thus becomes the dominant charge of the



complex. The number of SDS molecules that bind is simply proportional to the size of the protein. Therefore the charge to mass ratio should not change with size. In solution (water), in principle all different sized proteins covered with SDS would run at about the same mobility. However, the proteins are not run through water. Instead they are run through an inert polymer, polyacrylamide. The density and pore size of this polymer can be varied by just how you make it (concentration of monomer and of cross-linking agent). Thus, the size of molecules that can pass through the matrix can be varied. This determines in what molecular weight range the gel will have the highest resolving power.

**Notes**

### 21.4.6 Native Gels

It is also possible to run protein gels without the SDS. These are called native gels in that one does not purposely denature the protein. Here, the native charge on the protein (divided by its mass) determines how fast the protein will travel and in what direction.

### 21.4.7 Electrofocusing Gels

Another variation of gel electrophoresis is to pour a gel that purposely has a pH gradient from one end to the other. As the protein travels through this pH gradient, its various ionizable groups with either pick up or lose protons. Eventually, it will find a pH where its charge is zero and it will get stuck (focused) at that point.

### 21.4.8 DNA Agarose Gels

A simple way of separating fairly large fragments of DNA from one another by size is to use an agarose gel. Agarose is another type of matrix used for many purposes (such as the support for the growth of bacteria on plates). DNA does not need a detergent, since it already has a large number of negative phosphate groups evenly spaced. Thus, as with SDS-PAGE, the charge to mass ratio is constant. Also like SDS-PAGE, the separation results from the matrix itself. The range of size sensitivity can be varied by changing the density of the agarose.

DNA denaturing polyacrylamide gels (often called sequencing gels). To look at smaller DNA molecules with much higher resolution, people generally denature the DNA via heat and run it through a thin polyacrylamide gel that is also kept near the denaturing temperature. These gels usually contain additional denaturing compounds such as Urea. Two pieces of DNA that differ in size by 1 base can be distinguished from each other this way.



Notes

**INTEXT QUESTIONS 21.3**

1. Expand the following abbreviations:
  - SDS
  - PAGE
  - CE
2. DNA denaturing gels are also called as .....
3. Mention the correct type of electrophoresis for the following:
  - Gel has a pH gradient
  - Separate large DNA fragments
  - Separate protein without SDS
  - Separate proteins based on electrophoresis and reaction with antibodies
  - separate ionic species by charge, friction force and hydrodynamic radius

**21.5 APPLICATIONS**

Gel electrophoresis is used in forensics, molecular biology, genetics, microbiology and biochemistry. The results can be analyzed quantitatively by visualizing the gel with UV light and a gel imaging device. The image is recorded with a computer operated camera, and the intensity of the band or spot of interest is measured and compared against standard or markers loaded on the same gel. Depending on the type of analysis being performed, other techniques are often implemented in conjunction with the results of gel electrophoresis, providing a wide range of field-specific applications.

**WHAT HAVE YOU LEARNT**

- Electrophoresis plays a vital role in the separation of nucleic acids and proteins in the field of genomics and proteomics
- The techniques are very simple but has its role in advanced studies
- The electrophoretic devices are economical and can be explored to the core to analyze the complexity of biomolecules



## ANSWERS TO INTEXT QUESTIONS

## 21.1

1. The movement of particles under spatially uniform electric field in a fluid is called **electrophoresis**.
2. Polyacrylamide and agarose
3. 1903, Smoluchowsky
4. electrophoretic retardation force

## 21.2

1. ethidium bromide (Et Br)
2. It is used to visualize the Et Br stained DNA or RNA in gel through ultraviolet light of specific wavelength.
3. Bromophenol blue and xylene cyanol
4. TAE (tris Acetate EDTA) or TBE (Tris Borate EDTA)

## 21.3

1. SDS – Sodium dodecyl sulphate  
PAGE – polyacrylamide gel electrophoresis  
CE – capillary electrophoresis
2. Sequencing gels
3.
  - Gel has a pH gradient                      electrofocusing gels
  - Separate large DNA fragments        Agarose gels
  - Separate protein without SDS        Native gels
  - Separate proteins based on            Immunoelectrophoresis  
    electrophoresis and reaction  
    with antibodies
  - separate ionic species by charge,    capillary electrophoresis  
    friction force and hydrodynamic  
    radius



Notes