MSC S2: TECHNIQUES IN RESEARCH : 16P2EVST05 -DR. T J JAMES COORDINATOR, ENVIRONMENTAL SCIENCE DEPARTMENT, SACRED HEART COLLEGE THEVARA

TOPIC :**ELECTROPHORESIS**

MODULE – 3

ELECTROPHORESIS

- **“Electrophoresis” literally means running in the electric field.**
- **Electrophoresis means Electro = electric field + Phoresis = migration.**
- **“electrophoresis is a method of separation where in charged molecules migrate in differential speeds in an applied electric field.“**

- **Electrophoresis is a physical method of analysis which involves separation of the compounds that are capable of acquiring electric charge in conducting electrodes.**

- **Electrophoresis may be defined as the migration of the charged particle through a solution under the influence of an external electrical field.**

- **Ions that are suspended between two electrodes tends to travel towards the electrodes that bears opposite charges.**

- 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.

- It is one of the highly efficient techniques of analysis and sole method for separation of proteins for western blot, RNA studies etc.

PRINCIPLE

- Biological molecules exist in a solution as electrically charged particles at a given pH.

- anionic (+vely charged/basic)
- cationic (-vely charged / acidic)

} " Zwitterions " or "amphoteric molecules".

- pH greatly influences the total charge of molecules.

- All types of electrophoresis are governed by the single set of general principles illustrated by Equation

$$\text{Mobility of a molecule} = \frac{(\text{applied voltage})(\text{net charge on the molecule})}{(\text{friction of the molecule})}$$

- The mobility, or rate of migration, of a molecule increases with increased applied voltage and increased net charge on the molecule.

- Conversely, the mobility of a molecule decreases with increased molecular friction, or resistance to flow through the viscous medium, caused by molecular size and shape.

- Total actual movement of the molecules increases with increased time, since mobility is defined as the rate of migration.

- Most electrophoretic systems employ an equal and constant voltage on all of the cross-sectional areas of the paper strips, gels, or solutions employed in the

electrophoretic separation. These electric fields are best defined in terms of volts per linear centimeter.

- The resistance of the system is important because it will determine the amount of heat generated during electrophoresis.

- The electrophoretic mobility is also a function of temperature, If significant heating occurs during electrophoresis, it will be necessary to provide some means of cooling the apparatus so as to maintain a constant temperature

- When electricity is applied to the medium containing biological molecules, depending on their net charge & molecular size, they migrate differentially, thus different proteins/DNA can be separated.

- The velocity (v) of charged molecule in an electric field-

$$v = Eq/F$$

Where F = frictional coefficient, which depends upon the mass and shape of the molecule.

E = electric field (V/ cm)

q = the net charge on molecule v = velocity of the molecule.

- **If the voltage or current applied to an electrophoresis system is constant throughout an electrophoretic separation, the mobilities of the molecules being resolved will reflect the net charge and frictional characteristics of the molecules in the sample.**

- **Electrophorsis will depend on the charge on a macromolecule rather than its mass, it provides an additional 'handle' for the analysis and separation of mixtures.**

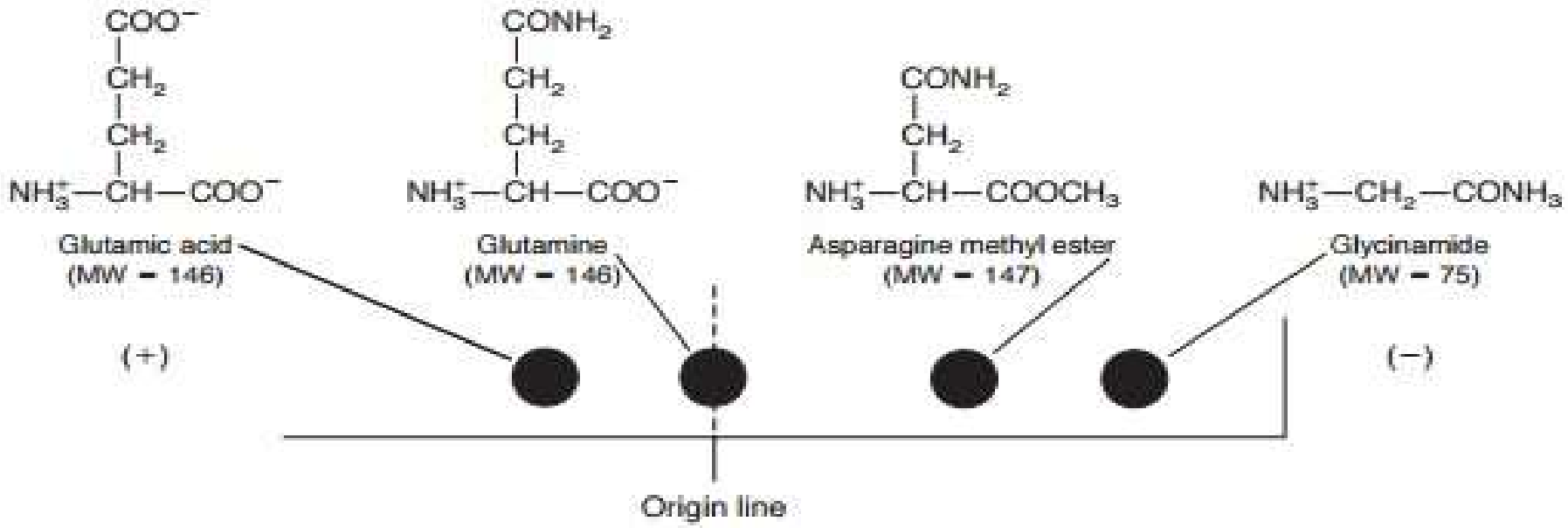

Figure 4-3 Paper electrophoretic separation of glutamic acid, glutamine, asparagine, methyl ester, and glycinamide at pH 6.0. The glycinamide, with a charge of +1 and a molecular weight of 75, may not necessarily migrate twice the distance migrated by asparagine methyl ester, with the same charge and −2 times larger size. Specifically, the character of the buffer can influence the expression of the electrophoretic frictional contribution of small molecules. Higher ionic strength decreases the frictional contribution.

Types of Electrophoresis

- **1) Zone Electrophoresis**

- **It involves the migration of the charged particle on the supporting media.**

- **Eg:**

a) Paper Electrophoresis
b) Gel Electrophoresis
c) Thin Layer Electrophoresis
d) Cellulose acetate Electrophoresis

2) Moving Boundary Electrophoresis

- The moving boundary method allows the charged species to migrate in a free moving solution without the supporting medium.

- Eg:

a) Capillary Electrophoresis
b) b) Isotachophoresis
c) c) Isoelectric Focussing
d) d) Immuno Electrophoresis

- **Electrophoresis is often classified according to the presence or absence of a solid supporting medium or matrix through which the charged molecules move in the electrophoretic system.**

- **Solution electrophoresis systems employ aqueous buffers in the absence of a solid support medium.**

- **Such systems can suffer from sample mixing due to diffusion of the charged molecules, with resultant loss of resolution during sample application, separation, and removal steps.**

- **Thus, solution electrophoresis systems must employ some means of stabilizing the aqueous solutions in the electrophoresis cell.**

- **For example, soluble-gradient electrophoresis systems use varying densities of a non-ionic solute (e.g., sucrose or glycerol) to minimize diffusional mixing of the materials being separated during electrophoresis.**

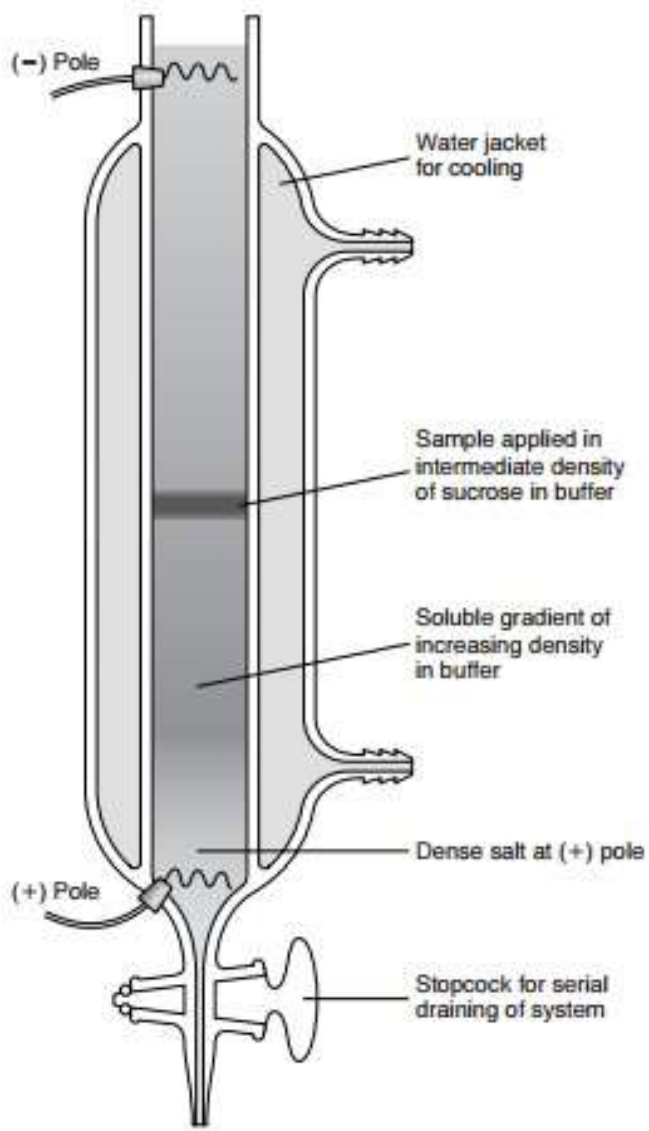

Figure 4-1 A solution electrophoresis system.

- Even with these refinements, solution electrophoresis systems have only limited application, usually when preparative scale electrophoretic separation is required.

- Most practical applications of electrophoresis in biochemistry employ some form of **zonal electrophoresis**, in which the aqueous ionic solution is carried in a solid support and samples are applied as spots or bands of material.

- **Paper electrophoresis, cellulose acetate strip and cellulose nitrate strip, and gel electrophoresis are all examples of zonal electrophoresis systems.**

- **Such systems are typically employed for analytical, rather than preparative scale, separations.**

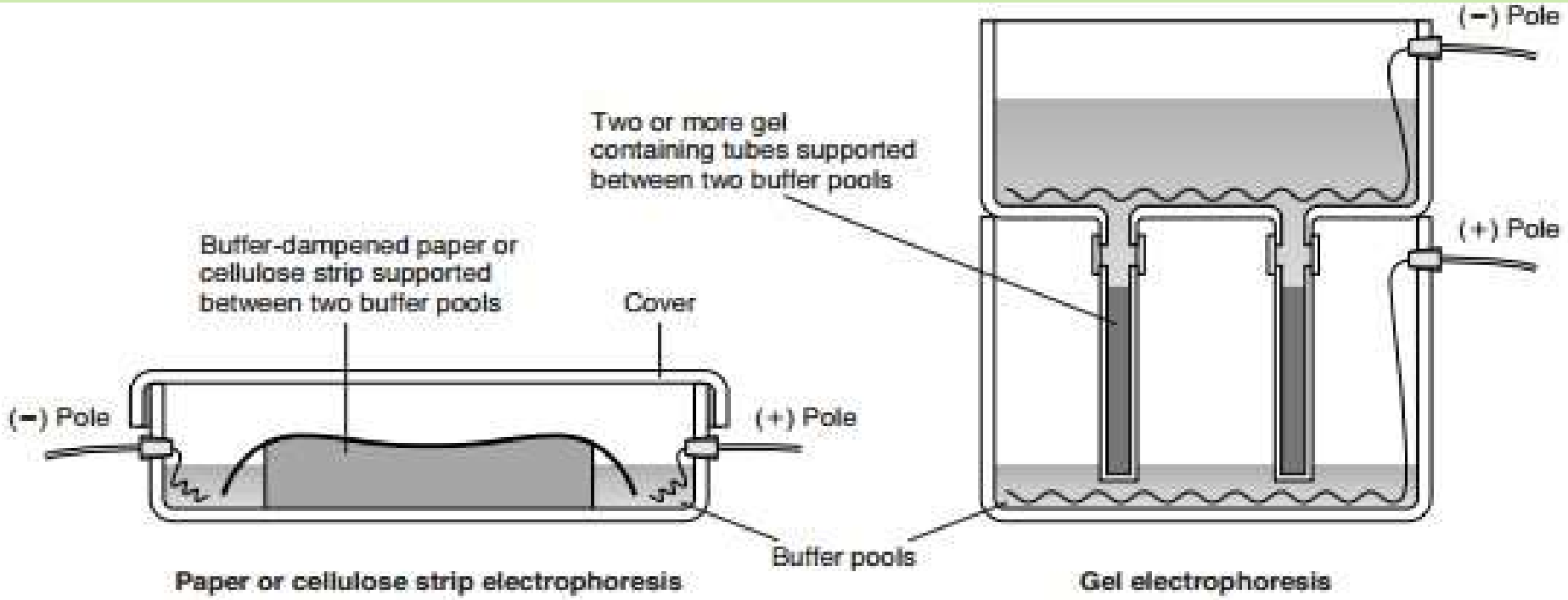

Figure 4-2 Two zonal electrophoresis systems.

- **The friction experienced by molecules during electrophoretic migration reflects both molecular size and molecular shape.**

- **If the electrophoresis is carried out in a medium that offers significant barriers to the movement of macromolecules through it (as is the case with polyacrylamide and agarose), *molecular size may prove to be the most important determinant of mobility.***

- **If the charge to mass ratio on the macromolecules being separated is approximately equal, molecular size becomes the sole determinant of electrophoretic mobility.**

- These conditions are exploited for the determination of the molecular weight of protein subunits by electrophoresis in polyacrylamide gels containing sodium dodecyl sulfate (SDS-PAGE), and in the electrophoretic separation of oligonucleotide "ladders" during DNA sequencing.

- Molecular shape is not very significant in small molecules, in which bonds are free to rotate, so size alone defines their friction.

- However, macromolecules often have defined shapes with specific axial ratios (i.e., length to width ratios). As a result, both size and shape influence migration.

- Molecules with high axial ratios demonstrate lower electrophoretic mobility than more spherical molecules that have equal weight and equal charge.

- In addition, macromolecules may deviate from the electrophoretic principles of Equation-

$$\text{Mobility of a molecule} = \frac{(\text{applied voltage})(\text{net charge on the molecule})}{(\text{friction of the molecule})}$$

because of interaction with ions or because of charge-dependent intermolecular associations.

1. Gel Electrophoresis

- In contrast to proteins, which can have either a net positive or net negative charge, nucleic acids have a consistent negative charge imparted by their phosphate backbone, and migrate toward the anode.

- Proteins and nucleic acids are electrophoresed within a matrix or "gel".

- Most commonly, the gel is cast in the shape of a thin slab, with wells for loading the sample.

- **The gel is immersed within an electrophoresis buffer that provides ions to carry a current and some type of buffer to maintain the pH at a relatively constant value.**

- **In gel electrophoresis, molecules are separated in aqueous buffers supported within a polymeric gel matrix.**

- **Separation is brought about through molecular sieving technique, based on the molecular size of the substances. Gel material acts as a "molecular sieve".**

- **Gel is a colloid in a solid form (99% is water).**

- The character of the gel matrix can be altered at will to fit a particular application.
- This is possible because the gel enhances the friction that governs the electrophoretic mobility.
- Low concentrations of matrix material or a low degree of cross-linking of the monomers in polymerized gel systems allow them to be used largely as a stabilizing or anticonvection device with relatively low frictional resistance to the migration of macromolecules.

- **Alternatively, higher concentrations of matrix material or a higher degree of cross-linking of monomers are used to generate greater friction, which results in molecular sieving.**

- **Molecular sieving is a situation in which viscosity and pore size largely define electrophoretic mobility and migration of solutes.**

- **As a result, the migration of macromolecules in the system will be substantially determined by molecular weight.**

- It is important that the support media is electrically neutral. When charged molecules are placed in an electric field, they migrate toward either the positive or negative pole according to their charge.

- The gel itself is composed of either agarose or polyacrylamide, each of which have attributes suitable to particular tasks:

1. Agarose

- Agarose is a polysaccharide extracted from seaweed.
- It is typically used at concentrations of 0.5 to 2%.
- The higher the agarose concentration the "stiffer" the gel.

- Agarose gels are extremely easy to prepare: you simply mix agarose powder with buffer solution, melt it by heating, and pour the gel. It is also non-toxic.

- Agarose gels have a large range of separation, but relatively low resolving power.

- By varying the concentration of agarose, fragments of DNA from about 200 to 50,000 bp can be separated using standard electrophoretic techniques.

2. Polyacrylamide

- It is a cross-linked polymer of acrylamide.
- The length of the polymer chains is dictated by the concentration of acrylamide used, which is typically between 3.5 and 20%.
- Polyacrylamide gels are significantly more annoying to prepare than agarose gels.
- Because oxygen inhibits the polymerization process, they must be poured between glass plates (or cylinders).

- **Polyacrylamide gels have a rather small range of separation, but very high resolving power.**
- **In the case of DNA, polyacrylamide is used for separating fragments of less than about 500 bp.**
- **However, under appropriate conditions, fragments of DNA differing is length by a single base pair are easily resolved.**
- **In contrast to agarose, polyacrylamide gels are used extensively for separating and characterizing mixtures of proteins.**

- **The acrylamide monomer and cross-linker are stable by themselves or mixed in solution, but polymerize readily in the presence of a free-radical generating system.**

Table 4-1 Recipe for Polyacrylamide Gels with Various Percent Acrylamide Monomer for Use with SDS-PAGE

	% Acrylamide in Resolving Gel				
Component (ml)	7.5	10	12	15	20
Distilled water	9.6	7.9	6.6	4.6	2.7
30% acrylamide solution	5.0	6.7	8.0	10.0	11.9
1.5 M Tris chloride (pH 8.8)	5.0	5.0	5.0	5.0	5.0
10% (wt/vol) SDS	0.2	0.2	0.2	0.2	0.2
TEMED	0.008	0.008	0.008	0.008	0.008
10% (wt/vol) ammonium persulfate	0.2	0.2	0.2	0.2	0.2

Prepare the ammonium persulfate fresh and add last to induce the polymerization process (polymerization of the resolving gel will take approximately 30 min).