A study material for M.Sc. Biochemistry (Semester: III) Students
on the topic (CC-14; Unit C)

Gel Electrophoresis I

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Electrophoresis:

Electrophoresis is defined as the separation (migration) of charged particles through a solution or gel, under the influence of an electrical field.The rate of movement of particle depends on the following factors.

a. The charge of the particle
b. Applied electric field
c. Temperature
d. Nature of the suspended medium.

Gel Electrophoresis:

Gel electrophoresis is a method that separates macromolecules—either nucleic acids or proteins—on the basis of size, electric charge, and other physical properties. A gel is a colloid in a solid form. The term electrophoresis describes the migration of charged particles under the influence of an electric field. “Electro” refers to the energy of electricity. “Phoresis,” from the Greek verb phoros, means “to carry across.” Thus, gel electrophoresis refers to the technique in which molecules are forced across a span of gel, motivated by an electrical current. Activated electrodes at either end of the gel provide the driving force. A molecule’s properties determine how rapidly an electric field can move the molecule through a gelatinous medium.

Electrophoresis

- Electrophoresis is a method whereby charged molecules in solution, chiefly proteins and nucleic acids, migrate in response to an electrical field.
- Their rate of migration through the electrical field, 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 the medium in which the molecules are moving.
- As an analytical tool, electrophoresis is simple, rapid and highly sensitive.
- It can be used analytically to study the properties of a single charged species or mixtures of molecules. It can also be used preparatively as a separating technique

Electrophoresis

- Electrophoresis is usually done with gels formed in tubes, slabs, or on a flat bed.
- In many electrophoresis units, the gel is mounted between two buffer chambers containing separate electrodes, so that the only electrical connection between the two chambers is through the gel.

History: Overview

- **1920's**
  - Erich Huckel and M. Smoluchowski are among the pioneers of electrophoresis.
  - Huckel developed the Huckel equation
  - D. C Henry – provided a theory spherical polyions.
- **1930's**
  - A. Tiselius: Nobel Prize for Chemistry in 1948
    - Introduced idea of moving boundaries
- **1960's**
  - A. L. Shapiro, E. Vinuela and J. V. Maizel: developed relationship between electrophoretic migration of proteins and their molecular weight.

Erich Huckel

Arne Tiselius

History: Overview

- **1975**
  - Farrell and J. Klose: developed 2D electrophoresis
- **1981**
  - J. W. Jorgensen and K. D. Lukas: performed electrophoretic amino acid separation at high efficiency
- **1990**
  - B. L. Karger's group: discovered a matrix that could be used to separate DNA at high resolution

- All these improvements led to the use of electrophoresis in mapping the human genome.

- **2000 to now**
  - widely used high-resolution techniques for analytical and preparative separations

Key application of Nucleic Acid Electrophoresis

Technique	Separation principle	Application	Reference
Large fragment DNA and RNA separation	Size; due to the negatively charged phosphate backbone, the nucleic acids have a constant charge-to-mass ratio	Purification, determination of purity and size, genetic analysis, and preparation of DNA probes	*CP Molecular Biology* *UNIT 2.7* (Chory and Pollard, 1999) and *UNIT 2.8* (Smith and Nelson, 2004)
Small fragment DNA and RNA separation	Size; see above	Purification, determination of purity and size, genetic analysis, and preparation of DNA probes	*CP Molecular Biology* *UNIT 2.5A* (Voytas, 2000) and *UNIT 2.5B* (Finney, 2000)
DNA sequencing	Size of single-stranded oligonucleotide; denaturing gels and high temperature keep DNA single stranded	DNA sequence determination; mutation and polymorphism analysis	*CP Molecular Biology* Chapter 7 (Ausubel et al., 2012)

[a]Table adapted from Gallagher (1999a).

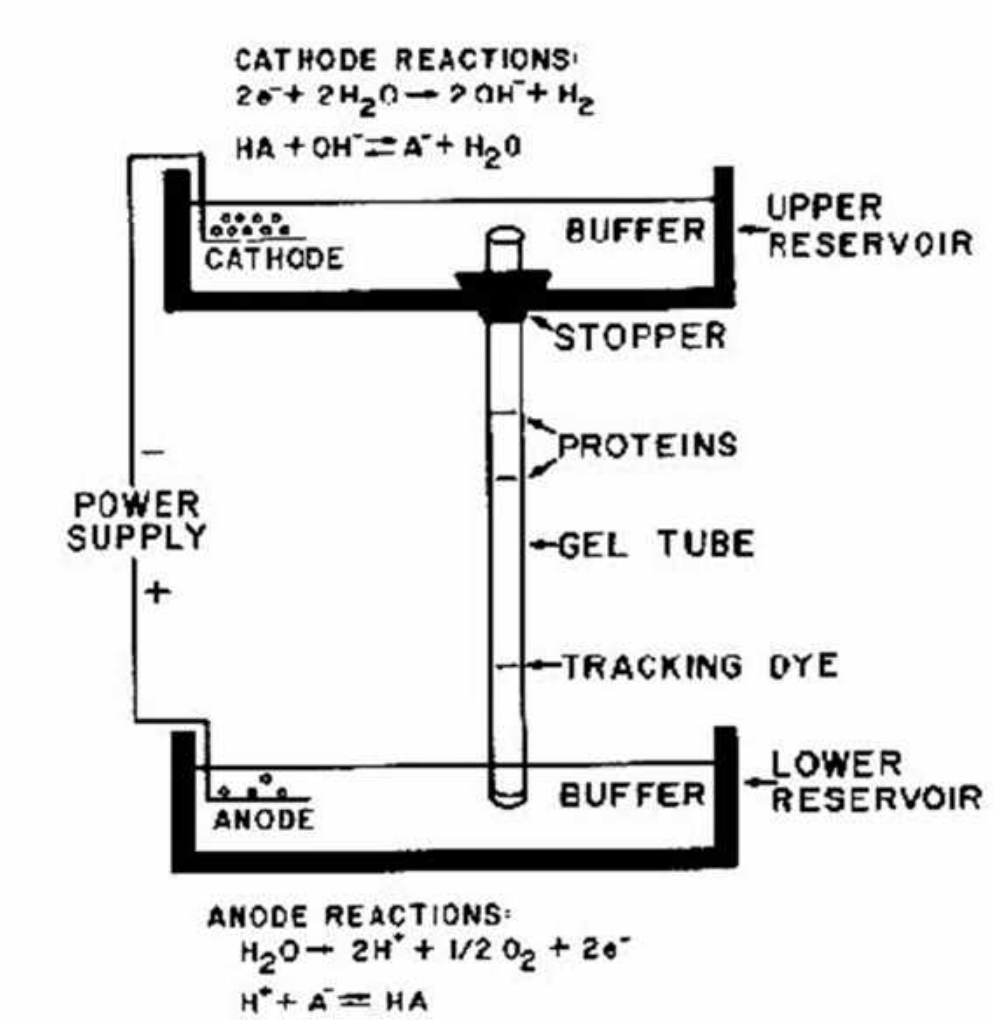
Key application of Protein Electrophoresis

Technique	Separation principle	Application	Reference
Native PAGE	Native charge, size, shape	Purification, size of native protein and protein complex, isoenzyme analysis	*CP Molecular Biology* *UNIT 10.2B* (Gallagher, 1999b)
SDS-PAGE	Size dependent; SDS imparts negative charge to proteins, giving a constant charge-to-mass ratio	Size estimation, purity, purification, subunit composition, protein expression and turnover, post-translational modifications	*CP Molecular Biology* *UNIT 10.2A* (Gallagher, 2006)
IEF	Intrinsic charge (both native and denatured proteins)	Purification, purity check, isoelectric point analysis, isoenzyme detection	*CP Molecular Biology* *UNIT 10.3* (Adams, 1996) and *UNIT 10.4* (Adams and Gallagher, 2004)
2-D SDS-PAGE	Isoelectric point in the first dimension and size in the second	Protein expression, purification, post-translational analysis, proteomics, MS analysis of separated protein	*CP Molecular Biology* *UNIT 10.3* (Adams, 1996) and *UNIT 10.4* (Adams and Gallagher, 2004)

[a]Table adapted from Gallagher (1999a).

Abbreviations: SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; IEF, isoelectric focusing; 2-D, 2-dimensional.

The Technique



Interrelation of Resistance, Voltage, Current and Power

- Two basic electrical equations are important in electrophoresis
  - The first is Ohm's Law, $I = E/R$ (I: current in AMP, R: resistance, E: voltage or potential gradient)
  - The second is $P = EI$ (P: Power in watts)
  - This can also be expressed as $P = I^2R$
- In electrophoresis, one electrical parameter, either current, voltage, or power, is always held constant

Consequences

- Under constant current conditions (velocity is directly proportional to current), the velocity of the molecules is maintained, but heat is generated.
- Under constant voltage conditions, the velocity slows, but no additional heat is generated during the course of the run
- Under constant power conditions, the velocity slows but heating is kept constant

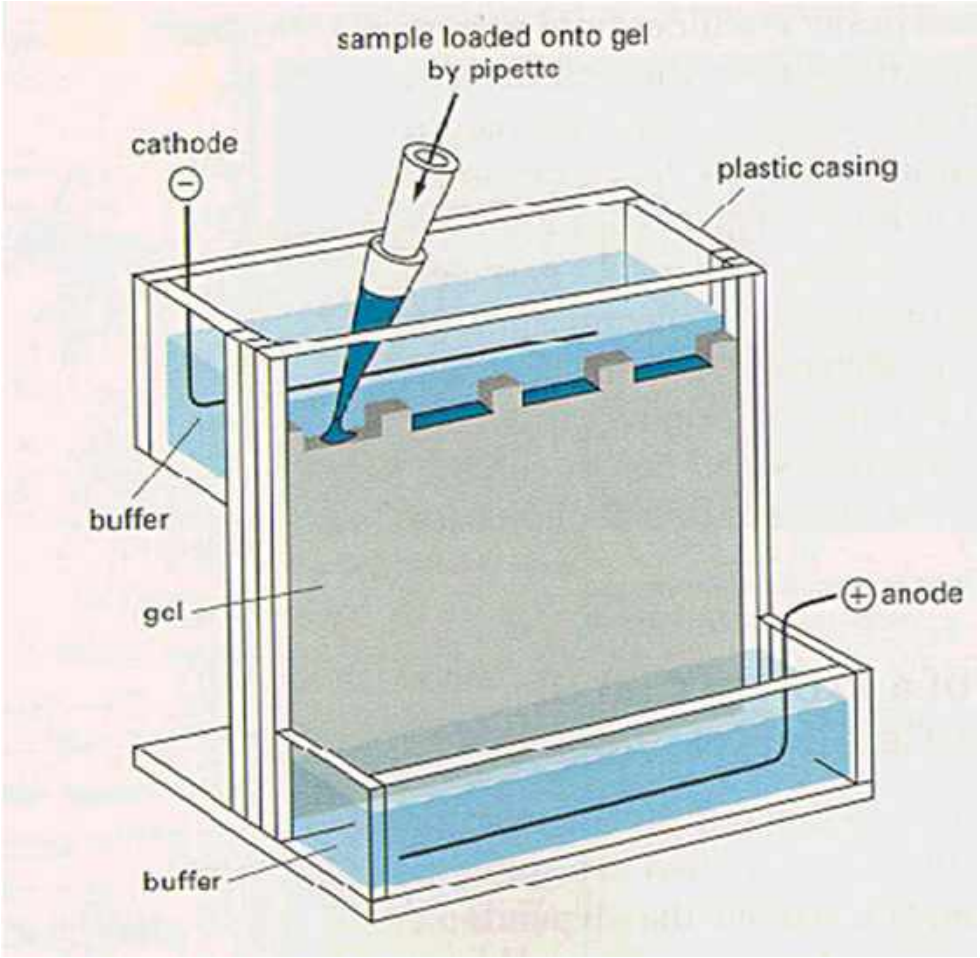
The Net Charge is Determined by the pH of the Medium

- Proteins are amphoteric compounds, that is, they contain both acidic and basic residues
- Each protein has its own characteristic charge properties depending on the number and kinds of amino acids carrying amino or carboxyl groups
- Nucleic acids, unlike proteins, are not amphoteric. They remain negative at any pH used for electrophoresis

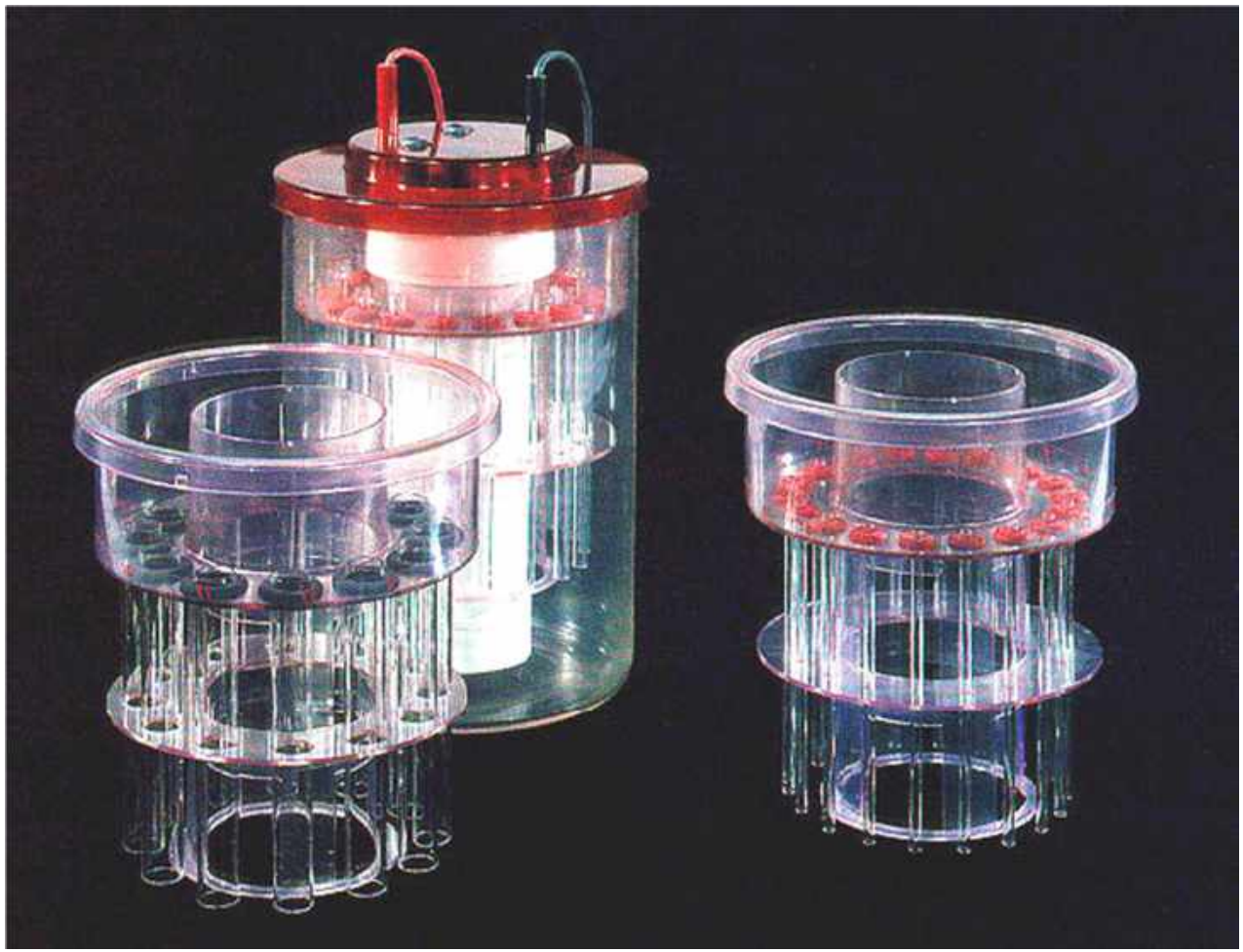
Temperature and Electrophoresis

- Important at every stage of electrophoresis
  - During Polymerization
    - Exothermic Reaction
    - Gel irregularities
    - Pore size
  - During Electrophoresis
    - Denaturation of proteins
    - Smile effect
    - Temperature Regulation of Buffers

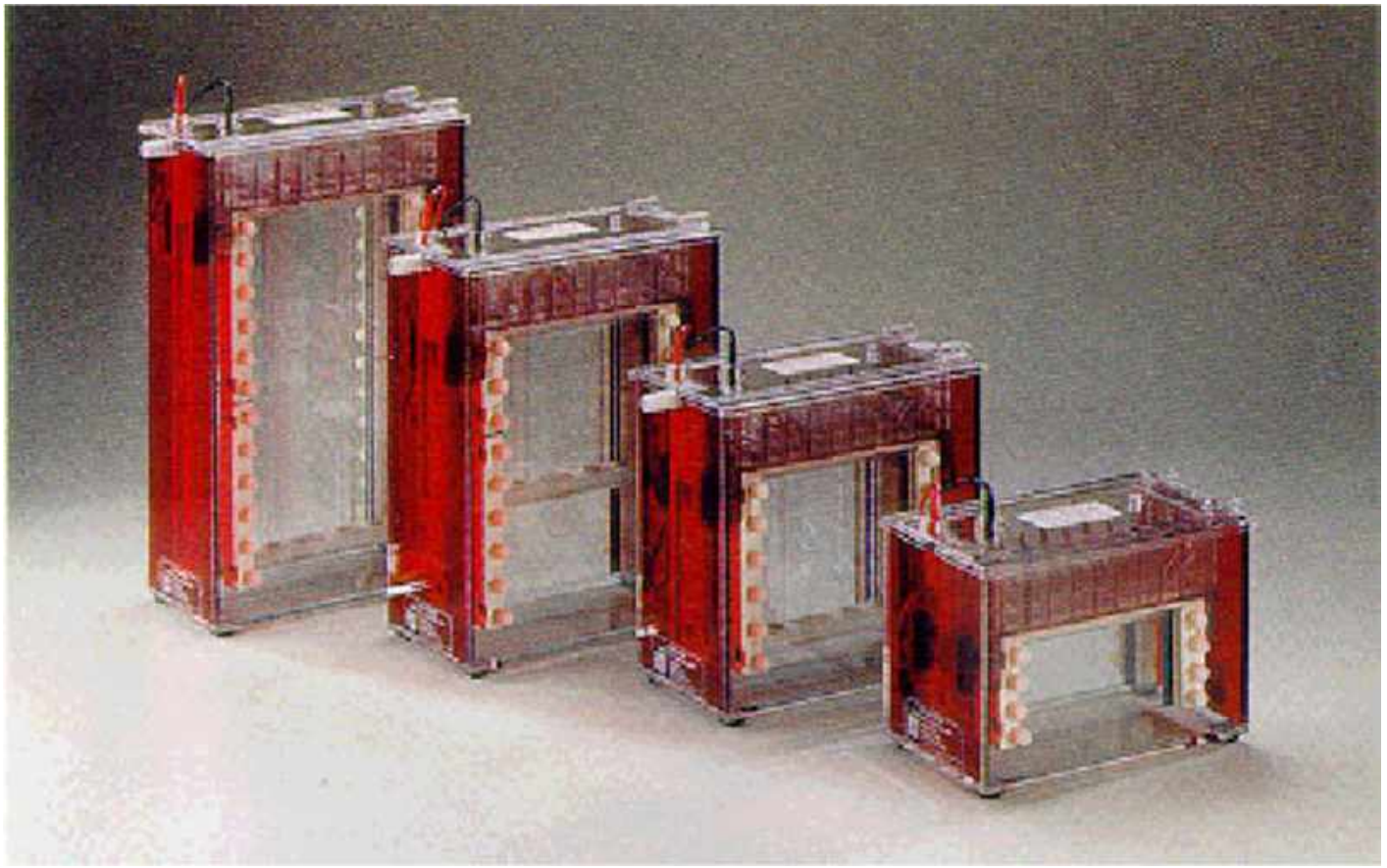
In most electrophoresis units, the gel is mounted between two buffer chambers containing separate electrodes so that the only electrical connection between the two chambers is through the gel.



Tube Gel Units

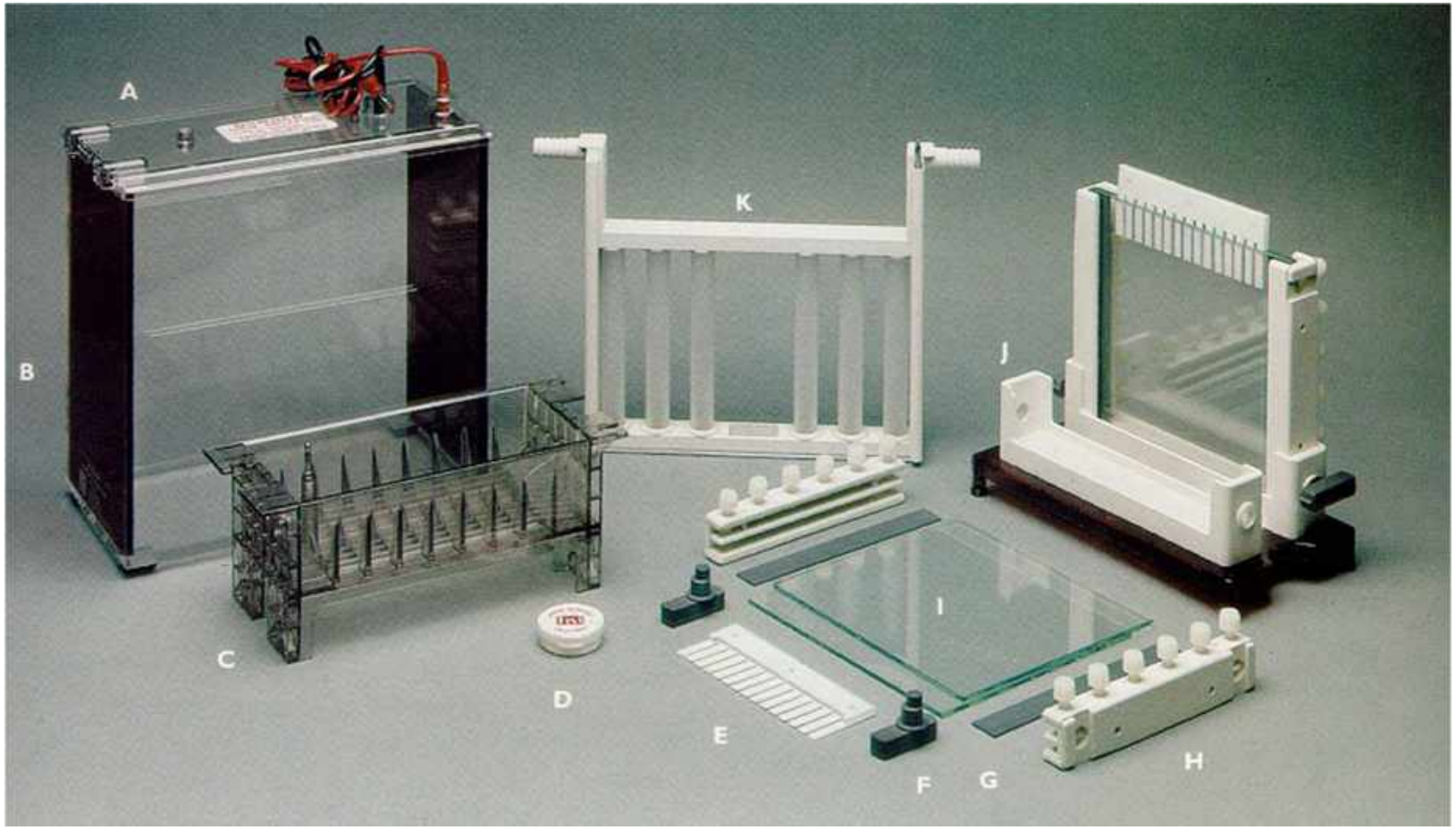


Slab Gel Units

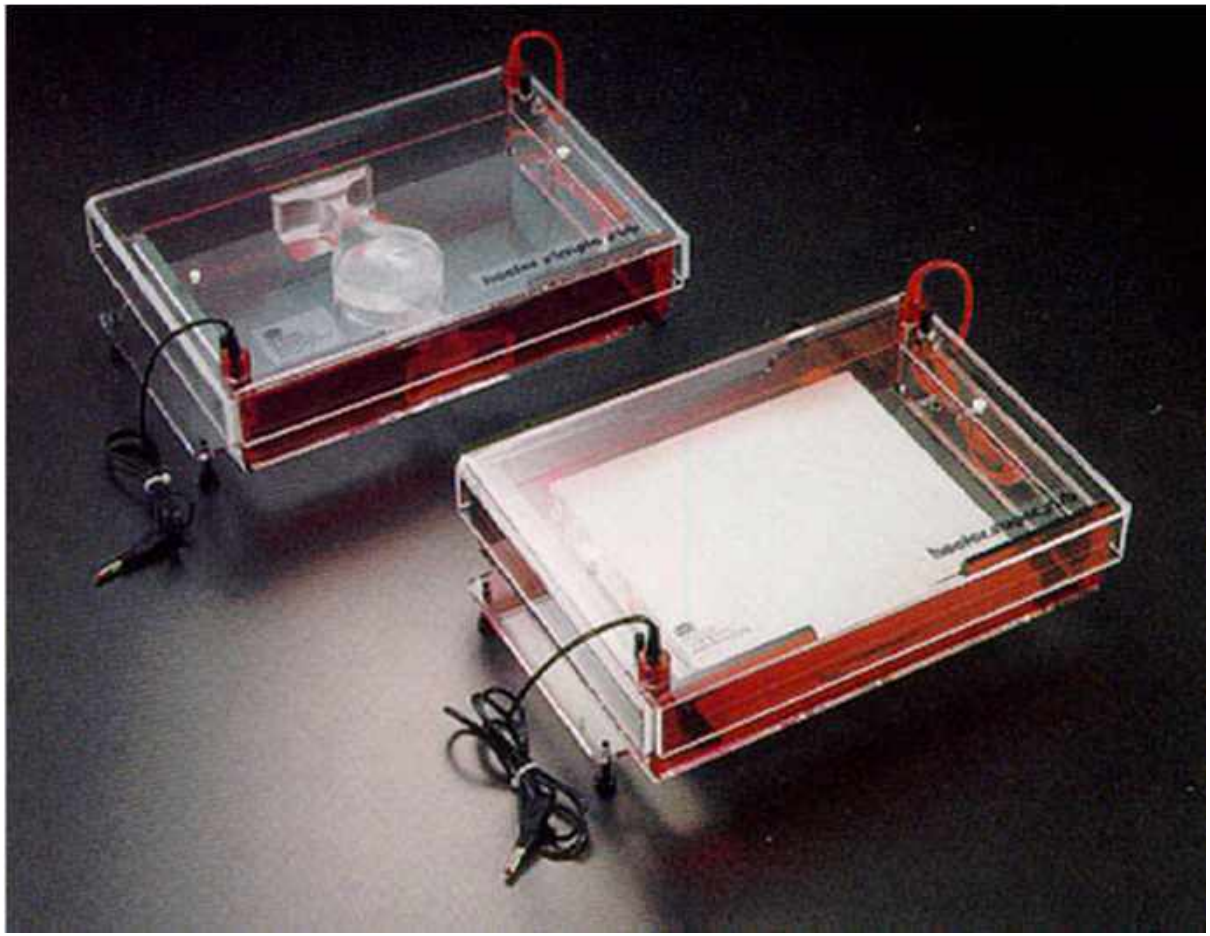


Slab Gel Unit

Slab Gel Unit



Flat Bed Unit



Role of the Solid Support Matrix

- It inhibits convection and diffusion, which would otherwise impede separation of molecules
- It allows a permanent record of results through staining after run
- It can provide additional separation through molecular sieving

Important formula used in Electrophoresis

Formula	Definition
QE	The driving force of the charged protein or DNA is a product of the charge Q and the voltage or potential gradient E in the electrophoresis gel
$F = 6\pi r v \eta$	F is the resistance to the movement of protein or DNA. r represents the molecule radius, v is the velocity in solution, and η is the viscosity of the liquid surrounding the molecule.
$V = IR$	Ohm's Law: useful for understanding how to use power supplies and electrophoresis separation equipment for optimum separation. V is voltage in volts, I is current in amps (A), and R is resistance in Ohms.
$I = V/R$	Typically, SDS-PAGE is performed at a constant current (I) of 10 to 30 milliamps (mA) per gel.
$P = VI$; $P = I^2R$	P is power in watts (W) and is defined as the product of current and voltage, or the product of current squared and resistance. Excess power generates heat that will cause distortion and overall loss of resolution. Typically, SDS-PAGE electrophoresis is performed at <5 W per gel.
R_f = Distance migrated by band/distance migrated by reference marker	Relative mobility (R_f) is a convenient way to compare gels and sample migrations from approximate molecular weight relative to the tracking dye.
$\%T$ = g total monomer (acrylamide + bisacrylamide)/ 100 ml final gel solution	$\%T$ (compared to % acrylamide) is a more accurate way (together with $\%C$) to express the exact composition of the gel.
$\%C$ = g bisacrylamide/g (acrylamide + bisacrylamide) × 100	$\%C$ is an important value to report so the exact composition of the gel can be reproduced.

[a]Table adapted from Gallagher (1999a).

Acknowledgement and Suggested Readings:
1. Biotechnology Procedures and Experiments Handbook; S. Harisha; Infinity science Press LLC
2. Molecular cloning, A laboratory Manual; Sambrook and Russell; Third Edition; Cold Spring Press

Thanks