ADVANCED LAB TECHNIQUES LECTURE 8

Electrophoresis

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Electrophoresis-

•Electro=Electric; phoresis=Migration;Carry accross.

•A kind of separation technique based on the differential migration features of charged molecules in an electric field.

• An analytical method frequently used in molecular biology, biochemistry and medicine.

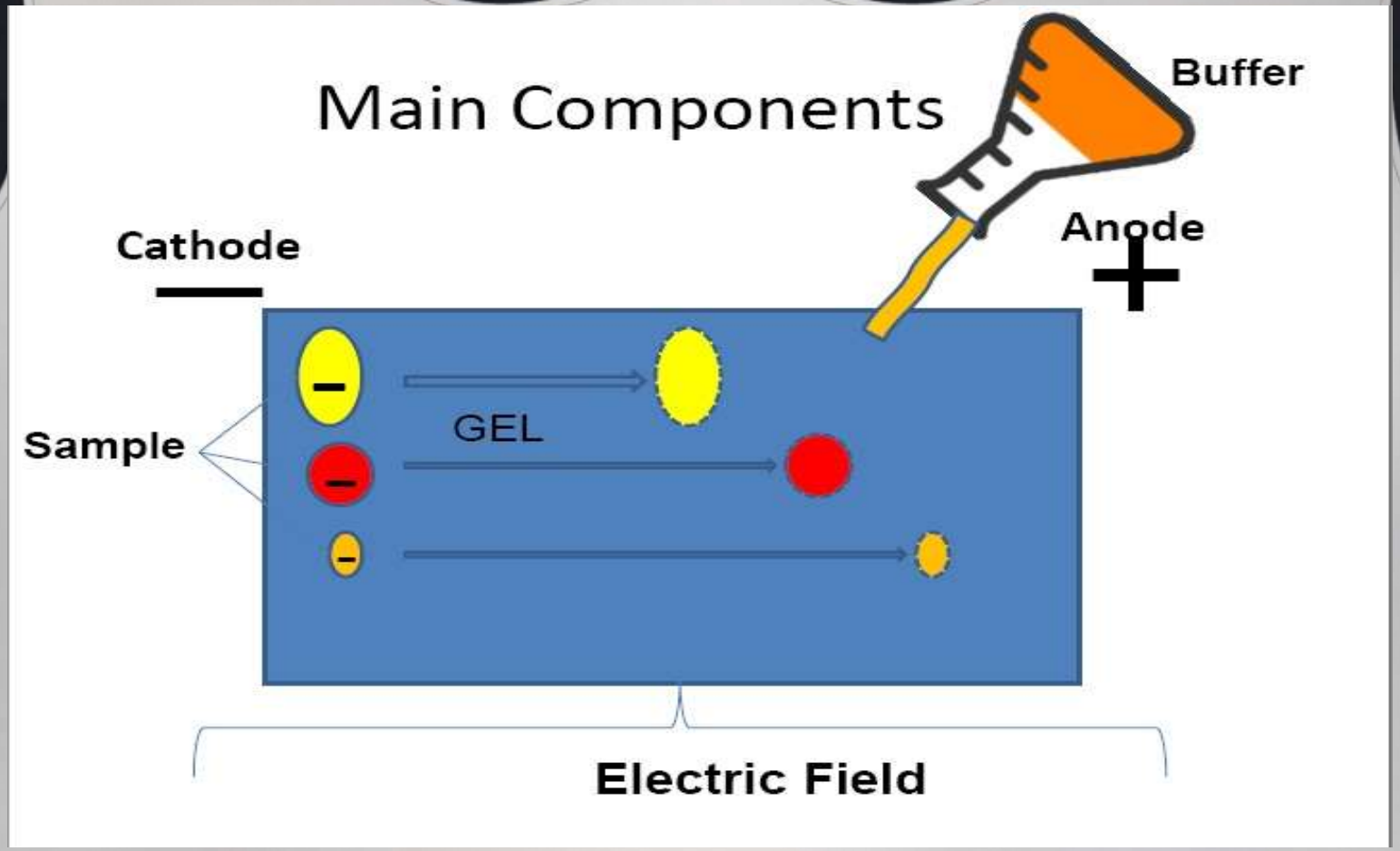
Principle:

– In an electrical field charged molecules and particles migrate to the opposite charge.

–Usually in aqueous solution(Buffer).

–Due to their varying charges and masses, different molecules and particles in the mixture are migrate at different speeds.

–As a result; separated into single fractions(bands).


Main Components
Buffer
Cathode
Anode
Sample
GEL
Electric Field

Migration Depends on

- Strength of electric fields.
- Temperature
- Features of the molecule
  - Net charge of molecule
  - Size of molecule
  - Shape of molecule
- Features of the Gel
  - Gel type
  - Gel concentration
- Buffer Type/pH.

Electrophoresis Separates :

–Nucleic acids

–Proteins

–Peptides

–Amino acids

–Organic acids/bases

–Drugs

–Pesticides

–Inorganic anions/cations.

– Everything that can carry a charge.!

Electrophoresis Types

- Gel electrophoresis
  - –Agarose gel
  - –Polyacrylamide gel
  - –Others.
- Pulsed Field Gel Electrophoresis
- Capillary Electrophoresis
- Isoelectric focusing
- 2D electrophoresis

Gel Electrophoresis

- Use of a gelatinous material.
- The gel acts as a support medium
- Used to separate proteins or nucleic acids.

Gel Types

- Starch-Rarely used
- Polyacrylamide-Protein, small nucleic acid fragments
- Agarose-Nucleic acids, large proteins
- Cellulose acetate-Proteins

Agarose Gel Electrophoresis

- Easy, fast, well established method for separating DNA fragments.
- Agarose, a polysaccharide derived from seaweed.

Dissolves in boiling water, and hardens, becomes gel when cooling.

- Bigger pore size than polyacrylamide

Polyacrylamide Gel Electrophoresis (PAGE)

- Synthetic polymer
- Formed from acrylamide subunits.
- Acrylamide with a cross linker, methylene bis-acrylamide .
- Polymerization catalysts:
- Ammonium persulfate (APS)

+Tetramethylethylenediamine (TEMED)

- Light
- 3.5–20% concentration.
- High resolution.
- Acrylamide is a dangerous neurotoxin

Buffer

•Provides ions in solution for electrical conductivity.

•Prevents the pH changing.

•Common using buffers:

•Tris Borate EDTA (TBE)-Stable, expensive,PAGE, long separation time.

• Tris Acetate EDTA (TAE)-Inexpensive,short separation time.

• Tris Phosphate EDTA (TPE)

•RNA

–Sodium phosphate Buffer

–MOPS Buffer (-3-(N-morpholino) propanesulfonic acid)

Do not forget!

- DNA molecule is an organic acid.
- Negatively charged.
- Migrate toward the positive electrode(Anode) in an electromagnetic field.
- Do not forget "Running of the gel "
- Cut off electricity before taking gel from apparatus.

Protein Electrophoresis

- Simple to use and highly reproducible technique.
- Provide information of the molecular weight, charged, subunits, purity of protein mixture.

Other Protein Electrophoresis Techniques

•IEF(Isoelectric focusing)

– Separates proteins by their isoelectric points (pI) by using pH gradient of the gel.

•2D PAGE(Two dimensional gel electrophoresis)

–Separates proteins are by two properties (eg: pI and size) in a mixture.

•Western blotting:

–Separating proteins first by size then staining with specific antibody-antigen reactions.

–Technique gives molecular weight and identifies specific protein.

Pulsed Field Gel Electrophoresis (PFGE)

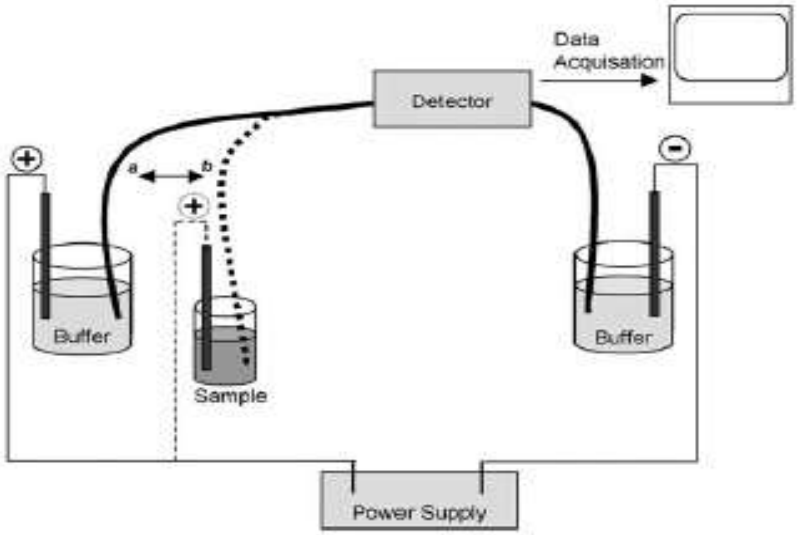
- Used for separating very large DNA molecules.

Based on the periodically changes of directions in the electric field. used for genotyping.

Capillary Electrophoresis

Principle

- Power supply.
- The anode and cathode buffer reservoirs with corresponding electrodes.
- The separation chamber(capillary tube).
- The injection system.
- The detector



Capillary Electrophoresis

- Applications

–Analyzing proteins in physiological matrices (eg.Serum, urine)

–DNA analysis

–Drug screening.

–Analysis of pesticides, food content, pollutants.