Benha University
Faculty of Agriculture
Biochemistry Department

Instrumental Practical Part

(Chemistry 5)

Biotechnology Program (Level 2)

(Section 4)

Course Professors

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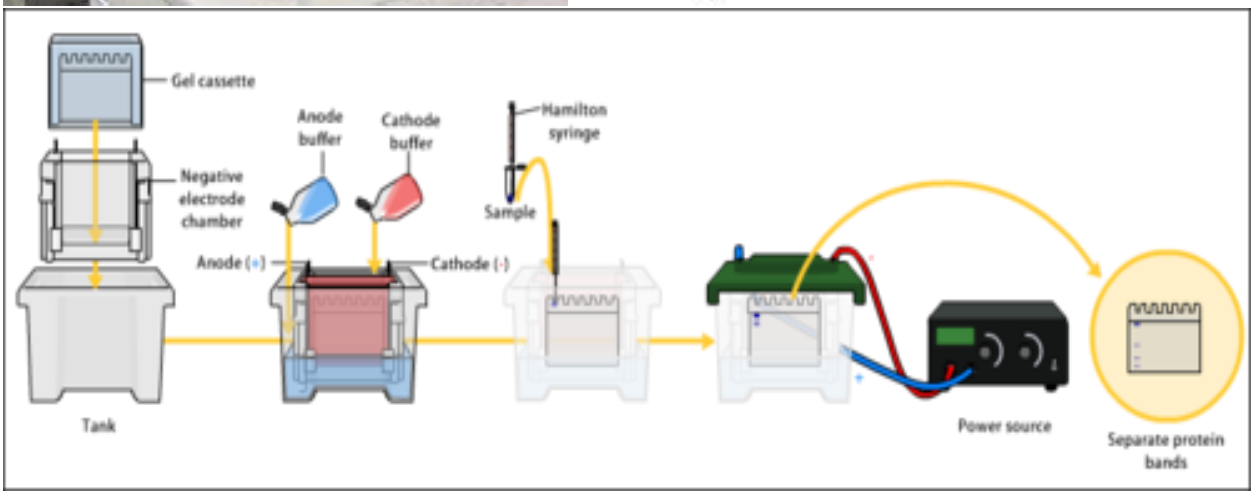
Instrumental (section 4)

Electrophoresis



Electrophoresis-Definition

• Electro=Electric; phoresis= Migration;Carry

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

Principle

When we place any charged molecules in an electric field, they move toward the positive or negative pole according to the charge they are having. Proteins do not have any net charge whereas nucleic acids have a negative charge so they move towards the anode when electric field is applied. 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.

Basis of separation

- Proteins are separated on the basis of two properties.
- Firstly they are separated on the basis of their net charge.
- After that, PAGE separates the proteins on the basis of their mass.
- Using this method small changes in charge and mass can be detected, as it is not possible that two different proteins will resolve to the same place in both dimensions.

Isoelectric Point

- There is a pH at which there is no net charge on a protein and this point is called isoelectric point (pI).
- A protein has a net negative charge above its isoelectric point, and it migrates toward the anode in an electrical field.
- The protein is positive below its isoelectric point, and it migrates toward the cathode.

Molecular Pathology

- **Nucleic acids.**

– Determining quality of DNA/RNA
– Analyses of PCR products
– Mutation detection
– Southern and Northern blotting
– Sequencing

- **Proteins**

– Western blotting
– Protein purification

Electrophoresis Types

- Gel electrophoresis

– Agarose gel
– Polyacrylamide gel
– Others.

- Pulsed Field Gel Electrophoresis
- Capillary Electrophoresis

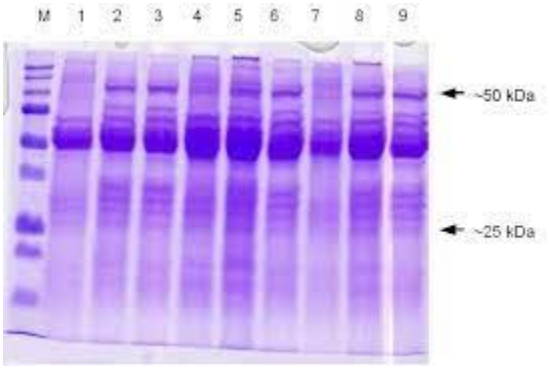
• Isoelectric focusing
• 2D electrophoresis

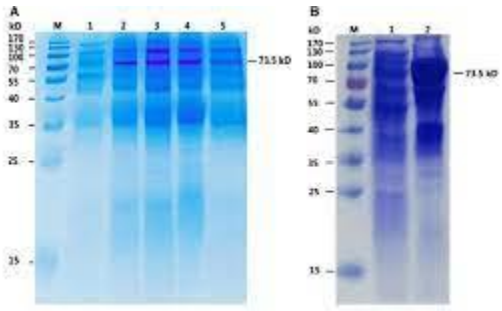
Gel Electrophoresis

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

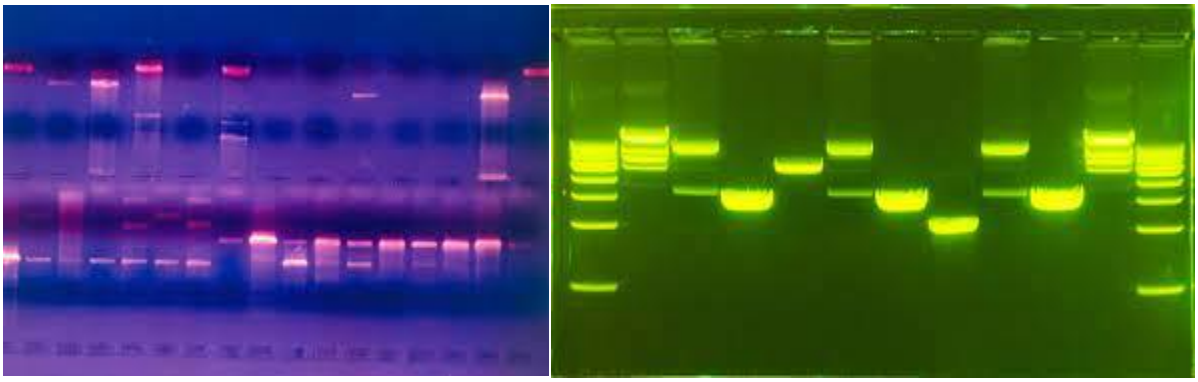
Gel Types

• Starch-Rarely used
• Polyacrylamide-Protein, small nucleic acid fragments.





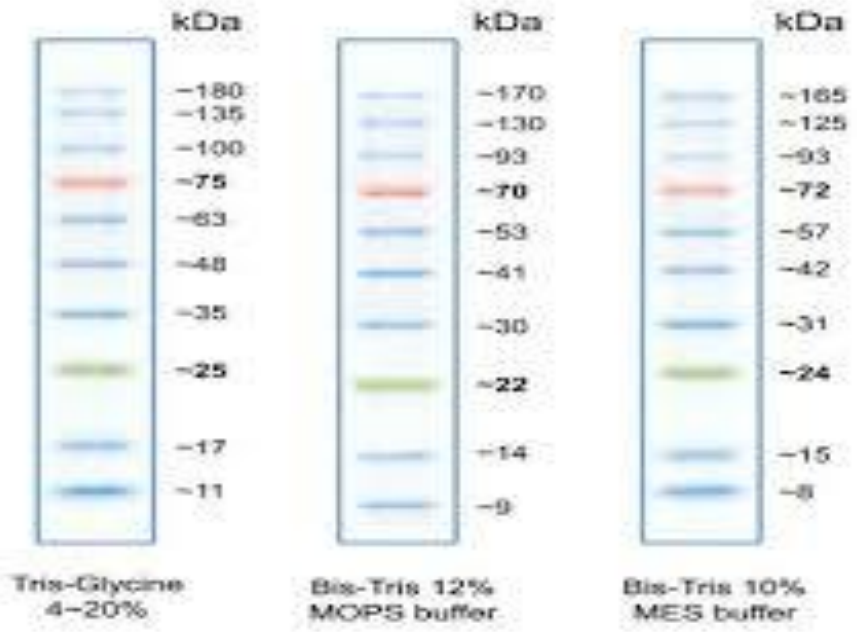
• Agarose-Nucleic acids, large proteins.



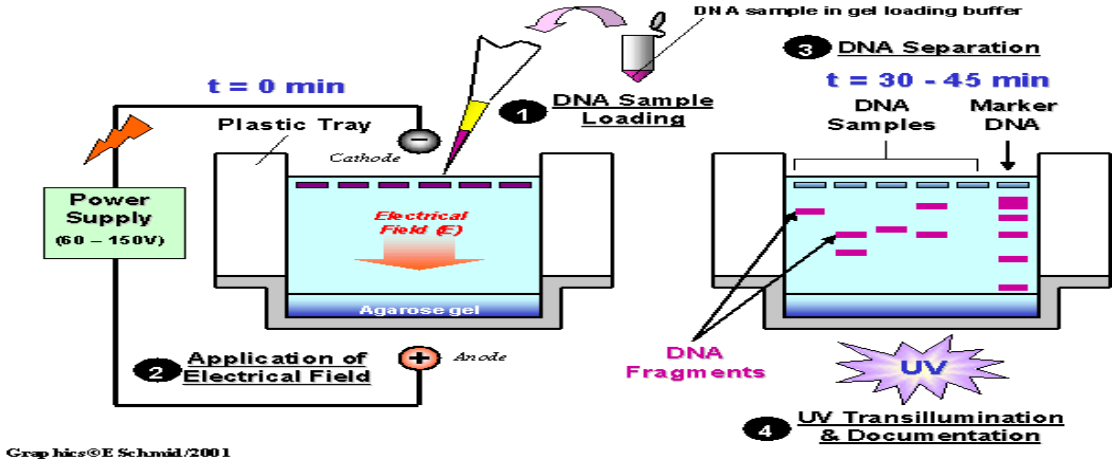
• Cellulose acetate-Proteins

Protein Markers:

Protein markers may also be categorized into molecular markers though the latter are more referred to DNA markers.



Steps of analysis



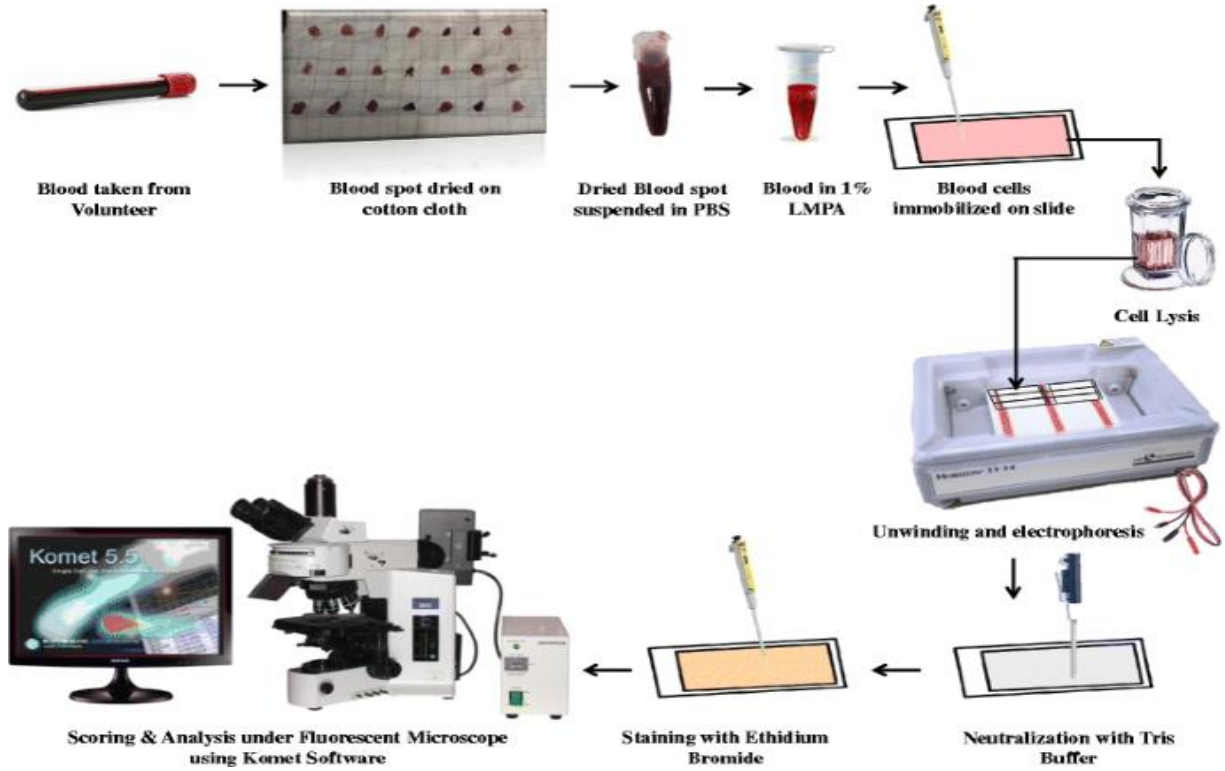
Steps of analysis

– Sample preparation
– Gel,buffers, etc. preparation.
– Load markers
– Load samples
– Running of the gel
– Staining of the gel

– Photography, gel documentation
– Interpret/analysis of gel

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

Capillary Electrophoresis



Contents:

• Power supply.
• The anode and cathode buffer reservoirs with corresponding electrodes.
• The separation chamber (capillary tube).
• The injection system.
• The detector
• **Applications**
– Analyzing proteins in physiological matrices (eg.Serum, urine)
– **DNA EXPERIMENTS**
• To visualize Products of animal, plant or bacterial DNA Extractions
• To study RFLP FORENSIC SCIENCE
• Paternity test

• DNA Fingerprinting
– Drug screening.
– Analysis of pesticides, food content, pollutants.

Specific Applications

• Neoplastic disorders
– Detection of tumor-related mutation.
– Microsatellite instability
– Analysis of monoclonality.
• Diagnosis of hereditary diseases and prenatal testing
• Diagnosis of infectious diseases
• Identity testing.

HUMAN HEALTH & DISEASE

- To study Human Genomics & Bioinformatics
- Restriction Enzyme Mapping
- Diagnostics (Human and plant pathogens)
- DNA Profiles of 'Infectious Diseases'

Applications of SDS PAGE

- Molecular weight of the proteins can be determined
- To perform western blotting SDSPAGE is required

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