A review on paper electrophoresis techniques

M Reshma kaja1* shaik mohammed zaidh2, Abhimannu Yadav3, Mohammad Mueen4, Mulla shabana4

K Reshma1*Department of Pharmaceutical Analysis Nimra College of Pharmacy Vijayawada 521456 AP INDIA

Corresponding Author: **E-Mail**: Shaikzaidh872@gmail.com.

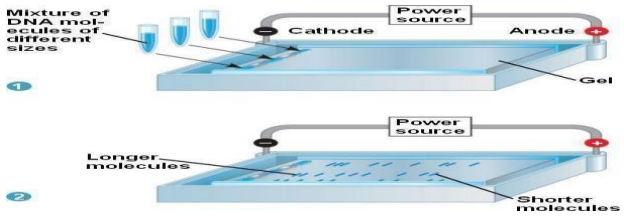
ABSTRACT

Electrophoresis is a commonly used laboratory technique that facilitates the movement of charged particles through a medium, often a gel or liquid, when exposed to an electric field. This method relies on the distinct migration rates of molecules, such as proteins, nucleic acids, and other charged bio molecules, which vary according to factors like size, charge, and shape. It is primarily employed for the separation, identification, and purification of bio molecules, making it crucial in fields like molecular biology, biochemistry, and clinical diagnostics. Different forms of electrophoresis, including gel electrophoresis, capillary electrophoresis, and two-dimensional electrophoresis, provide various levels of resolution and sensitivity, with the choice of method based on specific research requirements. Electrophoresis is indispensable for tasks such as DNA fragment analysis, protein profiling, and gene expression studies. A strong understanding of the underlying principles and the careful optimization of experimental parameters are essential for obtaining reliable results in both research and medical applications.

Key Words: Electrophoresis, Principles, types, Applications ,Uses.

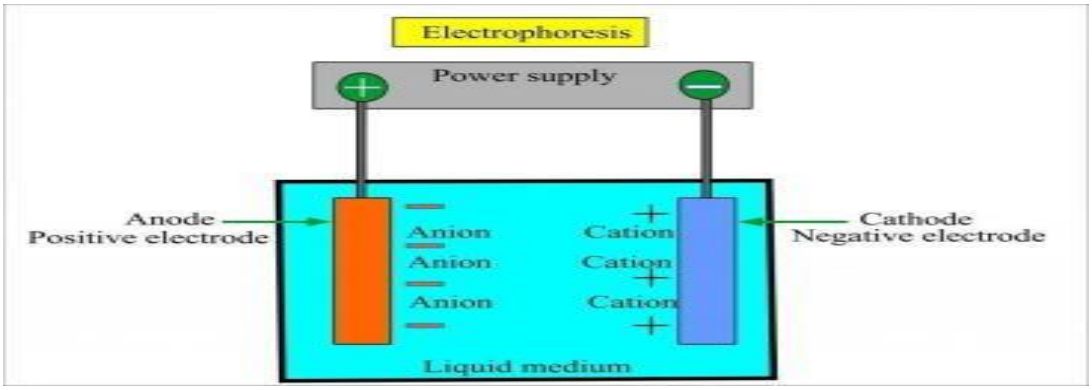
INTRODUCTION

Electrophoresis is a laboratory technique used to separate charged molecules, such as DNA, RNA, and proteins, based on their size and charge. This technique relies on the movement of charged particles through a gel or liquid medium under the influence of an electric field.



Principle of Electrophoresis

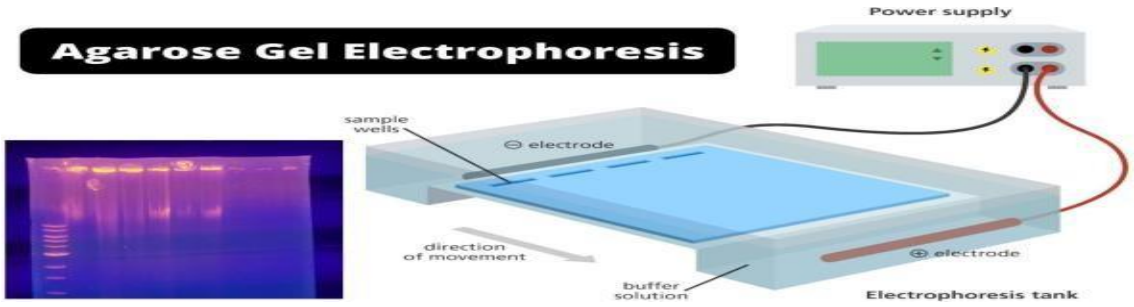
When an electric field is applied, negatively charged molecules (such as DNA) migrate towards the positively charged electrode (anode), while positively charged molecules move towards the negatively charged electrode (cathode). The rate of movement depends on factors like the molecule's charge, size, shape, and the composition of the medium.



Types of electrophoresis

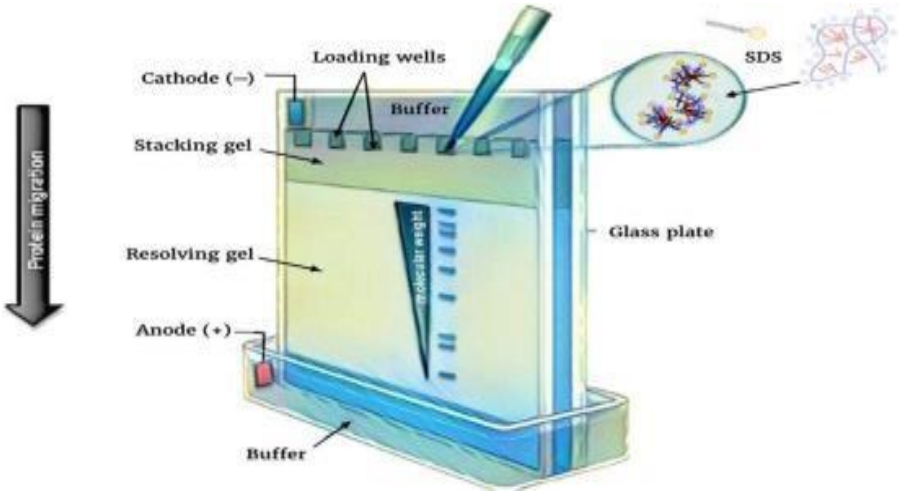
1. **Agarose Gel Electrophoresis-**

   Agarose Gel Electrophoresis is a widely used technique in molecular biology to separate DNA and RNA fragments based on their size. It utilizes an agarose gel matrix and an electric field to drive negatively charged nucleic acids towards the positive electrode (anode).



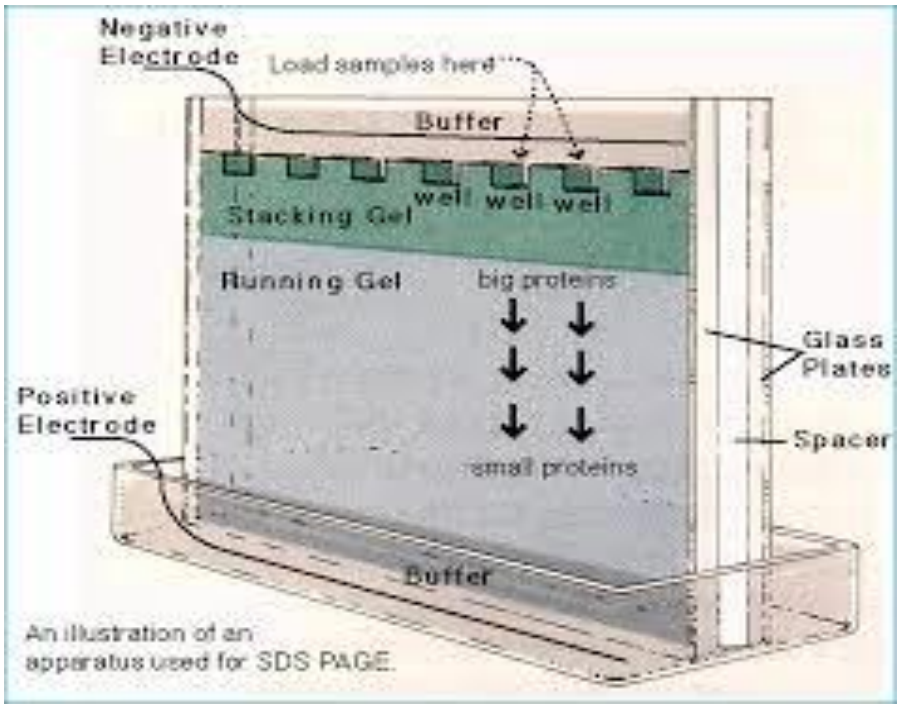
2.Polyacrylamide Gel Electrophoresis (PAGE) –

Polyacrylamide Gel Electrophoresis (PAGE) is a powerful technique used to separate proteins and nucleic acids based on their size and charge. It provides higher resolution than agarose gel electrophoresis, making it ideal for analyzing smaller molecules like proteins and short DNA fragments



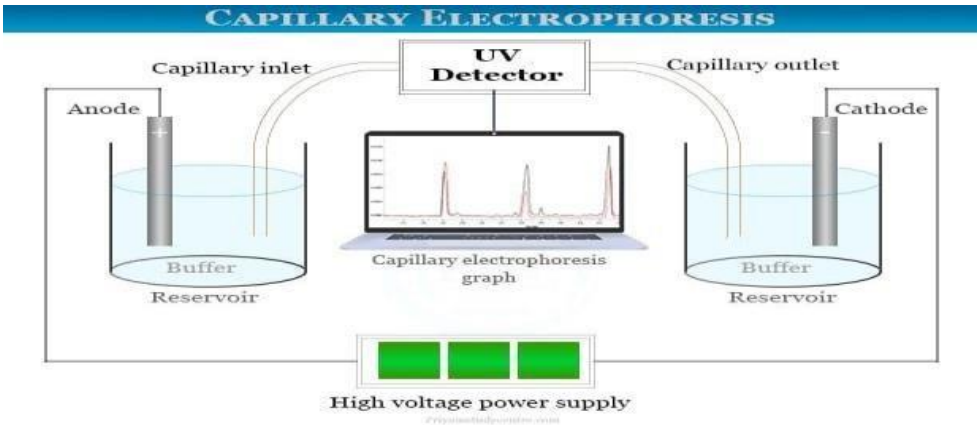
3.SDS-PAGE

SDS-PAGE is a widely used electrophoresis technique for separating proteins based on their molecular weight. It utilizes Sodium Dodecyl Sulfate (SDS), an anionic detergent, to denature proteins and provide them with a uniform negative charge, ensuring separation solely by size rather than shape or charge.



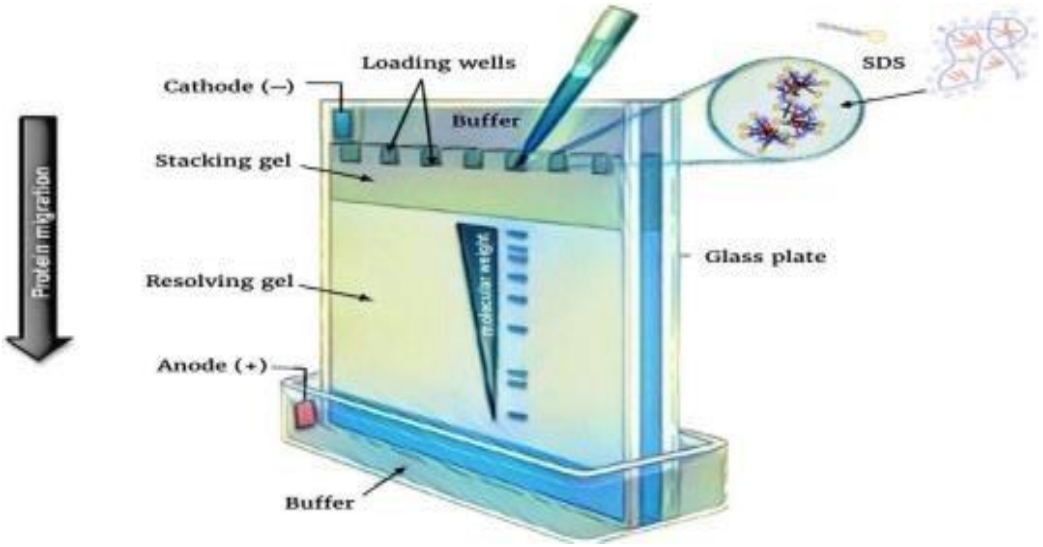
4.Capillary Electrophoresis

Capillary Electrophoresis (CE) is a high-resolution analytical technique used to separate charged molecules such as DNA, proteins, and small ions based on their size, charge, and mobility in an electric field. Unlike traditional gel electrophoresis, CE utilizes a narrow capillary tube filled with an electrolyte solution, providing faster and more efficient separation.



5. Focusing (IEF)

Isoelectric Focusing (IEF) is a specialized electrophoresis technique used to separate proteins based on their isoelectric point (pI)—the pH at which a protein carries no net charge. This method provides high-resolution separation, making it essential for proteomics, protein.



➢ PROTEIN ANALYSIS

Electrophoretic analysis of proteins is a widely used technique to separate, identify, and study proteins based on their size, charge, and conformation under an electric field. This method is essential for understanding protein structure, function, and interactions in biochemistry, molecular biology, and clinical diagnostics.

➢ PRINCIPLE OF PROTEIN ELECTROPHORESIS

Proteins migrate in an electric field based on their charge-to-mass ratio.Different electrophoresis techniques separate proteins based on molecular weight, charge, or isoelectric point (pI).Staining and detection methods allow visualization and quantification of separated proteins.

❖ WORKING OF PROTEIN ANALYSIS

Protein analysis through electrophoresis involves multiple steps, from sample preparation to visualization and data interpretation. The most commonly used method is SDS-PAGE, but other techniques like Native PAGE, Isoelectric Focusing (IEF), and Capillary Electrophoresis (CE) also play crucial roles in protein characterization.

❖ Step by step process

1.Gel Preparation

Acrylamide Gel Preparation (For SDS-PAGE or Native PAGE):
Separating Gel (Resolves proteins based on molecular weight).
Stacking Gel (Concentrates proteins into sharp bands before entering the separating gel).

2.pH Gradient Formation (For IEF): A stable pH gradient is created using ampholytes to separate proteins by their isoelectric points.

3. Loading the Sample into the Gel

Protein samples are loaded into wells along with a protein ladder (molecular weight marker) for size comparison**.**

4. Running the Electrophoresis

Electric Field Application:
SDS-PAGE & Native PAGE: Proteins migrate toward the positively charged anode because SDS imparts a uniform negative charge. IEF: Proteins migrate until they reach their isoelectric point (pI), where they stop moving.
Capillary Electrophoresis: Uses high voltage in a narrow tube for high-resolution protein separation.

5. Staining and Visualization

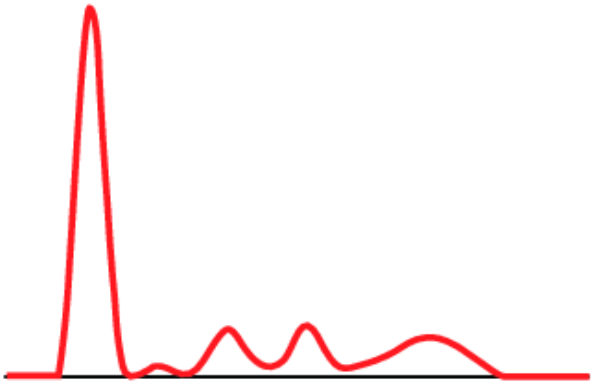
After electrophoresis, proteins are visualized using staining techniques:
coomassie Brilliant Blue (CBB): General protein staining.
Silver Staining: More sensitive detection for low-abundance proteins.
Fluorescent Dyes: Used for advanced detection with imaging systems.
Western Blotting: Proteins are transferred to a membrane and detected using antibodies.

6. Data Analysis and Interpretation

Compare Protein Bands:Molecular weight is determined by comparing bands to the protein marker.Protein purity is assessed by the presence of single or multiple bands.

❖ **GRAPHICAL REPRESENTATION**

Protein EP (serum) B3000006



Fraction	Result (g/dL)	Percentage (%)	Normal	Unit
Albumin	4.4	60.08	3.3−4.8	g/dL
Alpha1	0.1	1.46	0.1−0.3	g/dL
Alpha2	0.7	9.86	0.7−1.1	g/dL
Beta	0.8	10.39	0.8−1.2	g/dL
Gamma	1.3	18.21	0.7−1.8	g/dL
A/G ratio	1.51	1.51		
Total SPE	7.3	100.00	6.3−8.3	g/dL

❖ **EVALUATION TEST OF PROTEIN GEL ELECTROPHORESIS**

1. Visual Inspection of the Gel

After running electrophoresis, the gel is stained and examined:
Sharp, well-defined bands → Indicate good separation and sample quality.
Smearing or streaking → Suggests protein degradation, overloading, or incomplete denaturation.

Multiple bands → May indicate protein contamination, degradation, or presence of protein isoforms.
Uneven migration → Could result from buffer inconsistency, sample overloading, or improper gel polymerization.

❖ Conclusion

- Electrophoresis is a fundamental technique in molecular biology, biochemistry, and analytical chemistry, enabling the separation and analysis of biomolecules such as DNA, RNA, and proteins. Different electrophoresis methods, including Agarose Gel Electrophoresis, SDS-PAGE, Capillary Electrophoresis, and Isoelectric Focusing (IEF), provide researchers with powerful tools for molecular characterization, forensic analysis, medical diagnostics, and drug development.
- The technique's effectiveness lies in its ability to separate molecules based on size, charge, and shape under an electric field. With advancements in technology, modern electrophoresis methods offer higher resolution, automation, and faster analysis, making them indispensable in scientific and industrial applications.
- Overall, electrophoresis continues to be a versatile, precise, and essential tool for biomolecular research and diagnostics, contributing significantly to advancements in genetics, medicine, and biotechnology.

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