



# ELECTROPHORESIS TECHNIQUE AND ITS APPLICATIONS IN FORENSIC SCIENCE – A REVIEW

<sup>1</sup>Anthara R, <sup>2</sup>Jinju Mariam Immanuel, <sup>3</sup>Komaljeet Kaur

<sup>1</sup>M.Sc. Student, <sup>2</sup>M.Sc. Student, <sup>3</sup>Research Scholar  
Department of Forensic Science,  
Chandigarh University, Gharuan, Punjab, India

*Abstract:* Electrophoresis is the separation of charged molecules in an electric discipline used. The relative mobility of man or woman molecules depends on quite a number of factors. The most vital of which can be the total rate, charge / weight ratio, cellular shape and temperature, porosity and viscosity of the matrix from which the molecule moves. used for class of tracked samples collected at the crime scene, in particular if the sample size is too small. This paper is dedicated to presenting a brief overview of electrophoresis, its kinds, and its uses. Electrophoresis is the nice method or analytical method used in forensic technological know-how due to the fact it is an invaluable device used for the separation of traced samples collected from crime scene, especially whilst the sample size may be very small. This paper is devoted to presenting the brief explanation approximately electrophoresis, its kinds, and application.

*Index Terms* - Electrophoresis, Porosity, Viscosity, Anaphoresis, Electrokinetic, Fingerprinting, Paternity.

## I. INTRODUCTION

Each molecule has an opposing charge, when those molecules are located in an electric powered subject, the charged molecules pass closer to an electrode with a negative rate because of the occurrence of electrostatic appeal. Electrophoresis is the separation of charged molecules in an electric powered field used. Electrophoresis can be defined as the migration of charged particles the usage of a solution underneath the influence of the external electric powered area. Without the unique association of the apparatus cloth and no matter wherein the molecules are allowed to migrate, all electrophoretic separation depends. inside the fee distribution of the separated molecules.

Electrophoresis technique is frequently used to separate proteins from nucleic acids- DNA and RNA. Electrophoresis of debris is occasionally referred to as cataphoresis, at the same time as electrophoresis of terrible particles (anions) is called anaphoresis. The electrokinetic phenomenon of electrophoresis became first observed by means of Russian professors Peter Ivanovich Strakhov and Ferdinand Frederic Reuss in

Moscow in 1807, who found out that the usage of continuous electric subject reasons dissolved clay debris in water to evaporate.

## II. HISTORY

The history of electrophoresis had all started with the work of Arne Tiselius within the year of Nineteen Thirties, and new separation techniques and chemical analysis techniques based on electrophoresis continued into the twenty first century. With help from the Rockefeller foundation, the "Tiselius equipment" for moving boundary electrophoresis changed into advanced by Tiselius, which changed into defined in 1937, then after a brand new equipment for electrophoretic evaluation named as "colloidal combinations" is formed. This method unfold slowly till the arrival of powerful zone electrophoresis methods in the Nineteen Forties and Fifties, in which clear out paper or gels are used as assisting media. with the aid of the Sixties, state-of-the-art gel electrophoresis strategies expanded widely. The method made it viable to split organic molecules based totally on minute physical and chemical differences, supporting to pressure the upward push of molecular biology. For a wide variety of biochemical methods, together with protein fingerprinting, Southern blot and similar blotting strategies, DNA sequencing, and plenty of extra, gel electrophoresis and other related strategies have turn out to be an vital foundation. This sort of apparatus designed via Arne Tiselius enabled wide tiers of latest applications of electrophoresis in analyzing chemical mixtures.

## III. PRINCIPLE

When charged molecules are located in an electric powered field, they migrate towards either the advantageous or poor pole according to their rate. The fee of migration depends upon its internet fee, size, shape and the applied electric powered modern-day.

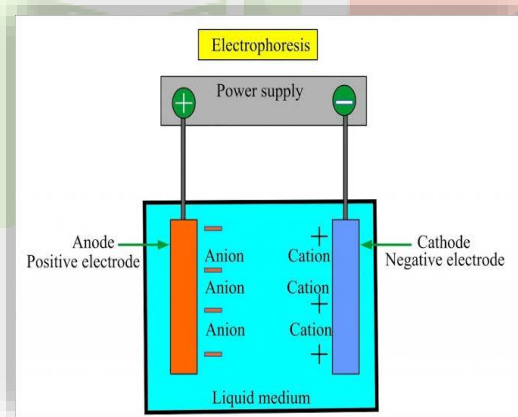


Figure 1 - Instrumentation of Electrophoresis

#### IV. INSTRUMENTATION OF ELECTROPHORESIS

1. Electricity supply - Electrophoresis is achieved below regular voltage or cutting-edge. The migration charge must be steady via a consistent strength supply.
2. Buffers - Buffer establishes the electric price on the solution and pH of the machine. It's miles used in wearing the electric contemporary.

An appropriate buffer:

- Need to no longer intrude inside the detection of favoured analytes
- Retaining the solubility of analytes
- Keeping the buffering capability all through the analysis procedure.
- Producing the desired variety of separation.

For preserving high pH tris, tricine, borate are primary buffers and for keeping low pH citrate, acetate, formate and phosphate are acidic buffers used for low pH.

3. Aid media - Slabs, sheets, columns of starch, polyacrylamide, agarose, the membrane of cellulose acetate are used as assisting media. it's far in a colloid form, made of greater than 90p.cwater. Agarose gel is in particular used as a help medium to carry out electrophoresis.

4. Agarose - The separation of molecules is primarily based handiest o mass to charge ration as nicely proteins can bypass thru the pores of the gel. it's far able to separating proteins into five zones most effective. there is a lower affinity for proteins, which affords precise readability after drying.

5. Polyacrylamide - It isn't charged and no endosmosis takes vicinity. it could accommodate a big quantity of protein. Separation is based totally on both molecular weight and mass to price ratio.

6. Electrophoresis chamber - It's far a plastic box or tank that is packed with a buffer for the migration of biomolecules. It has a transparent cowl, which enables smooth viewing of the migration process. It's miles linked to a strength source.

7. Packing containers for staining and destaining gel - The trays and boxes are used for the functions of staining and destaining gels. each open shape and lidded packing containers are available. commonly, they've a polypropylene base. they're tightly equipped and clear. they may be resistant to stains and solvents.

8. Electrodes - There are platinum electrodes, which facilitate the separation of molecules by attracting opposite prices. An anode is used for binding to high-quality ions and a cathode binds bad ions.

9. Gel caster and comb - The gel is poured after dissolving in the solvent into the gel caster, which holds the gel and is kept in the equipment. A comb is used to put the wells for the loading of samples.

## V. METHODOLOGY OF ELECTROPHORESIS

- The gel is prepared by using dissolving in boiling water.
- The solution is cooled to a warm temperature and poured into the pores and skin or caster.
- After the gel has hardened, a comb is used to insert the springs into it.
- The gel is then positioned in an electrophoretic chamber.
- The bath is stuffed to a room now not exceeding 1/three of the overall quantity of the room.
- The pattern is blended with loading dye, either with a fluorescent tag or ethidium bromide as the dye provides density to the pattern and gives color to it.
- The pattern is loaded into sources the use of a clean micropipette.
- Cells migrate to excessive-strength electrodes, as a result DNA, while properly charged, movements towards the anode. The motion is observed via the arrival of the dye.
- After the give up of the system, the gel is infected and seen the usage of a gel illustration tool.
- Sizes of pattern fragments are calculated by using cell-weighted logarithm, relative to standard.

## VI. TYPES OF ELECTROPHORESIS

1. Paper electrophoresis
2. Cellulose acetate membrane electrophoresis
3. Gel electrophoresis
4. Capillary electrophoresis
5. Immunoelectrophoresis

### 1. Paper Electrophoresis

Paper electrophoresis (PE) is beneficial for the separation of small charged molecules, together with amino acids and small proteins, using a strip of paper (chromatographic paper). For this motive, the strip paper this is used need to incorporate at the least  $\alpha$ cellulose at a percent of (95%) and must have a moderate adsorption capacity. on this technique, the occurrence movement of a colloidal particle of answer ends in next separation along the paper strip. Paper electrophoresis is simpler in comparison to gel electrophoresis because it does no longer require lots matrix preparation, and it does now not contain fees that intervene with the separation of compounds. A strip of filter out paper is moistened with buffer, and the ends of the strip are immersed into buffer reservoirs containing the electrodes. A high voltage is carried out to the stop of the paper strip and the pattern is located inside the middle of the paper. applying a high voltage reduces diffusion of small molecules, improving decision and decreasing the time it takes to complete the process. Spots are migrated in keeping with fees. The separated components after electrophoresis may be detected through diverse staining strategies, which include ethidium bromide staining, submit-imaging, fluorescamine chloride staining, chloride five(dimethylamino)naphthalene-1-sulfonyl chloride staining, and so forth. relying on the chemical composition utility:

- Serum evaluation
- Muscle proteins, egg white proteins, and snake, insect venom analysis.
- Used in the separation and identification of alkaloids.
- Utilized by the drug-testing enterprise to decide the presence of unlawful or leisure capsules in process candidates and crime suspects.

## 2. Cellulose Acetate Membrane Electrophoresis

Cellulose acetate membranes are composed of huge pores, so they have little sieving effect on proteins. because of this those electrophoretic separations are totally rate-based totally. The matrix has little effect on diffusion. for this reason, the segmentation vicinity is noticeably huge, and the decision and detection limits appear like low. then again, it is straightforward to split and stains quickly. Cellulose acetate strips are suspended in a tank on a leveling tool, both ends immersed in a buffer answer.

No refrigeration is required in the course of separation. Then the pattern is located at one give up and then the electrical subject is carried out; in order that it lets in the migration of molecules to their respective poles relying upon their price. because the resolution and reproducibility of separations in agarose and polyacrylamide gels are higher, cellulose acetate membranes are greater regularly changed through gel electrophoresis.

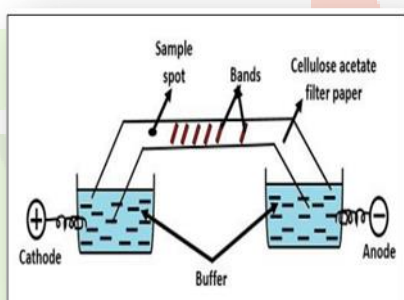


Figure 2 - Cellulose acetate membranes

### Application:

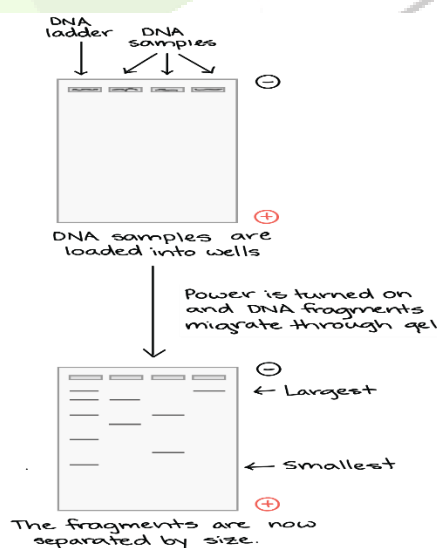
- Habitual clinical evaluation
- Evaluation of serum or isoenzymes.
- The analysis of scientific and organic protein samples (albumins and globulins).
- Used within the separation of glycoprotein, haemoglobin, and lipoprotein.

### 3. Gel Electrophoresis

A technique used to split pieces of DNA (or other macromolecules along with RNA and proteins) based totally on their length and fee. Electrophoresis involves passing an electric powered modern-day through a gel containing a molecule of interest. relying on their size and price, molecules can move thru the gel in unique instructions or at specific speeds, allowing them to break away every different. All DNA molecules have the identical fee per unit mass. due to this, gel electrophoresis is only based totally on length. Gel electrophoresis involves a gel: a plate of jelly-like fabric. DNA isolation gels are frequently made from a polysaccharide called agarose, that is a dry powder flake. Heating the agarose in a buffer (barely salted water) and cooling it bureaucracy a tough, barely viscous gel. at the molecular stage, a gel is a matrix of agarose molecules held together with the aid of hydrogen bonds and forming tiny pores. At one end of the gel, there's a pocket-fashioned despair called a well into which the DNA pattern may be placed. before including the DNA sample, the gel should be located in a gel field. One cease of the field is hooked up to the high quality pole and the other end is attached to the poor pole. the main a part of the field in which the gel is located is full of a saline buffer which could conduct an electric present day. Although you may not be able to see within the photograph above (way to my great inventive talents), the buffer fills the gel box to a level where it simply slightly covers the gel.

The end of the gel with the wells is positioned in the direction of the poor electrode. The cease without wells (in the direction of which the DNA fragments will migrate) is positioned towards the superb electrode.

After setting the gel in the field, cautiously transfer every DNA pattern that you want to investigate (e.g., every PCR response or each plasmid digested with restriction enzymes) into one of the wells. One properly is reserved for the DNA ladder, a widespread reference containing DNA fragments of recognised period. business DNA ladders are available in a diffusion of sizes, so you need to pick out one that "covers" the dimensions range of the anticipated fragments well.



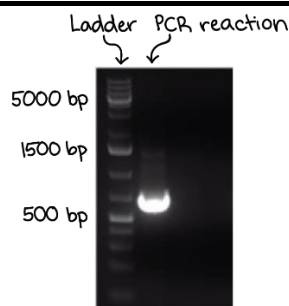


Figure 3 – A figure about gel electrophoresis

The gel box is then powered on and an electric powered present day flows through the gel. DNA molecules start to migrate undoubtedly through the gel matrix as they may be negatively charged because of the phosphate businesses within the sugar-phosphate backbone. whilst the strength is turned on and an electric current flows through the gel, the gel is stated to work. as the gel moves, shorter DNA fragments will skip thru the pores of the gel matrix quicker than longer ones. After the gel has run for a while, the shortest DNA fragments are towards each ends of the gel and the longest DNA fragments stay close to the wells.as soon as the fragments had been separated, we are able to examine the gel and see what sizes of bands are determined on it. when a gel is stained with a DNA-binding dye and located underneath UV mild, the DNA fragments will glow, allowing us to see the DNA present at unique places alongside the duration of the gel.

A nicely defined “line” of DNA on a gel is known as a band. every band incorporates a large variety of DNA fragments of the identical length which have all travelled as a collection to the identical function. A single DNA fragment (or even a small group of DNA fragments) might not be visible by way of itself on a gel.

through comparing the bands in a sample to the DNA ladder, we will determine their approximate sizes. as an example, the brilliant band on the gel above is kind of 700700700 base pairs (bp) in size.

#### 4. Capillary Electrophoresis

It's miles an analytical method used to split ions primarily based on their electrophoretic mobility with using carried out voltage. If ions have the same size, the ion with greater fee will circulate fastest.

Principle: While a pattern is injected into capillary field with buffer and a voltage is carried out, solute will migrate thru capillary as zones. Solute migrate based totally on their fee of migration, which relies upon on the electrophoretic mobility.

#### 5. Immuno electrophoresis

It is defined because the separation and identification of proteins based totally on variations in electric charge and reactivity with antibodies. The term immune electrophoresis turned into first coined by using Grabar and Williams in 1953. it is the technique of combination of immunodiffusion and electrophoresis.

Principle: While the electric modern applied to slide having gel, the antigen combination positioned in wells is separated into man or woman antigen additives in line with their length and rate. Then it's miles observed by using an immunodiffusion in opposition to the antiserum resulting in precipitin formation.

## VII. APPLICATION OF ELECTROPHORESIS TECHNIQUES IN FORENSIC SCIENCE

1. Analysis of explosives compound and residue: Capillary electrophoresis are used within the trace evaluation of organic and inorganic gunshot residues and explosives. organic additives of gunpowder like ethylcentralite, diphenylamine and nitroglycerin may be analysed through the use of Miceller Electro kinetic capillary chromatography.

2. Analysis of drug of abuse: One of a kind organic samples like tissue ,hair, nail and frame fluids are broadly speaking vital in detection of illicit tablets. For the detection of drug capillary electrophoresis is used.

3. Ink analysis: Ink include one of a kind additives which facilitates to become aware of the writing device utilized in committing crime. Capillary electrophoresis is specifically used.

4. Used to examine proteins.

5. Capillary electrophoresis is rapid and effective in private identity and paternity trying out.

## VIII. CONCLUSION

From the beyond a long time, electrophoresis has been provide accurate and effective method for the separation of the massive kind of substances. The distinctive types of electrophoresis have its very own position in identity of trace evidences in Forensic science. The reagents are special for one-of-a-kind electrophoresis. Capillary electrophoresis is mainly used inside the identity or separation in forensic technology.

## IX. ACKNOWLEDGMENT

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## X. REFERENCES

[1]<https://www.genome.gov/geneticsglossary/Electrophoresis#:~:text=Electrophoresis%20is%20a%20laboratory%20technique,move%20faster%20than%20larger%20>

[2]<https://en.wikipedia.org/wiki/Electrophoresis>

[3]<https://www.yourgenome.org/facts/what-is-gel-electrophoresis>

[4]<https://www.cleaverscientific.com/what-is-electrophoresis/>

[5] <https://www.thoughtco.com/electrophoresis-definition-4136322>