Study of Electrophoresis Techniques and its Types

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ABSTRACT: ***Electrophoresis is a simple yet sensitive analytical technique for separating proteins, nucleic acids, and other biological components in sample fluids. Electrophoresis is used to separate complicated protein mixtures (e.g., from cells, fractions, column fractions, subcellular or immune-precipitants), study subunit compositions, and validate protein homogeneity. Electrophoresis is a chromatography technique by which a mixture of charged molecules is separated according to size when placed in an electric field. It can also be used to purify proteins so that they can be used in other applications. Protein migrate through holes in a polyacrylamide gel matrix in response to an electrical field in polyacrylamide gel electrophoresis; pore size decreases with increasing acrylamide concentration. The combination of pore size and protein charge, size, and shape determines the migration rate of the protein. The main objective of this paper to learn more about different types of Electrophoresis Techniques. In future electrophoresis techniques will give more accurate outcomes as needed in diverse fields according to their applications.***

KEYWORDS: ***DNA, Electrophoresis, Gel Electrophoresis, Protein, RNA.***

1. INTRODUCTION

Electrophoresis is a separation technique for liquid molecules based on their ability to travel in an electric field. Electrophoresis, in its numerous forms or kinds, has become the most widely used method for analyzing biomolecules in biochemistry or molecular biology, including genetic components like DNA or RNA, proteins, as well as polysaccharides. While charged particles are immersed in a solution buffer, all kinds of electrophoresis separate them. An electrophoresis unit, also known as an electrophoresis chamber, is required for all types of electrophoresis. Figure 1 shows the electrophoresis unit. which is used for the separation of molecule[1].

Electrophoresis is a widely used method that works by passing an electric current through biological molecules (typically DNA, but it may also be protein or RNA) and separating them into bigger or smaller bits. It's utilised in a wide range of applications, from forensics to establishing the identification of people who may have been engaged in a crime by comparing their DNA pattern, their electrophoresis pattern, to one in a database.

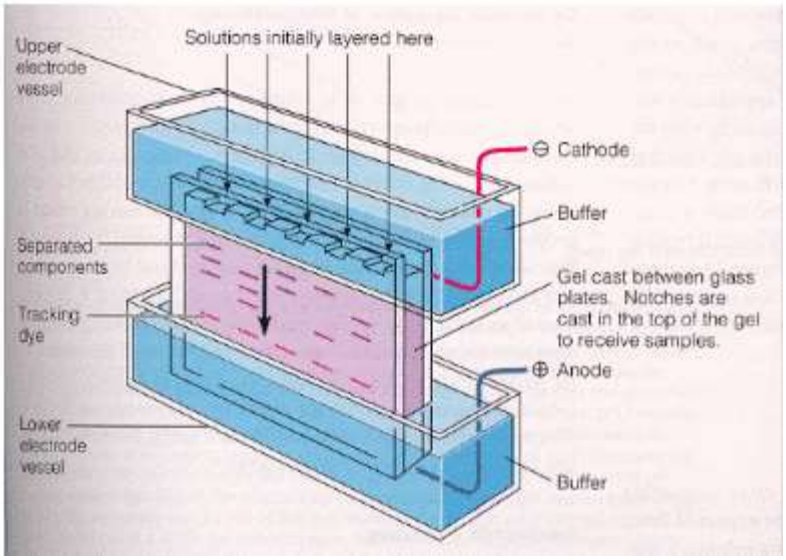

Figure 1: Illustrated diagram showing the electrophoresis separation technique unit[2].

1.1. Electrophoresis Principle:

Electrophoresis is based on the fact that the majority of biomolecule exists as electrically charged particles with ionizable functional groups This means that biomolecule in a solution can either have positive (positive charge) or negative (negative charge) Ionized biomolecules move at various speeds depending on their mass as well as net charge when exposed to an electric field Positively charged particles, termed cations, migrate towards a negatively charged electrode, called an anode, whereas the reverse is true for negatively charged particles called anions, as well as anions. The variations in the speed and direction of every charged particles will result in a migration pattern that is unique to each particular attribute, allowing apparatuses of biomolecules with comparable possessions to be isolated.

1.2. Forms of Electrophoresis:

Electrophoresis may be classified into four types as shown in figure 2, based on the kind of buffer solution used or how it affects the mobility of charged particle.

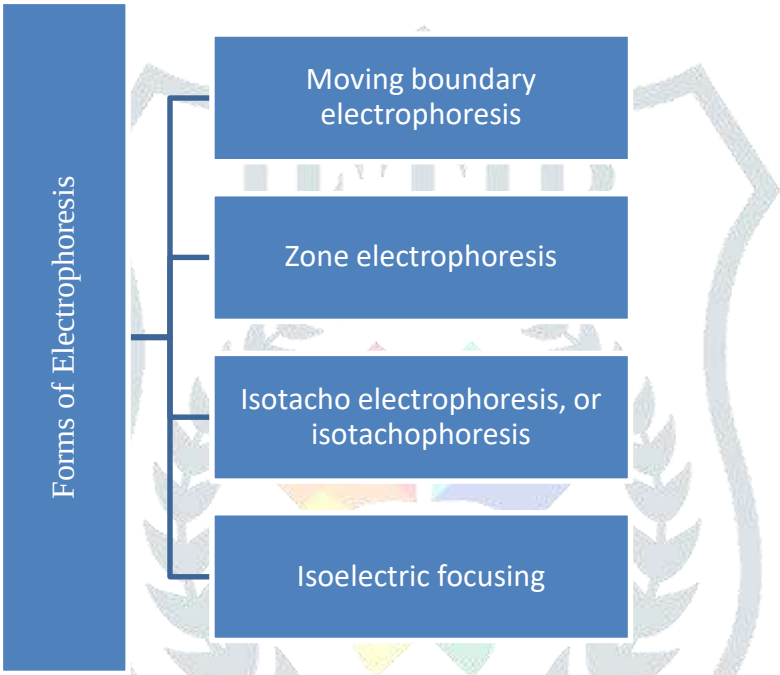

Figure 2: Illustrate the forms of electrophoresis, which is used in separation of molecule.

1.2.1. Moving boundary electrophoresis:

Moveable boundary electrophoresis is the oldest electrophoretic technique. A constant pH value during the procedure allows for separations in free solution, as well as tubes or capillary tubes. An important feature of electrophoresis in a free solution is the ability to monitor the mobilization of distinct particles without the interference of any intervening factors unrelated to the separation A low separation resolution and convection current are a result of the samples being mixed in the buffer, which may result in comparable components or particles being mixed together.

1.2.2. Zone electrophoresis:

Zone electrophoresis is equivalent to moving boundary electrophoresis in that it uses a homogeneous buffer solution to separate proteins. To reduce convection current and avoid uncontrolled sample diffusion, this format frequently employs a support medium or matrix. In most situations, the matrix also has an extra sieving action that influences electrophoretic separation. ZE-separation samples are encased in electrophoretic solution buffer and separated in the matrix for a set amount of time. The sample move at various speeds depending on their mass and charge when an electric current is introduced (q). Following the completion of the separation procedure, components of the sample with comparable properties are separated into a separate zone.

Gel electrophoresis, which employs a polymer-sieving matrix as a support medium, is an example of ZE. Because of its simplicity and adaptability, the method is frequently utilised in biochemistry and molecular biology research and regular work. Because of the use of a support medium, ZE is not appropriate for determining the isoelectric point of peptides or proteins or analyzing the mobility of charged particles of interest.

1.2.3. Isotacho electrophoresis and isotachophoresis:

All ions travel at the same speed in isotachophoresis, often known as ITP. A leading electrolyte or a terminating electrolyte are sandwiched between 2 non-homogeneous solution buffers in ITP. Particles in a sample of interest are charged in the same way by both electrolytes An electric current will have a greater effect on a leading electrolyte than a charged particle in the sample or even a terminating electrolyte. During the ITP, charged particles will be displaced in turn reducing electrical mobility, resulting in a continuous area of charged particle with equivalent characteristics wedged between regions occupied by the leading as well as terminating ions during the process. A graph showing the intensity of the electric field over time is used to depict the isotachophoresis data, where each region represents the identity of a charged particle and each region represents its concentration.

1.2.4. Isoelectric focusing :

It's an electrophoresis technique that uses a pH gradient to concentrate the sample from anode to cathode. Due to the fact that they are able to both give and absorb protons, the IEF only applies to amphoteric substance Amphoteric biomolecules include amino acids and carboxylic acid groups found in peptides or proteins, as well as Once a pH gradients and an electric current have been generated, an amphoteric sample will migrate towards the cathode or even the anode, depending on how much net charge it has. Velocity (v) and electrical mobility of amphoteric molecules both cease to exist when net charge equals zero at the isoelectric point, therefore stopping migration[3].

Every kind of electrophoresis may be done either one-dimensionally or two- (2D). This is followed by a second separation that is perpendicular to the first dimension. For clinical or field sample that require thorough analysis and characterization but are only accessible in limited amounts, 2D-electrophoresis can give more information or resolution[4].

1.3. Types of Electrophoresis:

Different types of electrophoresis as shown in figure 3, have been developed to separate various types of biomolecules, assess their properties, and research their interaction with a molecule of interest. The following are several electrophoresis methods that are based on distinct electrophoresis formats.

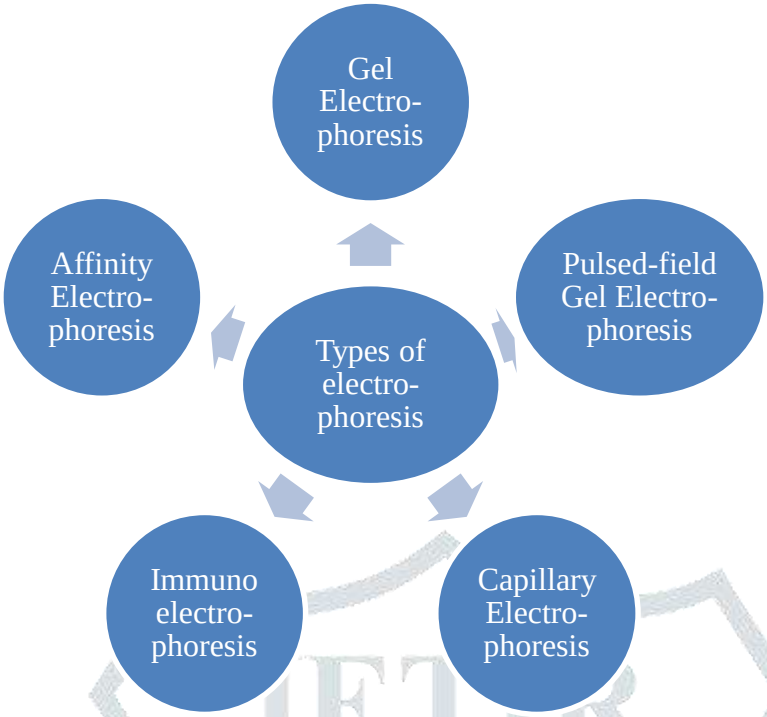

Figure 3: Illustrate the types of electrophoresis, such as capillary ,immuno, affinity electrophoresis.

1.3.1. Gel Electrophoresis:

If you're interested in using gel electrophoresis to maintain the pH of your solution buffer consistent, electrophoresis is for you. As a separation matrix, it's porous nature filter large particles while prevents smaller ones from electrophoretic separation. As a result, the gel is sliced or slabbed into thin strips and slabs with sample well Before electrophoresis separation can begin, the gel is either submerged in a buffer or samples are put in each well before the electric current is introduced. Gel electrophoresis will separate materials based on their mass. Because it is so easy to use and modify, gel electrophoresis is one of the most often used types of electrophoresis in research as well as routine diagnostics[5].

1.3.2. Pulsed field Gel Electrophoresis:

Pulsed-field As a kind of gel electrophoresis, electrophoresis involves the application of two electrical fields to the gel electrophoresis at various angles on a rotating basis. For instance, chromosomal separation involves the use of high-molecular-weight DNA molecules with a weight of more than twenty (kilobases).

1.3.3. Capillary Electrophoresis (CE):

An electrophoresis technique called High-Performance Capillary Electrophoresis uses a tiny capillary dipped in an electrolyte solution. Is it possible to do all four types of an electrophoresis with this method? In most cases, the capillary is between 20 and 30 centimetres in length, with an inner diameter of 25 to 75 It is electrophoretic separation that happens when a sample is either high-voltage injected into a capillary as well as high pressure is given to the capillary. Based on the capillary length and the electrophoresis technique, the components in a sample are separated. An opposite end capillary detector detects separated components and automatically records their detection time and retention duration in a logbook-style format Due to its narrow capillary, CE requires a very little sample quantity for separation. This technology permits high-throughput analysis and is automated at CE[6], [7].

1.3.4. Immuno electrophoresis:

Immuno electrophoresis is a kind of electrophoresis that separates antigens, such as proteins or peptides, depending on their interaction with antibodies or immunoglobulins or their specificity (Ig). The

precipitation of antigen-antibody complex occurs when antigen binds to its matching antibody at a particular antigen/antibody ratio, or even the equivalent point.

1.3.5. Affinity Electrophoresis:

Affinity A form of electrophoresis that separates a biomolecule that interacts with and binds to another molecule with which it has an affinity is called electrophoresis. It takes use of the fact that when a biomolecule, such as nucleic acids, proteins, peptides, or polysaccharides, attaches to another molecule, its electrical mobility changes, and this shift in electrical mobility is represented in the electrophoretic pattern[8].

2. LITERATURE REVIEW

Xinwang Wang et al. studied about separating microsatellite alleles with a novel electrophoresis method. Large-scale SSR (simple sequence repeats: microsatellites) response analysis can be laborious, time-consuming, as well as costly. The goal of this study was to report on a new electrophoresis method for rapidly and correctly analyzing or visualizing SSR data, as well as compare it to the capabilities of four existing electrophoresis methods. DNA from multiple Cornus Florida was used in individual PCR procedures. Using the three techniques of agarose gel, polyacrylamide gel, or QIAxcel System, cultivars and two SSR primer pairs were constructed for analysis. The CEQ TM 8000 Genetic Analysis System or the ABI 3130xl DNA Sequencer were used to build up two independent PCR reactions using the identical components plus a fluorescent-labeled primer for analysis. When compared to the two most commonly used capillary sequencers, the QIAxcel System was faster and less expensive, and it also offered a computer-generated gel picture[9].

Manuel J. et al discussed about the capillary electrophoresis. Electrophoretic separations in capillary tubes allow for fast and automated analysis of tiny quantities of complicated mixtures with remarkable sensitivity and resolution. This novel instrumentation's developing advancements are discussed. Narendra P. Singh et al. studied about a technique for directly quantifying DNA damage or repair in individual fibroblasts grown on microscope slides using microgel electrophoresis. Trypsinization as well as scraping, the two primary techniques for collecting normal diploid mammalian cells cultured in monolayer, produce DNA damage, they reveal using single-cell microgel electrophoresis. A single-cell gel electrophoresis (SCG) method was developed to quantify DNA single-strand breaks and alkali-labile sites in human fibroblasts. Scientists used human infant foreskin-derived fibroblasts, which adhered to frosted microscope slides, to illustrate the efficacy of this method. As a result of either medication treatment, DNA movement increased in a dose-Hydrogen peroxide-treated cells had more diversity in DNA migration than X-irradiated cells with the same degree of DNA damage. An X-ray generated DNA damage time course study found that the damage was completely repaired within 2 hours. In vitro toxicity applications of this method are studied[10].

3. DISCUSSION

Electrophoresis is a separation technique for liquid molecules based on their ability to travel in an electric field. Electrophoresis, in its numerous forms and kinds, has become the most widely used method for analyzing biomolecules in biochemistry or molecular biology, including genetic components like DNA and RNA, proteins, as well as polysaccharides. Electrophoresis is a simple yet sensitive analytical technique for separating proteins, nucleic acids, or other biological components in sample fluids. The major goal of this study is to learn more about various electrophoretic methods. In the biological sciences, electrophoresis has become one of the most widely used analytical methods. Due to their simplicity of use and improved design, manual electrophoresis systems have achieved a high degree of reliability, resolution, and accuracy after more than 30 years of research and improvement. Automation is the most recent step in this progression, and it offers unrivalled simplicity of use and repeatability, as well as less hands-on time and faster outcomes.

4. CONCLUSION

Electrophoresis is a simple yet sensitive analytical technique for separating proteins, nucleic acids, or other biological components in sample fluids. Electrophoresis is a separation technique for liquid molecules based on their ability to travel in an electric field. Electrophoresis, in its numerous forms and kinds, has

become the most widely used method for analyzing biomolecules in biochemistry or molecular biology, including genetic components like DNA and RNA, proteins, as well as polysaccharides. The major goal of this study is to learn more about various electrophoretic methods. Electrophoresis is a method for separating biomolecules and charged particle of interest depending on their mobility in an electric field. Electrophoresis is a simple yet sensitive analytical technique for separating nucleic acids, protein, and other biological components in sample fluids. The major goal of this study is to learn more about diverse electrophoretic systems.

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