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An Exploratory Review on Pharmaceutical and Bio-allied Applications of Electrophoresis and Recent Advancements

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Abstract: Electrophoresis is a technique that separates and analyzes molecules based on their size, shape, and charge. It has a wide range of applications in the pharmaceutical and bio-allied industries. This abstract will discuss the different applications of electrophoresis in these industries. One of the most important applications of electrophoresis is in the separation and analysis of proteins. Proteins are complex molecules that play a vital role in biological processes. Electrophoresis separates proteins based on charge and size, a process crucial for identifying and characterizing protein structures. This technique is widely used in the development of new drugs and the quality control of existing ones. Another important application of electrophoresis is in the analysis of DNA and RNA. Electrophoresis can separate DNA and RNA fragments based on size, which is essential for genetic research and diagnostic testing. This technique is used to identify gene mutations, which can help diagnose genetic disorders and design targeted therapies. Electrophoresis is also used in the purification of biomolecules. This technique can isolate specific molecules from complex mixtures, which is important for the development of biologics and other therapeutic agents. Electrophoresis is also used to analyze carbohydrates, lipids, and other biomolecules. Overall, electrophoresis has numerous applications in the pharmaceutical and bio-allied industries. It is an essential technique for the separation, identification, and characterization of biomolecules. Electrophoresis plays a critical role in drug discovery, diagnostic testing, and the development of biologics and other therapeutic agents. With the development of new electrophoresis technologies, this technique will continue to be a valuable tool in these industries.

Keywords: Electrophoresis; Proteins; Nucleic acid; Purification; Biomolecules; Pharmaceuticals.

1. Introduction

In 1807, Russian scholars Peter Ivanovich Strakhov and Ferdinand Frederic Reuss made the first scientific discovery of electrophoresis in Moscow when they realized that clay particles scattered in water move when an electric field is applied continuously. The presence of a charged contact between the particle surface and the surrounding fluid mostly brings it on. The movement of particles suspended in a fluid under a constant electric field is known as

electrophoresis (Figure 1). Positively charged particles (cations) undergo an electrophoresis process known as cataphoresis, whereas negatively charged particles (anions) undergo an electrophoresis process known as anaphoresis. The analytical methods used in chemistry to separate molecules according to their size, charge, or binding affinity are based on electrophoresis [1].

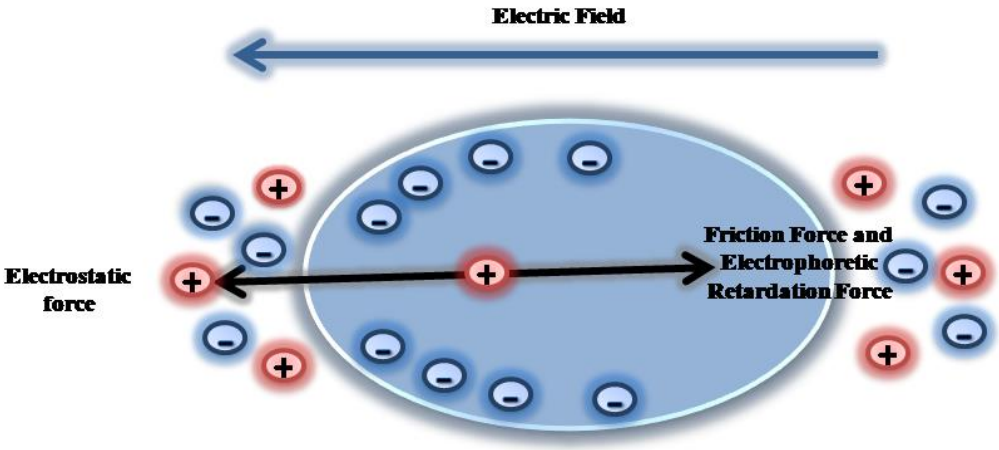

Figure 1. Illustration of electrophoresis.

Based on the size and charge of macromolecules, particularly proteins and nucleic acids, this approach is frequently used for analysis in some applications to separate or purify them. Using this method, a negative charge is applied to cause molecules like proteins, ribonucleic acid, or DNA to migrate toward a positive charge. These separated molecular components are visible in an image of the gel after separation [2]. Consequently, a purification method with a small sample requirement and high resolution is electrophoresis, a potent and affordable molecular separation technique [3].

2. Principle of Electrophoresis

The principle behind electrophoresis is that most biomolecules are electrically charged particles with ionizable functional groups. This means that a biomolecule in a solution can either have a positive (positive charge) or negative (negative charge) charge (negative charge). In an electric field, ionized biomolecules travel at different rates based on their mass and net charge. Cations, which are positively charged particles, migrate towards a negatively charged electrode, known as the anode, whereas anions, which are negatively charged particles, do the opposite. Each charged particle's varying speed and direction will produce a migration pattern specific to its characteristic, enabling devices to identify biomolecules with similar properties [4]. When an electric field is applied, charged species in a conductive liquid medium migrate toward the electrode of opposite charge (e.g., in electrophoresis). The molecules and ions are subjected to frictional forces as they move through the surrounding fluid. The net mobility of a molecule is estimated by the equation given below:

$$m=q/f \quad \text{.....i}$$

m is the electrophoretic mobility.....ii

q is the net charge..... iii

and f is the friction coefficient.....iv

(which is equal to 6ZR, where R is the particle radius and Z is viscosity).

An analyte's mobility is directly governed by its charge-to-size ratio since the frictional forces are proportional to its size. This straightforward formula was developed for spherical particles (often molecules) [5].

3. Application of Electrophoresis in Pharmaceutical Science

3.1. Detection of microbial contamination.

Due to its versatility, selectivity, sensitivity, and speed of analysis, electrophoresis is a separation-based approach for identifying and characterizing microorganisms. Detecting bacteria or their complete absence is a related field of investigation that is crucial for scientific and economic reasons. Microbe detection techniques often rely on capillary electrophoresis. All cell types are encouraged to unite into a single zone (peak)isolated from the electroosmotic flow front and any other interfering molecular components by CE. This procedure can be carried out using a segment of diluted cetyltrimethylammonium bromide, which temporarily reverses the direction of the cells' migration, and another segment of the solution containing a" blocking agent," which stops the migration of the cells and concentrates them into a small area. It is possible to employ relatively wide-bore capillaries to increase the sample size. The analyses take less than 10 minutes, and this method seems to work for various microorganisms [6].

3.2. Analysis of vaccine.

Vaccines against infectious diseases are urgently needed. So, the development of modern analytical methods should be as efficient as possible to speed up vaccine development. CE-SDS method for vaccine protein analysis based on a commercially available gel buffer [7]. The applicability of capillary electrophoresis for viral vaccine characterization, release, and stability testing of seasonal influenza virosomal vaccines, universal subunit influenza vaccines, Sabin inactivated polio vaccines (sIPV), and adenovirus vector vaccines was successfully demonstrated by this technique [8].

3.3. Determination of active compounds in medicinal plants and herbal formulations.

The measurement of active components (such as phenolic compounds, coumarins, protoberberines, curcuminoids, iridoid glycosides, alkaloids, and triterpene acids) in medicinal plants and herbal preparations using capillary electrophoresis. The most widely used capillary electrophoresis method for the selective separation and measurement of bioactive substances is capillary electrophoresis with UV detection. For specific applications, capillary electrophoresis is as selective as high-performance liquid chromatography. Capillary electrophoresis is an appealing and environmentally friendly alternative to more expensive methods for the quality control of drugs or raw plant material because of its short analysis time, high efficiency, versatility in separation modes, and low solvent and sample consumption, without any appreciable loss in sensitivity [9].

3.4 Carbohydrate analysis.

Capillary electrophoresis has become a potent method for analyzing carbohydrates. The technique offers great resolution and can separate carbs according to the charge-to-size ratio. N-glycans, which significantly impact the study of biomarkers and biological therapies, are the primary focus of principal applications. Exoglycosidase, lectin, and migration time indexing

are employed for N-glycan structure identification. Methods for sorting glycans with the same monosaccharide sequence but different positional isomers using capillary electrophoresis have been developed. These methods can also determine whether the monosaccharides that make up a glycan are alpha- or beta-linked. With a brief discussion of carbohydrate studies of glycosaminoglycans and mono-, di-, and oligosaccharides related to food and plant products, the importance of capillary electrophoresis to the investigation of N-glycans in biomarker development and biological therapies is highlighted [10].

3.5.Bacterial analysis.

Capillary electrophoresis is frequently used in the investigation of microorganisms (CE). They showed how *Lactobacillus casei* and the tobacco mosaic virus travelled through a capillary in response to an electric field. Due to the physiological diversity of the microbial community, CE analysis of bacteria has been shown to be more difficult than the analysis of small molecules. Microorganisms cannot be analyzed by capillary zone electrophoresis (CZE) in a repeatable, quantitative manner. Applications based on CE techniques have been developed for numerous microbiological assays. Common applications for CE include quick sterility testing, detecting microbial contamination in real biological samples, and estimating bacterial numbers in natural rivers [11].

3.6. Separation of human insulin, insulin lispro, and their degradation products.

The CZE and CGE could be used to evaluate the quality of pharmaceutical formulations, including lispro, a short-acting insulin analog. Pharmaceutical formulations must be evaluated for stability regarding deamidation-related degradation of the active component. These methods are appropriate for identifying their deamidated breakdown products. The deamidation products were effectively separated using CZE and identified with CE-MS. For identifying charge variances, CZE can be a straightforward, quick, and reliable tool for evaluating the quality of insulin formulations. Conversely, CGE can be useful for analyzing the molecular mass variations of lispro, an insulin analog [12].

3.7. Quantification of organic aids from tissue culture.

Having one or more terminal carboxyl (COOH) functional groups, carboxylic acids are a type of organic acid. The efficient operation of numerous biological systems depends on short-chain carboxylic acids (SCCAs; carboxylic acids with three to six carbons), including malate and citrate. SCCAs play a role in cellular respiration and can act as markers of cell health. Increased SCCA levels provide an "acid" or "sour" taste in foods, which can significantly impact the flavor of certain items. The food and beverage sectors are particularly interested in techniques for rapid analysis of organic acid levels. Sometimes capillary electrophoresis (CE) is preferred for the identification and quantification of organic acids in plant and food samples, using free-zonal capillary electrophoresis (CZE). CZE offers a method for measuring SCCAs with a low detection limit (0.005mg/mL). This approach focuses on measuring SCCAs in coffee beans; however, it can also be used to measure SCCAs in other plant-based foods [13].

3.8. Analysis of vitamins in food and beverages.

Capillary zone electrophoresis (CZE) and micellar electrokinetic capillary chromatography are the two CE techniques most frequently employed in studying food (MEKC). Both methods have been applied to identify a variety of vitamins in pharmaceutical preparations and biological fluids, but CE methods for vitamin identification in food are only able to identify vitamin C in fruits, vegetables, and beverages, niacin in a variety of foods, and thiamine in samples of meat and milk. Fish feed, plant, and animal tissue have all been found to contain vitamin C esters. The determination of niacin in cereal bars and vitamin C in blueberries was carried out using CE [14].

3.9. Analysis of alkaloids.

The simplest CE mode, known as capillary zone electrophoresis, separates analytes based on variations in their charge-to-mass ratios. This method can be useful in various situations; in particular, quaternary alkaloids are ideal solutes in CZE because of their persistent charge, making them suitable regardless of the pH of the operating buffer. With a focus on the pertinent elements of the study of alkaloids in herbal medicines and medicinal plants, the CZE separation will be considered. Potato calystegines A3 and B2 were identified using capillary zone electrophoresis (CZE) and capillary isotachophoresis (cITP) [15].

3.10. Chiral separation of a drug.

Drugs are chiral-separated at an analytical scale using CE, which enables rapid method development, high separation efficiency, relatively quick analysis times, low analyte, reagent, and chiral selector (CS) consumption, and high flexibility in CS selection and replacement [1617]. Typically, a direct chiral separation technique is used in CE, which only requires the addition of CS to the background electrolyte (BGE). There are a fair number of CSs that can be used in CE, including crown ethers, macrocyclic antibiotics (aminoglycosides, glycopeptides), chiral ionic liquids, and chiral surfactants, but the cyclodextrin (CD) derivatives (native and derivatized; neutral and ionised) are by far the most effective and frequently used [1819]. The antipsychotic medication risperidone's primary active metabolite, 9-hydroxyrisperidone, has already undergone a CE chiral separation process. The technique was used to simultaneously determine the enantiomers of risperidone and 9-hydroxyrisperidone, and it may be used to analyze biological samples to characterize risperidone metabolism [20].

4. Application of Electrophoresis in Bioallied Science

4.1. Determination of ions of saliva.

Saliva is a biofluid that is easy to obtain and informative, making it ideal for identifying various illnesses, including cancer, viral and bacterial infections, cardiovascular, renal, and autoimmune diseases, as well as other conditions. A promising clinical technique for identifying the relationship between salivary analytes and a specific disease is saliva-based diagnostics, particularly those built on metabolomics technology [21]. The analysis of biological materials, including DNA, has been successfully carried out using capillary electrophoresis (CE), a high-throughput analytical technique with excellent resolution for detecting trace levels of chemicals. Because collecting saliva is less intrusive than collecting

blood, CE is applied to salivary ion analysis. After filtering and dilution, ion analysis of saliva samples is possible. Similar to blood, saliva contains a variety of biochemical markers that can be used to detect diseases and stress, including cortisol, amylase, testosterone, and immunoglobulin A. Additionally, inorganic and organic ions are utilized as indicators of work stress, smoking, plaque control, and biorhythm [22].

4.2. Diagnosis of surgical site infections.

The majority of SSIs result from surgical incisions becoming contaminated with bacteria from the patient's own body. Due to the complexity of these samples and the low analyte concentrations, a system with great sensitivity and efficiency is necessary from an analytical standpoint. New analytical methods are critically needed to enable the rapid detection of pathogenic organisms in biological materials. High hopes are attached to the therapeutic application of CE for rapid screening and identification of specific etiological factors causing symptomatic postoperative wound infection. A quick method of microbiology based on the CZE system appears to provide at least a partial answer to this issue. Although its initial characterization does not allow specific identification of each pathogen, it does confirm the presence of microorganisms and narrow antibacterial treatment options [23].

4.3. Separation of DNA fragments.

Based on their size, DNA fragments are separated by gel electrophoresis in an agarose gel or another solid support. Introducing an electric current at the nodal, negative end causes the negatively charged DNA to move towards the bottom (cathodal, positive) end after the sample (DNA) has been pipetted into the sample wells. Smaller fragments migrate more quickly and settle at the bottom of the gel in direct proportion to their size. Ethidium bromide, an intercalating dye, is used to see DNA. The color is absorbed by DNA fragments as it moves through the gel. The intercalated dye fluoresces when it is illuminated with UV light. Larger pieces glow more strongly. It is possible to estimate the sizes of many other unknown DNA pieces by simultaneously running a "ladder" type set of DNA fragments with known sizes [24].

4.4. Detection of serum lipoproteins.

Lipoprotein electrophoresis analysis of serum lipoprotein fractions reveals lipid metabolism abnormalities and provides multiple types of bioinformation that support the diagnosis and therapy of dyslipidemia and related diseases (e.g., coronary artery disease or chronic kidney disease). Lipoprotein electrophoresis tests are too inexpensive [25]. Only a portion of the serum lipoproteins may be analyzed using total lipid staining and electrophoresis; however, trophoresis of Sudan black-pretreated samples on polyacrylamide gel offers sufficient separation and is beneficial for identifying mid-band or lipoprotein subfractions. The quantitative analysis of lipids is nonetheless restricted since staining agents cannot effectively penetrate the gel [26].

4.5.Antigen-antibody interactions.

Changes in the electrophoretic mobility of the affinity ligand (e.g., an antibody) and the target are the basis for affinity measurements by CE (e.g., antigen). Premixing the affinity ligand and target before injecting them into a CE system enables the separation of the affinity ligand and/or target from the affinity ligand-target complex if slow kinetics are present. The gradual

change in the mobility of the affinity ligand or target due to changing affinity ligand/target concentrations in the capillary can be used to estimate binding constants when interaction kinetics are relatively fast (i.e., the on- and off-rates are fast compared to the time spent within the capillary)—studying bimolecular interaction through CE. This technique has often been employed to investigate antibody-antigen interactions [27].

4.6.Assessment of PCR product yield.

When working with DNA, evaluating PCR product yield may be required, since several post-PCR analysis procedures, such as sequencing or fragment length analysis using capillary electrophoresis, depend on PCR product levels falling within a specific range to be effective. Agarose gel electrophoresis is a technique for calculating PCR product yield. This technique was first developed independently about 50 years ago to separate restriction enzyme-digested DNA fragments and mt DNA topoisomers. It is reasonably affordable and simple to use. The effectiveness of PCR reactions is frequently evaluated using agarose gel electrophoresis. In an agarose matrix, nucleic acid fragments are separated by their length. These fragments can be visualized under ultraviolet light using a dye or an intercalating agent such as ethidium bromide (EtBr) [28].

5. Future Perspectives

Electrophoresis has played a vital role in pharmaceutical and bio-related applications, and with the emergence of new technologies, it is expected to gain even greater importance in the future [29-34]. The potential applications of electrophoresis are expanding, and new techniques are being developed to improve its efficiency, sensitivity, and accuracy. This section will discuss future perspectives for electrophoresis in pharmaceuticals and bio-allied applications. One of electrophoresis's most promising future perspectives is its use in personalized medicine [35-37]. With the development of high-throughput sequencing technologies, the ability to sequence a patient's genome has become more accessible. This has led to an increasing demand for personalized medicine, where treatments are tailored to an individual's genetic makeup. Electrophoresis can play a crucial role in this field by providing a quick, reliable way to analyze proteins and other biomolecules, thereby helping identify potential drug targets. By using electrophoresis to analyze individual patient samples, doctors can identify biomarkers for specific diseases and tailor treatments to the patient's individual needs [38].

Another promising future perspective of electrophoresis is in the development of new drugs. Electrophoresis can be used to study the interactions between drugs and their targets, such as proteins and nucleic acids. By analyzing drug-target binding, researchers can optimize drug design and improve drug effectiveness. Furthermore, electrophoresis can be used to study the metabolism of drugs and their by-products, thereby helping identify potential side effects and toxicities. Electrophoresis can also be used in the field of biotechnology. One of the most promising applications is in producing biopharmaceuticals, such as monoclonal antibodies. Electrophoresis can be used to purify and separate these molecules from other contaminants, ensuring the purity and quality of the final product. In addition, electrophoresis can be used to study protein folding and stability, which are critical for developing new biopharmaceuticals.

Another promising application of electrophoresis is in proteomics [39-40]. Proteomics studies the entire set of proteins expressed by an organism, tissue, or cell. Electrophoresis can

be used to separate and analyze these proteins, enabling researchers to identify potential disease biomarkers and develop new diagnostic tools. Furthermore, electrophoresis can be used to study protein-protein interactions, thereby helping identify potential drug targets. In conclusion, electrophoresis has been and will continue to be a crucial tool in pharmaceuticals and bio-allied applications. The future perspectives of electrophoresis in this field are promising, and the development of new technologies and techniques will continue to expand its potential applications. From personalized medicine to drug development, biotechnology, and proteomics, electrophoresis will be vital in advancing our understanding of biological systems and developing new treatments for diseases. As such, developing and implementing electrophoresis techniques will be critical in shaping the future of medicine and biotechnology.

6. Conclusions

Electrophoresis is a versatile technique with a wide range of applications in pharmaceutical science. Its effectiveness in detecting microbial contamination, analyzing vaccines, determining active compounds in medicinal plants, analyzing carbohydrates, and separating insulin, insulin lispro, and their degradation products has been well documented. Electrophoresis is also useful for quantifying organic acids from tissue culture, making it a valuable tool for studying cell health. Electrophoresis techniques are highly sensitive, selective, and rapid, making them an attractive option for researchers developing new drugs or analyzing existing pharmaceutical formulations. Furthermore, electrophoresis is environmentally friendly, as it requires low solvent and sample consumption without any appreciable loss in sensitivity. In addition, the determination of ions in saliva has been enabled by capillary electrophoresis, which allows the identification of biochemical markers indicative of various diseases and stress. The diagnosis of surgical site infections has become more efficient with CE-based microbiology, enabling the detection of microorganisms and narrowing down antibacterial treatment options. Separation of DNA fragments is possible through gel electrophoresis, making it a crucial technique in molecular biology. Lipoprotein electrophoresis analysis and assessment of PCR product yield are other areas where electrophoresis plays a significant role in medicine and research. Finally, the use of CE in the study of antigen-antibody interactions has provided invaluable information about biomolecular interactions. In summary, electrophoresis has revolutionized how scientists and medical professionals study and diagnose diseases, and its impact on various fields is set to grow as more research is conducted. With its high sensitivity and efficiency, electrophoresis will continue to drive significant advancements across various fields, improving our understanding of the world around us and the mechanisms that drive it.

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Conflicts of Interest

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