ISSN: 2249-7196
IJMRR/March 2021/ Volume 11/Issue 3/80-85

EXPLORING THE FUTURE POTENTIAL OF ELECTROPHORESIS IN SEPARATION TECHNOLOGY RESEARCH

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Abstract: Electrophoretic separation has long been a cornerstone technique in both chemistry and biology, dating back to its first demonstration in 1807. Over the past century, advancements in miniaturization, precision engineering, biochemistry, electrical engineering, and electronics have revolutionized electrophoresis systems, transitioning from rudimentary paper-based setups to sophisticated automated platforms. These advancements have been driven by the need for faster and higher-resolution results. This paper provides an overview of the historical evolution of electrophoresis technology and offers insights into potential future developments. It explores the designs, applications, separation phases, biological implications, and functionalities of electrophoresis systems. The increasing complexity of electrophoresis systems underscores the significant technological progress in this field. By identifying current technological gaps, the paper offers glimpses into the potential future of electrophoresis. Examining the potential benefits and challenges of this seemingly simple separation technology provides both fascination and complexity.
Keywords*:* Progress, History, Electrophoresis, Development, Future.

IINTRODUCTION

The evolution of electrophoresis throughout history

In 1807, Ruess (Ruess, 1809) conducted an experiment that laid the groundwork for electrophoresis, observing particle movement in a suspension of clay in water when subjected to an electrical current. However, it wasn't until 1942 that Coleman and Miller (Coleman and Miller, 1942) demonstrated the migration of neutral hexose towards the anode in a borax solution, marking the widespread adoption of electrophoresis as a scientific technique. Various experiments were conducted to explore the application and limitations of electrophoresis for separating compounds containing contiguous "-OH" groups and high concentrations of neutral sugars (Smith, 1955; Hashimoto et al., 1942; Foster, 1957). Electrophoresis gained traction for separating DNA and RNA following Consden and Stanier's successful separation of sugars in 1952 (Consden and Stanier, 1952).

In the 1970s, Richards et al. (Richards et al., 1965) conducted significant studies popularizing Tris media as the buffer solution in electrophoresis, including the use of Tris-acetate-EDTA (TAE) and Tris-borate-EDTA (TBE). Danna et al. (Danna and Nathans, 1971) further defined electrophoresis as a technique for DNA analysis by measuring the length and relative molarity of SV40 DNA fragments using restriction enzymes. Ethidium bromide was initially used as a DNA stain, later employed by Aaij and Borst in 1972 to distinguish between circular and linear DNA

in conductive media (Aaij and Borst, 1972).
Tris emerged as the dominant buffer for the conductive buffer, although other compounds were available. Tris-borate-EDTA (TBE) was introduced by Peacock et al. in 1968 (Peacock and Dingman, 1968), while Tris acetate acid EDTA (TAE) was introduced by Danna and Nathans in 1972 (Danna and Nathans, 1971; Aaij and Borst, 1972).

Electrophoresis has evolved from laborious DNA analysis processes to a method for separating molecules based on molecular mass and charge ratio, with applications ranging from molecular biology studies to chemical compound analysis of water, soil, air quality, food quality, processing hygiene, and medical forensic analysis (Kappes and Hauser, 1998).
Beyond separation, electrophoresis also serves as a purification method, particularly for specific targets of interest such as genes or membrane proteins. Various modifications and enhancements have been made to electrophoresis systems, from basic paper electrophoresis to automated microchip electrophoresis, each with distinct functions and applications (Voet and Voet, 1995).

Agarose gel electrophoresis

Agarose gel electrophoresis serves as a fundamental technique for separating DNA or RNA fragments of varying lengths. The pore size in agarose gel, ranging from 100 to 300 nm, directly correlates with the gel concentration, decreasing as the agarose concentration increases (Hame and Rickwood, 1998). Negatively charged DNA or RNA molecules migrate from the negative to the positive electrode in an electric field generated between the electrodes, leading to their separation based on size, shape, and length (Hame and Rickwood, 1998).
Polyacrylamide gel electrophoresis offers two types of gels: dissociating and non-dissociating. Non-dissociating gels preserve protein structures, activities, and roles, while dissociating gels denature proteins into their component polypeptides (Voet and Voet, 1995). Native gel electrophoresis, a non-denaturing gel, offers superior resolving capability compared to SDS-PAGE and is commonly used for protein separations (Hame and Rickwood, 1998).
Polyacrylamide gel electrophoresis employs acrylamide monomer chains cross-linked with N, N′-methylenebisacrylamide units, with the concentration of cross-linker affecting pore size in the gel (Brown, 2002). This method boasts a higher resolving capacity than agarose gel electrophoresis and is suitable for separating proteins and DNA strands of similar sizes (Brown, 2002).
SDS-PAGE enables rapid and reliable quantification, comparison, and characterization of proteins, peptides, and small molecular weight nucleic acids based on their molecular weight (Judd, 2002). Variants of SDS-PAGE include gradient gels and SDS-urea gels, offering higher resolving power depending on sample requirements (Hame and Rickwood, 1998).
Denaturing Gradient Gel Electrophoresis (DGGE) and Temperature Gradient Gel Electrophoresis (TGGE) facilitate the separation of PCR-generated DNA products for molecular fingerprinting (Zijnge et al., 2006). DGGE divides PCR products based on denaturing rate and sequence size, enabling discrimination of samples with similar-sized PCR fragments (Zijnge et al., 2006).
Isoelectric focusing separates proteins based on their isoelectric point, or net charge, by subjecting protein samples to an electric field-generated pH gradient slab (O'Farrell's, 1975; Bollag and Edelstein, 1999). Combining isoelectric focusing with SDS-PAGE in 2D gel

electrophoresis provides greater resolution by separating proteins based on their isoelectric point and size (Bollag and Edelstein, 1999). However, the high sensitivity of these methods requires careful handling of samples to prevent charge alterations that could affect results (Bollag and Edelstein, 1999).

Zymography

Zymography offers a valuable method for analyzing enzyme activity directly within the gel after electrophoresis, eliminating the need for band extraction and cleaning (Scadden and Naaby-Hansen, 2001; Coughland, 1985). Various staining methods can be employed for zymography, such as chemical soaking or using a second gel containing chromogenic reagents and auxiliary enzymes to facilitate band identification (Coughland, 1985).
Advantages of zymography include its ability to study enzyme activity based on physical properties like molecular weight or isoelectric point, along with investigating factors like posttranslational modifications and enzyme heterogeneity (Scadden and Naaby-Hansen, 2001). Zymography finds applications in analyzing ribonucleases, bacterial and fungal works, and detecting microbial proteases, while also serving to verify protein-protein or enzyme interactions (Kumbaya et al., 2007).
Pulsed-field electrophoresis addresses the limitation of standard electrophoresis in separating very large DNA molecules (Watson et al., 2004). By alternating the electrical voltage across multiple directions, pulsed-field electrophoresis prevents large DNA molecules from creating smears, allowing their effective separation (Watson et al., 2004).
Capillary electrophoresis employs extremely thin capillary tubes to separate molecules with high efficiency and low sample requirements (Voet and Voet, 1995). This method offers various forms, including micellar electrokinetic capillary chromatography, capillary electrochromatography, and capillary array electrophoresis, each suitable for different applications (Mathies and Huang, 1992; Carretero et al., 2004; Norman, 1999).
Detection methods in capillary electrophoresis include laser-induced fluorescence and electrically floating conductivity detection, providing high sensitivity and accurate data collection (Zare et al., 1998). Capillary electrophoresis finds applications in drug discovery, genetic studies, and protein characterization (Voet and Voet, 1995).
Microchip electrophoresis represents an advanced form of capillary electrophoresis, offering increased throughput and automation (Hammond, 2001). Fabricated from materials like glass, fused silica, PDMS, or PMMA, microchips enable rapid DNA separation with high precision (Osbourn and Lunte, 2003). Novel materials and electrode options continue to drive advancements in microchip electrophoresis technology (Liu et al., 2006; Wang et al., 2008; Prêt et al., 2001; Alves-Brito-Neto et al., 2005).

Fluorophore-assisted carbohydrate electrophoresis (FACE)

Fluorescent-Assisted Capillary Electrophoresis (FACE) is a technique utilized for identifying carbohydrates labeled with a fluorescent dye, allowing for their separation using polyacrylamide gel electrophoresis (AlvesBrito-Neto et al., 2005). This method is particularly crucial as carbohydrates lack charge, and FACE serves as the primary approach for analyzing various carbohydrate types, including plant and bacterial polysaccharides (AlvesBrito-Neto et al., 2005).

It streamlines complex processes, such as detecting lipid-linked oligosaccharides, eliminating the need for metabolic labeling with radioactive sugar precursors (Gao, 2005; Starr et al., 1996).

Affinity electrophoresis leverages capillary electrophoresis's resolving power to separate materials based on non-specific or specific affinity interactions occurring during electrophoresis (Heegard et al., 1998; Colton et al., 1998). This technique finds applications in various fields, including peptide and protein detection, drug development, and immuno-affinity works (Heegard et al., 2003; Progent et al., 2002; Rochu et al., 2002; Mito et al., 2000; Villareal et al., 2003; Kuroda et al., 2003; Iki et al., 2000; German and Kennedy, 2000; Ou et al., 1999; Shimura et al., 2002). Affinity electrophoresis is valuable for studying receptor-ligand interactions in free or immobilized forms (Heegaard et al., 2001). Automated electrophoresis systems, facilitated by computerized robotics and programming, enable the automatic execution of electrophoresis techniques (Michels et al., 2002; Kristensen et al., 2001). These systems range from automated 2D capillary electrophoresis for high-throughput protein analysis to devices used in projects like the Human Genome Project for detecting genetic variations (Michels et al., 2002; Kristensen et al., 2001). Automation ensures precision and accuracy, aiding in tasks such as identifying single-strand conformation polymorphisms in genetic samples.

The development of portable electrophoresis apparatuses is underway, capitalizing on advancements in engineering and science (Zhang et al., 2006). These systems aim to overcome limitations such as the need for an external power source, potentially expanding the use of electrophoresis beyond traditional settings (Zhang et al., 2006). Additionally, research efforts focus on improving the efficiency of existing systems, reducing background noise, and enhancing detection limits (Burns, M.A., 2003). Patents and studies are dedicated to refining buffer systems, gel types, and detection methods to achieve more efficient and accurate separation runs (Judd, R.C., 2002; Brody and Kern, 2004a; Brody and Kern, 2004b; Cole, 2001; Kawabata et al., 2004; Garrels, 1999; Osbourn and Lunte, 2003; Irie et al., 2000; Collier et al., 1993). These endeavors aim to advance electrophoresis technology, enhancing its repeatability, accuracy, and application scope.

CONCLUSION

Even after two centuries since its inception in the early 19th century, electrophoresis technology remains crucial in the field of separation research. While the techniques and tools used today have evolved significantly from those of the past, the underlying principles of electrophoresis endure. Looking ahead, the next phase of development in electrophoresis technology is likely to focus on enhancing mobility and miniaturization of systems.

The trend towards mobility and miniaturization in electrophoresis systems is driven by the increasing demand for portability, efficiency, and versatility in research and diagnostic settings. Portable electrophoresis systems would enable researchers to conduct analyses in diverse environments, including fieldwork, point-of-care settings, and resource-limited regions. Miniaturization of systems would not only reduce space requirements but also facilitate high-throughput screening and integration with other analytical techniques.

Advancements in microfluidics, nanotechnology, and materials science are expected to play key roles in the development of mobile and miniaturized electrophoresis systems. Microfluidic

devices offer precise control over sample manipulation and separation processes, allowing for rapid analysis with minimal sample and reagent consumption. Nanomaterials, such as nanoparticles and nanotubes, can enhance detection sensitivity and enable novel separation mechanisms. Furthermore, the development of novel materials and fabrication techniques will enable the construction of compact and robust electrophoresis platforms.

In addition to technological advancements, there is a growing emphasis on the integration of electrophoresis systems with automated sample preparation, detection, and data analysis platforms. Automated workflows would streamline experimental procedures, reduce human error, and increase throughput, making electrophoresis more accessible and efficient for a wide range of applications.

Overall, the future of electrophoresis technology lies in the continued pursuit of mobility, miniaturization, and automation. By harnessing these trends, researchers can unlock new opportunities for rapid, sensitive, and portable analysis in various fields, including biomedicine, environmental monitoring, and forensic science.

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