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Principles of Capillary Electrophoresis – A Small Synthesis

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Abstract

Electrophoresis defines the migration of charged particles in an electrical field in accordance with their molecular charge and size. In clinical and veterinary medicine, electrophoresis is used mainly to separate and thus differentiate between proteins in each sample, for example, blood, urine, saliva or other. Capillary electrophoresis is a simple, reliable, and specific method for separating different protein fractions and resembles an essential method for diagnosis some deficiencies, disorders, and other information about the subject. The use of DNA samples greatly expands the range of medical and ecological problems that can be studied with this technique. The selection of the most effective method based on the analysed samples, as well as the implementation of an efficient laboratory protocol in terms of sampling, storage, and mobility within the laboratory, are key aspects in the optimal development of any research project. Here we reviewed the most recent knowledge on different analysed biological samples to briefly describe the principles and scope of capillary electrophoresis.

Keywords: ***Capillary electrophoresis; DNA electrophoresis; Electrophoresis principles; DNA sequencing; Electrophoresis.***

1. Introduction

The study of DNA electrophoresis began in 1964, when three groups of researchers used moving boundary methods to measure mobility in free solutions. They discovered that for DNA molecules larger than 400 base pairs (bp), mobility was independent of size and varied with ionic strength as well as the identity and valence of the cation in the background electrolyte [1].

Capillary electrophoresis is now used to separate macromolecules based on size and to identify genes and mutations to confirm site-directed mutagenesis, and there have been many substantive improvements that allow the separation of much larger DNA molecules than was previously possible [2]-[4].

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Choices involving voltage, electric current, conductivity, temperature, and the concentration and identity of the ionic species tend to complicate the conductive properties in DNA electrophoresis. The differences throughout existing chemical recipes for common conductive media have an impact on central properties. Even in their best form, various buffers create a runaway positive feedback loop between heat generation and retention, temperature, conductivity, and current. This is undesirable because it limits the permissible electric field and impairs resolution, therefore strict protocols should be followed during the procedure [5].

Capillary electrophoresis is an analytical method that has been shown to offer a promising alternative to thin-layer chromatography and liquid chromatography in genetic analysis, drug development, analysis of drug impurities, including anti-cancer drugs, and protein characterization [6]-[8].

This article aimed to review the recent literature to extract the principles and scope of capillary electrophoresis as readily available knowledge reading DNA sequencing, laboratory protocols and capillary electrophoresis and to identify the most suitable procedures in the hand of science personnel.

2. Materials and Methods

This research was conducted using the most popular scientific databases – Google Scholar, Science Direct, and Scopus – to identify relevant literature. The keywords used for determining the necessary result were “electrophoresis”, “DNA electrophoresis”, “electrophoresis principles”, “DNA sequencing” as well as “capillary electrophoresis”.

Before screening the full text, the studies considered appropriate were selected by (1) title, (2) abstract and (3) keywords. In the second step, the inclusion/exclusion criteria were applied. The inclusion criteria were (a) studies focused on DNA sequencing, which include sampling detailing and samples preparation; (b) studies that included laboratory procedures; (c) studies published after 2000 and (d) studies published in English. The exclusion criteria were (A) studies in fields other than human and animal research; (B) studies published before 2000, with a few exceptions due to their complex results and theories.

This synthesis encompasses holistically helpful information about DNA, DNA sequencing, laboratory protocols and capillary electrophoresis used by different authors in their studies to monitor and understand the impact of macromolecule separation and to identify the most suitable procedures.

3. Results

Using the three databases and after conducting the assessment stages for each of the records based on the exclusion criteria, the present review consists of 36 relevant studies.

3.1 General Characteristics of the Included Studies

The studies included in this review focused on describing the principles and scope of capillary electrophoresis and review the most recent knowledge on different protocols and analysed biological samples.

3.2 Serum Protein Electrophoresis and Capillary Electrophoresis (CE)

Serum protein electrophoresis (SPE) is a technique that is widely used in human, veterinary, and avian medicine. This technique is useful in identifying patients with multiple myeloma and other serum protein disorders in human medicine. Many subspecialists use serum protein electrophoresis screening in the initial assessment of a variety of clinical conditions. The results of this examination can be puzzling or difficult to interpret sometimes [9], [10].

SPE is usually done manually, with cellulose acetate or agarose gel, or with automated systems that use capillary electrophoresis (CE). SPE is labour-intensive and technically demanding when performed manually; therefore, capillary electrophoresis is required for high sample throughput in laboratories [11].

CE techniques and equipment are distinct from other electrophoresis techniques. A CE consists of a variable high voltage power supply, which allows for rapid separation of DNA fragments (and determination of DNA sequence), a fused silica capillary, two buffer reservoirs, two electrodes, an on-capillary detector, and a sensor linked to a data evaluation unit [12], [13].

CE is a technique for separating and quantifying a wide range of molecules based not only on charge but also on size, hydrophobicity, and stereospecificity. Working in narrow-diameter tubes has the advantage of eliminating heat generated by other electrophoretic methods and is being used in genetic analysis, drug discovery, drug impurity analysis, anti-cancer drugs, and protein characterization [8].

CE is regarded as a powerful technique for biomolecule analysis and sample preparation because it allows for the automation of all analysis steps. It also has complementary and alternative characteristics to liquid chromatography. Each CE mode's technical and analytical considerations, from sample preparation to analysis conditions, are critically discussed [14].

Capillary electrophoresis separations are important because they provide fast separations of small sample volumes and are easily automated. Following reports of exceptional amine, amino acid, peptide, and DNA separation efficiencies [15], [16].

The gel in traditional gel electrophoresis serves two purposes: it acts as an anticonvective medium and as a sieving matrix for separation. Gel-free separation is possible with CE. However, there is an exception for biopolymers with a constant charge: size ratio, such as RNA, DNA, or SDS-loaded proteins. In this case, there is no electrophoretic separation in free solution. There are two potential solutions: modifying the biopolymer and using a separation matrix as in traditional electrophoresis [17].

Gel-filled capillary columns have several advantages over open-bed gel electrophoresis, including higher resolution, shorter analysis times, and increased sensitivity, as well as the ability to use larger potential fields and on-line sample detection without staining procedures [18].

The matrix in which separation is achieved, as well as the type of capillary used, the electrophoresis buffer, and the strength of the electric field applied during the process, all influence the efficiency of analyte separation [13].

3.3 Laboratory Procedures

Sample preparation is a critical component of any DNA sequencing protocol. For efficient reading of DNA sequences, accurate amplification of the studied DNA sequence and removal of impurities that make accurate base calling difficult are required [19].

Choosing the right protocols is a crucial factor in determining quality. The best protocol depends on the nature of the sample to be analysed and the properties of the constituent protein species to be resolved [20].

Purification of DNA sequencing products is mandatory before electrophoretic analysis. After thermal cycling, residual background ions (buffer, chloride, unincorporated dideoxynucleosides) in the sequencing extension reaction cocktail reduce injection efficiency, significantly degrading sequence signal-to-noise. Extension product purification requires ethanos precipitation or more stringent spin-column methods, which are frequently used [21].

Typically, blood is collected in plain tubes, and serum is obtained by centrifugation. If the serum is haemolyzed or lipemic, it can be visually inspected and stored at -20ºC until analysis [22], [23]. However, when urine samples are electrophoresed, the sample's stability lasts for a few hours or three days at 4ºC. Years of storage at -20ºC have been reported with no significant changes [24].

Sample preparation, including clean-up and pre-concentration, is a key factor in the widespread use of CE (capillary electrophoresis) in a wide range of fields and samples. All the above on-line sample cleaning and pre-concentration methods can significantly reduce the detection limit of CE [25].

Highly entangled solutions of hydrophilic and high molar mass polymers are required to achieve high DNA separation efficiency and long read length, which are system characteristics that are especially important for genomic sequencing. The strength of DNA-polymer interactions, as well as the robustness of the entangled polymer network, have a significant impact on the performance of a given polymer matrix for DNA separation [26].

Capillaries are conditioned to repeatedly rinse with methanol and buffer until efficient and reproducible separations are obtained using an argon-ion laser. Capillaries are stored in distilled water overnight. Prior to analysis, native DNA samples are diluted in deionized water. If there is an excess of salts in the sample, it can be dialyzed using specific pipetting protocols. After that, internal standards for native DNA separations are obtained. The PCR is used to amplify denatured, dye-labelled DNA samples, which are prepared by diluting parts of the sample in formamide. Samples are then heated for a few minutes, depending on the protocol, and quickly cooled before being analysed. Because the buffer used for these separations is viscous, CE systems capable of high-pressure capillary filling are required [27].

The composition and ionic strength of the electrophoresis buffer influence the electrophoretic mobility of DNA. Electrical conductivity is minimal in the absence of ions (for example, if water is substituted for electrophoresis buffer in the gel or reservoirs), and DNA migrates slowly or not at all. Electrical conductance is very efficient in high ionic strength buffers, and significant amounts of heat are generated even when moderate voltages are applied. In the worst-case scenario, the gel melts and the DNA become denatured [28].

There are two types of sample-loading solutions: (i) those for nondenaturing gels with ionic conditions that are roughly matched to the gel, and (ii) those with denaturing conditions, the solution often being deionized, and no attempt to match the gel ionic conditions. Dyes, detergents such as SDS or sodium sarcosine, EDTA (E buffer) to reduce intermolecular interactions mediated by divalent cations, dense and viscous additives such as ficoll, sucrose, and glycerol to allow sample settling in or on the gel, and RNase to reduce the size of RNA fragments that are frequently copurified with DNA are also included in these loading solutions. EDTA in the sample medium prevents DNA denaturation, which is otherwise aided by dyes with low ionic strength. These ingredients can add undesirable properties [5].

For electrophoresis of native, double-stranded DNA, several buffers are available. Tris-acetate and EDTA, Tris-borate (TBE), or Trisphosphate are included (TPE). Electrophoresis buffers are typically prepared as concentrated solutions that are kept at room temperature. All these buffers work well, and the choice is largely based on personal preference. TAE has the smallest buffering capacity of the three and will run out if electrophoresis is performed for an extended period [28].

3.4 DNA and DNA Sequencing

DNA samples are the most important sources for understanding the fundamental basis of life and disease. Thus, DNA samples collection, handling, and storage are critical for obtaining reliable data in disease association studies and developing disease treatments. Scientific progress in the field has relied heavily on advancing novel genetic technologies such as polymerase chain reaction, sequencing, and whole genome scanning [29].

Throughout its life, every living organism accumulates changes to its DNA material. These changes manifest themselves in a variety of ways: I replication errors; (ii) environmental changes (radiation, exogenous chemicals, toxins, hormones, or even diet); (iii) spontaneous DNA damage (depurination, depyrimidination, and deamination), resulting in the loss of a nucleotide base or a change in the base pairing properties of a base [30].

Modern sequencing gels have the advantage of running up to 96 samples in parallel, increasing the instrument's throughput. Slab-gel electrophoresis has numerous limitations. Because gel pouring is a manual process, there is always the risk of trapping air bubbles in the gel, which can reduce separation efficiency [26].

CE DNA sequencing is increasingly being reported to provide high reproducibility and significantly increased speed when compared to planar gels, with the elimination of problems associated with electrophoretic distortion and lane

tracking. Array capillary sequencing (ACE) enables simple handling of multiple sample changeovers as well as very high throughput, with sequence reads of more than 1000 bases completed in less than 80 minutes [27].

At the DNA level, sequence variations can be classified as I single nucleotide substitutions, also known as point mutations, (ii) single or multiple nucleotide deletions, and (iii) single or multiple nucleotide insertions, which can also have the appearance of duplications. A single nucleotide deletion or insertion can result in the translation of a faulty protein or none. This is because the sequence of three-nucleotide codons determines the amino acid sequence of a protein [30].

Detecting DNA mutations and natural variation has become essential to the characterization and diagnosis of human genetic diseases and a foundation for many aspects of molecular biology and medicine/genetics. Several recent reviews provide detailed descriptions of point mutation detection capillary electrophoresis applications. Most mutation detection methods can be divided into two categories: (A) methods for detecting known mutations and (B) methods for detecting unknown mutations [31].

Because of its ability to fully automate, capillary electrophoresis (CE) is quickly becoming the dominant technique in DNA sequencing centres. DNA separation is accomplished in a fused-silica capillary using CE. The small capillary tubes high surface area to volume ratio efficiently dissipates heat generated during electrophoresis, allowing for the use of higher electric fields, which reduces run time and improves DNA resolution [26].

4. Conclusions

Different molecules mobility varies depending on their average charge, size, and shape, as well as the properties of the solvent medium. Electrophoretic separation techniques are based on this effect. When a narrow zone of a molecule mixture is injected into a migration channel, and an electric field is applied, the mixture separates into different zones that migrate at different rates depending on their mobilities [18].

Using capillaries as the migration channel in electrophoresis holds the promise of putting electrophoretic separations on the same level as high-performance liquid chromatography in terms of instrumentation. Thus, the advantages of CE, such as rapid and automated analyses of multiple samples, accurate quantification, and improved reproducibility, enabled the study, mapping, and sequencing of entire genomes [18], [32]-[34].

CE is maturing as a clinical diagnostic tool. As new areas where CE outperforms traditional methodologies are discovered, it is expected that more and more CE-based assays will be introduced into clinical laboratories [35].

Further research and development in CE technology must concentrate on increasing the automation, throughput, reliability, and sensitivity of the CE analysis. Currently, the high reproducibility of CE runs allows for the analysis of genetic differences that compare difference profiles between runs, such as the detection of differences in gene expression in target tissues through analysis of specific gene expression (RT-PCR) or differential display scanning of

random genes. These methods employ multiple mutation analysis to construct a genetic landscape of genomes and throughput and highly reproducible electro chromatograms obtained between CE runs [27].

Although electrophoretic methods have been widely used to separate nucleic acids for several decades, numerous theoretical issues occurred that have important implications for the optimization of new and existing technologies [36]. Therefore, CE electrophoresis seems to be the most effective method for a high throughput along with a defined protocol for sampling, storage, and processing to have accurate results as well as minimal human involvement due to the error that come with it throughout the process.

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