

A Review on Capillary Electrophoresis Techniques in Forensic Analysis

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Abstract:

Electrophoresis is a technique that separates charged molecules based on their movement in an electric field. Capillary electrophoresis (CE) enhances this by using narrow capillaries filled with electrolyte solutions, offering faster, high-resolution separations with minimal sample requirements. Capillary electrophoresis includes several subtypes, each subtype is tailored to specific analyte types and separation needs, broadening CE's applicability across scientific fields. CE is widely used in forensic science for DNA profiling, drug analysis, and toxin detection, providing faster and more accurate results than traditional methods. Despite its advantages, CE has limitations such as sensitivity to sample matrix effects and the need for specialized equipment. However, ongoing advancements continue to expand its forensic and general applications.

Keywords: Capillary Electrophoresis, Forensic Analysis, Analytical, Drugs, DNA

1. Introduction

Electrophoresis is a powerful technique used to separate charged materials, especially biopolymers, based on their size and charge. The movement of molecules, colloids, and other particles is influenced by an electric field and occurs in a fluid medium [35]. This method is highly sensitive and preserves the molecular structure, making it valuable for various analyses in biotechnology. Different matrices, such as paper, capillaries, and polymer gels (e.g., agarose and polyacrylamide), are used in electrophoresis, each with unique properties suited to specific applications. The technique provides critical information on the identity, frequency, and physical characteristics of molecules in a sample, making it an essential tool in scientific research and analysis [55].)

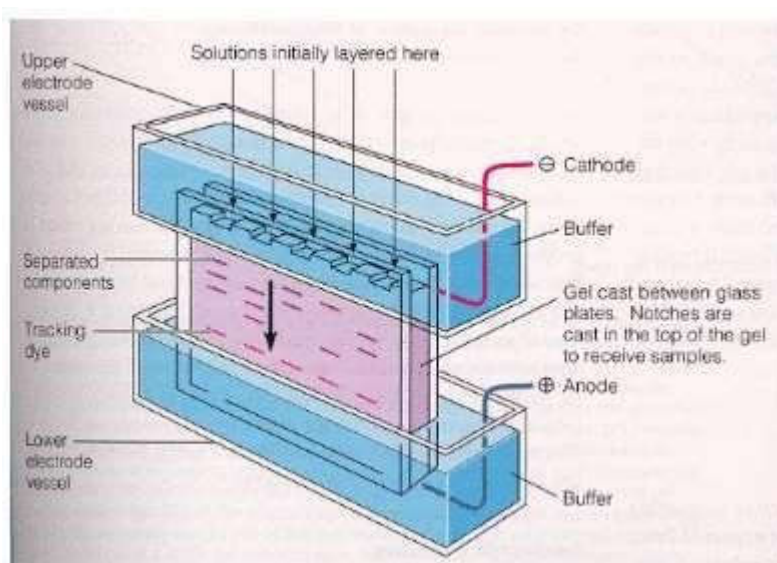


Figure 1. Diagrammatic Representation of The Electrophoresis Separation Technique

1.1. Principle

Electrophoresis uses an electric field to move charged molecules or particles through a matrix, with the strength of the field affecting their surface potential. The velocity of a particle is proportional to this potential, and over time, particles with different velocities will separate [1]. The cathode donates electrons while the anode absorbs them, completing the cycle [54]. Molecules with varying charges and sizes segregate due to differences in electrophoretic mobility and frictional forces. While some forms of electrophoresis separate molecules based on charge, others do so based on size [1, 2].

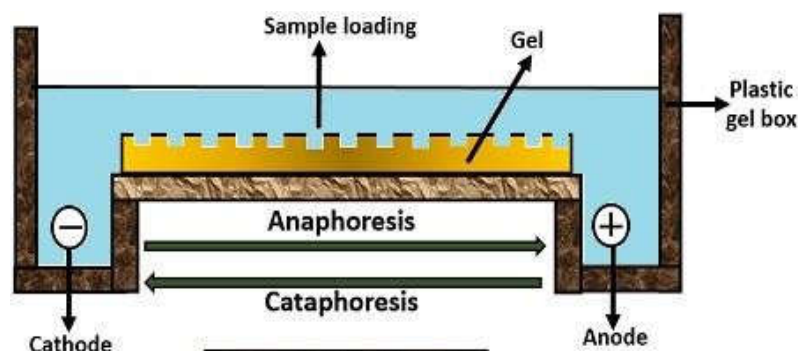


Figure 2. Electrophoresis Schematic Representation

Characteristics of CE

- Electrophoresis is performed on integrated pebbles with narrow diameters (25-75 nm inner diameter).
- The capillary is subjected to intense electric fields (ranging from 100 to 500 V/cm) and high voltages (between 10 and 30 kV).]
- High capillary resistance limits internal heating and current generation.
- Very good performance ($N > 10^5 - 10^6$) and fast analysis ($N = \text{No. of theoretical plates}$)
- A tiny sample volume (1 to 50 nl injected) is needed. (nl= Nano litre)
- Works with water-based media [25,29]

Because of its special qualities, which include high separation efficiency, quick analysis, minimal solvent and sample consumption, CE is a legitimate substitute for Liquid Chromatography (LC) in the identification of drugs of abuse. [42]

2. Capillary Electrophoresis (CE)

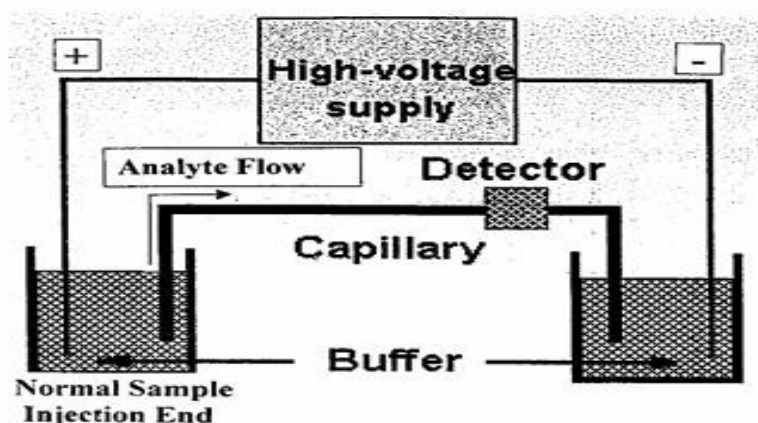


Figure 3. General Schematic of Capillary Electrophoresis Instrument

2.1 Introduction

Capillary Electrophoresis (CE) is an analytical method that separates charged particles based on their electrophoretic mobility using a system composed of a fused silica capillary, buffer chambers, a high-voltage power source, and a detector [37, 4, 16, and 34]. Modern CE systems may include auto samplers, temperature controls, programmable power, and computerized integration for enhanced performance. Known also as High-Performance Capillary Electrophoresis (HPCE), CE offers exceptional resolution, efficiency, and sensitivity, making it suitable for complex molecular analysis [38, 26].

2.2 Types of Capillary Electrophoresis-

a. Capillary Zone Electrophoresis (CZE):

The electrodes are mounted on both sides of high voltage performance. The electrodes contribute to the creation of an electric field that moves the sample from the anode to the cathode within the capillary. The capillaries are made of merged silica and sometimes coated with polyimide.

b. Capillary Gel Electrophoresis (CGE):

Capillary Gel Electrophoresis (CGE) is a separation technique in which invited molecules are separated into capillaries containing a porous gel matrix. CGE is a modification of traditional plate gel electrophoresis due to the advantages of capillary electrophoresis (CE). Among the many compounds that can be isolated by CG E are proteins, DNA, and RNA. Because the molecules have the same ratio from charge to mass, they have comparable electrophoretic mobility in the free solution.

To separate proteins according to their size, they must first be denatured and saturated with sodium sulfate (SDS). The dissolved material is subjected to an appropriate electrophoretic polymer that acts as a "molecular sieve" that allows for size separation.

c. Micellar Electrokinetic Chromatography (MEKC):

Micellar electrokinetic chromatography (MEKC) uses electrophoresis and chromatography. MEKC is the most commonly used type of CE in biopharmaceutical analysis. Since it was first discovered in 1984 by S. Terabe, it has been increasingly used in food, environmental and chemical analysis. Micellar Electrokinetic Chromatography (MEKC) Tensides are added to the electrolyte system at a concentration of micelles (CMC).

d. Capillary Isoelectric Focusing (CIEF):

High resolution" electrophoresis, called capillary isoelectrofocusing (CIEF), is used to separate amphiphilic molecules such as proteins and peptides based on isoelectric points (PI). CIEF operates with a pH gradient with a high pH value on the cathode and a low pH value on the anode. Trager Ampolytes is a class of hermaphrodites used to generate pH gradients. When current is applied to the Anpolites, the mixture separates in a tube.

e. Capillary Isotachophoresis (CITP):

Although the vessels are very large by today's standards (250-500 μm), the most popular capillary electrophoresis technique until 1981 was isotachophoresis (ITP). Similar to IEF, isotachophoresis is based on a non-uniform and zero electroosmotic flow. The idea is based on the different motions of different ions in an electric field.

In capillary isotachophoresis, a non-ionic solution of two electrolytes (called a leader and a terminator) is separated in a capillary tube of a certain size. The most mobile ions in the

separated mixture are found in the leader electrolyte, and the least mobile ions are found in the terminator electrolyte.

f. Capillary Electro Chromatography (CEC):

CEC is a hybrid technique that integrates the stationary phase of HPLC with electronic instrumentation. Generally speaking, the inner line of the CEC line is wider than the CE line, which makes MS detection easier. Alkyl silica is a stable phase in CEC, which can always be specifically modified or selectively retained.

Electrochromatography can produce very fine peaks that can cause plate numbers in the laboratory due to unexplained focusing effects in CEC. Neutral and anionic chemicals, as well as alkaline chemicals, have been shown to have this effect. Anticipating and managing [53].

2.3 Principles of Capillary Electrophoresis

There are two factors in capillary electrophoresis that determines separation. The first is the electrophoresis rate, which is the movement of the analyte within the capillaries under the influence of an electric field. The second is electroosmotic flow, which is the volumetric flow of the solution due to loading of the capillary walls [18].

3. Instrumentation

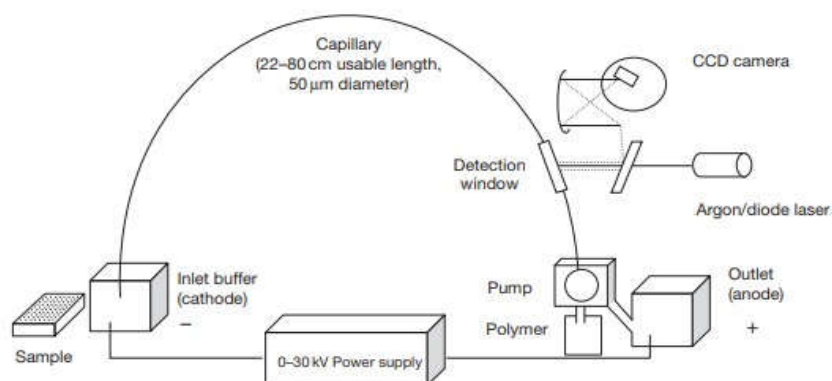


Figure 4. Schematic illustration of capillary electrophoresis system used for DNA analysis

3.1 Optimizing Electrophoresis Conditions

By optimizing electrophoresis conditions (running time, operating voltage, and operating temperature), data quality, operating sensitivity, and/or throughput can be significantly improved.

- **Power supply:** The core part of CE equipment is a high-voltage variable-current power supply. The power source generates the electric field needed for sample separation.
- **Buffer reservoirs:** Two identical buffer reservoirs containing anodic and cathodic solutions are needed for CE. These buffer reservoirs are crucial to the electric field's stability.

- **Electrodes:** The CE process requires an anode and a cathode, which must be placed in the cell buffer. The electrodes are connected to a power source and are an essential part of the process of generating electricity.
- **Capillary:** The capillaries used in CE are made up of combined silica and usually have diameters of less than 100 micrometres. The capillaries act as channels for rehearsals as they move towards separation.
- **Visual Display Window:** CE uses an optical display window that is properly aligned with the detector.
- **Injection system:** To load samples and buffers into the capillary during CE, a suitable injection system is required. For better precision, the injection might be automated. One of three techniques gravity pressure, vacuum, or electrokinetics is frequently used to inject samples.
- **Detector:** The amount of material flowing through the capillary at any particular time in CE can be tracked using mass spectroscopic, amperometric, or conductive fluorimetric absorption spectrophotometric detectors.
- **Thermostatic system:** Capillary environment regulation via a thermostat is crucial for the regulated temperature ensures the accuracy and dependability of the results.
- **Recorder:** For additional analysis, the data gathered during electrophoresis needs to be recorded. To enable later analysis, the data must be recorded.
- **Computer or adequate integration:** In addition, requires a suitable integrator or computer for digital data conversion. It is easier to assess and evaluate the digital data [54].

3.2 Sample Preparation and Injection

A sample must be prepared for analysis as soon as it is received. This step aims to stabilise the analyte, eliminate matrix particles that could obstruct the capillary, and increase the assay's specificity by eliminating interfering matrix chemicals while the analyte is concentrated [11]. Due to their ease of use and control, electrokinetic injections may frequently be the preferred technique [41]. In CE, injected sample amounts are naturally modest [17].

3.3 Sample Separation

In addition to the width of the injection site, capillaries, electrophoresis buffer, field strength, and polymers that help with separation are some of the other factors that affect DNA separation in the CE system. STR allele ladders is a useful tool for monitoring resolution.

a) The polymer separation matrix

Various sieving media are used in electrophoresis based on their physical properties, including chemical gels like polyacrylamide, physical gels like agarose, and entangled polymer solutions.

b) The buffer

Capillary electrophoretic separations depend on differences in electrophoretic mobility and are influenced by solvent flow. Soluble polymers play a key role in stabilizing DNA and improving current conduction and sample injection. Excessive heat or buffer degradation can reduce resolution, so regular buffer replacement is essential.

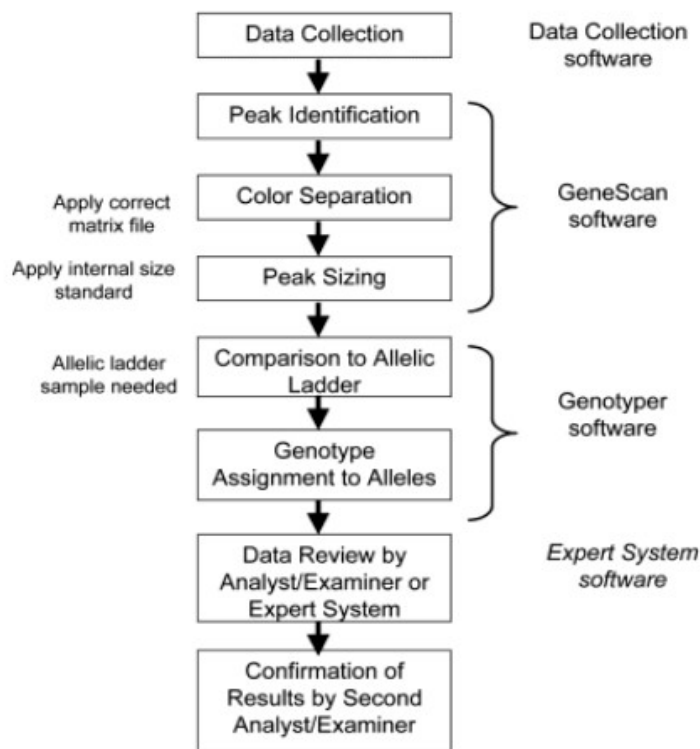


Figure 5. Sample interpretation and genotyping process for STR allele determination [11]

c) The capillary

Capillary performance is critical in CE, but uncoated columns can cause electroosmotic flow (EOF), disrupting DNA separation. To minimize EOF, capillaries are coated with charged or neutral polymers like POP-4 or POP-6, which also serve as separation matrices. Adsorption of DNA on capillary walls can reduce resolution and accuracy. Regular flushing and periodic replacement help maintain capillary efficiency. Manufacturers typically suggest replacing capillaries after around 100 uses to prevent performance decline [3].

3.4 Sample Detection

"Stacking" is a forthright method of enhancing the sensitivity in CE, which involves sample concentration on the capillary [22]. When an electric current passes through a capillary containing a sample with lower conductivity than the surrounding medium, a process known as sample stacking (or analyte concentration) takes place [32].

3.5 Sample Interpretation

a. Software used

The 310 data collection software performs three primary functions: it allows the creation of sample tables and short lists and displays sample names and operating procedures; it controls the electrophoresis operation; and the user enters the names of each pattern and the dye colours present in the sample.

The injection register controls the electrokinetic injection time and voltage, the electrophoresis voltage, the operating temperature, and (the sequence in which each sample is injected into the capillary).

b. Assessing resolution of DNA separations

Analysts can assess the performance of a CE system by calculating the resolution of the electropherogram. These resolution measurements are valuable for analyzing data and identifying changes that may affect electrophoresis. Troubleshooting can be incorporated into the testing process, alongside other tests, to evaluate system performance; for example, when analyzing conditions or evaluating system changes [3].

4. Applications

- I. A wide selection of dissolved dissolutions, including those that are strongly polar, thermally unstable, and/or non-fleet, can be separated with great efficiency and selectivity using the CE method [48].
- II. Capillary electrophoresis (CE) is a powerful analytical technique that has proven effective in analysing illegal substances in confiscated samples as well as in complex biological matrices, such as hair [19].
- III. The growing number of publications, books, and conferences focused on this approach reflects the widespread adoption of CE across various fields, including analytical chemistry, biochemistry, biotechnology, and pharmaceutical and chemical research [4].
- IV. In a homicide case, discovering alien biological material beneath a victim's or suspect's fingernails can be a useful piece of evidence in a criminal inquiry. [39].
- V. Often, multiple samples must be tested for the presence of chemical, toxic, or nontoxic residues. Identification of drugs and toxins in the body and tissues is necessary to determine the nature of the intoxication due to the crime and/or personal change [6].
- VI. Capillary electrophoresis is still a crucial research topic for the examination of medicines that have been confiscated. These solutes need to be analysed for intelligence and legal reasons [8].
- VII. This application is particularly important because sensitive spectroscopic techniques are not available to detect ammonium, monomethylamine, and other anions in pipe waste.
- VIII. The analysis of alkyl phosphonic acids with CZE was the last usage of anion analysis. Chemical warfare weapons degrade these compounds, which are separated using a pH 4-phenyl phosphonic acid buffer. Didodecyl dimethyl ammonium hydroxide was used by the authors as an EOF modifier, and conductivity or indirect photometric techniques were used to detect their samples [5].

4.1 Small-Molecular-Mass Molecules and Ions In Biological Samples

a. Drugs in biofluids and tissues

Capillary electrophoresis (CE) is widely used in drug monitoring, medical diagnostics, and toxicological testing. Techniques like CE-MS, immunoassays, and multi-wavelength detection support the analysis of drugs in biological samples. CE is effective for identifying both therapeutic and illicit drugs, including their metabolites. Methods like MEKC enable

analysis without extensive sample preparation. Drug metabolism studies often use selective reagents in CE buffers to explore stereoselectivity.

b. Endogenous small molecules and ions in biofluids and tissues

Sulfated cyclodextrins showed the highest solubility among tested types, enabling the detection of numerous urine components within a short timeframe using CE. Studies like Wessel et al. demonstrated CE's ability to identify purines, such as 2, 8-dihydroxyadenine, with and without SDS [5].

4.2 Analysis of Forensic DNA

As is known, analysis of DNA polymorphisms is the latest method for paternity testing and identification (for crime or mass murder). Forensic DNA polymorphism analysis is based on exponential amplification of individual loci by polymerase chain reaction (PCR) and subsequent comparisons of length or sequence changes.

Forensic genotyping is particularly suitable for short fragments (less than 1000 base pairs in length) or short fragments (up to 200 base pairs in length) with length differences as small as 1-2%.

Additionally, linear polyacrylamide has demonstrated excellent resolution of DNA fragments ranging from 51 to 23,130 base pairs within a concentration range of 4–10% [4].

4.3 Small-molecular-mass molecules and ions of forensic interest in non-biological samples

a. Drug seizures

Capillary zone electrophoresis (CZE), with or without additives like micelles or cyclodextrins, is widely used for analyzing seized drugs. Current research in CE covers areas like chiral separations, MEKC, NACE, CE-MS, and lab-on-a-chip technologies. CE has proven effective in detecting anorexia-related drugs such as methamphetamine and phentermine [5].

b. Analysis of explosives and gunshot residues

Capillary electrophoresis (CE) plays a growing role in analyzing gunshot and explosive residues. Beyond identifying suspects, it helps trace the origin and supply chains of explosives and ammunition. This analysis helps determine the type of explosive involved and supports criminal case development. Overall, CE is an emerging tool for the detection and management of various explosive materials and waste [40].

c. Ink analysis

To determine the writing instruments used in a crime, it is essential to verify and confirm the analysis of both printed and handwritten documents. Since different inks have different properties with certain characteristics, separate methods can be used to identify these properties [36].

d. Forensic drug analysis

A lot of research has been done on the use of CE in forensics to check urine for illegal substances such as morphine, benzodiazepines, barbiturates, opiates, and amphetamines [24].

Peptides, proteins, medications, and their metabolites, biological extracts, and environmental analysis are the main applications for CE [51].

Many different models of CE and HPLC have been reported previously. For example, the Weinberger and Lurie models separate raw materials, contaminants, defective products, and counterfeit products. The drugs CE originally used in screening for illegal drugs were psilocybin, morphine, phenobarbital, cocaine, methacryone, LSD, heroin, amphetamine, cocaine, methamphetamine, benzodiazepines, phencyclidine, cannabinoid s, and some of the degrees [40].

5. Drawbacks and overcomes

When creating STR samples, Formamide denaturates DNA. In place of formamide, water has also been effectively utilised to prepare STR samples for CE analysis. By using deionised water, formamide's health risks, expenses, and disposal issues can all be avoided.

Capillary lines play a crucial role in the separation efficiency of CE. In an uncoated capillary column, more liquid flows toward the negative electrode due to the charge retained on the silica surface, a phenomenon known as electroosmotic flow (EOF). This can complicate DNA separation, as the density of DNA molecules may vary between runs. To minimize EOF during DNA separation, capillaries and microchip channel walls are often dynamically coated or chemically modified with charged silanol groups. \

6. Conclusion

Diagnosing medical conditions through electrophoresis is most effective when carried out by an interprofessional team. The mobility of a molecule through an electric field will depend on the following factors: field strength, net charge on the molecule, size and shape of the molecule, ionic strength, and properties of the matrix through which the molecule migrates (e.g., viscosity, pore size). For the investigation of drugs of abuse, capillary electrophoresis proved effective, providing simultaneous determinations of different drugs without derivatization, with acceptable sensitivity. CE have proven to be advantageous and have thus been introduced in routine applications and clinical toxicology laboratories. It is clear that capillary electrophoresis offers significant operational advantages in terms of resolving power and analysis time in forensic analysis.

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