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Unit IV

Capillary Electrophoresis

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Capillary Electrophoresis

Capillary electrophoresis is a powerful analytical technique that separates and analyzes a wide range of molecules, from small ions to large biomolecules. This method utilizes the movement of charged particles under the influence of an electric field within a narrow capillary tube, allowing for highly efficient and sensitive separations.



Principles of Capillary Electrophoresis

1

Separation by Charge

Capillary electrophoresis separates analytes based on their charge-to-size ratio. Molecules with different charges and sizes will migrate at different velocities under the applied electric field, allowing for their separation and detection.

2

Electroosmotic Flow

The inner surface of the capillary carries a fixed charge, which generates an electroosmotic flow (EOF) of the buffer solution. This EOF can be used to control the direction and speed of analyte migration through the capillary.

3

High Efficiency

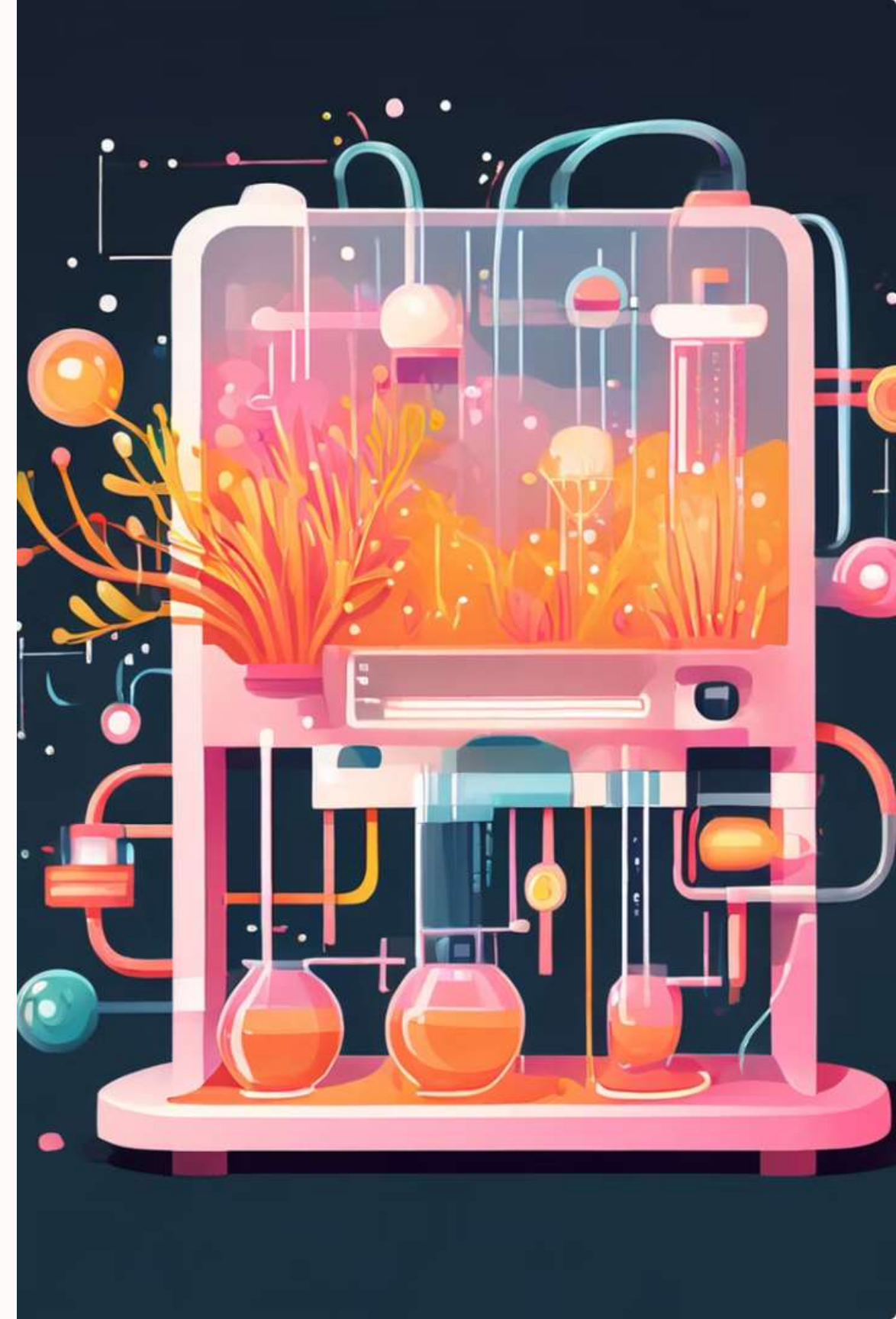
The small inner diameter of the capillary (typically 25-100 μm) and the high applied voltages (up to 30 kV) result in high separation efficiencies, allowing for the resolution of complex mixtures with minimal sample consumption.

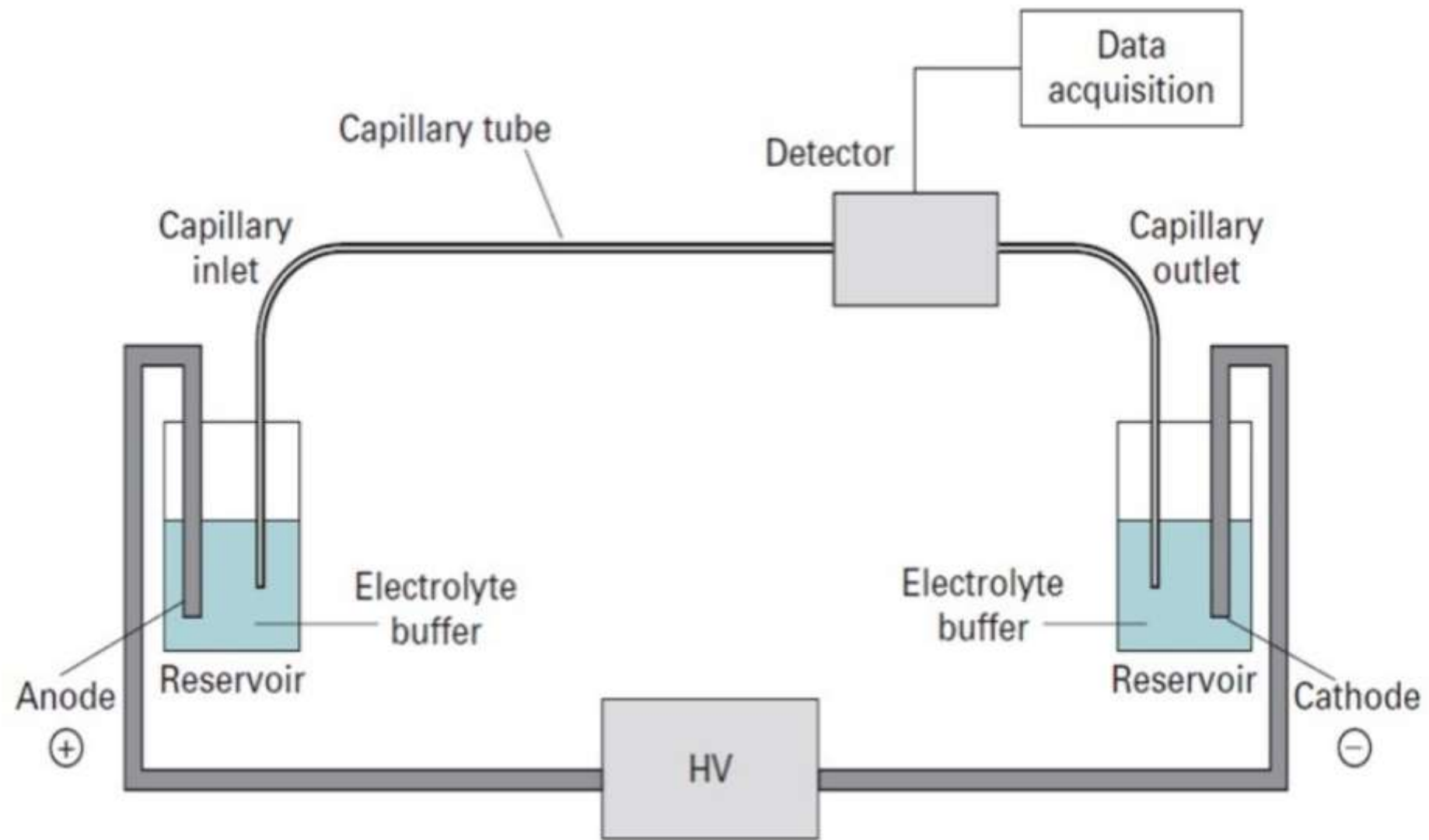


Instrumentation and Components

Capillary electrophoresis (CE) instruments consist of several key components that enable high-resolution separation and analysis of a wide range of analytes. The core of the system is the capillary, a narrow fused-silica tube typically 20-100 cm long and 50-100 μm in inner diameter, which serves as the separation column.

Sample introduction is achieved through a variety of injection methods, such as electrokinetic or pressure-driven injection, allowing precise control over sample volume and introduction. Specialized detectors, including UV-Vis absorbance, laser-induced fluorescence, and mass spectrometry, are used to monitor the separated analytes as they exit the capillary.





Capillary Design and Materials

Capillary Design

The capillary used in capillary electrophoresis is a narrow, cylindrical tube with a small internal diameter, typically ranging from 25 to 100 micrometers. This small size allows for efficient heat dissipation and high-voltage application, which are crucial for achieving effective separation of analytes.

Capillary Materials

The most commonly used materials for capillaries in CE are fused silica and polymers. Fused silica capillaries are known for their chemical inertness, mechanical strength, and thermal stability, making them well-suited for a wide range of applications. Polymer capillaries, such as those made from polyimide or polytetrafluoroethylene (PTFE), offer flexibility and durability, making them suitable for specific applications where silica capillaries may not be ideal.

Separation Mechanisms in Capillary Electrophoresis

1

Electrophoretic Mobility

The driving force behind analyte separation

2

Electroosmotic Flow

Controlling the bulk liquid movement

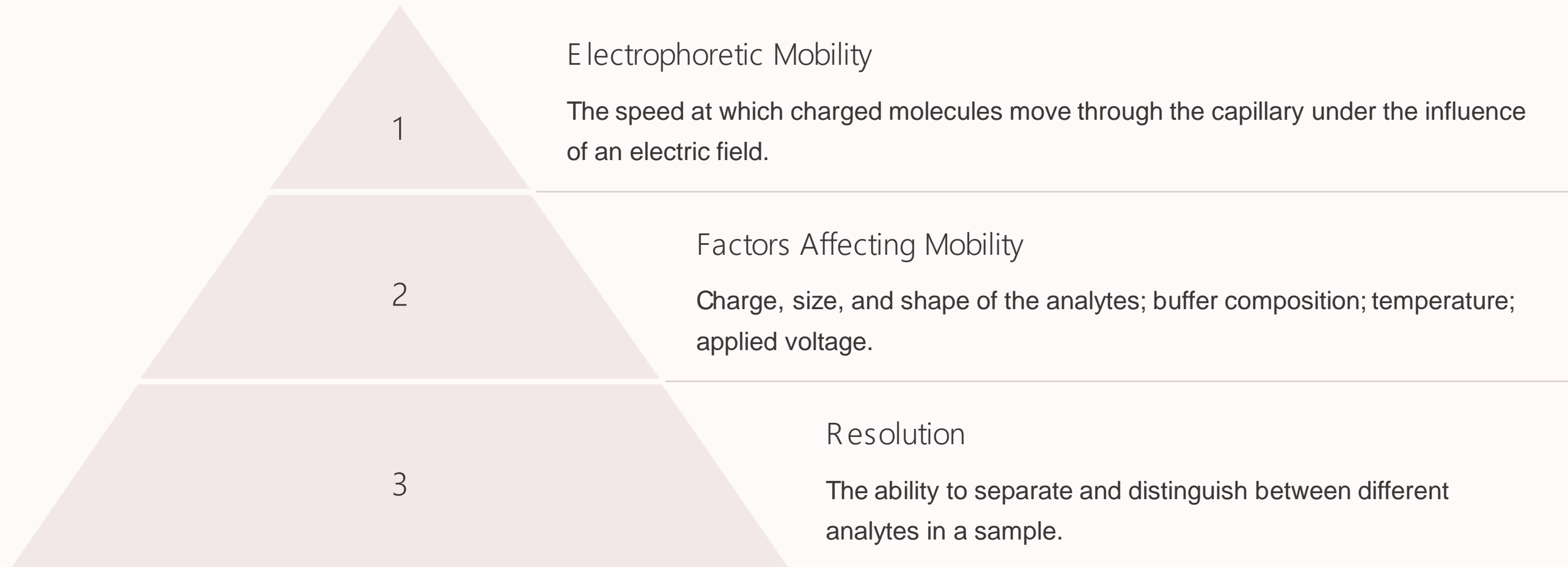
3

Analyte Interactions

Exploiting charge, size, and hydrophobicity

Capillary electrophoresis separates analytes based on their differential migration in an applied electric field. The key separation mechanisms include electrophoretic mobility, where charged analytes move at different velocities based on their charge-to-size ratio, and electroosmotic flow, which controls the bulk liquid movement. Additional interactions between the analytes and the capillary surface or buffer additives can further enhance the separation power.

Electrophoretic Mobility and Resolution



Electrophoretic mobility is a critical parameter in capillary electrophoresis, as it determines the speed and separation of analytes within the capillary. Factors such as the charge, size, and shape of the molecules, as well as the buffer composition, temperature, and applied voltage, all influence the mobility. Resolution, on the other hand, describes the ability to separate and distinguish between different analytes in a sample, and is a key measure of the performance and efficiency of the capillary electrophoresis system.



Sample Preparation and Injection

1

Sample Preparation

Proper sample preparation is crucial in capillary electrophoresis to ensure accurate and reproducible results. This may involve dilution, filtration, or other techniques to remove impurities and matrix interferences.

2

Sample Injection

Samples are introduced into the capillary through various injection methods, such as hydrodynamic injection using pressure or siphoning, or electrokinetic injection using an electric field. The injection parameters must be carefully controlled to maintain precise sample volumes and maintain separation efficiency.

3

Injection Modes

Common injection modes include gravity-fed injection, pressure-assisted injection, and electrokinetic injection. Each mode has its own advantages and considerations, such as sample loading volume, injection time, and the effect on analyte migration.



Buffers and Additives in Capillary Electrophoresis

Buffer Choice

Selecting the appropriate buffer is crucial in capillary electrophoresis. Buffers maintain the desired pH and ionic strength, which affects the separation efficiency and selectivity. Factors like buffer composition, pH, and ionic strength must be carefully optimized for each analysis.

Additives

Additives like organic solvents, surfactants, and cyclodextrins can be used to enhance separation, improve peak shape, and suppress unwanted interactions. These additives can modify the analyte charge, solubility, and hydrophobicity, leading to improved resolution and selectivity.

Compatibility

Buffers and additives must be compatible with the detection method, such as UV-Vis, fluorescence, or mass spectrometry. Careful selection and optimization of these components are essential to obtain high-quality, reproducible results in capillary electrophoresis.

Detection Methods in Capillary Electrophoresis

Capillary electrophoresis relies on sensitive detection methods to monitor the separation and identify the analytes of interest. The most commonly used detection techniques include UV-Vis absorbance, laser-induced fluorescence (LIF), and electrochemical detection.

UV-Vis absorbance detection is the simplest and most widely employed method, where the analytes are detected based on their ability to absorb light at specific wavelengths. LIF detection offers improved sensitivity, particularly for fluorescent or fluorescently labeled compounds.

Electrochemical detection, such as amperometric or conductivity detection, is suitable for the analysis of electroactive species and can provide excellent selectivity and sensitivity.





Applications of Capillary Electrophoresis



Biomolecular Analysis

Capillary electrophoresis is widely used for the separation and analysis of biomolecules such as DNA, RNA, proteins, and peptides. Its high resolution and sensitivity make it a powerful tool for genetic profiling, protein characterization, and drug discovery.



Environmental Analysis

Capillary electrophoresis is employed in the analysis of environmental samples, including water, soil, and air. It can detect and quantify a wide range of inorganic and organic pollutants, heavy metals, and trace elements with high accuracy and efficiency.



Pharmaceutical Analysis

Capillary electrophoresis plays a crucial role in the analysis of pharmaceutical compounds, including active ingredients, impurities, and metabolites. It helps ensure the quality, safety, and efficacy of drug products throughout the development and manufacturing process.

Capillary Electrophoresis in Biomolecular Analysis



Protein Separation

Capillary electrophoresis is a powerful tool for the separation and analysis of complex protein mixtures. By leveraging differences in charge-to-mass ratios, CE can efficiently resolve a wide range of protein isoforms, allowing researchers to study protein structure, function, and post-translational modifications with unparalleled precision.



DNA Sequencing

Capillary electrophoresis has become an indispensable technique in the field of genomics, enabling rapid and accurate DNA sequencing. The high-resolution separation capabilities of CE, combined with sensitive fluorescence detection, have revolutionized the way scientists analyze genetic information, paving the way for advancements in personalized medicine and evolutionary biology.



Metabolite Profiling

Capillary electrophoresis is also a valuable tool for metabolomic studies, allowing researchers to separate and identify a wide range of small molecules, such as amino acids, carbohydrates, and lipids. By providing a comprehensive snapshot of the metabolic state of a biological system, CE-based metabolite profiling has become instrumental in the understanding of disease mechanisms and the development of targeted therapies.



Capillary Electrophoresis in Environmental Analysis

1 Environmental Pollutants

Capillary electrophoresis has emerged as a powerful technique for the analysis of a wide range of environmental pollutants, including heavy metals, pesticides, and organic contaminants, enabling rapid and efficient separation and detection.

2 Water Quality Monitoring

CE can be used to analyze water samples from various sources, such as surface water, groundwater, and wastewater, providing valuable insights into the presence and levels of various ions, nutrients, and other water quality parameters.

3 Soil and Sediment Analysis

Capillary electrophoresis allows for the efficient analysis of soil and sediment samples, enabling the identification and quantification of contaminants, nutrients, and other important soil components, supporting environmental risk assessment and remediation efforts.



Capillary Electrophoresis in Pharmaceutical Analysis

Quality Control

Capillary electrophoresis is extensively used in the pharmaceutical industry for quality control of drug substances and drug products. It enables the separation and quantification of impurities, degradation products, and active pharmaceutical ingredients with high resolution and speed.

Chiral Separations

Many pharmaceutical compounds are chiral, and capillary electrophoresis is a powerful tool for the separation and determination of enantiomeric purity. It allows the analysis of chiral drug molecules with high efficiency and selectivity.

Protein and Peptide Analysis

Capillary electrophoresis is widely used for the analysis of proteins, peptides, and other biomolecules in the pharmaceutical field. It provides sensitive and high-resolution separations, enabling the characterization of protein therapeutics and monitoring of their quality.

Capillary Electrophoresis in Forensic Analysis

DNA Profiling

Capillary electrophoresis has become an indispensable tool in forensic DNA profiling. It enables the separation and analysis of DNA fragments with high resolution, allowing for the accurate identification of genetic markers used in criminal investigations and paternity testing.

Toxicology Screening

Capillary electrophoresis is used to detect and quantify a wide range of drugs, poisons, and other substances in biological samples such as blood, urine, and hair. This is crucial for toxicology investigations in forensic cases, providing critical evidence for criminal cases and medical examinations.

Trace Evidence Analysis

Capillary electrophoresis can be used to analyze minute traces of evidence, such as paint chips, fibers, and gunshot residue. The high separation efficiency and sensitivity of this technique allow for the identification and comparison of these tiny forensic samples, aiding in the reconstruction of crime scenes.

Explosives and Gunpowder Detection

Capillary electrophoresis is employed to detect and identify traces of explosives and gunpowder residues, which are crucial in the investigation of bombing and firearms-related incidents. This technique provides reliable and accurate data for forensic analysis in these high-stakes scenarios.



Advantages and Limitations of Capillary Electrophoresis



Speed and Efficiency

Capillary electrophoresis is known for its rapid separation capabilities, with analysis times often in the range of minutes. The high surface-to-volume ratio of the narrow capillaries allows for efficient heat dissipation, enabling the application of high voltages for fast separations.



High Sensitivity

Capillary electrophoresis is a highly sensitive technique, capable of detecting analytes at trace levels. The small sample volumes required, coupled with the use of sensitive detection methods, such as laser-induced fluorescence, make it suitable for analyzing a wide range of compounds, including proteins, nucleic acids, and small molecules.



Automation and Reproducibility

Capillary electrophoresis systems can be highly automated, allowing for unattended operation and improved reproducibility. The precise control of separation parameters, such as voltage, temperature, and buffer composition, contributes to the consistent and reliable performance of the technique.

Future Trends and Developments in Capillary Electrophoresis

As the field of capillary electrophoresis continues to evolve, researchers are exploring exciting new frontiers to push the boundaries of this powerful separation technique. From advancements in microfluidics to the integration of novel detection methods, the future of capillary electrophoresis holds great promise.

