A REVIEW ON CAPILLARY ELCTROPHORESIS WITH MASS SPECTROSCOPY

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ABSTRACT:

Capillary Electrophoresis coupled with Mass Spectrometry (CE-MS) is recognized as a highly effective analytical tool that integrates the precise separation ability of CE with the sensitive detection and quantification features of MS. This hybrid approach enables simultaneous acquisition of high-resolution separation and molecular mass data, offering both qualitative and quantitative insights in a single run. CE-MS provide unparalleled insights into complex biological mixtures and drug-related compounds. This hybrid method has made significant contributions to a variety of fields, including proteomics, genomics, metabolomics, and pharmaceutical development. This article aims to explore the fundamentals, advantages, challenges, and recent advancements in CE-MS, while also providing insight into its current and potential applications.

Keywords: Capillary Electrophoresis (CE), Mass Spectrometry (MS), CE-MS coupling, Electrospray Ionization (ESI), Proteomics, Metabolomics, Bioanalysis, Pharmaceutical analysis, Separation efficiency, Recent advancements.

1.GENERAL INTRODUCTION:

- Capillary Electrophoresis–Mass Spectrometry (CE-MS) is an integrated analytical technique that enables real-time separation of analytes based on variations in their electrophoretic mobilities, while also providing structural and mass-related information.
- By merging the high-resolution separation capabilities of CE with the sensitive detection and fragmentation analysis offered by MS, this combined approach enables comprehensive molecular characterization in a single run.
- Initially introduced in the late 1980s, CE-MS has since seen substantial advancements in both its instrumental design and its range of applications, particularly in the field of protein analysis.
- Pioneering work in the 1980s and 1990s laid the groundwork for its recognition as a valuable technique. The increasing demand for sensitive and selective protein analysis, driven by advancements in protein chemistry, proteomics, and biotechnology, has propelled CE-MS to the forefront of analytical techniques.
- CE-MS plays a crucial role across various applications, including **disease diagnostics**, **pharmaceutical development**, the study of **biomolecular interactions**, and **biomarker discovery.**
- Due to its capability to analyze a wide variety of biomolecules—including proteins, peptides, oligonucleotides, lipids, carbohydrates, and amines-CE-MS has become an essential tool in contemporary analytical chemistry.

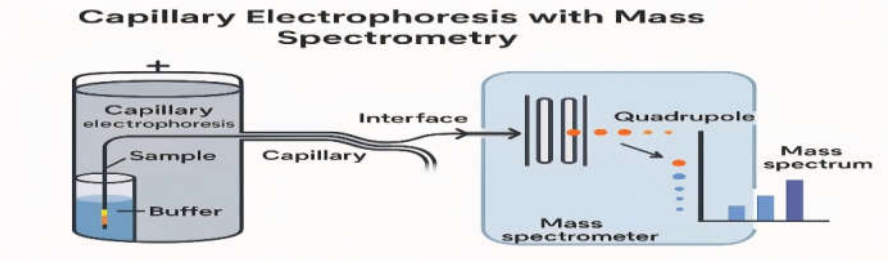

Figure 1. Capillary Electrophoresis with Mass Spectroscopy

The key features of CE-MS include:

- **High resolution:** Due to the narrow capillary, the electric field strength is uniformly distributed, resulting in high separation efficiency.
- **Small sample volume:** The capillaries used in CE require minimal sample amounts, making it ideal for rare or precious samples.

- **Versatility:** It is capable of separating a broad spectrum of analytes, ranging from proteins and peptides to nucleic acids, small molecules, and ionic specie

2.PRINCIPLE:

The core principle of CE-MS lies in the synergistic combination of two distinct analytical techniques.

Capillary Electrophoresis (CE): In Capillary Electrophoresis, analytes are separated according to their electrophoretic mobility, which depends on the ratio of their charge to size. This separation takes place within open tubular capillaries subjected to a high-voltage electric field. This fundamental aspect helps minimize peak broadening and sample carry-over issues commonly encountered with large biomolecules in techniques like liquid chromatography (LC). CE is a highly adaptable technique with numerous modes. Capillary Zone Electrophoresis (CZE) is the most widely applied mode, where analytes are differentiated based on their electrophoretic mobility. Another significant technique is Capillary Isoelectric Focusing (cIEF), which distinguishes proteoforms by their isoelectric points (pIs) through the establishment of a stable pH gradient inside the capillary, facilitated by ampholytes. Other modes include microchip CZE (mCZE), affinity CE (ACE), multi-segment injection (MSI)-CE, capillary isotachophoresis (cITP), and non-aqueous CE (NACE).
Mass Spectrometry (MS): Mass spectrometry serves as a highly selective and versatile detector for analytical separation techniques, offering detailed structural information about unknown compounds in complex mixtures with exceptional sensitivity and specificity.

The key benefit of integrating CE with MS lies in the ability to identify analytes through both their distinct migration behavior and their molecular weight or fragmentation profiles. Performing this separation prior to MS analysis is often crucial for accurate interpretation of spectral data. Achieving direct, online CE-MS coupling necessitates the use of specialized interface systems to facilitate the efficient transfer of analytes from the electrophoretic capillary to the mass spectrometer without compromising separation efficiency. These interfaces must also establish the electrical connection at the CE capillary terminus to complete the electrical circuits required for both CE separation and electrospray ionization (ESI).

3.INSTRUMENTATION:

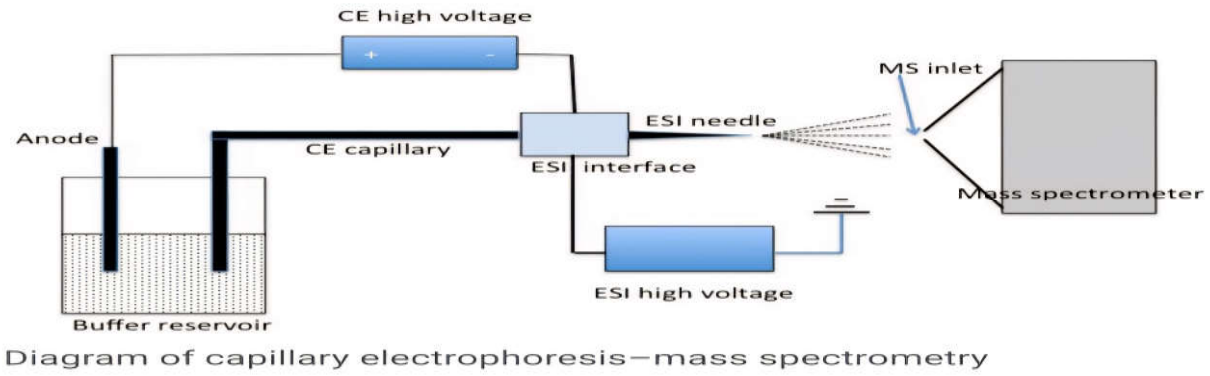

Figure 2: Instrumentation of CE-MS

A typical CE-MS instrumental arrangement involves a CE setup coupled to a mass spectrometer via an interface.

1.CE SETUP:
Fused silica (FS) capillaries are the most prevalent capillary material used in CE. They offer advantages such as cost-effectiveness, ease of surface modification, small internal diameter, excellent thermal stability, and high mechanical strength and flexibility. However, to prevent the adsorption of high molecular mass (Mr) molecules, particularly proteins, onto the inner capillary walls, **capillary coating** is often mandatory. Common coatings involve cationic polymers like polyethyleneimine (PEI) or neutral polymers such as polyvinyl alcohol (PVA) or linear polyacrylamide (LPA).

2.MS DETECTOR Mass spectrometers serve as powerful detectors for CE applications, offering exceptional sensitivity, high selectivity, and low detection limits. One of their standout capabilities is the ability to identify a vast array of biomolecules in a single analytical run.

Several types of mass spectrometers have been successfully integrated with CE systems, including:

- **Ion Trap (IT) mass spectrometers**, known for their ability to perform multi-stage MS experiments.
- **Fourier Transform Ion Cyclotron Resonance (FTICR) mass spectrometers**, valued for their superior sensitivity and resolving power.
- **Time-of-Flight (TOF) mass spectrometers**, which deliver fast scanning and enhanced resolution, particularly when equipped with a reflectron.
- **Triple quadrupole (QQQ)** mass spectrometers.

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3. <u>INTERFACING (SHEATH FLOW, SHEATHLESS)</u> Various ionization techniques have been utilized for interfacing CE with MS. Among them, **Electrospray Ionization (ESI)** is the most widely adopted method for biomolecular analysis due to its efficiency and compatibility with CE. Additional techniques include Ion Spray (ISP), a pneumatically assisted form of ESI, and Continuous-Flow Fast Atom Bombardment (CF-FAB). ESI is particularly advantageous as it facilitates the direct transfer of analytes from the liquid phase to the gas phase, enabling the generation of multiply charged ions-an essential feature for the analysis of large biomolecules such as peptide.

3.3.1<u>Sheath-Flow Interfaces:</u> Sheath-flow microelectrospray ionization (microESI) interfaces are among the most commonly used and well-established options for coupling CE with ESI-MS. They provide stable spray formation that is independent of the electro-osmotic flow (EOF) and offer greater flexibility in selecting the background electrolyte (BGE). Similar coaxial sheath-flow configurations are also used for ISP and CF-FAB systems Notable examples include the coaxial sheath-flow setup and the liquid junction interface, which are commonly employed in Ion Spray (ISP) and Continuous-Flow Fast Atom Bombardment (CF-FAB) techniques.

• **Advantages**: Stable spray formation, BGE flexibility

• **Drawbacks**: The use of sheath liquid can lead to considerable dilution of the sample, which can diminish separation efficiency and alter molecular conformation.

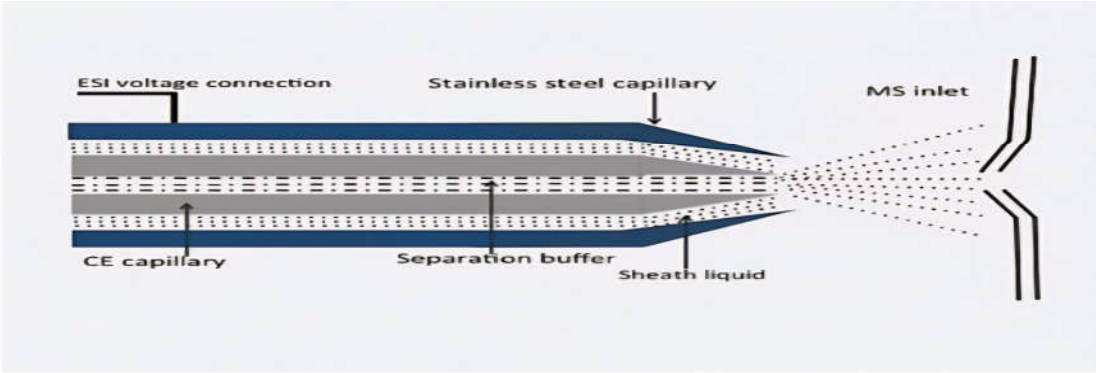

Figure 3: Sheath Flow Interface

3.3.2 <u>Sheathless Interfaces:</u> Sheathless interfaces offer superior sensitivity by eliminating the sheath liquid, thereby preserving the original sample concentration and enabling undiluted nanoflow for more efficient ionization. Various designs have been developed:

- One design featured an electropolished stainless-steel syringe needle with a tapered tip.
- Alternative configurations employed a **gold conductive coating** at the end of the CE capillary or placed a gold electrode within the capillary outlet to maintain electrical connectivity. The presence of gold in these setups enhances ESI spray stability, especially in aqueous environments.
- The high-sensitivity porous sprayer (HSPS) serves as a type of sheathless interface designed to enhance detection sensitivity commercialized by AB Sciex (CESI technique),

involves etching the capillary terminus to create a porous wall for electrical conductivity, increasing sensitivity by 10- to 100-fold.

- CMP has commercialized an **electrokinetically driven sheath-flow interface** for CE-MS, designed to enhance coupling efficiency and performance., commercialized by CMP Scientific (EMASS-II), reduces sample dilution with a low nL/min sheath liquid flow rate.
- The **microfluidic chip-based CE-MS interface**, commercialized by 908 Devices (Zip Chip technique), creates a sharp tip on a microfluidic chip for ESI.
- A **true sheathless metal-coated emitter interface** uses a tapered metal-coated capillary tip for improved robustness.
- The **spray capillary CE-MS technique** provides a new design for ultralow-volume sample handling by driving injection via ESI pressure drop.
- The vibrating sharp-edge spray ionization (VSSI) technique, which operates without the need for an external voltage, offers a novel approach to ion generation in CE-MS systems.

Limitations: Reduced stability, technical challenges in establishing reliable electrical contact, and higher costs associated with commercial setups.

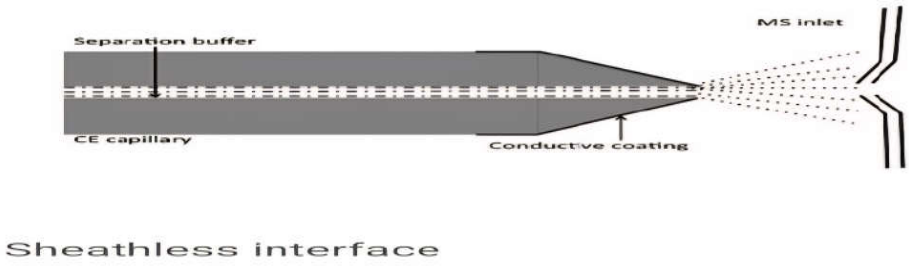

Figure 4: Sheathless Interface

3.3.3. **Liquid Junction Interfaces**: It involves a setup where the CE capillary and the mass spectrometer are connected via a junction filled with an electrically conductive liquid, allowing efficient transfer of analytes from the separation capillary to the MS inlet.

Advantages: Can compensate for different flow-rates required by the CE and MS systems. If properly constructed, they can provide high separation efficiencies with negligible peak broadening. They are relatively easy to set up and operate and allow for Larger-I.D. capillaries to improve sample load.

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Disadvantages: Can have a **large dead volume** and result in lower overall sensitivity compared to other interfaces if not optimally assembled.

Comparison of CE-MS Interfaces:

Interface Type	Main Ionizati	Key Advantage	Key Disadvantage	Sample Dilution	Sensitivity	Robustness	Flexibility (Buffer)

	on Method						
Sheath Flow	ESI, ISP, CF-FAB	Stable spray, wide buffer compatibility	Sample dilution, affected migration times	Significant	Moderate	High	High
Sheathless	ESI	Superior sensitivity (no dilution)	Stability, technical complexity, cost	None	High	Moderate	Limited (buffer must support ESI)
Liquid Junction	ISP, CF-FAB	Compensates flow rates, easy setup	Large dead volume, lower sensitivity	Present	Moderate-Low	Moderate	High

Table 1: Comparison of CE-MS Interfaces

3.3.4 Modern Commercial CE-MS Interfaces Numerous commercially available CE-MS interfaces have been developed to enhance sensitivity, operational stability, and ease of use.

- **CESI technique (SCIEX):** A sheathless configuration that employs a porous capillary tip as the electrospray ionization (ESI) emitter which avoids sample dilution and ensures high sensitivity for biomolecule measurement.
- **EMASS-II interface (CMP Scientific):** An electrokinetically driven sheath-flow interface for CE-MS has been developed to enhance analytical sensitivity.
- **ZipChip technique (908 Devices):** A microfluidic chip-based CE-MS interface that creates a sharp tip for ESI and generates sheath liquid flow via an electrokinetical pump.
- The **spray capillary** technique, developed by the Wu group, uses a porous capillary tip-based sheathless interface and pressure drop for extremely low volume (pL) sample injection, valuable for mass-limited omics applications.

3.3.4 Comparison of ESI and CF-FAB When comparing ESI and CF-FAB interfaces for CE-MS, ESI allows the use of **Larger-I.D. capillaries**, leading to greater loading capacity. It also provides **reduced background noise and higher sensitivity**. In contrast, CF-FAB typically results in greater peak broadening.

4.APPLICATIONS OF CE-MS:

CE-MS has found numerous applications across various scientific fields, with a majority in biological and biochemical studies.

4.1 PROTEOMICS: CE-MS serves as a versatile platform for protein analysis across various levels, including peptides in bottom-up proteomics, intact proteoforms in top-down approaches, and entire protein complexes in native proteomics.
Intact Proteins (Top-Down Proteomics, TDP): CE-MS-based TDP characterizes **proteoforms** in cells, tissues, and biological fluids to understand protein function and discover new protein biomarkers. It directly measures intact proteoforms, making it ideal for characterizing proteoforms with post-translational modifications (PTMs).

Applications in the analysis of:

- Identification and characterization of **protein biomarkers** present in biological fluids.
- **Biopharmaceutical products**, including monitoring quality control processes and drug development.
- **High Mr proteins** (e.g., antibodies, ~150 kDa) for investigating PTMs, particularly glycosylation.
- This includes monoclonal antibodies (e.g., cetuximab, bevacizumab, rituximab, trastuzumab), bispecific antibodies, Fc receptors, fusion proteins, and IgGs.
- **Low Mr therapeutic proteins** (e.g., insulin, insulin lispro, cobratide, 5000–7000 Mr) in drug formulations.
- **α-synuclein (α-syn)**, a critical protein associated with the pathology of Parkinson's disease.
- **Hemoglobin proteoforms** in clinical and veterinary samples.
- **Peptides (Bottom-Up Proteomics, BUP)**: CE-MS is indispensable for analyzing **peptide biomarkers.**
- The bottom-up approach typically involves enzymatic digestion of proteins into peptides, subsequently analyzed through separation, detection, and identification using CE-MS.
- **Urinary peptide biomarkers** are extensively studied for renal, urological, kidney, and cardiovascular diseases
- Urine is a rich source of stable and easily accessible biomarkers.
- **Tryptic digests** are characterized as model systems.
- Analysis of **synthetic mixtures of peptides** and their impurities
- Identification of **deoxynucleoside -polyaromatic hydrocarbon adducts.**
- **Glycopeptide glycoforms**, such as those from recombinant human erythropoietin (rhEPO).
- Characterization of **host cell proteins (HCPs)** in biotherapeutic products.
- **Native Proteomics**: This area directly characterizes **protein complexes.**
- CE-MS is highly promising for high-resolution separation and sensitive detection of intact protein complexes.

This technique has been applied in the:

- Investigation of **protein–protein interactions** as well as **protein–ligand binding studies.**
- **Protein-metal complexes** (e.g., carbonic Anhydrase-Zn complexes, hemoglobin tetramer).

- Characterization of **high-molecular-weight** protein assemblies up to mega Dalton levels, such as GroEL and ribosomes.
- **Phosphoproteomics**: CE-MS has been evaluated for **large-scale phosphoproteomics** to delineate protein phosphorylation sites, identifying thousands of phosphopeptides.

4.2 OLIGONUCLEOTIDES: CE-MS is gaining recognition as a promising technique for the detailed characterization of... oligonucleotides, which include small RNA molecules like **microRNAs (miRNAs)**. miRNAs regulate gene expression and are potential biomarkers for diseases such as cancer and neurodegenerative disorders.

Applications include:

- Analysis of **phosphodiester and phosphorothioate oligonucleotides**.
- Rapid analysis of **short oligonucleotides** (18 to 25 nucleotides) using mCZE-MS via a ZipChip interface.

4.3 METABOLOMICS: The use of CE-MS is expanding rapidly in the analytical investigation of metabolites and drugs in biological and environmental samples.

- **Lipids**: While less explored, CE-MS with NACE has been used for **lipid profiling** (e.g., serum from nonalcoholic steatohepatitis patients).
- **Amines**: Analysis of **polyamines** (e.g., in saliva for colorectal cancer biomarkers) and **biogenic amines** (e.g., in human urine for inflammatory bowel diseases).
- **Phosphoderivatives**: Determination of **inositol phosphates and pyrophosphates** in mammalian cells, crucial as second messengers in eukaryotic signaling processes.
- **Contaminant Metabolites**: Detection of 1-hydroxypyrene glucuronide in urine.
- **Neurotransmitters**: Determination of **serotonin** in urine.
- **Drugs and Drug Metabolism**: Analysis of low-molecular-weight pharmaceuticals such as **sulfonamides** and **benzodiazepines**
- It's used in studies of Chinese herbal medicine. It's also applied
- to facilitate the identification of **metabolites of lysergic acid diethylamide (LSD)** and for the analysis of **chiral drug mixtures**. Recent studies include the analysis of drugs like imatinib and β-lactam antibiotics in blood, urine, and plasma samples.
- **Environmental Analysis**: Analysis of **ionic textile dyes**, **quaternary ammonium herbicides**, **antibiotics in shellfish extracts**, **paralytic shellfish toxins**, and degradation products of **chemical warfare agents.**
- It can also be used for detecting **inorganic ions** and analyzing used aircraft engine oil.

4.4 BIOLOGICAL SCIENCES (PROTEINS, PEPTIDES, ENZYMATIC DIGESTS): Most CE-MS applications have been concentrated in the fields of biology and biochemistry.

- **Intact Proteins:** CE-MS is used for characterizing protein biomarkers in biological fluids and biopharmaceutical products
- **Low Molecular Mass (Mr) Proteins:** Therapeutic low Mr proteins (5,000-7,000 Mr) like insulin and cobratide have been analyzed in drug formulations using CZE-MS.

- **High Mr Proteins (Antibodies):** A major focus is on high Mr proteins (e.g., antibodies, around 150,000 Mr), primarily for investigating post-translational modifications (PTMs), especially glycosylation.
- Monoclonal antibodies (e.g., cetuximab, bevacizumab), bispecific antibodies, and IgGs are characterized using various CE modes coupled to high-resolution MS.
- **Peptides:** Widely characterized as model systems and for biomarker discovery.
- **Biomarkers:** Urinary peptide biomarkers are studied for renal, urological, kidney, and cardiovascular diseases.
- Peptides in urine can provide insights into specific diseases and PTMs.
- **Tryptic Digests:** Tryptic digests of proteins (e.g., bovine, equine, and tuna cytochrome c) are characterized to understand protein structure and modifications.
- **High-Throughput Analysis:** Automated spray capillary CE-MS
- Advanced platforms have been created to enable consistent microsampling and analysis of biological samples in the picoliter to nanoliter range, making them ideal for high-throughput proteomic studies.
- **Therapeutic Peptides:** CE-MS is used for determining synthetic mixtures of peptides, peptide standards, and peptide impurities in pharmaceutical development.
- **Enzymatic Digests:** CE-MS is used for the characterization of protein digests, including those from immobilized enzyme micro reactors.

4.5 PHARMACEUTICAL AND DRUG METABOLISM: CE-MS is increasingly being utilized for the analysis of pharmaceutical compounds, therapeutic agents, and metabolites of xenobiotics.

- **Drug Analysis:** It is employed for the quantification and identification of low-molecular-weight drug compounds, such as sulfonamides, benzodiazepines), macrolide antibiotics, and components in Chinese herbal medicines.
- Quantitative analysis is performed using internal standards.
- **Metabolites:** Demonstrated for the identification of drug metabolites (e.g., Mifentidine, lysergic acid diethylamide (LSD) metabolites) in biological incubations
- CE-ISP-MS serves as a complementary separation tool for identifying unknown metabolites.
- **Biopharmaceutical Characterization:** CE-MS is essential for quality control and characterization of biopharmaceutical formulations, including recombinant human insulin, growth hormone, and hemoglobin.
- It aids in studying glycosylation, impurity profiling, and characterizing metal-protein complexes.
- **Environmental Analysis:** CE-MS has applications in environmental analysis, such as the determination of antibiotics in shellfish extracts, β-agonists in calf urine, and fumonisins in corn samples.

4.6 OTHER APPLICATIONS:

- Studies have demonstrated the use of CE-MS for analyzing **paralytic shellfish toxins** in marine samples and for detecting textile **dyes.**
- CE-ESI-MS also plays a role in assessing the purity of laser dyes.

- **Chemical Warfare Agents:** The technique has been applied in identifying breakdown products of chemical warfare substances, including substituted organophosphorus acids.
- **Inorganic Ions:** Detection of inorganic ions, including high levels of lead and other metal ions (chromium, nickel) in used aircraft engine oil extracts
- **Metabolites:** Analysis of polyamines, biogenic amines, and 1-hydroxypyrene glucuronide in various biological samples.
- Inositol phosphates and pyrophosphates in mammalian cells are also analyzed due to their role as second messengers in eukaryotic signaling processes.

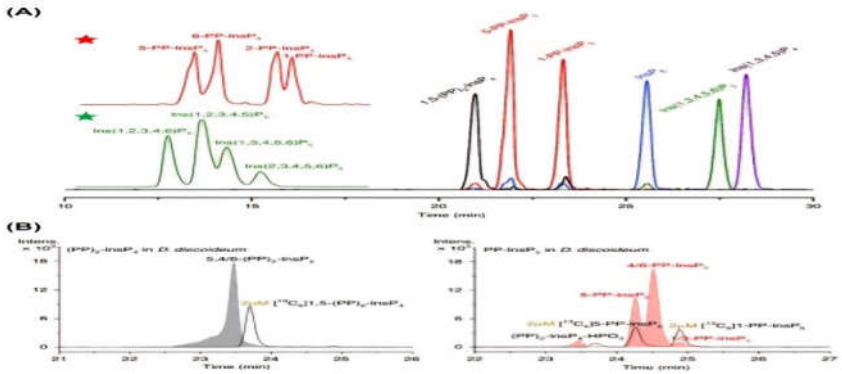

Figure 5:(A) Separation of inositol phosphates, pyrophosphates, and its regioisomers (star-enlarged insets) by CZE—ES–-QTOF. (B) inositol pyrophosphates regioisomers in Dictyostelium discoideum extracts.

5.ADVANTAGES AND LIMITATIONS OF CE-MS:

BENEFITS OF CE-MS:

- **Exceptional Separation Efficiency and Resolution**: Offers extremely high resolving power, separating analytes by differences in electrophoretic mobilities and structural information.

- **High Peak Capacity**: Capable of achieving high peak capacities for complex mixtures, such as 300 for *Escherichia coli* cell lysate.

- **High Sensitivity and Low Limits of Detection (LODs)**: MS provides high sensitivity and selectivity.

- CE-MS can achieve high sensitivity for large biomolecules as a result of its excellent separation capability and minimal sample requirements loss. ESI-MS also gives reduced background noise. For example, 1 zmol of angiotensin has been detected.

- **Low Sample and Reagent Consumption**: Offers economy of sample size, solvents, and reagents.

- **High Speed/Short Analysis Time**: Provides high speed and short analysis times.

- **Reduced Ion Suppression**: Less ion suppression compared to direct MS analysis**.**
- **Instrumental Simplicity and Automation**: Features instrumental simplicity and full automation.
- **Direct Proteoform Measurement**: Ideal for directly measuring intact proteoforms.
- **Complementarity to LC-MS**: CE-MS and RPLC-MS are orthogonal separation techniques and complement each other for identifying different pools of peptides and proteoforms, leading to improved proteome coverage when combine
- **Versatile Operating Conditions**: Can be operated under both denaturing and native conditions for analysis of proteoforms or protein complexes, using the same separation capillary by simply changing buffers

LIMITATIONS OF CE-MS:

- **Poor Concentration Sensitivity**: One of the main drawbacks is its comparatively low sensitivity for detecting dilute analytes.
- Current instrumentation often has detection limits exceeds practical limits for many real-world analytical scenarios.
- **Limited Sample Volume/Loadability**: CE has a restricted sample volume that can be processed without affecting the quality of separation.
- **Analyte Migration Time Fluctuation**: migration times can vary due to environmental temperature fluctuations.
- **Electrolyte Selection Limitations**: Constraints in selecting electrolytes for optimal performance.
- **Reproducibility and Ruggedness**: Still does not match the performance level of LC-MS in certain applications. in terms of reproducibility and ruggedness.
- **Protein Adsorption**: High molecular mass molecules, particularly proteins, can adsorb onto the inner capillary walls, causing band broadening and peak tailing.
- **Sheath Liquid Dilution**: Sheath-flow interfaces cause sample dilution, which can reduce sensitivity.
- **Sheathless Interface Challenges**: Sheathless interfaces face limitations related to reduced stability, technical challenges in establishing reliable electrical contact, and higher associated costs, hindering broader routine application.
- **Ampholyte Interference in cIEF-MS**: The high concentration of carrier ampholytes in cIEF-MS can lead to significant signal suppression of proteoforms, limiting its sensitivity
- **Routine Acceptance**: Despite advancements, CE-MS has yet to become a standard choice for routine analytical workflows.
- **Validation Gap**: Many studies still rely on small sample sizes and lack comprehensive validation steps, creating a gap between discovery and practical implementation

6.RECENT ADVANCEMENTS (2020–2024):

The period from 2020 to 2024 has seen significant strides in CE-MS, particularly in interface design, sample preconcentration, and automation, enhancing its applicability in biomolecular research.

Interface Development:

- **Commercialized Interfaces**: Advanced interfaces focused on improved sensitivity, robustness, and user-friendliness have been developed and commercialized
  These include:
- The **sheathless interface** incorporating a porous capillary tip—originally developed by Moini and later commercialized by SCIEX as CESI—eliminates sample dilution, thereby maximizing sensitivity.
- Similarly, the **electrokinetically driven sheath-flow CE-MS interface**, pioneered by the Dovichi group and commercialized as EMASS-II by CMP Scientific, represents another advancement aimed at improving coupling efficiency and performance.
- The **microfluidic chip-based CE-MS interface** (Ramsey group, commercialized as ZipChip by 908 Devices) integrates CE and ESI on a chip, offering shorter analytical times and minimal sample consumption.

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Novel Designs:

- A fully sheathless interface featuring a **metal-coated emitter**, developed by the Tang group, employs a tapered, metal-coated capillary tip to enhance durability.
- **The spray capillary CE-MS method,** introduced by the Wu group, allows for precise handling of ultra-small sample volumes by taking advantage of the pressure differential created during electrospray ionization.
- Additionally, a **voltage-free vibrating sharp-edge spray ionization (VSSI)** interface has demonstrated strong potential for protein analysis.
- To further improve robustness, innovative multi-capillary configurations for sheath liquid interfaces have been proposed.
- A novel design using a CO_2 laser-ablated opening has also emerged as a sheathless CE-ESI-MS interface, representing a significant step forward in interface development.

Online Sample Preconcentration Techniques:

To address the challenge of limited sample loading capacity, several on-line preconcentration techniques have been developed and refined over time.:

- **Solid-Phase Extraction (SPE)-CE-MS**: This technique offers a practical solution to reduce LODs and minimize sample handling.
- **Aptamer Affinity SPE–CE–MS (AA-SPE–CE–MS)** has been introduced for purification, preconcentration, separation, and characterization of protein biomarkers like α-synuclein, achieving 100 times lower LODs.

- This has been extended to **on-line aptamer affinity SPE and immobilized enzyme microreactor CE–MS (AA-SPE–IMER–CE–MS)** for sensitive targeted bottom-up analysis.
- **TiO2-SPE–CE–MS** is used for analyzing glycopeptide glycoforms, demonstrating LODsup to 100-fold lower compared to CE–MS.
- **Reversed-Phase Solid-Phase Micro extraction (RP-SPME)-CZE-MS** is a platform designed to facilitate bottom-up proteomic analysis of individual human cells, allowing for the injection of nearly 40% of the available peptide content.
- **Dynamic pH Junction**: Continues to be a highly efficient sample stacking technique, allowing microliter-scale sample loading. A modified approach known as the dynamic pH barrage junction has been introduced for the accurate quantification of amino acids, peptides, and digested monoclonal antibodies.
- **Polarity Switching Transient Isotachophoresis (PS-tCITP):** This technique enhances both sample loading efficiency and separation resolution.

Capillary Coatings:

Ongoing research focuses on developing more robust and protein-resistant capillary coatings to minimize non-specific adsorption:

- **Linear polyacrylamide (LPA) coating** remains widely used for CE-MS-based TDP, with recent studies focusing on its long-term reproducibility and efficient cleanup procedures to address protein adsorption.
- New **neutral and hydrophilic polymers** (e.g., carbohydrate-based neutral coating) are being explored.
- **PEI-coated capillaries** have been used for peptide analysis, demonstrating good separations and high reproducibility.

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Automation:

Increased automation of CE-MS systems is a significant trend:

- An **automated spray capillary CE–MS platform** has been developed for high-throughput proteomics, offering efficient and reproducible microsampling of picoliter and nanoliter-volume biological samples.
- **Automated cIEF-MS systems** have been designed to enable detailed characterization of charge variants in monoclonal antibodies allowing for fully automated focusing and mobilization steps, which were previously manual.

Diverse Applications:

Recent years have seen a broadening of CE-MS applications:

- On-capillary cell lysis facilitates top-down proteomic profiling at the single-cell level in mammalian systems.
- SPME-aided CZE-MS has demonstrated feasibility for single human cell proteomics.
- **Native Proteomics**: Proteome-scale measurement of protein complexes, including mega Dalton-level protein complexes like ribosomes, has been demonstrated.

- **Glycosylation Studies**: Detailed analysis of N-glycosylation of proteins and glycopeptides.
- **Drug Analysis**: Analysis of various drugs and their metabolites in complex biological matrices
- **Lipidomics**: Non-targeted serum lipid profiling, showcasing its potential in lipidomics and patient differentiation.

7.CONCLUSION AND FUTURE PERSPECTIVES:

CE-MS stands as a **flexible and powerful analytical technique** in biomolecular research, offering distinct advantages for the separation and analysis of a broad spectrum of biomolecules such as proteins, peptides, oligonucleotides, and various metabolites, oligonucleotides, and other metabolites. It effectively combines **fast separation, low reagent and sample consumption, high selectivity, and reduced ion suppression**, providing superior sensitivity compared to direct MS analysis. CE-MS particularly excels in handling intricate molecular profiles and plays a pivotal role in advancing diagnostic methods and facilitating biomarker discovery. Despite its significant progress, achieving the full potential of CE-MS requires addressing current challenges and continuing technological and methodological improvements.
Future developments are anticipated in several key areas:

- **Large-Scale Validation and Standardization**: There is a critical need for **larger-scale studies and standardization of methodologies** to bridge the gap between discovery and practical implementation in routine analysis.

- **Interface Refinement**: Future efforts will focus focused on developing more efficient and robust interfaces that enhance ionization efficiency, including robust and durable sheathless designs to avoid sheath flows.

- **Enhanced Automation**: Further advancements in automation are crucial to improve user-friendliness and throughput.

- **Integrated Preconcentration Techniques**: Continued integration of **chromatographic preconcentration techniques**, such as SPE-CE, will be essential for improving detection limits, especially for low-abundance analytes.

- **Expanded Analyte Range**: Research will continue to expand the range of detectable analytes and explore applications in emerging areas like **high-throughput screening and personalized medicine.**

- **Multidimensional Separations**: **Coupling LC prefractionation** (e.g., size exclusion chromatography) with CE-MS is essential to achieve **high proteome coverage** in complex biological systems.

- The advancement of multidimensional methods will be critical for the rising proteomics/peptidomics markets.

- **Improved Fragmentation**: To enhance the quality of proteoform characterization, more work is needed to boost the **backbone cleavage coverage of proteoforms**, especially large ones, by integrating electron- or photon-based fragmentation techniques (e.g., electron transfer dissociation or ultraviolet photodissociation).

- **Native Proteomics**: CE-MS, particularly cIEF-MS, is poised to be a **primary technique for native proteomics** due to its unique features precise separation combined with highly sensitive detection of protein complexes.

- However, improvements in sample loading capacity and capillary coatings for native separations are needed.

- **Addressing cIEF-MS Sensitivity**: Efforts are required to **reduce the interference of ampholytes** in cIEF-MS while maintaining high separation resolution for improved sensitivity.

- **Cross-Laboratory Studies**: More **cross-laboratory studies** on CE-MS-based applications will facilitate broader adoption of the technique.

- The advancement of **microchip-based CE-MS** technology systems will remain a key area of interest, contributing to miniaturization, lower costs, and increased efficiency

8.REFERENCES:

1. Loo JA, Udseth HR, Smith RD (June 1989). "Peptide and protein analysis by electrospray ionization–mass spectrometry and capillary electrophoresis–mass spectrometry"(https://zen odo.org/record/1253788). Analytical Biochemistry. 179 (2): 404–412. doi:10.1016/0003- 2697(89)90153-X(https://doi.org/10.1016%2F0003-2697%2889%2990153-X). PMID 2774189 (https://pubmed.ncbi.nlm.nih.gov/2774189).
2. Cai J, Henion J (1995). "Capillary electrophoresis–mass spectrometry" (https://doi.org/10.101 6%2F0021-9673%2894%2901178-h). Journal of Chromatography A. 703 (1–2): 667–692. doi:10.1016/0021-9673(94)01178-h (https://doi.org/10.1016%2F0021-9673%2894%2901178- h).
3. Maxwell EJ, Chen DD (October 2008). "Twenty years of interface development for capillary electrophoresis-electrospray ionization–mass spectrometry". Analytics Chimica

Acta. 627 (1): 25–33. doi: 10.1016/j.aca.2008.06.034 (https://doi.org/10.1016%2Fj.aca.2008.06.034). PMID 18790125 (https://pubmed.ncbi.nlm.nih.gov/18790125).

4. Zhang H, Caprioli RM (September 1996). "Capillary electrophoresis combined with matrixassisted laser desorption/ionization mass spectrometry; continuous sample deposition on a matrix-preloaded membrane target". Journal of Mass Spectrometry. 31 (9): 1039–1046. Bibcode:1996JMSp...31.1039Z (https://ui.adsabs.harvard.edu/abs/1996JMSp...31.1039Z). doi:10.1002/(SICI)1096-9888(199609)31:93.0.CO;2-F (https://doi.org/10. 1002%2F%28SICI%291096-9888%28199609%2931%3A9%3C1039%3A%3AAID-JMS398%3E3. 0.CO%3B2-F). PMID 8831154 (https://pubmed.ncbi.nlm.nih.gov/8831154).
5. Metzger J, Schanstra JP, Mischak H (March 2009). "Capillary electrophoresis–mass spectrometry in urinary proteome analysis: current applications and future developments". Analytical and Bioanalytical Chemistry. 393 (5): 1431–1442. doi:10.1007/s00216-008-2309-0 (h ttps://doi.org/10.1007%2Fs00216-008-2309-0). PMID 18704377 (https://pubmed.ncbi.nlm.ni h.gov/18704377). S2CID 23483338 (https://api.semanticscholar.org/CorpusID:23483338).
6. Ohnesorge J, Neusüss C, Wätzig H (November 2005). "Quantitation in capillary electrophoresis–mass spectrometry" (https://doi.org/10.1002%2Felps.200500398). Electrophoresis. 26 (21): 3973–3987. doi:10.1002/elps.200500398 (https://doi.org/10.1002%2 Felps.200500398). PMID 16252322 (https://pubmed.ncbi.nlm.nih.gov/16252322). S2CID 6897545 (https://api.semanticscholar.org/CorpusID:6897545).
7. Kolch W, Neusüss C, Pelzing M, Mischak H (2005). "Capillary electrophoresis–mass spectrometry as a powerful tool in clinical diagnosis and biomarker discovery" (https://doi.org/ 10.1002%2Fmas.20051). Mass Spectrometry Reviews. 24 (6): 959–977. Bibcode:2005MSRv...24...959K (https://ui.adsabs.harvard.edu/abs/2005MSRv...24...959K). doi:10.1002/mas.20051 (https://doi.org/10.1002%2Fmas.20051). PMID 15747373 (https://pubmed.ncbi.nlm.nih.gov/15747373) ...
8. Dakna M, He Z, Yu WC, Mischak H, Kolch W (May 2009). "Technical, bioinformatical and statistical aspects of liquid chromatography–mass spectrometry (LC–MS) and capillary electrophoresis–mass spectrometry (CE–MS) based clinical proteomics: a critical
9. assessment". Journal of Chromatography. B, Analytical Technologies in the Biomedical and Life Sciences. 877 (13): 1250–1258. doi: 10.1016/j.jchromb.2008.10.048 (https://doi.org/10.1016% 2Fj.jchromb.2008.10.048). PMID 19010091 (https://pubmed.ncbi.nlm.nih.gov/19010091).
10. Schmitt-Kopplin, P., Frommberger, M. (2003). Capillary electrophoresis – mass spectrometry: 15 years of developments and applications. Electrophoresis, 24, 3837-3867.
11. Breadmore MC (August 2009). "Electrokinetic and hydrodynamic injection: making the right choice for capillary electrophoresis". Bioanalysis. 1 (5): 889–894. doi:10.4155/bio.09.73 (http s://doi.org/10.4155%2Fbio.09.73). PMID 21083060 (https://pubmed.ncbi.nlm.nih.gov/21083 060).
12. Schaeper JP, Sepaniak MJ (April 2000). "Parameters affecting reproducibility in capillary electrophoresis". Electrophoresis. 21 (7): 1421–1429. doi:10.1002/(SICI)1522-2683(20000401)21:73.0.CO;2-7 (https://doi.org/10.1002%2F%28SICI%2 91522-

2683%2820000401%2921%3A7%3C1421%3A%3AAID-ELPS1421%3E3.0.CO%3B2-7). PMID 10826690 (https://pubmed.ncbi.nlm.nih.gov/10826690). S2CID 38448915 (https://api. semanticscholar.org/CorpusID:38448915).

13. Kuehnbaum NL, Kormendi A, Britz-McKibbin P (November 2013). "Multisegment injection capillary electrophoresis–mass spectrometry: a high-throughput platform for metabolomics with high data fidelity". Analytical Chemistry. 85 (22): 10664–10669. doi:10.1021/ac403171u (h ttps://doi.org/10.1021%2Fac403171u). PMID 24195601 (https://pubmed.ncbi.nlm.nih.gov/2 4195601).
14. Olivares JA, Nguyen N, Yonker CR, Smith RD. "On-line mass spectrometric detection for CZE". Analytical Chemistry. 59: 1230–1232. doi:10.1021/ac00135a034 (https://doi.org/10.1021%2Fa c00135a034).
15. Tomer KB (February 2001). "Separations combined with mass spectrometry". Chemical Reviews. 101 (2): 297–328. doi:10.1021/cr990091m (https://doi.org/10.1021%2Fcr990091 m). PMID 11712249 (https://pubmed.ncbi.nlm.nih.gov/11712249).
16. 15. Wang C, Lee CS, Smith RD, Tang K (August 2013). "Capillary isotachophoresisnanoelectrospray ionization-selected reaction monitoring MS via a novel sheathless interface for high sensitivity sample quantification" (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC37 44340). Analytical Chemistry. 85 (15): 7308–7315. doi:10.1021/ac401202c (https://doi.org/1 0.1021%2Fac401202c). PMC 3744340 (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC374 4340). PMID 23789856 (https://pubmed.ncbi.nlm.nih.gov/23789856).
17. Guo X, Fillmore TL, Gao Y, Tang K (April 2016). "Capillary Electrophoresis-Nano electrospray Ionization-Selected Reaction Monitoring Mass Spectrometry via a True Sheathless Metal Coated Emitter Interface for Robust and High-Sensitivity Sample Quantification" (https://www.n cbi.nlm.nih.gov/pmc/articles/PMC4854437). Analytical Chemistry. 88 (8): 4418–4425. doi: 10.1021/acs.analchem.5b04912 (https://doi.org/10.1021%2Facs.analchem.5b04912). PMC 4854437 (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4854437). PMID 27028594 (https://pubmed.ncbi.nlm.nih.gov/27028594).
18. Sun L, Zhu G, Zhang Z, Mou S, Dovichi NJ (May 2015). "Third-generation electrokinetically pumped sheath-flow nanospray interface with improved stability and sensitivity for automated capillary zone electrophoresis–mass spectrometry analysis of complex proteome digests" (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4416984). Journal of Proteome Research. 14 (5): 2312–2321. doi: 10.1021/acs.jproteome.5b00100 (https://doi.org/10.1021%2Facs.jproteo me.5b00100). PMC 4416984 (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4416984). PMID 25786131 (https://pubmed.ncbi.nlm.nih.gov/25786131).
19. 1. Fang P, Pan JZ, Fang Q (April 2018). "A robust and extendable sheath flow interface with minimal dead volume for coupling CE with ESI-MS". Talanta. 180: 376–382. doi: 10.1016/j.talanta.2017.12.046 (https://doi.org/10.1016%2Fj.talanta.2017.12.046). PMID 29332826 (https://pubmed.ncbi.nlm.nih.gov/29332826).
20. Höcker O, Montealegre C, Neusüß C (August 2018). "Characterization of a nanoflow sheath liquid interface and comparison to a sheath liquid and a sheathless porous-tip interface for CEESI-MS in positive and negative ionization". Analytical and Bioanalytical Chemistry. 410 (21): 5265–5275. doi:10.1007/s00216-018-1179-3

(https://doi.org/10.1007%2Fs00216-018-1179- 3). PMID 29943266 (https://pubmed.ncbi.nlm.nih.gov/29943266). S2CID 49409772 (https://api.semanticscholar.org/CorpusID:49409772).
21. Sauer F, Sydow C, Trapp O (August 2020). "A robust sheath-flow CE–MS interface for hyphenation with Orbitrap MS" (https://doi.org/10.1002%2Felps.202000044). Electrophoresis. 41 (15): 1280–1286. doi:10.1002/elps.202000044 (https://doi.org/10.1002%2 Felps.202000044). PMID 32358866 (https://pubmed.ncbi.nlm.nih.gov/32358866).
22. Cai, J., & Henion, J. (1995). Capillary electrophoresis-mass spectrometry. *Journal of Chromatography A, 703*(1-2), 667-692
23. Chen, D., McCool, E. N., Yang, Z., Shen, X., Lubeckyj, R. A., Xu, T., Wang, Q., & Sun, L. (2023). Recent advances (2019–2021) of capillary electrophoresis-mass spectrometry for multilevel proteomics. *Mass Spectrometry Reviews, 42*(2), 617-642
24. Colón Rosado, J. A., & Sun, L. (2024). Solid-Phase Microextraction-Aided Capillary Zone Electrophoresis-Mass Spectrometry: Toward Bottom-Up Proteomics of Single Human Cells. *Journal of the American Society for Mass Spectrometry, 35*(6), 1120-1127.
25. Lippens, J. L., Timmons, H. C., Welch, C., Kulkarni, A., & Flick, T. G. (2023). Rapid Intact Mass Analysis and Evaluation of the Separation Potential of Microfluidic Capillary Electrophoresis Mass Spectrometry for Oligonucleotides. *Journal of the American Society for Mass Spectrometry, 34*(11), 2491-2497.
26. Pont, L., Vergara-Barberán, M., & Carrasco-Correa, E. J. (2024). A Comprehensive Review on Capillary Electrophoresis–Mass Spectrometry in Advancing Biomolecular Research. *Electrophoresis*.
27. Shah, U., Patel, A., Patel, R., Patel, M., Patel, A., Kavad, M., & Desai, E. (2021). Overview on capillary electrophoresis with mass spectrometry: Application in peptide analysis and proteomics. *International Journal of Pharmaceutical Chemistry and Analysis, 8*(1), 6-15.
28. Wang, Q., Wang, Q., Zhu, G., & Sun, L. (2025). Capillary Electrophoresis–Mass Spectrometry for Top-Down Proteomics. *Annual Review of Analytical Chemistry, 18*(1), 125-147.
29. Zhao, T., Wang, L., & Chen, D. D. Y. (2020). Quantification of Imatinib and Related Compounds Using Capillary Electrophoresis-Tandem Mass Spectrometry with Field-Amplified Sample Stacking. *Electrophoresis, 41*(21-22), 1843-1850.
30. Zhu, G., Sun, L., & Dovichi, N. J. (2017). Simplified capillary isoelectric focusing with chemical mobilization for intact protein analysis. *Journal of Separation Science, 40*(4), 948-953.