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APPLICATIONS OF CE-MS IN PHARMACEUTICAL FIELD

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ABSTRACT: Capillary Electrophoresis–Mass Spectroscopy (CE-MS) is a hyphenated technique as it combines the advantages of both CE and MS. It provides high-resolution separations with high detection selectivity and sensitivity, which allows detailed characterization of many biomolecules in a single analysis. CE-MS improves the ability to deal with the analysis of complex biological samples containing trace amounts of analytes. CE coupled MS has become a widely used analytical technique in fields such as proteomics, metabolomics, and genomics. It is also increasingly used in various other fields like environmental science, food analysis, biotechnology and forensic science *etc*. However, the development of online CE-MS is not without challenges. Understanding of CE, the interface setup, ionization technique and mass detection system is important to tackle problems while coupling capillary electrophoresis to mass spectrometry. In this article, instrumentation details of CE-MS, the technical aspects of CE-MS interface, applications and the advancements made in recent years are discussed.

INTRODUCTION: Capillary electrophoresis (CE) is a versatile technique. The separation is based on their electrophoretic mobility and electro-osmotic flow with the use of applied voltage. It was first introduced by HJERTON in the year 1967. CE plays an ideal role in the analysis of highly polar charged analyte, analysis of both basic and clinical pharmaceutical and in analysis and characterization of macromolecules. CE uses tubes 20-100µm diameter and 20-100cm in length. Higher electric fields result in high efficiency and narrow peaks.

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Mass spectrometry is one of the most widely applied analytical techniques that is used for determining molecular mass and structure elucidation of a wide range of organic, inorganic compounds and many complex bio-molecules. In this technique, the sample under investigation is first vaporized and then ionized into rapidly moving positive ions and ionic fragments by bombarding with the beam of high energy electrons (50-100 eV). So to rupture the bonds present in the molecule. The entire process of ionization, separation, and detection results in a mass spectrum [1, 2].

CE-MS: CE-MS is an analytical technique. Mass spectroscopy is becoming popular as a detection method for capillary electrophoresis (CE-MS). The combination of CE's high efficiency and high selectivity offered by MS detection is very attractive.

CE is very tolerant of complex sample matrices, and therefore its combination with MS provides for highly variously complex mixtures. MS detection also helps to improve the general sensitivity of CE analyses in appropriate instances. The power of combining MS detection with any separation technique is that it provides a second dimension of separation.

2. Basic Principles:

2.1 Principle of Capillary Electrophoresis: The principle of capillary electrophoresis deals with the separation of a complex mixture of molecules based on the differential migration of analyte ions under the influence of an applied electric field.

Electrophoretic Mobility: It depends on the charge of molecule, viscosity & atom's radius. Electrophoresis a process in which sample moves under the influence of applied voltage. The ion undergoes a force that is equal to product 'f', transitional friction coefficient and velocity. This leads to an expression of electrophoretic mobility.

$$\mu EP = q/f = q/6\pi or\ s$$

f = spherical particle given by stokes law
q = charge
r = radius of ion
η = viscosity of the solvent.

The rate at which these ions migrate is dictated by a charge to mass ratio. The Actual velocity of ion is directly proportional to applied voltage; the magnitude of the electrical field can be determined by the following equation:

$$\upsilon = \mu_{EP} E$$

Electro Osmotic Flow: It is caused by applying high-voltage to an electrolyte-filled capillary. This flow occurs when the buffer running through the silica capillary tube has pH greater 3 and SIOH groups lose a proton to become SIO^-. The applied electric field causes free cations to move towards cathode creating a powerful bulk flow. The rate of electroosmotic flow is governed by

$$\mu_{EOF} = \varepsilon/4\pi\eta \times E\xi$$

ε = dielectric constant
η = viscosity
ξ = zeta potential
E = field strength [3, 4]

2.2 Principle of Mass Spectrometry: The basic principle behind this technique is that when a vaporized sample is exposed to a high voltage electric current, it loses electrons and forms positively charged ions (cations) that are deflected by means of magnetic and electrical fields along a circular path, the radius of which is a function of their mass-to-charge ratio (m/e).When a sample is exposed to the electron beam, it knocks out; it knocks out an electron from the sample molecule, resulting in the formation of a parent ion or molecular ion (M^+) with an unpaired electron.

$$M + e^- \longrightarrow M^+ + 2e$$

When energy supplied is more than the ionization energy of the molecule, the molecular ion (M^+) undergoes further fragmentation to give smaller ions and free radicals [5].

3. Modes of Capillary Electrophoresis:

Depending upon the buffer, types of the capillary as well as on nature of any incorporated additives, the techniques of CE may be divided:

3.1 Capillary Zone Electrophoresis (CZE): This is the most commonly used capillary electrophoresis method. CZE, also known as free solution capillary electrophoresis, is a separation technique that predominantly takes into account the ratio of the particle's charge to mass, where those with the large charge to mass ratio separate from the rest first; therefore, the larger the ratio, the quicker the separation. CZE is an excellent choice of technique to employ in cases where there are very small pI (isoelectric point) differences in protein isoforms.

3.2 Capillary Gel Electrophoresis (CGE): Like CZE, CGE requires constant field strength and is dependent on the pH of the buffer solution as the particles move through the gel and separate out based on the difference of their size and shape. It is a great choice for macromolecules such as DNA and proteins. Gels are additionally advantageous in reducing solute diffusion and heat transfer.

3.3 Capillary Electro Chromatography (CEC): This method resembles chromatography in many ways, as it is a combination of capillary

electrophoresis and liquid chromatography. Separation is based on a packed column similar to chromatography. CEC and CZE are similar in terms of having a plug-type of flowing comparison to the parabolic flow that is pumped, which increases band broadening.

3.4 Capillary Isoelectric Focusing (CIEF): This form of capillary electrophoresis is commonly employed to separate peptides and proteins. The determining factors in this technique are the charge of the protein groups involved and the pH of the solutions. Since the charge is altered with altering pH, this is the basis of the Mechanism for separating molecules in the mixture with this technique.

3.5 Capillary Isotachophoresis (CITP): Being the sole member of the discontinuous system, molecules with the CITP technique migrate in definitive zones. These zones can be measured for the quantification of sample purposes. The sample is essentially added between two different buffers, one of which has higher mobility in the separation process and is known as the leading electrolyte.

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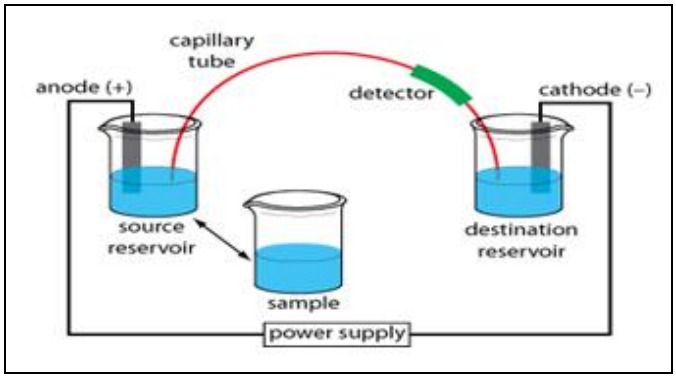

FIG. 1: SCHEMATIC REPRESENTATION OF INSTRUMENTATION OF CAPILLARY ELECTROPHORESIS

3.7 Micellar Electro Kinetic Capillary Chromatography (MEKC): MEKC uses surfactant to form micelles these micelles are imperative for their polar negativity, which causes a pull towards the positive pole. The molecules that are hydrophobic (water-hating) will tend to aggregate with the micelle.

4. Instrumentation of CE-MS: CE-MS is the hyphenated technique where CE is connected to the MS with the help of the long capillaries which will increase the analysis time also there is a lack of suitable volatile buffer which has to be compatible with the mass spectrometer.

4.1. CE: The CE apparatus consists of various major components they are:

i. Injector
ii. Capillary
iii. Detector
iv. BGE vessel
v. High voltage supply
vi. Electrode
vii. Recorder

4.1.1 Injector: Sample is injected into the capillary tube. The most common sample introduction is an electrokinetic and hydrostatic type of injection.

With pressure injection, the sample introduction end of the capillary is also placed momentarily into a small cup containing the sample, and a pressure difference is then used to drive the sample solution into the capillary. The pressure difference can end by pressurizing the sample or by elevating the sample end. HYDROSTATIC injections do not discriminate due to ion mobility, but cannot be used in gel-filled capillaries.

4.1.2 Capillary: A buffer filled fused silica capillary that is typically 10 to 100 µm in internal diameter and 40 to 100 cm long extends between two buffer reservoirs that also hold platinum electrodes. The sample is introduced at one end and detection at the other end. The detector should be placed at the right side *i.e.*, near the cathode electrode. A provision is made on the capillary tube to enter the injected sample *i.e.*, 0.5mm detector window. If a provision is absent, then the capillary tube is heated with 96-98% of conc.H_2SO_4 or conc. KOH at 130 °C.

4.1.3 BGE Vessel: Buffer plays an important role where the suitable buffer is selected for electro-

osmotic and electrophoretic mobility based on analyte behavior, pH constants. Buffers chosen should be of good quality and should be prepared under optimization concentration Phosphate buffer, ethanoate buffer and borate buffers are the most commonly used buffers in CE. Buffer additives like urea, surfactants, organic & inorganic salts are also used.

4.1.4 High Voltage Supply: High potentials can be applied in CE; with current technology, up to 30 kV can be applied for extremely fast and efficient separations. When the components are migrating at different rates along the length, although separated by the electrophoretic migration, the entire sample is drawn towards cathode by electro endosmosis. Cooling systems are maintained to reduce the temperature or reduce heat from the system.

4.1.5 Electrodes: An electrode in an electrochemical cell is referred to as either an anode or a cathode. The anode is now defined as the electrode at which electrons leave the cell and oxidation occurs and the cathode as the electrode at which electrons enter the cell and reduction occurs. Each electrode may become either the anode or the cathode depending on the direction of current through the cell. A bipolar electrode is an electrode that functions as the anode of one cell and the cathode of another cell.

4.1.6 Detectors: Separation by capillary electrophoresis can be detected by several detection devices. Different detector configurations are possible. Since the amount of each analyte passing the detector is very small, shooting the source lamp along a short section of the capillary increases the path length and, if absorbance is being used, decreases the detection limit.

The most commonly used are i) UV Absorption, ii) Fluorescence, iii) Conductivity, iv) Potential gradient detector, v) Amperometric detector, VI) Diode array detector, vii) Inductive coupled plasma detector, viii) Refractive index detector, ix) Raman spectroscopy detector, x) Chiral optical activity detector. xi) Thermo optical absorbance detector, xii) Atomic absorption.

4.1.7 Recorder: Like a chromatogram, the CE electropherogram is a plot of the time from injection on the x-axis *vs*. the detector signal on they. In the example below, indirect detection is being used. Note the y-axis [5].

5.2 Mass Spectrometry: A typical mass spectrometer contains the following components:

- **i.** Inlet system (sample handling system)
- **ii.** Ion source (ionization chamber)
- **iii.** Electrostatic accelerating system
- **iv.** Magnetic field
- **v.** Ion separator
- **vi.** Ion collector (detector & readout device)
- **vii.** Vacuum system

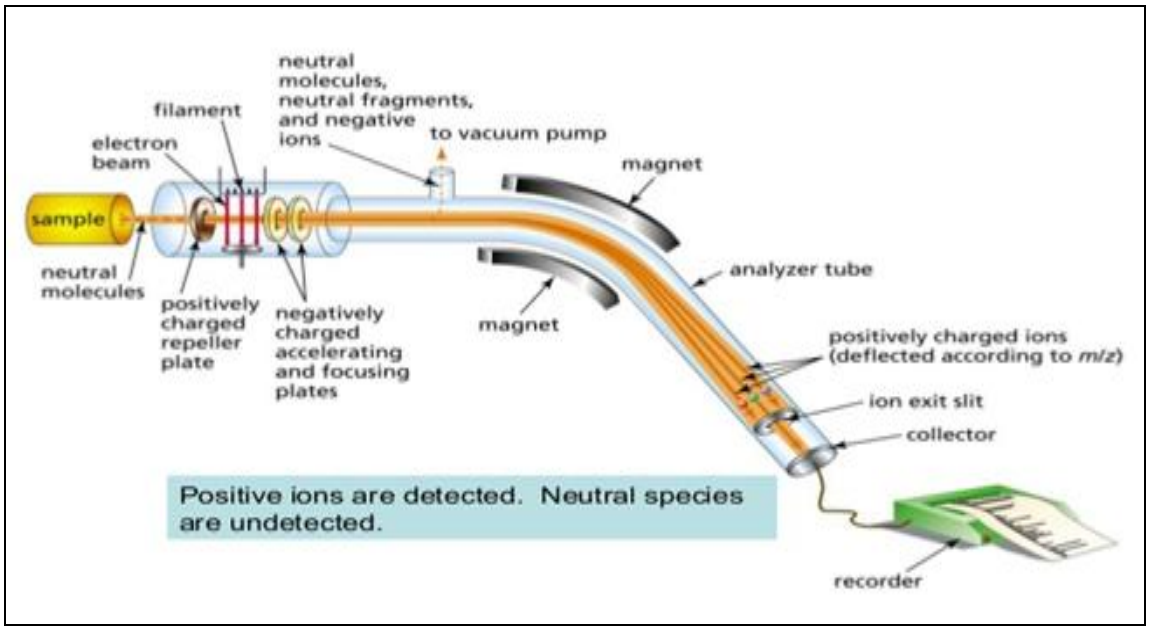

FIG. 2: MASS SPECTROMETER

5.2.1 Sample Inlet System: Inlet system is an interface between sample and ion source**.** The purpose of the inlet system is to introduce the sample into the ion source with minimal loss of vacuum. The inlet system can be as simple as a port through which the sample is injected or inserted into a chamber at high vacuum and heated to achieve vaporization. If the sample is a mixture of compounds, a GC inlet system will vaporize the sample and separate the mixture into its components. Mass spectra for each of the components are recorded in succession as the components enter the mass spectrometer. Hence, mass spectra of individual components of a complex mixture can be obtained without prior Separation.

5.2.2 Ion Source: The need of ionization arises since the mass analyzer utilizes only gaseous ions. Upon entering the mass spectrometer in the gas phase; the molecules are commonly ionized by either removing an electron or by adding a proton. Removal of an electron is achieved by bombarding the molecules with a high energy electron beam, commonly of 70 eV electrons. This electron beam is of much higher energy than the ionization energy of the molecules being bombarded. The resulting ion is a radical ion because it has one unpaired electron.

8.2.3 Electrostatic Accelerating Systems: positively charged ions are produced in the ionization chamber are accelerated by a strong electric field existing between the first accelerator plate and the second repeller plate owing to the potential difference between them. These ions attain their final velocities after passing through the second accelerator plate. A potential difference of about 400-4000Vis maintained between the first and second accelerating plates that accelerate the ions of masses m_1, m_2, m_3 *etc*., to their final velocities. The ions which come out through the slit consist of a collimated beam of ions having high velocities and kinetic energies.

5.2.4 Magnetic Field: The accelerated ions from the electric field enter the magnetic field where they take a curved path. The radius of the curvature (r) is dependent on the mass of the ion (m), accelerating Voltage (V), electron charge (e), and strength of the magnetic field (H). Mass spectrometry is based on the mass-to-charge ratio (m/e) and radius (r) of the curvature which in turn are inter-dependent. Any change in accelerating Voltage or strength of the magnetic field (H) in turn changes m/e and r values.

5.2.5 Ion Separator: Ion separator is also called as a mass analyzer. It is the heart of mass spectrometer at takes ionized masses and separates them based on charge to mass ratios. Based on the method of sorting, many commercial varieties of mass analyzers are available, few of which are:

5.2.5.1 Single Focusing Magnetic Sector Analyser: It has a horseshoe-shaped glass tube which is evacuated, consists of sample inlet, electron bombarding source, accelerating plates on one end & collector slit at the other end. At curvature of the tube, there is a provision to apply the electric/magnetic field. Sample in the formed vapor is allowed through an inlet and bombarded with an electron beam at 70eV. It knocked off one electron from every molecule then they become a positively charged ion. As these molecules become positively charged, they are accelerated by accelerating plates and travel in a straight path. By application of electric or magnetic fields, they travel in a curved path and molecular ions are separated according to their massed and collected. Different fragments fall on the detector then the mass spectrum is recorded.

5.2.5.2 Double Focusing Magnetic Mass Analyser: It is used to differentiate the small mass differences of the fragment. It contains two carefully selected magnetic and electrostatic devices for focussing a beam of ions. These provide a high resolution. To achieve better focussing, energy has to be reduced before ions are allowed to enter the magnetic field and increase resolving power can be obtained 2 mass analyzers in series. In double focussing, a mass analyzer beam is first passed the radial electrostatic field.

5.2.5.3 Quadra Pole Ion Trap Analyser: it is the most common type. The DC bias will cause all the charged molecules to accelerate and move away from the center line, the rate being proportional to their charge to mass ratio. If their course goes off too far they will hit the metal rods or the sides of the container and be absorbed. So the DC bias acts

as the magnetic field B of the mass spec and can be tuned to specific charge to mass ratios hitting the detector. The two sinusoidal electric fields at 90 orientation and 90 degrees phase shift will cause an electric field that oscillates as a circle over time. So, as the charged particles fly down toward the detector, they will be traveling in a spiral, the diameter of the spiral being determined by the charge to mass ratio of the molecule and the frequency and strength of the electric field. The combination of the DC bias and the circularly rotating electric field will be the charged particles will travel in a spiral which is curved. So, by timing the peak of the curved spiral to coincide with the position of the detector at the end of the Quadra pole, a great deal of selectivity to molecules charge to mass ratio can be obtained.

5.2.5.4 Ion Cyclotron Resonance: ICR is an ion trap that uses a magnetic field in order to trap ions into an orbit inside of it. In this analyzer, there is no separation that occurs rather all the ions of a particular range are trapped inside, and an applied external electric field helps to generate a signal. As mentioned earlier, when a moving charge enters a magnetic field, it experiences a centripetal force making the ion orbit. Again the force on the ion due to the magnetic field is equal to the centripetal force on the ion.

5.2.6 Ion Collector: After Ions after passing from the analyzer reaches the detector which produces a signal. The signal is further amplified to give the m/z value. The readout system usually employed is a direct writing recording oscilloscope consisting of 3-5 galvanometers.

5.2.7 Vacuum System: Vacuum is necessary to permit the ions to reach the detector without colliding with any extraneous materials including atmospheric gases which themselves undergo ionization and fragmentation giving their spectra [6, 7, 8].

6. Coupling of CE-MS: Capillary Electrophoresis (CE) coupled with Mass Spectrometry (MS) combining CE's high efficiency and high speed with the high sensitivity and high selectivity offered by MS detection is very attractive. There are several factors that must be considered when coupling the CE instrument to an MS detector.

Coupling CE with MALDI-MS: Off-line coupling of CE to MALDI, the CE effluent could be sprayed or added dropwise on the MALDI target plate then dried and analyzed by MS. For online coupling, a moving target with continuous contact with CE capillary end is required. The moving target takes analyte into MS where it is desorbed and ionized. Musyimi *et al*. developed a new technique where a rotating ball was used to transfer CE to MS. The sample from CE is mixed with matrix coming through another capillary. As the ball rotates the sample is dried before it reaches the ionization region. This technique has high sensitivity since no makeup fluid is used.

6.1. Interfacing with CE-MS: Capillary electrophoresis is a separation technique that uses a high electric field to produce electro-osmotic flow for separation of ions.

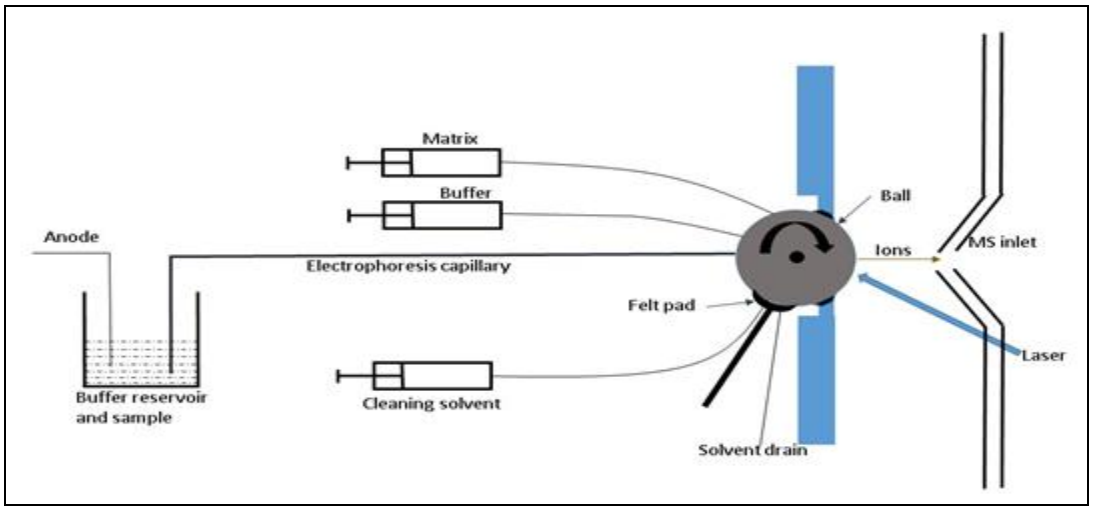

FIG. 3: SCHEMATIC DIAGRAM OF COUPLING OF CE-MS

Analyte migrates from one end of capillary to others based on their charge, viscosity and size. Higher the electric field, the greater is the mobility. The major problem faced when coupling CE to MS arises due to insufficient understanding of fundamental Processes when two techniques are interfaced. The separation and detection of the analyte can be improved with a better interface. The most used ionization technique is ESI. The three setups commonly used in CE-ESI-MS coupling are i) coaxial sheath liquid, ii) liquid junction, and iii) sheathless or nanospray. All these configurations involve closing the high voltage circuit at the outlet of the separation capillary.

6.1.1 Electrospray Ionization Interface: The first CE-MS interface had cathode end of CE capillary terminated within a stainless steel capillary. Electrical contact was made at that point completing the circuit and initiating the electrospray. This interface system had few drawbacks like mismatch in the flow rates of two systems. Since then, the interface system has been improved to have a continuous flow rate and good Electrical contact. At present, three types of interface systems exist for CE/ESI-MS which are discussed briefly.

6.1.2 Sheath Less Interface: CE capillary is coupled directly to an electrospray ionization source with a sheathless interface system. The electric contact for ESI is realized by using capillary coated with a conductive metal. Because no sheath liquid is used, the system has high sensitivity, low flow rates and minimum background. However, these interface designs; all have challenges including low mechanical robustness, poor reproducibility. The latest sheathless interface design features porous ESI emitter through chemical etching.

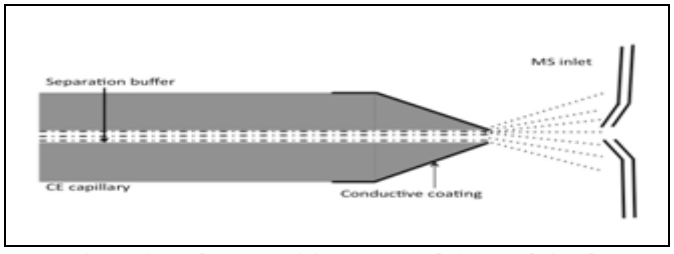

FIG. 4: SHEATH LESS INTERFACE DIAGRAM

This design effectively provides robust interfacing with mass spectrometry and addresses the reproducibility challenges associated with previous designs.

6.1.3 Sheath Flow Interface: With the sheath flow interface, the electrical connection is established when the CE separation liquid is mixed with sheath liquid flowing coaxially in a metal capillary tubing. Commonly used sheath liquid is 1:1 mixture of water-methanol with 0.1% acetic acid or formic acid. The system is more reliable and has a wide selection range of separation electrolytes. There might be some decrease in sensitivity due to sheath liquid.

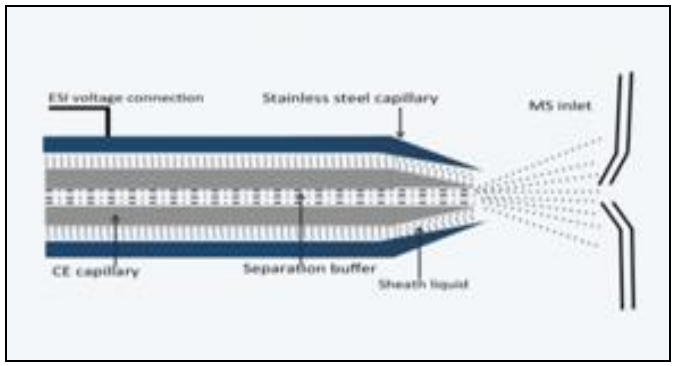

FIG. 5: SHEATHFLOW INTERFACE DIAGRAM

6.1.4 Liquid Junction Interface: In this setup, an electrode is placed at the injector extremity close to the MS inlet at the junction of two capillaries, and a voltage is applied to the system. An advantage of this setup is that the electrode's electrical contact is stable. This technique uses a stainless steel tee to mix separation electrolytes from CE capillary with makeup liquid. The CE capillary and ESI needle are inserted through opposite sides of the tee and a narrow gap is maintained. The electrical contact is established by makeup liquid surrounding the junction between two capillaries. This system is easy to operate. However, the sensitivity is reduced and the mixing of two liquids could degrade separation [9, 10].

7. Applications of CE-MS:

7.1. For Synthetic *in-vitro* Glycolysis Studies: A synthetic *in-vitro* glycolysis was reconstructed from ten purified *Escherichia coli* (*E. coli*) enzymes to obtain a better understanding of the regulation of sequential enzymatic reactions. The key to the success of this approach is the ability to perform direct and simultaneous determination of the diverse metabolic intermediates in the pathway by capillary electrophoresis-mass spectrometry.

7.2 For Long Term Comparable Assessment of the Urinary Metabolite: To demonstrate the use of this pipeline in clinical research, we compared the urinary metabolite of 34 new-borns with pelvic urethra junction (UPJ) obstruction and 15 healthy new-borns. A combination of the 32 compounds in an SVM classifier predicted with 76% sensitivity and 86% specificity UPJ obstruction in a separate validation cohort of 24 individuals.

7.3 Characterization of Monoclonal Antibodies: MAbs are highly heterogeneous proteins, thereby requiring a battery of sophisticated analytical technologies for their complete characterization. Mass spectrometry (MS) has become an essential analytical tool for the structural characterization of mAbs. As regards capillary electrophoresis (CE), it has become a routine tool for the analysis of recombinant protein therapeutics in the biotechnology industry [11].

7.4 For Chiral Separations: Chiral selectors such as modified cyclodextrin's (CDs) and polymeric surfactants (a.k.a. molecular micelles, MoMs) in electrokinetic chromatography (EKC), micelle electrokinetic chromatography (MEKC) and capillary electrochromatography (CEC) have been developed to address the need for high sensitivity by CE–MS.

7.5 For Forensic Analysis: Analytes of interest are divided into four main parts, namely, compounds with amine-containing side chains, compounds with N-containing saturated ring structures, other heterocyclic and peptides. Sample pre-treatments and direct injection modes used in CE-MS for forensic analysis are briefly discussed from a critical point of view. Special emphasis is placed to point out the advantages of mass spectrometric detection compared to UV- and laser-induced fluorescence (LIF) detections [12].

7.6 Analysis of O-Glycopeptides by Acetone Enrichment: The amount of ice-cold acetone added to the digests was optimized to maximize recoveries of O-Glycopeptides. Furthermore, the different behavior of peptides, N- and O-Glycopeptides was explained by studying with multivariate data analysis methods the influence of several physicochemical parameters and properties related to their composition and structure. Special attention was paid to O126-glycopeptide glycol forms of repos because of their applicability in biopharmaceutical quality control and doping analysis.

7.7 Identification of Anthraquinone Colouring Matters in Natural Red Dyes: for the identification of anthraquinone color components of cochineal, lac dye and madder, natural red dyestuffs often used by ancient painters. For the purpose of such analysis, ESI-MS was found to be a much more appropriate detection technique than DAD one owing to its higher sensitivity (detection limits in the range 0.1–0.5 $\mu g ml^{-1}$) and selectivity. The method developed made it possible to identify unequivocally carmine acid and laconic acids A, B and E as coloring matters in the examined preparations of cochineal and lac dye, respectively.

7.8 Determination of Drugs in Human Plasma: Using CE/MS techniques, the described API III Quadra pole system provided an acceptable ion current electropherogram from subpicomole levels of the targeted compounds loaded onto the chip. The corresponding electropherogram for the standard solution of carnitines at the 1−500 μg/mL level was obtained via SIM CE/MS techniques (R2 > 0.99). In addition, analyses of fortified samples of imipramine desipramine were measured relative to their corresponding d3 internal standards to obtain calibration curves ranging from 5 to 500 μg/mL in human plasma (R2 > 0.99). The intra-assay precision ranged from 4.1 to 7.3% RSD. The intra-assay accuracy ranged from 94.0 to 104% [13, 14].

7.9 Analysis of Inorganic Species: The selectable degree of ion-adduct DE clustering and molecular fragmentation in the MS interface region allows the system to be operated as an elemental analyzer or as a molecular detector suitable for oxidation state determinations. Both inorganic anions and cations (including alkalis, alkaline earth, transition metals, and lanthanides) are analyzed by CE−MS. A variety of CE separation buffers are evaluated for the cations analyses only one of the buffers (*i.e.*, creatinine) can be used for CE-indirect UV detection. A CE capillary permanently coated with strong anion exchange sites and a pyromellitic acid buffer (suitable for indirect UV detection) is used for the inorganic anion separations.

7.10 Developments for Profiling Metabolites of Steroid Hormone Metabolism: Thirteen steroids were included in the method development, and the selected were metabolites involved in major pathways of steroid biosynthesis. Although only eight of them could be separated and detected with UV, they could be identified by ESI-MS using selected ion monitoring (SIM) technique. Tandem MS spectra were also collected. The lowest limits of detection were 10-100 ng/mL for cortisone, corticosterone, hydrocortisone, and testosterone. The other steroids could be detected at 500-1000 ng/ml. The identification of cortisone, corticosterone, hydrocortisone, oestrogen and testosterone were made in patient urine samples and their concentrations were 1-40 µg/L.

7.11 Determination of Antioxidant Compounds of Propolis: Different Phenolic compounds (*e.g.* pinobanksin 3 acetate, Naringenin, pinocembrin, Chrysene, daidzein, Quarcetin 3′, 7 dimethyl ether, apigenin, and kaempferid) could be detected. To confirm the identity of the Phenolic compounds in propolis extracts, accurate mass data of the molecular ions were obtained by MS. Limits of detection ranging from 6 mg/100 g of raw propolis for chrysene to 58 mg/100 g of raw propolis for Luteolin, were obtained.

7.12 Amino Acid Analysis: To analyze free amino acids simultaneously a low acidic pH condition was used to confer positive charge on whole amino acids. The choice of the electrolyte and its concentration influenced the resolution and peak shape of the amino acids, and 1 M formic acid was selected as the optimal electrolyte.

CONCLUSION: Capillary electrophoresis is electrophoresis performed in a capillary tube. CE-MS establishes a new paradigm that separation methods together with MS detection can be used as comprehensive kinetic tools. Most previous attempts to use chromatography and electro-phoresis for studying nucleic acid interactions were restricted to assuming slow or no equilibrium between reactants.

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REFERENCES:

1. Kemp G: Capillary electrophoresis: a versatile family of analytical techniques. Biotechnology and Applied Biochemistry 1998; 27(1): 9-17.
2. Skoog DA, Holler FJ and Crouch SR: Principles of Instrumental Analysis. Thomson Brooks/Cole Publishing, Sixth edition 2007.
3. Janini GM and Issaq HJ: The buffer in capillary zone electrophoresis. Chromatographic Science 1993; 64: 119-60.
4. Cifuentes A and Poppe H: Rectangular capillary electrophoresis: some theoretical considerations. Chromatographia 1994; 39(7-8): 391-04.
5. Geiser L, Cherkaoui S and Veuthey JL: Simultaneous analysis of some amphetamine derivatives in urine by non-aqueous capillary electrophoresis coupled to electrospray ionization mass spectrometry. J Chromatography A 2000; 895(1-2): 111-21.
6. Wiley HE: mass spectroscopy principles and applications. Wiley-Interscience, Third edition 2007.
7. Ross GA: Capillary electrophoresis-mass spectrometry: practical implementations and applications. Agilent Technologies 2001.
8. Smith RD, Goodlet DR and Wahl JH: Capillary electrophoresis – mass spectrometry in handbook of capillary electrophoresis. Ed. J.P. Landers; CRC Press, 1994.
9. Guilhaus M: Principles and Instrumentation in Time-of-Flight Mass Spectrometry. Journal of Mass Spectrometry 1995; 30: 1519-32.
10. Price. P: Standard definitions of terms relating to mass spectrometry: A report from the committee on measurements and standards of the American Society for mass spectrometry. Journal for American Society Mass-Spectrometry 1991; 2(4): 336-48.
11. Jiang Y, He MY, Zhang WJ, Luo P, Guo D, Fang X and Xu W: Recent advances of capillary electrophoresis-mass spectrometry instrumentation and methodology. Chinese Chemical Letters 2017; 28(8): 1640-52.
12. Fekete S, Gassner AL, Rudaz S, Schappler J and Guillarme D: Analytical strategies for the characterization of therapeutic monoclonal antibodies. Trends in Analytical Chemistry 2013; 42: 74-83.
13. Huck CW, Huck-Pezzei V, Bakry R, Bachmann S, Najam-ul-Haq M, Rainer M and Bonn GK: Capillary Electrophoresis Coupled To Mass Spectrometry for Forensic Analysis. The Open Chemical Engineering Journal 2007; 1: 30-43.

14. Boatto G, Nieddu M, Dessi G, Manconi P and Cerri R: Determination of four thiophenethylamine designer drugs (2C-T-series) in human plasma by capillary electrophoresis with mass spectrometry detection. Journal of Chromatography A2007; 1159(1-2): 198-02.
15. Lin X, Cai Y, Yan J, Zhang L, Wu D and Li H: Determination of Gabapentin in Human Plasma and Urine by Capillary Electrophoresis with Laser-Induced Fluorescence Detection. Journal of Chromatographic Science 2015; 53(6): 986-92.
16. Corr JJ and Anacleto JF: Analysis of Inorganic Species by Capillary Electrophoresis - Mass Spectrometry and Ion Exchange Chromatography - Mass Spectrometry Using an Ion Spray Source. J of Anal Chem 1996; 68(13): 2155-63.
17. Gomes FP and Yates III JR: Recent trends of capillary electrophoresis-mass spectrometry in proteomics research. Mass Spectrometry Reviews 2019; 38(6): 445-60
18. Venkateswarlu N: A Review on capillary electrophoresis-mass spectrometry (CE-MS). Research & Reviews: Journal of Pharmaceutical Analysis 2015; 4(1): 23-28.
19. Lindon JC, Nicholson JK and Holmes E: The Handbook of Metabolic Phenotyping. Elsevier 2019.
20. Ramautar R, Mayboroda OA, Somsen GW and de Jong GJ: CE-MS for metabolomics: Developments and applications in the period 2008–2010. Electrophoresis 2011; 32(1): 52-65.
21. Zhang W, Hankemeier T and Ramautar R: Next-generation capillary electrophoresis–mass spectrometry approaches in metabolomics. Current Opinion in Biotechnology 2017; 43: 1-7.
22. Zhong X, Chen Z, Snovida S, Liu Y, Rogers JC and Li L: Capillary electrophoresis-electrospray ionization-mass spectrometry for quantitative analysis of glycanslabeled with multiplex carbonyl-reactive tandem mass tags. Analytical Chemistry 2015; 87(13): 6527-34.
23. Khatri K, Klein JA, Haserick JR, Leon DR, Costello CE, McComb ME and Zaia J: Microfluidic capillary electrophoresis-mass spectrometry for analysis of monosaccharides, oligosaccharides, and glycopeptides. Analytical chemistry 2017; 89(12): 6645-55.
24. Kleparnik K: Recent advances in combination of capillary electrophoresis with mass spectrometry: methodology and theory. Electrophoresis 2015; 36(1): 159-78.
25. Liu Y and Shamsi SA: Chiral Capillary Electrophoresis–Mass Spectrometry: Developments and Applications in the Period 2010–2015: A Review. Journal of Chromatographic Science 2016; 54(10): 1771-86.

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