

## *International Journal of Scientific Research and Reviews*

### **Capillary Electrophoresis - Mass Spectrometry (CE-MS) and its Applications A Review**

**K. Bhavyasri<sup>1\*</sup> and G.Mounika<sup>2</sup>**

<sup>1</sup>Associate Professor, RBVRR Women's college of pharmacy, Hyderabad.

<sup>2</sup>Research Scholar, RBVRR Women's college of pharmacy, Hyderabad.

#### **ABSTRACT**

Capillary Electrophoresis–Mass Spectroscopy (CE-MS) of capillary electrophoresis with mass spectroscopy. CE-MS combines advantages of both CE and MS to provide high separation efficiency and molecular mass information in a single analysis. Use of CE-MS has increased for protein and peptides analysis and other bio molecules. 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.

**KEYWORDS:** capillary electrophoresis, instrumentation, mass spectrometry, peptides, bio molecules.

#### **\*Corresponding author**

**Dr. K. Bhavyasri**

Associate Professor,

Department of Pharmaceutical Analysis,

RBVRR women's college of pharmacy,

Barkatpura-500027,Hyderabad.

Email: [bhavya.khagga@gmail.com](mailto:bhavya.khagga@gmail.com), mobile no: 9963713581.

## I. 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 characterisation of macromolecules. CE uses tubes 20-100 $\mu$ m diameter and 20-100cm in length. Higher electric fields result in high efficiency and narrow peaks.

**Mass spectrometry** is one of the most widely applied analytical techniques that is used for determining of 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 vaporised and then ionised 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 molecule. The entire process of ionisation, separation, and detection results in a mass spectrum.<sup>1, 2</sup>

### CE-MS

CE-MS is an analytical technique. Mass spectroscopy is becoming popular as 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 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 expression for Electrophoretic mobility.

$$\mu_{EP} = q/f = q/6\pi r\eta s$$

$f$  = spherical particle given by stokes law

$q$  = charge

$r$  = radius of ion

$\eta$  = viscosity of solvent.

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

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

*Electro osmotic flow:*

It is caused by applying high-voltage to an electrolyte-filled capillary. This flow occurs when the buffer running through silica capillary tube has  $p^H$  greater 3 and **SIOH** groups loose a proton to become **SIO<sup>-</sup>**. The applied electric field causes free cat ions to move towards cathode creating a powerful bulk flow<sup>3,4</sup>. The rate of electro osmotic flow is governed by

$$\mu_{EOF} = \frac{\epsilon}{4\pi\eta} \times E \xi$$

$\epsilon$  = dielectric constant

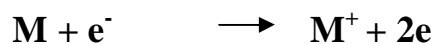
$\eta$  = viscosity

$\xi$  = zeta potential

$E$  = field strength

## 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 formation of a parent ion or molecular ion ( $M^+$ ) with an unpaired electron.



When energy supplied is more than the ionisation energy of the molecule, the molecular ion ( $M^+$ ) undergoes further fragmentation to give smaller ions and free radicals.<sup>5</sup>

## 3. MODES OF CAPILLARY ELECTROPHORESIS:

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

**3.6 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.

**3.7 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.

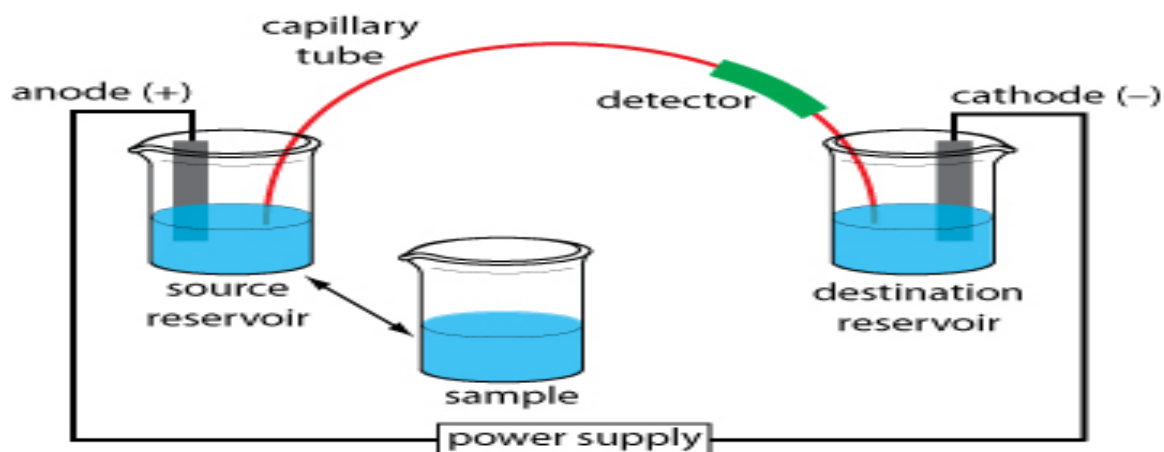


Fig.1 Schematic Representation Of Instrumentation Of Capillary Electrophoresis

#### 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 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 electro kinetic and hydrostatic type of injection. With pressure injection, the sample introduction end of the capillary is also placed momentarily into 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 does 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 $\mu$ m in internal diameter and 40 to 100cm long extends between two buffer reservoirs that also hold platinum electrodes. Sample is introduced at one end and detection at the other end. Detector should be placed at right side i.e., near cathode electrode. A provision is made on capillary tube to enter the injected

sample i.e., 0.5mm detector window. If provision is absent, then capillary tube is heated with 96-98% of CONC.H<sub>2</sub>SO<sub>4</sub> or CONC.KOH at 130<sup>0</sup>C.

**4.1.3 BGE vessel:** Buffer plays an important role where suitable buffer is selected for electro osmotic and Electrophoretic mobility based on analyte behaviour, pH constants. Buffers chosen should be of good quality and should be prepared under optimisation 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 rate 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 the y. In the example below, indirect detection is being used. Note the y axis<sup>6</sup>

## **5. 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

**5.1 Sample inlet system:** Inlet system is an interface between sample and ion source. The purpose of 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.

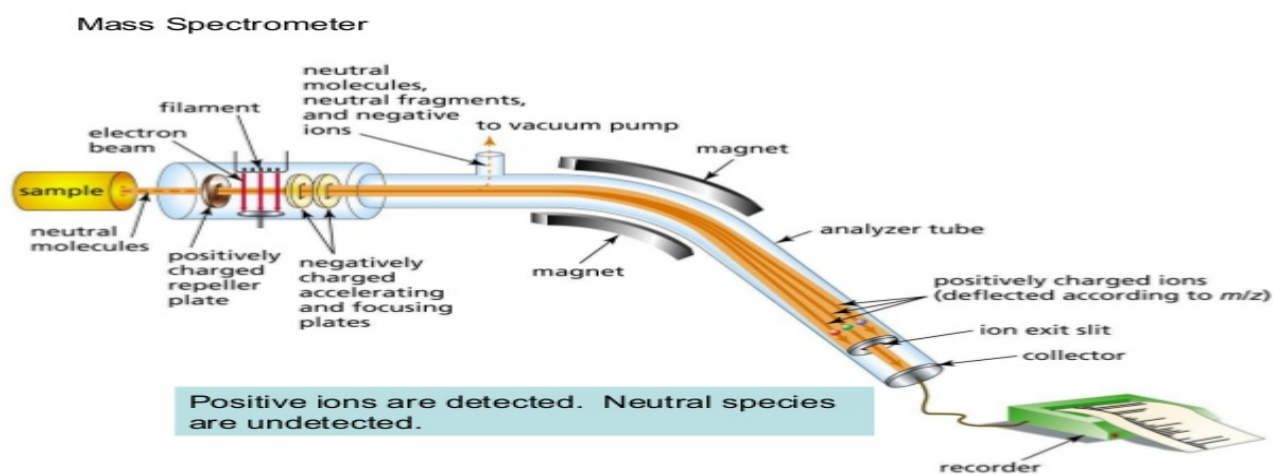


Figure: 2 Schematic Representation Of Instrumentation Of Mass Spectroscopy

**5.1.2 Ion source:** The need of ionisation arises since the mass analyser 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.

**5.1.3 Electrostatic accelerating systems:** positively charged ions are produced in the ionisation chamber are accelerated by strong electric field existing between the first accelerator plate and



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 400-4000V is 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.

**5.1.4 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 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 magnetic field ( $H$ ) in turn changes  $m/e$  and  $r$  values.

**5.1.5 Ion separator:** Ion separator is also called as mass analyser. It is the heart of mass spectrometer that takes ionised masses and separates them based on charge to mass ratios. Based on the method of sorting, many commercial varieties of mass analysers are available, few of which are:

**5.1.6 Single focussing magnetic sector analyser-** It has a horse shoe 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 tube there is provision to apply electric/magnetic field. Sample in the form of vapour is allowed through inlet and bombarded with electron beam at 70eV. It knocks off one electron from every molecule then they become +vely charged ion. As these molecules become +ve charged, they are accelerated by accelerating plates and travel in straight path. By application of electric or magnetic field they travel in curved path & molecular ions are separated according to their mass and collected. Different fragments fall on detector then mass spectrum is recorded.

**5.1.7 Double focussing 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 the high resolution. To achieve better focussing, energy has to be reduced before ions are allowed to enter the magnetic field and increasing resolving power can be obtained by using 2 mass analysers in series. In double focussing mass analyser beam is first passes radial electrostatic field.

**5.1.8 Quadrupole ion trap analyser:** it is most common type. The DC bias will cause all the charged molecules to accelerate and move away from the centre 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 like 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 which oscillates as a circle over



time. So as the charged particles fly down toward the detector, they will be travelling 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 charge 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.

**5.1.9 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 analyser 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.

**5.1.10 Ion collector:** After Ions after passing from the analyser reaches the detector which produces a signal. The signal is further amplified to give the  $m/z$  value. Readout system usually employed is a direct writing recording oscilloscope consisting of 3-5 galvanometers.

**5.1.11 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 ionisation and fragmentation giving their spectra.<sup>7,8</sup>

## 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 drop wise on MALDI target plate then dried and analysed by MS. For online coupling, a moving target with continuous contact to 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 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 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 which uses high electric field to produce electro osmotic flow for separation of ions. Analyte migrate from one

end of capillary to other based on their charge, viscosity and size. Higher the electric field, greater is the mobility.

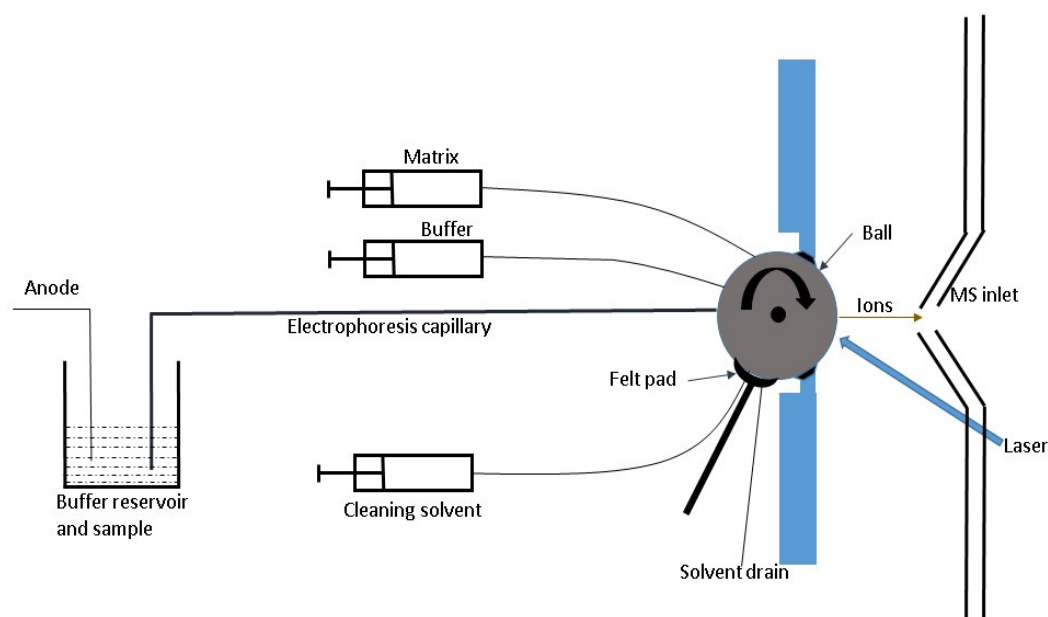


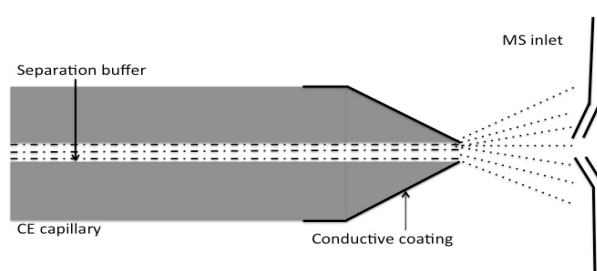
Figure., 3 Schematic Diagram Of Coupling Of CE-MS

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 analyte can be improved with 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) sheath less or Nano spray. All these configurations involve closing the high voltage circuit at the outlet of the separation capillary.

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

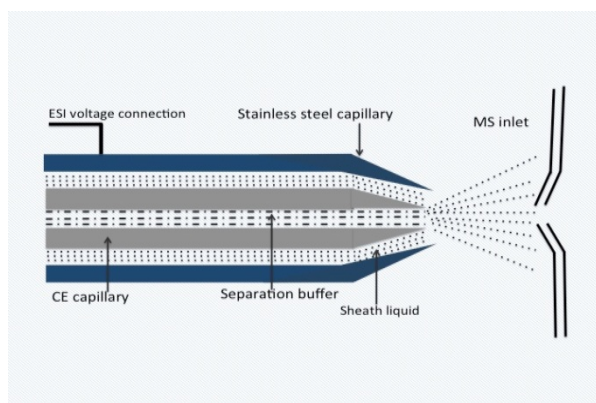
**6.1.2 Sheath less interface:** CE capillary is coupled directly to an electro spray ionization source with a sheath less interface system. The electric contact for ESI is realized by using capillary coated with conductive metal. Because no sheath liquid is used, the system has high sensitivity, low flow rates and minimum background. However, these interface designs; all have challenges including

low mechanical robustness, poor reproducibility. The latest sheath less interface design features porous ESI emitter through chemical etching. This design effectively provides robust interfacing with mass spectrometry and addresses the reproducibility challenges associated with previous designs.



**Figure, 4 Sheathless Interface Diagram**

**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 wide selection range of separation electrolyte. There might be some decrease in sensitivity due to sheath liquid.



**Figure,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 electrolyte 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.<sup>9, 10</sup>

## **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 urethra pelvic junction (UPJ) obstruction and 15 healthy new-borns. Combination of the 32 compounds in a 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:** Abs is 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.<sup>11</sup>

**7.4 For Chiral separations:** Chiral selectors such as modified cyclodextrin's (CDs) and polymeric surfactants (a.k.a. molecular micelles, MoMs) in electro kinetic chromatography (EKC), micelle electro kinetic chromatography (MEKC) and capillary electro chromatography (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 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.<sup>12</sup>

**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 behaviour 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 glycoforms 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 colour 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  $\mu\text{g ml}^{-1}$ ) and selectivity. The method developed made it possible to identify unequivocally carmine acid and laconic acids A, B and E as colouring 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 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  $\mu\text{g/mL}$  level were obtained via SIM CE/MS techniques ( $R_2 > 0.99$ ). 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  $\mu\text{g/mL}$  in human plasma ( $R_2 > 0.99$ ). The intra-assay precision ranged from 4.1 to 7.3% RSD. The intra-assay accuracy ranged from 94.0 to 104 %.<sup>13, 14</sup>

**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 analyser or as a molecular detector suitable for oxidation state determinations. Both inorganic anions and cations (including alkalis, alkaline earths, transition metals, and lanthanides) are analysed 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.<sup>14</sup>

**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.<sup>15</sup>

**7.11 Determination of Antioxidant compounds of propolis:** Different Phenolic compounds (e. g. pinobanksin 3-acetate, Naringenin, pinocembrin, Chrysenes, daidzein, Quercetin 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 chrysenes to 58 mg/100 g of raw propolis for Luteolin, were obtained.

**7.12 Amino Acid Analysis:** To analyse 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 resolution and peak shape of the amino acids, and 1 M formic acid was selected as the optimal electrolyte.<sup>16</sup>

## **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 electrophoresis for studying nucleic acid interactions were restricted to assuming slow or no equilibrium between reactants.

## **ACKNOWLEDGEMENT:**

I am most grateful to my Professor Khagga. bhavya, who gave me the opportunity to carry out this work at the Department of Pharmacy. I thank her for introducing me to the wonders of mass spectrometry and for the guidance, trust, and encouragement she provided throughout my work. Without her this would not have been possible. I am deeply grateful as well to Mogili. Sumakanth principal of Rbvvr women's college of pharmacy for being an inspiring supervisor, for the valuable daily discussions we shared, and for her constant support.

## **REFERENCES:**

1. Graham Kemp, Capillary electrophoresis: a versatile family of analytical techniques Archived April 27, 2006, at the Way back Biotechnology and Applied Biochemistry 1998; 27: 9–17.



2. Skoog, D.A.; Holler, F.J.; Crouch, S.R. "Principles of Instrumental Analysis" 6th ed. Thomson Brooks/Cole Publishing: Belmont, CA 2007.
3. Janine, G. M., and Issaq, H. J. the buffer in capillary zone electrophoresis, in *Capillary Electrophoresis Technology*, (Guzman, N.A., ed.), Marcel Dekker, Inc., New York, 1993; 119–160.
4. Cifuentes, A. and Poppe, H. Rectangular capillary electrophoresis: some theoretical considerations. *Chromatography* 1994; 39: 391–404.
5. L. Geiser, S. Cherkaoui and J.-L. Veuthey. *J. Chromatogr. A.*; Simultaneous analysis of some amphetamine derivatives in urine by non-aqueous capillary electrophoresis coupled to electrospray ionization mass spectrometry". 2000; 895: 111-121.
6. Edmondde Hoffmann, *Wiley mass spectroscopy principles and applications*. 3<sup>rd</sup> edition.
7. Gordon A. Ross, *aligenttechnologies, capillary electrophoresis-mass spectroscopy; practical implementations and applications*,
8. R.D. Smith, D.R. Goodlet and J.H. Wahl, in *Handbook of Capillary Electrophoresis*, Landers, J.P., Ed. (Marcel Dekker, New York, USA,) 1993; 185–206.
9. M. Guilhaus, *Journal of Mass Spectrometry*. 'Principles and Instrumentation in Time-ofFlight Mass Spectrometry' . 1995; 1519-32
10. P.Price, 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*. August 1991; 2(4): 336–48.
11. You Jianga, Mu-Yi Heb, Wen-Jing Zhangb , PanLuob , Dan Guob , Xiang Fanga,, Wei Xub, *elesiver, Recent advances of capillary electrophoresis-mass spectrometry instrumentation and methodology*. 2017; 28; 1640–1652.
12. SzabolcsFekete, Anne-Laure Gassner, Serge Rudaz, Julie Schappler, Davy Guillarme, *Trends in Analytical Chemistry*, Analytical strategies for the characterization of therapeutic monoclonal antibodies, 2013; 42: 74-83.
13. Christian W. Huck, Verena Huck-Pezzei, Rania Bakry, Stefan Bachmann, Muhammad Najam-ulHaq, Matthias Rainer and Günther K. Bonn, *The Open Chemical Engineering Journal*, *Capillary Electrophoresis Coupled To Mass Spectrometry for Forensic Analysis*, 2007; 1: 30-43.
14. Gianpiero Boatto\*, Maria Nieddu, Giuseppina Dess'1, Paola Manconi, Riccardo Cerri, *Journal of Chromatography A*, Determination of four thiophenethylamine designer drugs (2C-T-



- series) in human plasma by capillary electrophoresis with mass spectrometry detection 1159  
2007; 198–202.
15. Xia.Lin et.al Journal of Chromatographic Science, Determination of Gabapentin in Human  
Plasma and Urine by Capillary Electrophoresis with Laser-Induced Fluorescence Detection, ,  
July, 2015; 53(6): 986–992.
16. Jay.j.corr, joseph F.anacleto,journal of analytical chemistry,analysis of Inorganic Species by  
Capillary Electrophoresis–Mass Spectrometry and Ion Exchange Chromatography–Mass  
Spectrometry Using an Ion Spray Source,1996; 68(13):2155–2163