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Review Article

### AN OVERVIEW OF CAPILLARY ELECTROPHORESIS

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**Abstract:**

*Capillary electrophoresis is an effective separation technique where the ions are separated based on their electrophoretic mobility under an applied voltage. Capillary electrophoresis is most predominately used because it gives faster results and provides a high-resolution separation. It is one of the useful techniques as there is a large range of detection methods available. CE is an alternative for traditional methods such as gel electrophoresis and liquid chromatography and is employed to detect both high and low affinity molecular interactions, and separation of both charged and non-charged molecules. CE classified according to mode of separation on the basis of differences in charge, size and frictional force, offers fast separations with excellent efficiency. CE is an effective analytical tool for assay of pharmaceutical API including determination of drug related impurities. It possess other versatile applications like chiral, and bioanalysis of pharmaceutical API This review focuses on various aspects of capillary electrophoresis and CE-based separation modes with some advantages and disadvantages along with applications.*

**KEYWORDS:** Capillary Electrophoresis, High Resolution, Frictional Force, liquid chromatography

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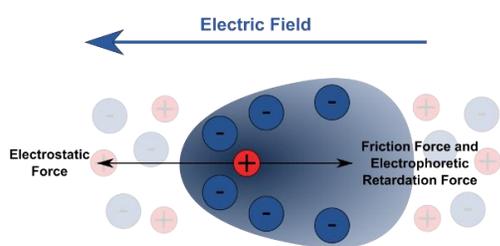


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## 1. INTRODUCTION:

Electrophoresis is the differential movement or migration of charged molecules (ions) in solution, under the influence of an electrical current. The separation of molecules occurs according to size and/or charge. Negatively charged molecules (anions) will be attracted towards anode and is called anaphoresis, positively charged molecules (cations) will move towards cathode and is called cataphoresis.

Electrophoresis is simple, rapid and highly sensitive. The rate of migration depends on: Molecular charge (net charge) Molecular shape and size Strength of the electrical field, Ionic strength, viscosity, and temperature of the medium.<sup>[1]</sup>



**FIG: 1 - ELECTROPHORESIS**

## 2. TYPES OF ELECTROPHORESIS: <sup>[2]</sup>

### 2.1. Zone Electrophoresis:

- Paper electrophoresis
- Cellulose acetate membrane electrophoresis
- Gel electrophoresis

### 2.2. Moving Boundary Electrophoresis:

- Capillary electrophoresis
- Isoelectric Focusing
- Isotachopheresis

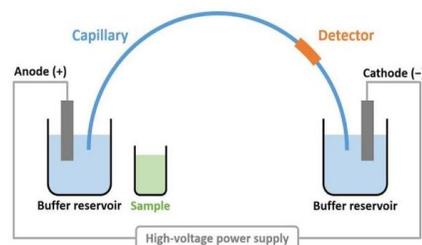
## 3. CAPILLARY ELECTROPHORESIS(CE):

Capillary electrophoresis is an analytical technique that is used to separate the ions based on their electrophoretic mobility with the use of a high applied voltage. It is dependent on the charge of the molecule, the viscosity, and the atom's radius. The rate at which the particle moves is directly proportional to the applied electric field. The Capillary electrophoresis is the most predominately used because it gives faster results and it provides a high-resolution separation. It is one of the useful techniques because there is a large range of detection methods available <sup>[3]</sup>

CE is an alternative to traditional methods such as gel electrophoresis and liquid chromatography and is employed to detect both high and low affinity molecular interactions, and separation of both charged and non-charged molecules. CE has proved to be an

efficient and versatile approach for physicochemical characterization of bioactive molecules and resolution for charged substances such as biomolecules, low molecular weight basic or acidic drugs and ions.<sup>[4]</sup>

After a long development, capillary electrophoresis techniques are now very strong in various fields. Separation is mainly based on charge-to-size ratio and high efficiencies can be obtained with short separation times. capillary zone electrophoresis (CZE) is used for different types of applications, capillary isoelectric focusing (CIEF) and capillary gel electrophoresis (CGE) are powerful for the analysis of biopolymers. The reproducibility and robustness of capillary electrophoresis (CE) was often less than that of liquid chromatography (LC) and gas chromatography (GC) but during the last decades this has been improved by reliable injection and stable electroosmotic flows (EOF) in the capillaries. The development and commercialization of suitable automated instruments played an important role in the progress of CE <sup>[5]</sup>



**FIG: 2 - CAPILLARY ELECTROPHORESIS**

## 4. HISTORY OF CAPILLARY ELECTROPHORESIS:

Endeavors in capillary electrophoresis (CE) began as early as the late 1800's. Experiments began with the use of glass U tubes and trials of both gel and free solutions <sup>[6]</sup>. In 1930, Arnes Tiselius first showed the capability of electrophoresis in an experiment that showed the separation of proteins in free solutions.<sup>[7]</sup> His work had gone unnoticed until Hjerten introduced the use of capillaries in the 1960's. However, their establishments were not widely recognized until Jorgenson and Lukacs published papers showing the ability of capillary electrophoresis to perform separations that seemed unachievable. Employing a capillary in electrophoresis had solved some common problems in traditional electrophoresis. For example, the thin dimensions of the capillaries greatly increased the surface to volume ratio, which eliminated overheating by high voltages. The increased efficiency and the amazing separating capabilities of capillary electrophoresis spurred a

growing interest among the scientific society to execute further developments in the technique.



FIG -3: JAMES JORGENSON



FIG - 4: STELLEN HJERTEN

### 5. INSTRUMENTATION:

A typical capillary electrophoresis system consists of a high-voltage power supply, a sample introduction system, a capillary tube, a detector and an output device. Some instruments consist temperature control device to ensure reproducible results because the separation of the sample depends on the electrophoretic mobility and the viscosity of the solutions decreases as the column temperature rises. Each side of the high voltage power supply is connected to an electrode. These electrodes help to induce an electric field to initiate the migration of the sample from the anode to the cathode through the capillary tube. The capillary is made of fused silica and is sometimes coated with polyimide.<sup>[8]</sup>

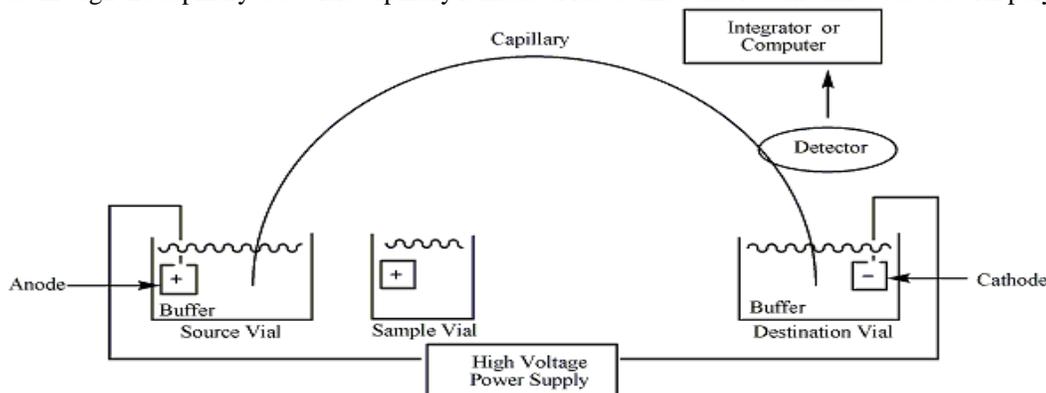


FIG 5: INSTRUMENTAL SETUP OF CE

Each side of the capillary tube is dipped in a vial containing the electrode and an electrolytic solution or aqueous buffer. Before the sample is introduced to the column, the capillary must be flushed with the desired buffer solution. There is usually a small window near the cathodic end of the capillary which allows UV-VIS light to pass through the analyte and measure the absorbance. A photomultiplier tube is also connected at the cathodic end of the capillary, which enables the construction of a mass spectrum, providing information about the mass to charge ratio of the ionic species.

### 6. PRINCIPLE:

The electrophoretic mobility of a solute ( $\mu_{ep}$ ) depends on the characteristics of the solute (electric charge,

molecular size and shape) and those of the buffer media in which the migration takes place (type and ionic strength of the electrolyte, pH, viscosity and

additives)<sup>[2]</sup>. This electrophoretic mobility called as electrophoresis. The electrophoretic velocity ( $v_{ep}$ ) of a solute, assuming a spherical shape, is given by the equation (1).<sup>[9]</sup>

$$(v_{ep}) = (\mu_{ep}) \times E$$

eq (1) Where, (E) electric intensity When an electric field is applied through the capillary filled with buffer, a flow of solvent is generated inside the capillary, called electro-osmotic flow. The velocity of the electroosmotic flow depends on the electro- osmotic flow depends on the electro-osmotic mobility ( $\mu_{eo}$ ) which in turn depends on the charge density on the capillary internal wall and the buffer characteristics.

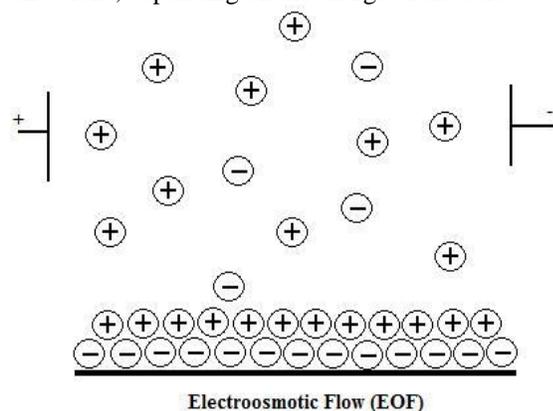
The electroosmotic velocity ( $v_{eo}$ ) is given by the equation (2).<sup>[9]</sup>

$$(v_{eo}) = (\mu_{eo}) \times E \text{ eq (2)}$$

This electrophoretic mobility called as electro-osmosis which refers to the movement of the buffer in the capillary under the influence of the electric field. The velocity of the solute ( $v$ ) is given by equation (3).<sup>[9]</sup>

$$(v) = (v_{ep}) + (v_{eo}) \text{ eq (3)}$$

The electrophoretic mobility and the electro-osmotic mobility may act in the same direction or in opposite directions, depending on the charge of the solute.



**FIG 6: ELECTRO OSMOTIC FLOW DUE TO APPLIED VOLTAGE**

**7. DIFFERENT SEPARATION MODES OF CE:** Based on the mode of the operation CE separated into following techniques:

- 7.1. Capillary zone electrophoresis (CZE)
- 7.2. Capillary Electrochromatography (CEC)
- 7.3. Capillary gel electrophoresis (CGE)
- 7.4. Capillary isoelectric focusing (CIEF)

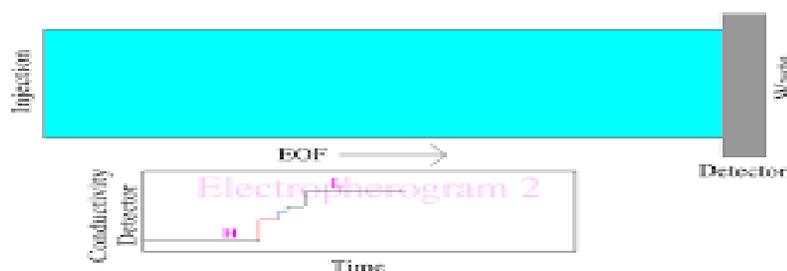
- 7.5. Non-Aqueous capillary electrophoresis (NAQCE)
- 7.6. Capillary Isotachopheresis (CITP)
- 7.7. Micellar electrokinetic chromatography (MEKC)
- 7.8. Micro-emulsion electrokinetic chromatography (ME-EKC)
- 7.9. Pressurized capillary electrochromatography (PCEC)
- 7.10. Affinity capillary electrochromatography (ACE)
- 7.11. Microfluidic Capillary electrophoresis (MFCE)
- 7.12. Immunoaffinity capillary electrophoresis (IACE)
- 7.13. Nano capillary electrophoresis (NCE)
- 7.14. Microchip-based capillary electrophoresis (Microchip-based CE)

### 7.1. Capillary zone electrophoresis (CZE)

In CZE capillary is filled with electrolyte (run buffer), the sample is introduced at the inlet and the voltage is applied. separation occurs as solutes migrate at different velocities and in discrete zones. One limitation of CZE is its inability to separate neutral species

**Applications:** CZE is very useful for the separation of proteins and peptides. It is used in the detection of biomaterials on a microscale. This method is used for analyzing the quality of antibiotics such as penicillin. It is applicable in the separation of smaller ions. It is used for the diagnosis of congenital hemoglobinopathies and used in the evaluation of serum protein abnormalities. It is also used for the analysis of monoclonal antibody charge variants.<sup>[10]</sup>

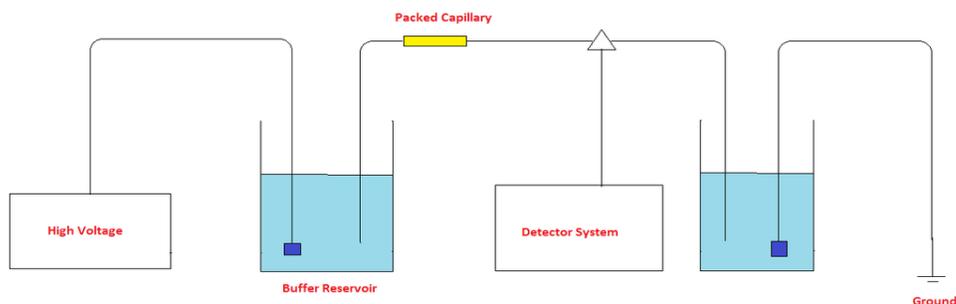
**Advantages:** The cost of maintenance is less. The sample size is very small. It has a greater speed and gives better resolution



**FIG 7: CAPILLARY ZONE ELECTROPHORESIS (CZE)**

### 7.2. Capillary Electrochromatography (CEC)

The separation mechanism is a packed column similar to chromatography. The mobile liquid passes over the silica wall and the particles. An electroosmosis flow occurs because of the charges on the stationary surface. CEC is similar to CZE in that they both have a plug-type flow compared to the pumped parabolic flow that increases band broadening.<sup>[6]</sup>



**FIG 8: CAPILLARY ELECTROCHROMATOGRAPHY (CEC)**

### 7.3. Capillary Gel Electrophoresis (CGE)

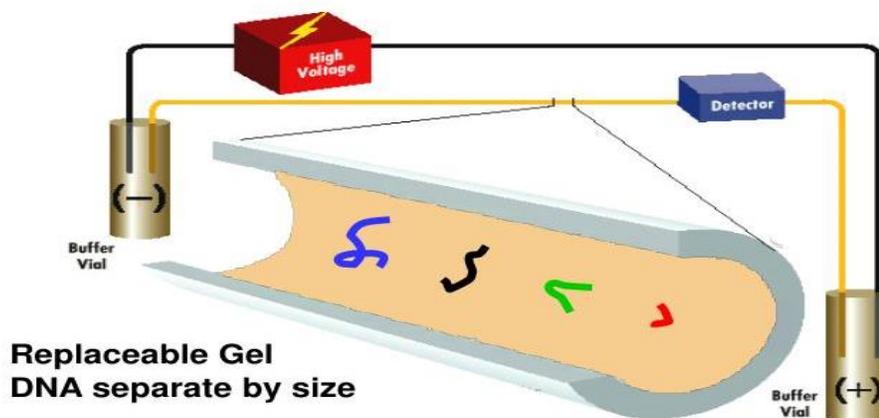
CGE uses separation based on the difference in solute size as the particles migrate through the gel. Gels are useful because they minimize solute diffusion that causes zone broadening, prevent the capillary walls from absorbing the solute, and limit the heat transfer by slowing down the molecules. A commonly used gel apparatus for the separation of proteins is capillary SDS-PAGE. It is a highly sensitive system and only requires a small amount of sample.<sup>[6]</sup>

**Applications:** Tomita M et al., stated that capillary gel electrophoresis is used for the diagnosis of aldehyde dehydrogenase-2 genotype. Capillary gel electrophoresis is used in the analysis of synthetic oligo deoxy ribo nucleotides. It is also used in the

oligomeric separation of ionic and nonionic ethoxylated polymers. Dong S. Zhao, Binayak Roy et al., demonstrated the rapid fabrication of a poly (dimethyl siloxane) microfluidic capillary gel electrophoresis system utilizing high precision machining. It is used in the characterization of antisense binding properties of peptide nucleic acids. It is also used for protein separation. CGE is used for the separation of anionic and cationic synthetic polyelectrolytes.

**Advantages:** Smaller sample volume. Consumption of buffer, gel, and reagent is less. It has shorter analysis time, higher resolution, and efficiency.

**Disadvantages:** Restriction in sample size. CGE gels are difficult to prepare. The capillary coating is often necessary to reduce electro-osmosis.<sup>[11]</sup>



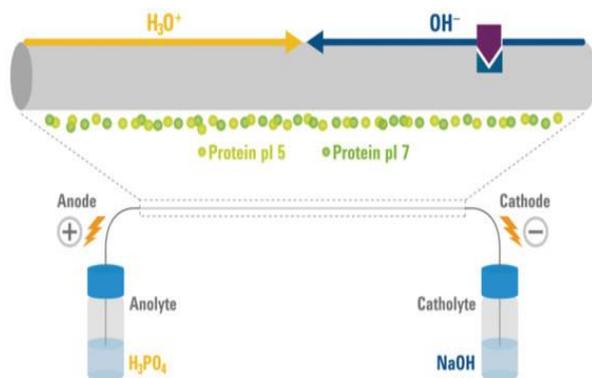
**FIG 9: CAPILLARY GEL ELECTROPHORESIS (CGE)**

#### 7.4. Capillary isoelectric focusing (CIEF)

CIEF is used for separation of amphoteric substances such as proteins, peptides, amino acids on the basis of their isoelectric point (pI). In CIEF after filling the capillary with a mixture of ampholytes and solutes, the pH gradient is formed. With a basic solution at the cathode and an acidic solution at the anode, upon application of the electrical field the charged ampholytes and proteins migrate through the medium until they reach a region where they become uncharged (pI point). This process is known as focusing.

**Applications:** Huanchun Cui *et al.* communicated about the application of ampholyte-based IEF in a poly (dimethyl siloxane) (PDMS) by using methyl cellulose (MC) to reduce the electro-osmosis and peak drift. Even though characteristics of the PDMS make it possible to fabricate microfluidic chips by using soft lithography, an unstable electroosmotic flow (EOF) and cathodic drift are significant problems when this medium is used. Thierry Backeljau reported the application of isoelectric focusing in molluscan systematics. This method was also used in the determination of apolipoprotein E phenotypes to characterize humic substances from different natural environments and for hydrophobic protein separations in glycerol-water media.<sup>[12]</sup>

**Advantages:** It has a high resolution, resulting in a greater separation of solutes. IEF is very easy to perform because the placement of the sample application is not important. It has a high capacity and resolution possible to 0.001 pH units. Disadvantage: A disadvantage of IEF is that minor bands and major bands are also seen and may cause confusion in interpretation.<sup>[13]</sup>



**FIG 10: CAPILLARY ISOELECTRIC FOCUSING (CIEF)**

#### 7.5. Non-Aqueous capillary electrophoresis (NAQCE)

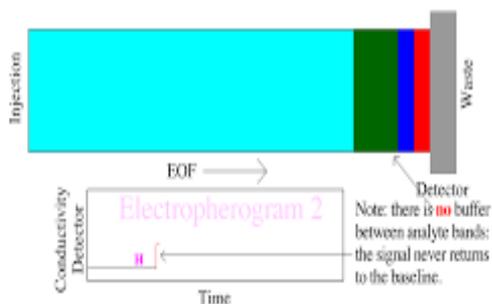
It is similar to other CE techniques in all aspects except the use of non-aqueous solvent used. CE can be performed using non-aqueous systems based on such solvents as acetonitrile, methanol, formamide, and dimethyl formamide, to which are added small amounts of anhydrous acid or buffer salts which utilized for hydrophobic analytes. Important factors which influence the choice of organic solvent for given separation are volatility, solubility parameter, viscosity, dielectric constant, UV transparency. Chiral separations of pharmaceutical racemic amines were achieved by NACE using various cyclodextrins. Separation of a ten-membered model mixture of aromatic compounds possessing a carboxylic moiety along with other functional groups performed in the pH range 4.5–8.5. non aqueous solvent composition Methanol-acetonitrile (1:1) containing equimolar concentration of sodium acetate and Tris as background electrolyte.<sup>[15]</sup> Cevdet Demir *et al* reported an optimized method for separation of metal ions such as Ag, Fe, Cr, Mn, Cd, Co, Pb, Ni, Zn, Cu in solvent acetic acid by using Imidazole as co-ion for indirect detection using UV.

#### 7.6. Capillary Isotachopheresis (CITP)

CITP is the only method to be used in a discontinuous system. The analyte migrates in consecutive zones and each zone length can be measured to find the quantity of sample present.

**Applications:** CITP method was developed by Juraj Jezek, Milan Suhaj *et al.*, and is applied for the determination of the anionic profile of orange juices to obtain some of the useful information on the authenticity or adulteration of imported and native beverage products. It is used in the determination of inorganic ions in food and feed samples and also in determining the amount of potassium present in seawater. This method helps in determining the efficiency of purification procedures during the isolation of peptides. Analysis of complex mixtures of peptides in biological fluids and tissue extracts. It also studies the interactions of peptides with low- and high molecular-mass ligands and their use for analytical and micro preparative purposes. It is also used in the evaluation of enzymatic reactions. D. Tsikas reported the applicability of the analytical capillary isotachopheresis technique to the analysis of GSH conjugates Koichi Inano *et al.*, developed a novel analytical method by using a carrier ampholyte as spacer ion for the analysis of serum lipoprotein. The application of the capillary isotachopheresis method to

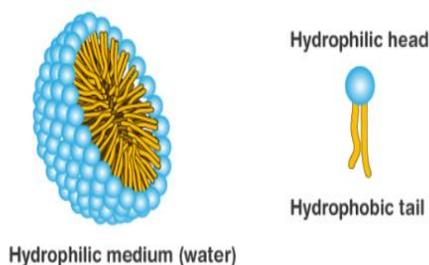
the simultaneous determination of nitrates and nitrites in meat products was studied by A. Jastrzbska [16]



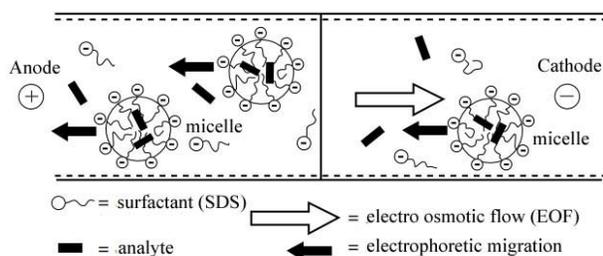
**FIG 11: CAPILLARY ISOTACHOPHORESIS (CITP)**

### 7.7. Micellar electrokinetic chromatography (MEKC) [17]

Individual surfactant molecules form micelles above CMC. The micelles are usually charged, anionic surfactants such as SDS migrate toward the anode that is in the opposite direction of the EOF. As the EOF velocity is larger than that of micelles (at the neutral or basic pH), they also move toward the cathode. During the electrophoretic migration the neutral species are partitioning in and out of the micelle. The stronger the interaction with the micelle the longer its migration time, since the micelle carries it against the EOF. The more hydrophobic compound interacts more strongly with the micelles and is retained longer. It is differential interaction between micelles and neutral molecules that causes the separation.



**FIG 12: MICELLE**



**FIG. 13: SEPARATION PRINCIPLE IN MEKC**

**Applications:** MEKC is used in the monitoring of atrazine sorption behavior on soil. It is used in the determination of ticarcillin and clavulanic acid in timentin intravenous preparation. It is also used in the separation of theophylline and its analogs in human plasma. R.H.H. Neubert *et al.*, stated an application of micellar electrokinetic chromatography for analyzing antiviral drugs in pharmaceutical semisolid formulations. The term MEKC is used for the evaluation of inhibitory effects on cytochrome P450 reaction. It is used in the determination of sultamicillin in oral pharmaceutical preparations. MEKC is used for the analysis of oligosaccharides with aminobenzoic alkyl esters as derivatization agents and also used in analyzing quercetin in plant materials.

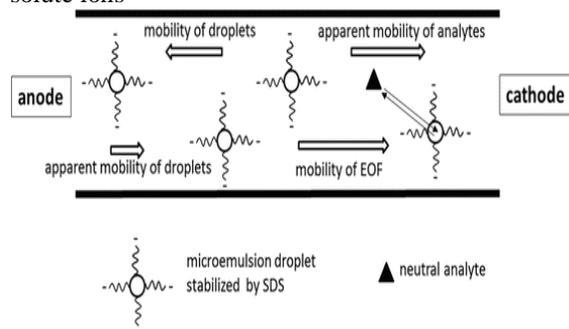
**Advantages:** Useful for biological samples. It can separate both ionic and neutral compounds with high efficiency and short retention time unlike in CE. It has high separation efficiency. Minimal consumption of the sample compared to HPLC since concentration is detected on ng/L scale. Have the ability to separate chiral compounds more efficiently. Low cost of equipment. Quicker than HPLC for separating complex samples.

**Disadvantages:** In some studies, MEKC suffers from poor reproducibility of electroosmotic flow between samples. It is generally limited to compounds that are reasonably soluble in the mobile phase. It cannot detect at low concentrations.

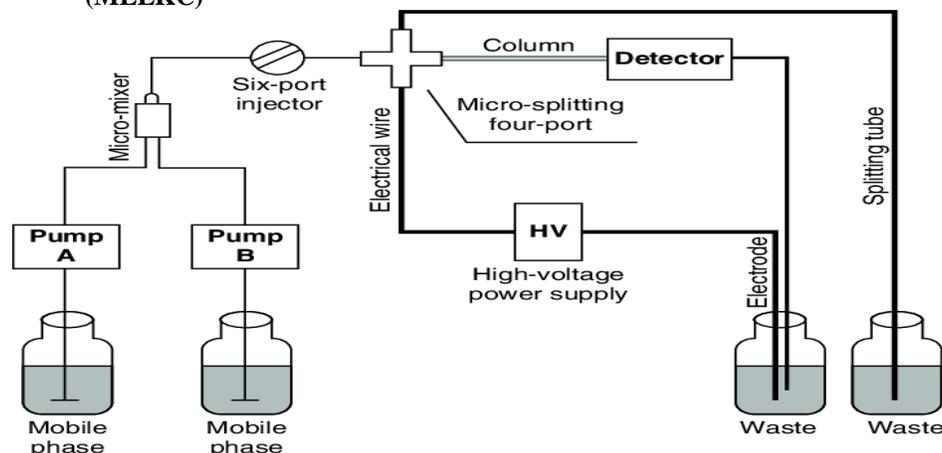
### 7.8. Micro emulsion electrokinetic chromatography (MEEKC) [18]

Micro emulsion Electrokinetic chromatography is a family of electrophoresis separation techniques which include electrophoresis and chromatography. The separation is based on a combination of electrophoresis and interactions of the analytes with additives such as surfactants that form a dispersed secondary phase moving at a different velocity, also called a pseudo stationary phase or separation carrier. A microemulsion containing ionic surfactant allows chromatographic separation to be obtained as solutes can partition between the charged oil droplets and the aqueous buffer phase. High-pH buffers such as borate and phosphate are generally used in MEEKC. These buffers generate a high electroosmotic flow (EOF) when the voltage is applied across the capillary filled with the buffer. Surfactant-coated oil droplets are negatively charged and, therefore, attempt to migrate towards the anode (away from the detector) when the voltage is applied. However, the EOF is sufficiently strong to eventually sweep the oil droplets through the detector to the cathode. If a solute is ionized then it

will electrophoretically migrate according to its size and number of charges when the voltage is applied. Repulsion from negatively charged droplets will occur if the solute is also negatively charged. Conversely if the solute is positively charged it may have ion-pair type interaction with the negatively charged droplets. The migration time obtained in MEEKC for ionized solutes reflects a combination of both the electrophoretic and chromatographic behavior of the solute ions



**FIG 14: MICRO EMULSION ELECTROKINETIC CHROMATOGRAPHY (MEEKC)**



**FIG 15: PRESSURIZED CAPILLARY ELECTROCHROMATOGRAPHY (PCEC)**

### 7.10. Affinity capillary electrochromatography (ACE)<sup>[20]</sup>

Affinity CE (ACE) is a versatile analytical technique to study a variety of bimolecular noncovalent interactions and in determining binding and dissociation constants of formed complexes. The technique uses the resolving power of CE to distinguish between free and bound forms of a receptor as a function of the concentration of free ligand. Since the initial reports in 1992 documenting the use of CE to study receptor ligand interactions ACE has been successfully used to examine a wide array of interactions including protein-drug, protein-DNA,

### 7.9. Pressurized capillary electrochromatography (pCEC)<sup>[19]</sup>

Pressurized capillary electrochromatography (pCEC) is a separation technology in which the retention mechanism is based on both chromatographic partition and electrophoresis. pCEC successfully makes use of columns with small particles of 1.5 $\mu$ m, and dramatically enhanced the efficiency, speed, peak capacity, reproducibility and sensitivity, compared to traditional HPLC and CE. CEC technology is widely applied in various fields, including pharmaceutical sciences. pCEC has several advantages over HPLC

- I. high separation efficiency and resolution using a column packed with extremely small particles
- II. high selectivity with a double separation mechanism
- III. high speed with driven force of both pressure and electroosmotic flow
- IV. quantitative sample injection with rotary type valve, and gradient solvent elution with binary solvent delivery. It has been widely applied in various fields, including pharmaceuticals.

peptide-carbohydrate, peptide-peptide, DNA dye, carbohydrate-drug, and antigen-antibody. Affinity

capillary electrophoresis (ACE) is an electrophoretic mode that takes advantage of the specific interactions of receptors, antibodies, or ligands with the analyte. In contrast to other CE modes, ACE is not dedicated to general analysis, but rather is focused on measuring molecular interactions of the solute with specific receptors.

### 7.11. Microfluidic Capillary electrophoresis (MF-CE)<sup>[21]</sup>

In practice, microfluidic systems are based on the principles of CE. A universal conductivity detector

was presented that allowed detection of charged species down to the  $\mu\text{M}$  level. Additionally, powder blasting was presented as a novel technique for direct etching of microfluidic networks. Micro fluidic system consists of an electrospray interface to a mass spectrometer was integrated with a capillary electrophoresis channel, an injector and a protein digestion bed on a monolithic substrate. This chip

provided a convenient platform for automated sample processing in proteomics applications. The design of most microfabricated electrophoresis systems is relatively simple, consisting of the following fundamental elements:

- (1) a sample injection zone,
- (2) an electrophoresis separation channel, and
- (3) a system for detection of the migrating analytes.

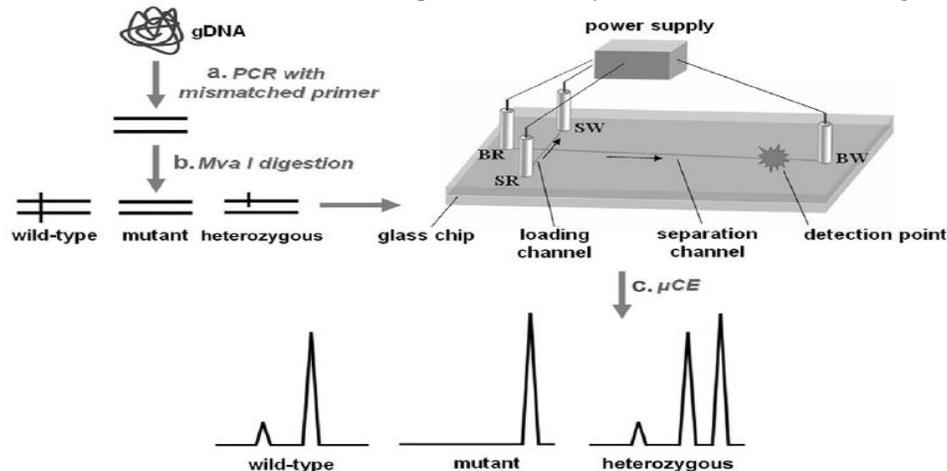


FIG 16: MICROFLUIDIC CAPILLARY ELECTROPHORESIS (MF-CE)

### 7.12. Immunoaffinity capillary electrophoresis (IACE):<sup>[22]</sup>

IACE combines the advantages of both immunoassay and capillary electrophoresis such as high specificity, high separation efficiency rapid and low consumption. IACE technology is rapidly emerging as the most promising method for the analysis of low-abundance biomarkers and involves a three-step procedure:

- bio selective adsorption
- subsequent recovery of compounds from an immobilized affinity ligand followed by
- separation of the enriched compounds. IACE has been employed for the secretion of pro-inflammatory cytokines and for determining protein biomarkers in inflammatory processes

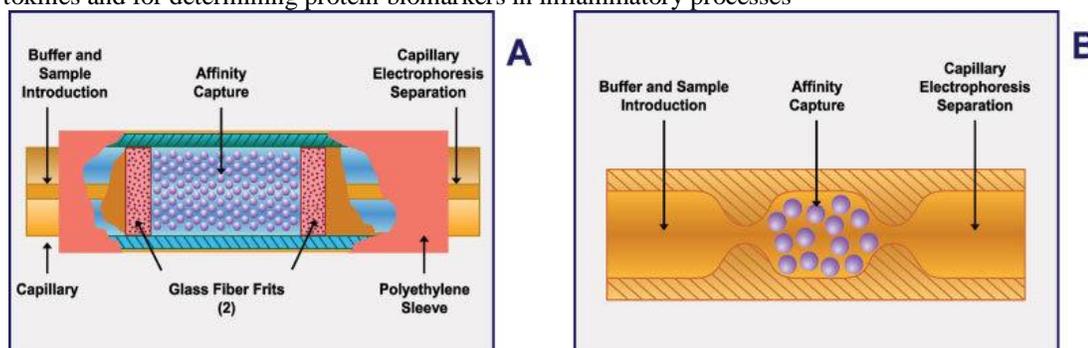


Fig 17 : Immunoaffinity capillary electrophoresis (IACE)

### 7.13. Nano capillary electrophoresis (NCE):<sup>[23]</sup>

NCE is employed especially in proteomics and genomics and it has gained increasing importance. Further, NCE is a suitable technique for samples that may be difficult to separate by Nano liquid chromatography as the principles of separation are entirely different. Moreover, lower detection limits of NCE lead to the possibility of separating and

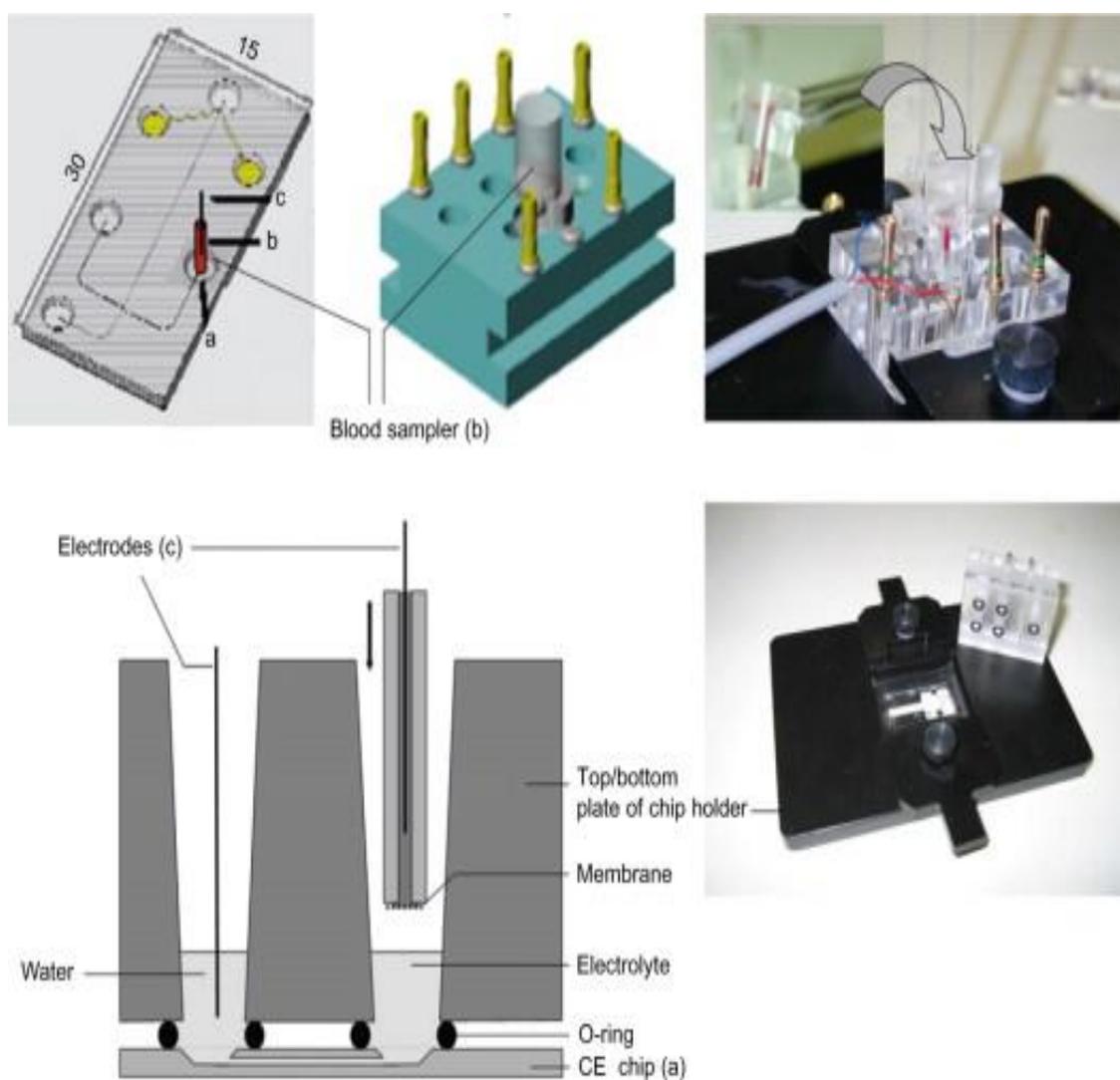
characterizing small quantities of materials, and the enzymatic reactions for analytical purposes can be conducted within the capillary. Furthermore, detection of drugs at low concentration can be achieved by NCE. The analyses of proteins and nucleic acids at low levels have been described using chip based nano-liquid chromatography and nano-capillary electrophoresis in genomics and proteomics. A

nanocapillary electrophoretic electrochemical (Nano-CEEC) chip with amperometric detection for directly capturing and analyzing zeptomole-level (30–75 zeptomoles) detection of catecholamines, including dopamine and norepinephrine (noradrenaline) that are released from coupled single PC-12 cells in an integrated fashion has been developed. This Nano-CEEC chip integrated a polydimethylsiloxane microchannel for cell sampling and biomolecule separation and a silicon dioxide nanochannel for sample pre-concentration and amperometric detection. The cell-capture voltage ranges from 0.1 to 1.5 V with a frequency of 1–10 kHz for PC12 cells, and the single cell-capture efficiency was optimized by varying the duration of the applied field. All of the processes, from

cell sampling to neurotransmitter detection were completed within 15 min

#### 7.14. Microchip-based capillary electrophoresis (Microchip-based CE):<sup>[24]</sup>

Microchip-based CE system is one of the techniques that have been developed in the area of separation on a microchip. Analysis of human serum proteins, monoclonal antibodies, determination of lactate dehydrogenase isoenzymes, pharmaceutical applications and clinical analysis applications were reported. A new method to combine chip-PCR and microchip-based capillary electrophoresis was developed and reported for detection of SARS virus



**Fig 18: MICROCHIP-BASED CAPILLARY ELECTROPHORESIS**

## 8. ADVANTAGES OF CAPILLARY ELECTROPHORESIS OVER HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

1. CE has a flat flow, compared to the pumped parabolic flow of the HPLC. The flat flow results in narrower peaks and better resolution (Figure 19)
2. CE has a greater peak capacity when compared to HPLC—CE uses millions of theoretical plates.
3. HPLC is more thoroughly developed and has many mobile and stationary phases that can be implemented.
4. HPLC has more complex instrumentation, while CE is simpler for the operator.
5. HPLC has such a wide variety of column lengths and packing, whereas CE is limited to thin capillaries.
6. Both techniques use similar modes of detection. Can be used complementary to one another.<sup>[25]</sup>

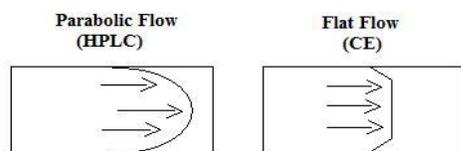


FIG 19: HPLC VERSUS CE FLOW PROFILES

## 9. CHARACTERISTICS OF CE

- CE is performed in narrow bore (25-75  $\mu\text{m}$ ) fused silica capillaries
- High voltages (10 to 30 kV) and high electric fields (100 to 500 V/cm) are applied across the capillary
- High efficiency ( $N > 10^5$  to  $10^6$ ) and short analysis time
- Detection performed as in HPLC: on-capillary (DAD, CCD) or on-line MS
- Small sample volume required (1 to 50 nL injected)
- Small buffer consumption
- Numerous modes to vary selectivity and wide application range
- Operates in aqueous and non-aqueous media
- Simple methods development
- Automated instrumentation

## 10. ADVANTAGES OF CE<sup>[26]</sup>

- Requires small sample in the range of 0.1 to 10 nL
- It yields high speed and high-resolution separations
- The separated components which exit from one end of capillary are immediately by detectors fixed at the other end of the tube

## 11. DISADVANTAGES OF CE

- Time consuming
- Expensive
- Technical skilled procedure is required

## 12. APPLICATIONS:

### 12.1. In Pharmaceutical Analysis<sup>[27]</sup>

CE is a powerful analytical technique which is increasing in utility in the pharmaceutical industry. It is used as an alternative or complementary technique to HPLC due to its high efficiency, speed of analysis, reduction in solvent and sample consumption, and low operating cost compared to HPLC methodology. CE is used for routine, particularly for analyzing serum proteins and disease markers in many hospitals and clinics. CE technique has also dramatically increased throughput for DNA profiling in criminal investigations and CE data have been shown to be credible evidence in law courts, and forensic testing laboratories have published validated procedures. Pharmaceutical companies make extensive use of CE, in particular for chiral separations, and the technique is widely accepted by regulatory authorities such as the US Food and Drug Administration. CE has been widely adopted for analyzing biomolecules such as DNA and proteins by CE gel, steroids by MECC. Applications of CE, CEC and their derived techniques to assay active pharmaceutical ingredients (APIs), drug impurity testing, chiral drug separation, determination of APIs in biological fluids and therapeutic drug monitoring have been reported. CE has contributed immensely to better understanding of affinity interactions between cyclodextrins and chiral drugs

### 12.2. Inorganic Nanoparticles Assisted CE Pharmaceutical Applications<sup>[28]</sup>

CE has seen used in the separation and characterization of inorganic nanoparticles (Ag, Au, TiO<sub>2</sub>, Al<sub>2</sub>O<sub>3</sub>, Fe<sub>2</sub>O<sub>3</sub>), quantum dots (QD), QD conjugates with bovine serum albumin and horse radish peroxidase, and QD-conjugates with Ulex europaeus and anti-von Willebrand factor

### 12.3. CE Application In Biotechnology<sup>[29]</sup>

CE separation technique is broadly used in the biotechnology industry for carbohydrate analysis and significant improvements for the standard CE sample preparation method of glycan analysis of glycoproteins by CE-LIF and CE-MS were reported. CE found applications for the separation of microorganisms as well as the detection and isolation of *Candida albicans* fungus in human blood. The applications of microchip-based CE to the detection

and separation of DNA fragments in biotechnological and clinical research were reported. CE methods showed promise for analysis of resveratrol and other flavonoid antioxidants (glycosides and aglycones) in wine.

#### 12.4. CE Application In Biopharmaceutical Analysis<sup>[30]</sup>

CE has demonstrated to be a complementary alternative to chromatographic techniques in biopharmaceutical analysis. The complete characterization of biopharmaceutical drugs such as erythropoietin and various therapeutic monoclonal antibodies for glycosylation compositions, IgG purity, and impurities for quality control purposes are of utmost importance. Various modes of CE offer several possibilities for biopharmaceutical analysis including glycosylated therapeutic proteins, monoclonal antibodies, pharmaceutical and biopharmaceutical impurities.

#### 12.5. Chiral Application of CE in Pharmaceutical Analysis.<sup>[31]</sup>

Chiral CE is a valuable tool for chiral analysis since most pharmaceutical entities are chiral and each Enantiomer expresses distinct pharmacological activity and toxicity. Chiral separation is, therefore, necessary in pharmaceutical analysis to obtain the safe and desirable enantiomer. CE is popular for chiral drug separation due to its high resolution, simplicity, and speed, chiral drug separation due to its high resolution, simplicity, and speed.

#### 12.6. Determination of Drug Related Impurity<sup>[32]</sup>

The use of capillary electrophoresis (CE) to determine drug-related impurities is becoming established within industrial pharmaceutical analysis laboratories. The three main separation mechanisms employed are low pH (for analysis of basic drugs), high pH (for analysis of acidic drugs) and Micellar electrokinetic capillary chromatography (for the analysis of neutral and/or charged compounds).

#### 12.7. Physicochemical profiling of analytes

Zhong jiang Jia reported utility of CE as an excellent tool for physico-chemical profiling of analytes, physicochemical properties of pharmaceuticals such as acid dissociation constant (pKa), octanol-water partition coefficient (logPo/w), protein binding constant, inclusion complex constant with cyclodextrin (CD), and self-association are determined with this which possess importance in drug design, candidate selection, and drug delivery.

### 13. CONCLUSION:

CE is a potential and well known automated analytical technique that separates cationic, anionic and neutral species covering both large and small molecules by applying voltage across buffer filled capillaries. It offers various advantages such as minimal organic consumption, fast analysis time, and high degree of resolution. CE is employed in quality control of pharmaceuticals, biopharmaceuticals, separation of pharmaceutical agents and drugs; pharmaceutical analysis, assay of active pharmaceutical ingredients, chiral drug separation, detection of impurities in drugs, analysis of metal and non-metal ions, carbohydrates and their derivatives, proteins, DNA, and assessment of potency and stability of drugs. CE is almost routine in many hospitals and clinics, particularly for analyzing serum proteins and disease markers. Moreover, CE technique is widely accepted by regulatory authorities.

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