

## CODEN [USA]: IAJPBB

ISSN: 2349-7750

# INDO AMERICAN JOURNAL OF PHARMACEUTICAL SCIENCES

SJIF Impact Factor: 7.187

Available online at: <u>http://www.iajps.com</u>

## **Research** Article

## **CAPILLARY ELECTROPHORESIS**

Arsha Anand<sup>1</sup>\*, Dr. Prasobh G.R<sup>2</sup>, Mrs. Athira A.S<sup>3</sup>, Ms. Ancy A.B<sup>4</sup>, Ms. Shibina Najeem<sup>5</sup>,

Ms. Simchu R.B<sup>6</sup>

<sup>1</sup>Sreekrishna college of Pharmacy and Research Centre, Parassala. Trivandrum Dist, Kerala

Article Received: January 2023 Accepted: February 2023 Published: March 2023

## Abstract:

Capillary electrophoresis [CE] is an analytical technique performed in a thin diameter glass tube that separate ions based on their electrophoretic mobility with help of an applied voltage. CE plays an ideal role in the analysis of highly polar charged analytes, analysis of both basic, clinical pharmaceutical, in the analysis and characterization of macromolecules like proteins and carbohydrates. It is often used when rapid results are desired. In this technique, separation is based on the size and charge of the molecules or ions. Capillary electrophoresis is most predominately used because it gives faster results and provide high resolution separation. The rate at which the particle moves is directly proportional to the applied electric field that is, the greater the field strength, the faster the mobility. It is a useful technique because there is a large range of detection methods available.

**Corresponding author:** 

## Arsha Anand,

8<sup>th</sup> semester B. Pharm student, Sreekrishna College of Pharmacy and Research Centre, Parassala, Thiruvananthapuram Kerala. India 695502 Email; <u>arisharsha74@gmail.com</u> QR code

Please cite this article in press Arsha Anand et al, Capillary Electrophoresis., Indo Am. J. P. Sci, 2023; 10(03).

## **INTRODUCTION:**

Capillary electrophoresis [CE] is an analytical technique that separate ion based on their electrophoretic mobility with use of an applied voltage. The electrophoretic mobility dependent upon the charge of the molecule, the viscosity, and atom's radius. [1]

The rate at which the particle moves is directly proportional to of the applied electric field. The greater is the field strength, the faster the mobility. Neutral species are not affected, where ions only move with electric field. If two ions are of same size, then the one with greater charge will move fastest. For the of same charge, the smaller particle has less friction and an overall faster migration rate. [2]

CE technique performed in a thin diameter glass tube or capillary tube that separates molecules and ions based on their mobility under the influence of an applied voltage. In this technique, a mixture of molecules and ions is separated based on their charge and size.

The term capillary electrophoresis consists of two terms: capillary and electrophoresis. Capillary refers to a very thin glass tube of submillimetre diameter. Electrophoresis is a separation technique that sorts the pool of ions based on their size and charges using electric current. [3]

Capillary electrophoresis [CE] is a very sensitive separation technique that has been developed based on the knowledge acquired from high performance liquid chromatography [HPLC]. CE allows the separation of biomolecules with high performance where HPLC fails, and CE allows the quantitation of small molecules that cannot be separated by gel electrophoresis.

Certain CE methods are a hybrid between electrophoresis and chromatography, such as electrochromatography, for which the theoretical bases are similar and will develop more fully in the next few years. [4]

With the development of High-Performance Capillary Electrophoresis [HPCE], CE has gained a superior position in the analysis of compounds. HPCE is a recently developed technique that makes use of capillary tubes of 25-100µm internal diameter with length ranging in between 10-75cm. Upon application of high voltage of about 500Vcm<sup>-1</sup>, there occurs the separation of analytes and the generated heat gets scattered through the walls of the capillary tube. Solutes then detected by suitable detectors. The separation process gets completed within 10-15 minutes. [5]

Capillary electrophoresis [CE] is one of the possible methods for analysing complex samples. In High Performance Liquid Chromatography [HPLC] and gas chromatography [GC] separation force is the difference in affinity or difference in boiling point of sample components with a stationary phase. The most important factor in both techniques is the polarity of a sample element. In CE the separation power is the difference in charge to size ratio. Not a flow through the column, but the electric field will do the separate. Analytes can be separated according to ionic mobility. [6]

## History of capillary electrophoresis:

Endeavors began capillary electrophoresis [CE] experiments as the early 1800s. He began with the use of glass tube and trials of both gel and free solutions. In 1930, Arnes Tiselius first showed the capability of electrophoresis in an experiment that showed the separation of proteins in free solutions. His worked unnoticed until Hjerten introduced the use the use of capillaries in 1960.<sup>7</sup> He separate some UV-absorbing compounds using a 300µm capillary tube.

Then, the technique has developed by Virtanen, Mikkers, Everaerts, Verheggen, who use small diameter Pyrex and Teflon tubing to separate inorganic and organic ions. In the late 1980s, Jorgenson and Luckas used narrowed silica capillary tubes; this is mostly where the term capillary electrophoresis was popularised. [8]

Since 1986 a new "form" separation science, namely capillary electrophoresis (CE) has been accepted as both as a routine and a research technique for the analysis of wide variety of analytes including drugs. By 1987 the first commercial instrument for the preparation has been introduced.

CE has gradually developed into a highly mature and versatile separation technique. In modern pharmaceutical laboratories, CE is still important technique, but its application in pharmaceutical analysis is less widespread. The rapid developments in liquid chromatography have a greater extent over taken CE techniques. [10]

#### Instrumentation of capillary electrophoresis:

Atypical CE system consists of a high-voltage power, a sample introduction system, a capillary tube, a detector and output device. Some instruments include a temperature control device to ensure reproducible results. This is because

the separation of the sample depends on the electrophoretic mobility and viscosity of the solutions decreases as the temperature rises. [9]



Figure; 1 Instrumentation of capillary electrophoresis

A quite simple in design instrumentation is required for capillary electrophoresis. The basic instrument of capillary electrophoresis includes sample introducer, buffer solution, capillary, detector, high voltage power supply, a computer.

Capillary Electrophoresis (CE) system consists of a fused silica capillary filled with an aqueous buffer electrolyte. The two ends of the capillary dip into containers of the electrolyte, one holding the anode and the other holding the cathode. (fig.1)

## Sample introduction:

The sample was introduced by inserting the anode end of the capillary into the sample vial and then applying an electric field to the sample vial leading to electrokinetic injection or by applying pressure on the vial to produce hydrodynamic injection. Other modes of hydrostatic injection, electromigration and electroosmotic injection.

#### **Buffer solution:**

The nature and consistency of the buffer solutions must be constantly monitored as it likely to be affected by the migration and accumulation of solutes.

#### **Capillaries:**

## **Fused Silica capillaries**

Fused silica capillaries have 30to 100 cm long with internal diameter of 50 to  $100 \mu m$  and external diameters of 200 to  $400 \mu m$  constitute the standard equipment although glass and Teflon capillaries are

also used. Fused silica are capillaries coated externally with polyamide for protection.

## The applied field

Capillary electrophoresis requires high strength electric fields to achieve rapid and efficient separation. The high voltage power supply needs to provide voltages between 20 and 100kV. Typical current should be between 50 to  $200\mu$ A at constants voltage.

#### **Detector:**

On column system are mostly used where the UV absorbance or fluorescence is measured while the material flows through a short length of the capillary. The standard around the capillary tube and to use it as the detector cell for a beam of light on one side pf capillary passing through to a detector on the other side. Absorption laws the apply and the signal form the photodetector can be coupled to a recorder. The system should operate at a range of wavelength or it should incorporate a photodide (a light sensitive semiconductor diode) array to obtain complete spectral zone pass through. The data sent from the detector can be displayed can be displayed as an electropherogram.

#### Other detection method Absorbance detectors:

Absorbance detector are the most commonly used detector of micro separations, particularly for CE, they are often used in universally applicable detection techniques because many organics can be detected at 195-210 nm. Essentially all organic molecules have appreciable absorption in the low UV (160- 180 nm)

nevertheless, accesses to this wavelength region are particularly problematic due to absorbance by optics and air gases. Therefore, while UV- visible absorption techniques are applicable to an abundance of solutes, they are not truly universal.

#### **Fluoresence Detectors:**

Sample components that are naturally fluorescent or that can be suitably modified can be detected fluorescence detectors. Laser- induced fluorescence (LIF) detector is commonly used as they offer improvised selectivity and high selectivity. It is most sensitive method, small volume detection method developed to data. Single molecules detection has been demonstrated in a capillary using LIF.

#### **Electrocemical Detection:**

Electrochemical detection for the CE can be divided into three main categories;

- Potentiometric detection
- Conductivity detection
- Amperometric detection

#### **Potentiometric detection:**

Potentiometric detectors are based on classical ionselective micro molecules and have able to detect extremely small quantities of inorganic and organic ions in small probe volumes. The signal is produced when the ion of interest is transferred from the flowing sample stream into a lipophilic membrane phase of the detector. The presence of the analyte generates a charge in the potential difference between the internal filling solution of the sensor and the sample stream. The potential difference is a measure of the ion's activity given by the Nernst equation and is directly related to the concentration.

#### **Conductivity detection:**

In general, conductivity detectors consist of two electrodes, in contact with the electrolyte solution, across which an electrical potential has been applied. When an analyte passes through the electrode gap, the conductivity between the electrodes changes by a quantity directly related to the concentration of the ionic analyte.

#### **Amperometric detection:**

Amperometric detection is based on electron transfer or from the analyte of interest at an electrode surrounding that is under the influence on applied DC voltage. The result of electron transfer is a redox reaction at the electrode that produce a current that is directly related to the analyte concentration.

#### Mass spectrometers:

Mass spectrometers may also be used for the detection of analytes. To use these detectors, the outlet of capillary tube is coupled with an ions source that uses electrospray ionization. The resultant ions are then detected in the mass spectrometer. The combination of mass spectrometer with CE is commonly preferred for the determination of macromolecules like proteins peptides, DNA fragments.

## **Raman-based detectors:**

Raman- based detected is useful for obtaining qualitative information (ie, structural details) about the analytes being separated. However, in order for solutes to be detected, they must be Raman-active (polarizable). In general, the signal is obtained by monitoring changes in intensity and frequencies of scattered of scattered light induced by solutes passing through the detection zone.<sup>12</sup>

#### **Procedure of capillary electrophoresis:**

- Fill the tube with the sample solution.
- Carry out the sample application by high voltage injection or by pressure injection.
- Apply a high voltage of 20kV.
- Two force acts on the proteins; The attraction of first the negatively charged proteins toward the positive electrode anode and second, an electro-osmosis force [EOF]
- EOF drags all the molecules towards the cathode irrespective of the charge.
- During this migration, positively charged moves faster than negatively charged ones. Detection of resolved molecule is done by a UV monitor and the signal is recording in a recorder.
- Capillary electrophoretogram can obtain by plotting the signal against time. [13]

#### Working:

The CE works using a glass U shaped capillary tube that is open at both ends. Both ends of the capillary tube are placed into two containers containing buffer and one of the electrodes. The capillary tube end dipped in the buffer with anode behaves as positive side and the another with cathode behaves as a negative side of the electrophoretic system.

Capillary tube walls have a negative charge and positive ions in the buffer get attracted and neutralise it. This forms an electric double layer on the entire wall surface of the capillary tube. A layer of buffer cations (positive ions) coats the wall of capillary tube. This allows the molecules to flow evenly through the middle of the tube generating electroosmotic flow (EOF). EOF is the flow of liquid that is contact with a charged surface induced by the electric field The buffer used here has a positive charge; when electricity is on, it moves from anode to cathode dragging molecules with it. Thus, buffer act as a vehicle used to carry molecules during capillary electrophoresis. This will lead the molecules to moves separated from the anode to cathode along with the buffer's movement when electric current is applied. A high voltage of up to 30 kV and ahigh electric field are applied across the capillary tube in the capillary electrophoresis. [14]

## Flow profile in capillary electrophoresis:

Another key features of EOF are that it has flat flow profile, is shown in figure alongside the parabolic flow profile generated by an external pump, as used for HPLC. EOF has a flat profile because its driving force (i.e., charge on the capillary wall) is uniformly distributed along the capillary, which means that no pressure drops are encountered and the flow velocity is uniform across the capillary.



Figure; 2 flow profile of EOF

In HPLC, in which frictional forces at the column walls cause a pressure drop across the column, vielding a parabolic or laminar flow profile. The flat profile of EOF is important because it minimizes zone broadening, leading to high separation efficiencies that allow separations on the basis pf mobility difference as small as 0.05 %. [15]

## Theory of capillary electrophoresis:

There are significant differences between the nomenclature of chromatography and capillary electrophoresis. For example, a fundamental term in chromatography is retention time. In electrophoresis, under ideal conditions, nothing is retained, so the analogous term become migration time. The migration time (t<sub>m</sub>) is the time it takes a solute to move from the beginning of capillary to the detector window

Other fundamental terms also included. electrophoretic mobility,  $\mu_{ep}$  $(cm^2/Vs)$ , the electrophoretic velocity, vep (cm/s), and the electric field strength, E (v/cm). The relationships between these factors are shown in Equation 1.

$$\mu_{ep} = \frac{v_{ep}}{E} = \frac{L_{d/tm}}{V/L_t}$$

## **Electrophoresis and capillary electrophoresis:**

The separation of compounds in capillary electrophoresis depends upon the velocity of individual compounds and the applied electric field, (E)

Velocity =  $\mu_{ep} E$ 

Where  $\mu_{ep}$  is Electrophoretic mobility

The electrophoretic mobility is measure of the particle's tendency to move through the medium at applied electric field and thus changes with the medium and the particle. In experiment, tabulated values of electrophoretic mobility often differ from experimental value, and experimentally determined value are called "effective mobility" and can change radically with different solvents and with different solutions pH. The electrophoretic mobility depends on the frictional drag  $(F_f)$  exerts on the particle medium and the electrical force  $(E_f)$  exerted to move the particle.

$$\mu_{ep} = \frac{E_f}{F_f}$$

Where the electrical force is dependent on the charge of the ion (q) and the strength of the electrical field (E).  $E_1$ 

$$f = E q$$

The frictional force can be described as

 $F_{\rm f} = -6\pi \eta r v$ 

Where r is the ion radius, v is the ion velocity and  $\eta$  is the solution viscosity.

In slab electrophoresis, the sample is placed on the gel and then the electrical field is applied. The force of electrical field causes the sample to progress down the slab, accelerating until it experiences intermolecular or friction interactions with medium which is equal to the force caused by the electrical field, and then sample proceeds at a constant rate. Once it reached a steady velocity, it can be shown that

$$\mu_{\rm ep} = \frac{q}{6\eta\pi rv}$$

This equation shows, that highly charged small particles moves quickly, especially with a low viscosity whereas less charged larger particles move slowly, especially through a more viscous medium. [17]

#### **Electroosmosis/ Electroosmotic flow:**

From Equation 1 only useful for determining the apparent mobility. For calculating actual mobility, electroosmosis phenomenon is used.

One of the fundamental processes that drive CE is electroosmosis. The process depends upon the surface charge on the wall of the capillary [18]. It is called electroendosmotic flow.

The electroosmotic flow, is the movement of liquid in a porous material (such as capillary tube) caused by a difference in a potential across material. When a charged particle is put in contact with a liquid in a capillary tube, a double layer or electrical double layer (EDL) form at the wall of capillary (figure;2). This occurs at the interface of the glass capillary wall and bulk solutions.

The first layer consists of surface charge and it can be positive or negative depending on the material. As the capillaries are generally borosilicate glass, the numerous silanol (SiOH) groups cause the charge of the first layer to be negative. This layer is sometimes called the Stern layer or Helmholtz layer.



Figure;4 Capillary double layer

by

The second layer is made up of ionic particles in solution that are electronically attracted to the charge of capillary surface. As the particle in this layer are not fixed, but moves as a result of electrical and thermal energy, it is called diffuse layer.<sup>19</sup> Under the influence of the electric field, the positive ions in the diffuse part of the double layer migrate towards the cathode; which results in electroosmotic flow. The equations of electroosmotic flow are identical to those developed for electrophoresis

The electroosmotic velocity  $v_{ep}$  is defined

 $V_{eo} = \mu_{eo} E$ 

Where  $\mu_{eo}$  is the electroosmotic mobility, a constant of proportionality between the electroosmotic velocity and electric field strength. Electroosmotic mobility proportional to the dielectric constant, ( $\varepsilon$ ) of the medium and the zeta potential  $\zeta$ , at the capillary buffer interface and inversely proportional to the viscosity ( $\eta$ ) of the medium.

$$\mu_{eo} = \frac{\epsilon \zeta}{4\eta \pi}$$



Figure;5 Electro-osmotic flow

The zeta potential is largely dependent on the electrostatic nature of the capillary surface, and to a small extent, on the ionic nature of the buffer. In fused silica capillaries, electroosmosis is diminished at low pH because protons convert the charged SiO- surface to SiOH, cause decreased the zeta potential. Electroosmosis also decreases with increasing ionic strength, due to collapse of the double layer. Electroosmotic flow can be reduced by coating the capillary with a material that suppresses ionization of the silanol groups, such as polyacrylamide or methylcellulose.

The first layer given above picture is a fixed layer. It is bound tightly to the silanol group. The mobile layer (outer layer) which further forms a silanol group. When an electric field is applied, the cations on the mobile phase flow to the negatively charged cathode. It causes the electro-osmotic flow of the buffer solution. Initially, most positive ions are detected while negative ions are detected later.

By this technique, we can easily separate and analytical DNA, RNA, and a mixture of 18 amino acids. It does not involve chromatographic distribution. Therefore, it is used for macromolecule separation.<sup>21</sup>

#### Types of capillary electrophoresis:

Depending upon the buffer, types of capillaries (forms and nature of gel) as well as on the nature of any incorporated additives, the techniques of CE may be divided in several types.<sup>21</sup> There are mainly classified into two, they are;

## Continuous system:

Continuous system has a background electrolyte acting through a capillary as buffer. This can be breakdown into kinetic process (constant electrolyte composition) and steady state processes (varying electrolyte composition). They are again classified into;

Kinetic process

- Capillary zone electrophoresis (CZE)
- Capillary electrochromatography (CEC)
- Capillary gel electrophoresis (CGE)
- Micellar electrokinetic capillary chromatography (MEKC)

Steady state process

• Capillary isoelectric focusing (CIEF)

Discontinuous system

It keeps the sample in distinct zones separated by two different electrolytes. It includes,

• Capillary isotachophoresis (CITP) [22]

## Capillary zone electrophoresis:

It is the simplest form of CE. The principle of separation in this mode is based on the differences in the charge to mass the ratio of analytes. The capillary tube is filled with electrolyte (buffer solution) of suitable pH and ionic strength. The composition of buffer solution should be homogenous throughout length of the capillary. [23] Buffer solution may be acidic (phosphate, citrate) or basic (borate) or amphoteric substance (a molecule that possesses both acidic or alkaline function). [24]

Capillary zone electrophoresis, [CZE] is also known as free solution capillary electrophoresis. It is the most commonly used technique of six methods. A mixture in a solution can be separated into its individual components quickly and easily. The separation is based on the difference in electrophoretic mobility, which is directly proportional to the charge on the molecules, and inversely proportional to the viscosity of the solvent and radius of the atom.<sup>25</sup>

#### Capillary gel electrophoresis (cge):

The mode of CGE is similar to the gel electrophoresis technique. The capillary is filled with a polymer solution composed of either agarose gel or polyacrylamide that creates a molecular sieve. polyacrylamide gel is usually used due to the greater stability to the applied electric field but agarose gel is unable to withstand heat produced applied electric field. The molecular sieve so formed is known as replaceable physical gel. The mixture of analytes when allow to flow through this sieve, get separated depending upon their mass to charge ratio that is, rate of migration of the larger analytes is slower through the sieve. CGE is used in the separation of macromolecules which bear similar charges but differ in their size. [26]

#### Micelluar electrokineticcapillary chromatography:

MEKC is a separation technique that is based on solutes partitioning between micelles and the solvent. Micelles are aggregates of surfactant molecules that form when a surfactant is added to solution above the critical micelle concentration. The aggregates have polar negatively charged surfaces and are naturally attracted to the positively charged anode. Because of the electroosmotic flow towards the cathode, the micelles are pulled to the cathode as well, but a slower rate. Hydrophobic molecules will spend the majority of their time in the micelle. While hydrophilic molecules will migrate quicker through the solvent. When micelles are not present, neutral molecules will migrate with the electroosmotic flow and no separation will occur. The presence of micelles results in retention time to where the solute has little micelle interaction and retention time where strongly interacts.

The principle of separation is based on the differential partition between the micelle and the solvent. The order of separation is as follows;

Cationic micelles or analytes > neutral analytes into partitioning into a cationic micelle > EOF > neutral analytes partitioning into anionic micelles > anionic micelles or analytes.

## Capillary electrochromography (cec):

The separation is a packed column similar to chromatography. The mobile liquid passes over the silica wall and the particles. An electroosmotic flow occurs because of the charge on the stationary surfaces. CEC is similar to CZE in that they have a plug- type flow compared to the pumped parabolic flow that increases band broadening.

## Capillary isoelectric focusing (cief):

CIEF is a technique commonly used to separate peptides and proteins. These molecules are called zwitter ion compounds because they contain both positive and negative charges. The charge depends upon functional groups attach to the main chain and the surrounding pH of the environment. In addition, each molecules have an isoelectric point (pI). When the surrounding pH is equal to this pI, the molecules carry no net charge. To be clear, it is not the pH value where all the protein bases are deprotonated and all acids are protonated, but rather the value where positive and negative charges cancel out to zero. At a pH below the pI, the molecules are positive, and then negative. During a CIEF separation, the capillary is filled with the sample solution typically no EOF is used (EOF is removed by coated capillary). When the voltage is applied, the will migrates to a region where they become neutral (pH=pI). The anodic ends of the capillary tube sit the acidic solution (low pH), while cathodic end sits in basic solution (high pH).

## Capilary isotachophoresis (citp):

CITP is the only method used in a discontinuous system. It is a variant of capillary electrophoresis (CE) which is also used in the separation of inorganic ions. However, it is measured either only cations or anions at a time. The technique demands two buffers, one (A) composed of ions whose mobility exceed than that of any of the sample ions while the other (B) composed of ions whose mobility is field, the ions move towards either cathode or anode depending upon their velocities. Therefore, fastest ions would lie after buffer A and slowest ions just before buffer B. At an equilibrium each of the sample components exist as a distinct band which is in turn is proceeded and succeeded by the next slow moving sample ion and next fast moving sample ion and next fast-moving ion respectively. CITP cannot be used to separate cations and anions at the same time. [29]

## Appication of capillary electrophoresis: Clinical application of capillary electrophoresis:

Various biological sample including blood, urine, cerebrospinal fluid and tissues lysates may be analysed in clinical laboratories using CE. Of these, blood and

urine have been extensively validated. Clinical applications of CE include;

## **Diagnosis of blood disorder:**

CZE can separate serum proteins into distinct bands  $\mu$  globulins,  $\beta$  globulin,  $\gamma$  globulin. The  $\gamma$  fraction provide information on disorder caused by clonal expansion of plasma cells. Monoclonal immunoglobulin produces a narrow band near the  $\gamma$  region. Large bands (>3g/dL) of these monoclonal proteins are usually present in patients with multiple myeloma, where low concentration (<3g/dL) occur in leukemia, lymphoma monoclonal gammopathy of undermined significance.

#### Therapeutic drug monitoring:

Drugs with narrow therapeutic index may be less effective or produce toxicity outside a certain concentration range. Therefore, the blood levels of such drugs need to be closely monitored. For example, aminoglycoside antibiotics such as gentamycin, kanamycin, amikacin and streptomycin are associated with the risk of ototoxity and nephrotoxicity. CE with borate buffer at pH 10 forms UV-absorbing borate complexes that are used to measure antibiotic levels. [32]

## Forensic apllication of capillary electrophoresis:

Forensic application across the world utilizes capillary electrophoresis to analyse a wide range of evidence collected from crime sciences. Capillary electrophoresis is considered to be a fast, sensitive, and reliable detection method of explosives that can work with high workload. MEKC is reliable method for characterizing organic gunpowder components. CZE is also relied upon for the investigation of post- blast and gunshot residues and low of explosives.

#### Application of ce in pharamaceutical analysis:

Capillary electrophoresis is also used in chiral separations, to determine the efficacy, toxicity and pharmacokinetics properties of racemates of chiral pharmaceuticals. This aids in the developments of new drug candidates that may be safer and more effective.

## **Biomedical applications of ce:**

Capillary electrophoresis is successfully in identifying drugs and disease markers when it is coupled with mass spectrometric detection. Recent research has developed mass spectroscopy in capillary electrophoresis (CE-MS) for use in determining metabolic and biochemical profiles, urine profiles, and electrophoretic and chromatographic profiles.

Application of ce for industrial analysis:

Capillary electrophoresis in industry is used to analyse the product such as food additives, herbicide, animal nutrition and detergents. CZE is used to separate and identify the small molecules existing in these types of samples. This aids product developers to design products with increased effectiveness with correct levels of enzymes, proteins, peptides, nutrients and minimise the levels of contaminants. [31]

## Application of ce for drug analysis:

CE is applicable in pharma for the analysis of and related drug compounds as discussed in CE: an attractive technique for chiral separations. Its high selectivity means that provides a good resolution to separations. When separating certain compounds, amines for example, CE can use a non-reactive capillary surface at a pH chosen by the technician to provide good separation.

## Application of ce for dna fingerprinting:

After DNA has been amplified, it can be separated by CE. Separation can be at a resolution of one base pair and individual nucleotides can be identified so allowing a high-resolution map of the DNA to be created.

## Application as molecular diagonsis tool:

It is used to identify and characterize the microorganisms that cause infectious disease in order to determine the best treatment strategies. It is used to identify gene polymorphisms associated with cancer diagnosis and prognosis.

## Microchip of capillary electrophoresis:

Microchip CE first developed in 1990s, is a miniaturized device with microfluidic chambers filled with various separation matrices. Microchip CE devices have been tested for diagnosis of cancer, cardiovascular, neurological and infectious diseases. Several microchip CE devices are commercially available and being increasingly used for routine analysis.

## Capillary electrophoresis of heamogolbin:

CE has been used in a variety of in-house capillary isoelectric focusing (CIEF), capillary zone electrophoresis (CZE) assays for detection of haemoglobin (Hb) variants and the quantitation of HbA<sub>2</sub> and HbF. A commercial kit has also been produced for the analysis of haemoglobin variants and thalassemia screening. [33]

#### **CONCLUSIONS:**

Capillary electrophoresis [CE] is an analytical technique used to separate ions based on their

electrophoretic mobility with help of an applied voltage. In this technique, separation is based on the size and charge of the molecules or ions. Capillary electrophoresis is most predominately used because it gives faster results and provide high resolution separation. The rate at which the particle moves is directly proportional to the applied electric field that is, the greater the field strength, the faster the mobility. It is a useful technique because there is a large range of detection methods available.

## **REFERENCE:**

- 1. S. Gummadi and V.N. Kandula. A Review of Electrophoresis, Capillary electrophoresis and Hyphenations: capillary electrophoresis; International journal of pharmaceutical science and research; Volume 11 Issue 12; 2020, Page no:6043
- 2. Capillary electrophoresis characteristics and example: what is capillary electrophoresis. https://study.com
- 3. Instrumental methods of analysis by N. Gray, M. Calvin, S.C, page no;175.
- 4. A Textbook of Instrumental Methods of Analysis by Dr. Manish kumar page no; 3.38
- 5. Introduction to capillary electrophoresis: Introduction. Prince Technologies' CE technologies.

https://www.princetechnologies.com

- 6. Pharmaceutical analysis by David C. Lee and Michael Webb, page no; 54-55
- 7. Clarke's Analysis of Drugs and Poisons by Moffat, Osselton and Widdop, page no. 535