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A REVIEW ON ELECTROPHORESIS, CAPILLARY ELECTROPHORESIS AND HYPHENATIONS

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ABSTRACT: Electrophoresis is an effective analytical tool for separation, as it does not affect the molecular structure, and it is also highly sensitive to small differences in molecular charge and size of the analytes. Capillary electrophoresis is a separation technique where the ions are separated based on their electrophoretic mobility with an applied voltage. So far, the research field of capillary electrophoresis remains very active, as exhibited by a steady and significant flow of scientific reports on theory, separation modes, new instrumentation, and applications of CE techniques in various areas. 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. This paper is devoted to providing a brief explanation of types of electrophoresis; capillary electrophoresis, and CE-based separation modes with some advantages and disadvantages along with applications. This paper also includes some of the hyphenation techniques based on capillary electrophoresis (CE-MS, CE-ICP-MS, CE-ESI-MS, CE-MALDI-MS, and CE-NMR).

INTRODUCTION: Electrophoresis is the movement of scattered particles comparative with a fluid under the influence of a uniform electric field **Fig. 1**. The electrophoresis of positively charged particles (cations) is sometimes called as cataphoresis, whereas the electrophoresis of negatively charged particles (anions) is called as anaphoresis. The electrokinetic phenomenon of electrophoresis was discovered for the first time by Russian professors Peter Ivanovich Strakhov and Ferdinand Frederic Reuss at Moscow in 1807, who noticed that the application of a constant electric field causes the clay particles dispersed in water to migrate.

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It is mostly caused by the presence of a charged interface between the particle surface and the surrounding fluid. Electrophoresis is the basis for analytical techniques used in chemistry for separating molecules by size, charge, or binding affinity.

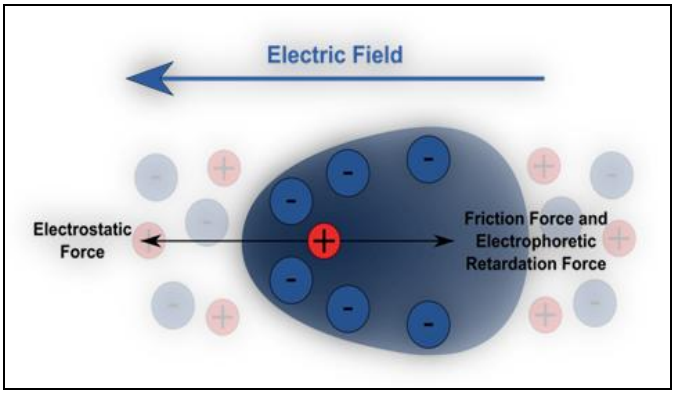

FIG. 1: ILLUSTRATION OF ELECTROPHORESIS

History of Electrophoresis: The history of electrophoresis had begun with the work of Arne Tiselius in the year of 1930s, and new separation

processes and chemical analysis techniques based on electrophoresis continued into the 21st century. With help from the Rockefeller Foundation, the "Tiselius apparatus" for moving boundary electrophoresis was developed by Tiselius, which was described in 1937 in the well-known paper "then after a new apparatus for electrophoretic analysis named as "colloidal mixtures" is formed.

This method spread slowly until the advent of effective zone electrophoresis methods in the 1940s and 1950s, where filter paper or gels are used as supporting media. By the 1960s, sophisticated gel electrophoresis methods increased widely. The method made it possible to separate biological molecules based on minute physical and chemical differences, helping to drive the rise of molecular biology. For a wide range of biochemical methods, such as protein fingerprinting, Southern blot and similar blotting procedures, DNA sequencing, and many more, gel electrophoresis and other related techniques have become an important basis. This kind of apparatus designed by Arne Tiselius enabled wide ranges of new applications of electrophoresis in analyzing chemical mixtures [1].

Types of Electrophoresis: The main types of electrophoresis in practice are:

1. Zone Electrophoresis: includes; (a) Paper electrophoresis; (b) Cellulose acetate membrane electrophoresis; (c) Gel electrophoresis

2. Moving Boundary Electrophoresis: includes; (a) Capillary electrophoresis; (b) Isoelectric Focusing; (c) Isotachophoresis

1. Zone Electrophoresis: Zone electrophoresis includes methods that produce more or less differentiated zones completely of individual components that are being separated. It involves the migration of the charged particles on the supporting media like (paper, cellulose acetate membrane, starch gel, polyacrylamide) [2]. Components that are separated are distributed into discrete zones on the support media. This supporting media is saturated with buffer solution in a small volume of samples and is being applied as a narrow band.

Advantages: It is useful in investigations of biochemical. The small quantity of sample can be analyzed. The cost is low and easy maintenance.

Disadvantages: It is unsuitable for accurate mobility and isoelectric point determination. Due to the presence of supporting medium, technical complications such as capillary flow, electro-osmosis can lead to adsorption and molecular sieving.

1.1. Paper Electrophoresis: Paper electrophoresis (PE) is useful for the separation of small-charged molecules, such as amino acids and small proteins, using a strip of paper (chromatographic paper). For this purpose, the strip paper that is used must contain at least α-cellulose at a percent of (95%) and should have a slight adsorption capacity. In this technique, the occurrence motion of a colloidal particle of solution leads to subsequent separation along the paper strip. Paper electrophoresis is easier in comparison to gel electrophoresis because it does not require much matrix preparation, and it does not contain charges that interfere with the separation of compounds. A strip of filter paper is moistened with buffer, and the ends of the strip are immersed into buffer reservoirs containing the electrodes. The samples are spotted in the center of the paper when a high voltage has been applied to the ends of the strip paper. Application of high voltage causes less diffusion of small molecules, which in raise giving better resolution, and this takes fewer periods to complete the process. Spots migrate according to their charges. After the electrophoresis process, the separated components can be detected by a variety of staining techniques like (staining with Ethidium bromide, subsequent visualization, fluorescamine chloride staining, and by dansyl chloride 5-(dimethylamino) naphthalene-1-sulfonyl chloride staining) depending upon their chemical composition.

Applications: Serum analysis for diagnostic purposes is routinely carried out by paper electrophoresis. Muscle proteins, egg white proteins, and snake, insect venom analysis is done by the technique of paper electrophoresis. Paper electrophoresis is used in the separation and identification of alkaloids. It can also be used to test the suitability of municipal water supplies, the toxicity of water, and other environmental components. Paper electrophoresis is used by the drug-testing industry to determine the presence of illegal or recreational drugs in job applicants and crime suspects.

Advantages: It is economical and inexpensive. It is easily available and easy to handle.

Disadvantages: Certain compounds like proteins, hydrophilic molecules cannot be resolved due to the adsorptive and ionogenic properties of paper, which result in tailing and distortion of component bands. Electro osmosis takes place. It is very time taking; it lasts around 14-16 h to complete the separation process.

1.2. Cellulose Acetate Membrane Electrophoresis: Cellulose acetate membranes consist of large pores and therefore hardly exert any sieving effect on proteins. This means that these electrophoretic separations are entirely based on the charges. The matrix has little effect on diffusion; because of that reason, the separated zones are relatively wide, whereas the resolution and limit of detection are seen as low. On the other hand, they are easy to handle the separation, and staining is rapid. The cellulose acetate strips are suspended in the tank of a horizontal apparatus, for that both ends are dipped into the buffer solution; no cooling is necessary during separation [3]. Then the sample is placed at one end **Fig. 2,** and then the electric field is applied; so that it allows the migration of molecules to their respective poles depending upon their charge. Because the resolution and reproducibility of separations in agarose and polyacrylamide gels are better, cellulose acetate membranes are more often replaced by gel electrophoresis.

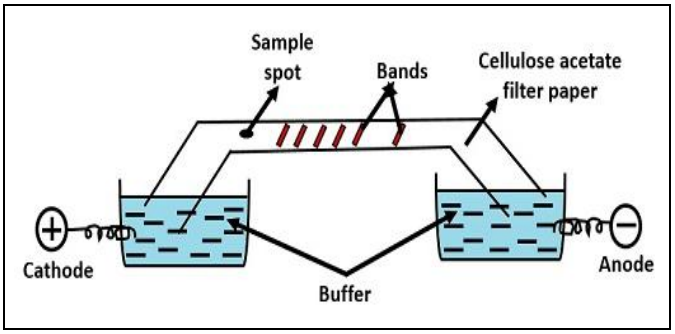

FIG. 2: CELLULOSE ACETATE ELECTROPHORESIS

Applications: This technique is widely used for routine clinical analysis and related applications for the analysis of serum or isoenzymes. It is widely used in the analysis of clinical and biological protein samples (albumins and globulins). It is also used in the separation of glycoprotein, hemoglobin, and lipoprotein.

Advantages: No tailing of proteins or hydrophilic materials. It is available in a wide range of particle sizes with suitable layer thickness. It gives sharp bands and offers a good resolution. A high amount of voltage can be applied, which will enhance the resolution.

Disadvantages: It is very expensive. The presence of sulphonic and carboxylic residue causes induced electro-osmosis during electrophoresis.

1.3. Gel Electrophoresis: Gel electrophoresis is used for separation and analysis of macromolecules (DNA, RNA, and proteins) and their fragments, based on their size and charge. It is also used in clinical chemistry to separate proteins by charge or size (IEF agarose, essentially size-independent) and also been used in biochemistry and molecular biology to separate a mixed population of DNA and RNA that fragments by length, to estimate the size of DNA and RNA fragments or to separate proteins by charge [4]. Molecules of nucleic acid are separated by applying an electric field to move the negatively charged molecules through a matrix of agarose and by other substances. The short molecules migrate farther than that of the longer ones and move faster because the shorter molecules migrate more easily through the pores of the gel. This phenomenon is called the sieving phenomenon. Proteins are separated by a charge in agarose because the pores of the gel are too large to sieve the proteins. This gel electrophoresis is also used for the separation of nanoparticles. Gel electrophoresis uses a gel as an anticonvective medium or sieving medium during electrophoresis, this gel medium which is used for the process is for the creation of the charged particle movement in an electrical field. Gels used to suppress the thermal convection which is caused by the application of the electric field, and can also act as a sieving medium, retarding the passage of molecules; gels can also simply serve to maintain the finished separation so that a post of electrophoresis stain can be applied. DNA Gel electrophoresis is usually performed for analytical purposes; often after amplification of DNA *via* polymerase chain reaction (PCR), sometimes it is also used as a preparative technique prior to the use of other methods such as mass spectrometry, RFLP, PCR, cloning, DNA sequencing or Southern blotting for further characterization.

Gel electrophoresis is of two types:

- ✓ Agarose gel electrophoresis
- ✓ Polyacrylamide gel electrophoresis

1.3.1. Agarose Gel Electrophoresis: Agarose gel electrophoresis is a method of gel electrophoresis in biochemistry, molecular biology, genetics, and clinical chemistry to separate a mixed population of macromolecules such as DNA, RNA, or proteins in a matrix of agarose. Agarose is a natural linear polymer that is extracted from seaweed, which forms a gel matrix by hydrogen-bonding when heated in a buffer and allowed to cool. They are the most popular medium for the separation of moderate and large-sized nucleic acids and have a wide range of separation.

Principle: Gel electrophoresis separates DNA fragments by its size in a solid support medium such as an agarose gel. Sample (DNA) is pipetted into sample wells, followed by the application of electric current at the anodal, negative end, which causes the negatively-charged DNA to migrate towards the bottom (cathodal, positive) end. The rate of migration is proportional to the size where smaller fragments move more quickly and wind up at the bottom of the gel. The rate of the migration depends upon,

- ✓ The strength of the field
- ✓ The hydrophobicity of the DNA
- ✓ The ionic strength of the buffer
- ✓ The size, shape of the DNA
- ✓ The temperature of the buffer

DNA is visualized by an intercalating dye called Ethidium bromide. DNA fragments take up the dye as it migrates through the gel. The illumination with the help of ultraviolet light causes the intercalated dye to fluoresce. The larger fragments fluoresce more intensely. Although each fragment of a single class of the molecule is present in an equimolar proportion, the smaller fragments include a less mass of DNA; it takes up less dye, and therefore fluoresce less intensely. A "ladder" kind set of DNA fragments of known size can be run simultaneously and used to estimate the sizes of many other unknown fragments.

Properties of Agarose: Agarose is a polysaccharide substance obtained from the red algae Porphyra umbilicalis. Its systematic name is (1 4)-3, 6-anhydro-a-L-galactopyranosyl-(1 3)-β-D-galactopyranan**.** Agarose gel is a three-dimensional matrix that is formed of helical agarose molecules in supercoiled bundles. This 3-D structure is held together with hydrogen bonds, which can, therefore, be disrupted by heating back to a liquid state. Agarose gel has a gelling temperature of 35-42 °C and with a melting temperature of 85-95 °C. Low-melting and low-gelling agarose made through chemical modifications are also available.

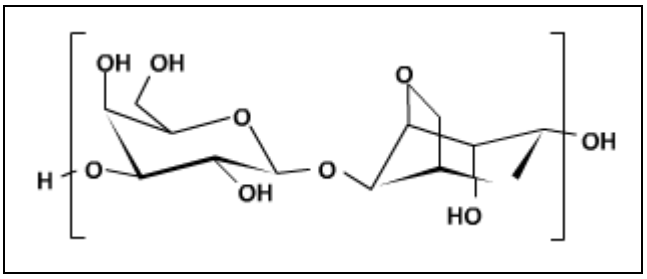

FIG. 3: STRUCTURE OF AGAROSE

The pore size of an agarose gel is large and has good gel strength, which makes it suitable for an anticonvection medium for the electrophoresis of DNA and large protein molecules. The pore size of a 1% gel has been estimated from 100 nm to 200–500 nm [5], and this gel strength allows gels to dilute as 0.15% to form a slab for gel electrophoresis. The low-concentration gels (0.1-0.2%) however, are fragile and therefore hard to handle. Agarose gel resolving power is lesser than the polyacrylamide gel for DNA but has a greater range of separation, and is therefore used for DNA fragments usually of 50-20,000 base pairs in size. The resolution limit for the standard agarose gel electrophoresis is around 750 kb, but the resolution of over 6 Mb is possible with pulsed-field gel electrophoresis (PFGE). It is also used to separate a large number of proteins, and it is the preferred matrix for the gel electrophoresis of particles with effective radii larger than 5-10 nm. A 0.9% agarose of the gel has pores large enough for the entry of bacteriophage T4[6].

Applications: It is used for the estimation of the size of DNA molecules**.** It is also used in the analysis of PCR products, *e.g.,* in molecular genetics diagnosis or genetic fingerprinting. It is used in the separation of restricted genomic DNA before Southern analysis or RNA before Northern

analysis. The agarose gel electrophoresis is widely employed to estimate the size of DNA fragments after digesting with the restriction enzymes, *e.g.*, in restriction mapping of cloned DNA. Agarose gel electrophoresis was used to resolve circular DNA with different supercoiling topology and to resolve fragments that differ due to DNA synthesis. It is commonly used in the diagnosis of several diseases such as thalassemia, sickle cell anemia, hemophilia, cystic fibrosis, and other mutation analysis.

Advantages: For most applications, only single-component agarose is needed and no polymerization catalysts are required. Therefore, agarose gels are very simple and rapid to prepare. The gel can be easily poured and does not denature the samples. Recovery of the samples can also be done.

Disadvantages: Gels can melt during electrophoresis. The buffer can become exhausted. Different forms of genetic material can be run in an unpredictable form.

1.3.2. Polyacrylamide Gel Electrophoresis: Electrophoresis through agarose gels or polyacrylamide gels is the standard method that has been used to separate, identify, and purify biopolymers since both these gels are porous. A polyacrylamide gel electrophoresis is a powerful tool used to analyze RNA samples. So, when a polyacrylamide gel is denatured after electrophoresis, it provides information on the sample composition of the RNA species [7]. The polyacrylamide gel with small pores helps to examine the smaller molecules better since the small molecules can enter the pores and travel through the gel while large molecules get trapped at the pore openings. The polyacrylamide gels are chemically cross-linked gels which are formed by the polymerization of acrylamide with a cross-linking agent, usually N, N'-methylene bisacrylamide. The reaction that formed is a free radical polymerization, usually carried out with ammonium persulfate as the initiator and N, N, N', N'-tetramethylethylendiamine (TEMED) as the catalyst. The technique of a polyacrylamide gel electrophoresis is a commonly and most widely used one in the place of biochemistry and forensic chemistry, in genetics, in molecular biology, and also in biotechnology for the separation of biological macromolecules, usually proteins or nucleic acids, according to their electrophoretic mobility. The most commonly used form of polyacrylamide gel electrophoresis is sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) used mostly for the separation of proteins.

Principle: There are two types of polyacrylamide gels, commonly named dissociating and non-dissociating gels. A non-dissociating gel is a gel that separates the proteins in their native form to conserve the protein structures, functions, and activities. A dissociating gel denatures the protein into its constituent polypeptides to determine the polypeptide composition of the sample. Native gel electrophoresis is a non-denaturing gel that has a higher resolving power than that of the SDS-PAGE when used for protein separations. SDS-PAGE (Polyacrylamide Gel Electrophoresis), this is an analytical method that is used to separate components of a protein mixture based on their size. The technique is based upon the principle that a charged molecule will migrate in an electric field towards an electrode stating with an opposite sign. Here the general electrophoresis techniques cannot be used to determine the molecular weight of biological molecules because the mobility of a substance in the gel depends on both charge and size. For this purpose, the biological samples need to be treated so that they acquire a uniform charge; then the electrophoresis mobility depends primarily on size.

For this purpose, different protein molecules with different shapes and sizes need to be denatured (done with the aid of SDS) so that the proteins lose their secondary, tertiary, or quaternary structure. The proteins which are covered by SDS are negatively charged, and when loaded onto a gel and placed in an electric field, it will migrate towards the anode (positively charged electrode) and are separated by a molecular sieving effect based on the size. After the visualization by the staining (protein-specific) technique, the size of the protein can be calculated by comparing its migration distance with that of a known molecular weight ladder.

There are two variants of polyacrylamide gel, namely the gradient gel and SDS-urea gel;

- Gradient gels are often employed to separate proteins in just a single resolving gel without the need for stacking gel.
- SDS-urea gels are used when the charge of the proteins is significantly similar to the mass of the protein, such as membrane protein and Immunoprecipitates.

Applications: Estimation of protein size. It is used in the determination of protein subunits or aggregation structures and estimation of protein purity.

Advantages: Stable chemically cross-linked gel. It has greater resolving power (sharp bands). It can accommodate large quantities of DNA without any significant loss in resolution. The recovery of the DNA from polyacrylamide gels is extremely pure. By changing the concentrations of the two monomers, the polyacrylamide gels (PAGE) pore size can be altered in an easy and controllable fashion. It is helpful for good separation of the low molecular weight fragments.

Disadvantages: It is more difficult to prepare and handle, involving a longer time for preparation than agarose gels. It contains toxic monomers. The preparation of gel takes more time and often leak. There is a need for new gels for each experiment**.** The gels taken should be chemically stable, and it should be of a cross-linked gel polymer.

2. Moving Boundary Electrophoresis: The moving boundary electrophoresis is the method that allows the charged species to migrate within a free moving solution without any need for a supporting medium.

Instrumentation: It consists of a U-shaped cell filled with a buffer solution in which the electrodes are immersed **Fig. 3** at its ends. The sample applied can be of any mixture of charged components such as a protein. On applying voltage, the compounds will migrate to the anode or cathode depending on their charges. The changes in the refractive index at the boundaries of the separated compounds are detected at both ends of the solution in the cell by using a 'schlieren'.

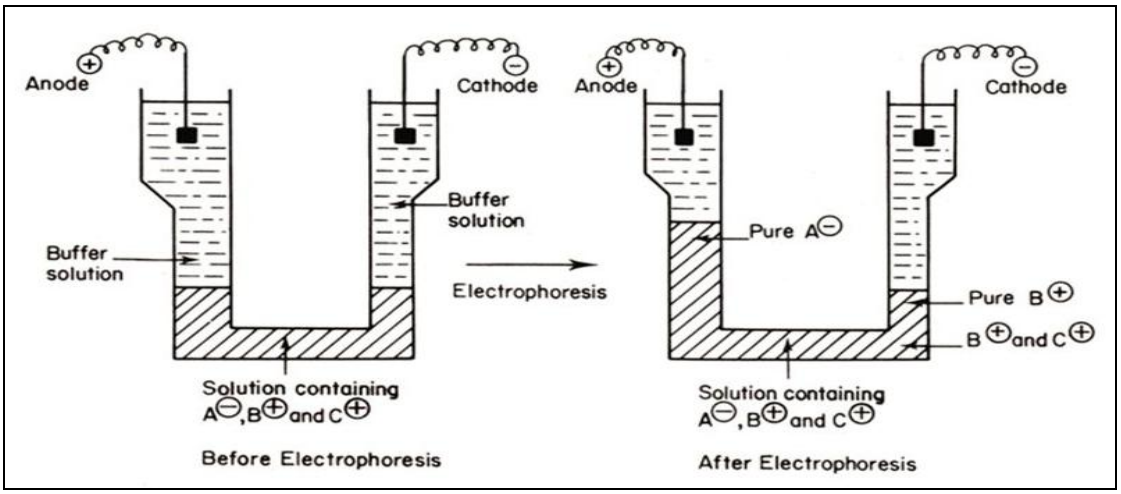

FIG. 4: THE MOVING BOUNDARY ELECTROPHORESIS

Applications: It is used to study homogeneity of a macromolecular system and in the analysis of complex biological mixtures.

Advantages: Its biologically active fractions can be recovered without the use of denaturing agents. It is a reference method for measuring electrophoretic mobility. Minute concentrations of the samples can be detected.

Disadvantages: It is a very expensive one. Very elaborative optical systems are required.

2.1. Capillary Electrophoresis: Capillary electrophoresis 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 upon the charge of the molecule, the viscosity, and the atom's radius. The rate at which the particle moves is directly proportional to that 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 the electric field.

If two ions are of the same size, then the one with the greater charge will move the fastest. For the ions of the same charge, the smaller particle has less friction and an overall faster migration rate. 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 [8].

Introduction: Endeavors in capillary electrophoresis (CE) began as early as the late 1800s. Experiments began with the use of glass U tubes and trials were done for 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 work had gone unnoticed until Hjerten introduced the use of capillaries in the 1960s.

Instrumentation: Typically, a capillary electrophoresis system **Fig. 4** consists of a high-voltage power supply, a sample introduction system, a capillary tube, a detector, and an output device. Some instruments also include a temperature control device to ensure reproducible results. This is because the sample separation 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 sometimes coated with polyimide. 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 or subjected to the column, the capillary must be flushed with the desired buffer solution.

There are two commonly used injection modes for CE-hydrodynamic and electrokinetic. The hydrodynamic injection is accomplished by the application of a pressure difference between the two ends of a capillary. In electrokinetic mode, a potential is applied to cause the sample to enter the capillary by a combination of ionic attraction and electroosmotic flow. 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.

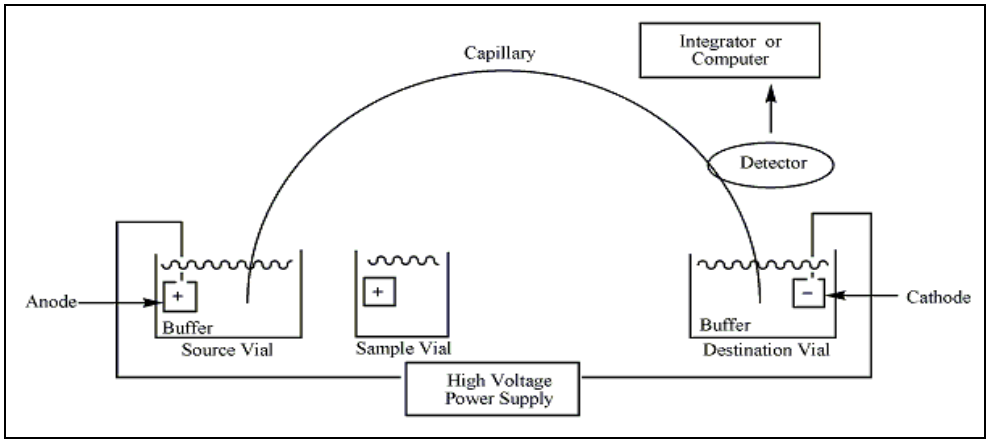

FIG. 5: INSTRUMENTAL SETUP OF CAPILLARY ELECTROPHORESIS

2.2. Modes of Capillary Electrophoresis:

2.2.1. Capillary Zone Electrophoresis (CZE): Capillary zone electrophoresis is the most widely used mode due to its simplicity. CZE allows the analysis of ionized or ionizable compounds. Analytes are simply separated according to their charge or hydrodynamic radius ratio and migrate towards anode or cathode according to their charges. Neutral compounds are not separated from this separation mode. The running buffer is extremely important in CZE and is the same in both separation vials. During a CZE analysis, the analyte apparent mobility is directly related to its migration in the capillary. The apparent mobility of an analyte is a vector sum of the electrophoretic mobility of the analyte plus the electroosmotic

mobility of the buffer. Capillary Zone Electrophoresis (CZE) is also known as a free solution capillary electrophoresis, and it is the most commonly used technique. A mixture in a solution can be separated into individual components quickly and easily. The separation mechanism is based on the differences in the charge-to-mass ratio. Fundamental to CZE is the homogeneity of the buffer solution and constant field strength throughout the length of the capillary. In CZE, separation selectivity can be achieved by simply optimizing the parameters of the carrier electrolyte, particularly pH, as well as ionic strength and the type and concentration of various EOF modifiers.

Although CZE is commonly performed with an aqueous buffer, it can also be implemented in a non-aqueous medium by using organic solvents and compatible conductive salts. The effect of replacing water with an organic solvent in CE can be understood from the fact that organic solvents possess significantly different viscosities and dielectric constants compared with water. Thus, changes in the magnitude of EOF and migration behaviors of charged analytes are expected in non-aqueous CE. Furthermore, organic solvents differ in their capacity to stabilize equilibria. Thus, in organic media, the charge status of organic analytes can be dramatically different from that in an aqueous medium, hence leading to quite different separation selectivity. Additionally, it is evidenced that organic media are capable of promoting certain mechanisms such as inclusion interaction and ion-pair formation, which enhance the possibilities of achieving the desired separation. Moreover, with a water dominated buffer, it is very difficult to conduct CE separations of hydrophobic analytes. Under such circumstances, switching to a non-aqueous buffer system would provide an efficient solution.

Finally, it is noted that, when an organic medium is utilized for CZE separations, the electrophoretic current is reduced considerably. As a result, even though capillaries of relatively wide inner diameter are employed, Joule heating is at a manageable level, thus enabling the enhancement of detection sensitivity through the usage of wide-bore capillaries. In short, non-aqueous CZE is an attractive means for extending the applicability of CE.

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.

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

2.2.2. Capillary Gel Electrophoresis (CGE): Capillary gel electrophoresis (CGE) has been resurrected in 1983 by Hjerten, albeit for protein separations. In capillary gel electrophoresis (CGE) the charged molecules are separated in capillaries that are filled with a porous gel matrix; this CGE is an analytical separation method. CGE is an adaptation of traditional slab gel electrophoresis to the capillary electrophoresis (CE) method for its advantageous features. CGE is used to separate a large number of biological molecules like protein, DNA, and RNA. In a free solution, these molecules have a similar electrophoretic migration rate which is due to similar charge-to-mass ratios. However, in CGE, the non-convective medium allows them to separate based only on their size. To separate proteins according to the size, they first have to be denatured, saturated with sodium dodecyl sulfate (SDS). The size separation is then obtained by electrophoresis of the solutes through a suitable polymer, which acts as a "molecular sieve". Here, two different gel types are used, “physical gel” and “chemical gel”. Physical gels are networks of molecular entanglements or secondary forces, including ionic, hydrogen bonding, or hydrophobic interactions. All the physical interactions that take place prevent dissolution but are reversible and can be disrupted by changes in physical conditions. Chemical gels are prepared by covalent cross-linking, have defined and controllable pore sizes, and produce high-resolution separations. Poly-acrylamide and PEG gels are commonly used with pore sizes that go as low as 1 nm, depending on the experimental requirements. Polyacrylamide gels are used widely and preferred due to their electro neutrality.

Applications: Tomita M *et al*., stated that capillary gel electrophoresis is used for the diagnosis of aldehyde dehydrogenase-2 genotype [9]. Capillary gel electrophoresis is used in the analysis of synthetic oligodeoxyribonucleotides [10]. It is also used in the oligomeric separation of ionic and nonionic ethoxylated polymers [11]. Dong S. Zhao, Binayak Roy *et al*., demonstrated the rapid fabrication of a poly (dimethylsiloxane) micro-fluidic capillary gel electrophoresis system utilizing high precision machining [12]. It is used in the characterization of antisense binding properties of peptide nucleic acids [13]. It is also used for protein separation [14]. CGE is used for the separation of anionic and cationic synthetic polyelectrolytes [15].

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.

2.2.3. Micellar Electrokinetic Chromatography (MEKC): Micellar electrokinetic chromatography (MEKC) is a hybrid of electrophoresis and chromatography. This was introduced by S. Terabe [16] in 1984; MEKC is now a widely practiced CE mode in biopharmaceutical analysis and increase in food, environmental, and clinical analysis. The main strength is that it is the only electrophoretic technique that can be used for the separation of neutral solute as well as charged ones.

In micellar electrokinetic chromatography (MEKC) a surfactant is added to the electrolyte system at a concentration above the critical micelle concentration (CMC). Surfactants are amphiphilic species, comprising both hydrophobic and hydrophilic regions. They can be anionic, cationic, zwitterionic, or nonionic. One of the most widely used surfactants in micellar electrokinetic chromatography (MEKC) is the anionic surfactant, sodium dodecyl sulfate, although other surfactants, such as cationic surfactant cetyl trimethyl ammonium salts, have also been used [17].

MEKC principle is based on the addition of a buffer solution to a micellar "pseudo stationary" phase, which interacts with the analytes according to partitioning mechanisms, just like in a chromatographic method. In this system, EOF acts like a chromatographic “mobile phase”. From a “chromatographic point of view”, the EOF’s “plug-like” flow profile is almost ideal as it minimizes band broadening, which can occur during the separation process.

The anionic SDS micelles are electrostatically attracted to the anode. The EOF transports **Fig. 5** to the bulk solution towards the negative electrode due to the negative charge on the internal surface of the silica capillaries. But the EOF is usually stronger than the electrophoretic migration of the micelles and therefore, the micelles will also migrate toward the negative electrode with a retarded velocity. The separation depends on the individual partitioning equilibrium of the different analytics between the micellar and the aqueous phase.

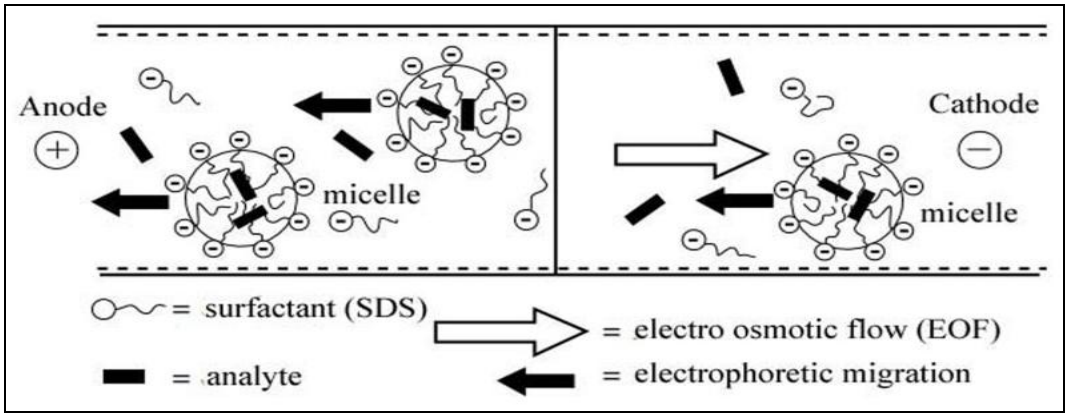

FIG. 6: SEPARATION PRINCIPLE IN MEKC

The greater percentage of the analyte is been distributed into the micelle, the slower it will migrate. Therefore, analytes that has a greater affinity for the micelles exhibit slower migration velocities compared with analytes that are mostly distributed in the bulk solution. A relatively recent

development in MEKC has been to perform separations in the absence of any EOF. This may be achieved using coated capillaries or at low pH values. This could be especially useful in the separation of acidic analytes, which would ionize at high pH values and would not interact with the negatively charged SDS micelle.

Cationic surfactants can be used in MEKC to reverse the charge on the capillary wall by absorption on the capillary wall surface through a mechanism involving electrostatic attraction between the positively charged ammonium moieties and the negatively charged Si-O-groups; when a reversal of the EOF takes place [18].

Applications: MEKC is used in the monitoring of atrazine sorption behavior on soils [19]. It is used in the determination of ticarcillin and clavulanic acid in timentin intravenous preparation [20]. It is also used in the separation of theophylline and its analogs in human plasma [21]. R.H.H. Neubert *et al.*, stated an application of micellar electrokinetic chromatography for analyzing antiviral drugs in pharmaceutical semisolid formulations [22]. The term MEKC is used for the evaluation of inhibitory effects on cytochrome P450 reaction [23]. It is used in the determination of sultamicillin in oral pharmaceutical preparations [24]. MEKC is used for the analysis of oligosaccharides with aminobenzoic alkyl esters as derivatization agents [25] and also used in analyzing quercetin in plant materials [26].

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.

2.2.4. Capillary Isoelectric Focusing (CIEF): Capillary isoelectric focusing (CIEF) is a "high resolution" electrophoretic technique used to separate amphoteric molecules such as peptides and proteins based on their isoelectric point (pI). IEF is run in a pH gradient where the pH is low at the anode and high at the cathode. The pH gradient is generated with a series of zwitterionic chemicals known as carrier ampholytes. When a voltage is applied to the ampholyte, the mixture separates in the capillary.

Ampholytes that are positively charged will migrate towards the cathode, while those negatively charged migrate towards the anode. The pH will then decrease at the anodic section and increase at the cathodic section. Finally, when the ampholyte migration ceases at some point, the ampholyte reaches its isoelectric point and is no longer charged. Initially, a solute with a net negative charge will migrate towards the anode where it encounters a buffer of decreasing pH.

Finally, the solute encounters a pH where its net charge becomes zero, the isoelectric point (pI), and migration halts. The pH gradient will be smoother if ampholytes in a solution are greater in number. The pH of the anodic buffer must be lower than that of the pI of the most acidic ampholyte to prevent migration into the analyte. Likewise, the catholyte also must have a higher pH than the most basic ampholyte. The EOF and other convective forces must be suppressed if IEF is to be effective. The capillary walls can be coated with methylcellulose or polyacrylamide to suppress EOF. The coating tends to suppress protein adsorption as well.

Applications: Huanchun Cui et al. communicated about the application of ampholyte-based IEF in a poly (dimethylsiloxane) (PDMS) by using methylcellulose (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 electro-osmotic flow (EOF) and cathodic drift are significant problems when this medium is used [27]. Thierry Backeljau reported the application of isoelectric focusing in molluscan systematics [28]. This method was also used in the determination of apolipoprotein E phenotypes [29], to characterize humic substances from different natural environments [30], and for hydrophobic protein separations in glycerol-water media [31].

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.

2.2.5. Capillary Isotachophoresis (CITP): Before 1981, isotachophoresis (ITP) was the most widely used instrumental capillary electrophoretic technique, although the capillaries were quite wide (250-500μm) by today's standards. Isotachophoresis is also a widely used technique in protein separations, where the operation is based on the development of a potential gradient rather than a pH gradient [32]. Like IEF, isotachophoresis relies on zero electro osmotic flow, and the buffer system is hetero-geneous. The principle is based on the difference in mobility of the separate ions in the electric field.

In the case of capillary isotachophoresis, separation occurs as a discontinuous system of two electro-lytes (called a leading and terminating) inside the capillary of suitable dimensions. Leading electrolyte contains ion with the highest mobility and terminating electrolyte contains ion with the lowest mobility across the separated mixtures. The sample is fed between these two electrolytes. After turning the direct current power on, the ions are partitioned in the capillary after a certain time closely to continuous zones in order of decreasing mobility (m). Zones move with a constant velocity. The conductivity detector positioned in the capillary records their passage, and the resulting record is a stair-like curve-isotachophoregram. The wave height is a qualitative characteristic of the separated substance, and its length is a quantitative indicator.

Analysis time in the capillary isotachophoresis varies according to the conditions and nature of the sample from 5 to 30 min. This technique is characterized by high accuracy and sensitivity (10^{-6} mol/l). Its other advantages are minimal sample preparation, low operating cost, and versatility. Unlike capillary electrophoresis, a simultaneous determination of anions and cations is not that much feasible by a single analysis carried on the capillary isotachophoresis.

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 [33]. It is used in the determination of inorganic ions in food and feed samples [34] and also in determining the amount of potassium present in seawater [35]. 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 [36]. D. Tsikas reported the applicability of the analytical capillary isotachophoresis technique to the analysis of GSH conjugates [37]. Koichi Inano *et al.*, developed a novel analytical method by using a carrier ampholyte as spacer ion for the analysis of serum lipoproteins [38]. The application of the capillary isotachophoresis method to the simultaneous determination of nitrates and nitrites in meat products was studied by A. Jastrzbska *et al.* [39]

2.2.6. Capillary Electro Chromatography (CEC): CEC is a hybrid technique combining electrokinetic pumping and stationary-phase retention capacity of HPLC. Columns for CEC typically have larger inner diameter than those used for CE methods, which more easily facilitates MS detection schemes. The CEC column can be packed with an alkyl silica stationary phase that may be modified for specific or selective retention. Under reversed-phases conditions, an uncoated capillary has a relatively high EOF due to the charged silanol surface and silica particles. Solvents with bulk flow move analyte through the stationary phase, producing high separation efficiencies. Frits are commonly used to prevent loss of the packed stationary phase, but often result in bubble formation and decreased EOF. In other cases, the capillary surface or packing material is chemically modified to reduce absorption effects and stabilize EOF [40]. Chiral separations are achieved in CEC by using stationary phases or by using conventional achiral stationary phases and adding chiral additives such as cyclodextrin to the mobile phase.

In electrochromatography unexplained focusing effects can occur in CEC, which can lead to the production of highly efficient peaks with theoretical plate counts in the millions. This effect has been reported for basic drugs, neutral and anionic compounds. Prediction and control of this effect would greatly enhance the possibilities in CEC.

Applications: Most of the applications of CEC reported till now concern the analysis of neutral species. Typical; LC stationary phases are mainly used. CEC has been successfully applied to separate a range of compounds from various biological matrices. Taylor et al. used the gradient elution method to separate mixtures of corticosteroids in extracts of equine urine and plasma. Lurie et al. demonstrated that CEC is a well-suited method for the analysis of cannabinoids in hashish and marijuana extracts [41]. Several enantiomeric compounds have been separated by CEC using protein encapsulated sol-gel monoliths [42]. Xin *et al.,* used CEC to monitor the chiral inversion of N-nitro-D-arginine (D-NNA), injected in rats, into L-NNA.

Bedair and E Rassi tested cationic stearyl-acrylate monoliths for the CEC separation of water-soluble and membrane proteins. Native silica monolith was used by Xie *et al.*, for the CEC separation of the basic alkaloids extracted from Coptis Chinensis. Feng and Zhu developed a CEC method for the determination of carbonyl compounds. Quaglia *et al.*, applied CEC in the quality control of the anti-inflammatory drug ibuprofen [43].

Advantages: It has high separation efficiencies. There is a possibility of a low UV detection wavelength. It can use high organic solvent contents and separation of neutral components.

Disadvantages: The main disadvantage is the formation of air bubbles, capillary fragility, analyte range, and lack of analyst experience.

Hyphenations of Capillary Electrophoresis:

Capillary Electrophoresis-Mass Spectrometry (CE-MS): Capillary electrophoresis–mass spectrometry is one of the analytical chemistry techniques which is formed by a combination of the liquid separation process of capillary electrophoresis with mass spectrometry [44]. CE-MS combines the advantages of both CE and MS to provide a high separation efficiency and molecular mass information in a single analysis [45]. It has high resolving power and sensitivity so, that it requires only minimal volume and can analyze at high speed. Ions are typically formed by electrospray ionization [46], but they can also be formed by matrix-assisted laser desorption/ionization [47] or other ionization techniques. Mass spectrometry (MS) is becoming increasingly popular as a detection method for capillary electrophoresis (CE). The combination of CE's high efficiency and high speed with the high sensitivity 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 selective detection of compounds in 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. CE coupled to MS has proven to be a powerful analytical tool for the characterization of intact proteins, as it combines the high separation efficiency of CE with the selectivity of MS.

Applications: In clinical metabolomics, capillary electrophoresis–mass spectrometry (CE-MS) has become a very useful technique for the analysis of highly polar and charged metabolites in complex biologic samples. CE-MS can be used for the profiling of polar and nonvolatile metabolites in complex aqueous sample matrices without pre-treatment or derivatization. Recently, D'Agostino *et al.,* developed a novel CE-MS approach for a comprehensive analysis of thiol-containing compounds in human plasma [48]. This technique is used in analyzing the heparin oligosaccharides [49]. Muller *et al.,* described the development of a CE-MS method aimed at the detection and quantification of bovine milk in either ovine or caprine milk samples for the analysis of the whey proteins, a-lactalbumin and various b-lactoglobulins [50]. Other pharmaceutical compounds have been analyzed by CE–MS using cyclodextrin as chiral selectors. Amino acids can be directly analyzed by CE/MS using acidic BGEs, mainly based on formic acid [51]. Gahoual *et al.,* recently reviewed the characterization of monoclonal antibodies and related products by CE including CE-MS

applications [52]. Ramoutar *et al.,* developed a CE-MS method using a sheathless low-flow porous tip interface for subnanomolar detection of polar and charged metabolites in human urine [53]. Jianjun Li *et al.,* reviewed an application to CE-MS for the characterization of bacterial lipopolysaccharides [54].

Capillary Electrophoresis-Inductive Coupled Plasma-Mass Spectrometry (CE-ICP-MS): In the 1980s, the spectroscopy and the separation science communities were witness to parallel development and maturation of two powerful instrumental techniques: capillary electrophoresis (CE) and inductively coupled plasma mass spectroscopy (ICP-MS). Capillary electrophoresis became commercially available in the latter part of the 1980s and rapidly emerged as a routine tool for many clinical and pharmaceutical applications. Another significant analytical development during the 1980s was the invention and commercialization of inductively coupled plasma mass spectrometry which was 1st described by Houk et al. This hybrid instrument exploits the energetic plasma in an ICP to destroy the sample matrix and efficiently ionize the constituent elements.

When an ICP source is combined with mass spectroscopic detection, one of the most sensitive element-specific detectors is obtained because the majority of the elements passing through the plasma are nearly completely ionized. ICP-MS can provide quantitative elemental and isotopic information with a dynamic range exceeding five orders of magnitude and instrumental limits of detection better than 1 ng for most elements [55].

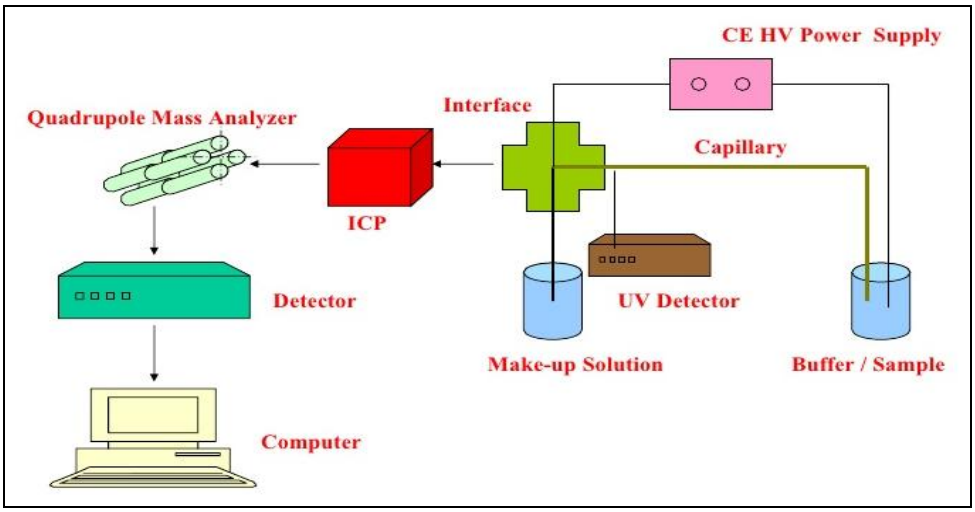

FIG. 7: CE-ICP-MS INSTRUMENT SETUP

The term CE coupled with ICP-MS has been improved rapidly upon by the sheath-flow interface design. It also includes another alternative interface based on volatile species generation for cadmium speciation in metallothioneins [56]. In recent years, capillary electrophoresis (CE) hyphenated with inductively coupled plasma mass spectrometry (ICP-MS) has become one of the preferred techniques for speciation analysis. This is the effect of many factors, including improvements in the methodology of interfacing CE and ICP-MS instruments, significant advances in the development of new approaches for the analysis of various element species in different types of samples, and the existence of a vast body of literature that supports understanding and implementation of this combined technique.

CE with ICP-MS used as an online detector has emerged as a recognized analytical tool to map metallodrug–protein interactions. Andrei R. Timerbaeva *et al.,* stated that how CE-ICP-MS, continually being developed in the past few years to streamline the existing procedures and contribute in the development of metal-based anticancer drugs via shedding light on the fate of the drug upon intravenous administration or entering the bloodstream [57].

Applications: CE-ICP-MS can be used for the separation of selenium compounds in a dynamically coated capillary applied to selenized yeast samples [58]. Some of the analytical features of CE-ICP-MS demonstrated in current research are mostly focused on gold nanoparticles. Lin *et al.,*

used gold nanoparticles as a tag to quantify albumin in human urine samples. Franze and Engelhard and Qu *et al*., adopted the combined method of interest for the characterization of dietary supplement products, with ingredients containing Au, Pt, and Pd nanoparticles [59]. Haiou Qu and Thilak K. Mudalige *et al.,* reported the development and optimization of a system consisting of capillary electrophoresis (CE) interfaced with inductively coupled plasma mass spectrometry (ICPMS) for rapid and high-resolution speciation and characterization of metallic (*e.g.*, gold, platinum, and palladium) nanoparticles in a dietary supplement [60]. CE-ICP-MS can also be used in the determination of platinum drug release and to study the liposomal stability in human plasma [61]. This technique is used to determine the stability constants of nitrate complexes [62] and to determine roxarsone and other transformation products in chicken manure [63.]

Capillary Electrophoresis-Ionization-Mass Spectrometry (CE-ESI-MS): Hyphenation of CE-MS is a powerful method to obtain high efficient, sensitive, and selective analyses. The successful coupling with electrospray ionization (ESI) source requires closed electric circuits for both the CE separation and the ESI processes. The electrospray ionization (ESI) method applies to broad classes of involatile and labile compounds. This hyphenation is widely used in a variety of fields, such as biomolecules, pharmaceuticals, metabolites, food analysis, environmental analysis, and in the investigation of technical products [64]. One of the major limitations of CE–ESI-MS is the lack of migration time reproducibility due to variations in electroosmotic flow (EOF) (CV > 10%) that serves as a natural electrokinetic pumping mechanism [65]. A wide range of interfaces has been proposed to satisfy this requirement [66]. In this sheath flow interface is one of the most common ionization techniques. This sheath liquid, containing a mixture of an aqueous solution and organic solvent, can absorb hydrogen or oxygen gas bubbles and diminish baseline noise, enabling stable CE-ESI-MS analysis. However, the flow rate of the sheath liquid is much higher than that of the electrolytes eluted from the separation capillary, leading to a large reduction in insensitivity. To overcome these problems, several novel interfaces that can reduce the sheath liquid flow rate have been developed.

Maxwell *et al*., developed a flow through microbial assisted CE-ESI-MS interface that reduced the sheath liquid flow rate to 100 nL/min and its sensitivity increases five times on average for tested amino acids compared with sheath-flow CE-MS. Sun *et al*., developed an improved electro kinetically pumped sheath-flow nano electrospray interface and applied it to proteome analysis. This interface could be used continuously for over 5000 min. and generate highly efficient and reproducible data [67].

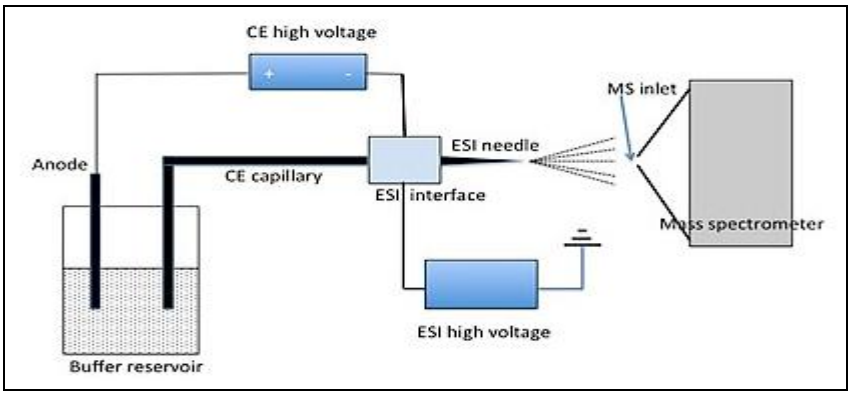

FIG. 8: CE-ESI-MS INSTRUMENTAL SETUP

Applications: Bailin Zhang *et al*., presented a new design for high-throughput of a microfabricated capillary electrophoresis/electrospray mass spectrometry (CE/ESI-MS) with automated sampling from a microwell plate for the analysis of peptides and protein digests [68]. W. Franklin Smyth has reviewed some of the applications on recent analytical methods to detect and determine the small molecular mass drug molecules [69]. Fernando Benavente, Rob van der Heijden *et al*., investigated the potential of CE coupled to ESI-MS in metabolite profiling of human urine by using

separation electrolytes at low pH [70]. This is used in the determination of stimulants, narcotics, and in-vitro metabolites [71] as well as peptide hormones of the brain and intestine [72].

CE-ESI-MS is used in the analysis of glycosaminoglycan [73] and bisphosphonates [74]. Juan Jose Berzas Nevado reviewed a new analytical method for the separation, identification, and quantification of seven phenolic acids [75]. It is also applicable in forensic toxicology [76].

Capillary Electrophoresis-Matrix Assisted Laser Desorption Ionization-Mass Spectrometry (CE-MALDI-MS): Capillary electrophoresis (CE) and matrix-assisted laser desorption/ionization-mass spectrometry (MALDI/MS) were combined in an off-line arrangement to provide separation and mass analysis of peptide and protein mixtures in the attomole range. A membrane target, precoated with the MALDI matrix, was used for the continuous deposition of effluent exiting from a CE device. A sample track was produced by linear movement of the target during the electrophoretic separation and this track was subsequently analyzed by MALDI/MS. The technique is effective for peptides and proteins, having limits of detection (signal-to-noise ratio >3) of about 50 Amol for neurotensin (1673 Da) and 250 Amol for cytochrome c (12361 Da) and apomyoglobin (16951 Da) [77]. The off-line coupling of CE with matrix-assisted laser desorption/ionization mass spectrometry (CE-MALDI-MS) offers an attractive alternative with increased flexibility for the independent optimization of CE and MS experiments and makes the CE fractions available for reanalysis or further biochemical characterization.

This offline arrangement has two main advantages: (i) better tolerance to salts and (ii) possibility to store samples/analytes. Recently, the continuous development in off-line CE-MALDI-MS has established it as a versatile technology for proteomics, metabolomics, and neuropeptidomics studies [78]. Off-line coupling of CE to MALDI, the CE effluent could be sprayed or added drop-wise on the MALDI target plate then dried and analyzed by MS. For online coupling, a moving target with continuous contact with CE capillary end is required. The moving target takes analytes into MS where it is desorbed and ionized.

Musyimi *et al*., developed a new technique where the rotating ball was used to transfer CE to MS [79]. The sample from CE is mixed with matrix coming through another capillary. As the ball rotates the sample is dried before it reaches the ionization region. This technique has high sensitivity since no makeup fluid is used.

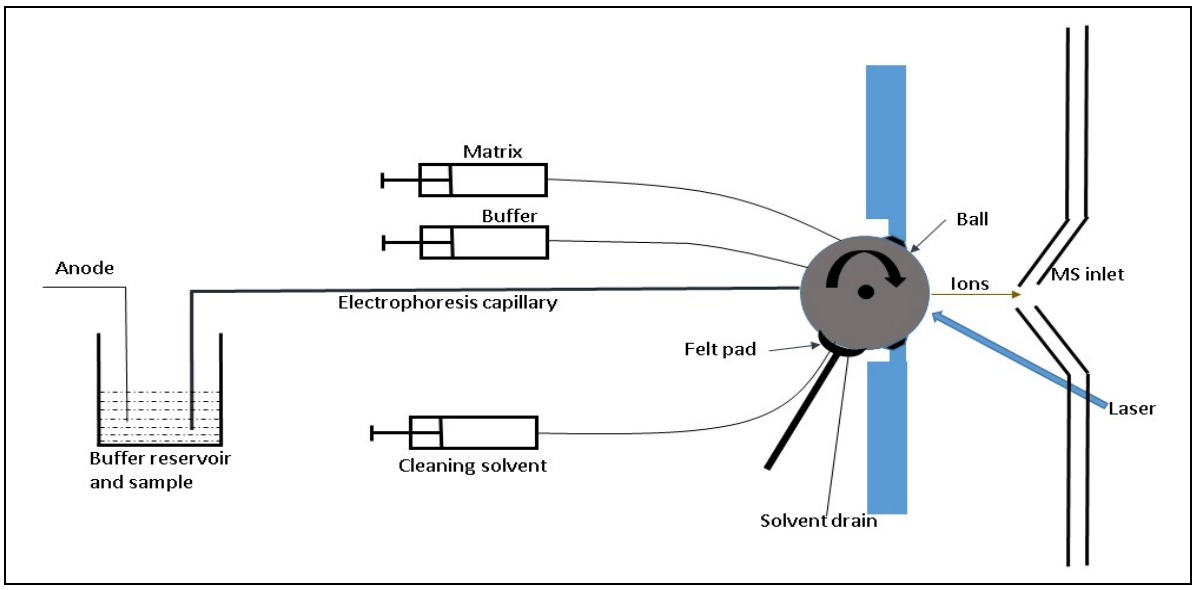

FIG. 9: CE-MALDI-MS INSTRUMENTAL SETUP

Applications: A capillary electrophoresis-matrix-assisted laser desorption/ionization time-of-flight mass spectrometry using a vacuum deposition interface was developed for the separation of a mixture of angiotensins and was found to significantly reduce ion suppression and enable

trace level detection [80]. Jason *et al*., performed direct cellular assays using off-line CE-MALDI-TOF-MS [81]. Junhua *et al*., combined CE-MALDI-MS with stable isotoping techniques for comparative crustacean peptidomics.

Capillary Electrophoresis-Nuclear Magnetic Resonance (CE-NMR): If analytes are present in relatively small amounts, hyphenated CE-NMR provides similar advantages as LC-NMR for the separation, chemical identification, and structural information. Both continuous and stopped-flow modes, similar to LC-NMR are used in CE-NMR. The typical problem associated with CE-NMR is the shorter residence time of the sample in NMR due to small sample volume output from CE that affects the detection sensitivity [82]. Although intensive innovative efforts have been made to improve this, only a few publications reported the application of CE-NMR to identification and characterization of trace amount of impurities and degradation products [83, 84]. The high theoretical plate numbers and the leveled flow profile of CE are optimal premises for a successful hyphenation of CE to NMR spectroscopy. Essential for this hyphenation is a probe with a detection volume that matches the dimension of the CE separation. In 1999, Schewitz *et al*., demonstrated the suitability of a hyphenated CE-NMR system. In their work, they investigated a crude adenosine dinucleotide and its side products, which were synthesized by solid-phase chemistry with continuous-flow CE-NMR. They showed the producibility of 2D CE-NMR spectra in the stopped-flow mode which also enabled the identification of more complex structures. Despite the very low LOD regarding stopped-flow measurements which are approximately in the range of 100 ng for 2D NMR experiments, less is known about the coupling of CE to NMR. Due to the optimal premises of CE in terms of the hyphenation to an NMR spectrometer, it is to expect that the CE-NMR technique will be rediscovered for some applications in the future [85]. In CE-NMR small diameter, capillaries are used so that the detection is typically accomplished using low volume micro-coil NMR probes. In CE-NMR experiments, to maintain the flow of charged species a voltage is applied across the capillary. With the use of a recently developed capillary NMR interface, the coupling of capillary electrophoresis with nuclear magnetic resonance spectroscopy has been achieved. This CE-NMR technique is used for the analysis of synthetic nucleic acids [86].

Applications: CE-NMR is used for the demonstration of drug metabolite identification. Continuous CE-NMR was used to separate the ingredients of caffeine and aspartame, which are commonly found in soft drink beverages. The other important application in this technique is to provide insights into processes that affect the separation. The first application of the coupling of capillary electrophoresis with NMR for static and online measurements was performed by Wu *et al*. They reported on the separation of some amino acids in the nanoliter scale (5-200 NL) and on the development of suitable detection cells and optimized coils [87].

CONCLUSION AND FUTURE ASPECTS: Capillary electrophoresis has proved to be a versatile tool for metalloproteins applications. Intensive develop-ment of CE-MS instrumentation and methodology is a subject of numerous research groups to satisfy the demands of analytical practice. CE-MS was successfully applied to the identification of disease biomarkers in fluids using both the top-down and the bottom-up approaches. CE with ICP-MS used as an online detector has emerged as a recognized analytical tool for metallodrug-protein interactions. The hyphenation of the different separation techniques based on electro migration with ESI-MS has grown up to a powerful analytical method. With the availability of instruments, the range of applications has increased.

The experience of this method can be extended to further miniaturization. Certainly, this growth will continue with increasing speed, and CE/ESI-MS will be an indispensable tool in analytical chemistry. CE-MALDI-MS has a wide scope in proteomics and metabolomics. Integrated CE-NMR instrumentation will provide data with comple-mentary separations.

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