REVIEW ARTICLE

RECENT ADVANCES IN CAPILLARY ELECTROPHORESIS

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ABSTRACT

Capillary electrophoresis is a rapid, flexible and effective separation technique with minimum requirement of sample and chemicals. This article focuses on recent advances in capillary electrophoresis, covering various preconcentration techniques, methods, injection techniques, detection techniques and applications of capillary electrophoresis in various fields.

Keywords: Capillary electrophoresis, injection, detection techniques, applications

INTRODUCTION

Electrophoresis[1] is the technique by which charged particles are migrated under the influence of an electric field. Ferdinand Frederic Reuss discovered this method in 1807. Anaphoresis is the name given to electrophoresis of negatively charged particles. Cataphoresis is the name given to electrophoresis of positively charged particle.

Electrophoresis is classified[2] into:

1. Zone electrophoresis[3] – It is the method by which charged particles are migrated on the supporting media. Supporting media used include paper, cellulose acetate, starch gel and polyacrylamide. Zone electrophoresis separates and distributes the components into various zones on the support media. Buffer solution is administered until supporting media is saturated, then a little amount of sample is applied in a narrow band. Potential difference is applied at the end of the strips, migration of components take place and rate of migration is estimated by electrophoretic mobility. Paper electrophoresis, gel electrophoresis, thin layer electrophoresis and cellulose acetate electrophoresis are few examples of zone electrophoresis types.
2. Moving boundary electrophoresis[4,5] - In this technique, migration of charged species take place in free moving solution without the supporting medium. U-shaped tube is filled with buffer and samples are fractioned in it. An electric field is applied and separation takes place due to difference in mobilities. Capillary electrophoresis, isotachophoresis, isoelectric focusing and immune electrophoresis are few examples of moving boundary electrophoresis. These methods allow for recovery of biological fractions without the use of denaturing agents and detection of concentration at micro-level.

The technique of capillary electrophoresis (CE) was first introduced by Jorgenson and Lukas in 1981. Capillary electrophoresis (CE) is one of the separation methods based on electro kinetic techniques. Separation by this technique is based on charge to mass ratio. This technique is highly versatile and is a mature technique. Compared to conventional gas and liquid chromatography, capillary electrophoresis has an important role in analysis. By this technique, molecules with a wide range of sizes, charges and hydrophobicity, proteins and peptides can be analysed[6]. Consumption of solvent in CE is small, since flow rate is less. The technique of electrophoresis is evaluated using narrow bore, fused silica capillaries. The current article focuses on recent advancements in techniques, procedures, detection techniques and applications of capillary electrophoresis.

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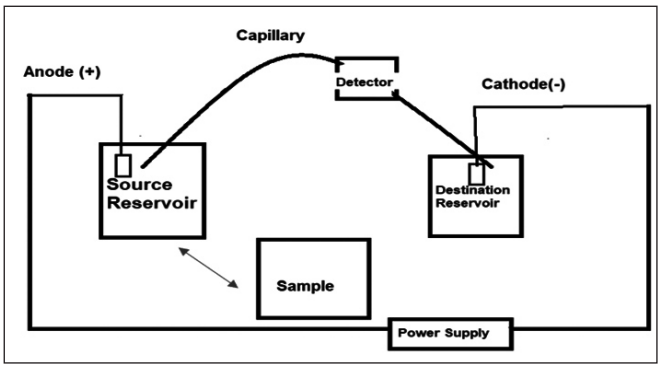

Fig. 1: Instruementation of CE[2]

One of the newest techniques in electrophoresis is capillary electrophoresis. It has two main advantages over gel electrophoresis[7]: (i) separation is more efficient as electroosmotic force is also working along with electrophoretic force (ii) applicable to small molecules is also possible. Several examples of small molecule drug analysis are available in literature.

Capillary electrophoresis is equipped with power supply, anode and cathode compartments, sample holder containing sample, capillary tube with internal diameter of 10-100 mm and 20-100 cm length, detector and output device [Fig. 1]. Electric field is applied with power supply. Anode and cathode compartments act as provisions of buffer solutions. Poor resolution, distorted peak width and peak shape is observed using fused silica capillary due to interaction of capillary surface with analyte. If sample matrix components like proteins are used, electroosmotic flow may change, which may affect migration time and reproducibility.

To improve this, Puolison et al's coating procedure was followed with polyethylene glycol. In this mechanism, transfer of atom takes place in capillary surface, as a result of which covalent binding takes place to capillary wall and aids in adsorption.

Factors affecting capillary electrophoresis[8]

Electrophoretic mobility

The electrical charge, molecular size and shape of the solute, pH, viscosity, additives as well as properties of buffer like type of buffer and its ionic strength, all affect the electrophoretic mobility. Solute's electrophoretic mobility is denoted by the symbol μep. Assuming solute to be spherical in shape, electrophoretic velocity (νep) is expressed as :

$$\nu ep = \mu ep \text{ X } E = (q/6\pi\eta r) \text{ X } (V/L)$$

where q is the effective charge of the solute, η is the viscosity of an electrolyte solution, r is the Stoke's radius of the solute, V is the applied voltage and L is the total length of capillary.

Electroosmotic velocity

When an electric field is applied to the capillary containing buffer, development of flow takes place inside the capillary and is referred as electro osmotic flow. The rate at which flow takes place is denoted by μ_{eo} which depends on charge density on capillary internal wall and the properties of buffer. Electroosmotic velocity is expressed by

$$\nu_{eo} = \mu_{eo} \text{ X } E = (\xi l/\eta) \text{ X } (V/L)$$

in which ξ is the dielectric constant of the buffer and l is the zeta potential of the capillary surface.

Methods and preconcentration techniques in CE

In capillary electrophoresis, sample injected is in minute quantity. When detection is performed using optical detectors, optical pathway is limited and concentration sensitivity of CE methods becomes poor. To improve the sensitivity detection levels sample preconcentration is performed online or offline.

Capillary zone electrophoresis

According to this method, when an electric field is applied, migration of analytes takes place according to their charge to mass ratio. Migration of analytes depends on voltage and electro osmotic flow. Faster analysis is obtained with higher voltage and a low electro osmotic flow, though voltage can be enhanced upto a level only, as beyond that Joule's heating may occur, giving a broad peak in the electropherogram.

Preconcentration techniques used[9]

Electrophoretic concentration**:** This preconcentration technique is based on difference in electrophoretic motilities of electrolyte by changing pH of supporting electrolyte and addition of organic or complex forming additives of supporting electrolyte.

Field amplified sample stacking (FASS)[10] – It is used for determination of low analyte concentration in clinical laboratories. This technique is based on difference in electrical conductivity of sample matrix and buffer. To avoid over electroosmotic flow, additives used are dimethylenetriamine, tetradecyltrimethylammonium bromide and are added to the buffer.

Head column field amplified sample stacking (HC-FASS) –Water plug is introduced before the introduction of sample. Charged analytes are injected into strong field zone which is created at the capillary inlet. At the boundary between buffer analyte and low conductivity zone, concentration of analytes take place. The preconcentration factor is higher than 1000.

Large volume sample stacking[11] (LVSS) – In this method, bulk solution is in direction opposite to that of electrophoretic migration of electrolyte. LVSS is available in two variations : LVSS with polarity and LVSS without polarity.

Extraction based preconcentration – This technique is a very efficient technique, producing high concentration factors. Volatile oils have been determined by online coupling headspace liquid phase micro extraction with capillary electrophoresis[12]. Preservatives benzoic acid and sorbic acid were determined by headspace liquid phase micro extraction techniques. Extraction was obtained with good enrichment along with limits of detection.

Hybrid preconcentration – It is the combination of different preconcentration techniques[13]. With the combination of anion and cation selective injections, acidic and basic components are detected in a single run by FASI and sweeping technology.

Chromatographic preconcentration[14,15] – Solid phase extraction is the most commonly used preconcentration technique in capillary electrophoresis. For analysis of yeast proteome, offline C5 reverse-phase liquid chromatography is employed prior to capillary zone electrophoresis coupled to electrospray ionization mass spectrometry.

For online SPE-CE, a small column is introduced before the initial part of separation capillary for analysis of alkaloid[16].

Dynamic pH junction[17] – Abersold and Morrison first introduced this method. In this method, pH is in the interface between sample and background electrolyte. Change in ionization state of ionic species produces change in analyte electrophoretic mobility. As a result, there is change in velocity, focusing effect is produced and there is accumulation of analyte at the boundary[17,18].

Micellar electrokinetic chromatography[19]

Separation of neutral compounds is performed by this technique. The buffer solution is micelle which acts as pseudo stationary phase.

Preconcentration techniques used are[20]

Micelle based preconcentration - Sample is prepared in matrix that is void of pseudo phase which is used for separation in electro kinetic chromatography. When the pseudo phase moves through the sample, it concentrates the analytes. Analytes are separated into a very sharp zone. Neutral compounds are estimated by micellar electro kinetic chromatography.

Chemometric approach - In micellar electrokinetic chromatography, chemometric approach is applied with hyphenation of field enhanced sample injection and sweeping [21].

Sweeping[22] – It is based on preconcentration using pseudo stationary phase using micelles and microemulsions. Sample is injected in fused silica capillary. The ends of capillary are immersed in the electrolyte, which acts as buffer with the solution of micelles. Upon application of voltage, analytes are concentrated by micelles, as a result there is penetration into sample zone on the cathodic side.

Micelle to solvent stacking –Quirino introduced the concept of micelle to solvent stacking. Its basis is the reversal in the effective electrophoretic mobility at the interface between micellar matrix and the background solution with the organic solvent. By using MSS-CZE, strychnine and brucine in traditional Chinese medicinal preparations were estimated. Concentration sensitivity was improved 50 times as compared to CZE-UV analysis.

Analyte focusing by micelle collapse - It relies on movement, release and accumulation of molecules contained in micelle carriers that are caused to collapse in liquid phase zone. More than a 22-order increase in detection sensitivity can be observed[23].

Microchip capillary electrophoresis

It is a small-scale capillary electrophoresis technique[24]. Using this method, microchip as rectangular channel is used which is about 10-100 µm in width and height and 10-100 mm in length. Separation time is faster, less than 1 min.

Capillary gel electrophoresis

Biomolecules are separated by this technique using sieve as a medium. For denaturation of proteins, sodium dodecyl sulphate is added to BGE. For separation of DNA fragments and protein, soluble polymers are used as slab gel and automated capillary array electrophoresis is performed[25].

Capillary isoelectric focusing[26]

Proteins are separated by this method based on the isoelectric point. Ampholytes and samples are mixed and are filled in the capillary. Sodium hydroxide acts as catholyte and phosphoric acid acts as anolyte. Upon application of the electric field, pH gradient is developed. Proteins are separated when pH of protein is equal to pI. Migration is stopped and net charge is zero.

Affinity electrophoresis

Capillary electrophoresis can be combined with affinity ligand which can bind, as a result there is shift in migration of analytes[27]. This was developed in 1990. Separation is performed by homogenous or heterogeneous methods. In homogenous method, interaction takes place between binding agent and analyte in a solution. In heterogeneous method, immobilization of binding agent takes place in inner surface of CE system.

It is one of the versatile techniques for study of non-covalent interactions. This method aids in determining the complex binding and dissociation constants. By this method, receptor in free and bound form can be distinguished versus concentration of free ligand[28].

Binding agents used are antibodies, aptamers, lectins, serum proteins, carbohydrates and enzymes.

Antibodies are used as binding agents in Capillary Electrophoresis immunoassay which is performed in homogenous mode. In this technique, a fixed amount of labeled analog of analyte and antibodies are mixed and the solution is incubated. Separation of mixture is performed by capillary electrophoresis and quantity of bound or unbound analog is estimated. CE immunoassays are performed by competitive and non-competitive techniques.

Aptamer[29] is composed of single stranded DNA or RNA or an oligopeptide which binds to a specific target like peptide or carbohydrate. Compared to the antibodies, aptamers are produced by special techniques like Selex and they are smaller in size compared to antibodies. They are also used in competitive[30] and non-competitive CE assay.

Lectins[31] are carbohydrate-binding proteins that react with compounds which contain glycans like glycoprotein and glycolipids. In mobility shift tests, lectins are used to evaluate how the binding proteins interact with carbohydrates.

Serum proteins used include human serum albumin, bovine serum albumin and alpha-1-acid glycoprotein. These are used as binding agents or chiral centers for mobile shift assays of drugs or enantiomers. Separation is performed by homogenous or heterogeneous methods.

They are used in analysis of glycans. Most commonly used is cyclodextrins. They are polymers with hydroxyl groups arranged on both the ends, giving them a toroidal form, which makes them good solubilizing agents and as a good chiral center.

In capillary electrophoresis, enzymes are used as stereoselective binding agents for chiral separations, study of enzyme substrate interactions and kinetics. Assays performed by CE are pre-capillary assays and in-capillary enzyme assays.

With affinity capillary electrophoresis[32], receptor-ligand interactions can be studied. Interactions like protein-small molecule, protein-drug, protein-DNA, DNA-DNA, metal-small molecule, antigen-antibody, antibiotics-drugs and oligosaccharides-drugs can be studied.

Electrokinetic chromatography

Non aqueous capillary electrophoresis – In this method organic solvents are used. The organic solvent improves the selectivity of the method and increases solubility of non-polar analyte. Analytes which are having same charge to size ratio or the analytes which are decomposed in aqueous media are separated using non aqueous media. This technique has been used for determination of fluoxetine, sertraline[33, 34] and citalopram in plasma.

Microemulsion electrokinetic chromatography[35]

In this technique, transparent stable microemulsion droplets are used as pseudo stationary phases. The droplets consist of non-polar and polar phases with the stabilization of surfactants and co surfactants. It is employed for the determination of Log P values and for determination of neutral and hydrophobic compounds Coenzyme Q10 in human plasma, DOPA, phenylalanine and tyrosine determined by this technique[36].

Micro fluidic capillary electrophoresis

It is also called as miniaturized CE or microchip CE. Compared to conventional CE, analysis can be performed with small sample volume, faster analysis, lower reagents, samples consumption, less time consumption and portability[37] and it is eco-friendly as it generates low waste.

Nano capillary electrophoresis[38,39]

It is also called microchip capillary electrophoresis with high versatility, speed, sensitivity, excellent separation efficiency, low sample volume and speed and low cost. Micro-channel network for sample pre- and post-handlings, chemical reaction, separation and detection make up the nano capillary electrophoresis chip.

Chips are composed of glass, silica, materials like poly (dimethyl siloxane), with cross sectional area of 50 X 20 µm and separation channel length of 5 cm.

Nano capillary electrophoresis[40] comprises of sample injection, background and separation. The microchips are made by techniques such as wet chemical procedures, dry etching or photolithography. Solvents used in nano capillary electrophoresis are of HPLC grade. Commonly used buffer is phosphate buffer of different pH and ionic strength. Acetate, borate and ammonium citrate buffers may be used. Acetonitrile and methanol may be used as organic modifiers for effective separation.

One of the most common system in micros chip electrophoresis is Lab Chip instrument. In this system, vacuum is used to pull sample from a multi well plate into the microfluidic separation channel with fluorescence detection.

Nanoparticle preconcentration is used. Nano particles[41] have high absorption capacity. With this technique, extraction of complex molecules can be performed. Metals are preconcentrated using carboxylic group functionalized nano particles prior to being estimated by CE. As extraction probes, boronate functionalized nano particles are utilized to isolate and concentrate biomolecules containing cis-1,2 diols.

CE- based PCR technique

The diagnostic techniques by CE consist of two steps. Based on the characteristics of pathologically altered genes, amplification of genomic area requires the design of PCR technique. The properties of mutant genes and conserved sections like microsatellite sequence are used to develop stragies. Capillary gel electrophoresis with fluorescence[42] detection, laser induced[43] fluorescence, CGE single strand confirmation[44] polymorphism have been used to investigate PCR products.

The techniques employed in CE- based PCR technique is Repeat flanking PCR-CE primer, Triple Repeat Primed -PCR-CGE-FD (detection of number of codon repeats), PCR-STR/VNTR/ PAHSTRCE-LIF for estimation of mutation in PAH gene and mutant related polymorphism), PCR-STRCE-LIF for autosomal dominant inherited disease, autosomal recessive inherited disease, autosomal dominant recessive inherited disease[45], as well as X-linked dominant inherited disease.

PCR in combination with CE is used for genetic modification. There is a neurogenerative disorder called scrapie which is found in sheep and goat. Single nucleotide polymorphism in bovine prion protein is the marker which can be detected by this technique[46].

Genetic modification in maize can be detected by CE-PCR technique by extraction and amplification of DNA fragment and detection by LIF[47].

Epigenetic modification is one of the genetic modifications which is not related to the change in the primary DNA sequence. One of the types of epigenetic modification is DNA methylation, the modification of which may lead to human diseases like cancer. CpG islandarae cytosine-phospho-guanine are base pair stretch of DNA. CpG are found in promoter regions which are unmethylated. Outside of promoter region is methylated[48]. Alteration in DNA methylation can lead to disorder which were detected by PCR or whole gene amplification but have drawbacks in terms of requirement of high-quality DNA and frequent incomplete restriction digests. CE-PCR overcomes these drawbacks.

Transient isotachophoresis[49]

It is a separation mode of capillary electrophoresis. It serves as temporary stage in the preconcentration step of capillary zone electrophoresis[50]. This technique employed on-line coupled ITP-CZE using UV detection with wavelength of 220 nm to estimate nine brominated phenols.

Injection In CE

Introduction of very small quantities of sample in a narrow bore capillary has always been a challenge. Several techniques can be used for sample injection.

Sequential injection CE –This approach allows for proper flow and voltage control as the sample and background electrolyte pass across the interface. It has twin syringe that is used to deliver background electrolyte and sample through the apparatus. An injector valve with two locations is used to alternately stream background electrolyte and sample[51].

Electrokinetic injection[52] – This method uses electric field created by applying voltage to an electrode that is sample in a sample solution as driving force. It is

selective for ions with high sensitivity. It is preferred in sample preconcentration, in gel electrophoresis. An additional apparatus is required for electrokinetic injection.

Pressure pinched[53] – In this technique pressure is applied to the vial having sample. Vial is pushed into the capillary. The drawback in this technique is control of pressure precisely. The performance of the system may be decreased as the equipment becomes older when the pressure lines become more rigid.

Hydrodynamic[54] - In this technique, the sample is forced into the capillary by a pressure which can be created by the applying vacuum to one side of detector or pressure to the other or by using the siphon method. Siphoning was the earliest sample injection method.

Hydrostatic pressure[55] - In this method, sample is loaded using hydrostatic pressure created by emptying the sample reservoir and sample is dispensed using electrokinetic force. Injection is performed on double cross microchip. Sample plug uses one cross is produced by sample and separation channels. For plug control, a different cross is created by the sample and controlling channels. The sample plug volume can be linearly controlled by altering the electric filed in regulating channel.

Push/pull[56] - In this procedure, separation channel receives a low voltage and the sample channel receives high voltage. There is ground on the buffer channel. Sample that was injected is returned to the buffer channel. In dispensing step, the sample channel receives a low voltage, the buffer channel receives high voltage and the separation plug is grounded. The separation channel is pushed with the sample plug.

Bias free pneumatic[57] - It is another kind of pressure injection. With the help of pressure, analyte is introduced into the separation system. It is based upon hydrophobicity or wettability of channel surfaces.

Portable capillary electrophoresis[58]

With capacitively coupled contactless conductivity detectors, anions and cations can be detected. While performing by this method, internal standard is required.

Post – blast residues containing inorganics were identified by in-house P-CE modified method. Using this technique, the residues containing cations and anions were determined. Test was also performed for detection of interferences present in the environment or added during sample preparation.

Separation media

Pseudo stationary phase[59] – Separations not possible with regular CZE are performed using pseudo stationary phase. Neutral surfactants are used. Thesit and Tween 20 are used to increase selectivity and reducing interaction with the capillary walls. For separation of insulin, negatively charged surfactant perfluoroctanoic acid was found to be suitable.

Capillary electro chromatography[60] – The advantage of capillary electro chromatography over other methods is that it offers a dual mechanism that includes analyte electrophoretic mobility and analyte portioning between stationary and mobile phase. One of the drawbacks is using frit of micro particulate column which may lead to bubble formation and there are issues of robustness which can be overcome by open tubular or monolithic columns.

Chiral media – Separation of enantiomers is performed by capillary electrophoresis using chiral selectors in aqueous background electrolyte. One of the most common chiral selectors is cyclodextrin. Other chiral selectors used are sodium dodecyl sulphate and potassium sodium tartarate.

Detection techniques In CE

Optical spectroscopy

UV detectors with CE are very well known but other spectroscopic detectors have also been introduced. One of the sensitive techniques of detection in CE is fluorescence. Aptamers are utilized as affinity probes in noncompetitive affinity CE assays with fluorescent assays with CE detection and they are mirror reflections of one another.

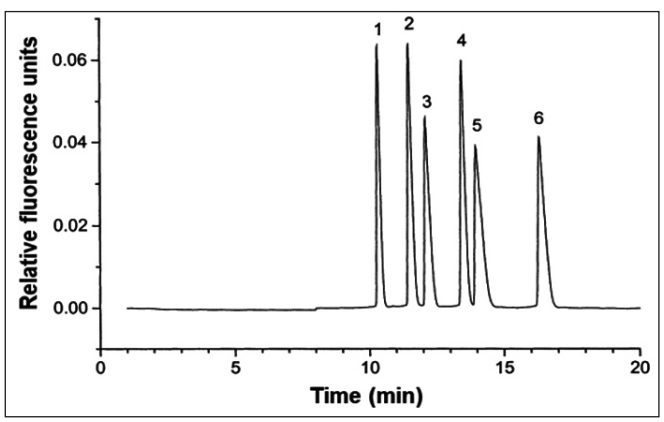

Fig. 2: Electropherogram of an aqueous standard solution containing 5 µg mL^{-1} of enantiomeric metabolites of ofloxacin[63]

Fluorescent detection[61,62]

With this technique, analytes can be detected at minute levels. Analytes which lack functional groups corresponding to fluoresce are derivatized with fluorescent reagents for the improvement of sensitivity. Ofloxacin[63] [Fig. 2] and urine containing metabolites which are enantiomers are estimated by using fluorescent detection.

Light emitting diodes

It possesses wide range of emission wavelengths, constant output, low energy consumption over a long lifetime, is inexpensive and is tiny in size. This technique is used for analysis of dopamine[64], octapamine in human plasma tablets and human plasma containing penicillamine.

Chemiluminescence detection

Its basis is the measurement of electromagnetic radiation resulting from a chemical reaction that yields electronically stimulated intermediates or products. It is classified as direct CL (luminescence) and indirect CL. Reagents used in CE-CL are ruthenium complexes, luminal and derivatives and peroxyoxalates. CE-CL is used in studies of clindamycin[65] [Fig. 3] interactions with hemoglobin, analysis of isoniazid[66] and p-amino salicylic acid in tablets and serum.

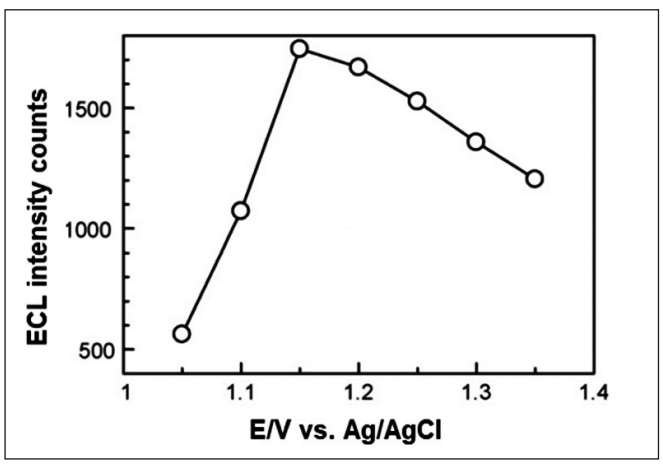

Fig. 3: Changes in the ECL intensity with the applied potential on the Pt electrode for clindamycin determination[65]

Capacitively coupled contactless conductivity detection

It is a universal detector. All absorbing, non-absorbing and neutral species are detected by this detection. Lecirelin and its impurities are analysed and non chromophore antibiotics (fosfomycin[67] [Fig. 4],[68] and tobramycin) in human body fluid are analyzed by CE-C4 detection[69].

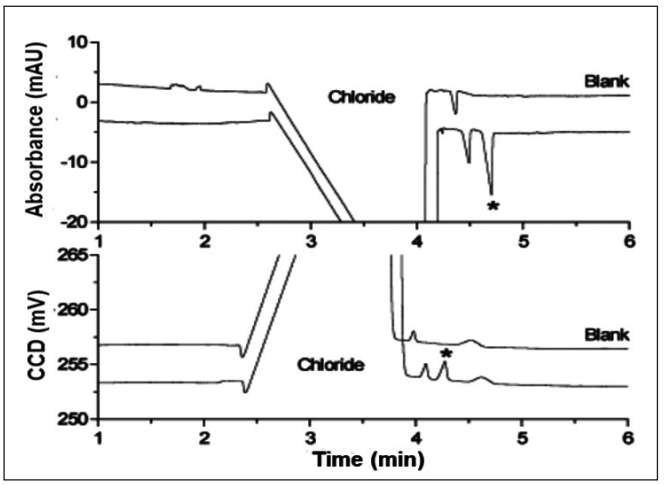

Fig. 4: Electropherogram of fosfomycin in Ringer's solution at pH 8.05 with indirect UV absorbance detection (upper trace) and CCD (lower trace)[67]

Mass spectrometry

CE-MS[70]

Sheath liquid interface

CE and MS are connected through capillary outlet and the stainless-steel needle of ESI. This was further modified with co-axial sheath liquid interface to increase the flow rate of electrospray[71]. Further stainless-steel needle is modified to platinum ESI needle to prevent oxidation and corrosion of the needle. Water and organic volatile organic solvents are combined with volatile acidic or alkaline additives to create formation of ions. For determination of phenolic compounds and free radicals, sheath liquid interface is used and also for estimation of exchangeable hydrogens in compounds[72] with deuterated solvents.

Sheathless interface

In this technique porous sheath interface is used, CE capillary is connected with hydrofluoric acid to make the porous end for transfer of ions and electrons. The end of the capillary end is attached to metal cylinder which functions as electrode and outlet vial. With sheathless interface technique, improvement in limit of detection of proteins and metabolites was obtained compared to the techniques of SL interface.

Liquid junction interface

CE effluent is mixed with outlet electrolyte that is connected to ground electrode at the liquid junction

interface. The interface is utilized with an asymmetrical stainless steel hollow needle. Miniaturized liquid junction interface with electrokinetically pushed outlet electrolyte and borosilicate glass emitter was created after further modification. With this technique, analysis with high speed is obtained.

CE-MALDI MS

In this technique CE is coupled to Matrix Associated Laser Desorption/ Ionization (MALDI). The interface used is sheath liquid interface with combination of spotting device for deposition of analytes on a target plate for MALDI. SL is used to apply MALDI matrix either before or after sample spotting. In other technique of CE-MALDI –MS tip is coated with silver and liquid free interfacing is used. The solution is deposited on MALDI plates for analytes to contact with capillary. Sheathless interface is prepared by coupling CE and capillary through porous polymer. With this technique, proteins can be analysed in presence of ampholytes and detergents.

Applications of Capillary electrophoresis

Biopharmaceuticals – Biopharmaceuticals[73] are the complex biomolecules which are produced by recombinant techniques and include hormones, vaccines and monoclonal antibodies. For separation of isoforms from variants and impurities, separation techniques used are capillary electro migration techniques. This method has increased capacity of separation and allows for the preservation of secondary protein's higher order structure while using an aqueous buffer.

Glycans[74] derived from proteins – The richest class of organic molecules on this system, carbohydrates are crucial to numerous biological processes. The monosaccharides that make up glycans attached to proteins are linked together in various sequences and connections. Glycans are released from proteins chemically or enzymatically. Since glycans lack UV activity, chromophore tagging of glycans is necessary.

Labelling agent used typically is used are 3-(4-carboxy-benzoyl)-2-quinolinecarboxyaldehyde for analyzing carbohydrates using LIF detection in conjunction with CE. Other derivatization agents used are 2-amino benzoic acid, amino naphthalene trisulphonic acids, ANTS consist of three sulphonic acid molecules.

Clinical Chemistry[75]– Capillary electrophoresis is the powerful analytical technique for characterization of hemoglobin in humans. With this technique, disorders related to abnormal blood composition, protein profiling in human serum can be determined.

Microchip Capillary Electrophoresis had been used for determination of protein biomarkers in diseases like sepsis and Crohn's disease. This technique has been used in different types of cancer by determination of different tumour biomarkers in biological samples. B Human Chronic Gonadotropin is biomarker in ovarian cancer diagnosis which can be determined by this technique. B-HCG binds to peroxidase antibody and complex Ag-Ab formed is separated from free unreacted Ab by this technique using chemiluminescence detection within 1 min[76].

Metabolomics - Capillary electrophoresis is a more powerful technique than MS in terms of separation efficiency, low solvent consumption, use of capillaries instead of expensive LC columns[77]. For application in metabolomics, capillary zone electrophoresis and micellar electrokinetic capillary chromatography is used. This technique is successful in detection of metabolites in urine, blood, bacteria metabolomics and plant metabolomics.

Proteins and peptides[78] – Peptide hydrolysis was monitored for amino acid detection and ladder sequencing. Two peptides which differ in the amino acid order were selected as model compounds for study of isomerization and enantiomerization of asparagines residues in both acidic and basic pH by CZE and CZE-MS/MS.

Carbohydrate analysis – Capillary electrophoresis has been used for separation of sugars based on the electrophoretic mobilities in strong alkaline solutions. Saccharide zones were detected with amperometric detection at constant potential using cylindrical copper wire electrode[79]. With this technique, 15 different sugars were separated in less than 45 min.

Inorganic compounds – CE-C4D is used for analysis of common cations. The evaluation was performed for comparison of conductivity detection compatible BGEs[80]. This demonstrated that adding 18-crown-6 and hydroxyisobutryic acid was required for raising detection's sensitivity. It is also used for detection of bromates in water.

CE has been developed for the estimation of metal ions like copper, iron, zinc, cobalt and nickel in tea. For estimation of these metals[77] the extract was reacted with chelating agent 4-(2-pyridylazo) resorcinol to form the complex with metal ions for their detection.

Affinity – With the techniques of capillary electrophoresis, biomolecular interactions can be studied. Affinity

capillary electrophoresis was applied for analysis of uranyl compounds which are bound by sulphated beta-CD in aqueous solutions. It was also utilized to analyse the complexes between acyclic nucleotide phosphonate and beta CD in an aqueous alkaline medium.

Assay of guanine-nucleotide-binding proteins (G-proteins) was performed using affinity probe CE. In this method, guanosine 5'-triphosphate analogue is fluorescently labeled and is used as affinity probe. Sample containing G-proteins and labelled analogue are subjected to incubation. By employing laser induced fluorescence[79], CE is used to distinguish from the resultant complexes from free analogue.

Nucleic acids-With capillary electrophoresis, study of DNA interactions was possible: analysis of DNA - ligand kinetics, micro RNA, nucleoside adenoside and inosine in brain sample[80].

Viruses and bacteria –Capillary gel electrophoresis was developed for characterization and quantification of viral protein in influenza vaccines, intact adenovirus type Ad26 and Ad35. Capillary electrophoresis has been evaluated for separation and characterization of living bacterial cells as well as characterization of intact phytopathogen bacteria by cIEF, CZE and MALDI-TOF.

Immunoassay – Immunoassays are based on antigen-antibody interaction. Conventional immunoassays are time-consuming, tedious with problems of reproducibility. Combination of CE with immunoassay helps in separation power with aided advantage of multianalyte analysis, low sample consumption, high speed and flexibility. These techniques are based on affinity probe CE. Capillary electrophoresis based immunoassays are of two types homogenous based and heterogeneous based. In homogenous based technique, all interacting partners are in solutions while in heterogeneous based technique, immobilized antigen or antibody is present on solid support. Homogenous method may be performed in competitive or non-competitive way.

Competitive CEIA is used for detection of insulin. It has been used for analysis of hormones, proteins and peptides, to check drugs and their abuse.

Noncompetitive CEIA is used for the systems in which difference in electrophoretic mobility of complex and antibody is high[81].

Heterogeneous CEIA is used for analysis of proteins, hormone, drugs and antibody. Extraction of ibuprofen, naproxene, angiotensin II and neurotensin by heterogenous CEIA has been done.

Biological applications – Microchip capillary[76] has been used for estimation of methyl parathion metabolites like 4-nitrophenol, its glucuronide form or sulphate ester form. This was performed by multi walled carbon nano tubes modified carbon fiber microelectrode using amperometric detection.

Environmental applications - Analysis of mercury in sewage water was performed using microchip analysis. This was obtained with low limit of detection less than 1 ppm. Substrate used was core shell surface enhanced Raman scattering. S-mercaptopyridine was used as internal standard. Substrates were coupled with micro fluidic techniques[76].

Escherichia coli was detected in water samples and food by microchip capillary electrophoresis. The detection was performed by bacteria specific aptamer which is conjugated MCE-LIF. *E. coli* is separated from sequence libraries.

Pharmaceutical applications[82] - It is one of the most effective technique in the separation of drug intermediates. Literature has shown separation of mixture of alicyclic amines on the basis of charge to mass ratio. Capillary zone electrophoresis is used in determination of kinetic and thermodynamic parameters. Binding of the broad-spectrum antibiotic vancomycin to the peptides containing sequence *N*-acetyl-D-Ala-D-Ala and *N*-acetyl-L-Ala-L-Ala is studied using this technique. Capillary electrophoresis[83] is used for analysis of active pharmaceutical ingredients, drug counter ions and impurities.

Food analysis – One of the applications of CE is analysis alpha lactalbumin in protein powders. Lactalbumin is present at less than 5 % in milk powders. This may undergo degradation during processing of milk powders. This analysis was performed by capillary zone electrophoresis with borate buffer of 50 mM at pH 8.0. Voltage applied was 15 kV using fused silica capillary 32 cm 650 mm inner diameter. Alpha lactalbumin was resolved in whey proteins using acetic acid. The separation was performed in less than 5 min[84].

Amino acids are one of the important nutrients. They lack of chromophores. They are detected by CZE[85] and micellar electrokinetic chromatography. Due to lack of chromophores, they can be derivatised and detected by laser[85] induced fluorescence or ESI-MS[86], electrochemical.

Analysis of explosives – For the study of anions and cations in post blast extracts from acid-aluminum burst residues[87], capillary electrophoresis has been developed. These were unable to be analysed by electrokinetic chromatography–UV technique. The background electrolyte used was 2,6 pyridine dicarboxylate and chelate was formed with aluminum. Anions did not interfere in this method, chloride was detected from the acid and aluminum was also detected. The outcome was attained under economical and low BGE consumption settings.

Microchip CE is used for measuring nitrate ester explosives[88]. With this technique analytes can be detected at pg level with the analysis time of 160 sec.

Identification of Atlantic Salmon and rainbow trout – Capillary zone electrophoresis was use for identification of replacement of higher species Atlantic Salmon[89] (*Salmo salar*) with lower quality species rainbow trout (*Onchorhynchus mykiss*). Analysis of raw species was performed using muscle sarcoplasmic (water soluble) proteins, canned species was evaluated with muscle proteins with solubilization of urea and sodium dodecyl sulphate.

With this technique, protein profiles were obtained for both species of fish samples and difference in change in quality during refrigerated, frozen, canned storage of the species under study.

Drug Discovery - Capillary electrophoresis is advantageous in high throughput screening. Decrease in separation time was observed. The migration time of an electrolyte depends upon its electrophoretic mobility under applied voltage[90,91] which is shown by the given equation: L2/(VX μep). In sample introduction, there are two techniques by which sample is introduced, direct injection from multi well plate and injection form small sample volumes.

Integration of segmented flow strategies are involved by techniques like parallelization separation including multichannel detection, connection to peripheral power supplies, sample pooling, multiplexing technique, to assay multiple targets simultaneously. With high throughput techniques enzymatic assays and affinity interactions non-covalent interactions, protein nucleic acid interactions, protein interactions have been successfully studied.

Enzyme assays - Enzymes assays are performed by capillary electrophoresis and are classified into three types – pre-capillary[92] (offline), in-capillary enzyme assays, post capillary assays, immobilized enzyme reactors and enzyme assays[93,94] by microfluidic devices.

Agrochemical analysis – Agrochemicals are charged compounds, Capillary zone electrophoresis with MS technique is used for analysis. Assays on environmental and biological samples are performed. With this technique simultaneous separation of two herbicides (glyphosate and glufosinate)[95,96] as well as their metabolites (methylphosphinic acid and methylphosphinicpropionic acid) were achieved with CZE with microelectrospray MS interface.

Separation of metallated and demetallated forms of transferring protein - Carbon dots[97] are fluorescent nano materials used in bioimaging, biosensing and biomedicine. They offer advantages in bioimaging, biosensing and biomedicine over inorganic quantum dots or organic dyes. From citric acid or other carbon precursors, they are created. They function as separation adjuvants for the mixtures of metallated and demetallated forms of transferrin. Carbon dots act as versatile tools for improvement of electrophoretic separation mainly for metalloprotein analysis.

Analysis of anticancer drugs – For the analysis of anticancer drugs[98] estimation is performed by bioanalytical method. The matrices used are blood, urine, saliva, tissue or cerebrospinal fluid. CEA is the glycoprotein related with certain cancers which is the indicator of tumor in the body. CEA is determined by non-competitive immunoassay based gold nanoparticles amplified CE-CL[99] detection. Tamoxifen and its metabolites are extracted from urine of the patients who are suffering from metastatic and locally confined breast cancers. These metabolite[100] are analysed by non-aqueous CE-ESI-MS.

Analysis of single cell omics[101] - Capillary electrophoresis with mass spectra is applied for the analysis of single cell omics. Reviews have been written about difficulties in single cell sampling[102] such as direct analysis, single cell dissociation culture and cell targeting. It is also focused on micro-chip single cell analysis for automation of sample and cell process. This method was created to analyse cells quickly and sensitively while also increasing analyte throughput and repeatability.

CONCLUSION

Capillary electrophoresis is a mature and versatile technique compared to conventional techniques like gas and liquid chromatography. It is one the robust techniques with rapid analysis of range of compounds

which are polar in nature, large biomolecules like DNA, proteins and peptides. The techniques like capillary zone electrophoresis, capillary isoelectric focusing and other similar techniques are used as routine analytical techniques in pharmaceutical industry for analysis of wide range of compounds. This technique involves various preconcentration techniques, separation media and various detection techniques for analysis of compounds ranging from biomolecules, pharmaceutical applications, analysis of explosives, agrochemicals, food analysis, environmental analysis to inorganic compounds and in drug discovery.

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