REVIEW ARTICLE

Capillary Electrophoresis-An insight into different modes and it's applications

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ABSTRACT

Electrophoresis is one among the separation science most useful as scientific tool which is used extensively in diagnostic and clinical science. CE is widely used in today's demanding pharmaceutical industry for its fast and robust separation methods, needed in order to reduce the time to market for new drugs. Compared to chromatography, electrophoresis uses an entirely different mechanism of separations. This technique is extensively used nowadays due to small size requirements, low cost, ideal for samples that are costly and time consuming challenges in chromatography. Even though CE is mainly applicable to R&D laboratories, it is migrating towards product testing laboratories, quality control and quality assurance departments, which indicates the technique does show unique benefits and can expect sustained growth in the near future. For long term sustainability of this technique, further development of capillaries suitable for protein analysis and development of other detection modules such as CE-MS is necessary. The present article is an overview of CE and its applications in various fields. Further developments in CE make this technique demanding in future for pharmaceutical industry.

Keyword: Capillary electrophoresis, Capillary electro chromatography, Micellar electro kinetic chromatography, Micro emulsion electro kinetic chromatography.

INTRODUCTION

Capillary electrophoresis is the most rapidly extended analytical technique which was employed in the recent years. It has been widely used in the analysis of inorganic ion and in the analysis of drugs, which has been already used for the bio polymer analysis. Tiny fused silica capillaries are available (< 100 µm I.D.) which was protected by a layer of polyimide to make physically resistant. [1-3] Capillary Electrophoresis is a powerful separation technique that is widely used in Research and Development (R&D), Quality Control (QC) and stability studies of Pharmaceuticals. CE offers several advantages over high performance liquid chromatography which include rapid analysis, automation, ruggedness, different mechanisms for selectivity and low cost. Further, it offers higher efficiency and thus greater resolution over HPLC, even if only a small sample size is available. These advantages are likely to lead to even greater use of CE in R&D, QC and stability studies of pharmaceuticals. CE has been found particularly useful for separations of peptides, proteins, carbohydrates, inorganic ions, chiral compounds and in numerous other pharmaceutical applications. [4-6]

The use of CE methods for pharmaceutical analysis has become increasingly popular in recent years. The advantages of CE for pharmaceutical analysis include its speed and cost of analysis, reduction in solvent consumption, disposal and the possibility of rapid method development. CE instruments can be coupled to a variety of detector types including mass spectrometers, for special applications and more detailed analysis. [7]

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Various Types of Separation Techniques are

1. Capillary Zone Electrophoresis

CZE is the simplest technique of the free solution capillary electrophoresis. The components are separated based on the differential migration generated by the differences in effective mobilities. The capillary is filled with a buffer electrolyte which ensures the controlling of the pH, current flow, viscosity and ionic strength. On injection of the

sample, they are separated electrophoretically into various individual pure zones on application of an electrical field. In CZE, the ionized compounds only can have a differential migration, whereas the neutral compounds cannot be separated. The neutral are carried to the detection site by the EOF and the neutral components move with the mobility and velocity as that of the EOF. The selectivity is increased by the use of several modifiers like complexing agents and organic solvents. [8, 9, 10]

The most commonly employed additives in CE are organic solvents (acetonitrile, methanol), to increase the solubility of the sample components in the running buffer, anionic (e.g. Sodium dodecyl sulfate, SDS), Cationic (e.g.Cetyl tri-methyl ammonium bromide) or neutral (e.g. Brij) surfactants (below their critical micelle concentration CMC), to increase solubility or to act as ion pairing agents, organic amines (triethylamine, triethanolamine) and linear polymers (PEG, Polyacrylamide, methylcellulose) to increase viscosity, to mask wall charges and to introduce a sieving selectivity into the system. [11]

2. Capillary Gel Electrophoresis

The Capillary is filled with an agarose gel or polyacrylamide in Capillary Gel Electrophoresis which forms a network of polymer acting as a molecular sieving mechanism especially for the separation of species by molecular size, where the analytes move through the pores of the gel. [12] CGE is mostly employed for the separation of proteins, large bio-molecules, DNA Fragments and polynucleotide's. CGE has reasonable advantages than the conventional slab-gel electrophoresis due to its automation, small sample requirement, high thorough-put, trace quantitation, efficient molecular mass determination, high sensitivity and is used in the analysis of wide variety of large bio-molecules. [13-16]

3. Capillary Isotachophoresis

The Capillary Isotachophoresis resembles the classical isotachophoresis which is an important separation mode in CE. In CITP the analytes migrate at the same velocity, hence the name 'iso' for same and 'tach' for speed. In this mode of separation, the sample is injected between two buffers a leading electrolyte which has the ions of higher mobility than any of the analyte ions and a terminating electrolyte with ions of a lower mobility than the sample ions. The ionic analyte tend to migrate at the same velocity, in discrete zones, after the leading electrolyte and before the terminating electrolyte, according to the individual mobilities. [17] The difference in migration rate results in the separation of the analyte ions into adjacent bands, where the fast species are located in a band directly adjacent to the leading buffer and the slowest species just ahead of the terminal buffer. After the bands have been formed, the ions then move at the same velocity. [18]

4. Capillary Isoelectric Focusing

CIEF is generally used to separate amphiprotic species, such as amino acids and proteins which contains a weak carboxylic acid group and a weak base amine group. The basic principle under -lied in the CIEF is the molecules tend to migrate till it is charged. The buffer in the CIEF is arranged in a pH gradient, which is available commercially as carrier ampholytes, where different zwitter ions with a range of isoelectric points and these molecules are very small, hence their electrophoretic mobilities are very high and can move quickly. When a potential is applied, all the molecules tend to migrate to their isoelectric point, whereas the carrier ampholytes move quickly because of their higher mobility when compared to the analytes and as a result the pH gradient is established in a short period of time. After the carrier ampholytes have reached their isoelectric points and after the establishment of the pH gradient, the analytes still move in order to get to their isoelectric points. At last in CIEF the peak can be identified by using chemical markers of known isoelectric points. The analytes are spaced before or after this marker in the electropherogram. Marker

compounds are added to the sample such that it helps to identify the electropherogram. CIEF is used to separate biological molecules mainly proteins or peptides based on difference between their isoelectric points. The pH at which known net migration occurs is called isoelectric point (PI) and it is an important physical constant for characterizing the amino acids. [18]

5. Capillary Electro Chromatography

CEC combines the principles of CE and Chromatography with the major difference that the micelles are replaced by very small, i.e., less than 3µm, solid or semi-solid particles in a packed or open column. The particles form a typical stationary phase and the mobile phase is obtained through the electrically driven flow resulting from the EOF. In CEC, separation of solutes is achieved by differential migration resulting from chromatographic partitioning, electro migration or a combination of both. The separations are performed using packed columns or in open tubular mode. [19] In open tubular mode, the stationary phase is coated on the inner surface of the capillary column. CEC is almost similar to HPLC, but results in a higher separation efficiency due to the flat profile of the EOF (mobile phase) and probably the stacking effect due to electro dispersion. Additionally, in CEC small particle sized phases can be applied since the EOF does not generate back pressure. The particles can be sol-gel, [19] molecularly imprinted polymers, [20] continuous monolithic beds [21] and polymer/ layered silicate nanocomposites [22] with reverse phase (e.g. ODS C_{18}), normal phase, ion-exchange (SCX) or size exclusion properties. [23]

6. Micellar Electro kinetic Chromatography

In this mode, the separation is based on chromatography like separation mechanism, where a micellar pseudo-stationary phase is introduced in the buffer, where it retains the solutes according to the partitioning between the stationary phase itself and the mobile phase (i.e. the running buffer).In this technique, a surfactant is employed which includes sodium dodecyl sulphate (SDS) at a concentration level where the micelles are formed. The micelles are formed in the aqueous solutions when the concentration of an ionic species having a long chain hydrocarbon tail which is increased above a certain level called as Critical micelle concentration (CMC). At this junction, the ions starts to form spherical aggregates which are made up to 40 to 100 ions, where the hydrocarbon tails are in the interior of the aggregate and its charged ends are exposed to water on the outside. Micelles constitute a stable second phase which is capable of absorbing non-polar compounds into the hydrocarbon interior of the particles, hence solubilizing the non-polar species. When the capillary electrophoresis is carried out in the presence of micelles, it is termed as micellar electro kinetic capillary chromatography and is termed as MEKC or MECC. [18] In this separation technique, addition of surfactant is made to the operating buffer in amounts that exceed the critical micelle concentration (Fig.1).

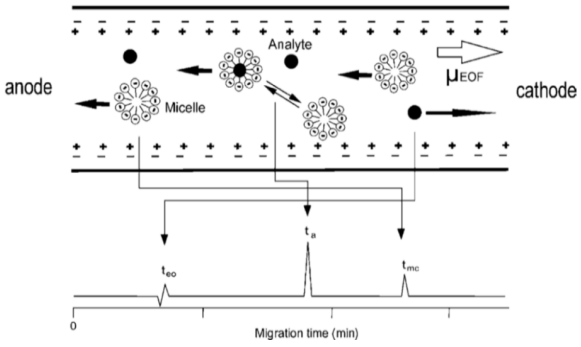

Fig.1: Schematic Representation of Micellar Electro kinetic Chromatography using anionic micelles, t_{eo} = migration time of a neutral "unretained" analyte, t_a = retention time in MEKC, t_{mc} = migration time of a micelle.

In brief, migration of polar molecules takes place faster than the less polar and hydrophobic compounds and the neutral substances elute within a time window, determined by the mobility of the electro osmotic flow and that of the micelles. Several other common surfactants that are employed in MECC in addition to SDS are bile salts and hydrophobic-chain

quaternary ammonium salts, to reduce the hydrophobic interactions between analyte and micelles and to increase the migration velocity similar to reverse phase chromatography. [24, 25]

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Table 1: Selected applications of Micellar Electro kinetic Chromatography [26, 27, 28, 29, 30]

Analyte	Buffer
Urinary porphyrins	85 mM SDS, 17 mM CAPS, 15% Me OH, pH 11
Cold Prep (14 drugs)	50 mM Sodium deoxycholate ,pH 9
Water-soluble vitamins	50 mM SDS, 20 mM $P0_4$, pH 9
β-lactam antibiotics	150 mM SDS, 20 mM $P0_4$ borate, pH 9
Penicillins	20 mM $P0_4$,100 mM SDS, pH 8.5

7. Micro emulsion Electro kinetic Chromatography

MEEKC is a mode of CE, where the separation is achieved by employing micro emulsion as a carrier electrolyte. In contrary to the other CE techniques, MEEKC is a specific method which can be used for the separation of neutral analytes based on their chromatographic retention factors. Micro emulsions are obtained by dispersion of two immiscible liquid which may be either of oil droplets suspended in water or of water droplets suspended in an oil phase. The separation system in the micro emulsion mode utilizes a high pH buffer, octane, butanol and SDS, where the solute is partitioned between the aqueous phase and the oil droplets, where it tends to move through the solution. [31] The oil in water micro emulsions are selected for the MEEKC separation and it consists of octane droplets which are dispersed in an aqueous buffer. Surfactant is added and it coats the octane droplets and the surface tension between the two liquids is lowered and forms a stable micro emulsion. The addition of a short chain alcohol like n-butanol further lowers the surface tension and also stabilizes the emulsion and acts as a co-surfactant. The diameter of the droplets in the micro emulsion is below 10 nm, where the micro emulsion formed is transparent optically and resembles like a single-phase solvent, although it is a two phase system. SDS is the most commonly employed surfactant and is used for the micro emulsion stabilization and due to the addition of an anionic surfactant, the oil droplets acquires a negative charge and exhibits a electrophoretic mobility and migrated towards the anode. The aqueous phase normally consists of buffers like phosphate or borate buffer which has alkaline pH [32] (Fig.2).

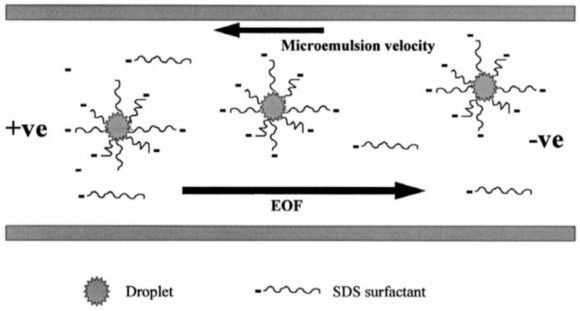


Fig.2: Schematic Illustration of the separation process in Micro emulsion electro kinetic chromatography for a neutral analyte in an alkaline micro emulsion stabilized by negatively charged Sodium dodecyl sulfate

Table 2: Pharmaceutical applications of Micro emulsion Electro kinetic Chromatography [33, 34, 35, 36]

Application	Micro emulsion Composition
Analysis of Betamethasone and derivatives.	1.44 % w/w SDS,0.81 % w/w octane ,6.6 w/w butan-1-ol, 91.4 % 20 mM sodium phosphate, pH 7.5
Analysis of 4-hydroxy benzoate preservatives in pharmaceuticals.	3.31 % w/w SDS,0.81 % w/w octane ,6.61 w/w but-an-1-ol 89.27 % w/w 50 mM phosphate buffer, pH 2.1
Analysis of formulated drug products	3.31 % w/w SDS,0.81 % w/w octane ,6.61 w/w butan-1-ol , 89.27 % w/w 10 mM borate buffer, pH 9.2
Analysis of ephedrine and pseudo-ephedrine.	23.3 mM SDS, 16.4 mM n-heptane,180.85 mM butan-1-ol 8% acetonitrile, 20 mM borate.

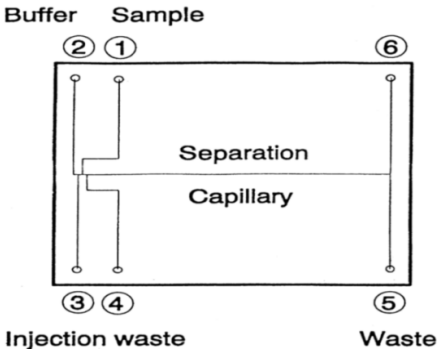
8. Non-aqueous Capillary Electrophoresis

Water insoluble basic drugs are troublesome to separate by Capillary Electrophoresis. Non aqueous capillary electrophoresis can be employed for the analysis of such type of samples. The electrolyte is made by the use of organic solvents which is a recent method in CE separation process. [37, 38, 39] A NACE electrolyte consists of organic solvents such as methanol and acetonitrile instead of water. Generally the electrolytes are prepared in 100 % organic solvents like methanol and acetonitrile, where the implication of non-aqueous solvents has an effect on the solvation of the ions and changes the pKa value of the solute. The selectivity can be altered by differing or changing the organic solvent employed or by using the solvent mixtures. The selectivity is altered by using additives like ion-pair agents, surfactants and cyclodextrins into the non-aqueous electrolyte. [41] NACE is the most commonly employed method for the analysis of basic drugs, which includes the analysis of opium alkaloids [40], tricyclic antidepressants, [42] a range of β-blockers [43] and different basic drugs. [44] NACE can also be used for the separation of polar acidic and basic drugs, calculating pka values of basic analytes in methanol [44] and chiral separation of pharmaceutical amines. [45]

9. Micro-chip Technology

The CE separations are made in a high speed manner by using micro fabricated devices or chips. There are number of devices which are recently employed for the miniaturized separations. The microchip CE offers great possibilities in the area of fast and inexpensive test equipment for clinical analysis, protein and DNA analysis. Mostly, the CE systems are performed or constructed on glass or plastic chips, which consists of appropriate set of rims where the capillary is replaced and is used to deliver the sample and wash solutions into the system. [46, 47] The lithographic technique has enabled the formation of network of channels on the micron scale which acts as a separation path for CE and also for the sample manipulation. The main advantage of chip technique is its capability to use electro kinetics for manipulation and to inject samples on the picolitre scale. [48] The sample is placed in the reservoir 1 and when the voltage is applied between the positions 1 and 3 for a short period of time, the sample tries to fill a small segment of the separation capillary. A very precise sample loading is obtained when the voltage is switched off and applied again between positions 2 and 6 [49, 50] (Fig.3).

Fig .3: Layout of Micro-chip



APPLICATIONS OF CAPILLARY ELECTROPHORESIS

1. Chiral Separation

CE plays an evident role in the separation of chiral compounds [51, 52, 53] and has started penetrating in the fields of environmental toxicant analysis and forensic drug analysis. [54, 55] CE has attained more attention in the chiral analysis and has proved to be the most successfully applied method for the separation of enantiomers. The most commonly employed chirally active selectors in CE includes Cu (II)-aspartame, natural and derivatised cyclodextrins, Cu (II)-L-histidine, bile salts, crown ethers, proteins (bovine serum albumin), α_1 –acid glycoprotein, ligand exchangers, glycosaminoglycan's, antibiotics and specific chiral additives like sulphated cyclodextrins and synthetic chiral surfactants, have also been developed for use in CE. [56]

Table 3: Selected Applications of Chiral Separation [57, 58, 59, 60, 61]

Application	Buffer
Enantiomeric separation of N-nitroso-nor nicotine found in tobacco smoke.	Citric acid buffer, pH 2.8, 30 mM HP-β-CD
Chiral antimalarial drug erythro-MQ and its analogues.	100 mM triethanolamine phosphate buffer, pH 3.0, 0.2-150 mg/ml HP-α-CD, acetyl-β-CD, HP-β-CD
Analgesic drug.	25 mM borate buffer, pH 9.0, 40 mM, SBE-β-CD
Non-steroidal Anti-inflammatory drug, S-Naproxen.	5 mM SB-β-CD, 20 mM TM-β-CD
Dopa enantiomers used to treat parkinson's disease.	10 mM Tris buffer, pH 2.5, 12 mM 18 C_6H_4

S-β-CD, Sulfate-β-cyclodextrin; HE-β-CD, Hydroxyethyl-β-cyclodextrin; HP-β-CD, Hydroxy propyl-β-cyclodextrin; SB-β-CD, Sulfobutyl-β-cyclodextrin; SU-β-CD, Sulfated-β-cyclodextrin ; TMCD, Trimethyl-β-CD; 18 C_6H_4 (18-Crown-6)-2,3,11,12-tetracarboxylic acid; L-ZGP,N-benzocarbonyl Glycyl-L-Proline.

Crown ethers are mostly used for the separation of chiral compounds which have a primary amino group. It is found to be specific for the analysis or separation of amino acids and basic drugs, where it is separated at low pH. A stabilized hydrogen-bond complex is formed by the crown ethers by protonating the primary amines. [62, 63] Most commonly employed crown ether is 18-Crown-6-tetra carboxylic acid and the combination of the crown ethers and cyclodextrins found to show enhanced chiral resolutions. [64]

2. DNA and Nucleic acid Analysis

CE has proved that it is a simple method to perform a simple and complex genetic test. Number of inherited genetic diseases like mitochondrial heteroplasmy, spinocerebellar ataxia, cystic fibrosis, fragile X and genetic variants of cytochrome P450 which is involved with the drug metabolism also can be detected by employing CE. The determination of the sequence of bases in DNA has become a big challenge and the slab gel electrophoresis has dominated the DNA sequencing, but recently the dominant technique employed is the CE. [65-70] The sophisticated CE attached with auto samplers can be used which can quantitatively inject and analyze more number of samples in an unattended sequence. Similar electrophoretic mobilities are shown by the DNA of different lengths as each increase in size is accompanied by increase in the number of negative charges. Hence, the separation cannot be done by mobility difference and the major separations are done by using a sieving mechanism, where the capillary is filled with a matrix of natural or synthetic polymer. [71, 72] The different DNA fragments move or migrate through the matrix and it becomes entangled or trapped in the matrix. The large DNA fragments migration is retarded and the separation is based on the size where, it is analogous to the gel permeation chromatography. A range of different polymers are employed which acts as a sieving media includes agarose, solutions of cellulose like hydroxyl ethyl cellulose and polyacrylamide. In the earlier stages, the CE had made the use of gels, where the capillary was filled with cross linked polyacrylamide gels, where it had faced problems like shrinkage, drying out and its inability to remove any contaminant. Therefore the use of polymer solutions or liquid gels have been used which offered improved performance and reliability. [73-75] CE is used to resolve and quantify the DNA species and the capillary arrays are used in the DNA sequencing of samples, where tens or hundreds of capillaries can be simultaneously analyzed to produce high throughout DNA sequences. [76, 77]

3. Ionic Analysis

CE is the most appropriate method for the determination of aqueous solutions and the solutes are small, which are highly charged ions. The solutes do not possess any chromophores and they are detected by indirect UV detection method. Commercially metal ion and inorganic anion test kits are available. [78]

Table 4: Application of CE for the separation of small molecules and ions [79, 80, 81, 82, 83, 84, 85]

Application	Electrolyte
Anions Indirect Detection TFA counter-ion of an Opioid peptide analgesic	Phthalate, CTAB
Drug inorganic counter ion determination	Chromate, TTAB
Drug organic acid counter ion determination	Phthalate, MES,TTAB
Anions- Direct detection Determination of residual Br in excess of chloride for local anaesthetic analysis	60:40 Me CN: Methane sulfonic acid buffer, pH1.3
Drug organic acid counter-ion determination e.g. Benzoate, Hydroxynapthoate	Borate, pH 9.5
Cations-Indirect Detection Ca in calcium acamprosate drug substance	Imidazole, sulfuric acid
Cations- Direct Detection K counter ion and inorganic cationic impurities of acidic drugs by conductivity determination	Creatine, acetic acid, 18- crown-6

In CE, the metal ions are generally determined by indirect UV detection method where a small positively charged UV active species like imidazole is added to the indirect detection buffer. The selectivity can be changed by the inclusion of complexing agents like small organic acids or crown ethers. A low pH buffer and uncoated capillaries are generally employed. [86] The inorganic anions and simple organic acids which are small, have high negative mobility's, where it opposes the EOF direction of an uncoated capillary, long migration times and a highly diffused peaks results due to this counter-migration. [87]

Hence the capillaries are coated to reverse the EOF direction. When a negative voltage is applied to the coated capillary, the solute migrates towards the detector which results in a fast and efficient separation. Generally, a cationic surfactant such as tetra decyl tri-methyl ammonium bromide (TTAB) or cetyl tri-methyl ammonium bromide (CTAB) is added to the electrolyte. The positively charged TTAB absorbs onto the capillary wall which results in the reversing of the EOF direction due to the capillary becoming positively charged. By the inclusion of UV active anions like chromate, phthalate or pyridine dicarboxylic acid the indirect UV detection is achieved. [88]

4. Pharmaceutical Substances

The pharmaceutical substances are mostly analyzed by HPLC, where it is applied for the determination of drug-related impurities, chiral separations, trace level determinations, quantification of the metal ions and the inorganic anions. The above specified applications are also possible by CE. Hence, CE is considered as a complementary technique to HPLC for the analysis of pharmaceutical substances. The CE methods have also been validated and it is also included in pharmacopoeias. [89, 90] Most of the pharmaceutical substances are bases and they are protonated at low pH. Hence, a low pH buffer like phosphate buffer of pH 2.5 can be used for the analysis of a wide range of basic drugs. For example phosphate buffer of low pH is used in the forensic analysis to analyze nearly 550 basic drugs. [91] Acidic drugs can be analyzed by employing buffers like borate or phosphate buffers at a pH between 7-10.A mixture of charged and neutral compounds can be analyzed by the use of MEKC or MEEKC methods, where CE has been applied for the analysis of major drug classes. [92] The CE method has also been validated similarly as HPLC which includes the assessment of validation parameters like accuracy, precision, specificity, linearity and sensitivity. [93]

CONCLUSION

CE has proved to be a sensitive, independent, reliable and versatile analytical technique, where the sample and reagent consumption and the running cost is lower than the chromatography. It is a new analytical tool which can be used for the investigation of both seized preparations of illicit drugs and biological samples too. CE has been routinely applied in the numerous fields, which includes the analysis of pharmaceuticals, DNA, chiral compounds, proteins, peptides, clinical and forensic samples, metal ions and inorganic anions.

Considerable research is being performed which is focused on the development of microchip CE. Capillary array CE is available commercially, which is used for the simultaneous analysis of multiple samples, where

the sample throughput is increased in the areas like serum protein analysis and DNA profiling. The recent advancements in CE include the non-aqueous CE, CE specific buffer additives such as sulphated cyclodextrins and novel capillary coatings.

CONFLICT OF INTEREST

None

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