CAPILLARY ELECTROPHORESIS

Technique first described by Lukacs (1980's)

Also referred as

- Jorgensen and High performance capillary electrophoresis (HPCE)
- Capillary zone electrophoresis (CZE)
- Free solution capillary electrophoresis (FSCE)
- Capillary electrophoresis (CE)

Electrophoresis:

The differential movement or migration of ions by attraction or repulsion in an electric field.

Capillary Electrophoresis:

- Capillary electrophoresis, then, is the technique of performing electrophoresis in buffer-filled, narrow-bore fused quartz silica capillaries, normally from 25 to 100 μm in internal diameter (ID) and 20-100 cm in length containing an appropriate electrolyte using a direct current (DC) high voltage source, capable of producing a current of 250 μA at voltage ranging from 1000 to 30,000volts and on-line detector that similar to those HPLC are involved (high voltage electrophoresis).
- Using narrow bore tube removes the Joule heating effect, which decreases band broadening, giving faster separations than gel.
- CE is used with/without gel.

Types of Molecules that can be Separated by Capillary Electrophoresis:

- Proteins
- Peptides
- Amino acids
- Nucleic acids (RNA and DNA)
- Inorganic ions
- Organic bases
- Organic acids
- Whole cells

Principle:

Capillary electrophoresis is an analytical technique that separates ions based on their electrophoretic mobility with the use of an applied voltage.

The electrophoretic mobility 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 the applied electric field i.e. the greater the field strength, the faster the mobility & vice versa.

Separations are facilitated by the use of high voltages, which may generate electro-osmotic and electro-phoretic flow of buffer solutions and ionic species, respectively within the capillary.

A positive (anode) and negative (cathode) electrode are placed in a solution containing ions.

Then, when a voltage is applied across the electrodes, solute ions of different charge, i.e., anions (negative) and cations (positive), will move through the solution towards the electrode of opposite charge.

Neutral species are not affected, only ions move with the electric field.

If two ions are the same size, the one with greater charge will move the fastest.

For ions of the same charge, the smaller particle has less friction and overall faster migration rate.

Capillary electrophoresis is used most predominately because it gives faster results and provides high resolution separation.

Sample's components migration depends on:

Electrophoretic mobility (μ): (the solute's response to the applied electrical field)

It is the process in which sample ions move under the influence of an applied voltage.

The flow of ions is toward the opposite charged electrode.

Electroosmotic flow (EOF): (occurs when the buffer moves through the capillary in response to the applied electrical field)

Osmosis under the influence of an electric field.

The speed of EOF can be adjusted by changing the buffer pH.

Bulk movement of solutes is caused by EOF.

EOF is usually sufficient to sweep all +ve, neutral, -ve species towards the same end.

Instrumentation:

Electrophoresis system consists of:

- Buffer solution (like sodium dihydrogen phosphate).
- sample vial
- source and destination vials
- electrodes
- High-voltage power supply (5 to 30 kV).
- A sample introduction system / sample injection (by pressure or vacuum).
- A capillary tube with internal diameter of 10-100µm & 20-100cm length.
- A detector.
- Output device.
- Some instruments include a temperature control device to ensure reproducible results. This is because the separation of the sample depends on the electrophoretic mobility and the viscosity of the solutions decreases as the column temperature rises.

The source vial, destination vial and capillary are filled with an electrolyte such as an aqueous buffer solution.

To introduce the sample, the capillary inlet is placed into a vial containing the sample.

Sample is introduced into the capillary via capillary action, pressure, siphoning, or electrokinetically, and the capillary is then returned to the source vial.

The migration of the analytes is initiated by an electric field that is applied between the source and destination vials and is supplied to the electrodes by the high voltage power supply.

In the most common mode of CE, all ions, positive or negative, are pulled through the capillary in the same direction by electroosmotic flow (EOF).

The analytes separate as they migrate due to their electrophoretic mobility, and are detected near the outlet end of the capillary.

The output of the detector is sent to a data output and handling device such as an integrator or computer.

The data is then displayed as an electropherogram, which reports detector response as a function of time.

Separated chemical compounds appear as peaks with different retention times in an electropherogram

The efficiency, N (Number of theoretical plates) may be expressed by the equation:

$N = \mu Ed/\ 2\ D$

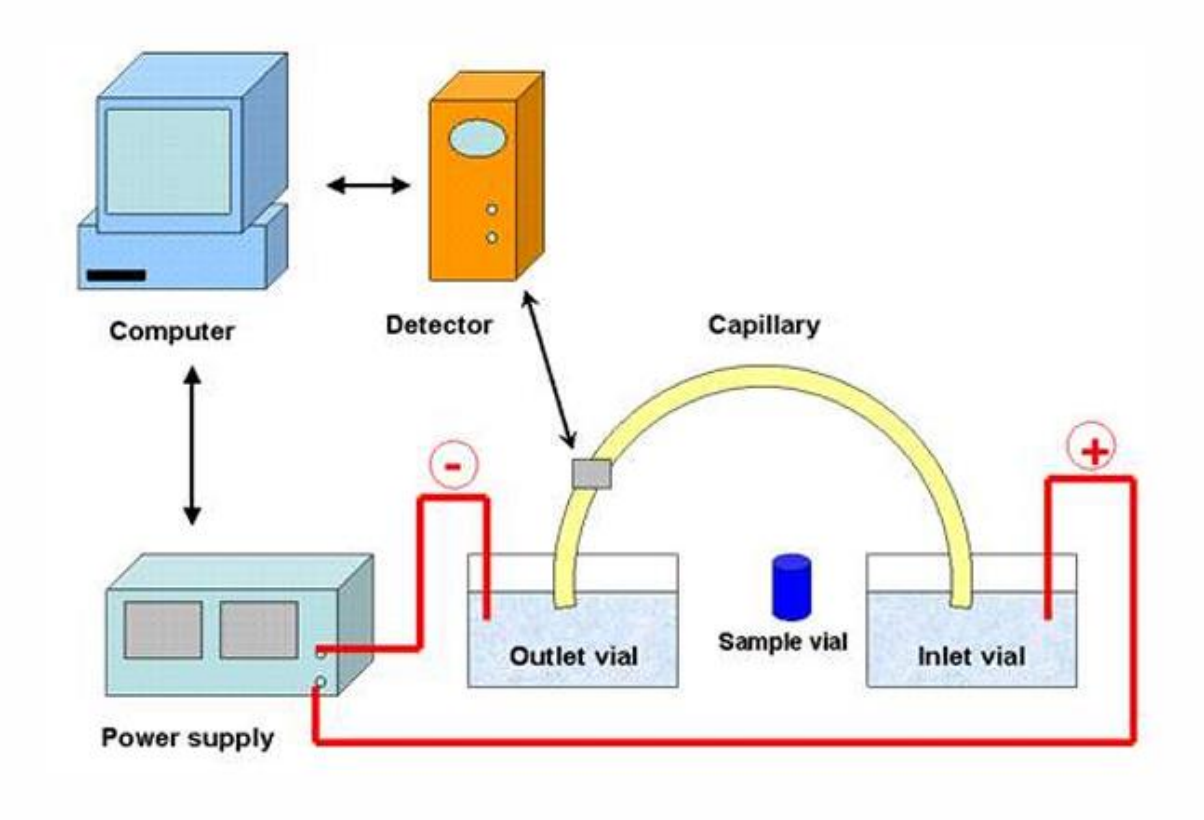
Where, D = the diffusion coefficients of migration species,

d = the distance travelled

μ = electrophoretic mobility of the species, and

E = the applied electric field

CE mechanism is entirely different from a chromatographic distribution mechanism, in that it is readily applicable smaller as well as macromolecules.Thus it is used for the separation of large biomolecules.



Sample injection:

Hydrodyanamic injection:

By applying pressure, by applying vacuum and by gravitation.

Electrokinetic injection:

By using Electric supply.

Detectors:

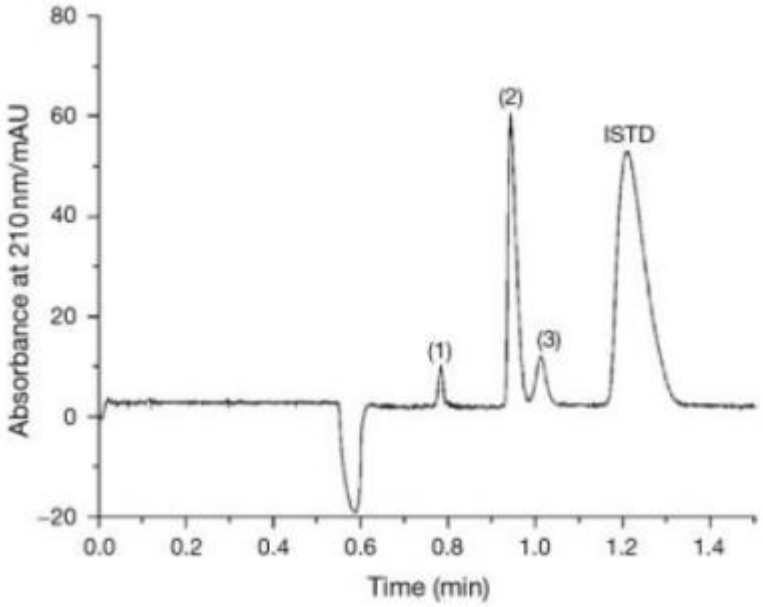
- UV detectors
- IR
- Fluorescence
- Laser induced fluorescence
- Conductivity and

- Indirect detection.
- The mass spectrometers is frequently used to give structural information on the resolved peaks.

The most commonly used detectors are a UV absorbance or a fluorescence monitor or a diode array spectrometer producing absorbance data at multiple wavelengths, on account of the very small volume (< 10-9 L) of the separated analytes, the detection is carried out on column (or on-line detection). For this a small outer part of the protective polyimide coating from the capillary surface is removed either by burning, dissolving or scratching. This small opening of the outer capillary surface then serves the purpose of a detector cell (optical window). The path length for the focused beam to be passed through such an opening is very small (50-100µm) which utilizes the small volumes.

Electropherogram:

The data output from CE is presented in the form of an electropherogram, which is analogous to a chromatogram. An electropherogram is a plot of migration time vs. detector response. The detector response is usually concentration dependent, such as UV-visible absorbance or fluorescence.



Modes Of CE:

- Capillary Zone electrophoresis (CZE).
- Capillary gel electrophoresis (CGE).
- Capillary isoelectric focusing (CIEF).
- Capillary isotachophoresis (CITP).
- Micellar Electrokinetic Capillary Chromatography (MECC)

Capillary gel electrophoresis

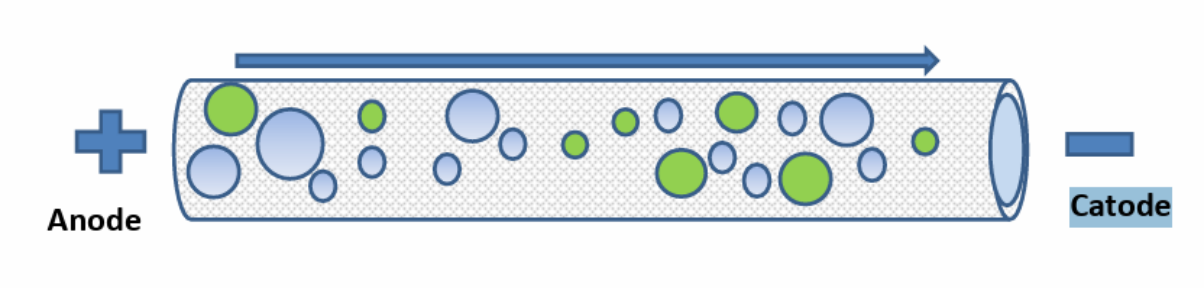
CGE is the adaptation of traditional gel electrophoresis into the capillary using polymers in solution to create a molecular sieve also known as replaceable physical gel.

Samples are injected electrokinetically.

CGE uses separation based on the difference in solute size as a particle migrate through the gel. Gels prevent the capillary walls from absorbing the solute.

This allows analytes having similar charge-to-mass ratios to also be resolved by size.

This technique is commonly employed in molecular weight analysis of proteins and in applications of DNA sequencing and genotyping.



Capillary Isoelectric Focusing

Separation of biological molecules mainly proteins, based on differences between the isoelectric points (pI)

These molecules are called zwitterionic compounds.

So, each molecule has a specific isoelectric point (pI).

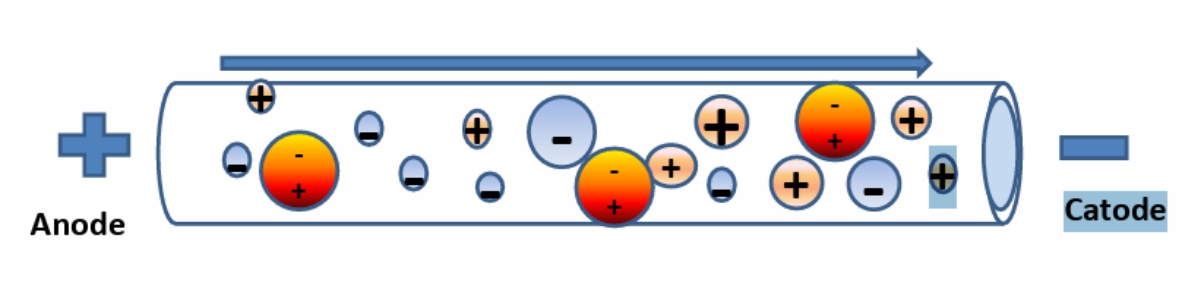
If pH = pI then molecule become a neutral.

A solute will migrate to a point where its net charge is zero.

At the solute's isoelectric point (pI), migration stops and the sample is focused into a tight zone.

In CIEF, once a solute has focused at its pI, the zone is mobilized past the detector by either pressure or chemical means.

This technique is commonly employed in protein characterization as a mechanism to determine a protein's isoelectric point.



Capillary Zone Electrophoresis

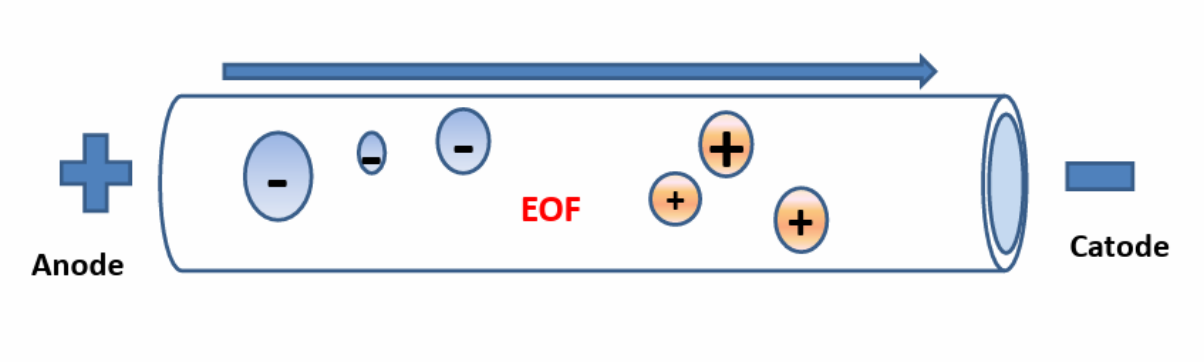
(CZE), also known as free-solution CE, is the most standard form of CE.

Buffer is flushed through the capillary by pressure, sample is injected and high voltage is applied.

Dependable on the polarity, the EOF is towards the inlet or the outlet.

Separation of charged molecules based on electrophoretic mobilities and migration velocities. Detection of charged cations species, including inorganic anions and , organic acids and amines, and large biomolecules such as proteins.

Fundamental to CZE are homogeneity of the buffer solution and constant field strength throughout the length of the capillary.



Capillary Isotachophoresis (CITP)

CITP is a focusing technique based on the migration of the sample components between leading and terminating electrolytes.

(isotach = same speed)

Solutes having mobilities intermediate to those of the leading and terminating electrolytes stack into sharp, focused zones.

Although it is used as a mode of separation, transient ITP has been used primarily as a sample concentration technique.

Micellar Electrokinetic Capillary Chromatography

MECC or MEKC is a mode of electrokinetic chromatography in which surfactants are added to the buffer solution at concentrations that form micelles.

Surfactants are surface active agents such as soap or synthetic detergents with polar and non-polar regions.

At low concentration, the surfactants are evenly distributed

At high concentration the surfactants form micelles

The separation principle of MEKC is based on a differential partition between the micelle and the solvent (a pseudo-stationary phase).

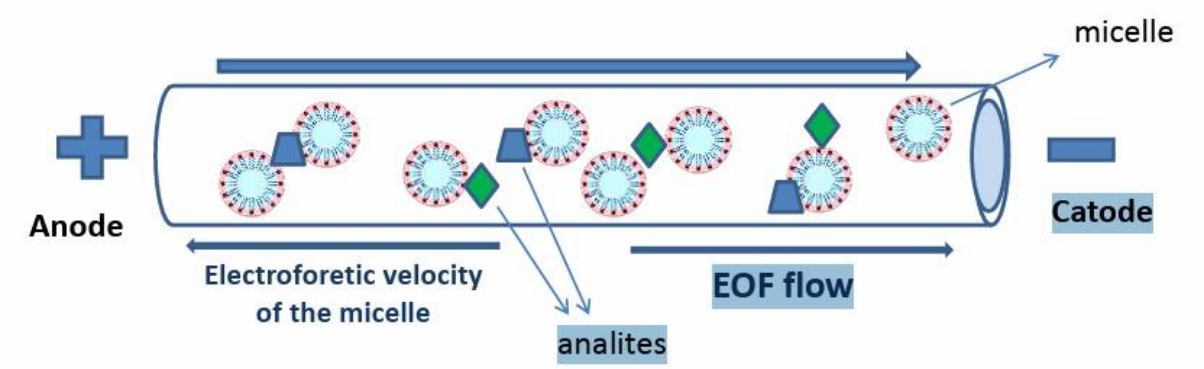
The most hydrophobic molecules will stay in the hydrophobic region on the surfactant micelle.

Less hydrophobic molecules will partition less strongly into the micelle.

Small polar molecules in the electrolyte move faster than molecules associated with the surfactant micelles.

This principle can be employed with charged or neutral solutes and may involve stationary or mobile micelles.

MEKC has great utility in separating mixtures that contain both ionic and neutral species, and has become valuable in the separation of very hydrophobic pharmaceuticals from their very polar metabolites.



Advantages:

- Simple
- Automated
- High efficiency of separation
- Short analysis time
- Low sample volume
- Ease of operation
- Ability to separate both charged and non-charged molecules
- Different mechanisms for selectivity
- Low cost
- Use aqueous rather organic solvents hence environment friendly

Disadvantages:

- Cannot do preparative scale separations, sticky compounds, Species that are difficult to dissolve.
- Aged, improperly stored blood samples.

Applications:

- Detecting bacterial/microbial contamination quickly using CE
- Direct inoculation (USP) requires a sample to be placed in a bacterial growth medium for several days, during which it is checked under a microscope for growth or by turbidity measurements.
- It is the most efficient separation technique available for the analysis of both large and small molecules.
- DNA Profiling, protein identification, inorganic metals and ions can be detected easily by this method.
- Capillary electrophoresis (CE) is the primary methodology used for separating and detecting short tandem repeat (STR) alleles in forensic DNA laboratories.
- Capillary electrophoresis may be used for the simultaneous determination of the ions NH_4^+, Na^+, K^+, Mg^{2+} and Ca^{2+} in saliva.

References:

A. H. Beckett, J. B. Stenlake, Practical Pharmaceutical Chemistry –Part Two Fourth edition CBS Publishers and Distributors, New Delhi (India); 2001

B. K. Sharma, Instrumental Methods of Chemical Analysis-Twenty Seventh Edition; 2011.