CAPILLARY ELECTROPHORESIS (CE)

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 and the atom's radius.

Electrophoretic techniques are divided into 3 groups:

1- Capillary electrophoresis

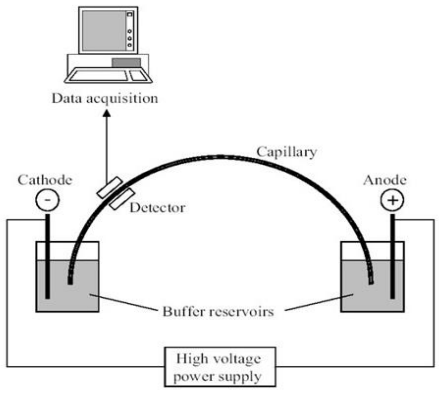
2- Disk electrophoresis

3- Gel electrophoresis

The rate at which the particle moves is directly proportional to the applied electric field--the greater the field strength, the faster the mobility. 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. Arnes Tiselius first showed the capability of electrophoresis.

A typical capillary electrophoresis system consists of a high-voltage power supply, a sample introduction system, a capillary tube, a detector and an output device. Some instruments include a temperature control device to ensure reproducible results.

In Capillary Electrophoresis a capillary is filled with a conductive fluid at a certain pH value. This is the buffer solution in which the sample will be separated. A sample is introduced in the capillary, either by pressure injection or by electrokinetic injection. A high voltage is generated over the capillary and due to this electric field the sample components move (migrate) through the capillary at different speeds. Positive components migrate to the negative electrode, negative components migrate to the positive electrode. When you look at the capillary at a certain place with a detector you will first see the fast components pass, and later on the slower components.



In High Performance Liquid Chromatography (HPLC) and Gas Chromatography (GC) the separating force is the difference in affinity of the sample components to a stationary phase, and or difference in boiling point. With both techniques the most important factor is the polarity of a sample component. In CE the separating force is the difference in charge to size ratio. Not a flow through the column, but the electric field will do the separation.

Electrophoresis in small diameter capillaries allows the use of high electric fields. Thus, small diameter vessels effectively distribute the produced heat. An increase in the electric field results in an effective separation and shortening of the separation time.

Capillary tubes have an inner diameter of 50-75 µm and a length of 0.25-1 m. The potential applied is 20-30 kV. Due to the electro-osmotic flow, all the sample components move in the direction of the negative electrode. A very small amount of the sample (at the level of picolitre and nanolitre) is injected into the positive tip of the capillaries and the separated components are diagnosed near the negative tip of the capillaries.

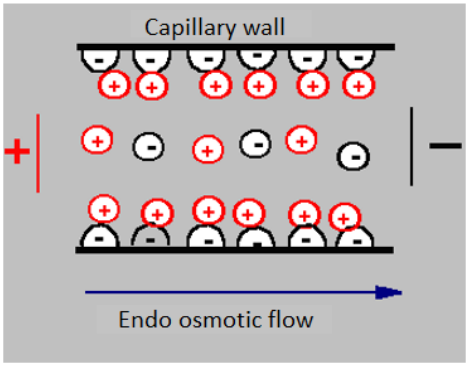
Capillary electrophoresis usually uses two injection methods: hydrodynamic and electrokinetic. Hydrodynamic injection is performed by applying a pressure difference between the two ends of the capillaries. In electrokinetic injection, the positive tip of capillary is immersed in a sample in a small container, and a voltage of 5 kV for a few seconds is applied. Thus, the effect of electroosmotic flow and ionic migration allows the sampling of the sample into the capillaries.

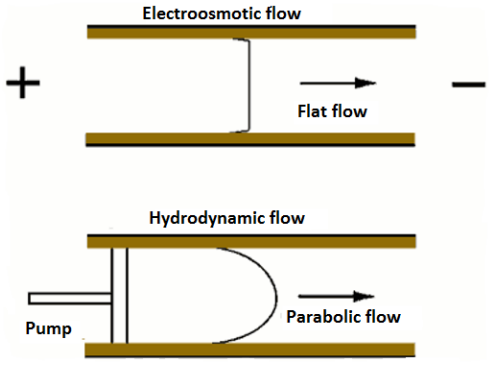
CE detectors are similar to those used in HPLC. These are absorbance, fluorescence, electrochemical detectors.

Endo Osmotic Flow / Electroosmotic Flow (EOF)

In most applications the capillary that is being used is made of bare fused silica. This material has at its surface silanol groups (Si - O - H). These groups are slightly acidic. In buffers at higher pH value there are a lot of negative charges at the capillary wall (Si – O-). In the buffer fluid positive charges will be present because of the law of electrical neutrality. When a high voltage is generated over the capillary, these positive charges will start to migrate through the capillary towards the negative electrode. They will drag along the buffer fluid with them. This flow is called the (Electro) Endo Osmotic Flow (EOF). The higher the pH, the more negative charges on the capillary wall and the more positive charges in the fluid. This will generate a stronger EOF.

Because the positive charges are all located close to the capillary wall, and there is no pressure force in the middle of the capillary, the flow profile of the EOF is completely flat. This will cause no peak broadening like the parabolic flow profile in HPLC and GC, and that is one of the reasons why such a high resolution can be achieved in CE.





Molecule Typing that can Separate by Capillary Electrophoresis

Proteins, peptides, amino acids, nucleic acids, inorganic ions, organic bases and organic acids.

Advantages of Capillary Electrophoresis

Capillary electrophoresis is used most predominately because it gives faster results (1-45 min.) and provides high resolution separation. Small sample volume is available (0.1-1 nl). It is a useful technique because there is a large range of detection methods available. Other advantages are high selectivity, automation, linearity, reproducibility and using with mass spectrometry.

Capillary Electroseparation Methods

Free Solution Capillary Electrophoresis (FSCE)

Separations are mostly association to the protonation of the basic groups or the pH-controlled dissociation of the acidic groups.. These ion species are separated depending on their differences in charge / mass ratios. For example, basic drugs are separated at low pH , although acidic drugs are separated at high pHs. Neutral molecules can not be separated.

Capillary Zone Electrophoresis (CZE)

The sample is applied as a narrow zone (strip) surrounded by the buffer. When an electric field is applied, each sample component migrates through the capillary at its own speed. All sample components are separated to form zones of pure material. Neutral molecules can not be separated.

Capillary Gel Electrophoresis (CGE)

With this technique there is a gel matrix inside the capillary. Components with different size but the same mobility are separated with this technique. Components of bigger size will be slowed down more by the gel, and will migrate later through the capillary. Especially with protein and DNA separations this technique is frequently used.

Micellar ElectoKinetic Chromatography (MECC)

Neutral particles, move in the capillar tube at the speed of electroosmotic flow. Therefore, it is unlikely that uncharged particles will be separated. However, with the addition of a small amount of surfactant, the micelle formation at low concentrations.and neutral substances can also be separated.

Capillary Isoelectric Focusing (CIEF)

The separation in the CIEF is based on differences in the isoelectric points rather than the speed of the components. CIEF is a technique commonly used to separate peptides and proteins. These molecules are called zwitterionic compounds because they contain both positive and negative charges. The charge depends on the functional groups attached to the main chain and the surrounding pH of the environment.

Capillary Electrochromatography (CEC)

With this technique a capillary is partly packed with silica based particles with a stationary phase. When high voltage is applied over the capillary, the buffer fluid will start to migrate due to the EOF that is present because of the silica. The sample will have, just as in HPLC, more or less affinity for the stationary phase. This is the separating force in this technique. The only difference between HPLC and CEC is that not a pressure pump is being used to force the mobile phase through the packed bed (HPLC), but a high voltage is used for this purpose.

Capillary Isotachorphoresis (CITP)

The analyte migrates in consecutive zones and each zone length can be measured to find the quantity of sample present. The separation at CITP is based on the difference in the velocities of the ions in the sample region.