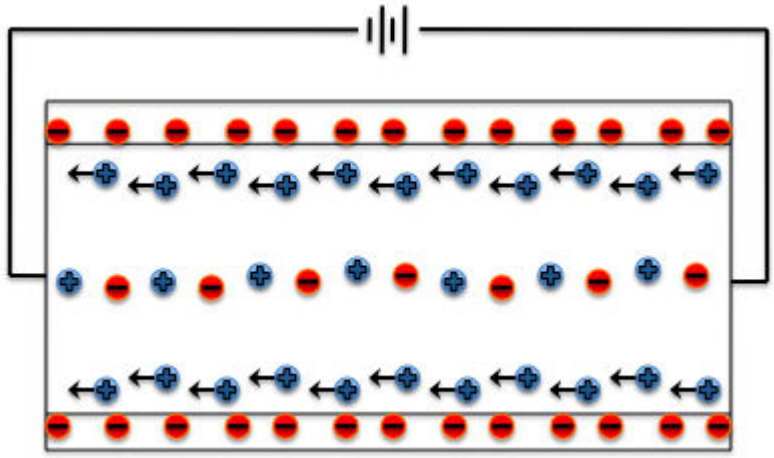
CAPILLARY ELECTROPHORESIS

Capillary electrophoresis (CE) is electrophoresis performed in a capillary tube. It is the most efficient separation technique available for the analysis of both large and small molecules. The main separation modes used in CE are capillary zone electrophoresis.

Electroosmosis refers to the movement of the buffer in the capillary under the influence of the electric field. The inner surface of a fused silica capillary is covered with silanol groups (Si-OH), which are ionized to SiO– at pH > 2. The negatively charged surface is counterbalanced by positive ions from the buffer, forming the so-called electric double layer. Under the influence of the electric field, the positive ions in the diffuse part of the double layer migrate towards the cathode; in doing so they entrain the waters of hydration, which results in electroosmotic flow.

Electroosmotic flow (or electro-osmotic flow) is the motion of liquid induced by an applied potential across a porous material, capillary tube, membrane, or any other fluid conduit.Electroosmotic flow is caused by the Coulomb force induced by an electric field on net mobile electric charge in a solution.



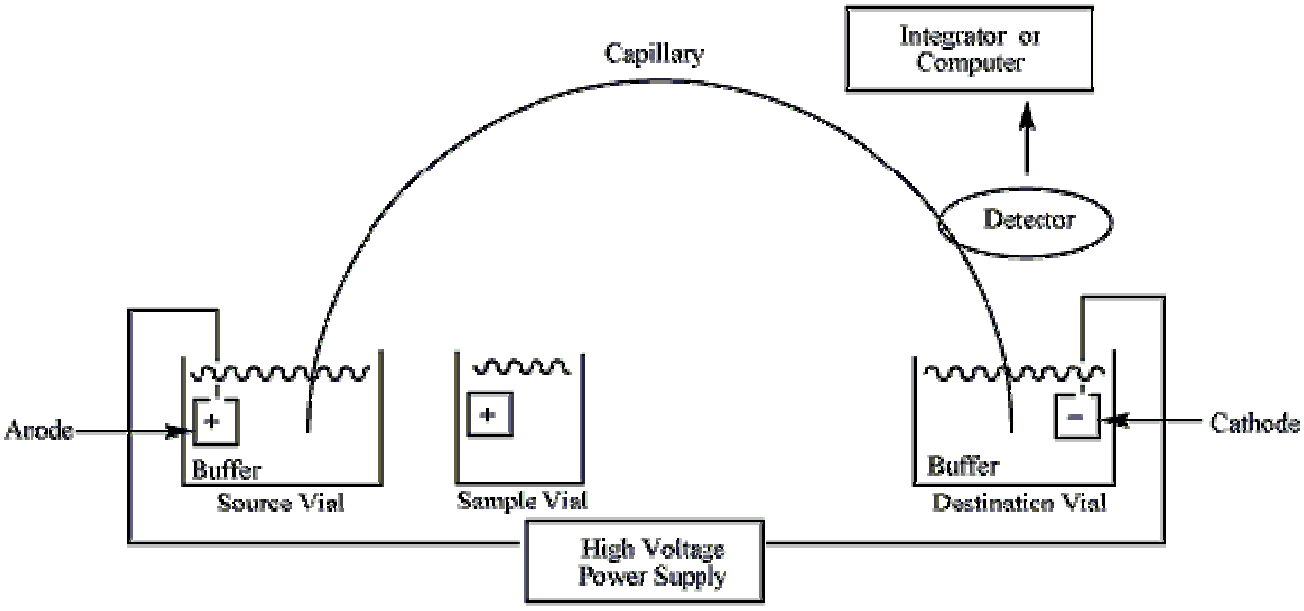
A double layer (also called an electrical double layer, EDL) is a structure that appears on the surface of an object when it is exposed to a fluid. The DL refers to two parallel layers of charge surrounding the object. The first layer, the surface charge (either positive or negative), comprises ions adsorbed onto the object due to chemical interactions. The second layer is composed of ions attracted to the surface charge via the Coulomb force. This second layer is

loosely associated with the object. It is made of free ions that move in the fluid under the influence of electric attraction rather than being firmly anchored. It is thus called the "diffuse layer".

CE Instrument Schematic:

A typical CE instrument uses the following components to achieve both EOF and Electrophoretic Mobility and therefore separations:

- Cathode (Negatively Charged Electrode)
- Anode (Positively Charged Electrode)
- Power Supply to generate Voltage/Current
- Catholyte (Buffer Solution at the Cathode End)
- Anolyte (Buffer Solution at the Anodic End)
- Capillary (25mm to 100mm ID)
- A Detection Method
- Data Acquisition Method



Capillary column:

The capillary column is a key element of the CE separation. Fused silica is by far the most frequently used material, although columns have been made of Teflon and borosilicate

glass.The widespread use of fused silica is due to its intrinsic properties, which include transparency over a wide range of the electromagnetic spectrum and a high thermal conductance.

Sample injection:

One of the main advantages of CE is its ability to inject extremely small volumes of sample. Typical injection volumes range from picoliters to nanoliters.

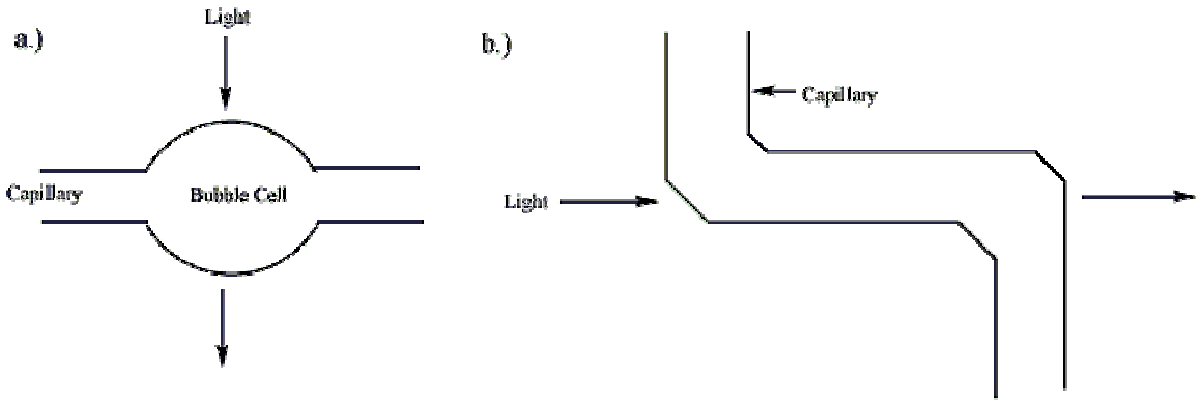
There are two commonly used injection methods for CE:

1. Electrokinetic Injection (Differs by analyte)
2. Hydrodynamic

Hydrodynamic injection is accomplished by the application of a pressure difference between the two ends of a capillary.Electrokinetic injection is performed by simply turning on the voltage for a certain period of time.

Detectors:

- UV Detector – Beer's Law
- Laser Fluorescence
- Chemiluminescence
- Diode Array Detector
- Refractive Index



Working:

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 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. 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.