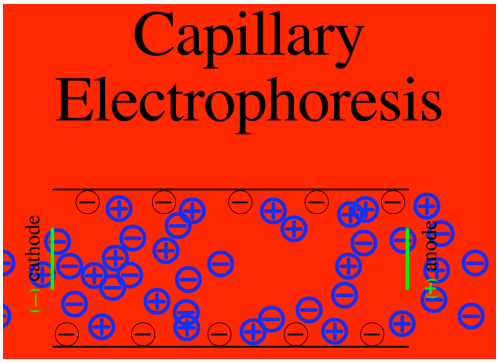
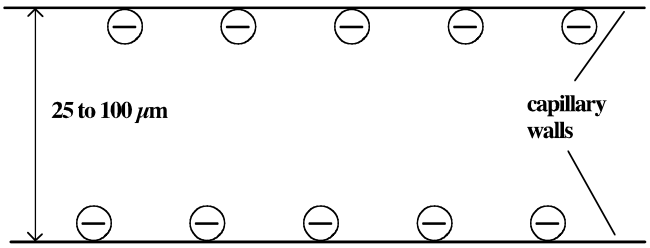
Capillary Electrophoresis



The heart of capillary electrophoresis (CE) is electroosmotic flow (EOF). This is the mobile phase "pump" in CE. Unlike gas chromatography (GC), there is no pressurized gas acting as the mobile phase in CE. Unlike high performance liquid chromatography (HPLC) these is no (high pressure) pumped mobile phase. And unlike paper chromatography, there is no capillary action that pulls the solvent through the stationary phase. Instead, the electrical potential maintained across the CE's capillary tube by the electrical circuit of the 1) capillary, 2) buffer, 3) reservoirs, 4) electrodes, and 5) power supply sets up some pretty interesting conditions that makes the buffer solution flow from one buffer reservoir to the other, just as if it were being pumped. This flow is called **electroosmotic flow**.

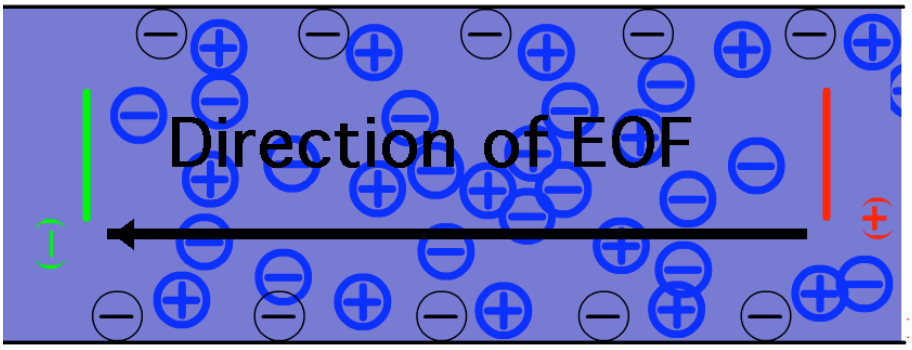
Here's what a naked fused silica capillary column looks like. They're often between 25 and 100 μm inside diameter and the walls—if they're uncoated, and this is important—are negatively charged because the inside wall has silanol groups (SO_x^-). This means that the inner wall has a net negative charge,



The buffer solution in each reservoir has equal amounts of cations and anions, and the capillary ends are each placed in a buffer reservoir. Each reservoir also has an electrode connected to the power supply.

When the voltage is applied to the circuit, one electrode become net positive and the other net negative. The (wall's) immobile silanol anions pair with mobile buffer cations, forming a double layer along the wall (wall--> buffer cations-->buffer anions-->bulk buffer solution). The remaining buffer cations are attracted to the negative electrode, dragging the bulk buffer solution with them. This is electroosmotic flow. <u>For an uncoated capillary, the EOF is toward the negative electrode</u>.

If the analyst wants the EOF (to flow) in the opposite direction then the capillary can be purchased coated with a cationic surfactant, or one is added to the buffer, and the capillary walls will be negatively charged and the electroosmotic flow will be reversed, that is, toward the positively charge electrode. This might be chose based on a specific analyte separation. In the case below the wall is uncoated, the wall is net negatively charged and the EOF is toward the negative electrode.

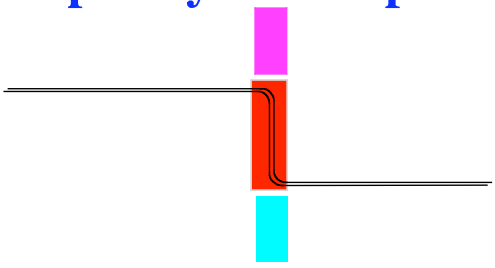


So everything injected into the buffer flows with the EOF. But, like the flow of analytes in a gas chromatographic carrier gas, separation wouldn't occur unless the analytes flow towards the detector at different speeds. In GC this occurs because of interaction with the GC columns stationary phase. In CE this occurs because analytes have different electrophoretic mobilities. In the simplest approximation, electrophoretic mobility can be because of analyte charge and size. Large, singly charged analytes will travel slower than small, singly charge analytes, and small, doubly charged ions will travel faster than larger, doubly charged analytes, etc. In other forms of CE (See www.shsu.edu/%7Echm_tgc/sounds/CEs.mov) separation is more complicated. The electrical potential also effects this process.

Like HPLC, capillary electrophoresis can use UV absorption detection. But because there are many CE separable analytes with poor UV absorbances (low molar absorptivities at UV wavelengths) a detection method called indirect detection is sometimes used. In indirect detection, a UV absorbing species is added to the buffer—it's there in a constant amount, producing a constant UV detector signal. Arrival of the poorly absorbing analyte at the detector—as the analytes are separated—yields a drop in absorbance. Voila! a signal.

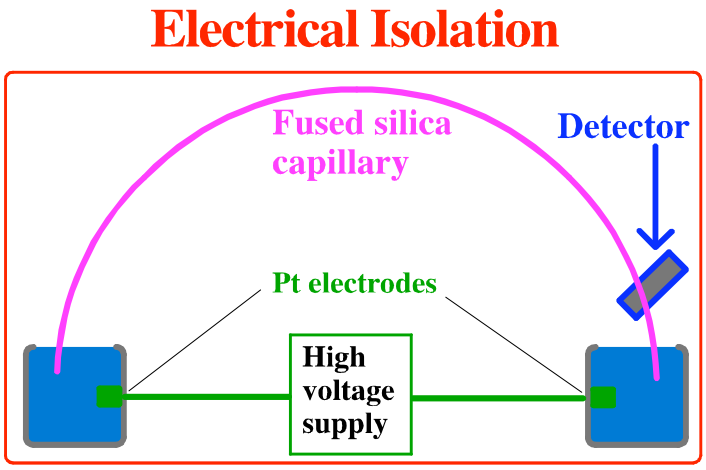
Different detector configurations are possible. Since the amount of each analyte passing the detector is very small, shooting the source lamp along a short section of the capillary increases the path length and, if absorbance is being used, decreases the detection limit (this is good).

Detection in Capillary Electrophoresis



To increase the absorbance, shining source light through a longer path length can be achieved by different configurations.

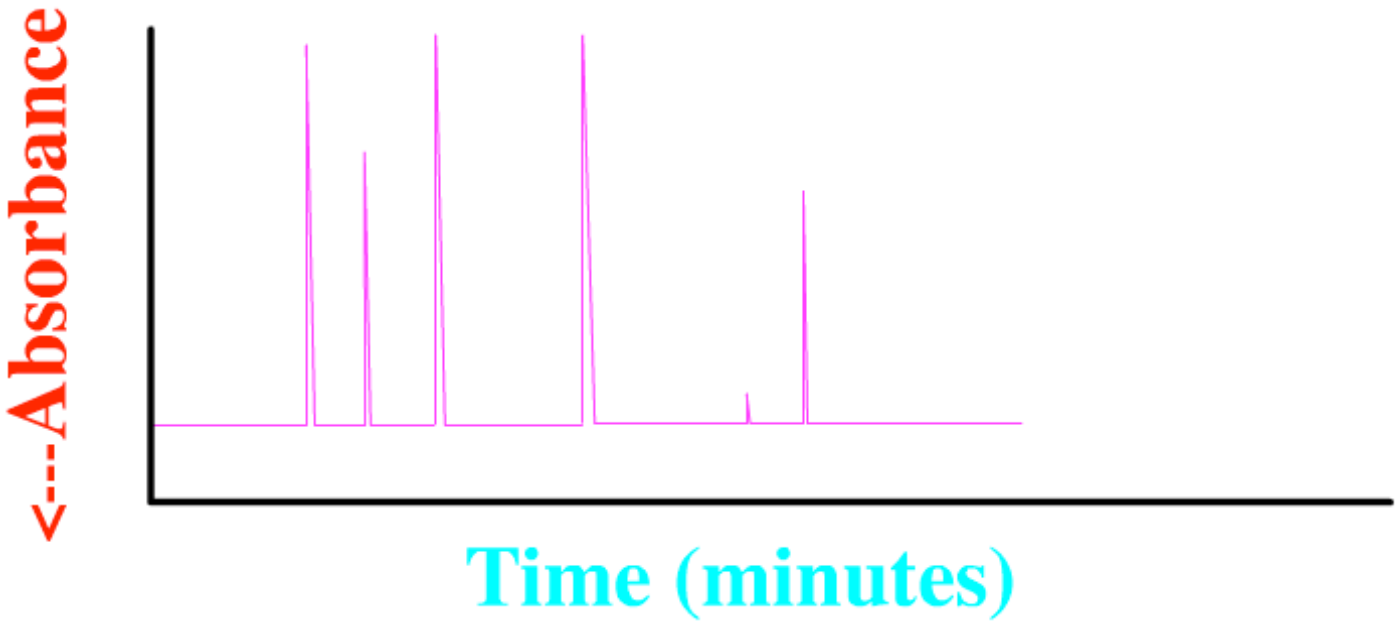
The final CE instrument's schematic looks like this (see below). Note that the entire system is isolated from foolish operator hands. Since typical CE potentials are 30,000 volts, this is smart and most modern CE instruments have computer-controlled safety systems that only apply the potential if the system is locked inside an insulated case.



Because of the small diameter of the typical CE capillary (25 to 100 μm), CE sample volumes are some of the smallest of any modern separation method. Sample volumes for HPLC are routinely 20 μL; those of capillary GC, 0.5 to 1 μL. But CE sample volumes are 50, 10, even 1 nanoliter (nL) or less. 1 nL!

The motive force that drives each of the analytes in CE is a function of each analyte's ionic charge, the ion's "mobility" in the buffer and the electrical potential across the capillary. Surprisingly this means that, unlike GC, where a longer column means more separating ability, in CE a longer capillary does not increase the number of theoretical plates. As a gas chromatographer this drives me crazy!

Like a chromatogram, the CE electropherogram is a plot of the **time from injection** on the x axis vs. **the detector signal** on the y. In the example below, **indirect detection** is being used. Note the y axis.



Capillary Electrophoresis and Forensic Chemistry References

"Capillary electrophoresis electrospray ionization ion trap mass spectrometry for analysis and confirmation testing of morphine and related compounds in urine". 2001; A. B. Wey and T. Wolfgang; J. Chromatogr. A.; v916; 225-238.
Detail: 2 to 5 ppm detection of morphine and codeine and metabolites via CE + Mass Spectrometric detection with no preconcentration.

"Simultaneous analysis of some amphetamine derivatives in urine by nonaqueous capillary electrophoresis coupled to electrospray ionization mass spectrometry". 2000; L. Geiser, S. Cherkaoui and J.-L. Veuthey. J. Chromatogr. A.; v895; 111-121.
Detail: 20 to 70 ppb determination of amphetamines via CE and mass spectrometric detection following liquid/liquid preconcentration.

"Blood group typing by electrophoresis based on isoelectric focusing"; 1999; M. Kanea, A. Nishimurab and K. Nishi; Anal. Chin. Acta; v383; 157-168.
Detail: Separation of blood proteins and enzymes using isoelectric focussing CE.

"Comparison of resolution of double-stranded and single-stranded DNA in capillary electrophoresis "; 1997; M. J. van der Schans A. W. H. M. Kuypers, A. D. Kloosterman, H. J. T. Janssen and F. M. Everaerts; J. Chromatogr. A,; V772; 255-264.
Detail: 200 base pair DNA fragments with differences of as little as 4 base pairs can be separated in under 7 minutes.