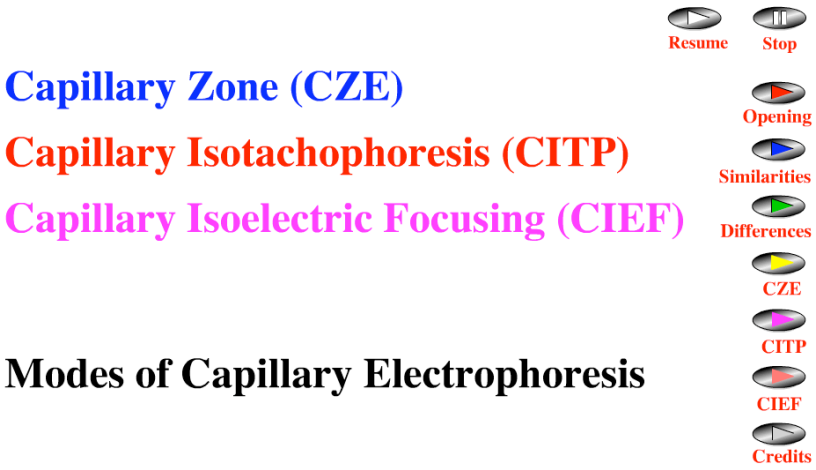
An animation detailing all three of these modes of capillary electrophoresis can be found here: www.shsu.edu/%7Echm_tgc/sounds/CEs.mov.



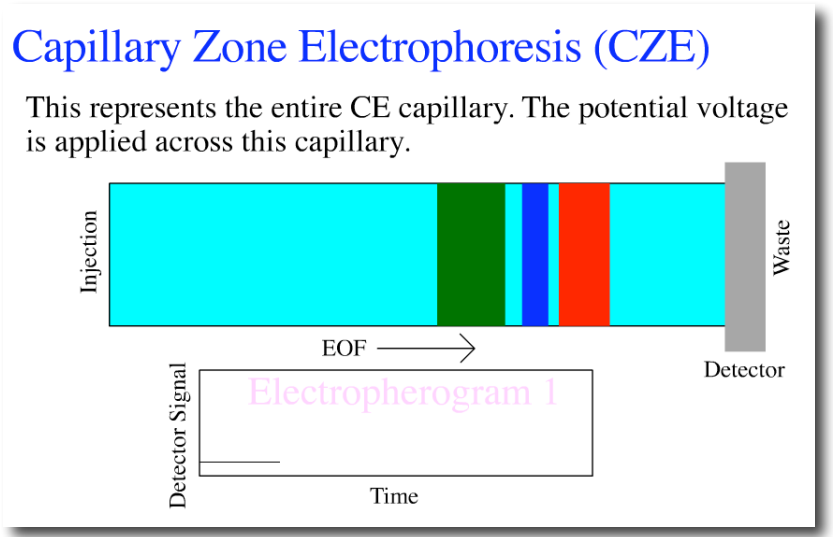
Similarities in these three modes of capillary electrophoresis

All analytes in the three methods I describe here, primarily move via electroosmotic flow (EOF, see my other animations on CE). That means, cations, anions, and neutral species move from the injector reservoir to the detector end of the capillary via EOF. CE methods in which analytes move against the EOF are less common because their analysis time is longer. With that said, it's only CZE that uses "simple" buffer solutions for separation. The other two methods described below use either conductivity gradients, when separating simple ions (CITP), or a pH gradient to separate amphiprotic compounds, molecules that can accept or donate a proton (CIEF). And one more point: although EOF is used in all methods, only CZE separates analytes during EOF. The other two methods use an electrical potential to achieve the separation, but EOF is only used in the last step to flow analytes pass the detector and off the column to waste.

In all of the following schematics, the long blue tube represents the entire CE capillary (whose diameter is really small). The potential voltage is applied across this capillary. Unlike capillary gas chromatography, the small diameter capillary used in CE is not very long at all, usually less than a meter, and modern CE instruments have an automatically controlled cooling system to remove joule heating that is produced by the electrophoretic process itself.

Capillary Zone Electrophoresis (CZE)

CZE analytes move in the EOF but separate into bands because of differences in their electrophoretic mobilities, μ. Differences in μ make each analyte's overall migration velocity slightly different, and difference in migration velocity = separation. μ's are roughly a function of analyte charge and frictional and size differences.

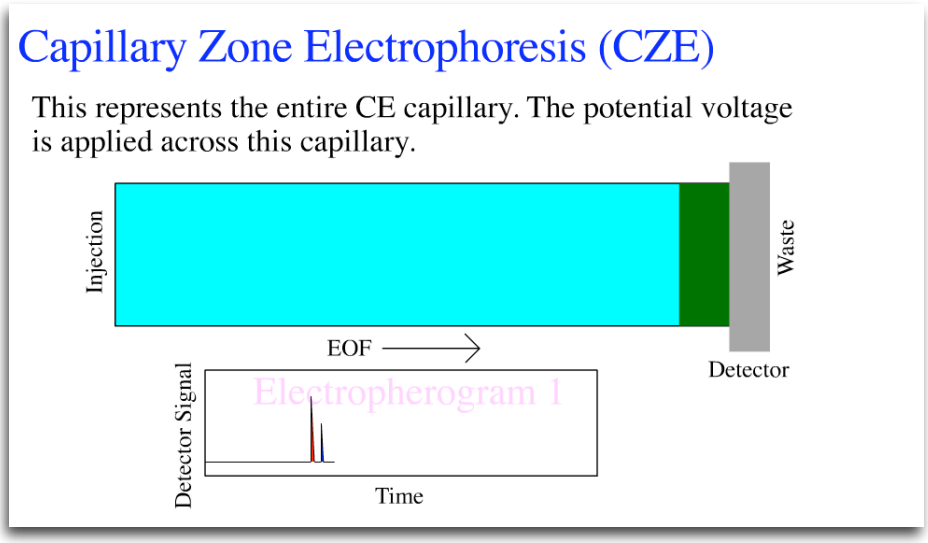


In the adjacent image, three peaks are traveling down the capillary from the beginning of the capillary on the left to the detector and exit reservoir on the right (again, this is a simple schematic). In this system, an absorption detector would work if the analytes have good molar absorption at wavelengths the detector has available (usually in the UV). If the analytes are poor absorbers then a strong UV absorber can be added to the run buffer and the **decrease** in absorption—when the analytes pass the detector—can be used to detect the

analytes. This last is called **indirect absorption**.

In the image above while the three analytes are still on the capillary, the electropherogram at the bottom of that image, which plots time versus detector signal, shows a flat line. Only when the analytes arrive at the detector will peaks appear in the electropherogram. Note: In CZE there is buffer between analyte bands.

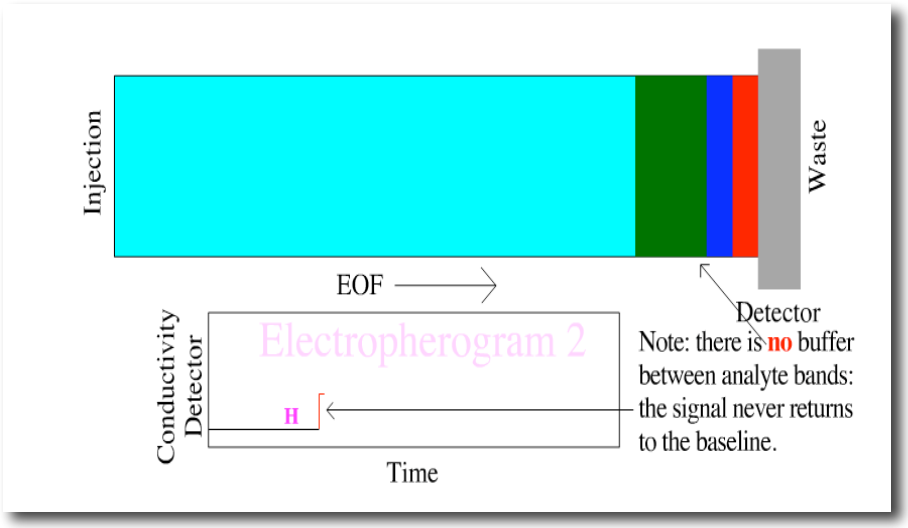
"All" that's required in the CZE method is a well-chosen buffer in the initial buffer reservoir. Separation occurs because of relatively simple interaction of the analytes with the pH of the buffer. **This technique is also called free solution capillary electrophoresis (FSCE)** for that reason. The capillary is often pre-washed with the buffer; the sample—dissolved in the same buffer—is injected; EOF is established; and you're off. This should be contrasted with the methods discussed below. In the adjacent figure, two of the peaks have already passed the detector and the third is about to.



Capillary Isotachophoresis (CITP)

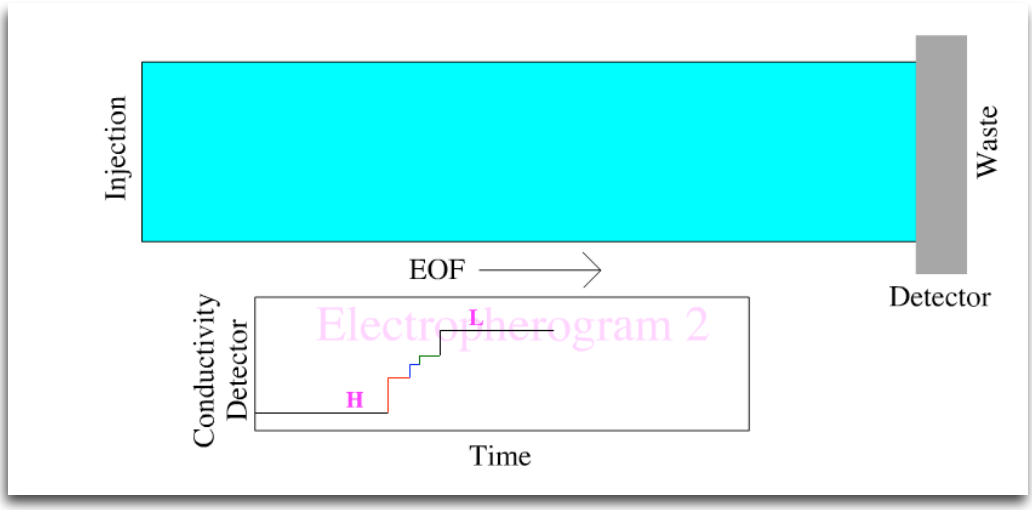
CITP sample injections are preceded by high mobility ions (H) and followed by low mobility ions (L) chosen so that analyte conductivities/mobilities lie between μ of H and L. This means that the H electrolyte must have a higher mobility than any of the analyte ions and L must have a mobility lower than any of the analyte ions. This is one step more complicated than CZE in that more careful solutions must be prepared than merely the run buffer of CZE.

The capillary is first filled with a solution containing the leading electrolyte, **H**. Leading, that is high mobility, ions can be small, completely dissociated ions, such as Cl^- for anionic separations or K^+ for cationic separations. Then analyte ions (the sample) are injected. Next a solution containing the trailing, that is low mobility, electrolyte (**L**) is introduced into the capillary's entrance reservoir and the capillary inserted. Trailing ions can be a weak acid in the case of anions separations or a weak base for cationic separation. In this way the analytes are sandwiched between H and L (as adjacent electropherograms show). When EOF is established, the analytes achieve a separation order based upon their mobilities, which also corresponds to their relative conductivities: highest mobility ions (H) are most conducting and lowest (L) are least conductive, analytes in between.



One of the most interesting feature about this method of CE is that the analytes order themselves immediately next to each other; that is, **there is no buffer between analyte bands**. This makes the CITP electropherogram very interesting (well, interesting for "traditional" chromatographers). So after the quick ordering of the "ion sandwich" EOF moves all ions past the detector and off the column. CITP analytes also move in the EOF—after their quick separation—all at the same speed (isotach = same speed).

Unlike CZE and CIEF, there's no buffer between analyte bands in CITP. Since the current (I) is consistently maintained across the entire capillary, the resistance of each analyte establishes a different potential, V, in each analyte band—the separating force. To satisfy V=IR, bands quickly form, ordered from bands of lowest to highest R. Band broadening is minimized in CITP because there is no buffer between analyte bands, and if analytes near a band's edge diffuse into the adjacent band (longitudinal diffusion) they experience a different voltage (V = IR again) and move back into their own band. Neat eh?



And finally the "identity" of each analyte is a function of its conductivity (the conductivity detector's signal intensity, y axis) and the amount of the analyte is a function of the width of the analyte band (x axis). Note the stair step feature of the CITP data: the fastest mobility and highest conductivity ions elutes first (H) and each analyte after that is more resistant (less conductive) and less mobile until the least mobile solution (L) elutes. **This is an easy way to recognize CITP data from a conductivity detector**. If the detector were not a conductivity detector then the electropherogram would look more "normal" (with baseline return of the detector signal after a peak) but there's a good chance that many of the analyte's would have no measurable UV absorption and so would be missing from the electropherogram. That's why CITP's power is best released by a conductivity detector for many applications.

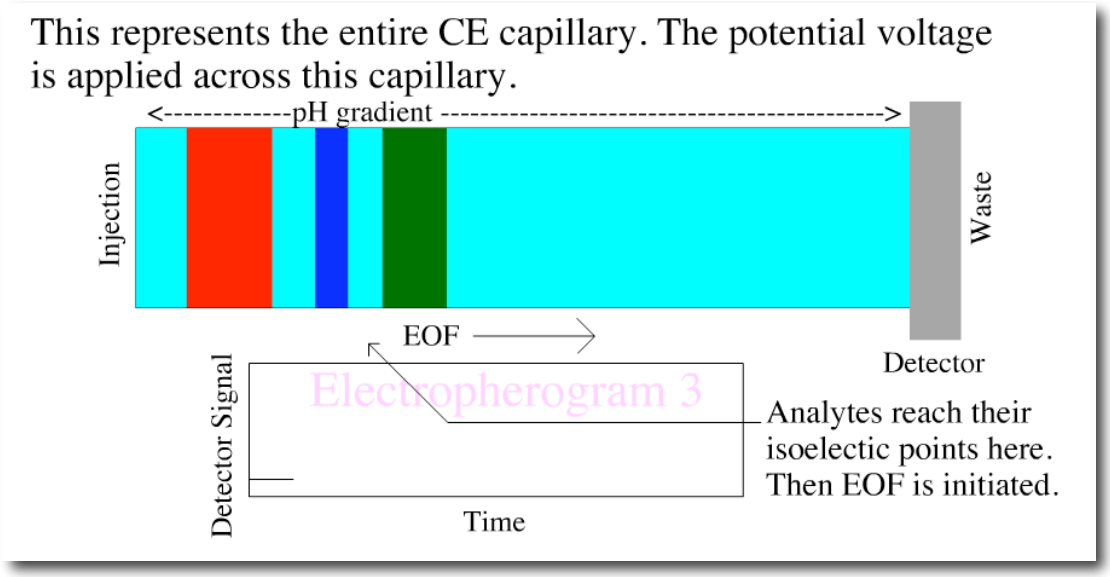
Capillary Isoelectric Focusing (CIEF)

Of the three methods we've talked about the buffer in this method is CIEF most complex: The buffer in CIEF is arranged in a pH gradient; it is usually a commercial mixture of so-called **carrier ampholytes**, many different zwitterions with a range of isoelectric points; these molecules are also small so their electrophoretic mobilities are high and they can move quickly. The more individual (that is different) ampholytes there are in the buffer, the smoother the pH gradient will be; the smoother the pH gradient, the better the separation between closely eluting peaks.

After filling the capillary with a mixture of the ampholytes and analyte molecules, a strong base is placed in one "buffer" reservoir and a strong acid in the other. When the system's electrical potential is applied, just as if we were trying to establish EOF (but wait), all molecules start to migrate to their isoelectric point, but the carrier ampholytes move quickest because their mobility is very high compared to the analytes' (let's say the analytes are proteins…), and this means that the pH gradient is established after a short time; the time length is a function of the carrier ampholytes characteristic and the voltage applied. This also applies to the focus step next too: higher voltages decreases analysis time.

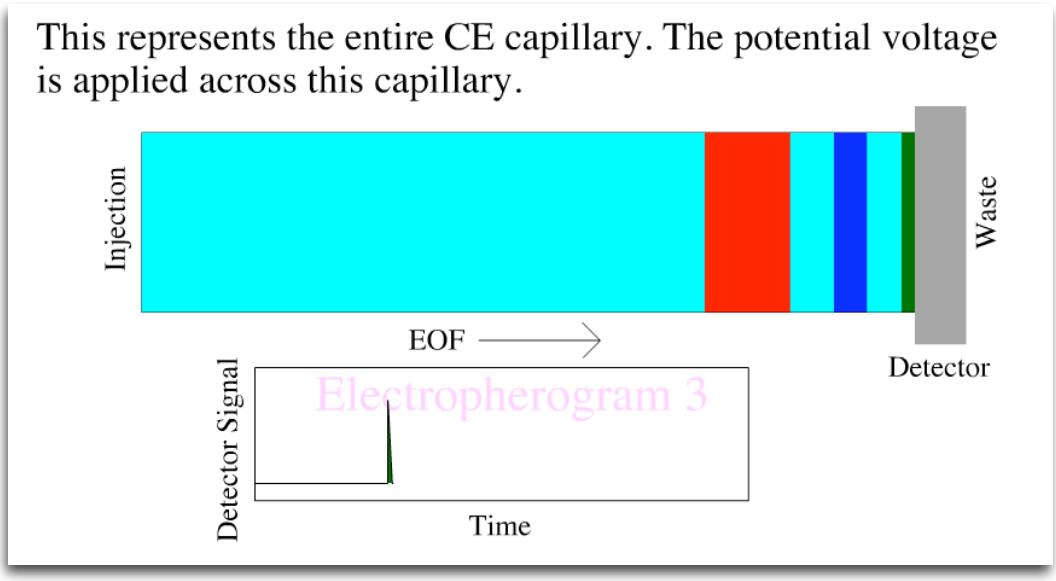
But after the carrier ampholytes have reached there isoelectric points and the pH gradient has been established, the (slower moving) analytes are still moving to get to *their* isoelectric points. Each analyte molecule also has a different isoelectric point (combination of multiple K_as or K_bs for each of these

macromolecules) so analyte bands focus at different capillary locations in the pH gradient during this, the so-called "focusing step." In theory the process is complete when "everyone" has reached their respective isoelectric points. And also in theory, this can be followed by watching the current in the system drop to zero.



Remember that current is a measure of charge flow in the circuit and when all ions are at their isoelectric point—and stop moving—the charge flow in the electrophoretic circuit stops, *so the current should fall to zero*. In reality, analysts seldom wait for this; based upon previous experience they may wait until the initial current has dropped to, say, 20% of the initial value and then the run is stopped. Running at the highest voltage possible yields the fastest run as long as the heat generated can be dissipated. Modern instruments can also be programmed to run at a constant power, so as the current drops as ions stop moving, the voltage ramps up automatically to compensate, but this too is also usually run until some predetermined low current flow is achieved, and then the run stopped.

After analyte focusing, the EOF is begun by changing the ionic strength in one of the buffer reservoirs; focused analytes and all ampholytes move to the detector. Note in the figure above that the peaks have reached their isoelectric points and are still far away from the detector, and therefore the electropherogram shows no eluted peaks, yet. They will then move at that same spacing/separation toward the detector. In the adjacent figure, the first peak is just about to finish eluting and is reflected in the ~~chromatogram~~, I mean electropherogram.



Finally, peak identification can be accomplished in CIEF by using chemical markers of known isoelectric points. Analytes will then be spaced before or after these marker in the electropherogram. Marker compounds can be added to the sample in amounts that help them to be identified in the subsequent electropherogram.

CZE, CITP and CIEF

Modes of Capillary Electrophoresis

T.G. Chasteen

Opening