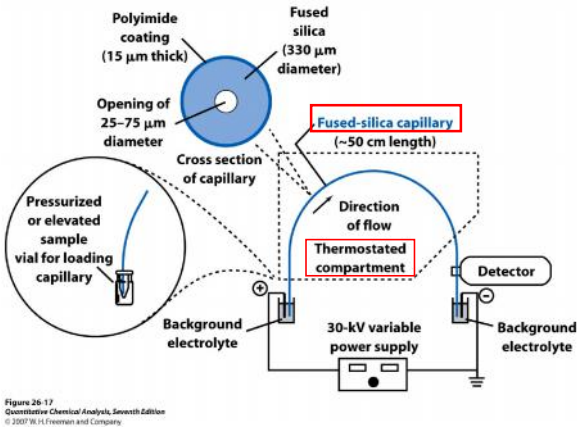
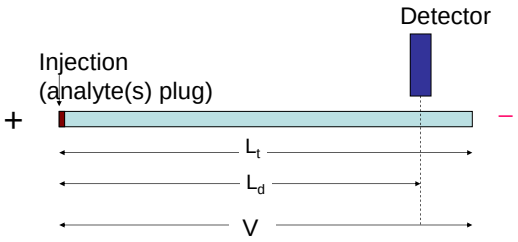
Capillary Electrophoresis

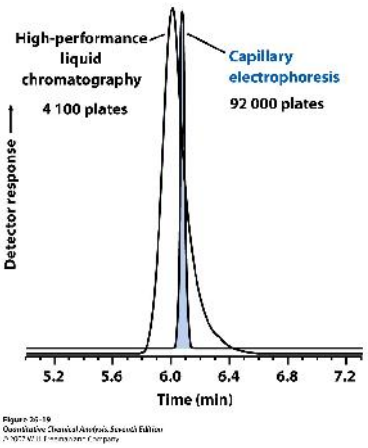
Electrophoresis is a separation technique based on the differential transportation velocities of charged species in an electric field through a conductive medium.

Primary candidates for CE separation are *ions*.

The basic instrumental set-up consists of a high voltage power supply (0 to 30 kV), a fused silica (SiO_2) capillary filled with a (background) buffer solution, two buffer solution reservoirs, two electrodes, and an on-column detector.







The very high resolution of CE is a consequence of the technique's extremely high efficiency.

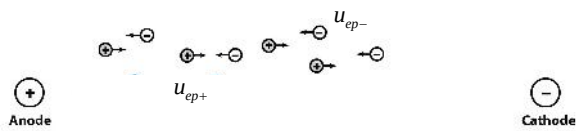
$$H = A + \frac{B}{u} + Cu$$

A lower value of *H* corresponds to a higher separation efficiency — when the plate height is reduced, more theoretical plates (*N* ~ 50,000 to 500,000) can be packed into a given length along the separation axis.

Resolution is proportional to $\sqrt{N}$.

In CE, *two terms* in Van Deemter Equation are *zero*; multiple-path term, *A,* mass-transfer term, *C*, because the separation is carried out in a single phase of uniformly flowing carrier liquid.

Only source of band broadening under ideal conditions is from the longitudinal diffusion term, *B*.



Electrophoretic Velocity: The velocity of a charged analyte under the influence of an electric field relative to the background electrolyte.

http://goldbook.iupac.org/ET06925.html

Electrophoretic Velocity

$F_{drag} = 6\pi\eta r u_{ep}$ (+) $+z$ $F_{elect} = zeE$ (-)

u_{ep}

$\leftarrow 2r \rightarrow$

$$F = 6\pi\eta r u_{ep} - zeE$$

At steady state: $0 = 6\pi\eta r u_{ep} - zeE$

u_{ep}: Electrophoretic velocity

$$u_{ep} = \frac{zeE}{6\pi\eta r} = \frac{eE}{6\pi\eta}\frac{z}{r}$$

z = charge on ion
r ~ size and shape

$$\mu_{ep} = \frac{u_{ep}}{E} = \frac{ze}{6\pi\eta r} = \frac{e}{6\pi\eta}\frac{z}{r}$$

Electrophoretic mobility

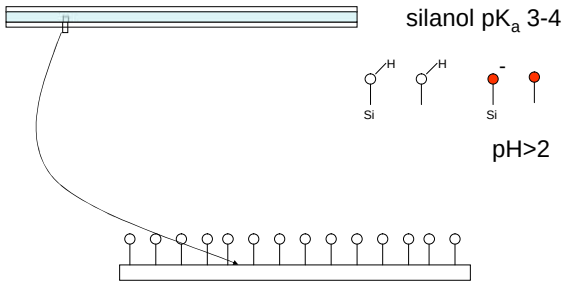
$$u_{ep} = \mu_{ep} E$$

Electro-osmotic velocity u_{eo}

The *mobility due to* a species being swept along in a flow arising from a *buffer solution's response* to an applied electric field (*electro-osmotic flow*).

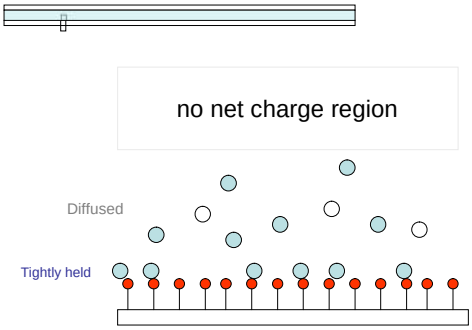
In the case CE, negative charges on the *silica* capillary wall create a double layer of charge.

The layer next to surface is rich in mobile, solvated cations which move towards the cathode and drag along anions and neutrals.



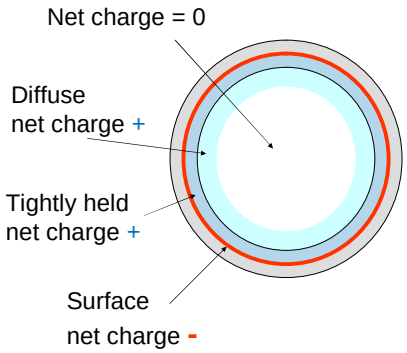
Tightly bound layer and surface charges

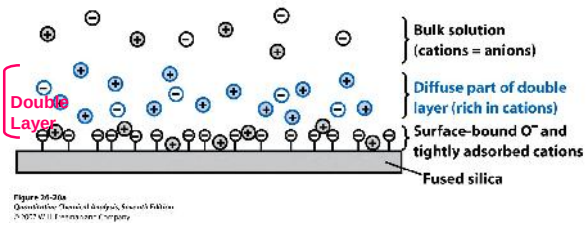
Structure of the capillary surface in contact with a buffer solution is electrically charged when in contact with buffer. In a basic buffer, for example the surface is negatively charged.



Surface charges, tightly bound layer, diffused layer and no net charge regions.

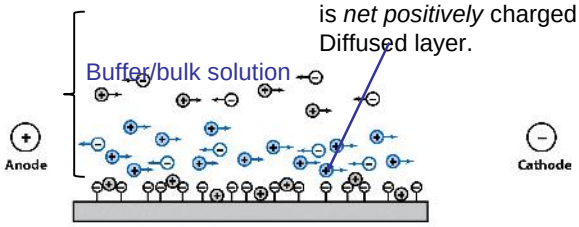
Cross-sectional view





Diffused layer positively charged

Solution next to surface is *net positively* charged. Diffused layer.



The bulk solution containing the buffer moves toward an electrode, here toward (-); electro-osmotic flow (EOF).

$$u_{eo} = «_{eo} E$$

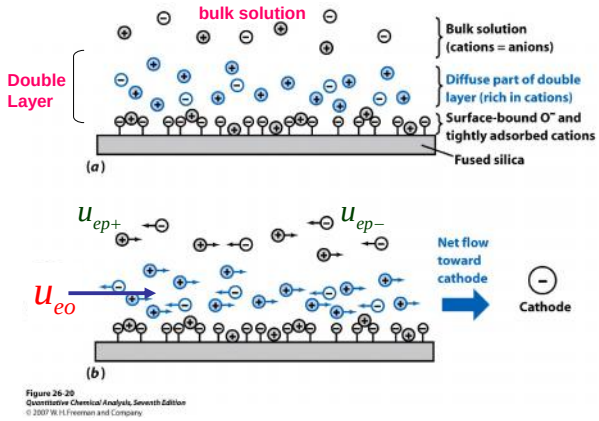


Figure 26-20
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$u_{eo} >> u_{ep}$ In general for pH>3

$u_{app+} = u_{eo} + u_{ep+}$ Positive ions, *apparent* velocity

$u_{app-} = u_{eo} - u_{ep-}$ Negative ions, *apparent* velocity

$u = u_{eo}$ Neutral molecules, *apparent* velocity

$u_+ > u > u_-$ (pH<=2, surface neutral; EOF=0) (pH>=11, highly charged)

Apparent mobilities

$\mu_{eo} >> \mu_{ep}$

$\mu_{app+} = \mu_{eo} + \mu_{ep+}$

$\mu_{app-} = \mu_{eo} - \mu_{ep-}$

$\mu = \mu_{eo}$

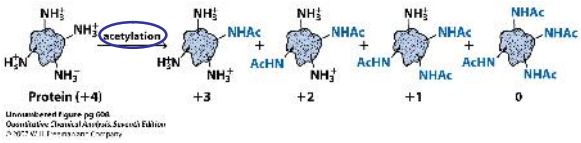
$\mu_{app+} > \mu > \mu_{app-}$

$$\mu_{app\pm} = \frac{u_{app\pm}}{E} = \frac{L_d / t_{\pm}}{V / L_t}$$

$$\mu_{eo} = \frac{L_d / t_{neutral}}{V / L_t}$$

The electro-osmotic mobility and electrophoretic mobility can cause *anions, cations and neutrals* to have a net migration towards the cathode because generally the bulk solution has a net positive charge.

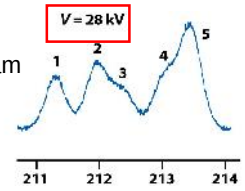
Depending on the charge and size, the molecules/ions move through at different speeds; separation is achieved.

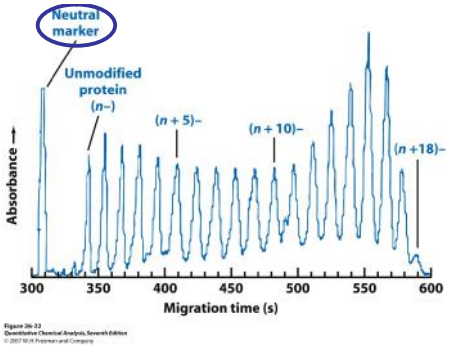


Example: Determine the charge of unmodified protein

r ~ nearly same for all species.

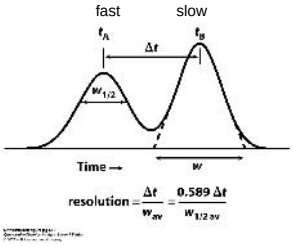
Electropherogram





Protein charge ladder. Acetylated bovine carbonic anhydrase

Resolution of (consecutive) peaks:



$$R = \frac{\Delta t}{w_{av}} = \frac{0.589\Delta t}{w_{1/2av}}$$

$$R = \frac{\sqrt{N}}{4}(\alpha - 1)$$

$$\alpha = \frac{u_{fast}}{u_{slow}} = \frac{t_{slow}}{t_{fast}}$$

$= separation\ factor$

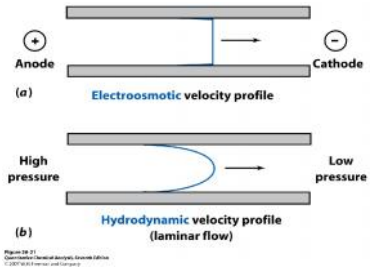
Efficiency in CE

The only contributor to peak broadening, practically, is diffusion.

A plug of analyte diffuses out to produce a Gaussian curve of distribution of concentration. The σ of it, in this case is given by the Einstein's Law;

$$\pm^2 = 2Dt \quad and \quad H = \frac{\pm^2}{L_d}$$

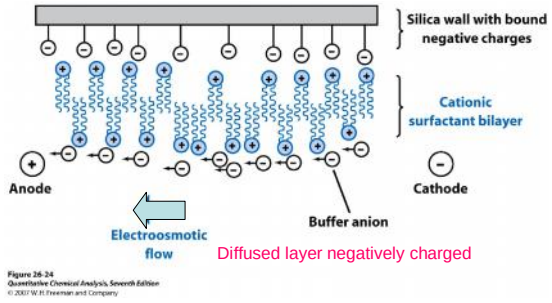
$$N = \frac{L_d^2}{2Dt} = \frac{«_{app}}{2D} \cdot V \cdot \frac{L_d}{L_t}$$

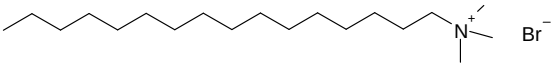


Electro-osmotic flow as opposed to laminar flow decreases broadening .

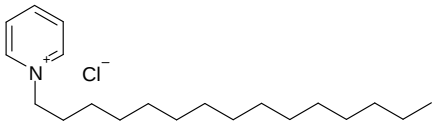
Controlling the nature of EOF:

The direction of the EOF can be changed with a cationic surfactant bilayer.



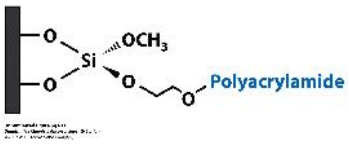


Hexadecyl-trimethyl-ammonium bromide

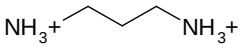


1-Hexadecylpyridinium chloride

Wall charge can be manipulated by derivatization of silanol groups on the wall surface, and also by adding modifiers to the (running) buffer solution

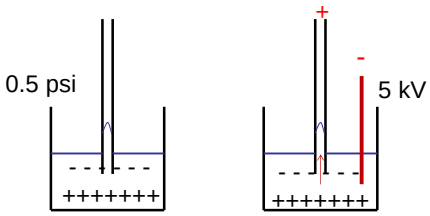


derivatization



modifier

Sample Injection:



Hydrodynamic

Uses the pressure difference between capillary ends

Electrolytic

Electric field drives sample into capillary

Hydrodynamic injection is accomplished by the application of a pressure difference between the two ends of a capillary. The amount of sample injected can be calculated by the Poiseuille equation.

Hydrodynamic injection volume:

$$Volume = \frac{\Delta P \cdot d^4 t}{128 \eta L_t}$$

ΔP is the pressure difference between the ends of the capillary, d is the inner diameter of the capillary, t is the injection time, η is the sample viscosity, and L_t is the total length of the capillary.

Electro-kinetic injection is performed by simply turning on the voltage for a certain period of time. The moles of each analyte injected, determined by the apparent mobility of each analyte, μ_{app}; the injection time, t; and the ratio of conductivities of the separation buffer and sample, concentration of the analyte ion C.

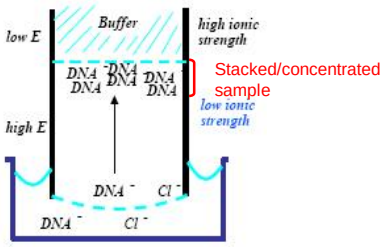
Electro-kinetic injection amount:

$$Moles = \mu_{app}\left(E\frac{\sigma_b}{\sigma_s}\right)t \cdot r^2 C$$

V/m mol/m^3

Concentrations (optimum):

Sample buffer ~ 1/10 background electrolyte (buffer)
Sample ~1/500 background electrolyte (buffer)

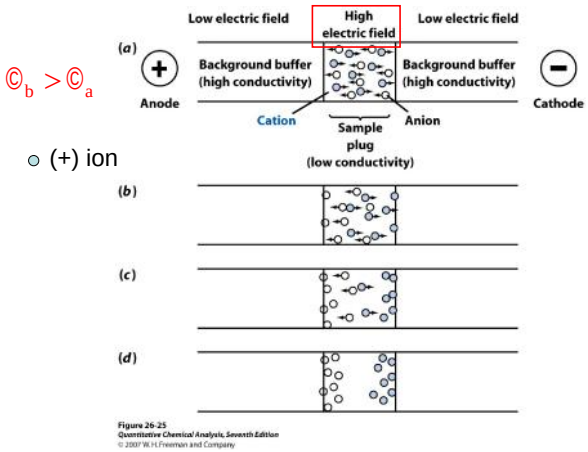


Because each analyte has a different mobility, electro-kinetic injection is *biased*. For qualitative analysis, this is not usually a problem. For quantitative analysis, the concentration/composition of the injected sample can be different than that of the original sample.

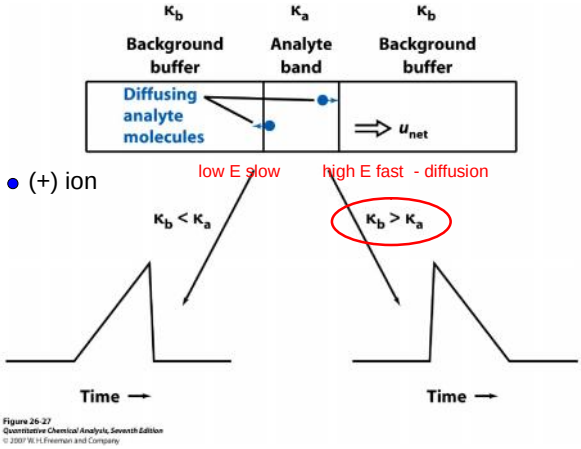
One of the main advantages of CE is its ability to inject extremely small volumes of sample. Typical injection volumes range from pico-liters to nano-liters.

Band skewing:

If the conductivities of the run electrolyte (background) and that of the analyte region are very different, distortions of the peaks occur.



- (+) ion



- (+) ion

*Joule heating***.** Joule heating is a consequence of the resistance of the solution to the flow of current.

$$H=VIt$$

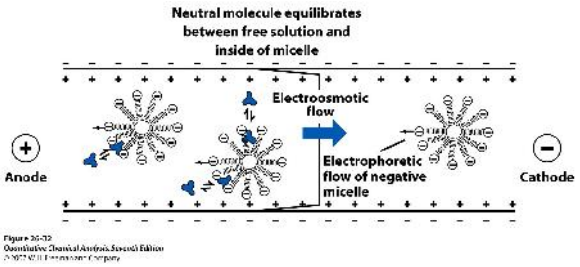
If the heat is not sufficiently dissipated from the system the resulting temperature and density gradients can reduce the separation efficiency. The capillary walls used in CE must dissipate efficiently.

- Detectors
  - **UV/Visible absorption**
  - **Fluorescence**
  - Radiometric (for radioactive substances)
  - Mass Spec.

	LOD (mol)
Spectrophotometric	
Absorption	$10^{-15} - 10^{-}$
Fluorescence	
Precolumn derivatization	$10^{-20} - 10^{-17}$
On-column derivatization	8×10^{-18}
Postcolumn derivatization	2×10^{-17}
Indirect	
UV	$10^{-13} - 10^{-12}$
Fluorescence	5×10^{-17}

Mass spectrometric	1×10^{-17}
Electrochemical	
Conductivity	1×10^{-16}
Amperometric	7×10^{-19}

Micellar Electro-kinetic Chromatography



Mobile phase = ‘micelles”

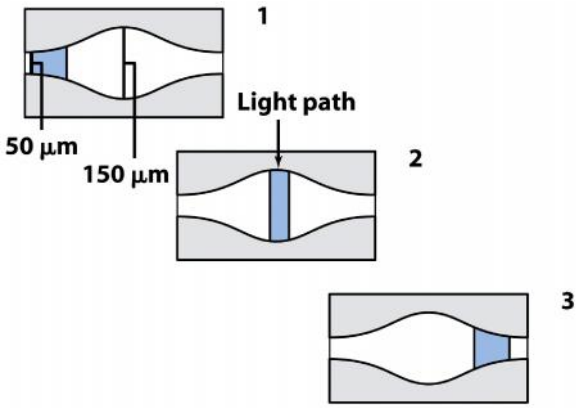


Figure 26-28a
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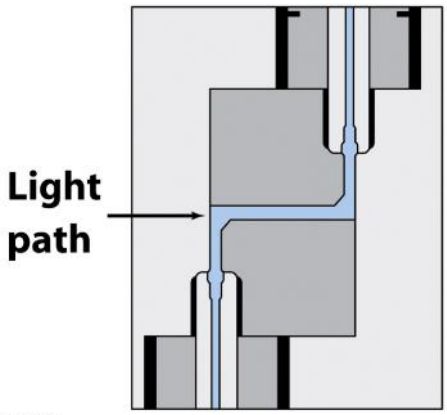

Figure 26-28b
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