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 (SiO₂) 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.

A lower value of $H$ corresponds to a higher separation efficiency — when the plate height is reduced, more theoretical plates ($N \sim 50,000$ 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, $B$. 

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

\[ u_{ep} = \frac{zeE}{6\pi\eta r} = \frac{eE}{6\pi\eta} \]

\[ z = \text{charge on ion} \]
\[ r \sim \text{size and shape} \]

\[ \mu_{ep} = \frac{u_{ep}}{E} = \frac{ze}{6\pi\eta r} = \frac{e}{6\pi\eta} r \]

**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.

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.
Tightly held
Diffused
no net charge region
Surface charges, tightly bound layer, diffused layer and no net charge regions.

Cross-sectional view
Net charge = 0
Diffuse net charge +
Tightly held net charge +
Surface net charge -

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).
\[ u_{eo} = \mu_{eo} E \]

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)

Diffused layer positively charged

Double Layer
Figure 14.19
Buffer/bulk solution
Anode
Cathode
Figure 14.20
Double Layer
bulk solution
Buffer/bulk solution
Solution next to surface is net positively charged. Diffused layer.
Apparent mobilities

\[ \mu_{eo} \gg \mu_{ep} \]

\[ \mu_{app}^+ = \mu_{eo} + \mu_{ep} \]

\[ \mu_{app}^- = \mu_{eo} - \mu_{ep} \]

\[ \mu = \mu_{eo} \]

\[ \mu_{app}^+ > \mu > \mu_{app}^- \]

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

\[ r \sim \text{nearby same for all species.} \]

Resolution of (consecutive) peaks:

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

\[ \gamma = \frac{u_{fast}}{u_{slow}} = \frac{t_{slow}}{t_{fast}} = \text{separation factor} \]
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 $\sigma$ of it, in this case is given by the Einstein’s Law:

$$\sigma^2 = 2Dt$$

and

$$H = \frac{\sigma^2}{L_d}$$

$$N = \frac{L_d^2}{2Dt} = \frac{\mu_{app}}{2D} \cdot V \cdot \frac{L_d}{L_i}$$

Controlling the nature of EOF:

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

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

Sample Injection:

Uses the pressure difference between capillary ends

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

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

Hexadecyl-trimethyl-ammonium bromide

1-Hexadecylpyridinium chloride

Sample Injection:

Hydrodynamic

Electrolytic

Uses the pressure difference between capillary ends

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:

\[
Volume = \frac{\Delta P \pi d^4 t}{128 \eta L}
\]

\(\Delta P\) is the pressure difference between the ends of the capillary, \(d\) is the inner diameter of the capillary, \(t\) is the injection time, \(\eta\) is the sample viscosity, and \(L\) is the total length of the capillary.

Electro-kinetic injection amount:

\[
Moles = \frac{4 \mu \ell r^2 C}{V m \text{ mol/m}^3}
\]

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.

Concentrations (optimum):

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

Band skewing:

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

<table>
<thead>
<tr>
<th>Spectrophotometric</th>
<th>LOD (mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absorption</td>
<td>(10^{-10})</td>
</tr>
<tr>
<td>Fluorescence</td>
<td>(10^{-10})</td>
</tr>
<tr>
<td>Precolumn derivatization</td>
<td>(10^{-10} - 10^{-7})</td>
</tr>
<tr>
<td>On-column derivatization</td>
<td>(5 \times 10^{-10})</td>
</tr>
<tr>
<td>Postcolumn derivatization</td>
<td>(2 \times 10^{-7})</td>
</tr>
<tr>
<td>Indirect UV</td>
<td>(10^{-9} - 10^{-12})</td>
</tr>
<tr>
<td>Fluorescence</td>
<td>(5 \times 10^{-7})</td>
</tr>
</tbody>
</table>

Micellar Electro-kinetic Chromatography

Mobile phase = ‘micelles’