Fundamentals of Capillary Electrophoresis

- Separation is driven by electric field
  - Combination of effects
- Several modes of operation
  - Depends on primarily buffer composition.

Flow through capillary is result of superposition two processes:

- **Electrophoretic Flow**
  - Cations are drawn toward cathode (-)
  - Anions are drawn toward anode (+)
  - Neutrals are unaffected
  - Mobility is determined by mass to charge ratio
    - Contributes through velocity and drag

- **Electroosmotic Flow**
  - Because cations are highly solvated, solvent is also drug toward cathode
  - Since solvation is a dynamic process, the result is general flow of all components (cations, anions, and neutrals) to toward cathode
  - $v_{\text{electro}} > v_{\text{electrophor}}$
  - Electrical double-layer causes cations to congregate near walls
    - Flat flow profile
Separation Efficiency in CE

- Migration velocity: Depends on voltage and mobility
  \[ v = (\mu_e + \mu_{eo})E = (\mu_e + \mu_{eo})V/L \]

- Electrophoretic mobility may be positive (cations), zero (neutrals), or negative (anions).

- Electroosmotic mobility is generally positive
  - Everything is being drawn to the cathode
  - Can be reversed by altering surface chemistry of the capillary
    - add cationic surfactant
  - Can be minimized by "neutralizing" the surface of the capillary
    - convert charged silanol groups to neutrals (like Si(CH₃)₃Cl)

- Plate Height:
  \[ N = \frac{(\mu_e + \mu_{eo})V}{2D} \]
  - Independent of capillary length
  - High potential is better...ideally
    - fast separations
    - can lead to heating in capillary
    - accelerates diffusion \( \rightarrow \) increased broadening (not terrible)

Basic CE Instrument Components

- Capillary: \~50-75 μm i.d., tens of cm long
  - Care and feeding of capillaries is critical

- High voltage power supply: kV potentials
  - Velocity \( \propto \) voltage

- Buffer
  - Need conductive solution

- Potential challenges
  - Particulates
  - Poor conductivity – heating

- Injection: Small capillaries require small injection volumes (nL to pL)
  - Electrokinetic Injection
    - non-uniform sampling due to mobility
  - Pressure Injection
    - uniform sampling
Basic CE Instrument Components

• **Detection:** Similar to LC, but smaller sample so sensitivity is an issue.
  – Mobility also plays a role in peakshape since materials elute at different rates (different than LC)
  – Indirect detection is becoming more common

<table>
<thead>
<tr>
<th>Type of Detector</th>
<th>Representative Detection Limit (attomoles detected)</th>
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<tbody>
<tr>
<td>Spectrometry</td>
<td>1–1000</td>
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<tr>
<td>Absorption</td>
<td>1–1000</td>
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<td>Fluorescence</td>
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<td>Amperometry</td>
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Electrophoresis vs. Electrochromatography

• In CE there are still plenty of materials that are difficult to separate
  – Try to improve separation by modifying buffer composition or separation voltage

• **Zone Electrophoresis (CZE):** single composition buffer, “classic” CE

• **Isotachophoresis:** two buffer compositions. Analytes distribute themselves between the extremes

• **Isoelectric Focusing:** Buffer composition changes throughout capillary. Amphiprotic materials migrate toward their isoelectric point (pI).
  – At pI, amphiprotic material is “uncharged”
Electrophoresis vs. Electrochromatography

- **Electrochromatography**: Hybrid technique
  - Two-phase separation driven by electroosmotic flow
  - Separation is a result of partitioning of analytes between the two phases (chromatography!)

- How to introduce second phase?
  - Packed capillaries - fairly uncommon, tough to prepare
  - Pseudo-stationary phase - component present in buffer that can allow partitioning of analyte
    - easier to do, much more flexible

- **Micellar Electrokinetic Chromatography (MECC)**
  - Surfactant is added to buffer (SDS, etc)
  - If surfactant concentration is appropriate, micelles form

  ![Exceed CMC](image)

  - Nonpolar interior of micelle acts as second phase
    - analytes partition into the phase on the basis of their distribution coefficients (K)
    - Micelles themselves have electrophoretic and electroosmotic mobility (negatively charged)
  - More efficient than HPLC (more plates)
  - Easy to change micellar phase!

- Chiral separations: chiral pseudo-stationary phase

MS Detection for CE

- **CE-MS**

  - CE is probably best suited for coupling to MS
    - low volume flow rates

  - ESI is most common
    - “End” of the capillary is metalized
    - Allows application of potential for both separation and ionization
      - E(injection)>E(ionization)>ground