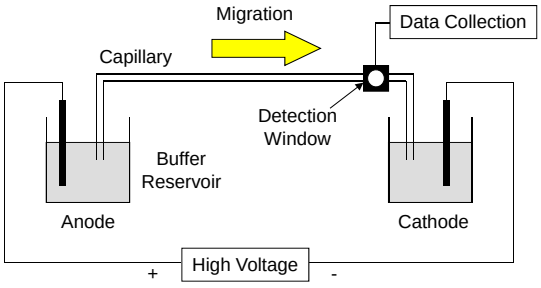
1

# Fundamentals of Capillary Electrophoresis



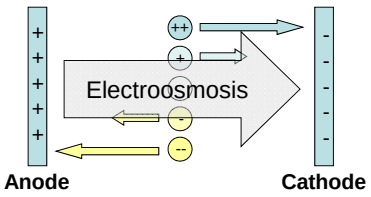

- **Separation is driven by electric field**
  - Combination of effects
- **Several modes of operation**
  - Depends on primarily buffer composition.

2

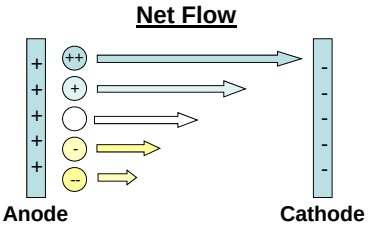
# Fundamentals of Capillary Electrophoresis

- Flow through capillary is result of superposition two processes:

- **<u>Electrophoretic Flow</u>**
  - 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




- **<u>Electroosmotic Flow</u>**
  - 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
  - $v_{electroos.} > v_{electrophor.}$
  - Electrical double-layer causes cations to congregate near walls
    - Flat flow profile



3

# 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 $Si(CH_3)_3Cl$)
- 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 → increased broadening (not terrible)

4

# 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 ∝ 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 30-1 Detectors for CE

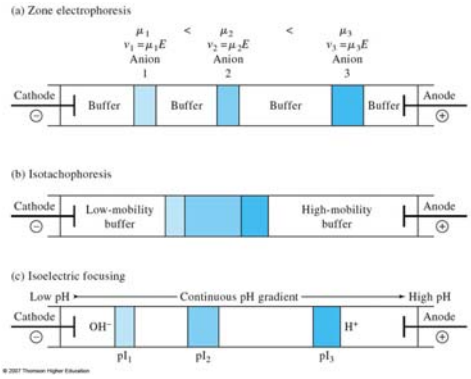
| Type of Detector | Representative Detection Limit* (attomoles detected) |
|---|---|
| Spectrometry | |
| Absorption† | 1–1000 |
| Fluorescence | 1–0.01 |
| Thermal lens† | 10 |
| Raman† | 1000 |
| Chemiluminescence† | 1–0.0001 |
| Mass spectrometry | 1–0.01 |
| Electrochemical | |
| Conductivity† | 100 |
| Potentiometry† | 1 |
| Amperometry | 0.1 |





# Electrophoresis vs. Electrochromatography

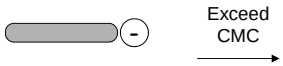
- In CE there are still plenty of materials that are difficult to separate
  - Try to improve separation by modifying buffer composition or separation voltage
- Zone Electrophoresis (CZE): single composition buffer, "classic" CE
- Isotachhophoresis: two buffer compositions. Analytes distribute themselves between the extremes
- Isoelectric Focusing: Buffer composition changes throughout capillary. Amphiprotic materials migrate toward their *isoelectric point* (pI).
  - At pI, amphiprotic material is "uncharged"



7

# 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




  - 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

8

# MS Detection for CE

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

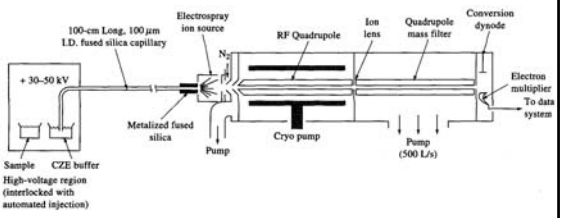



**Figure 30-7** An instrument for capillary electrophoresis/mass spectrometry. The voltage between the buffer solution on the left and the metalized silica capillary is 30 to 50 kV. The flow of nitrogen is 3 to 5 kV. The flow of nitrogen at ~70°C for desolvation is 3 to 6 L/min. *(From R. D. Smith, J. A. Olivares, N. T. Nguyen, and H. R. Udseth,* Anal. Chem., *1988, 60, 437. With permission.)*

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