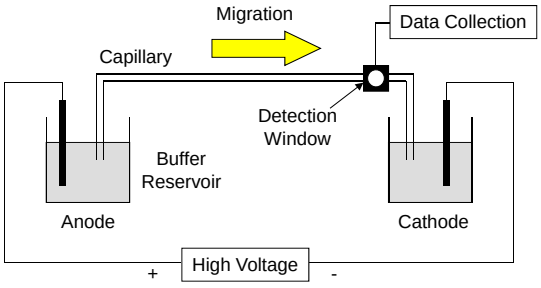
1

Fundamentals of Capillary Electrophoresis



- **Separation is driven by electric field**
  - **Combination of effects**
- **Several modes of operation**
  - **We'll focus on Capillary Zone Electrophoresis (CZE)**

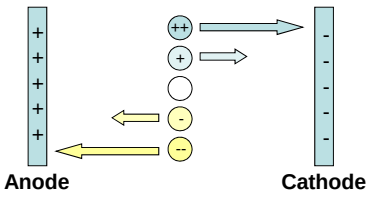
2

Fundamentals of Capillary Electrophoresis

- Flow through capillary is result of superposition two processes:

1. 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



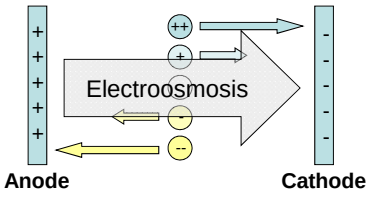
3

Fundamentals of Capillary Electrophoresis

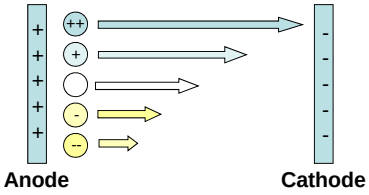
- Flow through capillary is result of superposition two processes:

2. Electroosmotic Flow

- Electrical double-layer causes cations to congregate near walls
  - Flat flow profile
- 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
- $v_{electroos.} > v_{electrophor.}$



Net Flow



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Basic CE Instrument Requirements

- 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
- Detection scheme: i.e. UV absorbance
  - Several others possible
- Injection scheme: pressure or "electrokinetic"
- Potential challenges
  - Particulates
  - Poor conductivity - heating

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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)

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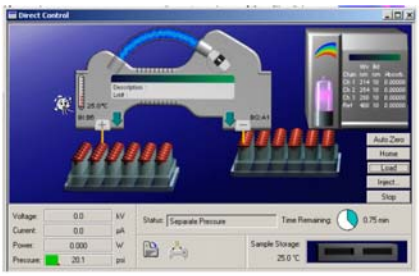
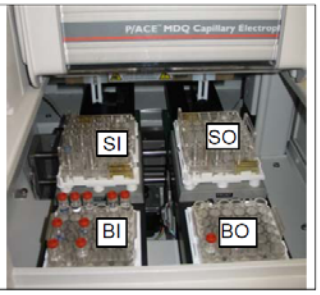
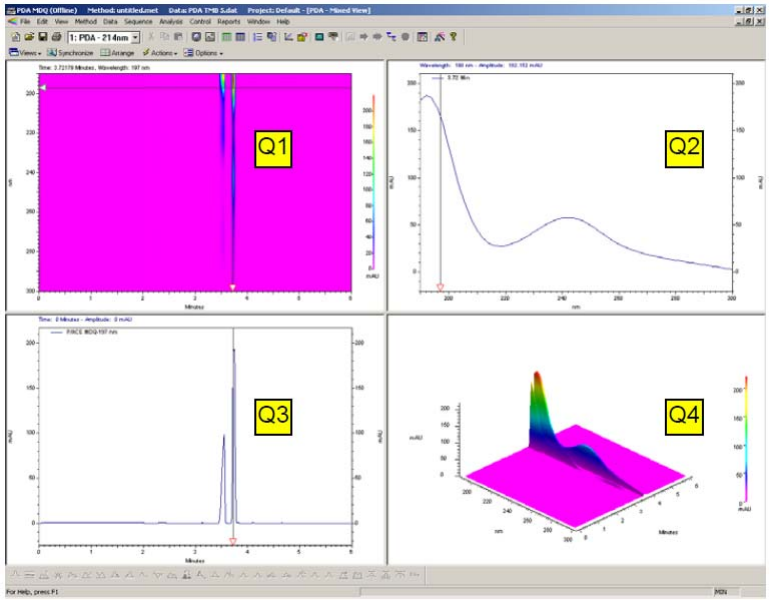
Beckman MDQ



Vial Location	Vial Contents	Use
BO:C1	buffer	during separation
BO:A1	waste	during rinsing
BI:C1	buffer	during separation
SI:A1	sample	during separation
BI:A6	0.1 N NaOH	during rinsing
BI:B6	18 MΩ water	during rinsing
BI:A1	buffer	during rinsing



Beckman MDQ Data Output



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

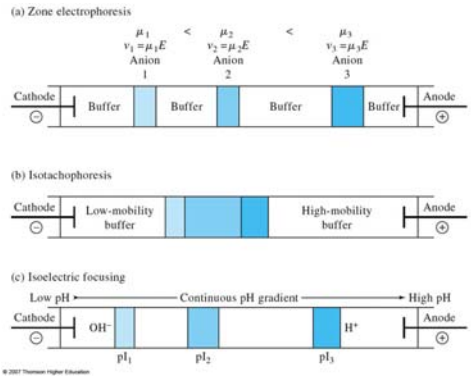
Electrophoresis vs. Electrochromatography

- 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"

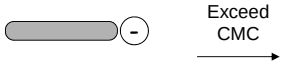


Electrophoresis vs. Electrochromatography

- **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