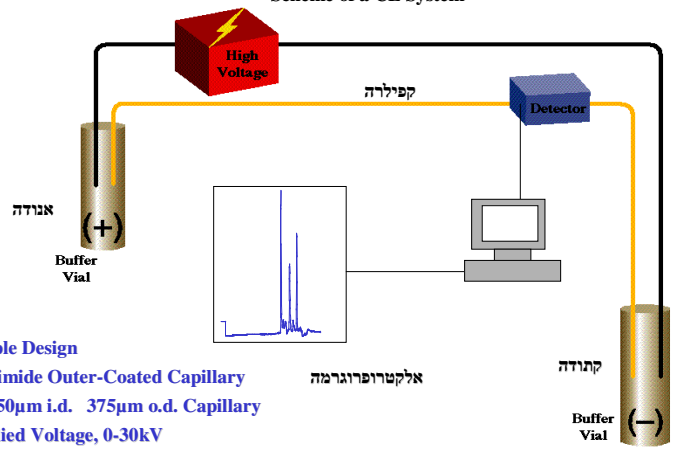
Basics of Capillary Electrophoresis

Acknowledgments: Beckman

Animation

http://www.shsu.edu/%7Echm_tgc/sounds/pushmovies/CE.gif

Scheme of a CE System



- Simple Design
- Polyimide Outer-Coated Capillary
- 20-150µm i.d. 375µm o.d. Capillary
- Applied Voltage, 0-30kV
- Detection On-Line Through Capillary
- No Pump

אלקטרופרוגרמה

CE/Mass Spectrometry

Co-axial

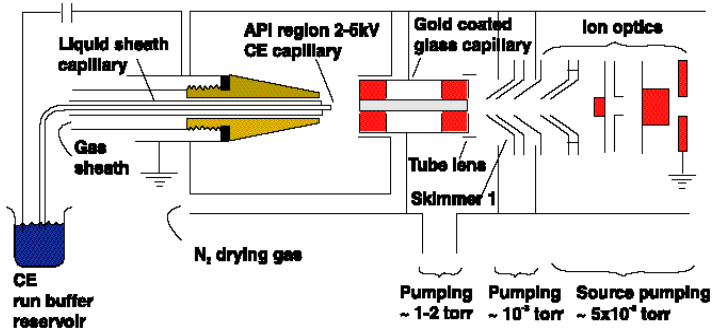
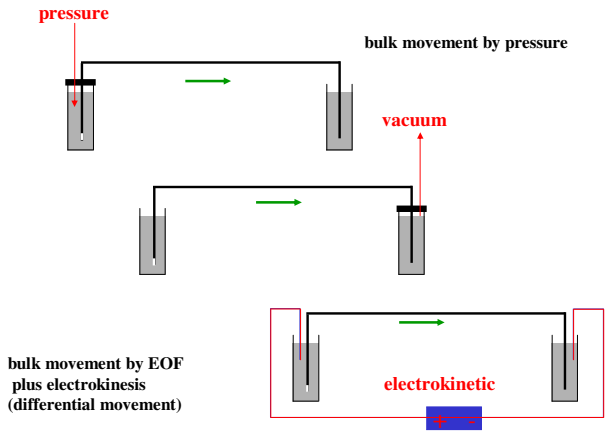


Diagram Courtesy of A.Tomlinson, S. Naylor, L.Benson, Mayo Clinic

Sample Injection

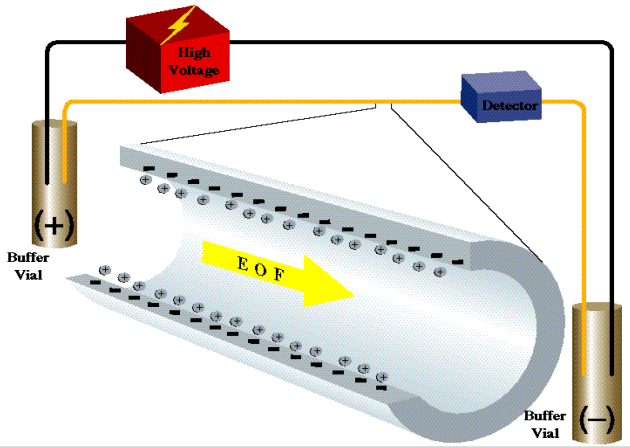


Electrophoretic Mobility

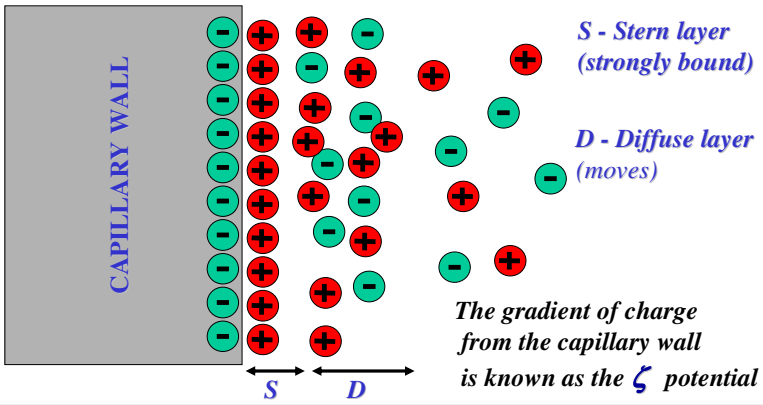
$$\mu = \frac{q}{6\pi r \ \eta}$$

- *q - charge (fixed for strong acids and bases pH dependant for weak acid and bases)*
- *6πr - effective ionic volume (N.B. complexation and counterion)*
- *η - viscosity*

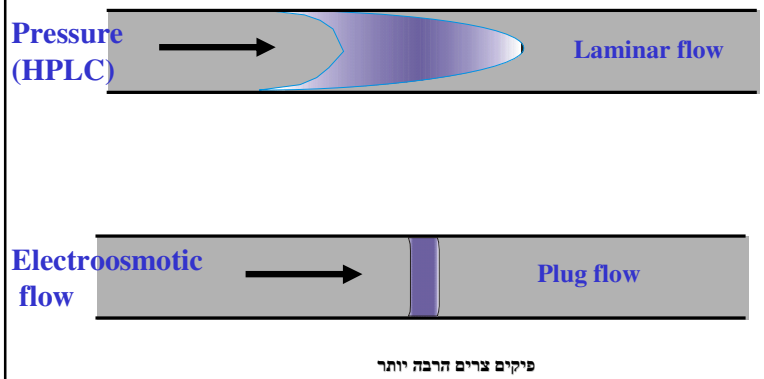
Creation of Electroosmotic Flow



Electroosmotic Flow

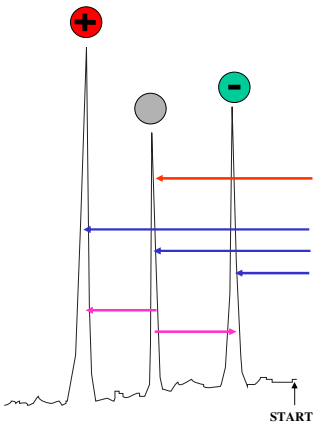


Electroosmotic Flow



פיקים צרים הרבה יותר

Mobility



μ_e can be used to correct for any inconsistencies in EOF. It can also be useful for compensating for area changes due to eof shifts.

EOF

Apparent mobility μ_a

Effective mobility μ_e

Detection Limits (Mols/inj)

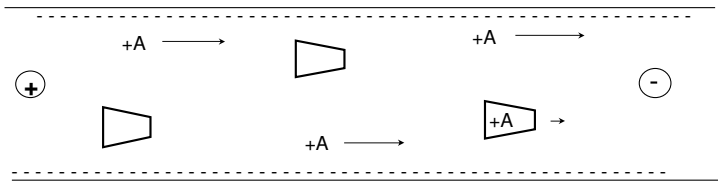
- Optical
  - Absorption 10^{-16} to 10^{-13}
  - Indirect Absorption 10^{-14} to 10^{-11}
  - Laser induced Fluorescence 10^{-20} to 10^{-17}
  - Indirect LIF 10^{-17} to 10^{-15}
- Mass spectometric 10^{-17} to 10^{-8}

General Approaches to Chiral CE

- Cavity or Inclusion-Complex Based Chiral Selectors

- A Cyclodextrins
- B Crown Ethers
- C Coiled Polysaccharides

MECHANISM OF SEPARATION

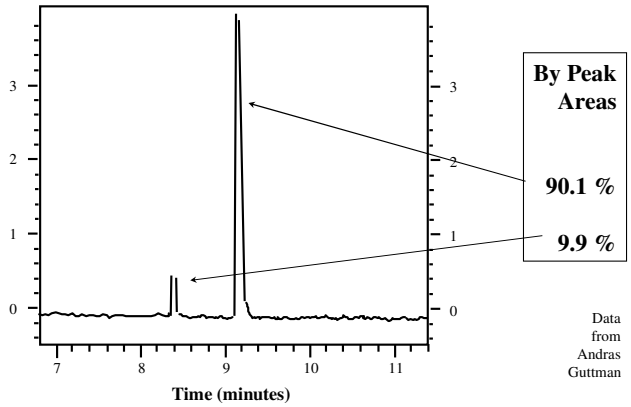


- +A = POSITIVELY CHARGED ANALYTE
- - - - - = NEUTRAL COATING
- = CYCLODEXTRIN

Example of Naproxen

- 1:10 Mixture of R and S Naproxen

$R_{R,S} = 3.75$

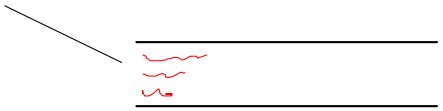


Capillary Gel Electrophoresis

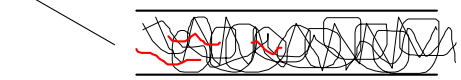
- Gels serve as a *molecular sieve* for size based separation
- Biopolymers are run under *denaturing conditions*
- Plot of log M.W. vs. migration time(or mobility) shows a linear relationship
- Generally use *electrokinetic* injections to protect the gel against extrusion

Size Based Separation in Gel

Denatured proteins with constant charge to mass ratio



Longer protein molecules move more slowly through gel



SDS-CGE of Protein Standards

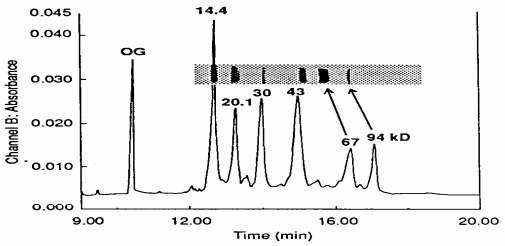


Figure 1. SDS-CGE of six protein standards on a 47-cm length (40 cm to detector) × 100 µm i.d. capillary. Run buffer, 100 mM TRIS-CHES, pH 8.8, 0.1% SDS. Sample buffer, 60 mM TRIS-HCL, pH 6.6. Sample injection by pressure for 60 s. Peaks: (1) α-lactalbumin; (2) soybean trypsin inhibitor; (3) carbonic anhydrase; (4) ovalbumin; (5) bovine serum albumin; (6) phosphorylase B. A tracking dye, Orange G (OG), was added to the sample. Protein concentration, 0.1 mg/mL. Detection, 214 nm. Run temperature, 20°C. Field strength, 300 V/cm. Current, 25-30 µA.