Capillary Electrophoresis-Mass Spectrometry (CE-MS)

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Capillary Electrophoresis

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- Separation based on electrophoretic mobility
- Simple instrumentation
- Primary applications in bioanalysis
  - DNA sequencing
  - DNA fragment analysis
- Multiple modes for improved selectivity of neutrals
  - MEKC
  - CEC

Advantages and Disadvantages of CE

Advantages

Offers new selectivity, an alternative to HPLC
Easy and predictable selectivity
High separation efficiency (10^5 to 10^6 theoretical plates)
Small sample sizes (1-10 ul)
Fast separations (1 to 45 min)
Can be automated
Quantitation (linear)
Different "modes" (to be discussed)

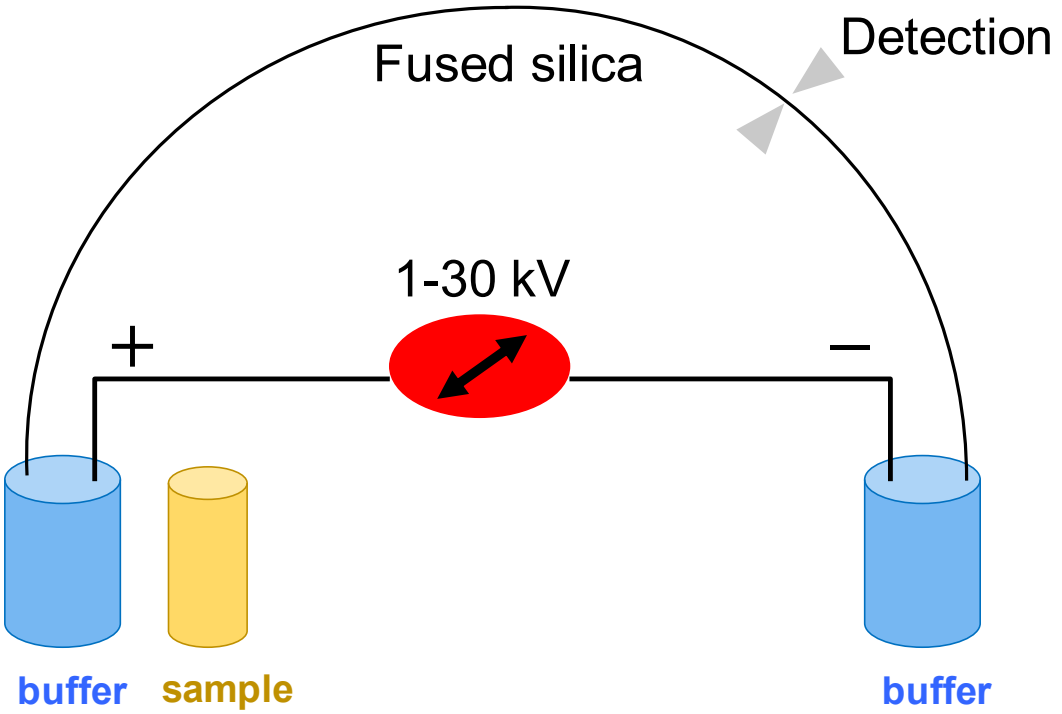
Disadvantages

Cannot do preparative scale separations
Low concentrations and large volumes difficult
"Sticky" compounds
Species that are difficult to dissolve
Reproducibility problems

Applications of CZE

- Wide variety of applications
  - Small molecules
  - Macromolecules (proteins, peptides)
- Limitations
  - Must have different charges
  - Low ionic strength sample
- Advantages
  - Simple
  - Direct analysis of complex systems

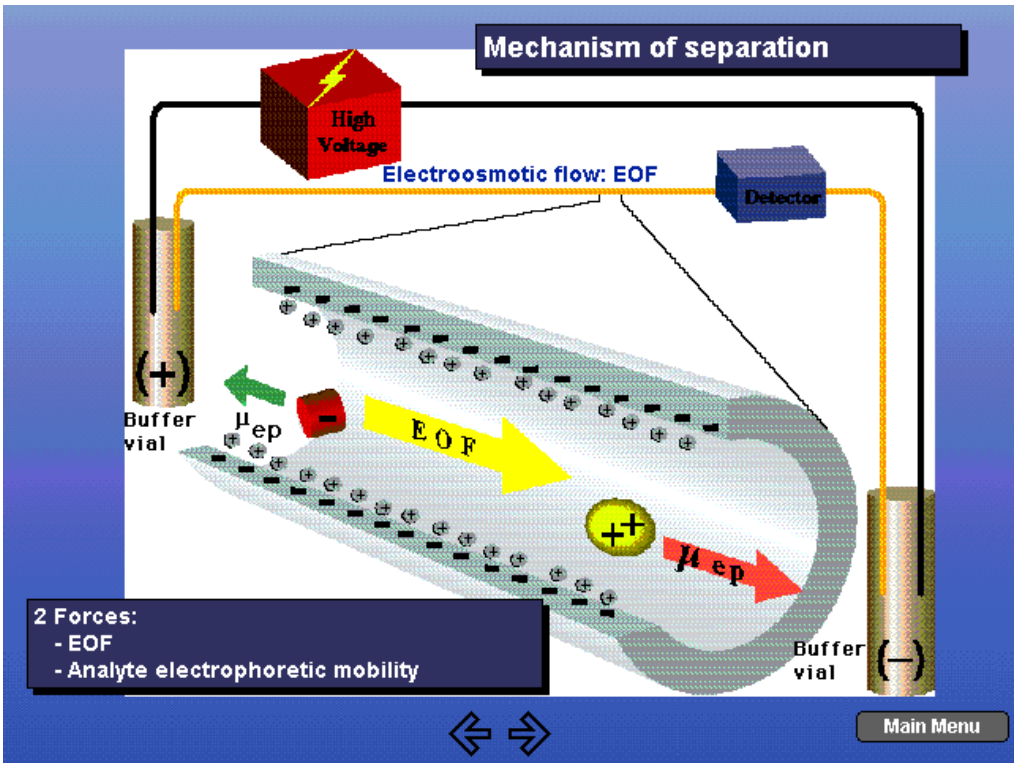
Capillary Electrophoresis (CE)



Modes of CE

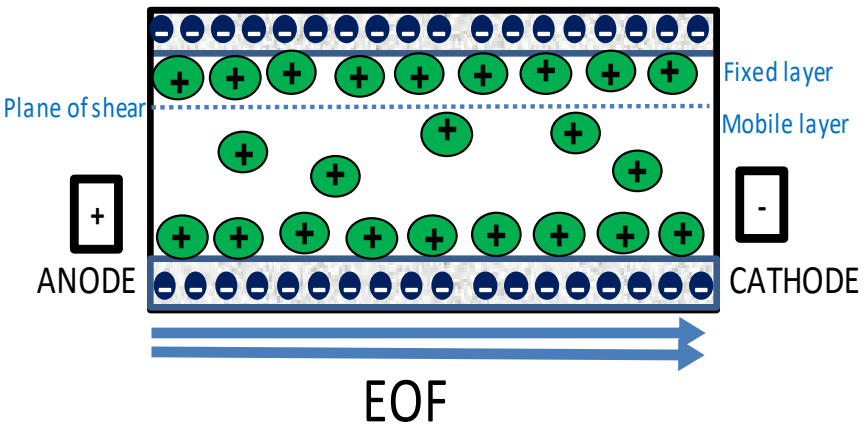
- Capillary Zone Electrophoresis (CZE)
  - Basic mode using open channels
- Micellar Electrokinetic Chromatography (MEKC)
  - Separates compounds with micelles
- Capillary Gel Electrophoresis
  - Size exclusion using sieving gels
- Capillary Electrochromatography
  - Hybrid of CE and HPLC
- Capillary Isoelectric Focusing
- Enantiomeric CE

Capillary Zone Electrophoresis (CZE)



Electroosmotic Flow

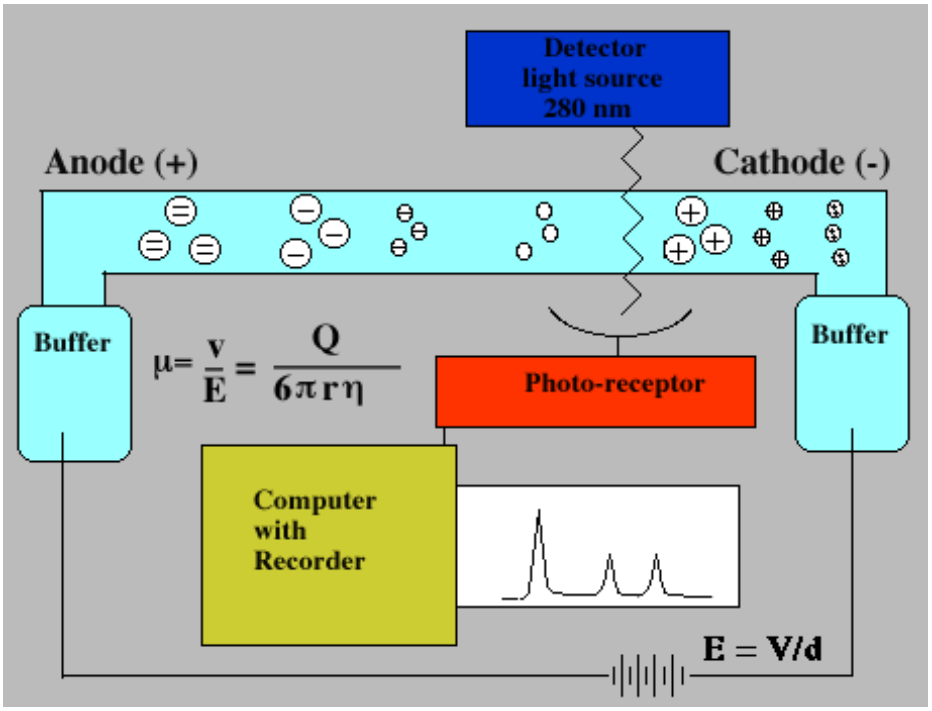
- Capillary flow mechanism based on applied potential and pH
- Provides bulk solution flow in capillaries with moderate to low concentration buffers are used
- Very dependent on solution ionic strength and surface chemistry



Electrophoretic Mobility

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

μ= electrophoretic mobility
Q= charge on the particle
η= solution viscosity
r = Stokes radius of the particle

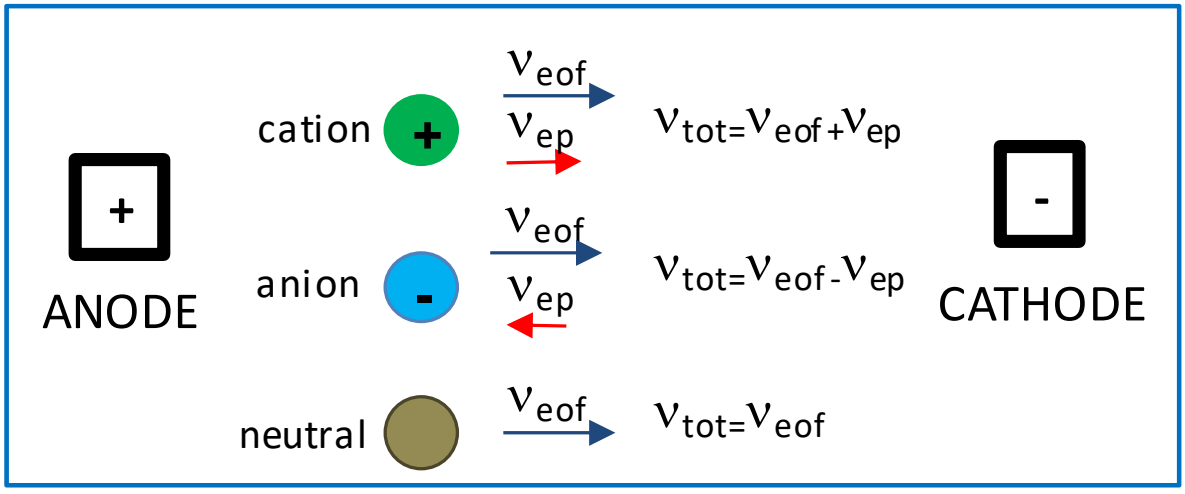


Electrophoresis and Electroosmosis

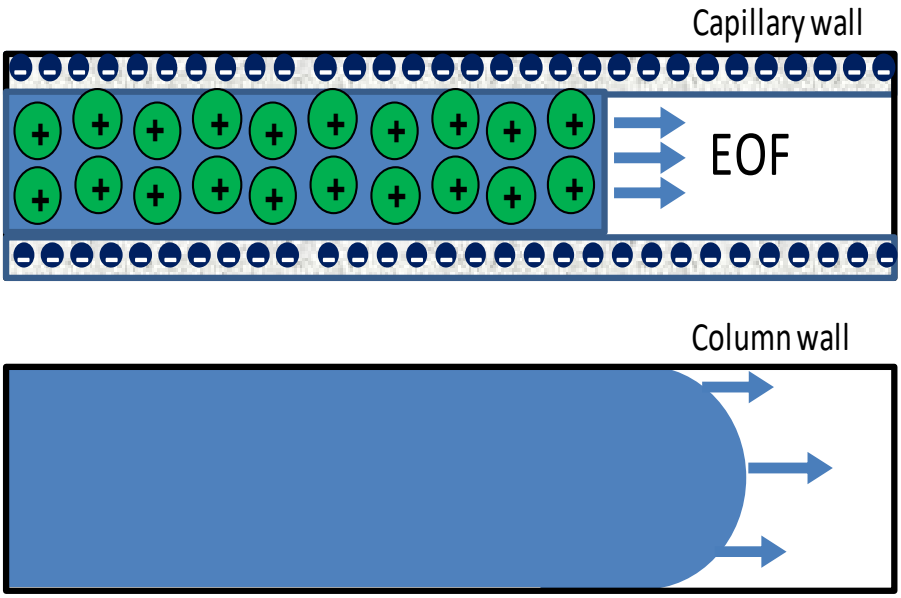
A pictorial representation of the combined effect in a capillary, when EO is faster than EP (the common case):

$$\nu = (\mu_{ep} + \mu_{eo})E = (\mu_{ep} + \mu_{eo})\frac{V}{L}$$

Figure from R. N. Zare, Stanford

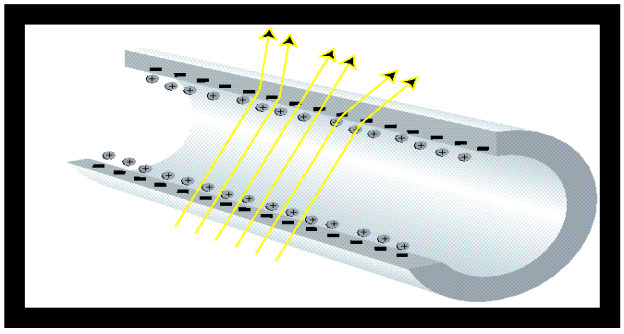


Diagrams of flow in CE and HPLC



Detection Options

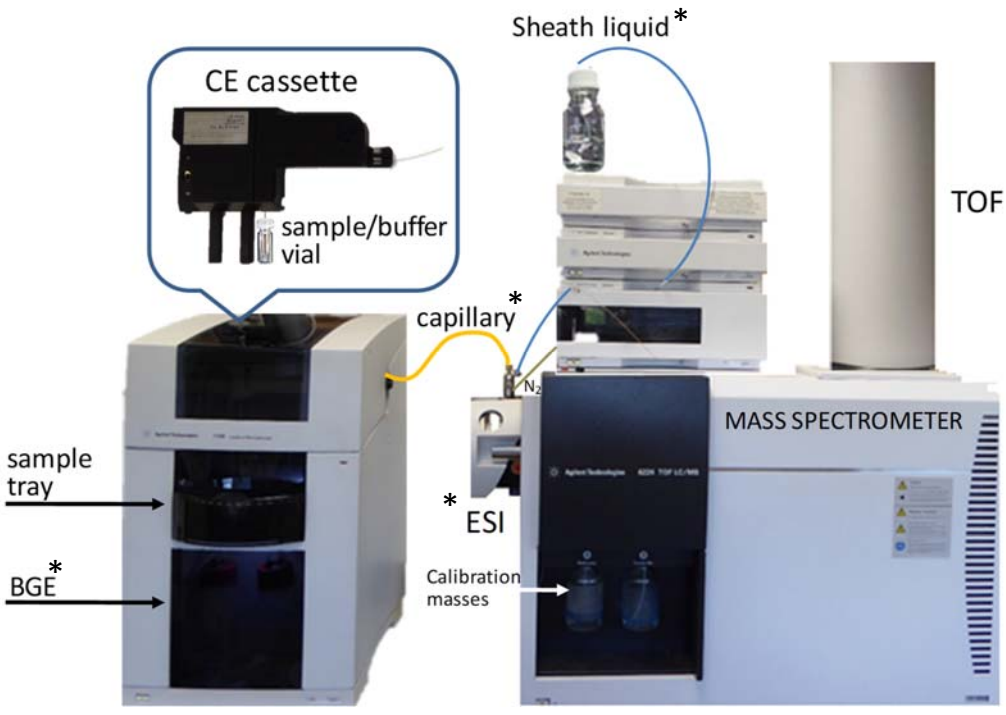
Detector	Advantages	Characteristics	Limit the range of detection (M)
UV/vis absorbance	-The possibility of direct and indirect detection -Very common detector	-Universal	-10^{-3} -10^{-6} for the detection of aromatic compounds
LIF (Laser-induced fluorescence)	-Highly sensitive and highly selective -Used for fluorescent compounds or derivatives	-Selective	-10^{-6} - 10^{-9}
MS	-Qualitative and quantitative information -Highly sensitive and highly selective	-Universal -Selective	-≈10^{-5} (it depends on the type of MS and metabolites)



Optimizing CE Separations PARAMETERS

- pH
  - First paramater to control
  - Effects EOF and mobility (charge)
- Organic Solvent
  - Analyte solvation
- Interacting agent
  - Ion-pairing, solvation, etc.
- Non-aqueous Conditions
  - Solvation and charge
- Temperature
  - Solvation, chemical equilibria

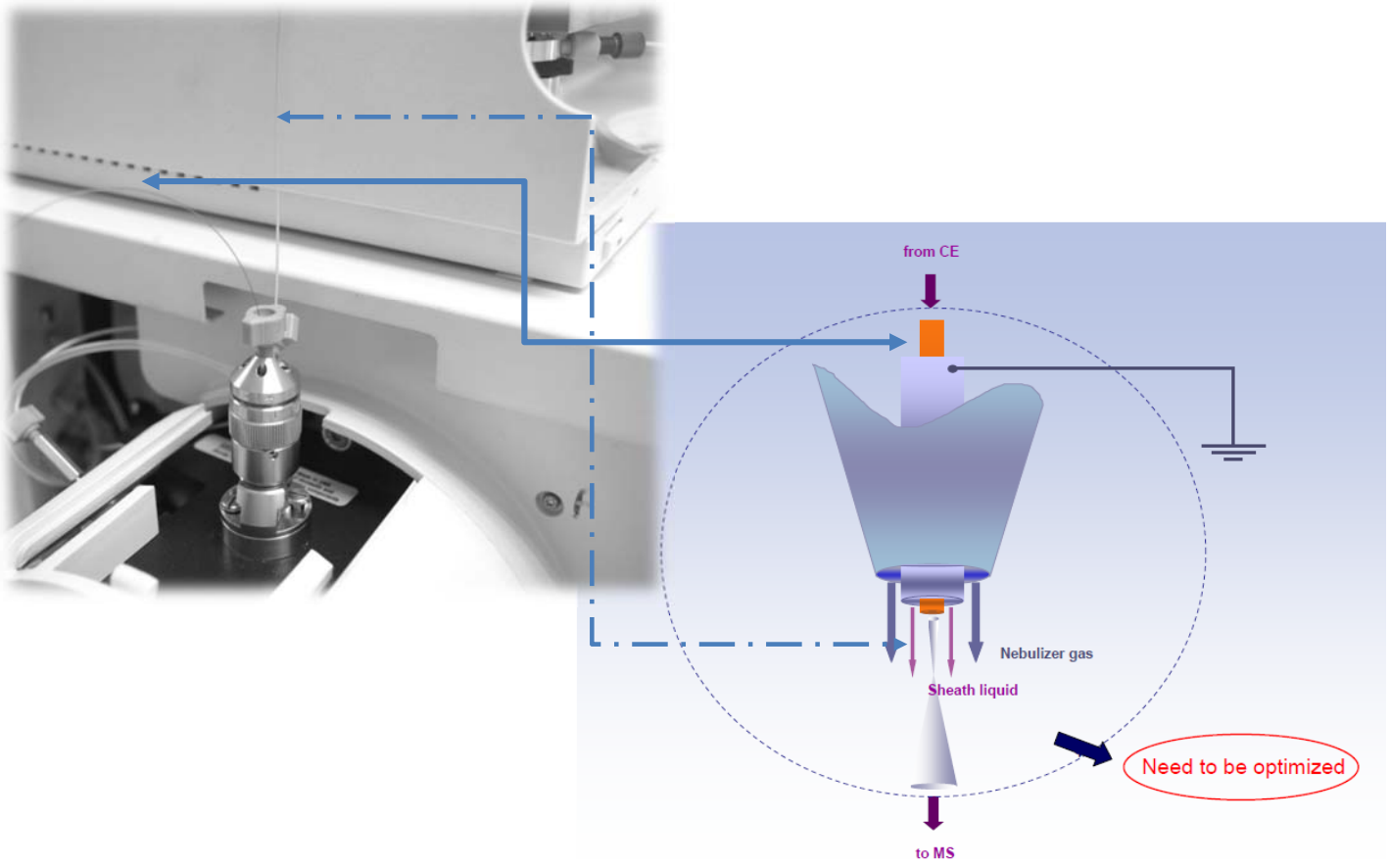
INSTRUMENTATION CE-MS



MS-TOF

* OPTIMIZATION REQUIRED

ESI SOURCE CE-MS



CE-MS: Electrical interfacing

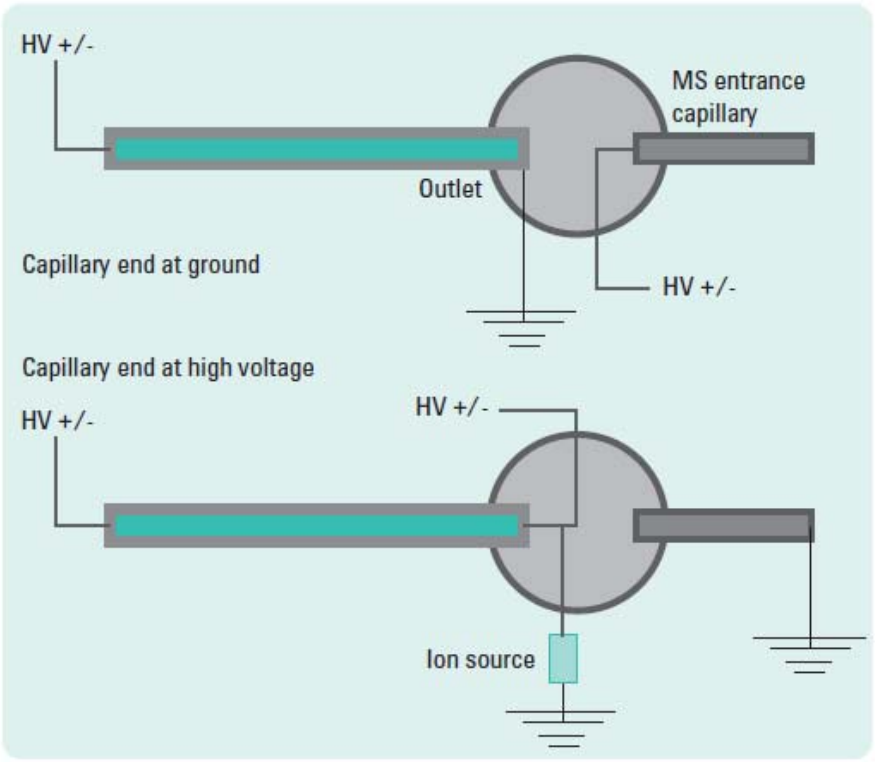


image provided by Agilent Technologies

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