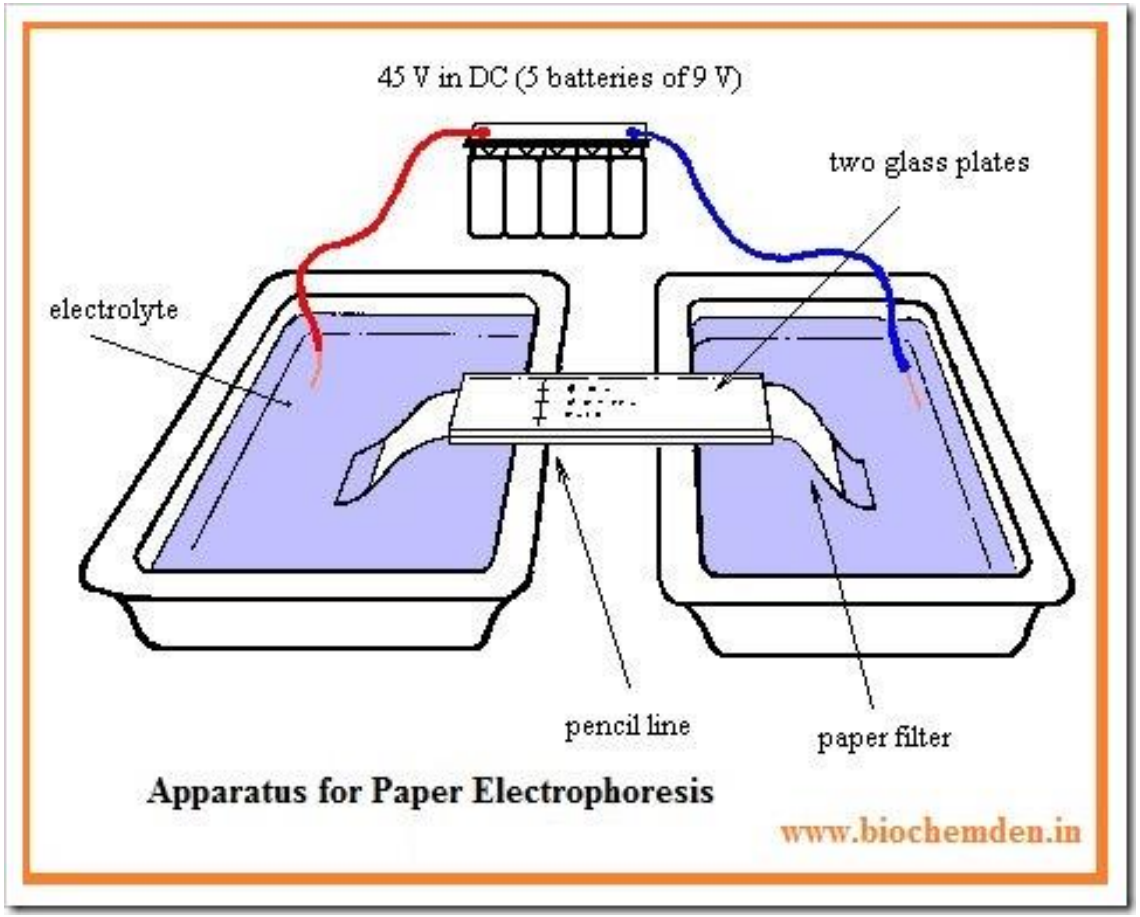
Capillary Electrophoresis

Intro: paper electrophoresis



http://biotechniquesden.blogspot.ca/2013/04/what-is-principle-procedure-and.html

Concepts of electrophoresis

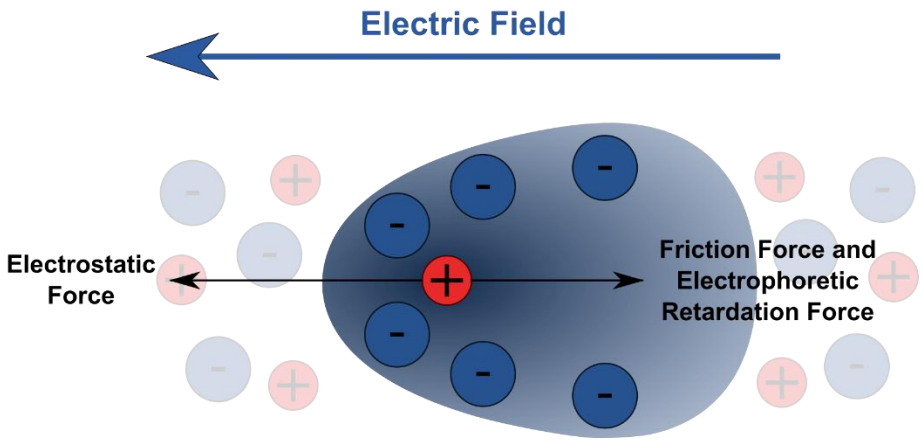
Electrophoresis: migration of ions under the influence of an electric field.

The force F_E imparted by the electrical field is proportional to an ion's effective charge, q, and the electric field strength, E.

$$F_E = qE$$

The translational movement of analyte ions is opposed by a retarding frictional force F_f proportional to the velocity of the ion, ν_{ep}, and the friction coefficient, f.

$$F_f = f\nu_{ep}$$


Electric Field
Electrostatic Force
Friction Force and Electrophoretic Retardation Force

The ions reach a steady state velocity where the electric force equals the frictional force.

$$qE = f\nu_{ep}$$

or

$$\nu_{ep} = \frac{q}{f}E = \mu_{ep}E$$

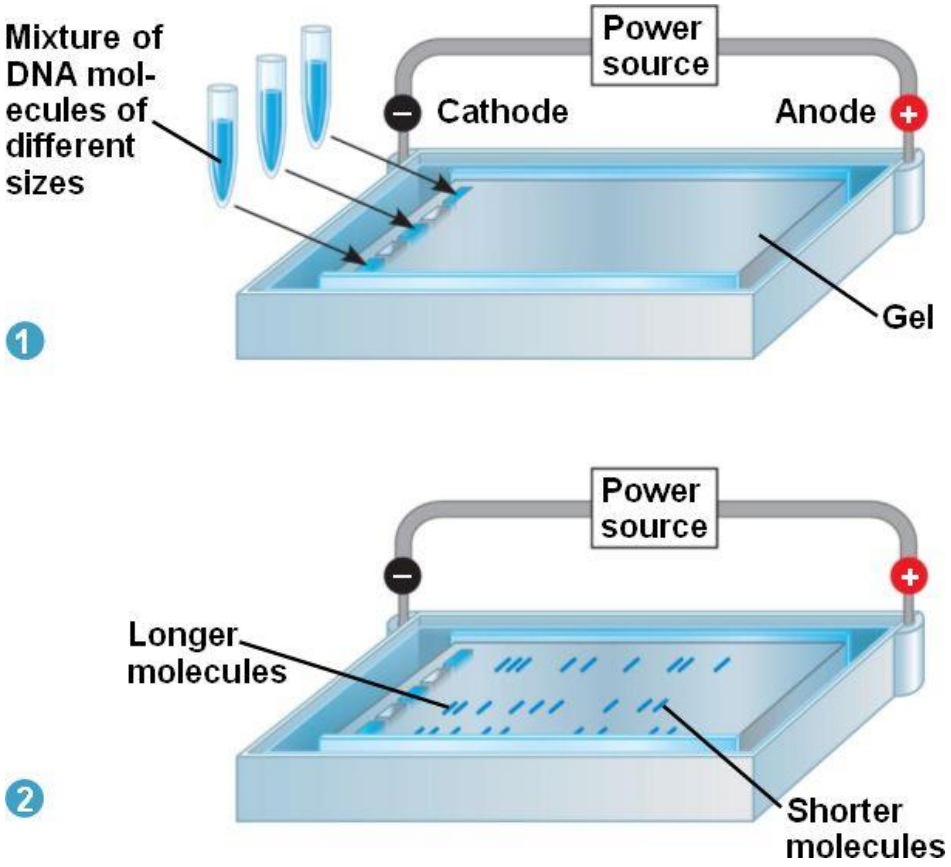
μ_{ep} = electrophoretic mobility of the ion

The friction coefficient of ions in motion is related to the hydrodynamic radius r of the ions and to the viscosity η of the surrounding medium.

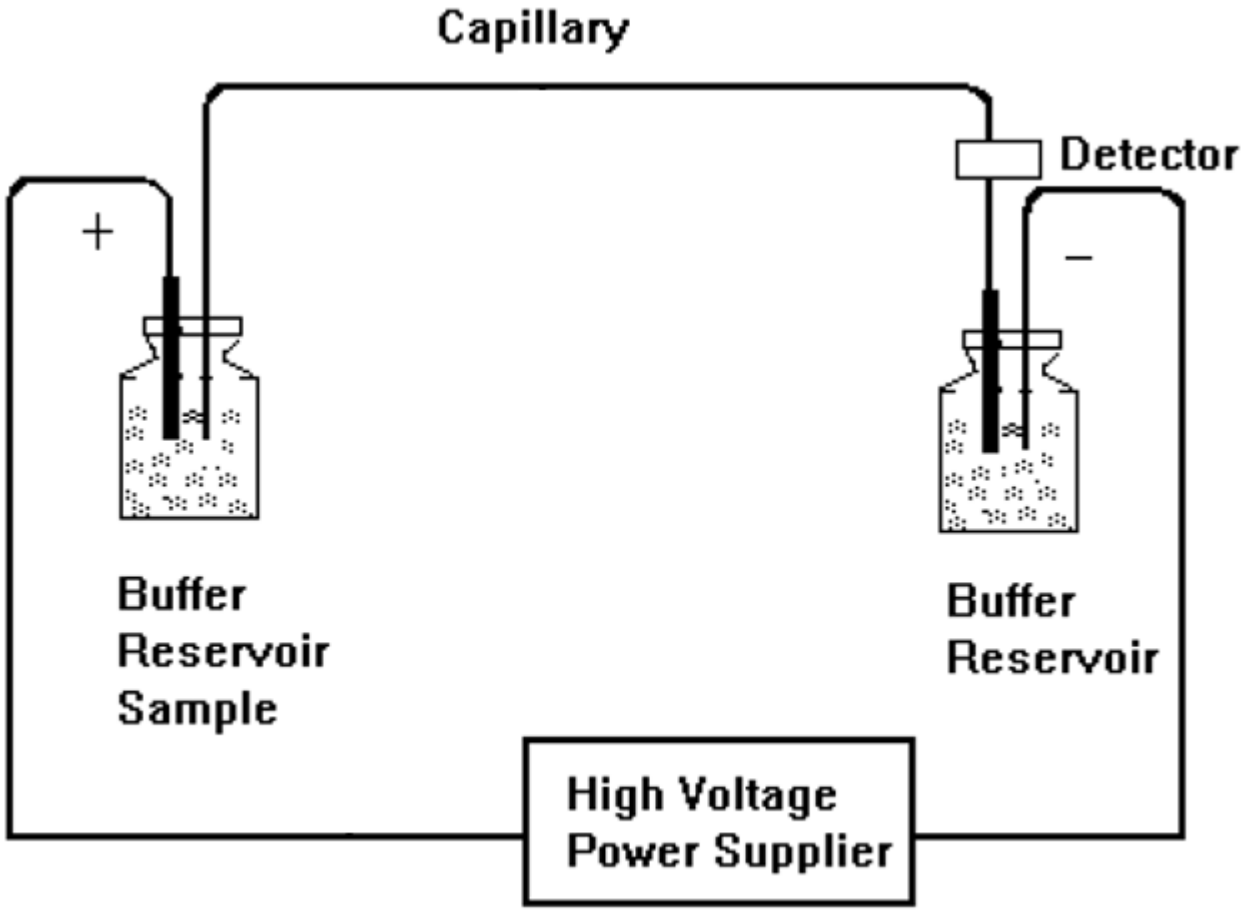
$$f = 6\eta\pi r$$

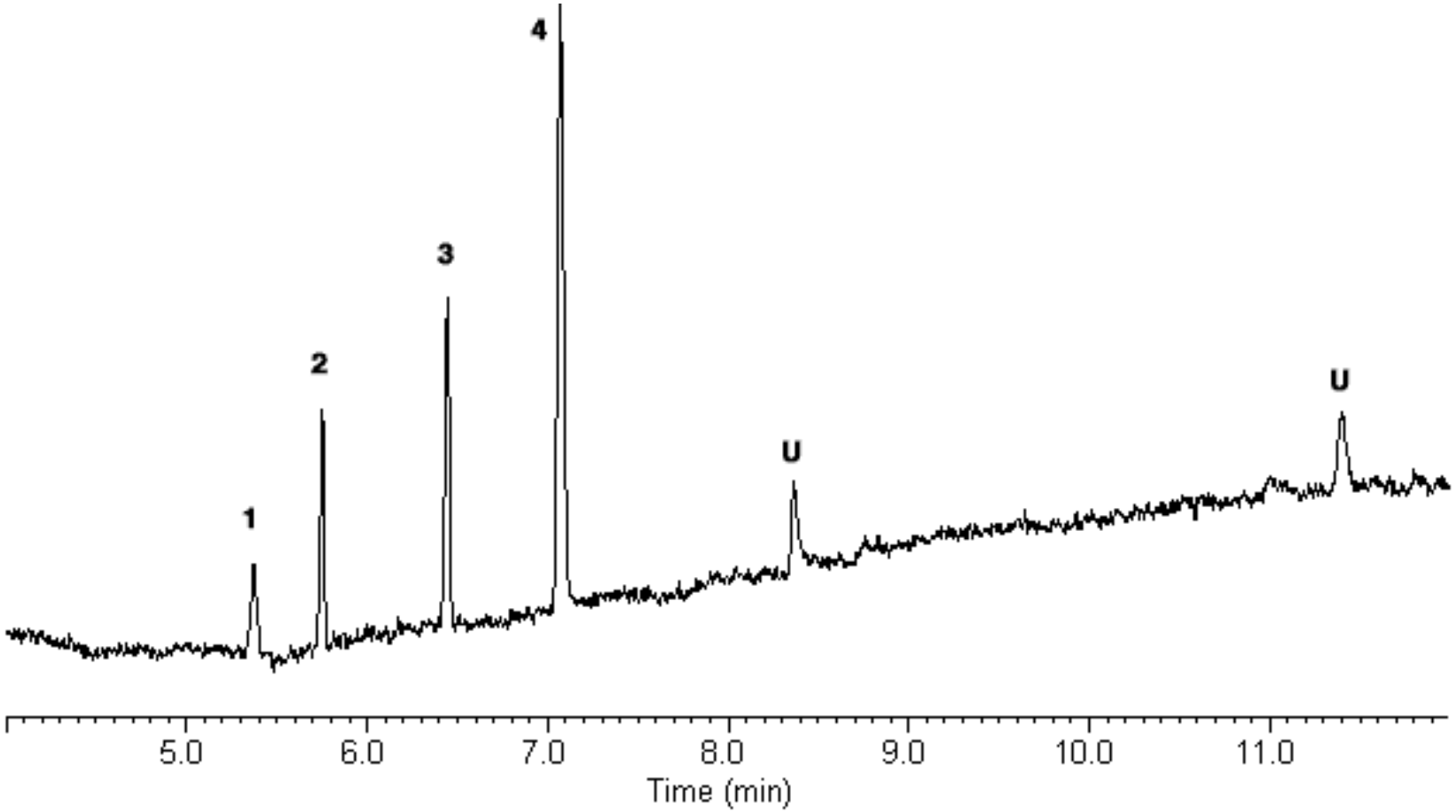
Because $\mu_{ep} = q/f$, a larger hydrodynamic radius means a lower electrophoretic mobility.

Gel electrophoresis



Capillary electrophoresis





Capillary electrophoresis analysis of black tea with sugar (diluted 20×) and spiked with less than 10 ppm of sodium azide (trace at 250 nm; *y*-scale in mV). Peak assignments: (1) Cl^-, (2) SO_4^{-2}, (3) $C_2O_4^{-2}$, (4) N_3^-, and (U) Unknown.

https://www.fbi.gov/about-us/lab/forensic-science-communications/fsc/april2001/index.htm/mmiller.htm

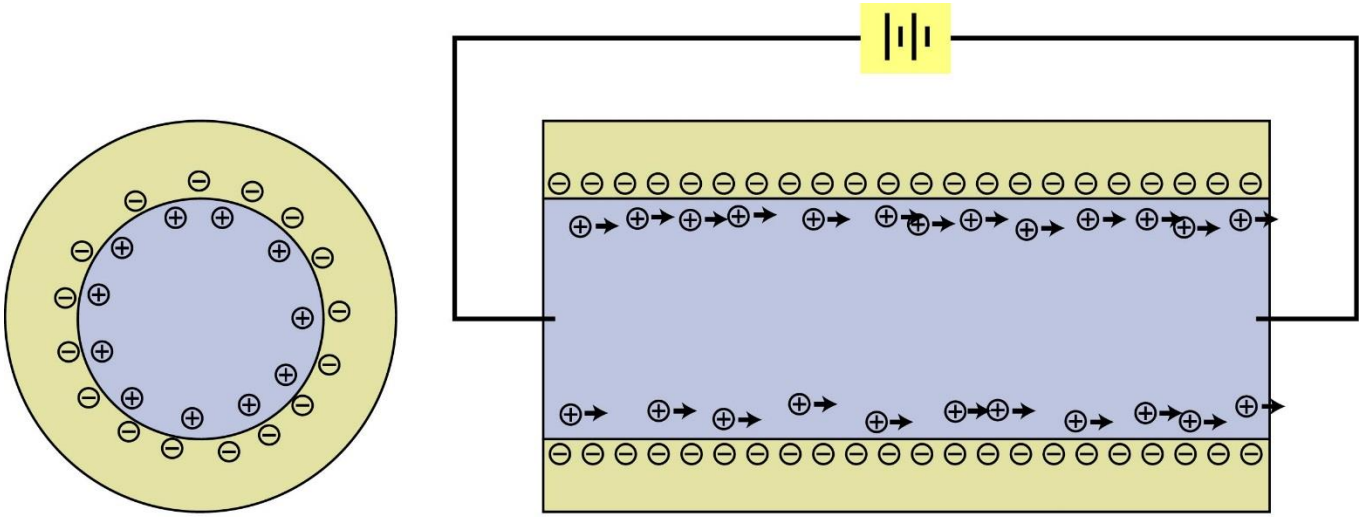
Electroosmosis

Refers to the movement of buffer in the capillary under the influence of E.

The inner surface of a fused silica capillary is covered with silanol groups (Si-OH), SiO^- at pH > 2.

The negatively charged surface is counterbalanced by positive ions from the buffer (Na^+, K^+, NH_4^+), forming an electric double layer.

Under the influence of E, these + ions migrate towards the cathode; in doing so they carry H_2O of hydration, resulting in electroosmotic flow.



[cross-section]

[inside of capillary, cut along length]

The electroosmotic velocity is defined as: $\nu_{eo} = \mu_{eo} E$

where μ_{eo} = electroosmotic mobility.

μ_{eo} is proportional to the dielectric constant ε of the medium and the zeta potential ζ at the capillary–buffer interface. It is inversely proportional to η.

ζ largely depends on the electrostatic nature of the capillary surface and on the ionic nature of the buffer.

Electroosmotic flow (EOF) can be reduced by coating the capillary with a material that suppresses ionization of the silanol groups, such as polyacrylamide.

Apparent mobility. The apparent mobility, μ_{app}, of a solute is a vector sum of the electrophoretic mobility, μ_{ep}, of the solute plus the electroosmotic mobility, μ_{eo}, of the solution.

$$\mu_{app} = \mu_{ep} + \mu_{eo}$$

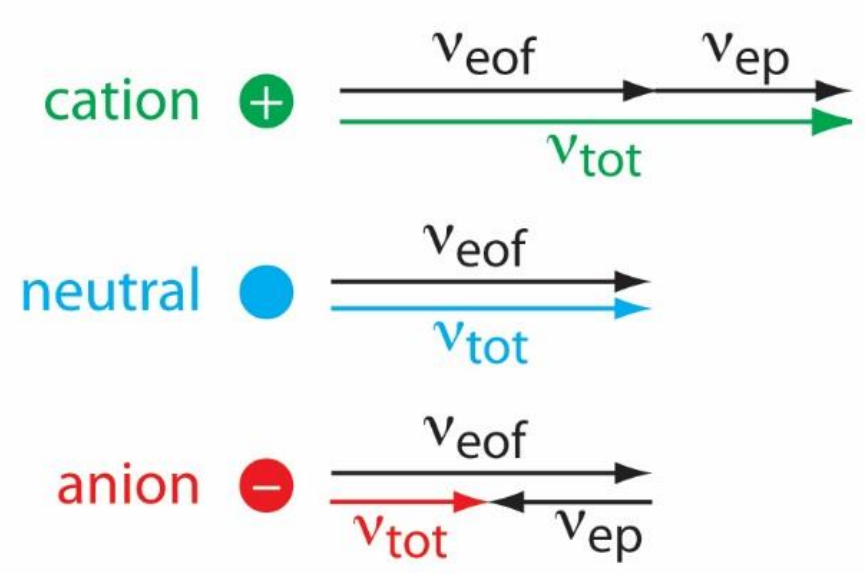
The apparent velocity ν_{app} of a solute is directly proportional to μ_{app} and the electric field strength, *E*, across the capillary.

$$\nu_{app} = \mu_{app} E$$

Neutral solutes migrate in the same direction and velocity as the EOF and are not separated or very little.

Cations and anions are separated based on differences in their apparent mobilities.

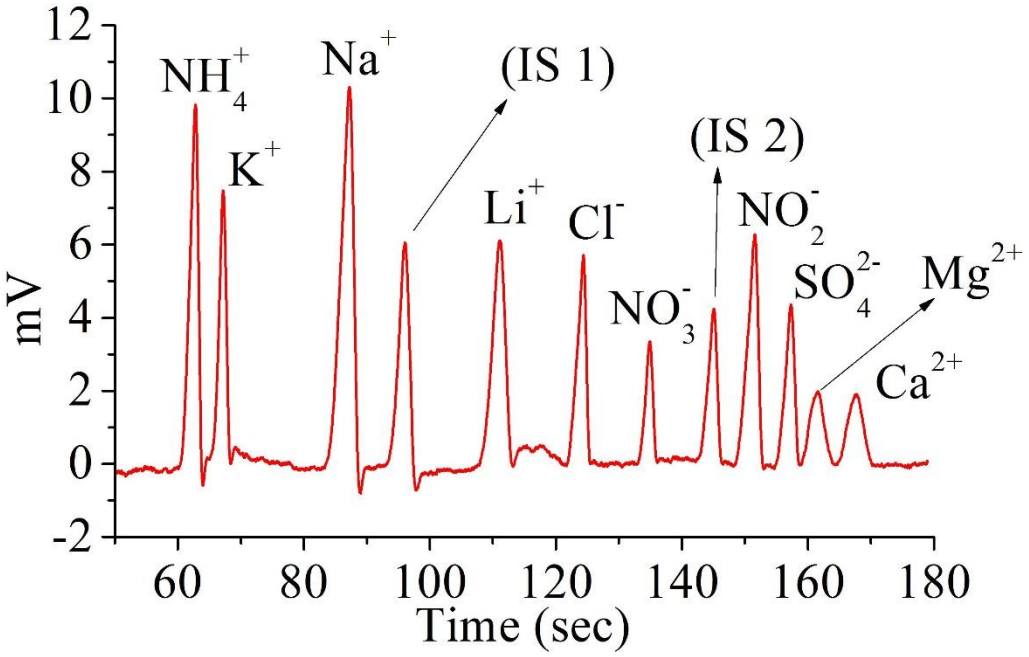
For cations, which move in the same direction as the electroosmotic flow, μ_{ep} and μ_{eo} have the same sign, so $\mu_{app} > \mu_{ep}$.



The electrophoresis of anions is in the opposite direction of electroosmosis, so for anions μ_{ep} and μ_{eo} have opposite signs.

At moderate pH values (pH > 3), EOF is generally higher than eplectrophoretic flow, causing anions to migrate towards the cathode, where the detector is typically located.

At lower pH, EOF is weak *(why?)* and anions may never reach the detector unless the polarity of the instrument is reversed.



Instrumentation: General Aspects

*Capillary column***.** Fused silica is by far the most frequently used material due to its transparency over a wide range of the electromagnetic spectrum and a high thermal conductance. It is also easy to manufacture into capillaries with diameters of a few μm.

Many reports describe the covalent attachment of silanes with neutral or hydrophilic substituents to the inner wall of the capillary in order to reduce EOF and prevent adsorption of the analyte.

Injection

Typical injection volumes range from picoliters to nanoliters.

Two commonly used injection methods for CE: hydrodynamic and electrokinetic.

Hydrodynamic injection is accomplished by the application of a pressure difference between the two ends of a capillary. The amount of sample injected can be calculated by the Poiseuille equation:

$$V_c = \frac{\Delta P \pi d^4 t}{128 \eta L t}$$

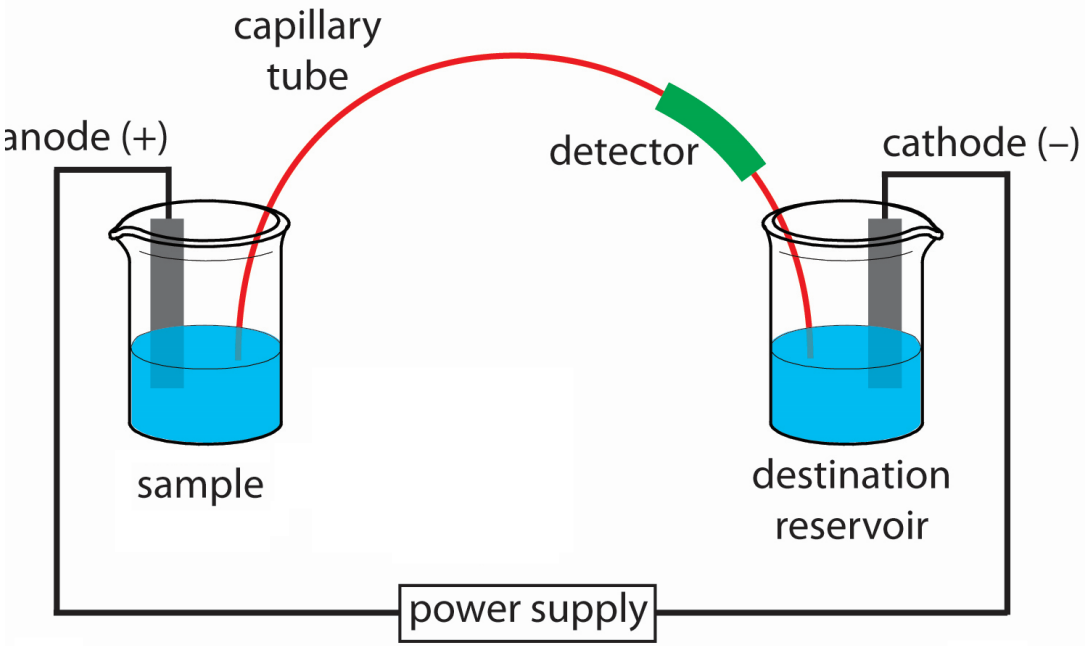
Where V_c = *calculated injection volume*

ΔP = *pressure difference between the ends of the capillary*

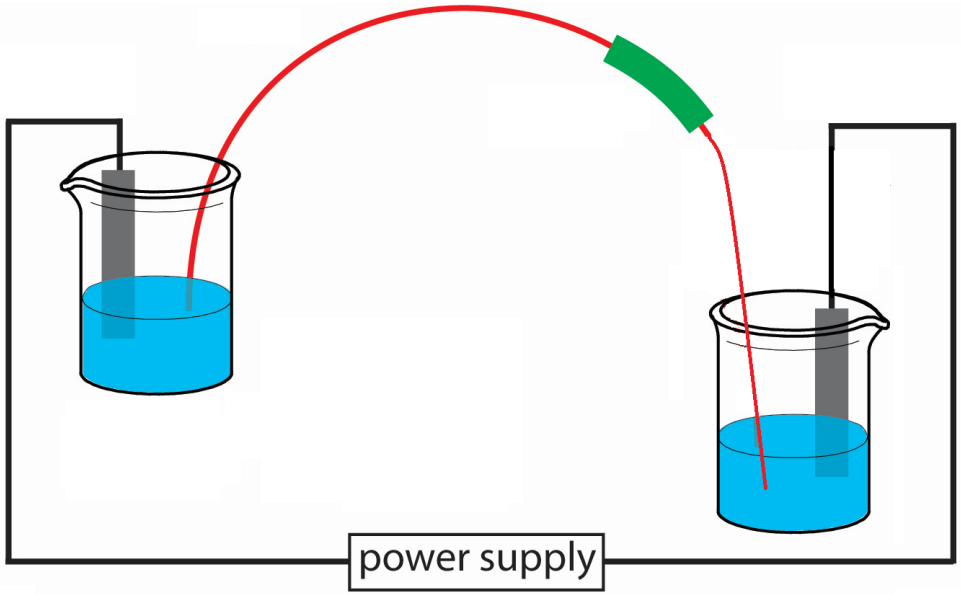
d = *inner diameter of the capillary*

t = *injection time*

L_t = *total length of capillary*



…or by siphoning



$$V_c = \frac{\rho g \pi d^4 \Delta h t}{128 \eta L T}$$

Electrokinetic injection is performed by simply turning on the voltage for a certain period of time.

The moles of each analyte injected, Q_i, are determined by the apparent velocity of each analyte, ν_{app}; the injection time, t; and the ratio of conductivities of the separation buffer and sample, k_b/k_s.

$$Q_i = \left(\frac{k_b}{k_s}\right)\nu_{app}\pi r^2 t C_i$$

r = inner radius of capillary
C_i = molar concentration of analyte i

Because each analyte has a different mobility, electrokinetic injection is biased. For qualitative analysis, this is not usually a problem. For quantitative analysis, the concentration of the injected sample may be different from that of the original sample.

Electrokinetic injection is useful for *capillary gel electrophoresis* in which the polymer inside the capillary is too viscous for hydrodynamic injection.

***Joule heating*:**

Consequence of the resistance of the solution to the flow of current. The heat produced, H, is directly proportional to the applied voltage between the electrodes V, the electric current I, and the time t.

$$H = VIt$$

If the heat is not sufficiently dissipated from the system the resulting temperature can reduce the separation efficiency.

The capillary walls used in CE can dissipate heat much more efficiently than the slab gels used in conventional electrophoresis due to the large ratio of surface area to volume.

As a result, high potentials can be applied in CE; with current technology, up to 30 kV can be applied.

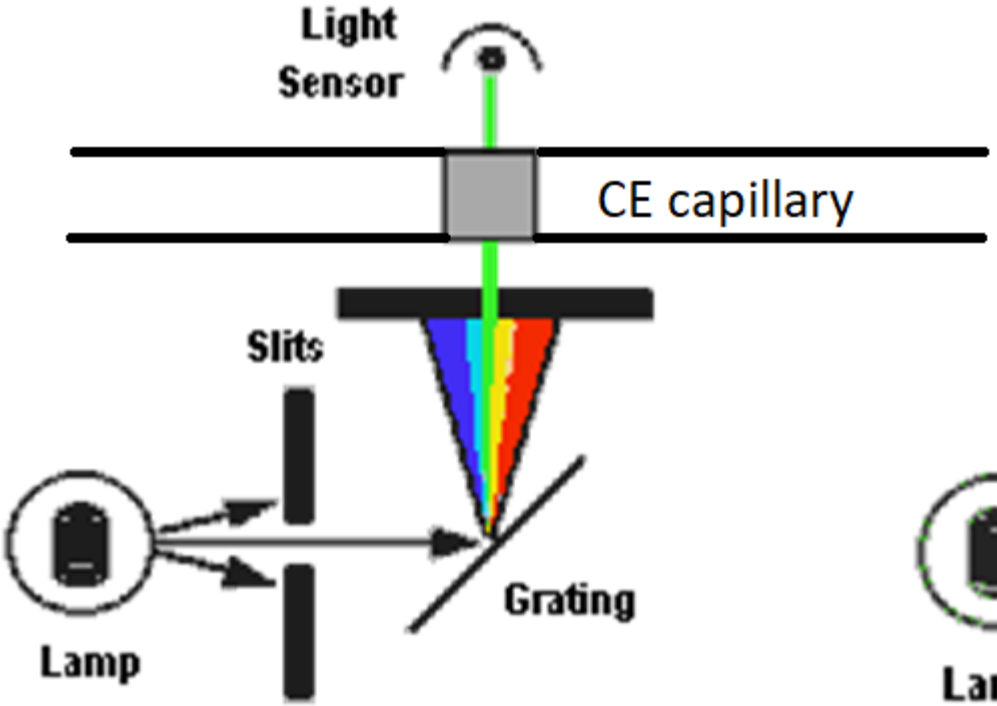
Separation Modes

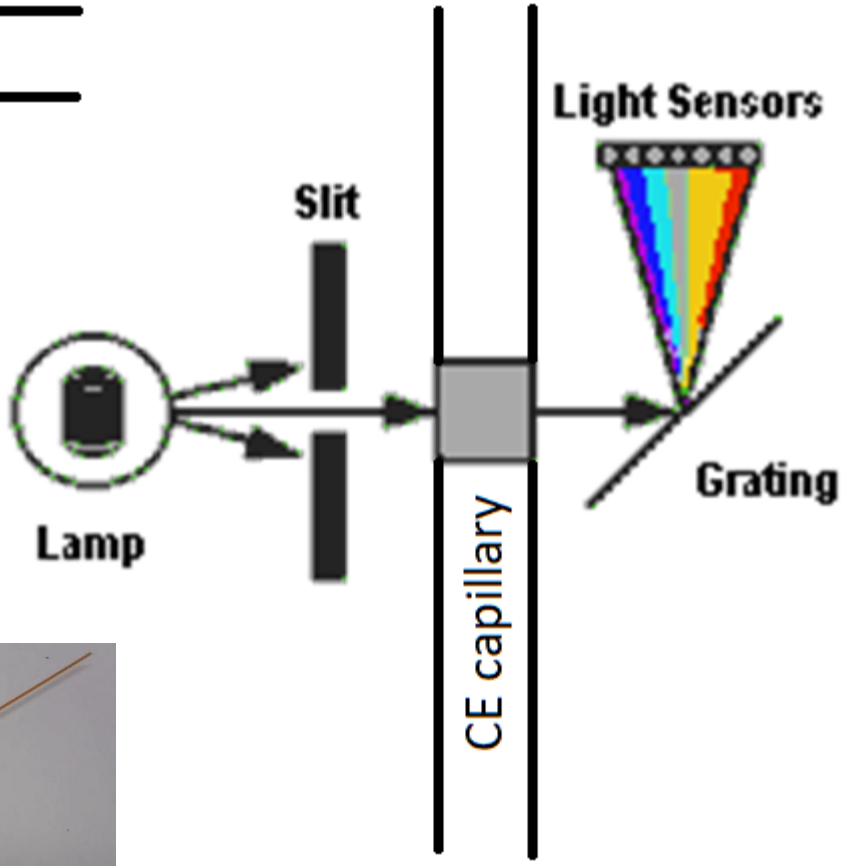
- capillary zone electrophoresis (CZE)
- Micellar electrokinetic capillary chromatography (MEKC)
- capillary isotachophoresis
- capillary gel electrophoresis
- capillary isoelectric focusing

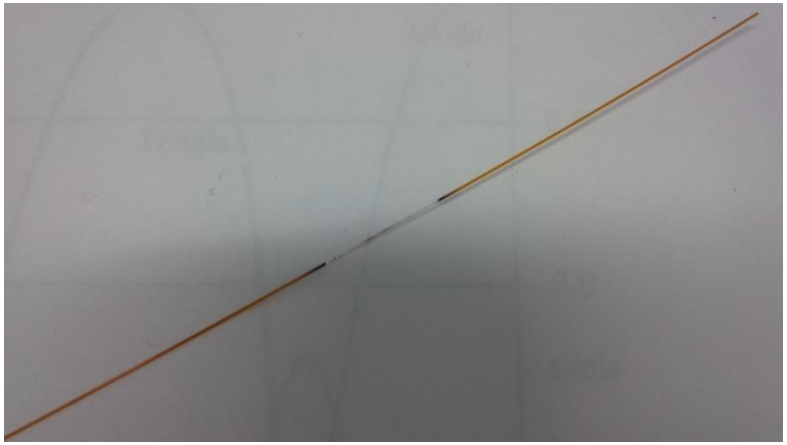
Detection Modes

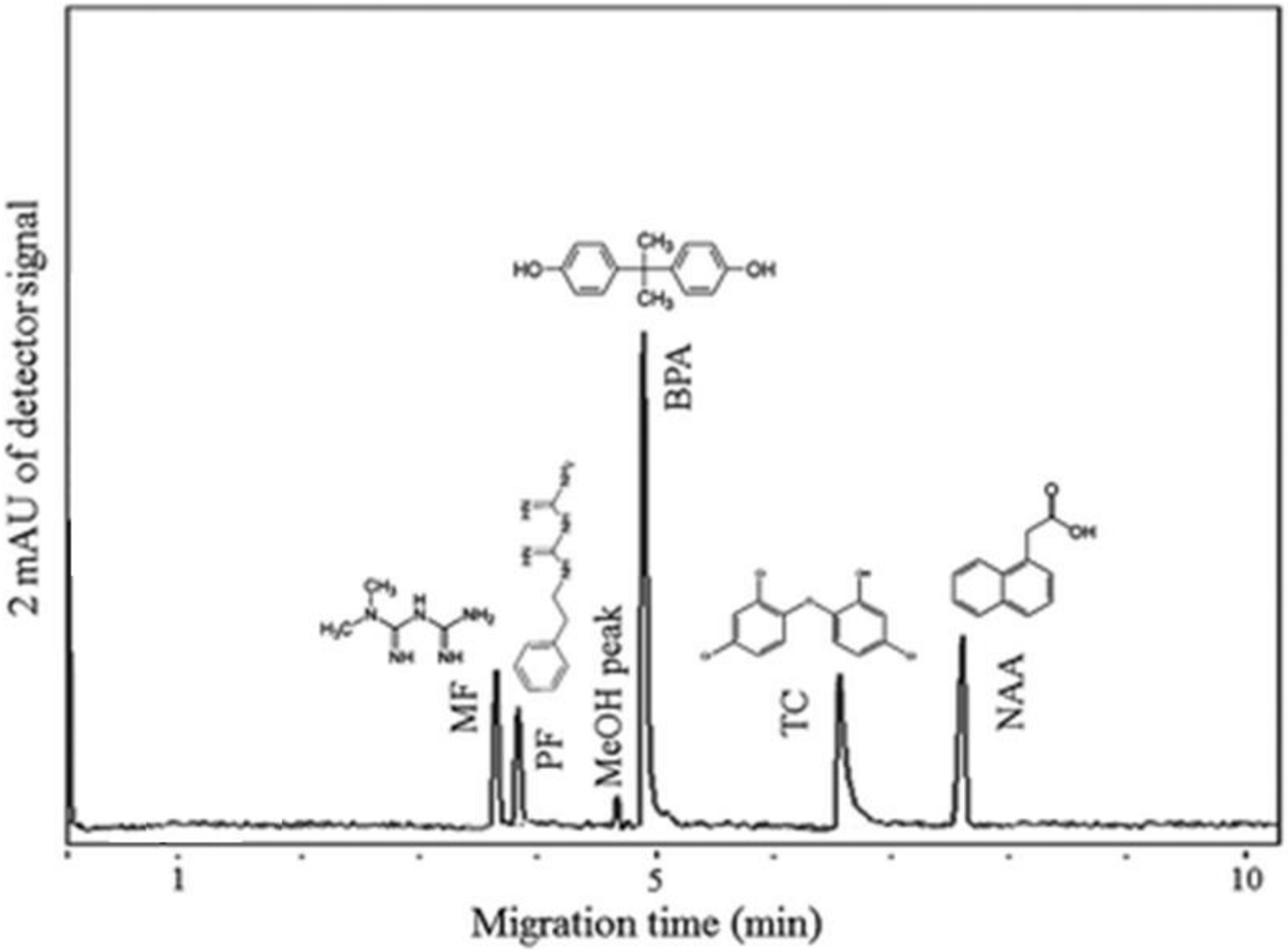
- UV-vis
- Laser induced fluorescence (LIF)
- MS
- Electrochemical

UV-Vis detection

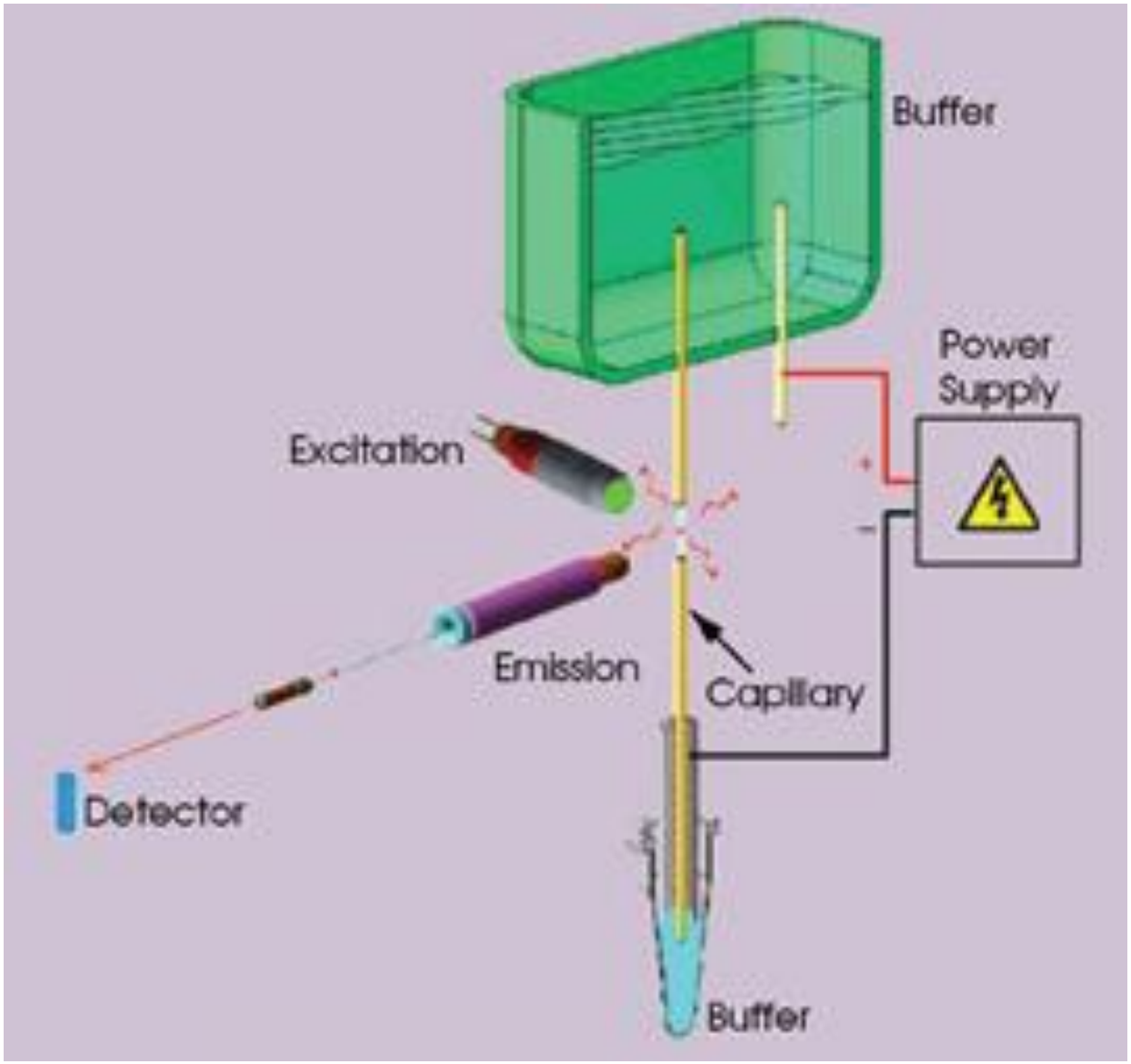






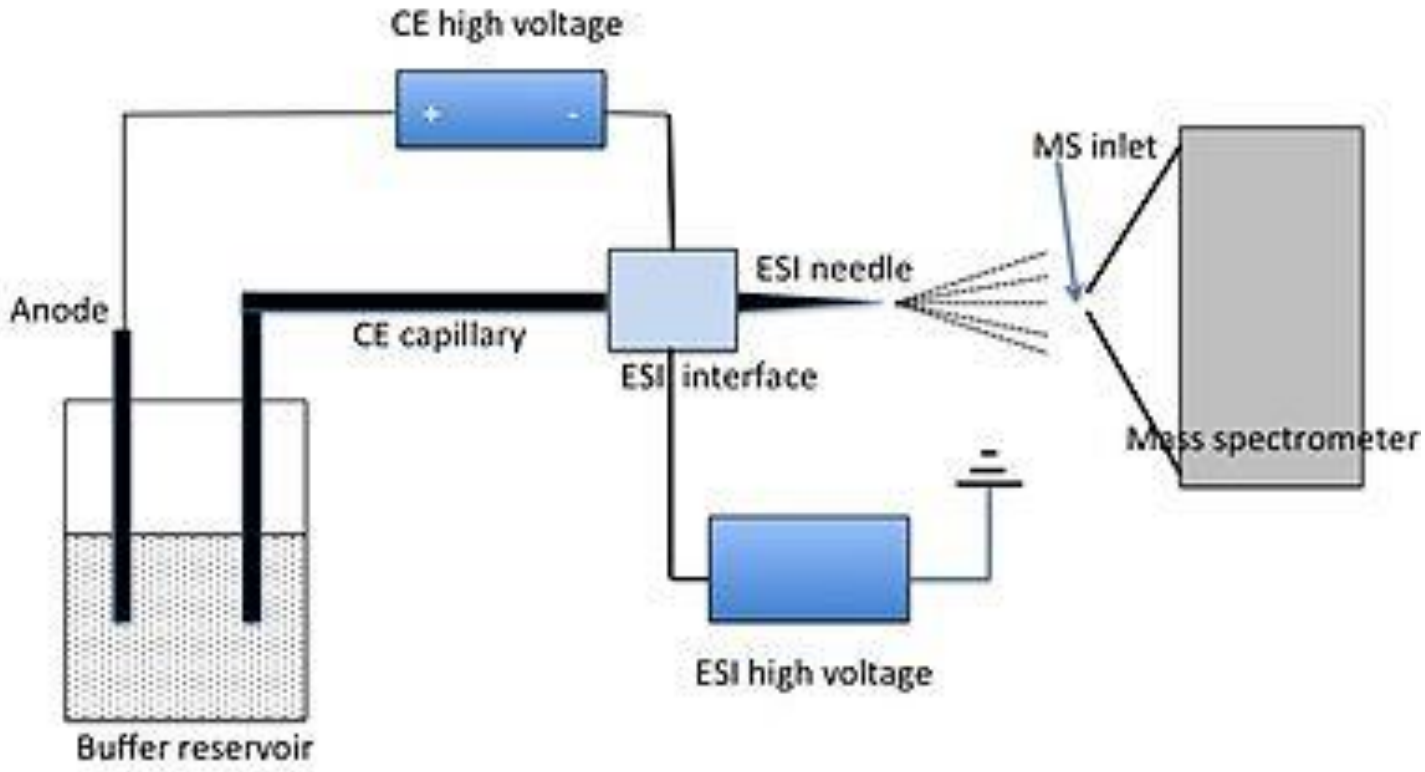

2 mAU of detector signal
MF
PF
MeOH peak
BPA
TC
NAA
1
5
10
Migration time (min)

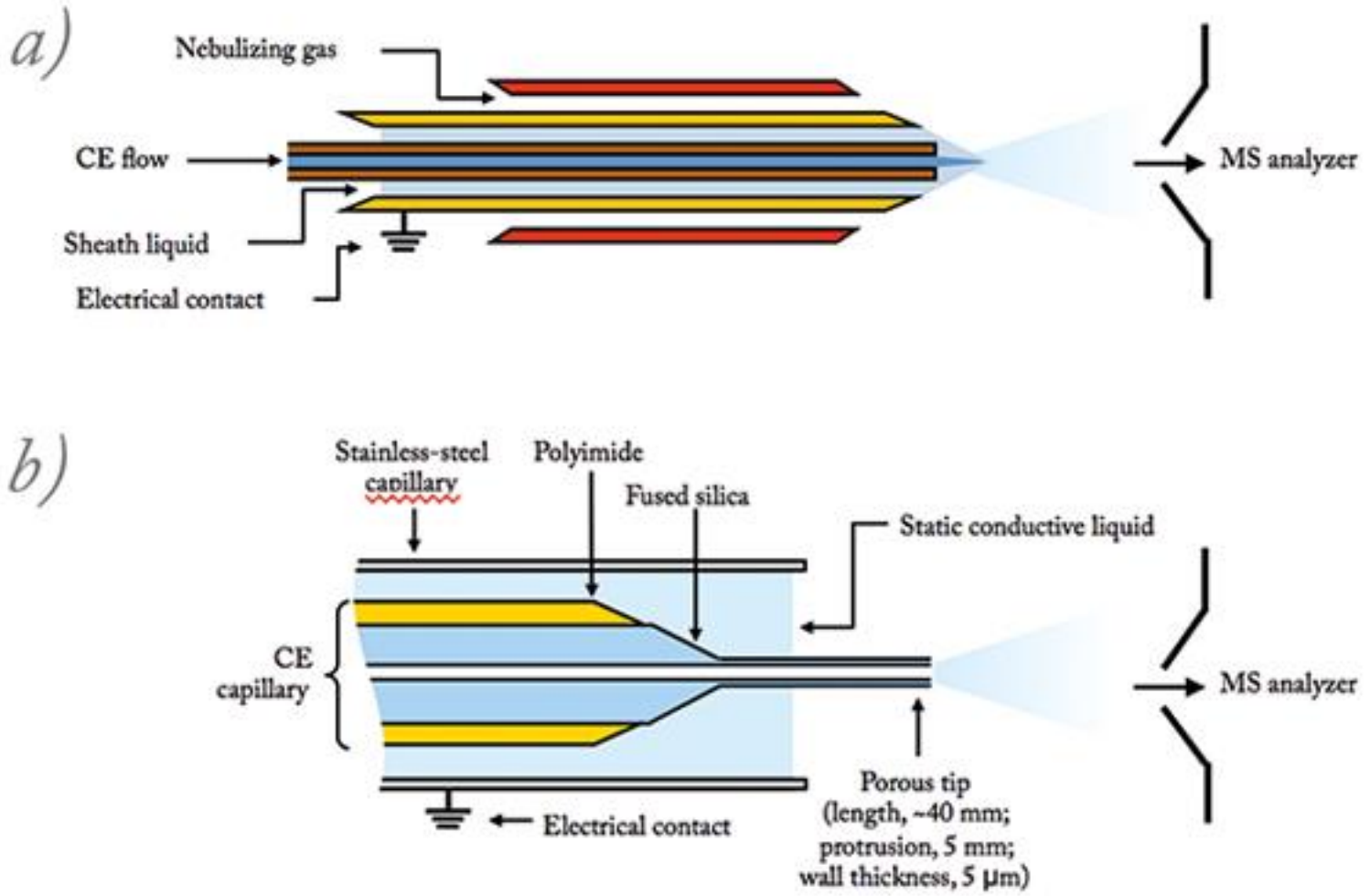
LIF detection



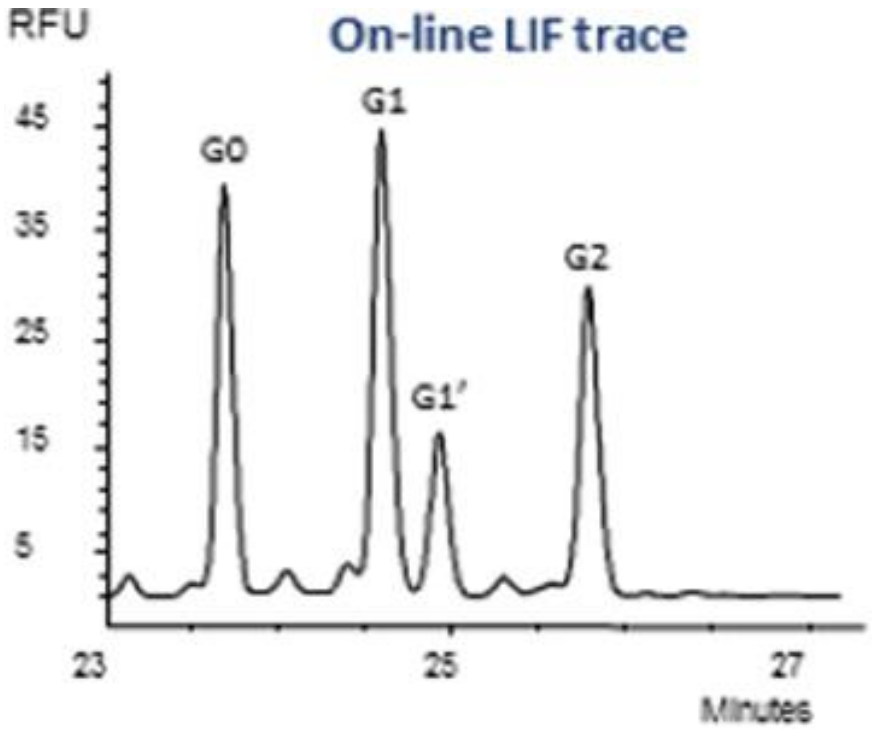
https://www.photonics.com/Article.aspx?AID=38236

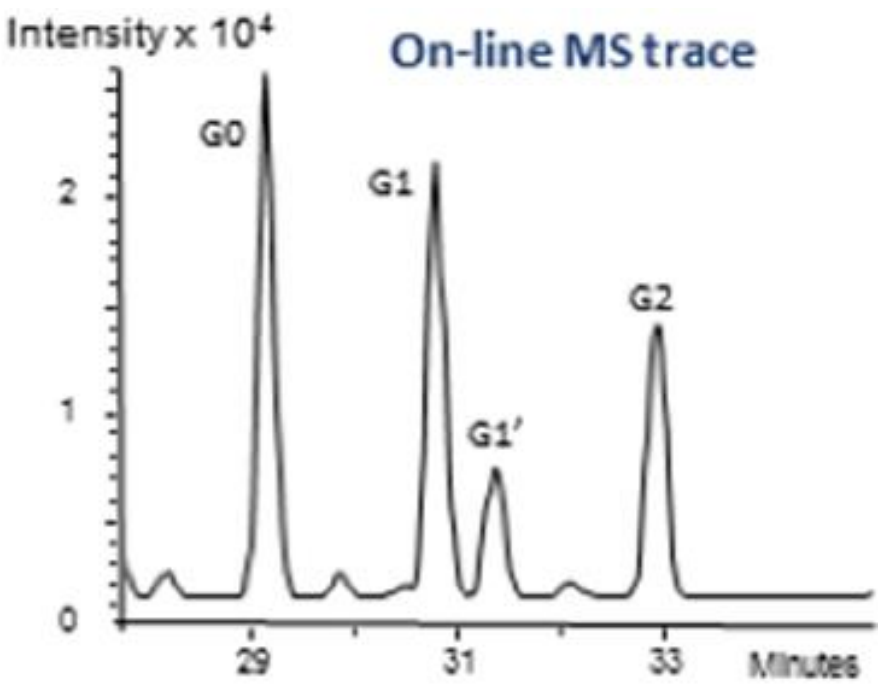
CE-MS

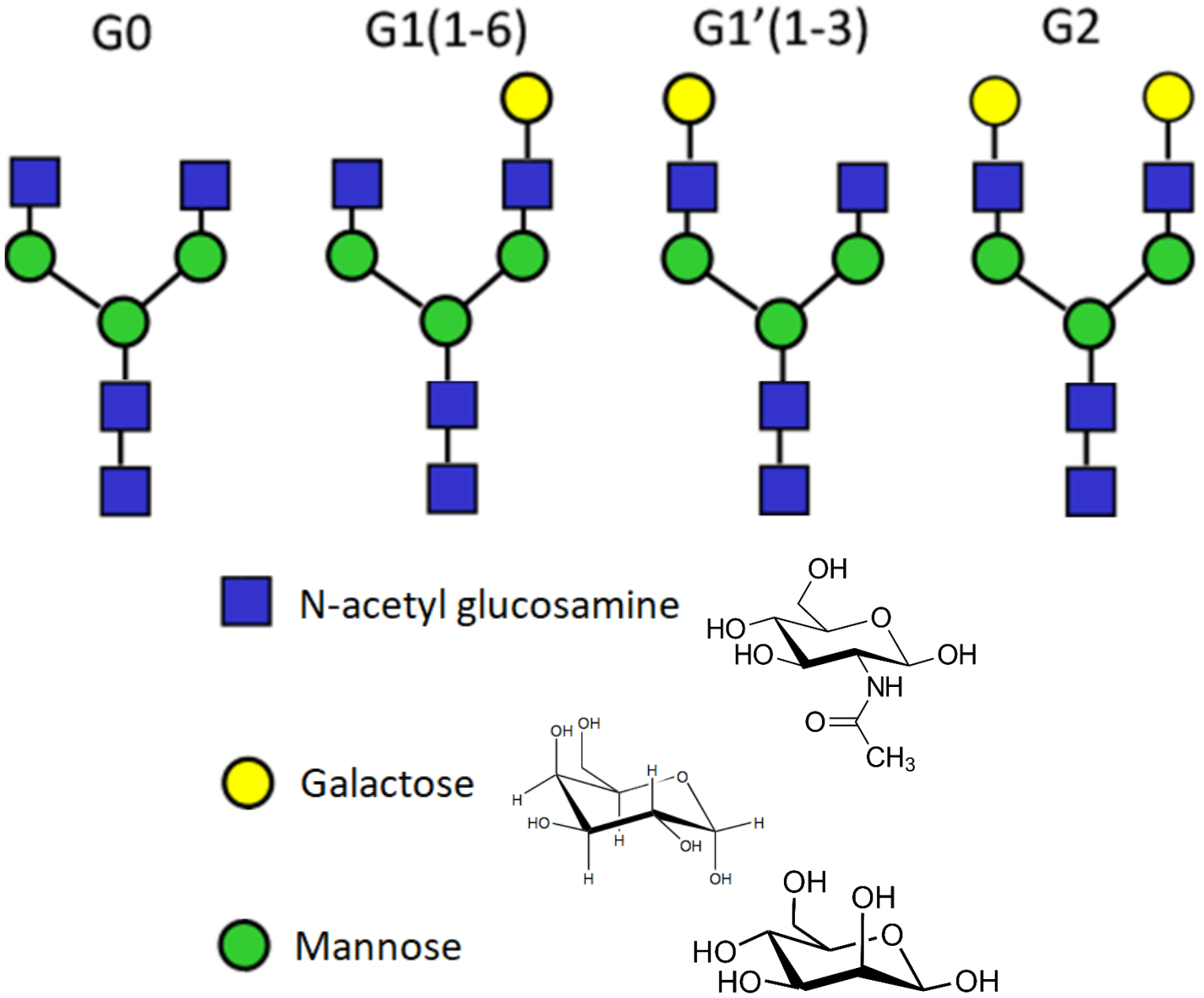

CE high voltage
+
-
MS inlet
ESI needle
Anode
CE capillary
ESI interface
Mass spectrometer
ESI high voltage
Buffer reservoir



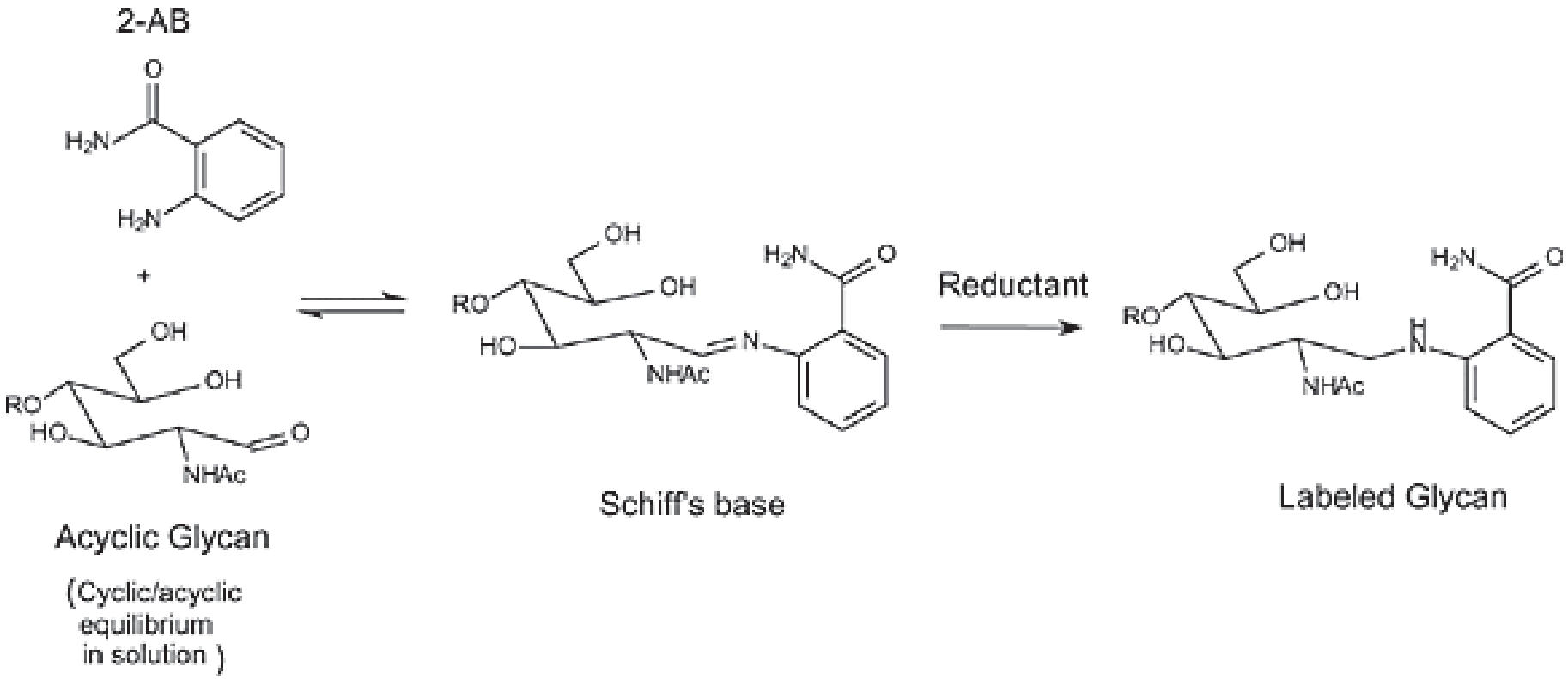
https://theanalyticalscientist.com/issues/1115/gurus-of-ce-ms/


RFU
On-line LIF trace
45
35
25
15
5
G0
G1
G1'
G2
23
25
27
Minutes


Intensity x 10⁴
On-line MS trace
2
1
0
G0
G1
G1'
G2
29
31
33
Minutes



https://www.agilent.com/cs/Satellite?assettype=GSA_C&assetid=1404943494990&d=&formassembly=false&pagename=Agilent/GSA_C/GenericInnerTemplateWithFourZones_New


2-AB
+
Acyclic Glycan
(Cyclic/acyclic equilibrium in solution)
Schiff's base
Reductant
Labeled Glycan

Recap on CE

Advantages:

- Easy to build home made system
- Fast analysis
- Low sample amounts
- Narrow peaks: high efficiency (N)
- Several possible modes of operation
- Low detection limits
- Interfaceable with MS, electrochemical detectors
- UV, fluorescence detectors possible

Disadvantages:

- Capillary easily plugged
- High voltages (10-25 kV)
- Detection window limited in optical methods
- Easier for analytes soluble in aqueous phases
- Electrolyte may be a problem for detection
- Reproducibility sometimes an issue
- Not as rugged as HPLC
- Sample collection difficult

Questions

1.

Given the following anions: Cl^-, Br^-, $C_6H_6O_7^{2-}$ (citrate) and HCO_3^- (bicarbonate), predict their migration order in capillary zone electrophoresis (CZE), if the apparatus is operated from + to – at high pH. The order is given from first to last to reach the detector.

a) Cl^-, Br^-, HCO_3^-, and $C_6H_6O_7^{2-}$

b) Br^-, Cl^-, $C_6H_6O_7^{2-}$ and HCO_3^-

c) $C_6H_6O_7^{2-}$, HCO_3^-, Br^-, and Cl^-

d) $C_6H_6O_7^{2-}$, Br^-, HCO_3^-, and Cl^-

e) These ions would not come out of the capillary

2.

This picture shows a detection window used in capillary electrophoresis. Which of the following CE detection methods could use such a window? (More than one answer may be possible; you may circle more than one).

- UV-visible
- Infrared
- Raman
- Amperometric
- Fluorescence

3. Why is a sheath flow necessary in the CE-MS interface?
There are two possible reasons.