Electrophoresis (Chapter 30):

Separation of charged species (ions) based on their migration rate in electric field

Slab or planar electrophoresis

porous layer 2-10 cm long - paper, cellulose acetate, polymer gel

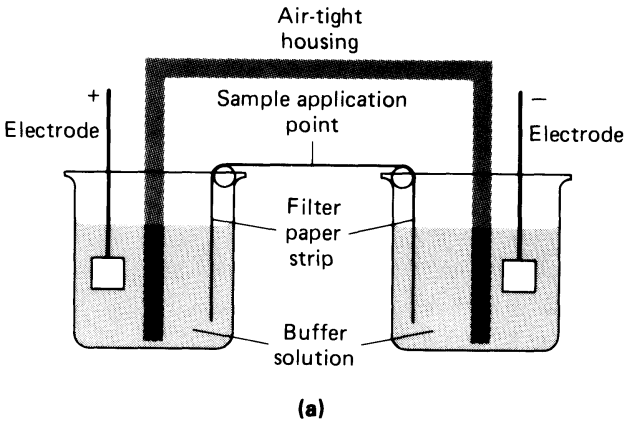
soaked in electrolyte buffer

slow

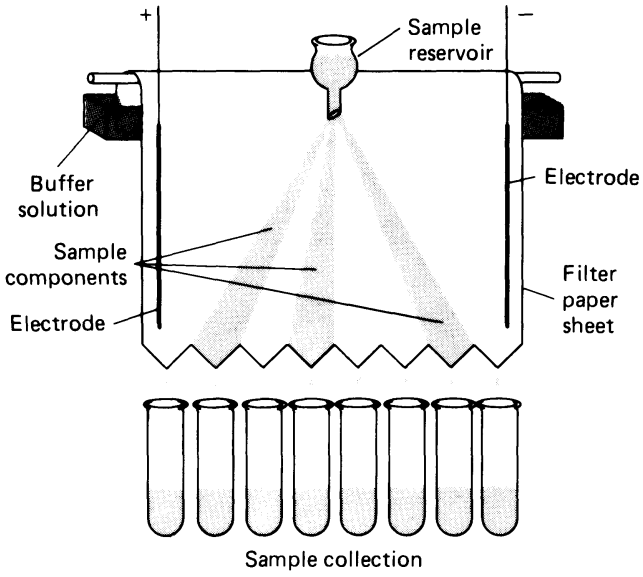
simple but difficult to automate

poor quantitation

large quantities (μL)



(a)



(b)

Capillary electrophoresis

narrow (25-75 μm diameter) silica capillary tube 40-100 cm long

filled with electrolyte buffer

fast

complex but easy to automate

quantitative

small quantities (nL)

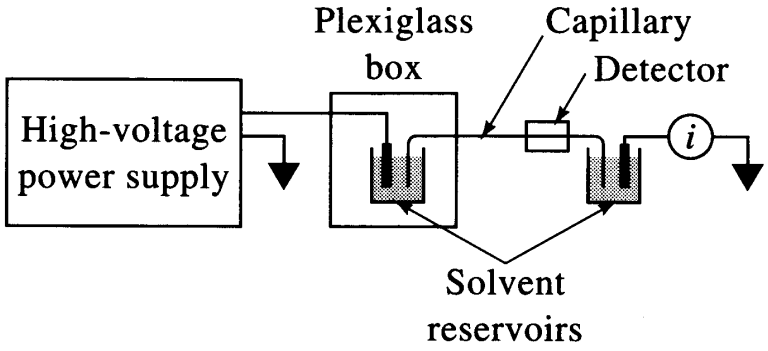


Fig 30-1

Migration rate depends on

movement in electric field

- molecular weight
- charge - small/highly-charged species migrate rapidly
  - affected by pH

    $HA \leftrightarrow H^+ + A^-$
  - affected by ionic strength

    $\mu = \frac{1}{2}\left([A]\ Z_A{}^2 + [B]\ Z_B{}^2 ...\right)$

    low μ, few counter-ions, low charge shielding

    high μ, many counter-ions, high charge shielding

Migration Rate:

$$v = \mu E = \mu \frac{V}{L}$$

migration rate ($cm \cdot s^{-1}$) electric field ($V \cdot cm^{-1}$)

electrophoretic mobility (-ve for anions) ($cm^2 \cdot V \cdot s$)

Electrophoretic mobility depends on net charge and frictional forces (size/molecular weight of analyte)

- Only ions separated

Plate Height and Number:

$$N = \frac{\mu V}{2D} \qquad H = \frac{L}{N} = \frac{2D L}{\mu V}$$

diffusion coefficient ($cm^2 \cdot s^{-1}$)

Increase number of plates (better resolution) by increasing V

- Slab electrophoresis large cross-sectional area, short length

  low electrical resistance, high currents

  Sample heating V_{max}=500 V

  N=100-1000 low resolution

- Capillary electrophoresis small cross-sectional area, long length

  high resistance, low currents

  V_{max}=20-100 kV

  N=100,000-10,000,000 high resolution

  (HPLC N=1,000-20,000)

Zone broadening?

- Single phase (mobile phase) - no partitioning

In chromatography three zone broadening phenomena (i) longitudinal diffusion (ii) transport to/from stationary phase (iii) multipath

- In planar EP no stationary phase
- In capillary EP no stationary phase or multipath

Transport Processes in Electrophoresis:

Electromigration

ions migrating in electric field

cations → cathode (-ve)

anions → anode (+ve)

Electroosmosis movement of entire fluid near wall of capillary in *one direction!*

anode (+ve) → cathode (-ve)

(A) Analyte dissolved in background electrolyte and pH buffer

(B) Silica capillary wall coated with silanol (Si-OH) and $Si\text{-}O^-$

(C) Wall attracts cations - double-layer forms

(D) Cations move towards cathode and sweep fluid in one direction

Electroosmotic flow proportional to V - usually greater than electrophoretic flow

(Fig 18-12 Harris cf Fig 30-2):

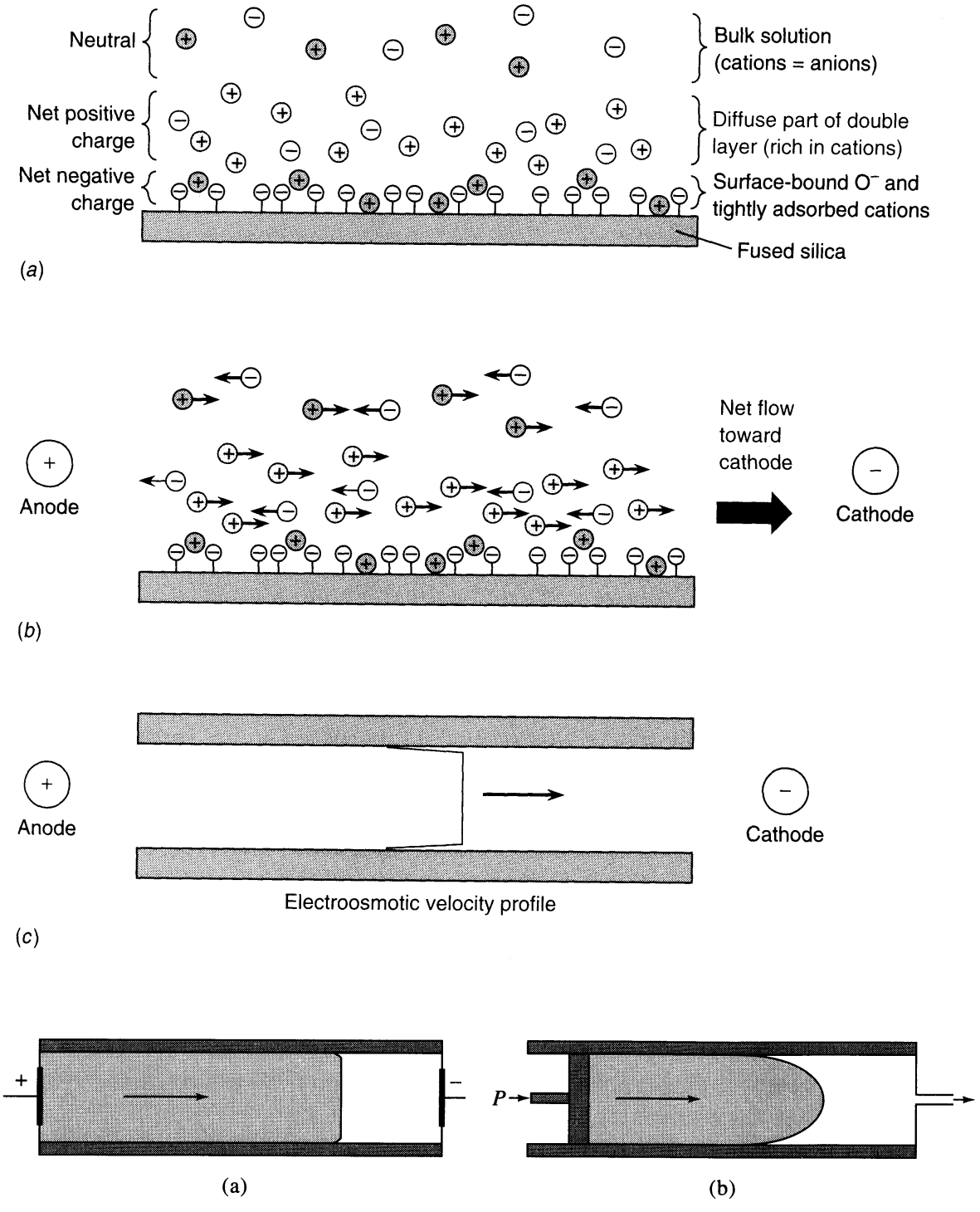
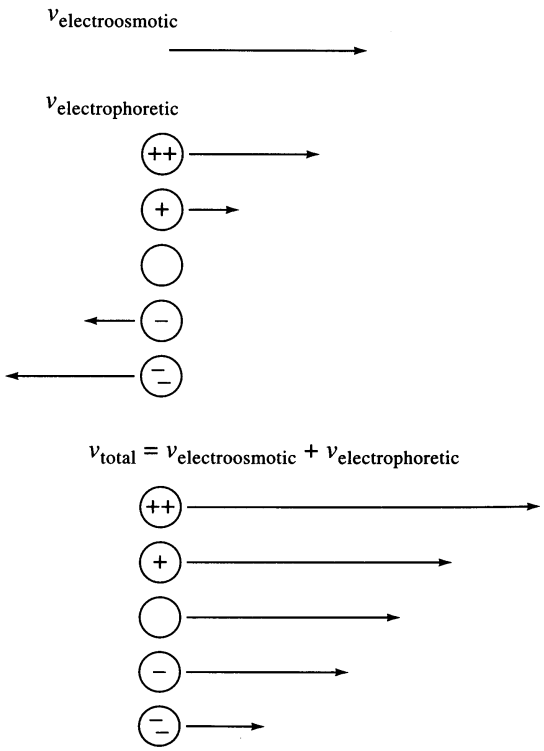


Fig 30-3



migration rate

electrophoretic mobility

$$v = (\mu + \mu_{eo})E$$

electric field

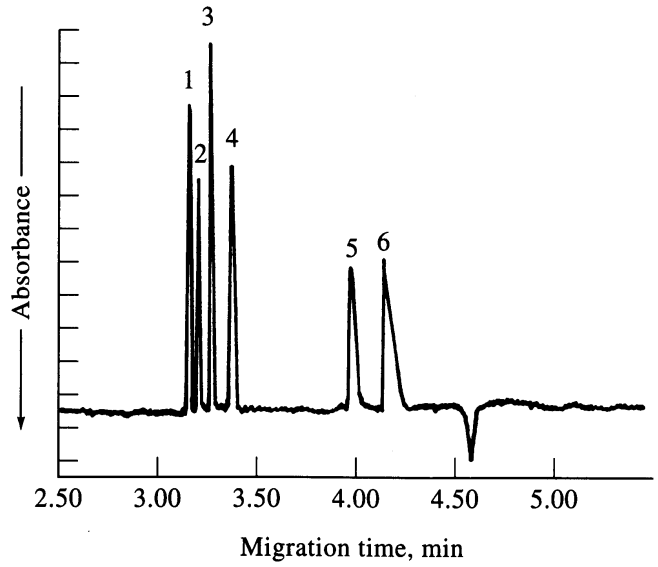
electroosmotic mobility

Typical Elution Order in EP:

(detector at cathode)

High mobility cations	first
Low mobility cations	
All neutrals (v_{eo})	
Low mobility anions	
High mobility anions	last

Electropherogram - migration time analogous to retention time in chromatography



(Fig 30-6)

Isoelectric focusing (IEF):

pH affects charge

$$HA \leftrightarrow H^+ + A^- \qquad K_a = \frac{[H^+][A^-]}{[HA]}$$

weak acid: $pH > pK_a \quad HA \rightarrow H^+ + A^-$

$pH < pK_a \quad HA \leftarrow H^+ + A^-$

$$H_3N - X - CO_2H \xrightarrow{\text{low pH}} H_3N - X - CO_2^-$$

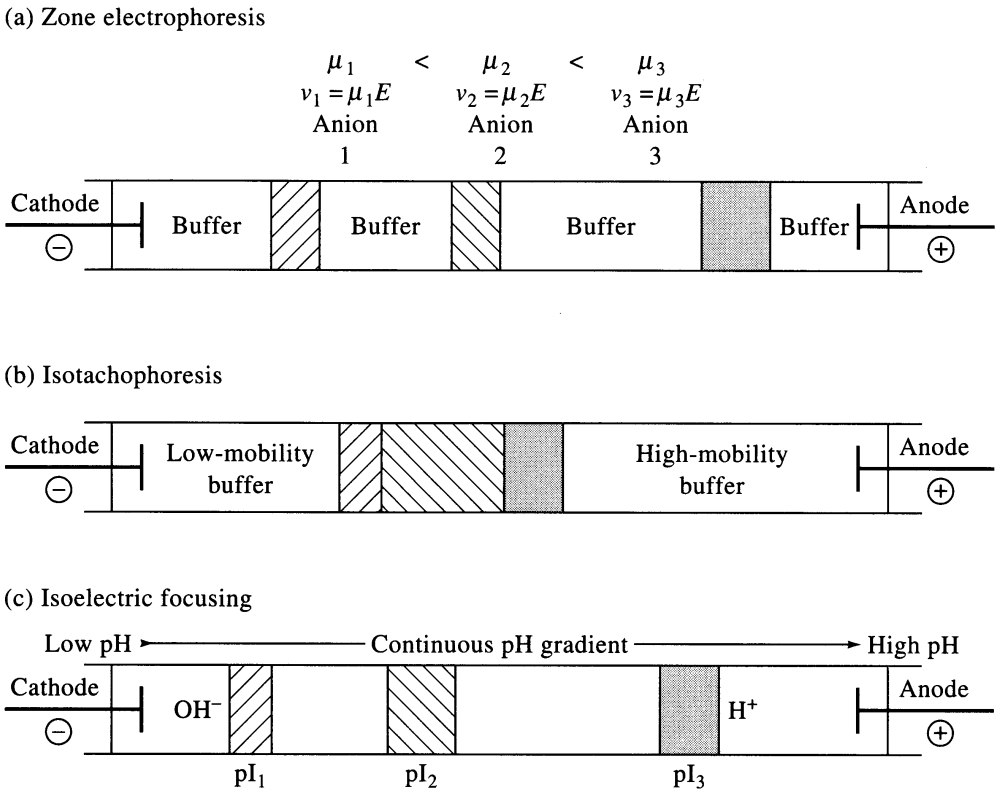
protein:

$$\xrightarrow{\text{low pH}} {}^+H_4N - X - CO_2H$$

$$\xrightarrow{pI} {}^+H_4N - X - CO_2^-$$

pI - isoelectric point (pH at which net charge on molecule is zero)

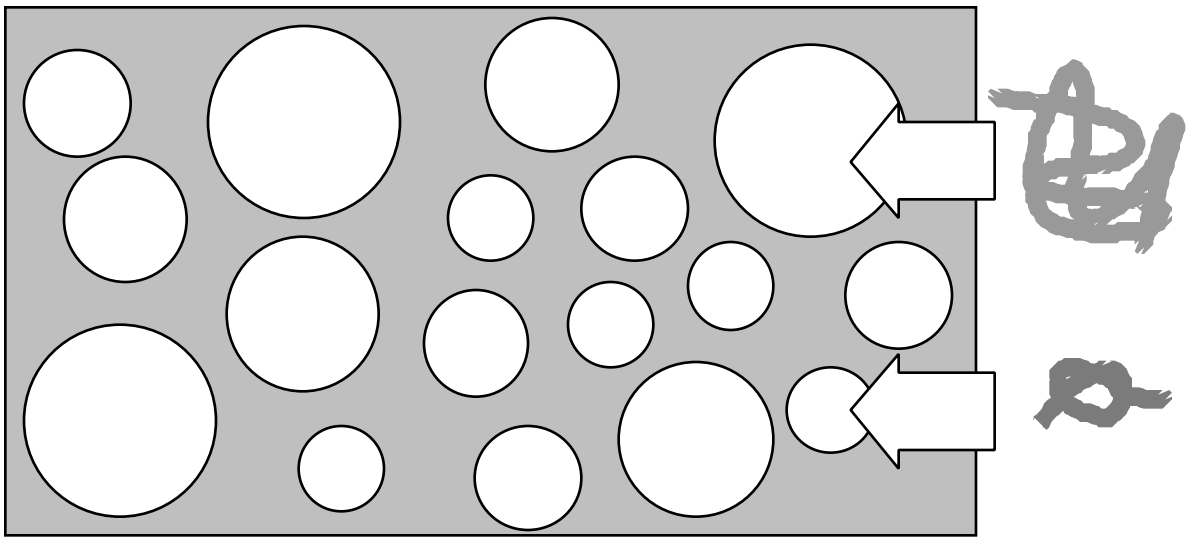
If plate has pH gradient, protein migrates until local pH = pI



Planar (gel) Electrophoresis:

Especially useful for *proteins*

Gel is random copolymer - polyacrylamide



In addition to charge and field, mobility can also be changed by adding pores

- small molecules pass quickly through pores - high overall mobility
- large molecules have to squeeze through pores - lowers overall mobility
- adds a *sieving component* to electrophoresis

Size of pores can be changed - determines range of sizes/weights over which sieving is effective

Detection

Staining - visual

Fluorescence

Absorption/UV-vis

Radiolabelling - use photographic film

Capillary electrophoresis:

In principle instrumentation is simple BUT difficult to

introduce sample

detect analyte in small volume (entire column <5 μL analyte zone <10 nL)

Injection:

1. remove anode end capillary from buffer
2. place end of capillary in sample
3. apply field for short time (electrokinetic injection - discriminates against low migration rate analytes)

or apply pressure for short time (pressure injection)

4. replace in buffer

Detection

Can use many HPLC detectors - absorbance, fluorescence, electrochemical, mass spectrometry, AAS, ICP-AES...

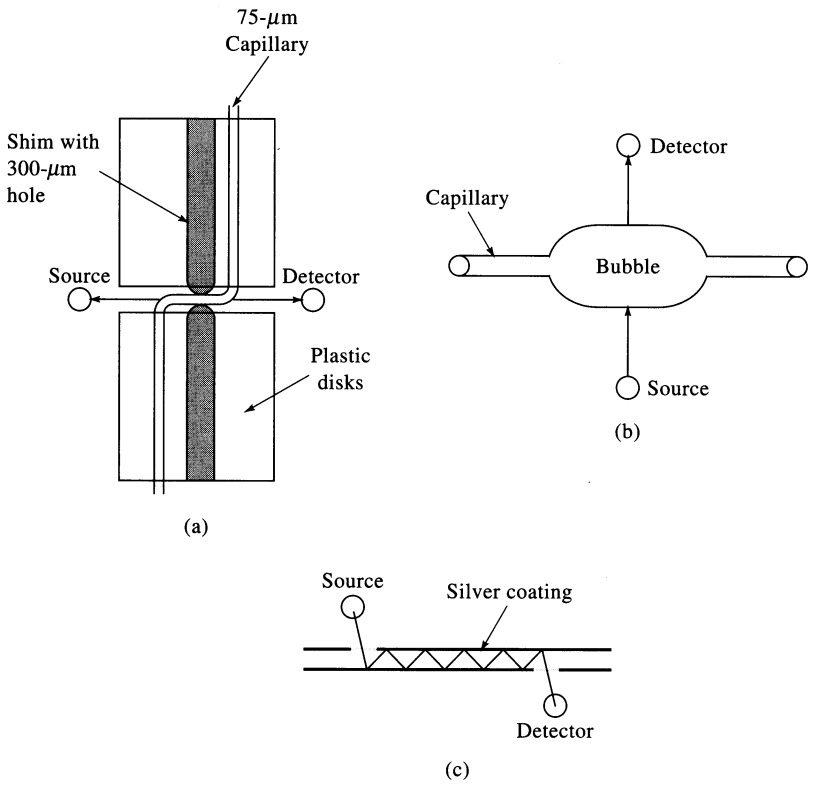


Fig 30-5

Indirect Detection:

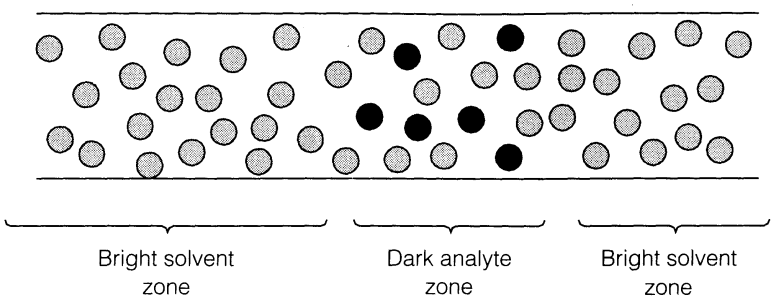
Can be used with many instrumental methods

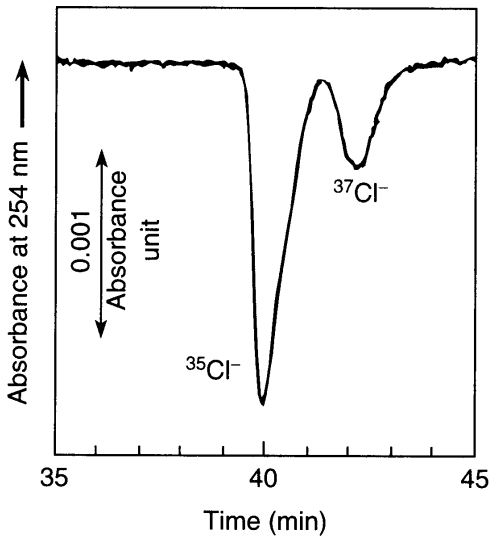
Example

- Addition of fluorescent molecule to buffer
- During EP, buffer concentration is decreased in analyte zone
- Detector measures high signal for buffer zone, low signal for analyte

Fluorescent molecule can be replaced with absorbing molecule, electroactive molecule ...

(Fig 18-13, 18-14 Harris)





Properties of EP

- Only works for ions - neutral species entrained in electroosmotic flow but not separated
- Planar instrumentation cost similar to UV-vis
- Capillary instrumentation cost comparable to HPLC
- Can be automated
- Especially useful for separation of macromolecular ions (polymers) and large biomolecules (amino acids, proteins, oligonucleotides) with charge/mass ratios differing by <1%