Separation Sciences

1. Introduction: Fundamentals of Distribution Equilibrium

2. Gas Chromatography (Chapter 2 & 3)

3. Liquid Chromatography (Chapter 4 & 5)

4. Other Analytical Separations (Chapter 6-8)

    a. Planar chromatography

    b. Supercritical fluid chromatography

    c. Electrophoresis

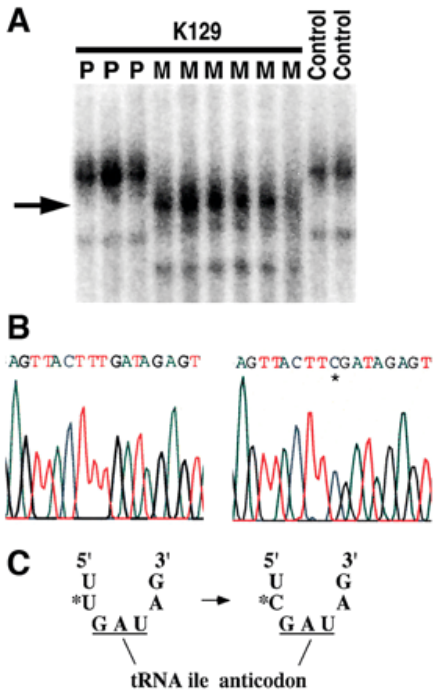
    d. Centrifugation

    e. Field Flow Fractionation

Electrophoresis

1. Capillary Electrophoresis

2. Gel Electrophoresis in Bio-applications



Science, Nov. 12, 2004

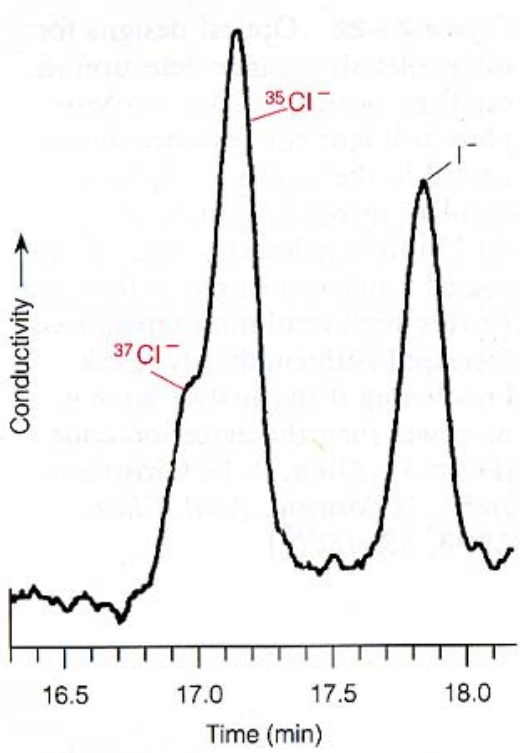
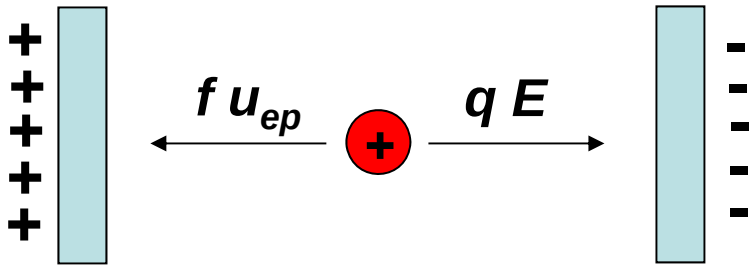


Figure 24-22 Partial separation of isotopes of 1 μM chloride by capillary electrophoresis with conductivity detection. Prior to detection, eluate is passed through a cation-exchange membrane to convert the conductive 2 mM sodium borate background electrolyte into poorly conductive boric acid. [From N. Avdalovic, C. A. Pohl, R. D. Rocklin, and J. R. Stillian, *Anal. Chem.* **1993**, *65*, 1470.]

Capillary Electrophoresis

1. Introduction

a. electrophoresis: the migration of ions in solution under the influence of an electric flied.



qE = $f\,u_{ep}$

Accelerating force

Frictional force

q: charge
E: electric field strength
f: friction coefficient
u_{ep}: *velocity of the ion of electrophoresis migration*

b. Electrophoretic mobility

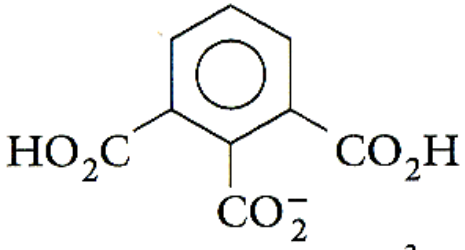
$$u_{ep} = \frac{q}{f} E \equiv \mu_{ep} E$$

Electrophoretic mobility

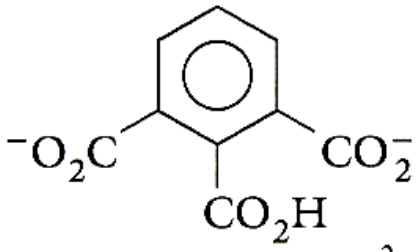
$$\mu_{ep} = \frac{q}{f} = \frac{u_{ep}}{E}$$

Electrophoretic mobility (μ_{ep}) is a constant and a intrinsic property of an ion. It is dependant on the charge and 3-D structure of the ion.

i. Molecules of similar size, the magnitude of the mobility (μ_{ep}) increases with charge

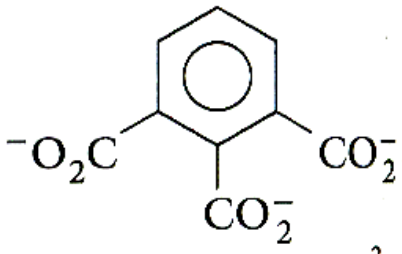


$\mu_{ep} = -2.54 \times 10^{-8} \frac{m^2}{V \cdot s}$



$\mu_{ep} = -4.69 \times 10^{-8} \frac{m^2}{V \cdot s}$

(solvent is H_2O at 25°C)



$\mu_{ep} = -5.95 \times 10^{-8} \frac{m^2}{V \cdot s}$

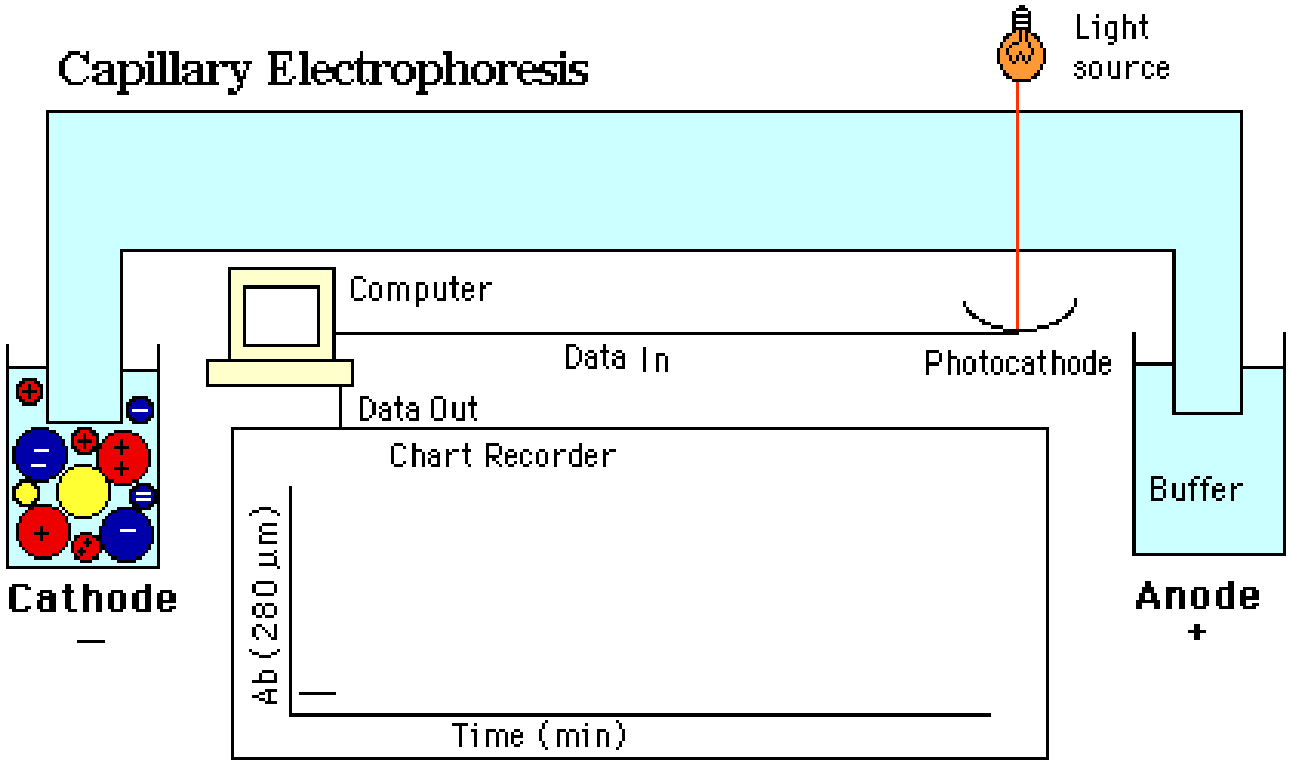
ii. For a spherical particle of radius *r* moving through a fluid of viscosity η, the friction coefficient *f* is

Stokes equation: $f = 6\pi\eta r$

$$\mu_{ep} = \frac{q}{6\pi\eta r}$$

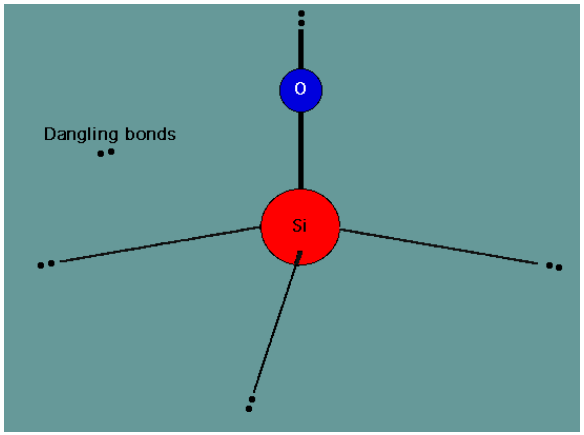
C. Capillary electrophoresis

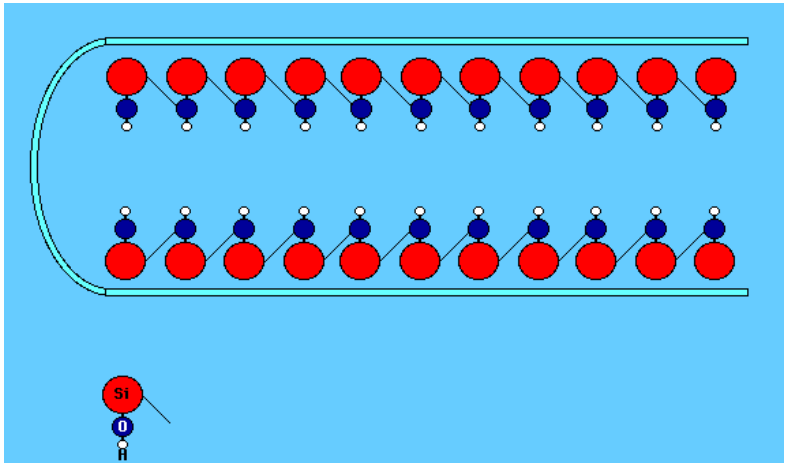
Capillary electrophoresis is formed in fused SiO_2 capillary tube long ~ 0.5 m, inner diameter: 25-75 μm.

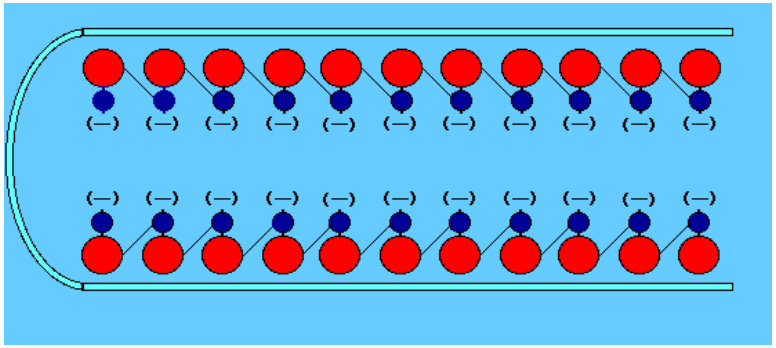


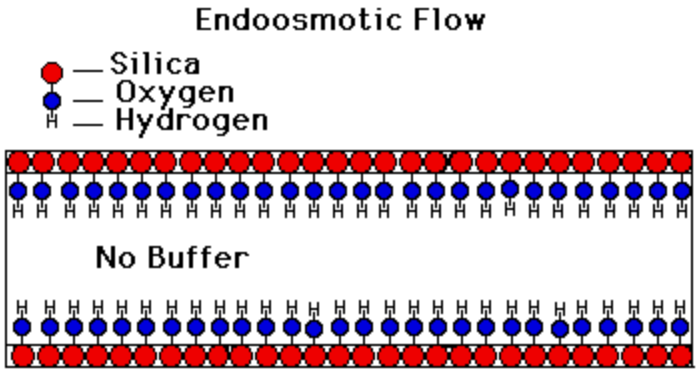
Fused SiO_2 Capillary Tube?

Fused silica capillary has exposed silanol groups which have a pKa ~ 2 which means that at pH's above this there is a negative surface charge ($-SiO^-$)



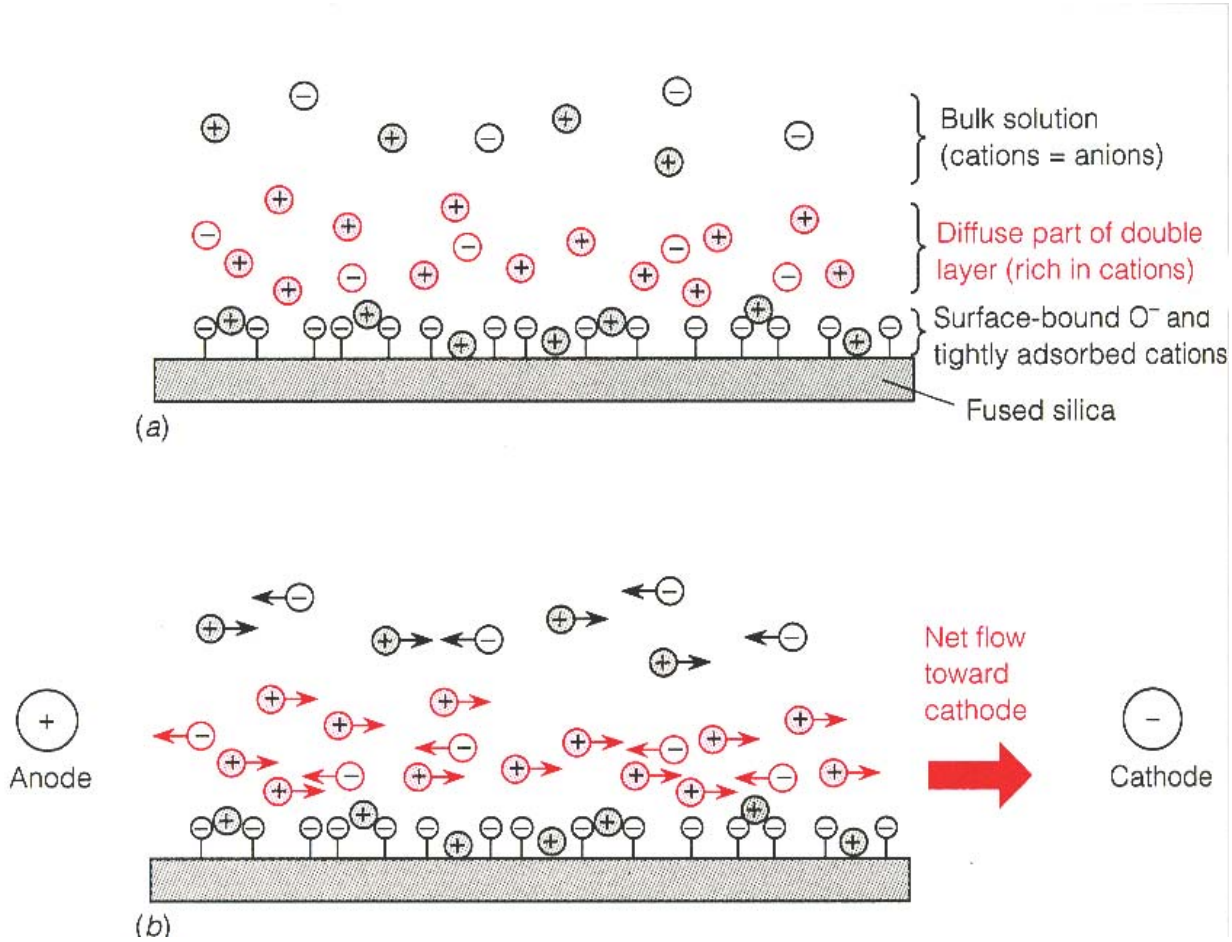






Electroosmosis

Electroosmosis

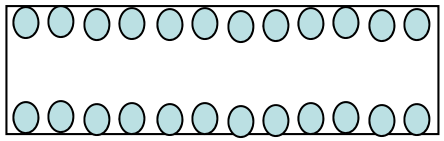


When DC voltage is applied, the excess positive charge ions (cations) migrate toward cathode. This migration is called <u>electroosmosis</u>. The resulted flow of bulk solvents is called <u>electroosmotic flow (EOF)</u>

Electroosmotic flow (EOF)

Electroosmotic mobility is the constant of proportionality between electroosmotic velocity (u_{eo}) and applied field E

$$u_{eo} = \mu_{eo} E$$



Electroosmotic mobility (is proportional to surface charge density on the silica)

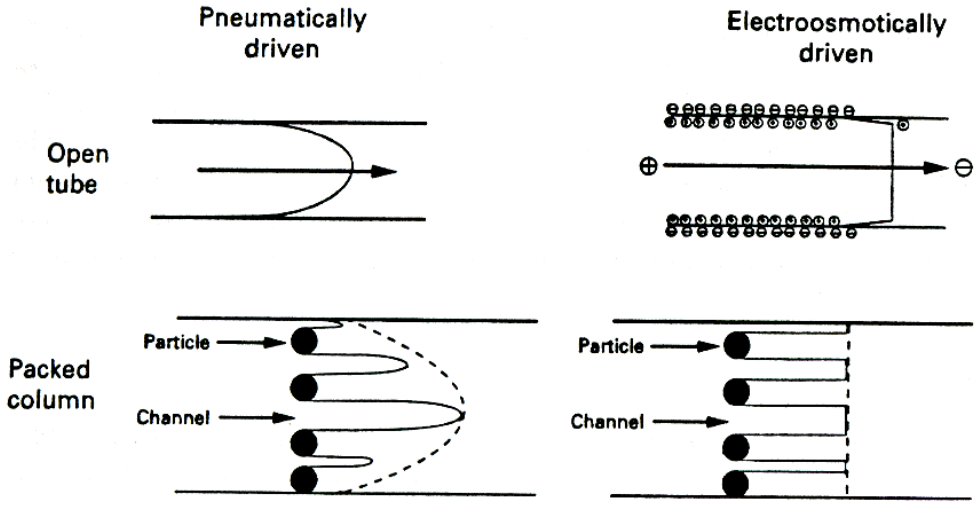


Figure 1.5. Mobile phase flow profile for an open tube and a packed column with pressure-driven and electroosmotic flow.

This is one reason why capillary electrophoresis has better separation efficiency.

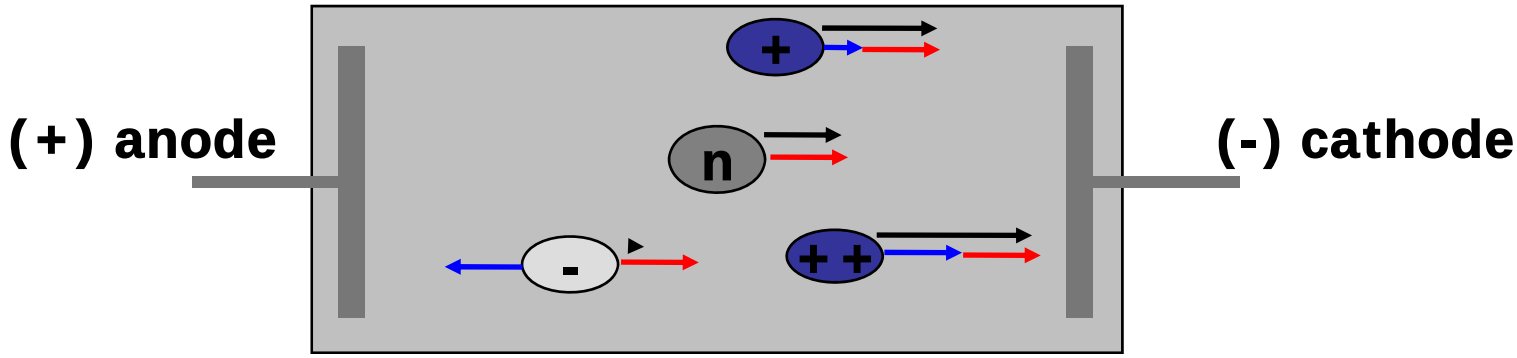
Capillary Electrophoresis

Apparent Mobility

Electroosmosis **Electrophoresis**

u_{app} u_{eo} u_{ep}

Direction is dependent on the sign of charge



Spherical particles $\mu_{ep} = \frac{q}{6\pi\eta r}$

The electroosmotic force is not necessary always stronger than the electrophoretic force.

Anion: negative charged ion (-)

Cation: positive charged ion (+)

Anode: is an electrode through which *electric current* flows *into* a polarized electrical device.

Positive charged electrode in electrophoresis (+)

Cathode: cathode is an electrode through which *electric current* flows *out of* a polarized electrical device:

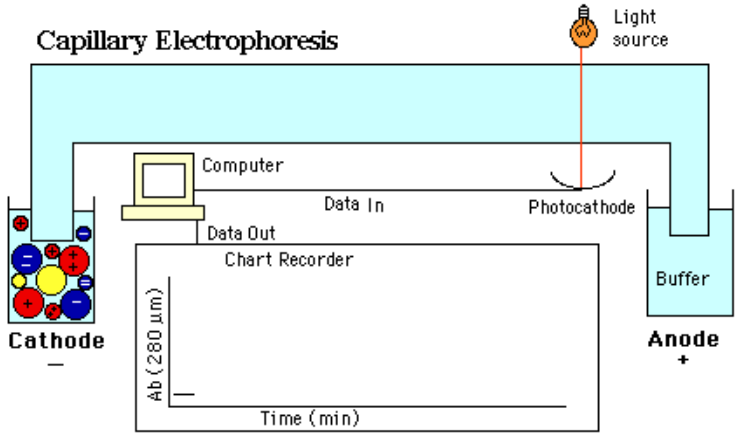
negative charged electrode in electrophoresis (-)

Apparent Mobility of An Ion

Apparent Mobility (μ_{app})

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

$$\mu_{app} = \frac{u_{net}}{E} = \frac{L_d/t}{V/L_t}$$



L_d is the length from the injector to the detector
L_t is the length from one end to another
V the voltage applied
t is the time required for solute to migrate from the injector to the detector

Measurement of Electroosmotic mobility

$$\mu_{eo} = \frac{u_{nutral}}{E} = \frac{L_d/t_{neutral}}{V/L_t}$$

Capillary Electrophoresis

Separation Efficiency

Recall $$H = A + \frac{B}{u_x} + C u_x$$

No particles ⇒ no multiple paths term (A = 0)

No stationary phase ⇒ no resistance to mass transfer term (C = 0)

$$H = \cancel{A} + \frac{B}{u_x} + \cancel{C u_x} = \frac{B}{u_x}$$

Increase velocity by increasing applied voltage, but due to solution resistance this generates heat and increases longitudinal diffusion (B)

Capillary Electrophoresis

$$H = \frac{B}{u_x} = \frac{2D}{u_x} = \frac{2D}{\mu_{app}E} = \frac{2DL}{\mu_{app}V}$$

where D = diffusion coefficient (m^2/s)

Number of Plates: $$N = \frac{L}{H} = \frac{\mu_{app}V}{2D}$$

i. Plate number is independent of capillary length at constant !

ii. The higher the voltage, the greater the number of plates.

iil. The smaller the diffusion coefficient, the greater the number of plates.

How many theoretical plates might we hope to attain?

Using typical values μ_{app} = 2 x 10^{-8} $m^2/V\bullet s$ which corresponds to a 10 minute migration time in a 55 cm long capillary with 25 kV (Serum albumin (a protein) with D = 0.059 x 10^{-9} m^2/s, and K^+ with D = 2 x 10^{-9} m^2/s).

$$N = \frac{\mu_{app} V}{2D}$$

For K^+: N = 125, 000 plates, H = L/N = 4.4 μm

For serum albumin: N = 4.2 x 10^6 plates
H = L/N = 0.13 μm

A greater plate number means a sharper peak!

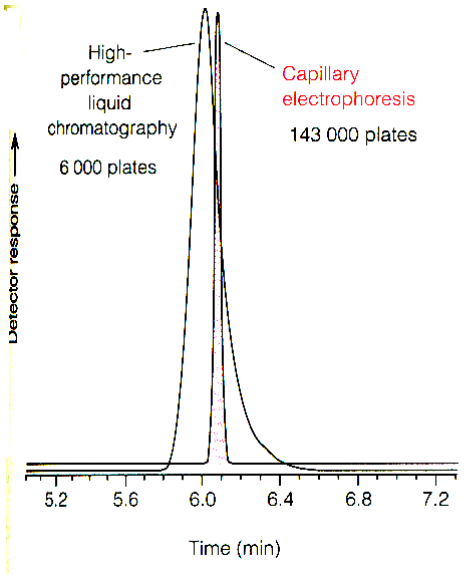


Figure 24-13 Comparison of peak widths for benzyl alcohol ($C_6H_5CH_2OH$) in capillary electrophoresis and HPLC. [From S. Fazio, R. Vivilecchia, L. Lesueur, and J. Sheridan, *Am. Biotech. Lab.* January 1990, p. 10.] Separations with ~3 million plates have been demonstrated [R. D. Smith, J. O. Olivares, N. T. Nguyen, and H. R. Udseth, *Anal. Chem.* **1988,** *60,* 436].

Other on-column band-broadening

i. <u>Joule heating</u> , ii. Mixing due to unstable density gradients

a. Joule heating **is an uneven heating thermal effect caused by electric field (I^2Rt). This effect occurs throughout the packed bed or open tube and results in solvent at different point in the system having different temperature. There thermal gradients are produced because solution at the edges of the system can give off heat more easily than that near the center.**

TABLE 24-6 Heat generation in capillary electrophoresis[a]

Buffer	Current density (A/cm^2)	Temperature difference (K) (capillary center to capillary wall)
100 mM sodium phosphate, pH 7.0	4.0	0.30
50 mM sodium citrate, pH 2.5	0.90	0.066
20 mM 3-(cyclohexylamino)propane sulfonate (CAPS), pH 11.0	0.31	0.024

a. Fused silica capillary with 50-μm diameter and electric field of 2.5×10^4 V/m.

SOURCE: Data from P. D. Grossman and J. C. Colburn, *Capillary Electrophoresis: Theory and Practice* (San Diego: Academic Press, 1992), Chapter 1.

b. The result of these thermal gradients is that solute and solvent molecules at different points in the system mix unevenly. This gives rise to band-broadening.

c. Ways of decreasing band-broadening due to Joule heating

(1) low current (optimizing).

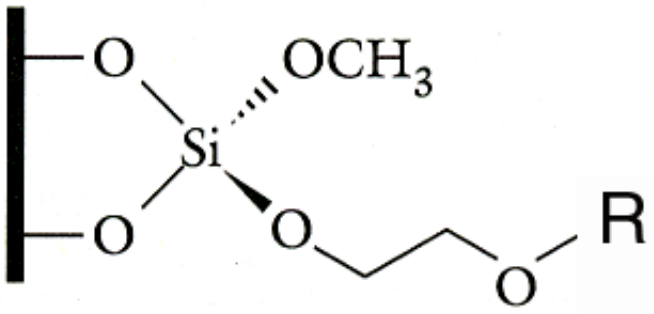
Although plate number is independent of capillary length at constant. Increasing length allows applying a higher voltage!

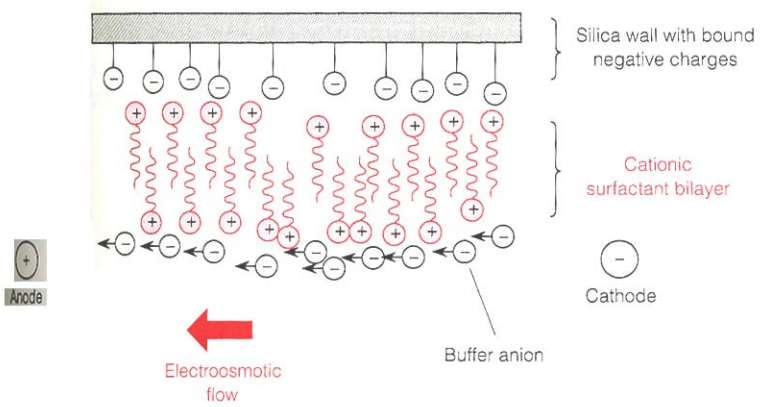
(2) the use of packed-bed system prevents mixing of solvent from different regions of the system.

(3) the use of more efficient cooling prevents the formation of the thermal gradients.

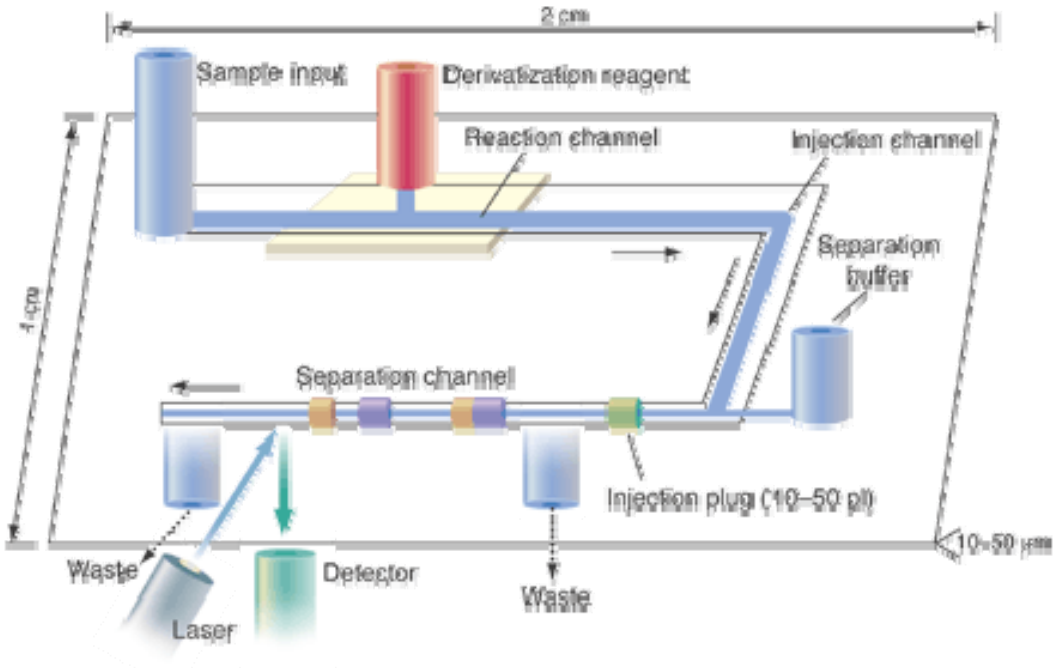
Column modification

Example of covalent wall coating:





Instrumentation

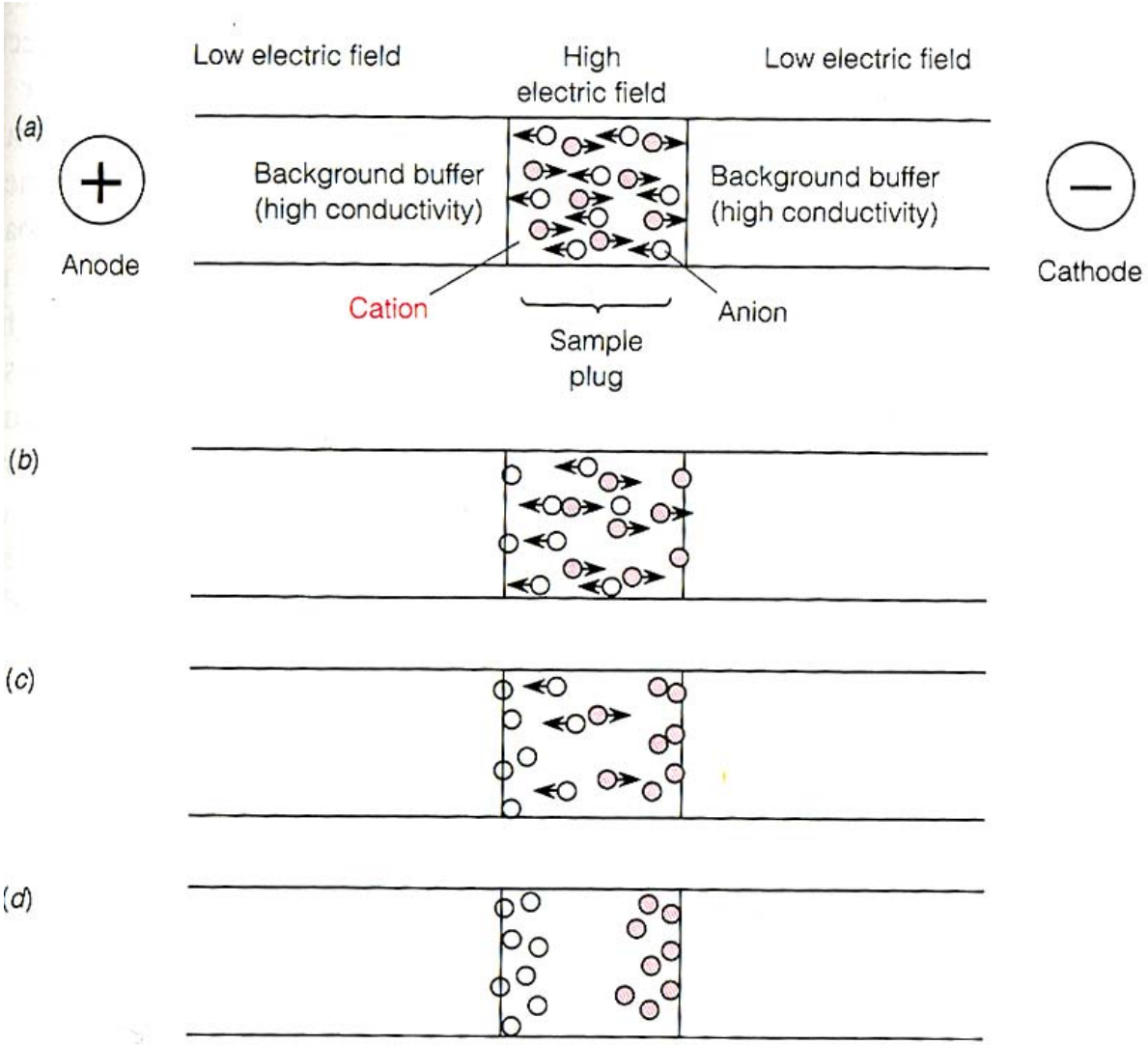


1. Injection (Sample volumes are typically in the nL range) :

a. Hydrodynamic injection-use of a pressure differential between the ends of the capillary

b. Electrokinetic-injection based on the m_{app} which means that different analytes have different mobilities and the injected sample has different composition than the original sample. It allowing pre-separation sample focusing.

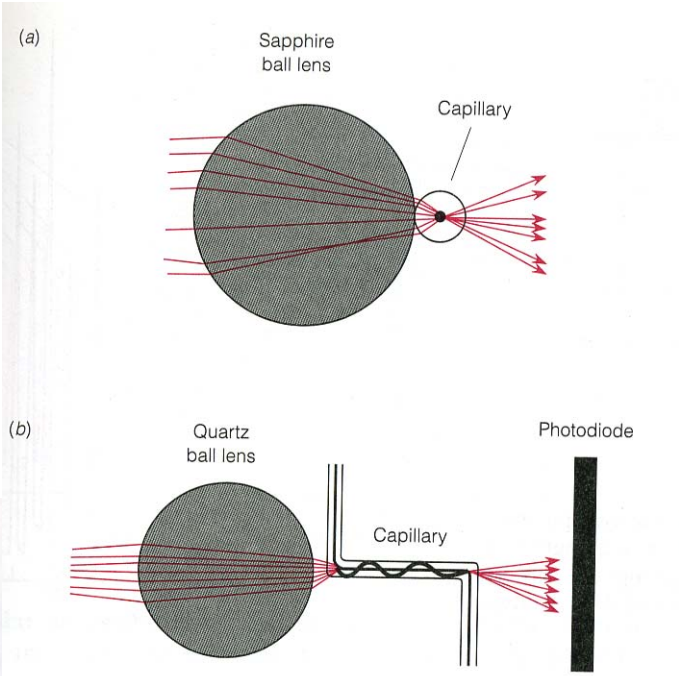
pre-separation sample focusing



Detection:

1. Absorption:

2. Fluorescence:



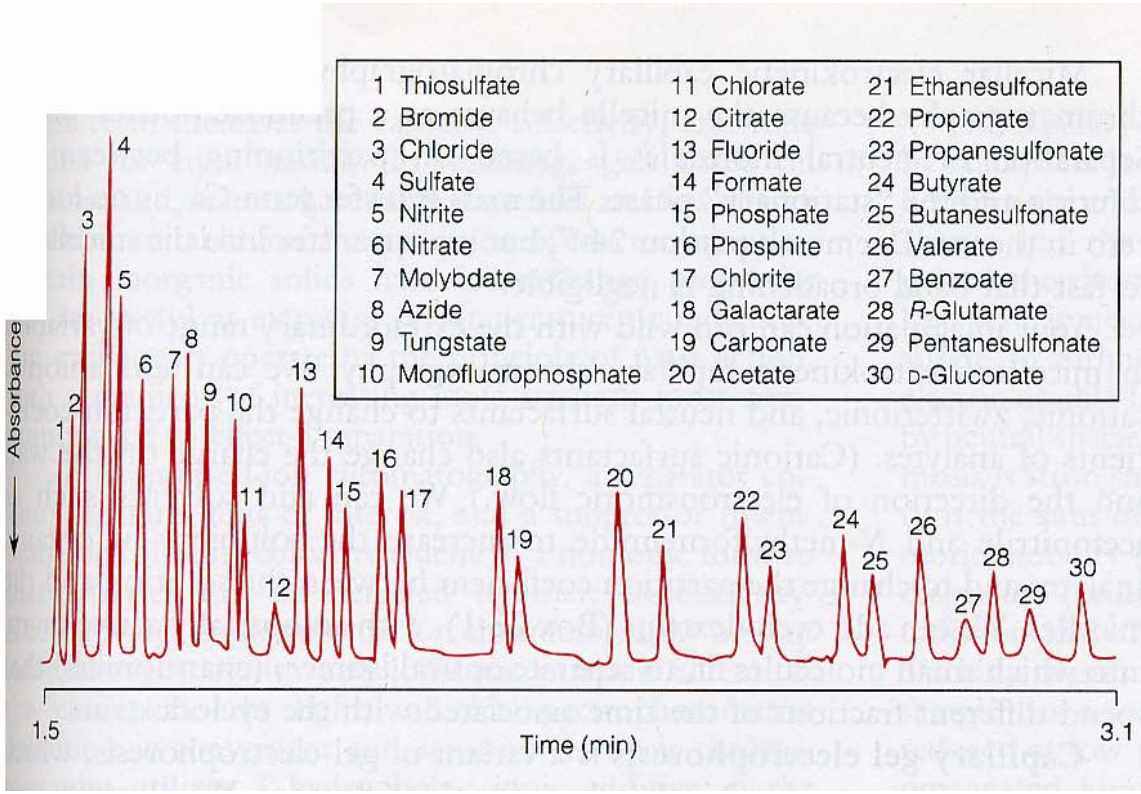
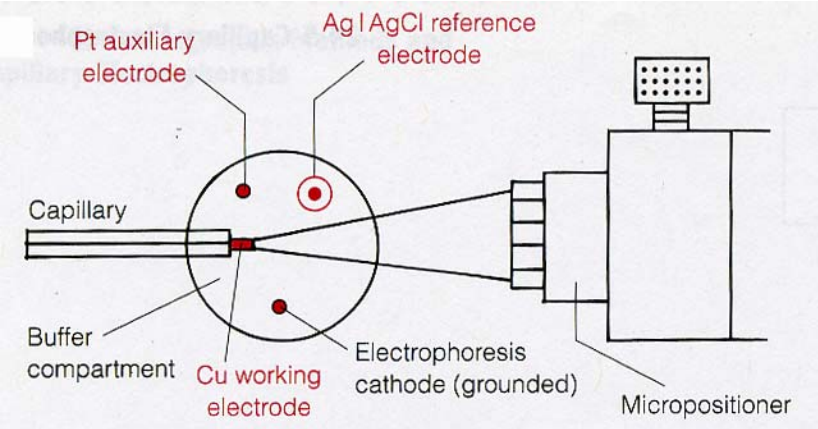
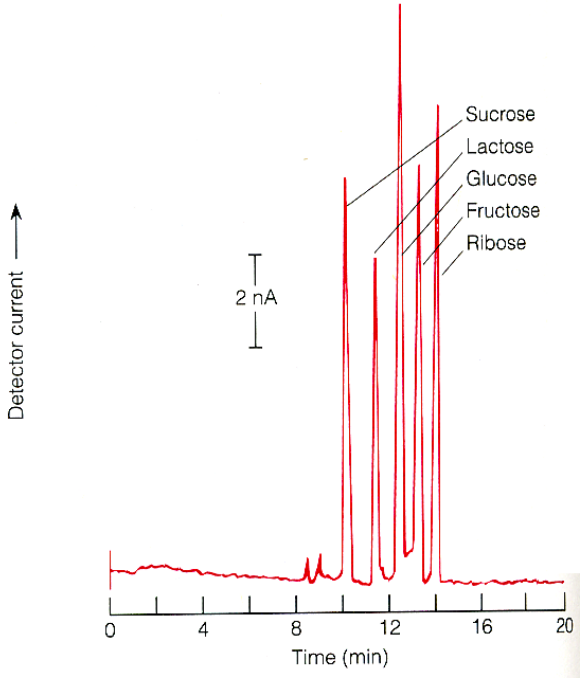


Figure 24-24 Anion separation with indirect ultraviolet absorbance detection (254 nm) of CrO_4^{2-} in the background electrolyte. Thirty anions were separated in 3 min on a 50-μm-diameter × 60-cm-long capillary at 30 kV. [From W. R. Jones, P. Jandik, and R. Pfeifer, *Am. Lab.* May 1991, p. 40.]

3. Conductivity

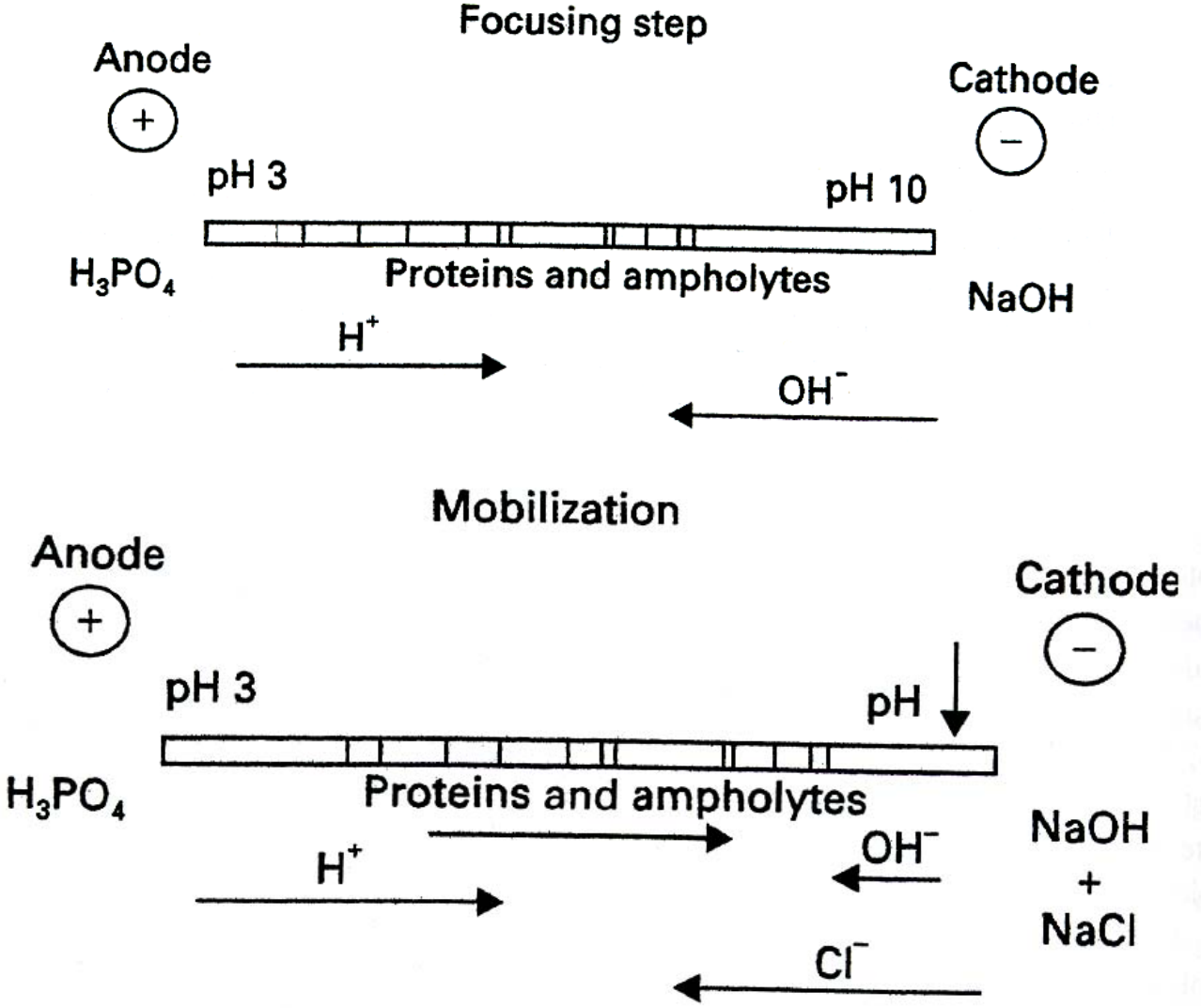




Modes of separation

1. Capillary Zone electrophoresis
2. Capillary iso-electric focusing
3. Micellar electrokinetic Capillary chromatography
4. Capillary gel electrophoresis
5. Capillary electrochromatography

Capillary iso-electric focusing

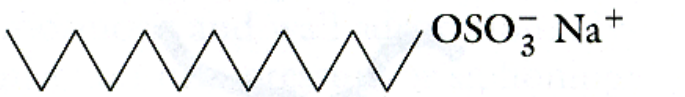


In this case, the electroosmotic force is weaker than elctrophoretic force.

Micellar electrokinetic Capillary chromatography

Separation of neutral solute

Psuedo-stationary phase



Sodium dodecyl sulfate (n-$C_{12}H_{25}OSO_3^-Na^+$)

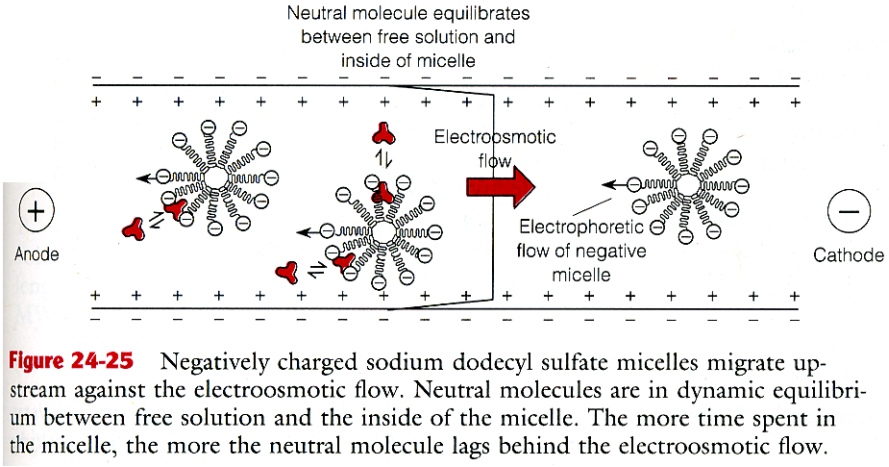
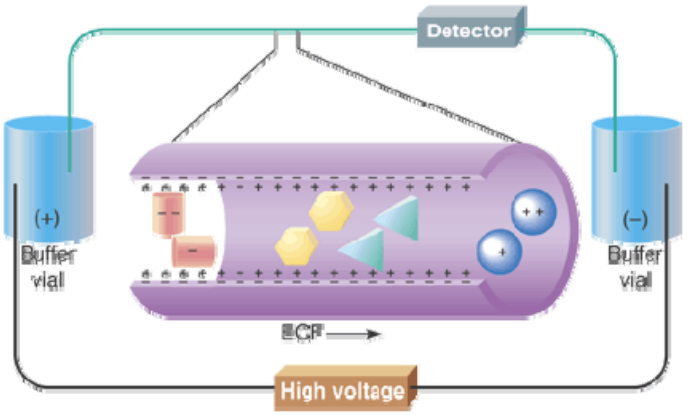


Figure 24-25 Negatively charged sodium dodecyl sulfate micelles migrate upstream against the electroosmotic flow. Neutral molecules are in dynamic equilibrium between free solution and the inside of the micelle. The more time spent in the micelle, the more the neutral molecule lags behind the electroosmotic flow.



Important Concepts:

Electrophoresis

Electrophoretic mobility

Capillary electrophoresis

Electroosmosis and Electroosmotic flow

Apparent Mobility

Separation Efficiency

Separation modes