Chapter 30 – Capillary Electrophoresis

Read: pp. 867-877 Optional Problems: 30-1,2,4,5,6 and 7

Charged analytes migrate in the presence of an electric field. *Separation is based on differential rates of migration.*

Separation method carried out in a buffer-filled capillary tube that is typically 10 to 100 μm in internal diameter and 40 to 100 cm in length. The tube extends between two buffer reservoirs that also hold Pt electrodes. The sample is introduced into one end of the tubing, and a dc potential in the 10 to 30 kV range is applied between the two electrodes throughout the separation. The separated analytes are observed by a detector at the end of the capillary opposite the end where the sample was introduced.

Instrumentation

Electrophoretic separations use two platforms: *slab* electrophoresis and *capillary* electrophoresis.

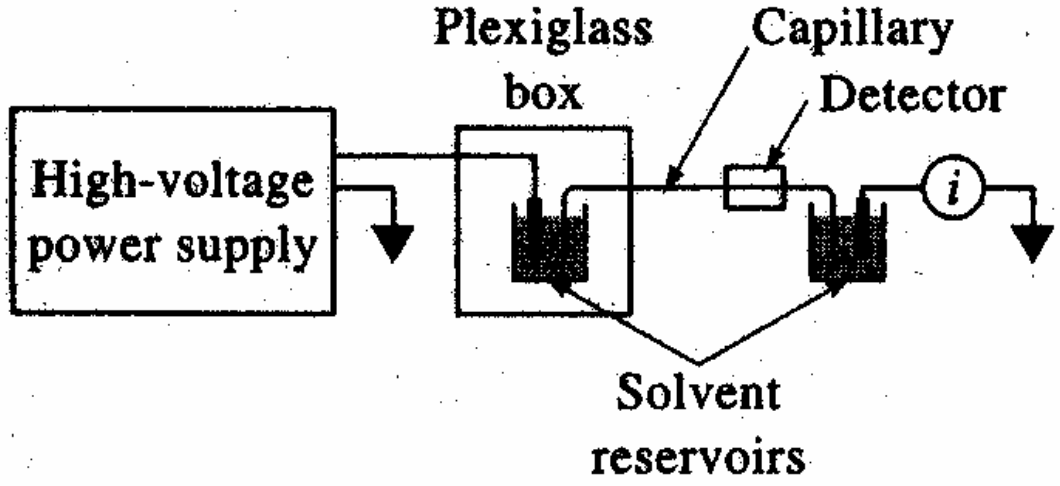
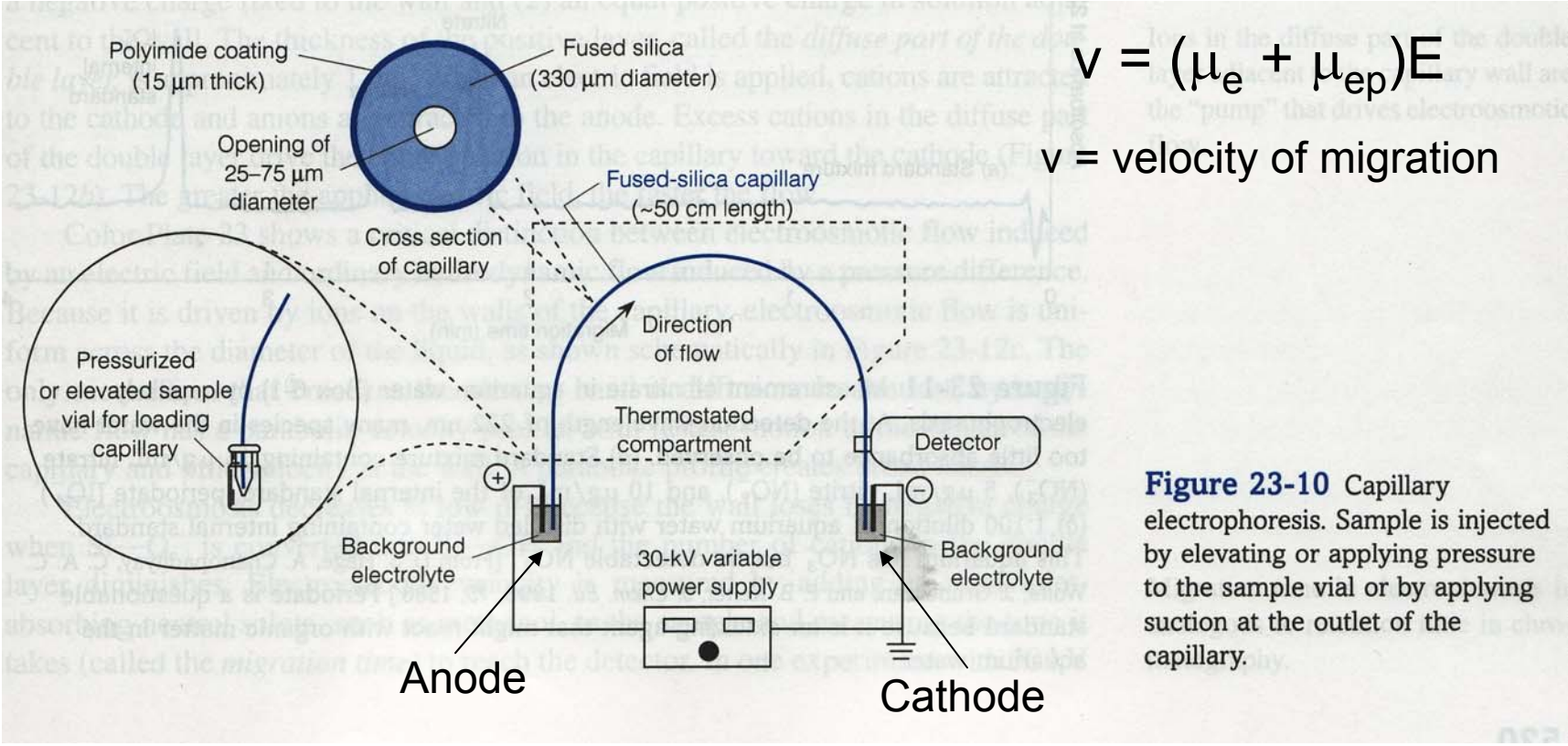


Figure 30-1 Schematic of a capillary zone electrophoresis system.

0.1 to 10 nL injection volume. No pump for the mobile phase!

Instrumentation

Analytes migrate in a run buffer (equivalent of a mobile phase in LC) due to their ***electrophoretic mobility*** and the ***electroosmotic flow*** of the solution.



$$v = (\mu_e + \mu_{ep})E$$

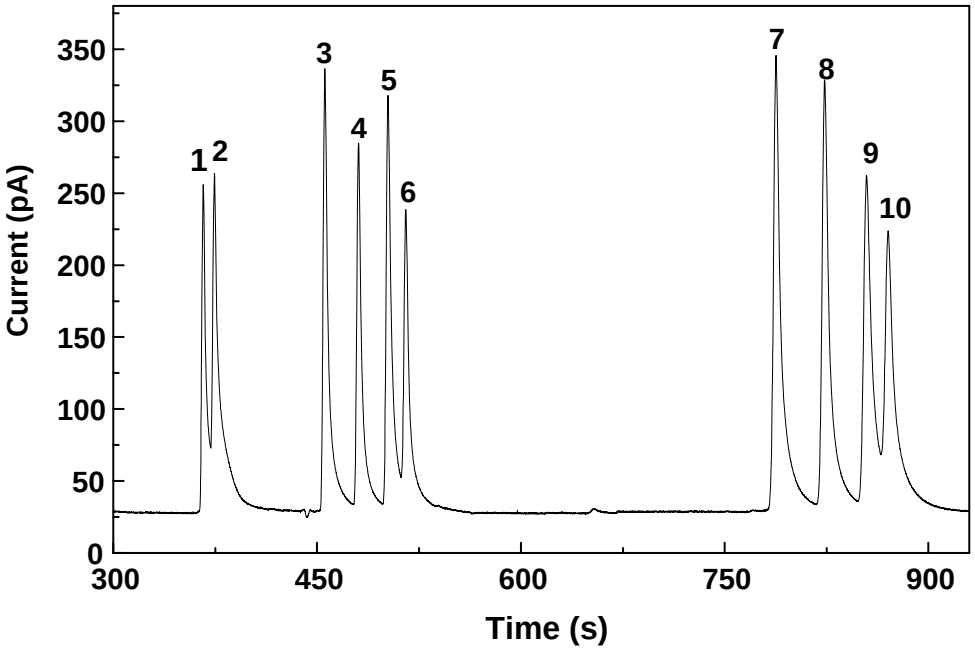
v = velocity of migration

Figure 23-10 Capillary electrophoresis. Sample is injected by elevating or applying pressure to the sample vial or by applying suction at the outlet of the capillary.

Two types of injection: ***electrokinetic*** and ***pressure*** injection.

Separation

- Fused silica capillary (~25 μm diam. x ~70 cm length)
- Injection volume = 1-10 nL (electrokinetic or pressure)
- Run buffer = mobile phase (0.01 M salt solution, pH > 4)
- Separation = 20-35 kV or (100'sV/cm)



electropherogram

Method useful for separating anions and cations but not neutral molecules. A fancier method is needed for neutrals – micellar electrokinetic capillary chromatography.

Nature of the Flow Profile

Flat Hyperbolic

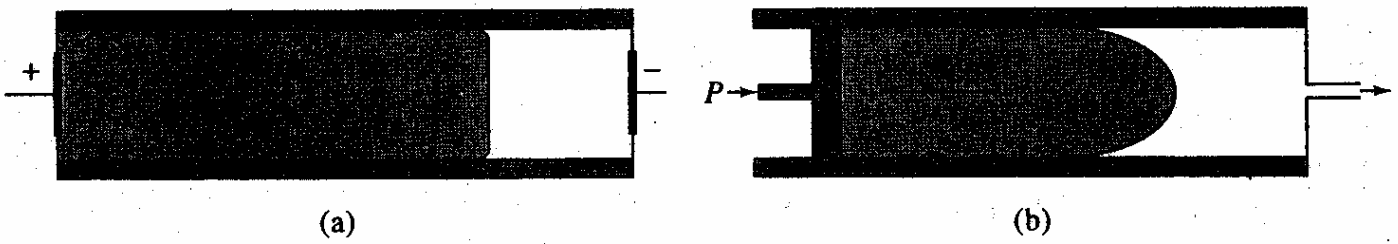


Figure 30-3 Flow profiles for liquids under (a) electroosmotic pressure and (b) hydrodynamic pressure.

Very high efficiency (N, plate number) results!
Sometimes on the order of 1 x 10^6 plates

$$N = (\mu_{ep} V)/2D$$

$$N = 16\,(t_m/w)^2$$

μ_{ep} = electrophoretic mobility (cm^2/V-s)
V = separation voltage (V)
D = diffusion coefficient (cm^2/s)

Solute Transport Down Capillary

$$v_{Total}\ (cm/s) = v_{eo} + v_e$$

- **Electroosmotic Flow** ($v_{eo} = \mu_{eo}E$) – mechanism by which entire run buffer moves from the inlet to the outlet of the capillary. Solution front is moved by an electric field.

- **Electrophoretic mobility** ($v_e = \mu_e E$) – transport of a charged solute in an electric field. Mobility is proportional to the q/r ratio (q = charge and r = solute size).

Electroosmotic Flow

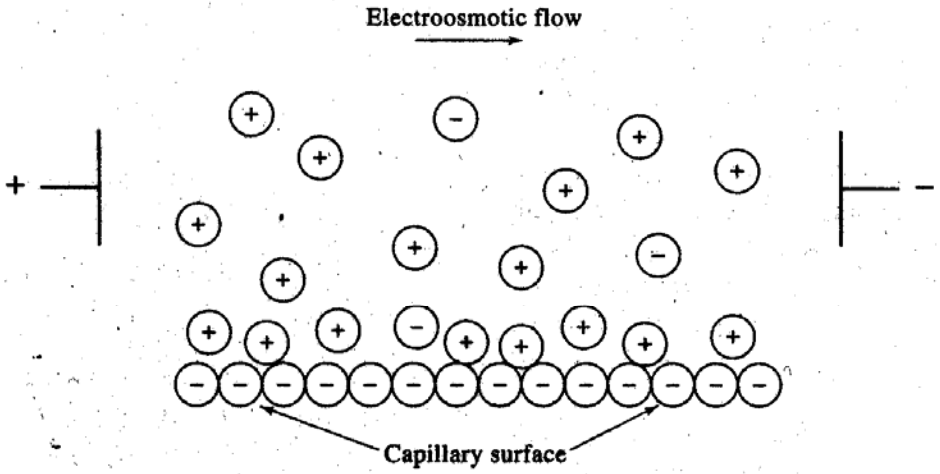
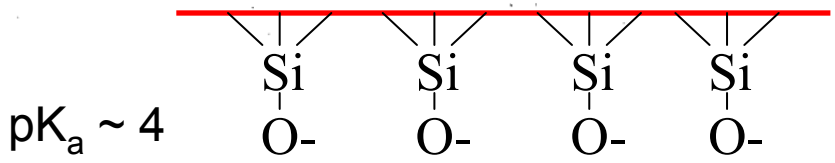


Figure 30-2 Charge distribution at a silica/capillary interface and resulting electroosmotic flow. *(From A. G. Ewing, R. A. Wallingford, and T. M. Olefirowicz, Anal. Chem., 1989, 61, 294A. With permission.)*



$pK_a \sim 4$

$$\nu = (\mu_e + \mu_{eo})\, E$$

Solvated cations drag water molecules during the migration, hence there is net solution movement from anode toward cathode.

$$\mu_{eo} = \varepsilon\zeta/6\pi\eta \ (cm^2/V\text{-}s)$$

ε = dielectric constant

ζ = zeta potential

η = viscosity

Nature of the Fluid Movement

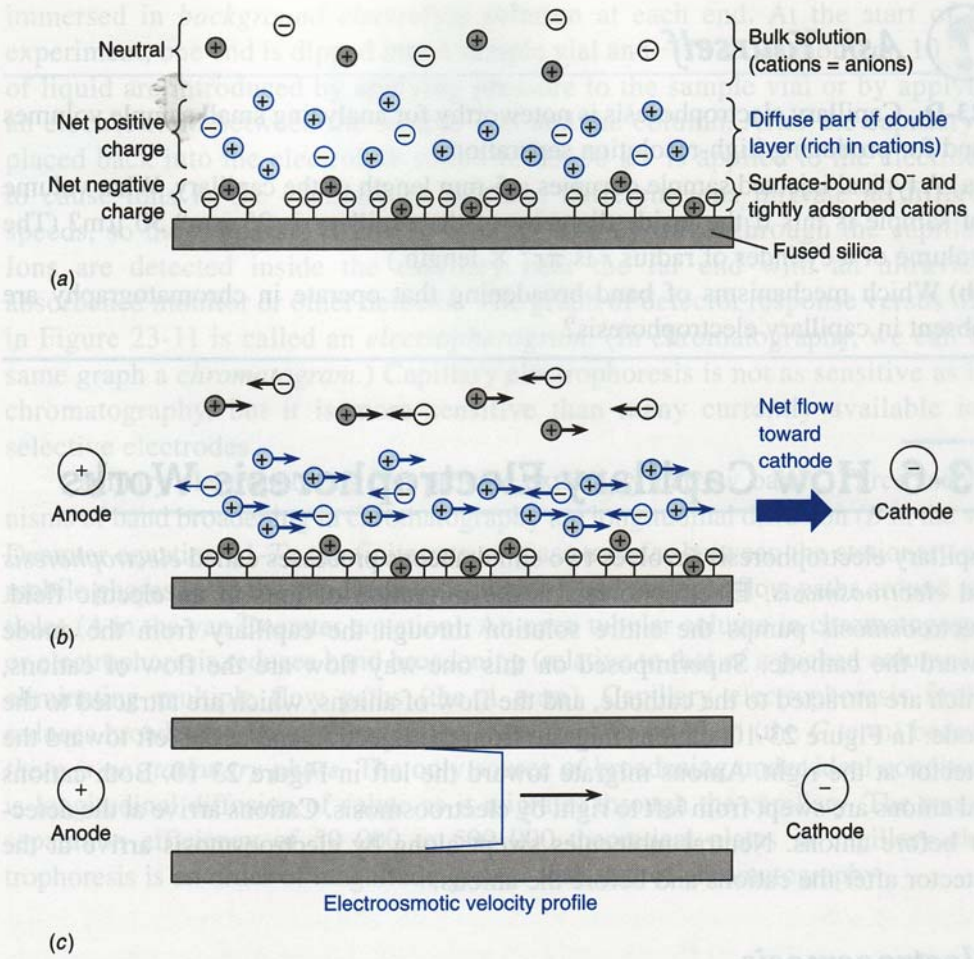


Figure 23-12 (*a*) Electric double layer is created by negative silica surface and excess cations in the diffuse part of the double layer in the solution near the wall. The wall is negative and the diffuse part of the double layer is positive. (*b*) Predominance of cations in diffuse part of the double layer produces net electroosmotic flow toward the cathode when an external field is applied. (*c*) Electroosmotic velocity profile is uniform over more than 99.9% of the cross section of the capillary. A capillary is required to maintain constant temperature in the liquid. Temperature variation in larger-diameter tubes causes bands to broaden.

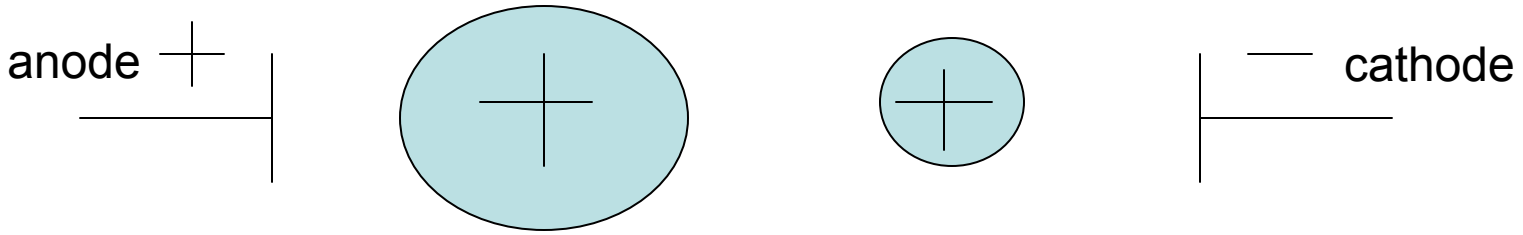
Electroosmotic flow results because of the electric double layer than forms near the capillary wall. This is the mechanism by which fluid movement is from anode (+) toward cathode (-) end of capillary where detector is positioned.

Electrophoretic Flow or Mobility

$$\nu_{Total} = (\mu_e + \mu_{eo})\, V$$

$$\nu\ (cm/s) = \mu_e\ (cm^2/V\text{-}s) \times E\ (V/cm) = \mu_e \times V/L$$

$\mu_e \propto q/r$ {charge/size}



Ion's velocity is the sum of the electrophoretic mobility and the velocity of electroosmotic flow.

Elution Order

Neutrals are not separated!

Specialized CZE methods are necessary – micellar electrokinetic chromatography (MEKC)

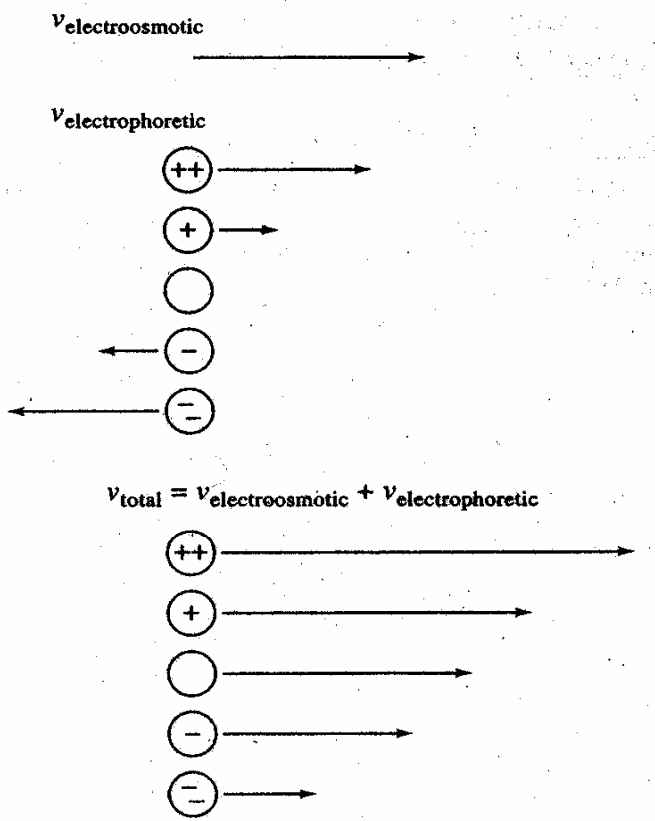


Figure 30-4 Velocities in the presence of electroosmotic flow. The length of the arrow next to an ion indicates the magnitude of its velocity; the direction of the arrow indicates the direction of motion. The negative electrode would be to the right, and the positive electrode to the left of this section of solution.

Detectors

TABLE 30-1 Detection Modes Developed for Capillary Electrophoresis[a]

Detection Principle	Representative Detection Limit[b] (moles detected)
Spectrometry	
Absorption[c]	10^{-15}–10^{-13}
Fluorescence	
Precolumn derivatization	10^{-17}–10^{-20}
On-column derivatization	8×10^{-16}
Postcolumn derivatization	2×10^{-17}
Indirect fluorescence	5×10^{-17}
Thermal lens[c]	4×10^{-17}
Raman[c]	2×10^{-15}
Mass spectrometry	1×10^{-17}
Electrochemical	
Conductivity[c]	1×10^{-16}
Potentiometry	Not reported
Amperometry	7×10^{-19}
Radiometry[c]	1×10^{-19}

[a]From A. G. Ewing, R. A. Wallingford, and T. M. Olefirowicz, *Anal. Chem.*, **1989**, *61*, 298A. With permission.
[b]Detection limits quoted have been determined with a wide variety of injection volumes that range from 18 pl to 10 nL.
[c]Mass detection limit converted from concentration detection limit using a 1-nL injection volume.

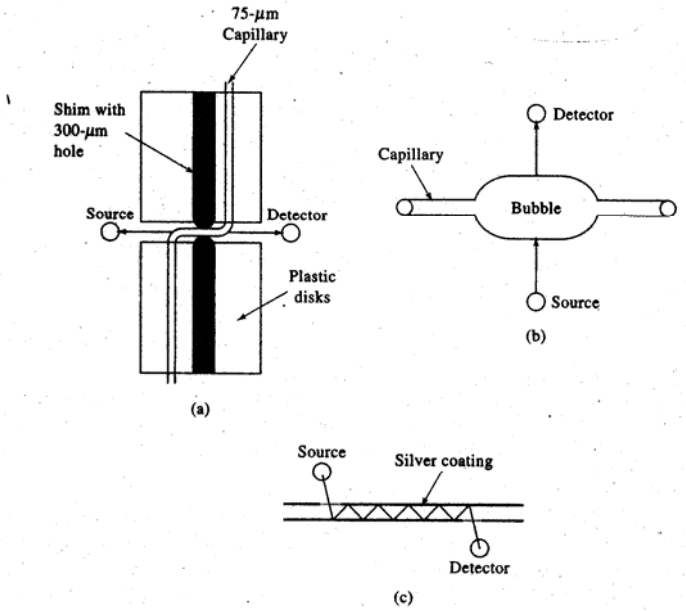


Figure 30-5 Three types of cells for improving the sensitivity of detection by absorbance measurements: (a) the 3-mm z cell, (b) the 150-μm bubble cell, (c) the multireflection cell.

Wide linear dynamic range, good reproducibility (<5% RSD), low limits of detection (μM or 1 femtomole (10^{-15}))

Detectors

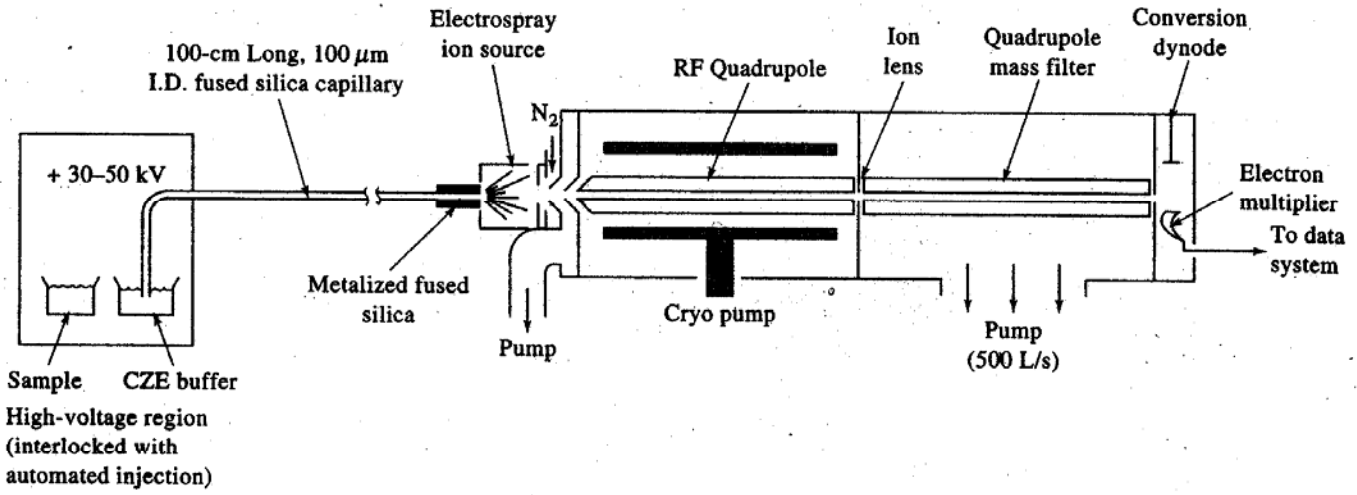


Figure 30-7 An instrument for capillary electrophoresis/mass spectrometry. The voltage between the buffer solution on the left and the metalized silica capillary is 30 to 50 kV. The flow of nitrogen is 3 to 5 kV. The flow of nitrogen at ≈70°C for desolvation is 3 to 6 L/min. *(From R. D. Smith, J. A. Olivares, N. T. Nguyen, and H. R. Udseth,* Anal. Chem., *1988, 60, 437. With permission.)*

Separation Examples

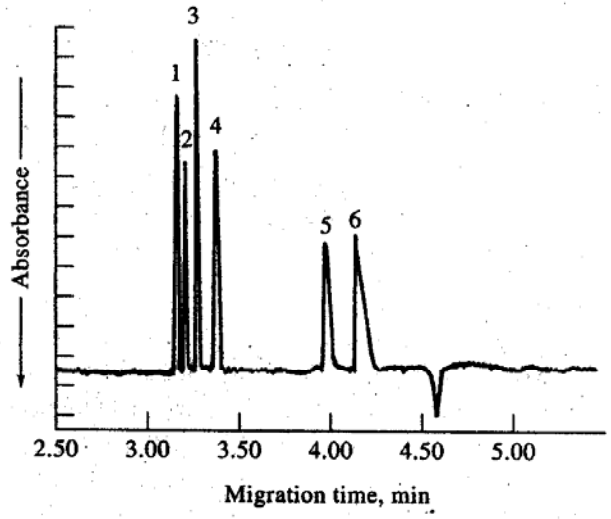


Figure 30-6 Electropherogram of a six-anion mixture by indirect detection with 4-nM chromate ion at 254 nm. Peak: (1) bromide (4 ppm), (2) chloride (2 ppm), (3) sulfate (4 ppm), (4) nitrate (4 ppm), (5) fluoride (1 ppm), (6) phosphate (6 ppm).

Electropherograms

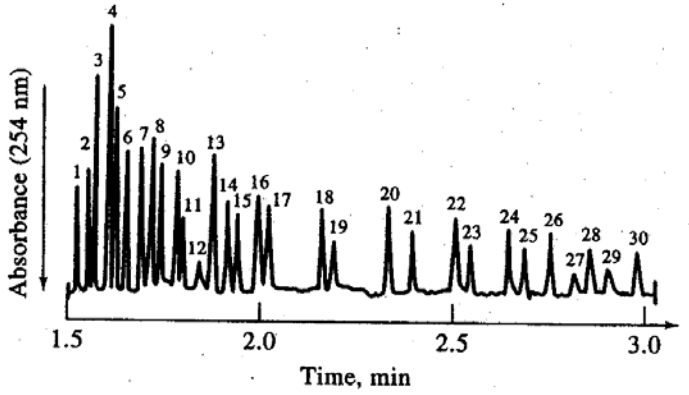


Figure 30-10 Electropherogram showing the separation of 30 anions. Capillary internal diameter: 50 μm (fused silica). Detection: indirect UV, 254 nm. Peaks: 1 = thiosulfate (4 ppm), 2 = bromide (4 ppm), 3 = chloride (2 ppm), 4 = sulfate (4 ppm), 5 = nitrite (4 ppm), 6 = nitrate (4 ppm), 7 = molybdate (10 ppm), 8 = azide (4 ppm), 9 = tungstate (10 ppm), 10 = monofluorophosphate (4 ppm), 11 = chlorate (4 ppm), 12 = citrate (2 ppm), 13 = fluoride (1 ppm), 14 = formate (2 ppm), 15 = phosphate (4 ppm), 16 = phosphite (4 ppm), 17 = chlorite (4 ppm), 18 = galactarate (5 ppm), 19 = carbonate (4 ppm), 20 = acetate (4 ppm), 21 = ethanesulfonate (4 ppm), 22 = propionate (5 ppm), 23 = propanesulfonate (4 ppm), 24 = butyrate (5 ppm), 25 = butanesulfonate (4 ppm), 26 = valerate (5 ppm), 27 = benzoate (4 ppm), 28 = *l*-glutamate (5 ppm), 29 = pentanesulfonate (4 ppm), 30 = *d*-gluconate (5 ppm). *(From W. A. Jones and P. Jandik,* J. Chromatogr., ***1991,*** *546, 445. With permission.)*