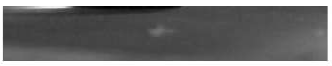
15th Week



25-6 Principles of Capillary Electrophoresis[20,21]

Late in 2007, more than 200 people receiving the anticoagulant *heparin* suffered acute, allergic reactions and died.[22] Heparin is a complex mixture of sulfate-substituted polysaccharides that have molecular masses of 2 to 50 kDa and are isolated from pig intestines. As soon as the

problem was recognized in January 2008, U.S. distributors recalled heparin products and the U.S. Food and Drug Administration launched an investigation. Heparin is administered thousands of times every day to manage life-threatening conditions, so an immediate understanding and solution to the problem were required.

When exposed to the enzyme heparinase, heparin is cleaved into disaccharide units. Tainted heparin contained 20 to 50 wt% of macromolecular components that did not react with heparinase. *Capillary electrophoresis* proved to be the tool of choice to observe two contaminants (Figure 25-21).[23] One was dermatan sulfate, which was not known to cause allergic reactions. The other was identified by nuclear magnetic resonance as oversulfated chondroitin sulfate. An animal study verified that oversulfated chondroitin sulfate caused the allergic reaction. By March 2008, deaths from contaminated heparin had ceased and emergency regulations were issued to incorporate capillary electrophoresis and nuclear magnetic resonance into required testing of heparin imported into the U.S. Contaminated heparin had been prepared in China. Oversulfated chondroitin sulfate might have been added because it has anticoagulant activity and costs less than heparin.

Electrophoresis is the migration of ions in solution under the influence of an electric field. The technique was pioneered in the 1930s by the Swedish biophysical chemist A. Tiselius, who received the Nobel Prize in 1948 for his work on electrophoresis and "discoveries concerning the complex nature of serum proteins."

In **capillary electrophoresis**, shown in Figure 25-22, components of a solution are separated by applying a voltage of ~30 kV from end to end of a fused-silica (SiO_2) capillary tube that is 50 cm long and has an inner diameter of 25–75 μm. Different ions have different *mobilities* and migrate through the capillary at different speeds.[25] Modifications of this experiment described later allow neutral molecules, as well as ions, to be separated. Electrophoresis can separate whole cells for medical diagnosis and detection of food contamination.[26] Electrophoresis can analyze single cells, nuclei, vesicles, or mitochondria.[27] Single-cell enzyme assays have detection limits at the zeptomol (10^{-21} mol) level.[28]

Capillary electrophoresis provides high resolution. When we conduct chromatography in a packed column, peaks are broadened by three mechanisms in the van Deemter equation (22-33): multiple flow paths, longitudinal diffusion, and finite rate of mass transfer. An open tubular column eliminates multiple paths and thereby reduces plate height and improves resolution. Capillary electrophoresis reduces plate height further by knocking out the mass transfer term that comes from the finite time needed for solute to equilibrate between the mobile and stationary phases. In capillary electrophoresis, *there*

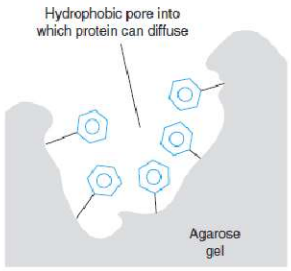


FIGURE 25-20 Stationary phase for hydrophobic interaction chromatography has ~10–20% as many bonded phenyl or alkyl groups per unit volume as a reversed-phase stationary phase.

Cations are attracted to the negative terminal (the cathode).

Anions are attracted to the positive terminal (the anode).

Electric potential difference = 30 kV

$$\textit{Electric field} = \frac{30\ \text{kV}}{0.50\ \text{m}} = 60\ \frac{\text{kV}}{\text{m}}$$

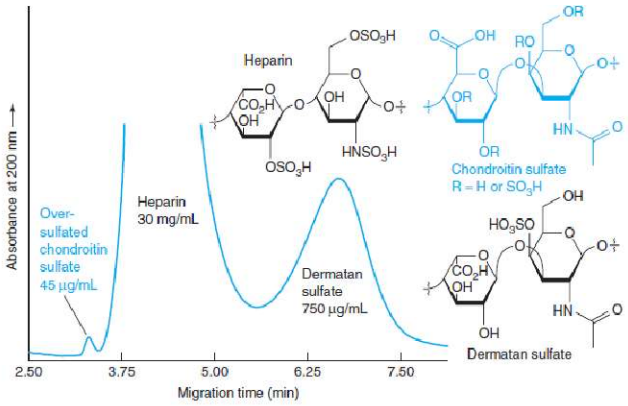


FIGURE 25-21 Electropherogram of heparin (30 mg/mL) spiked with oversulfated chondroitin sulfate and dermatan sulfate. Tainted heparin had ~200 times more oversulfated chondroitin sulfate than shown here. Conditions: −16 kV, 20°C, 25 μm × 30 cm capillary, detector at 21.5 cm. Background buffer was made by adding 0.60 M H_3PO_4 to 0.60 M Li_3PO_4 to reach pH 2.8. [Courtesy Robert Weinberger, CE Technologies and Todd Wielgos, Baxter Healthcare. For details, see T. Wielgos, K. Havel, N. Ivanova, and R. Weinberger, "Determination of Impurities in Heparin by Capillary Electrophoresis using High Molarity Phosphate Buffers," *J. Pharma. Biomed. Anal.* **2009**, *49*, 319.]

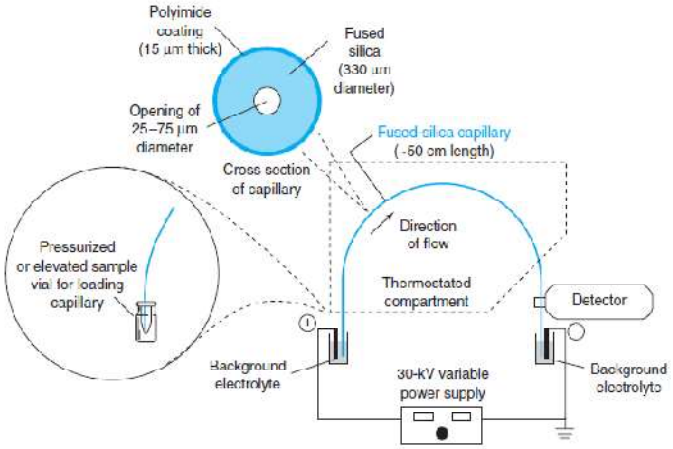


FIGURE 25-22 Apparatus for capillary electrophoresis. One way to inject sample is to place the capillary in a sample vial and apply pressure to the vial or suction at the outlet of the capillary. The use of an electric field for sample injection is described in the text.

Electrophoresis in glass capillaries was first described by J. W. Jorgenson in 1981.[24]

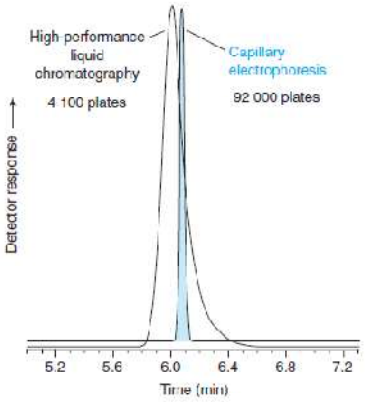


FIGURE 25-23 Comparison of peak widths for benzyl alcohol ($C_6H_5CH_2OH$) in capillary electrophoresis and HPLC. [From S. Fazio, R. Vivilecchia, L. Lesueur, and J. Sheridan, *Am. Biotech. Lab.*, January 1990, p. 10.]

is no stationary phase. The only fundamental source of broadening under ideal conditions is longitudinal diffusion:

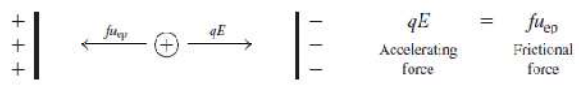
$$H = \cancel{A} + \frac{B}{u_x} + \cancel{C_x} \qquad (25\text{-}7)$$

A: Multiple path term eliminated by open tubular column. C_x: Mass transfer term eliminated because there is no stationary phase.

(Other sources of broadening in real systems are mentioned later.) Capillary electrophoresis routinely produces 50 000–500 000 theoretical plates (Figure 25-23), a performance that is an order of magnitude better than that of chromatography.

Electrophoresis

When an ion with charge q (coulombs) is placed in an electric field E (V/m), the force on the ion is qE (newtons). In solution, the retarding frictional force is fu_{ep}, where u_{ep} is the velocity of the ion and f is the *friction coefficient*. The subscript "ep" stands for "electrophoresis." The ion quickly reaches a steady speed when the accelerating force equals the frictional force:



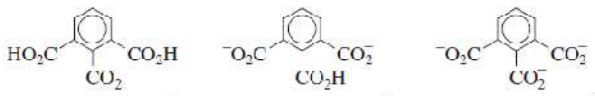
Electrophoretic mobility.

$$u_{ep} = \frac{q}{f}E = \mu_{ep}E \qquad (25\text{-}8)$$

μ_{ep}: Electrophoretic mobility

We encountered *mobility* earlier in connection with junction potentials (Table 14-1).

Electrophoretic **mobility** (μ_{ep}) is the constant of proportionality between the speed of the ion and electric field strength. Mobility is proportional to the charge of the ion and inversely proportional to the friction coefficient. For molecules of similar size, mobility increases with charge:



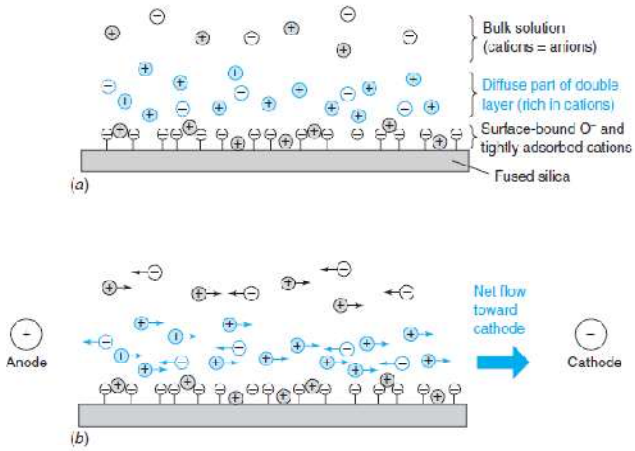


FIGURE 25-24 (*a*) Electric double layer created by negatively charged silica surface and nearby cations. (*b*) Predominance of cations in diffuse part of the double layer produces net electroosmotic flow toward the cathode when an external field is applied.

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

Stokes equation:
$$f = 6\pi\eta r \tag{25-9}$$

Mobility is q/f, so the greater the radius, the lower the mobility. Most molecules are not spherical, but Equation 25-9 defines an effective *hydrodynamic radius* of a molecule, as if it were a sphere, based on its observed mobility.

Viscosity measures resistance to flow in a fluid. The units are kg m^{-1} s^{-1}. Relative to water, maple syrup is very viscous and hexane has low viscosity.