Electrophoresis (*Capillary Electrophoresis*)

Electrophoresis *is a separation method based on the differential rates of migration of charged species in an applied dc electric field.* This separation technique for macro size samples was first developed by the Swedish chemist Arne Tiselius in the 1930s for the study of serum proteins; he was awarded the 1948 Nobel Prize for his work.

Electrophoresis on a macro scale has been applied to a variety of difficult analytical separation problems:

- inorganic anions and cations,
- amino acids,
- catechol amines,
- drugs,
- vitamins,
- carbohydrates,
- peptides,
- proteins,
- nucleic acids,
- nucleotides,
- polynucleotides &
- numerous other species.

A particular strength of electrophoresis is its unique ability to separate charged macromolecules of interest to biochemists, biologists, and clinical chemists. For many years, *electrophoresis has been the powerhouse method for separating proteins (enzymes, hormones, and antibodies) and nucleic acids (DNA and RNA) for which it offers unparalleled resolution.*

Historical Background

Until the appearance of capillary electrophoresis, electrophoretic separations were not carried out in columns but were ***performed in a flat stabilized medium such as paper or a porous semisolid gel.*** Remarkable

separations were realized in such media, but the technique was slow, tedious, and required a good deal of operator skill.

In the early 1980s, scientists began to explore the feasibility of performing these same separations on micro amounts of sample in fused-silica capillary tubes. Their results proved promising in terms of resolution, speed, and potential for automation. *As a result, capillary electrophoresis (CE) has developed into an important tool for a wide variety of analytical separation problems* and is the only type of electrophoresis that we will consider.

Instrumentation for Capillary Electrophoresis

As shown in Figure 1, the instrumentation for capillary electrophoresis is simple. A buffer-filled fused-silica capillary, which is typically 10 to 100 µm in internal diameter and 40 to 100 cm long, extends between two buffer reservoirs that also hold platinum electrodes. Sample introduction is performed at one end and detection at the other. A potential of 5 to 30 kV dc is applied between the two electrodes. The positive polarity of the high voltage in Figure 1 can be reversed to allow separation of anions.

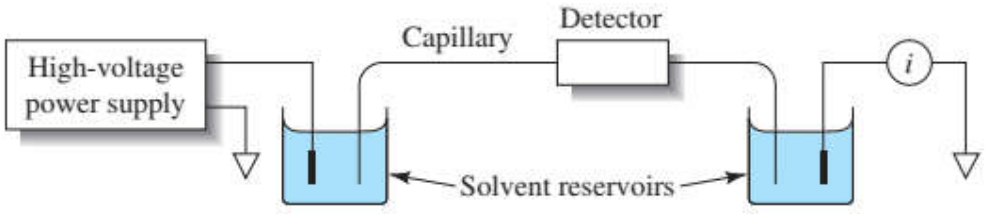

Fig 1: Schematic of a capillary electrophoresis system

Sample introduction is accomplished by electrokinetic or pressure injection. In electrokinetic injection, one end of the capillary and its electrode are removed from their buffer compartments and placed in a small sample cup. A voltage is then applied for a measured time, causing the sample to enter the capillary by a combination of ionic migration and electroosmotic flow (see next

section). *In hydrodynamic injection, the sample introduction end of the capillary is also placed in a small cup containing the sample, but in this case, a pressure difference drives the sample solution into the capillary.* The pressure difference can be caused by applying a vacuum at the detector end or by elevating the sample (hydrodynamic injection).

Because the separated analytes move past a common point in most types of capillary electrophoresis, detectors are similar in design and function to those described for HPLC. Table 1 lists several of the detection methods that have been reported for capillary electrophoresis. The second column of the table shows representative detection limits for these detectors.

Table 1. Detectors for Capillary Electrophoresis

Type of Detector	**Representative Detection Limit** (attomoles detected)
Spectrometry	1–1000
Absorption	1–0.01
Fluorescence	10
Termal lens	1000
Raman	1–0.0001
Chemiluminescence	1–0.01
Mass spectrometry	
Electrochemical	
Conductivity	100
Potentiometry	1
Amperometry	0.1

Electroosmotic Flow

A unique feature of capillary electrophoresis is electroosmotic flow. When a high voltage is applied across a fused-silica capillary tube containing a buffer solution, electroosmotic flow usually occurs in which the solvent migrates toward the cathode.

The rate of migration can be substantial. For example, a 50-mM pH 8 buffer has been found to flow through a 50-cm capillary toward the cathode at approximately 5 cm/min with an applied potential of 25 kV.

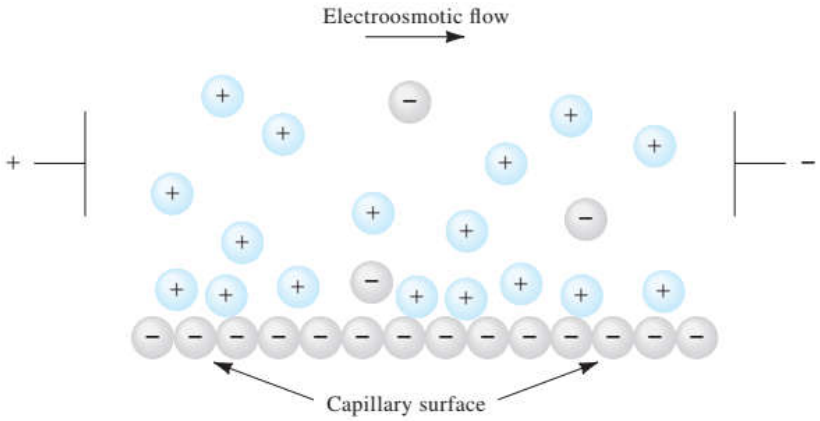

Figure 2. Charge distribution at a silica/capillary interface and resulting electroosmotic flow.

As shown in Figure 2, the cause of electroosmotic flow is the electric double layer that develops at the silica/solution interface. At pH values higher than 3, the inside wall of a silica capillary is negatively charged due to ionization of the surface silanol groups (Si - OH). Buffer cations congregate in an electrical double layer adjacent to the negative surface of the silica capillary. *The cations in the diffuse outer layer to the double layer are attracted toward the cathode, or negative electrode, and since they are solvated, they drag the bulk solvent along with them. As shown in Figure 3, electroosmosis leads to bulk solution flow that has a flat profile across the tube because flow originates at the walls of the tubing.* This profile is in contrast to the laminar (parabolic) profile that is observed with the pressure-driven flow encountered in HPLC. Because the profile is essentially flat, electroosmotic flow does not contribute significantly to band broadening the way pressure-driven flow does in liquid chromatography.

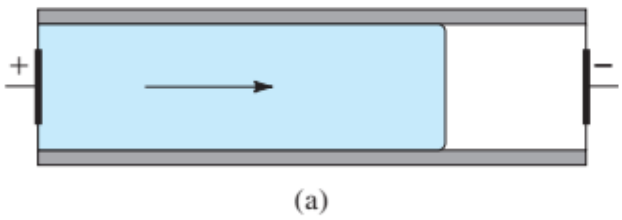

Figure 3a. Flow profiles for liquids under electroosmotic flow.

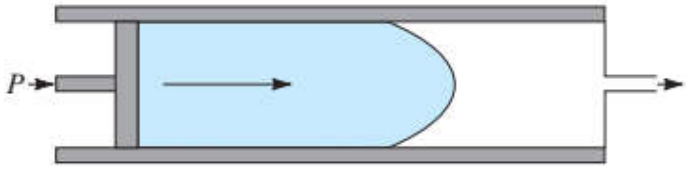

Figure 3b. Flow profiles for liquids under pressure-induced flow.

The rate of electroosmotic flow is generally greater than the electrophoretic migration velocities of the individual ions and effectively becomes the mobile-phase pump of CE. Even through analytes migrate according to their charges within the capillary, the electroosmotic flow rate is usually sufficient to sweep all positive, neutral, and even negative species toward the same end of the capillary so that all can be detected as they pass by a common point (Figure 4). *The resulting electropherogram looks like a chromatogram but with narrower peaks.*

Electroosmosis is often desirable in certain types of capillary electrophoresis, but in other types, it is not. It can be minimized by coating the inside capillary…

The Basis for Electrophoretic Separations

The migration rate v of an ion in an electric field is given by;

$$v = \mu_e E = \mu_e \times \frac{V}{L}$$

where

- ***E*** *is the electric field strength in volts per centimeter,*
- ***V*** *is the applied voltage,*
- ***L*** *is the length of the tube between electrodes, and*

- μ_e *is the electrophoretic mobility, which is proportional to the charge on the ion and inversely proportional to the frictional retarding force on the ion.*

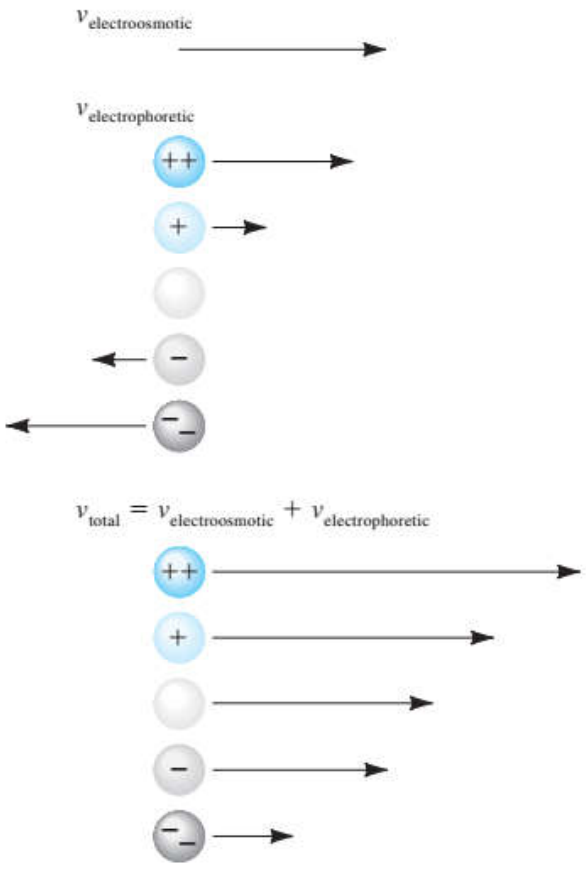

Figure 4. Velocities in the presence of electroosmotic flow. The length of the arrow next to an ion indicates the magnitude of its velocity; the direction of the arrow indicates the direction of motion. The negative electrode would be to the right and the positive electrode to the left of this section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

The frictional retarding force on an ion is determined by the size and shape of the ion and the viscosity of the medium.

Although CE is not a chromatographic process, separations are often described in a manner similar to chromatography. For example, in electrophoresis, we can calculate the plate count N by;

$$N = \frac{\mu_e V}{2D}$$

where

D *is the diffusion coefficient of the solute (cm$^{2/s}$).*

Because resolution increases with plate count, it is desirable to use high applied voltages in order to achieve high resolution separations. Note that for electrophoresis, contrary to the situation in chromatography, the plate count does not increase with the column length. Typically, capillary electrophoresis plate counts are 100,000 to 200,000 at the usual applied voltages.

Applications of Capillary Electrophoresis

Capillary electrophoretic separations are performed in several ways called modes. These modes include;

- *isoelectric focusing,*
- *isotachophoresis, &*
- *capillary zone electrophoresis (CZE).*

We shall consider only capillary zone electrophoresis in which the buffer composition is constant throughout the region of the separation. The applied field causes each of the different ionic components of the mixture to migrate according to its own mobility and to separate into zones that may be completely resolved or may be partially overlapped. Completely resolved zones have regions of buffer between them. The situation is analogous to elution column chromatography, where regions of mobile phase are located between zones containing separated analytes.

Separation of Small Ions

For most electrophoretic separations of small ions, the smallest analysis time results when the analyte ions move in the same direction as the electroosmotic flow.

Thus, for cation separations, the walls of the capillary are untreated, and the electroosmotic flow and the cation movement are toward the cathode. For the separation of anions, on the other hand, *the electroosmotic flow is usually reversed by treating the walls of the capillary with an alkyl ammonium salt, such as cetyltrimethylammonium bromide (CTAB).* The positively charged ammonium ions become attached to the negatively charged silica surface and, in turn, create a negatively charged double layer of solution, which is attracted toward the anode, reversing the electroosmotic flow.

In the past, *the most common method for analysis of small anions has been ion-exchange chromatography.* For cations, the preferred techniques have been atomic absorption spectroscopy and inductively coupled plasma emission or mass spectrometry. In recent years, capillary electrophoretic methods have begun to compete with these traditional methods for small ion analysis. Several major reasons for adoption of electrophoretic methods have been recognized: lower equipment costs, smaller sample size requirements, much greater speed, and better resolution. *However, because variations in electroosmotic flow rates make reproducing CE separations difficult, LC methods and atomic spectrometric methods are still widely used for small inorganic ions.*

The initial cost of equipment and the expense of maintenance for electrophoresis is generally significantly lower than those for ion chromatographic and atomic spectroscopic instruments. The cost of CE instruments varies significantly depending on the type of detection system desired. Simple CE instruments with UV-visible detection can cost in the

$10,000-to-$20,000 range, but instruments with mass spectrometry detection can cost substantially more.

Sample sizes for electrophoresis are in the nano liter range, but microliter or larger samples are usually needed for other types of small ion analysis. Thus, electrophoretic methods are more sensitive than the other methods on a mass basis (but usually not on a concentration basis).

Separation of Molecular Species

A variety of small synthetic herbicides, pesticides, and pharmaceuticals that are ions or can be derivatized to yield ions have been separated and analyzed by CE. This type of application in which anti-inflammatory drugs, which have acidic properties with characteristic pKas, are separated in less than 15 min.

Proteins, amino acids, and carbohydrates have all been separated in minimum times by CZE. In the case of neutral carbohydrates, the separations are preceded by formation of negatively charged borate complexes. The separation of protein mixtures is illustrated in Table 2.

Table 2. *Model Proteins Separated at pH 2.7*

Peak No.	Proteins Molecular Mass	Isoelectric Point	pH
1	Cytochrome c	12,400	10.7
2	Lysozyme	14,100	11.1
3	Trypsin	24,000	10.1
4	Trypsinogen	23,700	8.7
5	Trypsin inhibitor	20,100	4.5