

CHAPTER 1

Background Theory and Principles of Capillary Electrophoresis

1 Introduction

This chapter describes the basic theoretical concepts and principles of capillary electrophoresis (CE). The depth of discussion should provide enough background to understand the basic operation of CE instruments and the principles by which CE separates analytes. This is complemented in the next chapter by a discussion of the most common modes of separation operated by CE. For a more comprehensive explanation of the theoretical aspects of CE, please refer to one or more of the reference books listed in the Bibliography. In addition, a list of definitions for terms and abbreviations is given in the Glossary.

2 What is Capillary Electrophoresis?

The process of electrophoresis is defined as 'the differential movement or migration of ions by attraction or repulsion in an electric field'. In practical terms, a positive (anode) and negative (cathode) electrode are placed in a solution containing ions. Then, when a voltage is applied across the electrodes, solute ions of different charge, *i.e.*, anions (negative) and cations (positive), will move through the solution towards the electrode of opposite charge. Capillary electrophoresis, then, is the technique of performing electrophoresis in buffer-filled, narrow-bore capillaries, normally from 25 to 100 μm in internal diameter (ID).

3 Instrumentation

The instrumentation required for CE is remarkably simple in design, as Figure 1.1 illustrates. The ends of a capillary are placed in separate buffer reservoirs, each containing an electrode connected to a high-voltage power supply capable of delivering up to 30 kV. The sample is injected onto the capillary by temporar-

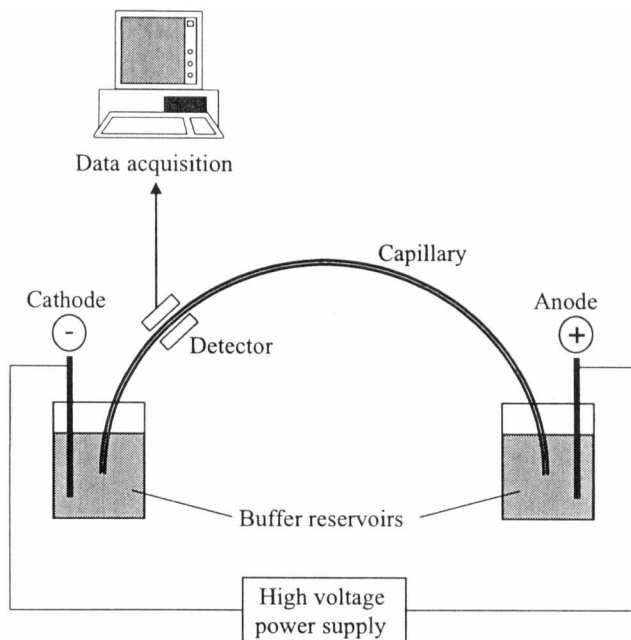


Figure 1.1 *A schematic representation of the arrangement of the main components of a typical CE instrument*

ily replacing one of the buffer reservoirs (normally at the anode) with a sample reservoir and applying either an electric potential or external pressure for a few seconds. After replacing the buffer reservoir, an electric potential is applied across the capillary and the separation is performed. Optical (UV-visible or fluorometric) detection of separated analytes can be achieved directly through the capillary wall near the opposite end (normally near the cathode).

CE is very suited to automation, and the arrangement of commercial CE instruments will seem familiar to those with knowledge of modern HPLC. Basic features of a CE instrument include an autosampler, a detection module, a high-voltage power supply, the capillary and, of course, a computer to control everything. So, if we consider that the power supply is equivalent to an HPLC pump and the capillary is equivalent to a column, the instrumentation is completely analogous. This is especially so as the software packages used to control most commercial CE instruments are based heavily on existing HPLC software.

4 Electrophoresis Theory

The theory that governs electrophoresis is directly applicable to CE and can be dealt with very briefly, with reference to a few equations. As mentioned earlier, electrophoresis is the movement or migration of ions or solutes under the influence of an electric field. Therefore, separation by electrophoresis relies on

differences in the speed of migration (migration velocity) of ions or solutes. Now, ion migration velocity can be expressed as:

$$v = \mu_e E \quad (1.1)$$

where v is ion migration velocity (m s^{-1}), μ_e is electrophoretic mobility ($\text{m}^2 \text{V}^{-1} \text{s}^{-1}$) and E is electric field strength (V m^{-1}).

The electric field strength is a function of the applied voltage divided by the total capillary length. Electrophoretic mobility is a factor that indicates how fast a given ion or solute may move through a given medium (such as a buffer solution). It is an expression of the balance of forces acting on each individual ion; the electrical force acts in favour of motion and the frictional force acts against motion. Since these forces are in a steady state during electrophoresis, electrophoretic mobility is a constant (for a given ion under a given set of conditions). The equation describing electrophoretic mobility is:

$$\mu_e = \frac{q}{6\pi\eta r} \quad (1.2)$$

where q is the charge on the ion, η is the solution viscosity and r is the ion radius. The charge on the ion (q) is fixed for fully dissociated ions, such as strong acids or small ions, but can be affected by pH changes in the case of weak acids or bases. The ion radius (r) can be affected by the counter-ion present or by any complexing agents used. From equation (1.2) we can see that differences in electrophoretic mobility will be caused by differences in the charge-to-size ratio of analyte ions. Higher charge and smaller size confer greater mobility, whereas lower charge and larger size confer lower mobility.

Electrophoretic mobility is probably the most important concept to understand in electrophoresis. This is because electrophoretic mobility is a characteristic property for any given ion or solute and will always be a constant. What is more, it is the defining factor that decides migration velocities. This is important, because different ions and solutes have different electrophoretic mobilities, so they also have different migration velocities at the same electric field strength. It follows that, because of differences in electrophoretic mobility, it is possible to separate mixtures of different ions and solutes by using electrophoresis.

5 Electroosmotic Flow (EOF)

A vitally important feature of CE is the bulk flow of liquid through the capillary. This is called the electroosmotic flow and is caused as follows.

An uncoated fused-silica capillary tube is typically used for CE. The surface of the inside of the tube has ionisable silanol groups, which are in contact with the buffer during CE. These silanol groups readily dissociate, giving the capillary wall a negative charge. Therefore, when the capillary is filled with buffer, the negatively charged capillary wall attracts positively charged ions from the buffer

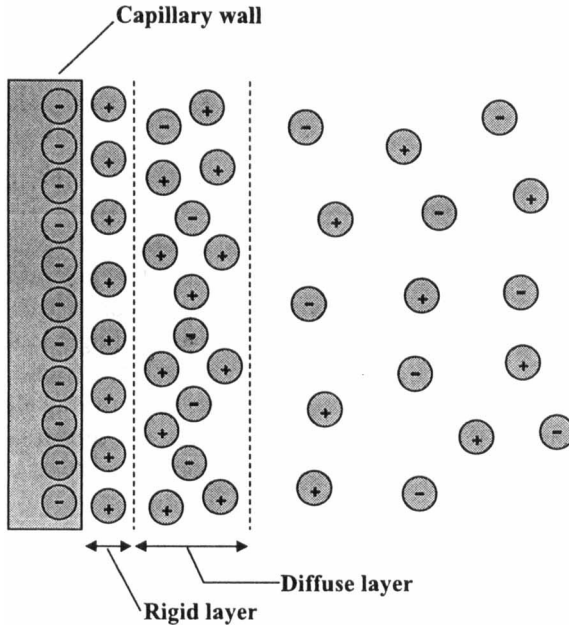


Figure 1.2 Stern's model of the double-layer charge distribution at a negatively charged capillary wall leading to the generation of a zeta potential and EOF

solution, creating an electrical double layer and a potential difference (zeta potential) close to the capillary wall, as described according to Stern's model in Figure 1.2. Stern's model for an electrical double layer includes a rigid layer of adsorbed ions and a diffuse layer, in which ion diffusion may occur by thermal motion. The zeta potential is the potential at any given point in the double layer and decreases exponentially with increasing distance from the capillary wall surface.

When a voltage is applied across the capillary, cations in the diffuse layer are free to migrate towards the cathode, carrying the bulk solution with them. The result is a net flow in the direction of the cathode, with a velocity described by

$$v_{\text{EOF}} = \left(\frac{\varepsilon_0 \varepsilon \zeta}{4\pi\eta} \right) E \quad (1.3)$$

where ε_0 is the dielectric constant of a vacuum, ε is the dielectric constant of the buffer, ζ is the zeta potential, η is the viscosity of the buffer and E is the applied electric field. The terms enclosed in brackets equate to the mobility of the EOF (μ_{EOF}).

The relationship between EOF mobility and EOF velocity is analogous to that between electrophoretic mobility and migration velocity. Indeed, the units for EOF mobility are the same as those for electrophoretic mobility.

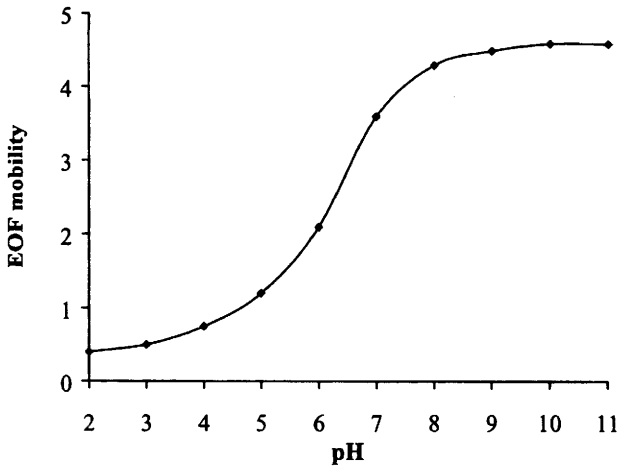


Figure 1.3 *The variation of EOF mobility with changing pH for a typical uncoated fused-silica capillary (simulated data)*

Factors Affecting EOF Mobility

The main variables affecting EOF mobility are the dielectric constant and viscosity of the buffer and the size of the zeta potential. The use of buffer additives and/or other modifications of the buffer composition may influence the dielectric constant and viscosity of the buffer. Buffer viscosity will also depend on the temperature at which the CE separation is performed.

Zeta Potential

The zeta potential is proportional to the charge density on the capillary wall, which itself is pH dependent. Therefore, EOF mobility will vary according to the buffer pH, such that at high pH the EOF mobility will be significantly greater than at low pH. Figure 1.3 depicts the variation of EOF mobility with pH for a typical fused-silica capillary. Above pH 9, silanols are completely ionised and the EOF mobility is at its greatest. Below pH 4, the ionisation of silanols is low and the EOF mobility is insignificant. The zeta potential will also depend upon the ionic strength of the buffer, because as ionic strength increases, the double layer will become compressed, which results in a decreased zeta potential and reduced EOF mobility.

At $\text{pH} > 7$, the EOF mobility is sufficient to ensure the net migration of most ions towards the cathode, regardless of their charge. Therefore, the observed migration velocity of a solute may not be directly related to its electrophoretic mobility. Instead, it is related to a combination of both its electrophoretic mobility and the EOF mobility. Therefore, a solute's apparent electrophoretic mobility (μ_a), that is calculated from its observed migration

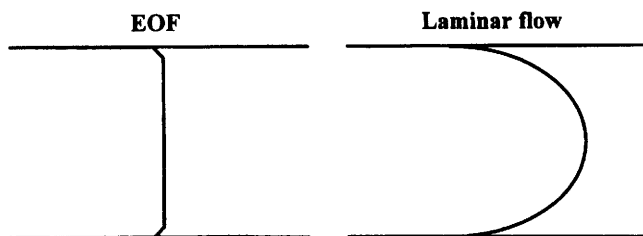


Figure 1.4 Flow profiles of EOF and laminar flow

velocity, is the vector sum of its real (or effective) electrophoretic mobility (μ_e) and the EOF mobility (μ_{EOF}), *i.e.*,

$$\mu_a = \mu_e + \mu_{\text{EOF}} \quad (1.4)$$

Since samples are normally introduced at the anode and EOF moves from the anode to the cathode, cations have positive μ_e , neutrals have zero μ_e and anions have negative μ_e . In other words, cations migrate faster than the EOF and anions migrate more slowly than the EOF. Neutrals migrate with the same velocity as the EOF.

Flow Profile in CE

A further key feature of EOF is that it has flat flow profile, which is shown in Figure 1.4, alongside the parabolic flow profile generated by an external pump, as used for HPLC. EOF has a flat profile because its driving force (*i.e.*, charge on the capillary wall) is uniformly distributed along the capillary, which means that no pressure drops are encountered and the flow velocity is uniform across the capillary. This contrasts with pressure-driven flow, such as in HPLC, in which frictional forces at the column walls cause a pressure drop across the column, yielding a parabolic or laminar flow profile. The flat profile of EOF is important because it minimises zone broadening, leading to high separation efficiencies that allow separations on the basis of mobility differences as small as 0.05%.

6 The Electropherogram

The data output from CE is presented in the form of an electropherogram, which is analogous to a chromatogram. An electropherogram is a plot of migration time *vs.* detector response. The detector response is usually concentration dependent, such as UV-visible absorbance or fluorescence. The appearance of a typical electropherogram is shown in Figure 1.5 for the separation of a three-component mixture of cationic, neutral and anionic solutes.

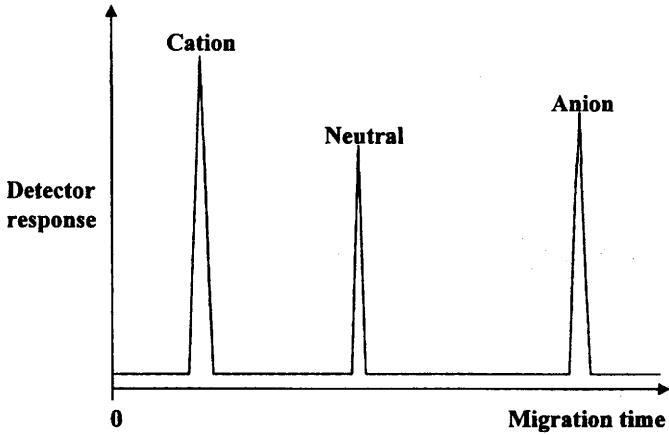


Figure 1.5 A typical electropherogram showing the separation of a cation, a neutral and an anion

7 Summary

- CE is based on the principles of electrophoresis.
- The speed of movement or migration of solutes in CE is determined by their size and charge. Small, highly charged solutes will migrate more quickly than large, less charged solutes.
- Bulk movement of solutes is caused by EOF.
- The speed of EOF can be adjusted by changing the buffer pH used.
- The flow profile of EOF is flat, yielding high separation efficiencies.
- The data output from CE is called an electropherogram.