Capillary Electrophoresis

By Nicole James

4.1: Introduction

The separation of compounds based on their movement when exposed to an electric field was first observed in 1807 by Ferdinand Friedrich Reuß, who noticed the movement of clay particles in water when a constant electric field was applied. The theory of electrophoresis was refined in the early 1900s by Marian Smoluschowski, and further refined in 1937 by Arne Tiselius, who won the 1948 Nobel Prize in Chemistry for his work.

Electrophoresis was initially conducted in polyacrylamide or agarose gels on a slab—a technique called *slab gel electrophoresis*—where charged molecules are applied to one end of the slab and an electric field is applied over the length of the slab. The molecules migrate down the slab at different rates according to the charge-to-size (m/z) ratio. Slab gel electrophoresis is still widely used in the fields of biology and biochemistry on large molecules such as nucleic acids and proteins to show relative concentrations of molecules, the purity of a sample, or, when used in conjunction with a standard, to identify compounds. Slab gel electrophoresis generally has long analysis times (often 20-40 minutes per sample), low efficiency, difficulties in analysis, and is unable to definitively identify compounds in a sample as migration time is not necessarily unique to each compound. Additionally, slab-gel electrophoresis is difficult to automate, making it very time-intensive to run multiple samples.

Capillary electrophoresis (CE) is widely used as an alternative to slab gel electrophoresis. Gel media are not necessary in capillary electrophoresis as capillary tubes are themselves anti-convective. Stellan Hjérten preformed the first work in capillary electrophoresis in 1967, using millimeter-diameter capillary tubes. By the early 1980s, the diameter of capillary tubes had been reduced to 75µm. Capillary electrophoresis generally runs faster than slab electrophoresis, provides better precision and accuracy, uses fewer reagents and is more easily automated. Capillary electrophoresis can also analyze smaller molecules than slab electrophoresis, thus expanding the range of possible analytes. By pairing capillary electrophoresis with mass spectroscopy, it is possible to obtain confirmatory results. This makes capillary electrophoresis an intensely useful and powerful instrument for many scientific disciplines. An overview of a CE system is shown in Figure 4.1.

Figure 4.1 Overview of a modern CE system.

4.2: Electrophoresis and Capillary Electrophoresis

The separation of compounds in capillary electrophoresis depends on the velocity of individual compounds. The velocity is given by the electrophoretic mobility (μ_e) of the compound and the applied electric field, (E)

$$\text{velocity} = \mu_e E \qquad \text{Eqn 4.1}$$

The electrophoretic mobility is a measure of the particle's tendency to move through the medium given the applied electrical field and thus changes with the medium and the particle; tabulated values of electrophoretic mobility often differ from the experimentally observed electrophoretic mobility. Consequently, experimentally determined mobilities are called "effective mobilities," and can change radically with different solvents and with different solution pH. The electrophoretic mobility depends on the frictional drag (F_f) the medium exerts on the particle and the electrical force (E_f) exerted to move the particle such that

$$\mu_e = \frac{E_f}{F_f} \qquad \text{Eqn 4.2}$$

where the electrical force is dependent on the charge of the ion (q) and the strength of the electrical field (E)

$$E_f = qE \qquad \text{Eqn 4.3}$$

The frictional force can be described as

$$F_f = -6\pi\eta r v \qquad \text{Eqn 4.4}$$

where r is the ion radius, v is the ion velocity, and η is the solution viscosity.

In slab electrophoresis, the sample is placed on the gel, and then the electrical field is applied. The force of the electrical field causes the sample to progress down the slab, accelerating until the drag--or friction--experienced by intermolecular interactions with the medium equals the force caused by the electrical field, and then the sample proceeds at a constant rate. Once the sample has reached a steady velocity, it can be shown that

$$\mu_e = \frac{q}{6\pi\eta r} \qquad \text{Eqn 4.5}$$

This equation shows that highly charged, small particles move quickly—especially with a low viscosity fluid—whereas less-charged, larger particles move more slowly, especially through a more viscous medium.

4.2.1: Electro-Osmotic Flow (EOF)

Also called electroendosmotic flow, electro-osmotic flow is the movement of liquid in a porous material (such as a capillary tube) caused by a difference in potential across the material.

When a charged particle is put in contact with a liquid in a capillary tube, a double-layer--or electrical double layer (EDL)--forms at the wall of the capillary (see Figure 4.2); this occurs at the interface of the glass capillary wall and the bulk solution. The first layer is surface charge, and can be positive or negative depending on the material. As capillaries are generally borosilicate glass, the numerous silanol (SiOH) groups cause the charge of the first layer to be negative. This layer is also sometimes called the *Stern layer* or *Helmholtz layer*. The second layer is made up of ionic particles in solution that are electronically attracted to the charge of the capillary surface. As the particles in this layer are not fixed, but move as a result of electrical and thermal energy, it is called the diffuse layer.

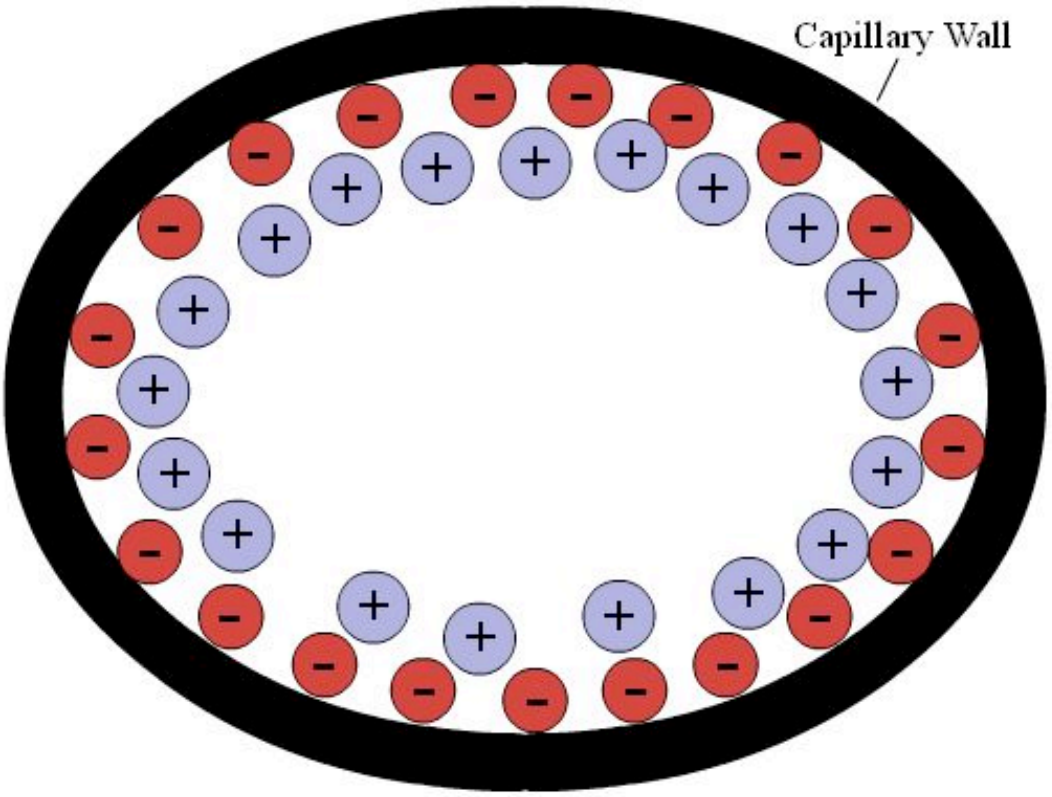


Figure 4.2: Capillary double-layer

The diffuse layer has a net charge, whereas the bulk, uncoordinated solution generally does not. When an electrical potential is placed across the capillary tube, the diffuse layer is pulled to one side. As the diffuse layer progresses to one side of the capillary tube, it drags the bulk solution along with it, creating a flow (specifically, the electro-osmotic flow) of the solution through the cathode. Because the species in the solution are charged,

they separate in solution as discussed above; for most applications*, positively-charged analytes move toward the anode given normal (negatively charged capillary) conditions.

The electro-osmotic flow (EOF) can be described in terms of velocity or mobility. The mobility can be calculated from Equation 4.6, where: ε (unitless) is the dielectric constant, a measure of a material's ability to respond to a dialectric's ability to store charge; ζ (V) is the zeta potential, discussed later; and η ($Pa\ s = \frac{kg}{s\ m}$) is the solution viscosity.

$$\mu_{EOF} = \frac{\varepsilon\zeta}{\eta} \qquad \text{Eqn 4.6}$$

Since the mobility is the ability to respond to electric potential, the velocity is the mobility multiplied by the electric potential:

$$V_{EOF} = E\frac{\varepsilon\zeta}{\eta} \qquad \text{Eqn 4.7}$$

The zeta potential (ζ) is the potential due to the double layer on the capillary wall, and is directly correlated with the charge on the capillary. Because the charge on the capillary varies as a function of pH, the zeta potential also varies with pH, meaning the mobility and velocity of the EOF are highly pH dependent. At high pH, the silanol groups are deprotenated, meaning the net charge of the capillary wall is greater. Thus, the EOF is significantly greater at high pH than at low pH; in fact, the EOF can sometimes vary more than an order of magnitude between a pH of 2 and a pH of 12.

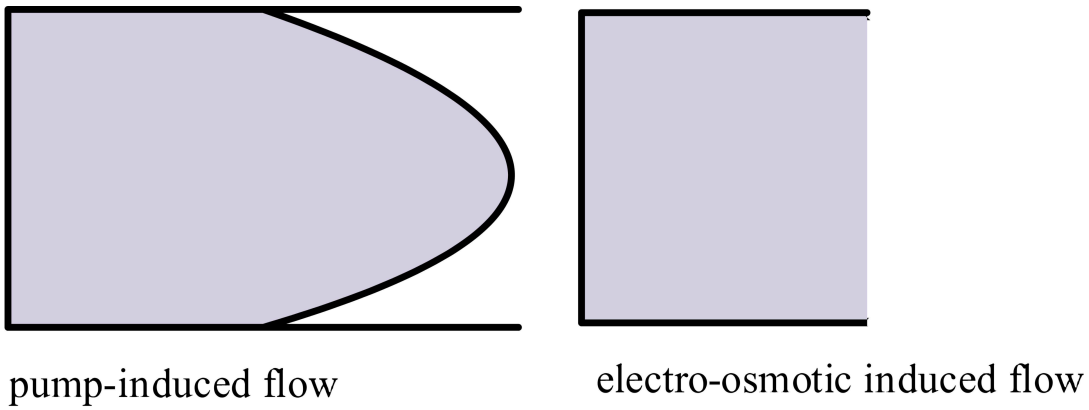


Figure 4.3: Pump induced flow vs. electro-osmotic induced flow

* For small monoatomic ions, the solute mobility can sometimes be greater the EOF; for these conditions, anions and cations will move in opposite directions.

A marked difference between electro-osmotic flow and flow induced by a pump is the cross section of the flow. Because a pump applies a force across the entire cross sectional area of the liquid in a capillary or tube, the friction between the wall and the liquid will cause the liquid at the center of the cross section to move faster than the liquid closer to the wall. Thus, the liquid velocity gradient flows parabolically down through the capillary. However, if a liquid is moved by electro-osmotic flow, the bulk flow is caused primarily by the acceleration of the cations near the capillary wall, and the force on the liquid is uniformly distributed. This results in a flat flow profile. As seen in (Figure 4.3), the flat flow profile drops off sharply at the capillary wall; this is caused by friction against the direction of flow, but to a much lower extent than observed in laminar flow caused by pumps. An even (or flat) flow results in compact peaks whereas a parabolic laminar flow results in a broad peak because the analyte is spread over a larger area. "Tighter" peaks are easier to integrate and facilitate the isolation of peaks that elute at similar times.

Table 4.1 summarizes the variables that control electro-osmotic flow.

Variable	**Effect on EOF**	**Possible Problems**
pH	directly proportional	can alter structure and charge of analyte
Ionic Strength	inversely proportional	sample heating and adsorption
Temperature	inversely proportional	changes in viscosity
Electric Field	directly proportional	can cause heating
Organic Modifiers	usually inversely proportional	can alter selectivity of instrument
Hydrophilic Polymer	usually inversely proportional	dependent on polymer
Covalent Coating	dependent on coating	coating often unstable

Table 4.1: EOF variables

4.2.2: Peak Resolution and Solute Zones

Peak resolution in capillary electrophoresis is dependent on many variables, including longitudinal diffusion, injection length, sample adsorption to capillary walls, electrodispersion, Joule heating, and detector cell size. The loss of peak resolution is often referred to as "zone broadening" in CE. As in other forms of chromatography, this results in reduced separation of compounds and increased difficulty in accurate peak integration.

4.2.2.1: Longitudinal Diffusion

Under normal conditions, diffusion along the capillary tube (longitudinal diffusion) is one of the more substantial contributors to zone broadening. A solute's propensity to diffuse throughout the solvent depends on the solute and the solvent. A longitudinal diffusion coefficient can be provided for a given system, with a lower coefficient meaning the solute will form narrower zones.

The equation for the number of theoretical plates in a capillary tube is given in Equation 4.8, where N is the number of theoretical plates, μ_a is the solute mobility, E is the electric field, I is the effective capillary length, and D is the diffusion coefficient of the solute.

$$N = \frac{\mu_a EI}{2D} \qquad \text{Eqn 4.8}$$

This equation only accounts for longitudinal diffusion, although there are often other variables that affect zone broadening.

4.2.2.2: Injection Length

When introducing a sample to a CE system, the volume of liquid that enters the capillary is called the sample plug (See Figure 4.4). If the sample plug is very spread out, analytes will not separate into tight peaks and tailing or broadening can occur. A practical injection should be between 1% and 2% of capillary length.

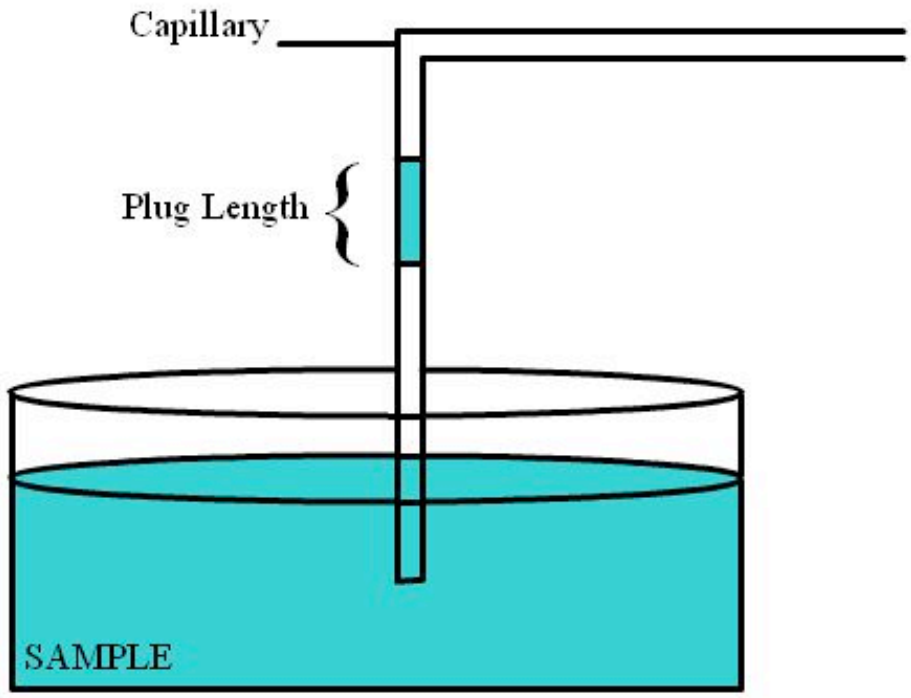


Figure 4.4: Diagram of sample introduction and plug length

4.2.2.3: Sample Adsorption

Interactions of the analyte with the capillary wall negatively affect zone broadening. In some cases, complete adsorption of the analyte to the capillary wall can occur. As most

capillary tubing is made of borosilicate glass, this is most substantial with positively charged analytes that can interact strongly with the hydroxide groups on the surface of the glass. Prolonged sample-surface interactions can result in peak tailing and zone broadening.

4.2.2.4: Electrodispersion

The capillary in a CE system contains an ambient level of ions, called the background electrolyte (BGE). For ideal separation, the BGE should be uniform in and between all solute zones. Therefore, the background solvent is generally always a buffer.

4.2.2.5: Joule Heating

Joule heat is generated by passing electric current through a conductor and is directly proportional to the amount of power used. Joule heating can result in non-uniform temperature gradients throughout the capillary tube. Temperature increases are often around 10ºC, although increases of up to 70ºC or higher can occur. While uniform increases in temperature generally do not affect zone broadening, uneven increases in temperature leading to temperature gradients can cause substantial peak broadening and/or tailing. Heat can relatively easily dissipate through capillary walls, which can result in a large difference in temperature between the center of the capillary and the internal edges of the capillary. These gradients can result in differences in solvent viscosity, and thus air or liquid cooling of the capillaries is often necessary. Using thicker capillary tubes with a large outer radius and a smaller inner radius can also help combat this problem. Smaller sample volumes and higher surface-to-volume ratios limit the Joule heating, even when hundreds of volts per centimeter are applied.

4.2.2.6: Detector Cell Size

Detectors on CE systems average a signal over a finite volume. Naturally, if the volume averaged is too large, it can combine signals from different analytes. In this way, zone broadening due to the size of the detector cell is similar to zone broadening due to a long injection plug. Generally, any variance due to detector cell size can be ignored if the length of the detection cell is roughly one third the sample zone length.

4.3: Samples

4.3.1: Sample Introduction

For loading a sample, a stable DC power supply of about 30kV (200-300mA) is required. Instruments are made with the ability to switch polarity to facilitate a variety of methods of operation. Usually, current is monitored and maintained to compensate for some temperature-induced changes in buffer viscosity.

Sample volumes are generally incredibly small. Volumes as low as 5μL can be sufficient for several injections. This can be disadvantageous if the sample is very dilute, because being limited on the loading volume of a dilute sample can reduce sensitivity. Loading too much of a sample can result in bad peak shapes due to field inhomogeneities if the conductivity of the sample is different from that of the buffer. Additionally, if the injection length is greater than the diffusion-controlled zone, peak broadening will occur.

4.3.1.1: Types of Sample Injection

There are several methods for introducing samples into CE systems. It is important to note that some sample is always injected when inserting a capillary into the sample reservoir, due to capillary action. This volume is called the Zero-Injection, and the effect of this initial injection on sample migration and analysis is called the Zero-Injection Effect. In most cases, this phenomenon is insignificant, but it can be important in systems being employed for quantitative analysis.

4.3.1.1.1: Hydrodynamic Injection

Hydrodynamic injection introduces a sample by applying a pressure on the sample reservoir. This physically forces solution through the capillary. The volume of sample loaded is independent of the sample matrix; instead, the volume depends on the details of the system, such as capillary dimensions, the viscosity of the buffer, the pressure applied, and the amount of time that pressure was applied. Pressures and times generally range from 25-100mbar and 0.5-5 seconds.

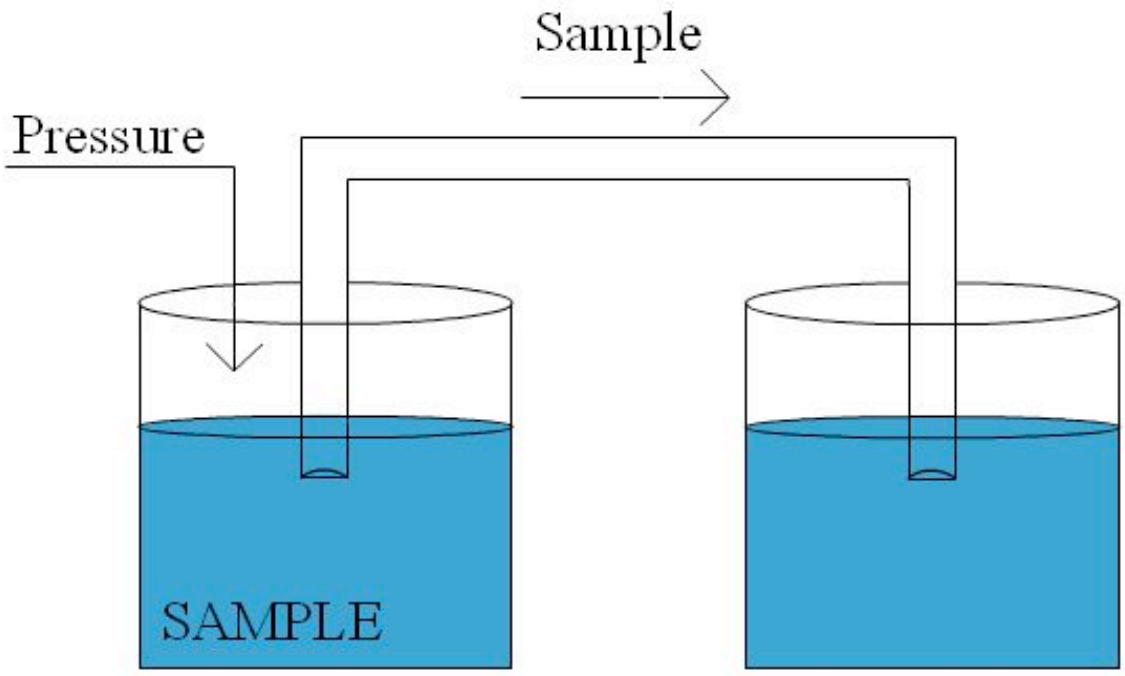


Figure 4.5: Hydrodynamic injection setup

Similarly, hydrodynamic injection can be performed by siphoning, or raising the input reservoir 5-10cm above the output reservoir while keeping the levels of each equal. This technique is generally employed when the equipment necessary for pressure injection is unavailable.

4.3.1.1.2: Electrokinetic Injection

In electrokinetic injection, a voltage 3-5 times lower than that required for separation is applied between the input and output reservoirs for approximately 10-30 seconds. Sample is loaded according to the difference in voltage across the capillary and the amount of each individual analyte that is loaded is dependent on the analyte's electrophoretic mobility. As such, electrokinetic injection cannot be used quantitatively. This technique is useful in situations where the media in capillary is very viscous making hydrodynamic injection difficult. Additionally, it requires no extra instrumentation.

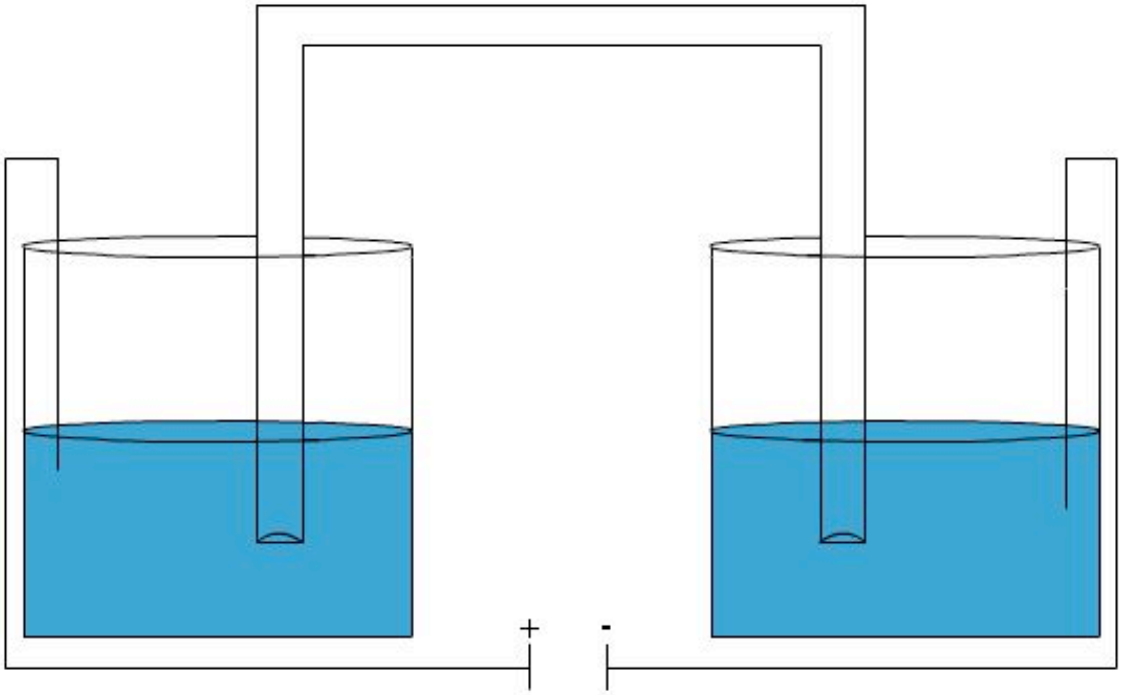


Figure 4.6: Electrokinetic injection setup

4.3.1.1.3: Field Amplified Sample Stacking (FASS) and Field Amplified Sample Injection (FASI)

Samples zones are sharpened when the conductivity of the sample matrix is substantially lower than that of the buffer. Essentially, this is the reverse of electro-dispersion, described previously. Thus, samples with low salt content can be actively sharpened and/or large quantities of them can be loaded onto a capillary before zone-broadening effects begin to occur. When this phenomenon is utilized to allow larger sample lengths to be loaded hydrodynamically, the concentration is called field amplified sample stacking (FASS). When the injection is performed electrokinetically, it is called field amplified sample injection (FASI).

In the case of FASI, if the conductivity of the sample varies or is unstable, results can be unreliable. In such a case, FASS can be used instead and, while peak resolution may be affected by variability in sample conductivity, the peak areas are still reliable.

Often 100 times more sample can be loaded by FASS before separation is greatly affected. The actual amount of sample that can be loaded by FASS is regulated by the sample's conductivity and factors such as Joule heating. As the conductivity of the sample must necessarily be much lower than that for the running buffer, the applied electric field can induce a large degree of heating within the sample plug, which can change the viscosity of the sample and result in degassing and/or boiling.

Additionally, due to the difference in ionic strength between the sample plug and the running buffer, the electro-osmotic mobility will vary between the sample plug and the buffer. This can result in a parabolic flow profile between the plug and the running buffer that may cause zone broadening. Similarly, a substantially different pH between the sample plug and the buffer can also result in differences in analyte mobility, so a buffer with a similar pH to the sample solution should be chosen.

4.3.2: Sample Purification

CE is attractive as a preparatory purification source because of its separatory strength. However, CE can only be used to purify small amounts of analyte, as the sample loading limit is low. However, small-volume fractions can be collected for sequencing DNA fragments, establishing biological activity of substances, and ascertaining the purity of a collected peak by reinjection.

Autosamplers can be programmed to run a sequence of samples so that, in the event that multiple runs must be made and fractions collected and combined, the instrument does not need to be attended for the entire period.

4.4: Methods of Operation

4.4.1: Capillary Zone Electrophoresis (CZE)

Capillary Zone Electrophoresis is the standard method of CE. Separations are performed by a difference in migration time as previously described.

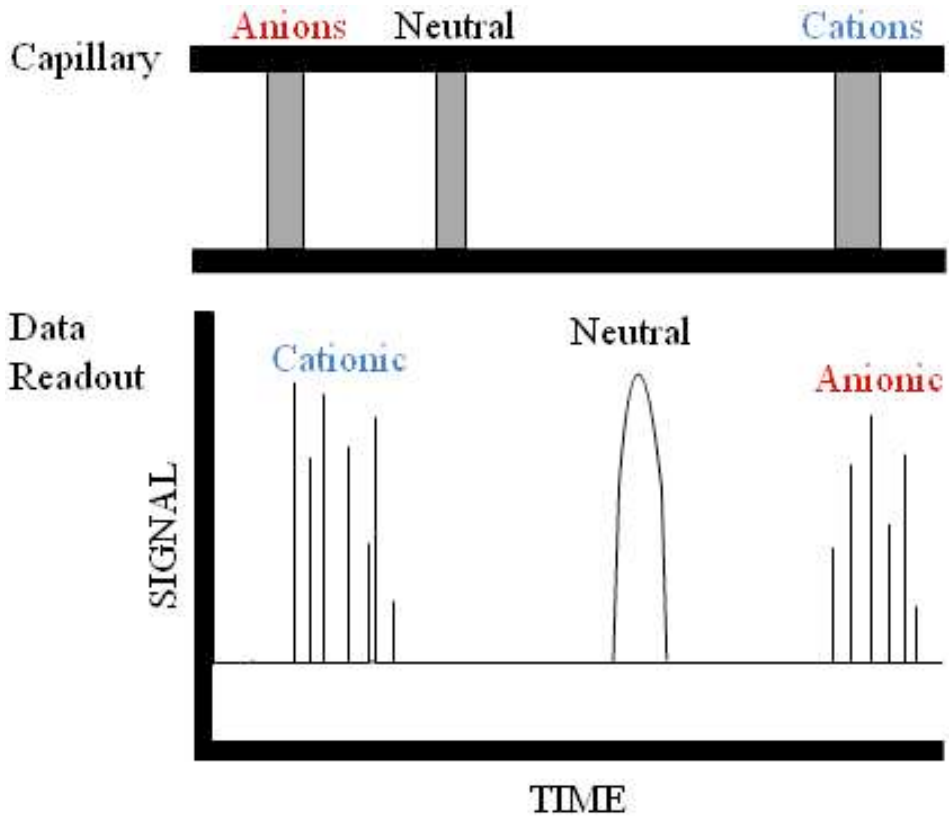
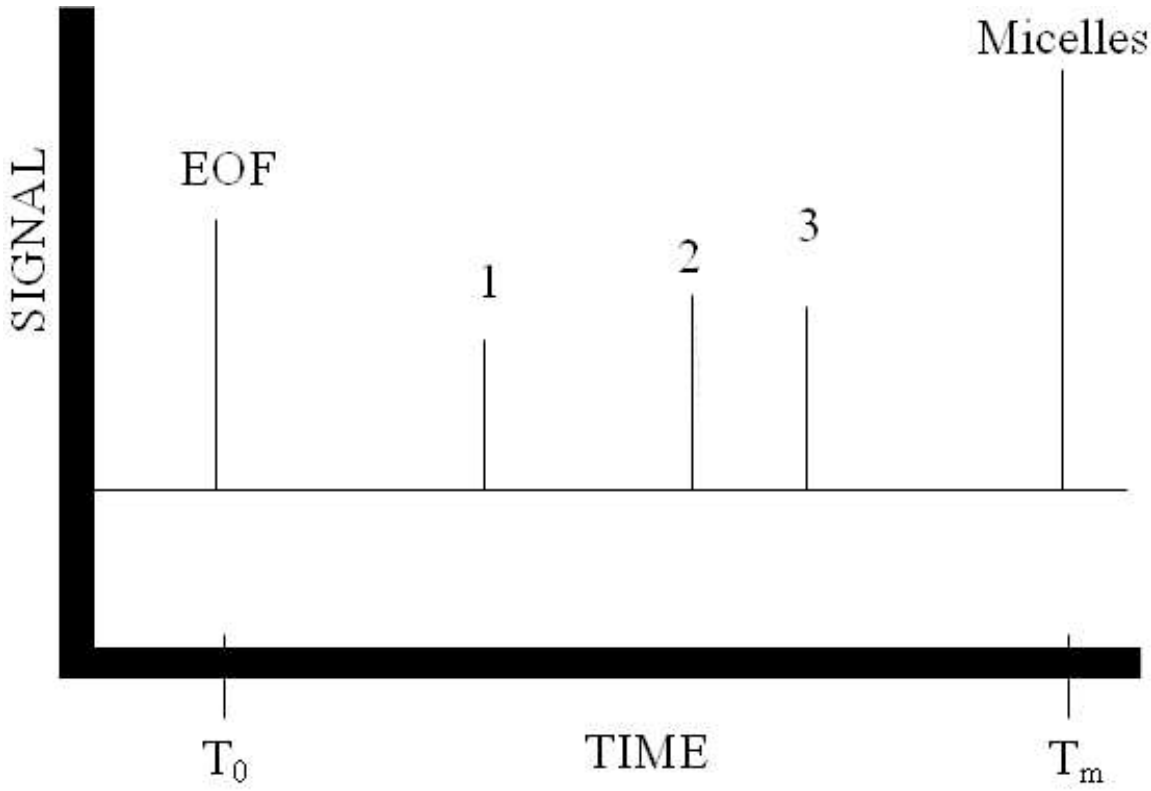


Figure 4.7 Diagram of a CZE system.

4.4.2: Miceller Electrokinetic Chromatography (MEKC)

Miceller electrokinetic chromatography is a combination of electrophoresis and chromatography. If one dissolves surfactants (for example, 8-9mM SDS) in the running buffer, micelles--often anionic--form and travel against the EOF. The EOF, however, is still faster and the net motion of the micelles is in the same direction as the EOF. Solutes partition between the micelles and the buffer through hydrophobic and electrostatic interactions. In this way, the micelles act as a stationary phase which is not necessarily stationary, but slower with respect to the EOF. The more hydrophobic compounds migrate more slowly through the capillary due to their interactions with the micelles. All neutral compounds will elute between the EOF, which elutes first, and the micelles, which elute last. (See Figure 4.8).

The surfactants can be anionic, cationic, non-ionic, zwitterionic, mixtures of these, salts, or microemulsions. Deciding which surfactant to use is based on the specific interactions necessary to separate target compounds.



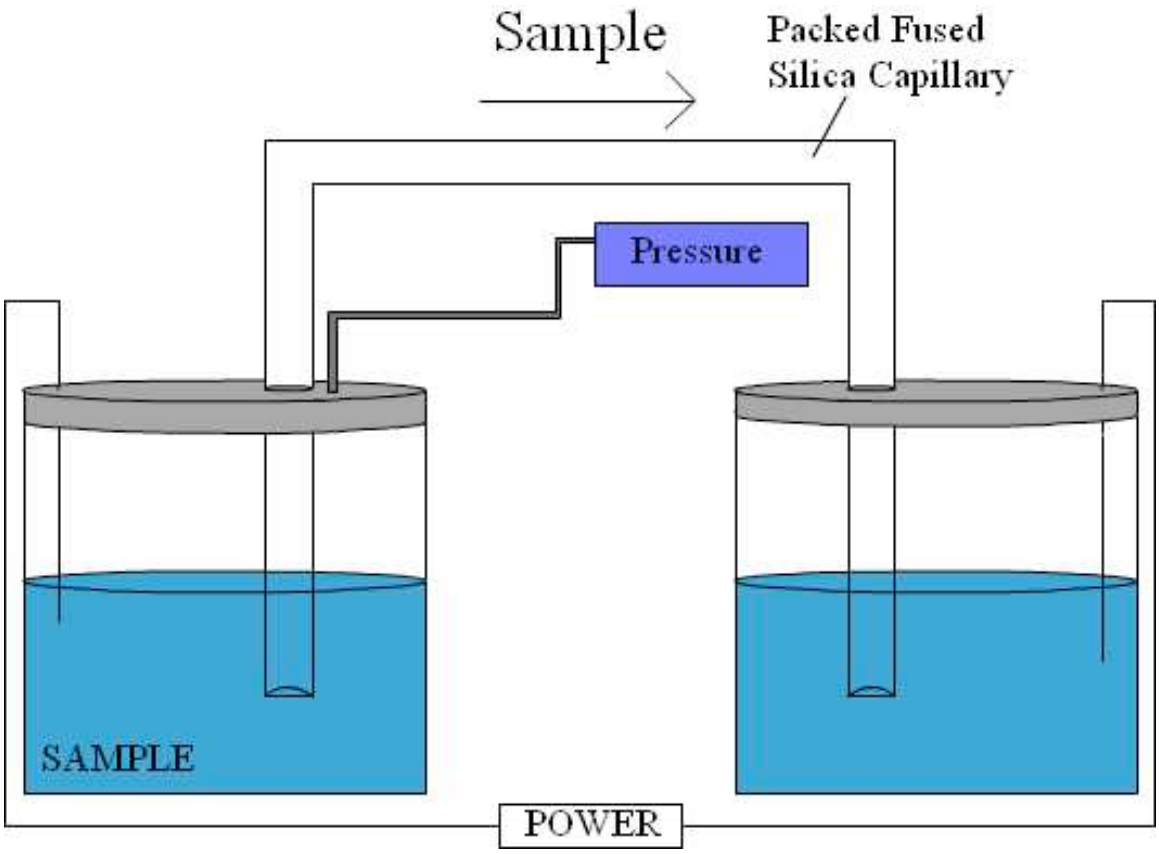
4.8: Example miceller electrokinetic chromatogram

Surfactants may affect the EOF and solute-wall interactions. Generally, using a high pH buffer will maintain the EOF and diminish changes in solute-wall interactions. Additionally, a consistent temperature is especially important as changes in temperature will affect the concentrations of surfactant required to form micelles as well as the partition coefficient(s) of the system.

4.4.3: Capillary Electro-Chromatography (CEC)

Capillary electro-chromatography is miniaturized liquid chromatography. An applied electric field pumps liquid through a packed capillary column. The silica capillaries are packed with silica-based reversed-phase particles of 1-5µm diameter. The migration times of different solutes vary as a function of their partitioning between the mobile and stationary phases. If the packed phase is charged, there is also an electrophoretic component to the partitioning. On average, there are 100,000 theoretical plates per peak in a CEC system.

As the only difference in a CEC setup is the capillary tubing used, CZE systems can be easily set up for CEC analysis.



4.4.4: Capillary Gel Electrophoresis (CGE)

Capillary gel electrophoresis is essentially gel electrophoresis conducted in a capillary tube. The "gel" is not necessarily stiff and solid as it is in slab gel electrophoresis. To avoid confusion, it is usually referred to as a polymer matrix. Many polymer matrices are available and offer different separating properties.

The polymer is dissolved in buffer and loaded into the capillary tube. Generally, the polymer concentration necessary for adequate separation is inversely proportional to the size of the analyte. As low viscosity polymer solutions do not experience the same capillary action as most regular buffer solutions, the capillary gel is often pumped with pressure and the capillary wall is coated to eliminate EOF.

There are generally 10 million theoretical plates per meter of capillary tubing. CGE is most commonly used to separate single-stranded oligonucleotides, proteins, and DNA fragments.

As in CZE, it is possible to add agents such as chiral selectors and ion-pairing agents by covalently bonding them to the gel or the running buffer. This can greatly increase the separation possibilities of a single system.

4.4.5: Capillary Isoelectric Focusing (CIEF)

Capillary isoelectric focusing separates peptides and proteins based on their isoelectric point (pI)--the point at which they carry no net charge. A pH gradient is formed within the capillary using ampholytes—zwitterionic molecules with both an acidic and basic moiety.

The capillary is filled with a mixture of solutes and ampholytes, and a pH gradient is formed with a basic solution at the cathode and an acidic solution at the anode. When an electric field is applied, ampholytes and solutes migrate through the pH gradient until they reach the pH that corresponds to their isoelectric point, where they become uncharged. The solute zones will maintain a narrow breadth as any solute which migrates into the zone of a different pH becomes charged and migrates back into the pH of its isoelectric point. After this equilibrium occurs, a steady state is reached and hydraulic pressure is used to pass the zones through to the detector.

EOF must be minimized to prevent flushing ampholytes through the capillary.

4.4.6: Capillary Isotachophoresis (CITP)

Capillary isotachophoresis is a moving boundary technique using two buffer systems. The buffer systems trap the analyte zones and all zones lay in between "leading" and "terminating" electrolytes, where the leading electrolytes are those in the buffer with a higher EOF and the terminating electrolytes are those in the buffer with the lower EOF.

This bracketing affects the electric field experienced by each zone, creating different electric fields for each analyte. The electric field varies such that each analyte moves at the same velocity as that established by the leading electrolyte. Because velocity is defined as the product of the mobility and the electric field (velocity=mobility•field), this varying field acts to compliment the mobility such that all analytes move at the same velocity. Zones are formed and focused much in the same way as they are in CIEF; as soon as an analyte drifts away from its zone, it experiences a new electric field such that it either accelerates or decelerates until it migrates back to its zone and the steady state is reestablished.

According to the Kohlrausch Regulating Function, the concentration of analyte in a zone is determined by the concentration of the leading electrolyte. Analytes with a higher concentration than the leading electrolyte experience broadened until the concentrations match, whereas analytes with a lower concentration than the leading electrolyte sharpen. In this way, CITP can be used as a preconcentration step prior to other methods of analysis. Generally, up to 30-50% of the capillary can be filled with sample while still maintaining good separation and concentration.

Also due to the Kohlrausch Regulating function, increasing the concentration of the leading and/or trailing electrolytes can result in better separation and can improve

detection of analytes that can be difficult to separate. However, when applying CITP, it can be difficult to identify a buffer system with leading and trailing electrolytes that have the appropriate mobilities while still maintaining the appropriate pH for the sample analysis. Additionally, only cations *or* anions can be analyzed by a given buffer system.

4.5: Detectors

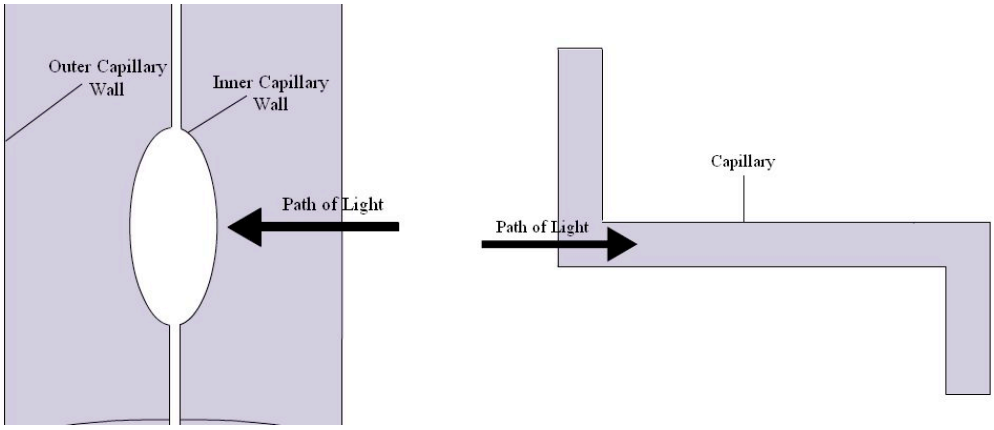
Detection in CE can be a significant challenge, as the small injection volumes require the samples to be relatively concentrated for reasonable detection. Many of these techniques are similar to those employed in liquid chromatography.

4.5.1: UV-vis Spectrophotometry

UV-visible absorption is the most common detection technique for CE. Detection is nearly universal, and with fused-silica as the sample holder, wavelengths from 200nm through the visible spectrum can be used without interference. Generally, the capillary itself is used as the sample holder and passes through a UV-vis detector.

Because the capillary tube is so thin compared to conventional path lengths, care needs to be taken in the design of the detector. The beam of light must be of small radius and focused directly on the capillary for accurate results. A disadvantage of this is that, correspondingly, few photons reach the detector and a generally high baseline is obtained. The optical beam is generally passed through a slit to restrict its width and center it on the capillary tube.

One approach to decrease the detection limit is by increasing the path length of the flow cell. This can be done by increasing the diameter of the capillary, but by doing this there is also an increase in the current and resulting Joule heating. Two ways of increasing path length without increasing capillary diameter are depicted in Figure 4.10. The method shown in 4.10A, called the “bubble cell” does not require an increase in current, as the detection occurs after separation. When a zone enters the bubble, the velocity decreases as it expands to fill the capillary, it contracts along the capillary and overall concentration remains the same, even with increasing path length. There is a third method, which is a combination of 4.10a and 4.10b; the capillary bends to increase the path length, but the capillary is also bubbled along the path.



4.10: a, left) Capillary bubble cell for UV-Vis detection; b, right) capillary cell.

When trying to detect a given analyte, care must be taken to ensure the running buffer is not optically active in the range of the analyte. Most peptides and carbohydrates absorb around 200nm, and consequently many biological buffers, such as HEPES, CAPs, and tris, should not be used in systems analyzing peptides and carbohydrates.

A normal S/N for a 75mm id capillary is 62.5, and up to 650 on an increased path length cell.

4.5.2: Indirect Photometry

Indirect photometry utilizes a monitoring ion that is UV-active to trace the presence of a non UV-active compound. An ion of the running buffer is chosen, generally to have a high UV absorbance intensity, and its absorbance is monitored. When the sample zone passes through the detector, it will be accompanied by a corresponding drop in the UV absorption due to the monitoring ion.

A very simplified explanation for this phenomenon is that the monitoring ion is displaced by an analyte with the same charge based on electroneutrality. However, the displacement ratio can be greater than one and analytes with charges opposite to the monitoring ion can be detected. This can be better explained by a closer analysis of the Kohlrausch Regulation Function.

4.5.3: Laser-Induced Fluorescence (LIF)

Laser-induced fluorescence adapts fluorescence spectrometry to CE in the same manner as UV-vis spectrometry. A laser is used to excite the analyte, and fluorescence is measured via a PMT. Often the laser is aligned non-collinearly, and a ball lense is used to focus the light on the center of the capillary. Generally, a reflective ellipsoid is glued to the capillary to direct fluorescence toward the PMT.

A drawback of LIF is that most compounds of interest in CE are not fluorescent. However, a number of derivatizing agents can be used to act as markers for the compound. These are generally organic aromatics and require excitation between 250nm and 500nm.

4.5.4: Contactless Conductivity

Contactless conductivity detectors (CCDs) place an actuator electrode and receiver electrode along the capillary tube, as depicted in Figure 4.11. An oscillating frequency is applied at the actuator electrode and detected at the receiver electrode with the liquid inside acting as a resistor. As a sample zone passes, the conductivity changes. Since analytes are charged, the conductivity generally increases--except for when samples have a lower ionic strength than the running buffer.

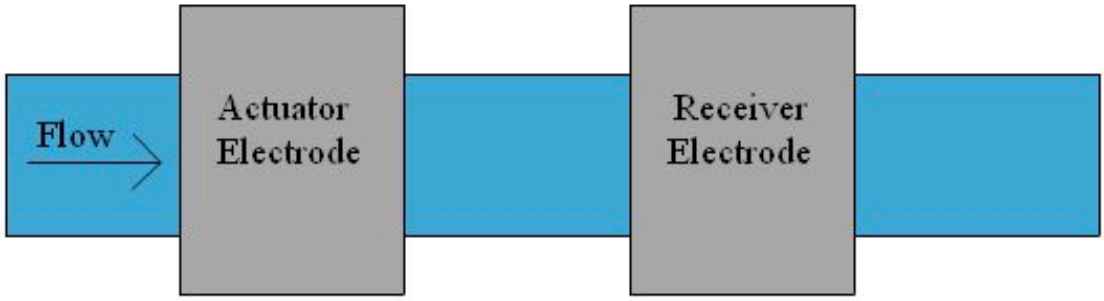


Figure 4.11 CCD setup.

Contactless conductivity detectors are universal and work well for inorganic analytes that are difficult to measure by other methods. CCDs function well with low sample volume, which is advantageous for use in CE. Additionally, it generally has a detection limit in the ppm-ppb range for small organic ions.

4.5.5: Mass Spectrometry

Mass spectrometry is a particularly attractive detecting strategy as it is confirmatory and can give valuable structural information about analytes. However, it is difficult to transition from a liquid-phase separatory technique to a gaseous, vacuum-based detection technique. To an extent, this problem is alleviated by the small amount of solvent and the low flow rate associated with CE.

4.5.5.1: Electro-Spray Ionization (ESI)

Electro-spray ionization is an ideal technique for interfacing CE and MS, as it accepts small volumes at a low flow rate and results in gaseous, ionized particles (for a detailed description go to section 5.3.1). Additionally, ESI is well-suited to larger, biological molecules, which are frequently analyzed by CE. However, CE and ESI are both dependent on electric fields. In order to couple them in the simplest of cases, the end electrode of the CE instrumentation is shared with the ESI electrode. However, in some situations the difference in the magnetic field can be several orders of magnitude and the electrodes may be of the opposite sign.

Some situations can be remedied by grounding the end electrolyte of the CE system, and providing voltage to the electro-spray system from the MS. If the potential applied to the ESI is negative, then positive ion will enter the MS and this is called positive ion mode. If the CE voltage is much greater than the ESI voltage, a ground path for the current is provided by a resistor sink. In general, when the ESI voltage is much different from the CE voltage, the variability in the electric field can affect the sample separation in the CE system.

4.5.5.1.1: Hydraulic Interfacing

A hydraulic flow rate is necessary to form the electro-spray. The amount of hydraulic flow necessary is dependent on many variables, including the EOF and capillary dimensions. In general, there are two methods for introducing a hydraulic flow: sheath flow and sheathless flow.

4.5.5.1.1.1: Sheath Flow Interface

Smith, et al. invented the tri-axial capillary sprayer, similar to the one shown below.

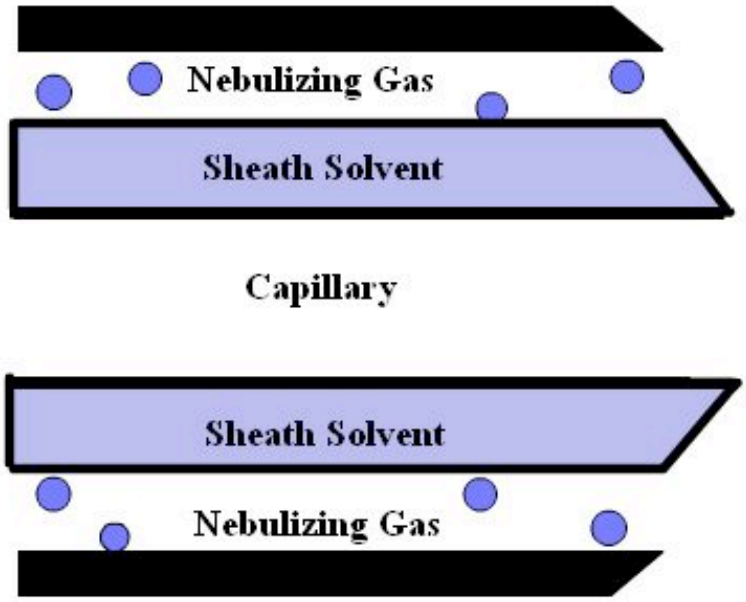


Figure 4.12: Sheath flow interface

Solvent flows between the capillary and the sprayer tip, and combines with the capillary solution immediately before the nebulizing gas. In this way, a hydraulic flow independent of the EOF is provided by the sheath solvent.

The major disadvantage of the sheath flow interface is the fact that the sample is necessarily diluted by the sheath solvent. Despite this, the sheath flow interface is one of the most reliable methods of interfacing CE and MS, although several alternative methods exist but are not "as robust," according to Agilent literature. (It should be noted that Agilent possesses the distribution rights on the tri-axial sheath.)

4.5.5.1.1.2: Sheathless Flow Interface

Several methods of uniting the CE capillary with the ESI system exist However, in all of these systems, electrochemical processes such as oxygen and hydrogen formation can occur. The formation of gas bubbles that are not frequently vented can cause increased resistance. In the tri-axial system, evolved gases are frequently flushed due to sheath flow and/or the nebulizer gas, but in sheathless systems increased resistance can form a substantial problem.

In 2007, Moini published a technique that does not require sheath flow and does not create electrochemical processes at the electrodes. A thin, porous segment of glass acts as a liquid and electrical contact outside the capillary. The tube is either grounded or placed at the ESI voltage. This system has recently become available commercially through Beckman-Coulter.

4.5.5.2: Atmospheric Pressure Chemical Ionization (APCI) and Atmospheric Pressure Photo Ionization (APPI)

ESI is highly efficient for many systems. However, for systems where the running buffer is not especially volatile and/or the molecules are not strongly charged, alternative ionization techniques may be more efficient. Atmospheric pressure chemical ionization (APCI) and atmospheric pressure photo ionization (APPI) can both be used for systems like these.

APCI relies on the evaporation of an analyte-containing aerosol, where a charged reagent gas collides with analyte molecules and confers the charge. In APPI, an analyte aerosol is again evaporated to a vapor, and then photons ionize the analyte. In situations where the analyte is not easily photoionized, a buffer gas may be photoionized and then transfer the charge to the analyte.

APCI and APPI both require substantial heating of the sample and therefore are not appropriate for analytes that easily degrade at higher temperatures. Typically, APCI and APPI systems are heated to between 200-300ºC. Additionally, low molecular weight (<1kD) analytes are better suited to APPI and APCI as they are more volatile.

4.6: Cited and Consulted References

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