Chapter 23

Electrophoresis

Chapter Outline
23.1 Introduction: The Human Genome Project
   23.1A What Is Electrophoresis?
   23.1B How Is Electrophoresis Performed?
23.2 General Principles of Electrophoresis
   23.2A Factors Affecting Analyte Migration
   23.2B Factors Affecting Band-Broadening
23.3 Gel Electrophoresis
   23.3A What Is Gel Electrophoresis?
   23.3B How Is Gel Electrophoresis Performed?
   23.3C What Are Some Special Types of Gel Electrophoresis?
23.4 Capillary Electrophoresis
   23.4A What Is Capillary Electrophoresis?
   23.4B How Is Capillary Electrophoresis Performed?
   23.4C What Are Some Special Types of Capillary Electrophoresis?

23.1 INTRODUCTION: THE HUMAN GENOME PROJECT

February 2001 saw one of the greatest achievements of modern science. It was at this time that two scientific papers appeared, one in the journal *Science* and the other in *Nature*, reporting the sequence of human DNA (or the “human genome”). These papers were the result of a major research effort known as the Human Genome Project, which was formally begun in 1990 under the sponsorship of the U.S. Department of Energy and the National Institutes of Health.

Although it was anticipated to take 15 years to finish, this project was “completed” in about a decade. This early completion was made possible by several advances that occurred in techniques for sequencing DNA. One common approach for sequencing DNA is the Sanger method (see Figure 23.1). In the Sanger method, the section of DNA to be examined (known as the “template”) is mixed with a segment of DNA that binds to part of this sequence (the “primer”). This mixture is placed into four containers that have the nucleotides and enzymes needed to build on the template. These containers also have special labeled nucleotides that will stop the elongation of DNA after the addition of a C, G, A, or T to its sequence. The DNA strands formed in each container are later separated according to their size. By comparing the length of these strands and by knowing which labeled nucleotides are at the end of each strand, the sequence of the DNA can be determined.

The Sanger method was originally developed as a manual technique that took long periods of time to perform. Thus, one thing that had to be addressed early in the Human Genome Project was the creation of faster, automated systems for sequencing DNA. Both traditional and newer systems for accomplishing this sequencing utilize a separation method known as electrophoresis. In this chapter we learn about electrophoresis, look at its applications, and see how improvements in this technique made the Human Genome Project possible.

23.1A What Is Electrophoresis?

Electrophoresis is a technique in which solutes are separated by their different rates of migration in an electric field (see Figure 23.2). To carry out this method, a sample is first placed in a container or support that also contains a background electrolyte (or “running buffer”). When an electric field is later applied to this system, the ions in the running buffer will flow from one electrode to the other and provide the current needed to maintain the applied voltage. At the same time, positively charged ions in the sample will move toward the negative electrode (the cathode), while negatively charged ions will move toward the positive electrode (the anode). The result is a separation of these ions based on their charge and size. Because many biological compounds have charges or ionizable groups (e.g., DNA and proteins), electrophoresis is frequently utilized in biochemical and
medical research. This approach can also be adapted for work with small ions (like Cl\(^-\) or NO\(_3\)^-) or for large charged particles (such as cells and viruses).

Even though it has been known for one hundred years that substances like proteins and enzymes have a characteristic rate of travel in an electric field,\(^{11-13}\) electrophoresis did not become a routine separation method until around the 1930s. One notable advance occurred in 1937 when a scientist named Arne Tiselius (Figure 23.3) used electrophoresis for the separation of serum proteins.\(^{3,14}\) Tiselius conducted this separation by employing a U-shaped tube in which he placed his sample and running buffer. When he applied an electric field, proteins in the sample began to separate as they migrated toward the electrodes of opposite charge. However, the use of a large sample volume gave a series of broad and only partially resolved regions that contained different mixtures of the original proteins.\(^{15}\)

The method employed by Tiselius is now known as moving boundary electrophoresis, because it produced a series of moving boundaries between regions that contained different mixtures of proteins, as shown in Figure 23.3.\(^{10,16}\) Today it is more common to use small samples to allow analytes to be separated into narrow bands or zones, giving a method known as zone electrophoresis.\(^{8-10,16}\) An example of zone electrophoresis is shown in Figure 23.1, where DNA is sequenced by separating its strands of various lengths into narrow bands on a gel.

There are many ways in which electrophoresis is used for chemical analysis. These include the sequencing of DNA, as well as the purification of proteins, peptides, and other biomolecules. In clinical chemistry, electrophoresis is an important tool for examining the patterns of amino acids, serum proteins, enzymes, and lipoproteins in the body. Electrophoresis is also used in the analysis of organic and inorganic ions in foods, commercial products, and environmental samples. In addition, electrophoresis is an essential component of medical and pharmaceutical research for the characterization of
23.1B How Is Electrophoresis Performed?

Electrophoresis can be performed in a variety of formats (see Figure 23.4). One format is to apply small amounts of a sample to a support (usually a gel) and allow the analytes in this sample to travel in a running buffer through the support when an electric field is applied. This approach is known as *gel electrophoresis* (a method we will discuss in Section 23.3). It is also possible to separate the components of a sample by using a narrow capillary that is filled with a running buffer and placed into an electric field. This second format is called *capillary electrophoresis* (discussed in Section 23.4).

Depending on the type of electrophoresis being used, the resulting separation can be viewed in one of two ways. In the case of gel electrophoresis, the separation is stopped before analytes have traveled off the support. The result is a series of bands where the migration distance \( d_m \) characterizes the extent to which each analyte has interacted with the electric field. This approach is similar to that used to characterize the retention of analytes in thin-layer chromatography and paper chromatography (see Chapter 22). Because the migration distance of an analyte through a gel for electrophoresis will depend on the exact voltage and time used for the separation, it is common to include standard samples on the same support as the sample to help in analyte identification. The intensity of the analyte band is then used to measure the amount of this substance in the sample.

In capillary electrophoresis all analytes travel the same distance, from the point of injection to the opposite end where a detector is located. The analytes will differ, however, in the time it takes them to travel this distance, in a manner similar to what occurs in the chromatographic methods of gas chromatography (GC) and high-performance liquid chromatography (HPLC). In this situation the migration time \( t_m \) for each analyte is measured and recorded. The resulting plot of detector response versus migration time is called an...
The migration times in this plot can be used to help in analyte identification, while the peak heights or areas are used to determine the amount of each analyte. An internal standard is usually injected along with the sample to correct for variations during injection or small fluctuations in the experimental conditions during the separation.

23.2 GENERAL PRINCIPLES OF ELECTROPHORESIS

The separation of analytes by electrophoresis has two key requirements. The first requirement is there must be a difference in how analytes will interact with the separation system. In electrophoresis this requirement means the analytes must have different migration times or migration distances. The second requirement is that the bands or peaks for the analytes must be sufficiently narrow to allow them to be resolved.

23.2A Factors Affecting Analyte Migration

Electrophoretic Mobility. Electrophoresis is similar to chromatography in that both involve the separation of compounds by differential migration. Chromatography brings about differential migration through chemical interactions between analytes with the stationary phase and mobile phase. In electrophoresis, differential migration is produced by the movement of analytes in an electric field, where their rate of migration will depend on their size and charge.

The overall rate of travel of a charged solute in electrophoresis will depend on two opposing forces (see Figure 23.5). The first of these forces \( F_+ \) is the attraction of a charged solute toward the electrode of opposite charge. This force depends on the strength of the applied electric field \( E \) (units of volts per distance) and the charge on the solute \( z \). The second force acting on the solute is resistance to its movement, as created by the surrounding medium. The force of this resistance \( F_- \) depends on the "size" of the solute (as described by its solvated radius \( r \)), the viscosity of the medium \( \eta \), and the solute’s velocity of migration \( v \) (in units of distance per time).

When an electric field is applied, a solute will accelerate toward the electrode of opposite charge until the forces \( F_+ \) and \( F_- \) become equal in size (although opposite in direction). At this point a steady-state situation is produced in which the solute begins to move at a constant velocity. This velocity can be found by setting the expressions for \( F_+ \) and \( F_- \) equal to each other and rearranging the resulting equation in terms of \( v \).

\[
6\pi\eta v = Ez \quad \text{or} \quad v = \frac{Ez}{6\pi\eta} \tag{23.1}
\]
To see how this velocity will be affected by only the strength of the electric field, we can combine the other terms in Equation 23.1 to give a single constant ($\mu$),

$$v = \mu E$$  \hspace{1cm} (23.2)

where $\mu = z/(6 \pi r \eta)$. This new combination of terms is known as the **electrophoretic mobility**, which is represented by the symbol $\mu$. The value of $\mu$ is often expressed in units of $\text{m}^2/\text{V} \cdot \text{s}$ or $\text{cm}^2/\text{kV} \cdot \text{min}$ and is constant for a given analyte under particular set of temperature and solvent conditions. The value of $\mu$ also depends on the apparent size and charge of the solute, as represented by the ratio $z/r$ in Equation 23.1. This last feature means that any two solutes with different charge-to-size ratios can, in theory, be separated by electrophoresis.

**EXERCISE 23.1**  **Determining the Electrophoretic Mobility for an Analyte**

The apparent electrophoretic mobility for an analyte in capillary electrophoresis can be found by rewriting Equation 23.2 in the form shown.

$$\mu = \frac{v}{E} = \frac{(L_d/t_m)}{(V/L)}$$  \hspace{1cm} (23.3)

In this equation, $V$ is the voltage applied to the electrophoretic system over a length $L$, and $L_d$ is the distance traveled from the point of application to the detector by the analyte in migration time $t_m$.

A sample of several proteins is applied to a neutral-coated capillary with a total length of 25.0 cm and a distance to the detector of 22.0 cm. Two of the proteins in the sample give migration times of 15.3 min and 16.2 min when using an applied voltage of 20.0 kV. What are the migration velocities and electrophoretic mobilities of these proteins under these conditions? What will their electrophoretic mobilities and migration times be at an applied voltage of 10.0 kV?

**SOLUTION**

The electrophoretic mobility of the first protein can be found by substituting the known values for $L_d$ (22.0 cm), $t_m$ (15.3 min), $V$ (20.0 kV), and $L$ (25.0 cm) into Equation 23.3.

Protein 1:  \hspace{1cm} $\mu = \frac{(22.0 \text{ cm}/15.3 \text{ min})}{(20.0 \text{ kV}/25.0 \text{ cm})} = 1.80 \text{ cm}^2/\text{kV} \cdot \text{min}$

A similar calculation for the second protein gives an electrophoretic mobility of $1.70 \text{ cm}^2/\text{kV} \cdot \text{min}$. The lower electrophoretic mobility of the second protein makes sense because it takes longer for this protein to migrate through the system. The migration velocities for these proteins can be found by simply dividing their distance of travel by their migration times ($v = L_d/t_m$), which gives (22.0 cm/15.3 min) = 1.44 cm/min and (22 cm/16.2 min) = 1.36 cm/min for proteins 1 and 2.

If we lower the applied voltage from 20 kV to 10 kV (a twofold change), the migration times will increase and the migration velocities for these proteins will decrease (also by twofold), but their electrophoretic mobilities will remain exactly the same. This situation occurs because the electrophoretic mobility is independent of voltage and electric field strength, while migration times and velocities are not. Thus, if there is a decrease in $V$ and $E$, Equation 23.3 indicates there must be a proportional decrease in $v$ and $t_m$ to keep $\mu$ constant.

**Secondary Interactions.** To obtain good separations in electrophoresis it is often necessary to adjust the conditions of this method to change the electrophoretic mobility of a solute. We can accomplish this goal by using side reactions that alter the charge or apparent size of the analyte. If an analyte is a weak acid or weak base, for example, its net charge can be varied by changing the pH. In the case of a weak monoprotic acid, the main species at a pH well below the $pK_a$ will be the neutral form of the acid (HA), while the dominant species at a pH much greater than the $pK_a$ will be the negatively charged conjugate base (A$^-$). At an intermediate pH, we will have a mixture of these two forms and the average charge for all of these species will be somewhere between “0” and “-1.” As a result, the overall observed electrophoretic mobility for such a compound (as well as for other weak acids and weak bases) can be adjusted by varying the pH.

It is also possible to use side reactions to change the effective size or charge of the analyte. This effect occurs in a method known as sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), which is a technique for separating proteins according to their size (see Section 23.4C). This analysis begins by first denaturing the proteins and coating them with sodium dodecyl sulfate, a negatively charged surfactant. The coating process can be thought of as a type of complexation reaction. The negative coating not only alters the overall charge but helps convert a protein into a rod-shaped structure, which alters its size and shape.

Another approach for altering the apparent electrophoretic mobility of an analyte is to use a solubility equilibrium. As an example, we could include a second phase within the running buffer into which the analyte can partition as it moves through the system (such as through the use of micelles, a method we will examine in Section 23.4C). Because the analyte in such a system will usually have different mobilities when it is present in the running buffer or in the second phase, the partitioning of an analyte between these regions leads to a change in the analyte’s rate of travel through the electrophoretic system. Physical interactions can also affect analyte migration. For instance, DNA sequencing by gel electrophoresis uses a porous support to separate DNA strands of different lengths. The same strategy is used in SDS-PAGE for protein separations.
Electroosmosis. Up until now we have examined only the direct movement of an analyte in an electric field. It is also possible for the running buffer to move in such a field. This phenomenon can occur if there are any fixed charges present in the system, such as on the interior surface of an electrophoretic system or on a support within this system (see Figure 23.6). The presence of these fixed charges attracts ions of opposite charge from the running buffer and creates an electrical double layer at the surface of the support. In the presence of an electric field, this double layer acts like a piston that causes a net movement of the buffer toward the electrode of opposite charge versus the fixed ionic groups. This process is known as electroosmosis and results in a net flow of the buffer and its contents through the system.\(^7\)

The extent to which electroosmosis affects the buffer and analytes in electrophoresis is described by using a term known as the electroosmotic mobility \(\mu_{eo}\).\(^7\) This term has the same units as the electrophoretic mobility \(\mu\). The value of \(\mu_{eo}\) depends on such factors as the size of the electric field, the type of running buffer that is being employed, and the type of charge that is present on the support. This relationship is described by Equation 23.4,

\[
\mu_{eo} = \frac{(e \xi E)}{\eta}
\]

where \(E\) is the electric field, \(e\) and \(\eta\) are the dielectric constant and viscosity of the running buffer, and \(\xi\) is the zeta potential (which represents the charge on the support).

Depending on the direction of buffer flow, electroosmosis can work either with or against the inherent migration of an analyte through the electrophoretic system. The overall observed electrophoretic mobility \(\mu_{Net}\) for an analyte will be equal to the sum of its own electrophoretic mobility \(\mu\) and the mobility of the running buffer due to electroosmotic flow \(\mu_{eo}\).

\[
\mu_{Net} = \mu + \mu_{eo}
\]

In gel electrophoresis, electroosmotic flow is often small compared to the inherent rate of analyte migration. This is not usually true in capillary electrophoresis, where the support has a relatively large charge and high surface area compared to the volume of running buffer (see Section 23.3).

23.2B Factors Affecting Band-Broadening

The same terms used to describe efficiency in chromatography (e.g., the number of theoretical plates \(N\) and the height equivalent of a theoretical plate \(H\)) can be used to describe band-broadening in electrophoresis. Two particularly important band-broadening processes in electrophoresis are (1) longitudinal diffusion and (2) Joule heating.

Longitudinal Diffusion. You may recall from Chapter 20 that longitudinal diffusion occurs when a solute diffuses away from the center of its band along the direction of travel, causing this band to broaden over time and to become less concentrated. One factor that affects the extent of this band-broadening is the “size” of the diffusing solute, or its solvated radius. Because larger analytes have slower diffusion, they will be less affected by longitudinal diffusion than smaller substances. The rate of this diffusion will also decrease as we increase the viscosity of the running buffer or lower the temperature of the system.

![Figure 23.6](image-url) The production and effects of electroosmosis. This particular example shows a support that has a negatively charged interior. Such a situation is often encountered when working with a support that is an uncoated silica capillary. The interior wall of this capillary has silanol groups at its surface, which can act as weak acids and form a conjugate base with a negative charge. The extent of electroosmosis in this case will depend on the pH of the running buffer, because this will affect the relative amount of the silanol groups that are present in their neutral acid form or charged conjugate base form.
The extent of longitudinal diffusion will depend on the amount of time that is allowed for this process to occur.\cite{10} This time, in turn, will be affected in electrophoresis by the size of the electric field, because lower electric fields result in smaller migration velocities and longer migration times.\cite{22} Electroosmosis will also affect the time needed for an electrophoretic separation and diffusion. If electroosmosis moves in a direction opposite to that desired for the separation of analytes, the effective rate of travel for these analytes is decreased and the time allowed for longitudinal diffusion is increased. If electroosmosis instead occurs in the same direction as analyte migration, longitudinal diffusion is decreased.

One way we can minimize the effects of longitudinal diffusion in electrophoresis is to have an analyte move through a porous support. If the pores of this support are sufficiently small, they will inhibit the movement of analytes due to diffusion and help provide narrower bands. If the pore size becomes too small, a size-based separation will also be created. Although this last feature is not always desirable, in some cases it can be an advantage, such as in the sequencing of DNA by gel electrophoresis.

**Joule Heating.** The most important band-broadening process in electrophoresis is often *joule heating*.\cite{21-23} This process is caused by heating that occurs whenever an electric field is applied to the system. According to *Ohm’s law* (see Chapter 14), placing a voltage $V$ across a medium with a resistance of $R$ requires that a current of $I$ be present to maintain this voltage across the medium.\cite{10}

\begin{equation}
V = I \cdot R \tag{23.6}
\end{equation}

As current flows through the system, heat is generated. This heat production depends on the voltage, current, and time $t$ the current passes through the system, as shown below.

\begin{equation}
\text{Heat} = V \cdot I \cdot t \tag{23.7}
\end{equation}

As heat is produced, the temperature of the electrophoretic system will begin to rise. This rise in temperature will increase longitudinal diffusion and lead to increased band-broadening. In addition, if the heat is not distributed uniformly throughout the electrophoretic system, the temperature will not be the same throughout the system. An uneven temperature will lead to regions with different densities (causing mixing) and different rates of diffusion, which results in even more band-broadening. Other problems created by an increase in temperature include possible degradation of the analytes or components of the system and the evaporation of solvent from the running buffer, the latter of which can alter the pH and composition of the buffer. All of these factors lead to a loss of reproducibility and efficiency in the system.

One way Joule heating can be decreased is by using a lower voltage for the separation. A lower voltage, however, will lower the migration velocities of analytes and give longer separation times. An alternative approach is to use more efficient cooling for the system, which would allow higher voltages to be used and provide shorter separation times. Another possibility is to add a support to the electrophoretic system that minimizes the effects of joule heating due to uneven heat distribution and density gradients in the running buffer. Examples of these approaches will be given later when we examine the methods of gel electrophoresis and capillary electrophoresis.

Another factor that affects Joule heating is the ionic strength of the running buffer. A lower ionic strength for this buffer will lower heat production, because at low ionic strengths there are fewer ions in this buffer. This lower ionic strength creates a greater resistance $R$ to current flow at any given voltage because fewer ions are available to carry the current. We can see from Ohm’s law in Equation 23.6 that as $R$ increases a smaller current is needed at voltage $V$. This smaller current, in turn, will create lower heat production, as shown by Equation 23.7.

**Other Factors.** Eddy diffusion (a process we discussed in Chapter 20 for chromatography) is another factor that can sometimes lead to band-broadening in electrophoresis. This type of band-broadening can occur if a support is used to minimize the effects of Joule heating, a situation that creates multiple flow paths for analytes through the support. If the support interacts with analytes, band-broadening due to these secondary interactions will be introduced as well; this extra band-broadening also occurs when secondary interactions are used to adjust analyte mobility, such as complexation reactions or partitioning into a micelle. These latter effects are similar to those described in Chapter 20 for stationary phase mass transfer in chromatography. Broadening of the peaks before or after separation can be another issue when dealing with highly efficient systems, such as those used in capillary electrophoresis.

*Wick flow* is another source of band-broadening that occurs in gel electrophoresis.\cite{19} In such a system, the gel is kept in contact with the electrodes and buffer reservoirs through the use of wicks. Because this support is often open to air, the presence of any Joule heating will lead to some evaporation of solvent in the running buffer from the support. As this solvent is lost, it is replenished by the flow of more solvent through the wicks and from the buffer reservoirs. This flow leads to a net movement of buffer from each reservoir towards the center of the support. The rate of this flow depends on the rate of solvent evaporation, so it will increase with the use of a high voltage or high current. The extent of this flow varies across the support, with the fastest rates occurring furthest from the center of the support.

### 23.3 GEL ELECTROPHORESIS

#### 23.3A What Is Gel Electrophoresis?

One of the most common types of electrophoresis is the method of *gel electrophoresis*. This technique is an electrophoretic method that is performed by applying a sample...
to a gel support that is then placed into an electric field.\textsuperscript{17–20} Typical separations obtained by gel electrophoresis were shown previously in Figures 23.1 and 23.4. In this type of system, several samples are usually applied to the gel and allowed to migrate along the length of the support in the presence of an applied electric field. The separation is stopped before analytes have left the end of the gel, with the location and intensities then being determined.

It is important to remember in gel electrophoresis that the velocity of an analyte's movement will be related to the distance it has traveled in the given separation time (as represented by the migration distance). The farther this distance is from the point of sample application, the higher the migration velocity is for the analyte and the larger its electrophoretic mobility. This migration distance will, in turn, be related to the size and charge of the analyte and can be used in identifying such a substance.

\subsection*{23.3B How Is Gel Electrophoresis Performed?}

\textbf{Equipment and Supports.} Some typical systems for carrying out gel electrophoresis are shown in Figure 23.7. These systems may have a support that is held in either a vertical or horizontal position. This support contains a running buffer with ions that carry a current through the support when an electric field is applied. To replenish this buffer and its components as they move through the support or evaporate, the ends of the support are placed in contact with two reservoirs that contain the same buffer solution and the electrodes. Once samples have been placed on the support, the electrodes are connected to a power supply and used to apply a voltage across the support. This electric field is passed through the system for a given amount of time, causing the sample components to migrate. After the electric field has been turned off, the gel is removed and examined to locate the analyte bands.

The type of support we use in such a system will depend on our analytes and samples.\textsuperscript{17,19} Cellulose acetate, filter paper, and starch are useful supports for work with relatively small molecules, like amino acids and nucleotides. Electrophoresis involving large molecules can be carried out on agarose, a support that we discussed in Chapter 22. The resulting approach is known as “agarose electrophoresis.” In addition to its low nonspecific binding for many biological compounds, agarose has a low inherent charge. Agarose also has relatively wide pores that allow it to be employed in work with large molecules, such as during the sequencing of DNA.

The most common support used in gel electrophoresis is polyacrylamide. This combination is often referred to as \textit{polyacrylamide gel electrophoresis}, or PAGE.\textsuperscript{17–19} Polyacrylamide is a synthetic, transparent polymer that is prepared as shown in Figure 23.8. It can be made with a variety of pore sizes that are smaller than those in agarose and of a size more suitable for the separation of proteins and peptide mixtures. Like agarose, polyacrylamide has low nonspecific binding for many biological compounds and does not have any inherent charged groups in its structure.

\textbf{Sample Application.} The samples in gel electrophoresis are applied to small “wells” that are made in the gel during its preparation (see Figures 23.4 and 23.7). A sample volume of 10–100 µL is then placed into one of these wells by using a micropipette. These sample volumes help provide a sufficient amount of analyte for later detection and collection, but they also create a danger of introducing band-broadening by creating a large sample band at the beginning of the separation.

A common approach to create narrow sample bands is to employ two types of gels in the system: a “stacking gel” and a “running gel.”\textsuperscript{19} The running gel is the support used for the electrophoretic separation of substances in the sample. In a vertical gel electrophoresis system, this gel is formed first and is located throughout the middle and lower section of the system (see right-hand portion of Figure 23.7). The stacking gel has a lower degree of cross-linking (giving it larger pores) and is located on top of the running gel. The stacking gel is also the section of the support in which the sample wells are located. After a sample has been placed in the wells and an electric field has been applied, analytes will travel quickly through the stacking gel until they reach its boundary with the running gel.
Analytic ability of these substances can be employed for their absorbance measurements and a scanning device known as a densitometer.20

Detection Methods. There are several ways analytes can be detected in gel electrophoresis. Analyte bands can be examined directly on the gel or they can be transferred to a different support for detection. Direct detection can sometimes be performed visually (when dealing with intensely colored proteins like hemoglobin) or by using absorbance measurements and a scanning device known as silver staining to detect low concentration proteins. DNA bands can be detected by using ethidium bromide (see Chapter 2). When separating enzymes, the natural catalytic ability of these substances can be employed for their detection, as occurs when using the fluorescent compound NAD(P)H to detect enzymes that generate this substance in their reactions.19,20

Another possible approach for detection in gel electrophoresis is to transfer a portion of the analyte bands to a second support (such as nitrocellulose), where they are reacted with a labeled agent. This approach is known as “blotting.”19 There are several blotting methods. One such method is a Southern blot (named after its discoverer Edwin Southern, a British biologist).24 A Southern blot is used to detect specific sequences of DNA by having these sequences bind to an added, known sequence of DNA that is labeled with a radioactive tag (³²P) or with a label that can undergo chemiluminescence. A Northern blot (which was developed after the Southern blot) is similar, but is instead used to detect specific sequences of RNA by using a labeled DNA probe.25

Another type of blotting method is a Western blot.26,27 A Western blot is used to detect specific proteins on an electrophoresis support. In this technique, proteins are first separated on a support by electrophoresis and then blotted onto a second support like nitrocellulose or nylon. The second support is then treated with labeled antibodies that can specifically bind the proteins of interest. After the antibodies and proteins have been allowed to form complexes, any extra antibodies are washed away and the remaining bound antibodies are detected through their labels, indicating whether there is any of the protein of interest present. This method is used to screen blood for the HIV virus by looking for the presence of proteins from this virus in samples.

There also has been growing interest in the use of instrumental methods for analyzing bands on electrophoresis supports. For instance, mass spectrometry is becoming a popular method for determining the molecular mass of a protein in a particular band. Such an analysis is accomplished by removing a portion of the band from the gel (or sometimes by looking at the gel directly) and examining this band by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) (see Box 23.1). This approach makes it possible to identify a particular analyte (such as a protein) by its molecular mass even when there are many similar analytes in a sample.

23.3C What Are Some Special Types of Gel Electrophoresis?
Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis. Whenever a porous support is present in an electrophoretic system, it is possible that large analytes may be separated based on their size as well as their electrophoretic mobilities. This size separation occurs in a manner similar to that which occurs in size-exclusion chromatography and can be used to determine the molecular weight of biomolecules. This type of analysis is accomplished for proteins in a technique known as sodium dodecyl sulfate polyacrylamide gel electrophoresis, or SDS-PAGE (see Figure 23.10).18,19

![Fig 23.8 Preparation of a polyacrylamide gel. In this reaction, acrylamide is used as the monomer and bisacrylamide is used as a cross-linking agent. The reaction of these two agents is begun by adding ammonium persulfate, where persulfate (SO₄²⁻) forms sulfate radicals (SO₄⁻) that cause the acrylamide and bisacrylamide to combine. N,N,N',N'-Tetramethylethylenediamine (TEMED) is added to this mixture as a reagent that stabilizes the sulfate radicals.](image-url)
Chapter 23 • Electrophoresis

In SDS-PAGE, the proteins in a sample are first denatured and their disulfide bonds broken through the use of a reducing agent. This pretreatment converts the proteins into a set of single-stranded polypeptides. These polypeptides are then treated with sodium dodecyl sulfate (SDS), a surfactant with a nonpolar tail and a negatively charged sulfate group. The nonpolar end of this surfactant coats each protein, forming roughly linear rods that have an exterior layer of negative charge. The result for a mixture of proteins is a series of rods with different lengths but similar charge-to-mass ratios. Next, these protein rods are passed through a porous polyacrylamide gel in the presence of an electric field. The negative charges on these rods (from the SDS coating) cause them to all move toward the positive electrode, while the pores of the gel allow small rods to travel more quickly to this electrode than large rods.

At the end of an SDS-PAGE run, the positions of protein bands from a sample are compared to those obtained for known protein standards applied to the same gel. This comparison is made either qualitatively or by preparing a calibration curve. The calibration curve is typically prepared by plotting the log of the molecular weight (MW) for the protein standards versus their migration distance ($d_m$) or retardation factor ($R_f$). The retardation factor for an analyte band in SDS-PAGE is calculated by using the ratio of a protein’s migration distance over the migration distance for a small marker compound ($d_s$), where $R_f = d_m/d_s$. The resulting plot of log(MW) versus $d_m$ or $R_f$ gives a curved response with an intermediate linear region for proteins with sizes that are neither totally excluded from the pores nor able to access all pores in the support.

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) is a type of mass spectrometry in which a special matrix capable of absorbing light from a laser is used for chemical ionization. The term “MALDI” was first used in 1985 to describe the use of a laser to cause ionization of the amino acid alanine in the presence of tryptophan (the “matrix” in this case). In 1988 it was shown almost simultaneously by two research groups, one in Germany and one in Japan, that MALDI-TOF MS could also be employed in work with large biomolecules, such as proteins. The value of this method was recognized in 2002 when members of both these groups shared the Nobel Prize in chemistry for the development of this technique.

Figure 23.9 shows the typical way in which a sample is analyzed by MALDI-TOF MS. First, the sample is mixed with a matrix that can readily absorb UV light. This mixture is then placed on a holder in the MALDI-TOF instrument, where pulses of a UV laser are aimed at the sample and matrix. As the matrix absorbs some of this light, it transfers its energy to molecules in the sample, causing these to form ions. These ions are then passed through an electric field into a time-of-flight mass analyzer, where ions of different mass-to-charge ratios will travel at different velocities. The number of ions arriving at the other end is measured at various times, allowing a mass spectrum to be obtained for analytes in the sample.

MALDI-TOF MS is a soft ionization approach that results in a large amount of molecular ions and few, if any, fragment ions for most analytes. This method also has a low background signal, a high mass accuracy, and can be used over a wide range of masses. These properties make MALDI-TOF MS valuable in the study and identification of proteins after they have been separated by techniques like SDS-PAGE or 2-dimensional (2-D) electrophoresis (see Section 23.3). MALDI-TOF MS can also be used to look at peptides, polysaccharides, nucleic acids, and some synthetic polymers.

In SDS-PAGE, the proteins in a sample are first denatured and their disulfide bonds broken through the use of a reducing agent. This pretreatment converts the proteins into a set of single-stranded polypeptides. These polypeptides are then treated with sodium dodecyl sulfate (SDS), a surfactant with a nonpolar tail and a negatively charged sulfate group. The nonpolar end of this surfactant coats each protein, forming roughly linear rods that have an exterior layer of negative charge. The result for a mixture of proteins is a series of rods with different lengths but similar charge-to-mass ratios. Next, these protein rods are passed through a porous polyacrylamide gel in the presence of an electric field. The negative charges on these rods (from the SDS coating) cause them to all move toward the positive electrode, while the pores of the gel allow small rods to travel more quickly to this electrode than large rods.

At the end of an SDS-PAGE run, the positions of protein bands from a sample are compared to those obtained for known protein standards applied to the same gel. This comparison is made either qualitatively or by preparing a calibration curve. The calibration curve is typically prepared by plotting the log of the molecular weight (MW) for the protein standards versus their migration distance ($d_m$) or retardation factor ($R_f$). The retardation factor for an analyte band in SDS-PAGE is calculated by using the ratio of a protein’s migration distance over the migration distance for a small marker compound ($d_s$), where $R_f = d_m/d_s$. The resulting plot of log(MW) versus $d_m$ or $R_f$ gives a curved response with an intermediate linear region for proteins with sizes that are neither totally excluded from the pores nor able to access all pores in the support.
**Sample pretreatment**

- Denature proteins and reduce disulfide bonds
- Coat proteins with SDS

![Diagram of Sample pretreatment]

**Protein separation**

- Standard
- Sample 1
- Sample 2

<table>
<thead>
<tr>
<th></th>
<th>High MW</th>
<th>Low MW</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sample 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sample 2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

![Diagram of Protein separation]

**FIGURE 23.10** Preparation of proteins and their separation by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE).

**EXERCISE 23.2 Using SDS-PAGE for Estimating the Molecular Mass of a Protein**

The proteins in the standard in Figure 23.10 have molecular weights (from top-to-bottom) of 200, 116, 97, 66, 45, 31, 23, and 14 kDa. What are the molecular weights of the proteins in sample 1?

**SOLUTION**

The first band in sample 1 is at approximately the same location as the 66 kDa band in the standard sample. The second band in sample 1 appears between the 45 kDa and 31 kDa bands in the standard, giving this second protein a mass of roughly 38 kDa. A similar analysis for the second sample gives proteins with estimated masses of 31 and 97 kDa.

**Isoelectric Focusing.** Another type of electrophoresis that often employs supports is **isoelectric focusing (IEF)**. IEF is a method used to separate zwitterions (substances with both acidic and basic groups, as discussed in Chapter 8). Zwitterions are separated in IEF based on their isoelectric points by having these compounds migrate in an electric field across a pH gradient. In this pH gradient, each zwitterion will migrate until it reaches a region where the pH is equal to its isoelectric point. At this point, the zwitterion will no longer have any net charge and its electrophoretic mobility will become zero, causing the analyte to stop migrating. The result is a series of tight bands, where each band appears at the point where pH = pI for a given zwitterion.

The reason isoelectric focusing produces tight bands for these analytes is that even if a zwitterion momentarily diffuses out of the region where the pH is equal to its pI, the system will tend to “focus” the zwitterion back into this region (see Figure 23.11). This focusing occurs because of the way the pH gradient is aligned with the electric field. High pH’s occur toward the negative electrode, so as solutes diffuse out of their band and...
toward this region they will take on a more negative charge and be attracted back to the positive electrode. At the same time, zwitterions that move toward the positive electrode and region of lower pH will acquire a more positive charge and be attracted back toward the negative electrode. It is this focusing property that makes it possible for IEF to separate zwitterions with only very small differences in their pI values.

To obtain a separation in IEF, it is necessary to have a stable pH gradient. This pH gradient is produced by placing in the electric field a mixture of small reagent zwitterions known as ampholytes. These are usually polyprotic amino carboxylic acids with a range of pKₐ values. When these ampholytes are placed in an electric field, they will travel through the system and align in the order of their pKₐ values. The result is a pH gradient that can be used directly or by cross-linking the ampholytes to a support to keep them stationary in the system.

IEF is a valuable tool for separating proteins or other compounds that contain both positive and negative charges. These include some drugs, as well as bacteria, viruses, and cells. Applications of this method range from biotechnology and biochemistry to forensic analysis and paternity testing. IEF is particularly useful in providing high-resolution separations between different forms of enzymes or cell products. For instance, it is possible with this method to separate proteins with differences in pI values as small as 0.02 pH units.

2-Dimensional Electrophoresis. Another way gel electrophoresis can be utilized is in two-dimensional (or 2-D) electrophoresis, which is a high-resolution technique used to look at complex protein mixtures. In this method, two different types of electrophoresis are conducted on a single sample. The first of these separations is usually based on a isoelectric point, as accomplished by using isoelectric focusing. The second separation method (SDS-PAGE) is according to size.

A typical 2-D electrophoresis method is illustrated in Figure 23.12. First, a small band of sample is applied to the top of a support for use in isoelectric focusing. The support used in this case is typically agarose or a polyacrylamide gel with large pores. After this first separation has been finished, some proteins will have been separated based on their pI values, but there may still be many proteins with similar isoelectric points and overlapping bands. A further separation is obtained by turning this first gel on its side and placing it at the top of a second support (a polyacrylamide gel) for use in SDS-PAGE. This process gives a separation according to size, in which each band from the first separation has its own lane on the SDS-PAGE gel. The result is a series of peaks that are now separated in two dimensions (one based on pI and the other on size) across the gel. The fact that two different characteristics of each protein are used in their separation makes it possible to resolve a much larger number of proteins than is possible by either IEF or SDS-PAGE alone.

![Figure 23.12](image-url) Two-dimensional gel electrophoresis, using a combination of isoelectric focusing and SDS-PAGE as an example.

After a 2-D separation has been finished, the protein bands can be detected using the methods discussed in Section 23.3B. Staining with Coomassie blue or silver nitrate is often used in the location and measurement of these bands. Analysis by mass spectrometry is another option. Other issues to consider are the interpretation and analysis of the many protein bands that can occur in a single sample. This analysis requires the use of computers to help image and catalog the location of each band and to correlate this information with that obtained by other methods, such as mass spectrometry.

23.4 CAPILLARY ELECTROPHORESIS

23.4A What Is Capillary Electrophoresis?

Another type of electrophoresis is the method of capillary electrophoresis (CE). CE is a technique that separates analytes by electrophoresis and that is carried out in a capillary. This method was first reported in the late 1970s and early 1980s and is sometimes known as “capillary zone electrophoresis.” CE in its current form is typically conducted in capillaries with inner diameters of 20–100 µm and lengths of 20–100 cm. The use of these narrow-bore tubes provides efficient removal of Joule heating by allowing this heat to be quickly dissipated to the surrounding environment. This removal of heat helps to decrease band-broadening and provides much more efficient and faster separations than gel electrophoresis (see Figure 23.13).

One reason capillary electrophoresis is more efficient than gel electrophoresis is that Joule heating is greatly reduced as a source of band-broadening. Also, capillary electrophoresis is often used with no gel or support present, which eliminates eddy diffusion and secondary interactions with the support (other than the capillary wall). The result is that longitudinal diffusion now becomes the main source of band-broadening. Under these conditions,
20.0 kV and at 30.0 kV? What factors may cause lower values for \( N \) to be obtained?

**SOLUTION**

We can use Equation 23.8 along with the conditions given in Exercise 23.1 and the electrophoretic mobility calculated earlier for protein 1 to get the expected value for \( N \) at 20.0 kV.

\[
N = \frac{(1.80 \text{ cm}^2/\text{K} \cdot \text{min}) \cdot 20.0 \text{ kV} \cdot 22.0 \text{ cm}}{2 \cdot (2.0 \times 10^{-7} \text{ cm}^2/\text{s}) \cdot (60 \text{ s/min}) \cdot 25.0 \text{ cm}}
\]

\[
= 1.3 \times 10^6 \text{ theoretical plates}
\]

If we increase the applied voltage from 20.0 to 30.0 kV (or by 1.5-fold), Equation 23.8 indicates we will see a proportional increase of 1.5-fold in \( N \) from 1.3 \( \times \) 10\(^6\) to 1.9 \( \times \) 10\(^6\) plates. Factors that might give lower plate numbers include the presence of adsorption between the protein and capillary wall, extra-column band-broadening, or an increase in Joule heating as the voltage is increased.

Besides providing efficient separations, we have seen that the use of high electric fields in capillary electrophoresis also reduces the time needed for a separation. This relationship can be shown by rewriting Equation 23.3 to give the expected migration time for an analyte in terms of the electric field, the electrophoretic mobility of the analyte, and the length of the capillary.

\[
t_m = \frac{L_d L}{\mu V} = \frac{L_d}{\mu E}
\]

For instance, Equation 23.9 indicates that the migration time for the protein in Exercise 23.3 will decrease by 1.5-fold (from 15.3 to 10.2 min) if we increase the applied voltage from 20.0 to 30.0 kV. The result is a situation in which we can improve both the efficiency and speed of a separation by increasing the voltage. This feature has made capillary electrophoresis popular for the analysis of complex samples, such as those used in DNA sequencing. Unfortunately, there is a limit to how high the voltage can be increased before Joule heating again becomes important. Most CE systems are capable of using voltages of up to 25–30 kV, but significant Joule heating can appear at lower voltages.

### EXERCISE 23.3

**The Effect of Electric Field Strength on Efficiency in Capillary Electrophoresis**

The protein 1 in Exercise 23.1 has a diffusion coefficient of approximately \( 2.0 \times 10^{-7} \text{ cm}^2/\text{s} \) in its running buffer. If longitudinal diffusion is the only significant band-broadening process present during the separation of this protein by capillary electrophoresis, what is the maximum number of theoretical plates that would be expected for this protein’s peak at an applied voltage of 20.0 kV and at 30.0 kV? What factors may cause lower values for \( N \) to be obtained?

**SOLUTION**

We can use Equation 23.8 along with the conditions given in Exercise 23.1 and the electrophoretic mobility calculated earlier for protein 1 to get the expected value for \( N \) at 20.0 kV.

\[
N = \frac{(1.80 \text{ cm}^2/\text{K} \cdot \text{min}) \cdot 20.0 \text{ kV} \cdot 22.0 \text{ cm}}{2 \cdot (2.0 \times 10^{-7} \text{ cm}^2/\text{s}) \cdot (60 \text{ s/min}) \cdot 25.0 \text{ cm}}
\]

\[
= 1.3 \times 10^6 \text{ theoretical plates}
\]

If we increase the applied voltage from 20.0 to 30.0 kV (or by 1.5-fold), Equation 23.8 indicates we will see a proportional increase of 1.5-fold in \( N \) from 1.3 \( \times \) 10\(^6\) to 1.9 \( \times \) 10\(^6\) plates. Factors that might give lower plate numbers include the presence of adsorption between the protein and capillary wall, extra-column band-broadening, or an increase in Joule heating as the voltage is increased.

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For instance, Equation 23.9 indicates that the migration time for the protein in Exercise 23.3 will decrease by 1.5-fold (from 15.3 to 10.2 min) if we increase the applied voltage from 20.0 to 30.0 kV. The result is a situation in which we can improve both the efficiency and speed of a separation by increasing the voltage. This feature has made capillary electrophoresis popular for the analysis of complex samples, such as those used in DNA sequencing. Unfortunately, there is a limit to how high the voltage can be increased before Joule heating again becomes important. Most CE systems are capable of using voltages of up to 25–30 kV, but significant Joule heating can appear at lower voltages.

### 23.4B How Is Capillary Electrophoresis Performed?

**Equipment and Supports.** Besides being faster and more efficient than gel electrophoresis, capillary electrophoresis is easier to perform as part of an instrument system. An example of a CE system is shown in Figure 23.14.8,21 Along with the capillary, this system includes a power supply and electrodes for applying the electric field, two containers that create a contact
between these electrodes and the solution within the capillary, an on-line detector, and a means for injecting samples onto the capillary. Because these instruments can use voltages of up to 25–30 kV, they include safety features that protect the user from the high-voltage region and that can turn off this voltage when the system is opened for maintenance or the insertion of samples and reagents.

The capillary in a CE system is typically made of fused silica. This capillary can be used directly or it can be modified to place various coatings on its interior surface. An uncoated silica capillary can lead to a significant amount of flow due to electroosmosis when working at a neutral or basic pH, due to deprotonation of the silica’s surface silanol groups. One useful feature of this electroosmosis is it tends to cause all analytes, regardless of their charge, to travel in the same direction through the CE capillary. This effect means that a sample containing many types of ions can be injected at one end of the capillary (at the positive electrode), with electroosmosis then carrying these through to the other end (to the negative electrode) and past an on-line detector. This format is called the “normal polarity mode” of CE. In addition, electroosmotic flow can be altered by changing the pH (which changes the degree of deprotonation and charge on silica), or by placing a coating on the surface of the support. In this second case, a neutral coating helps to reduce electroosmosis while a positively charged coating will reverse the direction of this flow toward the positive rather than negative electrode.

**Injection Techniques.** There are two features of capillary electrophoresis that place special demands on how samples can be injected. First, the small volume of a CE capillary must be considered. A typical 50 µm I.D. × 25 cm long capillary for CE will contain only 0.5 µL of running buffer. Another factor to consider is the high efficiency of capillary electrophoresis. Both of these factors restrict the sample volumes that can be injected without introducing significant band-broadening (< 10 nL for a 0.5 µL volume capillary).

There are two techniques that make it possible to inject these small sample volumes onto a CE system. The first technique is **hydrodynamic injection**, which uses a difference in pressure to deliver a sample to the capillary. This method can be carried out by placing one end of the capillary into the sample in an enclosed chamber and applying a pressure to this chamber for a fixed period of time, where the amount of injected sample will depend on the size of the pressure difference and the amount of time that this pressure is applied. Once the sample has entered the capillary, the separation is begun after the capillary
has been put back into contact with the running buffer and electrodes. A second technique that allows the injection of small sample volumes is electrokinetic injection. This method again begins by placing the capillary into the sample, but an electrode is also now in contact with the sample. When an electric field is applied across the capillary, electroosmotic flow and the electrophoretic mobility of the analytes cause them to enter the capillary. The amount of each analyte that is injected in this method will depend on the analyte’s electrophoretic mobility, the electric field, and the time over which this field is applied.8

There are various methods for concentrating samples and providing narrow analyte bands in CE. One such method is sample stacking (see Figure 23.15).21 Sample stacking occurs when the ionic strength (and therefore the conductivity) of the sample is less than that of the running buffer. When an electric field is applied to such a system, analytes will migrate quickly through the sample matrix until they come to the boundary between the sample and running buffer. Because the running buffer has a higher ionic strength than the sample, the rate of analyte migration decreases at this boundary. This decrease in migration rate causes the analytes to concentrate into a narrower band as they enter the running buffer. The overall effect is similar to what occurs when using stacking gels in traditional electrophoresis.

Detection Methods. Examples of detection methods that are used for capillary electrophoresis are shown in Table 23.1. Many of these methods are also used in liquid chromatography (see Chapter 22).8,21 An important difference between detection in LC and CE is the need in CE for methods that can work with very small sample sizes. This need is a result of the small injection volumes that are required in capillary electrophoresis to avoid excessive band-broadening. Selective monitoring methods that work well for this purpose are electrochemical and fluorescence detection. Ultraviolet-visible (UV-vis) absorbance, conductance, and mass spectrometry detection are also often employed in CE.

Another difference between detection in LC and CE concerns how their signals vary with analyte retention or migration. In LC, all analytes pass at the same flow rate (that of the mobile phase) through the detector and spend the same amount of time in this device. This effect makes it possible to directly compare the peak areas of two analytes with different retention times. However, in capillary electrophoresis analytes with different migration times also spend different amounts of time in the detector. A correction must be made for this difference if we wish to compare the areas of two analytes in the same CE run. We can make this adjustment by using a corrected peak area ($A_c$), which is equal to the ratio of the measured peak area ($A_m$) for an analyte divided by its migration time.

$$A_c = \frac{A}{t_m} \quad (23.10)$$

<table>
<thead>
<tr>
<th>Detector Name</th>
<th>Compounds Detected</th>
<th>Detection Limits</th>
</tr>
</thead>
<tbody>
<tr>
<td>General detectors</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ultraviolet-visible (UV/vis) absorbance detector</td>
<td>Compounds with chromophores</td>
<td>$10^{-13}$–$10^{-16}$ mol</td>
</tr>
<tr>
<td>Selective detectors</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fluorescence detector</td>
<td>Fluorescent compounds</td>
<td>$10^{-15}$–$10^{-17}$ mol</td>
</tr>
<tr>
<td>Laser-induced fluorescence detector</td>
<td>Fluorescent compounds</td>
<td>$10^{-18}$–$10^{-20}$ mol</td>
</tr>
<tr>
<td>Conductivity detector</td>
<td>Ionic compounds</td>
<td>$10^{-15}$–$10^{-16}$ mol</td>
</tr>
<tr>
<td>Electrochemical detector</td>
<td>Electrochemically active compounds</td>
<td>$10^{-18}$–$10^{-19}$ mol</td>
</tr>
<tr>
<td>Structure-specific detectors</td>
<td>Compounds forming gas-phase ions</td>
<td>$10^{-16}$–$10^{-17}$ mol</td>
</tr>
</tbody>
</table>

*These data are for commercial instruments.
This correction allows areas for different analytes to be compared, as well as areas that are obtained for the same analyte under different electrophoretic conditions.

**EXERCISE 23.4 Correcting Peak Areas for Analyte Migration Times**

Proteins 1 and 2 in Exercise 23.1 have measured areas of 1290 and 1360 units at 20.0 kV when examined by an absorbance detector. If it is known that these two proteins have a similar response to the detector, what is the corrected area and relative amount of each protein in the given sample?

**SOLUTION**

The migration times for these proteins (given in Exercise 23.1) are 15.3 min and 16.2 min. Placing these data into Equation 23.10 along with the measured peak areas gives the following results.

- Protein 1:  \[ A_{c,1} = \frac{1290}{15.3 \text{ min}} = 84.3 \]
- Protein 2:  \[ A_{c,2} = \frac{1360}{16.2 \text{ min}} = 84.0 \]

If these proteins have a similar response to the detector, then we can say from these corrected areas that there is approximately the same amount of both proteins in the sample. If we had used the uncorrected areas for this calculation, we would have incorrectly concluded that protein 2 was present at a greater level.

Along with the various detection methods we discussed for liquid chromatography in Chapter 22, another detection approach that is used in capillary electrophoresis is **laser-induced fluorescence (LIF)**. This method employs a laser to excite a fluorescent compound, allowing the detection of this agent through its subsequent emission of light. There are several advantages to using a laser as the excitation source. First, the laser is monochromatic and has a high intensity, which allows for the selective and strong excitation of a compound with an excitation spectrum that overlaps with the emission wavelength of the laser. Also, the laser beam can be focused as a very narrow beam. This feature is extremely valuable in work with the small-bore capillaries found in capillary electrophoresis. One limitation of LIF detection is that it does require an analyte that is naturally fluorescent or that can be converted into a fluorescent derivative. This second option makes use of a fluorescent tag like fluorescein or rhodamine (see Chapter 18).

LIF detection with CE was used in the automated DNA sequencing systems that made early completion of the Human Genome Project possible. This detection involved the use of several fluorescent dyes, one for each of the four terminating nucleotides present during the Sanger reaction. These labeled DNA strands were then separated based on their lengths by capillary electrophoresis (see Figure 23.16). It was possible to further increase the speed of this analysis by using a single laser beam to simultaneously examine a whole array of capillaries, each sequencing a different segment of DNA. The utilization of multiple capillaries is called **multiplexing**, and this has been a major contributor to the completion of the Human Genome Project.

**FIGURE 23.16** DNA sequencing by a capillary electrophoresis system. The top panel shows the original electrophoretic data, and the bottom panel shows the same data after they have been processed to determine the nucleotide sequence of the DNA segment being examined (as given by the symbols A, C, T, and G). (Based on data from J. Bashkin, in Capillary Electrophoresis of Nucleic Acids, K.R. Mitchelson and J. Cheng, Eds., Humana Press, Totowa, NJ, 2001, Chapter 7.)
capillaries in a single CE system is known as capillary array electrophoresis (CAE). Such a system can examine many DNA sequences at the same time, which increases sample throughput and lowers the cost per analysis.

23.4C What Are Some Special Types of Capillary Electrophoresis?

The main capillary electrophoresis method that has been discussed up to this point is zone electrophoresis, in which differences in the charge/size ratio of analytes is the only means employed for their separation. It is possible to include other chemical and physical interactions in a CE system to give additional types of separations. Examples include CE methods that separate analytes based on their size, isoelectric points, or interactions with additives in the running buffer. The use of CE in microanalytical systems has also been a topic of great interest (see Box 23.2).

Capillary Sieving Electrophoresis. One useful feature of gel electrophoresis is the ability of some supports to separate analytes based on size, as occurs for proteins in SDS-PAGE. The same effect can be obtained in capillary electrophoresis by including an agent in the CE system that “sieves” the analytes, or separates them based on size. This approach is known as Capillary Sieving Electrophoresis.

BOX 23.2
Analytical Chemistry on a Chip

The development of silicon microchips created a revolution in the computer and electronic industries. The result over the past few decades has been a continuous decrease in the size of electronic devices and an increase in their capabilities. A similar change is occurring in analytical chemistry. This change began in 1979, when methods developed for the creation of microchips where used to make a gas chromatographic system on a silicon wafer. In 1990 it was proposed that all of the components of a chemical analysis could even be placed onto a miniaturized system. The resulting device is now known as a “lab-on-a-chip” or micro total-analysis system (μTAS).

Capillary electrophoresis was one of the first analytical methods that was adapted for use on a microchip. An example of such a device is given in Figure 23.17. There are now many reports that have used microchips for CE. One feature that makes CE and microchips a good match is the need in CE for narrow channels to avoid the effects of Joule heating. In addition, the elimination of Joule heating allows CE to work with short separation channels and high electric fields, as is used in Figure 23.17. The creation of an electric field to separate analytes and to generate electroosmotic flow for CE is relatively easy to obtain with a microchip by including electrodes as part of this device. The availability of detection schemes like LIF that are capable of working with small detection volumes is also valuable when placing a CE system on a microchip.

CE is not the only analytical method that has been carried out on microchips. Other methods have included liquid chromatography, gel electrophoresis, biosensing, water analysis, flow injection analysis, and solid-phase extraction. There are several potential advantages of using microchips with these techniques. One is the small sample requirements of these devices. The ability to make these systems fully portable or disposable are additional advantages. The possibility of making microchips on a large scale and at a low cost are other attractive features.

as capillary sieving electrophoresis (CSE). A comparison of the results of CSE and a size separation by gel electrophoresis (e.g., by SDS-PAGE) is given in Figure 23.18.

There are several ways we can perform capillary sieving electrophoresis. The first way is to place a porous gel in the capillary, like the polyacrylamide gels employed in SDS-PAGE. This method is called capillary gel electrophoresis (CGE). One problem with these gels is they are not always stable in the high electric fields used in capillary electrophoresis and must frequently be replaced. A second approach, and the one now used in DNA sequencing by CE, is to add to the running buffer a large polymer that can entangle with analytes and alter their rate of migration. This approach provides a system with better reproducibility and stability than those using gels, because the polymer is continuously renewed as the running buffer passes through the capillary.

Electrokinetic Chromatography. Ordinary capillary electrophoresis works well for separating cations and anions, but it cannot be used to separate neutral substances from each other. Instead, these substances migrate as a single peak that travels with the electroosmotic flow. It is possible to employ CE with such compounds if we place in the running buffer a charged agent that can interact with these substances. This approach is called electrokinetic chromatography. One common way of carrying out this method is to employ micelles as additives, giving a subset of electrokinetic chromatography known as micellar electrokinetic chromatography (MEKC).

A micelle is a particle formed by the aggregation of a large number of surfactant molecules, such as sodium dodecyl sulfate (SDS). We saw earlier that SDS has a long nonpolar tail attached to a negatively charged sulfate group. When the concentration of a surfactant like SDS reaches a certain threshold level (known as the “critical micelle concentration”), some of the surfactant molecules come together to form micelles. If these micelles form in a polar solvent like water, the nonpolar tails of the surfactant will be on the inside of the aggregate (giving a nonpolar interior), while the charged groups at the other end will be on the outside by the solvent (see Figure 23.19).

When micelles based on SDS are placed into the running buffer of a CE system, they will be attracted toward the positive electrode. If a sample with several neutral compounds is now injected into this system, some of these neutral substances may enter the micelles and interact with their nonpolar interior. This interaction involves a partitioning process similar to that found in liquid–liquid extractions and some types of liquid chromatography (see Chapters 20 and 22), in which the micelles act as the “stationary phase.” Although these neutral compounds normally travel with the electroosmotic flow through the capillary, while they are in the micelles they migrate with the micelles in the opposite direction. The result is a separation of neutral compounds based on the degree to which they enter the micelles. Micelles can also alter the migration times for charged substances through partitioning and charge interactions between the analytes and micelles.

Other Methods. There are many other types of CE that have been explored for use in chemical analysis and separations. For example, isoelectric focusing can be carried out in a capillary, creating the method of capillary isoelectric focusing (CIEF). This technique involves the production of a pH gradient across the capillary for the separation of proteins. The figure on the left shows an electropherogram obtained for a series of proteins with molecular masses ranging from 14 to 200 kDa. The inset shows an SDS-PAGE gel for the same proteins. The calibration curve on the right shows how the migration times for these proteins in CE are related to their molecular masses. (Adapted with permission from Bio-Rad Laboratories.)

![Figure 23.18](image_url)
zwitterions. One way CIEF can be conducted is shown in Figure 23.20. In this example, the electrodes are in contact with two different electrolyte solutions: (1) the "catholyte," which is a basic solution located by the cathode, and (2) the "anolyte," which is an acidic solution located by the anode. The capillary contains a mixture of ampholytes that will create a pH gradient when an electric field is applied between these electrodes. A coated capillary is also used in this case to minimize or eliminate electroosmotic flow. When a sample (generally a mixture of proteins) is injected onto this system, its zwitterions will migrate until they reach a region where the pH is equal to their pI. Once these bands have formed, they are pushed through the capillary and past the detector by applying pressure to the system.

Another type of capillary electrophoresis occurs when biologically related agents are placed as additives in the running buffer. As analytes travel through this buffer, their overall mobility will be affected by their binding to these agents (see Figure 23.21). The result is a method known as **affinity capillary electrophoresis** (ACE).\(^7,21,44,45\) One common use of ACE is in the separation of chiral analytes through the use of binding agents like cyclodextrins or proteins (see Chapters 8 and 22). This method can also be used in clinical and pharmaceutical assays and for the study of biological interactions.
WHAT IS ELECTROPHORESIS?

1. Define “electrophoresis” and explain how this method is used to separate chemicals.
2. What is “zone electrophoresis”? How does this technique differ from “moving boundary electrophoresis”? Which of these methods is more common in modern laboratories?

HOW IS ELECTROPHORESIS PERFORMED?

3. Define each of the following terms and explain how they are used in electrophoresis.
   (a) Migration distance
   (b) Migration time
   (c) Electropherogram
4. Chloride is found to migrate a distance of 35 cm in a capillary electrophoresis system with a migration time of 5.63 min. What is the migration velocity of chloride under these conditions? At the same velocity, what distance would chloride have traveled in 2.5 min?
5. A protein is found to migrate a distance of 3.2 cm in 30 min when 100 V is applied to a 10 cm long polyacrylamide gel. What is the migration velocity of this protein? If an applied voltage of 200 V is used instead, how long will it take the same protein to migrate a distance the entire length of the 10 cm gel?

FACTORS AFFECTING ANALYTE MIGRATION

6. Explain why a charged substance will tend to move at a constant velocity through an electric field. What forces are involved in this process?
7. What is “electrophoretic mobility”? How is this term related to the movement of a substance in an electric field? What are some general factors that affect the size of the electrophoretic mobility for an analyte?
8. A peptide is found to have a migration time of 8.31 min at an applied voltage of 10.0 kV and on a capillary electrophoresis system with a total length of 25.0 cm. The detector is located at 21.5 cm from the point of sample injection and conditions are used so that electroosmotic flow is negligible. What is the migration velocity of the peptide? What is its electrophoretic mobility?
9. How would the migration velocity and migration time for the peptide in the previous problem change if the voltage...
What is the "electroosmotic mobility"? What factors affect its magnitude in an electrophoretic system?

What is "electroosmosis"? What causes electroosmosis to occur? How does electroosmosis affect the movement of analytes in an electrophoresis system?

Dicamba is a herbicide commonly used on broadleaf weeds. Its major metabolite is dichlorosalicylic acid (DCSA). Both compounds are weak acids. Dicamba has a single weak-acid group, which is a carboxylic acid group with a $pK_a$ of 1.94. DCSA has two weak-acid groups: a carboxylic acid group with a $pK_a$ of 2.08 and a phenol group with a $pK_a$ of 8.60. A separation of these compounds at pH 7.4 by capillary electrophoresis (CE) gives a migration time of 2.05 min for dicamba and 2.35 min for DCSA. The same compounds had migration times of 2.06 min and 4.1 min at pH 10.0.46 Explain why there is a large shift in the migration time for DCSA over this pH range but no significant shift in the migration time for dicamba.

Two chromatic forms of a drug have identical electrophoretic mobility for a particular separation. What is the electroosmotic mobility for a particular separation? How is it calculated? What factors affect it?

The electroosmotic mobility for a particular separation is assumed that the observed electrophoretic mobility for an analyte in a CE system. However, it is possible to separate these forms when $\beta$-cyclodextrin (a complexing agent we discussed in Chapter 7) is placed as an additive into the running buffer. Based on your knowledge of chemical reactions, explain why the presence of $\beta$-cyclodextrin might lead to such a separation.

What is "electroosmotic mobility"? What factors affect this mobility?

A neutral compound injected onto a capillary electrophoresis system has a migration time of 1.52 min through a 50 cm long capillary at 30.0 kV, with the detector being located 30.0 cm from the point of injection. If it is assumed that the observed electrophoretic mobility for this compound is equal to the electroosmotic mobility, what is the value of $\mu_e$ under these conditions?

The electroosmotic mobility for a particular separation is found to be $8.3 \times 10^{-10} \text{m}^2/\text{V} \cdot \text{s}$. A 25 cm long capillary is used for this separation at an applied voltage of 20.0 kV, with the detector being located 20 cm from the point of injection. What is the expected migration time for a neutral analyte in this system (i.e., an analyte that travels through the system only due to electroosmotic flow)?

FACTORS AFFECTING BAND-BROADENING

Use the same equations as given in Chapter 20 for chromatography to calculate the following values. (Note: You can assume Gaussian peaks are present.)

(a) The plate number of a peak in capillary electrophoresis that has a migration time of 7.30 min and baseline width of 0.12 min
(b) The plate height of the system in Part (a) for a capillary with a total length of 35.0 cm
(c) The plate number of a band in gel electrophoresis with a migration distance of 4.2 cm and baseline width of 2.1 mm

Use the equations given in Chapter 20 to calculate the following values.

(a) The resolution of two peaks in capillary electrophoresis with migration times of 10.1 min and 10.4 min with baseline widths of 0.15 min and 0.16 min, respectively
(b) The resolution of two bands in gel electrophoresis with migration distances of 2.3 cm and 2.6 cm with an average baseline width of 1.5 mm

How does longitudinal diffusion affect band-broadening in electrophoresis? How is this type of band-broadening related to the time of the separation and the size of the analytes? Why can a porous support help minimize the effects of this process?

What is "Joule heating"? What causes this heating? Why does Joule heating result in band-broadening in electrophoresis?

What are some approaches that can be used to minimize the effects of Joule heating in electrophoresis?

One useful tool in optimizing a separation for capillary electrophoresis is an "Ohm’s law plot." This graph is prepared by plotting the measured current of the electrophoretic system at various applied voltages. Explain why there is a large shift in the migration time for DCSA over this pH range but no significant shift in the migration time for dicamba.

Under what conditions will eddy diffusion be present during electrophoresis?

What is "wick flow"? How does wick flow create band-broadening? In what types of electrophoresis can wick flow be important?

WHAT IS GEL ELECTROPHORESIS?

What is “gel electrophoresis”? How is this technique used for analyte identification and measurement?

A biochemist looking for a particular protein in a cell sample obtains the following results when using gel electrophoresis and a Western blot to compare this sample with standards containing the same protein. What is the approximate amount of this protein in the unknown cell sample?

<table>
<thead>
<tr>
<th>Amount of Protein (ng)</th>
<th>Relative Band Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>50</td>
</tr>
<tr>
<td>5.0</td>
<td>560</td>
</tr>
<tr>
<td>10.0</td>
<td>1120</td>
</tr>
<tr>
<td>20.0</td>
<td>2040</td>
</tr>
<tr>
<td>Unknown sample</td>
<td>980</td>
</tr>
</tbody>
</table>

Describe how each of the following items can be used for detection in gel electrophoresis.

(a) Densitometer
(b) Coomassie Brilliant Blue
592 Chapter 23 • Electrophoresis

(c) Silver staining
(d) Blotting

32. What is the difference between a Southern blot and a Northern blot? What is the difference between a Southern blot and a Western blot? How is each of these methods performed?

33. What is MALDI-TOF MS (matrix-assisted time-of-flight mass spectrometry)? Explain how this method can be used for identifying the contents of bands in gel electrophoresis.

WHAT ARE SOME SPECIAL TYPES OF GEL ELECTROPHORESIS?

34. Explain how SDS-PAGE (sodium dodecyl sulfate polyacrylamide gel electrophoresis), is performed. Describe why SDS-PAGE can provide information on the molecular mass of a protein.

35. The molecular mass of a protein is to be estimated by SDS-PAGE. The following migration distances are obtained for proteins of known mass on the gel: 200 kDa, 0.33 cm; 116.3 kDa, 0.37 cm; 66.3 kDa, 0.91 cm; 36.5 kDa, 1.63 cm; 21.5 kDa, 1.96 cm; 14.4 kDa, 2.24 cm. The unknown protein has a migration distance of 1.25 cm on the same gel. What is the approximate molecular weight of this protein?

36. A biochemist uses the same conditions as in the last problem to look for proteins with approximate masses of 18.5 kDa, 40.2 kDa, and 91.8 kDa. What are the expected migration distances for these proteins in this gel?

37. What is “isoelectric focusing”? Describe how this method separates analytes.

38. What is an “ampholyte”? How is an ampholyte used in isoelectric focusing?

39. What is “2-D electrophoresis”? What types of electrophoresis are often used in this method? What advantages are there in the use of 2-D electrophoresis for complex samples?

WHAT IS CAPILLARY ELECTROPHORESIS?

40. What is meant by the term “capillary electrophoresis”? How does this method differ from gel electrophoresis?

41. The amount of a nitrate in an unknown sample is to be quantitated by capillary electrophoresis. Another anion is added to all samples and standards as an internal standard (IS) prior to injection. The following results are obtained. What is the amount of the nitrate in the unknown sample?

<table>
<thead>
<tr>
<th>Concentration of Nitrate (mg/L)</th>
<th>Peak Height</th>
<th>Concentration of Internal Standard (mg/L)</th>
<th>Peak Height</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>0.2</td>
<td>2.5</td>
<td>9.8</td>
</tr>
<tr>
<td>5.0</td>
<td>18.8</td>
<td>2.5</td>
<td>10.2</td>
</tr>
<tr>
<td>10.0</td>
<td>43.1</td>
<td>2.5</td>
<td>11.5</td>
</tr>
<tr>
<td>15.0</td>
<td>55.2</td>
<td>2.5</td>
<td>10.1</td>
</tr>
<tr>
<td>Unknown sample</td>
<td>15.1</td>
<td>2.5</td>
<td>9.7</td>
</tr>
</tbody>
</table>

42. Capillary electrophoresis and laser-induced fluorescence detection were used to determine the amount of a fluorescein-labeled peptide in a biological sample. A fixed amount of non-conjugated fluorescein was added to each sample as an internal standard. The following results were obtained in this method for a series of standards.

<table>
<thead>
<tr>
<th>Concentration of Peptide (nM)</th>
<th>Peak Area — Peptide</th>
<th>Peak Area — Fluorescein</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>109</td>
<td>546</td>
</tr>
<tr>
<td>15.0</td>
<td>2185</td>
<td>598</td>
</tr>
<tr>
<td>25.0</td>
<td>3174</td>
<td>532</td>
</tr>
<tr>
<td>50.0</td>
<td>7046</td>
<td>601</td>
</tr>
</tbody>
</table>

The unknown sample gave measured peak areas of 4098 and 556 for the labeled peptide and fluorescein. What was the concentration of this peptide in the unknown sample?

43. What helps give capillary electrophoresis high efficiency? What processes are normally the most important in CE in determining the band-broadening of this method?

44. A small anion with a diffusion coefficient of $3.0 \times 10^{-5}$ cm$^2$/s and an electrophoretic mobility of 1.58 cm$^2$/kV·s is to be analyzed at 20.0 kV by a capillary electrophoresis system. The system has a total length of 40.0 cm and the detector is located 33.0 cm from the point of injection. In the absence of electroosmotic flow, what is the maximum number of theoretical plates that can be obtained for this anion (i.e., assuming longitudinal diffusion is the only band-broadening process)? What will the migration time of the analyte be under these conditions?
45. A chemist wishes to use the plate number measured for a CE to estimate the diffusion coefficient for a new drug. This drug is injected onto a 42.5 cm long neutral coated capillary, which has no binding for the drug and produces negligible electroosmotic flow. The detector is located 38.0 cm from the point of injection. The drug has a measured migration time of 14.8 min and a baseline width of 26 s, when a voltage of 15.0 kV is applied across the capillary.

(a) What is the number of theoretical plates for the peak due to the drug, if it is known that this peak has a Gaussian shape?

(b) What is the diffusion coefficient for the drug (in units of cm²/s)? What assumptions did you make in reaching your answer?

HOW IS CAPILLARY ELECTROPHORESIS PERFORMED?

46. What are the main components of a capillary electrophoresis system? How does this system differ from the equipment used in gel electrophoresis?

47. Why does the use of an uncoated silica capillary lead to electroosmotic flow in capillary electrophoresis? What is the direction of this flow? How does electroosmosis affect the apparent migration of analytes through the CE system?

48. What is the “normal polarity” mode of CE? What is the “reversed polarity” mode? In what general situations are these two modes utilized?

49. Explain why it is necessary in capillary electrophoresis to use small injection volumes. What are some difficulties in working with these small volumes? What are some advantages?

50. What is “hydrodynamic injection”? What is “electrokinetic injection”? How does each of these methods work?

51. What is “sample stacking”? Describe one way sample stacking can be accomplished in capillary electrophoresis.

52. List some general and some selective detectors that are used in capillary electrophoresis. How does this list compare to that given in Chapter 22 for liquid chromatography?

53. Two isoforms of a protein are found to elute with migration times of 20.3 min and 24.5 min from a capillary electrophoresis system. These protein peaks have measured areas of 3430 and 1235 units, respectively. What is the relative amount of each protein in the sample?

54. A drug is found to have a peak area of 11,250 units and it migrates through a 50.0 cm long capillary in the presence of an applied voltage of 15.0 kV. The detector is located at a distance of 45.0 cm from the point of injection. What will the expected area be for this sample if it is injected onto the same system, but now using an applied voltage of 20.0 kV?

55. What is “laser-induced fluorescence”? Explain why this technique is useful in capillary electrophoresis.

WHAT ARE SOME SPECIAL TYPES OF CAPILLARY ELECTROPHORESIS?

56. What is “capillary sieving electrophoresis”? What are two ways in which this method can be performed?

57. A protein is injected onto the same system used in Figure 23.18. What is the molecular weight of this protein if it has a normalized migration time of 2.69?

58. Define each of the following terms.

(a) Electrokinetic chromatography
(b) MEKC
(c) Micelle
(d) Critical micelle concentration

59. What is “capillary isoelectric focusing”? Describe one way this method can be performed.

60. What is “affinity capillary electrophoresis”? What are some applications of this method?

CHALLENGE PROBLEMS

61. Compare the capillary electrophoresis system in Figure 23.14 with the electrochemical cells that are discussed in Chapter 10.

(a) What similarities can you find in these two types of systems? Based on this comparison, which part of the electrochemical cell would be equivalent to the capillary in a CE system? Which part of an electrochemical cell do you think would be equivalent to the gel or support in a gel electrophoresis system?

(b) Describe how current is carried from the power supply and throughout an electrophoresis system. What is the role of the running buffer in this regard? What are the roles of the electrodes?

(c) One tool we used in Chapter 6 for solving chemical problems was to use the method of charge balance, which says that the number of positive and negative charges in a system must be equal. And yet, in electrophoresis we use an electric field to separate analytes with positive and negative charges. Why do you think this separation is possible? (Hint: Consider your answers to Parts (a) and (b).)

62. The effect of electroosmotic flow on the overall observed electrophoretic mobility (μωl) and migration velocity (v) for an analyte in electrophoresis is given by the following equations,

\[ v = \mu_{\text{eff}} E = \frac{(\mu + \mu_{\text{osm}}) V}{L} \]  
\[ t_{\text{in}} = \frac{L d_{\text{avg}}}{(\mu + \mu_{\text{osm}}) V} = \frac{L d_{\text{avg}}}{\mu_{\text{eff}} E} \]

where all terms are the same as described earlier in this chapter.21,23

(a) A cation has an electrophoretic mobility of 2.50 cm²/kV·min on a CE system containing a 30.0 cm long coated, neutral capillary with a detector located 25.0 cm from the point of injection. What migration time and migration velocity would be expected for this cation when using an applied voltage of 15.0 kV?

(b) What migration time and velocity would be obtained for the same cation as in Part (a) if a switch was made from the neutral capillary to a negatively charged capillary of an identical size, but that gives an electroosmotic mobility of −2.50 cm²/kV·min? Compare your results with those obtained for the cation. What does this comparison tell you about the role electroosmotic flow plays in the analysis of cations and anions in CE?

63. The effect of electroosmotic flow on the efficiency and resolution of a separation in electrophoresis is given by the equations shown,23

\[ N = \frac{(\mu + \mu_{\text{osm}}) V}{2D} \]  
\[ R_s = 0.177 (\mu_1 - \mu_2) \sqrt{\frac{V}{D(\mu_{\text{avg}} + \mu_{\text{osm}})}} \]

(a) What is the number of theoretical plates for the peak due to the drug, if it is known that this peak has a Gaussian shape?

(b) What is the diffusion coefficient for the drug (in units of cm²/s)? What assumptions did you make in reaching your answer?
where $\mu_1$ and $\mu_2$ are the electrophoretic mobilities of the first and second eluting solutes, $\mu_{\text{avg}}$ is the average electrophoretic mobility of solutes 1 and 2, and $D$ is their diffusion coefficient.

(a) The same protein as in Exercise 23.1 is examined on a CE system with a 25.0 cm long negatively charged capillary (22.0 cm to the detector) at 20.0 kV. This new capillary has an electroosmotic mobility of 3.0 cm$^2$/kV·min. If the protein still has an inherent electrophoretic mobility of 1.70 cm$^2$/kV·min and a diffusion coefficient of $2.0 \times 10^{-7}$ cm$^2$/s, how many theoretical plates are possible for this system? How does this result compare with that in Exercise 23.1?

(b) Make a plot showing what resolution would be expected at various values for $\mu_{\text{osm}}$ in the case where the applied voltage is 10.0 kV and two analyte peaks have electrophoretic mobilities of 1.70 and 1.72 cm$^2$/kV·min, with an average diffusion coefficient of $2.0 \times 10^{-7}$ cm$^2$/s. At what values of $\mu_{\text{osm}}$ versus $\mu_{\text{avg}}$ will the largest resolutions be obtained? What values of $\mu_{\text{osm}}$ will give the smallest resolutions?

64. The amount of sample applied to a capillary by hydrodynamic injection can be determined by the following form of the Hagen–Poiseuille equation:

$$\text{Sample volume} = \frac{\Delta P d^4 \pi t}{128 \eta L}$$  \hspace{1cm} (23.15)

where $\Delta P$ is the pressure applied across the capillary during injection, $d$ is the inner diameter of the capillary, $t$ is the time over which the pressure is applied, $\eta$ is the viscosity of the applied solution, and $L$ is the total length of the capillary.\(^8\)

(a) What volume of sample would be applied to a 50 $\mu$m ID $\times$ 20 cm long capillary if a pressure of 0.5 psi is applied for 1 s to a solution with a viscosity of 0.01 poise?

(b) How much sample would be applied under the same conditions but using a 1 s pulse on a 25 $\mu$m ID $\times$ 20 cm long capillary?

65. The amount of an analyte that is applied to a capillary by electrokinetic injection is described by Equation 23.16,

$$Q = \frac{(\mu + \mu_{\text{osm}}) V A C t}{L}$$  \hspace{1cm} (23.16)

where $Q$ is the quantity of analyte injected, $\mu$ is the electrophoretic mobility of the analyte, $\mu_{\text{osm}}$ is the mobility due to electroosmosis, $V$ is the applied voltage, $A$ is the cross-sectional area of the capillary, $C$ is the analyte concentration in the original sample, $t$ is the time over which the electric field is applied for injection, and $L$ is the distance over which the voltage is applied.\(^8\)

(a) Based on Equation 23.16, which types of analytes will have the largest injected quantities in electrokinetic injection: those that move with electroosmotic flow or against it?

(b) How does the value of $Q$ change with the size of electroosmotic flow? Are small values or large values for $\mu_{\text{osm}}$ desirable in this method?

66. Compare and contrast the following analytical methods in terms of the way in which they separate analytes:

(a) Electrophoretic and reversed-phase chromatography

(b) Capillary electrophoresis and ion-exchange chromatography

(c) SDS-PAGE and capillary gel electrophoresis

(d) Affinity capillary electrophoresis and affinity chromatography

67. Obtain more information on the Human Genome Project. Discuss the challenges this project presented to analytical chemists. What changes in DNA sequencing methods were made to make this project possible?

68. Now that human DNA has been sequenced, scientists have begun to examine the vast number of proteins that are encoded by this DNA. This research has lead to an area known as “proteomics.” Obtain more information on proteomics and the challenges that are presented by this field to chemical analysis. Describe some analytical methods that are being used in this field.

69. Contact or visit a local hospital or biochemical laboratory. Report on how electrophoresis is used in these laboratories.

70. Compare and contrast the advantages and disadvantages for each of the following pairs of methods:

(a) Gel electrophoresis versus capillary electrophoresis

(b) Gel electrophoresis versus HPLC

(c) Capillary electrophoresis versus HPLC

71. Use the Internet to obtain material safety data sheet (MSDS) information for the various chemicals that are shown in Figure 23.8 for the preparation of a polyacrylamide gel. Identify any chemical or physical hazards that are associated with these reagents.

72. Work with Northern and Southern blots in gel electrophoresis often involves the use of phosphorus-32 ($^{32}$P) as a radiolabel. Obtain further information on any special requirements, training or facilities that are needed for dealing with this agent. In addition, find out why phosphorus-32 is used as a label for these applications. Write a report discussing your findings.

73. There are several additional types of electrophoresis besides those that were discussed in this chapter. A few examples are listed below.\(^1,12,13\) Write a report on one of these methods. Include a description of how the method separates analytes, its applications, and its advantages and disadvantages.

(a) Isochrochromatography

(b) Pulsed-field electrophoresis

(c) Dielectrophoresis

(d) Immunoelctrophoresis

74. The need for small sample sizes originally created several challenges in the design of CE instruments. This same feature has made capillary electrophoresis attractive for the analysis of samples for which only small volumes are available. For instance, it has been shown that CE can be used to analyze the content of single cells. Obtain a recent research article or review on this topic. Write a report that discusses how CE was used to analyze single cells in the subject paper.

75. Some early examples of capillary electrophoresis can be found in References 23 and 34. Look up these articles and examine how CE was used to analyze single cells. Obtain a report discussing your findings.

76. Capillary electrophoresis is a method in which the movement of an analyte through a stationary phase is achieved through the use of electroosmotic flow rather than by only a difference in pressure.\(^7,47,48\) Obtain more information on this method. Report on how this method works and on some of its recent applications. Discuss how this method is related to both traditional liquid chromatography and capillary electrophoresis, even though it is usually classified as a chromatographic method.
Four chemicals that can be used as matrices in MALDI-TOF MS are nicotinic acid, sinapinic acid, α-cyano-4-hydroxycinnamic acid, and 2,5-dihydroxybenzoic acid. Obtain more information on one or more of these chemicals and learn about how they are used in MALDI-TOF MS. What chemical or physical properties make these chemicals used in this method? What are some analytes that can be examined with these matrices?

References