First Week

22-2 What Is Chromatography?

In 1903 in Warsaw, the botanist M. Tswett invented adsorption chromatography to separate plant pigments, using a hydrocarbon solvent and inulin powder (a carbohydrate) as stationary phase. The separation of colored bands led to the name *chromatography*, from the Greek *chromatos* ("color") and *graphein* ("to write")—"color writing." Tswett later found that $CaCO_3$ or sucrose could also be used as stationary phases.[6]

Chromatography lay dormant until Tswett's methods were applied, beginning in 1931, to biochemical separations by E. Lederer and R. Kuhn in Heidelberg, P. Karrer in Zurich, and L. Zechmeister in Hungary.[7] During the 1930s, adsorption chromatography became an established tool in biochemistry.

eluent–in
eluate–out

Chromatography operates on the same principle as extraction, but one phase is held in place while the other moves past it.[8,9] Figure 22-5 shows a solution containing solutes A and B placed on top of a column packed with solid particles and filled with solvent. When the outlet is opened, solutes A and B flow down into the column. Fresh solvent is then applied to the top of the column and the mixture is washed down the column by continuous solvent flow. If solute A is more strongly adsorbed than solute B on the solid particles, then solute A spends a smaller fraction of the time free in solution. Solute A moves down the column more slowly than solute B and emerges at the bottom after solute B. We have just separated a mixture into its components by *chromatography*.

The **mobile phase** (the solvent moving through the column) in chromatography is either a liquid or a gas. The **stationary phase** (the one that stays in place inside the column) is most commonly a viscous liquid chemically bonded to the inside of a capillary tube or onto the surface of solid particles packed in the column. Alternatively, as in Figure 22-5, the solid particles themselves may be the stationary phase. In any case, the partitioning of solutes between mobile and stationary phases gives rise to separation.

Fluid entering the column is called **eluent.** Fluid emerging from the end of the column is called **eluate:**

eluent in → COLUMN → eluate out

The process of passing liquid or gas through a chromatography column is called **elution.**

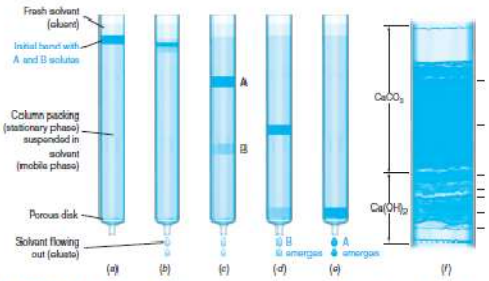

FIGURE 22-5 The idea behind chromatography: solute A, with a greater affinity than solute B for the stationary phase, remains on the column longer. Panel *f* is a reconstruction of the separation of pigments from red paprika skin from the work of L. Zechmeister in the 1930s. Bands marked by horizontal lines are different pigments. The lower stationary phase is $Ca(OH)_2$ and the upper stationary phase is $CaCO_3$. [Panel *f* from L. S. Ettre, "The Rebirth of Chromatography 75 Years Ago," *LCGC* 2007, *25*, 640.]

Columns are either **packed** or **open tubular.** A packed column is filled with particles of stationary phase, as in Figure 22-5. An open tubular column is a narrow, hollow capillary with stationary phase coated on the inside walls.

Types of Chromatography

Chromatography is divided into categories on the basis of the mechanism of interaction of the solute with the stationary phase, as shown in Figure 22-6.

Adsorption chromatography. A solid stationary phase and a liquid or gaseous mobile phase are used. Solute is adsorbed on the surface of the solid particles. The more strongly a solute is adsorbed, the slower it travels through the column.

This form of chromatography was invented by Tswett in 1903.

Partition chromatography. A liquid stationary phase is bonded to a solid surface, which is typically the inside of the silica (SiO_2) chromatography column in gas chromatography. Solute equilibrates between the stationary liquid and the mobile phase, which is a flowing gas in gas chromatography.

For their pioneering work on liquid-liquid partition chromatography in 1941, A. J. P. Martin and R. L. M. Synge received a Nobel Prize in 1952.

Ion-exchange chromatography. Anions such as $—SO_3^-$ or cations such as $—N(CH_3)_3^+$ are covalently attached to the stationary solid phase, usually a *resin*. Solute ions of the opposite charge are attracted to the stationary phase. The mobile phase is a liquid.

B. A. Adams and E. L. Holmes developed the first synthetic ion-exchange resins in 1935. *Resins* are relatively hard, amorphous organic solids. *Gels* are relatively soft.

Molecular exclusion chromatography. Also called *size exclusion*, **gel filtration,** or **gel permeation** chromatography, this technique separates molecules by size, with the larger solutes passing through most quickly. In the ideal case of molecular exclusion, there is no attractive interaction between the stationary phase and the solute. Rather, the liquid or gaseous mobile phase passes through a porous gel. The pores are small enough to exclude large solute molecules but not small ones. Large molecules stream past without entering the pores. Small molecules take longer to pass through the column because they enter the gel and therefore must flow through a larger volume before leaving the column.

Large molecules pass through the column *faster* than small molecules.

Affinity chromatography. This most selective kind of chromatography employs specific interactions between one kind of solute molecule and a second molecule that is covalently attached (immobilized) to the stationary phase. For example, the immobilized molecule might be an antibody to a particular protein. When a mixture containing a thousand proteins is passed through the column, only the one protein that reacts with the antibody binds to the column. After all other solutes have been washed from the column, the desired protein is dislodged by changing the pH or ionic strength.

The Chromatogram

Solutes eluted from a chromatography column are observed with detectors described in later chapters. A **chromatogram** is a graph showing the detector response as a function of elution

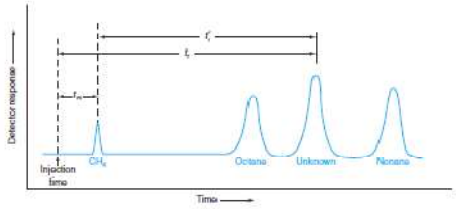

FIGURE 22-7 Schematic gas chromatogram showing measurement of retention times.

time. Figure 22-7 shows what might be observed when a mixture of octane, nonane, and an unknown are separated by gas chromatography, which is described in Chapter 23. The **retention time,** t_r, for each component is the time that elapses between injection of the mixture onto the column and the arrival of that component at the detector. **Retention volume,** V_r, is the volume of mobile phase required to elute a particular solute from the column.

Mobile phase or an unretained solute travels through the column in the minimum possible time, t_m. The **adjusted retention time,** t_r', for a retained solute is the additional time required to travel the length of the column, beyond that required by solvent:

Adjusted retention time:
$$t_r' = t_r - t_m \tag{22-14}$$

In gas chromatography, t_m is usually taken as the time needed for CH_4 to travel through the column (Figure 22-7).

For two components 1 and 2, the **relative retention,** α (also called *separation factor*), is the ratio of their adjusted retention times:

Relative retention:
$$\alpha = \frac{t_{r2}'}{t_{r1}'} \tag{22-15}$$

where $t_{r2}' > t_{r1}'$, so $\alpha > 1$. The greater the relative retention, the greater the separation between two components. Relative retention is fairly independent of flow rate and can therefore be used to help identify peaks when the flow rate changes.

For component 2 eluted after component 1, the *unadjusted relative retention*, γ, is the ratio of their unadjusted retention times:

Unadjusted relative retention:
$$\gamma = \frac{t_{r2}}{t_{r1}} \tag{22-16}$$

Unadjusted relative retention
$$= \frac{\text{retention time of component 2}}{\text{retention time of component 1}} = \frac{\text{speed of component 1}}{\text{speed of component 2}}$$

The unadjusted relative retention is the inverse of the ratio of the speeds at which the two components travel.

For each peak in the chromatogram, the **retention factor,** k, is the time required to elute that peak minus the time t_m required for mobile phase to pass through the column, expressed in multiples of t_m.

Retention factor:
$$k = \frac{t_r - t_m}{t_m} \tag{22-17}$$

Retention factor is also called *capacity factor, capacity ratio,* or *partition ratio* and was formerly written as k' instead of k.

The longer a component is retained by the column, the greater is the retention factor. It takes volume V_m to push solvent from the beginning of the column to the end of the column. If it takes an additional volume $3V_m$ to elute a solute, then the retention factor for that solute is 3.

Relation Between Retention Time and the Partition Coefficient

The retention factor in Equation 22-17 is equivalent to

$$k = \frac{\text{time solute spends in stationary phase}}{\text{time solute spends in mobile phase}} \tag{22-18a}$$

Let's see why this is true. If the solute spends all its time in the mobile phase and none in the stationary phase, it would be eluted in time t_m. Putting $t_r = t_m$ into Equation 22-17 gives $k = 0$, because solute spends no time in the stationary phase. Suppose that solute spends equal time in the stationary and mobile phases. The retention time would then be $t_r = 2t_m$ and $k = (2t_m - t_m)/t_m = 1$. If solute spends three times as much time in the stationary phase as in the mobile phase, $t_r = 4t_m$ and $k = (4t_m - t_m)/t_m = 3$.

If solute spends three times as much time in the stationary phase as in the mobile phase, there will be three times as many moles of solute in the stationary phase as in the mobile phase at any time. The quotient in Equation 22-18a is equivalent to

$$\frac{\text{Time solute spends in stationary phase}}{\text{Time solute spends in mobile phase}} = \frac{\text{moles of solute in stationary phase}}{\text{moles of solute in mobile phase}}$$

$$k = \frac{c_s V_s}{c_m V_m} \tag{22-18b}$$

where c_s is the concentration of solute in the stationary phase, V_s is the volume of the stationary phase, c_m is the concentration of solute in the mobile phase, and V_m is the volume of the mobile phase.

The quotient c_s/c_m is the ratio of concentrations of solute in the stationary and mobile phases. If the column is run slowly enough to be at equilibrium, the quotient c_s/c_m is the *partition coefficient*, K, introduced in connection with solvent extraction. Therefore, we cast Equation 22-18b in the form

Partition coefficient $= K = \dfrac{c_s}{c_m}$

Relation of retention time to partition coefficient:

$$k = K\frac{V_s}{V_m} \overset{\text{Eq. 22.17}}{=} \frac{t_r - t_m}{t_m} = \frac{t_r'}{t_m} \tag{22-19}$$

which relates retention time to the partition coefficient and the volumes of stationary and mobile phases. Because $t_r' \propto k \propto K$, relative retention can also be expressed as

Physical basis of chromatography:
The greater the ratio of partition coefficients between mobile and stationary phases, the greater the separation between two components of a mixture.

Relative retention:

$$\alpha = \frac{t_{r2}'}{t_{r1}'} = \frac{k_2}{k_1} = \frac{K_2}{K_1} \tag{22-20}$$

That is, the relative retention of two solutes is proportional to the ratio of their partition coefficients. This relation is the physical basis of chromatography.

Retention volume, V_r, is the volume of mobile phase required to elute a particular solute from the column:

Retention volume:

$$V_r = t_r \cdot u_v \tag{22-21}$$

where u_v is the volume flow rate (volume per unit time) of the mobile phase. The retention volume of a particular solute is constant over a range of flow rates.

volume is proportional to time, so any ratio of times can be written as the corresponding ratio of volumes. If V_m is the elution volume for unretained solute,

$$k = \frac{t_r - t_m}{t_m} = \frac{V_r - V_m}{V_m}$$

where V_r is the retention volume for solute