Second Week

Scaling Up

We normally carry out chromatography for *analytical* purposes (to separate and identify or measure the components of a mixture) or for *preparative* purposes (to purify a significant quantity of a component of a mixture). Analytical chromatography is usually performed with thin columns that provide good separation. For preparative chromatography, we use fatter columns that can handle more load (Figure 22-8).[10] Preparative chromatography is especially important in the pharmaceutical industry, which can afford the high cost of separating compounds such as *optical isomers* of drugs (Box 23-1).

If you have developed a procedure to separate 2 mg of a mixture on a column with a diameter of 1.0 cm, what size column should you use to separate 20 mg of the mixture? The

most straightforward way to scale up is to maintain the same column length and to increase the cross-sectional area to maintain a constant ratio of sample mass to column volume. Cross-sectional area is πr^2, where r is the column radius, so the desired diameter is given by

Scaling rules:
- Keep column length constant
- Cross-sectional area of column ∝ mass of analyte:

$$\frac{\text{Mass}_2}{\text{Mass}_1} = \left(\frac{\text{radius}_2}{\text{radius}_1}\right)^2$$

(The symbol ∝ means "is proportional to.")
- Maintain constant linear flow rate:

$$\frac{\text{Volume flow}_2}{\text{Volume flow}_1} = \left(\frac{\text{radius}_2}{\text{radius}_1}\right)^2$$

- Sample volume applied to column ∝ mass of analyte
- If you change column length, mass of sample can be increased in proportion to total length

Scaling equation:

$$\frac{\text{Large mass}}{\text{Small mass}} = \left(\frac{\text{large column radius}}{\text{small column radius}}\right)^2 \qquad (22\text{-}22)$$

$$\frac{20 \text{ mg}}{2 \text{ mg}} = \left(\frac{\text{large column radius}}{0.50 \text{ cm}}\right)^2$$

$$\text{Large column radius} = 1.58 \text{ cm}$$

A column with a diameter near 3 cm would be appropriate.

To reproduce the conditions of the smaller column in the larger column, the *linear flow rate* (not the volume flow rate) should be kept constant. Because the area (and hence volume) of the large column is 10 times greater than that of the small column in this example, the volume flow rate should be 10 times greater to maintain a constant linear flow rate. If the small column had a volume flow rate of 0.3 mL/min, the large column should be run at 3 mL/min.

The mass of sample (g) that can be run in preparative chromatography on a reversed-phase (page 603) silica-based column is *roughly*

$$\text{Column capacity (g)} \approx (2.2 \times 10^{-7}) L d_c^2 \sigma_g$$

where L is column length in mm, d_c is column diameter in mm, and σ_g is the surface area (m^2) per gram of stationary phase.[11] For $L = 250$ mm, $d_c = 50$ mm, and $\sigma_g = 200$ m^2/g, we estimate the column capacity as $(2.2 \times 10^{-7})(250)(50)^2(200) = 28$ g. This calculation presumes that the band will occupy the entire volume of the column, so it is surely an upper limit estimate. If you want the band to occupy just 20% of the column, so there is room for chromatography, the mass of sample would be (0.2)(28 g) = 5.6 g.

22-4 Efficiency of Separation

Two factors contribute to how well compounds are separated by chromatography. One is the difference in elution times between peaks: The farther apart, the better their separation. The other factor is how broad the peaks are: the wider the peaks, the poorer their separation. This section discusses how we measure the efficiency of a separation.

Resolution

Solute moving through a chromatography column tends to spread into a Gaussian shape with standard deviation σ (Figure 22-9). The longer a solute resides in a column, the broader the band becomes. Common measures of breadth are (1) the width $w_{1/2}$ measured at a height

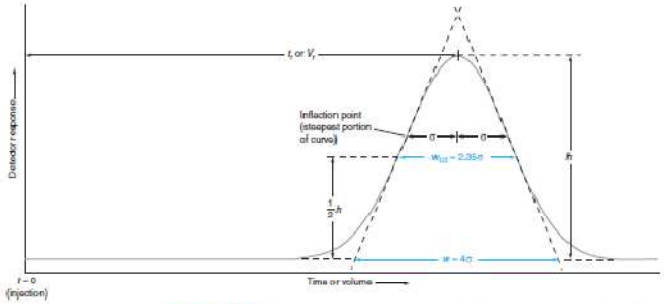

FIGURE 22-9 Idealized Gaussian chromatogram showing how w and $w_{1/2}$ are measured. The value of w is obtained by extrapolating the tangents to the inflection points down to the baseline.

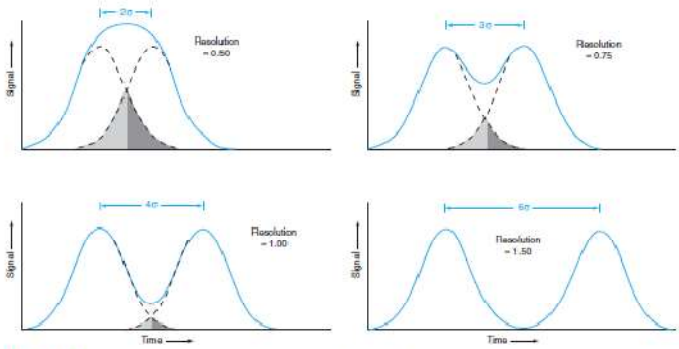

FIGURE 22-10 Resolution of Gaussian peaks of equal area and amplitude. Dashed lines show individual peaks, and solid lines are the sum of two peaks. Overlapping area is shaded.

equal to half of the peak height and (2) the width w at the baseline between tangents drawn to the steepest parts of the peak. From Equation 4-3 for a Gaussian peak, it is possible to show that $w_{1/2} = 2.35\sigma$ and $w = 4\sigma$.

In chromatography, the **resolution** of two peaks from each other is defined as

Resolution:

$$\text{Resolution} = \frac{\Delta t_r}{w_{av}} = \frac{\Delta V_r}{w_{av}} = \frac{0.589\,\Delta t_r}{w_{1/2av}} \qquad (22\text{-}23)$$

where Δt_r or ΔV_r is the separation between peaks (in units of time or volume) and w_{av} is the average width of the two peaks in corresponding units. (Peak width is measured at the base, as shown in Figure 22-9.) Alternatively, the last expression in Equation 22-23 uses $w_{1/2av}$, the width at half-height of Gaussian peaks. The width at half-height is usually used because it is easiest to measure. Figure 22-10 shows the overlap of two peaks with different degrees of resolution. For quantitative analysis, a resolution >1.5 is highly desirable.

EXAMPLE **Measuring Resolution**

A peak with a retention time of 407 s has a width at half-height of 7.6 s. A neighboring peak is eluted 17 s later with $w_{1/2} = 9.4$ s. Find the resolution for these two components.

Solution

$$\text{Resolution} = \frac{0.589\,\Delta t_r}{w_{1/2av}} = \frac{0.589(17\text{ s})}{\frac{1}{2}(7.6\text{ s} + 9.4\text{ s})} = 1.18$$

Test Yourself What difference in retention times is required for an adequate resolution of 1.5? (***Answer:*** 21.6 s)

Diffusion

A band of solute broadens as it moves through a chromatography column (Figure 22-11). Ideally, an infinitely narrow band applied to the inlet of the column emerges with a Gaussian shape at the outlet. In less ideal circumstances, the band becomes asymmetric.

One main cause of band spreading is **diffusion,** which is the net transport of a solute from a region of high concentration to a region of low concentration caused by the random

TABLE 22-1 Representative diffusion coefficients at 298 K

Solute	Solvent	Diffusion coefficient (m^2/s)
H_2O	H_2O	2.3×10^{-9}
Sucrose	H_2O	0.52×10^{-9}
Glycine	H_2O	1.1×10^{-9}
CH_3OH	H_2O	1.6×10^{-9}
Ribonuclease (FM 13 700)	H_2O (293 K)	0.12×10^{-9}
Serum albumin (FM 66 000)	H_2O (293 K)	0.059×10^{-9}
I_2	Hexane	4.0×10^{-9}
CCl_4	Heptane	3.2×10^{-9}
N_2	CCl_4	3.4×10^{-9}
$CS_2(g)$	Air (293 K)	1.0×10^{-5}
O_2 (g)	Air (273 K)	1.8×10^{-5}
H^+	H_2O	9.3×10^{-9}
OH^-	H_2O	5.3×10^{-9}
Li^+	H_2O	1.0×10^{-9}
Na^+	H_2O	1.3×10^{-9}
K^+	H_2O	2.0×10^{-9}
Cl^-	H_2O	2.0×10^{-9}
I^-	H_2O	2.0×10^{-9}

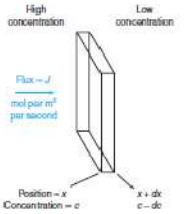

FIGURE 22-13 The flux of molecules diffusing across a plane of unit area is proportional to the concentration gradient and to the diffusion coefficient: $J = -D(dc/dx)$.

Table 22-1 shows that diffusion in liquids is 10^4 times slower than diffusion in gases. Macromolecules such as ribonuclease and albumin diffuse 10 to 100 times slower than small molecules.

If solute begins its journey through a column in an infinitely sharp layer with m moles per unit cross-sectional area of the column and spreads by diffusion as it travels, then the Gaussian profile of the band is described by

Broadening of chromatography band by diffusion:

$$c = \frac{m}{\sqrt{4\pi Dt}} e^{-x^2/4Dt} \tag{22-25}$$

where c is concentration (mol/m^3), t is time, and x is the distance along the column from the current center of the band. (The band center is always $x = 0$ in this equation.) Comparison of Equations 22-25 and 4-3 shows that the standard deviation of the band is

Standard deviation of band:

$$\sigma = \sqrt{2Dt} \tag{22-26}$$

Bandwidth = $\sqrt{t}$. If elution time increases by a factor of 4, diffusion will broaden the band by a factor of 2.

Plate Height: A Measure of Column Efficiency

Equation 22-26 tells us that the standard deviation for diffusive band spreading is $\sqrt{2Dt}$. If solute has traveled a distance x at the linear flow rate u_x (m/s), then the time it has been on the column is $t = x/u_x$. Therefore,

u_x = linear flow rate (distance/time)
u_v = volume flow rate (volume/time)

$$\sigma^2 = 2Dt = 2D\frac{x}{u_x} = \underbrace{\left(\frac{2D}{u_x}\right)}_{\text{Plate height} = H} x = Hx$$

Plate height:

$$H = \sigma^2/x \tag{22-27}$$

Plate height, H, is the constant of proportionality between the variance, σ^2, of the band and the distance it has traveled, x. The name came from the theory of distillation in which separation could be performed in discrete stages called plates. Plate height is also called the *height equivalent to a theoretical plate*. It is approximately the length of column required for one equilibration of solute between mobile and stationary phases. We explore this concept later in Box 22-2. *The smaller the plate height, the narrower the bandwidth.*

The ability of a column to separate components of a mixture is improved by decreasing plate height. An efficient column has more theoretical plates than an inefficient column.

As a teenager, A. J. P. Martin, convenor of partition chromatography, built distillation columns in discrete sections from coffee cans. (We don't know what he was distilling!) When he formulated the theory of partition chromatography, he adopted terms from distillation theory.

Small plate height → narrow peaks → better separations

Different solutes passing through the same column have different plate heights because they have different diffusion coefficients. Plate heights are ~0.1 to 1 mm in gas chromatography, ~10 μm in high-performance liquid chromatography, and <1 μm in capillary electrophoresis.

Plate height is the length σ^2/x, where σ is the standard deviation of the Gaussian band in Figure 22-9 and x is the distance traveled. For solute emerging from a column of length L, the number of plates, N, in the entire column is the length L divided by the plate height:

$$N = \frac{L}{H} = \frac{Lx}{\sigma^2} = \frac{L^2}{\sigma^2} = \frac{16L^2}{w^2}$$

because $x = L$ and $\sigma = w/4$. In this expression, w has units of length and the number of plates is dimensionless. If we express L and w (or σ) in units of time instead of length, N is still dimensionless. We obtain a useful expression for N by writing

Choose a peak with a retention factor greater than 5 when you measure plate height for a column.

Number of plates on column:

$$N = \frac{16t_r^2}{w^2} = \frac{t_r^2}{\sigma^2} \tag{22-28a}$$

where t_r is the retention time of the peak and w is the width at the base in Figure 22-9 *in units of time*. If we use the width at half-height, also called the *half-width*, instead of the width at the base, we get

Challenge If N is constant, show that the width of a chromatographic peak increases in proportion to retention time. That is, successive peaks in a chromatogram should be

Number of plates on column:

$$N = \frac{5.55t_r^2}{w_{1/2}^2} \tag{22-28b}$$