FUNDAMENTALS OF CHROMATOGRAPHY

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Many "real-world" samples are mixtures of dozens, hundreds, or thousands of chemicals. For example, medication, gasoline, blood, cosmetics, and food products are all complex mixtures. Common analyses of such samples include quantifying the levels of drugs – both legal and illegal – in blood, identifying the components of gasoline as part of an arson investigation, and measuring pesticide levels in food.

Chromatography is a technique that separates the individual components in a complex mixture. Fundamental intermolecular interactions such as dispersion, hydrogen bonding, and dipole–dipole forces govern the separations. Once separated, the solutes can also be identified and quantified. Because of its ability to separate, quantify, and identify components, chromatography is one of the most important instrumental methods of analysis, both in terms of the number of instruments worldwide and the number of analyses conducted every day.

1.1. THEORY

Chromatography separates components in a sample by introducing a small volume of the sample at the start, or head, of a column. A mobile phase, either gas or liquid, is also introduced at the head of the column. When the mobile phase is a gas, the technique is referred to as gas chromatography (GC) and when it is a liquid, the technique is called liquid chromatography (LC). Unlike the sample, which is injected as a discrete volume, the mobile phase flows continuously through the column. It serves to push the molecules in the sample through the column so that they emerge, or "elute" from the other end.

Two particular modes of LC and GC, known as reversed-phase liquid chromatography (RPLC) and capillary gas chromatography, account for approximately 85% of all chromatographic analyses performed each day. Therefore, we focus on these two techniques here and leave discussions of specific variations to the chapters that describe LC and GC in greater detail.

In GC, the mobile phase, which is typically He, N_2, or H_2 gas, is delivered from a high-pressure gas tank. The gas flows through the column toward the low-pressure end. The column contains a stationary phase. In capillary GC, the stationary phase is typically

Chromatography: Principles and Instrumentation, First Edition. Mark F. Vitha.

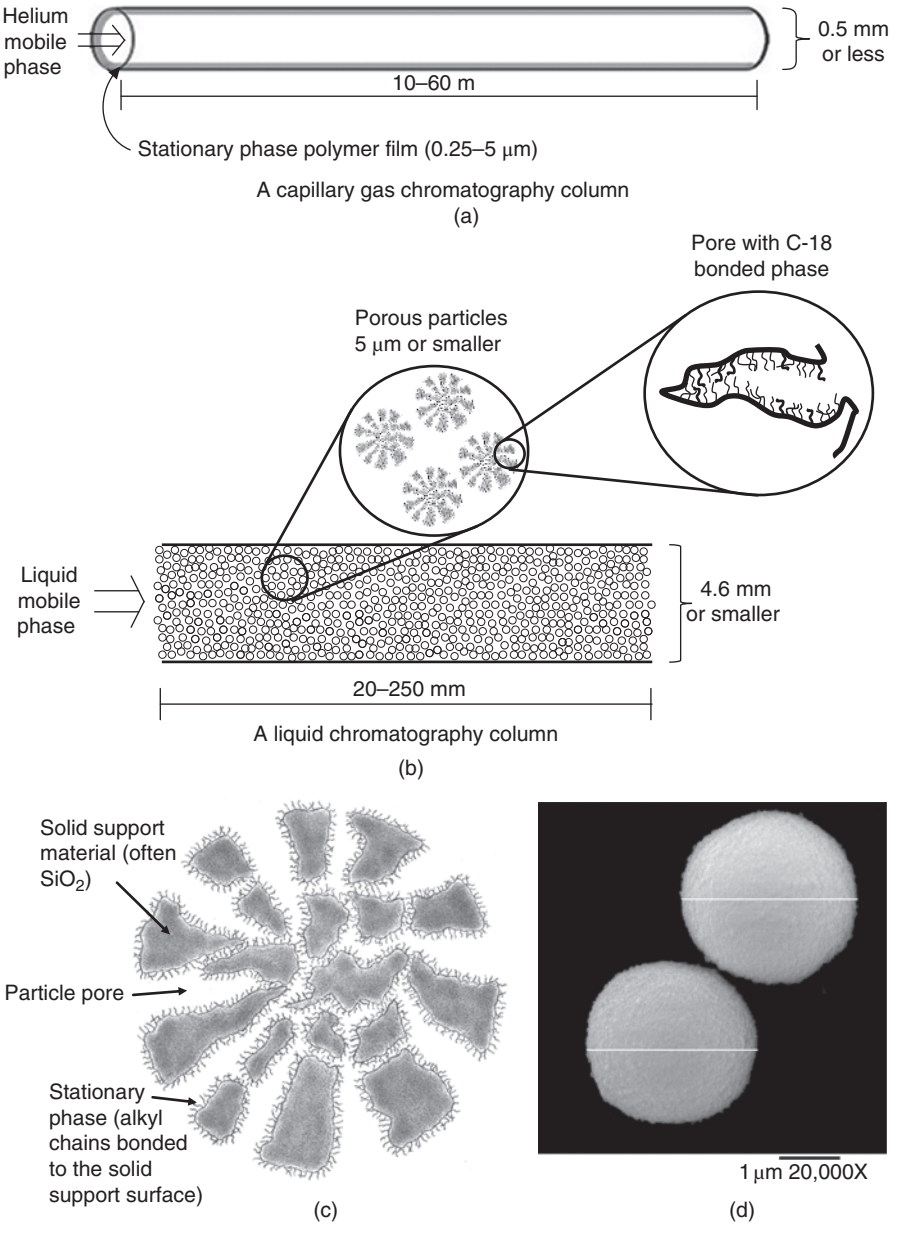


FIGURE 1.1 Representations of typical capillary gas (a) and liquid (b) chromatography columns. Figure (c) is a depiction of a cross section of a porous particle (shaded areas represent the solid support particles, white areas are the pores, and the squiggles on the surface are bonded alkyl chains. Figure (d) is an scanning electron microscope (SEM) image of actual 3 μm liquid chromatography porous particles. Note that the lines across the particle diameters have been added to the image and are not actually part of particles. (*Source:* Alon McCormick and Peter Carr. Reproduced with permission of U of MN.). It is worth taking time to note the different dimensions involved. For the GC columns, they range from microns (10^{-6} m) for the thickness of the stationary phase, to millimeters (10^{-3} m) for the column diameter, up to tens of meters for the column length. Note also that LC columns are typically much shorter than GC columns (centimeter versus meter).

a polymer film that is 0.25–5 μm thick (see Figure 1.1a). It is coated on the interior walls of a fused silica capillary column with an inner diameter of approximately 0.5 mm or smaller. The column is usually 10–60 m (30–180 ft) long.

RPLC is the most common mode of liquid chromatography. In RPLC, the mobile phase is a solvent mixture such as water with acetonitrile (CH_3CN) that is forced through the column using high-pressure pumps. The column is typically made of stainless steel, has an inner diameter of 4.6 mm or smaller, and is only 20–250 mm (1–10 in.) in length (see Figure 1.1b). However, unlike most GC columns, most LC columns are packed with tiny spherical particles approximately 5 μm in diameter or smaller, as shown in Figure 1.1c and d. When rubbed between your fingers, the particles feel like talc or other fine powders. The particles are not completely solid, but rather are highly porous, with thousands of pores in each particle. The pores create cavities akin to caves within the particle. The pores create a large amount of surface area inside the particles. A stationary phase, typically an alkyl chain 18 carbon atoms long, is bonded to the surface of these pores. A more specific discussion of the important aspects of these particles, and variations in the kinds of stationary phases bonded to them, is provided in Chapter 3. For now, it is simply important to have an image of a stainless steel column packed with very fine porous particles that have an organic-like layer bonded to the surface of the pores.

Some of the important RPLC and capillary GC column characteristics are summarized in Table 1.1. We also point out here that a chromatographic analysis is conducted with an instrument called a *chromatograph* and results in a *chromatogram*, which is a plot of the detector's response versus time (see Figure 1.2). Subsequent sections describe how retention and separation of molecules are quantified.

1.1.1. Component Separation

Different types of molecules are separated within the column because they have different strengths of intermolecular interactions with the mobile and stationary phases. To help

TABLE 1.1 Common RPLC and GC Characteristics

	RPLC	GC (open tubular)
Column construction	Stainless steel	Quartz with a polyimide coating
Column length	20–250 mm	10–60 m
Column inner diameter	2.1– 4.6 mm	0.1–0.5 mm
Particle composition	Porous silica (SiO_2) particles	No particles – open tube
Particle size	1.8–5 μm	No particles – open tube
Mobile phase	Solvent mixture (e.g., water mixed with acetonitrile)	He, N_2, or H_2
Stationary phase location	Alkyl chains (C-8 and C-18) bonded to particle surface	Liquid-like polymer film bonded to capillary walls
Stationary phase chemistry	Relatively nonpolar and organic in nature	Polysiloxane polymer derivatized with organic moieties

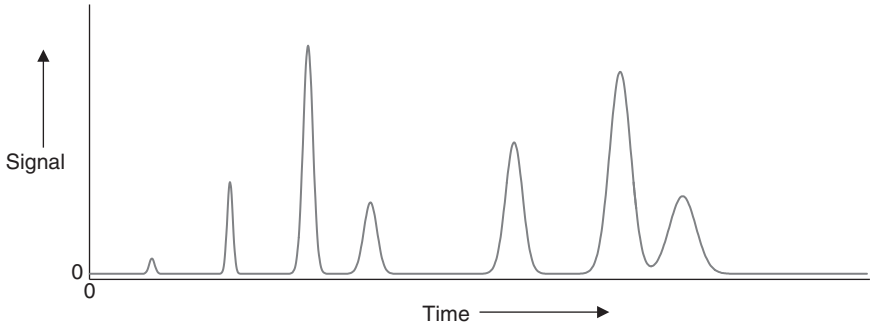


FIGURE 1.2 An example of a chromato*gram* – a plot of signal versus time – measured using a chromato*graph* (the instrument). Each peak represents a different solute that emerges from the column at a different time than the others. The peak width and height are related to the amount of each solute present.

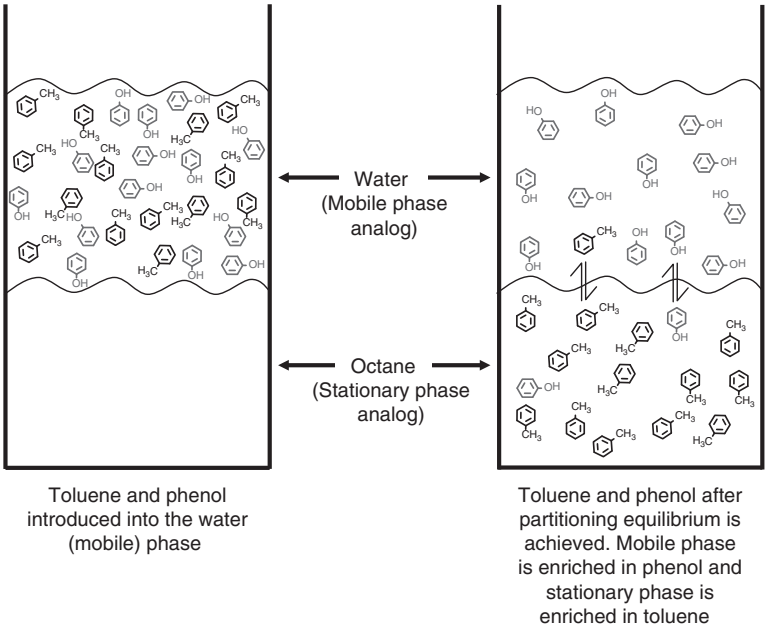


FIGURE 1.3 This figure depicts the behavior of phenol and toluene (solutes) partitioning between water and octane (bulk solvents). The water and octane serve as models for the mobile and stationary phases, respectively, in liquid chromatography. The left image depicts the system right after solutes are added to the aqueous phase before equilibrium is established. Once equilibrium is established (right), more toluene than phenol partitions into the nonpolar octane phase. Similarly, more phenol resides in the water due to hydrogen bonding and dipole-dipole interactions.

understand chromatographic separations, we first use a simplified model of liquid chromatography with water as the mobile phase and octane (C_8H_{18}) as the stationary phase. Imagine that a mixture of toluene and phenol is introduced as solutes into the mobile phase as depicted in Figure 1.3.

In this static image, given enough time, the solute molecules diffuse through the water and into the octane. They eventually reach equilibrium, being distributed to different extents between the water (mobile) and octane (stationary) phases. This equilibrium process is described in Equation 1.1

$$A_{mobile} \rightleftharpoons A_{stationary} \tag{1.1}$$

with the associated equilibrium constant

$$K = \frac{[A]_s}{[A]_m} \tag{1.2}$$

where "A" represents a specific analyte such as phenol or toluene, and K, by IUPAC definition, is known as the distribution constant. Many chromatographers refer to it as the partition coefficient or distribution coefficient. We will treat all of these as synonymous in this and the following chapters.

Because phenol is more polar than toluene and capable of hydrogen bonding with water, it does not partition into the octane to the extent that the toluene does. When looked at from a temporal perspective, phenol molecules spend less time in the octane, on average, than do the toluene molecules, which are attracted to the octane by dispersion interactions. It is important to understand that phenol is also attracted to the octane by dispersion interactions, and in fact, toluene is attracted to water through dispersion and dipole-induced dipole interactions. However, because phenol can participate in dipole–dipole and hydrogen-bonding interactions with water, and toluene cannot, phenol has a greater affinity for the aqueous phase than does toluene. As a consequence, phenol stays in the water more and partitions less into the stationary phase than does toluene.

It is clear from Figure 1.3 that what was once a mixture of an equal number of phenol and toluene molecules separates by differential partitioning between the mobile and stationary phases. It is easy to imagine that if the water phase were now drawn off and allowed to equilibrate with a fresh volume of octane, further purification of the phenol from the toluene would occur. Done repeatedly, eventually the phenol and toluene would be completely separated from one another.

In the actual practice of chromatography, these individual, discrete steps such as just described are not actually performed, but the effect of partitioning within a column is the same. As the mobile phase is continuously introduced into the column, the solutes continuously partition between it and the stationary phase. Because the molecules do not move down the column when they are in the stationary phase, those with higher affinities for the stationary phase relative to the mobile phase, meaning those with high distribution constants (i.e., partition coefficients), lag behind those with smaller distribution constants. In other words, some molecules elute from the column relatively quickly because their affinity for the mobile phase is greater than that for the stationary phase. Others, whose partitioning favors the stationary phase, take more time to make it through the column. For the molecules considered in Figure 1.3, phenol elutes before toluene. In this way, different molecules are separated within the column based on their intermolecular interactions with

the stationary and mobile phases, which ultimately depends on the structure of the solutes and the chemical composition of the phases. The separated molecules are detected at the end of the column using a variety of detectors that can quantify, and in some cases, identify them as they elute. The detectors used in GC and LC are described in detail in the following chapters.

It is important to note that all molecules spend the same amount of time in the mobile phase. Therefore, *the separation occurs because of different times spent in the stationary phase*. To understand this, think about two canoes that start down a river at the same time, being carried along solely by the current. The time it takes them to go from one end of the river to the other, without stopping, is dictated simply by the speed of the current and the length of the river (distance = velocity × time). If along the way, the canoes pull into a number of ports (i.e., the stationary phase), they stop their progress down the river. If one canoe stops more than another, and stays in port for longer periods of time (i.e., has stronger interactions with the ports), then it reaches the end of the river later than the canoe that did not stop as often or for as long. Thus, a separation has occurred because the *total* time for the journey is different for the two canoes. But still, the time spent *on the river* (i.e., in the mobile phase) is the same because both canoes covered the same distance and moved at the same velocity when on the river. Therefore, the difference in the canoes' affinities for being in port (i.e., in the stationary phase) caused the separation.

It should be noted that the simplified mobile and stationary phases depicted in Figure 1.3 are rough approximations to actual liquid chromatography systems, which are discussed in more detail in Chapter 3. In addition, if the water is replaced with a gas such as helium or nitrogen, the system approximates conditions in gas chromatography. Thus, separations in both GC and LC depend on the relative strength of intermolecular interactions of solutes with the mobile and stationary phases. The only difference is that in GC, because molecules do not interact with the gaseous mobile phase, the separation is dictated solely by the relative strengths of interactions of solutes with the stationary phase.

1.1.2. Retention Factor

In the preceding section, we established that different molecules spend different amounts of time traveling through the column. The total amount of time that a molecule spends in the column, from the time of injection to the time of detection, is called the retention time, t_r. The name indicates that we think of the molecules as being retained by the column – specifically by the stationary phase into which the solutes partition. In Figure 1.4, the retention time of phenol is 3.3 min and toluene's retention time is 5.2 min. While in the column, molecules spend their time in two places – the mobile phase and the stationary phase. Thus, the total time they are retained is simply the sum of the time they spend in each. Hence,

$$t_r = t_s + t_m \tag{1.3}$$

where t_s and t_m are the time spent in the stationary and mobile phases, respectively.

While the retention time is the most fundamental quantity measured, we often convert it into a dimensionless quantity called the retention factor, k, where

$$k = \frac{t_r - t_m}{t_m} \tag{1.4}$$

Here, t_m is a measure of the time it takes the mobile phase to flow from the start of the column to the end of the column. It is often referred to as the "dead time," or "hold-up time." A solute that has no affinity for the stationary phase and therefore travels down the column at the same rate as the mobile phase is used to measure the dead time. This peak is marked as t_m in Figure 1.4.

Converting retention times into retention factors normalizes for some operating conditions that vary between columns. For example, longer columns produce longer retention times even if everything else such as particle size, column diameter, and mobile phase flow rate are the same. More specifically, suppose one laboratory uses a column that is twice as long as the one used by another laboratory. In this case, t_r and t_m double because the molecules have twice the distance to travel. However, Equation 1.4 shows that k is the same in both laboratories because

$$\begin{aligned} k &= \frac{2t_r - 2t_m}{2t_m} \text{ (lab with longer column)} \\ &= \frac{2(t_r - t_m)}{2t_m} = \frac{t_r - t_m}{t_m} \text{ (lab with shorter column)} \end{aligned} \tag{1.5}$$

Another reason for focusing on retention factors rather than retention times is that k is directly related to the distribution constant, which, as described above, fundamentally controls the separation process. To derive the relationship between the retention factor, k, and the distribution constant, K, note that Equation 1.3 can be rewritten as shown in Equation 1.6,

$$t_r - t_m = t_s \tag{1.6}$$

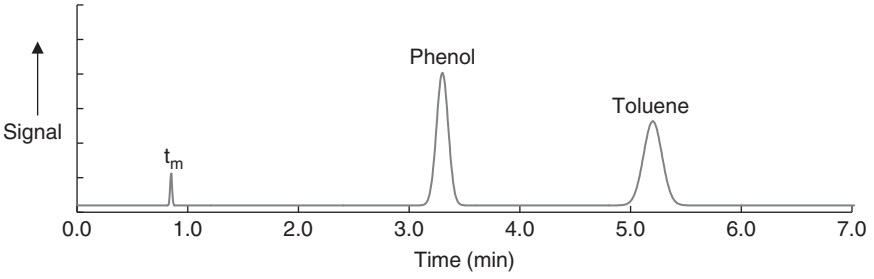


FIGURE 1.4 Chromatogram of phenol and toluene. The retention times of phenol and toluene are 3.30 and 5.20 min, respectively. The dead time, the time it takes an unretained solute to pass through the column, is labeled as t_m.

so that Equation 1.4 can be rewritten as

$$k = \frac{t_s}{t_m} \tag{1.7}$$

such that k reflects the ratio of the time one type of molecule (e.g., phenol) spends in the stationary phase relative to the time it spends in the mobile phase. The longer the molecule spends in the stationary phase relative to the time it spends in the mobile phase, the greater the value of k.

For a collection of identical molecules – for example, 100 phenol molecules – the average time spent in the stationary phase relative to the mobile phase also reflects the instantaneous distribution of molecules between the phases. If molecules spend more time, on average, in the stationary phase than in the mobile phase, then a snapshot taken at a discrete point in time shows more molecules in the stationary phase (n_s) than in the mobile phase (n_m). Thus,

$$k = \frac{t_s}{t_m} = \frac{n_s}{n_m} \tag{1.8}$$

Because the molar concentration of a solute, A, is given by n_i/V_i where n is the number of moles of A in phase i, V is the volume of phase i, and "i" is either the mobile or stationary phase, combining Equations 1.2 and 1.8 results in

$$k = \frac{\left[\frac{n_s}{V_s}\right] V_s}{\left[\frac{n_m}{V_m}\right] V_m} = \frac{[A]_s V_s}{[A]_m V_m} = K\left(\frac{V_s}{V_m}\right) = K/\beta \tag{1.9}$$

where $\beta = V_m/V_s$ and is called the "phase ratio." It is important to note that this definition of the phase ratio is the one given by the International Union of Pure and Applied Chemistry (IUPAC), but because many people define the phase ratio as $\phi = V_s/V_m$ such that $k = K\phi$, it is important to understand which definition is being used in different publications. In this and subsequent chapters, we will follow the IUPAC definition.

The phase ratio plays a role in retention that can be understood using chemical reasoning and logic. Going back to Figure 1.3, if more octane is added to the beaker, the ratio of octane to water increases. Furthermore, just out of sheer probability, toluene and phenol molecules respond to the addition of octane by partitioning out of the water into the octane. Taken to the extreme, as the mobile phase volume goes to zero, all of the molecules *have to* partition into the stationary phase, with the consequence that t_s increases and t_m decreases as shown in Figure 1.5. The result is that t_r increases for all solutes. Similarly, as V_s decreases relative to V_m, the distribution of solutes shifts toward the mobile phase and t_r decreases.

In practice, the physical characteristics of the column that one purchases dictate the phase ratio and cannot be manipulated easily. To change the phase ratio, a different column must be purchased and installed in the instrument. Manipulating retention in this manner is more frequently a consideration in gas chromatography than it is in liquid chromatography. In GC, stationary phases with thicker polymer films bonded to the capillary

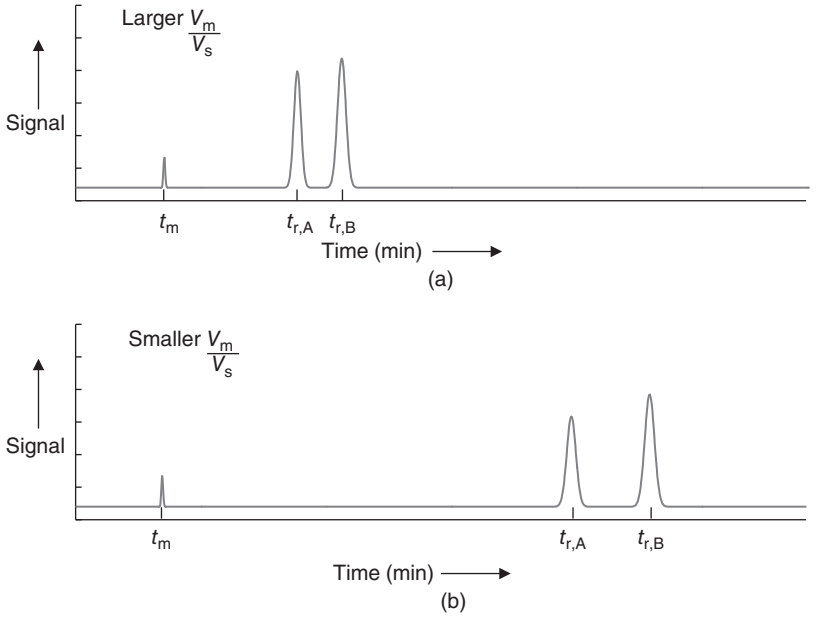


FIGURE 1.5 Effect of decreasing the phase ratio (β, V_m/V_s) by increasing V_s and decreasing V_m on retention (assuming constant V_{tot}). Solute retention times, t_r, increase as the volume of stationary phase (V_s) in the column increases due to solutes spending more time in the stationary phase.

walls that increase retention are available. In LC, most columns have bonded phases that are 18 carbon atoms long, although shorter chains that produce less retention are available.

EXAMPLE 1.1

We have used water and octane as models of a mobile and stationary phase in the text and we use it again in this question. Suppose 10,000 molecules each of butylamine and methyl hexanoate are added to a closed system containing 500 mL each of water and octane. The system is then shaken and allowed to come to equilibrium. The water-to-octane distribution constant for butylamine is 0.260 and 171.8 for methyl hexanoate.[1]

(a) How many molecules of butylamine and methyl hexanoate are in the aqueous phase at equilibrium?
(b) Based on the calculations, which solute is more concentrated in the aqueous phase at equilibrium?
(c) Which in the stationary phase?
(d) Based on the structures of the molecules and using arguments regarding their intermolecular interactions, rationalize the differences in the distribution constants for the two molecules.
(e) If more octane than water was used in the system, would the number of moles of each solute in the water phase increase or decrease?

Answer:

(a)

$$K = \frac{[\text{butylamine}]_{\text{octane}}}{[\text{butylamine}]_{\text{water}}} = \frac{\left(\frac{n_{\text{octane}}}{V_{\text{octane}}}\right)}{\left(\frac{n_{\text{water}}}{V_{\text{water}}}\right)} = 0.260$$

$$n_{\text{octane}} + n_{\text{water}} = 10{,}000 \text{ so } n_{\text{octane}} = 10{,}000 - n_{\text{water}}$$

And we know $V_{\text{octane}} = V_{\text{water}}$ so the volumes cancel. Substituting yields:

$$K = \frac{10{,}000 - n_{\text{water}}}{n_{\text{water}}} = 0.260$$

$$10{,}000 = 0.260 n_{\text{water}} + n_{\text{water}} = 1.26 n_{\text{water}}$$

$$7940 = n_{\text{water}} \text{ for butylamine (approximately after rounding to three significant figures)}$$

Following the same procedure yields ~58 molecules of methyl hexanoate in the aqueous phase at equilibrium, meaning the other 9942 methyl hexanoate molecules are in the octane.

(b) Butylamine is much more concentrated in the aqueous (mobile) phase, while methyl hexanoate is much more concentrated in the octane/stationary phase.

(c) Octane is being used to represent a stationary phase.

(d) Butylamine is polar, can donate hydrogen bonds to water, can accept hydrogen bonds from water, and has fewer carbon atoms, so it can interact well with water. Methyl hexanoate is a larger compound with more carbon atoms, giving it stronger dispersion interactions with the octane. It is also polar (although less so than butylamine) and can hydrogen bond, but these effects are not as strong and therefore water does not compete as well as the octane to attract the methyl hexanoate.

(e) The phase ratio would shift in favor of the octane, so more molecules of both solutes would be found in the octane phase at equilibrium, meaning fewer than what was calculated in part (a) would be in the aqueous phase.

Another question:

The retention time of a solute is measured to be 23.76 min using gas chromatography. Under the same conditions, the dead time is 0.88 min.

(a) What is the ratio of the moles of solute in the stationary phase relative to the moles of solute in the mobile phase at any point in time during the analysis?

(b) If the column were changed to one with a larger phase ratio (i.e., larger V_m/V_s), would this ratio increase or decrease?

(c) Would the retention time increase or decrease?

Answer:

(a) $k = \frac{n_s}{n_m} = 26.0$ (or 26 : 1)

(b) Equation 1.9 shows that as β increases, k decreases, so the ratio of solutes in the stationary phase relative to those in the mobile phase also decreases. An increasing phase ratio means greater volume of mobile phase relative to stationary phase. Therefore, decreasing retention makes sense because there is less stationary phase present to retain the molecules and/or more mobile phase present for solutes to partition into.
(c) As a consequence, the retention time of the solute decreases.

1.1.3. Separation

The *retention* of any single component is typically not of primary importance to a chromatographer. The idea of *separation* is much more important. In other words, do different types of molecules elute at sufficiently different times so that they can be individually quantified and identified?

As Equations 1.4–1.9 show, molecules that have higher distribution constants (larger K)

1. have higher retention factors (larger k), and hence
2. are retained longer, causing them to
3. have higher retention times, t_r,

than those with smaller distribution constants. In Figure 1.4, toluene has a higher distribution constant as established in Figure 1.3 and thus elutes later than phenol. Molecules that are retained for a long time are likely to be well separated from those that are retained for a short time.

The degree of separation between any two solutes, A and B, is quantified using a parameter called the separation factor, α.

$$\alpha = \frac{k_B}{k_A} \tag{1.10}$$

where "B" is the solute with the longer retention time and higher k. The "separation factor" is also frequently called "selectivity," although IUPAC discourages this use. The words "separation factor" and "selectivity" are quite descriptive and convey the idea that the ratio measures the extent of separation between two solutes – in other words, how selectively one compound is retained relative to another on the same column.

Molecules that are well separated from one another have high separation factors, while solutes that elute close to each other have small separation factors. However, the difference in retention times is not the only factor that has to be considered when measuring separation.

Consider the two chromatograms in Figure 1.6. The retention factors of both solutes are the same in the two different chromatograms. Because the retention factors are the same, α is the same. Yet, the separation in Figure 1.6b is incomplete – baseline separation has not been achieved – but in Figure 1.6a the solutes are fully resolved. Clearly, the separation factor is not the only parameter that dictates how well resolved one component is from another.

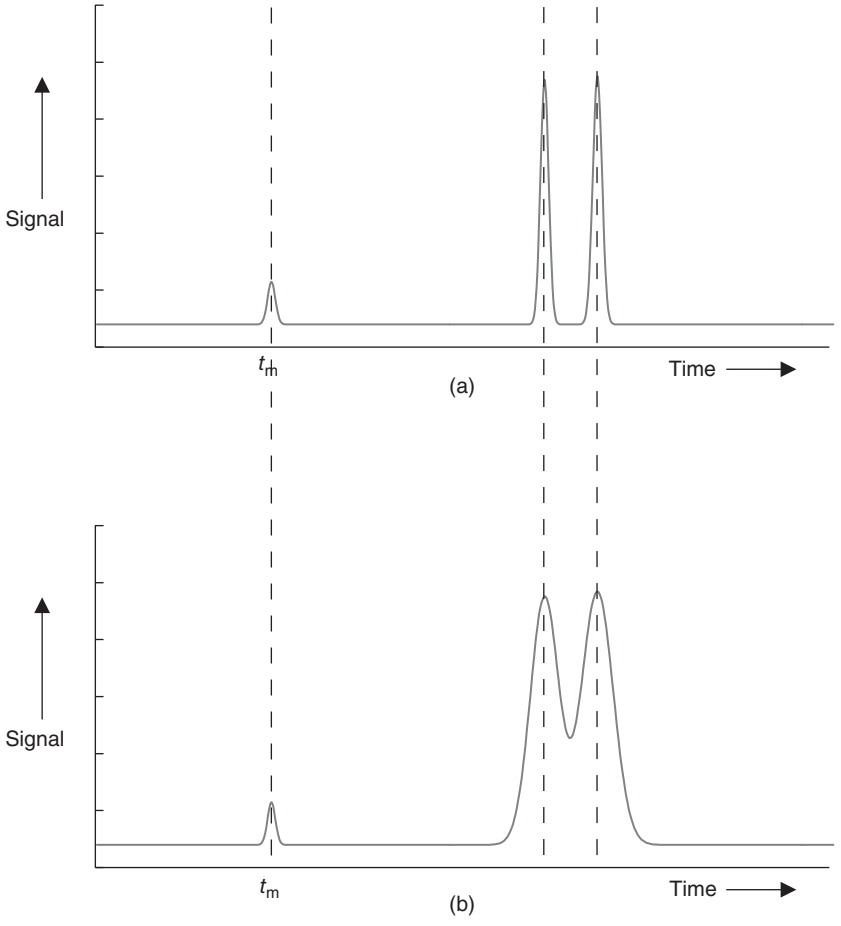


FIGURE 1.6 The effect of peak width on resolution. The dead time and solute retention times are the same in both chromatograms, meaning that the separation factor is the same in both chromatograms. The lack of resolution in (b) compared to (a) is therefore due to the widths of the peaks.

EXAMPLE 1.2

(a) Estimate the retention factors of phenol and toluene in Figure 1.4.
(b) What is the separation factor of the separation?

Answer:

(a)
$$k_{\text{phenol}} = \frac{t_r - t_m}{t_m} = \frac{3.30\,\text{min} - 0.90\,\text{min}}{0.90\,\text{min}} = 2.7$$
$$k_{\text{toluene}} = \frac{t_r - t_m}{t_m} = \frac{5.20\,\text{min} - 0.90\,\text{min}}{0.90\,\text{min}} = 4.8$$

(b) $\alpha = \frac{4.8}{2.7} = 1.8.$

Another question:

(a) What is the new value for the separation factor if the separation is repeated under conditions that lead to $k = 7.73$ and $k = 9.14$ for phenol and toluene, respectively?
(b) Which separation has better (i.e., higher) selectivity?

Answer:

(a) $\alpha = 1.18$.
(b) The first separation has better selectivity.

1.1.4. Resolution and Theoretical Plates

Resolution between peaks is important because it makes it possible to quantify each individual component in a mixture more accurately and precisely than when peaks overlap. In the extreme case of complete overlap, quantitation is typically completely impossible (except when a mass spectrometer or an analyte-selective detector is used), and the scientist might not even be aware that the observed peak is the result of multiple components.

Figure 1.6b makes it clear that in addition to the separation factor, the width of the peaks is important in terms of resolution. Thus, a consideration of the processes contributing to peak widths follows.

In order to fully understand the physical processes that occur within a chromatographic column that lead to peak broadening, also known as "band broadening," it is first important to be able to quantify peak widths. Ideally, chromatographic peaks elute with a Gaussian profile, as shown in Figure 1.7. Two measures of peak widths are commonly

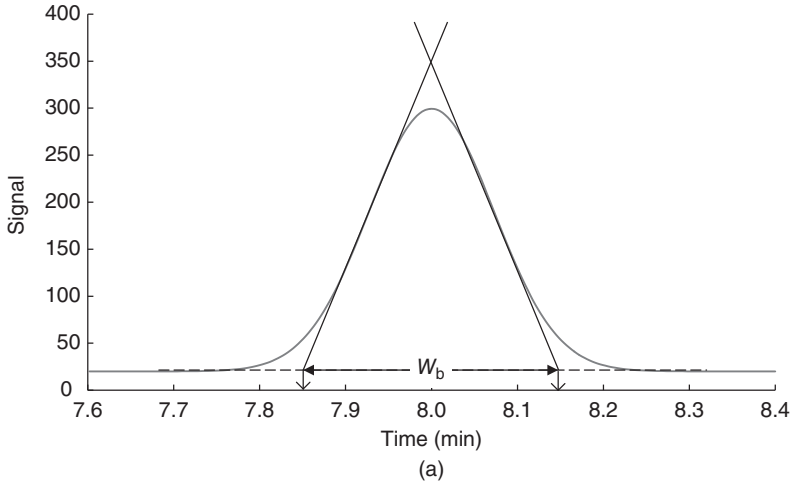


FIGURE 1.7 (*Continued*)

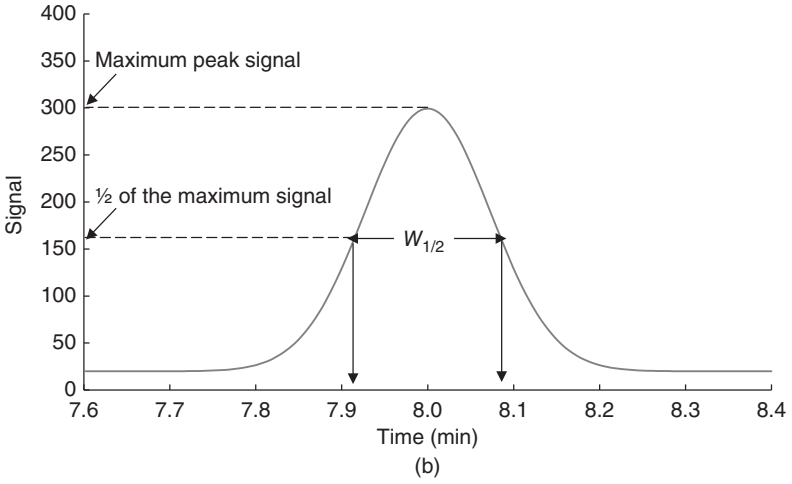


FIGURE 1.7 Illustration of the two different measurements of width: (a) baseline width (W_b) and (b) full width at half maximum (FWHM, $W_{1/2}$).

used, the baseline peak width (W_b) and the peak width at half maximum height ($W_{1/2}$). The baseline peak width is found by drawing tangents to the curve as shown in Figure 1.7a and determining the distance between the two tangents at the baseline of the peak. To find $W_{1/2}$ the highest signal reached by the peak is found (300 in Figure 1.7b) and divided in half (note however, that while the maximum signal is 300, the baseline is at 20, so the actual signal height is 280); hence, the "half maximum" refers to half the maximum signal. The width of the peak, in time units, is then found at the corresponding $^1/_2$-height as shown (i.e., at 140 + 20, or 160). Naturally, broader peaks have greater values of $W_{1/2}$ and W_b than do narrower peaks, as depicted in Figure 1.8.

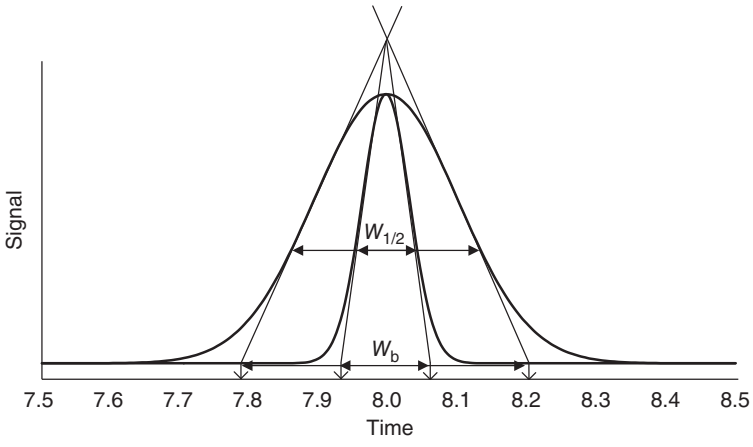
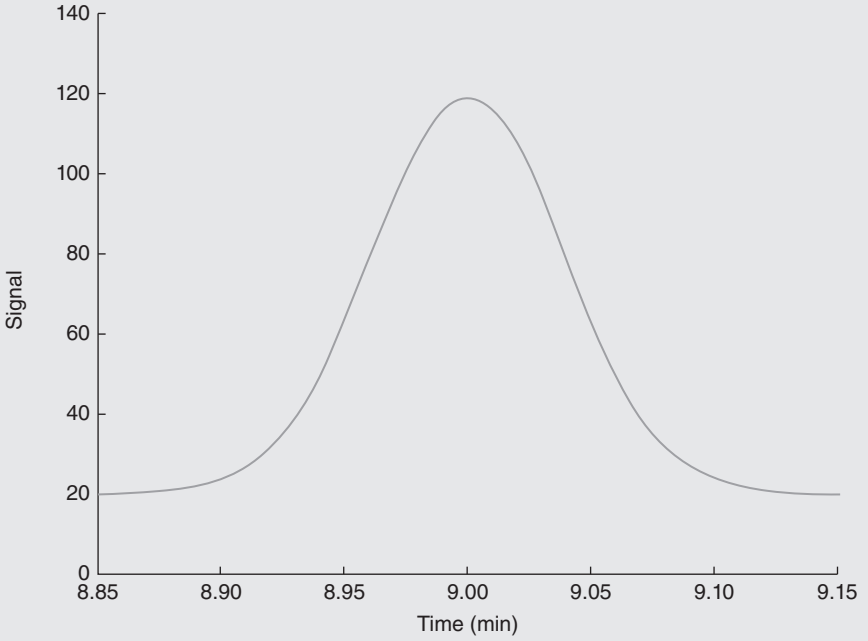


FIGURE 1.8 Illustration showing that narrower peaks have smaller W_b and $W_{1/2}$ than do broader peaks.

EXAMPLE 1.3

(a) Estimate $W_{1/2}$ and W_b for the peak shown (hint: note that the signal baseline is already at 20).



Answer:
$W_{1/2} = 0.093$ min, $W_b = 0.16$ min.

The half-width should be measured at a signal of 70 because the total height is 120, but the baseline is at 20. So the actual peak height is 100 signal units tall. Half of this is 50, added to the baseline of 20, indicates that the half-width occurs at a signal strength of 70. The answers given here were obtained by blowing up an image of the peak and estimating the results using a ruler, so values differ slightly depending on the methodology used. The important points are (1) understanding the two measures and how they differ and (2) that the width at half-height is not simply half of the baseline width.

(b) This question is aimed at showing why peak width is important. Imagine a chromatogram with the following four peaks:

Peak	t_r (min)	$W_{1/2}$
1	3.00	0.10
2	4.00	0.10
3	6.00	0.50
4	7.00	0.50

Notice that peaks 1 and 2 are separated from each other by 1.00 min, as are peaks 3 and 4. Accurately sketch the chromatogram. Which peaks are better separated (i.e., not overlapping), peaks 1 and 2 or peaks 3 and 4? What do you conclude about peak widths and their influence on separation?

Answer:
Peaks 1 and 2 are better separated because they have narrower peak widths. While both sets of peaks are separated by the same time difference, wider peaks can overlap, making the peaks poorly resolved and therefore difficult to quantify. This is why we measure peak widths in practice and devote a lot of discussion in the sections below to factors that affect peak widths.

To make comparisons between different columns, the concept of "theoretical plate number," symbolized by N, was introduced. The concept of "plates" is borrowed from distillation columns that have actual plates in the interior (see Figure 1.9). A mixture is heated to boiling at the bottom and a temperature gradient develops along the vertical axis of the column, with cooler temperature at the top. The vapor created by the boiling liquid is enriched in the more volatile components. As this vapor mixture rises, it cools and condensese on the plates. Hot vapors rising up through the column reheat the condensed liquid, causing it to vaporize again, further enriching the vapor in the more volatile components. This process of volatilization and condensation occurs continuously. The result is that the more volatile components are increasingly enriched near the top of the column, leaving the higher-boiling species enriched near the bottom. In industrial distillation processes, the vapor exiting the top of the column, now significantly enriched or composed entirely of the most volatile component, can be condensed. As the temperature of the remaining mixture continues to increase, the less volatile components also exit the column and can be collected. Collecting different fractions at different points in time results in separation or at least partial purification of the components. *Columns with more plates, and thus plates that are closer together, produce better separations* that result in purer components being isolated from the original mixture. It should be noted that the fractional distillation columns that are used in chemical laboratories use glass beads, glass protrusions, or other packing material to provide the surface on which the vapors condense as they rise. In this case, the column does not have plates *per se*, but its performance is still described by the concept of "theoretical plates," as discussed in the following in the context of chromatography.

Chromatography columns do not have discrete, individual plates either and do not separate components based on a temperature gradient along the length of the column, but the theory of plates was borrowed as a way to quantify a chromatographic column's ability to separate the chemicals in a mixture and to compare columns to each other.

The number of theoretical plates for a chromatographic column is given by

$$N = \left(\frac{t_r}{\sigma}\right)^2 = 5.54\left(\frac{t_r}{W_{1/2}}\right)^2 \tag{1.11}$$

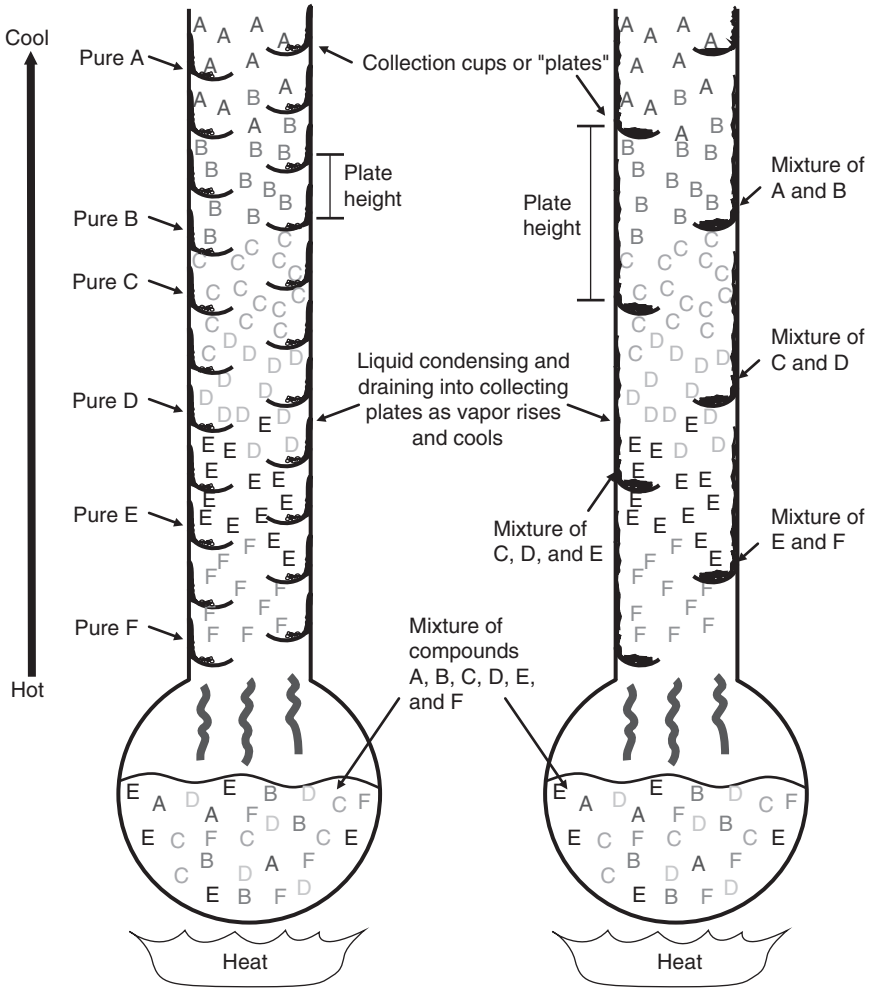


FIGURE 1.9 A mixture of compounds A, B, C, D, E, and F separated with two different distillation columns. The compound volatility follows the order: volatility of A > volatility of B > · · · > volatility of F. The column on the left has more plates and smaller plate heights (the distance between plates). This leads to a more complete separation of the components of the mixture compared to that achieved with the column on the left with fewer plates and larger plate heights. *Chromatography columns do not have actual, physical plates inside them like the distillation columns pictured here*, but the concept is borrowed as a way to measure and compare the separation ability of different columns. The columns depicted here have around 10–20 actual plates, whereas GC and LC columns have thousands or hundreds of thousands of "theoretical plates."

where σ is the standard deviation of the solute peak. The second equality in Equation 1.11 is used because $W_{1/2}$ is easier to measure quickly from a chromatogram than is the standard deviation of the peak. The factor 5.54 assumes that the peak is Gaussian in shape.

Equation 1.11 is essentially a measure of how broad a solute band gets for a given time it spends in the column (hence, $t_r/W_{1/2}$). Columns in which solutes can reside for a long time (large t_r) but still produce narrow peaks (small $W_{1/2}$) have high N values. Columns

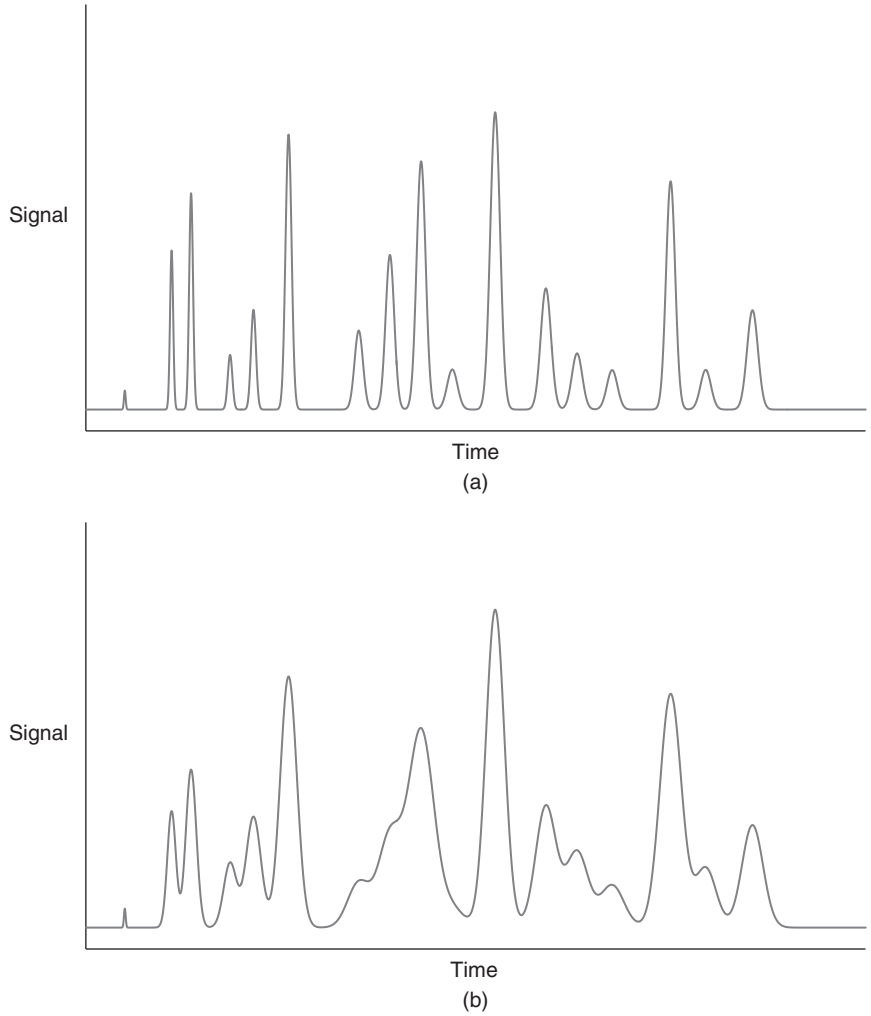


FIGURE 1.10 Illustration of the effects of broad peaks on peak overlap. The peak maxima occur at the same time in both plots, but the bottom plot has broader peaks resulting from a less efficient column with fewer theoretical plates. It is easier to quantify the peaks in the top chromatogram than in the bottom because in the top, all of the peaks are baseline resolved.

with high plate numbers are favored because more sample components can be completely resolved and quantified (see Figure 1.10).

N is also referred to as the "efficiency" of the column, in addition to being called plate number. If peaks remain narrow even after the solutes have been in the column for a long time, then many components in a mixture can be resolved in a single analysis, providing a highly efficient system.

In practice, the number of theoretical plates is directly proportional to the column length, so long columns naturally have higher *N* values than shorter columns, all else being equal (i.e., the same stationary phase, column dimensions, particle characteristics). In order

to compare columns of different lengths, L, a parameter known as the "height equivalent to a theoretical plate (HETP)" or more simply "plate height (H)," is introduced:

$$H = \frac{L}{N} \tag{1.12}$$

In terms of plate theory, this relates to the distance, or height, between the collection plates in a distillation column (see Figure 1.9). Smaller plate heights mean more plates for a given column length, resulting in better separations. *Thus, high numbers of theoretical plates (large N) and small plate heights (small H) are associated with narrow peaks* and generally better resolution than columns with smaller N and correspondingly larger plate heights.

Plate theory as developed by Martin and Synge[2] is useful for discussing peak width and providing a way to compare different columns. It also correctly predicts the elution and separation of compounds based on their equilibrium distribution coefficients. At its heart, however, it uses a series of multiple, discrete extraction steps to model chromatography. It also assumes that complete equilibrium is achieved in each step. Chromatography, however, is a continuous process; columns do not have discrete plates, and equilibrium of the solute partitioning between the two phases is never completely established.

Plate theory also ultimately predicts that the solute distribution (i.e., peak width) at any particular point in the column is dictated solely by the column characteristics and is therefore the same for all solutes. This means that the widths of all of the solute zones are predicted to be the same at the end of the column. We know from observation, however, that this is not entirely true. Different solutes elute with different widths on the same column when analyzed under identical conditions. Solute properties, such as their ability to diffuse and their adsorption and desorption kinetics, contribute to peak width. These kinetic effects are not considered in the plate model – a fact explicitly acknowledged by Martin and Synge.[2] So while plate theory is useful in some ways, a different theory known as "rate theory" – which considers these kinetic factors – is used to describe peak broadening. Rate theory, therefore, is used as the basis for our discussion of broadening in the following sections.

EXAMPLE 1.4

Suppose the drug warfarin (aka Coumadin) is chromatographed on two different liquid chromatography columns under different conditions and the following data collected:

	t_r (min)	$W_{1/2}$ (min)	Column length (cm)
Column 1	10.350	0.160	25.0
Column 2	8.721	0.130	10.0

(a) Calculate the number of theoretical plates on both columns (assume Gaussian peak shapes were obtained).

(b) Calculate the HETP (in cm/plate) for both columns.
(c) Which column is better in terms of HETP?

Answer:

(a) Plates for column 1:

$$N = 5.54\left(\frac{t_r}{W_{1/2}}\right)^2 = 5.54\left(\frac{10.350}{0.160}\right)^2 = 23{,}182 \approx 23{,}200\,\text{plates}$$

Plates for column 2:

$$N = 5.54\left(\frac{t_r}{W_{1/2}}\right)^2 = 5.54\left(\frac{8.721}{0.130}\right)^2 = 24{,}932 \approx 24{,}900\,\text{plates}$$

(b) HETP for column 1

$$\text{HETP} = \frac{L}{N} = \frac{25.0\,\text{cm}}{23{,}200\,\text{plates}} = 0.00108\,\text{cm/plate}$$

HETP for column 2

$$\text{HETP} = \frac{L}{N} = \frac{10.0\,\text{cm}}{24{,}900\,\text{plates}} = 0.000402\,\text{cm/plate}$$

(c) While the total number of plates is comparable on both columns, because column 2 is shorter, it has a smaller plate height. When comparing columns in terms of HETP values, smaller plate heights are better.

Another question:
What is the HETP for conditions under which warfarin elutes at $t_r = 3.715$ min, $W_{1/2} = 0.083$ min, with a 15-cm column.

Answer:
0.0013 cm/plate.

1.2. BAND BROADENING

The previous section describes ways to quantify peak width and general column performance, but it does not describe the physical processes that lead to band broadening. As mentioned, a model of chromatography known as rate theory is used to examine the movement of molecules in a column and the factors that affect peak width. Rate theory specifically considers four main contributions to band broadening:

1. *Axial* diffusion, also called *longitudinal* diffusion;
2. *Radial* diffusion, also called *lateral* or *transverse* diffusion;
3. The existence of multiple paths with different linear velocities within the column; and
4. The rate of mass transfer (i.e., analyte transport) within and between the stationary and mobile phases, which is influenced by both diffusion and convective (flow) processes.

We develop our understanding of broadening dynamics starting with the simplest system and applying what we learn from it to more complex ones, discussing

1. open tubes with no stationary phase and hence no retention of solutes (not practical for chromatography but a useful place to start);
2. open tubes with a thin stationary phase coating on the walls that causes retention – the situation that exists in capillary gas chromatography; and lastly,
3. packed columns, which applies to virtually all liquid chromatography separations and to gas chromatography separations that are conducted in columns packed with particles coated with a stationary phase (in contrast to capillary columns where the stationary phase is coated on the walls of the columns).

1.2.1. Diffusion

An explicit discussion of diffusion is important because it plays a critical role in chromatography. Diffusion is the completely random movement of molecules driven by their translational kinetic energy. This leads to their movement from a region of higher concentration to lower concentration. In other words, solutes diffuse in response to concentration gradients. Diffusion operates in the absence of bulk motion (i.e., no stirring or mechanical mixing).

To picture diffusion and how it relates to band broadening in chromatography, imagine a very narrow plug, or band, of solute molecules that is introduced into an open tube, as shown in Figure 1.11, where the dot density indicates solute concentration. The solute concentration in the areas in front of and behind the band is *initially* zero. Over time, the solute molecules diffuse out from the concentrated plug in both directions due to random molecular motion, as shown in Figure 1.11b and c. The longer the period of time they diffuse, the further they travel. Ultimately, the entire tube will reach the same concentration throughout, assuming it is capped at the ends.

Because diffusion is based on random movements of molecules, Gaussian statistics apply and the broadening of the solute plug over time can be related to the standard deviation in distance units (i.e., the spread of molecules) through Equation 1.13 (the Einstein diffusion equation):

$$\sigma^2 = 2Dt \tag{1.13}$$

where t is time and D is the diffusion coefficient of the diffusing molecules (with units of length^2/time). This equation shows that the standard deviation of molecules that diffuse rapidly (higher D) is larger than for molecules that diffuse more slowly when given the same amount of time to diffuse. It also shows that broadening increases over time. The effect of time and diffusion coefficients on the spread of molecules is depicted in Figure 1.12.

Diffusion coefficients measure how rapidly the molecules spread out in the medium they are in. Small molecules generally have larger diffusion coefficients than large molecules. Also, diffusion coefficients measured in the gas phase are 10,000–100,000 times greater than in the liquid phase – these are important relationships that should be noted.

It is important to note also that diffusion is rigorously the movement of molecules in response to a concentration gradient. This is in contrast to convection, which is the transport of molecules caused by bulk flow arising from sources such as stirring, pushing liquids and

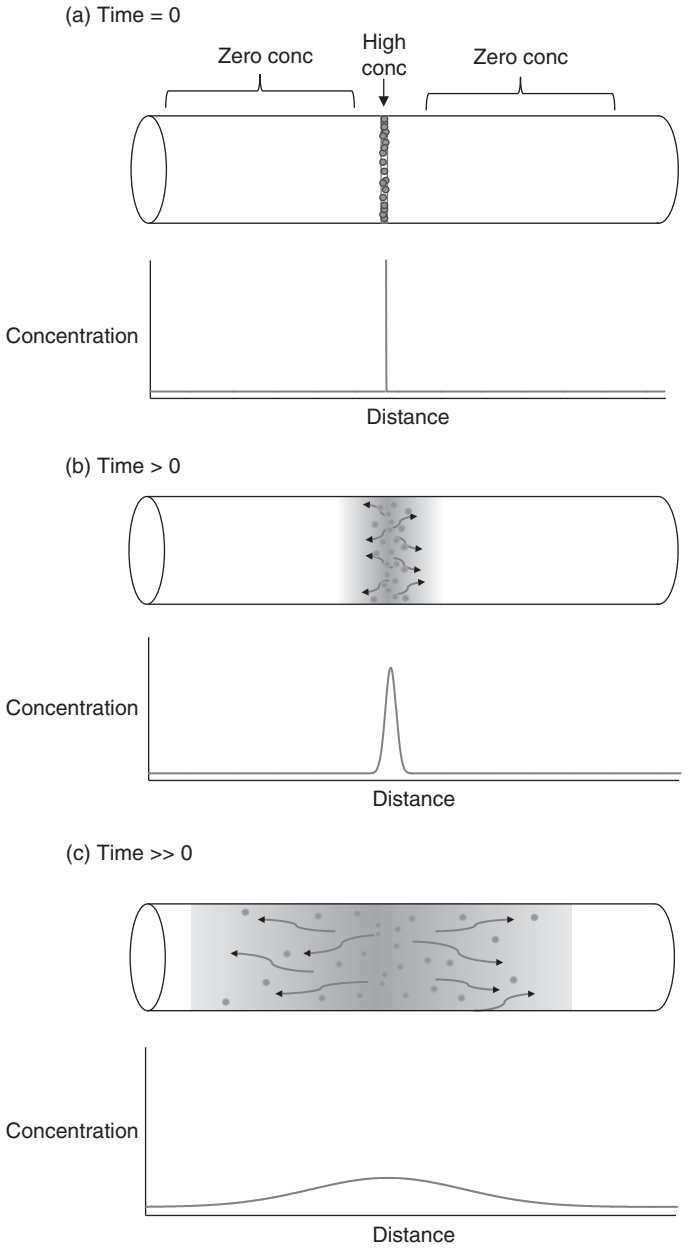


FIGURE 1.11 Depiction of solute diffusion over time in an open tube. (a) An infinitely narrow plug of solute molecules. (b, c) Solutes diffuse toward regions of lower concentration down the long axis of the column (i.e., longitudinally). The more time that is allowed for diffusion, the broader the distribution of solute molecules, as depicted below each column.

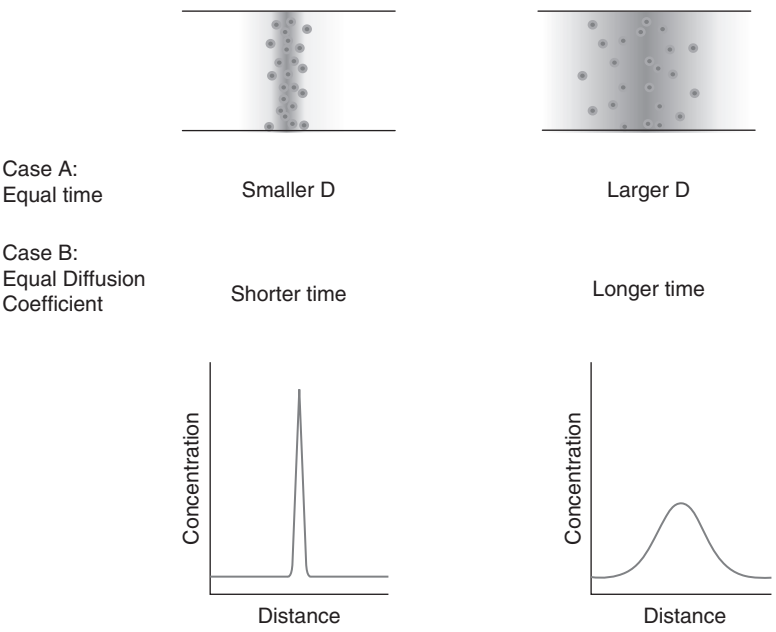


FIGURE 1.12 The effect of time and diffusion coefficient on band spreading. In case A, the time allowed for diffusion is assumed to be equal. In this case, molecules with higher diffusion coefficients spread out more than do ones with smaller diffusion coefficients. In case B, the diffusion coefficients of the two sets of molecules are assumed to be the same. In this case, the longer the time the molecules are allowed to diffuse, the more broadening occurs. So increased time and higher diffusion coefficients both lead to increased band spreading. Conversely, shorter times allowed for broadening and smaller diffusion coefficients reduce the amount of band broadening.

gases through tubes using higher pressure on one side than the other, or from density gradients. Many of the band-broadening factors we consider below are linked to diffusion, so diffusion coefficients and time are important variables in a number of equations. Diffusion is rather efficient at moving molecules over short distances but bulk flow (convection) is much more effective than diffusion over long distances.

1.2.2. Linear Velocity

In this discussion, we talk a lot about velocity, and in all cases we really mean the linear velocity of the mobile phase. It has the same definition here as it does when considering cars, planes, etc. It is simply the distance traveled over the time it took to travel that distance. In chromatography, there are really three distance scales that are important. The first is the length of the column, and the relevant time is the time it takes a completely unretained species to travel the length of the column. The major transport mechanism on this scale is the flow (convection) of mobile phase through the column. So below, when we use the word velocity, we are envisioning the speed with which molecules move when they are in the mobile phase. The second and third distance scales are the radius of the open tube and

the diameter of a particle in a packed column. The major transport mechanism on these last two scales is diffusion.

Molecules can travel through the column on different paths. The paths have different velocities, so all of the molecules are not moving at the same speed all the time. These different velocity paths are a source of band broadening because the molecules that move faster than others elute slightly ahead of the rest while those that are on slower paths elute slightly later. We discuss the consequences of these different velocity paths in the following sections.

We also limit our discussion below to situations in which the mobile phase flow is "laminar" as opposed to "turbulent." Laminar flow means that the mobile phase flows with a regular, "nonviolent" motion. For laminar flow, picture a river with a brisk flow but where the water curves around rocks without creating swirls or foam. In contrast, turbulent flow is comparable to a stretch of the river that has rapids.

1.2.3. Broadening in Open Tubes with No Stationary Phase and No Retention

As indicated earlier, we first visualize processes occurring in simple open tubes with no stationary phase and no solute retention. In the following section, we add stationary phase considerations, and then we fill the column with particles and examine the contributions of each of these additions to broadening.

1.2.3.1. Parabolic Flow Profile and Radial Diffusion in the Mobile Phase. To begin our considerations, in this section, we examine the effects that parabolic flow and diffusion have on broadening in open tubes. When a solute plug is introduced in an open tube and then pushed down the column using a carrier fluid (gas or liquid), a parabolic flow profile – also called laminar flow – develops inside the column as shown in Figure 1.13. In parabolic flow, molecules at the center of the column move faster than the molecules near the column walls. In fact, molecules right at the walls are in a region of zero velocity (i.e., not moving) and molecules at the center of the column move at twice the average velocity of all the molecules. In three dimensions, the shape of the flow resembles a bullet. As the pressure is increased, the parabolic flow profile becomes more pronounced and the differences in the velocities of the molecules from the center to the walls increase. In the absence of any other molecular motion, the solute zone spreads out across the entire length of the column, resulting in incredibly broad peaks.

But we know from experience that this does not happen, so what keeps the solute zone from being infinitely broad? The answer is radial diffusion. Consider Figure 1.14. Solutes that are at the center of the column and therefore out ahead of the other solutes experience a radial concentration gradient (i.e., a gradient in the direction of the radius of the column). The concentration at the wall is zero, whereas it is high in the center. So molecules diffuse from the center toward the wall. This is called radial diffusion because it occurs in the direction of the radius of the column. In doing so, the molecules move from a faster flow path to a slower path. Solutes at the wall at the rear of the zone also experience a radial concentration gradient. So solutes near the wall diffuse radially toward the center, and in doing so move from a slower flow path to a faster one. This occurs throughout the parabolic flow profile, so molecules are constantly randomly diffusing between different velocity

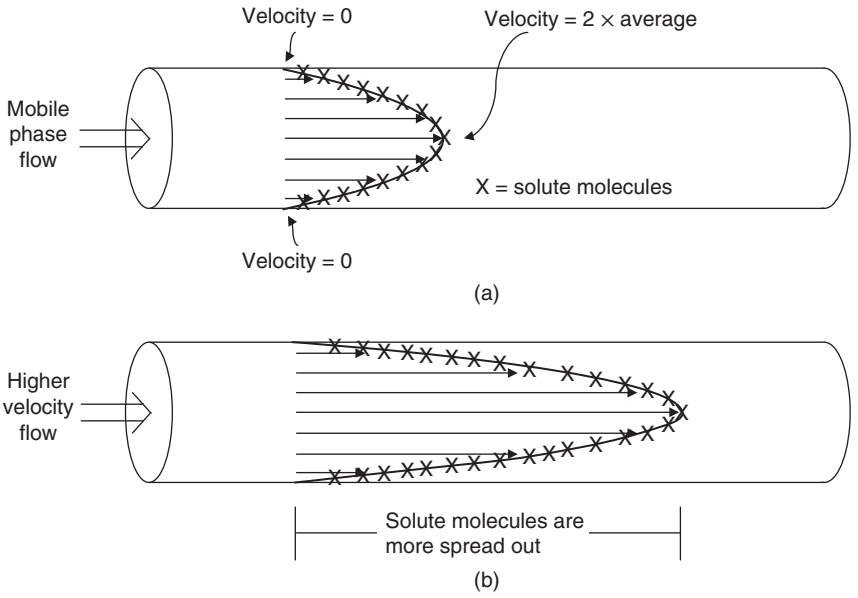


FIGURE 1.13 Depiction of the parabolic flow profile. (a) Parabolic flow at a lower mobile phase velocity. (b) With a higher mobile phase velocity, the parabolic flow profile becomes more pronounced and solutes are spread out over a greater distance (assuming at this point that there is no mechanism for combating the spread of molecules).

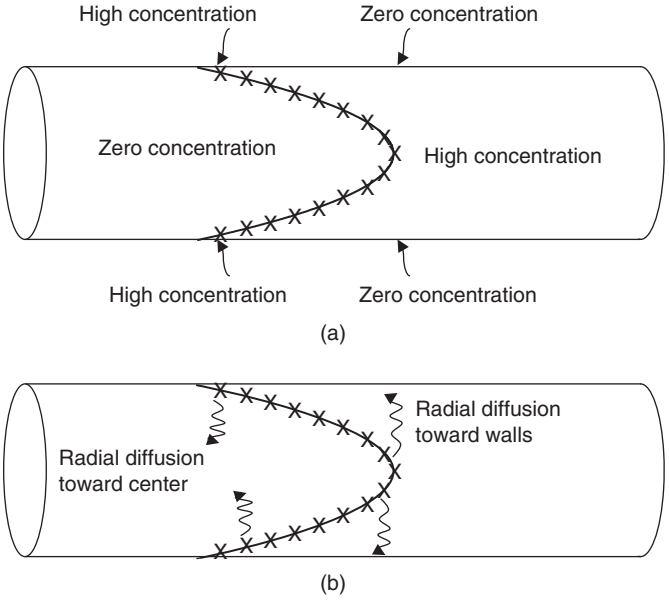


FIGURE 1.14 Depiction of radial diffusion in response to concentration gradients caused by parabolic flow. X = solute molecules. Note that in (a), on the far right side, the solute concentration is high in the center of the column and zero at the walls. Conversely, on the far left side, the solute concentration is high near the walls and zero in the center of the column. In both cases, a radial concentration gradient exists. In (b), radial diffusion acts to decrease these concentration gradients.

flow paths. Thus, for the entire collection of molecules within the zone, there is an overall *average axial velocity* at which the zone travels through the column.

As with any process dictated by random movements, like diffusion, most of the molecules travel at or close to the average velocity, while a few travel faster and a few slower. The molecules that move slightly faster elute from the column at slightly shorter retention times, those that travel slightly slower elute at longer times, and most of them elute around the center of the peak. This is consistent with the Gaussian shape of chromatographic peaks.

It is important to note that radial diffusion keeps the solute zone together, which decreases broadening, and thus decreases the plate height, H. Chromatographically, this is a good thing. In fact, broadening due to parabolic flow and its relaxation by diffusion is given by[3–7]

$$H = \frac{R^2\overline{u}}{24D_m} \tag{1.14}$$

where H is the plate height introduced earlier in the chapter (recall that small H values are favorable), $\overline{u}$ is the average linear velocity of the mobile phase through the column (and therefore also of solutes when they are in the mobile phase), D_m is the diffusion coefficient of the solute in the mobile phase (liquid or gas in the case of LC or GC, respectively) and R is the radius of the tube or column.

From this, we see that molecules that diffuse rapidly (larger D_m) produce narrower peaks associated with smaller H values than molecules that diffuse more slowly. This is because solutes that diffuse quickly randomly sample more velocity paths, increasing the averaging that takes place and reducing the spread of solute velocities present within the zone. As stated earlier, smaller molecules tend to have higher diffusion coefficients than do larger ones, so something like benzene (single ring) diffuses more rapidly than pyrene (four fused rings) and produces narrower peaks, all else being equal.

From Equation 1.14, we also see that higher mobile phase velocities lead to broader peaks associated with higher plate heights. This arises from two effects:

1. When the zone is moved through the column faster, an exaggerated parabolic flow profile exists, as seen in Figure 1.13, which naturally increases the breadth of the peak.
2. Furthermore, because the solutes are traveling faster, they spend less time in the column. This means there is less time for individual solute molecules to diffuse radially and experience many different velocity paths, so less averaging occurs. If less averaging occurs, a broader range of solute velocities exists and solutes elute over a broader range of time, resulting in broader chromatographic peaks.

Lastly, from Equation 1.14 we see that for open tubes, bigger column radii lead to bigger plate heights (broader peaks), and conversely, smaller radii lead to narrower peaks. Bigger radii mean longer distances that the molecules have to diffuse in order to randomize the velocity paths they experience. With bigger radii, solutes experience fewer velocity paths and thus less randomization and more broadening. For this reason, narrower columns produce smaller H values, and subsequently higher N (theoretical plates) and more efficient separations than do wider bore columns.

1.2.3.2. Effects of Longitudinal Diffusion. While diffusion acting in the radial direction is a good thing in terms of keeping the solute zone together, solute diffusion in the direction of the long axis of the column, also known as longitudinal diffusion, must also be considered. As shown when we first considered diffusion (see Figures 1.11 and 1.12), longitudinal diffusion – also called axial diffusion – spreads out the molecules along the length of the column. This occurs because the solute concentration in front of and behind the solute zone is lower than it is in the center of the zone. In contrast to the effects of diffusion in the radial direction, the longer the molecules remain in the column, and the faster they diffuse, the broader the peak gets due to longitudinal diffusion. In fact, the broadening arising solely from longitudinal diffusion can be computed as[8]

$$H = \frac{2D_m}{\overline{u}} \tag{1.15}$$

where D_m is again the diffusion coefficient of the solute in the mobile phase and $\overline{u}$ is the average mobile phase velocity. It is important to keep in mind that we are considering the situation in which there is no stationary phase and therefore no retention. So the average mobile phase velocity is the only factor dictating the length of time a solute zone spends in the column. Lower velocities result in longer times spent in the column and do so equally for all solutes, regardless of structure. Similarly, higher velocities result in shorter times and also affect all solutes equally. Equation 1.15 shows that at low velocities (long time spent in the column), H is large because the molecules have more time to diffuse along the axis of the column. Also, molecules that diffuse faster (higher D_m) produce broader peaks. This makes sense because the faster the molecules diffuse away from the center of the peak, the broader the peak gets.

The two sources of broadening can be added together with the result that

$$H = \frac{2D_m}{\overline{u}} + \frac{R^2\overline{u}}{24D_m} \tag{1.16}$$

for solutes being forced through an *open tube* with *no stationary phase* and *no retention* under conditions in which parabolic flow exists.

EXAMPLE 1.5

These problems are designed to get at the effects of diffusion as well as the relative contributions of the two terms in Equation 1.16.

What value of H is expected for *n*-octane (as a solute) traveling in hydrogen gas as the mobile phase with an average linear velocity of 60.0 cm/s in a 30.0-m column with a 0.250-mm inner diameter, with no stationary phase? In H_2, the diffusion coefficient of *n*-octane is 0.211 cm^2/s.[9] These parameters mimic those found in gas chromatography (except that a stationary phase is present when actually doing chromatography). To see the relative contributions of longitudinal (axial) diffusion and the parabolic flow profile, *explicitly calculate the value of the two terms in Equation* 1.16 *separately*.

Answer:

$$H = \frac{2D_m}{\overline{u}} + \frac{R^2\overline{u}}{24D_m}$$

$$H = \frac{2(0.211\,\text{cm}^2/\text{s})}{60.0\,\text{cm/s}} + \frac{\left[\frac{1}{2}\left(0.250\,\text{mm} \times \frac{\text{cm}}{10\,\text{mm}}\right)\right]^2 (60.0\,\text{cm/s})}{24(0.211\,\text{cm}^2/\text{s})}$$

$$H = 0.00703\,\text{cm} + 0.00185\,\text{cm}$$

$$H = 0.00888\,\text{cm}$$

Note that the factor of $\frac{1}{2}$ in the second term is introduced because we have been given the inner diameter of the column but the equation calls for the radius.

It is clear that the longitudinal diffusion term makes a much bigger contribution to broadening than does the parabolic flow profile *in this situation*. But as the average velocity is increased, the first term decreases and the second increases in magnitude.

Another question:

(a) To see the effect of diffusion coefficients on H, repeat the calculation using the diffusion coefficient of *n*-octane in nitrogen (0.0460 cm^2/s).
(b) What effect did the diffusion coefficient have on the contributions to broadening from each of the two terms?

Answer:

(a) $H = 0.00153\,\text{cm} + 0.00849\,\text{cm} = 0.0100\,\text{cm}$.
(b) Slower diffusion significantly decreases the broadening arising from the first term (longitudinal diffusion), and significantly increases the broadening due to the second term, which accounts for the broadening due to the parabolic flow profile and its reduction by radial diffusion.

1.2.4. Broadening in Open Tubes with a Stationary Phase

When a stationary phase is present, Equation 1.16 for broadening in open tubes gets modified in two ways:

1. The second term on the right-hand side becomes dependent on the amount of retention the solutes experience (i.e., dependent on the retention factor, k); and
2. A whole new term gets added to account for slow mass transfer (i.e., diffusion) that occurs in the stationary phase.

The result is the following:

$$H = \frac{2D_m}{\overline{u}} + \frac{1}{24}\left(\frac{1 + 6k + 11k^2}{(1+k)^2}\right)\frac{R^2\overline{u}}{D_m} + \frac{2}{3}\left(\frac{k}{(1+k)^2}\right)\frac{d_f^2\overline{u}}{D_s} \tag{1.17}$$

where d_f is the thickness of the stationary phase film and D_s is the diffusion coefficient of the solute in the stationary phase. This equation, which is referred to as the Golay equation,[10]

is often written more simply where $f(k)$ and $f'(k)$ just represent the respective functions of retention factor that they replace:

$$H = \frac{2D_m}{\overline{u}} + \frac{f(k)}{24}\frac{R^2\overline{u}}{D_m} + \frac{2f'(k)}{3}\frac{d_f^2\overline{u}}{D_s} = B/\overline{u} + C_m\overline{u} + C_s\overline{u} \tag{1.18}$$

where $B = 2D_m$, $C_m = \frac{f(k)}{24}\frac{R^2}{D_m}$, and $C_s = \frac{2f'(k)}{3}\frac{d_f^2}{D_s}$.

The change in the second term compared to the equation for open tubes without a stationary phase results from the fact that solutes near the wall randomly sorb onto or into the stationary phase and thus temporarily stop moving down the column. In the meantime, solutes that remain in the mobile phase continue down the column as shown in Figure 1.15.

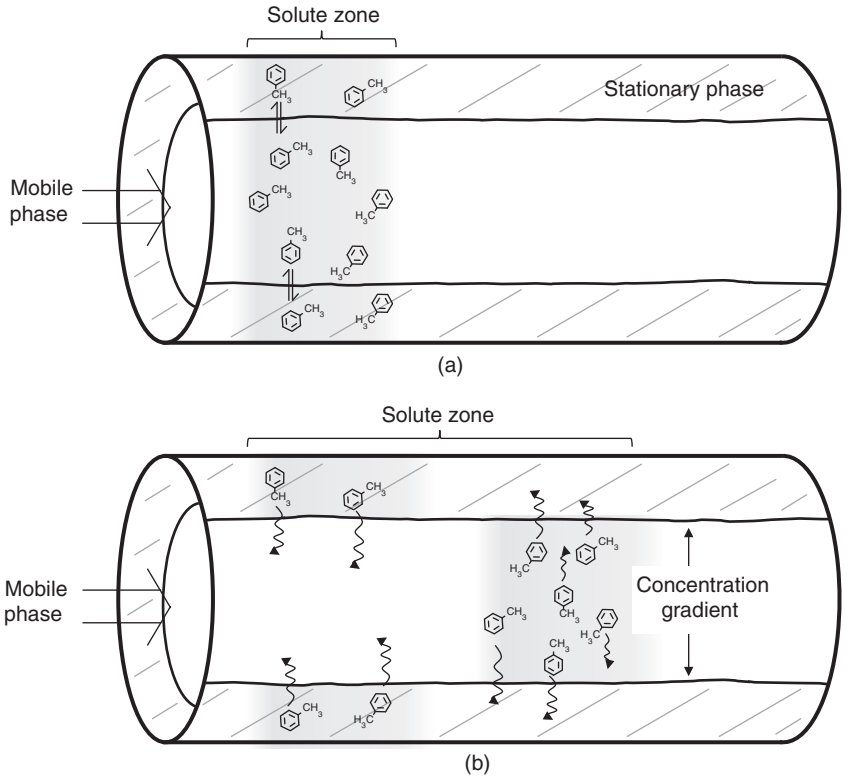


FIGURE 1.15 Effect of radial diffusion in the presence of a stationary phase to help reduce zone broadening. In (a), the solute is in equilibrium between the stationary and mobile phases. In (b), the solutes in the mobile phase have moved down the column, meaning that those in the stationary phase lag behind. This causes the solute zone to broaden. Radial diffusion (signified by the squiggly arrows) in response to the concentration gradients that get created mitigates this effect by averaging out the rate of travel. This process of solutes diffusing into and out of the stationary phase in response to concentration gradients gets repeated the entire time that the solutes spend in the column. Note that the stationary phase thickness in these images has been grossly exaggerated. This has been done simply to help the reader picture the dynamic processes occurring in the column. In reality, the stationary phase thickness is nearly negligible compared to the column diameter.

It is clear that this broadens the solute zone because the molecules are now spread over a greater distance in the column and thus elute over a broader range of time.

The broadening is mitigated by the fact that solutes that move ahead in the mobile phase experience a radial concentration gradient. The gradient is created because some solute molecules that had been in the mobile phase are now interacting with the stationary phase (see Figure 1.16). This means that the solute concentration in the region near the stationary phase is lower than at the center of the column. In response, solutes diffuse radially from the middle toward the stationary phase. By diffusing into this region, these molecules can now also be retained by sorbing to the stationary phase (either *ab*sorbing *into* the stationary phase or *ad*sorbing *onto* the surface). In the meantime, the solutes that had been left behind can move back into the mobile phase and catch up with the zone.

More diffusion radially toward the stationary phase leads to more sorption events per molecule, leading to more averaging of velocities. More averaging leads to narrower peaks and smaller H values. Resistance to mass transfer (i.e., solute motion) therefore leads to broader peaks. For this reason, the second term in Equation 1.17 is often referred to as the "slow mass transfer in the mobile phase" term. As Equation 1.17 shows, if diffusion in the mobile phase is rapid (large D_m), solutes diffuse radially faster and increase the opportunity for averaging the retention of all solute molecules. This reduces the broadening that arises from retention caused by the stationary phase.

Thus, the second term in Equation 1.17 incorporates three different effects:

1. broadening due to parabolic flow (i.e., different velocity flow paths);
2. relaxation of broadening associated with the different flow paths by radial solute diffusion; and
3. the need for rapid radial mass transfer in the mobile phase via diffusion to counteract the broadening effects of retention.

The new term in Equation 1.17 compared to Equation 1.16 arises from slow mass transfer in the stationary phase. To understand this, picture a molecule that is absorbed in a thin film of stationary phase with a given thickness, d_f. As stated, a retained molecule does not move down the column and therefore temporarily lags behind molecules in the mobile phase. In order to catch up, it must first diffuse through the stationary phase in order to get back to the stationary/mobile phase interface. Once it is at the interface, it can then reenter the mobile phase and resume its journey down the column. The faster it diffuses in the stationary phase, the faster it reaches the interface and the less it lags behind, resulting in a narrower peak. This is consistent with the third term in Equation 1.17, which shows that solutes that diffuse quickly (large D_s) produce narrower peaks (smaller H). Conversely, slow diffusion in the stationary phase (i.e., resistance to mass transfer) produces broader peaks.

The film thickness also plays a role. It takes a lot less time for molecules to diffuse through a thin film than a thick film. Reducing the time it takes for the solutes to reenter the mobile phase helps keep the solute zone together. Thus, columns with thin films produce narrower peaks than those with thicker films, all else being equal. It should be noted that the film thickness (d_f) is squared in the third term, so small changes in d_f can produce large changes in H. While thin films are advantageous from a broadening perspective, they also offer less overall retention and less overall solute capacity, so there are legitimate reasons for using thicker films in some circumstances.

The last terms also depend on the mobile phase velocity. Increasing velocity means the solutes spend less time in the column. This means less time for all of the molecules

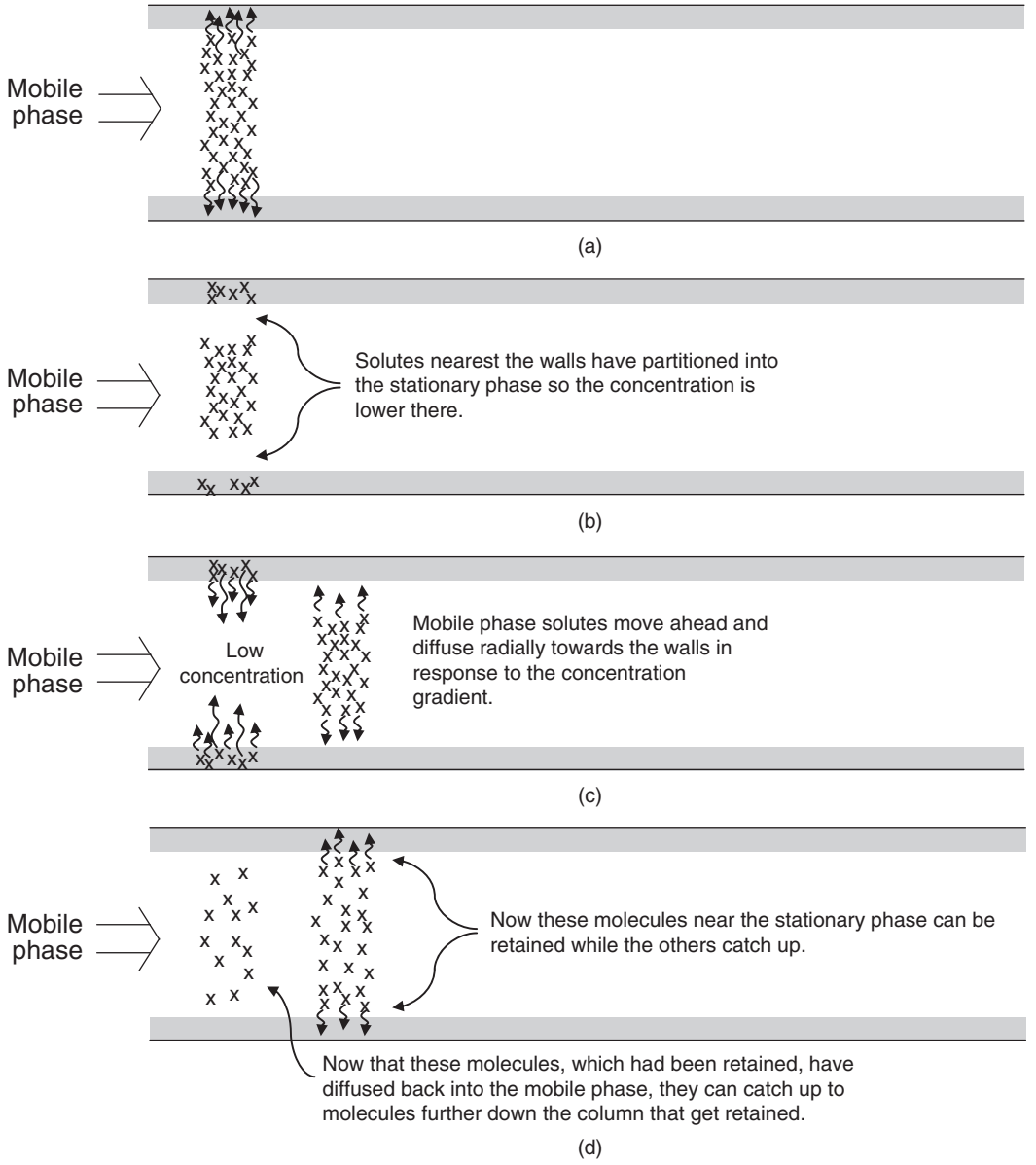


FIGURE 1.16 An illustration of the process of radial diffusion in response to solute retention caused by the presence of a stationary phase. (a) Solutes in the mobile phase initially partition into the stationary phase. (b) Solutes nearest to the stationary phase are those most likely to partition. When they do, a radial solute concentration exists in the mobile phase, with high solute concentration in the center of the column and low concentration near the walls. (c) Solutes in the mobile phase continue down the column and diffuse to reduce the radial concentration gradient. Similarly, solutes in the stationary phase diffuse out in response to the low concentration in the center of the column that arises because the mobile phase has pushed the rest of the molecules down the column. (d) The molecules that have reentered the mobile phase will now be pushed down the column, while molecules out in front are retained as they approach and interact with the stationary phase. It is important to note that these are static pictures, but in reality, all of these processes, in addition to parabolic flow and longitudinal diffusion, are happening in the column continuously and simultaneously. But breaking them up into discrete steps makes it easier to understand the effects of each process on band broadening.

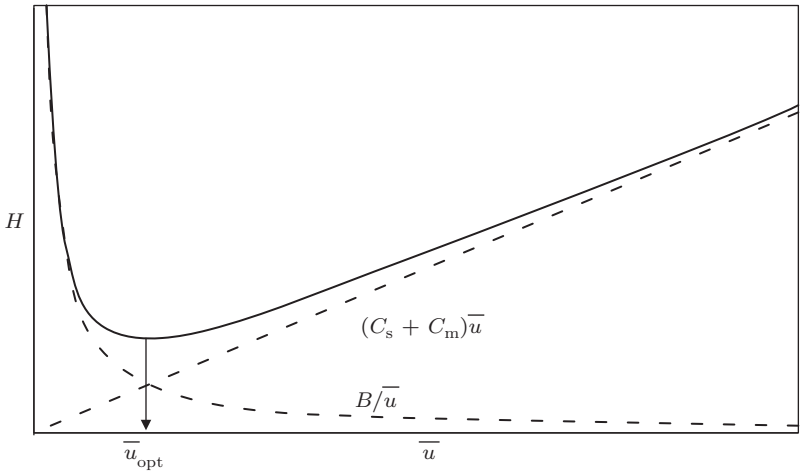


FIGURE 1.17 *H* versus $\overline{u}$ plot for an open tube with a thin stationary phase. There are three main contributions: the *B*, C_s, and C_m terms as shown in Equation 1.18. The C_s and C_m terms are both linear with $\overline{u}$ and their contribution to *H* can be combined as shown. The contribution to *H* of the $B/\overline{u}$ term approaches zero at high average linear velocities. The solid black line is the sum of the two contributions, or the total *H*. Note that at high average linear velocities, the $(C_s + C_m)$ term makes the vast majority of the contribution to the overall *H*. Conversely, at low average linear velocities, the $B/\overline{u}$ term dominates. The arrow indicates the optimum average linear velocity ($\overline{u}_{opt}$), meaning the velocity that produces the lowest plate height and therefore the narrowest peaks. As noted in the text, however, we frequently operate at linear velocities higher than the optimum, accepting the slightly broader peaks that result in order to decrease the total analysis time.

to experience multiple adsorption events and thus less averaging of velocities. So as $\overline{u}$ increases, broadening – as measured by *H* – does too.

It is worth stopping here to point out that the first term in Equation 1.17 indicates that high velocities (associated with high mobile phase flow rates) are favored to reduce *H*, whereas the second and third terms indicate that low velocities are favorable. Combined, the three terms yield an *H* versus $\overline{u}$ plot (Figure 1.17) that indicates that for any column or tube there is an optimal velocity ($\overline{u}_{opt}$) that produces the narrowest peaks (smallest *H*). While it might seem logical to operate at this optimal velocity, in practice we generally operate at higher velocities and accept a slight increase in broadening for the sake of increased speed and faster analyses. This is discussed in more detail later in the chapter.

EXAMPLE 1.6

These problems build on the calculation in Example 1.5 to explore the effect of retention and slow mass transfer in the mobile phase on broadening that were just discussed.

We will use the same conditions as in **Example** 1.5:

- *n*-Octane as the solute with $D_m = 0.211\ cm^2/s$
- Hydrogen gas as the mobile phase

- Average linear velocity of 60.0 cm/s
- Column: 0.250-mm inner diameter, 30-m long.

Now, in this problem, let there be a stationary phase coated on the wall that is 0.25-μm thick and let the retention factor for *n*-octane be 3.25. Let the diffusion coefficient of *n*-octane in the stationary phase be 1.20×10^{-5} cm^2/s (note how much lower this is compared to the value in the gaseous hydrogen mobile phase). Calculate the HETP that results from these conditions. Note that these parameters reasonably mimic those found in gas chromatography.

Suggestion: Explicitly calculate the value of the three terms in Equation 1.17 separately and compare them to the related terms in Example 1.5. This will be more informative and interesting and help provide a sense of the relative importance of the three different terms.

Answer:

$$H = \frac{2D_m}{\overline{u}} + \frac{1}{24}\left(\frac{1+6k+11k^2}{(1+k)^2}\right)\frac{R^2\overline{u}}{D_m} + \frac{2}{3}\left(\frac{k}{(1+k)^2}\right)\frac{d_f^2\overline{u}}{D_s}$$

$$H = \frac{2(0.211\,\text{cm}^2/\text{s})}{60.0\,\text{cm/s}} + \frac{1}{24}\left(\frac{1+6(3.25)+11(3.25)^2}{(1+3.25)^2}\right)\left(\frac{(0.0125\,\text{cm})^2 60.0\,\text{cm/s}}{0.211\,\text{cm}^2/\text{s}}\right)$$

$$+ \frac{2}{3}\left(\frac{3.25}{(1+3.25)^2}\right)\left(\frac{\left(0.250\,\mu\text{m} \times \frac{10^{-4}\,\text{cm}}{\mu\text{m}}\right)^2 60.0\,\text{cm/s}}{1.20 \times 10^{-5}\,\text{cm}^2/\text{s}}\right)$$

$$H = 0.00703\,\text{cm} + (7.57)0.00185\,\text{cm} + 0.000375\,\text{cm}$$

$$H = 0.00703\,\text{cm} + 0.0140\,\text{cm} + 0.000375\,\text{cm}$$

$$H = 0.0214\,\text{cm}$$

The factor of 7.57 in the second term is shown explicitly to show the impact that solute retention has on broadening. Solutes in the stationary phase are not moving down the column, while the solutes that are in the mobile phase move ahead of them. The higher the retention factor, the bigger the effect.

Comparing these results to Example 1.5 shows that the first term due to longitudinal diffusion is the same. The second term, which incorporates the effects of retention and slow mass transfer in the mobile phase, is now the largest contributor to broadening, and slow mass transfer in the stationary phase adds a whole new term but not a significant one because the given film thickness is quite thin.

Another question:

(a) Now, as you did in Example 1.5, repeat the calculation but with nitrogen as the mobile phase. Recall that the diffusion coefficient of *n*-octane in N_2 was given as 0.0460 cm^2/s.
(b) By comparing the results in this example, which represent actual GC practice fairly well, which mobile phase is better, H_2 or N_2? Why?

Answers:

$$H = \frac{2D_m}{\overline{u}} + \frac{1}{24}\left(\frac{1+6k+11k^2}{(1+k)^2}\right)\frac{R^2\overline{u}}{D_m} + \frac{2}{3}\left(\frac{k}{(1+k)^2}\right)\frac{d_f^2\overline{u}}{D_s}$$

$$H = \frac{2(0.0460\,\text{cm}^2/\text{s})}{60.0\,\text{cm/s}} + \frac{1}{24}\left(\frac{1+6(3.25)+11(3.25)^2}{(1+3.25)^2}\right)\left(\frac{(0.0125\,\text{cm})^2 60.0\,\text{cm/s}}{0.0460\,\text{cm}^2/\text{s}}\right)$$

$$+ \frac{2}{3}\left(\frac{3.25}{(1+3.25)^2}\right)\left(\frac{\left(0.250\,\mu\text{m} \times \frac{10^{-4}\,\text{cm}}{\mu\text{m}}\right)^2 60.0\,\text{cm/s}}{1.20\times10^{-5}\,\text{cm}^2/\text{s}}\right)$$

$$H = 0.00153\,\text{cm} + (7.57)0.00849\,\text{cm} + 0.000375\,\text{cm}$$

$$H = 0.00153\,\text{cm} + 0.0643\,\text{cm} + 0.000375\,\text{cm}$$

$$H = 0.0662\,\text{cm}$$

(a) $H = 0.0662$ cm.

(b) H_2 is better because it leads to smaller plate heights. This is due to the higher diffusion coefficient of the solute in H_2, which means solute molecules diffuse radially toward the stationary phase faster than they do in nitrogen. This reduces the broadening caused by retention in H_2 more than in N_2 as demonstrated by the relative magnitudes of the second terms. This counteracts and outweighs the decrease in the longitudinal diffusion contribution (i.e., the first term).

1.2.4.1. A Note About Diffusion Before Considering Packed Columns. In the treatment above, we account for broadening of the solute zone caused by solute diffusion in the mobile phase along the axis of the column, but not by solutes retained in the stationary phase. Molecules do, in fact, also diffuse longitudinally while in the stationary phase. However, we typically apply Equation 1.17 to capillary gas chromatography. In this particular case, the broadening caused by longitudinal diffusion in the stationary phase is negligible compared to that in the mobile phase because diffusion coefficients in the gas phase are 10,000–100,000 times greater than in condensed, or liquid-like phases (such as the polymeric phases used in GC). Thus, broadening due to longitudinal diffusion in the stationary phase is negligible compared to that in the mobile phase in GC. This is not so in LC and modifications to Equation 1.17 are required to account for longitudinal diffusion effects in the stationary phase.[11]

1.2.5. Broadening in a Packed Column

Capillary gas chromatography is generally conducted in open, narrow tubes and therefore follows the broadening phenomena discussed above. But some gas chromatography, and all of liquid chromatography, is conducted in columns packed with particles coated with a stationary phase. While many of the sources of broadening discussed above are still at work in packed columns, the presence of the packing requires modifications to the H versus $\overline{u}$

equation. Using what is known as the "random walk" model of chromatography, Giddings developed the following equation[12]:

$$H = \frac{2\gamma D_m}{\overline{u}} + \sum\left(\frac{1}{2\lambda_i d_p} + \frac{D_m}{\omega_i {d_p}^2 \overline{u}}\right)^{-1} + q\frac{k}{(1+k)^2}\frac{d^2\overline{u}}{D_s} \tag{1.19}$$

The first and third terms on the right-hand side are essentially identical to those in the Golay equation (Equation 1.17) and arise from the same fundamental phenomena. The middle term therefore receives the bulk of our attention. So in the following sections, we briefly address the first and third terms, followed by a more detailed discussion of the second term.

1.2.5.1. The First Term. The first term $\left(\frac{2\gamma D_m}{\overline{u}}\right)$ accounts for the broadening arising from longitudinal diffusion in the mobile phase. Compared to this term in the Golay equation, an additional factor, γ, is included. This is referred to as the obstruction factor and accounts for the fact that solid particles block or disrupt the diffusion of molecules down the axis of the column. Because of this, solutes do not diffuse as far away from the center of the zone as they would in an open tube. In a typical column, γ is between 0.6 and 0.8, whereas $\gamma = 1.0$ for an open tube. Inserting a value of less than 1.0 into the first term decreases its magnitude, resulting in a decrease in H, corresponding to narrower peaks in packed columns compared to open tubes when considering this term only.

More importantly, D_m is the diffusion coefficient of the solute in the mobile phase, which, in the case of LC, is a liquid. As noted earlier, diffusion coefficients in liquids are 10,000–100,000 times lower in liquids than in gases.

1.2.5.2. The Third Term. The third term $\left(q\frac{k}{(1+k)^2}\frac{d^2\overline{u}}{D_s}\right)$ in the packed column equation (Equation 1.19) is identical to the third term in the open tube equation except for the factor q. This factor accounts for the fact that in a packed column, the stationary phase is bonded to or coated on the surface of the pores of the particle and thus is not a uniformly thin film. In the case of regular films coated on a wall, $q = 2/3$ as seen in the Golay equation. With less regular geometry, q needs to be determined experimentally, but its actual value is of little importance here, where our focus is on visualizing the sources of band broadening and the dynamics that occur inside a chromatography column.

Another change is that d_f has been replaced by simply d, which is a measure of the depth of the stationary phase through which the solute can diffuse. If the stationary phase is coated on hard particles, d is essentially the film thickness. In developing this equation, however, Giddings tried to be as general as possible, envisioning not only solid spheres but also allowing for particles that have pores that are completely filled with stationary phase. With such particles, d represents the depth of the pores and the value of q depends on their shape (conical, cylindrical, etc.). In all cases, however, regardless of the specific mathematical form, the third term accounts for the broadening due to solute retention and resistance to mass transfer in the stationary phase.

1.2.5.3. The Second Term. To understand the middle term in Equation 1.19, it is necessary to consider the velocity profile in a packed column. We saw that in open tubes, the velocity varies regularly from the wall to the center of the column. The only mechanism

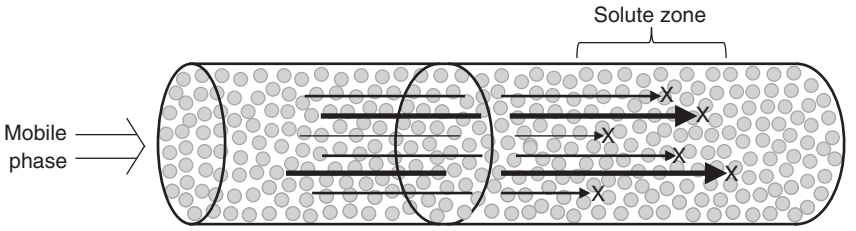


FIGURE 1.18 Velocities of the mobile phase vary in different regions of a column packed with particles (gray dots). The different thicknesses of the arrows represent the differing velocities. The existence of multiple flow paths, along which solutes are carried at different velocities, is a source of band broadening in packed columns. This is illustrated by the solutes (X) at the front of each arrow. They are spread out in their location within the column because of the velocity variations. This means that they will also elute at slightly different times, resulting in band broadening. Note: The size of the particles is greatly exaggerated relative to the diameter of the column. Recall that the most common particle sizes are 5 μm and smaller.

by which an individual molecule can change its velocity is by diffusing radially into a different velocity path. The situation in packed columns is more complicated because the velocity profile across the column is irregular due to the presence of the particles. Velocities are higher along some paths and lower along others, depending on how the particles are packed together – more rigorously, the "packing density" – in different regions of the column. This is depicted in Figure 1.18.

The existence of different velocity paths creates broadening because molecules that reside in faster paths elute before those in slower paths, as shown in Figure 1.18. This phenomenon is quite comparable to the different velocities present in parabolic flow in open tubes, except that the velocities do not vary in a regular way across a packed column as they do in an open tube. The broadening created by the velocity irregularities due to the presence of the particles is often referred to as eddy dispersion or eddy diffusion.

1.2.5.4. Mechanisms That Reduce Broadening. Solute diffusion between different velocity paths is the only mechanism that counteracts the broadening due to radial velocity variations in open tubes. Two mechanisms work in tandem in packed columns to reduce the effects of different velocity paths:

1. Changes in velocity within a given path; and
2. Diffusion of solute molecules between different paths.

Consider Figure 1.19 that shows two different paths that molecules can take through a region of a packed column. A molecule on Path 1 initially moves slowly because the particles are packed tightly together. The velocity in that region will be low due to the obstruction caused by densely packed particles, but the packing "opens up" down the column and the velocity increases in the more open region. On the other hand, molecules on Path 2 initially move at a higher velocity but then slow down where the particles are packed more closely together.

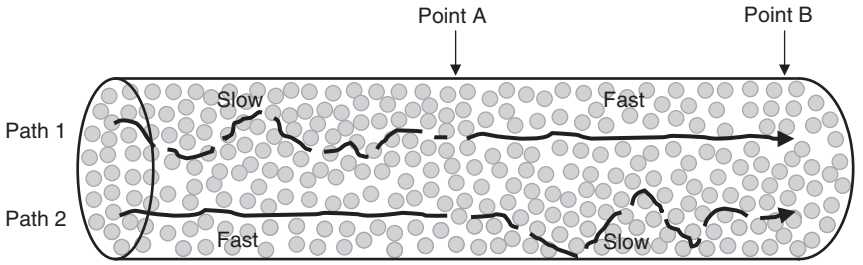


FIGURE 1.19 Depiction of the "flow mechanism" of broadening relaxation. A solute on Path 1 initially experiences a slow velocity due to a high packing density in a particular region of the column. As the packing density decreases, it then experiences a faster velocity. Conversely, a solute on Path 2 initially experiences faster velocity, but then slows as it enters a region of higher packing density. The solute on Path 2 reaches Point A first, but the two solutes might still elute close together in time due to the averaging of velocities that they experience as the move through the column. Again, the size of the particles is exaggerated relative to the column dimensions.

It is clear that solute molecules on Path 2 will get to Point A in the column ahead of solute molecules on Path 1. However, it is equally clear that as the mobile phase and the solute molecules in it on Path 2 slow down between Points A and B, molecules on Path 1 speed up. So the *average* velocity of solute molecules on the two paths might be quite comparable. As a result, solutes on the two paths might elute quite close in time to each other despite the existence of different velocities within the column. Furthermore, note that in this situation, the averaging of the velocities does not occur because of diffusion. In fact, in this extreme example, the molecules never leave their flow paths. The averaging results from variations in the velocity at different axial positions within the flow paths themselves.

There are clearly more than two flow paths through a column, and in fact some paths intersect with other paths, but the general principle of random variation of the velocities of the paths down the length of the column provides a mechanism for averaging out the velocities experienced by the solute molecules. This reduces, but does not entirely eliminate, the broadening that occurs because of the presence of multiple flow paths. Giddings called this mechanism of broadening relaxation the "*flow mechanism*."[13]

The second mechanism that causes averaging of velocities is one we have already seen – radial diffusion of solutes through the mobile phase from one path to another. This

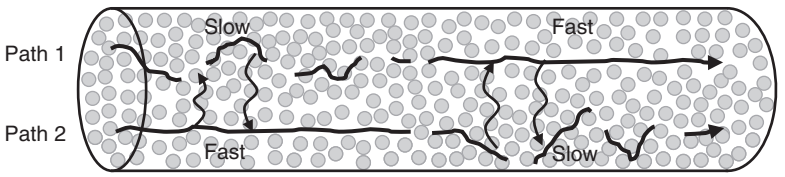


FIGURE 1.20 Depiction of the "diffusion mechanism" of broadening relaxation. Solutes diffuse in the radial direction (depicted by arrows). In doing so, solutes that had been in fast paths move into slower ones, and conversely, solutes that had been in slow paths move into faster ones. In this way, the velocities experienced by all solutes average out, decreasing the effects of band broadening caused by the existence of different velocity paths through a packed column.

is depicted in Figure 1.20 and is called the "*diffusion mechanism*." Just as in parabolic flow, molecules diffuse radially from one flow path to another and in this way experience many different velocities. The more the molecules do this, the more they tend to travel at or near the average velocity and thus elute in a narrow band.

In addition, we saw in the open tube case that diffusion through the mobile phase also brings solutes to the stationary phase where they can be retained. Slow solute diffusion (i.e., slow mass transfer) in the mobile phase toward the stationary phase reduces the number of times a solute is retained. This limits the amount of averaging of the velocities that occurs over all the solutes, leading to a wider distribution of velocities and broader peaks.

1.2.5.5. Mathematical Description of the Flow and Diffusion Mechanisms. In this section, we will first look at two extremes:

1. The case in which the flow mechanism is the only mechanism combating the broadening due to multiple flow paths; and
2. The case in which the diffusion mechanism is the only mechanism at work.

The first case dominates at high average velocities. At high velocities, the molecules spend little time in the column and thus have very little time to diffuse between flow paths, essentially eliminating the potential for the diffusion mechanism to reduce broadening. In contrast, the diffusion mechanism dominates at low velocities, where the molecules have extensive time to experience all of the various velocities. In this case, the reduction in broadening due to diffusion swamps any reduction arising from the flow mechanism.

After having established these two extremes, we address the more realistic situation in which both mechanisms are acting in parallel.

1.2.5.6. Case 1 – Flow Mechanism Only. If the flow mechanism is the dominant cause of the solute velocity averaging, then the contribution to plate height arising from different velocity paths is given by

$$H_{\mathrm{f}} = 2\lambda d_{\mathrm{p}} \tag{1.20}$$

where the subscript "f" indicates the flow mechanism, d_{p} is the diameter of the particles used to pack the column, and λ is a constant related to the range of velocities present relative to the average velocity ($\Delta u/\overline{u}$) and to the packing structure within the column. The λ parameter characterizes how well packed a column is, with smaller values being associated with narrower peaks and smaller plate heights. It is also important to note that H is dependent on d_{p}, with smaller particles leading to narrower peaks (lower H values). This is one of the reasons manufacturers of chromatography columns and instruments have continuously tried to produce systems that can use smaller and smaller particles.

1.2.5.7. Case 2 – Diffusion Mechanism Only. If the diffusion mechanism is the only mechanism reducing the broadening caused by the presence of different velocity paths, then the contribution to H from the different paths is given by

$$H_{\mathrm{d}} = \frac{\omega d_{\mathrm{p}}^2 \overline{u}}{D_{\mathrm{m}}} \tag{1.21}$$

where the subscript "d" indicates the diffusion mechanism, and ω depends on the range of velocities present, the average velocity, and the packing structure (similar but not identical to λ in the equation above).

Here again, we see that smaller particles lead to smaller H values associated with narrower peaks. In fact, the particle size is squared, so using smaller particles leads to a significant reduction in broadening.

This term is also dependent on the mobile phase velocity for the same reasons discussed above in association with broadening in open tubes. As the velocity increases, less time is allowed for the solutes to diffuse between and experience multiple velocities. Therefore, less averaging occurs and solutes elute over a larger range of times.

Equation 1.21 also shows that solutes that diffuse faster (higher D_m) have narrower peaks than those that diffuse more slowly. This makes sense because rapidly diffusing molecules transfer between different velocity paths more frequently, thus experiencing more velocities, leading to more averaging of solute velocities throughout the column and concomitantly narrower peaks.

1.2.5.8. Combining the Flow and Diffusion Mechanisms – The Coupling Term. While in the preceding sections we looked at two separate mechanisms for reducing broadening due to multiple flow paths, it is typical for both mechanisms to be operating at the same time – that is, in parallel. It might be intuitively appealing to think that when they are both contributing we simply add the two equations together. This idea of linear additivity, however, does not apply because the two mechanisms are not independent of each other. For example, a radial diffusion event can cut short, or eliminate, a molecule's experience of a random change in velocity within a given velocity path, and similarly, a random change in velocity along a path can preempt a solute's diffusion into a different velocity path.

When both mechanisms are acting, the combined contribution to the plate height from the existence of different velocity paths is given by

$$H_c = \frac{1}{\dfrac{1}{2\lambda d_p} + \dfrac{D_m}{\omega d_p^2 \overline{u}}} = \left(\frac{1}{2\lambda d_p} + \frac{D_m}{\omega d_p^2 \overline{u}} \right)^{-1} \tag{1.22}$$

where the subscript "c" stands for "combined" or "coupled." (As an aside, we note that this equation mirrors that for determining the net resistance of two resistors in parallel.)

1.2.5.9. Complete Equation for H in a Packed Column. Combining all of the mechanisms that lead to broadening in packed columns leads to Equation 1.23:

$$H = \frac{2\gamma D_m}{\overline{u}} + \left(\frac{1}{2\lambda d_p} + \frac{D_m}{\omega {d_p}^2 \overline{u}} \right)^{-1} + q \frac{k}{(1+k)^2} \frac{d^2 \overline{u}}{D_s} \tag{1.23}$$

which is nearly but not quite the same as Equation 1.19 (the second term is the sum over "i" processes in Equation 1.19), which was introduced at the start of the packed column discussion.

For the sake of completeness, we note that our discussion above focused on broadening arising from different velocity paths caused by differences in obstruction and openness of

the paths. Giddings describes four additional sources of velocity differences within packed columns, each contributing to zone broadening in a manner that is mathematically similar to that discussed above for the middle term.[12] These effects sum together, so Equation 1.23 can be recast more broadly as

$$H = \frac{2\gamma D_m}{\overline{u}} + \sum\left(\frac{1}{2\lambda_i d_p} + \frac{D_m}{\omega_i {d_p}^2 \overline{u}}\right)^{-1} + q\frac{k}{(1+k)^2}\frac{d^2\overline{u}}{D_s} \tag{1.24}$$

which is the equation presented at the start of the packed column discussion, in which the summation is taken over all five broadening mechanisms (i.e., $i = 1$–5) identified by Giddings.

It is common to see this equation written more simply as

$$H = \frac{B}{\overline{u}} + \sum\left(\frac{1}{A_i} + \frac{1}{C_{m,i}\overline{u}}\right)^{-1} + C_s\overline{u} \tag{1.25}$$

where

$$A_i = 2\lambda_i d_p \tag{1.26}$$

$$B = 2\gamma D_m \tag{1.27}$$

$$C_{m,i} = \frac{\omega_i d_p^2}{D_m} \tag{1.28}$$

$$C_s = q\frac{k}{(1+k)^2}\frac{d^2}{D_s} \tag{1.29}$$

This equation is often presented in a simplified, approximate form and is known as the van Deemter equation.[14]

$$H = A + \frac{B}{\overline{u}} + C_m\overline{u} + C_s\overline{u} \tag{1.30}$$

or

$$H = A + \frac{B}{\overline{u}} + C\overline{u} \tag{1.31}$$

Table 1.2 summarizes the physical effects that are accounted for by each term.

In open tubes, $A = 0$ because there are no particles, and in situations in which the diffusion mechanism dominates the second term of Equation 1.25 and Equation 1.30 becomes

$$H = \frac{B}{\overline{u}} + C_m\overline{u} + C_s\overline{u} \tag{1.32}$$

which we saw earlier in Equation 1.18. This equation particularly applies to gas chromatography. In capillary GC with open tubes, there are no particles, and in GC with packed

TABLE 1.2 Physical Significance of the Terms in the van Deemter Equation

Term	Accounts for broadening due to
A	Multiple velocity paths in packed columns (also called eddy diffusion or eddy dispersion)
B	Longitudinal diffusion (also called axial diffusion)
C_m	Slow radial mass transfer of solutes in the mobile phase between different velocity paths and toward the stationary phase. Also accounts for broadening from the parabolic flow profile in open tubes
C_s	Slow mass transfer in the stationary phase allowing solutes in the mobile phase to advance down the column ahead of retained solutes

columns, the condition that the diffusion mechanism dominates normally applies because the diffusion coefficients in gases are so high.

When the flow mechanism dominates (high velocity situations), the equation can be written as

$$H = A + \frac{B}{\overline{u}} + C_s\overline{u} \tag{1.33}$$

which is consistent with the form of the van Deemter equation (Equation 1.30).

1.2.5.10. Specific Considerations for Modern Liquid Chromatography. As mentioned earlier, when Giddings developed his treatment of band broadening that we presented in the previous sections, he largely had in mind GC in wall-coated open tubes or columns packed with particles coated with a stationary phase. Many of the underlying phenomena such as diffusion in the mobile and stationary phases are also relevant to LC, but Equation 1.25 as formulated is only a rough approximation to the band-broadening behavior in liquid chromatography as it is practiced today. The most significant difference is that LC is commonly performed using particles that are porous rather than being completely solid (see Figure 1.21).

The pores in the particle are quite small (60–120 Å pores are typical) and mobile phase does not flow through them even though an elaborate network of channels connect the pores throughout the particle. The pores are, however, filled with the mobile phase that is stagnant inside the pores. This nonmoving mobile phase is therefore referred to as the stagnant mobile phase to differentiate it from the mobile phase that flows around the particles. Both phases, however, have the same composition. The pore walls are also coated with a stationary phase. In the case of RPLC, the stationary phase is typically composed of long alkyl chains chemically bonded to the surface of the pores (see Figure 1.21).

As depicted in Figure 1.21, for solutes to be retained in LC with porous particles,

1. solute molecules must first be brought to the solid support particle through convection (i.e., flow) and diffusion;
2. then they must diffuse into the stagnant mobile phase inside a pore (so solute diffusion coefficients in the mobile phase, D_m, are important);
3. then they must diffuse through the stagnant mobile phase (also dependent on D_m) to the stationary phase where they can be retained by the stationary phase;

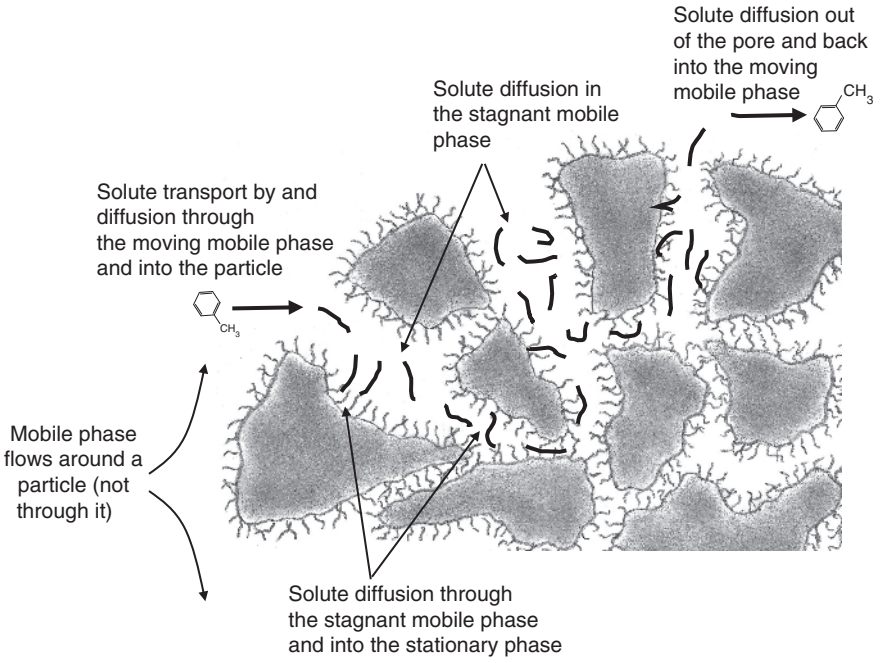


FIGURE 1.21 A depiction of the events involved in solute retention. A quadrant of a cross section of a particle is shown. Shaded areas represent the silica support particle. White spaces represent the pores, which are filled with stagnant mobile phase. In order to be retained, solutes in the flowing mobile phase must (i) be brought to the solid support particle, (ii) diffuse into the particle pores by diffusing through the stagnant mobile phase that fills the pores, and (iii) diffuse into the stationary phase. The solute must diffuse back through the stagnant mobile phase and out of the pores in order to get back into the flowing mobile phase. When they do, they catch up to other molecules of the same kind that have moved down the column with the mobile phase.

4. there they interact with and diffuse through the stationary phase for some time (this is dependent on k and D_s); and
5. finally, the molecules have to diffuse out of the stationary phase back into the stagnant mobile phase, diffuse through the pores in the stagnant mobile phase to a pore exit, and diffuse out of the particle back into the moving mobile phase, all in order to catch up with those molecules that had remained in the flowing mobile phase while they were in the particle.

It is reasonable to deduce that the faster the solutes diffuse in the mobile and stationary phases, the less zone broadening occurs due to the time solutes spent inside the particles. Not surprisingly then, specific mathematical formulations that account for these phenomena include terms related to D_s and D_m and have been treated in the literature.[11,15,16] However, a discussion of them is beyond the scope of this chapter. We do note, however, that Knox and Scott suggested that experimental data acquired over a very wide range of velocities fit equations better if the A-term is allowed to be weakly dependent on velocity

as well.[11] They therefore modified the van Deemter equation as

$$H = A\bar{u}^{0.33} + \frac{B}{\bar{u}} + C\bar{u} \tag{1.34}$$

This equation provides quite good fits to LC data in particular.

1.2.6. Putting It All Together

The overall point of the discussion above is that a lot is going on as solute molecules travel through a chromatographic column. The molecules are carried down the column by the flow of the mobile phase. The mobile phase velocity they experience varies from point to point depending on the path they take, and the existence of different velocities is a major source of broadening. Radial diffusion of solutes through the mobile phase reduces the broadening to some extent. These effects are incorporated in the A-term for packed columns and in the C_m-term for packed and open tubes.

Diffusion of solutes through the mobile phase also transports them to the stationary phase where they can be retained. In capillary GC, this diffusion is toward the coated wall of the column, and in LC with columns packed with porous particles, this diffusion is through both the moving mobile phase and through the stagnant mobile phase inside the pores, toward the stationary phase bonded to the particles. These diffusion processes allow molecules to experience multiple retention events and thus reduce the severe broadening that retention would otherwise cause. These effects are incorporated in the C_m-term in the equations presented above.

In addition, diffusion of the retained molecules through the stationary phase helps the retained molecules get back to the stationary/mobile phase interface. Once at the interface, they can move back into the mobile phase and begin to catch up to the solutes that have moved down the column in the mobile phase, which themselves may have been retained as others are catching up. The effects of solute diffusion through the stationary phase are incorporated in the C_s-terms in the equations presented above (e.g., Equations 1.17–1.19, 1.23–1.25, and 1.30–1.33).

For the effects just described, the slower the mobile phase velocity, the better in terms of broadening. Slower velocities allow more time for radial diffusion, and more sampling or averaging of the various velocity paths that solutes experience. For these effects, then, lower velocities lead to narrower peaks (desirable) and higher velocities lead to broader peaks (undesirable).

However, we also saw that longitudinal diffusion down the long axis of the column also broadens peaks. The slower the mobile phase velocity, the worse the broadening gets. Thus, when considering only this effect, higher velocities are favored over lower velocities. The effect of longitudinal diffusion is incorporated in the B-term in the equations above.

Table 1.3 examines the effects of decreasing the key variables embedded in the A, B, and C terms and the velocity regime (high versus low) in which they are more important. In all cases, increasing instead of decreasing the key variables reverses the effects described in the table.

TABLE 1.3 Factors That Impact Band Broadening

Factor	Impact	Term impacted	Especially important at	Reasons
Smaller D_m	Smaller H	B-term	Low mobile phase velocities	Decreased broadening due to longitudinal diffusion
	Larger H	C_m-term	High mobile phase velocities	Increased broadening due to less averaging of velocities because of fewer radial transfers between different mobile phase velocity paths. Also less averaging of retention events due to slow transport of solutes to the stationary phase
Smaller D_s	Larger H	C_s-term	High mobile phase velocities	Increased broadening due to slower transfer of solutes through the stationary phase and back to the mobile phase allows molecules in the mobile phase to move further ahead down the column. High velocities exacerbate the distance between molecules in the stationary and mobile phases
Smaller d or d_f	Smaller H	C_s-term	High mobile phase velocities	Decreased broadening because solutes in thin stationary phases can diffuse through the thin layer and reenter the mobile phase faster than solutes in thicker stationary phases
Smaller d_p	Smaller H	A-term	High mobile phase velocities	The presence of particles creates multiple velocity paths in the column. The velocity within a path varies with longitudinal distance along the column, and the length of the variations (slow versus fast segments within a particular stream) scales with particle size. This "flow mechanism" term becomes more important at high mobile phase velocities relative to the "diffusion mechanism" term because molecules do not have enough time to diffuse radially between flow paths to average out the velocities
	Smaller H	C_m-term	High mobile phase velocities	Decreased broadening because smaller particles lead to shorter radial distances that solutes have to diffuse in order to join a different velocity path, so more averaging of velocities occurs

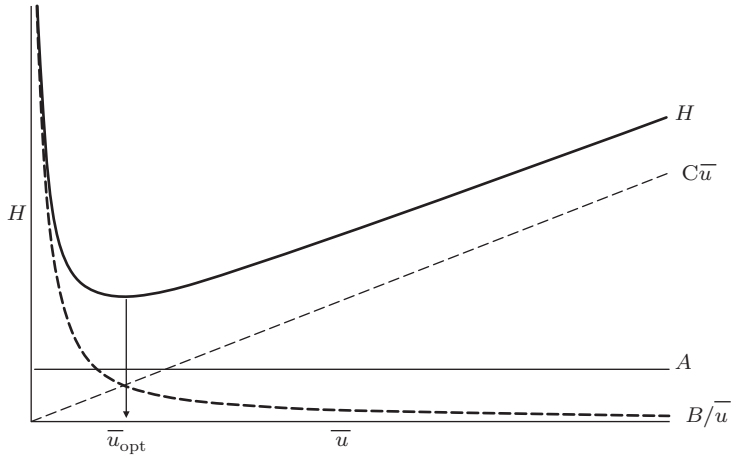


FIGURE 1.22 *H* versus $\overline{u}$ plot for packed columns. There are three main contributions: the *A*-, *B*-, and *C*- terms as shown in Equation 1.33. The *A*-term accounts for multiple velocity paths, the *B*-term accounts for longitudinal diffusion, and the *C*-term includes the contributions of slow mass transfer in the stationary and mobile phases. This plot is similar to that in Figure 1.17 for flow through an open column coated with a stationary phase, except that here, with a packed column, the *A*-term is present and makes a constant contribution at all velocities. As in Figure 1.17, the *B*-term dominates at low average linear velocities, and the *C*-term dominates at high average velocities. The arrow indicates the optimum average linear velocity ($\overline{u}_{opt}$), meaning the velocity that produces the lowest plate height and therefore the narrowest peaks. As noted in the text, however, we frequently operate at linear velocities higher than the optimum, accepting the slightly broader peaks that result in order to decrease the total analysis time.

1.2.7. Practical Consequences of Broadening Theory

Having looked at the physical processes represented by each term, it is possible to combine the *A*-, *B*-, and *C*-terms and plot broadening as a function of linear velocity. Figure 1.22 shows the dependence of each individual term in the van Deemter equation on flow rate and the result when all of the terms are combined. From this plot, it is clear that the *B*-term – broadening due to longitudinal diffusion – makes the largest contributions to *H* at low linear velocities. At higher linear velocities, the *C*-term dominates in regard to the contribution they make to broadening.

The lowest point on the curve corresponds to the linear velocity (and hence the experimental flow rate) at which *H* is minimized, meaning the flow rate that generates the highest efficiency, *N*, possible for the system and conditions being studied. While this is the optimum flow rate in terms of broadening effects, it is common to operate at flow rates above the optimum, because higher flow rates mean faster analyses and more samples analyzed in a given period of time (e.g., more samples per day). For this reason, current emphasis in chromatography is being placed on minimizing the *A*- and *C*-term contributions to broadening. By keeping the slope in the high linear velocity region of the curve low, the flow rate can be increased significantly while incurring an acceptable increase in broadening, as demonstrated in Figure 1.23, curve "*a*." If the slope at high velocities is steep, as it is in curve "*c*," then increasing the flow rates leads to dramatic increases in *H* and much broader peaks,

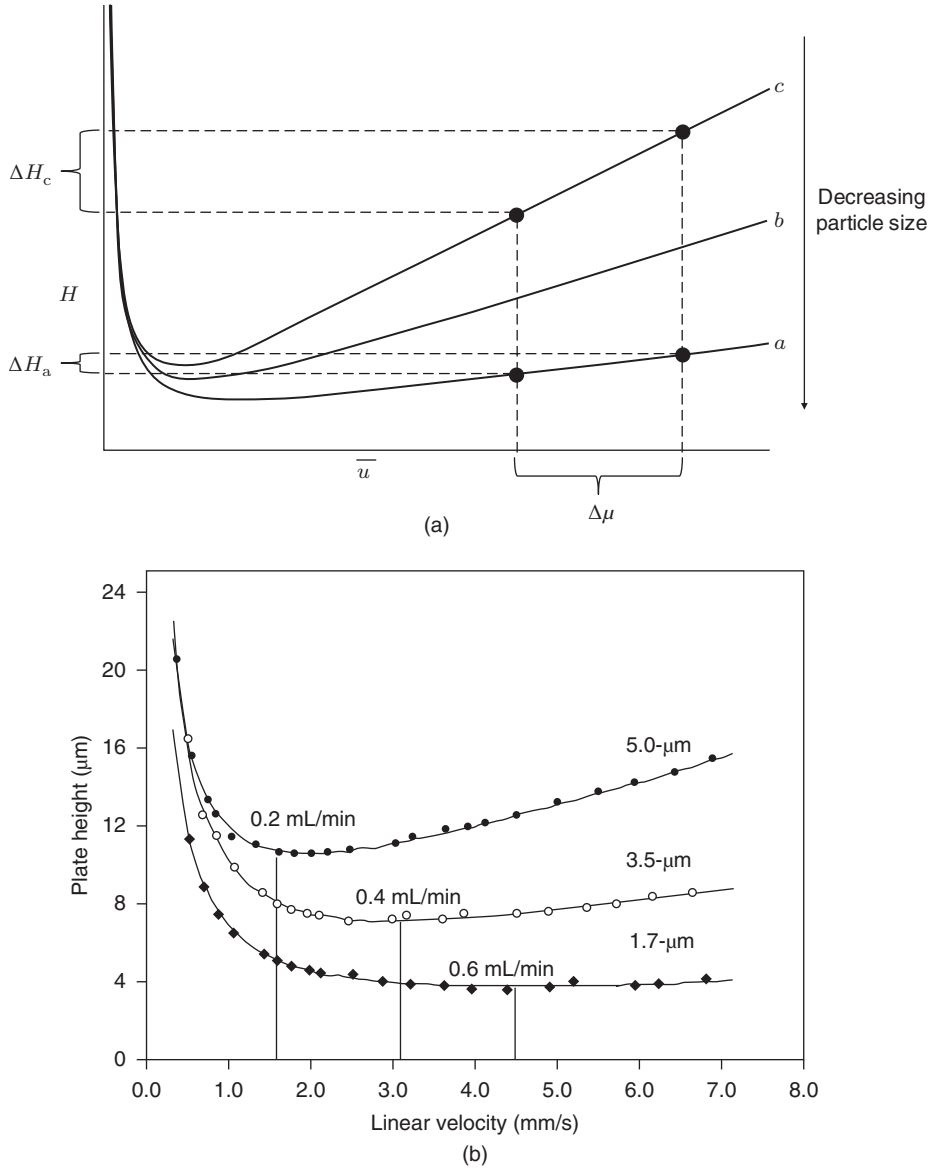


FIGURE 1.23 (a) Depiction of the effect of decreasing particle size on plate height, in which the curve labeled (c) corresponds to larger particles and (a) corresponds to smaller particles. The vertical lines show that with smaller particles, a given increase in the linear velocity ($\Delta\mu$) produces a much smaller corresponding increase in plate height (ΔH) compared to larger particles. This means that smaller particles can be used at higher velocities without significantly increasing band broadening. (b) Experimentally determined van Deemter curves for 5, 3.5, and 1.7 μm particles. The vertical lines correspond to the optimum linear velocity for each column (i.e., the velocity that produces the smallest plate height and thus the narrowest peak). Again we see that the *C*-term in the high linear velocity region is the least steep for the smallest particles, allowing for higher velocities with minimal additional band broadening. Conditions: 2.1 mm inner diameter column; 50/50 v/v (ACN/water); 210 nm detection; 45 °C, benzylphenone as analyte; ultra-high performance LC system. (*Source:* Reprinted with permission from *Amer. Pharm. Rev.* 2008, 11, 24–33, from the authors of that article, and from Dr. Michael Dong who modified the original image for publication in *LC/GC North Am.*, 2014, 32, 553–557, which is the version shown here.)

which can lead to peak overlap, ruin the separation, and make quantitation of solutes less reliable or impossible. These considerations drive much of the practical work being done in chromatography today, including the manufacturing of smaller particles and particles that minimize broadening contributions arising from slow mass transfer. Figure 1.23b shows the dramatic difference in plate height obtained with particles of different diameter. The 1.7 μm particles produce significantly smaller plate heights than either the 3.5 or 5 μm particles.

Current work is also aimed at other instrumental design aspects that minimize broadening that occurs when the molecules are *outside* of the column, such as broadening due to the injection process and broadening that occurs in the tubes that transfer the solutes from the end of the column to the detector (so-called extra-column broadening effects).

Careful consideration of all of these factors has led to significant advances in the speed of LC and GC separations, making it possible to analyze complex samples containing tens or hundreds of compounds in just seconds or minutes.

1.3. GENERAL RESOLUTION EQUATION

In the previous sections, we have seen all of the factors that affect the ability to separate two components, A and B, from one another, including overall retention (k), separation factor (α), and band broadening as measured by the number of theoretical plates (N). These factors can be combined in an overall equation known as the general resolution equation:

$$R = \frac{\sqrt{N}}{4}\left(\frac{\alpha - 1}{\alpha}\right)\left(\frac{k_B}{1 + k_{ave}}\right) \tag{1.35}$$

where k_B is the retention factor of the more retained solute of interest and k_{ave} is the average retention factor for the two compounds whose resolution is being measured. In practice, the resolution between two peaks is only of interest when the two peaks elute near one another – in other words, when $k_A \approx k_B$ such that $k_B \approx k_{ave}$. Given this, for simplicity, k_B or k_{ave} can be used in both the numerator and denominator in the third term in Equation 1.35. An R value of 1.5 typically produces peaks that are just baseline resolved for peaks of approximately the same height.

From Equation 1.35, it can be concluded that high N values (narrow peaks), high α values (large differential retention), and high k values (long retention) increase resolution. Figures 1.24–1.26 show the dependence of resolution on each of these three variables in this equation. Several practical conclusions can be reached by analyzing these graphs:

1. Because of the square root dependence of R on N, increasing N, if possible, reaches a point of diminishing returns (Figure 1.24). Attention to the band-broadening details discussed above can contribute to increasing N by decreasing band broadening, but N is largely determined by the quality and characteristics of the column, including particle diameter, film thickness, and packing quality. So in practice, improvements in N that are large enough to influence R often cannot be made without investing in better columns or instrumentation.
2. Resolution can be improved by increasing retention (k) when retention is low, but at higher retention factors, this effect quickly levels off (Figure 1.25). In fact, the third

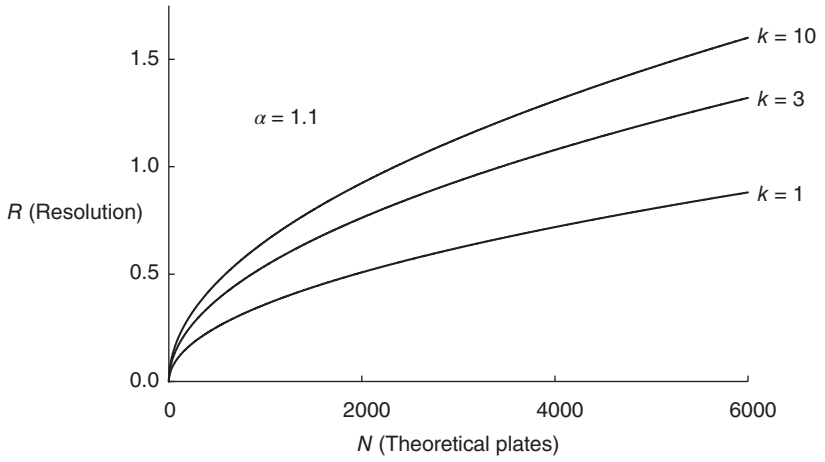


FIGURE 1.24 Resolution (R) as a function of the number of theoretical plates (N) on a column for three different values of the retention factor, using the general resolution equation (Equation 1.35). The separation factor, α, is set at 1.1. Note that R rapidly increases as N increases at low plate numbers, but R increases more slowly at higher N. The square root dependence of R on N mitigates the effect on R of increasing N as N approaches high values.

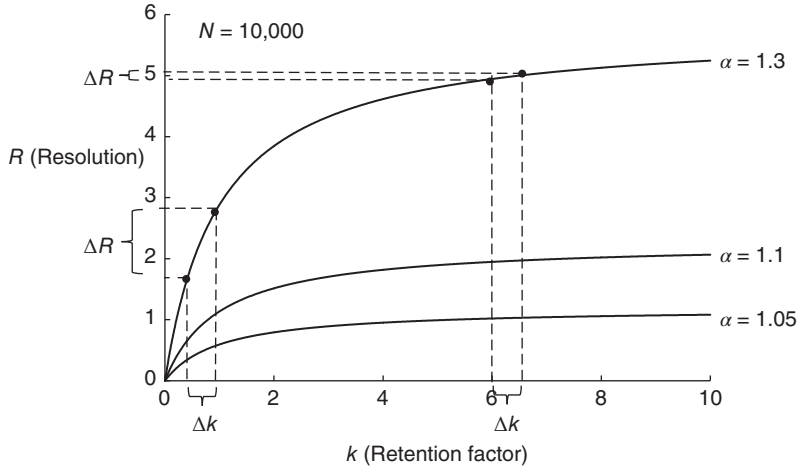


FIGURE 1.25 Effect of retention factor (k) on resolution (R) at three different separation factors (α), calculated using the general resolution equation (Equation 1.35) with $N = 10{,}000$. As illustrated by the dashed lines, increasing the retention factor (Δk) when the retention factor is low increases R (ΔR) considerably. When retention is already high ($k = 6$–10), increasing retention has little effect on improving resolution, but it makes the analyses take longer. It is also clear from this graph that small increases in the separation factor even from 1.05 to 1.10, improve resolution substantially at every retention factor. When viewing this plot, recall that a resolution of 1.5 is generally associated with baseline resolution for peaks of the same height. Values under 1.5 suggest overlapped peaks.

term in Equation 1.35 approaches a maximum value of 1 at high k. In addition, the way to increase k is to increase the retention times of the solutes, thus making the analysis times longer, resulting in fewer analyses per day.

3. Small changes in separation factor (α), particularly near $\alpha \approx 1$ (perfectly overlapped peaks) make large improvements in resolution (Figure 1.26). Thus, by changing the relative retention (i.e., the extent to which one solute is retained relative to another) by a small amount, large gains in resolution can be made. *Thus, in practice, the most*

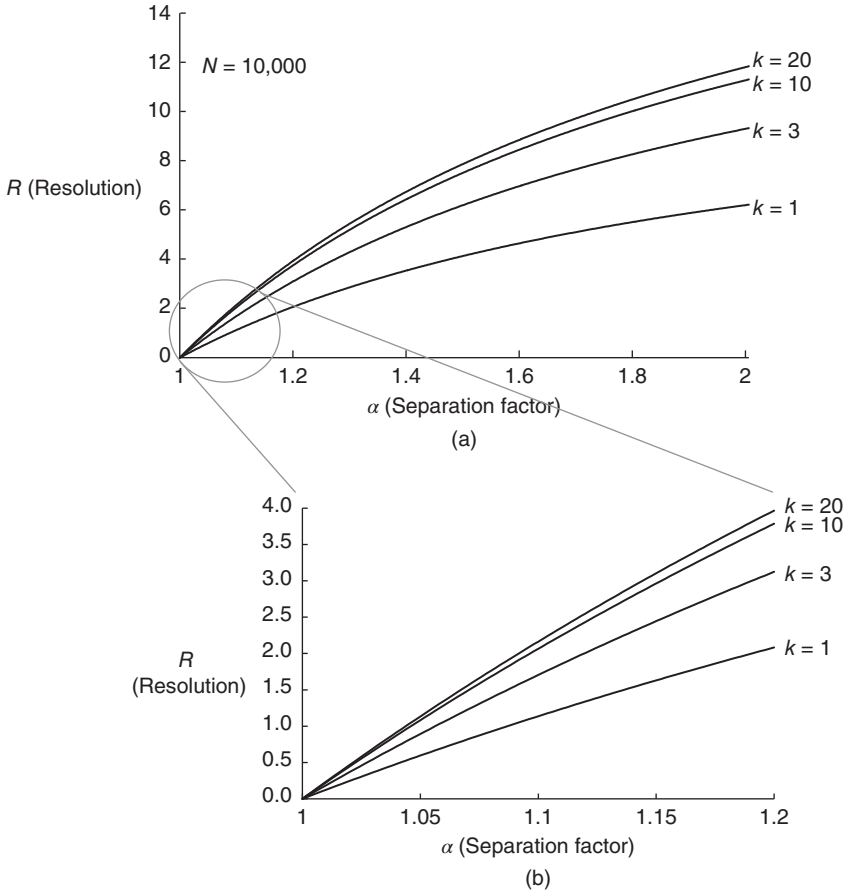


FIGURE 1.26 Effect of separation factor (α) on resolution (R), using the general resolution equation with $N = 10{,}000$ at varying values of retention factor (k). (a) This plot shows that as separation factors increase, so does resolution. However, the effect begins to level off at high separation factors. The plot also reinforces that there is little gain in resolution once retention factors exceed $k = 10$, but significant increases in analysis time result. The inert (b) shows an expanded view of the circled region of plot A. It is clear that small improvements in the separation factor can lead to relatively large improvements in resolution when the separation factor is initially low (i.e., between 1.0 and 1.2). Separation factors are often changed by varying the mobile phase composition (common in LC) or the temperature (common in GC). Recall, baseline resolution is generally associated with $R = 1.5$. These plots show that even small values of the separation factor can achieve baseline resolution of peaks if the solute retention factors are high enough and N is large enough.

important variable in the general resolution equation (assuming adequate N and k) is the separation factor. The operating parameters that affect separation factors in GC and LC are discussed in detail in their respective chapters.

The theory underpinning the general resolution equation provides guidance for changing important operating variables to improve resolution. It drives the way we conduct chromatography. To measure resolution, however, we use data taken from the chromatogram and the equation

$$R = \frac{t_{r,B} - t_{r,A}}{\frac{1}{2}(W_A + W_B)} \tag{1.36}$$

where compound B elutes later than compound A and W is the baseline width of the peaks determined graphically as shown earlier in Figure 1.7.

Again in this equation, we see that bigger differences in retention time (i.e., large $t_{r,B} - t_{r,A}$) and narrow peaks (small W's) lead to higher resolutions, just as we saw in the general resolution equation. The two equations complement each other in that Equation 1.36 provides a way *to measure* resolution while the general resolution equation shows which variables to change *to improve* it.

EXAMPLE 1.7

If *p*-xylene and *m*-xylene elute at 11.38 and 11.53 min, respectively, with a dead time of 3.23 min, on a column with 124,000 theoretical plates, what is the resolution of the peaks? Are the peaks baseline resolved? Assume the peaks are the same height.

Answer:

$$k_{p\text{-xylene}} = \frac{11.38\,\text{min} - 3.23\,\text{min}}{3.23\,\text{min}} = 2.523$$

$$k_{m\text{-xylene}} = \frac{11.53\,\text{min} - 3.23\,\text{min}}{3.23\,\text{min}} = 2.570$$

$$\alpha = \frac{k_{m\text{-xylene}}}{k_{p\text{-xylene}}} = \frac{2.570}{2.523} = 1.019$$

$$R = \frac{\sqrt{124{,}000}}{4}\left(\frac{1.019 - 1}{1.019}\right)\left(\frac{2.570}{1 + 2.547}\right)$$

$$R = 1.189 \approx 1.19$$

Baseline resolution is associated with $R = 1.50$, so these peaks are not baseline resolved and therefore overlap to some extent.

Another question:
How many plates are necessary to achieve baseline resolution (i.e., $R = 1.50$) for these compounds assuming retention times and the dead time do not change?

Answer:
$N = 197{,}000$ plates.

1.4. PEAK SYMMETRY

For a variety of reasons, symmetric peaks are generally favored over asymmetric peaks in chromatography. Examples of asymmetric peaks are shown in Figure 1.27. Peaks that rise gradually but then fall off sharply are referred to as being "fronted," whereas peaks that rise quickly but trail off more slowly over time are referred to as "tailed."

Asymmetric peaks generally indicate that some aspect of the analysis is not optimal. This can arise from a range of phenomena that are beyond the scope of this text. If observed in practice, the source(s) of the asymmetry should be identified and corrected if possible.

The degree of peak asymmetry is measured by the peak asymmetry factor (AF), defined as the ratio of the peak $^1/_2$-widths at a given peak height, often taken at 10% of the total height, leading to

$$\mathrm{AF} = \frac{b}{a} \tag{1.37}$$

where "a" and "b" are defined in Figure 1.27.

EXAMPLE 1.8

Estimate the asymmetry factor for peak (b) in Figure 1.27.

Answer:

$$\mathrm{AF} = \frac{b}{a} \approx \frac{8.040 - 8.028}{8.028 - 7.978} \approx 0.24$$

Another Question:
Estimate the asymmetry factor for peak (c) in Figure 1.27.

Answer:

$$\mathrm{AF} = \frac{b}{a} \approx \frac{8.024 - 7.972}{7.972 - 7.960} \approx 4.3$$

Note: Answers will vary depending on how the times are estimated. It is important, however, to recognize that the times should be estimated from the position of the curve at 10% of the maximum height (not at the baseline) as shown in Figure 1.27.

1.5. KEY OPERATING VARIABLES

From the theory above, it is evident that some of the key variables that must be controlled in chromatography include

1. retention;
2. separation factor (i.e., selectivity);
3. flow rate; and

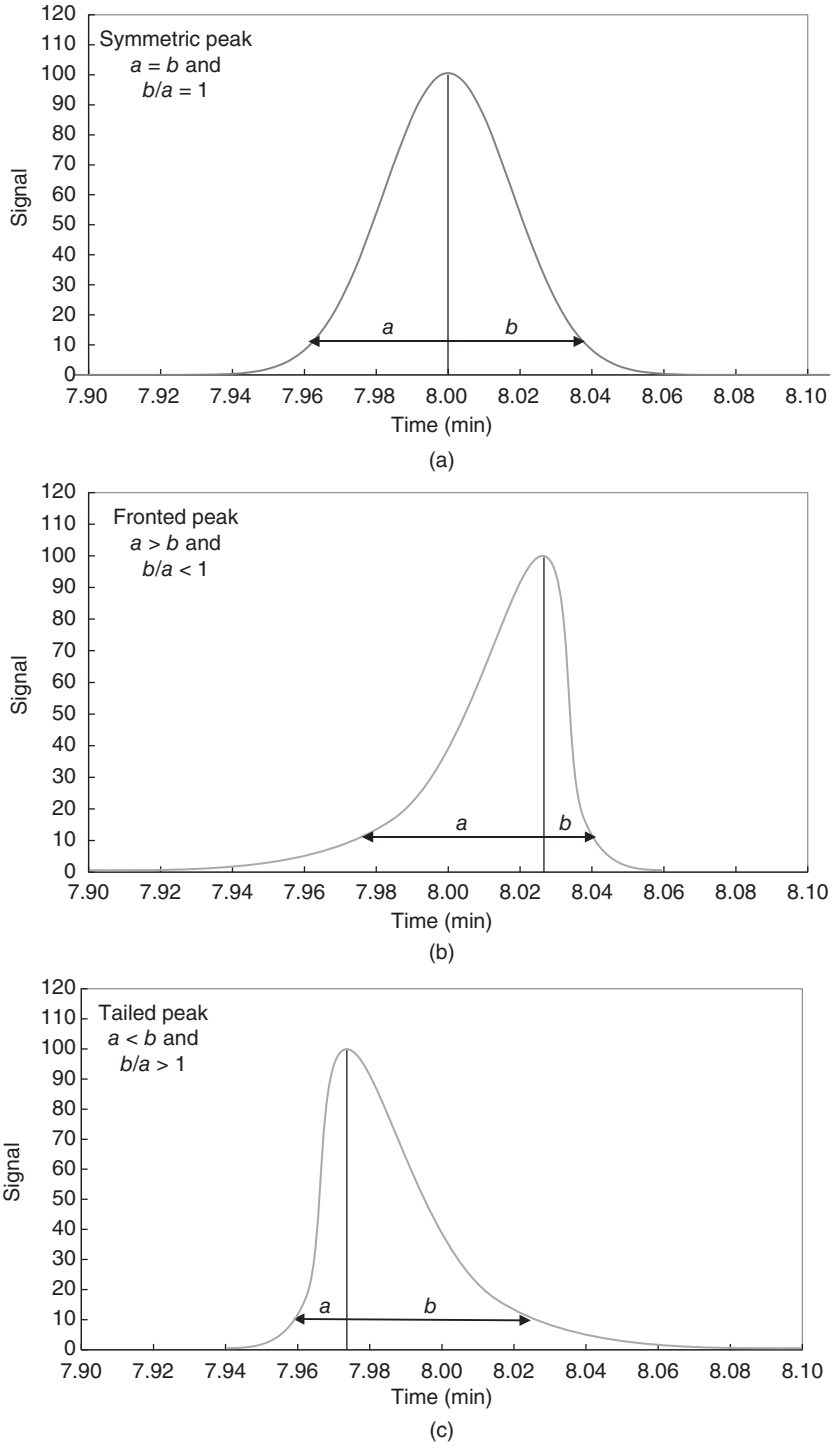


FIGURE 1.27 Depiction of symmetric, fronted, and tailed peaks. The asymmetry factor (AF) is measured as the ratio of *b*/*a*, where *b* and *a* are determined as shown in the figure at 10% of the peak height. Asymmetric peaks often indicate that something is not optimal and the source of the asymmetry should be investigated.

4. factors that affect broadening (particle size, stationary phase thickness, etc.)

The retention and separation factor in GC analyses are generally altered by changing the *temperature* at which the separation is done. The nature of the stationary phase impacts the fundamental intermolecular interactions that cause retention.

In liquid chromatography, the *composition of the liquid mobile phase* plays an important role in the retention and separation factor of solutes. The stationary phase material and the nature of the particles are also key variables in controlling LC separations.

These variables and specifically how they are adjusted in GC and LC are described in detail in the following chapters.

1.6. INSTRUMENTATION

While both GC and LC are fundamentally based on solute partitioning, the instrumentation used in the techniques is different due to the different demands arising from pumping gases versus liquids through columns. The methods of detection are also quite different. Because of these differences, the instrumentation used in each technique warrants its own discussion. Therefore, just like the key operating variables described above, the specific issues of instrumentation are described in more detail in the chapters that deal with GC and LC specifically.

1.7. PRACTICE OF THE TECHNIQUE

In the following two sections, we focus on one of the main reasons to use chromatography, namely to quantify the concentrations of various components in a mixture. As with many analytical techniques, this requires the construction of a calibration curve and often benefits from the use of internal standards or the method of standard additions to improve the accuracy and precision of the results.

1.7.1. Quantitation

A primary use of chromatography is the quantitation of individual components in a mixture, such as the amount of dextromethorphan (a cough suppressant) in a liquid cold medication or the concentration of an organic pollutant in a lake. While specific detection methods and their pros and cons are discussed in the GC and LC chapters, we note here that peak heights and peak areas, regardless of detector type, typically correlate with analyte concentration. Peak areas are generally favored for quantitative purposes because they produce better precision. However, in some cases, peak heights may be preferable. Based on the correlation of peak area with concentration, calibration curves are constructed by injecting solutions with known concentration of the analyte(s), measuring peak areas or heights, and plotting signal versus concentration as is typically done for calibration curves (see Figure 1.28). The sample, containing the analyte of unknown concentration is then injected under the same conditions, and the slope and intercept of the best fit line through the calibration data is used to calculate the concentration of the analyte in the sample.

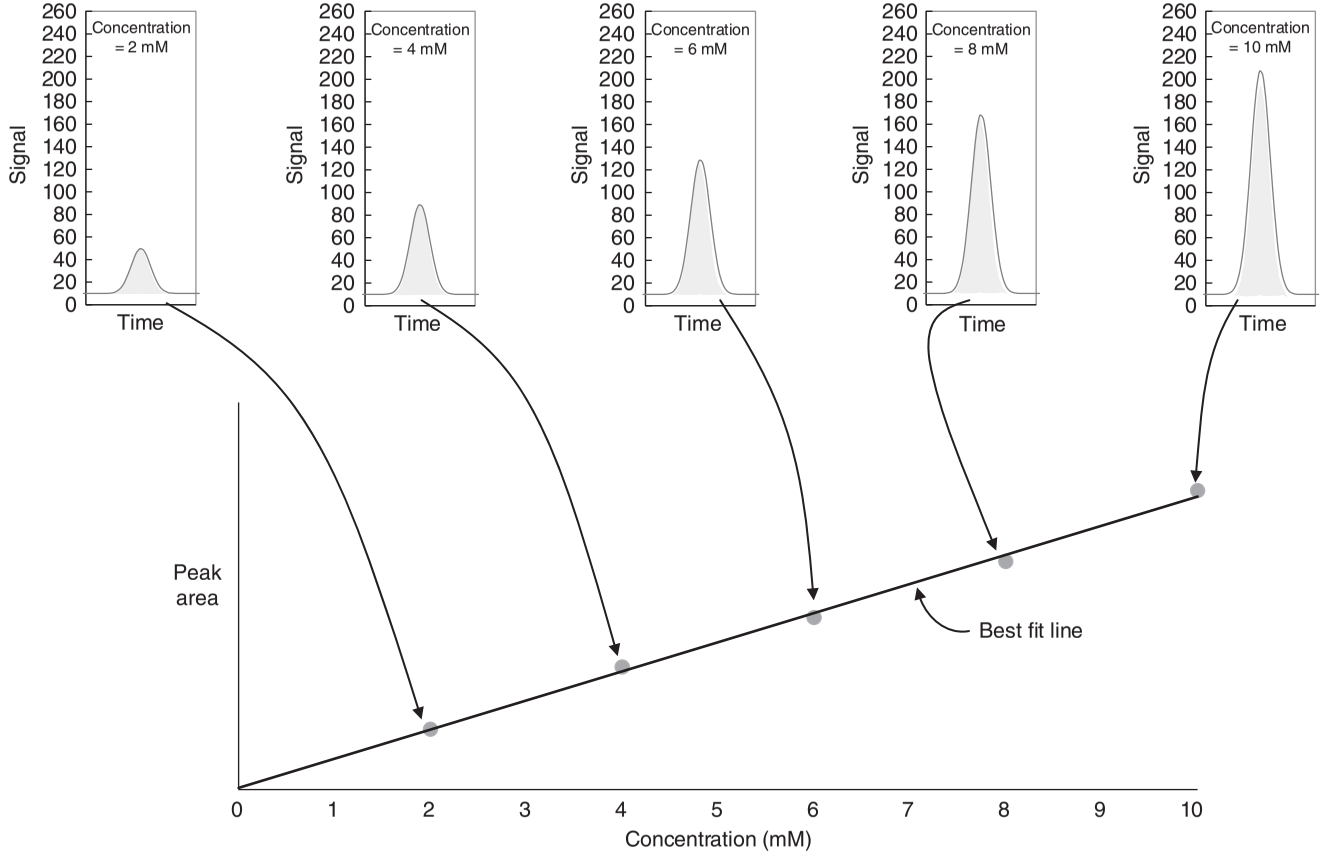


FIGURE 1.28 Example of a calibration curve based on chromatographic peak area of the analyte.

Peak areas are generally obtained via computer software that computes the area under the curve, using peak recognition parameters that determine where a peak starts and ends. These parameters are set by the user and it is important for the user to visually inspect the chromatograms to ensure that these parameters lead to satisfactory recognition and integration of peaks.

1.7.2. Internal Standards and the Method of Standard Additions

Internal standards are frequently used in chromatography to compensate for variability arising from the injection process. As is always the case, the internal standards in chromatography are selected so that they generally mimic the behavior of the analyte(s). This means they should have comparable retention (although not identical as this would cause peak overlap) and generally produce similar detector responses as the analyte(s). In cases where the sample matrix may affect the response of the analyte, the method of standard additions is used to improve the accuracy of analyses.

1.8. EMERGING TRENDS AND APPLICATIONS

As hinted at in some of the earlier sections, one of the trends in chromatography is toward faster separations, driven by improvements in particle manufacturing and instrument design – particularly in the ability to create systems that can pump liquids at high pressures that are created by columns packed with small particles. In some ways, it is misleading to give the impression that the quest for faster separations is a recent one. Theory has driven practice in this regard for decades, and chromatographers continue to push the boundaries as new columns and instruments become available.

Another area that has received much consideration recently is the use of coupled columns that have significantly different stationary phases that maximize separation factors (i.e., selectivity).

Both of these areas, other work currently being pursued in both GC and LC, and applications of the techniques are described in greater detail in their respective chapters.

1.9. SUMMARY

Many areas of science, including biochemistry, biotechnology, chemical manufacturing, forensic science, environmental science, and the pharmaceutical industry, use chromatography to analyze samples such as blood, air, water, gasoline, food, medications, and crime scene evidence.

This chapter examined the fundamental concepts of partitioning, retention, separation, and resolution that are common to all modes of chromatography. In the following chapters, we describe how these concepts are put into practice in GC and LC specifically. We also look at how instruments are designed to introduce samples into the column, how mobile phase is pumped through the column, how the chemical composition of the stationary and mobile phases are manipulated to control retention, separation factors, and resolution, and how detectors are designed to sense the presence, concentration, and in some cases, the identity of the molecules as they elute from the column. There's a lot to look forward to!

PROBLEMS

1.1 (a) The water-to-hexadecane distribution coefficients for valeronitrile and octanal are 3.236 and 462.4, respectively. Which partitions out of water into hexadecane more favorably? Rationalize this based on the solute structures using arguments related to intermolecular interactions.

(b) Suppose 1,000,000 molecules of each are placed in a container with 500.0 mL of water and 500.0 mL of hexadecane. The container is shaken and allowed to come to equilibrium. How many molecules of valeronitrile and octanal are in the water phase at equilibrium? How many of each are in the stationary phase? Note that here again we are modeling a reversed-phase separation where water is the mobile phase and the hexadecane represents a less polar, organic-rich environment.

(c) Suppose that after equilibrium is achieved, the 500.0 mL of aqueous phase from this system are drawn off and added to a second container that has 500.0 mL of fresh, pure hexadecane in it already. This new system is shaken and allowed to come to equilibrium. How many molecules of valeronitrile and octanal are in the water phase at equilibrium? How many of each are in the stationary phase?

(d) What has happened to the mixture of valeronitrile and octanal that at the beginning had an equal number of both molecules in it?

(e) What would happen if this process of moving the mobile phase onto fresh stationary phase were repeated multiple times?

(f) Repeat the calculation in part (b), but this time let the aqueous phase be 950.0 mL and the hexadecane phase be just 50.0 mL. How does this affect the distribution of the solutes?

1.2 (a) Calculate the retention factors for dichlorvos and mevinphos (two pesticides) that elute at $t = 22.84$ and 31.53 s, respectively, with a dead time of 3.80 s in a fast GC separation.

(b) Based on these retention factors, for every one molecule of each solute in the mobile phase at any point in time, how many are in the stationary phase?

(c) Which solute spends more time in the stationary phase?

(d) Which solute spends more time in the mobile phase? Hint: think carefully about this.

1.3 Calculate the phase ratio for a 30.0-m column with 0.530-mm inner diameter and 5.00-μm film thickness. It is quite helpful to sketch a picture to visualize the different volumes involved and how to calculate them.

1.4 Which would increase retention for all solutes in a GC separation – using a column with a thinner film or a thicker one while keeping the inner diameter constant?

1.5 (a) Calculate the separation factor given the following data:

$t_m = 1.56$ min

$t_{bromobenzene} = 3.87$ min

$t_{iodobenzene} = 4.57$ min

(b) If conditions were changed such that (1) the dead time remained the same, (2) the retention time of iodobenzene decreased more than that of bromobenzene,

and (3) iodobenzene still eluted after bromobenzene, would the separation factor increase or decrease?

1.6 Calculate the HETP for a 25.0-cm RPLC column based on a peak that elutes at 17.456 min with a peak width at half maximum of 0.276 min.

1.7 Which process, longitudinal diffusion or slow mass transfer in the mobile phase, contributes more significantly to band broadening at high mobile phase velocities? Which dominates at low velocities?

1.8 Name and explain the two mechanisms that reduce band broadening arising from multiple flow paths through a packed bed.

1.9 Assuming all else is equal, which parameter in each of the following pairs would you choose in order to decrease broadening? Assume that the average linear velocity being used is above the optimum in each set.

(a) N_2 versus H_2 as the mobile phase in GC.
(b) $d_p = 2.0\,\mu m$ versus $5.0\,\mu m$ particles in RPLC.
(c) $0.25\,\mu m$ versus $1.00\,\mu m$ film thickness in GC.
(d) Increase the linear velocity versus decrease the linear velocity in either GC or RPLC (recall that the problem states that the velocity is already above the optimum velocity).

1.10 (a) Use the following conditions for octane as a solute, diffusing in He gas as the mobile phase, in a wall-coated capillary GC column, to calculate H at the following average velocities: 2, 5, 10, 15, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, and 100 cm/s.

Variable	Value
d_f	$0.25\,\mu m$
D_s	$1.20 \times 10^{-5}\ cm^2/s$
D_m	$0.191\ cm^2/s$. This is the diffusion coefficient of octane in He, which is frequently used as the mobile phase in GC
k	3.25
i.d. (inner diameter)	0.250 mm
Length	30.0 m

It is helpful to use a spreadsheet and *calculate each of the three terms in the relevant equation explicitly* so you see how they each vary as a function of linear velocity. Be careful with units.

(b) Make a plot of the contribution of each of the three terms versus linear velocity.
(c) Make a plot of H versus linear velocity.
(d) Use the plot to approximate the optimum linear velocity for this given set of conditions.
(e) Using the same spreadsheet, substitute the D_m for octane in N_2 ($D_m = 0.046\ cm^2/s$). Compare the two plots of H versus linear velocity and compare the optimum velocities found for both gases.

1.11 Make a plot of H versus k by calculating H at $k = 0.1, 0.25, 0.50, 0.75, 1.0, 1.5, 2, 3, 4, 5, 6, 7, 8, 9, 10$ using Equation 1.23 and the following parameters (be careful with units).

Parameter	Value
γ	0.700
D_m	1.00×10^{-5} cm^2/s
$\overline{u}$	0.300 cm/s
λ	0.500
d_p	2.00 μm
ω	0.500
d	2.00 nm
D_s	3.00×10^{-6} cm^2/s
q	0.133

1.12 If a peak has an asymmetry factor of 0.73, is it tailed or fronted?

1.13 (a) Calculate the resolution for the following two antihistamines separated by RPLC. Assume equal peak heights and Gaussian peak shapes.

	t_r (min)	$W_{baseline}$ (min)
Imipramine	6.912	0.095
Amitriptyline	7.028	0.099

(b) If the dead time of this separation is 1.44 min, using the general resolution equation, how many theoretical plates does the column have?

(c) If the column is a 10.0 cm RPLC column, what is the plate height?

1.14 The same solute was analyzed on two GC columns and yielded the following data:

	t_r (min)	$W_{1/2}$ (min)
Column 1	10.20	0.072
Column 2	24.965	0.138

(a) Which column produced the broader peak?

(b) Which column has more theoretical plates?

(c) Based on theoretical plates, which column is better (i.e., more efficient)?

(d) What does this problem illustrate about evaluating columns? Can evaluations be based on peak width alone?

1.15 In GC we make the approximation that the contribution to zone broadening from solute diffusion longitudinally down the column while *in the stationary phase* is negligible compared to that arising due to longitudinal diffusion in the mobile phase.

(a) Why is this a good approximation when the mobile phase is a gas?

(b) Is the approximation equally valid if the mobile phase is a liquid instead of a gas? Explain why or why not.

1.16 Diffusion constants for solutes depend on the viscosity of the medium in which they are diffusing. In that regard, does band broadening increase or decrease as the temperature of the column, and hence the mobile and stationary phase in it, is increased in a liquid chromatography system? Assume one is already operating at flow rates above the optimum.

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