Third Week

22-5 Why Bands Spread[14]

A band of solute invariably spreads as it travels through a chromatography column (Figure 22-11) and emerges at the detector with a standard deviation σ. Each individual mechanism contributing to broadening produces a standard deviation σ_i. The observed variance (σ^2_{obs}) is the sum of variances from all contributing mechanisms:

Variance is additive, but standard deviation is not.

Variance is additive:
$$\sigma^2_{obs} = \sigma^2_1 + \sigma^2_2 + \sigma^2_3 + \cdots = \sum \sigma^2_i \qquad (22\text{-}31)$$

Broadening Outside the Column

Solute cannot be applied to the column in an infinitesimally thin zone, so the band has a finite width even before it enters the column. If the band is applied as a plug of width Δt (measured in units of time), the contribution to the variance of the final bandwidth is

Variance due to injection or detection:
$$\sigma^2_{injection} = \sigma^2_{detector} = \frac{(\Delta t)^2}{12} \qquad (22\text{-}32)$$

The same relation holds for broadening in a detector that requires a time Δt for the sample to pass through. Sometimes on-column detection is possible, thereby eliminating the problem of band spreading in a detector.

EXAMPLE Band Spreading Before and After the Column

A band from a column eluted at a rate of 1.35 mL/min has a width at half-height of 16.3 s. The sample was applied as a sharp plug with a volume of 0.30 mL, and the detector volume is 0.20 mL. Find the variances introduced by injection and detection. What would $w_{1/2}$ be if broadening occurred only on the column?

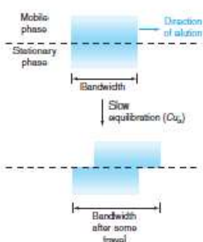


FIGURE 22-18 The finite time required for solute to equilibrate between mobile and stationary phases gives rise to Cu_x in the van Deemter equation. The slower the linear flow, the more complete equilibration is and the less zone broadening occurs.

where D_m is the diffusion coefficient of solute in the mobile phase, t is time, and H_D is the plate height due to longitudinal diffusion. The time needed to travel the length of the column is L/u_x, where L is the column length and u_x is the linear flow rate.

Finite Equilibration Time Between Phases

The term Cu_x in Equation 22-33 comes from the finite time required for solute to equilibrate between mobile and stationary phases.[15] Although some solute is stuck in the stationary phase, the remainder in the mobile phase moves forward, spreading the overall zone of solute (Figure 22-18).

Plate height from finite equilibration time, also called the *mass transfer term*, is

Plate height due to finite equilibration time:
$$H_{mass\ transfer} = Cu_x = (C_s + C_m)u_x \qquad (22\text{-}35)$$

where C_s describes the rate of mass transfer through the stationary phase and C_m describes mass transfer through the mobile phase. Specific equations for C_s and C_m depend on the type of chromatography.

For gas chromatography in an open tubular column, the terms are

Mass transfer in stationary phase:
$$C_s = \frac{2k}{3(k+1)^2}\frac{d^2}{D_s} \qquad (22\text{-}35a)$$

Mass transfer in mobile phase:
$$C_m = \frac{1 + 6k + 11k^2}{24(k+1)^2}\frac{r^2}{D_m} \qquad (22\text{-}35b)$$

where k is the retention factor, d is the thickness of stationary phase, D_s is the diffusion coefficient of solute in the stationary phase, r is the column radius, and D_m is the diffusion coefficient of solute in the mobile phase. Decreasing stationary phase thickness, d, reduces plate height and increases efficiency because solute can diffuse faster from the farthest depths of the stationary phase into the mobile phase. Decreasing column radius, r, reduces plate height by decreasing the distance through which solute must diffuse to reach the stationary phase.

Mass transfer plate height is also decreased by increasing temperature, which increases the diffusion coefficient of solute in the stationary phase. In Figure 22-19, raising the temperature allows the linear flow rate to be increased by a factor of 5 while maintaining acceptable resolution. Resolution is maintained because of the increased rate of mass transfer between phases at elevated temperature. Many common silica-based stationary phases for liquid chromatography are not stable at elevated temperature. The zirconia (ZrO_2)-based material in Figure 22-19 is used because it is stable.

Multiple Flow Paths

The term *A* was formerly called the *eddy diffusion* term.

The term A in the van Deemter equation (22-33) arises from multiple effects for which the theory is murky. Figure 22-20 is a pictorial explanation of one effect. Because some flow paths are longer than others, molecules entering the column at the same time on the left are

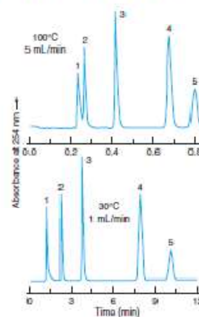


FIGURE 22-19 Liquid chromatography showing decreased analysis time when temperature is raised from 30° to 100°C. 1, uracil; 2, *p*-nitroaniline; 3, methyl benzoate; 4, phenetole; 5, toluene. The 4.6-mm-diameter × 100-cm-long column was packed with 4.5-μm-diameter zirconia (ZrO_2) coated with 2.1 wt% polybutadiene and eluted with 20 vol% acetonitrile in water. [From J. Li, Y. Hu, and P. W. Carr, "Fast Separations at Elevated Temperatures on Polybutadiene-Coated Zirconia Reversed-Phase Material," *Anal. Chem.* 1997, 69, 3884.] For a silica-based stationary phase, temperature is usually kept below 60°C to prevent hydrolysis of the silica.

FIGURE 22-20 Band spreading from multiple flow paths. The smaller the stationary phase particles, the less serious this problem is. This process is absent in an open tubular column. [Adapted from H. M. McNair and E. J. Bonelli, *Basic Gas Chromatography* (Palo Alto, CA: Varian Instrument Division, 1968).]

eluted at different times on the right. For simplicity, we approximate many different effects by the constant A in Equation 22-33.

Advantages of Open Tubular Columns

Compared with packed columns, open tubular columns can provide

- higher resolution
- shorter analysis time
- increased sensitivity
- lower sample capacity

In gas chromatography, we have a choice of using open tubular columns or packed columns. For similar analysis times, open tubular columns provide higher resolution and increased sensitivity to small quantities of analyte. Open tubular columns have small sample capacity, so they are not useful for preparative separations.

For a given pressure, linear flow rate is proportional to cross-sectional area of the column and inversely proportional to column length:

$$u_x \propto \frac{\text{area}}{\text{length}}$$

Particles in a packed column resist flow of the mobile phase, so the linear flow rate cannot be very fast. For the same length of column and applied pressure, the linear flow rate in an open tubular column is much higher than that of a packed column. Therefore, the open tubular column can be made 100 times longer than the packed column and still achieve a similar pressure drop and linear flow rate. If plate height is the same, the longer column provides 100 times more theoretical plates, yielding $\sqrt{100} = 10$ times more resolution.

Compared with packed columns, open tubular columns allow

- increased linear flow rate or a longer column or both
- decreased plate height, which means higher resolution

Plate height is reduced in an open tubular column because band spreading by multiple flow paths (Figure 22-20) cannot occur. In the van Deemter curve for the packed column in Figure 22-16, the A term accounts for half of the plate height at the most efficient flow rate (minimum H) near 30 mL/min. If A were deleted, the number of plates on the column would be doubled. To obtain high performance from an open tubular column, the radius of the column must be small and the stationary phase must be as thin as possible to ensure rapid exchange of solute between mobile and stationary phases.

Table 22-3 compares the performances of packed and open tubular gas chromatography columns with the same stationary phase. For similar analysis times, the open tubular column gives resolution seven times better (10.6 versus 1.5) than that of the packed column. Alternatively, speed could be traded for resolution. If the open tubular column were reduced to 5 m in length, the same solutes could be separated with a resolution of 1.5, but the time would be reduced from 38.5 to 0.83 min.

A Touch of Reality: Asymmetric Bandshapes

c_s = concentration of solute in stationary phase
c_m = concentration of solute in mobile phase

A Gaussian bandshape results when the partition coefficient, K (= c_s/c_m), is independent of the concentration of solute on the column. In real columns, K changes as the concentration of solute increases, and bandshapes are skewed.[16] A graph of c_s versus c_m (at a given temperature) is called an *isotherm*. Three common isotherms and their resulting bandshapes are shown in Figure 22-21. The ideal center isotherm gives a symmetric peak.

The upper isotherm in Figure 22-21 arises from an *overloaded* column in which too much solute has been applied to the column. As the concentration of solute increases, the solute becomes more and more soluble in the stationary phase. There is so much solute in the stationary phase that the stationary phase begins to resemble solute. (There is a rule of thumb in chemistry that "like dissolves like.") The front of an overloaded peak has gradually increasing concentration.

TABLE 22-3 Comparison of packed and wall-coated open tubular column performance[a]

Property	Packed	Open tubular
Column length, L	2.4 m	100 m
Linear gas velocity	8 cm/s	16 cm/s
Plate height for methyl oleate	0.73 mm	0.34 mm
Retention factor, k, for methyl oleate	58.6	2.7
Theoretical plates, N	3 290	294 000
Resolution of methyl stearate and methyl oleate	1.5	10.6
Retention time of methyl oleate	29.8 min	38.5 min

a. Methyl stearate ($CH_3(CH_2)_{16}CO_2CH_3$) and methyl oleate (cis-$CH_3(CH_2)_7CH{=}CH(CH_2)_7CO_2CH_3$) were separated on columns with poly(diethylene glycol succinate) stationary phase at 180°C.

SOURCE: L. S. Ettre, Introduction to Open Tubular Columns (Norwalk, CT: Perkin-Elmer Corp., 1979), p. 26.

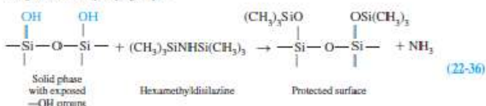
Overloading produces a gradual rise and an abrupt fall of the chromatographic peak.

As the concentration increases, the band becomes overloaded. The solute is so soluble in the overloaded zone that little solute trails behind the peak. The band emerges gradually from the column but ends abruptly.

A long tail occurs when some sites retain solute more strongly than other sites.

The lower isotherm in Figure 22-21 arises when small quantities of solute are retained more strongly than large quantities. It leads to a long "tail" of gradually decreasing concentration after the peak.

Sites that bind solute strongly cause tailing. Silica surfaces of columns and stationary phase particles have hydroxyl groups that form hydrogen bonds with polar solutes, thereby leading to serious tailing. **Silanization** reduces tailing by blocking the hydroxyl groups with nonpolar trimethylsilyl groups:



(22-36)

Glass and silica columns used for gas and liquid chromatography can also be silanized to minimize interaction of the solute with active sites on the walls.

Now that you have been exposed to many concepts, you might want to read about a microscopic model of chromatography in Box 22-2.

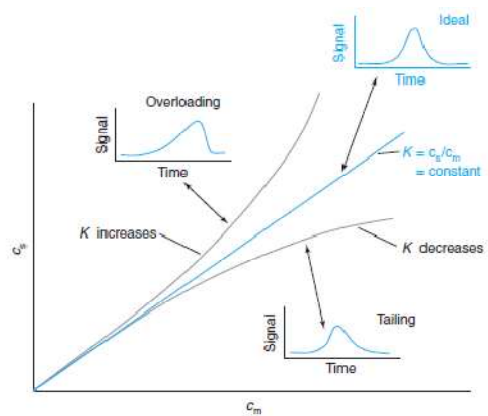


FIGURE 22-21 Common isotherms and their resulting chromatographic bandshapes.