- Consider a liquid chromatography experiment
  → in which the column has an inner diameter of 0.60 cm
  → and the mobile phase occupies 20% of the column volume.

- Each centimeter of column length
  → has a volume of $\pi r^2 \times \text{length} = \pi(0.30\ \text{cm})^2(1\ \text{cm}) = 0.283\ \text{mL}$,
  → of which 20% (0.056 5 mL) is mobile phase (solvent).

- For example, the volume flow rate = 0.30 mL/min

- Because 1 cm of column length contains 0.056 5 mL of mobile phase,
  → 0.30 mL would occupy (0.30 mL)/(0.056 5 mL/cm) = 5.3 cm of column length.

- The linear flow rate corresponding to 0.30 mL/min
  → 5.3 cm/min.

The Chromatogram

- Solutes eluted from a chromatography column
  → observed with detectors

- A chromatogram
  → a graph showing the detector response as a function of elution time

See Figure 23-7

- Figure 23-7 shows what might be observed
  → when a mixture of octane, nonane, and an unknown are separated by gas chromatography (Ch. 24).

- The retention time, t_r, for each component

  → the time that elapses between injection of the mixture onto the column and the arrival of that component at the detector.

- Retention volume, V_r,

  → the volume of mobile phase required to elute a particular solute from the column.

See Figure 23-7

- Retention volume, V_r,

$$\textit{Retention volume:} \quad V_r = t_r \cdot u_v$$

→ where

u_v : the volume flow rate (volume per unit time) of the mobile phase.

→ The retention volume of a particular solute is constant over a range of flow rates.

- Mobile phase or an unretained solute

  → travels through the column in the minimum possible time, t_m.

- In gas chromatography,

  → t_m is usually taken as the time needed for CH_4 to travel through the column (Figure 23-7).

- The adjusted retention time, t_r', for a retained solute

  → the additional time required to travel the length of the column, beyond that required by solvent or carrier gas:

Adjusted retention time: $$t_r' = t_r - t_m$$

See Figure 23-7

For two components 1 and 2,

- the relative retention, α (also called separation factor),

  → the ratio of their adjusted retention times:

Relative retention:

$$\alpha = \frac{t'_{r2}}{t'_{r1}}$$

→ where $t'_{r2} > t'_{r1}$, so $\alpha > 1$.

- The greater the relative retention,

  → the greater the separation between two components.

- Relative retention

  → fairly independent of flow rate

  → can therefore be used to help identify peaks when the flow rate changes.

For component 2 eluted after component 1,

- the unadjusted relative retention, γ,

  → the ratio of their unadjusted retention times:

$$\textit{Unadjusted relative retention:} \qquad \gamma = \frac{t_{r2}}{t_{r1}}$$

- The unadjusted relative retention

  → the inverse of the ratio of the speeds at which the two components travel.

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

For each peak in the chromatogram,

- the retention factor, k,

  → 'the adjusted retention time' over 'the time t_m required for mobile phase to pass through the column',

Retention factor:

$$k = \frac{t_r - t_m}{t_m} \qquad (23\text{-}16)$$

- The longer a component is retained by the column,

  → the greater is the retention factor.

- It takes volume V_m to push solvent from the beginning of the column to the end of the column.

- If it takes an additional volume $3V_m$ to elute a solute,

  → then the retention factor for that solute is 3.

Relation Between Retention Time and the Partition Coefficient

- The retention factor in Equation 23-16 is equivalent to

$$k = \frac{t_r - t_m}{t_m} \quad \Rightarrow \quad k = \frac{\text{time solute spends in stationary phase}}{\text{time solute spends in mobile phase}} \qquad (23\text{-}17)$$

- If the solute spends 'all its time in the mobile phase' and 'none in the stationary phase',

  → it would be eluted in time t_m.

  → solute spends no time in the stationary phase

  → $t_r = t_m$, so $k = 0$

- If solute spends three times as much time 'in the stationary phase' as 'in the mobile phase',

  → $t_r = 4t_m$, so $k = (4t_m - t_m)/t_m = 3$.

- If solute spends three times as much time 'in the stationary phase' as 'in the mobile phase',

  → there will be three times as many moles of solute 'in the stationary phase' as 'in the mobile phase' at any time.

- The quotient in Equation 23-17 is equivalent to.

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

$$k = \frac{c_s V_s}{c_m V_m} \qquad (23\text{-}18)$$

→ where

c_s : the concentration of solute in the stationary phase,

V_s : the volume of the stationary phase,

c_m : the concentration of solute in the mobile phase,

V_m : the volume of the mobile phase.

$$k = \frac{c_s V_s}{c_m V_m}$$

- The quotient c_s / c_m

  → the ratio of concentrations of solute in the 'stationary' and 'mobile' phases.

- If the column is run slowly enough to be at equilibrium,

  → the quotient c_s / c_m = the partition coefficient, K, $K = \frac{c_s}{c_m}$

  → introduced in connection with solvent extraction.

- Therefore, we cast Equation 23-18 in the form.

Relation of retention time to partition coefficient:

$$k = K\frac{V_s}{V_m} \overset{\text{Eq. 23-16}}{=} \frac{t_r - t_m}{t_m} = \frac{t'_r}{t_m} \quad (23\text{-}19)$$

→ relates 'retention time' to 'the partition coefficient and the volumes of stationary and mobile phases'.

$$k = K\frac{V_s}{V_m} \overset{\text{Eq. 23-16}}{=} \frac{t_r - t_m}{t_m} = \frac{t'_r}{t_m}$$

- Because $t'_r \propto k \propto K$, relative retention can also be expressed as

$$\textit{Relative retention:} \qquad \alpha = \frac{t'_{r2}}{t'_{r1}} = \frac{k_2}{k_1} = \frac{K_2}{K_1}$$

- The relative retention of two solutes

  → proportional to the ratio of their partition coefficients.

- This relation is the physical basis of chromatography.

23.4 Efficiency of Separation

- Two factors contribute to how well compounds are separated by chromatography.

1) The difference in elution times between peaks:
   → The farther apart, the better their separation.

2) The other factor is how broad the peaks are:
   → the wider the peaks, the poorer their separation.

- This section discusses how we measure the efficiency of a separation.

Resolution

- Solute moving through a chromatography column tends to spread into a Gaussian shape with standard deviation σ (Figure 23-9).
- The longer a solute resides in a column,

  → the broader the band becomes.

See Figure 23-9

- Common measures of breadth

(1) the width $w_{1/2}$ measured at a height equal to half of the peak height

(2) the width w at the baseline between tangents drawn to the steepest parts of the peak.

- From Equation 4-3 for a Gaussian peak (also refer to Table 4-1),

  → it is possible to show that $w_{1/2} = 2.35\sigma$ and $w = 4\sigma$.

See Figure 23-9

- In chromatography,

  → the resolution of two peaks from each other is defined as

Resolution:

$$\text{Resolution} = \frac{\Delta t_r}{w_{av}} = \frac{\Delta V_r}{w_{av}} = \frac{0.589\Delta t_r}{w_{1/2av}}$$

- where

$\triangle t_r$ or $\triangle V_r$: the separation between peaks (in units of time or volume)

w_{av} : the average width of the two peaks in corresponding units.

(Peak width is measured at the base, as shown in Figure 23-9.)

$w_{1/2av}$: the width at half-height of Gaussian peaks.

- The width at half-height is usually used

  → because it is easiest to measure.

- Figure 23-10 shows

  → the overlap of two peaks with different degrees of resolution.
- For quantitative analysis,

  → a resolution 1.5 is highly desirable.

See Figure 23-10

23.5 Why Bands Spread

- A band of solute broadens as it moves through a chromatography column (Figure 23-11).
- Ideally, an infinitely narrow band applied to the inlet of the column emerges with a Gaussian shape at the outlet.
- In less ideal circumstances, the band becomes asymmetric.

See Figure 23-11

- As a solute travels through a chromatography column (Figure 23-11),
  → A band of solute invariably spreads
  → 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})
  → the sum of variances from all contributing mechanisms:

Variance is additive: $$\sigma^2_{obs} = \sigma^2_1 + \sigma^2_2 + \sigma^2_3 + \cdots = \sum \sigma^2_i$$

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 △t (measured in units of time),
  → the contribution to the variance of the final bandwidth is

Variance due to injection or detection:

$$\sigma^2_{\text{injection}} = \sigma^2_{\text{detector}} = \frac{(\Delta t)^2}{12}$$

Longitudinal Diffusion

- If you could apply a thin disk-shaped band of solute to the center of a column,

  → the band would slowly broaden as molecules diffuse from the high concentration within the band to regions of lower concentration on the edges of the band

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

  → then the Gaussian profile of the band is described by

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

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

- where

c : concentration (mol/m^3),

t : time,

x : the distance along the column from the current center of the band.

(The band center is always x = 0 in this equation.)

$$y = \frac{1}{\sigma\sqrt{2\pi}} e^{-(x-\mu)^2/2\sigma^2}$$

Standard deviation of band: $\sigma = \sqrt{2Dt}$

Plate Height: A Measure of Column Efficiency

- If solute has traveled a distance x at the linear flow rate u_x (m/s), then the time it has been on the column is $t = x/u_x$ therefore:

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

$$H = \sigma^2/x$$

- *Plate height*, H, is the proportionality constant between the variance, σ^2 of the band, and the distance it has traveled, x.
- Plate height is approximately the length of column required for one equilibration of the solute between the mobile and stationary phases.

- It is different for different analytes on the same column due to differences in diffusion coefficients.

- The smaller the plate height, the narrower the band and the better the separation.

- Plate heights are ~0.1-1 mm for gas chromatography, 10 mm for high-performance liquid chromatography, and <1 mm for capillary electrophoresis.

Number of plate on column: $N = \frac{L}{H} = \frac{Lx}{\sigma^2} = \frac{L^2}{\sigma^2} = \frac{16L^2}{w^2}$ w (unit in length)

Recall that $x = L$ and $\sigma = w/4$ (because $w = 4\sigma$).

The number of plates on a column:

$$N = \frac{16t_r^2}{w^2} = \frac{t_r^2}{\sigma^2}$$

w (unit in time)

If we use the width at half-height:

$$N = \frac{5.55t_r^2}{w_{1/2}^2}$$

w (unit in time)

For two closely spaced, symmetric peaks, resolution is governed by the **Purnell** equation:

$$\text{Resolution} = \frac{\sqrt{N}}{4}\frac{(\alpha - 1)}{\alpha}\left(\frac{k_2}{1 + k_2}\right)$$

N: the number of theoretical plates

α: the relative retention of the two peaks

k_2: the retention factor for the more retained component

Resolution is proportional to the square root of N, so doubling the colu mn length increases the resolution by $2^{1/2}$.

Back to "Longitudinal diffusion"

- Diffusional broadening of a band occurs

  → while the band is transported along the column by the flow of solvent

- called longitudinal diffusion

  → because it takes place along the axis of the column

See Figure 23-18

Finite Equilibration Time Between Phases

- Another broadening comes from the finite time required for solute to equilibrate between mobile and stationary phases

- Although some solute is stuck in the stationary phase, the remainder in the mobile phase moves forward, spreading the overall zone of solute (Figure 23-19).

See Figure 23-19

Multiple Flow Paths

- Band spreading from multiple flow paths.

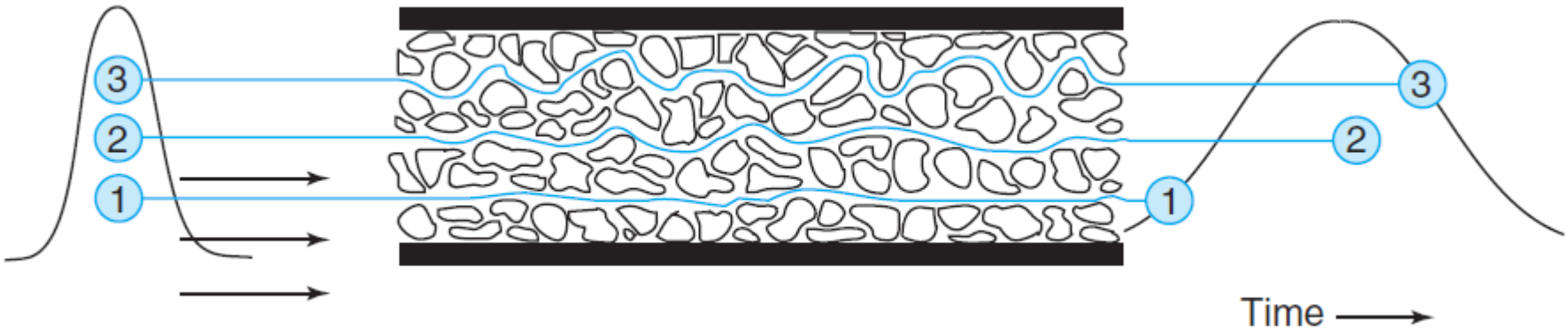
- Because some flow paths are longer than others,

  → molecules entering the column at the same time on the left are eluted at different times on the right.

- The smaller the stationary phase particles,

  → the less serious this problem is.

- This process is absent in an open tubular



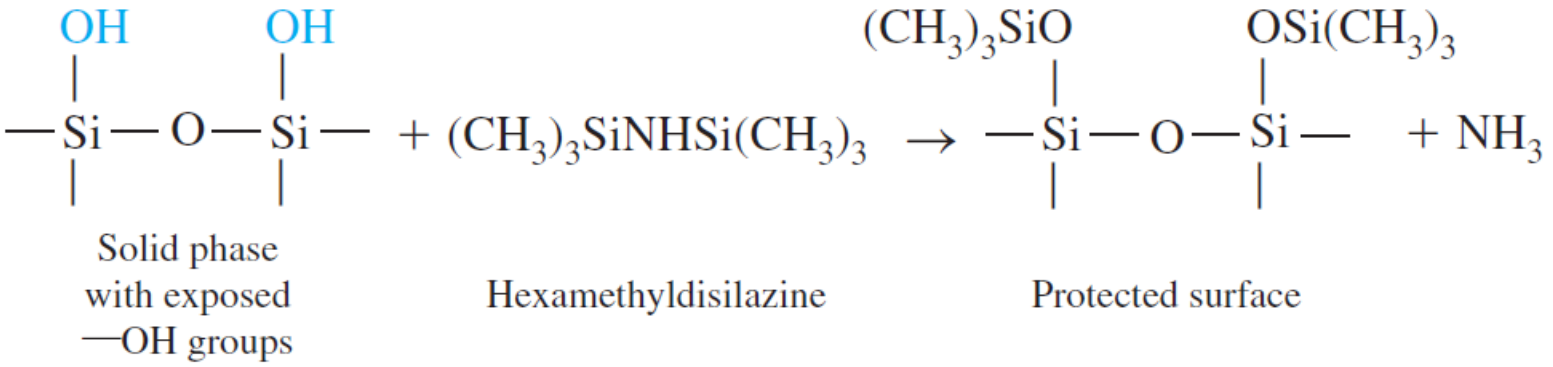
Asymmetric Bandshapes

- A long tail occurs when some sites retain solute more strongly than other sites.
- Silica surfaces of columns and stationary phase particles have hydroxyl groups that form hydrogen bonds with polar solutes,

  → thereby leading to serious tailing.

See Figure 23-14

- Silanization reduces tailing by blocking the hydroxyl groups with nonpolar trimethylsilyl groups:



Analytical Chemistry

Chapter 24. Gas Chromatography

- This chapter discusses

  → discuss specific chromatographic methods and instrumentation.

- The goal

  → to understand how chromatographic methods work

  → and what parameters you can control for best results.

24.1 The separation process in gas chromatography

- Gas chromatography:
  → mobile phase: gas
  → stationary phase: usually a nonvolatile liquid, but sometimes a solid
  → analyte: gas or volatile liquid

- In gas chromatography,
  → gaseous analyte is transported through the column by a gaseous mobile phase, called the carrier gas.

In **the schematic gas chromatograph**,

- volatile liquid or gaseous sample

  → injected through a septum (a rubber disk) into a heated port,

  → in which it rapidly evaporates.

- Vapor is swept through the column by He, N_2, or H_2 carrier gas,

See Figure 24-1