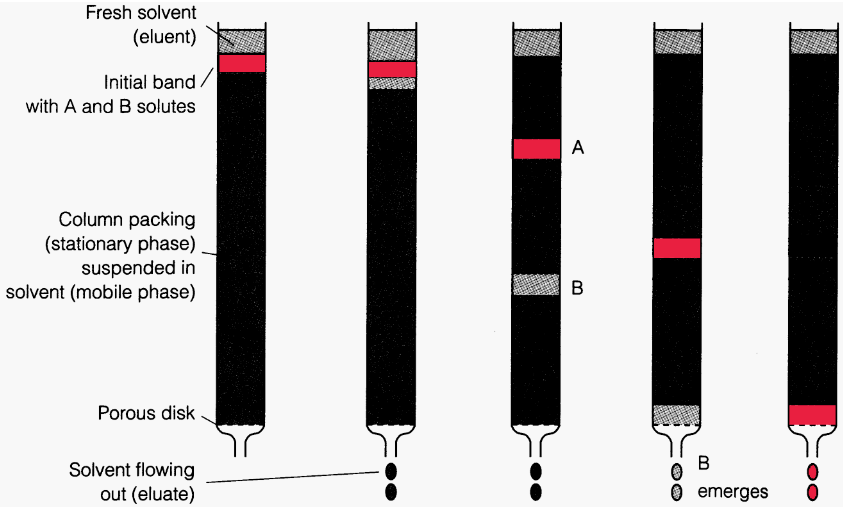
Chromatography

Outline

- What is Chromatography?
- The Chromatogram
- Optimization of Column Performance
- Why Do Bands Spread?
- Gas Chromatography
- High-Performance Liquid Chromatography
  - Ion-Exchange Chromatography

What is Chromatography?



In **chromatography**, separation is achieved by utilization of differences in the degree to which various substances are adsorbed onto the surface of an inert material

Example of a separation shown at left

eluent - fluid entering column
eluate - fluid exiting column

Types of Chromatography

There are many types of chromatography but all are based on the principle that the **mobile phase** carries the compounds to be separated and a **stationary phase** binds these compounds through intermolecular forces

Adsorption chromatography uses a solid stationary phase and a liquid or gaseous mobile phase. Solute is adsorbed on the surface of the solid particles.

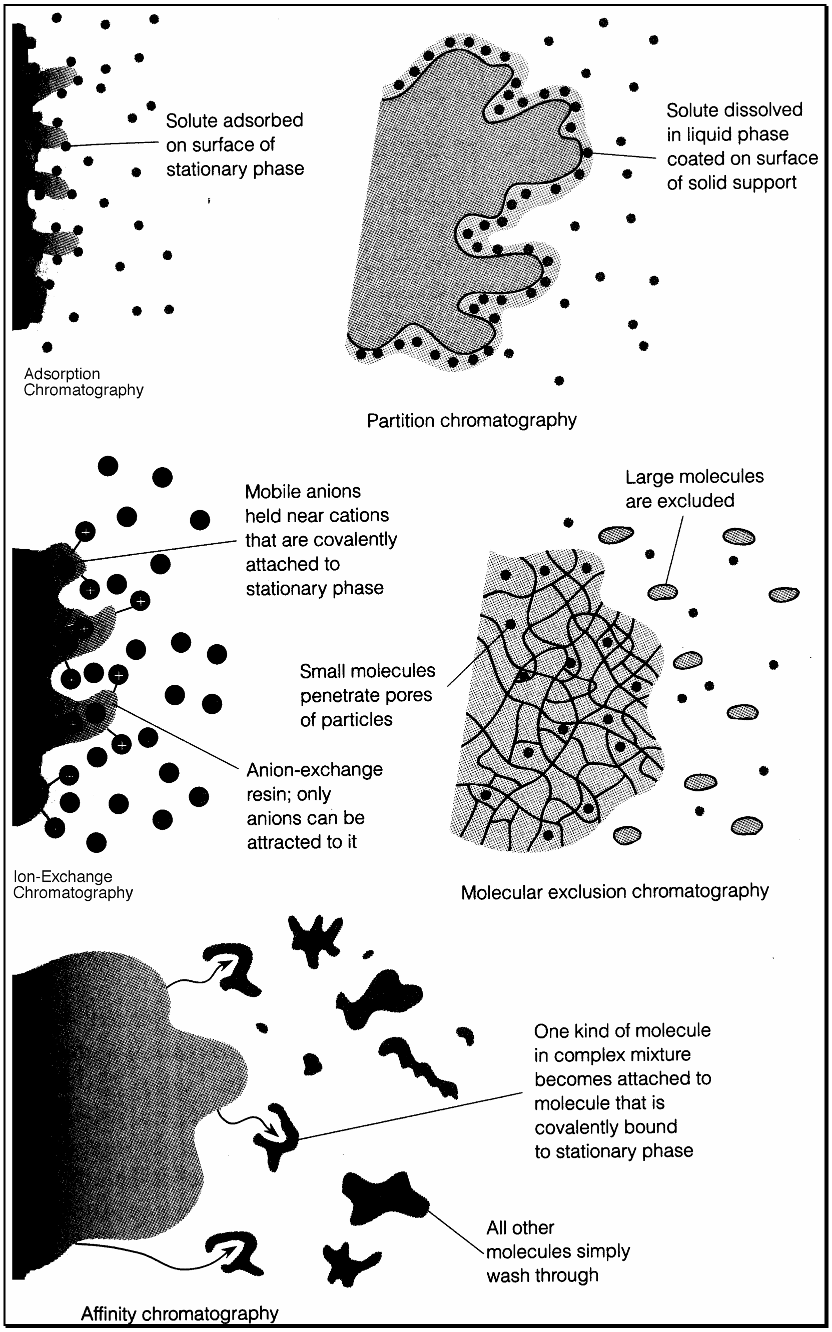
Partition chromatography involves a thin liquid stationary phase coated on the surface of the solid support. Solute equilibrates between the stationary liquid and the mobile phase.

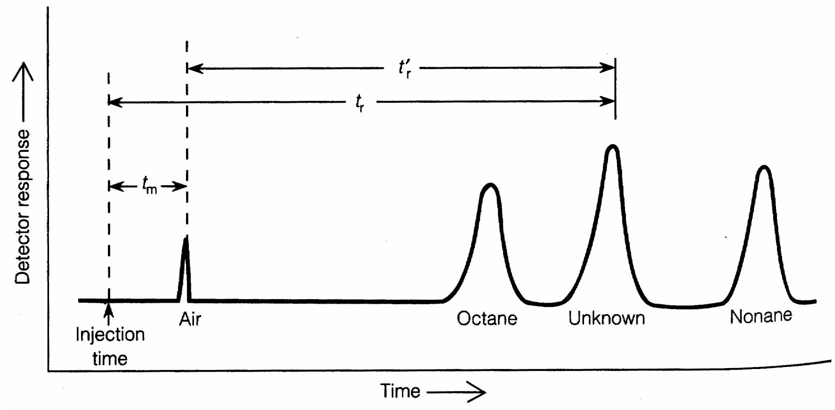
Ion-exchange chromatography features ionic groups such as $–SO_3^-$ or $–N(CH_3)_3^+$ covalently attached to the stationary solid phase. Solute ions are attracted to the stationary phase by electrostatic forces. The mobile phase is a liquid.

Molecular exclusion chromatography separates molecules by size, with larger molecules passing through most quickly. The stationary phase has pores small enough to exclude large molecules but not small ones.

Affinity chromatography employs specific interactions between one kind of solute molecule and a second molecule that is covalently attached to the stationary phase.

Look at the types of interactions shown below:





The Chromatogram

The **volume flow rate** tells us how many milliliters of solvent per minute travel through the column.

A **chromatogram** is a graph showing the detector response as a function of elution time.

The **retention time**, t_R, is the time needed for a component to reach the detector after injection.

Unretained mobile phase travels through the column in the minimum possible time, designated t_m.

The **capacity factor**, k', for a peak in a chromatogram is defined as

$$\boxed{k' = \frac{(t_R - t_m)}{t_m}}$$

the longer a component is retained by the column, the greater is the capacity factor.

Resolution

Two factors contribute to how well compounds are separated by chromatography

1. Difference in elution times between peaks
2. How broad the peaks are

The **selectivity factor**, α, of a column for two solutes is defined as

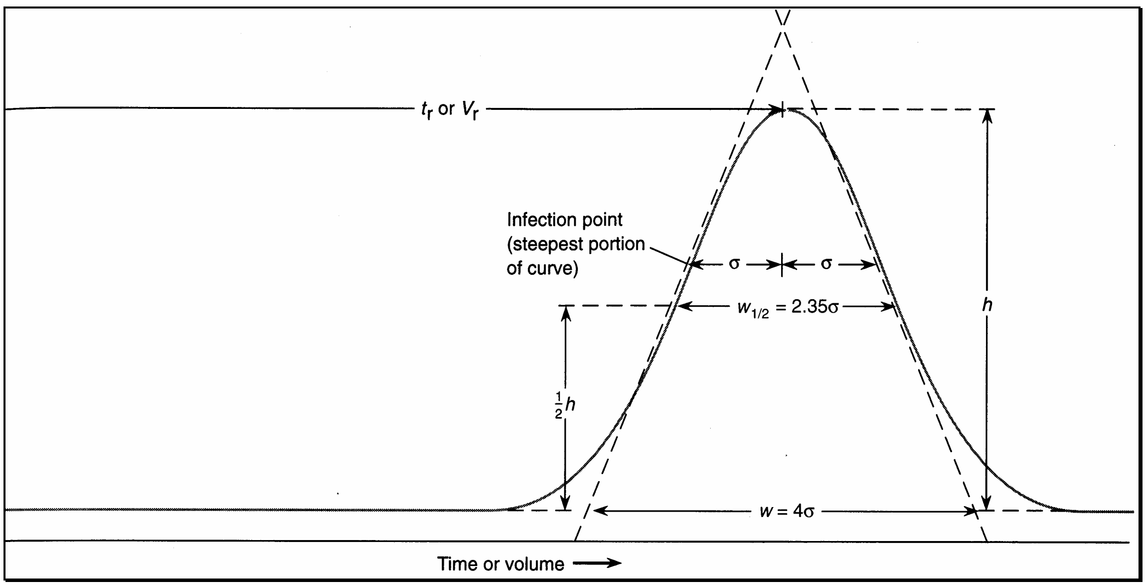
$$\boxed{\alpha = \frac{((t_R)_y - t_m)}{((t_R)_x - t_m)}}$$

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

$$\boxed{\text{Resolution} = \frac{\Delta t_R}{w_{av}}}$$

where

Δt_R = separation between peaks in time, $((t_R)_y - (t_R)_x)$
w_{av} = average width of the two peaks in units of time, $((W_x + W_y)/2)$



Example

A solute with a retention time of 407 s has a width at the base of 13 s. A neighboring peak is eluted at 424 s with a width of 16 s. Find the resolution for these two components.

$$\text{Resolution} = \frac{\Delta t_R}{w_{av}} = \frac{(424\text{ s} - 407\text{ s})}{0.5(13\text{ s} + 16\text{ s})} = 1.1$$

Peak Separation and Resolution

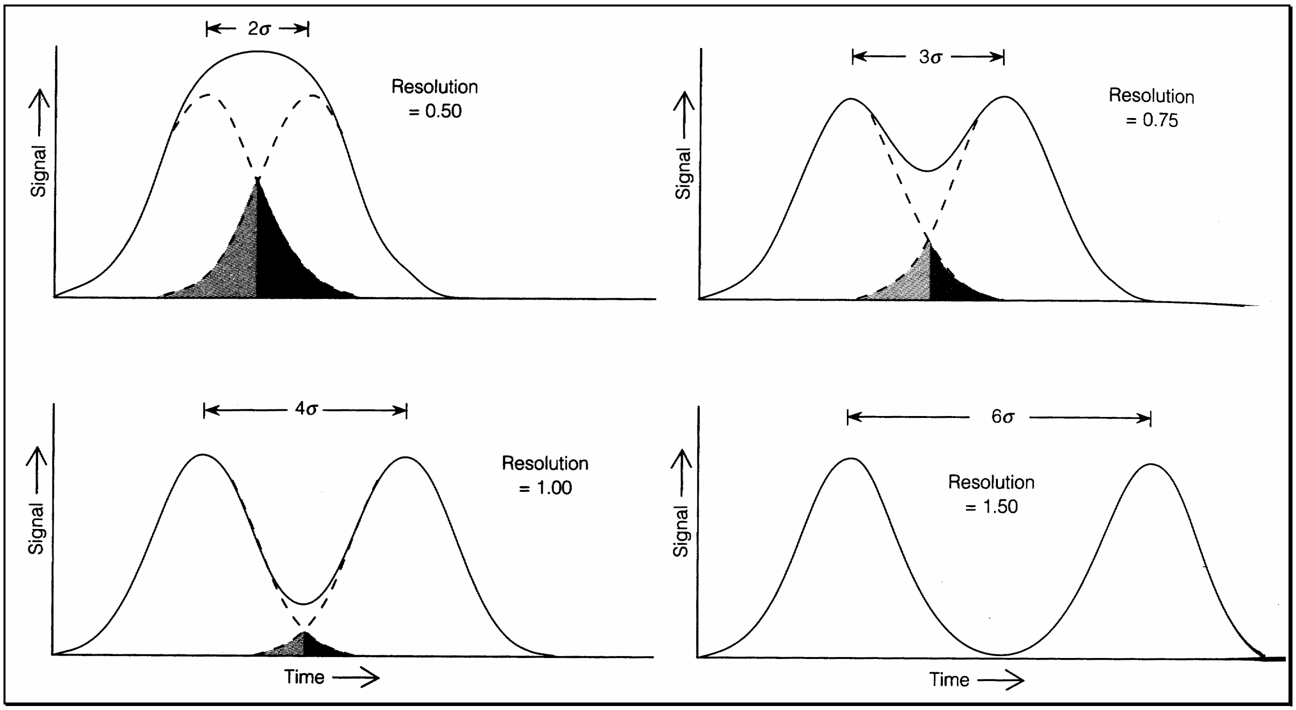


Plate Height: A Measure of Column Efficiency

Plate height (aka height equivalent to a theoretical plate) is the constant of proportionality between the variance (^{2}) of the band and the distance it has travelled, x.

$$H = \frac{\sigma^2}{x}$$

small plate height ⇒ narrow peaks ⇒ better separations

The number of plates on a column is defined as

$$\boxed{N = \frac{16\ t_R^2}{w^2}}$$

this leads to the convenient equation

$$\boxed{H = \frac{L}{N}}$$

where L is the column length and N is the number of plates on the column.

Example

A solute with a retention time of 407 s has a width at the base of 13.76 s on a column 12.2 m long. Find the number of plates and the plate height.

$$N = \frac{16\ t_R^2}{w^2} = \frac{(16)\ (407\ s)^2}{(13.76\ s)^2} = 1.40 \times 10^4$$

$$H = \frac{L}{N} = \frac{12.2\ m}{1.40 \times 10^4} = 8.7 \times 10^{-4}\ m = 0.87\ mm$$

Example

A solute with a retention time of 407 s has a width at half-height of 8.1 s on a column 12.2 m long. Find the number of plates and the plate height.

$$N = 5.55\ (t_r)^2\ /\ (w)^2{}_{@1/2bht} = 1.40 \times 10^4$$

$$H = \frac{L}{N} = \frac{12.2\ m}{1.40 \times 10^4} = 8.7 \times 10^{-4}\ m = 0.87\ mm$$

Optimization of Column Performance

The previous two equations are of considerable importance because they serve as guides to the choice of conditions that will lead to a desired degree of separation with a minimum expenditure of time.

Each of these equations is made up of three parts:

1. The first describes the efficiency of the column (N or H)
2. The second is a selectivity term and depends on the properties of the solutes
3. The third component is the capacity term which depends on the properties of the column, the mobile phase, temperature and the solute.

Column Efficiency

Smaller plates will lead to better separations

Selectivity Factor

Resolution is improved as the selectivity factor becomes larger. However, increasing the selectivity factor generally results in much longer times for separation

Capacity Factor

The easiest component to change. Either mobile phase or stationary phase can be altered, although experimentally the mobile phase is the easiest to change. Also a tradeoff in terms of separation time.

Resolution expected with a given number of plates:

$$\boxed{R_s = \frac{\sqrt{N}}{4}\left(\frac{\alpha - 1}{\alpha}\right)\frac{k'_y}{(1+k'_y)}}$$

Time required for separation to be completed:

$$\boxed{(t_r)_y = \frac{16\, R_s^2\, H}{u}\left(\frac{\alpha}{\alpha - 1}\right)^2 \frac{(1+k'_y)^3}{(k'_y)^2}}$$

where $(t_r)_y$ is the time required to elute the more strongly held species

Example

Substances A and B were found to have retention times of 16.40 and 17.63 min, respectively, on a 30.0-cm column. An unretained species passed through the column in 1.30 min. The peak widths (at base) for A and B were 1.11 and 1.21 min, respectively. Calculate: (a) the column resolution; (b) the average number of plates in the column; (c) the plate height; (d) the length of column required to achieve a resolution of 1.5; and (e) the time required to elute substance B at a resolution of 1.5.

(a) $$R_s = \frac{2[(t_r)_y - (t_r)_x]}{W_x + W_y}$$

$$R_s = \frac{2(17.63 - 16.40)}{1.11 + 1.21} = 1.06$$

(b) $$N = 16 \left(\frac{t_r}{W}\right)^2$$

$$N_A = 16 \left(\frac{16.40}{1.11}\right)^2 = 3493 \qquad N_B = 16 \left(\frac{17.63}{1.21}\right)^2 = 3397$$

$$N_{avg} = (3493 + 3397)/2 = 3345$$

(c) $H = L/N = 30.0 \text{ cm} / 3445 = 8.7 \times 10^{-3} \text{ cm}$

(d) k' and α do not change with increasing N and L, so by using

$$R_s = \frac{\sqrt{N}}{4} \left(\frac{\alpha - 1}{\alpha}\right) \frac{k'_y}{(1 + k'_y)}$$ we can solve for N at a given R_s

$$\frac{R_{s1}}{R_{s2}} = \frac{\sqrt{N_1}}{\sqrt{N_2}}$$ where 1 and 2 are the initial and longer columns, respectively

$$N_2 = N_1 \left(\frac{R_{s2}}{R_{s1}}\right)^2 = 3445\,(1.5/1.06)^2 = 6.9 \times 10^3$$

$$L = N \times H = (6.9 \times 10^3)(8.7 \times 10^{-3} \text{ cm}) = 60 \text{ cm}$$

(e) Since $$(t_r)_y = \frac{16 R_s^2 H}{u} \left(\frac{\alpha}{\alpha - 1}\right)^2 \frac{(1 + k'_y)^3}{(k'_y)^2}$$ we get

$$\frac{t_{r1}}{t_{r2}} = \frac{R_{s1}^2}{R_{s2}^2} \qquad \text{or} \qquad t_{r2} = t_{r1} \left(\frac{R_{s2}^2}{R_{s1}^2}\right)$$

t_{r2} = (17.63 min) x $(1.5/1.06)^2$ = 35 min