Principles of Chromatography
Chapter 26

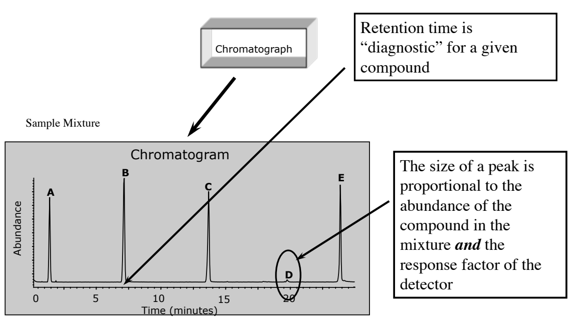
Chromatography - the ***separation*** of an analyte from a mixture

Classification of Chromatographic Methods

TABLE 26-1 Classification of Column Chromatographic Methods

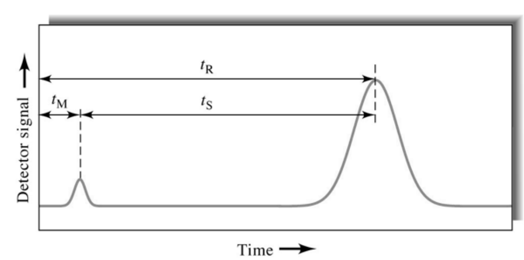
General Classification	Specific Method	Stationary Phase	Type of Equilibrium
1. Gas chromatography (GC)	a. Gas-liquid chromatography (GLC)	Liquid adsorbed or bonded to a solid surface	Partition between gas and liquid
	b. Gas-solid	Solid	Adsorption
2. Liquid chromatography (LC)	a. Liquid-liquid, or partition	Liquid adsorbed or bonded to a solid surface	Partition between immiscible liquids
	b. Liquid-solid, or adsorption	Solid	Adsorption
	c. Ion exchange	Ion-exchange resin	Ion exchange
	d. Size exclusion	Liquid in interstices of a polymeric solid	Partition/sieving
	e. Affinity	Group specific liquid bonded to a solid surface	Partition between surface liquid and mobile liquid
3. Supercritical fluid chromatography (SFC; mobile phase: supercritical fluid)		Organic species bonded to a solid surface	Partition between supercritical fluid and bonded surface

The Chromatogram



Chromatographic - Terms

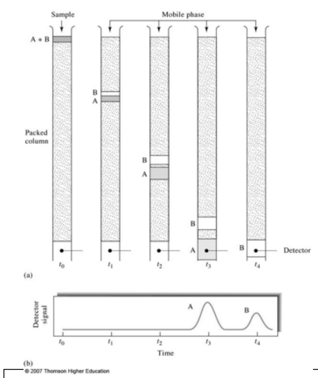
- Retention Time (t_R) = the time it takes after injection for a solute to reach the detector
- Dead time (t_M) = the time for an un-retained species to reach the detector
- Mobile phase velocity (u) = L/t_M; the average linear rate of movement of molecules in the mobile phase
- L = length of chromatographic column
- t_S= time in stationary phase



Basic Separation Principles

Column elution chromatography

Introduce two solutes (A & B) onto a packed column through which a mobile phase (i.e. solvent) or eluent is continuously pumped



Basic Separation Principles cont'd

- Separations depend on the extent to which solutes *distribute* or *partition* between the mobile and stationary phases

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

- We define this interaction with a ***distribution constant*** or ***partition coefficient***:

$$K = c_s/c_m$$

Where:

- c_s = stationary phase concentration (molar)
- c_m = mobile phase concentration (molar)

Elution chromatography simulation

Chromatographic Terms cont'd

Retention Factor or ***Retention Factor*** (k'_A) - a term used to describe the migration rate of solutes (analytes) on columns

$$k'_A = \frac{K_A V_S}{V_M}$$

Where:

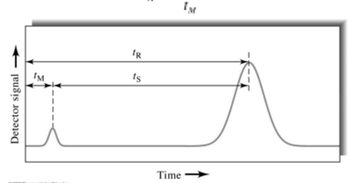
K_A = is the ***partitioning coefficient*** or ***distribution constant*** for species A

V_S = volume of stationary phase

V_M = volume of the mobile phase

The ***retention factor*** or ***capacity factor*** can be determined directly from a chromatogram using:

$$k'_A = \frac{t_R - t_M}{t_M}$$



Chromatographic Terms cont'd

Interpreting the ***retention factor*** or capacity factor

- If $k'_A < 1$; the elution is too rapid for accurate determination of t_R.
- If $k'_A >$ approx. 20-30; the elution is too slow to be practical
- The preferred range for k'_A is between 1 and 10

Chromatographic Terms cont'd

- ***Selectivity Factor*** $\alpha = K_B/K_A$
  - Where K_B is the ***partition coefficient*** or ***distribution constant*** of the more strongly retained species (so that $\alpha > 1$)
- The ***selectivity factor*** can also be defined in terms of ***retention factors*** or ***capacity factors*** and retention times:

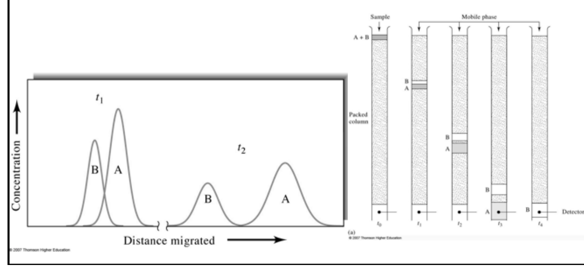
$$\alpha = \frac{k'_B}{k'_A} = \frac{(t_R)_B - t_M}{(t_R)_A - t_M}$$

Band Broadening in Chromatographic Separations

Zone Broadening or Band Broadening

As a solute migrates through a chromatographic column, it will "spread out" and "shorten in height"

By minimizing zone broadening, we can maximize resolution!



Chromatographic Terms cont'd

FACTORS EFFECTING ZONE OR BAND BROADENING

Plate Height (H) and Theoretical Plates (N)

Terms used to quantitatively describe chromatographic column efficiency

Column "efficiency" increases as N increases

$$N = L/H$$

Where:

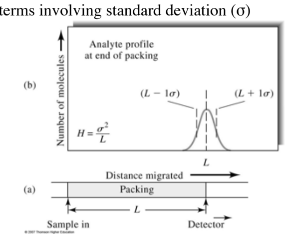
N = the number of interactions (i.e. transitions between mobile and stationary phases) that a solute has during its residence in the column

H= the distance through the column a solute travels between interactions (typically given in centimeters)

Chromatographic Terms cont'd

If the shape of a chromatographic peak is assumed to be Gaussian, then the plate height (***H***) can be defined in statistical terms involving standard deviation (σ)

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



H is defined as variance per unit length of column

H is the length of column that contains the fraction of analyte between L and L – σ
This is 34% of the analyte (1/2 of 1 std. dev.)

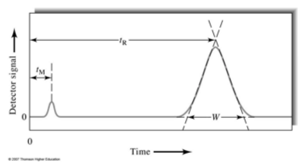
Calculation of N (and H) from a chromatogram

Where:

W = magnitude of the base of the triangle (in units of time)

t_M = retention time of an unretained solute

t_R = retention time of the solute



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

Recall that N = L/H so once N is known, H can be determined

Chromatography Terms cont'd

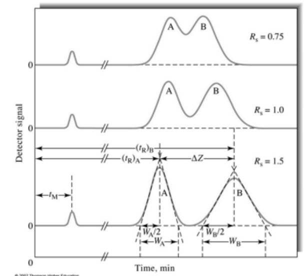
Column resolution (R_S) is a quantitative measure of the ability of a column to separate two analytes:

$$R_S = \frac{2[(t_R)_B - (t_R)_A]}{W_A + W_B}$$

To increase resolution increase column length (L)

- Limitation: longer time and broader bands

Hmmm…can we make the peaks sharper???



Variables Effecting Separation Efficiency in Column Chromatography

In general, Separation (or Column) Efficiency ↑ , as N ↑ and H ↓

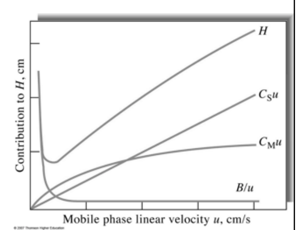
$$H = A + B/u + Cu$$

- Particle Size of Packing (as size ↓, N↑ and H ↓)
- Immobilized Film Thickness (as film thickness ↓, N↑ and H↓ due to faster diffusion rates in film)
- Viscosity of Mobile Phase (as viscosity ↓, N↑ and H↓)
- Linear Velocity of Mobile Phase; $u = L/t_M$
- Column Length (as L ↑, N↑, but H = constant, and separation efficiency ↑)

Variables Effecting Separation Efficiency cont'd

Mobile Phase Flow Rate

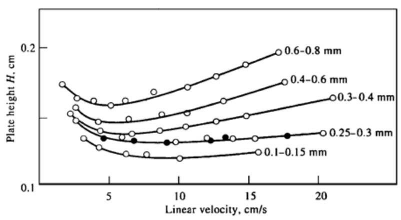
- Optimum flow rate corresponds to minimum H
- H is much smaller for HPLC than for GC (more efficient), but in practice GC columns are much longer than for HPLC - hence greater N for GC.



Variables Effecting Separation Efficiency cont'd

Particle Size of Column Packing

The smaller the packing particles, the greater the column efficiency.

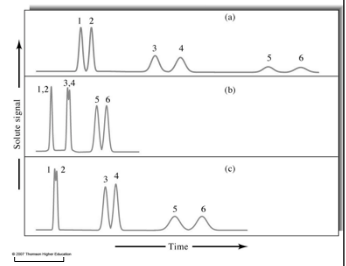


The General Elution Problem

It is often difficult to find a set of conditions which will resolve all peaks to the same degree and also permit reliable quantification

Solutions

- Multiple runs at different conditions
- Programmed elution (i.e. change conditions during the run)



Summary of Important Terms

TABLE 26-4 Important Chromatographic Quantities and Relationships

Name	Symbol of Experimental Quantity	Determined From
Migration time, unretained species	t_M	Chromatogram (Figure 26-7)
Retention time, species A and B	$(t_R)_A, (t_R)_B$	Chromatogram (Figures 26-7 and 26-12)
Adjusted retention time for A	$(t'_R)_A$	$(t'_R)_A = (t_R)_A - t_M$
Peak widths for A and B	W_A, W_B	Chromatogram (Figures 26-7 and 26-12)
Length of column packing	L	Direct measurement
Volumetric flow rate	F	Direct measurement
Linear flow velocity	u	F and column dimensions (Equations 26-6 and 26-7)
Stationary-phase volume	V_S	Packing preparation data
Concentration of analyte in mobile and stationary phases	c_M, c_S	Analysis and preparation data

Summary of Important Terms

TABLE 26-5 Important Derived Quantities and Relationships

Name	Calculation of Derived Quantities	Relationship to Other Quantities
Linear mobile-phase velocity	$u = \frac{L}{t_M}$	
Volume of mobile phase	$V_M = t_M F$	
Retention factor	$k = \frac{t_R - t_M}{t_M}$	$k = \frac{KV_S}{V_M}$
Distribution constant	$K = \frac{kV_M}{V_S}$	$K = \frac{c_S}{c_M}$
Selectivity factor	$\alpha = \frac{(t_R)_B - t_M}{(t_R)_A - t_M}$	$\alpha = \frac{k_B}{k_A} = \frac{K_B}{K_A}$
Resolution	$R_s = \frac{2[(t_R)_B - (t_R)_A]}{W_A + W_B}$	$R_s = \frac{\sqrt{N}}{4}\left(\frac{\alpha - 1}{\alpha}\right)\left(\frac{k_B}{1 + k_B}\right)$
Number of plates	$N = 16\left(\frac{t_R}{W}\right)^2$	$N = 16R_s^2\left(\frac{\alpha}{\alpha - 1}\right)^2\left(\frac{1 + k_B}{k_B}\right)^2$
Plate height	$H = \frac{L}{N}$	
Retention time	$(t_R)_B = \frac{16R_s^2 H}{u}\left(\frac{\alpha}{\alpha - 1}\right)^2\frac{(1 + k_B)^3}{(k_B)^2}$	