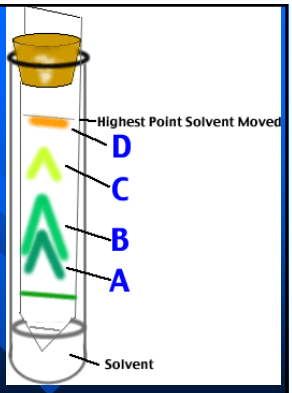
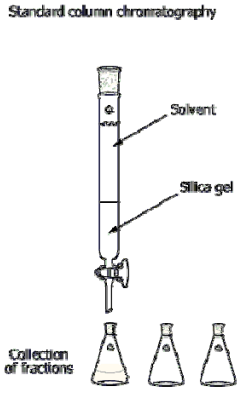
Section VI
Kinetics and Separation

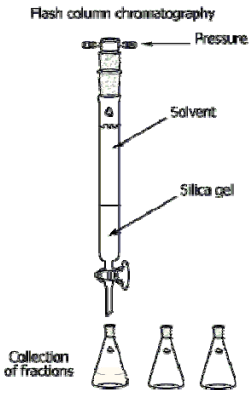
Chapters 31-33

Chapter 30
An Introduction to
Chromatographic Separations



Column Chromatography Paper Chromatography





I. Introduction

- Chromatography - Techniques for separation of components in a mixture based upon the distribution of the components between a mobile phase and a stationary phase that has a large surface area.
- Mobile Phase - A liquid or gas that moves the analytes through a solid or liquid stationary phase.
- Stationary Phase - A solid or immobilized liquid upon which analyte species are partitioned (separated) during passage of the mobile phase.

A. Classification of Methods

1. Column Chromatography - The stationary phase is packed in a narrow tube (column) and the mobile phase is forced through the column under pressure.
2. Planar Chromatography -The stationary phase is on a flat plate or is paper and the mobile phase moves through the stationary phase by capillary action or gravity.

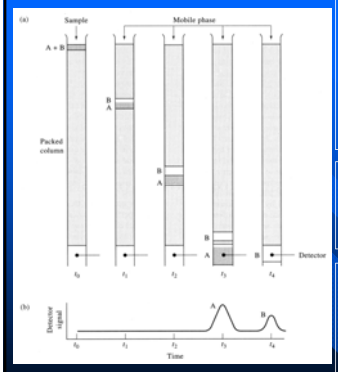
B. Column Chromatography

- Three Basic Types:

1. Liquid Chromatography
2. Gas Chromatography
3. Supercritical Fluid Chromatography

TABLE 26-1 Classification of Column Chromatographic Methods

General Classification	Specific Method	Stationary Phase	Type of Equilibrium
Liquid chromatography (LC) (mobile phase: liquid)	Liquid-liquid, or partition	Liquid adsorbed on a solid	Partition between immiscible liquids
	Liquid-bonded phase	Organic species bonded to a solid surface	Partition between liquid and bonded surface
	Liquid-solid, or adsorption	Solid	Adsorption
	Ion exchange	Ion-exchange resin	Ion exchange
	Size exclusion	Liquid in interstices of a polymeric solid	Partition/sieving
Gas chromatography (GC) (mobile phase: gas)	Gas-liquid	Liquid adsorbed on a solid	Partition between gas and liquid
	Gas-bonded phase	Organic species bonded to a solid surface	Partition between liquid and bonded surface
	Gas-solid	Solid	Adsorption
Supercritical-fluid chromatography (SFC) (mobile phase: supercritical fluid)		Organic species bonded to a solid surface	Partition between supercritical fluid and bonded surface



- Components of the mixture that are strongly retained on the stationary phase move slower than weakly retained components.
- Components are separated into discrete bands.
- The rate that a band migrates depends upon the fraction of time it spends in the mobile phase.

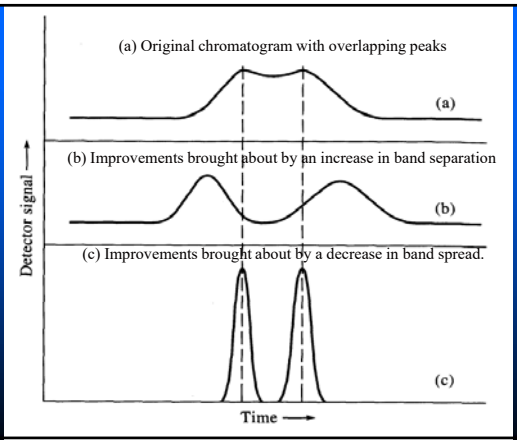
Important:

- chromatogram (concentration versus elution time)
- more strongly retained species elutes last (elution order)
- analyte is "diluted" during elution (dispersion)
- zone broadening proportional to elution time

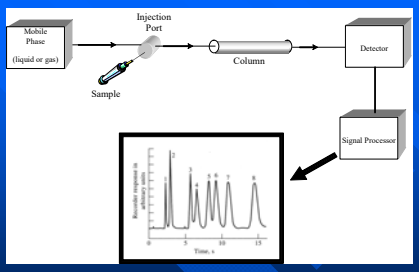
By changing experimental conditions, non-separated bands can be separated

(A) adjust migration rates for A and B (increase band separation)

(B) adjust zone broadening (decrease band spread)



- General Instrument Schematic



- Chromatograph - A plot of detector response vs. time. The detector responds to analyte as the analyte is eluted from the end of the column.

II. Migration of Analytes in a Column

- The rate of migration of an analyte through a column depends on the equilibrium of the analyte between the stationary phase and the mobile phase (i.e. the rates of association and dissociation of the analyte to the stationary phase).

A) Distribution Constant (K)

- The analyte is constantly binding to and dissociating from the stationary phase as it moves through the column.

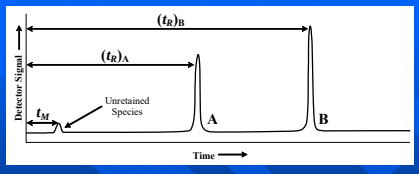
$$A_{stationary} \rightleftharpoons A_{mobile}$$

- The distribution constant (***K***) is an equilibrium constant that describes the ratio of the analyte concentrations in the mobile phase and the stationary phase.

$$K = \frac{c_s}{c_m}$$

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

B) Retention Time (t_R) - length of time from sample injection until the analyte reaches the detector.

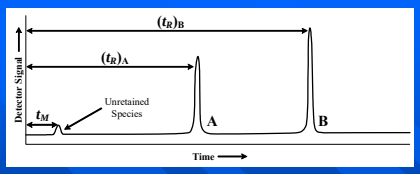


- Dead Time (t_M) - length of time for an unretained species to pass through the column.

- Adjusted Retention Time - Actual time the analyte is retained on the column.

$$t_R - t_M$$

C) Rate of Analyte Migration: The Retention Factor

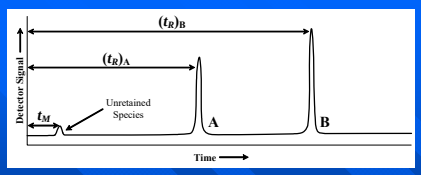


- Retention Factor (k') (also called Capacity Factor)
  - An important parameter commonly used to describe the migration rates of analytes in a column.

For analyte A: $k'_A = \frac{(t_R)_A - t_M}{t_M}$ For analyte B: $k'_B = \frac{(t_R)_B - t_M}{t_M}$

When k' is ≤ 1.0, separation is poor; When k' is > 30, separation is slow; When k' is 2-10, separation is optimum.

D) Relative Rates of Migration: The Selectivity Factor



- Selectivity Factor (α) - A figure of merit that reflects the selectivity (separation) between analytes in a mixture.

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

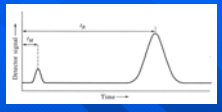
k'_A = retention factor for analyte A
k'_B = retention factor for analyte B

- The retention and selectivity factors are used to calculate the resolving power (resolution) of a column. larger α = better separation.

III. Band broadening and Column Efficiency

A) Peak Shape

- Peaks are shaped like a Gaussian distribution due to the random variations of the residence times of the analyte in the mobile phase.

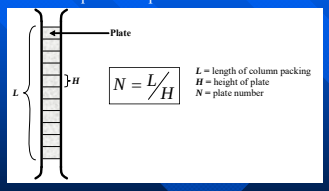


- Some analyte molecules spend more overall time in the mobile phase while others spend less. The peak of the curve represents the average time an analyte spends on the column.
- The width of the peak is directly proportional to the length of the column. The longer the column, the wider the peak.
- Peak width is inversely proportional to the flow rate (velocity) of the mobile phase. The higher the flow rate, the narrower the peak.

B) Column Efficiency

- Column efficiency is a performance characteristic that describes how well a column separates components in a mixture. Plate theory is commonly used to quantify column efficiency.

1) Plate Theory - the column is considered to contain a series of plates in which the analyte moves from one plate to the next through an equilibrium process.



$$N = L/H$$

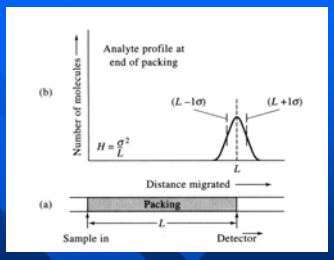
L = length of column packing
H = height of plate
N = plate number

- ***H*** can vary from 0.1 cm to 0.001 cm, ***N*** can be 100's to 100,000's
- Column efficiency increases as ***H*** decreases and ***N*** increases

a) Definition of plate height (*H*)

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

σ^2 = **variance**



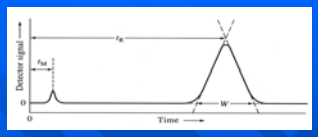
- Not particularly useful since chromatograms are plotted as a function of time, not length.

b) Experimental determination of *H* and *N*

- Standard deviation (σ) as a function of time.

$$\tau = \frac{\sigma}{L/t_R}$$

τ = time standard deviation
t_R = retention time



- The area of the triangle constitutes ~96% of the area under the peak and 96% of a Gaussian curve is within ±2σ of the mean. So the width at the base of the peak equals four standard deviations.

$W = 4\tau$ or $\tau = W/4$

So:

$$H = \frac{LW^2}{16t_R^2}$$

or

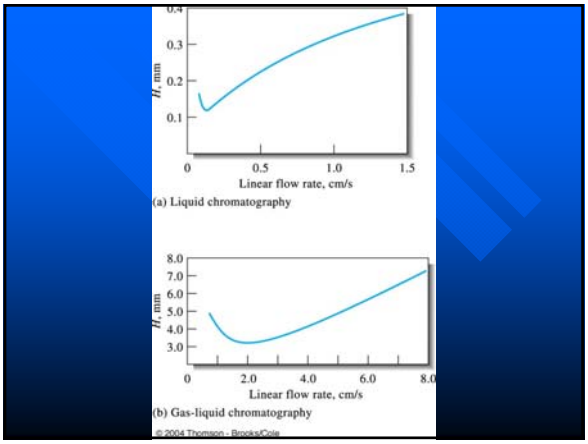
$$N = 16\left(\frac{t_R}{W}\right)^2$$

TABLE 30-5

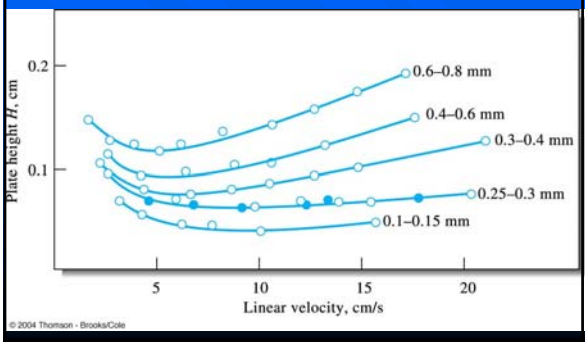
Variables That Influence Column Efficiency

Variable	Symbol	Usual Units
Linear velocity of mobile phase	u	cm s^{-1}
Diffusion coefficient in mobile phase*	D_M	cm^2 s^{-1}
Diffusion coefficient in stationary phase*	D_S	cm^2 s^{-1}
Retention factor (see Equation 30-18)	k	unitless
Diameter of packing particles	d_p	cm
Thickness of liquid coating on stationary phase	d_f	cm

*Increases as temperature increases and viscosity decreases.



Effect of Particle Size on Plate Height for a Packed GC Column



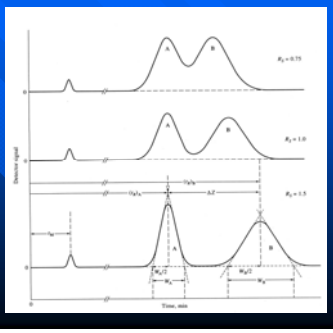
Optimization of Column Efficiency:

2. Column Resolution (R_S) - also commonly referred to as *resolving power*

- Standard deviation (σ) as a function of time.

$$R_S = \frac{\Delta Z}{W_A/2 + W_B/2} = \frac{2\Delta Z}{W_A + W_B}$$

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



Chapter 31
Gas Chromatography

Gas Chromatography (GC)

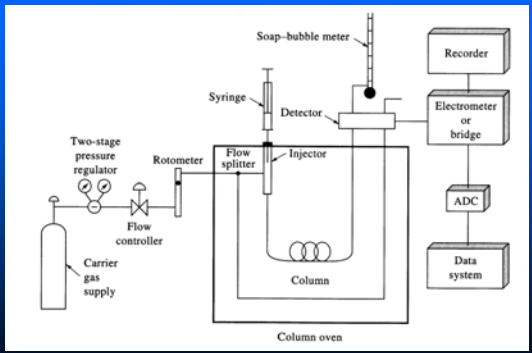
- GC involves a **vaporized sample** being injected into the head of a chromatographic column.
- an inert gas as mobile phase.
- *In contrast to most other types of chromatography, the mobile phase does not interact with molecules of the analyte.* Its only function is to transport the analyte through the column.

Two major types

- Gas-solid chromatography
  (stationary phase: solid)
- Gas-liquid chromatography
  (stationary phase: immobilized liquid)

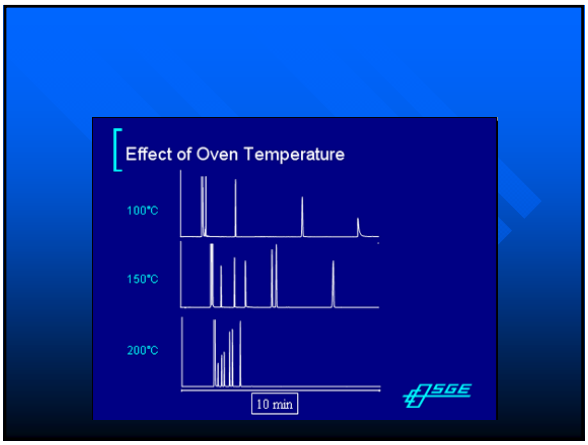
Separation is based on differences in boiling points of the solutes and the solutes' interaction with the stationary phase.

Instrumentation



Carrier gas:	He (common), N_2, H_2
	P_{inlet} 10-50 psig
	F=25-150 mL/min packed column
	F=1-25 mL/min open tubular column
Column:	2-50 m coiled stainless steel/glass/Teflon
Oven:	0-400 °C ~ average boiling point of sample
	accurate to <1 °C
Detectors:	FID, TCD, ECD, (MS)

Flame Ionization Detector, Thermal Conductivity Detector, Electron Capture Detector



Chapter 32

High Performance Liquid Chromatography

HPLC

originally referred to:
High Pressure Liquid Chromatography
currently refers to:
High Precision Liquid Chromatography

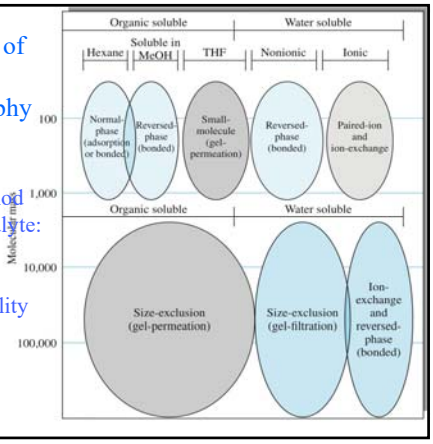
– high pressure to be able to use small particle size to allow proper separation at reasonable flow rates

Four Types of HPLC

- Partition
- Adsorption (liquid-solid)
- Ion exchange
- Size exclusion or gel

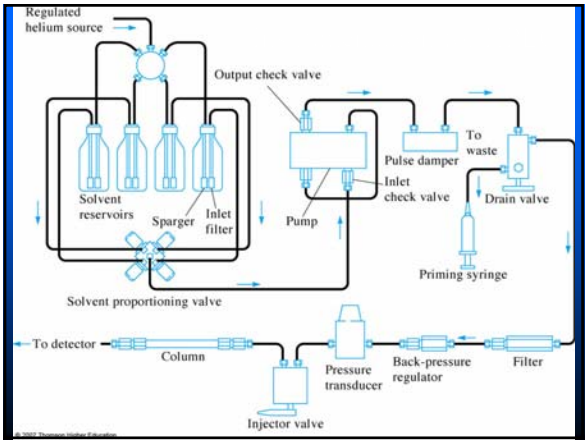
Applications of Liquid Chromatography (LC)

Choice of method depends on analyte:
1. Mol weight
2. Polarity
3. Water solubility
4. Net charge

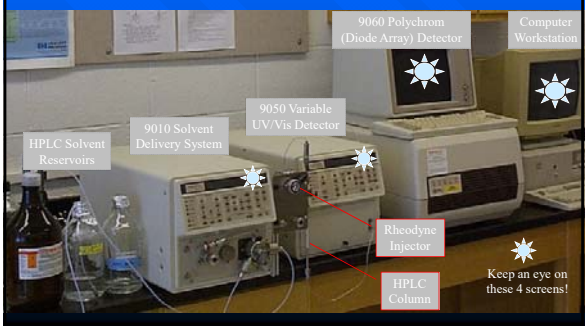


Normal vs. Reversed Phase Chromatography

	Normal Phase	Reversed Phase
Stationary phase	Polar (silica gel)	Non-polar (C18)
Mobile phase	Non-polar (organic solvents)	Polar (aqueous/organic)
Sample movement	Non-polar fastest	Polar fastest
Separation based on	Different polarities (functionality)	Different hydrocarbon content



Varian HPLC System



Normal- (polar column) versus Reversed Phase (nonpolar) elution