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**Ministry of Higher Education & Scientific research**
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# Analytical Techniques

## For

## Second Year Student/course 1

## Lecture 8

**By**

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# Introduction to Chromatography

- Chromatography is a physical method of separation in which the components to be separated are distributed between two phases
- one of which is stationary (stationary phase) while the other (the mobile phase) moves through it in a definite direction.
- **Chromatography** is a technique in which the components of a mixture are separated based on differences in the rates at which they are carried through a fixed or **stationary phase** by a gaseous or liquid **mobile phase.**

## Classification of Chromatographic Methods

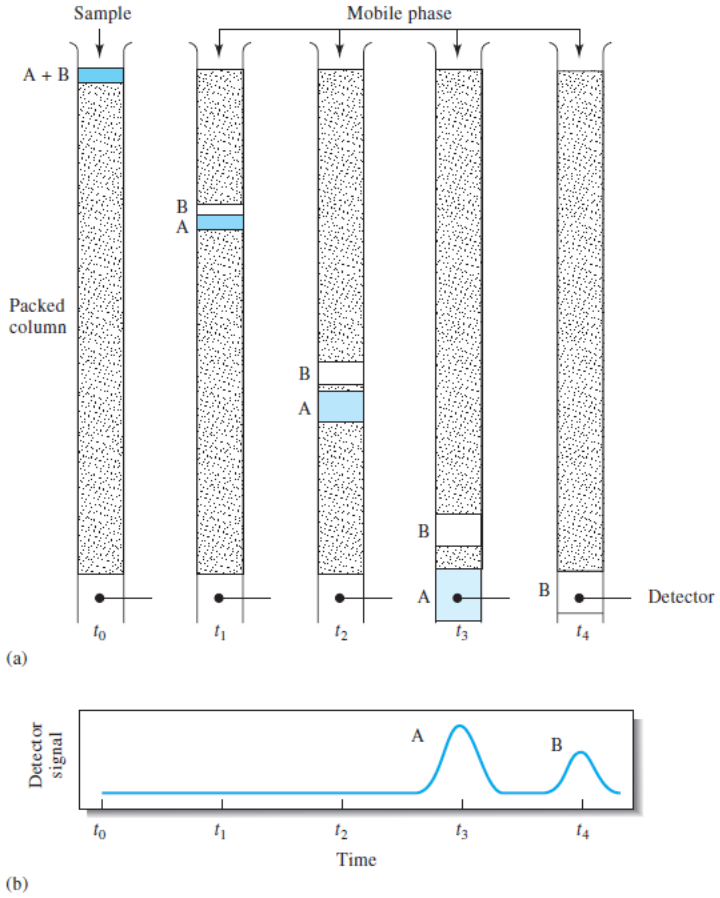
Chromatographic methods are of two basic types.

1. In column chromatography, the stationary phase is held in a narrow tube, and the mobile phase is forced through the tube under pressure or by gravity.
2. In planar chromatography, the stationary phase is supported on a flat plate or in the pores of a paper, and the mobile phase moves through the stationary phase by capillary action or under the influence of gravity.
3. We consider here only column chromatography. As shown in the first column of Table 1, chromatographic methods fall into three categories based on the nature of the mobile phase: liquid, gas, and supercritical fluid. The second column of the table reveals that there are five types of liquid

Classification of Column Chromatographic Methods

| General Classification | Specific Method | Stationary Phase | Type of Equilibrium |
|---|---|---|---|
| 1. Gas chromatography (GC) | a. Gas-liquid (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 |

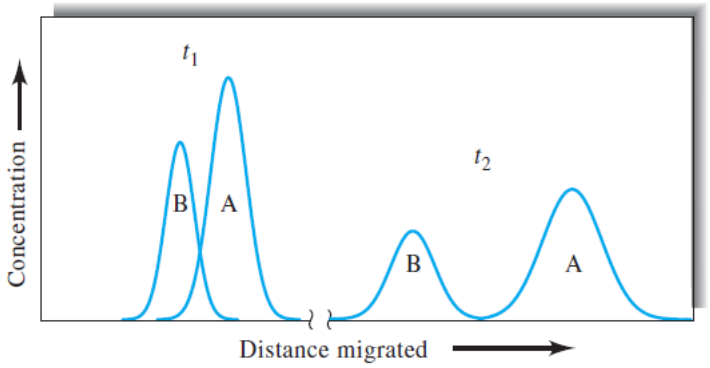
In column chromatography, a sample mixture containing components A and B is placed at the top of a packed column containing a stationary phase and a mobile phase. As the mobile phase (eluent) flows through the column, components repeatedly partition between the two phases. Substances that spend more time in the mobile phase (like A) move faster, while those more strongly retained by the stationary phase (like B) move slower. This difference in migration rates causes the components to separate into distinct bands, which can then be eluted and collected separately.




a) Diagram showing the separation of a mixture of components A and B by column elution chromatography. (b) The detector signal at the various stages of elution shown in (a).

- **Elution** is a process in which solutes are washed through a stationary phase by the movement of a mobile phase. The mobile phase that exits the column is termed the **eluate**.
- An **eluent** is a solvent used to carry the components of a mixture through a stationary phase.

Chromatograms: A **chromatogram** is a plot of some function of solute concentration versus elution time or elution volume.




Concentration profiles of solute bands A and B at two different times in their migration down the column

Distribution Constants: The **distribution constant** for a solute in chromatography is equal to the ratio of its molar concentration in the stationary phase to its molar concentration in the mobile phase., the equilibrium is described by the equation

$$\mathrm{A}(mobile) \rightleftharpoons \mathrm{A}(stationary)$$

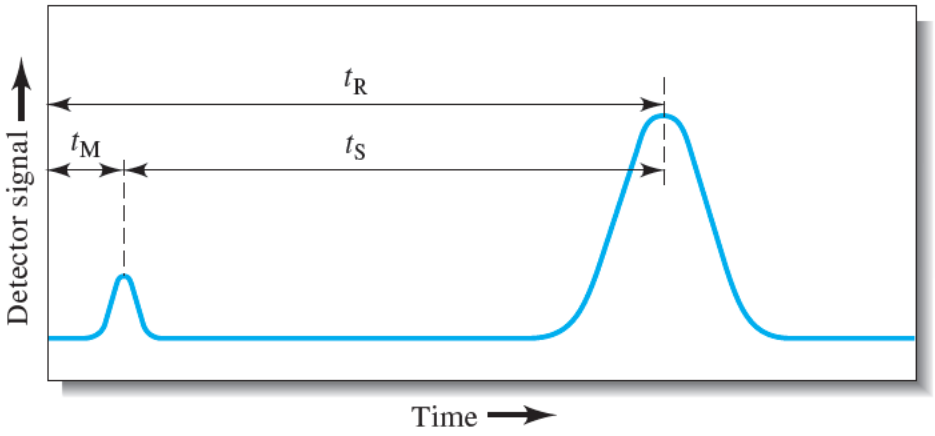
The equilibrium constant *K*c for this reaction is called a **distribution constant**, which is defined as

$$K_c = \frac{(a_A)_S}{(a_A)_M}$$

where (*a*A)S is the activity of solute A in the stationary phase and (*a*A)M is the activity in the mobile phase. We often substitute *c*S, the molar analytical concentrations of the solute in the stationary phase, for (*a*A)S and *c*M, the molar analytical concentration in the mobile phase, for (*a*A)M.

$$K_c = \frac{c_S}{c_M}$$

Retention Times: The **retention time**, *t*R, is the time between injection of a sample appearance of a solute peak at the and the detector of a chromatographic column




The **dead time** (void time), *t*M, is the time it takes for an unretained species to pass through a chromatographic column. All components spend at least this amount of time in the mobile phase. Separations are based on the different times, *t*S, that components spend

the **retention time** and is given the symbol *t*R. The analyte has been retained because it spends a time *t*S in the stationary phase. The retention time is then

$$t_R = t_S + t_M$$

The average linear rate of solute migration, *v* (usually cm/s), is

$$\overline{v} = \frac{L}{t_R}$$

where $L$ is the length of the column packing. Similarly, the average linear velocity, $u$, of the mobile phase molecules is in the stationary phase.

$$u = \frac{L}{t_M}$$

$$\bar{v} = u \times \text{fraction of time solute spends in mobile phase}$$

$$\bar{v} = u \times \frac{\text{no. of moles of solute in mobile phase}}{\text{total no. of moles of solute}}$$

$$\bar{v} = u \times \frac{1}{1 + K_c V_S / V_M}$$

$$k_A = \frac{K_A V_S}{V_M}$$

where $K$A is the distribution constant for solute A

$$\bar{v} = u \times \frac{1}{1 + k_A}$$

$$\frac{L}{t_R} = \frac{L}{t_M} \times \frac{1}{1 + k_A}$$

$$k_A = \frac{t_R - t_M}{t_M} = \frac{t_S}{t_M}$$

The **retention factor**, $k$A, for solute A is related to the rate at which A migrates through a column. It is the amount of time a solute spends in the

stationary phase relative to the time it spends in mobile phase.

**The Selectivity Factor:** The selectivity factor, α, for solutes A and B is defined as the ratio of the distribution constant of the more strongly retained solute (B) to the distribution constant for the less

$$\alpha = \frac{K_B}{K_A}$$

where *K*B is the distribution constant for the more strongly retained species B and *K*A is the constant for the less strongly held or more rapidly eluted species A. According to this definition, α is always greater than unity.

$$\alpha = \frac{k_B}{k_A}$$

where *k*B and *k*A are the retention factors for B and A, respectively

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

**Band Broadening and Column Efficiency**

As a solute moves through a chromatographic column, the separated bands tend to spread out and become wider — this phenomenon is called **band broadening**. It reduces the resolution between closely eluting compounds.

Band broadening occurs due to several factors:

1. **Eddy diffusion:** Different paths through the packing cause molecules to travel different distances.
2. **Longitudinal diffusion:** Solute molecules diffuse along the column length from high to low concentration.
3. **Mass transfer:** Slow equilibrium between the stationary and mobile phases causes lag in solute movement.

**Column efficiency** measures how well a column minimizes band broadening. It is expressed by the number of **theoretical plates (N)**:

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

$$N = 5.54 \left( \frac{t_R}{W_{1/2}} \right)^2$$

where:

- tR = retention time of the peak
- W = width of the peak at the base
- W1/2 = width of the peak at half height

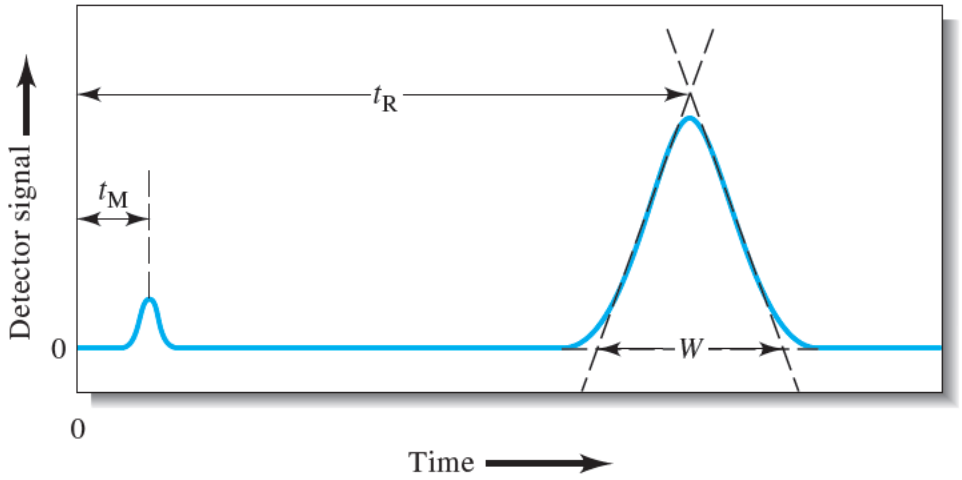
The relationship between column length L and efficiency is given by:

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

A smaller H value indicates higher column efficiency and narrower peaks.

**Factors affecting column efficiency include:**

- Particle size of the stationary phase
- Flow rate of the mobile phase
- Temperature
- Diffusion and mass transfer rates




**Column Resolution:** The resolution of a chromatographic column is a quantitative measure of its ability to separate analytes A and B.

$$R_s = \frac{2(t_{R2} - t_{R1})}{W_1 + W_2}$$

**where:**

- $tR_1$ and $tR_2$= retention times of the two peaks
- $W_1$ and $W_2$= baseline widths of the two peaks

**Interpretation:**

- Rs<1.0: Poor separation (peaks overlap)
- R_s = 1.0–1.: Partial separation
- Rs≥1.5: Good baseline separation

**Example:**

A chromatographic analysis for the chlorinated pesticide Dieldrin gives a peak with a retention time of 8.68 min and a baseline width of 0.29 min.

Solution

Using equation 12.17, the number of theoretical plates is

$$N = 16\left(\frac{t_r}{w}\right)^2 = 16\left(\frac{8.68\,\text{min}}{0.29\,\text{min}}\right)^2 = 14,300\,\text{plates}$$

Given that the column used in this analysis is 2.0 meters long, the height of a theoretical plate is found by solving equation 12.12 for $H$, giving the average height of a theoretical plate as

$$H = \frac{L}{N} = \frac{(2.0\,\text{m})(1000\,\text{mm/m})}{14,300\,\text{plates}} = 0.14\,\text{mm/plate}$$

**Example:** Substances A and B have retention times of 16.40 and 17.63 min, respectively, on a 30.0-cm column. An unretained species passes through the column in 1.30 min. The peak widths (at base) for A and B are 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 on the column that gives an *R*s value of 1.5.

**Solution**

(a) Using Equation 31-29, we find

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

(b) Equation 31-24 permits computation of $N$:

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

$$N_{avg} = \frac{3493 + 3397}{2} = 3445$$

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

(d) The quantities $k$ and $\alpha$ do not change greatly with increasing $N$ and $L$. Thus, substituting $N_1$ and $N_2$ into Equation 31-30 and dividing one of the resulting equations by the other yield

$$\frac{(R_s)_1}{(R_s)_2} = \frac{\sqrt{N_1}}{\sqrt{N_2}}$$

where the subscripts 1 and 2 refer to the original and longer columns, respectively. Substituting the appropriate values for $N_1$, $(R_s)_1$, and $(R_s)_2$ gives

$$\frac{1.06}{1.5} = \frac{\sqrt{3445}}{\sqrt{N_2}}$$

$$N_2 = 3445\left(\frac{1.5}{1.06}\right)^2 = 6.9 \times 10^3$$

But

$$L = NH = 6.9 \times 10^3 \times 8.7 \times 10^{-3} = 60 \text{ cm}$$

(e) Substituting $(R_s)_1$, and $(R_s)_2$ into Equation 31-32 and dividing yield

$$\frac{(t_R)_1}{(t_R)_2} = \frac{(R_s)_1^2}{(R_s)_2^2} = \frac{17.63}{(t_R)_2} = \frac{(1.06)^2}{(1.5)^2}$$

$$(t_R)_2 = 35 \text{ min}$$

So, to obtain the improved resolution, the column length and thus the separation time must be doubled.

**Example:**

Substances A and B have retention times of 12.50 and 13.85 min, respectively, on a 25.0-cm column. An unretained species passes through the column in 1.20 min. The peak widths (at base) for A and B are 0.95 and 1.05 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 1.50, and (e) the time required to elute substance B on the column that gives an RsR_sRs value of 1.50.

Example: The following data were obtained by gas-liquid chromatography

on a 40-cm packed column:

| Compound | $t_R$, min | $W$, min |
|---|---|---|
| Air | 1.9 | — |
| Methylcyclohexane | 10.0 | 0.76 |
| Methylcyclohexene | 10.9 | 0.82 |
| Toluene | 13.4 | 1.06 |

Calculate

(a) an average number of plates from the data.

(b) the standard deviation for the average in (a).

(c) an average plate height for the column.