Lecture 2: General Chromatography Theory
IUPAC Definition of Chromatography

A method, used primarily for separation of the components of a sample in which the components are distributed between two phases, one of which is stationary while the other moves. The stationary phase may be a solid, liquid supported on a solid, or a gel. The stationary phase may be packed in a column, spread as a layer, or distributed as a film. The mobile phase may be gaseous or liquid.

[Notice that the definition neglects supercritical fluid chromatography (SFC)].
General Principles: Definition

**IUPAC Terms**

- **Chromatography**: 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 in a specific direction.

- **Chromatogram**: a graphical or other presentation of detector response, concentration of analyte in the effluent or other quantity used as a measure of effluent concentration versus effluent volume or time.
General Principles: Definition

IUPAC Terms

Stationary Phase: one of the two phases forming a chromatographic system. It may be a solid, a gel or a liquid. If a liquid, it may be distributed on a solid. This solid may or may not contribute to the separation process. The liquid may also be chemically bonded to the solid (bonded phase: covalently bonded to the support particles or to the inside wall of the column tubing) or immobilized onto it (immobilized phase)

Mobile Phase: a fluid which flows through or along the stationary phase, in a specific direction. It may be a liquid (liquid chromatography) or a gas (gas chromatography) or a supercritical fluid (supercritical-fluid chromatography)
General Principles: Stationary Phases

- Stationary phases come in several arrangements: in columns or on plates (used in thin layer chromatography).

- In columns, open tubular (coated walls), packed columns and monoliths are possible means of attaching stationary phase.

- Packed columns contain packing material with the stationary phase either being the surface or being a coating on the surface.

- Porous packing material is common.

- Most common stationary phase is a liquid-like material chemically bonded to packing material (typically HPLC) or to wall (typically GC in open tubular chromatography).
Open Tubular (end on, cross section view)

- Column Wall
- Mobile phase
- Stationary phase (wall coating)

Packed column (side view) (e.g. Silica in normal phase HPLC)

- Packing Material
- Stationary phase is outer surface (although influenced by adsorbed solvents)

Expanded View

- Stationary Phase
- Chemically bonded to packing material

Bonded phase (liquid-like)

- Packing Material

Note: true representation should include micropores in sphere
Elution

A solute partitions between two phases (equilibrium).

Separation is based on relative retention.

Making the column longer will increase the degree of separation, but also increase band (peak) broadening.
Elution

- During the solute transfer in the column, it shows typical broadening due to the diffusion phenomena (transversal and longitudinal)

- The solute band width increases with the retention time giving typical peak broadening
Behavior of Solutes in Columns:

- **t = 0**: Solutes are injected into the column.
- **t = 5mn**: Most solutes have interacted with the stationary phase, while some are still mobile.
- **t = 10mn**: Solutes are further separated, with some showing the least interaction with the stationary phase.

The diagram shows the flow of mobile phase from the injector to the detector, with solutes experiencing different degrees of interaction with the stationary phase over time.
Behavior of Solutes in Columns: Parameters Defined
Behavior of Solutes in Columns: Parameters Defined

Reflects the distribution of a solute between the mobile and stationary phases.
• A - Injection Point
• B - Unretained Solute Peak
• C, D - Solute Peaks (Analyte)
• \( V_r \) - Retention Volume - the volume of mobile phrase necessary to convey a solute band from the point of injection, through the column, to the detector.
• \( t_r \) - retention time of analyte to move from injection to detector
• \( V_m \) – retention volume of the unretained solute
• \( t_m \) – retention time of unretained solute
• \( V'_{r} \) - adjusted retention volume
• \( t'_{r} \) - adjusted retention time
Notice the retention time an analyte spends in the system is composed of two components: \( t_r' \) and \( t_m \)

\( t_m \) - is the time it takes a solute to pass through the space occupied by the mobile phase (dead space, column void volume).

\( t_m \) is the same for all analytes in a given system – the solutes migrate with the same velocity of the mobile phase.

\( t_m \) represents no separation process.

The adjusted retention time, \( t_r' \) - represents the time the analyte spends retained by the stationary phase.

\( t_r' \) represents the separation process or interaction of analyte with stationary phase.
One goal of chromatography is to achieve sharp symmetrical peaks, thus optimizing analyte separation and improving detection. The sharpness of the peak represents the efficiency of the chromatographic column (actually the entire system). Two general approaches have been developed to measure column efficiency – plate theory and rate theory.
Column Efficiency

Two general approaches have been developed to measure column efficiency.

Plate theory
Proposed by Martin and Synge in 1941

Rate theory
Proposed by van Deemter in 1956
Plate Theory

• Models a chromatographic column as a series of narrow, discrete sections called theoretical plates.

• Theoretical plate is a term coined by Martin & Synge. It is based on a study in which they imagined that chromatographic columns were analogous to distillation columns and made up or numerous discrete but connected narrow layers or plates.

• Movement of the solute down the column then could be treated as a stepwise transfer.
Plate Theory

• Models a chromatographic column as a series of narrow, discrete sections called theoretical plates.

• Assume that at each plate equilibrium of the analyte is established between mobile and stationary phase.

• Movement of analyte and mobile phase is viewed as a series of transfers from one plate to the next. Efficiency of a column increases as the number of equilibrations (i.e. theoretical plates) increases.
Partition Coefficient, Distribution Coefficient

K – concentration of the analyte in the stationary and mobile phase

\[ K = \frac{C_s}{C_m} \]

\( C_s \) – concentration of the analyte in stationary phase
\( C_m \) – concentration of the analyte in mobile phase
Partition Coefficient, Distribution Coefficient

$K$ – concentration of the analyte in the stationary and mobile phase

$$K = \frac{C_s}{C_m}$$

$K = 1$ when the analyte is distributed equally

$K$ is assumed to be independent of concentration but can be altered by such factors as temperature.
Capacity Factor, Partition Ratio, $k'$ (Capacity Ratio)

• $k'$ – solute partition ratio is an important parameter routinely used in HPLC but not as much in GC.

• $k'$ – the ratio of the total amount of a solute in the stationary phase to the amount in the mobile phase at equilibrium.

$$k' = \frac{m_s}{m_m}$$
Relationship between $k'$ and $K$

$$K = \frac{C_s}{C_m} = \frac{m_s}{m_m} \times \frac{V_s}{V_m} = \frac{m_s}{m_m} \cdot \frac{V_m}{V_s}$$

$$K = k' \times \frac{V_m}{V_s}$$

$V_s$ – volume of the stationary phase
The ratio \( \frac{V_m}{V_s} \) is the phase ratio called \( \beta \).

\[ K = k' \times \frac{V_m}{V_s} \]

\( \beta \) is a measure of the “openness of the column” and is used to characterize columns, especially in GC.

\( K = k' \beta \) – relates the equilibrium distribution of the analyte within the column to the thermodynamic coefficient, \( K \).
K is:
• independent of the particular column
• depends on temperature and nature of the stationary and mobile phase.
• difficult to measure $V_m / V_s$ in practice, so $k'$ is related to a probability ratio.

\[ k' = \frac{P_s}{P_m} \]

$P_s$ – probability of a single solute molecule being in the stationary phase.
$P_m$ – probability of a single solute molecule being in the mobile phase.
Retention volume, $V_R$
Volume of mobile phase required to elute a solute from a column.

Retention time, $t_R$
Time required to elute a solute from a column.

$V_R = t_R \times \text{flowrate}$

Time is easier to measure.
Retention Volume

\[ V_r = V_m \left(1 + K \frac{V_s}{V_m}\right) \]

\[ V_r = V_m + K V_s \]
This probability can be related to time, which is easier to measure.

\[ k' = \frac{t_r - t_m}{t_m} = \frac{t_r'}{t_m} \]

In practice, accurate measure of column void volume is difficult in HPLC (difficult to find marker compounds), but for GC the practice is simple and accurate.

- when \( k < 1.0 \), separation is poor
- when \( k > 30 \), separation is slow
- when \( k \) is 2-10, separation is optimum
Retention Time

\[ k' = \frac{t_r - t_m}{t_m} \]

rearrange \( t_r = t_m (1 + k') \)

and substitute for \( k' \)

\[ K = k' \cdot \frac{V_m}{V_s} \quad k' = K \cdot \frac{V_s}{V_m} \]

\[ t_r = t_m (1 + K \frac{V_s}{V_m}) \]
Average linear mobile phase velocity is $u$

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

$L$ – length of the column

$$t_r = \frac{L}{u} \left(1 + \frac{K V_s}{V_m} \right)$$

• equation used to predict the effect of change in column length, linear velocity of mobile phase, phase ratio and distribution coefficient on retention time of an analyte.

• equation is only valid when the mobile phase velocity is constant throughout the column.
Liquid mobile phase velocity will stay constant, however gas and supercritical fluid are compressible, so the linear velocity (cm/s) varies along the column length – higher at column outlet than at inlet.
For GC and SFC, where the mobile phases are compressible, the average linear mobile phase velocity,

\[ \bar{u} = j \cdot u \]

\( j \) – correction or compressibility factor

\[ j = \frac{3}{2} \left( \frac{P_i}{P_o} \right)^2 - 1 \]

\[ j = \frac{3}{2} \frac{P_i^3}{P_o^3} - 1 \]

\( P_i \) – column inlet pressure
\( P_o \) – column outlet pressure
\[ j = \frac{3}{2} \left( \frac{P_i}{P_o} \right)^2 - 1 \]

\[ \frac{P_i}{P_o} \]

- is closer to unity for open tubular columns

\[ t_r = \frac{L}{u} \left(1 + KV_s / V_m \right) \cdot 2 \left( \frac{P_i}{P_o} \right)^3 - 1 \]

\[ \frac{2}{3} \left( \frac{P_i}{P_o} \right)^2 - 1 \]
Plate Theory

According to plate theory, a column is mathematically equivalent to a distillation plate column.

The total length is divided into segments each representing one equilibrium stage (or theoretical plate).

Equilibrium is established at each stage.
Plate Theory

When a peak is produced – it can be used to determine the number of theoretical plates in a column.

Knowing the width and retention time of the peak (assuming Gaussian distribution), the number of plates can be determined.

Theoretical plates (N) measure how efficiently a column can separate a mixture into its components. This efficiency is based on the retention time of the components and the width of the peaks.
Plate Theory

Efficiency of a column increases as the number of equilibrations (i.e. theoretical plates) increases.

\[ n = 5.54 \left( \frac{t_r}{w_{1/2}} \right)^2 \]  
(assumes a Gaussian peak)

\[ n = 16 \left( \frac{t_r}{w_b} \right)^2 \]  
n is a dimensionless quantity
Plate Theory

Efficiency of a column increases as the number of equilibrations (i.e. theoretical plates) increases.

Example: Can have analytes that have same elution time but different number of plates.
Plate Theory

N or \( N_{\text{eff}} \) or \( n_{\text{eff}} \) – Effective plate number or number of effective theoretical plates. Used especially if void volume is large or for early eluting peaks.

\[
N = 5.54\left(\frac{t_r - t_m}{w_{1/2}}\right)^2 = 5.54\left(\frac{t'_r}{w_{1/2}}\right)^2
\]

\[
N = 16\left(\frac{t_r - t_m}{w_b}\right)^2 = 16\left(\frac{t'_r}{w_b}\right)^2
\]
Plate Theory

If the peak is asymmetrical then calculation of $N$ is more complex.

$$N = \frac{41.71(t_r / w_{0.1})^2}{A_s + 1.25}$$  
(for asymmetrical peak)

$A_s$ – asymmetry factor

$w_{0.1}$ – peak width at 10% of peak height
Plate Theory

Plate number and effective plate number depend on the length of the column, so other parameters have been developed.

- $h$ or $h_{	ext{etp}}$ – the height equivalent to a theoretical plate or plate height

\[
h = \frac{L}{n}
\]

$L$ – length of the column
Plate Theory

- H or HETP – the effective plate height or height equivalent to an effective theoretical plate.

\[ H = \frac{L}{N} \]
Plate Theory

Figure 26-7  Effect of mobile-phase flow rate on plate height for (a) liquid chromatography and (b) gas chromatography.
Assignment

- Read Chapter 2: Principles and Practice of Modern Chromatographic Methods, Peter E. Jackson, Academic Press.