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1. What is chromatography?

**IUPAC definition** (International union of pure and applied chemistry)

Chromatography is a physical method of separation in which the components to be separated are distributed between two phases, one of which is stationary while the other moves in a definite direction.

The stationary phase: a solid, a liquid,
The mobile phase: a gas, a liquid.
2. History of chromatography

Mikhail Tsvet and column chromatography

In 1901, invented the first true chromatograph to separate plant pigments.

In 1906, first used the term *chromatography* in print in his two papers.

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<thead>
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<th>Born</th>
<th>1872</th>
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<td>Died</td>
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<td>Nationality</td>
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<td>Known for</td>
<td>adsorption chromatography</td>
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2. History of chromatography

Martin and Synge and partition chromatography

In 1941, Martin and Synge developed partition chromatography by combining the techniques of chromatography and countercurrent solvent extraction.

In 1952, the Nobel Prize in Chemistry was awarded jointly to Martin and Synge "for their invention of partition chromatography".
In chromatographic separations, the sample is transported in a mobile phase. The mobile phase is forced through a stationary phase held in a column or on a solid surface. When the sample moves past the stationary phase, it interacts with the stationary phase. Component having great interaction appears to move slowly. Component having weak interaction appears to move fast. Because of the difference of moving rate, the components are separated by passing the chromatographic column.
4. Classification of chromatography

According to mobile phase

- Gas chromatography
  - The mobile phase is inert gas, nitrogen or helium.
  - Gas-solid chromatography (GSC): the stationary phase is solid
  - Gas-liquid chromatography (GLC): the stationary phase is liquid

- Liquid chromatography
  - The mobile phase is liquid.
  - Liquid-solid chromatography (LSC): the stationary phase is solid
  - Liquid-liquid chromatography (LLC): the stationary phase is liquid

- Supercritical fluid chromatography
4. Classification of chromatography

According to mechanism of separation

- **Adsorption chromatography**
  The stationary phase is a solid with adsorption power. Sample will be adsorbed on the surface of the stationary phase with different powers.

- **Partition chromatography**
  The stationary phase is a liquid film supported by an inert solid. The stationary liquid is usually more polar than the mobile liquid.

- **Ion exchange chromatography**
  The stationary phase is an ion exchange resin. Ions of opposite charges (counter ions) in the mobile phase will be attracted to the resin. It is used for separation of charged molecules.

- **Molecular exclusion chromatography (Size exclusion)**

- **Affinity chromatography**
4. Classification of chromatography

According to the method of holding the stationary phase

- Column chromatography (CC)
  The stationary phase is held in to a tube.

- Plane chromatography
  The stationary phase is used in the form of layer.
  - Thin layer chromatography (TLC): the stationary phase in the form of fine powder is spread on glass or plastic or aluminum sheets.
  - Paper chromatography (PC): a specific type of papers is used as stationary phase.
5. Chromatography terms

- **Partition coefficient (distribution constant)**
  \[ K = \frac{C_S}{C_M} \]
  \( C_S, C_M \): component concentrations in stationary and mobile phases

- **Retention factor (capacity factor)**
  \[ k = \frac{V_S}{V_M} \frac{C_S}{C_M} = K \frac{V_S}{V_M} = \frac{t_R - t_M}{t_M} \]
  \( V_S, V_M \): volume of stationary and mobile phases
  \( k \) describes the ability of a stationary phase to retain components.

- **Separation factor**
  \[ \alpha = \frac{K_B}{K_A}, \quad \alpha = \frac{k_B}{k_A}, \quad \alpha = \frac{(t_R)_B - t_M}{(t_R)_A - t_M} \]
  \( \alpha \) indicates the ability of a stationary phase to separate two components.
5. Chromatography terms

- **Retention times**

- $t_M$: retention time of mobile phase (dead time)
- $t_R$: retention time of analyte (solute)
- $t_R' = t_R - t_M$: time spent in stationary phase (adjusted retention time)
5. Chromatography terms

- **Resolution** ($R_s$)
  
  $R_s$ indicates the degree of separation of two peaks.

  $$R_s = \frac{2(t_{R2} - t_{R1})}{(w_{R2} + w_{R1})} = \frac{\sqrt{N}}{4} \frac{\alpha - 1}{\alpha} \frac{k_2}{1+k_2}$$

  - $t_{R1}, t_{R2}$: retention time
  - $w_{R1}, w_{R2}$: width of peak

  $R_s \geq 1$, sufficient for separating peaks
  $R_s \geq 1.5$, peaks totally separated
It is supposed that the chromatographic column can be divided into a number (N) of imaginary, adjacent, and separate layers called theoretical plates.

Within each theoretical plate, sample can reach complete equilibration between stationary and mobile phase.

- Molecules of the sample interact with the molecules of the MP and SP.
- Each molecule interacts differently with MP and SP.
- Retardation of rate of movement of molecules.
- Different distribution coefficients and different net rates of migration.

Equilibrium established at each plate.
6. Principles of chromatography

Plate model theory (By Martin and Synge)

How to calculate the number (N) of plates?
Empirical equation for N from a chromatogram

\[ N = 5.54 \left( \frac{t_R}{W_{1/2}} \right)^2 = 16 \left( \frac{t_R}{W} \right)^2 \]

- \( t_R \): retention time;
- \( W_{1/2} \): width of peak at half of the peak height
- \( W \): width measured at base line

The larger the N, the higher the separation potential of the column. N varies directly with column length and inversely with column diameter.

How to calculate the plate height (H)?

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

- \( L \): the length of column
6. Principles of chromatography

Rate theory

Peak broadening effects:

- molecular diffusion (axial and longitudinal)
- different pathways within the column
- mass transfer kinetics between stationary and mobile phases
6. Principles of chromatography

Rate theory

Van Deemter equation for plate height

\[ H = A + \frac{B}{\mu} + \mu(C_M + C_S) \]

A: Eddy-diffusion
B: molecular diffusion
C: mass transfer kinetics
u: average linear velocity
7. Schematic & main components of GC
8. Advantages of GC

- High resolution, \( N \sim 1.3 \times 10^6 \)
- Small samples (μl or µg needed)
- Fast analysis, results are rapidly obtained (1 to 100 minutes)
- Reliable, good repeatability & reproducibility
- Non-destructive analysis, allows on-line coupling, e.g. to MS
- Sensitive detectors (easy ppm, often ppb)
- Equipment is not very complex
9. Column selection for GC

- Packed column or capillary column?
  - Packed column: gas samples, high sample capacity
  - Capillary column: a general term for columns with a small diameter, almost all other samples, good efficiency (narrow peaks), time-saving

- Column material
  - Packed column:
    metal: non-polar samples
    glass: polar samples
  - Capillary column: fused silica
    WCOT (Wall Coated Open Tubular): liquid film stationary phase, the most widely used columns
    PLOT (Porous Layer Open Tubular): usually solid stationary phase
9. Column selection for GC

- **Stationary phase (for capillary columns)**
  
  WCOT: “*likes dissolve likes.*” The more you know about your sample, the easier it is to find the optimum stationary phase.
  - Non-polar capillary columns: non-polar samples
  - Intermediate polar or polar capillary columns: polar samples
  - Highly polar capillary columns: polarizable samples

- **PLOT:**
  - Molecular sieve: fixed gases (refer to N₂, O₂, Ar, CO₂, and CO), sensitive to water
  - Divinylbenzene (DVB): complete resolution of C₁ to C₃ isomers, only partial resolution of isomers of C₄ and higher (up to C₁₄), polar compounds, tolerate water
  - Alumina Al₂O₃: separation of isomers of C₁ to C₁₀, sensitive to water
9. Column selection for GC

- **Film thickness**
  - Increase the film thickness, improve resolution, but long retention time.
  - Thin film: $0.1 \mu m$, low volatility, high-boiling point, or temperature sensitive components
  - Standard film: $0.25\sim0.5 \mu m$, most samples eluting up to $300 ^\circ C$
  - Thicker film: $1.0\sim1.5 \mu m$, low-boiling samples, between $100 \sim200 ^\circ C$
  - Extremely thick films: $3\sim5 \mu m$, for gases, solvents, and purgeables to increase their interaction with the stationary phase
9. Column selection for GC

- Column length
  - 15m: for fast screening, simple mixtures, or very high molecular weight samples
  - 20~30m: the most popular one for most samples
  - 60 m or longer: for extremely complex samples

Note: resolution only increases according to the square root of the column length.
9. Column selection for GC

- Inside diameter
  - Small column: better resolution, but require a split injection because of the low sample capacity
  - Large column: high sample capacity, low resolution, for gas samples, very volatile samples, and purge and trap or headspace sampling
10. Requirements for GC samples

General requirements for samples: volatile & thermally stable

Never be injected: metals, strong acids or bases, salts, oligomeric and polymeric material.

Sampling methods:
- Solid samples using pyrolysis or dissolve the solid in volatile solvents, usually methylene chloride
- Volatile samples using direct sampling/pre-concentration
- Liquid sampling using split/splitless injection
11. Applications of GC

- Qualitative analysis:
  - Confirm the presence or absence of a suspected compound
  - Determine an unknown: hyphenated GC with other methods
  - Retention index: Normal standards

- Quantitative analysis:
  - Area (preferred) of a peak vs. calibration: linearly
  - Calibration of standards: straight line through origin
  - Internal standard method
Questions?

Thank you!