Chromatography

- Principle, Classification, Distribution coefficient, Retention time, resolution, Retardation factor
- Column chromatography
- Thin layer chromatography
- HPLC – Principle, instrumentation, applications
- GC – Principle, instrumentation, applications

Principle of Chromatography:

Chromatography is a separation technique based on the **differential distribution of components between two phases**:

- **Stationary phase**: The phase that stays fixed in place (e.g., paper, silica gel).
- **Mobile phase**: The phase that moves and carries the mixture components with it (e.g., solvent like water or ethanol).

Different substances in a mixture move at **different rates** depending on:

- Their **solubility** in the mobile phase.
- Their **affinity** (attraction) to the stationary phase.

This leads to the **separation** of individual components.

Key Concept: Partition and Adsorption

- **Partition chromatography** (e.g., paper chromatography) is based on **differences in solubility** between the mobile phase and the stationary phase.
- **Adsorption chromatography** (e.g., column chromatography) depends on how strongly substances **adhere to the surface** of the stationary phase.

Principle of Differential Migration (in Chromatography)

Differential migration is the **core principle** behind chromatography, which explains how different components in a mixture move at **different speeds** through a medium, leading to their **separation**.

Differential migration refers to the differential rate of movement of solute particles on the stationary **phase** under the influence of a **mobile phase**. The rate at which each component moves depends on its **relative affinity** (attraction) toward the stationary and mobile phases.

- Each component in a mixture interacts **differently** with the stationary and mobile phases.
- A substance that has a **higher affinity for the mobile phase** moves **faster**.
- A substance that has a **higher affinity for the stationary phase** moves **slower**.

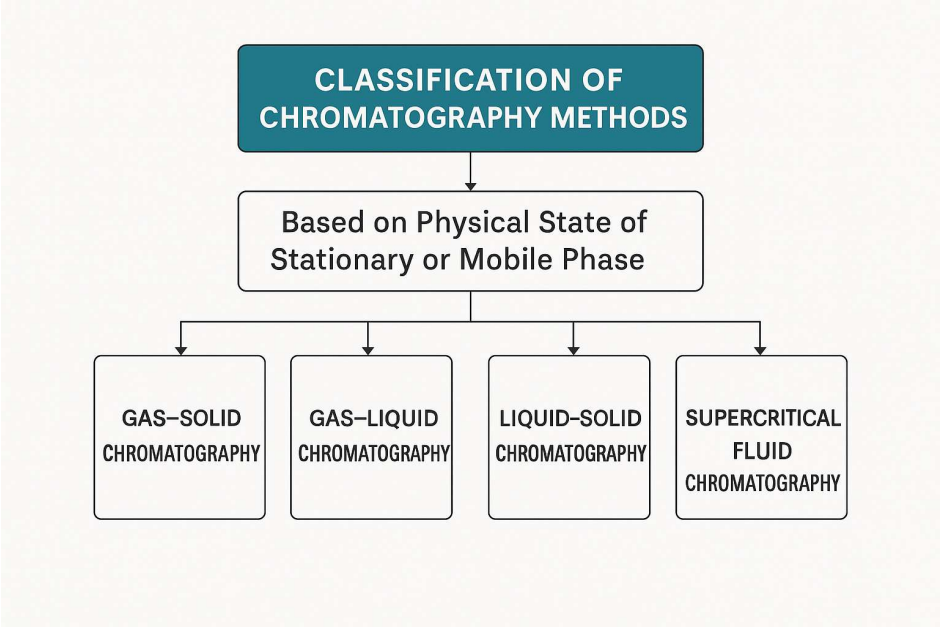
This difference in speed /migration / rate is what causes **separation**.

https://youtu.be/0m8bWKHmRMM?feature=shared

Classification of chromatography methods:

1. Based on Physical State of stationary or Mobile Phase
2. Based on the basic principle involved

1. **Based on Physical State of stationary or Mobile Phase:** This classification considers the physical state (solid or liquid state) of the stationary phase and (liquid or gas state) of the mobile phase involved in the chromatographic process.



I. Gas–Solid Chromatography (GSC)

- **Stationary Phase**: Solid
- **Mobile Phase**: Gas
- **Mechanism**: **Adsorption** of analytes on solid surface
- **Example**: Separation of permanent gases like O_2, N_2, CO_2

✔ **Used for** low molecular weight volatile substances.

II. Gas–Liquid Chromatography (GLC) ***(Most common form of GC)***

- **Stationary Phase**: Liquid (coated on inert solid support inside the column)
- **Mobile Phase**: Gas (usually He or N_2)
- **Mechanism**: **Partition** between gas and liquid phases
- **Example**: GC analysis of essential oils, alcohols, hydrocarbons

✔ **Widely used** in industries and forensic labs.

III. Liquid–Solid Chromatography (LSC)

- **Stationary Phase**: Solid (e.g., silica, alumina)
- **Mobile Phase**: Liquid
- **Mechanism**: **Adsorption** of analytes on solid
- **Examples**: Column chromatography, Thin-Layer Chromatography (TLC)

✔ Used for **separating non-polar or slightly polar organic compounds**.

IV. Liquid–Liquid Chromatography (LLC)

- **Stationary Phase**: Liquid (adsorbed on an inert support)
- **Mobile Phase**: Liquid
- **Mechanism**: **Partition** between two immiscible liquids
- **Examples**: Paper Chromatography, Partition Column Chromatography, HPLC

✔ Useful for **polar and water-soluble compounds**.

V. Supercritical Fluid Chromatography (SFC)

- **Stationary Phase**: Solid or liquid
- **Mobile Phase**: Supercritical fluid (commonly **CO_2**)
- **Mechanism**: **Partition** and **diffusion**
- **Example**: Separation of thermally unstable or chiral compounds

✔ Combines advantages of both GC and LC.

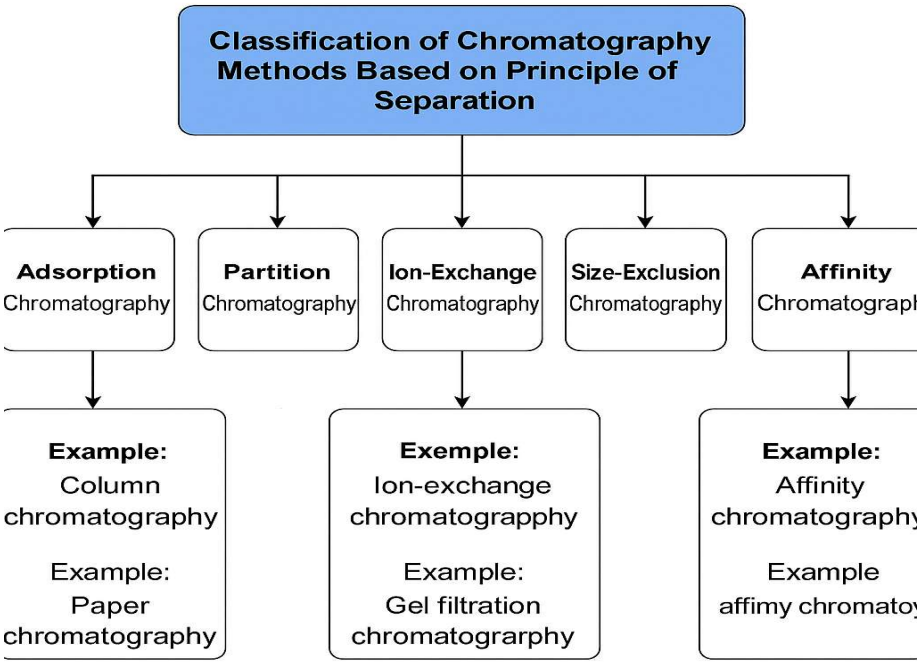
Summary Table

Type	Stationary Phase	Mobile Phase	Example Technique
GSC	Solid	Gas	Separation of O_2, CO_2, N_2
GLC	Liquid	Gas	Gas Chromatography (GC)
LSC	Solid	Liquid	Column chromatography, TLC
LLC	Liquid	Liquid	Paper chromatography, partition
SFC	Solid/Liquid	Supercritical Fluid	Supercritical Fluid Chromatography

2. **Based on the basic principle involved in the separation:**

Chromatography methods can be classified **based on the principle of separation** — that is, how the components in a mixture are separated during the chromatographic process. Each method utilizes a different interaction between the components of the mixture and the stationary or mobile phases. The main principles include

- **Adsorption**,
- **Partition**,
- **Ion-exchange**,
- **Size-exclusion**, and
- **Affinity**.



1. Adsorption Chromatography:

Principle: Separation is based on the adsorption of solutes on the surface of the solid stationary phase. The more strongly a compound adsorbs to the surface, the slower it moves.

- Stationary phase: Solid (e.g., silica gel, alumina)
- Mobile phase: Liquid or gas

Examples:

- Column chromatography (using silica or alumina as adsorbent)
- Thin Layer Chromatography (TLC)

2. Partition Chromatography

Principle: Separation occurs due to differential partitioning of components between two liquid phases — one stationary and one mobile. It is based on differences in solubility.

- Stationary phase: Liquid film on inert solid support (e.g., water on silica)
- Mobile phase: Liquid or gas

Examples:

- Paper chromatography
- Gas-liquid chromatography (GLC)
- High-performance liquid chromatography (HPLC)

3. Ion-Exchange Chromatography

Principle: Separation is based on the electrostatic attraction between charged analyte ions and oppositely charged sites on the stationary phase (ion-exchange resin).

- Stationary phase: Ion-exchange resin (cationic or anionic)
- Mobile phase: Liquid (buffered solution)

Examples:

- Cation-exchange chromatography (for separating metal ions or amino acids)
- Anion-exchange chromatography (for separating anions like phosphate, nitrate)

4. Size-Exclusion Chromatography (also called Gel Filtration or Gel Permeation)

Principle: Separation is based on molecular size. Larger molecules are excluded from entering the pores of the stationary phase and elute first, while smaller molecules enter the pores and elute later.

- Stationary phase: Porous polymer or gel beads
- Mobile phase: Liquid

Examples:

- Gel filtration chromatography (for proteins, polysaccharides)
- Gel permeation chromatography (for synthetic polymers)

5. Affinity Chromatography

Principle: Separation is based on specific biological interactions such as enzyme-substrate, antigen-antibody, or receptor-ligand binding.

- Stationary phase: Matrix with immobilized ligand
- Mobile phase: Buffered solution

Examples:

- Protein purification using antigen-antibody affinity
- DNA separation using specific binding proteins

Summary Table

Principle of Separation	Method Example	Stationary Phase	Mobile Phase
Adsorption	TLC, Column chromatography	Solid (e.g., silica)	Liquid
Partition	Paper chromatography, GLC, HPLC	Liquid on inert support	Liquid or Gas
Ion-exchange	Ion-exchange chromatography	Ion-exchange resin	Aqueous solution
Size-exclusion	Gel filtration chromatography	Porous beads (polymer/gel)	Liquid
Affinity	Affinity chromatography	Ligand-bound resin	Buffer

Distribution coefficient:

In chromatography, distribution coefficient, partition coefficient, and distribution ratio are essential parameters that describe how a compound distributes itself between two phases—typically the stationary phase and the mobile phase.

Partition Coefficient (K):

Definition: The partition coefficient is the ratio of the concentrations of a solute in two immiscible liquids (like water and an organic solvent) at equilibrium. When a solute is added to a system of **two immiscible liquids**, it **partitions** between the two layers. At **equilibrium**, the **ratio of its concentrations** in each solvent is **constant** (at a given temperature).

$$K = \frac{[S]_{organic}}{[S]_{aqueous}}$$

Where:

- [S]organic = concentration of solute in the organic phase
- [S]aqueous = concentration of solute in the aqueous phase
- Used when: The solute exists in only one chemical form (non-ionized) in both phases.

This ratio arises because the solute **distributes itself** between the two layers until **dynamic equilibrium** is reached. At this point:

- The **rate of solute transfer** from aqueous to organic phase = **rate from organic to aqueous**
- The system is in **equilibrium** (no net transfer of solute)
- The value of K becomes **constant** for that solute–solvent pair at a particular temperature.

Example: **Iodine in Water and CCl_4**

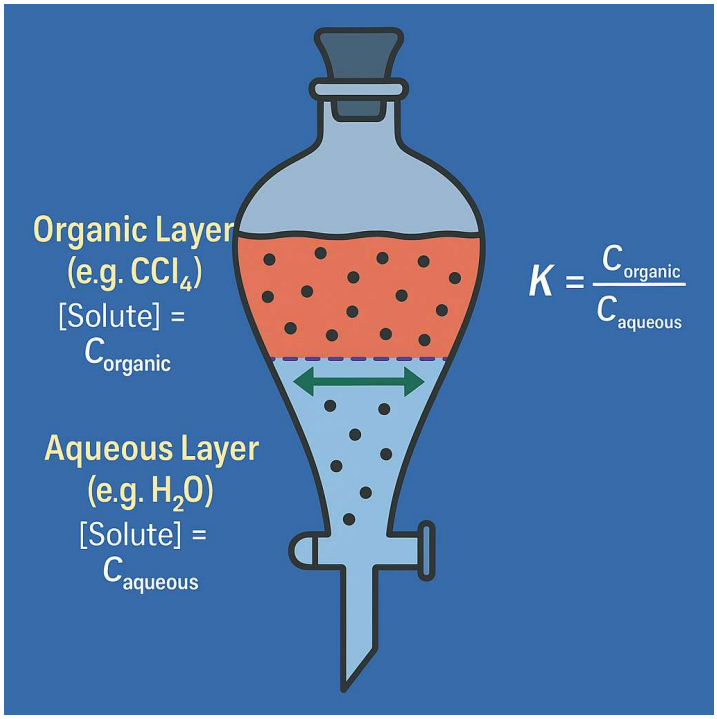
Iodine is more soluble in carbon tetrachloride (CCl_4) than in water. If iodine is added to a separatory funnel with water and CCl_4:

- Iodine partitions between the two layers
- After shaking and settling, equilibrium is reached
- Experimentally, suppose:

$$K = \frac{[I_2]_{CCl_4}}{[I_2]_{H_2O}} = 85$$

Key Points:

- The partition coefficient is an equilibrium constant for a solute between two immiscible solvents.
- Higher K → more solute in the organic phase
- Lower K → more solute in the aqueous phase
- It assumes the solute remains chemically unchanged in both solvents.



Retention time and Retention volume:

In chromatography, retention time and retention volume are key parameters used to describe how long and how far a compound travels through the chromatographic system before being detected.

Retention Time (t_R):

Definition:
The **retention time** is the **time taken** by a compound to pass through the column **from the point of injection to the detector**.

- t_R=Time when analyte peak appears.
- It is measured in **minutes or seconds**.

Key Points:

- Each compound has a **characteristic retention time** under fixed conditions (column type, temperature, flow rate, etc.).
- Helps in **qualitative identification** of compounds.
- Shorter retention time → compound interacts **less** with stationary phase.
- Longer retention time → compound interacts **more** with stationary phase.

Retention Volume (V_R):

Definition:
The **retention volume** is the **volume of mobile phase** required to elute a compound **from the column**.

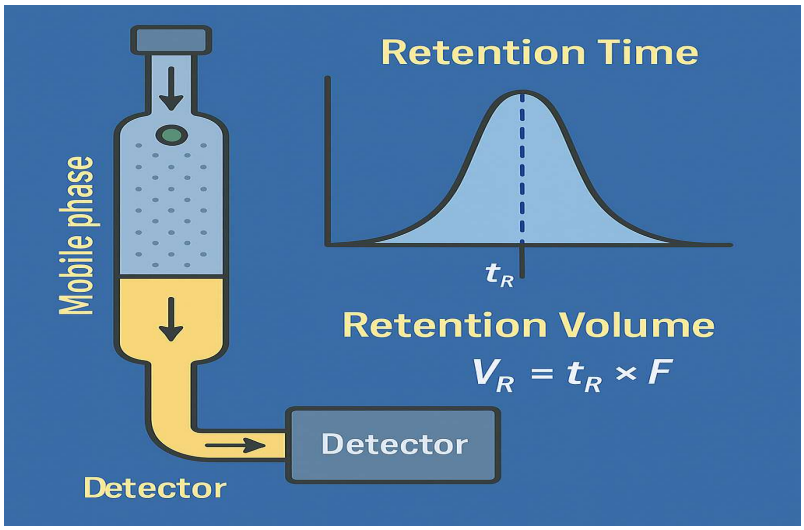
$$V_R = t_R \times F$$

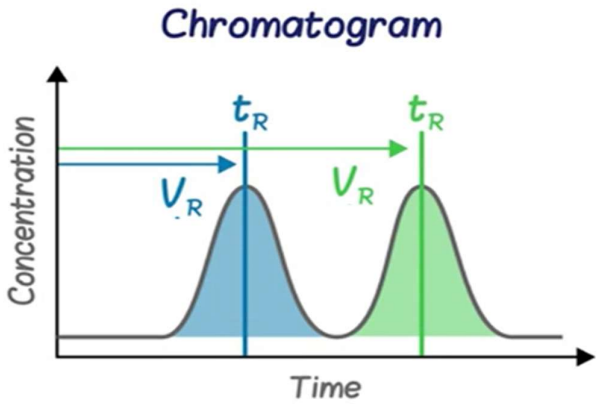
Where:

- t_R= retention time
- F = flow rate of the mobile phase (e.g., mL/min)

Key Points:

- It is measured in **millilitres (mL)**.
- It represents the **physical volume** of mobile phase needed to carry the analyte to the detector.
- Used in **preparative chromatography** and for quantitative work.





Resolution:

Resolution (R_s) is a measure of how well two chromatographic peaks (representing two different compounds) are separated from each other in a chromatogram.

Definition:

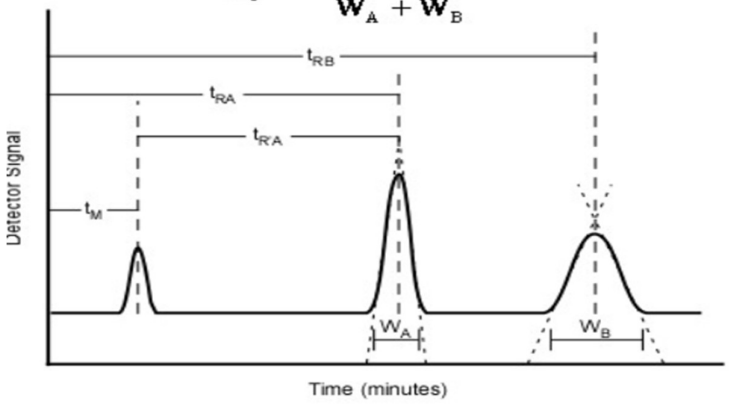
Resolution is defined as the difference in retention times of two peaks divided by the average of their peak widths (usually at the base).

$$R_s = \frac{2(t_{R2} - t_{R1})}{w_1 + w_2}$$

Where:

- t_{R1} ,t_{R2} = retention times of compounds 1 and 2
- w_1, w_2= widths of the peaks at their base

$$R_S = \frac{2(t_{R\cdot B} - t_{R\cdot A})}{W_A + W_B}$$



Factors Affecting Resolution:

Resolution depends on **three key parameters**:

$$R_s \propto \left(\frac{\sqrt{N}}{4}\right) \cdot \left(\frac{\alpha - 1}{\alpha}\right) \cdot \left(\frac{k'}{1 + k'}\right)$$

Where:

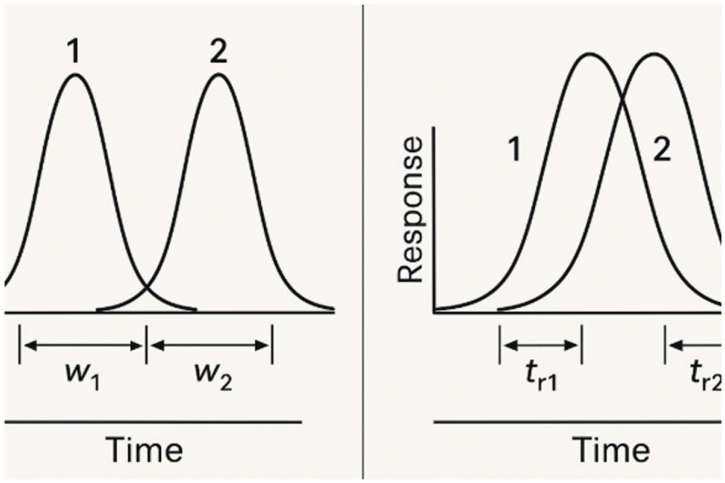
- N = number of theoretical plates (column efficiency)
- α = selectivity factor (ratio of retention factors)
- k' = capacity factor (retention factor)

How to Improve Resolution:

1. Increase column length → increases efficiency (N)
2. Use a different stationary phase → changes selectivity (α)
3. Change mobile phase composition → affects retention (k')
4. Optimize temperature (for GC) or pH (for LC)

Summary:

Term	Symbol	Meaning
Resolution	Rs	Degree of separation between two peaks
Good separation	Rs≥1.5	Baseline separation – ideal
Poor separation	Rs<1.0	Peaks overlap significantly



Good resolution **Poor resolution**

Retardation Factor (R_f) in Chromatography:

The retardation factor, also known as the retention factor in planar chromatography (especially in paper or thin-layer chromatography), is a measure of how far a compound travels relative to the solvent front.

Definition:

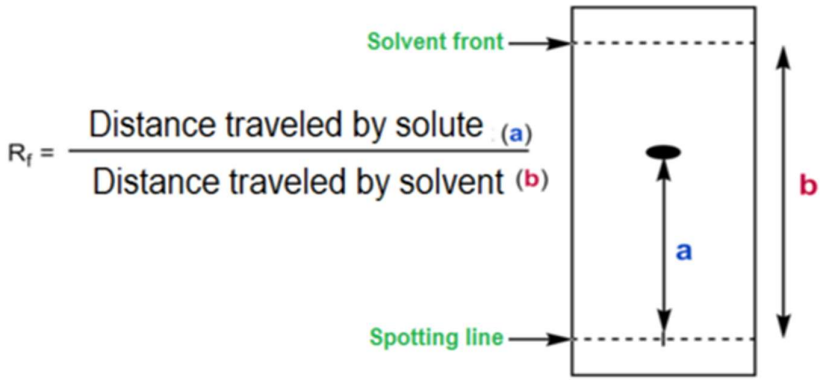
$$R_f = \frac{\text{Distance travelled by solute (spot)}}{\text{Distance travelled by solvent front}}$$

Where:

- The solute is the analyte spot
- The solvent front is the furthest point the mobile phase has reached on the stationary phase (paper or TLC plate)

Important Characteristics:

- R_f is always between 0 and 1
    - R_f=0: solute did not move
    - R_f=1: solute moved with solvent front
- The larger the R_f value, the less interaction the solute has with the stationary phase
- Used to identify compounds by comparing with known standards



Efficiency of a Chromatographic Column:

In chromatography, **column efficiency** indicates how well a column can **separate** different compounds. It is a measure of how **narrow** (sharp) and **well-resolved** the peaks are on a chromatogram.

Key Concept:

A **more efficient column** produces **sharper peaks** (less band broadening), leading to **better separation** of closely eluting compounds.

Measured by: Theoretical Plates (N)

The concept of **theoretical plates** comes from distillation but is applied in chromatography to describe the efficiency of solute movement.

$$N = 16\left(\frac{t_R}{w}\right)^2 \quad \text{or} \quad N = 5.54\left(\frac{t_R}{w_{1/2}}\right)^2$$

Where:

- N = number of theoretical plates
- t_R = retention time of the peak
- w = width of the peak at base
- $w_{1/2}$ = width at half height (for Gaussian peaks)

Interpretation:

- Higher NNN → **greater column efficiency**
- Narrower peaks → **less dispersion** → higher resolution
- Used to compare performance of different columns or same column over time

Plate Height (H) or Height Equivalent to a Theoretical Plate (HETP):

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

Where:

- H = plate height (cm)
- L = column length (cm)

🔹 **Smaller H means higher efficiency** (more plates per unit length)

Graphical Representation (Van Deemter Plot):

Van Deemter Equation explains how flow rate affects efficiency**:**

$$H = A + \frac{B}{u} + C \cdot u$$

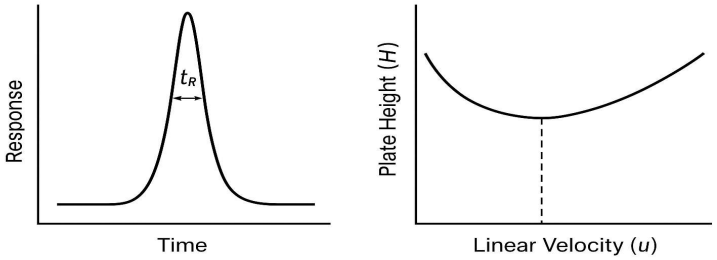
Where:

- A: eddy diffusion (path differences)
- B: longitudinal diffusion
- C: mass transfer resistance
- u: linear velocity of mobile phase

📄 The curve shows an **optimal flow rate** where H is minimum (maximum efficiency).

Summary Table:

Parameter	Symbol	Interpretation
Theoretical Plates	N	More plates = better efficiency
Plate Height	H	Smaller H = higher efficiency
Peak Width	w or $w_{1/2}$	Narrower = better separation
Flow Rate	u	Must be optimized for efficiency



Van Deemter Equation

$$\mathrm{HETP} = \mathrm{A} + \frac{\mathrm{B}}{\mathrm{u}} + \mathrm{Cu}$$

A = eddy diffusion
B = longitudinal molecular diffusion
C = mass transfer