Chapter:

Column Chromatography

Liquid Chromatography

HPLC

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Chemistry with MJS

Chemistry Preparation by MJS

Column Chromatography

The chromatographic separations occured in column known as column chromatography.

- Liquid chromatography
- Gas chromatography

* For Air Sensitive compounds, we use the column chromatography bic of closed system we can not use the paper chromatography For Air Sensitive compounds bic of open system.

* Generally, we use the gas chromatography for non-polar compounds.

• GC Also called vapour phase chromatography

Liquid Chromatography

A) Classification: (Based on performance)

① LPLC (low performance liquid chromatography)
② Flash (Liquid chromatography)
③ HPLC (High performance LC)
④ UPLC (ultra performance LC)

* If partical size > 40 um ⟶ LPLC & Flash used
* If 10–40 um ⟶ ~~Flash~~ HPLC
* If < 10 um ⟶ UPLC

* LPLC > Flash > HPLC > UPLC (Retention time)

↓
peak shows the good separation

not good separation bic base line is not present

* After every separation Base line occured which shows that our Analyte has been separated and oney M·phase is left.

* Retention time: → time which analyte spends in column with the stationary phase.

* Back pressure: → Resistance in Flow in column

* Reproducibility: → two, three or many times repeated the separation

* % Recovery: → How much our sample Recovered After purification

• When porosity of stationary phase is more then back pressure will be less.

* Mesoporous → 2–50 nm

* Microporous → >50 nm

Q: Why we preferred Silica?

Silica is available in every particle size & pore size so widely used - Best known stationary phase its pore size from nano to el. size available

* 2DLC (two Dimentional Liquid chromatography)

If we use two diff. stationary phases in two different columns - M·P of 1st column should be different than M·P of 2nd column.

↳ 95% separation occured by Only one column One hundred components can be separated through one column so no need of 2D/3D LC

* Mobile phase:

The Basic purpose of M·P is to elute the Analyte - it do elution.

- Isocratic elution
- Gradient elution
- Stepwise elution

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* Isocratic elution:

in this, M.P composition Remains same throught the column separation e.g either totally polar or non-polar etc.

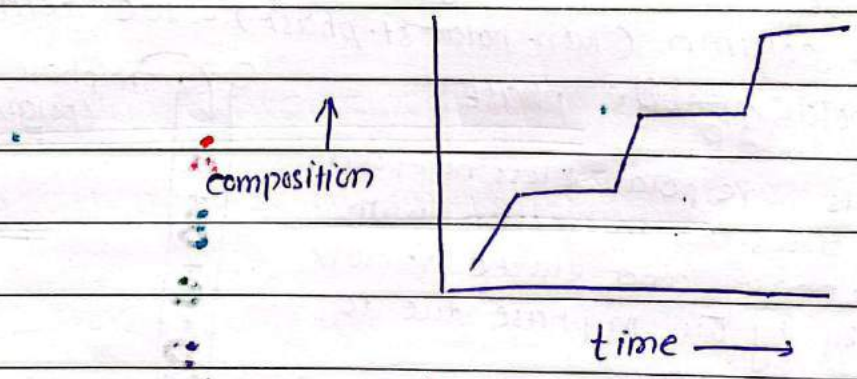
* Gradient elution:

in this, M.phase composition changed during the separation.

* We move from weaker to stronger composition of M.phase so that it gave good time to Analyte with stationary phase and better separation can occured.

* Step-wise elution:

steps are clearly increases then become constant & then increases. Here hundreds of compositions can be made



* Normal phase chromatography: (Generally polar stationary phase used)

Strong St. phase + weak M.phase → Non-polar Analytes

(strong St. phase: more polar; weak M.phase: more non polar)

* Reverse phase chromatography (Non-polar St.phase)

weak St. phase + Strong M.phase → polar Analytes

(weak St. phase: more non-polar; Strong M.phase: more polar)

Note: Most often used in Reverse phase chromatography

* We use mostly the Reverse phase chromatography bic every Analyte has some polar components & Non-polar components
↓
70–85% separations through this

* Totaly polar molecules can Also be separated by the Reverse phase chromatography bic this polar molecules have a few character of non-polarity for interaction with non-polar stationary phase.
↳ e.g silica / Alumina etc

Example;
let we have 4 unknown components of Analyte A, B, C & D having different polarity

A > B > C > D polarity order
↓ more polar
↓ less polar

Q: Which type of chromatography used?

Ans: We can Separate these components through the Reverse phase chromatography - silica is packed in the column (Non-polar st. phase) - we will use the polar mobile phase

- A is more polar & less non-polar so it shows less interaction with st. phase and eluted rapidly firstly by the m.phase due to highly interaction
- D is less polar & more non polar so it shows strong interaction with the stationary phase & eluted later.

So, elution order;

A > B > C > D
↓ 1st eluted
↓ later eluted

B) Classification Based on Flow Rate: *

- Conventional LC (mL min⁻¹) — Flow rate
  - conventional column / packed column is used
- µ-LC (µL/min)
- n-LC (nL/min)
  - Capillary column is used
    - monolith is used for packing

* If column diameter ↑ then its Length ↓
  - ↳ conventional column (broad peak occur)
  - thus less efficiency than capillary column.
    - ↳ lower Resolution
* If diameter ↓ length ↑ (capillary column)
  - sharp peak → more efficiency → higher Resolution

⇒ We can make the capillary column from conventional column by lower. its diameter and increasing its length

Column Efficiency:

* Column efficiency depends upon;
  - (i) S·phase in column
  - (ii) Inner diameter vs Length of column
  - (iii) M·phase
  - (iv) packed material (st·phase)
  - (v) No·of theoretical plates

Packed column	Difference	Capillary Column (open tubular column)
① S. phase is packed		① S. phase is coated in the inner surface of cavity of column
② Required large sample (10 mg) compared to capillary		② Required small amount (100 ng)
③ High pressure inside column due to packing material		③ Low pressure inside column
④ Short length (0.5–5m)		④ long length 5–100 m
⑤ internal diameter can be several mm (2–4 mm)		⑤ internal diameter (0.1–0.7 mm)
⑥ Efficiency low as compared to open-tubular		⑥ high efficiency
⑦ poor Resolution compairitively		⑦ High Resolution
⑧ Less expensive		⑧ mere expensive
⑨ Flow rate required ↓ 10–60 ml/mint		⑨ Flow Rate required 0.5–15 ml/mint
⑩ Total Theoretical plates ↓ 4000		⑩ Total theoretical plates ↓ 250,000
⑪ Generally used in HPLC (Liquid chromatography)		⑪ Generally used is Gas chromatography

lumber Name

Theory of Chromatography

column chromatography

Basically two theories are developed to discuss the chromatographic separations & column Efficiency

① Plate theory → old Theory

② Rate theory → modern theory

1) Plate Theory:

This Theory was developed by Synge- This states that;

"Separation efficiency of a column can be expressed in terms of the no. of theoretical plates in the column."

★ A theoretical plate represents a single equilibrium step like Excel cell. The more Theoretical plates, more will be the equilibrium steps, Thus more will be the efficiency of Column.

★ No. of theoretical plates depends upon the column length (L) & height of theoretical plate (H)

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

N → no. of theoretical plates; L → length of column; H → height of theoretical plate

★ By decreasing the heigth of theoretical plate of fixed ~~increasing~~ length of column → no. of theoretical plates can be increased thus efficiency of column can be increased

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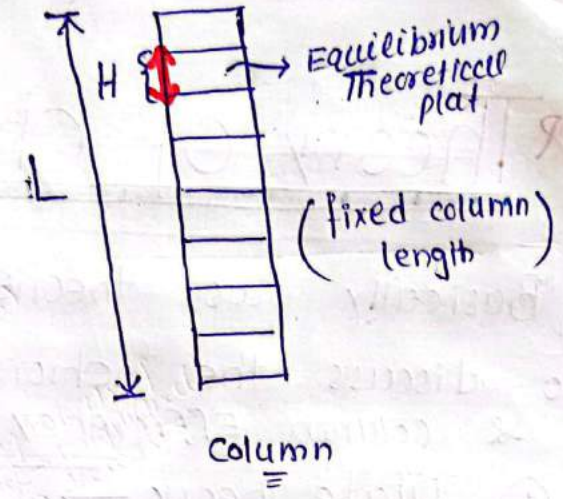
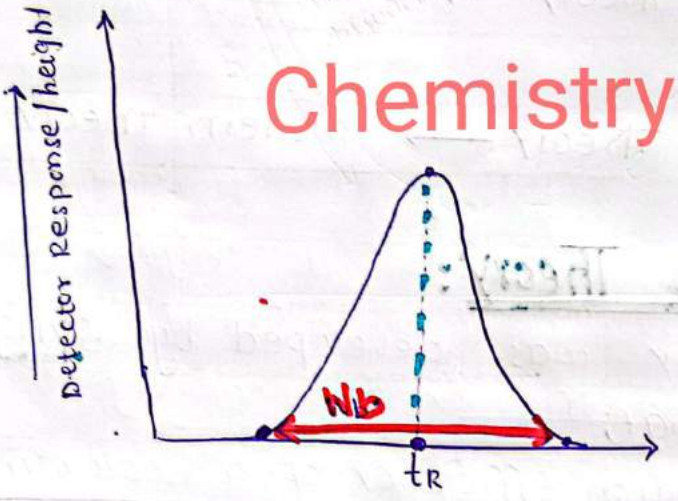
$$N = \frac{L}{H}$$

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

t_R → Retention time

W_b → Base line of peak





2) Rate Theory: (Flow of velocity)

Van Deemter, developed the famous Equation to describe the efficiency of column known as Van-Deemter Equation

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

- H → HETP Height Equivalent to theoretical plates
- A → Eddy diffusion → multiple path diffusion
- B → longitudnal diffusion
- u → Flow rate
- C → mass transfer

$\therefore$ H ↓ $\Rightarrow$ no. of theoretical plates ↑ $\Rightarrow$ column efficiency ↑

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$A = 2\lambda d_p$ → Average particle size

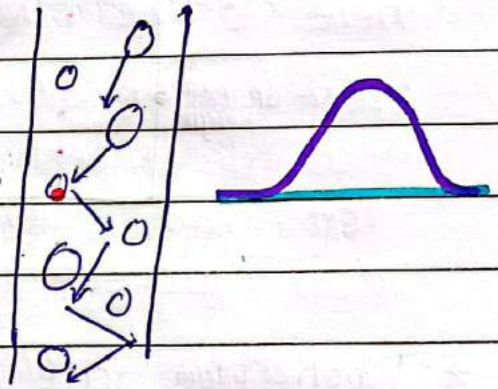
packing factor

① **A → term:**

↳ This is called the Eddy diffusion which shows the diffusion of multiple paths.

∴ value of A ↑ multiple paths ↑ Broad peak ↑

∴ If multiple paths, then Analyte spent more time in the column thus broadening of peak occur.



* A → term depends upon two factors

(i) particle size ↓ A term can be ignored

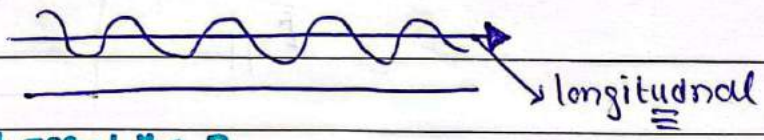
(ii) type of Geometry of packing

* A term does not depend upon the flow rate (velocity)

② **B → term:**

↳ Shows the Longitudnal diffusion (axial/ molecular

Analyte diffuses out from centre to the edges-



* particle size does not Effect the B-

③ **C → term:**

↳ Shows the mass transfer. shows the movement of Analyte from one end of column to the last end.

∴ flow rate ↑ of m.p C ↑ (mass transfer)

∴ m-p interaction with s-phase ↓ C ↑

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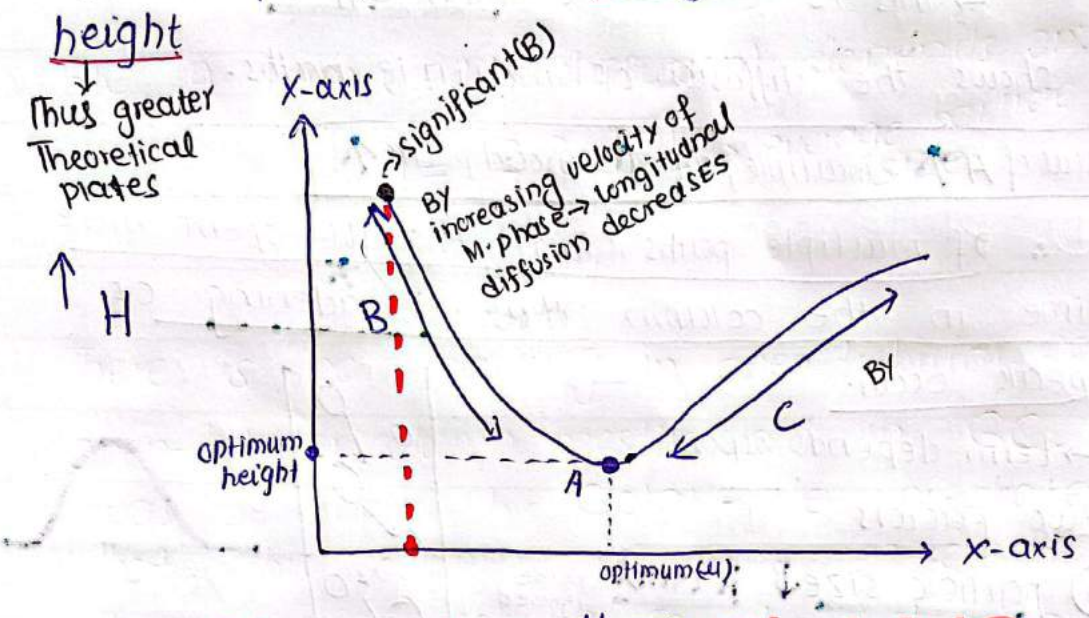
④ Flow rate : (u)

Flow Rate affect the values of A, B & C

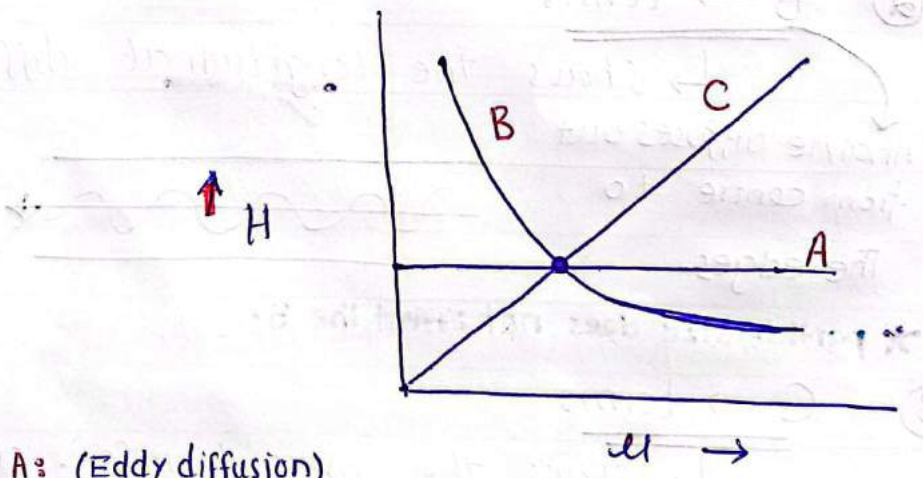
- Flow Rate should be control at some extent optimum point, where we get the optimum height

↓
Thus greater Theoretical plates



* When Flow rate is more than optimum, slower will be separation & lower will be efficiency

★ Effect of Flow Rate on A, B & C :



- Effect on A: (Eddy diffusion)
  * By increasing (or decreasing) flow rate, due to multiple diffusion, height does not change / no effect on height of theoretical plates-

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* Effect on B: (Longitudnal diffusion) / molecular diffusi

→ If the velocity of M.phase is high then Analyte spends less time with S.phase, thus decreases the Effects of longitudnal diffusion

- By increasing Flow rate → height decreases and then become constant
- At low velocity → molecular diffusion become Significant and height increases.

* Effect on C: (mass transfer)

* As Flow Rate increases → thus mass transfer increases, thus height increases which cause the decreases of no. of theoretical plates so it is necessary to optimize the height of theoretical plates.

✓ **Van- Deemter Equation:** (USED For packed Column)

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

✓ **Golay Equation:** → (when open tubular column OR capillary column)

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

→ A term is Neglected.

* There is no Eddy Diffusion in open-tubular column, so here A term is not present. Thus van-deemter Equation is converted into Golay Equation.

* BIC A term (Eddy diffusion) depends on;

$$A = 2\lambda d_p$$

λ → packing Factor

d_p → Average particle size

* There is no packing in open-tubular column.

* Open-tubular column mostly used in GC. It was developed by Golay. He predicted that, this column is more efficient than packed column. So, he developed Equation called- Golay Equation

So,

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

↳ Golay Equation.

Packing OF Column:

(*) In Conventional Column (packed column) here we can packed the stationary phase easily by tapping bic its diameter is large.

(*) In Capillary column → we can not pack our stationary phase by tapping bic its diameter is very small and its length is very large.

- Here we use the packing device called column packing bomb device
- using this technique → we make the monolith of st.phase (polymer)

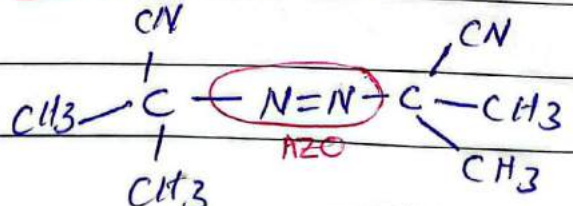
Q= How to make Monolith?

Monomers + initiator + porogen
↓ forms pores in monomers

* porogen → H_2O is used as a porogen For Monolith

* initiator → Free Radical initiator (AIBN)

Azobis iso butyro nitrile



(AIBN)

Name

Instrumentation of HPLC | UPLC

Necessary parts

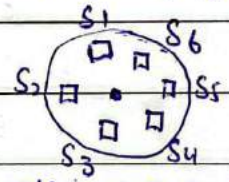
① columns: → Analytical column → Separation column ↓ more length ≈ 25cm
→ Guard column → small length → 1-2 cm
↳ optional
↳ used before Analytical column
↳ too much expensive
↳ prevent impurities

② Mobile phase Reservior: → storage of solvent

③ pressure pump:
↳ piston pump → push the solvent
↳ transfer the m.phase

④ loop injector:
↳ sample is injected.

⑤ Hexaport valve: → provide lines among Samples
↳ optional
↳ Sample selection through this valve.



↳ At a time 6 → samples can be Analyzed.

⑥ Back pressure Regulator:
↳ Regulate the back pressure.
• Generally used in UPLC bic highly pump pressure is given thus more back pressure occcur.

⑦ Auto sampler: (optional)
Generally use for UPLC ↓ bic U. small Amount of Samples
↳ itself take & inject Samples. → expensive
It is just a transfer tray, different positions are named • 1—10
• 11—20
• 21—30
→ Sampler pick the Sample and transfer
↓ If there is no sample in compart
↳ Autosampler move forward and choose another Sample

(8) pulse damper: → to remove the Air bubbles

↳ when we use the pump → pulses create which produces Air bubbles ↔ disturb the base line of peak so we have to use the pulse damper.

(9) Detectors: → Detect ~~the~~ Analytical Signal

↳ LC-UV detector is used best.

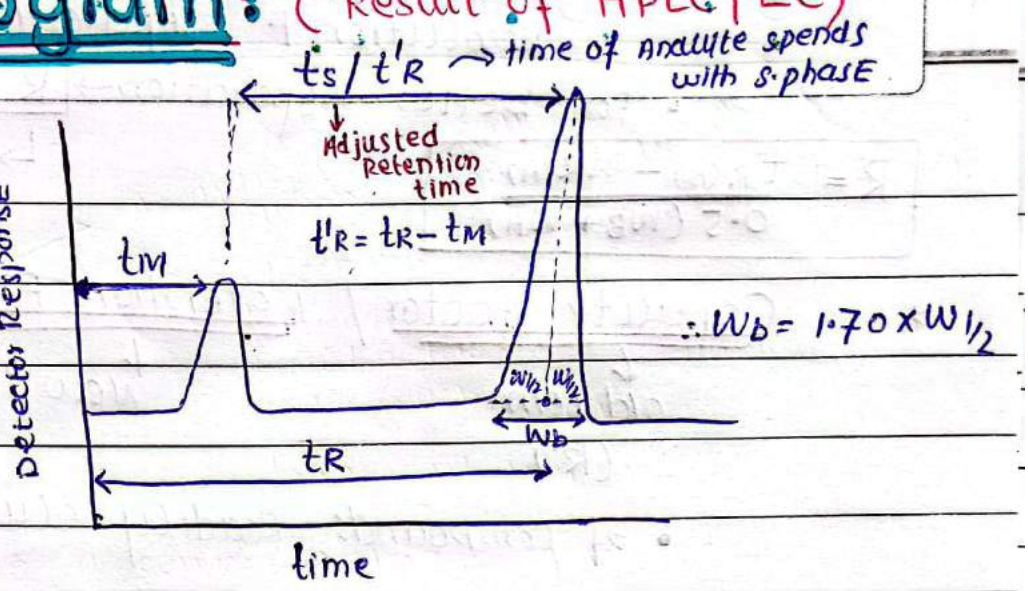
- we can Also use more than one detectors to increase the sensitivity.

(10) Vacuum Degassing:

↓ this is used in HPLC → bic here we do not need of gas so it is necessary to remove ~~the~~ gas bubbles by vacuum degasser.

- ✓ characteristics OF HPLC Detector
  - Sensitivity ↑
  - selectivity ↑
  - show linear Response
  - Qualitative information
  - Ease OF use
  - Universality

Chromatogram: (Result of HPLC | LC)



★ **Base line:**
↳ peak width → indication of good separation.

★ **injection time:**
zero time is the injection time

★ **Dead time:** (t_m) → void time
The time of no interaction of M-phase from injecting to ejecting. This cause ~~ww~~ jerk → which shows the only m-phase passed without interaction

★ **Retention time:** (t_R)
time of Analyte from injecting to ejecting

$$t_R = t_m + t_s$$

t_m ↓ m-phase; t_s ↓ Analyte with St-phase

. At this time peak maxima occur

★ peak height → good for Separation →

★ peak width → not good for Separation

★ Resolution:

How to peak Resolute / separated

- Resolution ↑ : separation good
- For better separation → $R > 1.5$ ↳ Rule of thumb

$$R = \frac{t_{R(B)} - t_{R(A)}}{0.5\,(W_B + W_A)}$$

★ Capacity factor / Retention factor:

Capacity factor (old term) (k') / Retention factor (New term) (R) — measure of Retention time

- If compounds Readily eluted → capacity Factor not good

$$k = \frac{\text{time of solute spends with s-phase}}{\text{time of solute spends with m-phase}} = \frac{t'R}{t_M} = \frac{t_R - t_M}{t_M}$$

★ Separation Factor / Selectivity factor (α)

→ Ratio of Retention factors of two Analytes

$$\alpha = \frac{k_2}{k_1} = \frac{t_{R_2} - t_m}{t_{R_1} - t_m}$$

- For better separation → $\alpha > 1$

★ No. of Theoretical plates:

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

OR

$$\therefore N = 5.545\left(\frac{t_R}{W_{h/2}}\right)^2$$

OR

$$\therefore N = \left(\frac{t_R}{\sigma}\right)^2$$

σ = standard deviation

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

* Effective plate Number (Neff)

$$N_{effective} = N\left(\frac{k}{k+1}\right)^2$$

∴ k = Retention Factor

* peak Capacity: (n_c)

↳ estimate how much amount of solutes can be resolved ~~separated~~ on a particular column.

$$n_c = 1 + \frac{\sqrt{N}}{4} \ln \frac{V_{max}}{V_{min}}$$

V_{max} → max. volume of M. phase which eluted solute

V_{min} → Min. volume of M. phase which eluted M. phase

Good Luck