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# Semester VI (CHB-601)

# Separation of molecules by chromatographic techniques

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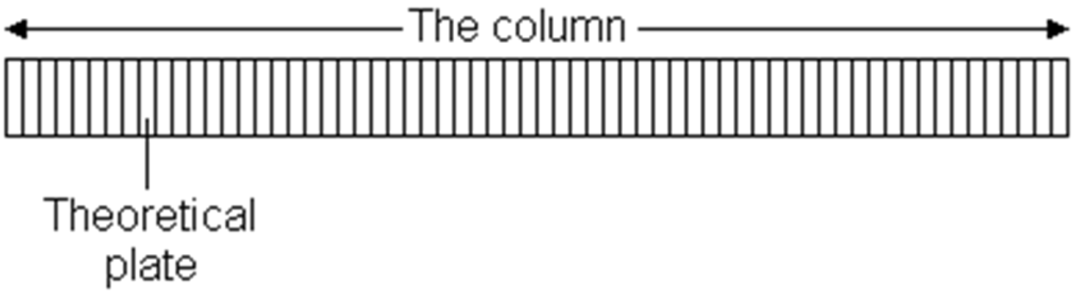
# Plate theory

- For a physical model, Martin & Synge utilized the theoretical plate approach which had been so successful in the elucidation of distillation column processes.
- A chromatographic column is subdivided into individual contact units (hypothetical plates).
- Each unit (plate) contains an amount of mobile phase ($\Delta V_M$) and of stationary phase ($\Delta V_S$).
- In the entire column, there are N of these units and because a complete stationary phase-mobile phase equilibration occurs in each, they are called theoretical plates.



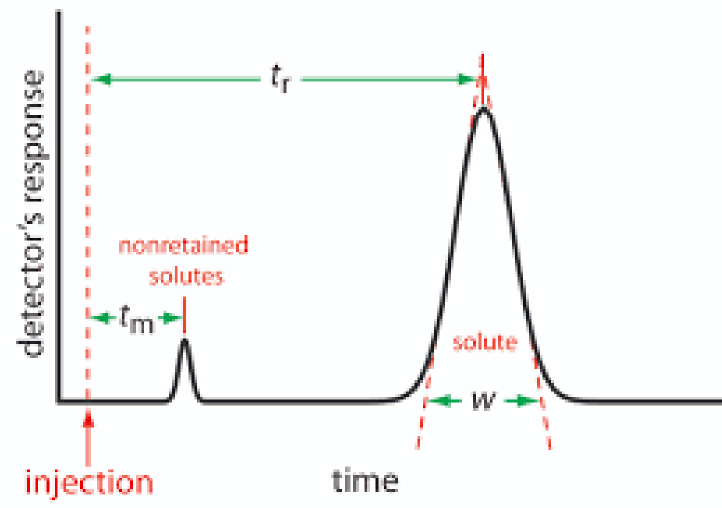
# Plate theory

- A sample is added to the column and enters the first theoretical plate.
- A fractional amount *p* remains in the mobile phase while a fractional amount *q* enters the stationary phase.
- After equilibration is complete in this plate, a small volume ($\Delta V_M$) of mobile phase is added to the column and the amount *p* is transferred to the second plate.
- As the process continues, a pattern of solute distribution develops and in general, the fractional amount of solute in any plate *r* after the addition of *n* volume increments ($\Delta V_M$)of the mobile phase is

$$F_{r,n} = [n!/(n-r)!r!]\, p^r q^{n-r}$$

# Relationship between N and exptal variables

- After various approximations and theoretical treatments, $N=16(t_r/t_w)^2$ (1)
- $t_w = 4\tau$ $\tau$ is standard deviation
- $N = (t_R/\tau)^2$ (2)



- Eq 1-2 furnish a way in which separation efficiency can be very easily determined from the basic exptal data.
- In addition, the direct relationship between peak width and retention time is revealed.

# Efficiency and theoretical plate

- Efficiency of any column is best judged by *H,* the height equivalent to a theoretical plate:

$H = L/N$ L-length of chromatography column

By substitution:

$$H = \tau^2 L / t_R^2$$

Exptal variables can be exploited to minimize H, maximize N and enhancing efficiency of column towards chromatographic separations.

# Limitations of plate theory

Incorrect assumptions

- Partition coefficient (K) is constant independent of solute conc in stationary and mobile phase
- Equilibration is rapid
- Diffusion (especially longitudinal) does not occur
- Column consists of a no of discrete volume elements and
- Moblie phase is added in $\Delta V_M$ increments rather than continuously.

In conclusion, mobile phase velocity has an important effect.

# Rate theory of band broadening

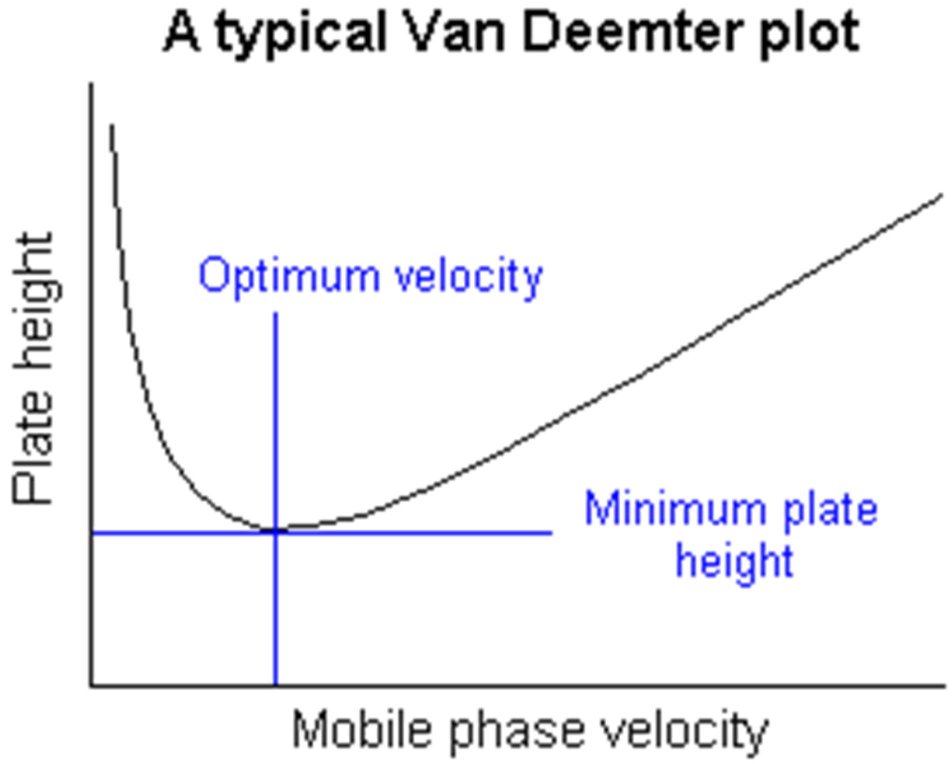
- Dutch chemists van Deemter, Klinkenberg and Zuiderweg in 1956 proposed rate theory of chromatography.
- Efficiency of chromatographic columns at low flow velocities can be approximated by the relation

  $H = B/u + C_S u + C_M u$ $C_S$ and $C_M$ are mass-transfer coefficients for the stationary and mobile phase, respectively.
- At high flow velocities,

  $H = A + B/u + C_s u$

  A,B, C- coefficients of multiple path effects, longitudinal diffusion and mass transfer respectively

# Relation between HETP and mobile phase velocity



# Band broadening

- Why do bands spread?
  - Eddy diffusion
  - Mobile phase mass transfer
  - Stagnant mobile phase mass transfer
  - Stationary phase mass transfer

- Band broadening reflects a loss of column efficiency.
- Slower the rate of mass transfer processes occurring while a solute migrates through a column, broader is the band.

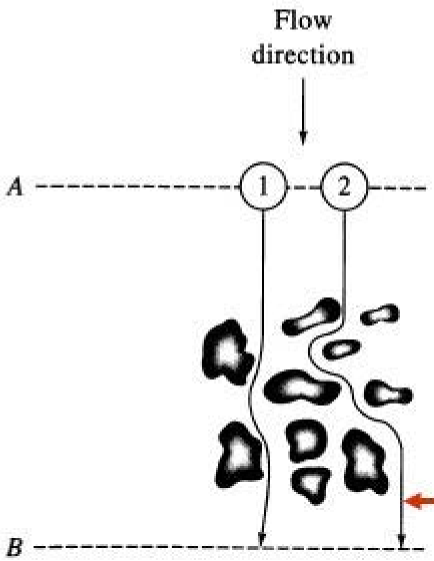
# Variables affecting column efficiency

- Mass transfer effects are the result of the finite time required for solute molecules to move from the mobile phase into the stationary phase and back again.
- Some of the variables that affect mass transfer rates caan be exploited to improve separations:
  - Linear velocity of mobile phase
  - Diffusion coefficient of analyte in mobile phase
  - Diffusion coefficient of analyte in stationary phase
  - Retention factor
  - Diameter of packing particles
  - Thickness of liquid coating on stationary phase

# Eddy diffusion in column

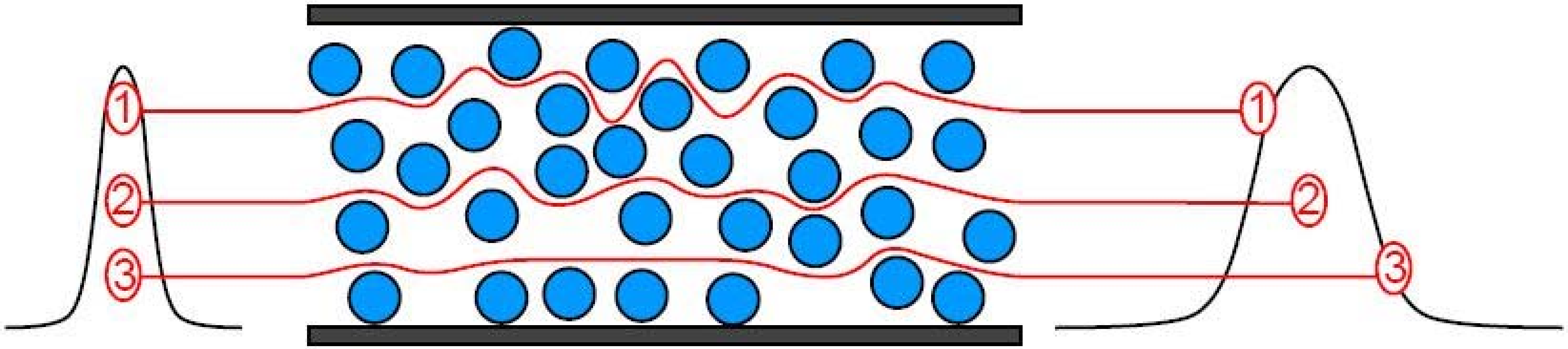
- Zone broadening in the mobile phase is due in part to the multitude of pathways by which a molecule (ion) makes its way through a packed column.
- Length of these pathways can differ significantly.
- Solute molecules reach the end of the column over a range of times leading to a broadened band.
- Multiple path effect, eddy diffusion is independent of mobile phase velocity

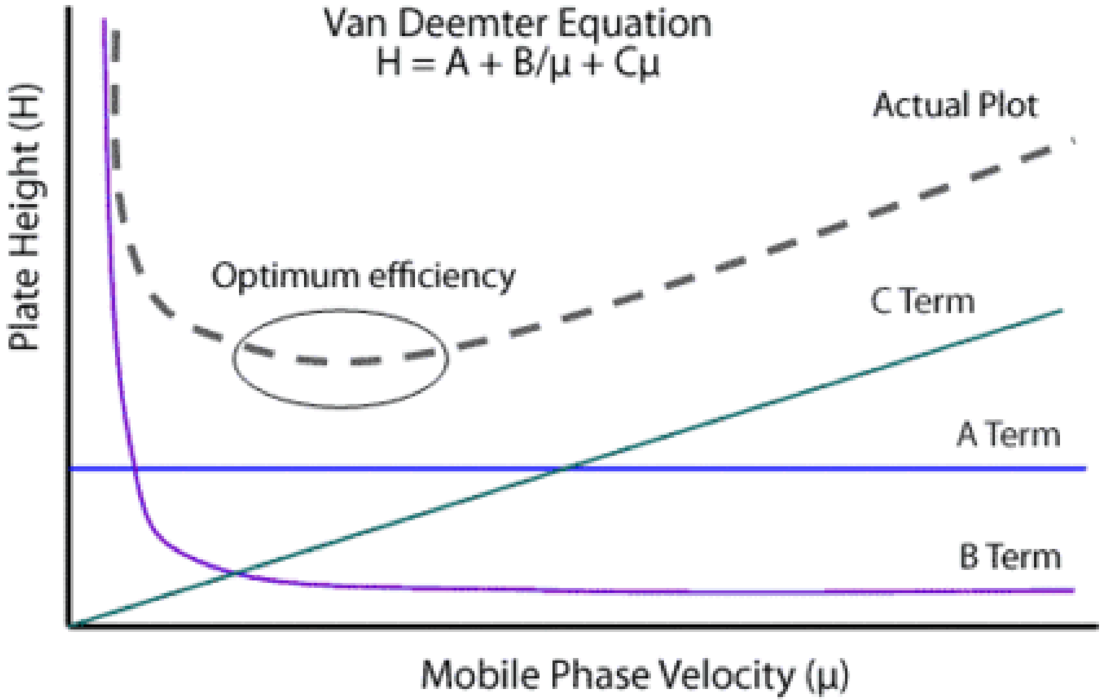
a.) *Eddy diffusion* – a process that leads to peak (band) broadening due to the presence of multiple flow paths through a packed column.



As solute molecules travel through the column, some arrive at the end sooner then others simply due to the different path traveled around the support particles in the column that result in different travel distances.

Longer path arrives at end of column after (1).




Van Deemter Equation
H = A + B/μ + Cμ
Actual Plot
Optimum efficiency
C Term
A Term
B Term
Plate Height (H)
Mobile Phase Velocity (μ)