

9/1/23
steps after
harvesting
the cells

→ Downstream Processing

90% of the time

→ we disrupt
the cells

→ we get many
macromolecules

- proteins
- nucleic acids
- polysaccharides
- glycoproteins, etc.

Purification of HSA

P. pastoris
(yeast)

eukaryotic
protein

Problems of large scale recombinant protein production

- ① folding, aggregation & solubility
- ② Loss of expression → cells do not produce the recombinant protein any more
- ③ Post-translational processing
- ④ Proteolytic processing

glycosylation
(e.g.: t-PA)

after prodⁿ
↓
degradⁿ
of proteins

metabolic load

cells get rid
of it

non-recombi.
orient, grow,
copy no.
fast

- ① Plasmid loss
- ② Chromosomal instability problems

↓
heterogeneous plasmid population

high copy no.
no plasmids

they still
grow in
antibiotic
medium

↓
genetic mutations

Stages in bioseparation:

• Removal of insolubles

• Product isolation → sep. from many other components

• Product Purification

or getting out
from somewhere

• Product polishing/
preparation

increasing conc. &
quality of product

- ① Cell disruption
- ② Cell removal → filtr², centrifug², coagul², floccul²
- ③ Primary isolation → packed bed, expanded bed
- ④ Purification → chromatography, ultrafiltration
- ⑤ Product preparation → ultrafiltr².

Filtration:

Dead-end filtration

may get clogged;
the retentate have
to be removed
from time to time

Tangential flow filtration

continuous process where
sample flows tangentially
over a membrane

↓
retentate gets automatically
washed away.

→ Rotary drum filtration

Centrifugation:

centrifugal force → measured as the relative centrifugal force (RCF) (or) (G)

↓
irrespective of the size/ diameter of centrifuge

unlike RPM which produced varied centrifugal forces, in centrifuges of different sizes

Svedberg's coefficient (S)

helps to separate using centrifuge

they do not add up { 60S + 40S → 80S
30S + 50S → 70S }

Precipitation

salt ppt^c → salting out / salting in

Adsorption:

Freundlich isotherm

Langmuir isotherm

Chromatographic Techniques:

- ✓ FPLC - first protein purification liquid chromatography.
- ✓ by gravity → takes a long time
- ✓ by pump/suction → shorter time

peristaltic pumps

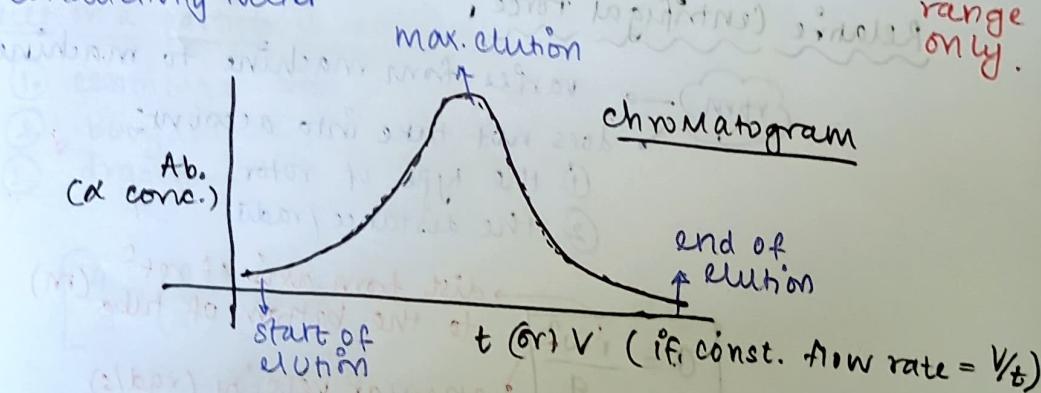
we can control the flow rate

- ✓ high pressure → HPLC - high performance LC

- ✓ even ↑ pressure → UHPLC - ultra high pressure LC

↑ stainless steel, alloy columns → can withstand high pressures

- Peristaltic pump → gentle squeezing action
- UV Detector → absorbance at 280 nm (proteins) → UV range only.
- Conductivity Meter



Column Chromatography:

(membrane proteins are hydrophobic)

- ① Size exclusion / Gel filtration → acc. to size/mol wt.
- ② Ion exchange (IEC) → separates by charge
- ③ Affinity chromatography → specific binding
- ④ Hydrophobic interactions e.g.: Ni²⁺-NTA - Histidine Antigen-antibody.

↳ by hydrophobic/hydrophilic characteristics

only when affinity const. is very high
Kd in nanomolar or picomolar range

✓ DNA-binding proteins → high PI → remain +ve charged ($n > 10$) in physiological pH

16/1/QA

Centrifugation

came from separating the cellular organelles → by applying differential centrifugal speeds

Laboratory centrifuges.

- Rotor → has a certain degree of inclination
- Axis

free to change
swing-bucket rotor

max. dist. the particle can travel varies

cannot be changed

fixed angle rotor

migrate to a fixed distance

- ✓ small-scale separn

✓ operate within G values ranging b/w 1000 - 20,000~

Relative Centrifugal Force,

rpm → varies from machine to machine

does not take into account:

- ① the type of rotor
- ② the distance/radū

$$\alpha = \frac{rw^2}{g}$$

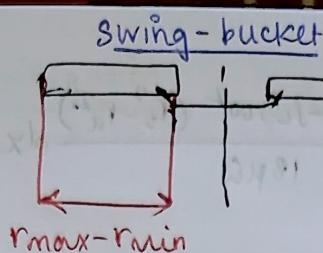
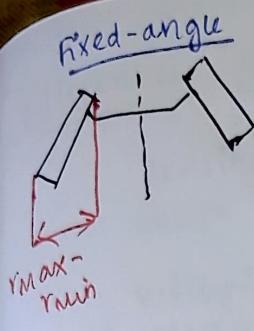
dist. from axis of rot² to the bottom of tube (m)
angular velocity (rad/s)

accelⁿ. due to gravity

$$w = 2\pi n$$

rot^b speed (rpm)

$$G = \frac{r(2\pi n)^2}{g}$$



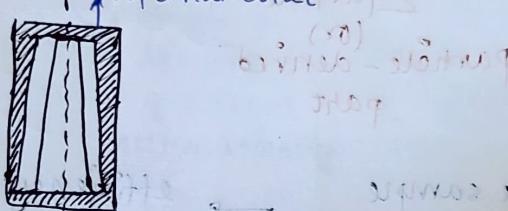
centrifuge factor

$$K = 2.53 \times 10^{11} \left(\frac{\ln(r_{\max} - r_{\min})}{\text{rpm}_{\max}^2} \right)$$

↳ helps to calculate time of centrifuging

Preparative Centrifuges

Tubular Bowl Centrifuge → continuous type → we do not have to stop it to remove the cake/pellet



feed → axial
movement of particle → radial

→ never have to stop it to remove the cake/pellet
at a particular volumetric flow rate

assumpⁿ: spherical particle

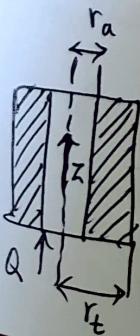
forces on a particle in centrifuging:

- ① centrifugal force → one dirⁿ
- ② buoyant force } → other dirⁿ
- ③ drag force

$$\left[\frac{4}{3} \pi \left(\frac{d}{2} \right)^3 (\rho_s - \rho_w) \right] r w^2 = 6 \pi \mu \left(\frac{d}{2} \right) v$$

centrifugal buoyant drag

$$v = \frac{d^2 (\rho_s - \rho_w) r w^2}{18 \mu} \rightarrow \text{velocity in the radial direction}$$



annular region → perfect cylinder (assumpⁿ, not really)

$$\frac{dz}{dt} = \frac{Q}{\pi (r_o^2 - r_i^2)}$$

$$\int_{r_a}^{r_t} \frac{dr}{r} = \int_0^x \frac{\pi d^2 (\rho_s - \rho_L) w^2 (r_t^2 - r_a^2)}{18 \mu Q} dx$$

(or) $Q = \left[\frac{d^2 (\rho_s - \rho_L)}{18 \mu} \right] \left[\frac{\pi (r_t^2 - r_a^2) w^2}{\ln(r_t/r_a)} \right]$

$$\frac{Q}{\pi} = \left(\frac{d^2 (\rho_s - \rho_L) g}{18 \mu} \right) \frac{1}{g} \left[\frac{(r_t^2 - r_a^2) w^2}{\ln(r_t/r_a)} \right]$$

terminal
velocity
(v_T)

\downarrow
particle

particle-derived
part

Σ machine

(or)
machine-derived
part

same sample
at same feed rate \rightarrow efficiency of
the centrifuge

Σ machine
determined in
preparative/
continuous centrifuge

Disk-Stack Centrifuge:

- 1 Disk CVD + fixed impurities
- 2 Disk CVD + fixed impurities
- 3 Disk CVD + fixed impurities

$$\left(\frac{1}{n} \right) \pi D^2 = \omega r \left[\left(\frac{r_b - r_a}{2} \right)^2 \left(\frac{D}{2} \right) \pi \frac{H}{S} \right]$$

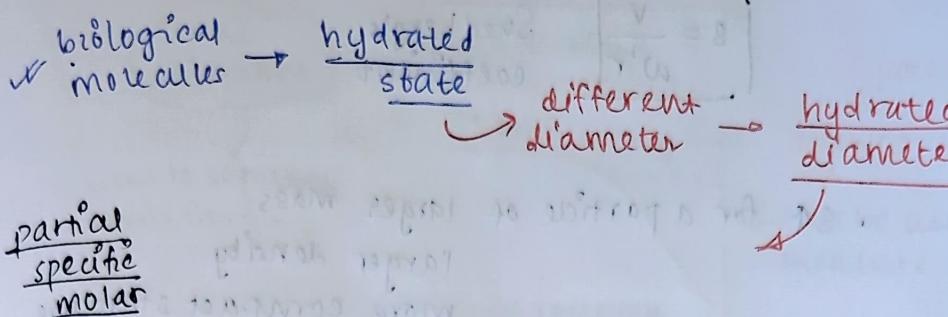
$$\text{mass of disk} = \frac{\omega r (r_b - r_a) H}{4 \pi S} = v$$



$$\frac{D}{(r_b - r_a) \pi} = \frac{v}{H}$$

Differential Centrifugation:

density
shape



ESCI density gradient

salt of a heavy metal

↳ forms a density gradient automatically

Latex particles

→ differential sedimentation

→ depend of. particle mass & size

→ crude method for fractionation of subcellular organelles.

centrifuging at diff. speeds

Svedberg Coefficient:

$$F_c = mw^2 r$$

$$F_B = -m_{\text{disp}} w^2 r$$

$$F_f = -fv$$

$$F_c + F_B + F_f = 0 \quad \text{challenge is to find this}$$

$$mw^2 r - m_{\text{disp}} w^2 r - fv = 0$$

$$\text{partial specific } = \left(\frac{\partial v_i}{\partial m_i} \right)_{T, P}$$

$$mw^2 r (1 - \bar{v}_{\text{psol}}) - fv = 0$$

$$-fv = -mw^2 r (1 - \bar{v}_{\text{psol}})$$

$$\frac{V}{w^2 r} = \frac{m(1 - \bar{V}_{psol})}{f}$$

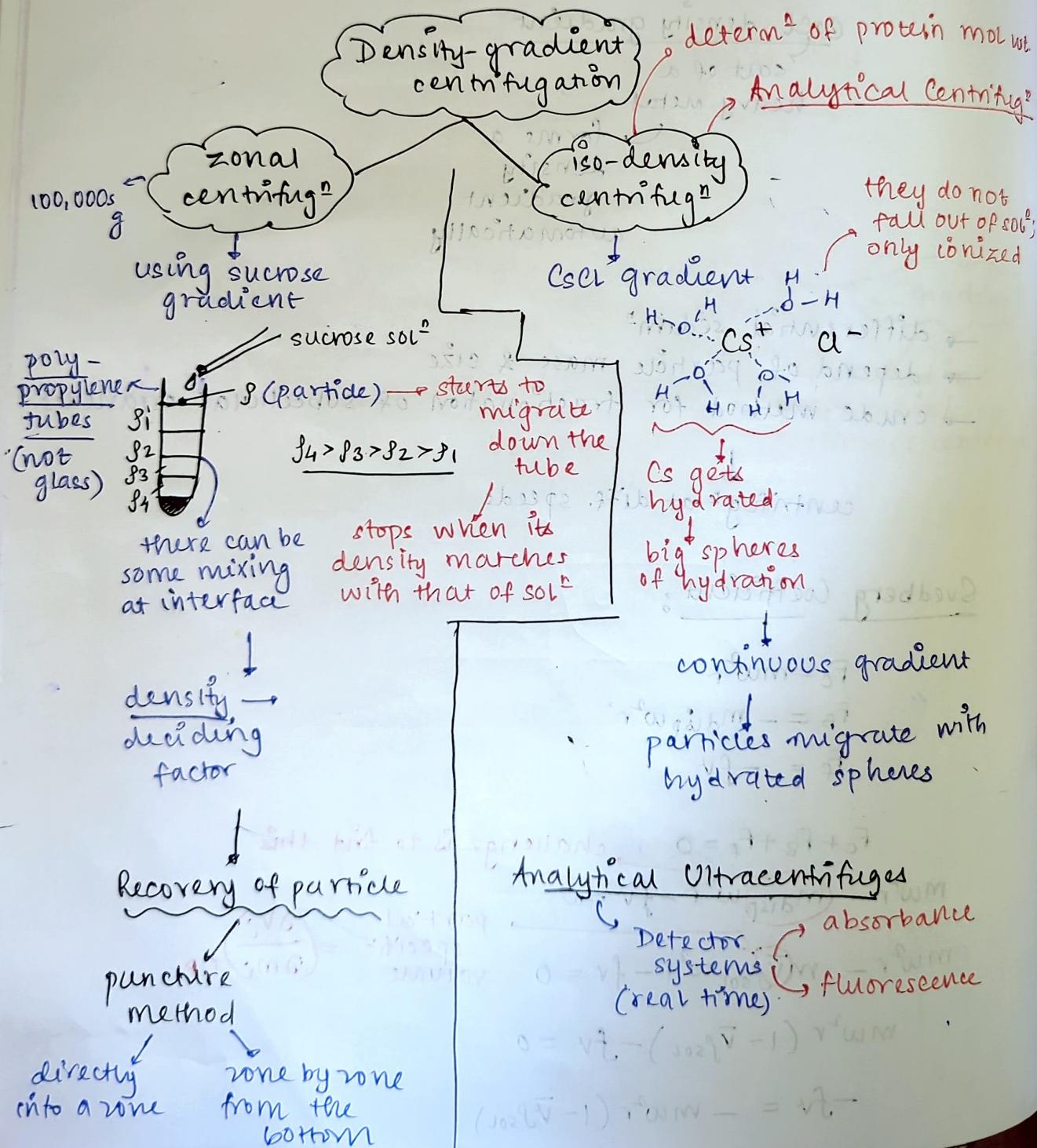
partial specific volume = $\frac{1}{\text{Sparticular}}$

$$m_{\text{disp}} = m \bar{V}_{psol}$$

$$s = \frac{V}{w^2 r}$$

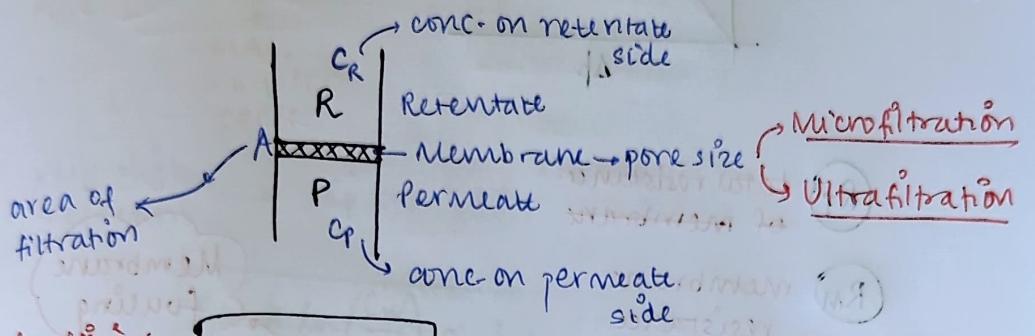
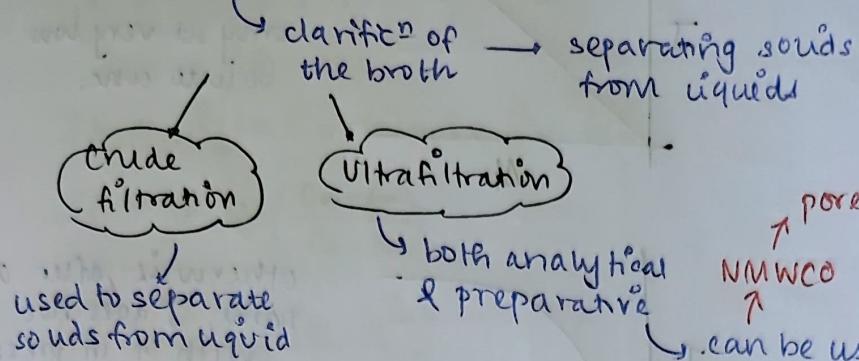
Svedberg's coefficient

- $s \uparrow$ for a particle of larger mass
larger density
more compact structure



Filtration

23/10/24



Coefficients of retention

$$R = \frac{C_R - C_p}{C_R}$$

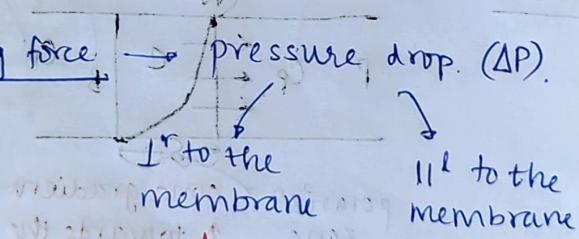
$$\therefore R \in [0, 1]$$

$$J = \frac{V}{A \cdot t}$$

volume
C.S. area
time

no reflection
equilibrium → everything is permeable
 $C_R = C_p$ (or) $R = 0$
 $C_p = 0$ (or) $R = 1$

main driving force → pressure drop. (ΔP)



Dead-end filtration

Tangential flow filtr² (FFF)

Hagen-Poiseuille Eqn.

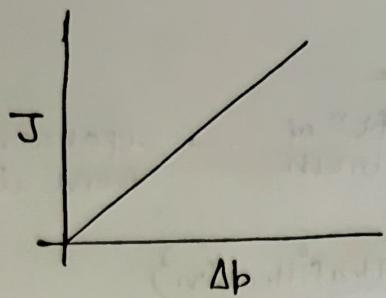
$$J = \frac{\Delta p / d^2 \varepsilon}{32 \times \mu_p}$$

particle diameter
porosity

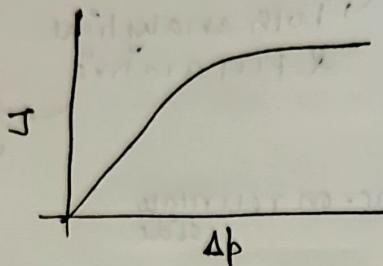
constant usually



$$\Rightarrow J \propto \Delta p$$



only at very low
solute conc.



otherwise, after a certain
point, J does not increase
with \uparrow in pressure.

(R_T)

total resistance
of membrane

(R_M)

membrane
resistance

also a resistance
due to the \uparrow conc.
of solute

(R_C)

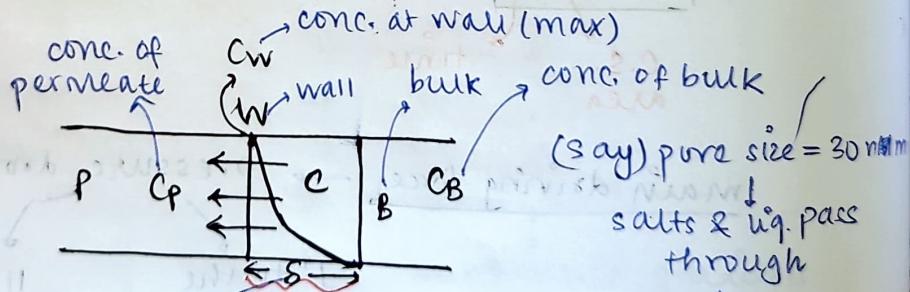
concentration
resistance

Membrane
fouling

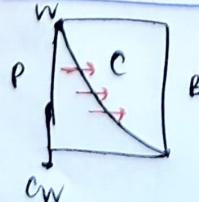
or due to the
deposition/cake
formation by
solid particles
on the memb.

choke & block
the memb.

concentration
polarization



polarizⁿ zone
conc. gradient
↑ towards the
membrane
chance of diff.
back diffusion



$$J_C - J_C^P - D \frac{dC}{dy} = 0$$

diffusivity const.
indep. of
conc. change

$$\int_0^S dy = D \int \frac{dc}{C_w - C_p}$$

$$JS = D \left[\ln(C_w - C_p) - \ln(C_B - C_p) \right]$$

$$JS = D \ln \left(\frac{C_w - C_p}{C_B - C_p} \right)$$

flux \downarrow
 mass transfer coeff.
 $J = \frac{D}{\delta} \ln \left(\frac{C_w - C_p}{C_B - C_p} \right)$

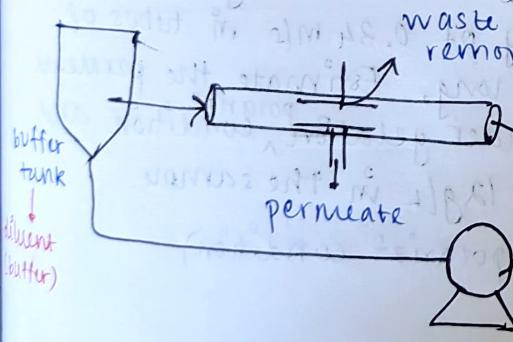
very high rejection $\rightarrow C_p \approx 0$ (or) $C_w - C_p \approx C_w$
 $C_B - C_p \approx C_B$

$$J = k \ln \left(\frac{C_w}{C_B} \right) \approx k \ln \left(\frac{C_a}{C_B} \right)$$

useful in selecting filtration memb.

gelation conc.
 conc at which solute starts to form a gel-like layer on memb.

Tangential flow UF \rightarrow post CG, ppt starts & Aitrn cannot proceed



Disadvantage
 Higher surf area is required as ΔP is not much

Advantage
 No membrane fouling.

$$Sh = M Re^\alpha Sc^\beta \left(\frac{dh}{L} \right)^w$$

Schmidt Number → hydraulic diameter
 $M, \alpha, \beta, w \rightarrow$ process constants

Sherwood number
contain mass transfer coeff. info.

Reynold's number

length of flow channel

hydraulic diameter

$$Re = \frac{dh u_p}{\mu}$$

velocity
viscosity

mass transfer coeff.

$$Sh = \frac{K dh}{D}$$

$$Sc = \frac{\mu}{SD}$$

$$dh = \frac{4A}{P_w}$$

cross-section area
wetted perimeter

$Re > 4000$: Turbulent

$2000 < Re < 4000$: Transient

$Re < 2000$: Laminar

- Q. A clarified fermentation broth contain a polysaccharide product which shows a gelatin conc. of 25 g/L. The fluid density is 1020 kg/m^3 . The viscosity is 1.8 cP. $D = 5.6 \times 10^{-11} \text{ m}^2/\text{s}$. Product is recovered by UF from the broth at a fluid velocity of 0.34 m/s in tubes of 2.4 cm diameter and 2m long. Estimate the permeate flux and if the filter is under gelation condition and the polysaccharide conc is 12 g/L in the sample.

$$C_G = 25 \text{ g/L} = C_W \text{ (gel polarization condition)}$$

$$\rho = 1020 \text{ kg/m}^3$$

$$\mu = 1.8 \text{ cP}$$

$$D = 5.6 \times 10^{-11} \text{ m}^2/\text{s}$$

$$u = 0.34 \text{ m/s}$$

$$dh = 2.4 \text{ cm}$$

$$L = 2 \text{ m}$$

$$C_B = 12 \text{ g/L}$$

$$Re = \frac{d_h u_s}{\mu} = \frac{(2.4 \times 10^{-2}) m (0.34 m/s) (1020 \text{ kg/m}^3)}{1.8 \times 10^{-3} \text{ kg/m/s}}$$

$$Re \approx 4624$$

at turbulent flow condⁿ:

$$M = 0.023$$

$$\alpha = 0.89$$

$$\beta = 0.3$$

$$w = 0$$

$$Sc = \frac{\mu}{\rho \cdot D} = \frac{1.8 \times 10^{-3}}{(1020)(5.6 \times 10^{-11})} = 31512.6$$

$$Sh = (0.023) (4624)^{0.89} (Sc)^{0.5} = 940$$

$$Sh = \frac{k d_h}{\theta} \Rightarrow k = \frac{(Sh)(\theta)}{d_h} = \frac{(Sh)(5.6 \times 10^{-11})}{(2.4 \times 10^{-2})}$$

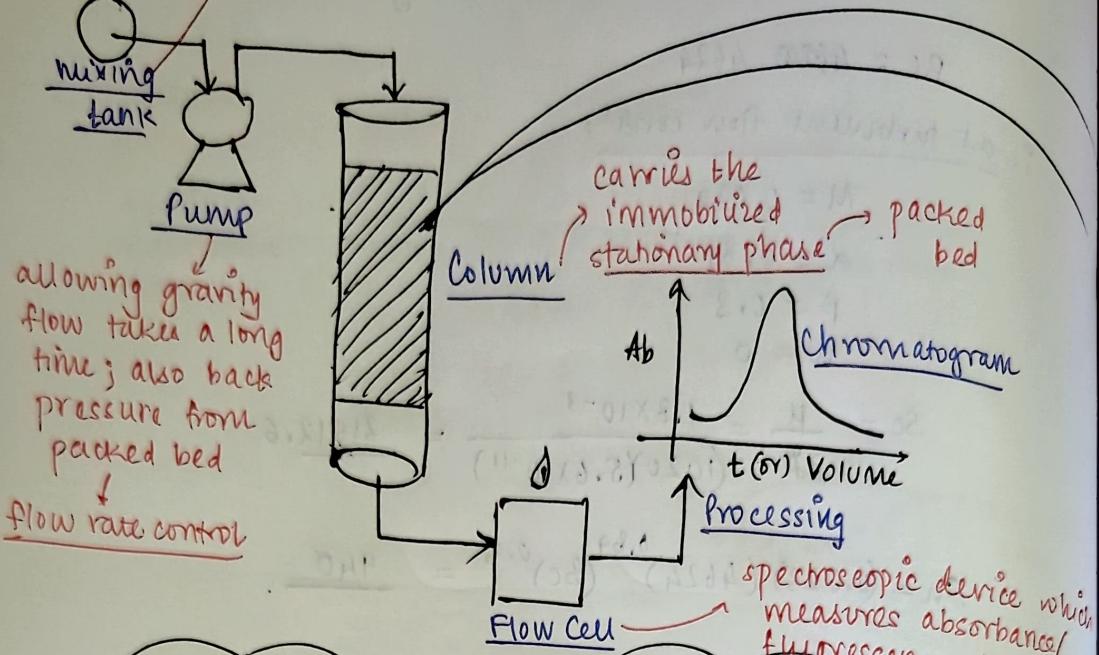
$$k = 2.19 \times 10^{-6}$$

$$J = k \ln \left(\frac{C_w}{C_B} \right) = k \ln \left(\frac{25 \text{ g/L}}{12 \text{ g/L}} \right) = 1.6 \times 10^{-6} \text{ m/s}$$

30/1/23

mixing of
sample +
buffer

Chromatography



Interactive chromatography

- affinity interaction
- hydrophobic
- electrostatic

Non-interactive chromatography

- Size-exclusion (path travelled by molecules)
- Get filtration chrom.

LC: Liquid Chromatography

FPLC

Fast Protein
Liq. Chrom.

HPLC

High pressure/
performance
Liq. Chrom.

Molecule of interest
in the mobile phase

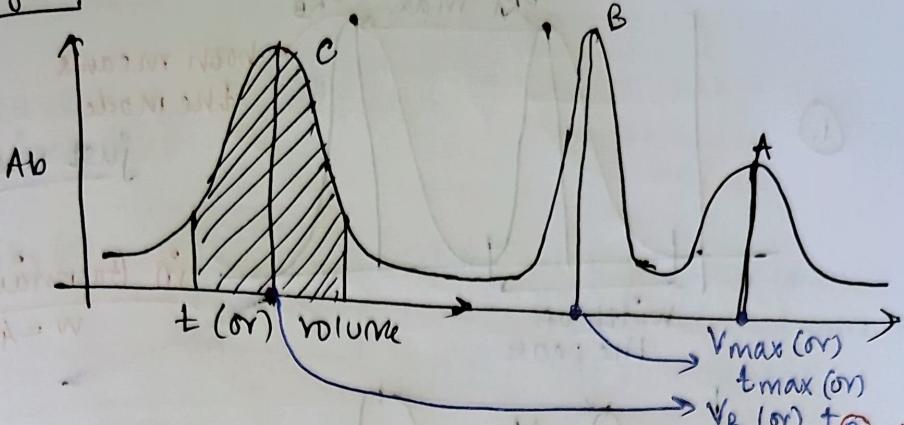
→ Reverse Phase LC

Elution Chromatography

→ separating the analyte b/w the MP & SP. Chosing the MP such that separation foll takes place

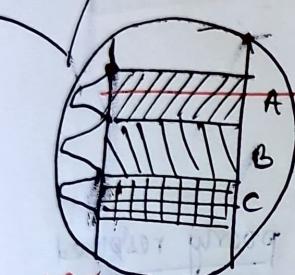
↓
change the property or characteristics of MP to allow elution.

chromatogram:



λ_{max} → λ at which absorption/emission is maximum.

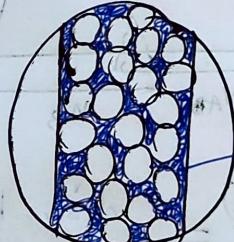
signifies how long the analyte is retained in the column



conc profiles

middle → conc. of the analyte is max. → absorbance is max.

Assumpⁿ: uniform beads



spaces b/w packing

void

void is always ~30% of total volume

$$\frac{\text{void}}{\text{vadage}} \leftarrow \text{voidage} = 0.3$$

time taken for the MP to travel the column

$$At - at = ?$$

Dead time

MP passes through

(tm) (or) dead void time.

volume that passes b/w the packings/stationary phase

void volume (V_m)

can be found experimentally

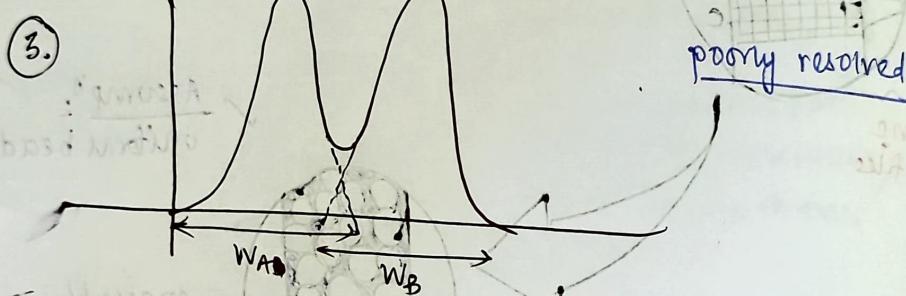
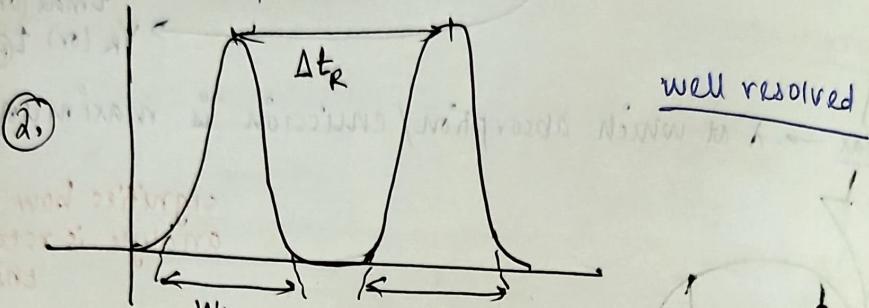
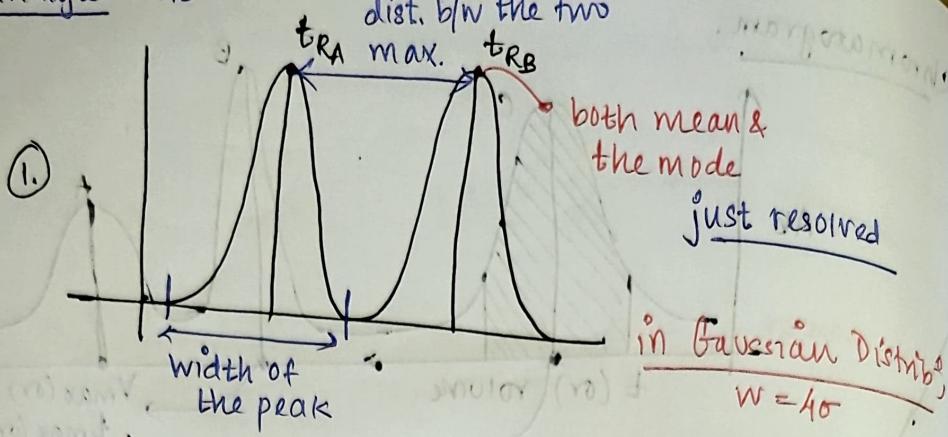
$$t_R' = t_R - t_m$$

adjusted
retⁿ time

(or)
adjusted
retⁿ volume

the actual time analyte spends in the stationary phase

2 analytes - A, B



Resolution (R) = dist. b/w the two maxima

$$\frac{1}{2}(\text{sum of the width of the two peaks})$$

$$R = \frac{t_{RB} - t_{RA}}{\frac{1}{2}(w_A + w_B)}$$

→ dimensionless

1. $R \Rightarrow t_{RB} - t_{RA} = \frac{1}{2}(w_A + w_B) \Rightarrow R = 1$ Baseline resolution

2. $R \Rightarrow t_{RB} - t_{RA} > \frac{1}{2}(w_A + w_B) \Rightarrow R > 1$ very good resolution

3. $R \Rightarrow t_{RB} - t_{RA} < \frac{1}{2}(w_A + w_B) \Rightarrow R < 1$ poor / bad resolution

Capacity factor (K):

how well an analyte is retained in SP

$$K = \frac{t_R - t_M}{t_M}$$

does not change for a particular system

Selectivity (α):

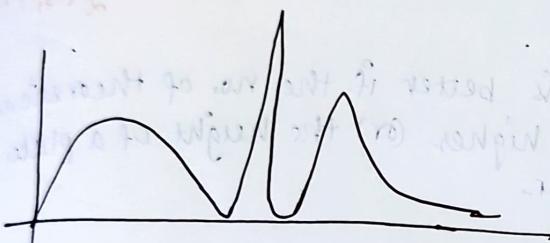
$$\alpha = \frac{K_B}{K_A}$$

$\alpha > 1$: B is retained more than A
 $\alpha < 1$: A is retained more than B
 $\alpha = 1$: equal retention

Info from chromatogram:

✓ how well column is packed

✓ how well analyte is getting separated



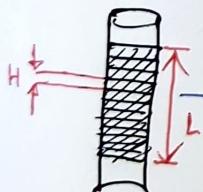
$$H \times R$$

Very often, we get peaks such as these.

for the same sample

under different conditions
and in different columns

Band broadening / Peak broadening:



we can divide the entire bed into numerous small plates

①

Plate theory of chromatography

Assumpⁿ

All plates are same

✓ \uparrow # of theoretical plates \rightarrow better resolution \rightarrow distribⁿ of analytes on theoretical plate

✓ \downarrow # of plates \rightarrow poor resolution

Longitudinal diffusion

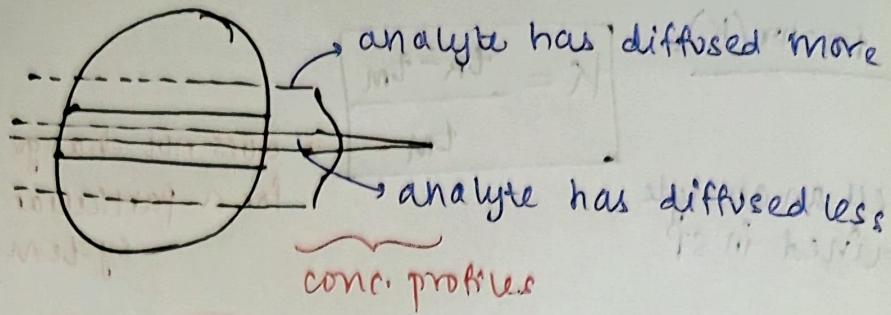
not considered
• flow rate of fluid

No. of theoretical plates,

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

$$N = 16 \frac{t_R^2}{W^2}$$

from chromatogram



② Rate theory of chromatography

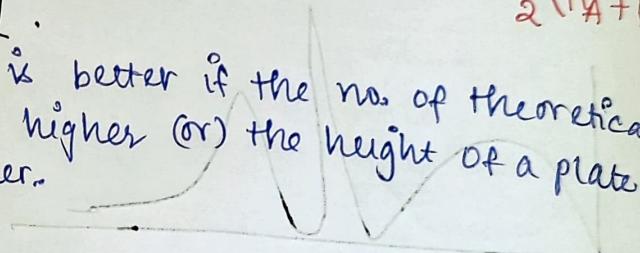
$$R = \frac{t_{RB} - t_{RA}}{\frac{1}{2}(w_A + w_B)} = \frac{\sqrt{N}}{4} \left(\frac{\alpha - 1}{\alpha} \right) \left(\frac{k_B}{1 + k_{av}} \right)$$

selectivity = $\frac{k_B}{k_{av}}$

$$\therefore R \propto \sqrt{N}$$

$$\frac{1}{2}(k_A + k_B)$$

Resolution is better if the no. of theoretical plates is higher (or) the height of a plate is smaller.



SW, NBP
more efficiency
300 ft vs 200

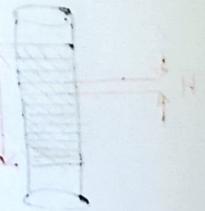
number of plates

efficiencies + efficiencies
increases in height in terms

private board 2009 | private board 2009

private board
private board for
private board

height has an
but with an
measure of
ability using



private board

private board