Separation techniques!

Separation ⟶ resolve the mixture into its components.

↘ resolve, characterize and estimate the component.

* Types of Separation Techniques:-

1. **Mechanical Methods** ⟶ based on size ⟶ Dialysis, Centrifugation, size exclusion chromatography.
2. **Physical Methods** ⟶ change in state ⟶ chromatographic technique, crystallization, filtration, sublⁿ
3. **Chemical method** ⟶ Precipitation, electrodeposition, etc.

Separation technique ⟶ Separating the component

↓ efficiency of separation

*Separation factor = Ratio of the parameter measured for the two solutes.

* Parameter is the crucial factor that controls the separation.

eg. Distillation ⟶ Boiling point.

glucose + urea :- $\beta = \frac{\text{B.P of glucose sol}^n}{\text{B.P of urea sol}^n}$.

eg. Solvent extraction :- $\beta = \frac{\text{distribution of solute in solvent 1}}{\text{distribution of solute in solvent 2}}$

1. Precipitation :-

→ oldest form of separation.

→ involves converting substance from soluble form into insoluble form by addition of suitable reagent.

sub 1 + sub 2 $\xrightarrow{\text{Reagent}}$ substance 1↓ + subst 2

→ Reaction of pptⁿ should go to completion

→ Ppt should have definite composition for quantitative analysis.

→ Ppt should be pure & free from impurities.

→ Ppt should be filterable.

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2. Filtration :-

→ mechanical method
→ separates solid from liquid
→ suspension ⟶ solid by coagulating it first.

@ Gravity filtration ⟶ own weight pulls its through the filter paper.
ⓑ Vacuum filtration ⟶ solvent is forced through filter by vacuum.

3. Distillation :-

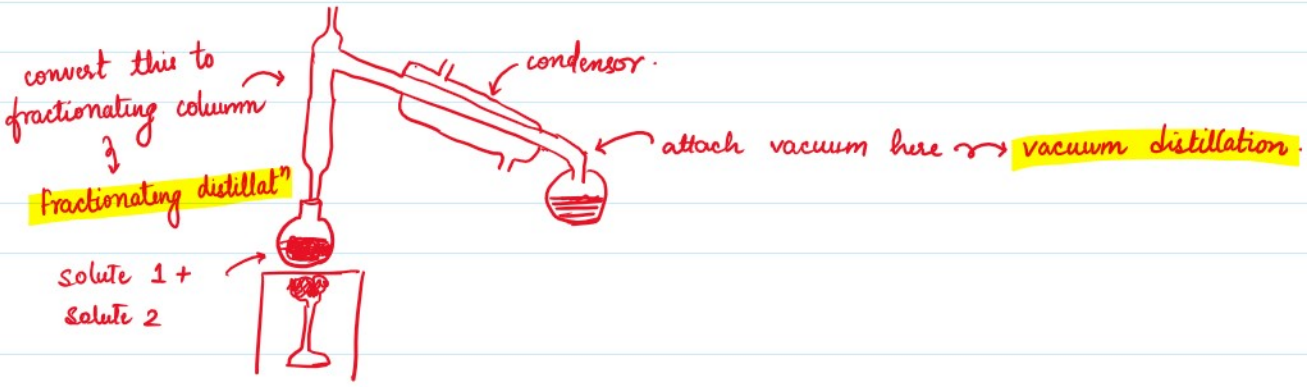
→ separate two liquid from one another.
→ liquid converted to vapour & condensed to liquid again.

@ Normal distillation :-
BP of the two liquids should differ by 25°C to 30°C
The liquids should boil below 150°C.
It should not form axeotropic mixture.

ⓑ Vacuum distillation :-
uses vacuum to decrease the BP of liquid.
BP of two liquids is over 150°C.
attach a vaccum to this.

ⓒ Fractional distillation :-
uses fractionating coloumn.
Diff. in BP is less than 25°C.
Fractioning coloumn ⟶ increases surface area that is available for vapor to condense.

convert this to  condensor.



4. Solvent Extraction :-

→ also called liquid - liquid extraction.
→ uses two immiscible liquids.
→ solute has more affinity towards one of the solvent.

Extractive Photometry :- metal ion —(ligand/chelate)→ complex (gives colour & checked for absorbance in photometry.

● Nernst's distribution law :-

When solute distributes itself in the two immiscible solvent, then the ratio of concentration of solute in the two phases remains constant, when the solute has same molecular condition.

$$\text{Partition coefficient} = K = \frac{C_1}{C_2}$$

✓ Distribution Ratio = D_A = $\frac{\text{Total concentrat}^n \text{ of solute A in (solvent 1) aqueous solvent}}{\text{Total concentration of solute A in (solvent 2) organic solvent}}$

$$\text{Separation factor} = \frac{D_A}{D_B}$$

W = amount of solute
W_1 = amount of solute in aqueous solvent.
$W - W_1$ = amount of solute in organic phase

solute (W) → aqueous (W_1)
solute (W) → organic

$$\therefore \text{conc. of solute in org phase} = \frac{W - W_1}{V_{org}}$$

conc. of solute in aq. phase = $\frac{w_1}{V_{aq}}$.

$$D = \frac{\left(\frac{w - w_1}{V_o}\right)}{\left(\frac{w_1}{V_{aq}}\right)}$$

$$D = \frac{(w - w_1)\, V_{aq}}{w_1 \; V_o}$$

$$D w_1 V_o = w V_{aq} - w_1 V_{aq}$$

$$(D V_o + V_{aq})\, w_1 = w V_{aq}$$

$$w_1 = \frac{V_{aq}}{(D V_o + V_{aq})}\, w$$

$$w_1 = \frac{1}{(Dx + 1)}\, w$$

Efficiency of extraction = $\frac{w - w_1}{w}$ = $\frac{\text{amt of solute in org solvent}}{\text{total amt of solute}}$.

$$E = \left(\frac{Dx}{Dx + 1}\right)$$

If n extractions are performed,

$$w_n = \left(\frac{1}{Dx + 1}\right)^n W$$

1 extract^n with $n\times$ Volume of solvent

$$w' = \left(\frac{1}{nDx + 1}\right) w$$

$w_n >> w'$

∴ extracting more times with less solvent is way better than extracting once with lot of volume.

* Metal ions are usually present in aqueous layer and we want to extract in organic layer then it is converted into non-polar covalent species.

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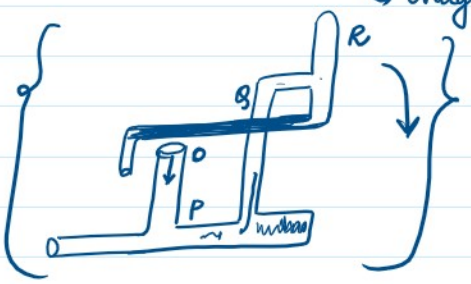
① Chelation
② Ion Pair formation
③ solvation
④ Synergistic extractions. —(use two complex reagent)

* Type of solvent extraction :-

① Batch extraction :- separation factor is large & amt to be extracted is small.
② Continuous extraction :- separation factor is small & amt to be extracted is large.
③ Counter current extration :- separation factor close to 1.
↳ Craig's counter current apparatus.



1 chamber of Craigs counter current apparatus.

* Crown ether is widely used in separation of metal.

5) Chromatography :-

General props. of chromatography :-

① has 2 phase
② one phase is stationary phase (static) and other phase is mobile phase (moves)
③ solute moves along with mobile phase.
④ separation of solute in 2 phases is brought about by differential solubilities / adsorption between the two phases.

*. Classification of Chromatographic Techniques :-

① Based on physical state of the stationary phase and mobile phase.

Mobile Phase	Stationary Phase	Examples
Gas	Solid	Gas-Solid Chromatography
Gas	Liquid	Gas-Liquid Chromatography
Liquid	Liquid	HPLC
Liquid	Solid	TLC, HPTLC, Coloumn Chromatography,

Liquid	Liquid	HPLC
Liquid	Solid	TLC, HPTLC, Column Chromatography, Paper Chromatography.

② Based on mechanism of separation.

ⓐ **Adsorption chromatography :-**
→ solute is adsorped by the solid surface.

ⓑ **Partition chromatography :-**
→ separation is brought about by differential solubilities of the solute in two phases.

③ Based on the dimension of phases involved :-

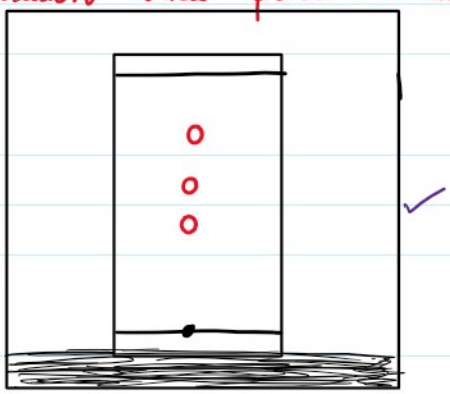
ⓐ **Planar chromatography** – Paper chromatography, Thin layer chromatography.
ⓑ **Non-planar chromatography**

① **Thin Layer Chromatography :-**

→ R_f = Retardation factor = Retention factor = $\frac{\text{distance covered by solute}}{\text{distance covered by solvent}}$

→ stationary phase → thin layer of inert solid
mobile phase → some solvent.

→ separation takes place on the basis differential adsorption.

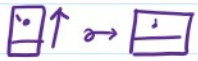


* Stationary phase → Silica gel, cellulose Kieselguhr, aluminium oxide, Magnesium silicate, PEI silicate
(Polar phase)
↓
impregnated on a plate ⇒ Aluminium, Glass, Plastic.
↓
fragile
↓
delicate, reactive

* Mobile phase → single solvent
mixture of solvent.

Arrangement of solvent based on their polarity is called Elutropic Series.

* Method of plate development :-
  - → Ascending development
  - → Two direction development → tilt plate by 90° after 1st run. → diagonal spot development.



  - → Continuous development → run the plate over & over again.
  - → Multiple development → run over and over again using different mobile phases.

* Detection of spots :
  - → Spot are coloured.
  - → indicator
  - → spraying agent → ninhydrin.
  - → I_2 spots
  - → UV light.

② Paper chromatography
- → stationary phase = cellulose paper, acetylated paper, carboxylic acid paper, ion exchange papers.
  (Polar phase)
- → Mobile phase = any solvent.
- → Development of paper.
  - → Ascending development
  - → Descending development
  - → Horizontal development — btw two steel/glass plate
  - → Radial development —

③ High Performance Liquid Chromatography :- (HPLC)

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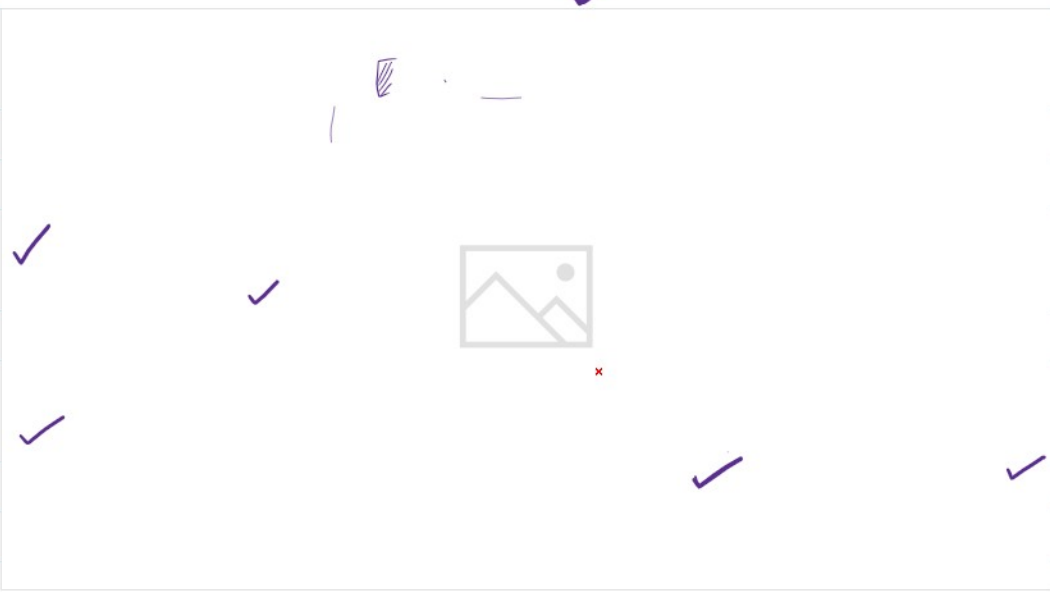
→ Both phases are liquid.

→ move one liquid over another ⟹ Require Pressure

∴ Also called as High Pressure Liquid Chromatography.

	Stationary Phase	Mobile Phase
Reverse HPLC	Inert, Non-polar	Polar liquid
Normal HPLC	Polar	Inert, Non-Polar Liquid



* General HPLC diagram.

1. Solvent Reservior

→ glass / steel

→ 200 mL to 1000 mL

→ store solvent.

→ degasing system & millipore attached.

degasing system → remove gases

millipore → remove suspended material.

Degassing system ⟶ will remove dissolved gases.

ⓐ vacuum pumping system

↓

evaporate solvent & condense again.

ⓑ Distillation system with heating & stirring

ⓒ Sparaging = bubble inert gas with low solubility.

Isocratic elution Tec. → single solvent
Gradient elution Tech. → mixture of solvents.

2. Pump :-
→ provide pressure to make liquid move over one another.
→ deliver pressure of 6000 psi.
→ reproducible, smooth and continuous pressure should be given.
→ reciprocating pumps, displacement pump, pneumatic pump.

3. Sample Injection System :-
→ injecting sample into the system.
→ Manual injection technique → Reproducibility is less.
→ Syringe should be able to withstand pressure.
→ Stop flow method → stop flow of solvent → open fritter → inject sample → close fritter.
Reproducibility is low.
→ Sample loop injection.

4. Precolumn :-
- Guard column / Sacrificial column
- helps to get rid of any suspended material that is present in sample & can get stuck in column.
- It helps to avoid the loss of stationary phase when mobile phase is introduced in column.
→ composition of pre-column is same as analytical column.
pre-column → larger sized particle
analytical column → smaller sized particle

5. Column :-

	Normal Column	Commercially available	Capillary column
Length →	10-30 cm	25 cm	4-7.5 cm
Internal dia →	4-20 mm	4-6 mm	1-4 mm
Particle size →	4-10 mm	5 mm	3-5 mm
Plate No.		40000 - 60000	100000

→ usually coloumn is operated room temp or ambient temp.
→ If higher temperature is to be used, jacket is provided.

6. Detector :-
detects the solution that is coming out of coloumn.
* UV detector
- measures the absorbance at diff. wavelength.
- UV cell has pressure 600 psi.
- A pressure reduction unit is required.

* Refractive detector :-
- made of 2 compartment
↙ Mobile Phase ↘ Solution from coloumn

- no bending of light → if only mobile phase is present in both compartment
- Bending of light → if different sol^n are present.

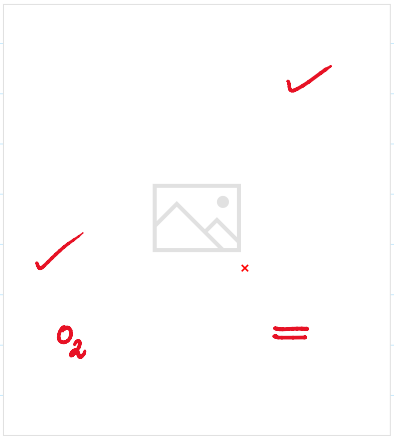
④ Gas Chromatography :-
→ mobile phase → gas
stationary phase → solid / liquid.

Imp :- sample should be volatile or should get converted into gaseous form.

→ All other components are same as HPLC except detector
→ Detector is of 3-types

- Thermal Conductivity Detector (TCD):-
  - measures the thermal conductance.
  - Wheatstone bridge concept ⇒ measure resistance.
  - carrier gas = He/H_2
  - Rate flow, temperature changes the response of conductor
  - Non-destructive, not very sensitive.
  - analyze organic & inorganic salts.

- Flame Ionization Detector (FID):-
  - Carrier gas - He/N_2
  - destructive gas, better than TCD, costly.
  - organic substance.



flame ionization detector.

OC's ⟶ ionized ⟶ ions ⟶ ion current ⟶ detector.

more ions ⟶ more ion current.

- Electron Capture detector
  - β rays are emitted
  - inert gas like 'Ar' is ionized

$$Ar^+ + e^-$$

Ions ⟶ Ion current

↳ If solute is present ⟶ they will absorb e^- ⟶ ion current changes.
↳ pesticides, halogenated compound containing substances.

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↳ pesticides, halogenated compound containing substances.

Electron-Capture detector.

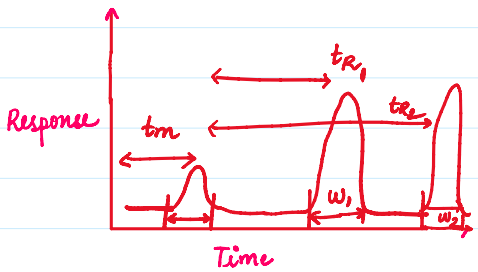
* ✓ $K = \frac{C_s}{C_m}$

✓ fraction of molecule = f_m = $\frac{\text{no. of molecule in mobile phase}}{\text{Total no. of molecules}}$

$$= \frac{C_m V_m}{C_m V_m + C_s V_s}$$

$$f_m = \frac{1}{1 + \frac{C_s}{C_m}\frac{V_s}{V_m}} = \frac{1}{1 + K\frac{V_s}{V_m}} = \frac{1}{1 + K'}$$

✓ Retention time :-



t_m = time required by mobile phase to come out

t_R = time required by solute to be eluted

$t_R = \frac{L}{v} = \frac{\text{length}}{\text{velocity}} = \frac{L}{u_m f} = \frac{L(1+K')}{v}$ $\quad v = v_m f_m$

$$t_R = \frac{L}{V} = \frac{\text{length}}{\text{velocity}} = \frac{L}{V_m f_m} = \frac{L(1+K')}{V_m}$$

$V = V_m f_m$

$V_R = t_R f_m$

$V_m = t_m f_m$

✓ Retention Volume - volume of mobile phase required to elute solute.

$$V_R = t_R f_m$$
$$= t_m (1+K) f_m$$
$$V_R = V_m (1+K)$$

✓ Relative Retention :- Ratio of retention time of solute and standard when they are subjected to same experimental condition

$$\alpha = \frac{t_R - t_m}{t'_R - t_m}$$

t_R = retention time of solution
t'_R = retention time of standard
t_m = retention time of mobile phase.

R_s = how well the peaks are separated

$$R_s = \frac{2(t_{R_1} - t_{R_2})}{w_1 - w_2}$$

w_1 & w_2 = width of peaks 1 & 2
t_{R_1} & t_{R_2} = retention time of 1 & 2.

* HETP :- Height equivalent to theoretical plate.

length ↑ width ↓ HETP ↓ efficiency ↑

width of each plate is called HETP.

$$HETP = \frac{L}{b}$$

L = length
b = no. of plates.

(Plate Theory) :-

HETP = (contribution by non-equal paths) + (contribution by longitudinal) + (contribution by non-equilibrium mass)

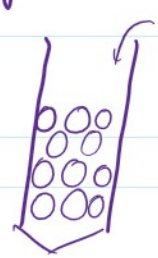
$$= A + \frac{B}{u} + Cu.$$

A = Eddy diffusion → donot move in straight line (This non-equal paths)

B = longitudinal → will not present at given point at the same time.

C = Non-equilibrium mass = diff. velocity of migrat" of front and back solute.

* Ion-exchange chromatography :-



1) Cation exchanger :- sulphonic acid - strong cation exchanger.
carboxylic acid - weak cation exchanger.

2) Anion exchanger :- quaternary ammonium salts – strong
secondary / tertiary ammonium salts - weak.

Thank-You!