Affinity Chromatography

ligand → binding site for protein.
matrix → material which hold the ligand with the help of covalent bond.

Affinity → attraction.

Principle

Based on the specificity and affinity to the ligand.

Matrix + ligand → Matrix ligand

matrix ligand + protein → complex

G.C → vapour/volatility.

PTGC → Program Temp. Gas Chromatography.
Temp. is controlled with the software.

S.P in affinity chromatography → matrix ligand

Eg- Matrix gel →
- Cyanogen bromide - activated agarose
- 6-amino hexanoic acid (CH) mix agarose and 1,6 diaminohexane - agarose
- Epoxy activated agarose

unbonded → protein doesn't bind to ligand

Chaotropic agents → Guanidine, urea.

Gel filtration + Size exclusion chromatography + Gel permeation chromatography

⇒ Semi-preparative process → partial separation.

Principle

Separation on the basis of difference in the molecular size. in some cases mol. wt.

Smaller particles → penetrates into gel.
Larger " → moves faster
buffer → acts as M.P.
Smaller particles → longer path.
Larger " → longer transit.

Gel - polyacrylamide

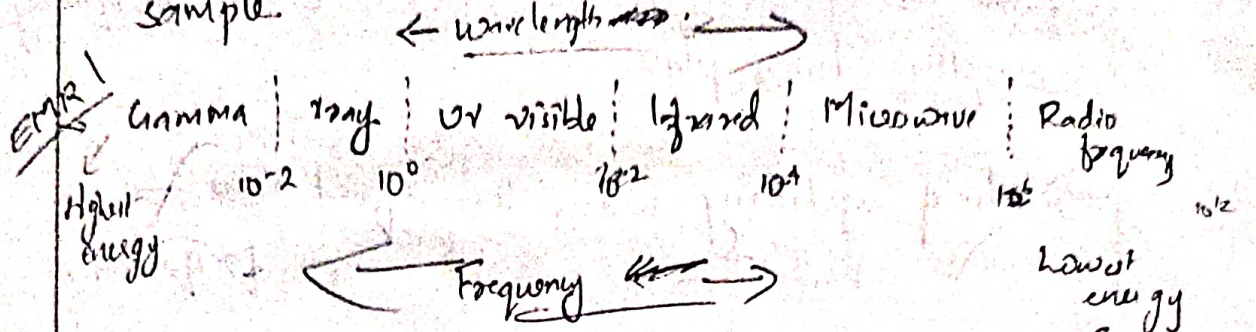
Detectors → Same as HPLC.

RI detector
UV "

} Eq. Construction Working merits demerits.

Spectroscopy

→ For identification purpose and to quantify the sample.



Highest energy → Highest frequency → highest photon. Low frequency.

Wave length (λ max) is opposite to frequency and energy.

energy $\propto$ frequency

wavelength $\frac{1}{\propto}$ frequency and energy

→ Crest also called as wavefront, peak.

→ Trough " " " valley.

Photon – energy packets present in Electromagnetic Radiation.

Amplitude – ½ of peak on base line.

OR

centre of the peak point.

ΔE – Energy
- Vibrational
- Rotational
- Electronic
- Translational

$\frac{1}{\lambda} = \nu$

Note on EMR

→ Wave form → Transmit energy at various velocities.

→ Consists of photon

→ Have both electric and magnetic properties.

In EMR, E represents Electric field and H represents magnetic field.

Natural Frequency of Organic molecule – When passing radiation, any of energy levels is ΔE, radiation will

select required frequency. Individual molecule have their own frequency. When the frequency of the molecule matches with the radiation frequency absorption takes place.

⇒ No: of repeating waves in a fixed time → frequency.

⇒ discrete particles → also called as photons.

Wavelength

Distance b/w two successive maxima or minima. Expressed in nm.

Frequency

No: of wavelength units passing through a given unit time is called as frequency.

⇒ unit expressed in cycles/second or hertz or frenel

Wavenumber (ν)

⇒ No: of number of waves/cm in vacuum.

$$\nu = \frac{1}{\lambda}$$

⇒ It is expressed in cm^{-1} or Kaiser(K).

Velocity (m/s)

⇒ Depends upon the passing medium

⇒ expressed in cm/sec

⇒ 3×10^8 m/s → velocity of light in vacuum.

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Amplitude :-

distance b/w the highest point (or lowest) on a wave and the centre of wave

Absorption Spectroscopy

Paired electrons in the ground state

↓ Radiation / energy

migrates to the excited state (Transition) (unpaired).

↓

To maintain the stability, the existing electrons in the excited state, pushes the electrons back to their ground state

↓

The electrons loses the absorbed / extra energy

↓

detected by detector and produce spectra.

↓ (detect absorbance from the sample)

↓

electrons comes to ground state and becomes paired.

σ (bonding) : GS

π (bonding) : GS

n (non-bonding) : GS

Infra-red Radiation

⇒ Energy to break single bond is less and unsaturated is more.
[Stretching single bond is easy]]

Beer-Lambert's Law :-

* Related to conc. of absorbing species.

"The intensity of radiation decreases with increase in concentration." of absorbing species.

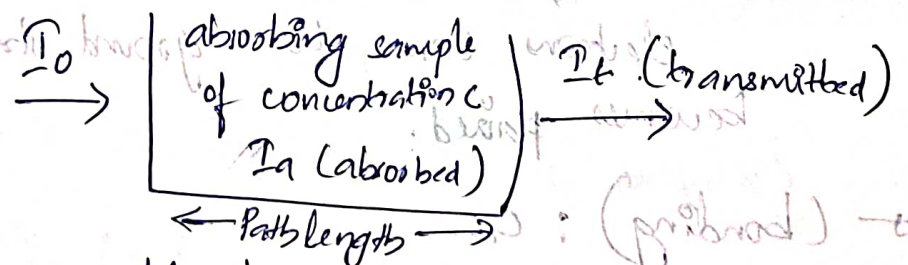
Beer's law

OR

~~Absorption~~ Absorbance ∝ concentration.

* Radiation should be single wavelength radiation (monochromatic light).

Polychromatic radiation — Radiation composed of different wavelengths.



⇒ Absorbance ∝ concentration

⇒ % transmittance $\frac{1}{\propto}$ absorbance

emission ∝ absorption.

24/2/21

Lambert's law

* Related to thickness and path length.
* The intensity of monochromatic light ↓s with ↑sc in the thickness of the medium.
* Distance travelled by radiation inside the medium —
  → Path length.
* ↑sc in pathlength → ↓s the transmittance

Beer Lambert's law

$$I_0 = I_a + I_t$$

I_0 → intensity of incident light
I_a → " " absorbed "
I_t → " " transmitted light

– No reflection / scattering of light takes place.
(This law is acceptable only)

* This is not applicable on colloidal sample where its thickness is more can cause reflection / scattering.

Equation of Beer Lambert's Law :

$$A = \varepsilon ct$$

where, A = absorbance / optical density.
ε = molecular extinction coefficient.
c = Concentration
t = path length. ('b' is also path length)

For calculating ε value → convert the absorbance obtained from mg into g. (ε = Mol. wt.)

* ε is fixed for particular drug.
* Can be used to analyse the purity of the drug.
* Also represent → $A^{1\%}_{1cm}$

[mg → mg → g → g% → 1%];

$$\varepsilon = \frac{A^{(1\%\ 1cm)} \times \text{mol. wt}}{10}$$

Absorbance of a specified conc in a cell of specific path length.

$A^{(1\%\ 1cm)}$

Application

* Only for dilute solution.
* Apply to multi component estimation. Also called as simultaneous estimation.
* Species present in the sample should not interact with each other.

Limitations

* Not applicable for suspension → (particles can settle down)
* Not for coagulated matter → radiation passes through the upper side and there will be no particle for absorption)
* No idea about the wave length.

Units of beer lambert's (decimolar) law:

$\varepsilon = dm^3 mol^{-1} cm^{-1}$

$c = mol\ dm^{-3}$

$t = cm.$

A = unit less, because epsilon is decimolar (dm^3) power of 3

gets cancelled with C and I. as well as mol^{-1} and cm^{-1}.

⇒ Absorbance ↑es → conc. ↑es to upto an extent.

Real limitations to Beer's Law:-

⇒ This law is good only to a appropriate concentration at, higher conc. & absorbance will be less, contradicted with law, as as $A \propto C$.

It can be prevented by dilution.

⇒ If beer's law is plotted on a graph, it gives straight line, A on y axis

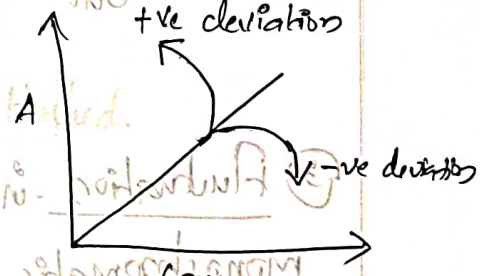
Conc. on x axis.

deviation of straight line → apparent failure of beer law.

Types of Deviation :-

a) +ve deviation :-

A small change in the conc, causes sudden jump in the absorbance (Conc. less, absorbance high)



b) -ve deviation :-

There will be a sudden ↓ce in the absorbance after a particular conc.

Reasons for deviation

⇒ Instrumental deviation.

⇒ Chemical deviation. (due to interaction b/w compounds, there can be a physicochemical change in soln.).

⇒ Instrumental deviation

① Stray Radiation :-

Any radiation apart from sample compartment is measured by the detector.

So that, absorbance is more a while conc. is less.

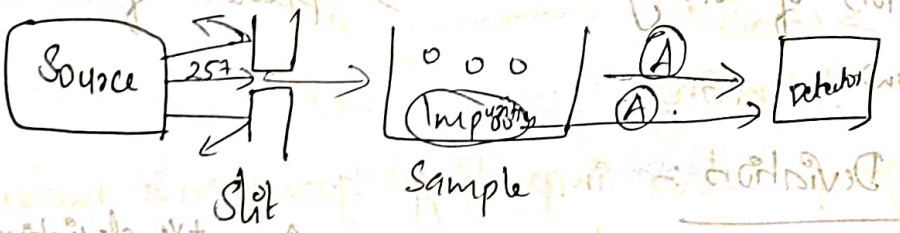
↓

give +ve deviation.

② Improper slit width :- (+ve deviation).

entry slit → before sample

exit " → " detector



Conc. is less but A is more.

③ Fluctuation - in a single beam and when monochromatic light is not used.

⇒ Chemical deviations :-

① Association.

Sample reacts with impurity / neighbouring particle forms its derivative. More wavelength is required, as λ is for sample, affects A.

↑se in conc. of sample → affects λmax

② Dissociation
Splitting of molecules into 2 & Ionizing molecule.

③ Ionization and change in pH.

④ Faulty development of colour (incompletion of reaction).

Wavelength → qualitative aspect.

⇒ Coloured radiation produced by impurity → fluorescence.

⇒ Absorption of UV radiation and release of visible radiation by the sample → Fluorescence.

Chromophore :- / Chromosphere :-

Chromo → colour
phore → Imparting.

⇒ The ~~group~~ colour imparting group on a molecule is called as chromophore.

⇒ Colour depends upon moiety attached.

Type of Chromophores :-

① Independent chromophore

The group in which have its own colour when a single ~~comp~~ chromophore is sufficient to impart colour to the compound. Eg. $-N=N-$ (Azo-electron)

② Dependent Chromophore

When more than one chromophore is required to impart colour to the compound.

Eg - $>C=O$ group, $>C=C<$ group etc.

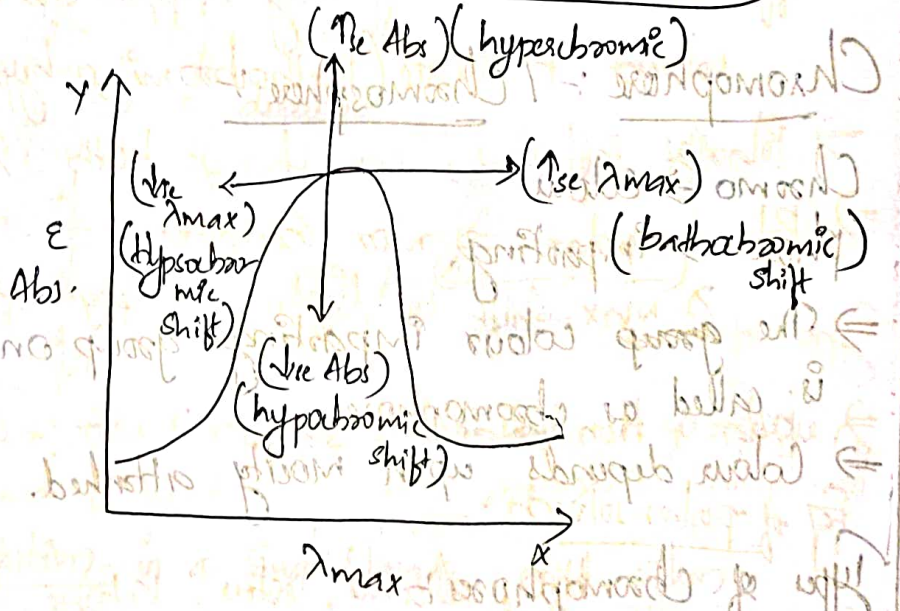
Auxochrome

Group of atom attached to chromophor which modifies the ability of the chromophor to absorb radiation from visible part by formation of covalent bond

Eg - CHO, $-OH$, $-NH_2$

hetro atom → consists of lone pair of electron easily binds to form covalent bond (S, N, O).

Absorption shift / bathochromic shift / Red shift



Bathochromic shift → Shifting of λ max. towards higher wavelength (800 nm).

↑se in chromophore. Due to addition of auxochrome.

Hypsochromic (blue shift) → Due to electron with drawing group, ↓se in intensity of particular group → effect

Hyperchromic → Due to the addition of electron

Hypochromic → group which reduce the intensity is added → ↓ie Abs.

hyperchromic ⟶ absorbance and epsilon ↑

$A = \varepsilon$

hypochromic ⟶ Opposite ↑

Effects of solvents

⇒ good solvents ⟶ < 210 nm.

cut off wavelength ⟶ removal of solvent wavelength.

⇒ 95% ethanol used as polar solvent because of its transparency (ethanol, methanol, H_2O).

⇒ effect of solvent → shift may happen due to type of solvent + solute (bathochromic or hypsochromic).

⇒ Polarity influence, can change both λ max & Abs.

⇒ Polar + polar → max interaction

λ max shift to higher wavelength. + change in polarity.

⇒ polar + non polar → same λ max. + change in polarity

Eg of polar solvents :-

H_2O, alcohol, ester, Ketone

Eg - for non polar solvents :-

n-hexane, CCl_4, C_2S_2

⇒ In n (non) - bonding electrons.

* $n - \pi^* + n - \sigma^*$; very sensitive to polarity.

Eg :- C = O.

⇒ In bonding electrons

$\pi - \pi^*$, lesser extent of red shift or bathochromic shift, if polarity of solvent is ↑ed.

[λ max of solvent should be less than sample

else, the peak of the sample will get deleted.

The Corresponding Absorption Band (R-band)

⇒ If a radical is present in non bonding unsaturated compound called as R-band (Radikalartig).
originated from $n-\pi^*$ transition.

⇒ Max. abs. wavelength > 270 nm.

⇒ Eg - Acetone.

K-band (Konjugierte)

forms $\pi-\pi^*$ transition.

Eg - • Diene
• Acetophenone.

B-band (benzene band)

forms $\pi-\pi^*$ transition.

This band is used to identify aromatic compound (270 - 300 nm).

E-band

forms $\pi-\pi^*$ transition of ethylenic band in benzene.

(E_1 and E_2 band)