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CH 2213 - ANALYTICAL CHEMISTRY

Introduction

Analytical chemistry is a set of tools & techniques that have been developed in laboratory & industry, especially for quantification & quality control. (Till 1960s)

- Analysis of chemical components
- Translates to large scale production in industry

Sampling size - grams to milligrams

Example – TiO_2 (used in make up) may have SiO_2 impurities

Polymers production

Here taking large no. of samples and checking for impurities is relatively easy.

Elemental analysis for ensuring the purity of the compound

it cannot differentiate isomers – we need advanced tools.

Tools – balances, NMR, X-ray

Spectro-analytical chemistry (1970 - 2000)

Tools – lasers, chromatography, electron microscope, MRI

Helped understand – fundamental understanding of molecules

larger systems : complexes / formulation

Sampling size – g to mg

Specialty materials : low volume - high performance - high cost

Nanotechnology – from 2000s

Smart materials – OLED TVs, computer chips

Materials science is at the interface of chem and physics

Imaging techniques (2000 onwards)

Tools : optical and electron microscopes

"Seeing is believing" – imaging techniques used for analysis.

Sampling size – nanograms

Mainly used in genetic medicine – DNA, mRNA, viral capsid technology

New analytical tools have been developed to help the "progress of science" in biomedical and energy field.

Molecules → Complex system → Actions

Taxol - drug used to treat ovarian & breast cancer.
It's extracted from Pacific Yew bark / leaves.

Role of AC - Extraction & purification
[10-15 yrs] Structural analysis
Biological studies in cancer
Clinical trial.

Extract : 1g of drug from 1kg of leaves - unfeasible to cut so many trees

Laboratory process - Holton process - 51 steps from a relatively simple starting material - takes 8 months

Extraction from plant -

1. Soxhlet extraction setup
   Vary the sequence of solvents & isolate multiple fractions
   Eg: water, methanol, ethanol.
2. Identification : Thin-layer chromatography
3. Purification : Liquid - Fractional distillation, column chromatography
   Solids - ~~re~~crystallization, sublimation
4. Characterization : BP - MP
   HPLC
   NMR spectroscopy
   Chiral HPLC
   Mass spectrometry
   Crystal structure

Nanotechnology platform for medicine
Taxol - when the drug is suspended in a formulation of ~~fatty~~ fat and ethanol
Abraxane - drug in a formulation of albumin (protein)

This is because the active molecule is very hydrophobic - to make it enter the body intravenously and reach the active site through blood, it needs to be combined with something that has hydrophobic & hydrophilic parts

Administration of medicine
- Lipid based delivery system
- Polymer based nanoparticle system
- Polymer-drug conjugate
- Self-assembling drug amphiphiles
- Hydrogel system for local delivery

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Lecture 2

Fundamental Concepts

⟹ Making of Solutions

Using volumetric flasks

★ Molarity : Molar conc : no. of moles of species present in 1L

1 mole of substance has 6.022×10^{23} molecules

✱ NaOH is hygroscopic – so dissolving 4g of NaOH in 1L will give us a solution lower than 0.1 M

★ Volumetric calculation

$$V_{conc} \cdot M_1 = V_{dil} \cdot M_2$$

No. of moles in dilute soln and concentrated solution must be equal.

Problem : Make 50 ml of 0.4 M from stock solution of 3M.

$\Rightarrow V_{conc} \times 3 = 50 \times 0.4$

$V = \frac{20}{3} = 6.67$ ml ⇒ Add 43.33ml of water to 6.67 ml of conc. solution

★ Specific gravity

For commercially available HCl, Assay 35.4%, SG 1.18

Mol. wt of HCl = 36.5 g

In the solution, 1 ml = 1.18 g (but its a 35.4% solution)

$\therefore$ 1 ml = 1.18×0.354 = 0.417 g of HCl

⇒ 1L of solution has 417 g of HCl

Concentration = $\frac{417}{36.5} = 11.4$ M.

Nitric acid : Assay 70%, SG = 1.47 ⇒ conc = 16.3 M.

✱ To prepare dilute acid – first add some water, then the V_{conc} of acid and then the rest of water

★ Primary Standard Solution

They're solutions of highly pure & atmospherically stable substance.

HCl – HCl vapors escape NaOH, KOH – Hygroscopic

So their molarity varies upon storage

④

Example : Oxalic acid

So to calculate the true conc. of solutions, they are titrated with primary std solution

→ Chemical Stoichiometry

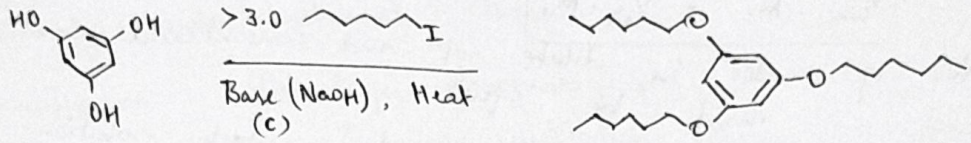
★ To prepare 11.2 g of Ag_2CO_3 from $AgNO_3$ and $CaCO_3$ — Actually Na_2CO_3

Product = $\frac{11.2\ g}{276\ g}$ = 0.04 M

$$2AgNO_3 + CaCO_3 \longrightarrow Ag_2CO_3 + Ca(NO_3)_2$$

So we need 0.04M of $CaCO_3$ = 4g 0.08 M of $AgNO_3$ = 13.6g

★ Organic Reaction



126 g >3 x 165 g

(A) (B)

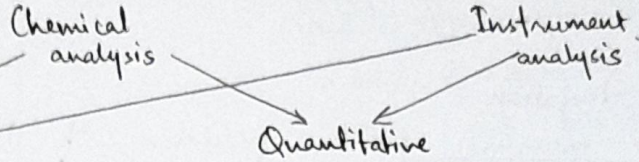
Compound	Mol. Weight	Weight Taken	No. of moles
A	126 g	12.6 g	0.1
B	165 g		0.4
C	40 g	12g	0.3

Solvents — EtOH | H_2O, CH_3CN, DMF etc.

★ Human plasma — total weight of chloride salts in 10 ml of plasma?

Ions	Conc (given) [mmol/L]	Product	Mol wt	In 10ml of plasma	Total weight (mg)
Na^+	143	NaCl	58.44	1.43 mmol	83.57
Ca^{2+}	2.38	$CaCl_2$	74.55	0.0238	1.774
K^+	4.51	KCl	110.99	0.0451	5.00
Mg^{2+}	1.32	$MgCl_2$	95.22	0.0132	1.26
					91.59 mg

→ Qualitative vs Quantitative Analysis



- Identification of analytes
Eg: Litmus test

- Determination of amt of analytes
Eg: pH & conc of soln

- Elucidate chemical structures

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Lecture 3

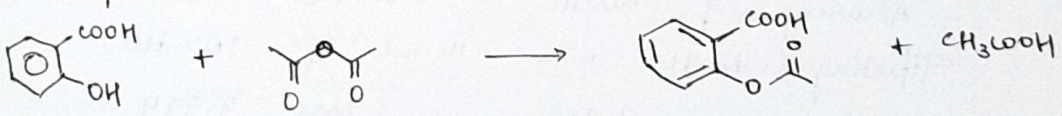
Data Handling: Types of Errors

Its important to write down the details and data accurately and exhaustively.

- 8.2 g is enough instead of 8.20 g. Don't overshoot the error bar to try being 'more accurate'.

- Report the yield in percentage.

Synthesis of Aspirin



Accuracy: degree of agreement b/w measured value and true value

Precision: degree of agreement between the replicate measurements of the same quantity.

Considering these, only shows the trend in data.

Quantifying Experiment errors

Absolute error: $E_A = |x_t - x_i|$

Relative error: $E_R = \frac{|x_t - x_i|}{x_t} \times 100\%$

But this doesn't say anything about the deviation from true value. (mean)

For very few data points, use median rather than mean as true value
Less sensitive to outliers.

Determinate or systematic error - instrumental errors, or due to chemicals

Indeterminate or random error - unpredictable variations (acceptable)

Gross error - due to personal error, leading to an outlier can be discarded.

Standard deviation

Gives a measure of the precision of the data set.

$$SD = \sqrt{\frac{\sum_{i=1}^{n}(x_i - \bar{x})^2}{n-1}} = \sqrt{\frac{\sum_{i=1}^{n} x_i^2 - \left(\sum_{i=1}^{n} x_i\right)^2 / N}{n-1}}$$ where $\bar{x} = \frac{\sum x_i}{n}$

For $n > 10$, n is replaced with $n-1$

Std deviation of mean : standard error $s_{mean} = \frac{s}{\sqrt{n}}$ Measures the discrepancy in sample mean compared to population mean

Use excel to do these calculations.

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Lecture 4

Data handling : Standard deviation

Consider : 50 trials of acid-base titrations

Accuracy of burette - 0.001 mL

Titrating NaOH with 10 mL of 1N HCl

Mean	: 9.982	Max	: 9.994
Median	: 9.982	Min	: 9.969
Std dev	: 0.0056	Spread	: 0.025

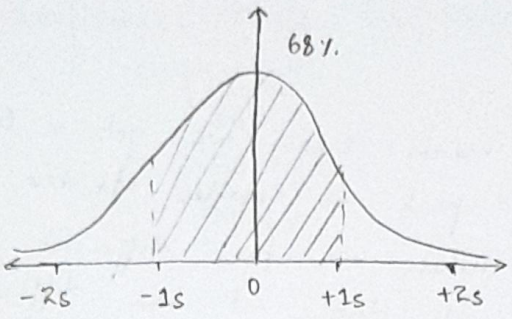
μ : Population SD that can be calculated only with infinite no of operations.

But $\mu = \bar{x} \pm \frac{s}{\sqrt{N}}$ # $\frac{s}{\sqrt{N}}$ is calculated as % error.

SD - measures the dispersion of datata set relative to its mean

Frequency distribution of data series in 0.003 mL ranges, from the plot we get a sort of Gaussian distribution.

Always true for a large set of data represented as freq. distribution



Confidence limit

Good approximation is that —

- 68% of data is in range $\bar{x} \pm 1s$
- 95% will fall within $\bar{x} \pm 2s$
- 99% will fall within $\bar{x} \pm 2.5s$

$$\text{Confidence limit} = \bar{x} \pm \frac{t\,s}{\sqrt{N}}$$

t-distribution – a statistical factor described on the no. of degrees of freedom & confidence level desired

Confidence limits defines a range of values either side of calculated mean that describes the probability of finding the "true mean"

Table of values of t for degrees of freedom ν

$$\boxed{\nu = N - 1}$$

Example : Mean = 93.5% N = 3 STD DEV = 0.075

Within what range are you 95% confident that true value lies?

$t = 4.303$ for $\nu = 3-1 = 2$ at 95% conf. level

$$\text{Confidence limit} = \boxed{\bar{x} \pm \frac{t\,s}{\sqrt{N}}} = 93.5 \pm \frac{4.303 \times 0.075}{\sqrt{3}}$$

$$= 93.5 \pm 0.19\%$$

∴ 95% confidence that it lies b/w 93.31% and 93.69%

Morphology of nanomaterial

To produce nanospheres of silver, we need to add surfactant to stabilise them so they don't form bigger aggregates

To determine the amount of surfactant needed to form nanospheres, she carries out the reaction at different concentrations.

Refer slides / video to see statistical distribution of different images of material formed

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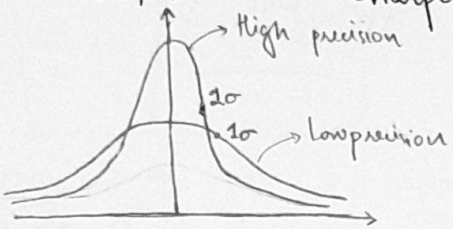
Lecture 5

Statistical data treatment

When we plot standard mean error, we get a Gaussian curve — Sharper peak ⇒ smaller std dev, high precision



When we take different samples, each data set has different σ-values and data centred around the mean.

So we take population deviation - σ. (calculated from infinite data)

Plot x-data deviation against $z = \frac{x \pm \mu}{\sigma}$ $\frac{x - \mu}{\sigma}$

$$\sigma = \sqrt{\frac{\sum_{i=1}^{n} (x_i - \mu)^2}{n}}$$ here, μ: population mean.

We can establish an interval surrounding $\bar{x}$ (expt mean) around which μ (population mean) will occur with a certain degree of probability. This interval is called Confidence Interval (CI).

If σ is known or s is a good estimate of σ, then we won't have a problem while analysing.

If σ is unknown and s is not a good estimation then we've to follow another process.

Here, s is std dev of sample and not standard error

⇒ Finding confidence interval when s is a good estimation of σ

$$z = \frac{x \pm \mu}{\sigma}$$

$\mu = x \pm z\sigma$ } CI for μ

↓ single measurement

Usually we estimate μ from multiple sample means where $s \simeq \sigma$

$\Rightarrow \mu = \bar{x} \pm z \frac{\sigma}{\sqrt{N}}$ } std error / STD of sample means

Confidence levels for various values of z —

Conf. level	z
68%	1
95.4%	2
99.9%	3.29

⟹ Finding CI when σ is unknown $s \neq \sigma$

To account for variability of sample std dev, a statistical parameter 't' is used

For single data point, $z = \frac{x \pm \mu}{\sigma}$ $\quad t = \frac{x \pm \mu}{s}$

For N measurements, $\boxed{t = \frac{\bar{x} \pm \mu}{s/\sqrt{N}}} \Rightarrow \mu = \bar{x} \pm t\frac{s}{\sqrt{N}}$

t depends on — desired confidence level
N (degree of freedom?)

t approaches z as no. of degrees of freedom (ν) becomes large.

$\nu = N - 1$

* When s is a good approximation of σ, and σ is known, z-test is performed.

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Usually all points don't lie on the ideal, normal error curve, so Gaussian curve has to be fitted

Hypothesis testing: Larger No. of datasets

When s is good approx of σ and σ is known or theoretically predicted, z-test can be performed.

→ Null Hypothesis (H_0)

$\mu \simeq \mu_0$

μ : Unknown — bcuz impossible to do ∞ expts & get ∞ data sets

μ_0 : Nearness to known [Theory / Prior knowledge]

$$z = \frac{\bar{x} \pm \mu}{\sigma} \qquad z = \frac{\bar{x} \pm \mu_0}{s/\sqrt{N}}$$ when $\sigma \sim s$.

$$\Rightarrow \quad \mu = \mu_0 = \bar{x} \pm \frac{z s}{\sqrt{N}}$$

- Determined μ_0 is almost similar or closer to true mean (μ)
- Probability of distribution exhibited by dataset is comparable and differences are result of random error. So the process maybe taken further

→ Alternate Hypothesis (H_a)

If there are more than 5 deviation for 100 trials, then 95% confidence level is not attainable.

$\mu \neq \mu_0$ The difference is significant.

Significant level is given by α.

Confidence level $= (1-\alpha) \times 100\%$

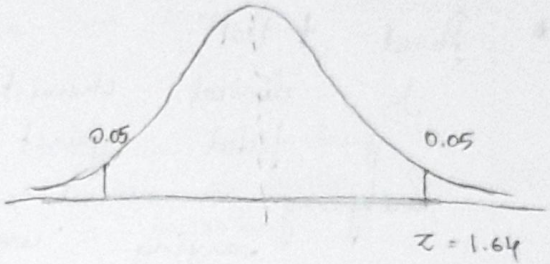
$\mu > \mu_0$ or $\mu < \mu_0$

$$z = \frac{\bar{x} \pm \mu_0}{s/\sqrt{N}}$$

Tail of the Gaussian: it contains data points that fall outside the confidence interval

To get a good estimation of μ_0 for experimental data, one can perform Two-tail test or single tail test.

To get a better estimate of μ_0 with 95% confidence level, 5% of data points can be excluded either from one tail or 2.5% of data from both sides.



Hypothesis testing for small no. of datasets.

When s is not a good approximation of σ, and σ is unknown, t-test is performed

Null hypothesis : $H_0 = \mu = \mu_0$

test-statistic : $$t = \frac{\bar{x} - \mu_0}{s/\sqrt{N}}$$

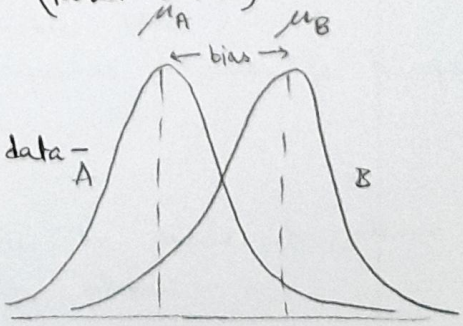
State alternative hypothesis and determine the rejection region

$\mu \neq \mu_0$,	reject H_0	if $t \geq t_{crit}$ or $t \leq t_{crit}$
$\mu > \mu_0$	reject H_0	if $t \geq t_{crit}$
$\mu < \mu_0$	reject H_0	if $t \leq -t_{crit}$

T test for differences in means (Pooled t-test)

Usually, $$\pm t = (\bar{x} - \mu)\frac{\sqrt{N}}{s}$$

But when there are two sets of data —



$$\pm t = \frac{\mu_A - \mu_B}{s_p}\sqrt{\frac{N_A N_B}{N_A + N_B}}$$

Pooled standard deviation → $$s_p = \left[\frac{\sum (x_{i_1} - \bar{x}_1)^2 + \sum (x_{i_2} - \bar{x}_2)^2 + \dots + \sum (x_{ik} - \bar{x}_k)^2}{*N-k}\right]^{1/2}$$

$N = N_1 + N_2 + \dots + N_k$ → total no. of measurements

$N - k$ → degrees of freedom

*

Comparing new method of measurement with the established method!

If obtained t value is less than the value in the table, then there is no significant difference

⟹ Paired t-test.

In clinical chemistry laboratory, a new method is tested against an accepted method by analyzing several different samples of slightly varying composition.

Difference b/w each of pair of measurements is computed Avg difference $\bar{D}$ is calculated and individual deviations of each from $\bar{D}$ are used to compute std dev s_d

t value is calculated from —

$$t = \frac{\bar{D}}{s_d}\sqrt{N}$$

$$s_d = \sqrt{\frac{\Sigma (D_i - \bar{D})^2}{N-1}}$$

Lecture 7

Antibiotic oral suspension

For young children, antibiotics are administered orally as a suspension. The powder is instructed to be added to sterilised water and form a suspension.

Anticancer drug formulation

Doxil - commercial name of doxorubicin. The drug is adminstered by pressing inside a vesicle. This formulation is stabilised with particles of ~ 100 nm.

Molecular self-organisation (soft materials)

Consider an amphipathic molecule. If conc is increased, they assemble to form a spherical micelle.
This process can be reversible or irreversible.

Based on the geometry of amphiphile, we can predict which if it forms i.e spherical / cylindrical / reversed micelle or spherical vesicle / planar bilayer.

This is based on the packing parameter.

$$P = \frac{v}{a_0 \times l_c}$$

v: volume of lipid molecule
a_0: area of lipid head group at lipid-water interface
l_c: length of lipid molecule

Making of vesicles

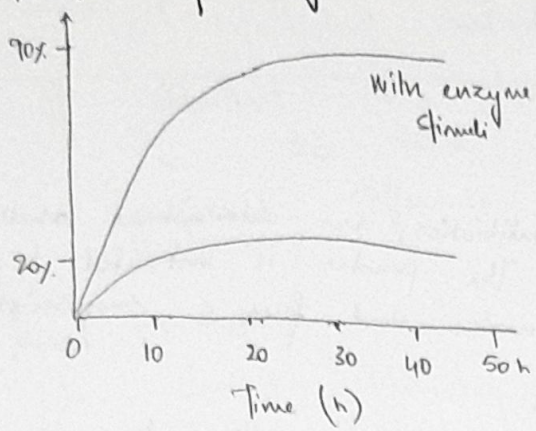
- Very high cooperative self-organisation is required
- Long range ordered bilayer assemblies in aqueous medium
- Efficient inter-digitisation of hydrophobic units
- Vesicles should be stable in aqueous medium for therapeutics

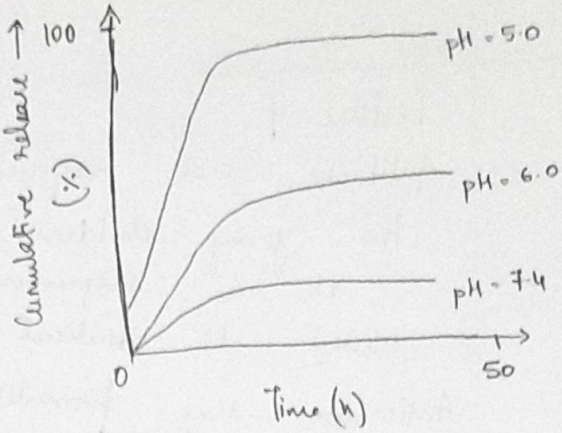
Stimuli-Specific responsiveness

The drug delivery systems can be made to cleave in response to different stimuli —

- pH responsiveness
- Redox responsiveness
- Enzyme responsiveness (lysosomal)

Kinetics of Drug release

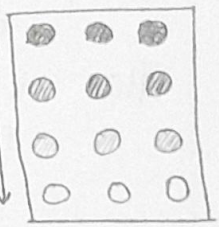


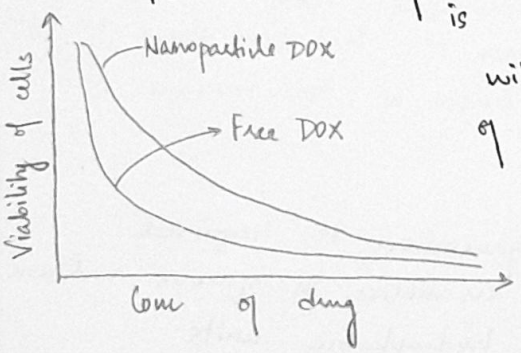


Such data is collected and plotted (with error bars) to prove that the drug is effective — that it will release only in certain conditions.

MTT Assay

- Its a colorimetric assay for assessing cell metabolic activity. It's done in a 96 well well plate
Equal amounts of cells are put in each well.
- Then MTT molecule is added Mitochondrial reductase acts on it and produces a colored compound
- The conc. of drug is increased over the column of wells. If the drug is effective, then cells will die and color of the wells will get paler because of decreased metabolic activity.





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Lecture 08

Student t-test in bioimaging

Cellular uptake of anticancer nanoparticles

This occurs through regular endocytosis or receptor mediated endocytosis (requires directed drug delivery).

In cancer cell line, the uptake of drug increases with time, while its levels remain low in normal cell line.

Here there are enough data pts to show the trend, so a t-test is not required.

Endocytosis of NP – can be observed by tagging the NP with a colored fluorophore. This endocytosis is energy driven by using ATP.

To confirm that the process is occuring through endocytosis, the process is carried out at 4°C so ATP production is suppressed

The image at 4°c and 37°c when compared show expected intensity. But we have only 2 data points So we have to factor in t test.

To check if data is significant, a software called GraphPad Prism software can be used get t and compare with the table

** in graph – error bar is in 0.01.

→ Estimation of bacterial population (alive or dead)

The E.coli are marked with HPTS (green fluorescent marker). In presence of green laser, the bacteria lights up. Then an agent is used to break the membrane and a red dye (propidium iodide) is used which enters the bacteria only when dead

When green + red laser is used the live bacteria light up green and the dead ones appear orange.

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Lecture 9

Separation of Chemical compounds

Based on Inorganic chemicals – Complexation & recrystallization

Organic chemicals –

< 500 Da	300 – 1000 Da	500 – 1500 Da	2 – 10 kDa	> 10 kDa
Distillation (Fractional, vacuum azeotropic) Extraction	Recrystallization Sublimation Chromatography	Chromatography	Size exclusion – Chromatography Precipitation → polymers, proteins	Precipitation

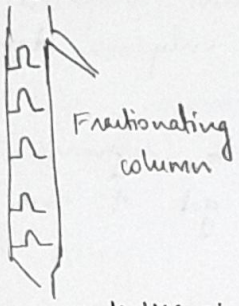
Petroleum compound – mostly hexanes (liquids) and octanes, so can be purified by distillation

C_{11} to C_{20} – solids.

⟹ Distillation of Organic compounds

★ Simple distillation – BP of miscible solvents are significantly different at atm. pressure

★ Fractional distillation



BP are close at atm. pressure

Crude oil is purified through this method.

★ Vacuum distillation – used for liquids with very high boiling point (140°C - 240°C) or they are thermally decomposing compounds at atm pressure

A vacuum pump is attached to the collecting flask and it has a trap to retain volatile compounds.

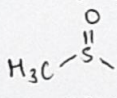
Because of lowering in P, the compound distills at a lower T because its BP decrease

Nomograph – a set of scales that can be used to calculate the pressure to be applied to distill something as a liquid.

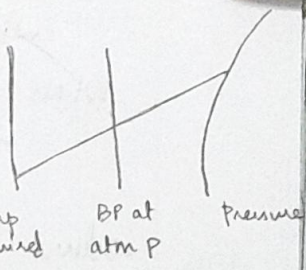
Based on the Clausius - Clapeyron equation.

Ethylene glycol - 250°C

Glycerol - 240°C



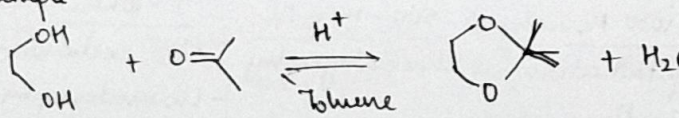
DMSO – 150°C breaks down



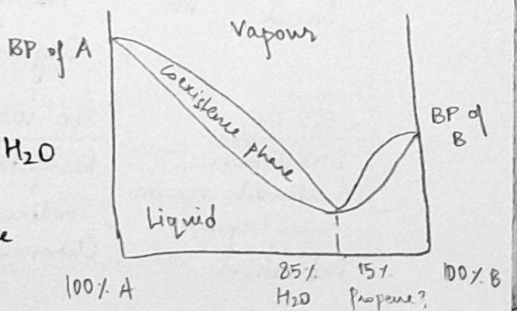
★ Azeotropic distillation

Constant boiling mixture.

Example –

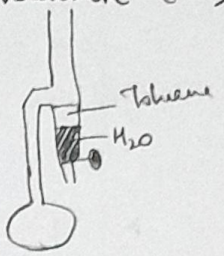


We can remove H_2O to drive the reaction forward



Dean - stark trap is used to remove the condensate (H_2O) and purify the liquids.

Toluene being lighter will float up after condensing, while water can be removed through tap.



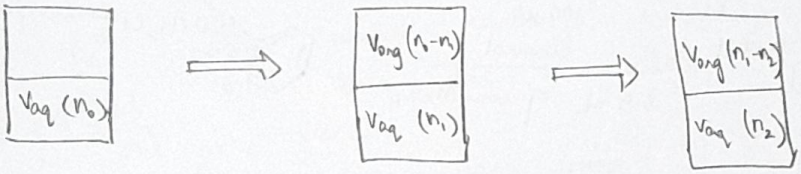
⇒ **Extraction**

Solute A in a liquid is extracted by shaking with an immiscible liquid in which the solute dissolves better. The partition of solute is based on phenomena governed by distribution law —

$$A_{(aq)} \rightleftharpoons A_{(org)}$$

$$K = \frac{[A]_{aq\,org}}{[A]_{org\,aq}}$$

Better to do multiple extractions with small portions of solvent to extract the solute efficiently.



Consider n_0 moles of solute in aqueous medium.
After one washing, n_1 moles will remain in aqueous —

$$[A]_{aq} = [A]_1 = \frac{n_1}{V_{aq}} \qquad [A]_{org} = \frac{n_0 - n_1}{V_{org}}$$

$$K = \frac{[A]_{org}}{[A]_{aq}} = \frac{(n_0 - n_1)}{V_{org}} \cdot \frac{V_{aq}}{n_1} \Rightarrow n_1 K V_{org} = (n_0 - n_1) V_{aq}$$

$$\Rightarrow n_1 (K V_{org} + V_{aq}) = n_0 V_{aq}$$

$$\therefore n_1 = \frac{V_{aq}}{K V_{org} + V_{aq}} \cdot n_0 \Rightarrow$$

$$n_k = \left(\frac{V_{aq}}{K V_{org} + V_{aq}}\right)^k \cdot n_0$$

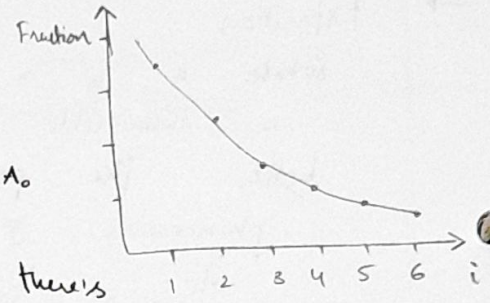
This eqn can be written in terms of initial and final concentrations of A in the aqueous layer

$n_i = [A]_i V_{aq}$ $\quad n_0 = [A_0] V_{aq}$

$$\Rightarrow \boxed{[A]_i = \left(\frac{V_{aq}}{K V_{org} + V_{aq}}\right)[A]_0}$$

▶ Eg: Consider $K = 2$ $\quad V_{aq} = 100\ mL$ $\quad$ Total volume of org = 100 ml

So, $V_{org} = \frac{100}{n_i}$

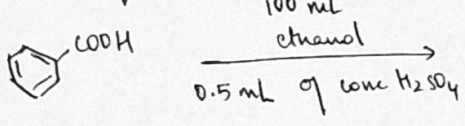


If $i = 2$ $\quad$ i.e. $V_{org} = 50\ ml$,

$$[A]_2 = \left[\frac{100}{50 \times 2 + 100}\right]^2 [A_0] = 0.25\, A_0$$

From the graph we can see there is not much to be gained by dividing the extracting solvent more than 5-6 times.

▶ Esterification of Benzoic acid



10g $\quad n = \frac{10}{150} = \frac{1}{12.2}$ $\quad$ 6.2g $\quad n_0 = \frac{6.2}{150}$

- After reaction, the ethanol is evaporated and residual slurry is extracted in diethyl ether (ester), washed with $NaHCO_3$ (unreacted acid reacts with this and goes into water) and brine solution (to remove H_2SO_4).
- Diethylether is evaporated to get 8.2g of crude product.
- It's further purified by recrystallizing from hot ethanol.

Yeild = 6.2g

$$\text{Pure Yeild \%} = \frac{\text{observed}}{\text{expected}} = \frac{6.2}{150} \times 12.2 \times 100 = 50.4\%$$

$$\text{Crude yeild \%} = \frac{8.2}{150} \times 12.2 \times 100 = 66.7\%$$

⇒ Purification by recrystallization

Imp: • Solute should not be soluble in solvent at room T
• Shouldn't be completely soluble in solvent at high T (conc. driven process)

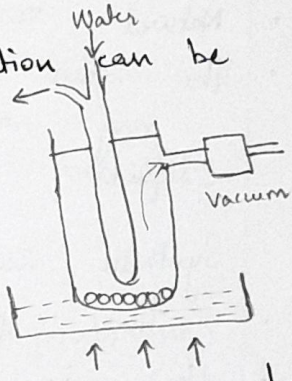
Steps —

- Take the solvent & bring it to a boil
- Add small portions of solute until you reach saturation
- Filter the hot solution using Whitman filter paper
- Allow the hot solution to cool
- Collect the crystalline solid by filtration

If crystal isn't formed, scratch the side of the beaker to initiate nucleation so crystal growth can occur

⇒ Purification by sublimation

The solids which undergo sublimation can be purified like this. This is carried out in vaccum



This process produces very pure compounds, even more than recrystallisation.

1/3/21

Lecture 10

Solid phase extraction: zeolites and porous silica

⇒ Solid seperation — Soxhlet extraction

Product as a mixture of solids — unreacted reactant, catalyst and product.

It is seperated based on solubility of solids in methanol, chloroform or water

Sequence: Methanol → Chloroform → Water
- Reactant - Product Catalyst

Zeolites and Silica

Zeolites have been known since 1800s, and silica gel since 1930s

- Liquid-liquid extractions require immisible solvent combinations and formation of emulsions should be avoided
- In solid phase extraction —

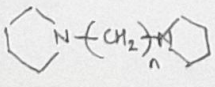
Advantages
- no solvent restriction
- solid phase absorbents can be reused
- readily expandable to industry

Natural zeolites

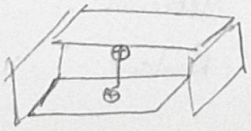
- Elementary building units are silica (SiO_4) and Alumina (AlO_4) and they are liked at their corner via a common oxygen atom
- There a positive free Na^+ ions
- Pore size — 0.2 nm to 1 nm — used for purification
- They occur in different shapes — rods, spheres
- Natural zeolites are extracted by mining
- The main limitation is that channel diameters are very small, so they can't be used to purify liquids with bigger molecules (organic solvents).

Synthetic zeolites — molecular seives

- Here, pore size varies from 0.1 to 5nm
- Used in vehicles to absorb toxic gases, used in chemical reactions to remove water in situ
- They are synthesized under hydrothermal - solvothermal conditions and reaction gel medium contains the framework atoms, (Al_2O_4, Si_2O_4) and structure-directing agents (SDAs)
- Also used to remove trace amt of water in $CDCl_3$ — deuterated chloroform used in NMR spectroscopy
- Also has applications in in situ H_2 production, CO_2 capture, radionuclide removal, biomass conversion



SDA n = 4, 5, 6



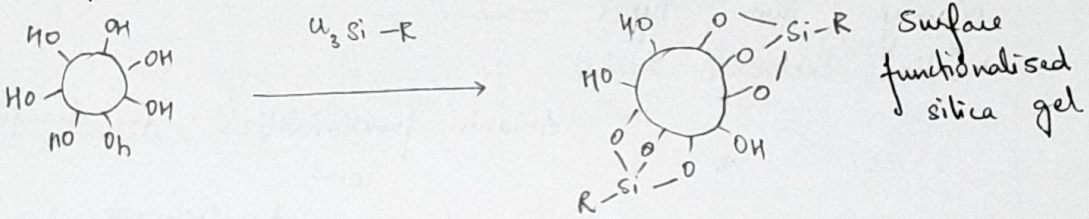
Different 3-D structures/cages can be created.

⟹ Silica Particles

- Silica gel preperation —

$NaSiO_3$ + distilled water $\xrightarrow[\text{Add HCl 1M}]{\text{Stir 4 hrs}}$ Sodium silicate solution $\xrightarrow[\text{Surfactant}]{\text{Add}}$ SiO_2 solution

- Silica gel particle size — 40 to 250 μm.



- There are used in determining organic constituents/pollutants in drinking water

Learn again:

These organic molecules are concentrated in solid phase and it can later be displaced by a solvent like methanol.

Alkyl chain – hydrophobic groups $-C\equiv N$: Phenol / Sulfonic acid (van der Waal)

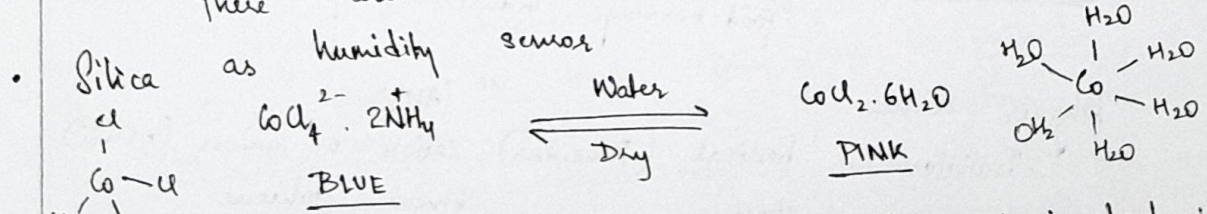
Pore size in zeolites are very constrained but silica gel pores can be functionalised easily

- Extraction of rare earth metals

Monolith – cm sized functionalized (by diglycolamine) silica gel with macropores (μm) and mesospores (nm) that helps in extraction of uranium, thorium through coordinate bond interactions.

Other ions in aqueous medium wash off, while these are retained

- Silica as humidity sensor



When producing dry N_2 (other gas), silica gel is kept in a chamber & gas passed over it so that water is absorbed selectively

(20) ⟹ Ion-exchange Resins

They are cross-linked, synthetic organic polymers. Can be functionalised : $R-SO_3^{\ominus}$: Anionic resins → (water purification)

$R-\overset{\oplus}{N}Me_3$: Cationic resin

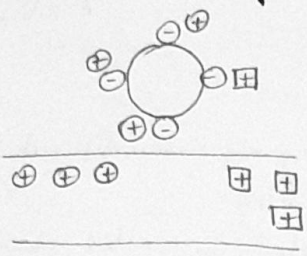
2/3

Lecture 11

Ion exchange resins and membranes

Mainly two types —

▸ Cation exchange resins



Anionin functionalities, +ve charged mobile ions

Strong / weak acid exchange

↓ ↳ Carbonic acid group

Sulfonic acid group

▸ Anion exchange resis — cationic functionalities

Strong (4° ammonium) / weak (NH_3) base exchange.

Conditions — { Electrostatic binding ability
Concentration gradient
3D porous network.

Pore dimensions and properties of resins should be retained for large no. of cycles.

Some resins can be make into a thin membrane while maintaining mechanical stability. This is done through cross-linked resins.

Common use — water purification, catalysis, metal recovery, food-beverage industries, pharmaceutical industry.

Polymer Science

Initiator — Radical (Peroxides), cation ($AlCl_3$) or anion ($nBuLi$)

Monomer — styrene

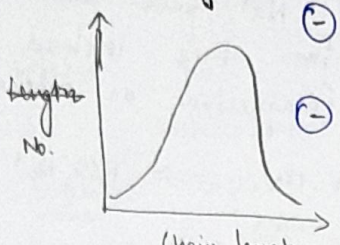
Solvent — toluene

Propogation → Termination ⇒ Polymer : Polystyrene

Vary the conc. of $[M]/[I]$

* activated double bond
(?) Low cross link → microporous gel structure
High cross link → macroreticular resins

Depending on conc and kinetics, the polymer chains vary in length and form a distribution.

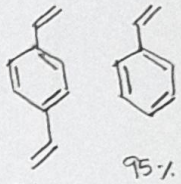


(-) Increase in viscosity → slows down chain movement → reduces the reactivity
(-) Slow down in kinetics due to decrease in monomer conc. with time ⇒ Produces different chain length

Formation of different chain length is an inherent limitation in synthetic chem.

Cross-linking process

- For cross linking you need molecule with two sensitive positions* from where polymer chains can grow. → easily filtered/decanted
- These resins form an insoluble mass but when put in a solvent, the swell because they have pores in them as the resin forms randomly.
- There are dangling free groups at the end which can be functionalized with sulfonic group to make it a cation exchange resin



- Less crosslinking molecules ⇒ larger pore size (?)
- These resins have an internal hydrophobic part and outer hydrophilic groups.

Carboxylic acid polymers :

Similarly, we can make phosphoric acid & cationic polymers

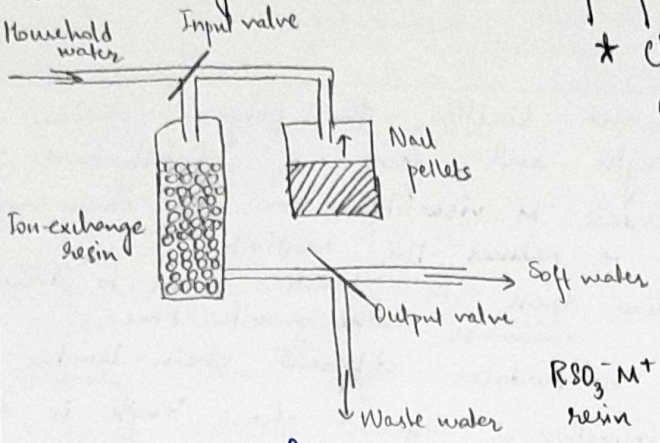
Polymer membranes can also be made.

Once the resins are made, they can be packed in a column and used for application

≠ lower cross-linking % — higher moisture content, equilibration rate, loading capacity and ability to accommodate larger ions

Porosity affects bulk properties — swelling, reactivity

Ion exchange resins in Water Purification



* Charging / Regeneration cycle
  Conc. NaCl from reservoir is flushed through the resin so Na^+ ions now occupy the space instead of M^+ (magnesium or calcium)

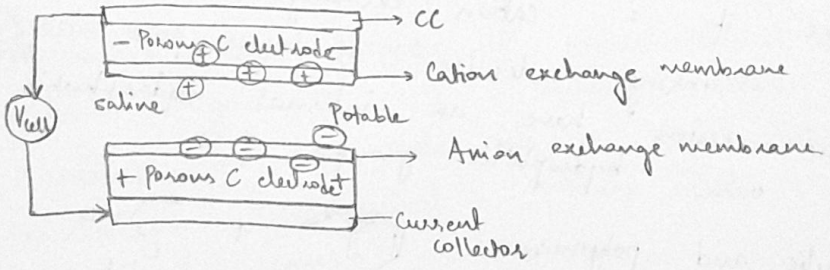
$$RSO_3^- M^+ + Na^+ \rightleftharpoons RSO_3^- Na^+ + M^+$$
resin (water) ⇌ resin (water)

$[Na^+] > [M^+]$

* Before use for drinking
  When household water is passed through resin, Na^+ ions are replaced by M^+ because they have more affinity.

$$RSO_3^- Na^+ + M^+ \rightleftharpoons RSO_3^- M^+ + Na^+$$
resin + hard water ⇌ resin + soft water

To purify sea water and make it potable, you need both cation and anion exchange resins that will run through electric current, by connecting electrodes and applying electric field.



TLC – made from silica gel + resin so compounds can be seperated and quantified

Lecture 12

Chromatography Techniques : TLC and Column

- Useful for molecules that can't be seperated by distillation, crystallization, sublimation or extraction
- Enables purification and estimation of purity level
- Enables continuous monitoring of the process

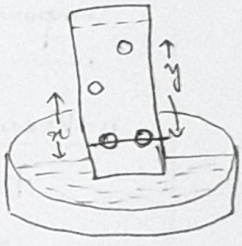
→ **Thin layer chromatography (TLC)**

Consider a reaction : 

A → B

We want to check whether reaction has occured and how far along it is.

- TLC paper is a sheetlike thing made out of silica gel. A & B are spotted on it and its dipped in solvent i.e mobile phase so that the liquid level should be below the spots.



- The beaker should be covered so the solvent doesn't evaporate.
- Low volative solvents like diethyl ether and dichloromethane should be avoided.

Retention factor : $R = \frac{\text{Distance traveled by compound}}{\text{Distance traveled by liquid}}$

$R_A = \frac{x}{z}$ $\quad R_{prod} = \frac{y}{z}$

If product had mixture of A + B, the we would have seen some spot at same level as A.

R is the same for a given compound + solvent.

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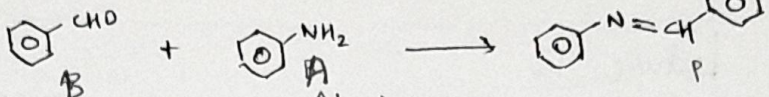
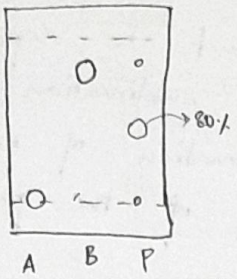
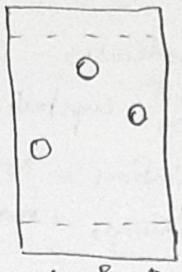
→ ~~Silica~~ TLC plates material — Silica gel is acidic and Alumina plate is basic

Consider 





Due to acidic condition, the product probably cleaved

→ Combination of solvent for mobile phase

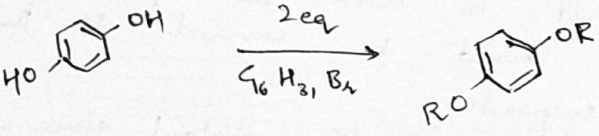
The combination should be considered so the compound moves — not too less, not too much.

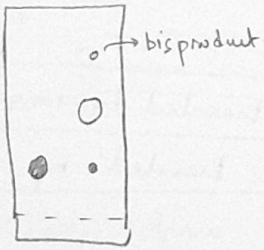
R_f should be calculable ie between 0 and 1.

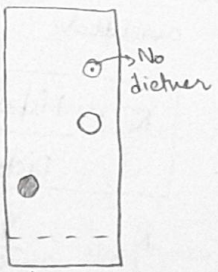
n-Hexane — very non-polar ethylacetate - very polar

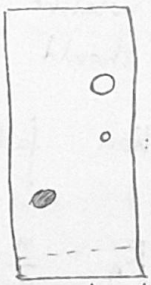
7 : 3 ratio probably okay.

→ TLC for reaction monitoring









Rxn condn: K_2CO_3 / CH_3CN Reflux, 24h

$NaOH$ / H_2O + EtOH Reflux 24h

K_2CO_3 / KI / DMF 80°C, 24h

Disubstituted product — less polar, won't get attached to silica plate

In c, KI is used ie R-Br → R-I activated.

So reaction occurs better.

→ Visualising TLC plates
Aromatic compounds — can be seen under UV light
Aliphatic compounds — staining agents are used, depending on the type of compounds formed

→ Limitations of TLC
- Para & meta products can't be distinguished
- Polymer chain reactions — the product forms a band rather than a spot on TLC

→ Seperation of small quantity products.
When reaction is of small quantity and gives distinct spots, then the product can be isolated and extracted from TLC
Its tedious and time-consuming; however useful of new compounds.

Column chromatography
Used for large scale seperation of compounds.
A column is packed with silica gel and sample is put on top. Then the mobile phase (hexane) is poured on top of it continuously.
Each fraction should be compared using TLC so we know when each of compound should be collected from column
Automated flash column chromatography and industrial column chromatography also exist.

Need for advanced chromatography techniques — to seperate chiral and isomeric compounds.

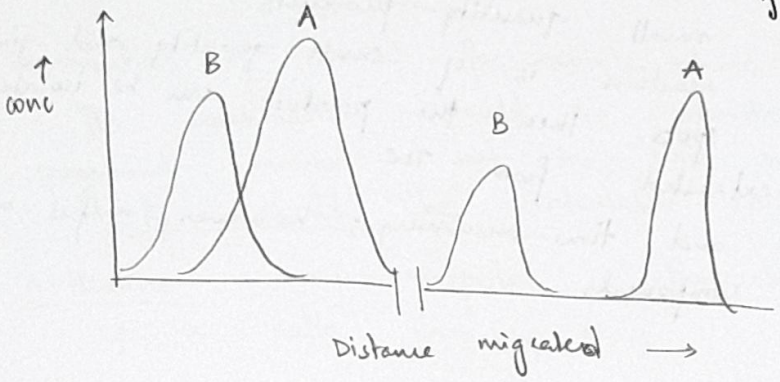
Lecture 13

LC and HPLC

- Until 1970s, classical LC was performed with gravity-fed glass columns. Column matrices are used and stable only at low P.
- In 1970s and 80s HPLC was developed – stainless steel column packed with silica and can run at pressure ~ 40 MPa

Separation of compounds in column

* Conc. bands A and B at two different times – in their migration down the column



There is a detector at the end of the column that can detect the required compound, so it can be isolated

* Retention time
  Is the time taken for the compound to pass through detector

Dead / void time – time taken for the solvent to pass through the column
All components take at least this amount of time to pass through column. This is calculated by by using a small molecule (acetone) that doesn't bind to the silica

$$t_R = t_s + t_m$$ → void time

t_R: Retention
t_s: retained in stationary phase

Avg linear rate of solute migration —

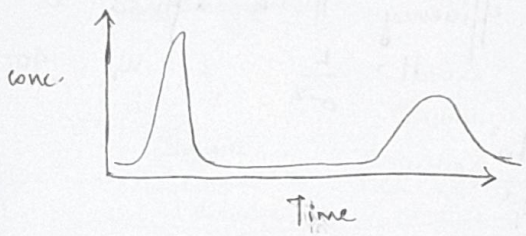
$$\bar{v} = \frac{L}{t_R} \quad cm\,s^{-1}$$

of mobile phase — $u = \frac{L}{t_m}$

Band broadening

★ All the molecules don't come together. So as the analyte passes through column, the peak broadens i.e takes longer time for all of sample to exit the column.

If the band is too broad, then the column is not very efficient. So this factor is used to analyze the quality of the column.



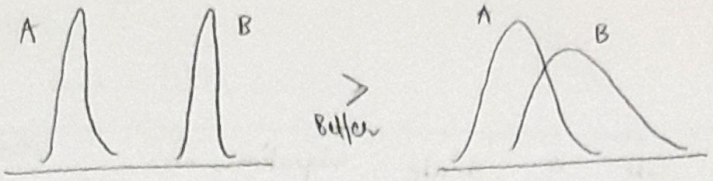
★ Why does it happen?

The stationary phase is packed with beads of silica or resin. If the stacking is uniform, then the molecule/analyte can travel easily.

But if bead sizes are different, the some molecules move faster than others and this results in peak broadening.

It can also happen because of mass transfer between mobile and stationary phase because of increased porosity of silica/resin.

★ When using HPLC for seperation purposes, if peaks are too broad, then there compounds can't be seperated efficiently because their exit times overlap.



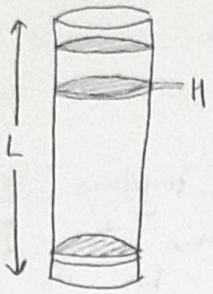
→ can't be separated

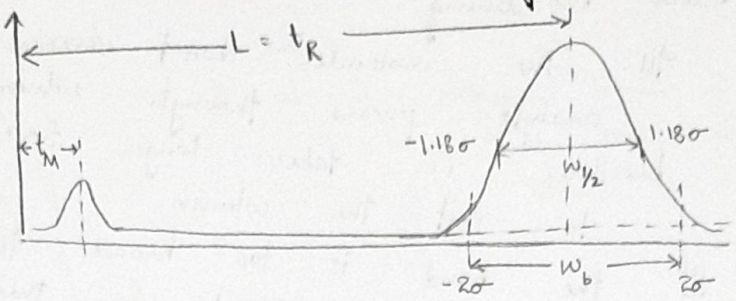
★ Efficiency of chromatography column through band broadening.

H : Plate height L : Length of column

N : theoretical number of plates

Can be measured chromatography graph.





W_b covers 96% of 'area of Gaussian'

$N = \frac{L}{H} = \frac{L^2}{\sigma^2}$ Column efficiency H is defined as $H = \frac{L}{\sigma^2}$, $W_b = 4\sigma \Rightarrow \sigma = \frac{W_b}{4}$

$$N = \frac{t_R^2}{(W_b/4)^2} = \frac{16\, t_R^2}{W_b^2}$$

$$N = \frac{t_R^2}{(W_{1/2}/2)^2} = \frac{4\, t_R^2}{W_{1/2}^2}$$

In the analyte is coming out in a narrow peak, the no. of theoretical plates will be more and conversely, when band broadening occurs. N goes down

Even when analytes have same retention time, N can vary significantly.

When conc. of solvent injected increases, the area under the peak will increase. If there are impurities, then area would decrease.

and quantification.

Seperation of solvent mixture — ≈ 53:00 min.

Lecture 14 — Not in portions 9/3

Chromatography Instruments

Gas chromatography – mobile phase & anyte both gases

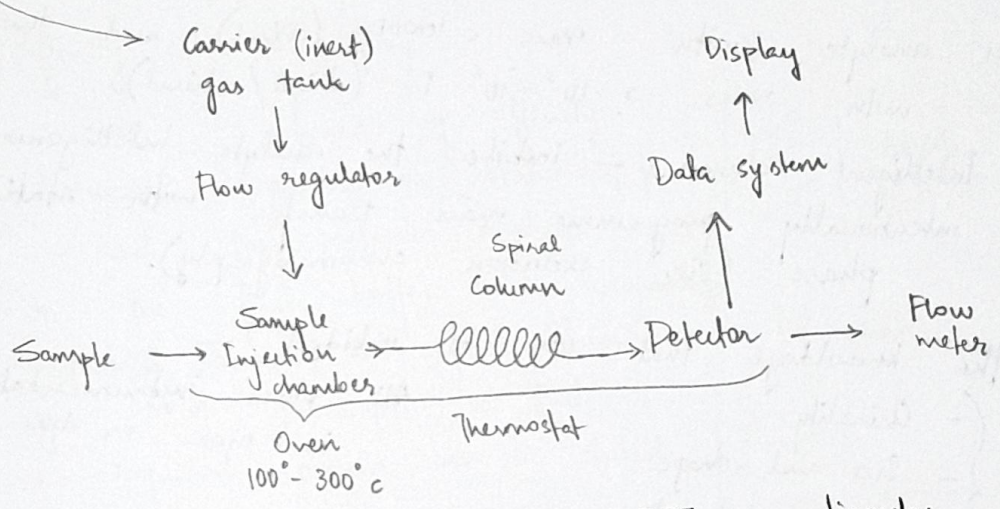
HPLC, Chiral, Ion exchange, Affinity — Liquid chromatography

Size exclusion chromatography

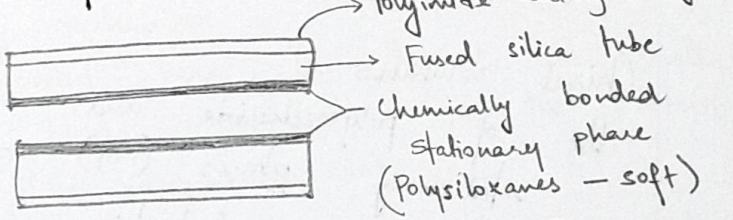
Two types —

Gas liquid (Partition) Chromatography [GC]

Gas solid (adsorption) chromatography — v. rare.



Fused silica column of 0.32 - 0.25 mm diameter



Different silanes (stationary phases) are used for analysing different kinds of molecules of analytes

Van Deemter Eqn : Theoretical plate height in GC predictions.

Flame ionization detector and other kinds in GC

— Gave up —

Lecture 15 (10/8)

Preparing a column — Imprinted, Chiral & Affinity

Chromatography Mass range

Mass : < 1000 Da

LC, HPLC — Analyte : liquid phase
Carrier : solvent
Stationary phase : silica
} L = 30 cm N ~ 100s

GC — Analyte : Gas phase
Carrier : inner gas
Stat. phase : silica (0.5 mm)
} L = 30 - 60 M N ~ 100,000s

→ Linear velocity of mobile phase
→ Mass transfer with stationary phase

For analyte with mass < 1000Da (chiral) and those with mass > $10^3 - 10^6$ Da (chiral / achiral)

- Intelligent column — imbibe the analyte intelligence in column
- intentionally programme mass transfer with stationary phase (size exclusion chromatography).

The knowledge that can be imbided —
- Chirality
- Size and shape
- Affinity interaction

All other instrumentation is more or less same.

Chiral columns

Use of polysaccharide and cyclodextrin (sugar) chiral stationary phases (CSP) are used for this → cyclic polymer

Three types of cyclodextrins : α (5 Å), β (7 Å), γ (9 Å) cavity size

The ~~silica~~ CD is attached to —OR and $-NR_2$ and further bonded to silicates / silica.

This film made of these molecules is 'intelligent' — if can seperate molecules according to chirality

Electron microscope image — porous with size of few μm

Because of pore shape, the retention time of R and L isomers differ.

To maximise seperation, we'll just have to design the matrix optimally

Enantiomeric excess : $\frac{A-B}{A+B} \times 100$

Catalyst 1 gives - 53 : 47 & Catalyst 2 - 95 : 4

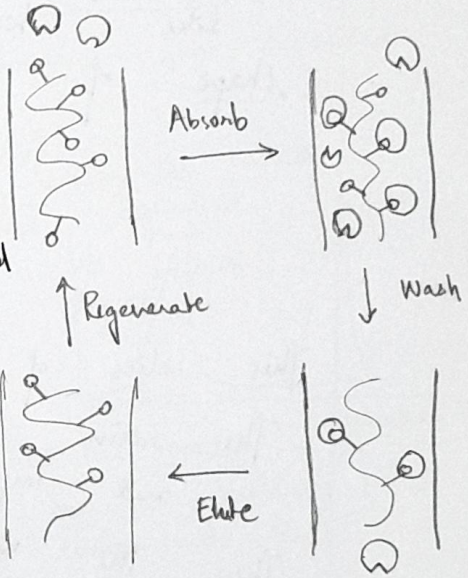
Only chiral HPLC can give this kind of quantification directly.

→ Affinity chromatography

This is based of affinity between ligands on stationary phase and the preferred analyte

After letting it absorb

→ In the first buffer wash, unwanted protein comes out first. Then you vary the pH so that required protein can be eluted and the stationary phase



mainly used for this

REVERSIBLE INTERACTION

The 'lock and key' mechanism in biology is exploited here. The ligands are usually antibodies that bind specifically to the protein of interest. (its recognition site)

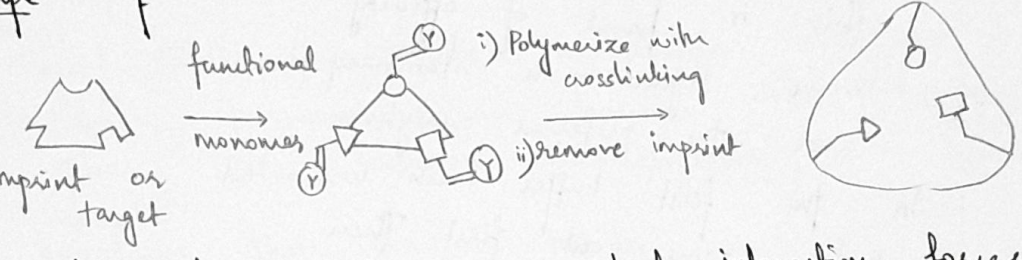
Expanded-bed adsorbents — the beads float ⇒ prevents clogging and leads to better absorption.

Then when pressure is exerted, the protein of interest can be repeated.

The antibody is bound to the column by making sure
- that single site is attached, not multiple
- proper orientation (light chain facing outwards)
- spacers are used (so more binding sites available)

Eg: Purification of Glutathione S-transferase —
glutathione is attached to beads so GST binds to it.
Then column is washed with free glutathione
so that GST comes out along with it.
Free glutathione competes for GST ⇒ competetion assay.

⟹ Molecular imprinting column
Affinity C is expensive So this method was developed to seperate commercially important compounds like steroids. This method only considers the shape of the molecule



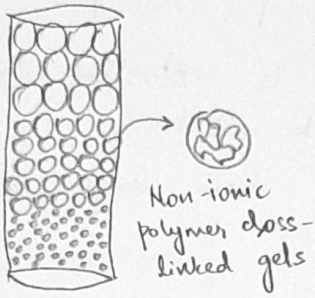
This relies of secondary non-covalent interaction forces
The resin is constructed using functional monomers and imprint, so that the cavity is perfect
Then the non-covalent interaction (H-bonding / dipole / π-stacking) is broken by changing pH or something
Then we get the required + resin which can only seperate required compound from everything else So a template is used for efficient seperation of compounds.
Specific example — last slide.

Lecture 16

Size Exclusion Chromatography

This is used for macromolecules — b/w 1000 - 10,000 Da like proteins, synthetic polymers etc.

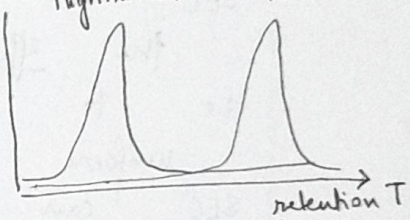
For Organic soluble — gel permeation chromatography
Water soluble — gel filtration & ion exchange C.



The smaller beads are packed at the bottom and larger ones at the top. So the pores at the top are larger and get smaller along the length. Pore size can be tailor made — from 5 nm to 1 mm

Here there are no chemical interactions — just separated based on shape and size

The size and shape of molecule in water is called hydrodynamic volume. Consider two protein molecules with different chain length A — n = 5000 High wt
B — n = 50 Low wt.



The smaller molecules can reside in the pores — so their retention time increases. So the molecule with higher wt / larger size actually comes out first.

Eg: lysozyme enzyme, different sizes of polyethylene glycol, polyethylene vs polystyrene (same wt but different shapes).

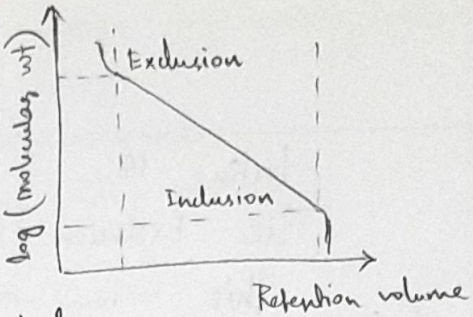
→ Working principle
• while in pores, molecules are effectively trapped and removed from flow of mobile phase.

Avg retention T ∝ 1/effective size ?

• Larger molecules — exclusion limit — they're excluded from the column and suffer no retention.
⇒ All larger molecules elute together.

Smaller molecules – inclusion limit – all smaller solutes elute together

∴ they stay in the pores for longer



Different columns with diff. pore sizes can be prepared so the limits vary.

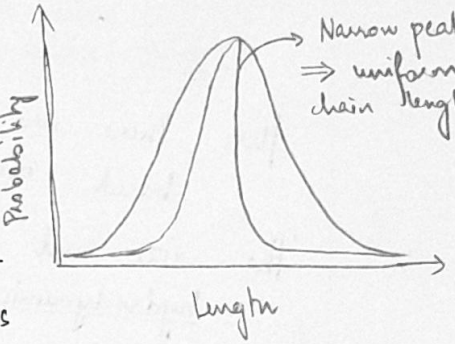
The intermediate molecules are seperated more efficiently.

Eg: Polymer chains, proteins
Fullerene is isolated and purified using SEC

In synthetic polymer, the size is controlled by varying the conc. of monomer-to-initiator.

Possible side reactions that vary chain length –

- hinder growth of chain
- inhomogeneity in reaction.
- introduce chain distribution



SEC can be used to quantify the efficiency of the reaction – i.e to see if chain length is uniform or varies a lot.

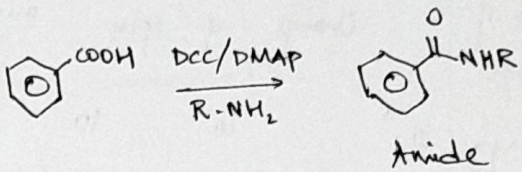
SEC can also be used to characterize different features – multimodal graphs, efficiency, uniform chain length etc.

16/3

Lecture 17 – Dismission – didn't attend.

Lecture 17

Spectroanalytical Chemistry

Consider this reaction: 

Amide

How to ensure that the product we've obtained is the one we desire?

$-COOH \longrightarrow -CONHR$

Spectroanalytical methods are based on measuring the amt of radiation produced or absorbed by molecular or atomic species of interest

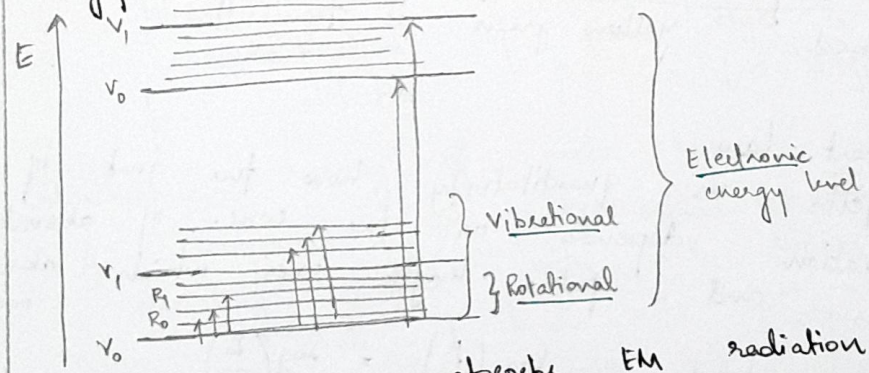
EM spectrum used: γ-ray, X-ray, UV, visible, infrared, radio waves.

Electromagnetic wave

$$E = h\nu = \frac{hc}{\lambda} = hc\bar{\nu}$$

$$c = \nu\lambda = 3\times10^{8}\ m\,s^{-1}$$

Energy level diagrams



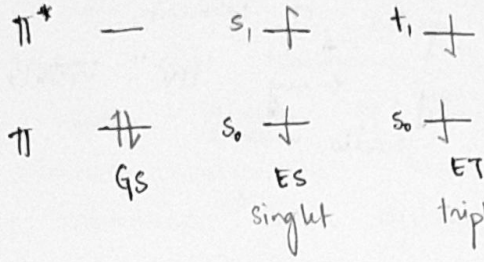
The species absorbs EM radiation and gets excited. Its energy level increases based on the amount of energy received

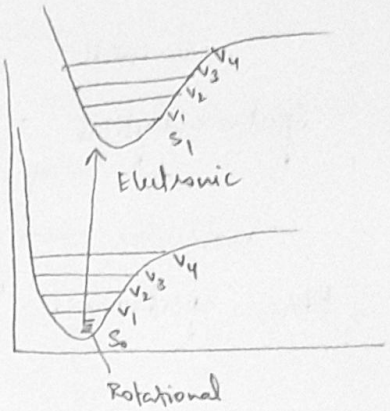
Depending on the nature of compound, what you want to focus on and the energy level in consideration, we can use different things — change of spin, change of orientation, change of configuration, change of e⁰ config, change of nuclear config.

Type of change	Change of spin	Change of orientation	Change of configuration	Change of e⁰ config	Change of nuclear config.
Energy	10^{-3} — 10^{-1} — 10 — 10^{3} — 10^{5} — 10^{7} — 10^{9}				
	NMR	ESR	Microwave	Infra	Visible + UV	X-ray	γ-ray

Electronic transition

2-butene





Absorption is a characteristic of the molecule — if the absorption spectra differs before and after the reaction, then we know that some product has been formed

Color of light absorbed and transmitted will complement each other i.e. if blue is absorbed, yellow-green is transmitted

Beer-Lambert law

It tells us quantitatively how the amt of attenuation depends on the conc. of absorbing molecules and path length over which absorption occurs

absorbance

$$A = -\log T_{10} = -\log_{10}\left(\frac{P}{P_0}\right) = \log_{10}\left(\frac{P_0}{P}\right)$$

P_0 : power of incident ~~solvent~~ light (on solvent)

P : power of transmitted light (on analyte)

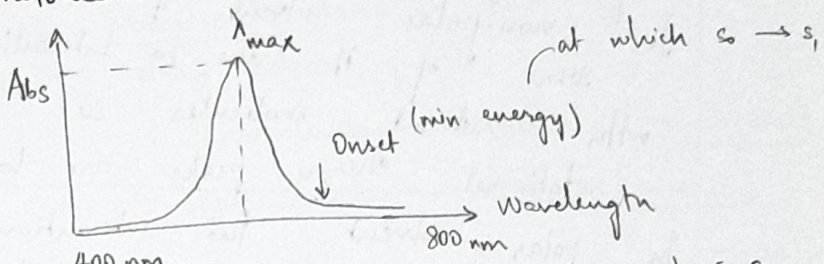
According to law —

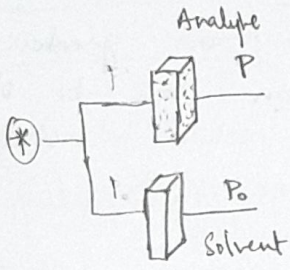
$$A = \log_{10}\left(\frac{P_0}{P}\right) = abc = \varepsilon bc$$

ε : molar absorptivity
b : path length (width of the cell)
c : concentration

Absorption spectrophotometer – Instrumentation

Output graph





A depends on ε when b & c are fixed

Determination of molar extinction coefficient (ε)

Its calculated by plotting A versus concentration

Path length 'b' is known. So calculating the slope of graph gives us ε.

Importance of ε

for compounds with high ε extinction coefficient, low concentration of probe dye is sufficient for detection

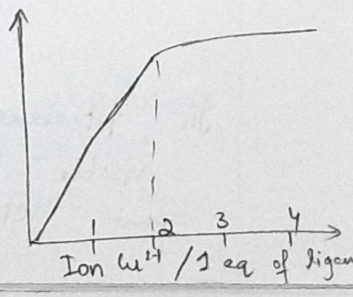
$\uparrow \varepsilon \Rightarrow \downarrow c$ sufficient

Colorimetric sensing of metal ions

A compound was synthesized which had a chelating effect with only copper. i.e if changes color when it binds with Cu^{2+}.

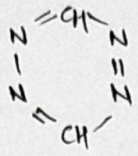
Absorbance reaches a maxima at $\frac{Cu}{ligand} = 2$

IK 2 ligands bind to 1 Cu

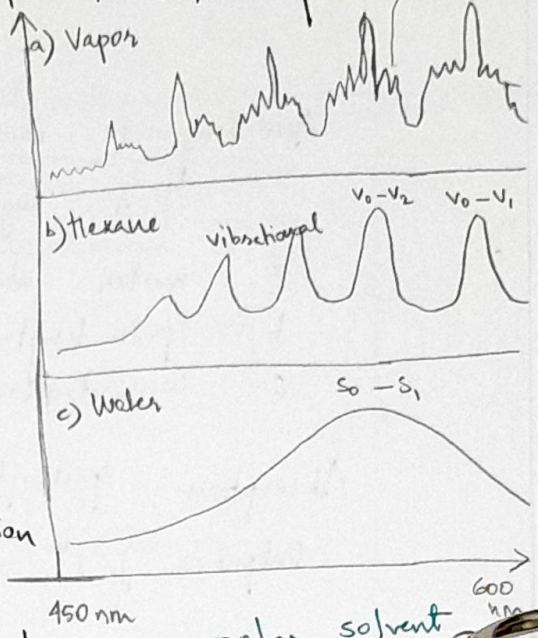


The absorption spectrum varies with the state of the analyte.

Example: Tetrazine



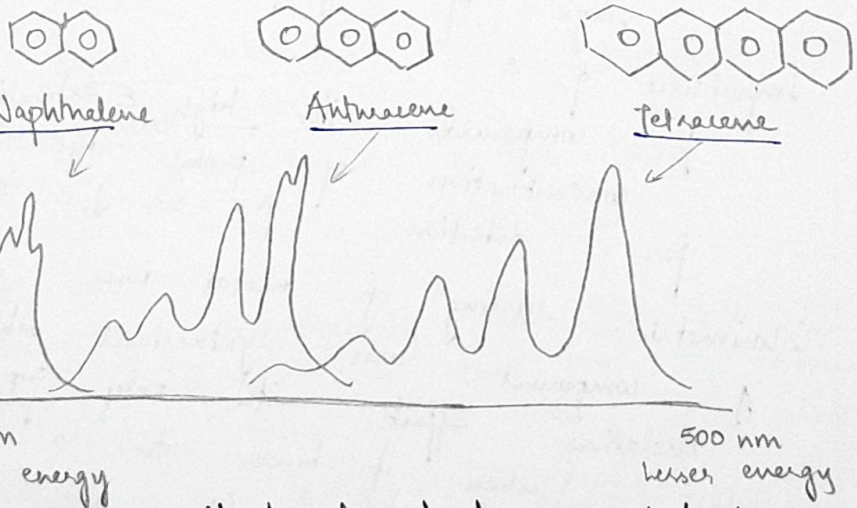
- In the gas phase, the molecules retain the memory of information of rotational and vibrational excitations. So the spectrum is very sharp and of high resolution



- In non-polar solvent, it loses some of it due to interaction with solvent molecules, so rotational energy peaks are lost — non-polar solvent
- In polar solvent, the interactions are greater still
  ⇒ only electronic excitation can be observed (as a broad band). Here, solute-solute interactions also comes into picture hence internal features of vibronic and rotational levels are also lost

Rigid aromatic structures

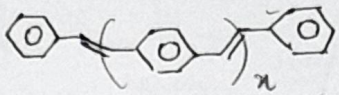
They're not prone to change their structures easily.



In tetracene, all vibrational levels are distinctly visible because those levels are energetically far apart. Whereas, in naphthalene, they're bunched together

vib levels

Thermal relaxation – energy used for vibrational & rotational transitions. not electric.

For eg: In  For these molecules, vibrational levels (transitions) can't be observed because they're very flexible. i.e. these levels can only be observed in rigid molecules

Applications

1. Detection of toxins, heavy-metal ions
2. Kinetics of reaction, drug release profile
3. Isomerisation
4. Structural changes
5. Intermolecular interactions (aggregates)
6. Supramolecular assemblies (micelles)

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Lecture 18

Spectroanalytical chem – Emission spectroscopy

Radiative decay
- Photoluminescence
- Electroluminescence
- Chemical reaction (chemiluminescence)
- Biological system

Non-radiative decay
- Isomerisation
- New product formation

When a molecules is excited to a higher energy level, it tries to come back to the ground state by releasing energy.

The emission depends on form of the energy given to the molecule – light (photons), electricity. given chemical reaction, biological system.

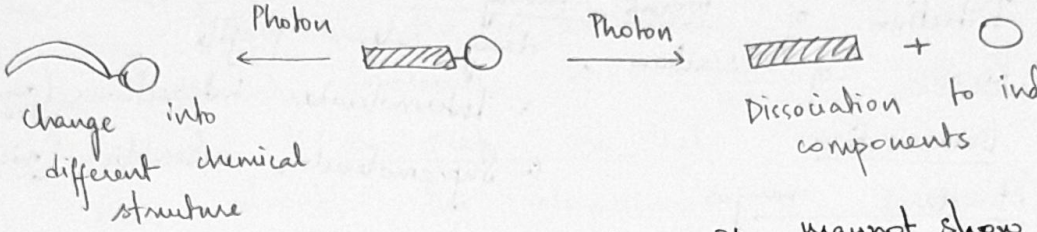
→ Non-radiative Process

Most metal salts have strong absorbance but they donot show No emission due to fast thermal relaxation, except some lanthanides

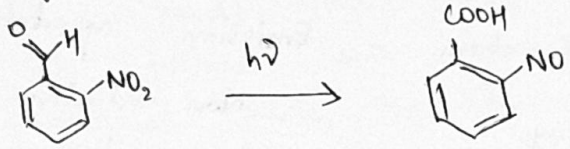
Some lanthanides (Eu, Sm, Tb) show emission due to electronic transitions among well-defined d-f orbital transitions.

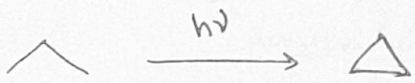
* Aliphatic or olefinic – non-luminescent
* Azo-benzene compounds – they're mostly non-luminescent, they use the excitation energy for isomerization

Photoswitching (non radiative)



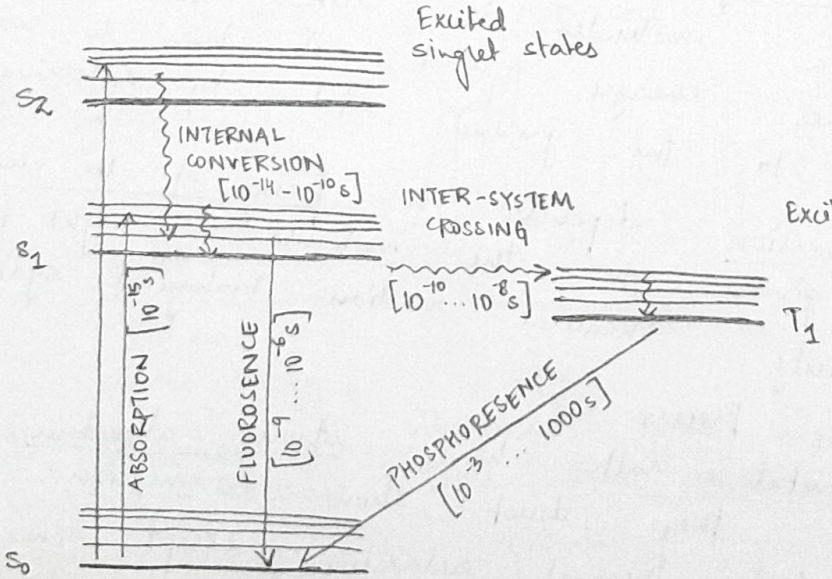
Newly generated species may or maynot show emission

Eg: 

Photocyclisation : 

luminescent — non-luminescent

Jablonski energy diagram

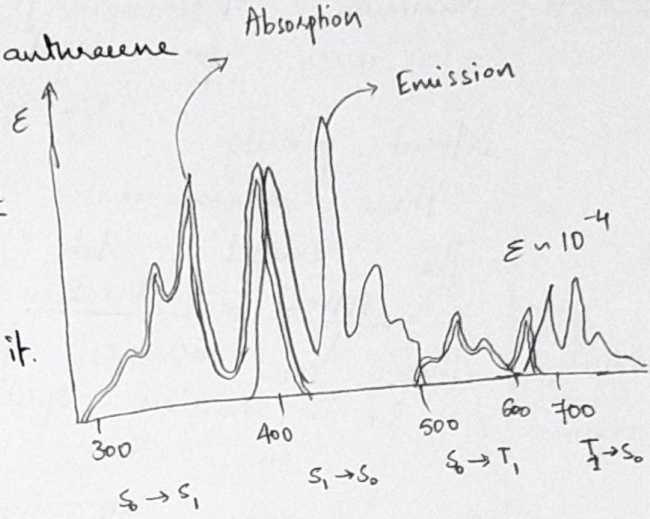


Note the difference in time scales of various processes

Phosphorescence occurs rarely, at very few intensity.

Eg: Emission / Absorption of anthracene

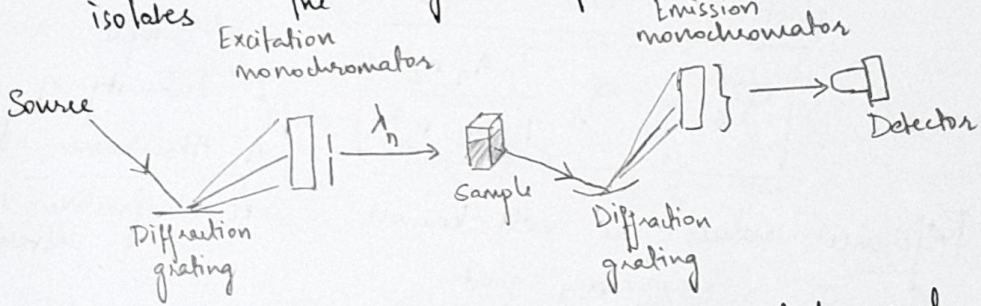
$\varepsilon_{max} \approx 10^4$



Intensity of phosphorescence is negligible unless the molecules is specifically prepared for it.

Instrumentation

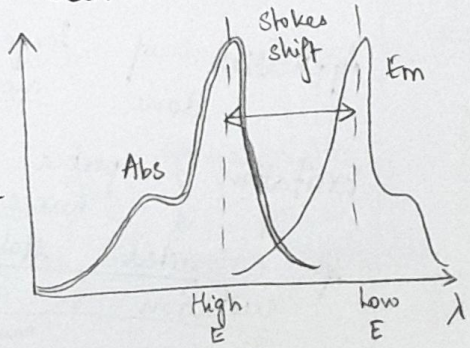
The instrument has 2 monochromators – that isolates the light of a particular λ.



First absorption spectrum is recorded and λ_{max} (wavelength of max absorption) is identified.

λ_{max} is isolated through excitation monochromator and passed through sample. The resulting emission (λ & intensity) is scanned and detected.

Stokes shift For a molecule which undergoes radiative decay, the difference b/w maxima of absorbance & emission is called Stokes shift.



The shift is a reflection of energy lost in internal conversion (i.e. lower vibrational level).

The abs & em spectra may or may not be symmetrical, depending on the molecule.

yes for rigid molecule

Minimum stokes shift ⇒ Max quantum yield
↳ lower for rigid molecules.

↑Rigidity – ↓Stokes shift – ↑ Q. yield

Solvent polarity

These measurements are carried out in some solvent.

The excited state loses some energy through solvent relaxation

↑Polarity – ↑ λ_{em} – ↑Stokes

The λ_{em} increases from apolar → polar solvent.
i.e. emission spectrum actually varies depending on the solvent ⇒ stokes shift increases.

Quantum Yeild – Efficiency of photon emission

Heuristically: If 100 photons are absorbed & 100 photons are emitted, then QY is 100%.

$$\Phi_s = \Phi_r \frac{I_s A_r n_s^2}{I_r A_s n_r^2}$$

s - sample r - reference
I - Intensity of emission (area) peak
A - Absorbance (optical density = 0.1)
n - refractive index of solvent

Reference values are well-known

$\Phi_s > 85\%$ very good

$\Phi_s > 20\%$ is acceptable

- Excitation λ of sample & reference should be same
  Emission collection region – preferably same
- Measurement – fresh samples should be used

Application of large stokes shift – measurements can be done more reliably. Max shift – 210 – 250 nm

Excitation spectra – similar to absorption spectra*
It is necessary to make sure that molecule in excited state is structurally similar. If it is so if excitation & absorbance spectra match – true for rigid molecules, not for photo-switchable molecules

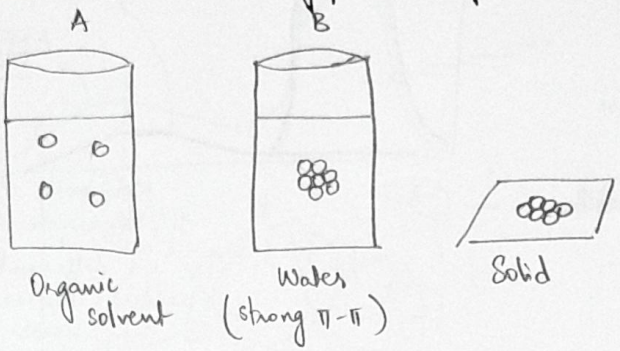
* measured by molecular spectrophotometry

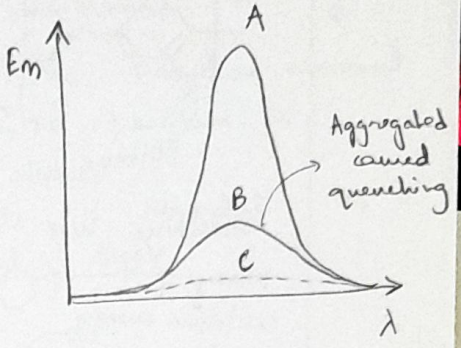
Lecture 19

Aggregates and Resonance Energy Transfer

Factors influencing emmission properties

- Intermolecular interaction (Aggregation)
  - ✓ Quenching
  - ✓ Induced emmission
- Resonance energy transfer

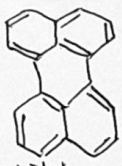




The aggregate traps the energy from radiation and quenches the emmission.

In solid state, almost no emission is observed

Example : Perylene



(poor solubility) → It dissolves in DMSO and has a bright red fluorosene, but it aggregates in water rich environment.

Most aromatic dyes undergo strong π-π stack interaction which promotes aggregates in aqueous medium.

They behave as quenching pockets and provide the pathway for decay of excitation (non-radiative pathway).

So one should be careful while using these dyes as indicators

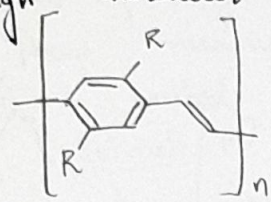
This feature is also important for polyaromatic anticancer drugs. (Eg. Doxorubicin)

So, low quantum yeild in water ⇒ bio-imaging application is limited

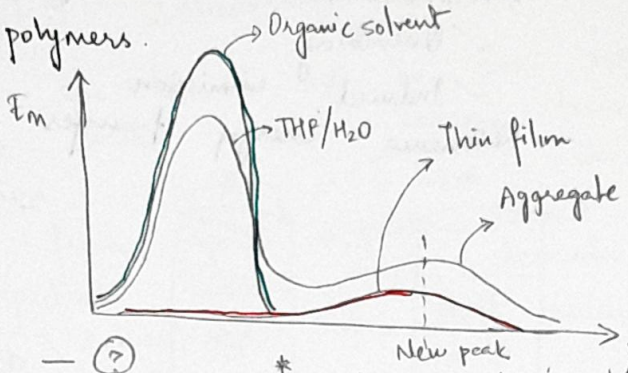
When the drug goes into the cell, it inserts into DNA and becomes isolated. So the Q. yield increases and imaging is useful

Note: Binding with intracellular species may enhance or decrease Fluorescence intensity.

High molecular wt. polymers.

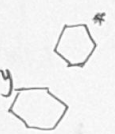


Stilbene

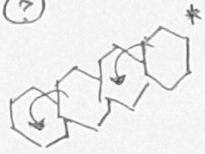


Something here in slides — (?)

Decay of excitation energy not easy



ISOLATED

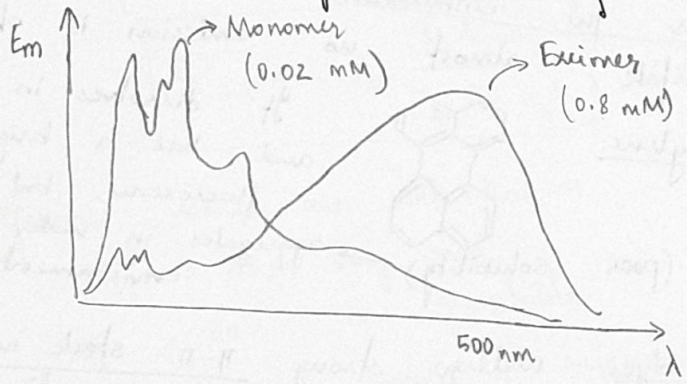


AGGREGATE

If aggregate is stable, its a different mol. than parent molecule. So they have their own emission — new peak

Pyrene – Increasing conc ⇒ forms excited dimer (Excimer)

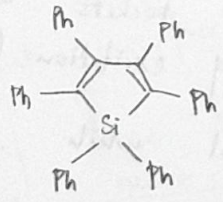
↑ conc.



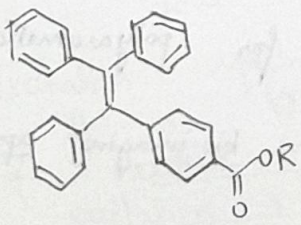
AGGREGATE INDUCED EMMISSION

* Emmission highly depends on molecular properties.

Another eg.



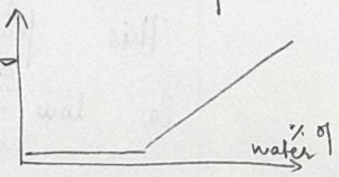
No fluorescence in DMSO (isolated) ∵ excitation energy is used for molecular rearrangement



Aggregate: Restriction of intramolecular rotation, so energy is used for radiative decay

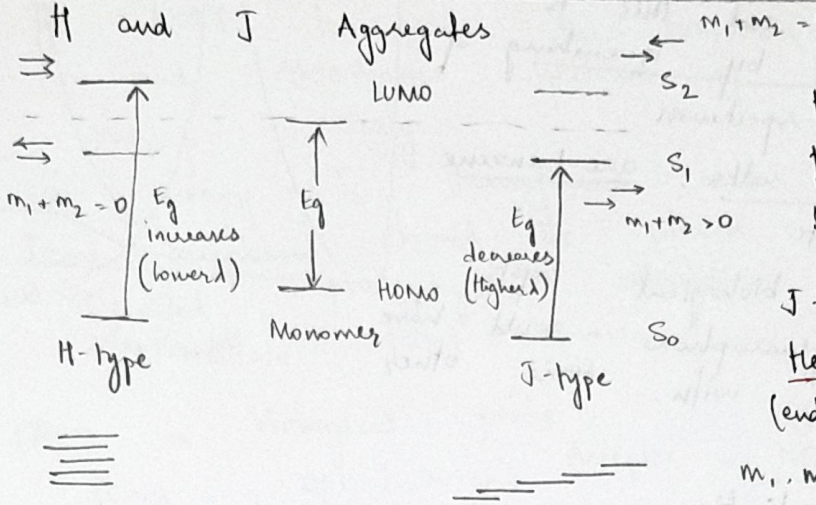
In high H_2O

v. important in biological studies



$m_1+m_2 > 0$

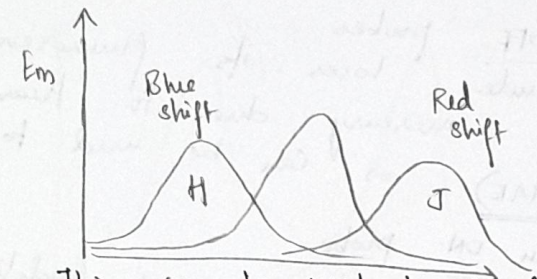
H and J Aggregates



H-type
Face-to-plane stacking like sandwich arrangement

J-type
Head to tail (end to end) stacking

m_1, m_2: transition moment
$m_1+m_2 > 0$ transition is allowed
Molecule is regarded as a point dipole



This can be used to classify aggregates

Eg: Thionine dye - H aggregate (1984 study ~~dye~~)
Bisazomethine dye - J aggregate

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Lecture 20

Resonance Energy Transfer

When you excite the molecule, it goes to higher energy state. This energy could be transferred to other molecules — RET.

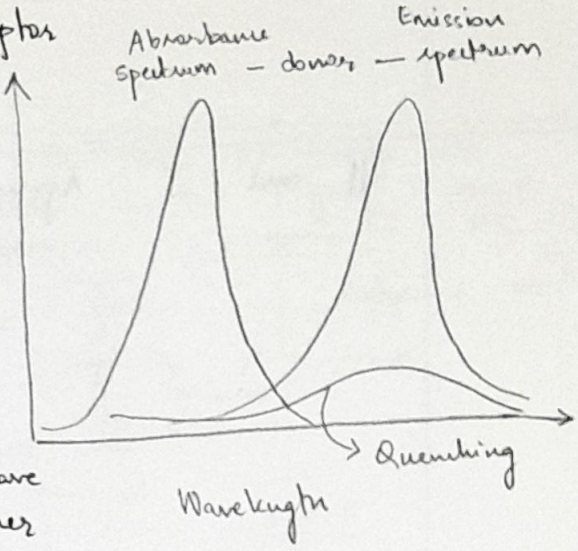
If a molecule (acceptor) is in the vicinity of excited molecule, then the energy can be trapped. If acceptor is —

- Non luminescent : no emmission from acceptor or donor
- Luminescent : No emission from donor
  Yes emission from acceptor.
  ↳ Higher wavelength region

Case I : Non luminescent acceptor

Excitation energy is transferred to acceptor and this is confirmed by quenching of emission spectrum

Eg: Metal salts, azo-benzene etc



Caution: In biological expts, the fluorophore could have integrated with some other molecules

Eg of application

★ Turn ON → Turn OFF probes

Here the molecule loses its fluorosence in presence of mercury due to Heavy Atom Effect (HAE). ⇒ Can be used to detect Hg

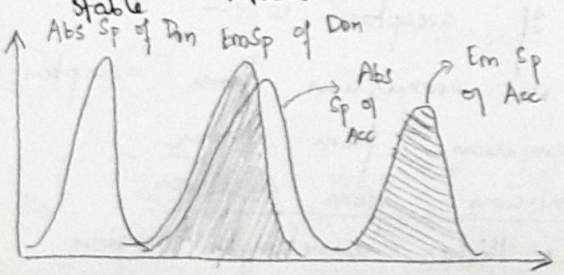
★ Turn OFF → Turn ON probe

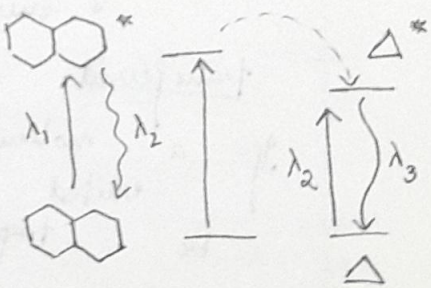
Doxorubicin in presence of conjugated Gadolinium doesn't show fluorosence and this compound is stable outside the cell.

When absorbed the conjugation is broken and the drug becomes fluoroscent thus indicating uptake efficiency of cell.

Case II - Luminescent acceptor

The S_1 (excited) state of donor and acceptor overlap significantly. But stable states need not overlap.





Fluoroscent Resonance Energy Transfer (FRET)

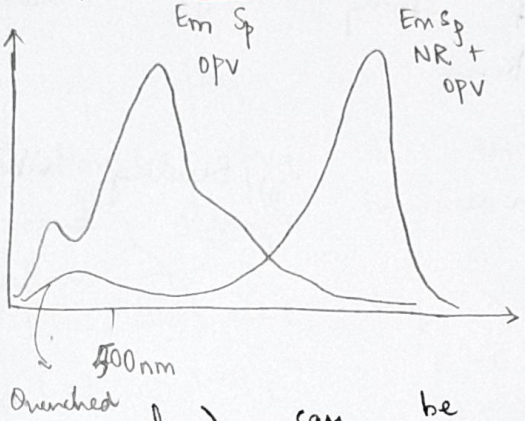
$\lambda_1 \ll \lambda_2 \ll \lambda_3$

FRET → Forster or Fluorescent
↳ Discovered FRET

For FRET to occur, the Emission spectrum of donor and Absorbance spectrum of acceptor should overlap nicely.
So energy is transferred and donor emission is not visible
Donor acceptor should be placed < 10 nm distance
Detailed photophysical calculations are done to validate FRET process.

FRET in biological probe

Donor – OPV (~401 nm) Acceptor – NR (~600 nm)



Em Sp of OPV overlaps well with Abs Sp of NR.
So this pair can be used to visualize cells
Similarly binding of analyte to a protein (physically close) can be visualised through FRET.
If analyte dissociates, then we'll see a different color than when bound

Two photon Excitation → Being nice to cells
Instead of using 1 photon of 300 nm, 2 photons of 600 nm are used so that total amt. of energy will be the same, and exitation will occur (in some molecules) and cells won't be damaged.
↳ Same emission spectrum.

(16)

Metal-to-ligand transfer, Some inorganic salts (of lantanide elements) show fluorescence, but their absorptivity is very low. So energy is transferred from ligand to metal and emission spectrum can be analysed to see D-F transitions.

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(?)

Lecture 21

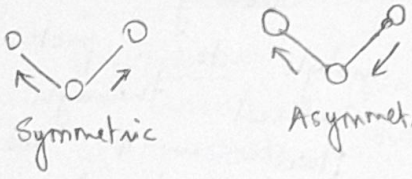
FTIR Spectroscopy

Fourier Transform IR Spectroscopy → (Vibrational)

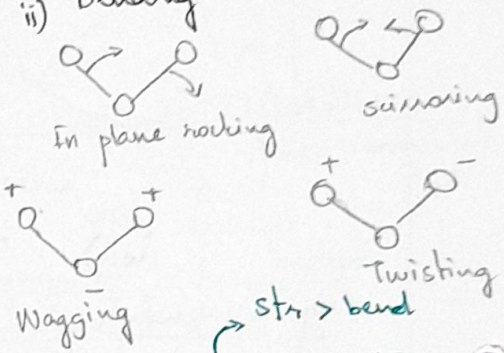
IR radiation mostly causes vibrational transitions, as they're not strong enough to cause electronic transitions

Types of transitions

(i) Stretching vibration



ii) Bending vibration



Stretching absorptions occurs at higher freq. in IR than bending absorption for same bond (str > bend)

Determination of Vibrational frequencies

Based on Hooke's law, correlates freq with bond strength & atomic masses

$$\nu = \frac{1}{2\pi}\left(\frac{k}{m_1 m_2/(m_1+m_2)}\right)^{1/2} \qquad \nu = \frac{1}{2\pi}\sqrt{\frac{K}{\mu}}$$

k: constant related to strength of bond

m_1, m_2: atomic mass of atoms

Reduced mass: $\mu = \frac{m_1 m_2}{(m_1+m_2)}$

In FTIR, stretching freq are represented in wavenumbers ($\bar{\nu}$) – in cm^{-1}

Theoretically, C-H : 3100 cm^{-1}

In benzaldehyde, aromatic C-H : 3073 cm^{-1}

Aldehyde C-H : 2827 cm^{-1}

Absorptions in 600 to 1800 cm^{-1} region is dependent on molecular environment – its called the Fingerprint region – unique to the molecule

Table of freq.

Vibrational freq is expected to increase when bond strength increases and when μ decreases

Predictions –

* C=O and C=C freq > C-O and C-C freq *
* C-H, O-H > C-C and C-O
* O-H > O-D

X-H freq in C-H, N-H, O-H & F-H

May be greater ∵ increased electronegativity

Without accurate values of k, we can't predict more.

FTIR instrumentation

Earlier – Sample + KBr pellets

Now – ZnSe crystal (reference) + 1 drop of sample

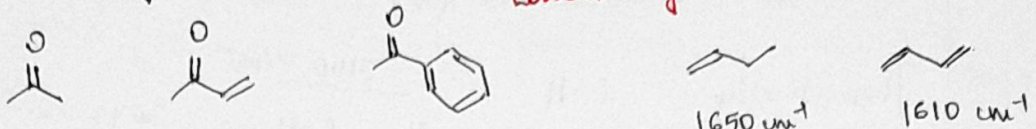
Now its very small & convenient.

(?) For gaseous phase analysis –

FTIR : Conjugation Effect

Conjugation lowers the stretching freq. of C=C and C=O, whether conjugation is brought about by α,β unsaturation or aromatic ring

Lowers by 20-30 cm^{-1}



1720 cm^{-1} 1700 cm^{-1} 1700 cm^{-1} 1650 cm^{-1} 1610 cm^{-1}

(iii)

Resonance effect

Any substituent that enhances resonance shift will decrease C=O str freq.

Conjugation in (iii) does that.

Reaction monitoring

Click chemistry reaction. Zoned out.

Rewatch : n.35 mins — Slide 12

Functional groups have specific peaks, so if they change ⇒ reaction is happening

Amides and esters

Conflict b/w -I to +M ~~gro~~ effect.

+M ~~increases~~ weakens C=O bound i.e decreases freq

but -I increases freq

Usually, +M effect is dominant

H-bonding interaction

H-bonding involves lengthening of original O-H bond. i.e bond is weakened ⇒ stretching freq is lowered

H-bonding is especially strong in enols and chelates, so O-H freq. are significantly lowered.

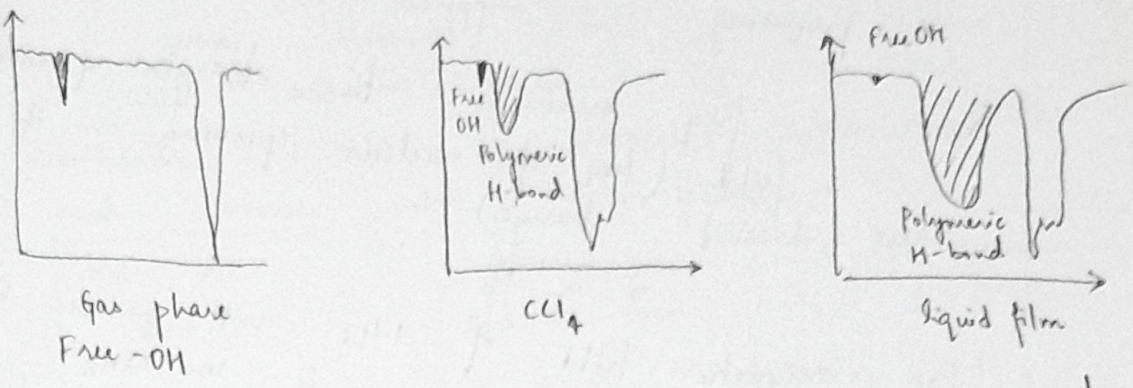
No variation of peak position

Intramolecular bonding will not change with conc, but intermolecular bonding will go down at lower freq ⇒ the peak will shift.

conc? at varying conc

→ only intensity of peak will change

1 - hexanol



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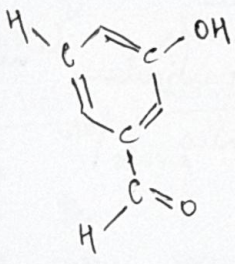
Lecture 22

NMR Spectroscopy — differentiates b/w ~~pots~~ isomers.

All other previous methods can't effectively differentiate between isomers.

NMR — most powerful method to figure out the structure of molecules



The protons are different ∵ the atoms they're connected to are different

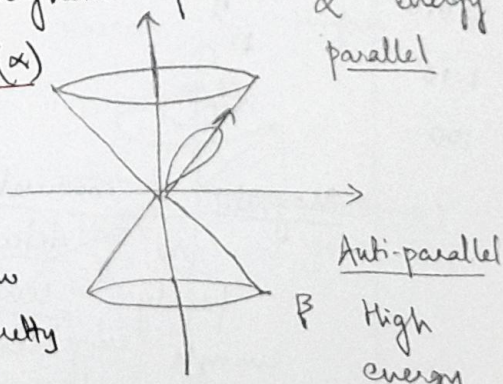
⇒ Their generation of magnetic field must be different

If we can differentiate, we can discern the structure

Theory

Like bar magnets, the proton will spin around the axis of B_0 applied magnetic field

The orientation can be along (α) or away (β) from direction of applied field



There's an energy differene b/w there two states — it's pretty small ($\sim 10^{-4}$ kJ mol^{-1}) — so population of protons in both states are pretty equal

Acc to Boltzmann distribution, at 1.4 T, α state is 0.0001% greater than β.
It will change if energy of correct frequency is supplied

Precession freq increases with strength of magnetic feild (freq of nucleus spinning on its own axis doesn't change) —

$$\nu \propto B_0$$

Ext magnetic feild of 1.4T will precess $60 \times 10^6\ s^{-1}$
For 2.3T, ν = 100 MHz
14.1T, ν = 600 MHz (Superconducting magnets)

Superconducting magnets were developed in 1940s. So NMR wasn't possible until then

$$\nu = \frac{\gamma B_0}{2\pi}$$

$$\gamma = \frac{2\pi \mu}{h I}$$

γ : Gyromagnetic ratio
μ : Nuclear magnetic moment
I : nuclear angular moment

γ is characteristic of a particular nuclei
So precessional freq (ν) varies for different nuclei.

Isotopic abundance	Atoms	γ value
99.985	^1H	26.8
0.015	^2H	4.1
1.10	^{13}C	6.72
100	^{19}F	25.2

$\Rightarrow$ ^1H : ^{13}C ≈ 4 : 1

$\frac{\gamma_H}{\gamma_C} \simeq 4$

Magnetic moments of ^1H and ^{19}F are relatively large, so detection of NMR with these nuclei is fairly sensitive, and less conc of sample is enough because isotopic abundance is great.
But for ^{13}C, high conc of sample should be provided to detect a good signal AND more no. of scans

NMR Active Nuclei

For every element, only some isotopes are active, because spin quantum number $I > 0$. Its associated with mass no. and atomic no. as follows —

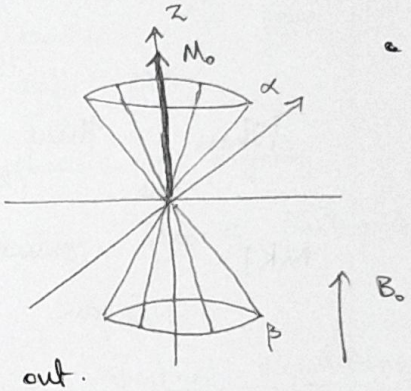
Mass no.	Atomic no.	Spin quantum no.
Odd	odd or even	$\frac{1}{2}, \frac{3}{2}, \frac{5}{2} \ldots$
Even	Even (NOT ACTIVE)	0
	Odd	1, 2, 3 ...

^{12}C and ^{16}O have $I = 0$ i.e. non-magnetic

Table of precessional freq for a set of magnetic feild values.

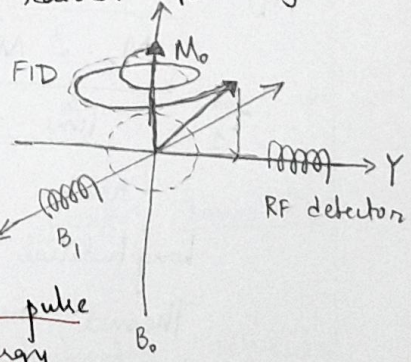
NMR Instrumentation

There is a slight excess of parallel states, so there's a net magnetization vector, M_0 along the direction of the feild.



XY plane components get cancelled out.

How to apply radiofrequency (rf) electromagnetic energy to protons and make sure its absorbed as protons are raised to higher spin state ?



Pulsed - Fourier Transform method

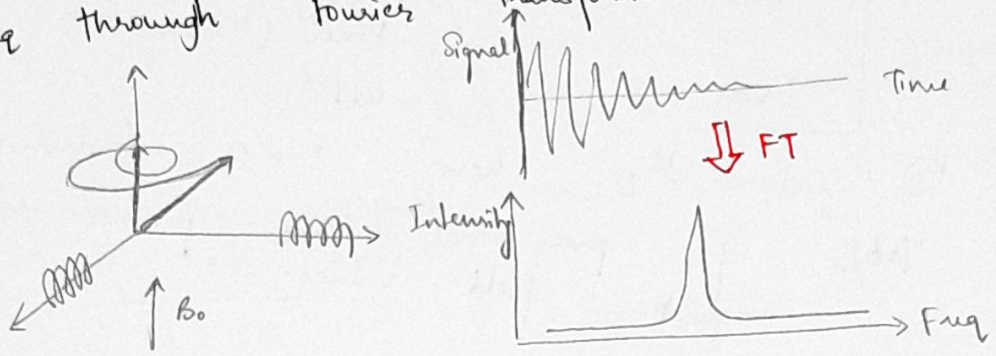
Sample is placed in a magnetic feild and irradiated with short pulse of high power radiofrequency energy

This excites all nuclei of given type.

When pulse is applied, a torque is exerted on M_0 and it'll be tipped towards XY plane

Magnetic component ← It is detected by a detector in XY plane as a function of time. The excited spin precesses around B_0 creating a current in the detector coil. The resulting signal, known as Free Induction Decay (FID) is recorded and digitized by a computer

FID is converted to a readable spectrum in freq through Fourier Transform



SINGLE PROTON FID

When there are multiple protons, the signals are extricated and spectrum is obtained.

MRI – Same principle, but instead of spectrum, an image is procured.

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Lecture 23

NMR – Chemical shift & anisotropic effect

FID can be solved by Bloch equations

$$M_z = M_0\left(1 - e^{-t/T_1}\right)$$

T_1 : Time required for z-component of M to reach $(1 - 1/e)$ or 63% of its max value (M_0)

Longitudinal relaxation
Thermal relaxation
Spin-lattice relaxation

[Highly influenced by viscous solution]

$$M_{xy} = M_0 e^{-t/T_2}$$

T_2 : spin - spin relaxation.

Chemical Shift

The frequencies of protons are measured in relation to Trimethylsilane (TMS - internal standard)

TMS - inert

Resonates at 60 MHz when $B_0 = 1.409$ T

-OH signal is 144 Hz higher in comparison

$-CH_2$ is 276 Hz higher in 60 MHz

2760 Hz higher in 600 MHz machine

$\therefore \nu \propto B_0$

To standardise this, δ units are used - Chemical shift is expressed in δ units, defined as proportional differences in ppm from an appropriate ref. std.

$$\delta_x = \frac{\nu_x - \nu_{TMS}}{\nu_0}$$

Chemical shift in ppm

Frequencies of signals

Operating freq of instrument ↑ MHz

Higher MHz instrument ⇒ Good spectral resolution even though there's no change in chemical shift.

Solvent selection & sample handling

- Sample must be soluble.
- 5 - 10 mg of compound in 0.5 mL of solvent in a 5 mm glass tube
- Ideal solvent — no protons, inert, low bp and inexpensive.

Eg: Chloroform - d & other deuterated solvents

Deuterated solvents

Modern instruments depend on deuterium signal to "lock" or stabilize the magnetic field.

Typically, ^1H NMR signals are in range of 0.1 to 5 Hz, so B_0 feild needs to be stabilized and uniform.

Modern instruments have deuterium channel that constantly monitors and adjusts B_0 to the freq of deuterated solvent.

Instruments use small electromagnets (20-40 'shims') to adjust B_0 so the feild is homogenous.

Peak intensities

Protons appear at different δ based on structure

Area under the peak $\propto$ no. of protons

The spectra is normally integrated, area under peaks is computed and line with steps corresponds to the area.

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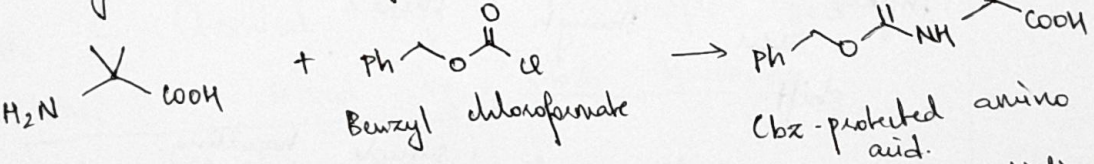
Lecture 24

Small peak at 7.2 – leftover amt. of $CHCl_3$ in deuterated chloroform.

Peak integration – confirmation of product



TLC and FTIR are not useful in differentiating the product.

So peak intensities allow us to characterize the molecule and hence confirm the product with much greater accuracy

Factors influence chemical shift

Inductive effect – with increase in χ of functional group attached to $-CH_3$, the protons become resonant at higher ppm values

This is because of change in electron density around the proton nuclei

The electron density, to some extent, shields the nucleus from the influence of B_0.

More χ functional group → Less electron density & sheilding → More chemical shift ↑χ ⇒ ↑δ

Extent of sheilding lowers the precessional frequency which in turn lowers δ.

Sheilding – they create a magnetic feild through the movement of electrons.

Greater ⊖ inductive effect ⇒ greater desheilding effect

Eg: F > Cl > Br > I

CH_3-Li : −1.94

CH_3-TMS : 0

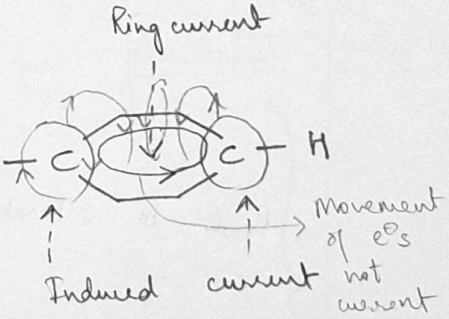
CH_3-OH : ~3.4

CH_4-F_3 : ~7.5

Anisotropic effect

Aromatic-ring system

π-electrons are delocalised & they are induced to circulate in presence of B_0, such that they produce an electric current.



Magnetic feild created – opposing B_0 in the ring centre whereas augmenting B_0 outside the ring.

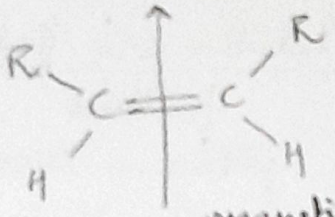
So the protons in the periphery are greatly desheilded because of increased B. δ = 7.26 ppm

Effect of function group –

-I & EWG : > 7.26

+I & EDG : < 7.26

★ Alkenes



Plane of alkene is 90° to the applied magnetic field.

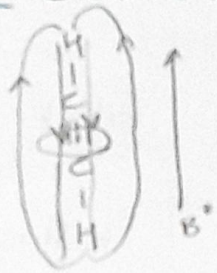
Circulation of π-electrons creates a magnetic field that shields C atom but deshields H.

So δ is greater.

★ Alkynes

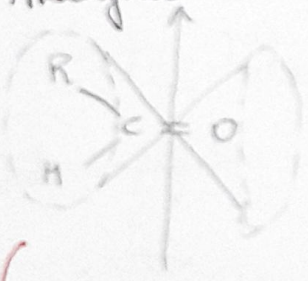
They is less shifted (more sheilded than alkenes)

This is because alkynes are linear and the molecule aligns itself along the external B_0.



The electrons circulate so that the induced magnetic field opposes the external. thus sheilding the protons.

★ Aldehydes



Here too, like alkenes, the proton falls in deshielded region.

Thus, oxygen is electronegative

So δ of this proton is pretty high

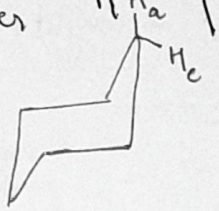
Bond to saturated C	Not next to O	0 - 3.0
	Next to O atom	3.0 - 4.5
	Alkenes	4.5 - 6.5
Bond to unsaturated carbon atom	Aromatic	6.5 - 8.5
	Aldehyde	8.5 - 10.5

Anisotropic effect

The geometry of the molecule can significantly affect δ of protons

Eg: Inner & outer H_a of annulenes

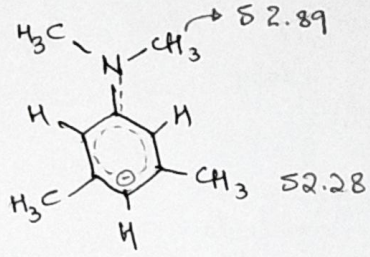
* Cyclohexane :



H_a : axial - shielded
δ 1.14

H_e : equatorial - deshielded
δ 1.62

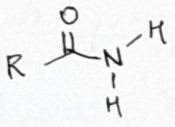
* Resonance effect :



* Acidic protons

The more acidic the proton, more the bond is polarised towards O atom in OH ⇒ no shielding

⇒ -OH chemical shift and acidity of OH are related



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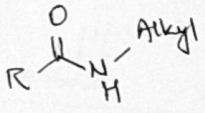

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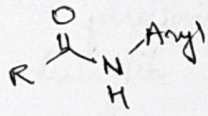

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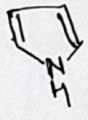

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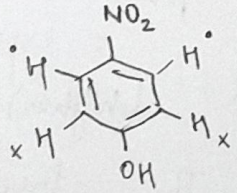
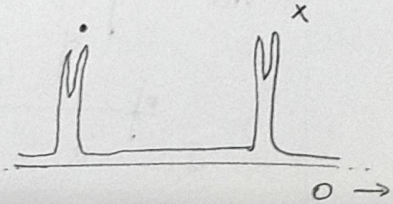
δ_{NH}

↑ Acidity ⇒ ↓ shielding ⇒ ↑ δ

Again, NMR is important for structural analysis.

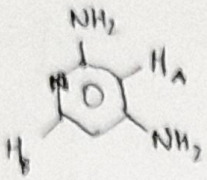
Spin-spin coupling : The magnetic moment / spin of neighboring proton interferes with the particular proton, so the peak appears as a doublet ie a split peak.



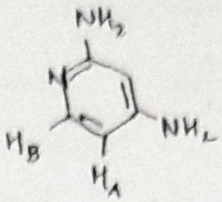


Lecture 25

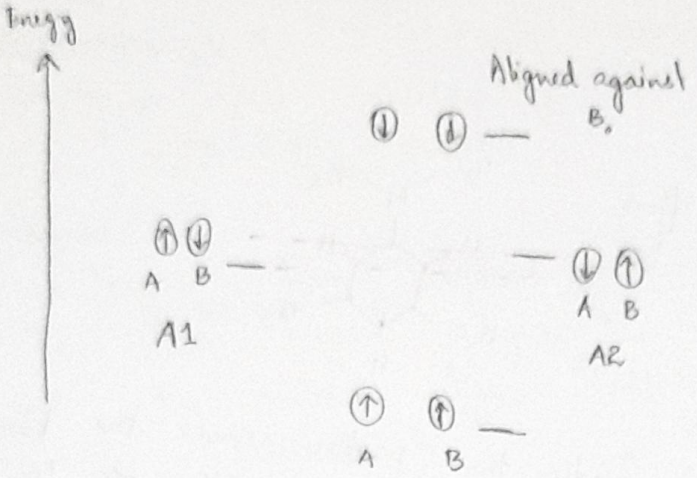
Spin - Spin coupling



Here, each H is far apart & doesn't influence each others' spin — singlets



Here, both protons produce doublets



Exciting H atoms can change alignment in 2 ways — A1 and A2.

Similarly for B also there a slightly different energy levels

Result is two resonances very close together in the spectrum.

So each proton gives 2 lines (doublet) in the NMR spectrum and splitting of two doublets is exactly the same.

For H_A, 50% go towards A1 (lower than expected resonance) and other 50% towards A2 (higher) but average remains the same.

The frequency difference (in Hz) between 2 peaks of a doublet is proportional to strength of coupling & denoted by coupling const. J

J is reported in Hz.

J depends on the machine MHz. To measure coupling const. measure distance b/w lines and multiply by ν

$$J = [0.136 \times 10^{-6}]_{ppm} \times [90 \times 10^{6}]_{MHz} = 12.3\ Hz$$

Aromatic coupling – 8.8 Hz
Trans (olefin) coupling – 12.3 Hz

J values <u>don't change</u> with applied frequency. i.e. for lesser MHz, the peaks are closer together.

The spin of neighboring electron affects the precessional freq of a particular proton if their spins donot align. (one ↑, one down)

In a proton has <u>n neighboring</u> protons, it will show <u>(n+1) splits</u> in its peak.

Doublet height : 1 : 1
Triplet height : 1 : 2 : 1

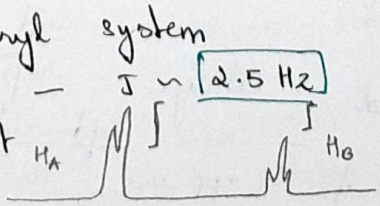
∵ 2 neighboring protons can have config – ↑↓ ↓↑ ↑↑ (x_1 x_2)

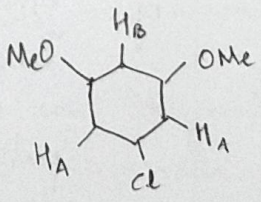
Pascal diagram – tells us about the intensity of peaks of spit / multiplicity. Pascal's triangle

Quadruplet – 1 : 3 : 3 : 1
Quintuplet – 1 : 4 : 6 : 4 : 1

Examples – assigning protons to peaks bis-substituted aryl compounds. (ortho-coupling) 8-9 Hz

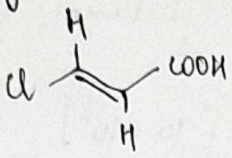
<u>Meta-coupling</u> in aryl system – J ~ 2.5 Hz
<u>Weak coupling</u>
Meta coupling might not be seen.



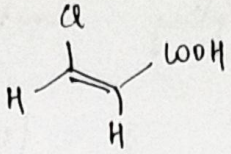


In meta-coupling, does coupling happen through space or bond?

Coupling is a through-bond effect



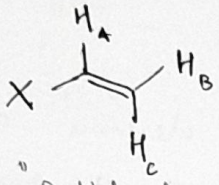
J = 15 Hz



J = 9 Hz

This has a extended conjugation system

If coupling were through space, the J_{cis} would be greater.

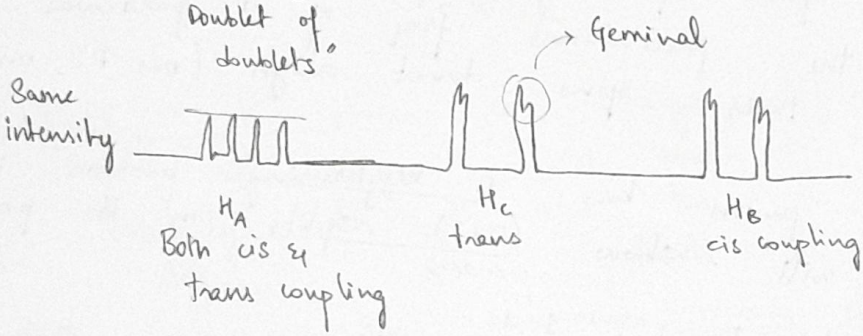


"Doublet of doublets"

$H_A - H_B$ = cis : 10 - 13 Hz

$H_A - H_C$ = trans : 14 - 18 Hz

$H_B - H_C$ = geminal : 0 - 2 Hz



Hydrogen bonding interaction

It decreases e⊖ density around H atom.

So its δ value will increases by 0.5 - 4 ppm in $CDCl_3$

because its getting deshielded.

Because of this phenomena, δ value depends upon concentration, temp. and polarity of solvent

Intramolecular H-bonding is not dependent on conc.

Eg: Ethanol in $CDCl_3$

There is some acid impurities develop in $CDCl_3$ and catalyze rapid exchange of hydroxylic proton — so hydroxylic H doesn't couple and it appears as a singlet.

Ethanol in DMSO — H bond is formed and hydroxylic -OH stays in place. So, the kind of peaks formed (of middle $-CH_2$) and δ of -OH are both in different

Keto-enol interconversion

Predominantly enol form exists. but abt 30% of keto form also exists. So both peaks show up in NMR — both forms are observed.

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Lecture 26

Mass spectrometry : small molecules

Mass spectrometer

1. Sample injection (which form)
2. Compound is ionised
3. Ions are seperated on basis of mass/charge
4. It is recorded as a spectrum.

Small organic/inorganic : 300 - 3000 Da
Synthetic polymers/proteins : 5k - 100k Da

Injection : Gas phase (GCMS)
Liquid phase (LC-MS)

Resolution of mass spectrometer

$$R = \frac{M_n}{M_n - M_m}$$

M_n : higher mass no. of 2 adjacent peaks
M_m : lower mass number

Low resolution instrument : R = 3000 - seperates unit mass of about 1 amu

High resolution instrument : R = 20,000 — can differentiate between isotopes in compounds

Gas Phase ionization methods

Uncharged molecule cannot be detected, so it has to be charged

* Electron bombardment — sometimes the molecule gets fragmented on impact

Bombareded with high energy electron (70 eV)

Breaks the covalent bonds (~15 eV) and create radical cation.

* Chemical ionization method.

Pre-ionized chamber of reagent gas (CH_4, NH_3)

The vaporised sample is mixed with reagent gas and gets ionised (~5 eV).

Here, fragmentation is minimised

You may get adducts.

When ionised, sample becomes M^+.

Importance of fragmentation spectrum — to identify and analyse the potential fragments to ensure they're not toxic / harmful

Mass spectrometry detects isotopes

Chlorine : 3:1 of ^{35}Cl and ^{37}Cl

Bromine : 1:1 of ^{79}Br and ^{80}Br

Desorption Ionization methods

This is for large, nonvolatile or ionic compounds

Here, samples are emitted directly from a condensed phase into vapor phase as ions.

* Feild desorption ionisation : Carbon microneedles activate surface, maintained at accelerating voltage & functions as anodes

* Fast atom bombardement (FAB) ionisation : High energy Xe / Ar to bombard samples dissolved in glycerol

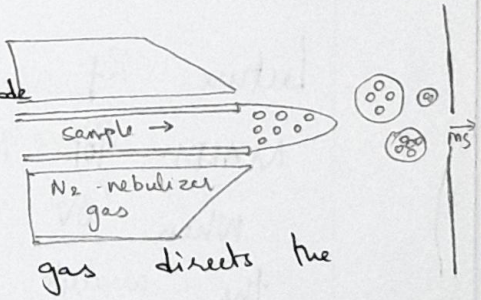
Protects sample from radiative damage

* Plasma desorption ionization : fission products from ^{252}Cf are used for bombardment.
There techniques are used to on molecules of ~ 1,500 amu weight. This was limitation till 1980s.
Nobel in 2002 - Fenn, Tanaka, Wüthrich for developing a process to analyse larges molecules.

→ MALDI - TOF - MS
Matrix assisted laser desorption ionisation - time of flight mass spectrometry.
Pulsed laser beam is used to ionise samples
Sample is on the matrix. Time of flight and m/e ratio are used for seperation & analysis. This method is not applicable for proteins.

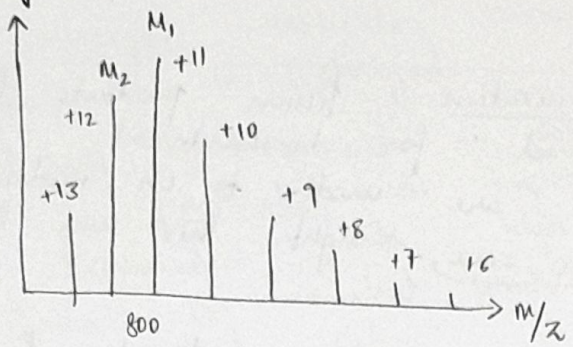
→ Electron spray injection techniques. (ESI) sample particles
Aerosol Droplets with excess charged on surface are sprayed from a nebulizer needle
No fragmentation involved ; works on proteins

Tip of capillary is maintained at high potential wrt counterelectrode
Potential gradient (5kV/cm) creates charged droplets as they exit the capillary. Flow of nebulizing gas directs the effluents towards MS.



Analysis of ESI spectrum of proteins
The droplets can have 2 or 3 or 4 species aggregate, so the graph has different peaks.
How to analyze the charge on the peak?

Highest peak : M_1 Next highest peak : M_2



$$MW = \frac{(M_1 - A)(M_2 - A)}{M_1 - M_2}$$

$$z = \frac{M_2 - A}{M_1 - M_2}$$

MW : mol. wt. of analyte
z : charge state of first ion i.e. highest peak
A : mass of adduct ion (usually H^+, also Na^+ or K^+)

Functionalization of proteins

No clue. Some functional group is added but z value is still the same

So MS is a powerful tool to analyse functionalizⁿ

In 1980s, simple proteins were analysed (17k Da)
Now, tissues also can be analysed (18 MDa)

Now the technology of ionisation method and detector has also been improved.

Lecture 27

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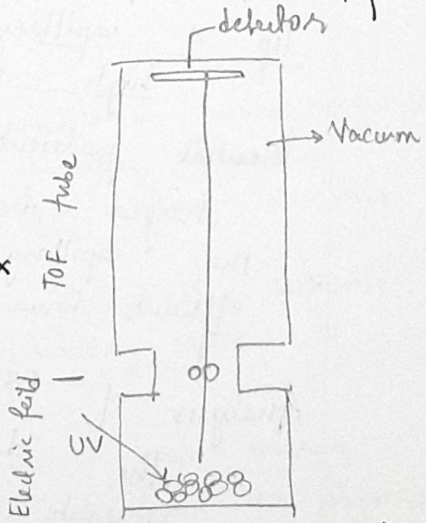
MALDI - TOF MS

When UV laser is incident on the analyte, it gets ionised and jumps off the matrix and travels through the tube. The detector at the end measures the TOF which is dependent on molecule's m/z ratio. Ionised sample jumps ∴ of electric field



Detection

1. Linear detection – its resolution is not good if molecules are of comparable size

2. Reflection detection – this increases the TOF, thus increasing the resolution of similarly sized molecules

$$KE \text{ of molecule} = zeU_e = \frac{1}{2}m\left(\frac{dx}{dt}\right)^2$$

z: charge on molecule; U_e: Electric field; $\frac{dx}{dt}$: velocity

Examples of matrix – THAP, dithranol, 2-5-DHB etc. Chosen mostly on trial & error basis

Introduction to biopolymers

Protein synthesis

L-amino acids $\xrightarrow[+\text{ ribosome}]{\text{mRNA}}$ Polypeptide strand $\longrightarrow$ Protein (3° or 4°)

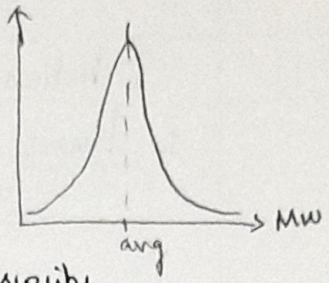
Features: Mono-disperse (uniform size)
Length specific
Sequence specific

Synthetic polymer

Monomers $\xrightarrow{\text{Radicle}}$ Polymer

Initiation
Propogation
Termination

Synthetic polymers are nearly monodisperse — the chain length of polymer varies.



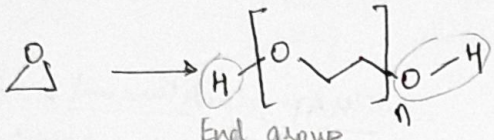
Chain length can be controlled by varying the [monomer] / [initiator] ratio.

Factors influencing chain length

* Increase in polymer conc increases viscosity, slows down chain movement & reduces reactivity
* Slow down the kinetics by decrease in monomer conc. with time produces diff. chain lengths.

Formation of diff chain lengths is an inherent limitation in synthetic chemistry.

MALDI - TOF is used to record the mass of polymer. First spectrum was recorded in 1982



$(44)_n + 18 + 23 = MW$

Na^+

$$n = \frac{\text{Molecular peak} - (\text{End group} + \text{ion})}{\text{Repeating unit mass}}$$

$$n = \frac{2067 - (18 + 23)}{44} = 46$$

The most probable chain length was 43.

End - group Analysis.

After reaction of end group with some reagent, then the peaks of MS spectrum will shift by the expected amount after the reaction. This confirms our end group reaction.

So, MALDI - TOF is a powerful tool in analysing and confirming ~~MALD~~ end groups.

More (lots!) examples of end-group analysis

Lecture 28

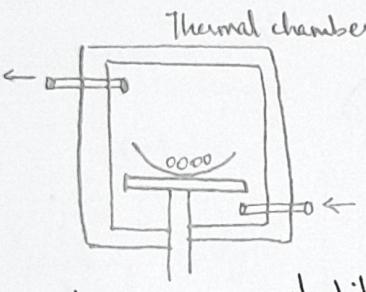
Thermogravimetric analysis

Thermoanalytical analysis — using heated forms to determine a particular quantity of the analyte.

Eg: Copper sulphate : Hydrated form (blue)
Anhydrate (white)

Thermogravimetric : compositional analysis by varying temperature (remove H_2O) and measure weight loss accurately.

$$CuSO_4 \cdot xH_2O \xrightarrow{\Delta} CuSO_4 + 5H_2O$$

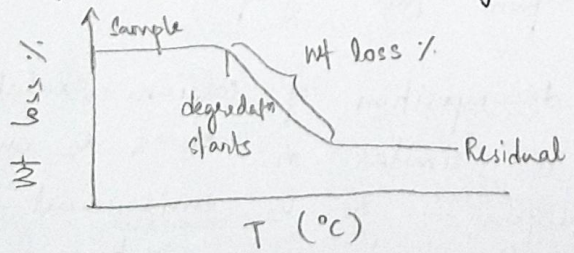


for this we need a programmable thermal chamber and a very sensitive balance

This is used to analyse —

- thermal stability carbon reinforced composites in rockets
- Co-crystal formulation (making a tablet of two drugs) in pharma industry — composition analysis is done
- Pt-drug loading content in cancer drug formulation

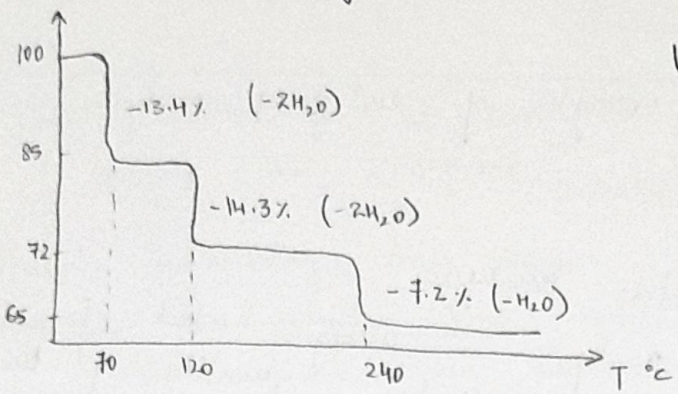
Instrumentation — Thermogravimetric analyzers (TGA)



This is done under N_2 or Ar / O_2 or air

Relatively cheap instruments

$CuSO_4 \cdot 5H_2O$ dehydration



We can see that water removal from hydrated $CuSO_4$ is a step-wise process

Similar graph for $Ca(COO)_2 \cdot H_2O$

$Ca(COO)_2 \cdot H_2O \rightarrow Ca(COO)_2 \rightarrow CaCO_3 \rightarrow CaO$

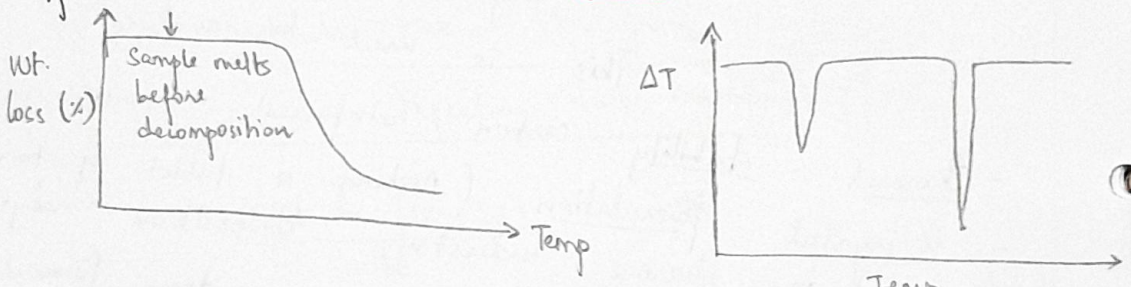
oxalate hydrate

At 900°C

Differential TGA Curve (DTG)

First derivative of TGA curve wrt T or time is plotted so the temp. of breaking down is properly visible

Differential thermal analysis (DTA)



Measures ΔT between sample & reference

Provides info on phase transitions

Enthalpy of phase transition (not accurate) can also be determined from the graph.

Examples: DTA of decomposition of calcium oxalate is similar in N_2 & O_2 environment.

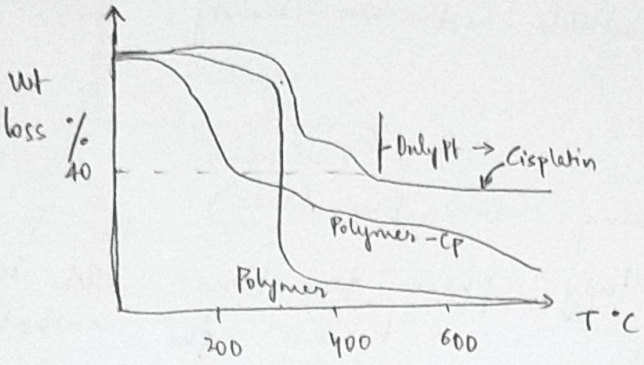
Refer to graphs

But DTA curve differs — In O_2 environment, CO burns to CO_2 which has an exothermic effect.

In N_2, evolution of CO is an endothermic process.

Another example — Aspartame

⇒ Drug core-shell mspr nanoparticles analysis



Polymer degrades at 300°C

At ~600°C, all polymer is lost, so whatever remains is cisplatin

Using TGA, we can analyse the content of the drug

⇒ Metal-organic frameworks

They are used to absorb gases. When TGA is done on absorbed MOF, we can see the temp at which gases are evolved So we know we can't use that MOF above a certain temperature.

Coupling with other instruments

TGA – MS : highly sensitive + very fast measurement extremely small amount can be detected & characterised

TGA – FTIR : high chemical specificity + fast measurement Characterises using functional group

Enthalpy of degradation & transition – quantitative analysis

DTA measures ΔT whereas Differential scanning calorimetry (DSC) measures the heat flow.

The molecule is not degraded here. The enthalpy of phase transition can be calculated

Eg: enthalpies of melting of pharma molecules

Also useful to calculate enthalpy of various molecules based on their packing efficiency.

For polymers, when packing efficiency is very good, ΔH_m will be high and mechanical properties are very good. $\uparrow H_m \Leftrightarrow \uparrow$ packing efficiency

Eg: Polyethylene. $T_m = 130^\circ C$
Gaining enormous mechanical strength
Flexible, expandable and low density

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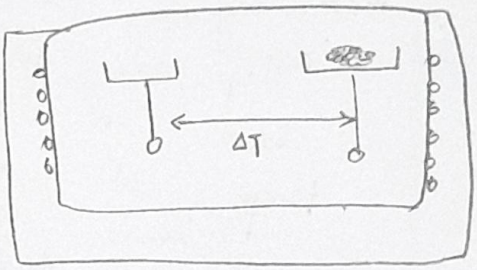
Lecture 29

Differential Scanning Calorimetry (DSC)

Its used to study phase transitions. TGA is a prequisite for DSC because the sample shouldn't degrade



Heat flow is amt of heat supplied per unit time

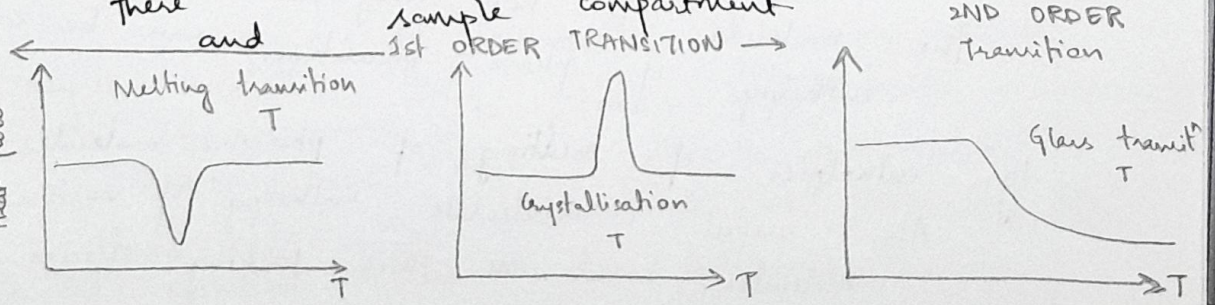
$= \boxed{\frac{q}{t}}$

Heating rate $= \boxed{\frac{\Delta T}{t}}$

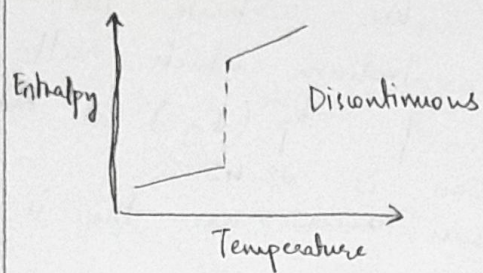
$$\text{Heat capacity} = \frac{\text{Heat flow}}{\text{Temp. increase}} = \frac{q}{\Delta T} = C_p = \left(\frac{dH}{dt}\right)_p$$

DSC allows heating & cooling rates with 0.1° accuracy
DSC is calibrated with highly pure Indium, tin and lead standards

In absence of phase changes in sample (melting / cryst) there is no thermal difference b/w reference and sample compartment

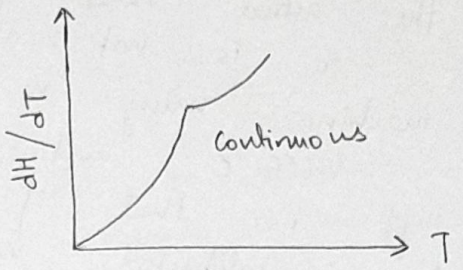


1st order plot



1st order phase transition
- discontinuous at transition

2nd order plot



Also 1st order phase transition
- continuous in 2nd order plot

DSC Instrumentation

DSC cell — Reference & Sample crucible in N_2 atmosphere
Its surrounded by heating coil and a cooling flange. so you can do a heating cycle
It can be programmed to heat/cool at a particular rate.

As per Ohm's law, heat flow can be measured by,

$$\phi_1 = \frac{T_s - T_c}{R_{th}}$$

T_s : sample T
T_c : furnace T
R_{th} : thermal resistance of sensor

$$\phi_R = \frac{T_R - T_c}{R_{th}}$$

DSC signal, $\phi = \phi_1 - \phi_R = \boxed{\frac{T_s - T_R}{R_{th}}}$

Since T is measured by thermocouples, we need the eqn that defines the sensitivity,

V: thermoelectric voltage

$$S = \frac{V}{\Delta T}$$

$$\Rightarrow \phi = \frac{V}{S \cdot R_{th}} = \boxed{\frac{V}{E} = \phi}$$

E : Calorimetric sensitivity of the sensor

DSC measures heat flow as function of T or time as is usually shown in units of mW on Y-axis. mW = mJ s^{-1}

The actual value of heat flow depends on reference & is not absolute. We calibrate the machine using v. pure Indium which melts at 156.6 °C and area of dip (J g^{-1}) at melting pt i.e Heat of fusion is 28.42.

One calibrated, we can use the instrument on other samples.

Phase transition in Solid state

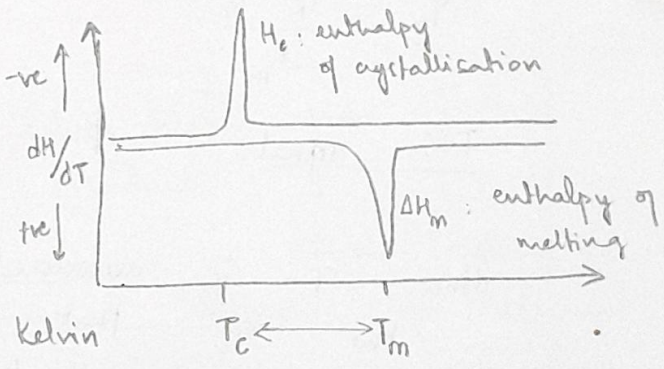
Highly crystalline	Semi-crystalline	Liquid-crystal
Metals	Synthetic polymer	LCD tech
Alloys	Inorganic glass	
Organic cmpds	Highly viscous liquid	
Metal complex	Amorphous solid	

DSC can be used to characterize a sample into these.

⇒ Small Molecule

Eg: Naphthalene — Ordered state / Normal solid ⇌ (Heat →, ← Cooling) Molten liquid

Crystal is isotropic



This is an isolated and reversible transition

$\Rightarrow \Delta G = 0$

$\therefore \Delta S = \frac{\Delta H}{T}$

$\Delta H_m > \Delta H_c$ and $T_m > T_c$

This effect is called Super-cooling effect : crystallization T is much lower than T_m. This is because of rate of cooling.

For naphthalene, $\Delta H_m = 30\ J\ g^{-1}$ $T_m = 80.73^\circ C$

ΔS in $J\ mol^{-1}$?

$$\Delta H = 30\ \frac{J}{g} \times \frac{128\ g}{1\ mol} \Rightarrow \Delta S = \frac{30 \times 128}{350} \approx 11\ J mol^{-1} K^{-1}$$

Thus DSC can be used to calculate ΔH and ΔS of organic & inorganic cmpds

→ Role of chain length on Crystallization

Consider polyethylene — $T_m = 130^\circ C$

Enormous mechanical strength i.e well packed ⇒ some crystalline domain

Flexible, expandable & low density

Long-chain systems — Silica (very ordered)

$[SiO_2]_n$ —Heat→ Molten liquid —Cooling→ Glass (AMORPHOUS Disorder state)

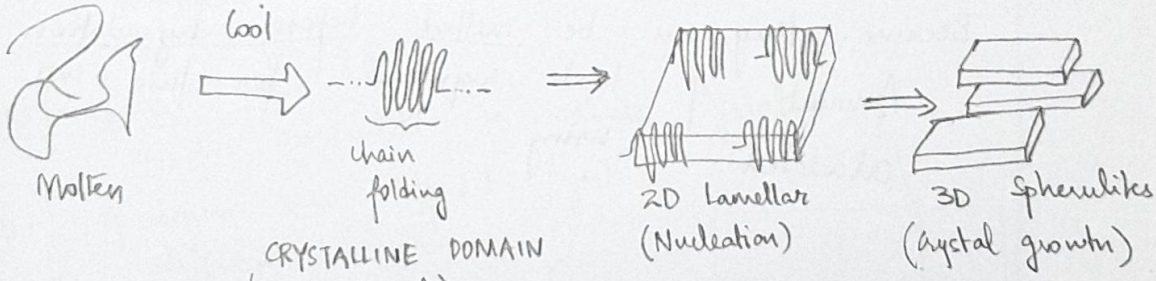
$[SiO_2]_n$ ←(Maybe apply infinitesimally slow cooling)— CRYSTALLINE

Since silica has lot of long chains, it can't crystallize quickly.

Glass is an intermediate, kinetically trapped state

This is a fundamental property (read: problem) of polymeric compounds.

Consider polymers



There's also a disordered domain i.e. amorphous domain in a polymer — can't be 100% crystalline

100% amorphous polymer is possible

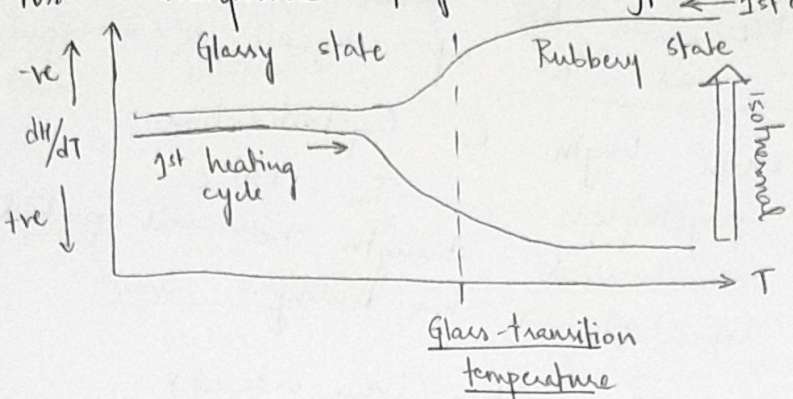
Amorphous domains arise due to interchain network in multiple - lamella.

∴ Polymers are <u>semi-crystalline</u>.

CD polycarbonate — 93% amorphous brittle

Bottle PTA — crystalline

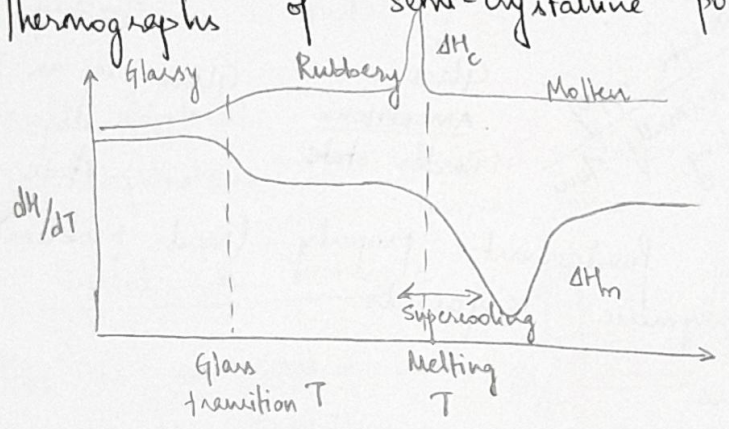
For amorphous polymers, typical thermographs —



Eg: rubber bands

There's only a transition from <u>rigidity to flexibility</u>.
This is called <u>2nd order</u> phase transition.

Thermographs of semi-crystalline polymers —



<u>Rubbery</u> = pouring hot water in plastic water bottle.

<u>Molten</u> = liquid state at ~100°C

These plastics are <u>recyclable</u> also called engineering plastic

Because they can be <u>melted</u> <u>before degradation</u> Temp.
Amount of heat required for this can be calculated using DSC.

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Lecture 30

Liquid Crystals

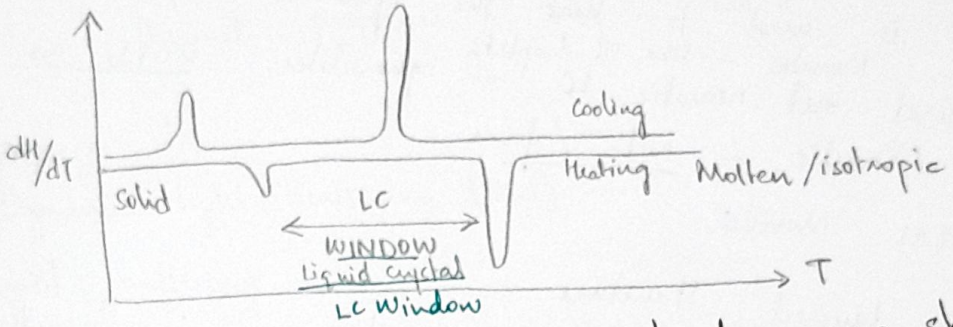
Crystal solids go from crystal (ordered) to isotropic (disordered) state. Liquid crystals go through an intermediate state.

Nematic – all units are arranged in one direction

Smectic – each mesogen is arranged in a lamellar way

Chokstric / spiral nematic – arranged as a spiral staircase

Typical thermograph –



To determine the microscopic structure, we should look at its Thermograph and Polarised Light Microscopy Texture (PLM)

In PLM, a polariser produces a plain polarised light which lights the specimen.

Birefringence in LC

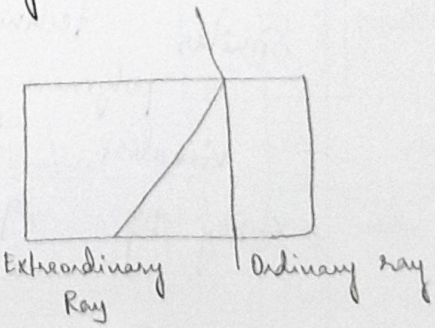
First observed in calcite crystal

The specimen can diffract light in different ways.

Ordinary ray (normal speed)

and extraordinary ray (less v)

This is due to anisotropy.



There two rays (that've passed through specimen) now go through polariser analyser (which polarises them again). There 2 rays undergo interference (destructive or constructive). Ultimately we get a characteristic spectrum from the analyser.

Different textures for nematic, smectic & cholesteric
1D 2D 3D

This is used for characterisation of solids.

Mobile phone / electronic device display use Liquid Crystal Display Technology.

If LC window is b/w 60° to 150°C then we'll have to heat the specimen So a hot plate (programmable) is used to heat the specimen while observing it

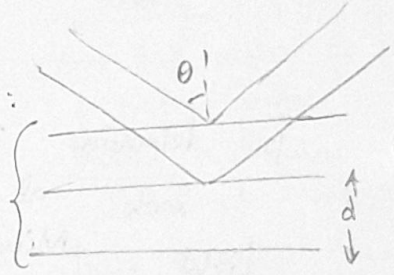
Nematic – like oil droplets
Chiral ~~not~~. nematic LC – spherulites : visible as disc.
Smectic – rods / fans.

PLM Movies

2D Layered Structure

X-ray diffraction Bragg's Law :

$$2d \sin\theta = n\lambda$$



d can be calculated since we know θ and λ

When we do X-ray diffraction, we can confirm that LC is in 2D layered structure

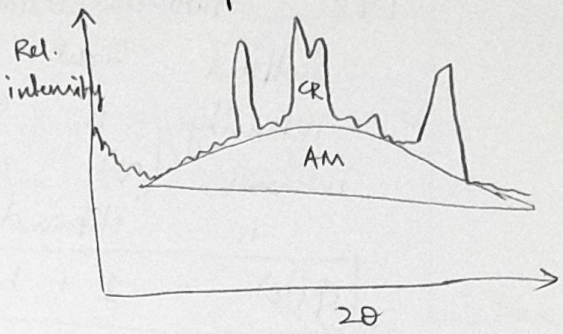
Similar techniques can be used on macroscopic polymers also. Microscope can be used to visualise the 3D structure of polymers.

X-Ray Dif. of crystals are very sharp and uniform even with varying Temp.

Wide angle X-Ray Diffraction (WXRD) of polymers.

Here, the spectrum is not sharp.

This is beuz they have crystalline & amorphous domains



$$\text{Crystallinity} = \frac{\text{Area of peaks}}{\text{Total area}}$$

After making a compound —

1. Purification
2. Structural characterisation — NMR, FTIR
3. Optical properties — Absorbance, emission
4. Thermal properties — TGA, X-Ray D.

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Lecture 31

Dynamic light Scattering (DLS)

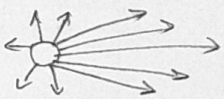
Light Scattering

Rayleigh scattering

- ✓ Particle size $< \lambda/10$
- ✓ Not angle dependent
- ✓ Elastic scattering

Mie scattering

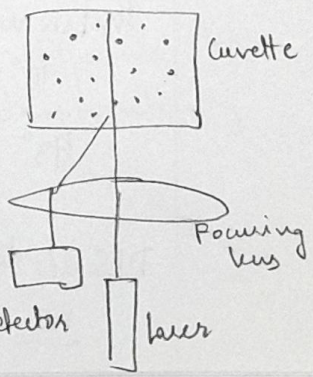
- ✓ size $> \lambda/10$
- ✓ Angle dependant
- ✓ Inelastic scattering which increases with size



Static light scattering — advanced

Dynamic light scattering

The particles are in brownian motion when light is passed through it.



We can find the size of particles through this
Larger the particle, greater the variation of scattering intensity wrt time

DLS : Auto-correlation function

Scattered light fluctuates over time. Fluctuation in intensity is correlated against short decay interval (τ) and intensity autocorrelation fn. (ACF) is obtained through mono-exponential Eqn:

$$G(\tau) = 1 + be^{-2D_t q^2 \tau}$$

b: constant

D_t: translational diffusion coeff

q: scattering vector

$$|q| = \frac{4\pi n_0}{\lambda_0 \sin\left(\frac{\theta}{2}\right)}$$

n_0: Refractive Index

λ_0: wavelength in vacuum

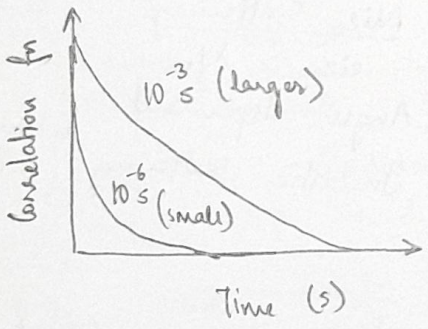
θ: scattering angle

Once we get the diffusion coefficient (D_t), we can relate it to hydrodynamic radius (R_H)

$$D_t = \frac{k_B T}{6\pi\eta R_H}$$

We can use Stokes-Einstein eqn to convert ACF to intensity vs size

η: absolute viscosity



Instrumentation

90° detector : for >50 nm

173° (low angle) detector : for much smaller particles and dispersed soln (like milk)

DLS data reporting - it can be reported in terms of no. of particles, volume of particles or scattering intensity.

Say 5nm & 50nm no. of particles are 50:50.

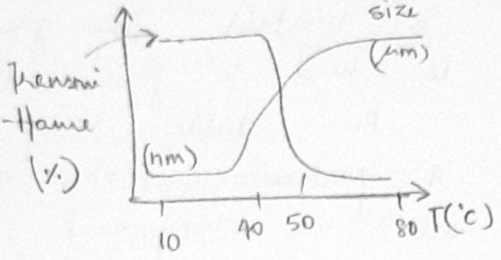
⇒ Volume = 1:1000 Intensity = $1:10^6$

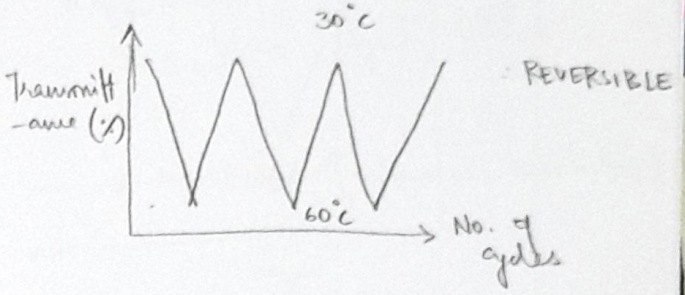
Usually, intensity based plot is used.

The medium of disperal also affects the size recorded, maybe due to aggregation.

Applications of DLS

⇒ Thermoresponsive materials - they precipitate when solution is heated, when cool it dissolves.





⇒ Enzyme responsive material

Polymer covered thing is broken down in presence of enzyme size increase when esterase

? # Then why does is present?

DLS can be used whenever there's size change

Charged nanoparticles

Zeta potential - electric potential at surface of nanoparticle. Its the potential diffrence b/w EDL (electrical double layer) of electrophoretically mobile size

Mobility : $\mu = \frac{V}{E}$

V: particle velocity

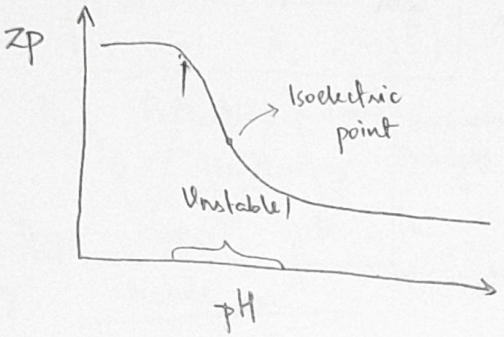
E: electric feild strength ($V\,cm^{-1}$)

From mobility of particle (measured), we measure ζp through Henry's formula, and through that, size of particle.

$$\mu_e = \frac{2\varepsilon_r \varepsilon_0 \zeta f(ka)}{3\eta}$$

ε_r : relative permittivity
ε_0 : permittivity of vacuum
$f(ka)$: Henry's fn
η : viscosity ζ : ζp.

Isoelectric point



When ζp changes, particle is unstable. This graph is used to figure out the stable range of a particular protein or aā or compound

Antibacterial Activity Assay

AMP – +ve charged drug
Bacteria – -ve charged

When they're mixed, charge is neutralised & bacteria killed To monitor and ascertain the mechanism, we can measure zeta potential

Stimuli responsiveness, DNA/RNA binding analysis – these are analysed through ζp.

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Lecture 32

Confocal microscopy analysis

Its mainly used in biological imagin whose spatial resolutions can vary from

Small molecule (1nm) - Bacteria/cell (5μm) - Mouse (10cm)

There are different kinds of microscopy developed for this range. Confocal microscopy is of range 200 nm to ~5mm

Difference b/w fluorescence and confocal microscopy

From 1970s, since development of lasers, the confocal microscope uses a laser light source

Instrumentation

1. Light source
2. Dichroic mirror
3. Aperture (influences resolution)

1. Light source

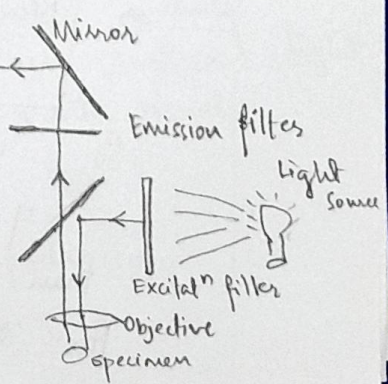
Illumination sources - mercury/xenon/metal halide are lamps. They have distinct spectrums - broad in some regions (not useful), with certain sharp peaks at a particular wavelength.

Since 1980s, we have LEDs and quantum dots that can be chosen to excite something at a particular wavelength.

2. Dichroic beam splitter

Excitation filter - to isolate required wavelength (not strictly necessary for LED source)

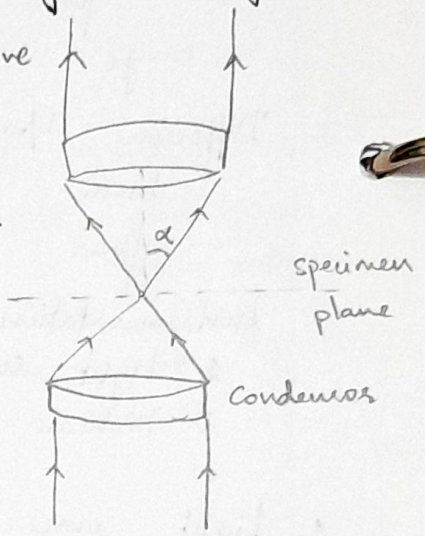
Dichroic beamsplitter - it's a lens which allows a narrow range of wavelength through it.



Source light (λ_1) doesn't go through beamsplitter, but the reflected, scattered light from the specimen passes through it - which can be detected. Different excitation filters and dichroic beamsplitters are built in. This filter combination produces crisp, bright images

03. Resolution - Aperture

Diffraction limit in microscope is governed by the fact that when imaging a point source of light, the instrument produces two points that can be distinguished The minimum distance b/w these two points at which they can be distinguished is called the resolution of the microscope



Numerical aperture $NA = \eta \cdot \sin\alpha$

$$\text{Resolution } (x,y) = \frac{\lambda}{2(\eta \cdot \sin\alpha)}$$

$$\text{Resolution } (z) = \frac{2\lambda}{(\eta \cdot \sin\alpha)^2}$$

η : Refractive index of medium

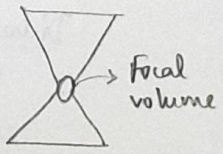
$\eta \cdot \sin\alpha$ → Objective NA.

Objectives (lens) have NA ~ 1.5, so α is restricted to ~ 70°

$$\Rightarrow \text{Resolution} = \frac{\lambda}{2(\eta \cdot \sin\alpha)} = \frac{400\text{ nm}}{2(1.4 \times \sin 70)} = 151\text{ nm}$$

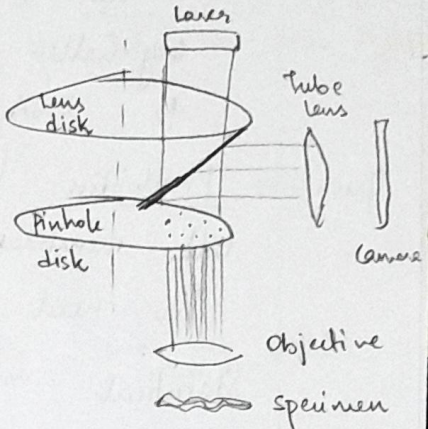
So confocal microscope has a resolution of ~ 150 - 200 nm. So structures smaller than 200 nm cannot be resolved

For $\alpha = 53°$, Resolution ~ 400nm



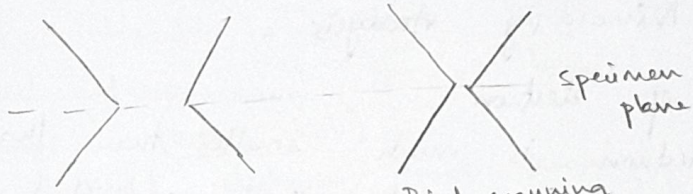
Modern Spinning-disc confocal microscope

there, the laser passes through 2 spinning disks — lens & pinhole very sharp individual laser beams hit the specimen, so it can be reconstructed in 3D at very high resolution.



Also useful for live-cell imaging because direct laser is not used.

Confocal volume
Fluorescent microscope



Not light — Widefield illumination — larger volume

Point-scanning — smaller volume ∵ laser light source

Size of illumination point ranges from 250 - 800 nm (depending on NA) and 500 - 1500 nm at bright intensity. It depends on microscope design, λ, objective NA, scanning unit settings and the specimen.

Out-of-focus fluorescence contributes to detail obscurity, limiting the depth of tissue that can be imaged. In confocal, there's a small pinhole before the detector so that other, scattered light can be blocked, allowing greater visual detail.

Excitation, emission etc - same as spectroscopy. there we do imaging.

PP : Pollen grain — 0.5 μm parallel planes.

Biological imaging — different fluorescent proteins/molecules have been developed that bind to specific organelles / components of cell. Eg: Blue DAPI binds to DNA — can be used to map the nucleus.

Green → Phalloidin (bicyclic peptin) — very selectively binds to actin

Cell membrane staining — "cell break"

You need optical chromophores to visualize diff. parts.

Statistical analysis of imaging.

PP : Live cell imaging

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Lecture 33

Electron Microscopy Analysis

Wavelength of electron

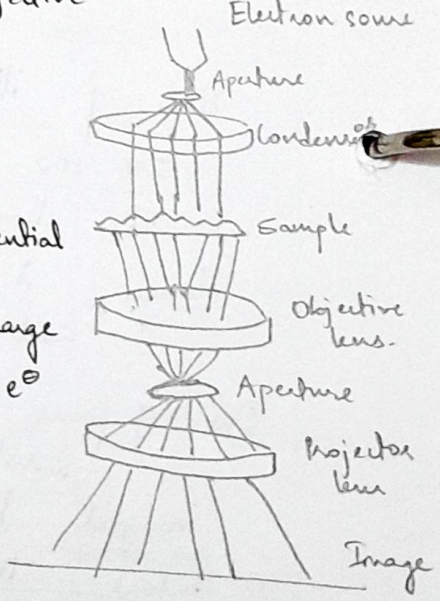
λ of electron is much smaller than that of photons, so resolution is theoretically unlimited

Practically, its ~0.1nm due to objective lens

$$\lambda = \frac{h}{p} = \frac{h}{m_0 v} = \frac{h}{\sqrt{2 m_0 e U}}$$

$$v = \sqrt{\frac{2eU}{m_0}}$$

U : electric potential
m_0 : mass
e : electron charge
V : velocity of $e^{\ominus}$



For applied voltage U,

$$\lambda = \frac{12.2D}{\sqrt{U}} \text{ Å}$$

Resolution in Electron microscope

$$\text{Resolution}_{x,y} = \frac{\lambda}{2(\eta \cdot \sin\alpha)}$$

Aperture angles are very small, so $\alpha \approx \sin\alpha$

Also, for some reason instead of 0.5, we take 0.61

$$r_{airy} = \frac{0.61\,\lambda}{\eta \cdot \alpha}$$

for air, $\eta = 1$

$$\lambda = \frac{12.2}{\sqrt{U}}\ \text{Å}$$

$$r_{airy} = \frac{7.5}{\alpha\sqrt{U}}\ \text{Å}$$

Using this and practical constraints, we get that max resolution is -

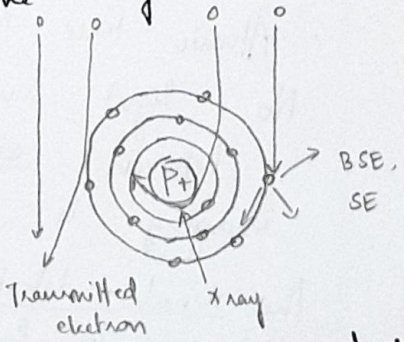
$$r_{airy} \approx 0.01\ \text{Å}$$

Interaction of electron beam with matter

It interacts in many ways — beam (20 keV)

- Direct transmission of e^- beam
- X-rays (0.007 keV)
- Back-scattered electrons and secondary electrons (0.4 & 0.6 keV)
  Auger electron are produced
- Inelastically scattered electrons

Analysis of BSE, SE or X-rays can be used to reconstruct the image



SE are scattered from surface
BSE are returned from some depth
If $e^\ominus$ penetrates the sample, X-rays are produced

Elastic scattering occurs when there's no loss of energy ⇒ $e^\ominus$ change direction, not d.

Scanning Electron Microscope (SEM)

The mounting plate can be rotated. There are detectors for BSE and SE.

Mixoporous of nanoporous structures can be clearly visualised and resolved. This gives surface morphology

↓ microscope

Main signals generated – low energy SE (<50 eV), high energy BSE (>50 eV) and characteristic X-rays

Energy Dispersive X-ray (EDX or EDS) detectors are also attached to SEM

Transmission Electron Microscope (TEM)

There, the sample holders are tiny (3mm) – they have a grid which is coated with a resin & carbon. There, sample preperation is v. important

This is v. advanced – the transmitted beams are detected and imaged

Its used to analyse nanoparticles & quantum dots

We can talk about packing of the crystals

It can also give high resolution 2D images.

SEM will only show the surface – can't distinguish a hollow or filled cylinder whereas TEM can because it analyses transmitted electrons.

TEM has been used to study organelles & macromolecules

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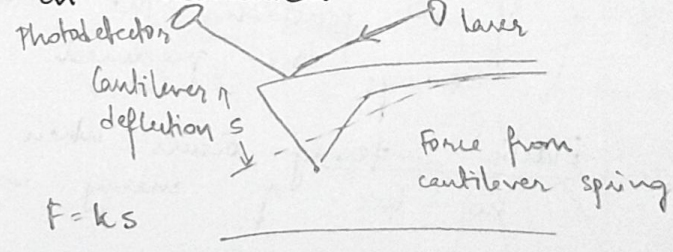
Lecture 34

Atomic Force Microscope

No light source involved – it measures the force between cantilever and the sample. The imaging is based on mechanical device

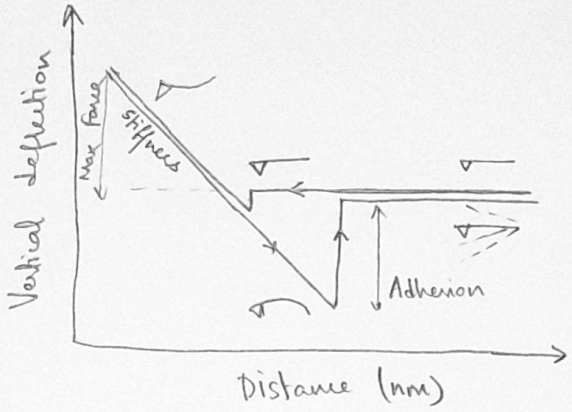


The minute detection of change in force is possible through a photodetector which measures the ~~no~~ reflected laser from cantilever which is being deflected or attracted based on the sample

there the sample doesn't get degraded ∵ of laser or electron beam ⇒ could be used for soft sample (biological ones) No degradation

Different types of microscopes (Scanning Tunneling microscope) are used for different types of samples.

AFM modes – Contact, Non-contact, Intermittent contact.



Sample preperation –
dry sample i.e solvent has to be evaporated

– Missed some stuff until ~24 mins –

Case study : Polysaccharide vesicles
pH responsive vesicle