Biochemistry 494

Spring Semester, 1975

OPTICAL SPECTROSCOPY OF BIOMOLECULES

Course of 24 lectures

Absorption spectroscopy

Photons and wave packets. Oscillators: free and forced behavior. Beer's law. Effects of scattering, fluorescence, molecular aggregation. Oscillator strength. Selection rules. Singlets and triplets. Potential energy curves. Franck-Condon principle. Absorption by linear conjugated molecules and by aromatics. π - π^* and n-π * transitions. Dichroic absorption. Environmental effects upon absorption: dipole in a dielectric. Solvent effects. Absorption of molecular complexes. Hypo and hyperchromism.

Fluorescence emission

Invariance of the emission spectrum. Fluorescence excitation spectrum: invariance of the relative yield. Absolute quantum yields; measurement. Fluorescence lifetime: determination by impulse response and by harmonic response. Polarization of fluorescence: dependence upon molecular rotations. Measurement of rotational rates by stationary polarization, polarized decay and phase fluorometry. Reactions of the excited state: thermalization, solvent relaxation, excited state ionization, chemical quenching. Stern-Volmer law. Static and dynamic quenching. Electronic energy transfer: Homo and hetero-transfer. Red edge effects.

Fluorescence of Biomolecules

NADH and the dehydrogenases. Flavins and flavoproteins. Intrinsic protein fluorescence. Fluorescence of protein conjugates and adsorbates. Fluorescent probes: their use in the investigation of membranes and cell surfaces. Limitations.

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8/28 **1st Lecture.**

Scope of Optical Spectroscopy. – Range of the electromagnetic spectrum ($0.2\mu - 1\mu$) involved.

Processes Involved:

Absorption → Excited State → Emission or Deactivation.

(Exclusion of Scattering and Retardation).

Preliminaries: Electromagnetic Radiation. Characterization

Frequency ν (sec^{-1}) (circular frequency $\omega = 2\pi\nu$).

Wavelength λ (cm).

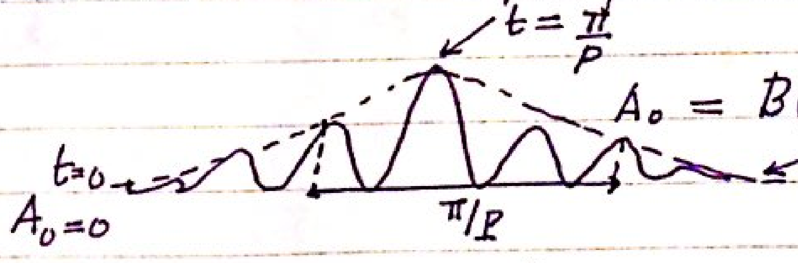
Wavenumber $\bar{\nu}$ (cm^{-1}).

Amplitude & Phase.

Coherent and non-coherent radiation.

$$A(t) = A_0 \sin(2\pi\nu t + \phi) = A_0 \sin(\omega t + \phi). \quad (1).$$

Infinite sinusoidal motion. Light waves are finite phenomena: The concept of the wave packet.



$A_0 = B(1 - \cos pt)$.

$0 < t < \frac{2\pi}{p}$

The phenomenon is defined over one period of the frequency p.

$$A(t) = B(1 - \cos pt) \sin \omega t$$
$$A(t) = B(\sin \omega t - \cos pt \sin \omega t).$$

$\sin(\omega t + pt) = \sin \omega t \cos pt + \cos \omega t \sin pt.$

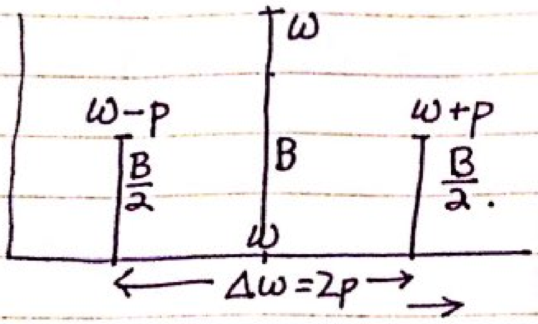
$\underline{\sin(\omega t - pt) = \sin \omega t \cos pt - \cos \omega t \sin pt}$

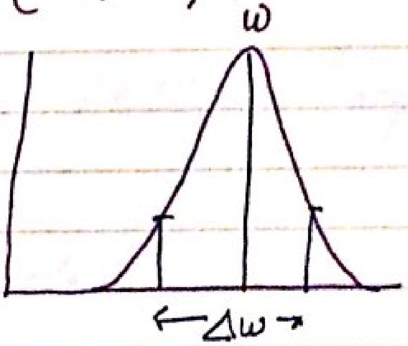
$\sin(\omega + p)t + \sin(\omega - p)t = 2 \sin \omega t \cos pt.$

or

$$\sin\omega t\cos pt = \frac{1}{2}\sin(\omega+p)t + \frac{1}{2}\sin(\omega-p)t.$$

$$A(t) = B\left(\sin\omega t + \frac{1}{2}\sin(\omega+p)t + \frac{1}{2}(\omega-p)t\right).$$





The simplest wave packet.

$$\Delta t\cdot\Delta\nu \sim 1$$

$$\Delta t\cdot\frac{\Delta\omega}{2\pi} \sim 1 \text{ or } \Delta t\cdot\Delta\omega \sim 2\pi.$$

Since $\Delta\omega = 2p$, $\Delta t \sim \frac{\pi}{p}$.

$A(t) = \frac{B}{2}$ at $\cos pt = \frac{1}{2}$.

Example of the television screen: Each dot takes $2\times10^{-}$

30 frames/sec.

525 lines/frame.

360 dots/line.

$30\times360\times525$ dots/second $= 567\times10^4$.

$$\Delta\nu\Delta t = \frac{\Delta\nu}{567\times10^4} = 1 \text{ or } \Delta\nu = 5.67\times10^6.$$

~ 6 MHz.

Photons

$$\epsilon = h\nu = \frac{hc}{\lambda} = \frac{6.65\times10^{-27}\times 3\times10^{10}}{\lambda} = \frac{19.95\times10^{-17}}{\lambda}.$$

$$\epsilon = \frac{2\times10^{-16}}{\lambda}.$$

$$N\epsilon = \frac{6.03\times10^{23}\times 2\times10^{-16}}{\lambda}\text{ ergs} = \frac{12.06\times10^{7}}{\lambda}\text{ ergs}$$

$$N\epsilon = \frac{12.06}{\lambda}\ J = \frac{28.6}{\lambda(\mu)}\text{ kcal/mole.}$$

or $\frac{1.24}{\lambda(\mu)}$ e.v.

$\lambda = 0.5\,\mu$. 57.2 kcal/mole or 2.48 e.v.

Notice that 57.2. Kcal is close to the smallest value of some bond energies. (Importance in photochemistry)

Bonds:			λcrit =
$C-NO_2$	(~ 60 kcal)	($\Delta H°$).	477 nm
$C-O$	(75 kcal)		381 "
$O-H$	(110 ")		260 "
$C-N$	(85 ")		336 "

Monochromatic Radiation.

Natural width of lines is determined by $\Delta t = 10^{-8}$

Therefore $\Delta\nu \sim 10^{8}$ Hz. Since $\nu = \frac{\cancel{\epsilon}c}{h} = \frac{\frac{2}{5}\times10^{-11}}{6.65\times10^{-2}}$

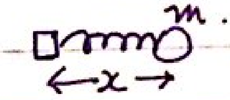
or $\nu = \frac{c}{\lambda} = \frac{3.10^{10}}{5\times10^{-5}} = \frac{3}{5}\times10^{15}$

$\underline{\Delta\nu}$ (Na light) $= \frac{5}{3}10^{-7}$. (lines are broadened by collision and by pressure)

2nd Lecture Oscillators.

1/23.

Simple harmonic.



$$m\frac{d^2x}{dt^2} = -kx \qquad (1)$$

$$x = A \sin \left(\sqrt{\frac{K}{m}}\right)t \qquad (2.)$$

The motion of the mass is periodic.

$$\text{Period} = T = \frac{2\pi}{\sqrt{\frac{K}{m}}} \qquad (3)$$

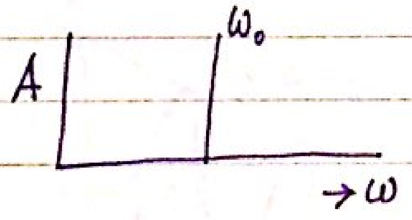
The circular frequency (or proper frequency) of the oscillation is

$$\omega_0 = \sqrt{\frac{K}{m}}. \qquad (4).$$

In terms of ω_0 Eq (1) may be written

$$m\frac{d^2x}{dt^2} + m\omega_0^2 x = 0. \qquad (5).$$

The solution is the undamped harmonic motion



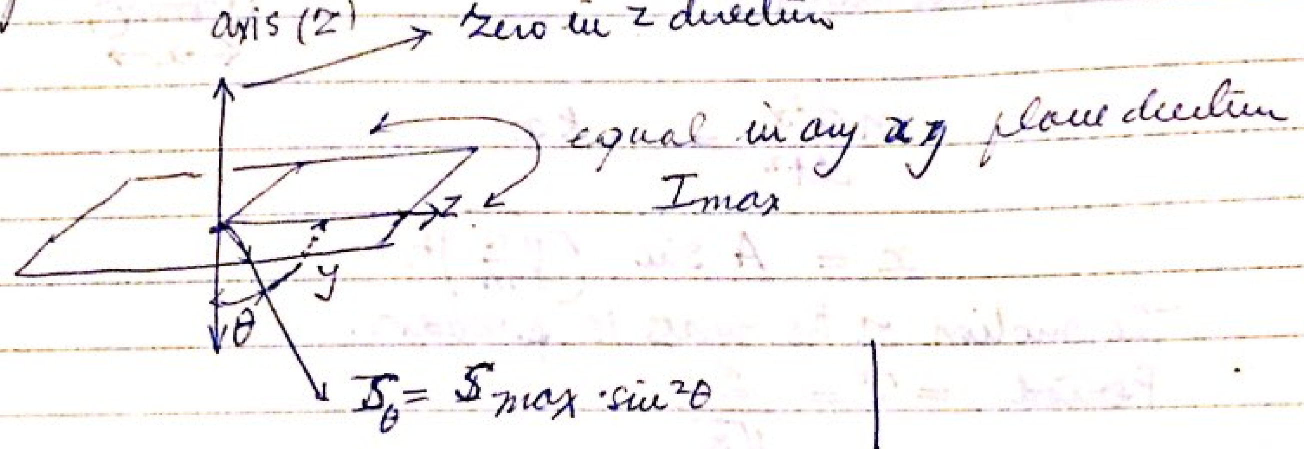
Fourier spectrum of S.H.O.

To obtain a damped wave we introduce a damping term proportional to the velocity of displacement.

$$m\mu\frac{dx}{dt}$$

$$m\frac{d^2x}{dt^2} + m\mu\frac{dx}{dt} + m\omega_0^2 = 0.$$

Spatial Distribution of the radiation from an electronic oscillator = Isotropic & anisotropic osc.

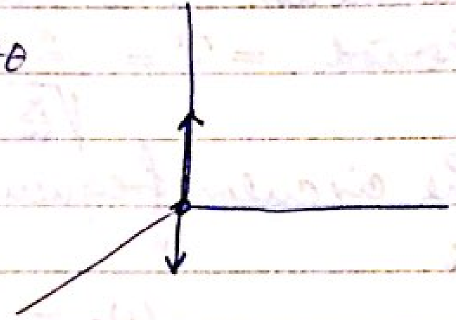


$S_y = S_{max}$

$S_x = S_{max}$

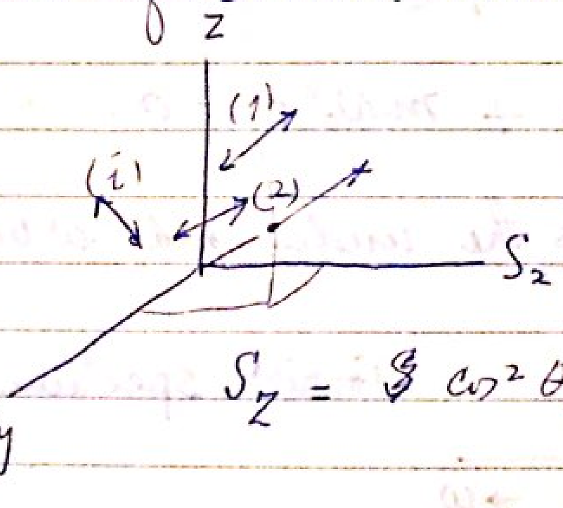
$S_z = 0$

$S_\theta = S_{max} \cdot \sin^2\theta$



For a collection of oscillators randomly distributed in space

$S_{max} = 1$



$S_Z = S_{Z1}$

$S_Z = S \cos^2\theta(1)$

The solution after $t \geq 0$. is.

$$x = C_1 e^{\gamma_1 t} + C_2 e^{\gamma_2 t}.$$

$$\gamma_1 = -\frac{\mu}{2} + \sqrt{\frac{\mu^2}{4} - \omega_0^2}.$$

$$\gamma_2 = -\frac{\mu}{2} - \sqrt{\frac{\mu^2}{4} - \omega_0^2}.$$

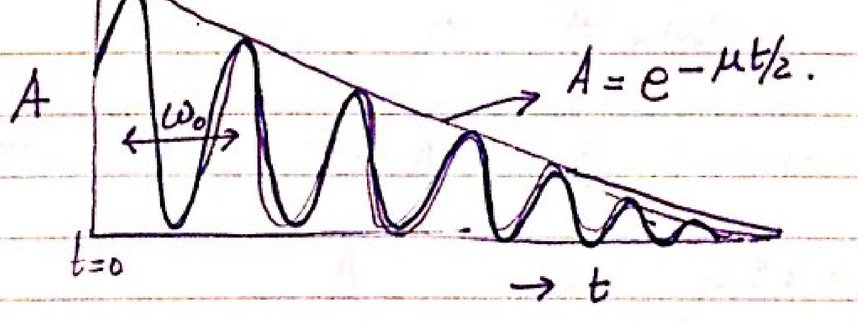
If $\mu/2 > \omega$. the amplitude decays in a time less than $1/\omega_0$, that is the oscillator is completely damped. If $\mu/2 < \omega_0$ (case of interest). γ_1 and γ_2 become imaginary

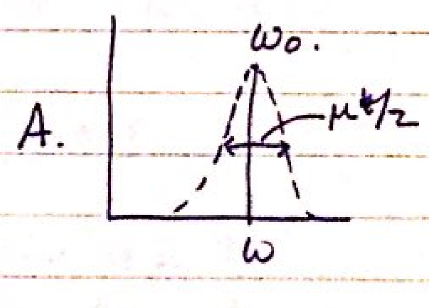
$$x = e^{-\frac{\mu}{2}t}\left\{C_1 e^{j\sqrt{\omega_0^2 - \frac{\mu^2}{4}}\,t} + C_2 e^{-j\sqrt{\omega_0^2 - \frac{\mu^2}{4}}\,t}\right\}.$$

$$C_1 e^{j\theta t} + C_2 e^{-j\theta t} = \cos(\theta t + \phi) \sim \cos(\omega_0 t + \phi)$$

$$\theta = \sqrt{\omega_0^2 - \mu^2/4} \; ; \quad \tan\phi = C_2/C_1.$$

Since $\mu^2/4 \ll \omega_0^2$ $\quad \theta \sim \omega_0$





This constitutes the free behaviour of the oscillator. when at $t=0$ it is subjected to no force. It corresponds to emission of the radiation, since the oscillator has maximal energy at $t=0$ and loses it over a damping time $\mu/2$.

Forced behaviour

Impressed electrical force $= F(t) = E_0 \sin \omega t$

Solution: The oscillator performs a motion given by

$$x(t) = A \sin(\omega t + \delta). \qquad (1)$$

$$A = \frac{E_0/m.}{\sqrt{(\omega^2-\omega_0^2)^2+\mu^2\omega^2}} \qquad (2).$$

$$\tan\delta = -\frac{\mu\omega}{\omega_0^2-\omega^2}.$$

When $\omega = \omega_0$ the amplitude is a maximum $\frac{E_0}{m\mu\omega_0}$ and the phase lags 90° to the excitation.

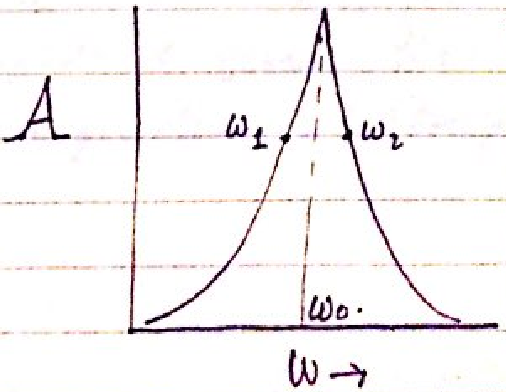
The amplitude falls to $1/\sqrt{2}$ when

$$-\omega^2+\omega_0^2 = \mu\omega \quad \text{or at values.}$$

$$\omega_1 = -\frac{\mu}{2} + \sqrt{\frac{\mu^2}{4}+\omega_0^2} \rightarrow \omega_0 - \frac{\mu}{2}$$

$$\omega_2 = -\frac{\mu}{2} - \sqrt{\frac{\mu^2}{4}+\omega_0^2} \rightarrow \omega_0 + \frac{\mu}{2}.$$

The plot of. (2) A vs. ω gives a Lorentzian curve.



The larger the value of μ the greater the width. Absorption is always of a band of frequencies not of monochromatic light! However $\omega_0 \sim 10^{15}\,s^{-1}$

$\mu \sim 10^{8}\,s^{-1}$ $\quad \frac{\omega_1-\omega_2}{\omega_0} \sim 10^{7}$

Practical Spectroscopy.

Light Sources. (Effect of intensity)
Detectors – Sensitivity of Cathode ~0.20
Pulses. Photon Counting
Monochromators
Parasitic Light.
Resolution & Rayleigh Criterion.
Static and Dynamic resolution.

$$\overline{\Delta\lambda} = \Delta\lambda_{st} + \underline{\underline{v\sigma}}$$

v = scanning speed nm/sec or Å/sec.
σ = response time of system

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The radiation may be considered pretty well monochromatic.

Free and forced behaviour are characteristics of <u>all</u> dynamic systems (Lord Rayleigh).

Practical Spectroscopy.

Light Source = Range, Continuity
Xe arc.
Dispersive element = Prisms, gratings.
Resolution limit by diffraction. $\lambda/\Delta\lambda \sim 10^3$-10
Energy at the slit.

3rd Lecture

Detectors = Photomultipliers = Photon efficiency of Cathode. Photon Counting: 10^5-10^6 counts/sec. Error.
Scanning = Dynamic resolution.

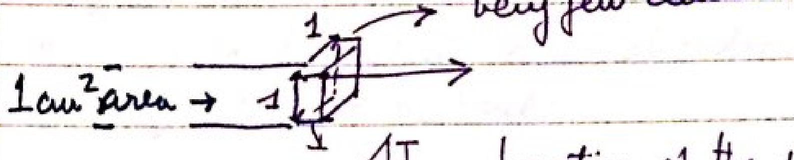
$\Delta\lambda = \Delta\lambda_s + \Delta\lambda_d$.

$\Delta\lambda_d = v\sigma$ v = scanning speed in nm/sec.
σ = response time in sec.

Often pen response time is limiting and app 1 sec. ~~e.o~~ thus if v = 1 nm/sec. Resolution (dynamic) is 1 nsec. Independent Sampling – Numerical Averaging.

Beer's law.
Derivation — Significance of molar absorptivity.
Departure from Beer's law.
Independence of absorptivity from light intensity.

σ_m = effective cross section. Area normal to photon direction such that absorption takes place if the photon passes through it but not otherwise.



$-\frac{\Delta I}{I}$ = fraction of the incident light I absorbed.

$\sigma_m L \Delta X$ = fraction of the area covered.

$-\frac{\Delta I}{I} = \sigma_m L \Delta X$. (L = molecules in unit volume).

$$L = [C] \times N \times 10^{-3} = [C] \times N'$$

$$-\frac{\Delta I}{I} = \sigma_m [C] N' \Delta X.$$

$$\int_0^X -\frac{dI}{I} = \int_0^X \sigma_m [C] N' dx.$$

$$-\ln I = [C]x[N']\sigma_m + C.$$

at $x = 0$. $C = -\ln I_0$.

or $\ln \frac{I_0}{I} = [C] \cdot N' \sigma_m x$

$\sigma_m N'$ = milimolar cross section

$$\log \frac{I_0}{I} = 0.434\, \sigma_{mM} [C] x = k [C] x$$

$\log \frac{I_0}{I}$ = absorbance or O.D. (decimal).

$\ln \frac{I_0}{I}$ = natural absorbance (old word extinction) or 'natural OD'

$k[C]x$ = dimensionless $\frac{\text{moles}}{10^3\,\text{cm}^3} \cdot \text{cm} = \frac{\text{moles}}{10^3}\,\text{cm}$

or k must cancel.

K dimensions is cm²/mM.

σ_m, the millimolar cross section equals 2.3 the ~~'molar absorption~~ molar absorption coefficient'.

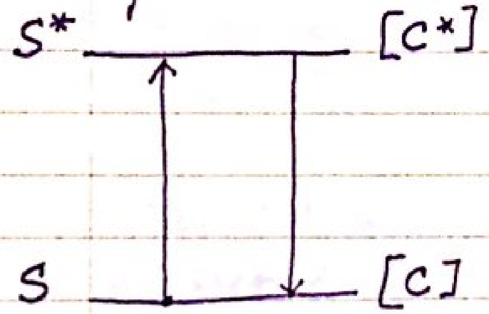
σ_{mM} (max) $\sim 10^5\ cm^2/mM$.

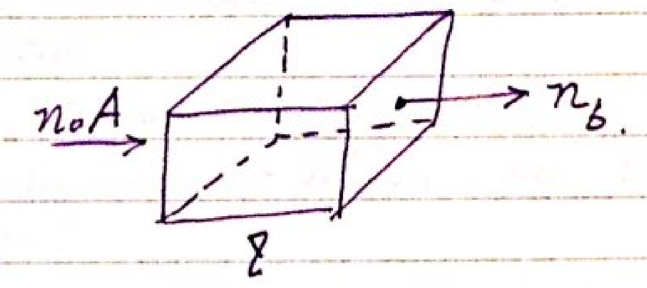
$\sigma_m = \sigma_{mM}/N' = 4 \cdot 10^{-16}\ cm^2$/molecule or $4\ \text{Å}^2$.

The physical section are of order. 16 Å

if $\sigma_m < 10^4\ cm^2\,mM$.

Independence of the absorbance from the intensity of the Source.





$$k_{S\to S^*}[C] = k_{S^*\to S}[C^*] \quad \text{(stationary state)}$$

<u>Calculation of $k_{S\to S^*}$</u> at the back per second.

number of quanta emerging

$$n_b = n_0 A\, e^{-\sigma_{mM}[C]l}. \qquad n_0 = \text{photons/unit } cm^2.$$

number absorbed per second is

$$n_a = n_0 A\left(1 - e^{-\sigma_m [C] l}\right)$$

the number of photons absorbed per second per molecule.

$$k_{S-S^*} = \frac{n_0 A\left(1 - e^{-\sigma_m [C] l}\right)}{A l\, [C] N'}$$

Maximum number is obtained when $\sigma_m [C] l$ is small.

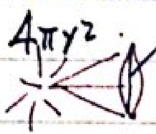
or

$$e^{-\sigma_m [C] l} = 1 - \sigma_m [C] l + \frac{(\sigma_m [C] l)^2}{2!}$$

$$k_{S-S*} = n_0 A \frac{\sigma_{mM}[C]l.}{Al[C]N'} \longrightarrow \frac{n_0 \sigma_{mM}}{N'} = n_0 \sigma_m$$

"1,000 watts" light ⟶ 200 watts appear as light output, the rest as heat.

fraction directed $= \frac{\pi d^2}{4\pi r^2} = \frac{1}{4}\left(\frac{d}{r}\right)^2 = \left(\frac{d}{2r}\right)^2$



5% of 200 watts = 10 watts. (1/16 – 1/25)

Dividing by 10^3 the region of the spectrum isolated. we have 10 miliwatts of ~~poor~~ "monochromatic" radiation through the solution. At the center of the visible spectrum we would have some $4 \cdot 10^{15}$ quanta. In the most favourable case $\sigma_m \sim 10^{-16}$ cm².

$$n_0 \sigma_m \approx 1 \text{ or less.}$$

Thus we have only in the most extreme case 1 transition per second per molecule.

$$k_{S \to S*} \sim 1.$$

On the other hand.

$$k_{S* \to S} \sim 10^8 \text{ sec}^{-1} \quad [C^*]/[C] = 10^{-8}$$

4th Lecture
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Departures from Beer's law.

Broad band. Illumination (I_0/I less than for narrow bands.)
Scattering (I_0/I more than predicted in absence of scattering
Fluorescence. (I_0/I less than predicted in absence of fluorescence
Molecular Aggregation. –

$$A_2 \rightleftharpoons A + A.$$

Effects at different wavelength according to whether

$$k_{(A_2)} \gtrless 2k(A).$$

Strength of the absorption

The concept of oscillator strength
'Energy density' in a homogeneous beam equal $\rho(\nu)$ ergs per unit interval in frequency (1cps). per cm^3.
Under steady state conditions

$$\left(\frac{dE}{dt}\right)^1_{ideal} = B\rho(\nu)$$

$$B = \frac{\pi^2 e^2}{3mn^2} = \frac{8.303 \times 10^8}{n^2}.$$

where e = electronic charge. 4.8×10^{-10} e.s.u
m = electronic mass. 9.1×10^{-28} gms.
n = refractive index of medium. (vacuum = 1)

A real oscillator differs by the parameter $f(\nu)$ = oscillator strength for frequency ν.

$$\left(\frac{dE}{dt}\right)^1_{real} = f(\nu)\left(\frac{dE}{dt}\right)_{ideal} = f(\nu)\,B\rho(\nu).$$

If the molar concentration is [C] the number of oscillators/cm^3 equals [C][N']

$$\left(\frac{dE}{dt}\right)_r = B\rho(\nu)\,f(\nu)\,[C]\,N'$$

From Bier's law under steady state conditions. the energy lost by the beam due to absorption is

$$\Delta E(\nu) = \rho(\nu)\left(1 - \exp\left(-\sigma_\nu [C]\right)\right)$$

Since $\rho(\nu)$ must be constant throughout the beam.
$\exp -(\sigma_\nu [C]) \ll 1$ or

$$\Delta E(\nu) = \rho(\nu)\,\sigma(\nu)[C]$$

$$\frac{dE}{dt} = \Delta E(\nu)\cdot\frac{c}{n} = \rho(\nu)\,\sigma(\nu)\,[C]\,\frac{c}{n}$$

where c = velocity of light in vacuum.
n = refractive index.

$$\Delta f(\nu) = \frac{c\,\sigma_\nu}{n B N'} = 2.302\,k_\nu\,c / n B N'^{-1}.$$

$$f = \int_{\Delta\nu} f(\nu)\,d\nu = \frac{2.302\,c^2}{B N' n}\int_{\Delta\bar\nu} k_{\bar\nu}\,d\bar\nu =$$

$$4.125\times10^{-9}\,n\int_{\Delta\nu} k_{\bar\nu}\,d\bar\nu$$

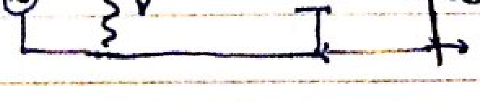
$$k_\nu = k_{max}\,e^{-(\bar\nu-\bar\nu_{max})^2/\varepsilon^2}$$

$$\int_{\Delta\nu} k_{\bar\nu}\,d\bar\nu = k_{max}\sqrt{\pi}\,\varepsilon.$$

[Origin of the variable oscillator strength.
Selection rules. (See Herzberg = Atomic Spectra and atomic structure).

Atomic absorption: Sharp line. (Damped oscillator).
Molecular absorption Broad Bands. due to vibrational and rotational levels.

The synchronous rectifier and the absorbing molecule.



$$V_0 = \frac{V_{in}}{\sqrt{(\omega^2-\omega_0)^2+\frac{1}{R^2C^2}}}$$

Example. f

Last band of Benzene = $10^{-3} f$ (very weak).
NADH (340) = 0.2 mod. allowed.
Haemoglobin (420) Soret = 1.05 fully allowed. (v. strong).

Values of 1.5 to 2 are well known.

— Differences in charge in the two states —

7th Lect → Singlets and triplets — Multiplicity.

Definition of Multiplicity

$S = n \times \frac{1}{2}$ n = number of unpaired spins

$M = 2S + 1$

$S = \frac{1}{2}$ doublet
$S = 0$ singlet
$S = 1$ triplet
$S = 2$ quintet etc.

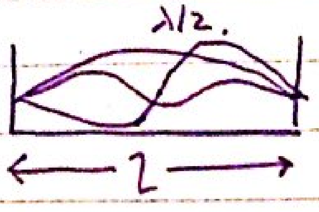
Intercombinations are forbidden. (Radiatively!).
To each singlet state there corresponds a triplet state of lower energy except for $S=0$. (Hund's rule).
Ratio of forbidden to allowed transition $(10^{-6} - 10^{-9})$.

Molecular Structure and light absorption in organic compounds.

Saturated hydrocarbons. Participation of outer electrons (valency electrons) in the transitions.

$CH_2 = CH_2$ 165 nm. $(\pi \rightarrow \pi^*)$. } X-ray absorp.
$(\sigma \rightarrow \sigma^*)$. } vacuum u.v
π u.v and visible

FEMO (Free-electron molecular orbitals).



$$E_n = \frac{n^2 h^2}{8ml}$$ (Particle in a box).

$$E_{n+1} - E_n = \frac{h^2}{8ml^2}\left((n+1)^2 - n^2\right) = \frac{h^2}{8ml^2}(2n$$

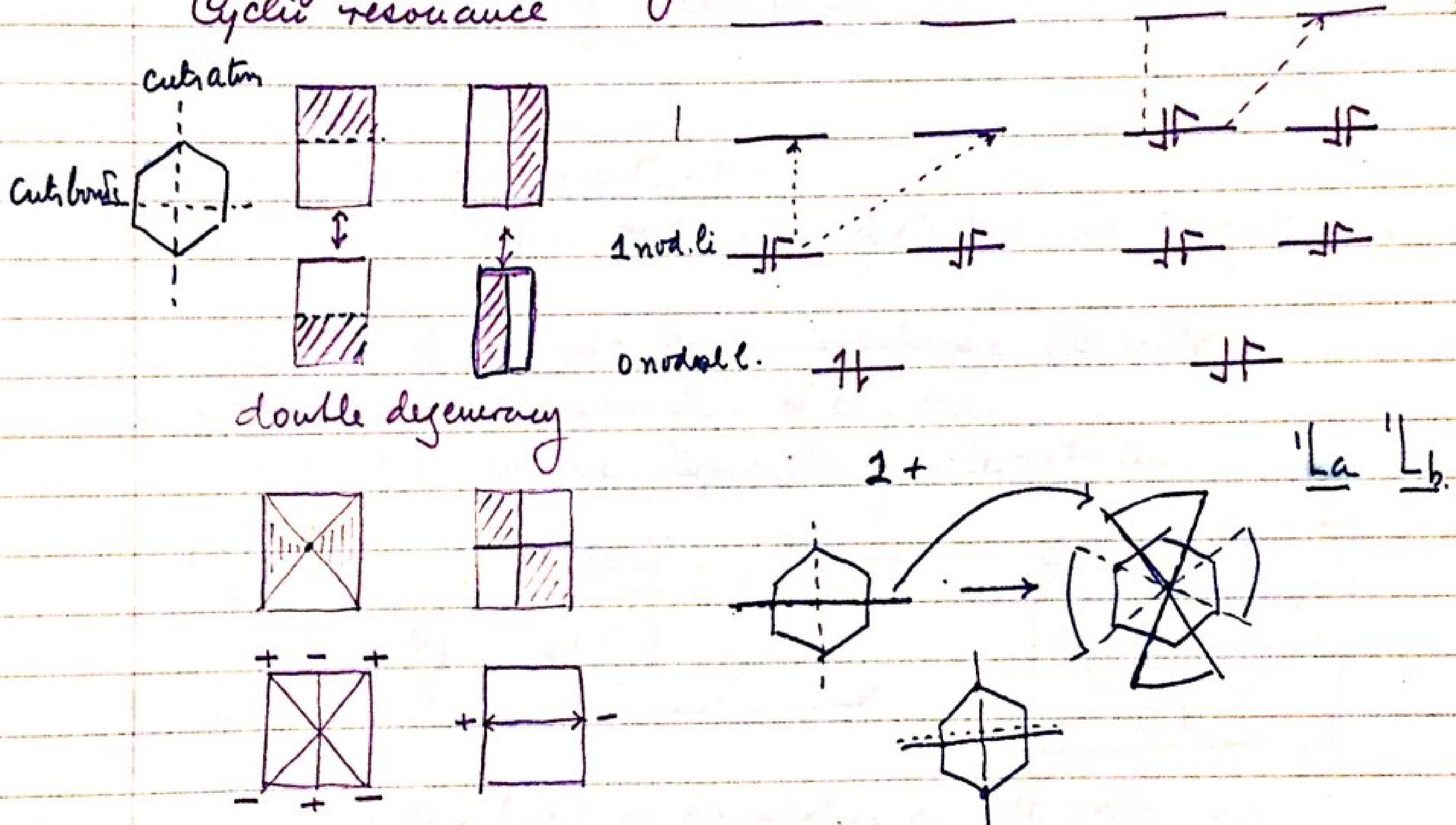
$$E_{n+1} - E_n = \frac{hc}{\lambda}.$$

$$\lambda = \frac{hc}{E_{n+1}-E_n} = \frac{8ml^2c}{2n+1} = \frac{33l^2}{(2n+1)}$$ nm if l in Å units.

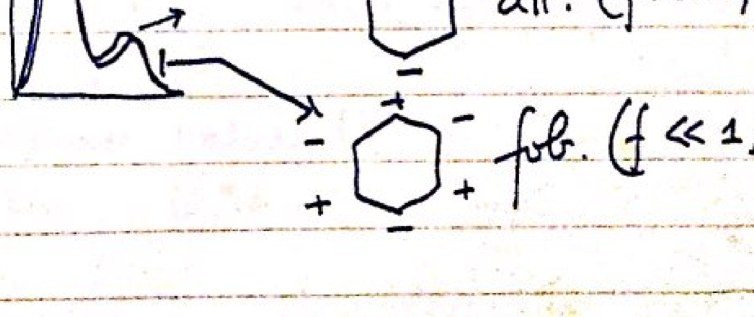
$n=4$ predicted = 471 Abs. max = 304 nm.

Case of the aromatic hydrocarbons.

Cyclic resonance

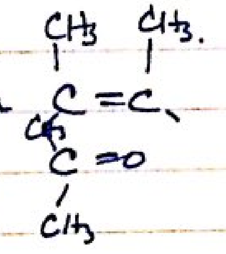


The effect of substituents = Comparison of toluene and phenol. Chromophores and auxochromes (OH, NH₂, S, O).



6th lecture n—π^* transitions.

Comparison of spectra of substances with a C=C double bond (ethylene, $\lambda_{abs} = \overset{200\text{ nm}}{165\text{ nm}}$) and C=O (acetone $\lambda_{abs} = 280$ nm) indicates a difference.



Other examples — [illegible]

- Blue shift. (Effect of hydrogen bonds).
- Protonation (fixing of lone pair).
- Substituents.

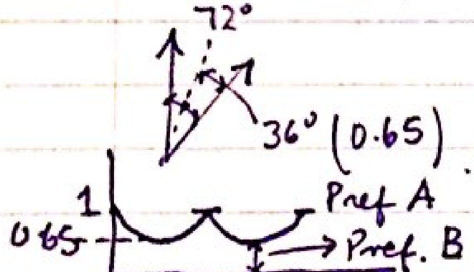
Polarization of transitions.—

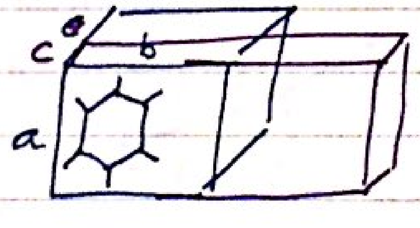
Experiments of Wiener on the effect of electric and magnetic vectors.

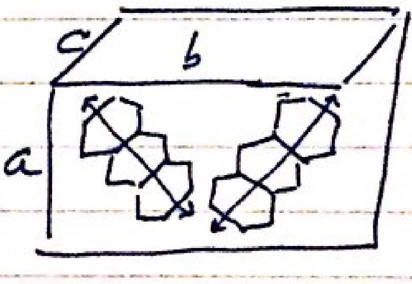
Definition of isotropic and anisotropic oscillators.

Experiments on the absorption of crystals:

Hexamethyl benzene. Difficulties of interpretation.







$\pi \rightarrow \pi^*$ transitions in aromatics are all polarized in the plane of the ring.

Environmental effects upon absorption.

Characterization of the medium by its macroscopic properties.

Significance of the quantity Δf.

Definition of shifts. Gas phase value U^*, U.

Change in energy upon solution $U^* + u^*$ $U + u$

$$U^* + u^* - (U + u) = \frac{hc}{\lambda} = hc\bar{\nu}$$

Problem calculate u^* and u.

Characterization of the medium.

$$f_{HF} = \frac{n^2-1}{2n^2-1} \qquad f_{LF} = \frac{D-1}{2D+1}$$

$$\Delta f = -\frac{n^2-1}{2n^2-1} + \frac{D-1}{2D+1}$$ = orientation polarizability

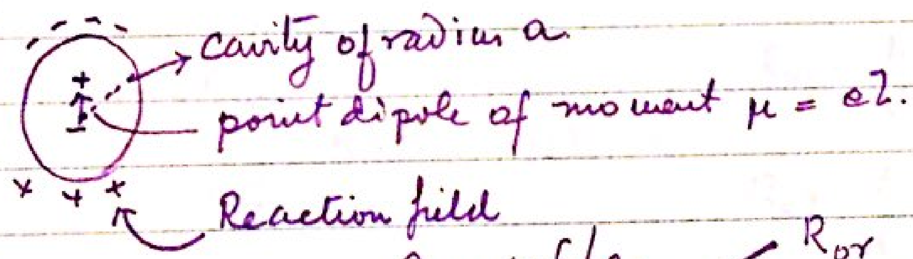
Polar liquids have $\Delta f > 0$.

Ideal non polar solvent has $\Delta f = 0$.

Hexane = 0.001

H_2O = 0.340.

7th Lecture = Dipole in a dielectric.



$$R = \mu f / a_3.$$

$$R = R_{or} + R_{el} = \frac{\mu^2}{a^3}\left[\overbrace{\Delta f}^{R_{or}} + \underbrace{\frac{n^2-1}{2n^2+1}}_{R_{el}}\right]$$

only electronic part changes upon excitation

Interaction energy.

$$u = -R\mu = -\frac{\mu^2}{a^3}\left[\Delta f + \frac{n^2-1}{2n^2+1}\right]$$

Upon absorption $\mu \rightarrow \mu^*$

$$\Delta u_{OR} = R_{OR}(\mu - \mu^*)$$ [Franck-condon!]

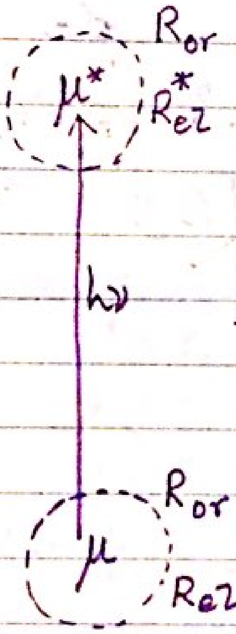
$$\Delta u_{OR} = -\frac{\mu^*}{a^3}\Delta f(\mu - \mu^*)$$

$$\Delta u_{el} = \left(\frac{\mu^2 - \mu^{*2}}{a^3}\right)(n^2-1)/2n^2+1$$

$$\Delta u = \mu(\mu^* - \mu)\frac{\Delta f}{a^3} + (\mu^{*2} - \mu^2)\{(n^2-1)/2n^2+1\}/a^3 .$$

$$\Delta \bar{\nu} = \Delta u/hc.$$

A better derivation



$R^* = R_{or} + R^*_{el}.$

$R_{or} = \frac{\mu}{a^3}\Delta f$

$R^*_{el} = \frac{\mu^*}{a^3}(n^2-1)/(2n^2+1).$

$$\boxed{\Delta u = \mu^*(R_{or} + R^*_{el}) - \mu(R_{or} + R_{el})}$$

$R = R_{or} + R_{el}.$

$R_{or} = \frac{\mu}{a^3}\Delta f$

$R_{el} = \frac{\mu}{a^3}(n^2-1)/(2n^2+1).$

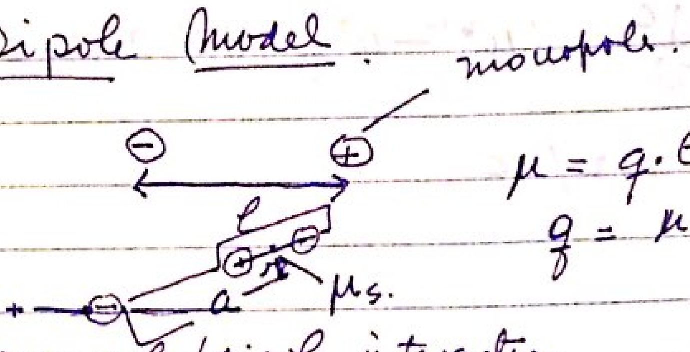
$$\Delta u = (\mu^*\mu - \mu^2)\frac{\Delta f}{a^3} + \frac{(\mu^{*2} - \mu^2)}{a^3}\frac{(n^2-1)}{2n^2+1}.$$

$$\Delta u = \mu(\mu^* - \mu)\frac{\Delta f}{a^3} + \left(\frac{\mu^{*2} - \mu^2}{a^3}\right)\left(\frac{n^2-1}{2n^2+1}\right)$$

non polar medium $\Delta f = 0$ $\quad \Delta u = \left(\frac{\mu^{*2} - \mu^2}{a^3}\right)\left(\frac{n^2-1}{2n^2+1}\right)$

$$\Delta u_{pol} - \Delta u_{non\ polar} \sim \mu(\mu^* - \mu)\Delta f/a^3.$$

oscopic Dipole Model.

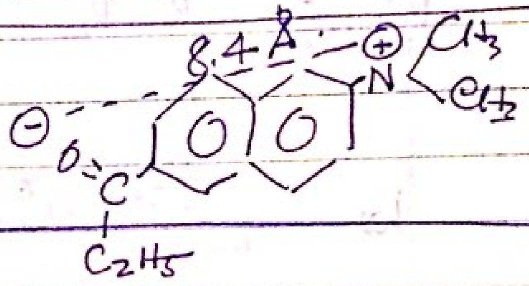


$\mu = q \cdot l$.
$q = \mu / l$.

monopole/dipole interaction

$$\boxed{u = \frac{\mu_s \cdot q}{a^2} = \frac{\mu_s \mu}{a^2 l} \cdot \cos\phi \qquad \phi =}$$

$$u = 2\frac{\mu_s \mu}{a^2 l}\cos\phi.$$



$a = 3.2 \times 10^{-8}$
$l = 8.4 \times 10^{-8}$.
$a^2 l =$

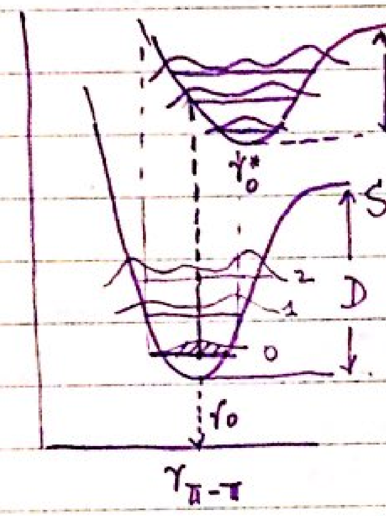
$$\langle\cos\phi\rangle = \coth\left(\frac{u}{KT}\right) - \left(\frac{KT}{u}\right).$$

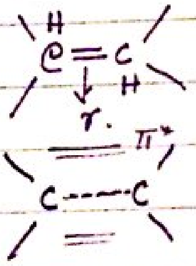
Langevin (1906).
ratio = 1.63 μ_s = 2.64 (ethanol) μ_s = 2.77 (prop. glycol).
ν_0 = 27,932. (ethanol)
ν_0 = 27,037. (P.G).
ν_0 = 29,154 (cyclohexane).

} Δ ratio = 1.73
} < (5%).

Before environmental effects

Franck-Condon principle.





Boltzmann : $n(j) = e^{-E_j/RT}$

Draw the corresponding curve for S*

$r_0^* > r_0$

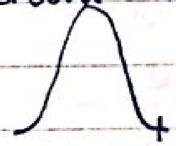
$D^* < D$.

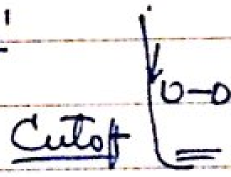
$dV/dr^* < dV/dr$.

Use of F-C principle.

Effect of temperature on absorption.

Definition of 0-0'





Franck Condon Principle immediately after Δf. and before analysis of change.

Applications to molecules.

Example from Lippert. (1955).

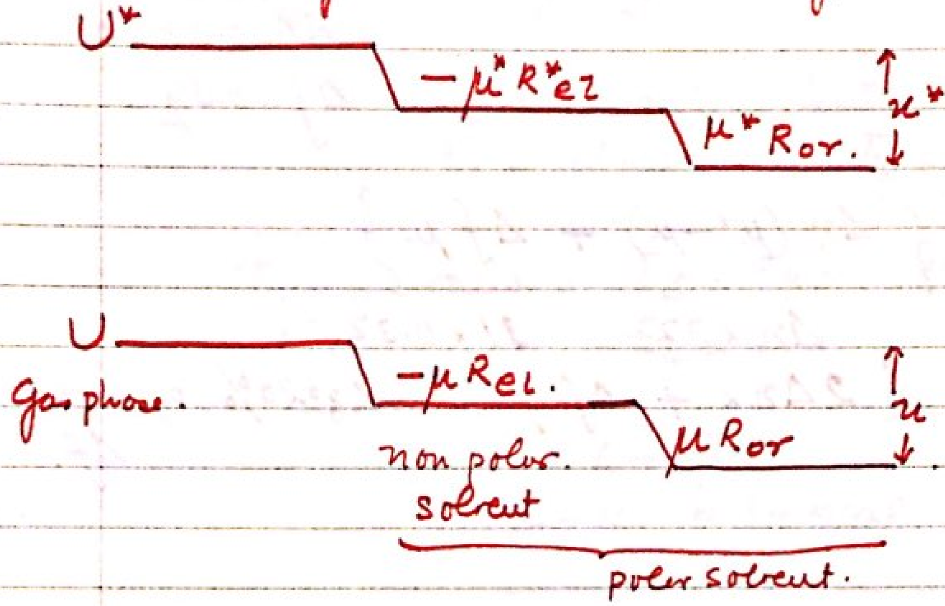
$\Delta u_{polar} - \Delta u_{nonpolar} \simeq \mu(\mu-\mu^*)\Delta f/a^3$.

$\mu = 33$.

$\Delta\mu = 11$.

5th Lecture.

Review of. Environmental effects.



$$u = u_{el} + u_{or} = -\frac{\mu^2}{a^3}\left(\frac{n^2-1}{2n^2+1}\right) - \frac{\mu^2}{a^3}\Delta f$$

$$u^* = u^*_{el} + u^*_{or} = -\frac{\mu^{*2}}{a^3}\left(\frac{n^2-1}{2n^2-1}\right) - \frac{\mu\mu^*}{a^3}\Delta f$$

$$u^* - u = \left(\frac{\mu^{*2} - \mu^2}{a^3}\right)\left(\frac{n^2-1}{2n^2+1}\right) + \mu(\mu^* - \mu)\frac{\Delta f}{a^3}.$$

Difference between $(n^2-1)/(2n^2-1)$ = ~~0.17/3.7~~5. 0.0

The first term accounts for 25% of the change and the second for 75% of the change in the example of Lippert's. –

Molecular Interactions = Effects upon absorption.
Change in the region where neither A or B absorbe.
Observation of Benesi & Hildebrand (1948).

A-Benzene band at 2900 Å Kmax = 9,000.
I-hexane λmax = 500 at Kmax – 1,000.
B-enzene-hexane " = 254 Kmax ~ 10.

η $CH_2Cl_2 = 1.4242$ $(n^2-1)/2n^2+1 = 0.3364$

$H_2O = 1.3334$ $(n^2-1)/2n^2+1 = \underline{0.3044}$

Δn 0.0320

$\Delta f = 0.32$.

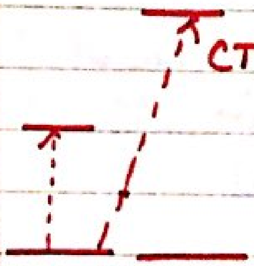
$$\frac{\overset{\mu}{(\mu^* - \mu)}}{a^3} \{ \Delta n (\mu^* - \mu) + \Delta f \mu \}.$$

$\Delta n \times 22$ 31×0.32

$(2 \Delta n \times + \Delta f)$ $\Delta n = 20\%$. of second term Δf.

$$E = I_D + E_A + f(r). = \frac{hc}{\lambda_{CT}}$$

Mulliken: $AB + h\nu \rightarrow A^-B^+$.



I_D = Ionization potential.
E_A = electron affinity of acceptor

Typical values = $I_D = 8$ eV or 174
$E_A = 2$ eV. 45

$I_D - E_A = 6$ eV. e^2/r = 65 kcal or 3 eV.

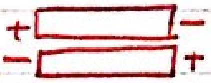
$6 - e^2/r$.

$174 - 110 = 64$ kcal.

$I_D = +8$ eV.
$E_A = -2$ eV.
$f(r) = -e^2/r = 3$ eV.

$E \doteq 3$ eV or 65 kcal.

Hypochromism and Hyperchromism.

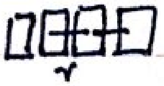


9th Lecture.

Hypochromism & Hyperchromism

Polymers.

Multimolecular interactions



$$2E\left(1+\frac{1}{2^3}+\frac{1}{4^3}\right) = \cdots 2E\left(1+\frac{1}{8}+\frac{1}{64}+\cdots\right).$$

$E \times 2.3E \sim$ ~~4.7E~~

$17 \times 2.3 =$ 51 40%.

34

$\boxed{391}$

Case of polynucleotides and polyaminoacids.

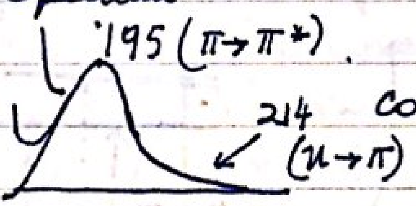
Helix coil transitions

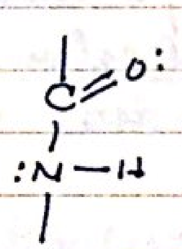
Denaturation

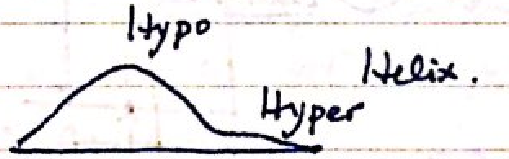
Polyglutamic: Coil = 7,000 cm^2/mMR.

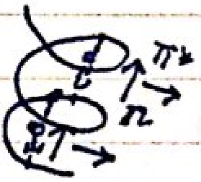
Hx = 4,500 " $^2/mMR$.

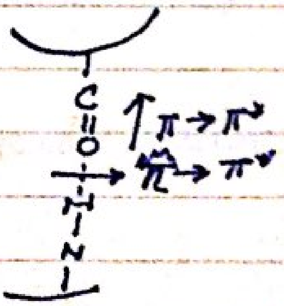
Polylysine Spectrum

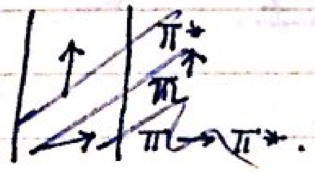












61 cal/mol

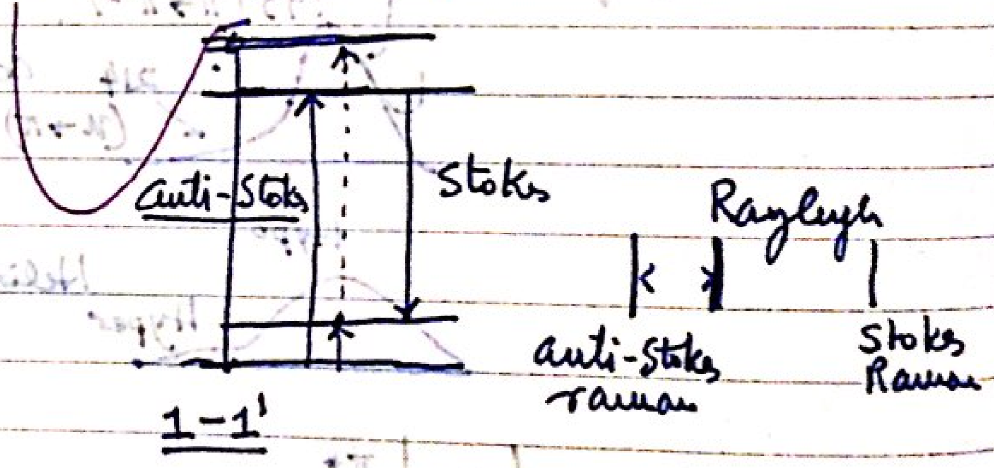
61 cal

$\Delta E = C_v \Delta T$

61 cal/mol

Displacement of Spectrum = F.C. principle.

Application to ~~dia~~



Fluorescence
General Remarks.
Fl. E. Spectrum.

10th lecture. Fl. Spectrum. | 0.1 mm Hg. = 0.1 Torr.
The three laws.

· Neporent experiments on Naphthylamine vapour

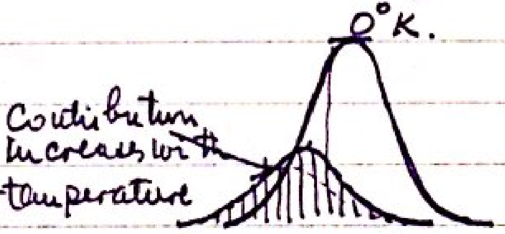
$\Delta E = C_v \Delta T_{vib}$. ($C_v$ = molar specific heat at const. volume)

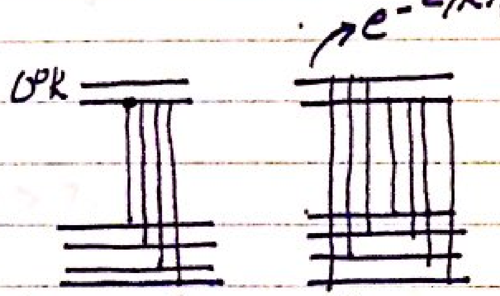
ΔE obtained from difference in energy between excitation wavelength and 0–0' transition

e.g 0–0' in Naphthylamine = 26,500 cm^{-1}

Exc. at 250 nm gives $\Delta E \sim 36$ kcal.

ΔT_{vib} = several hundred degrees.





— ΔT_{vib} may be calculated from the emitted spectrum. and C_v calculated and compared with the s.h. of $\phi\phi NH_2$.

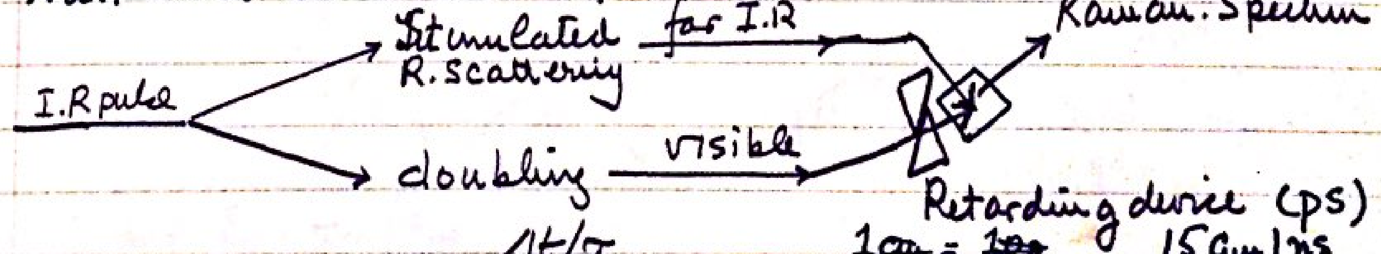
— Equipartition must be reached very rapidly 10^{-14}–10^{-13} s

— Thermalization requires collisions.

Experiments with He, H_2, pentene. (Boudart & Dubois)

Experiments of Kaiser with liquids.

Anti-stokes raman scattering.



1 cm = ~~10p~~ 15 cm/ns.

$$J = J_0 e^{-\Delta t/\tau}.$$

τ = 6, 20 psec.

$C_2H_4Cl_2$, CH_3-CH_2OH

Additions = SVL emission / observations on
~~Diff~~ the pure spectrum.
~~Franck Condon principle.~~

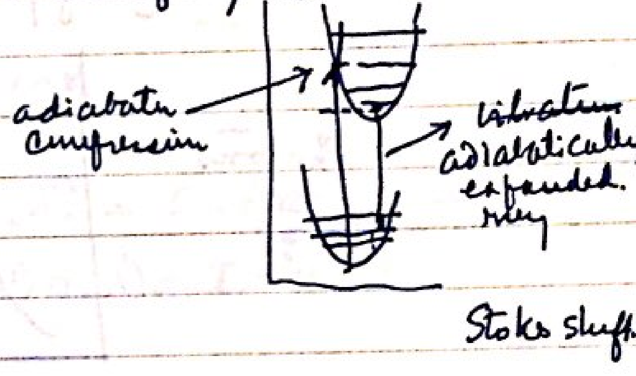
1. Constancy of the fluorescence spectrum.

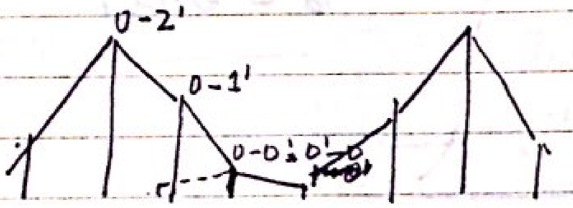
2 Displacement of the fluorescence spectrum.

F–C. principle. Solvent interactions. ($10^2 cm^{-1} - 10^4 cm^{-1}$).

3. Mirror image rule. → Geometries of S_0/S_1^*.

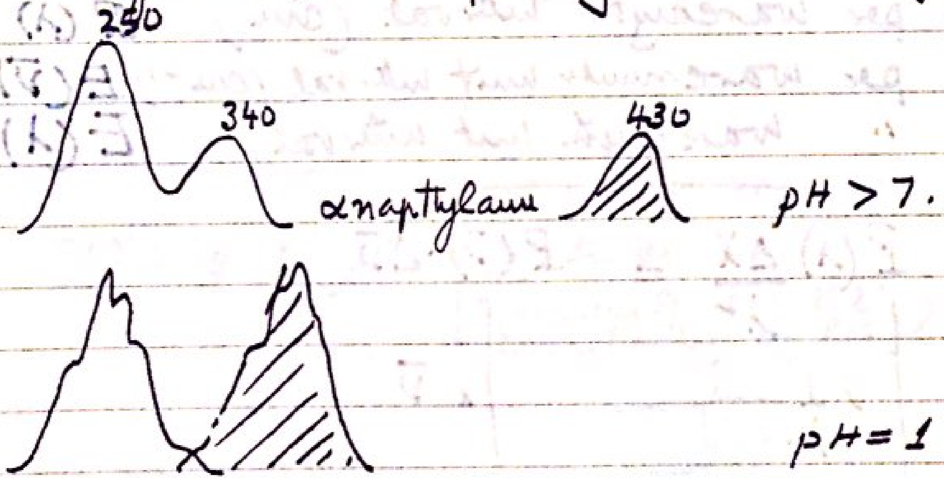
$P(0 \rightarrow n') = P(0' \rightarrow n)$.



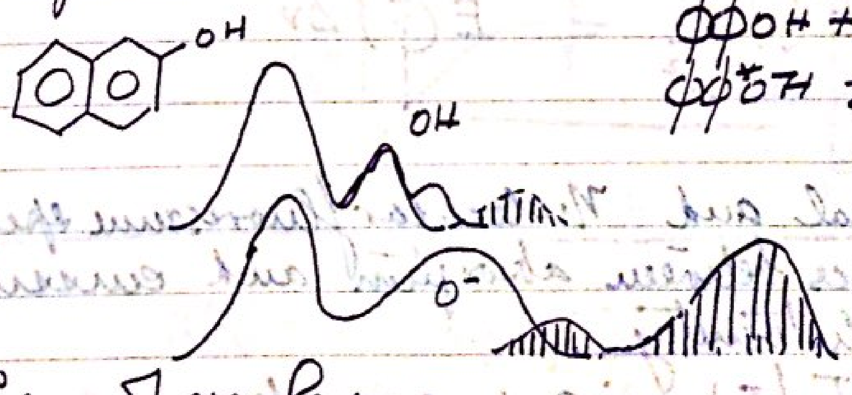


True and apparent exceptions

Case of two spectra appearing simultaneously.



Changes in the excited state.



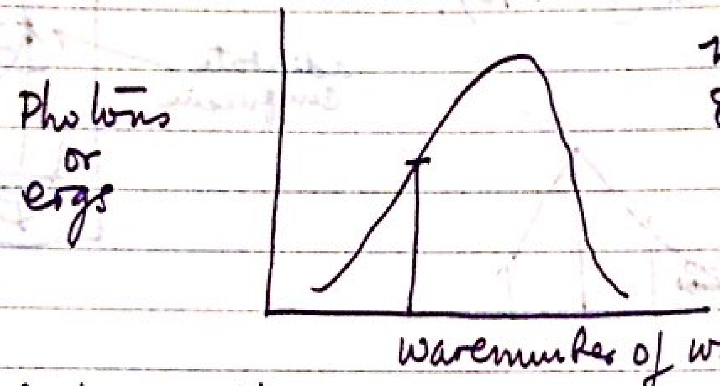
$\phi\phi OH + h\nu \rightarrow \phi\phi\overset{*}{O}H \rightarrow h\nu$

$\phi\phi\overset{*}{O}H \rightleftharpoons \phi\phi\overset{*}{O}^- + H^+$

$\downarrow h\nu''$

Case of azulene.

Lecture 11. Measurement of the fluorescence spectrum.
The units of the fluorescence may be photons or ergs (energy units) –



n = photons:
$\mathcal{E}$ = energy.

The height of the ordinate may represent one of four quantities

1. Photons per wavenumber unit interval (cm^{-1}) $P(\bar{\nu})$
2. Photons per wavelength unit interval (cm) $P(\lambda)$
3. Ergs per wavenumber unit interval (cm^{-1}) $E(\bar{\nu})$
4. " " wavelength unit interval. $E(\lambda)$.

$$\begin{tikzcd}
P(\lambda)\dfrac{\Delta\lambda}{\lambda^2} \arrow[r, phantom, "="] \arrow[d, "\times \frac{1}{\lambda}"'] & P(\bar{\nu})\cdot\Delta\bar{\nu} \arrow[d, "\times \bar{\nu}"] \\
E(\lambda)\dfrac{\Delta\lambda}{\lambda^2} \arrow[r, phantom, "="] & E(\bar{\nu})\,\Delta\nu
\end{tikzcd}$$

<u>Technical and Molecular fluorescence spectrum.</u>
Difference between absorption and emission spectrophotometry.

$(I_0/I)_\lambda$ is a pure number

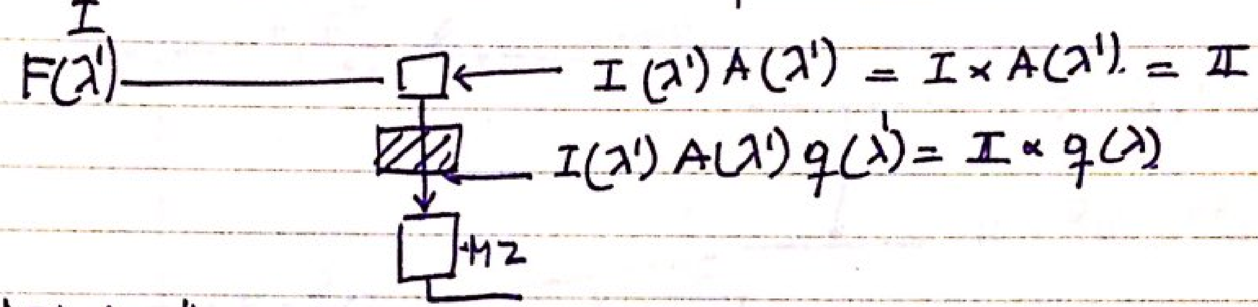
P or E are <u>not</u> dimensionless.

Lecture 11 17/ Fluorescence Excitation Spectrum.

Dependence of the exciting wavelength

Intensity of the exciting light at wavelength $\lambda' = I(\lambda')$.

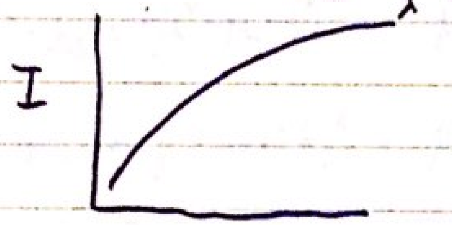
$$F(\lambda') = I(\lambda')\,A(\lambda')\,q(\lambda^*)$$



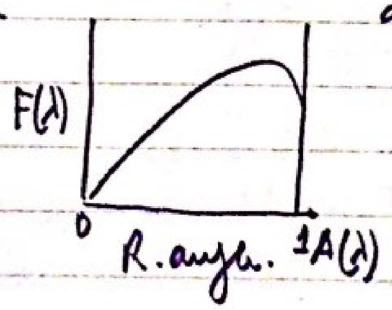
$I(\lambda')\,A(\lambda') = I \times A(\lambda') = II$

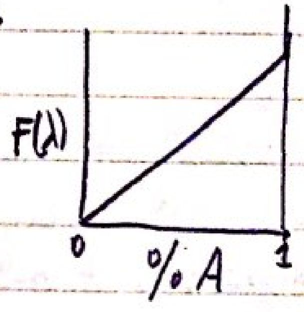
$I(\lambda')\,A(\lambda')\,q(\lambda') = II \times q(\lambda)$

'Photon" counting of Exciting light



$S(\lambda')$ is $\neq F(\lambda')$. { Geometry of exc. } λ'
Effective aperture
Transmission of MZ } λ
sensitivity of PM





$$A(\lambda) = 1 - \exp(\sigma_\lambda [c] z)$$

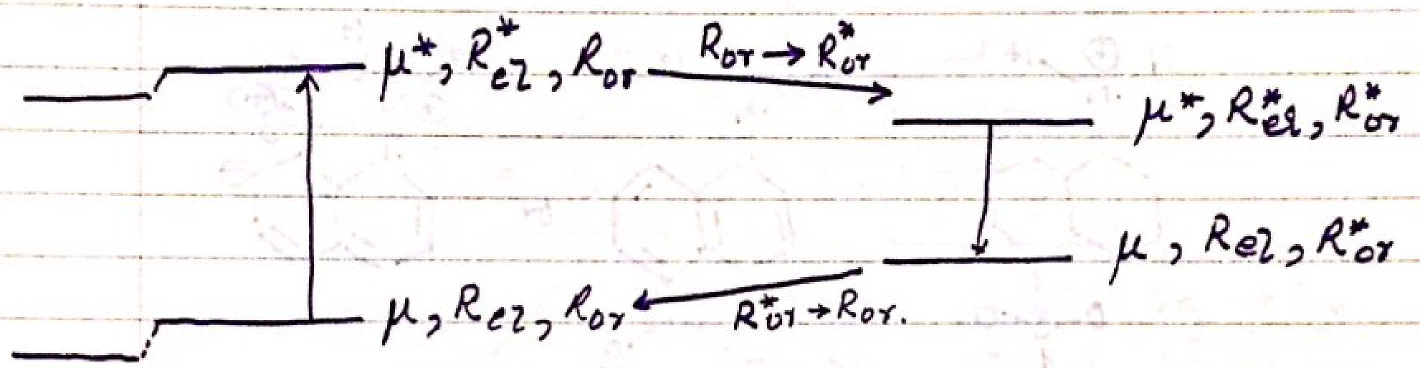
Observations by front face or at right angles.

Teclan

Lecture 12 Solvent effects upon the fluorescence.

Statement of problem.

$S_1 \rightarrow S_0^{\cdot} \quad \neq \quad S^* \rightarrow S_0$



$$\Delta u_{abs} = (\mu^* - \mu) R_{or} + \mu^* R^*_{el} - \mu R_{el}. = (U^* - U)_{abs}.$$

$$\Delta u_{em} = (\mu^* - \mu) R^*_{or} + \mu^* R^*_{el} - \mu R_{el}.$$

$$\Delta u_{abs} - \Delta u_{em} = hc(\Delta \nu_{abs} - \Delta \nu_{em}) = (\mu^* - \mu)(R_{or} - R^*_{or})$$

Notice no change in difference if Δ

$$R^*_{or} = \mu^* \Delta f / a^3 \; ; \; R_{or} = \mu \Delta f / a^3.$$

$$hc(\Delta \nu_{abs} - \Delta \nu_{em}) = (\mu^* - \mu)^2 \Delta f / a^3.$$
$$= \Delta\mu^2 . \frac{\Delta f}{a_3}.$$

$a = 4 Å \quad \Delta f = 0.34 \quad \Delta\mu = 6D. \quad \Delta\bar{\nu} = 900 cm^{-1}$.

Observations of Lippert. Naphthylamine derivative

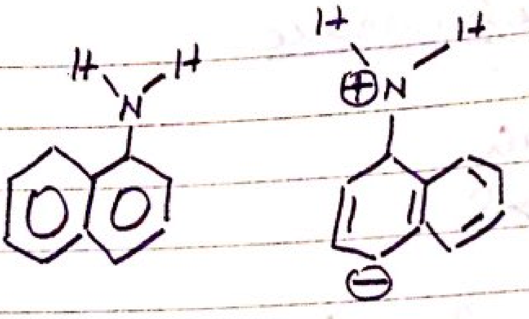
Rotational relaxation time of solvent molecules.

Einstein's relation gives =

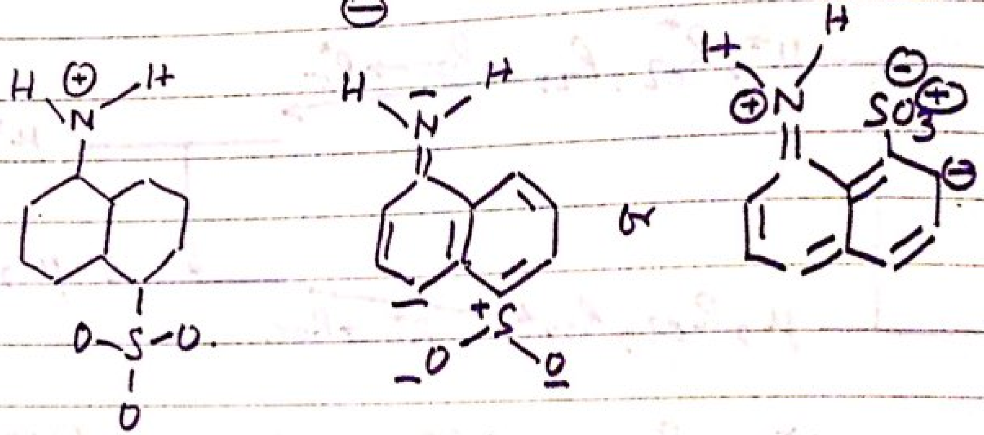
$$\rho = \frac{3\eta V}{RT.} = \frac{3V}{8.31 \times 293} \cdot 10^9 = \frac{V}{8} \times 10^{-11} \text{ sec.} \sim V \times 10^{-12} \text{ sec}$$

for water or $1 cp. = \eta \quad T = 20°. \quad T/\eta = 29,300 \quad R = 8.31 \times 10^7$.

for $V = 26 - 126 = 20 - \cancel{1}50$ p.s.



$\mu^* > \mu$.



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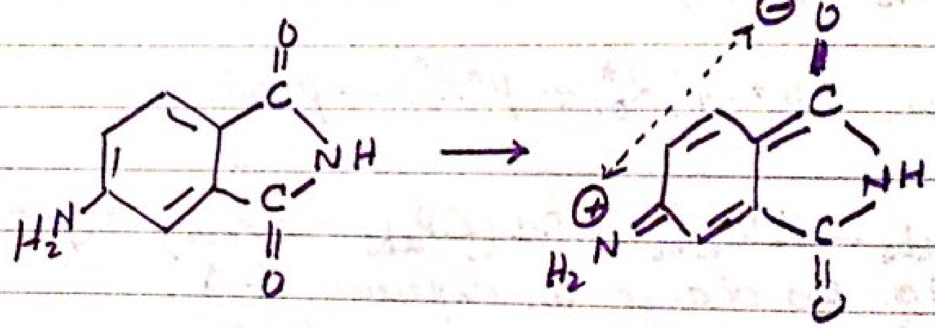


$$\bar{\nu}_g = \nu_B \cdot \frac{I(B)}{I(B)+I(R)} + \nu_R \frac{I(R).}{I(R)+I(B)}$$

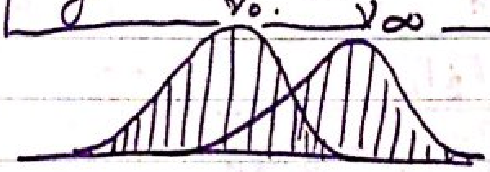
$I(B) = \Gamma_B \cdot B.$

$I(R) = \Gamma_R \cdot R.$

If $\rho/\tau \ll 1$ all motions of solvent are completed in 10% of τ.
If $\rho/\tau \gg 1$ no changes.
Bakshiev's equation. ν_g

Propylene glycol. Dielectric Constant. n Δf

$$\boxed{\nu_g = \bar{\nu}_0 - (\bar{\nu}_0 - \bar{\nu}_\infty)\frac{\tau}{\rho + \tau}}$$

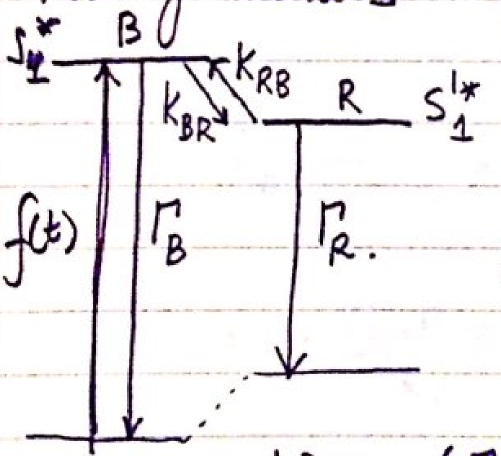


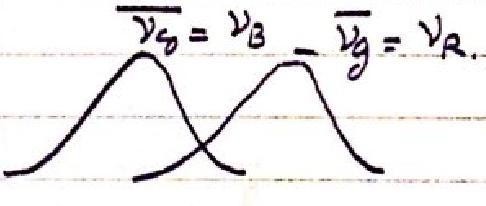
$\tau \ll \rho$ $\nu_g \to \nu_0$
$\tau \gg \rho$ $\nu_g \to \nu_\infty$

$$\nu_g = \frac{\sum \nu \cdot f(\nu)}{\sum f(\nu)}$$

$hc(\nu_0 - \nu_\infty) = (\mu^* - \mu)^2 \Delta f / a^3$.
for variable temperature

Transformations in the excited state.





Set of differential equations

$$\begin{cases} \dfrac{dB}{dt} = -(\Gamma_B + K_{BR}) \cdot B + K_{RB} \cdot R + f(t) \\ \dfrac{dR}{dt} = K_{BR} \cdot B - (\Gamma_R + K_{RB}) R \end{cases}$$

$$K_{BR} \cdot B = (\Gamma_R + K_{RB}) R$$

$$\boxed{\frac{B}{R} = \frac{\Gamma_R + K_{RB}}{K_{BR}};} \quad \bar{\nu}_g = \bar{\nu}_B \cdot \frac{B\Gamma_B}{B\Gamma_B + R\Gamma_R} + \bar{\nu}_R \frac{R\Gamma_R}{B\Gamma_B + R\Gamma_R}$$

$$\tau = \sqrt{\tau_R \tau_B}.$$

$$\rho = \frac{\frac{1}{\sqrt{\tau_R \tau_B}}}{\frac{K_{BR}}{\tau_R} + \frac{K_{RB}}{\tau_B}} = \frac{\sqrt{\tau_R \tau_B}}{\tau_B K_{BR} + \tau_R K_{RB}}$$

if $\tau_R \sim \tau_B$ $\qquad \rho = \frac{1}{K_{BR} + K_{RB}}$

$$\tau = \frac{1}{\Gamma}$$

$$\frac{1}{1 + \frac{\Gamma_B \Gamma_R}{\Gamma_B \Gamma_R + \Gamma_R K_{BR} + \Gamma_B K_{RB}}}$$

$$= \frac{\frac{1}{\cancel{\rho}}\,\tau\overset{2}{\cancel{\rho}}}{\rho + \tau.} \qquad \frac{\frac{1}{\tau\rho}}{\frac{1}{\tau^2} + \frac{\tau}{\tau\rho.}} = \frac{\frac{1}{\rho}}{\frac{1}{\tau} + \frac{1}{\rho}}$$

$$\nu_g = \bar{\nu}_B - (\bar{\nu}_B - \bar{\nu}_R)\,\frac{\Gamma_R K_{BR}}{\Gamma_B \Gamma_R + \Gamma_B K_{RB} + \Gamma_R K_{BR}}$$

if $K_{RB} \sim K_{BR} \ll \Gamma_B, \Gamma_R$ $\qquad \bar{\nu}_g \to \bar{\nu}_B$

therefore $\bar{\nu}_B = \nu_0$.

13th Lect. Continuation of Env. effects.

$$\bar{\nu}_g = \bar{\nu}_B \frac{\Gamma_B \cdot B}{\Gamma_B \cdot B + \Gamma_R \cdot R} + \bar{\nu}_R \frac{\Gamma_R \cdot R}{\Gamma_B B + \Gamma_R \cdot R}.$$

$$R = B \cdot \frac{K_{BR}}{\Gamma_R + K_{RB}}$$

~~$\bar{\nu}_g = \bar{\nu}_B \cdot \frac{\Gamma_B}{\Gamma_B + \frac{\Gamma_R K_{BR}}{\Gamma_R + K_{RB}}} + \bar{\nu}$~~

$$\bar{\nu}_g = \bar{\nu}_B - (\bar{\nu}_B - \bar{\nu}_R)\,\frac{\Gamma_R R.}{\Gamma_B B + \Gamma_R R.}$$

$$B = R \cdot \frac{\Gamma_R + K_{RB}}{K_{BR}}.$$

$$\bar{\nu}_g = \bar{\nu}_B - (\bar{\nu}_B - \bar{\nu}_R)\,\frac{\Gamma_R . R}{R \Gamma_B \frac{(\Gamma_R + K_{RB})}{K_{BR}} + \Gamma_R R}$$

$$\boxed{\bar{\nu}_g = \bar{\nu}_B - (\bar{\nu}_B - \bar{\nu}_R)\,\frac{\Gamma_R K_{BR}}{\Gamma_B \Gamma_R + \Gamma_B K_{RB} + \Gamma_R K_{BR}}}$$

if $K_{RB} \sim K_{BR} \ll \Gamma_B \Gamma_R$; $\bar{\nu}_g \to \bar{\nu}_B = \nu_0$.

if $\Gamma_R \sim \Gamma_B \ll K_{BR}, K_{RB}$. $\nu_g \to \nu_\infty$

$$\bar{\nu}_g = \bar{\nu}_B - (\bar{\nu}_B - \bar{\nu}_R)\frac{\Gamma_R K_{BR}}{\Gamma_R K_{BR} + \Gamma_B K_{RB}} = \nu_\infty$$

$$\bar{\nu}_B = \nu_0$$

$$\underline{\bar{\nu}_0 - \nu_\infty} = (\nu_B - \nu_R)\frac{\Gamma_R K_{BR}}{\Gamma_R K_{BR} + \Gamma_B K_{RB}}$$

$$\underline{\nu_g = \nu_B \mp (\nu_B - \nu_R)}$$

$$(\nu_B - \nu_R) = (\nu_0 - \nu_\infty)\frac{\Gamma_R K_{BR} + \Gamma_B K_{RB}}{\Gamma_R K_{BR}}$$

$$\bar{\nu}_g = \nu_0 - (\nu_0 - \nu_\infty)\frac{\Gamma_R K_{BR} + \Gamma_B K_{RB}}{\Gamma_R \Gamma_B + \Gamma_R K_{RB} + \Gamma_B K_{BR}}.$$

Two-state equation

Bakshiev. $\bar{\nu}_g = \nu_0 - (\nu_0 - \nu_\infty)\frac{\tau}{\tau + \rho}$.

let $\Gamma_R = \Gamma_B = \Gamma = \frac{1}{\tau}$.

$$\rho = \frac{1}{K_{RB} + K_{BR}}$$

Solution for the impulse response.

$$\frac{dB}{dt} = -(\Gamma_B + K_{BR})B + K_{RB}\cdot R.$$

$$(D+\Gamma_B+K_{BR})B - K_{RB}\cdot R = 0.$$

$$-K_{BR}B + (D+\Gamma_R+K_{RB})R = 0.$$

To make things easier let $\Gamma_B = \Gamma_R = \Gamma$.

$$(D+\Gamma+K_{BR})B - K_{RB}\cdot R = 0.$$

$$-K_{BR}B + (D+\Gamma+K_{RB})R = 0.$$

$$\Delta = (D+\Gamma+K_{BR})(D+\Gamma+K_{RB}) - K_{BR}K_{RB}.$$

$$= D^2 + (2\Gamma+K_{BR}+K_{RB}) + \Gamma^2 + \Gamma(K_{BR}+K_{RB}) =$$

$$\Delta = D^2 + (2\Gamma+K_{BR}+K_{RB}) + \Gamma(\Gamma+K_{BR}+K_{RB}).$$

$$B = \frac{\begin{vmatrix} 0 & -K_{RB}. \\ 0 & D+\Gamma+K_{RB} \end{vmatrix}}{\Delta} \; ; \quad R = \frac{\begin{vmatrix} D+\Gamma+K_{BR} & 0 \\ -K_{BR} & 0 \end{vmatrix}}{\Delta}$$

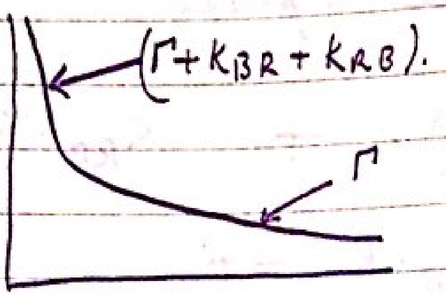
$$\Delta\cdot B = 0 \qquad \Delta\cdot R = 0$$

$$\frac{d^2B}{dt^2} + a\frac{dB}{dt} + b = 0 \;\Big|\; B(t) = A_1 e^{\lambda_1 t} + A_2 e^{\lambda_2 t}.$$

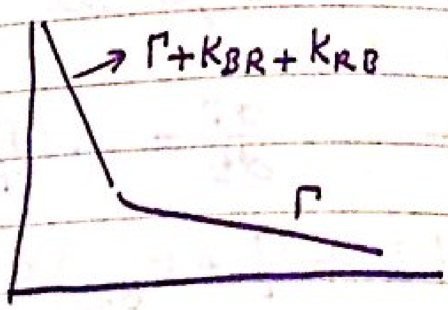
$$\lambda_1 + \lambda_2 = 2\Gamma + K_{BR} + K_{RB}.$$

$$\lambda_1\lambda_2 = \Gamma(\Gamma + K_{BR} + K_{RB}).$$

$$\left.\begin{aligned} \lambda_1 &= -\Gamma \\ \lambda_2 &= -(\Gamma + K_{BR} + K_{RB}) \end{aligned}\right\}$$



$\ln J_{\underline{\underline{B}}}$



Proton Dissociation in the excited state..

β-Naphthol.

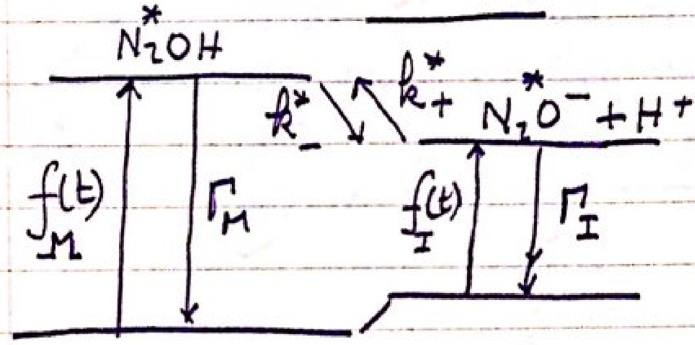
$$N\cdot OH + h\nu \rightarrow N^*OH.$$

$$N_2^*OH \rightleftarrows N_2^*O^- + H^+$$

$$N_2^*OH \xrightarrow{h\nu'} N_2^*OH. + h\nu'$$

$$N_2^*O^- \longrightarrow N_2O^- + h\nu$$

$$N_2O^- + H^+ \longrightarrow N_2OH$$



$$\frac{d\,NOH}{dt} = f_M(t) - (\Gamma_M + k_-)M + k_+[H^+]\,I = \frac{dM}{dt}.$$

$$\frac{d\,NO^-}{dt} = f_I(t) - (\Gamma_I + k_+[H^+])I + k_-M = \frac{dI}{dt}.$$

$$f_M(t) = \epsilon_M(1-\alpha)\,f(t)$$

$$f_I(t) = \varepsilon_I\,\alpha\, f(t). \qquad \underline{\alpha = 0}.$$

$$\frac{[I]}{[M]} = \frac{k_-}{k_+[H] + \Gamma_I + \varepsilon_I\alpha.}$$

$$\bar{\nu}_g = \bar{\nu}_M \frac{\overline{M.\Gamma_M}}{M.\Gamma_M + I\Gamma_I} + \nu_I \frac{I\Gamma_I}{M\Gamma_M + I\Gamma_I}$$

if $\alpha \rightarrow 0$ $\qquad \dfrac{[I]}{[M]} = \dfrac{k_-^*}{k_+^*[H] + \Gamma_I.}$

if $k_+[H] \gg \Gamma_I$ $\qquad \dfrac{[I]}{[M]} = \dfrac{k_-^*}{k_+^*[H]}$ then $\dfrac{[I]}{[M]}$

Conditions under which thermodynamic equilibrium is reached.

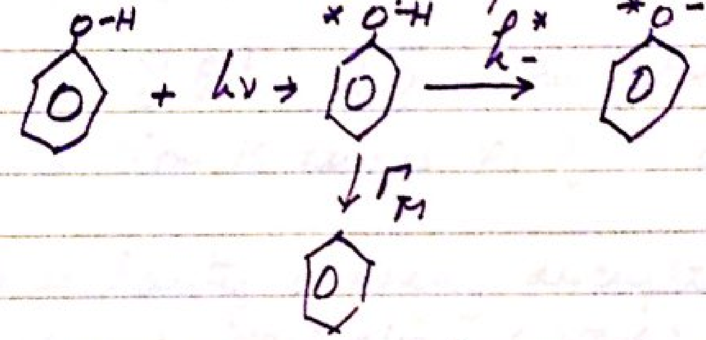
Necessary: $k_-^* \gg \Gamma_{M}$.

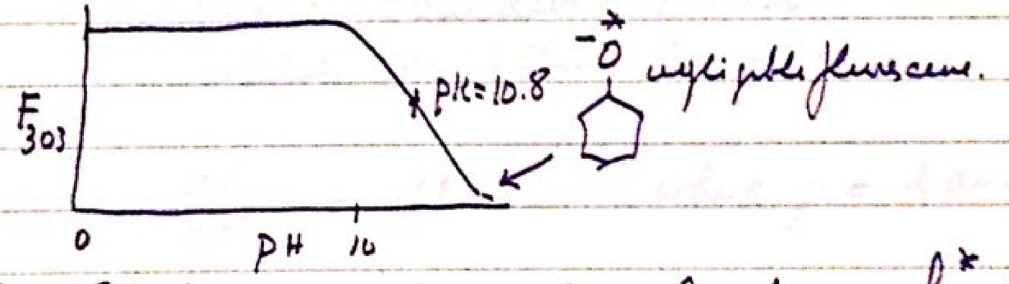
Necessary: $k_+^* [H^+] \gg \Gamma_I$.

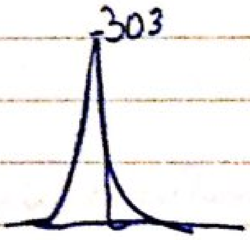
or $\rho = \dfrac{1}{k_-^* + k_+^* [H^+]} \gg \tau$. (if $(\Gamma_M \sim \Gamma_I)$)

ρ is the relaxation time of the chemical equilibrium.
In all other cases no true equilibrium is reached.

Ex.



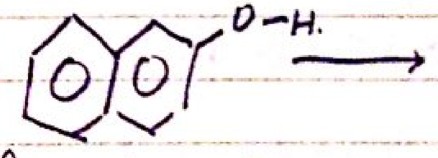




Elect. Titration gives pK = 10.8. Conclusion $k_-^* \ll \Gamma_M$.

$\Gamma_M = \frac{1}{2} \times 10^9 = 5 \times 10^8$. $k_-^* \ll 5 \times 10^{-1}$.

Naphthol



Close to observed pK*

Case of the naphthylamines.
α — N⁻ (quenched).
β — N⁻ (shifted).
anthryl amines shifted

Lecture 14.

Fluorescence lifetime.

Coherent and incoherent radiation

$$A_i = A_0 \sin(\omega t + \varphi_i)$$

A_i = amplitude of electric field

$\mathcal{E}_i = A_i^2$

$$\mathcal{E} = \left(\sum_i A_i\right)^2 = \sum_i A_i^2 + 2\sum A_i A_j$$

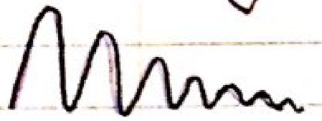
$$A_i^2 = A_0^2 \sin^2(\omega t + \varphi_i) = \mathcal{E}_i^2$$

$$\mathcal{E} = \sum \mathcal{E}_i^2 + 2\sum A_0^2 \sin(\omega t + \varphi_i)\sin(\omega t + \varphi_j).$$

The second term is zero is $\varphi_i \varphi_j$ are chosen at random.

Complementarity of decay description.

1. By a damped oscillator (wave)
2. By an excited population (particle).



$\frac{d\mathcal{E}}{dt} = -\mu \mathcal{E}$ where μ = damping constant

$\frac{dn^*}{dt} = -\Gamma n^*$ where Γ = rate of emission.

Solution $n_t^* = n_0^* e^{-\Gamma t}$.

Average lifetime in the excited state

$$\langle t\rangle = \frac{\int n_0^* e^{-\Gamma t} \times t\,dt}{\int n_0^* e^{-\Gamma t}} = \frac{\int_0^\infty n_t^* \cdot t\,dt}{\int n^* dt}$$

generalization of $\langle t\rangle = \frac{\sum t \times n_t}{\sum n_t}$.

$$\int_0^\infty t e^{-\Gamma t} = -\frac{1}{\Gamma} t e^{-\Gamma t}\Big|_0^\infty + \frac{1}{\Gamma}\int e^{-\Gamma t} dt.$$

$$\langle t \rangle = \frac{\frac{1}{\Gamma}\int_0^\infty e^{-\Gamma t} dt}{\int_0^\infty e^{-\Gamma t} dt} = \frac{1}{\Gamma}. = \tau.$$

τ the average lifetime is the reciprocal of the rate of emission
Methods of determination. The two general methods of investigation of dynamic systems. (Impulse response, or harmonic responce).

$$\frac{dn^*}{dt} = -\Gamma n^* + f(t)$$

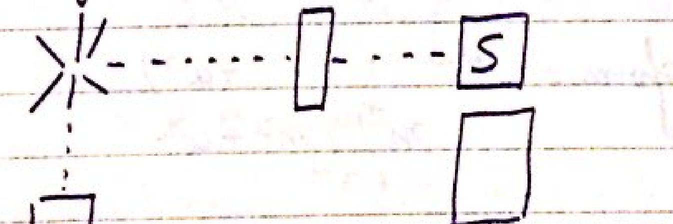
if $f(t)$ is a very brief ($t \ll \frac{1}{\Gamma}$) pulse of light. at all instants afterwards $f(t) = 0$. The response of the system is the so called impulse response. The solution is

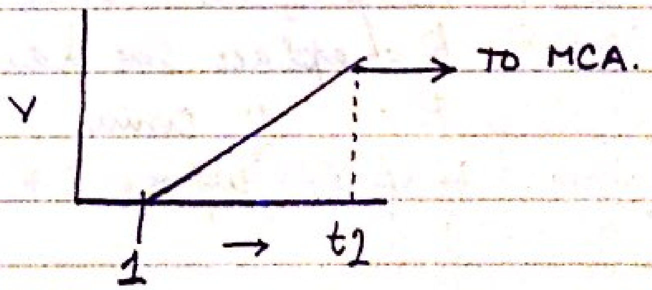
$$n^* = n_0^* e^{-\Gamma t}$$

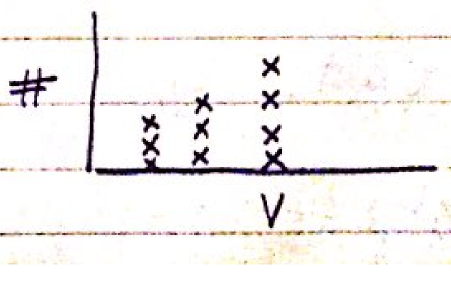
Paractical methods using the impulse response:

d ns. for conventional lamp. a few ps. for Laser. pulse.

Single photon technique







Sine and Cosine transforms.

Let $I = \beta . \cancel{\beta} e^{-mt}$. (normalized impulse response).

$$P = \int_0^\infty I(t) \cos \omega t \, dt = \frac{\beta m}{m^2 + \omega^2}$$

$$Q = -\int_0^\infty I(t) \sin \omega t \, dt = \frac{\beta \omega}{m^2 + \omega^2}$$

} Laplace. transform.

$$\tan \varphi = Q/P = -\omega/\Gamma$$

Rel. Modulation $\sqrt{P^2 + Q^2} = \sqrt{\frac{\beta^2 (m^2 + \omega^2)}{(m^2 + \omega^2)^2}} = \frac{\beta}{\sqrt{m^2 + \omega^2}}$.

$\beta = 1$. $\tan \phi = -\omega\tau$.

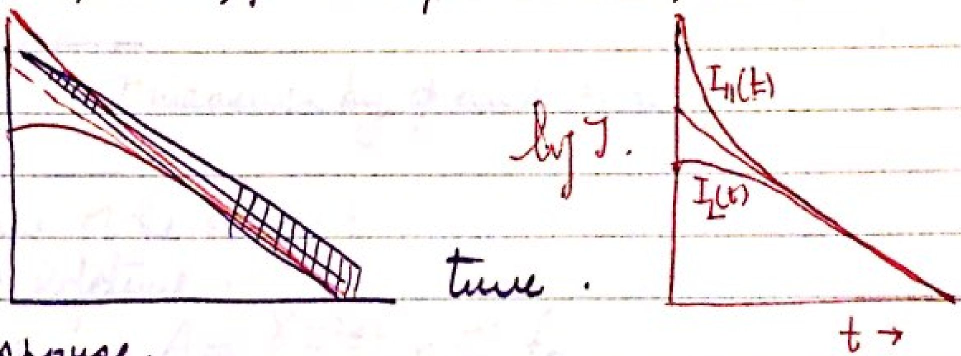
Rel mod $= \frac{1}{\sqrt{m^2 + \omega^2}}$

V. Solodovnikov. (Dover paper back).
Statistical Dynamics of automatic Control systems.

$$\tan \Delta = \frac{\tan \phi_1 - \tan \phi_2}{1 + \tan \phi_1 \tan \phi_2} = \frac{\frac{P_1}{Q_1} - \frac{P_2}{Q_2}}{1 + \frac{P_1}{Q_1}\frac{P_2}{Q_2}} = \frac{P_1 Q_2 - P_2 Q_1}{Q_1 Q_2 + P_1 P_2}$$

Q = Laplace sine transform = $\omega / (m^2 + \omega^2)$
P = " cosine " $m / (m^2 + \omega^2$.
$Q/P = \omega/m$.

<u>One photon technique</u> require approximately either 1 or 0 photon emitted by fluorescent light. Rep. Rate of lamp. = KHz 90% of pulse should not generate excitation or 100 - 1000 c.p.s. Error in any channel is prop. to $\sqrt{\text{cou}}$
For 100 counts 10% error, for 10^4 ajb $\pm 10^2$ or 1% error.



Harmonic response.

$$f(t) = a + b \sin \omega t \quad \text{when } a \geq b.$$

$$n^*(t) = A + B \sin(\omega t + \varphi) \quad (\text{forced response}).$$

$$n^*(t) = A + B \sin \omega t \cos \varphi + B \cos \omega t \sin \varphi.$$

$$\frac{dn^*}{dt} = \omega B (\cos \omega t \cos \varphi - \sin \omega t \sin \varphi).$$

$$\frac{dn^*}{dt} + \Gamma n^* = f(t).$$

$$\Gamma A + \Gamma B \sin \omega t \cos \varphi + \Gamma B \cos \omega t \sin \varphi + \omega B (\cos \omega t \cos \varphi - \sin \omega t \sin \varphi)$$
$$= a + b \sin \omega t.$$

$$\Gamma A + B \sin \omega t \left[\Gamma \cos \varphi - \omega \sin \varphi\right] + B \cos \omega t \left[\Gamma \sin \varphi + \omega \cos \varphi\right]$$
$$= a + b \sin \omega t$$

$$A = \frac{a}{\Gamma}; \quad \Gamma \sin \varphi + \omega \cos \varphi = 0 \quad -\frac{\omega}{\Gamma} = \tan \phi.$$
$$\Gamma \cos \varphi - \omega \sin \varphi = b/B.$$
$$\Gamma^2 (\sin^2 \varphi + \cos^2 \varphi) + \omega^2 (\sin^2 \varphi + \cos^2 \varphi) = (b/B)^2$$

$$A = a/\Gamma$$

$$b/B = \sqrt{\Gamma^2 + \omega^2} \quad : \frac{B}{p} = \frac{1}{\sqrt{\Gamma^2 + \omega^2}}$$

$$\text{Rel Mod} = \frac{A/B}{a/b} = \frac{\Gamma}{\sqrt{\Gamma^2 + \omega^2}} = \frac{1}{\sqrt{1 + (\omega/p)^2}}$$

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Lecture 15. Fluorescence Lifetime.

Experimental Results.

1. Value observed = Range.
2. Detection of heterogeneity.

Two components:

{Double decay.
{Difference in τ measured by ϕ and mod.

Significance of the values.

The emissive lifetime.

$$\frac{dE}{dt} = At \qquad A = \frac{8\pi^2 e^2}{mc} n \bar{\nu}^2 . f_e .$$

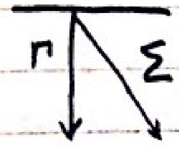
$$f_e \simeq f_a . \qquad A = 0.7667\,\bar{\nu}^2 f_e . \qquad \tau = A^{-1}$$

For $f_e = 1$ $\lambda = 350$ $\tau = 1.4$ ns.
$\lambda = 450$ $\tau = 2.2$ "
$\lambda = 650$ $\tau = 4.6$ "

Fluorescein $= f = \frac{1}{2}$
$f_e =$ Quinine $= f_a = 0.1$

High absorption ⇔ High emis

Competitive Processes.



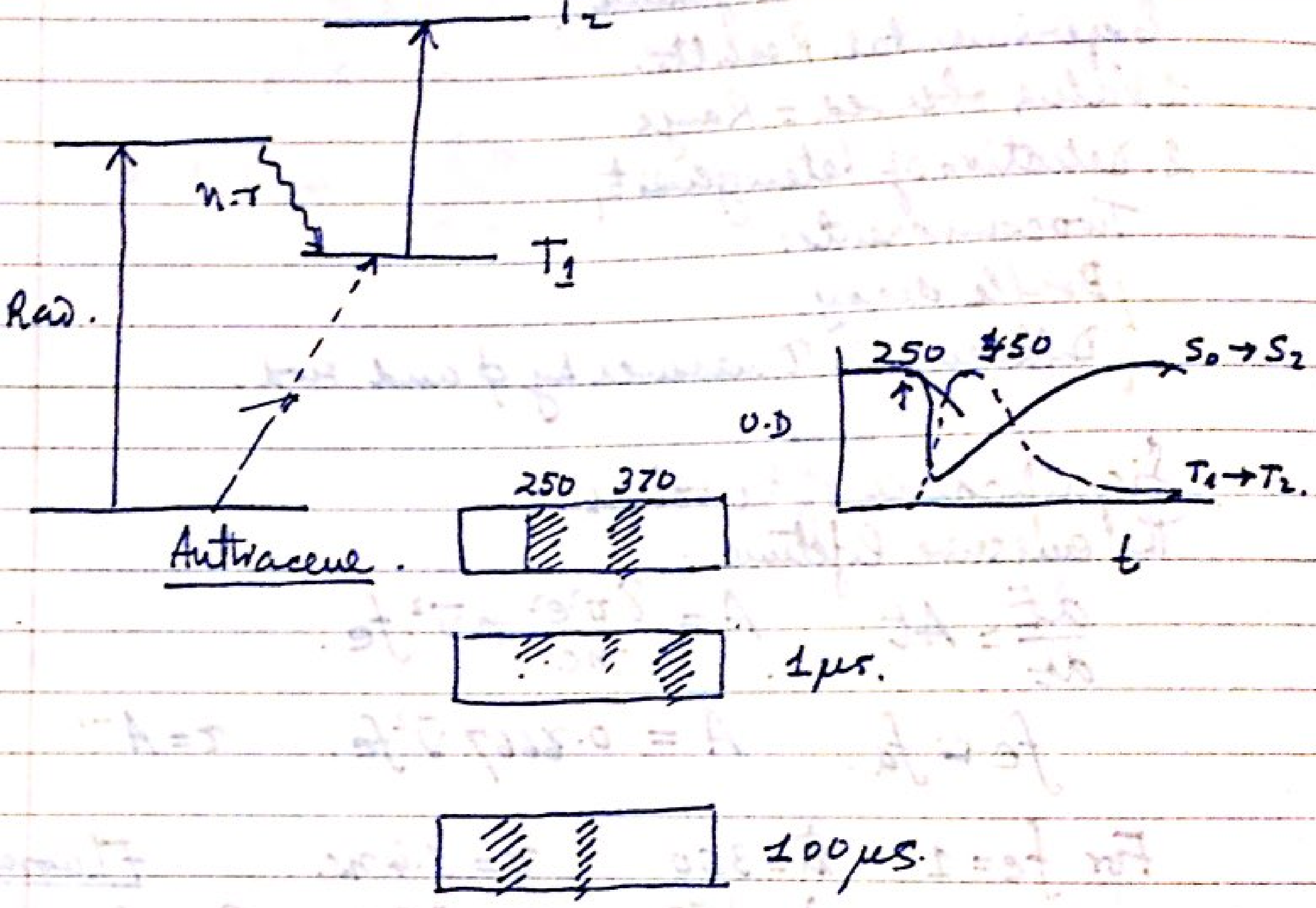
$$\frac{dn^*}{dt} = \varphi - (\Gamma + \Sigma) n^*$$

$$n^*_t = n^*_0 e^{-t(\Gamma+\Sigma)t}$$

$$\tau = \frac{1}{\Gamma + \Sigma} \qquad \frac{1}{\Gamma} = \tau_e .$$

rate of decay $= \Gamma + \Sigma$
" " emission $= \Gamma$ } $q = \frac{\Gamma}{\Gamma + \Sigma}$

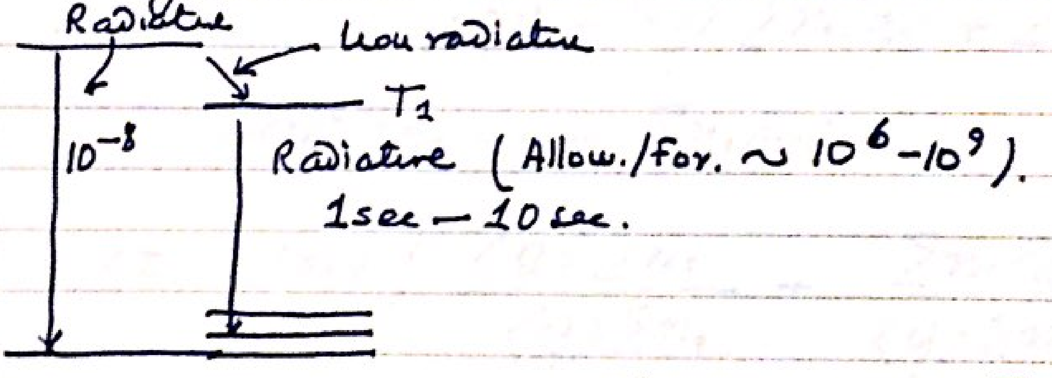
$$\boxed{\tau / \tau_e = q .}$$


T₂
n.r
T₁
Rad.
Anthracene.
250
370
1μs.
100μs.
250
450
S₀→S₂
O.D
T₁→T₂.
t

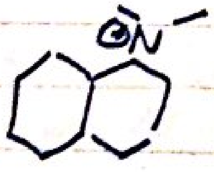
Lecture 16th Quantum Yield.

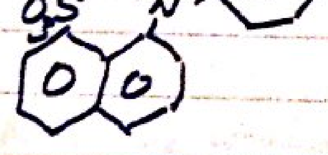
Definition: Energy yield and quantum yield.
Methods of measurement: By calorimetric methods.
By photometric methods.–
Relative value –v– Absolute value.
Measurements and values. Use of the rule
$\tau = \tau_e q$ where τ is observed, q calculated.
Quinine sulfate 0.55 or 0.70 ?. 0.63 ± 0.07 (10%)

Causes of variable yield.
Radiationless Transitions.
The triplet state. Demonstration by Flash photolysis and by phosphorescence. Additional causes?



$T_1 - T_n$ $(n \geq 2)$ is investigated by Flash Photolysis. This permits to calculate the rate of $T_1 - T_0$ (non radiative) in the fluid. It is seen to be app $10^{-3} - 10^{-6}$.
Therefore $q_{TS} = \underline{10^{-3} - 10^{-6}} \sim 10^{-4} - 10^{-7}$.
This very low yield[10] has in fact been observed.
Effects of temperature and solvent on yields. The Case of ANS and other amines.





$$\sum \tau_{ei} n_i q_i^2 = \langle q \rangle \sum n_i \tau_{ei} + 2\bar{q} \sum$$

Heterogeneous Population. –

$$\tau_i = \tau_{ei}\, q_i$$

$$\langle\tau\rangle = \frac{\sum \tau_i n_i q_i}{\sum n_i q_i} = \frac{\sum \tau_{ei}\, n_i q_i^2}{\sum n_i q_i}.$$

$$\langle q\rangle = \sum n_i q_i / \sum n_i$$

$$q_i = \langle q\rangle + \delta q_i \qquad \sum n_i \delta q_i = 0.$$

$$q_i^2 = (\langle q\rangle + \delta q_i)^2 = \langle q\rangle^2 + 2\langle q\rangle \delta q_i + \delta q_i^2.$$

$$\sum n_i q_i^2 = (\sum n_i)\langle q\rangle^2 + 2\langle q\rangle \sum n_i \delta q_i + \sum n_i \delta q_i^2.$$

$$= \langle q\rangle^2 \sum n_i + 0 + \sum n_i \delta q_i^2$$

$$\langle\tau\rangle = \langle\tau_{ei}\rangle \left\{ \frac{\langle q\rangle^2 \sum n_i}{\langle q\rangle \sum n_i} + \frac{\sum n_i \delta q_i^2}{\langle q\rangle \sum n_i} \right\}$$

$$\langle\tau\rangle = \langle\tau_{ei}\rangle\langle q\rangle + \frac{\langle\tau_{ei}\rangle}{\langle q\rangle}\, \overline{\delta q_i^2}$$

$$\langle\tau\rangle \geqslant \langle\tau_{ei}\rangle\langle q\rangle.$$

= homogeneous.

> heterogeneous population.

All with equal τ_{ei}!

The results would be different if τ_{ei} would be different.

Polarization of fluorescence

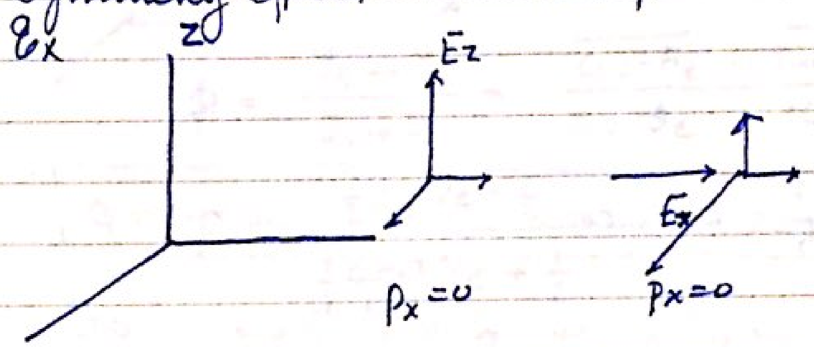
Coherent and Incoherent case.

Representation by dipoles. Definition of P and p.

$$P = \frac{P}{P+N} = \frac{J_z - J_y}{J_z + J_y}.$$

Lecture 17

Symmetry effects. Perrin's application of Curie's law.



Case 1 Excitation by light E_z. or E_x.

$E_z \longrightarrow I_z^z \neq I_y^z = I_x^z$. $p_z \neq 0, p_y = p_x = 0$

$E_x \rightarrow I_x^x \neq I_z^z = I_y^z$ $p_z = 0 = p_y \quad p_x \neq 0$

define $I_\parallel, I_\perp$ $\frac{I_\parallel}{I_\perp} = \frac{1+p_z}{1-p_z}$

Case 2

$E_z = E_x$ (natural light)

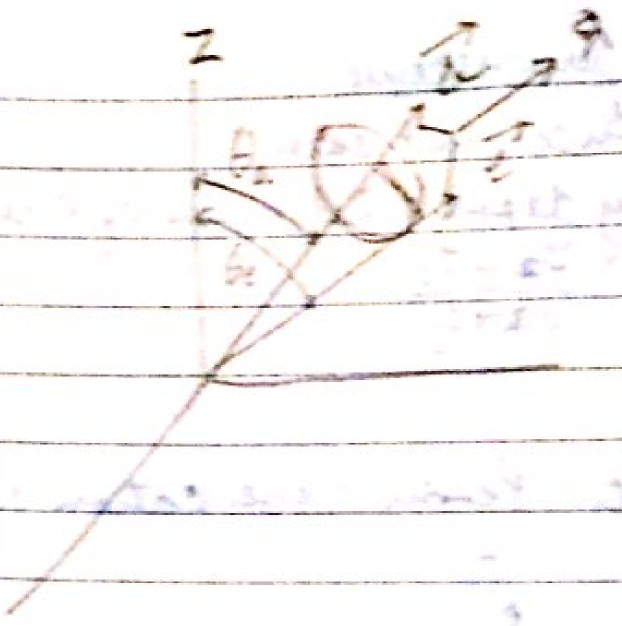
$I_{N_x} \quad I_{N_y} \quad I_{N_z}$. $p_{nx} = \frac{I_{N_z} - I_{N_y}}{I_{N_z} + I_{N_y}}.$

$$\left.\begin{aligned} I_{N_x} &= I_x^z + I_z^x \\ I_{N_y} &= I_y^z + J_z^x. \\ I_{N_z} &= I_y^z + I_y^x \end{aligned}\right\} P_{nx} ~~P_z~~ = \frac{I_z^z + I_z^x - (I_y^z + I_y^z)}{I_z^z + I_z^z + I_y^z + I_y^z}$$

$I_y^x = I_x^z = I_\perp$ $\frac{I_\parallel}{I_\perp} =$

$P_{nx} = \frac{I_\parallel + I_\perp - 2I_\perp}{I_\parallel + 3I_\perp}$ $I_z^z = I_x^x = I_\parallel$ $P_{nx} = \frac{p_x}{2-p_x}.$

$$p_{nx} = \frac{I_\parallel - I_\perp}{I_\parallel + I_\perp + 2I_\perp} = \frac{\frac{1+p_x}{1-p_x} - 1}{\frac{1+p_x}{1-p_x} + 3} = \frac{2p_x/(1-p_x)}{(4 - 2p_x)/(1-p_x)} =$$



$$\boxed{\cos\theta_e = \cos\theta_h \cos\lambda + \sin\theta_h \sin\lambda \cos\phi}$$

ϕ angle determined by $Z O \vec{h}$ and $a O \vec{e}$.

if $\phi = \pi$ reduces to usual formula
$= 0$

$\cos\theta_e =$

$$\cos^2\theta_e = \cos^2\theta_h \cos^2\lambda + \sin^2\theta_h \sin^2\lambda\, \overline{\cos^2\phi} \pm$$

$$2 \sin\theta_h \cos\theta_h \sin\lambda \cos\lambda\, \overline{\cos\phi}$$

$\cos^2\theta_e =$

$$\overline{\cos\phi} = 0, \quad \overline{\cos^2\phi} = 1/2$$

$$\cos^2\theta_e = \cos^2\theta_h \cos^2\lambda + \frac{1}{2} \sin^2\theta_h \sin^2\lambda.$$

$$(3\cos^2\theta_h - 1)(3\cos^2\theta_e - 1) =$$

$$9\cos^2\theta_h \cos^2\theta_e + 3\cos^2\theta_h - 3\cos^2\theta_e - 1$$

$$9\cos^2\theta_h (1 - \sin^2\theta_e) - 3\cos^2\theta_h - 3\cos^2\theta_e - 1 =$$

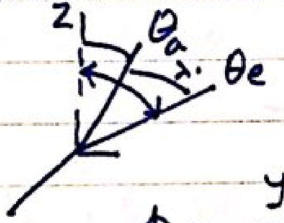
$\frac{3}{5} \times \left(\frac{3}{2}\cos^2\lambda - \frac{1}{3}\right)$

Limiting pol. Polarization Spectrum.

Demonstr. of $p_0 \leq \frac{1}{2}$

Lecture 18.—

Abs. and emission non colinear.



$$\frac{3}{2}\cos^2\theta_e - \frac{1}{2} = \left(\frac{3}{2}\cos^2\theta_a - \frac{1}{2}\right)\left(\frac{3}{2}\cos^2\lambda - \frac{1}{2}\right).$$

$$p = \frac{I_{\parallel} - I_{\perp}}{I_{\parallel} + I_{\perp}} = \frac{\overline{\cos^2\theta_e} - \overline{\sin^2\theta_e/2}}{\cos^2\theta_e + \sin^2\theta_e/2.}$$

$$p = \frac{\frac{3}{2}\cos\theta_e - \frac{1}{2}}{\frac{1}{2}\cos^2\theta_e + \frac{1}{2}.} = \frac{3\cos^2\theta_e - 1}{\cos^2\theta_e + 1}$$

24 $$\frac{1}{p} - \frac{1}{3} = \frac{\frac{1}{2}\cos^2\theta_e + \frac{1}{2}}{\frac{3}{2}\cos^2\theta_e - \frac{1}{2}} - \frac{1}{3} = \frac{\frac{3}{2}\cos^2\theta_e + \frac{3}{2} \mp \frac{3}{2}\cos^2\theta_e +}{\frac{3}{2}\cos^2\theta_e - \frac{1}{2}.}$$

$$\frac{1}{p} - \frac{1}{3} = \frac{2}{\frac{3}{2}\overline{\cos^2\theta_e} - \frac{1}{2}} = \qquad \cos^2\theta_e = 1.$$

$\cos^2\theta_e =$

~~$\cos^2\theta_e = 3/5.$ $\frac{1}{p} - \frac{1}{3} = \frac{2}{\frac{4}{10} - \frac{5}{10}} = \frac{2\times 10}{4} = 5$~~

$$\frac{1}{p} - \frac{1}{3} = \frac{1}{3}\left[\frac{3 + 3\cos^2\theta_e - 3\cos^2\theta_e + 1}{3\cos^2\theta_e - 1}\right] = \frac{2}{3\left(\frac{3\cos^2\theta - 1}{2}\right)} = \frac{2}{3\left(\frac{3}{5}\left(-\frac{1}{2}\right)\right)}$$

~~$\frac{1}{p} - \frac{1}{3} = \frac{4}{\frac{9}{5} - \frac{5}{5}} = \frac{4}{\frac{4}{5}} = \frac{5}{3}.$~~ $= -\frac{10}{3}.$

$\cos^2\theta_e = \frac{3}{5}$ $\quad \frac{1}{p} - \frac{1}{3} = \frac{5}{3}$ $\quad p = 1/2.$

$\cos^2\theta_e = -\frac{1}{5}$ $\quad \frac{1}{p} - \frac{1}{3} = \frac{4}{3}$

$$\frac{dF_\parallel}{dt} = -(\Gamma + 4k_r)F_\parallel + 4k_r F_\perp + f_\parallel(t).$$

$$\frac{dF_\perp}{dt} = 2k_r F_\parallel - (\Gamma + 2k_r)F_\perp + f_\perp(t).$$

$f_\parallel(t) = F_{0\parallel}$ $\quad$ $f_\perp(t) = F_{0\perp}$

$dF_\parallel/dt = 0$ $\quad$ $dF_\perp/dt = 0.$

$$\left.\begin{aligned}(\Gamma + 4k_r)F_\parallel - 4k_r F_\perp &= F_{0\parallel}\\ -2k_r F_\perp + (\Gamma + 2k_r)F_\perp &= F_{0\perp}\end{aligned}\right\}.$$

$$\frac{F_\parallel - F_\perp}{F_\parallel + 2F_\perp} = A \qquad A = \frac{A_0 \Gamma}{\Gamma + 6k_r}.$$

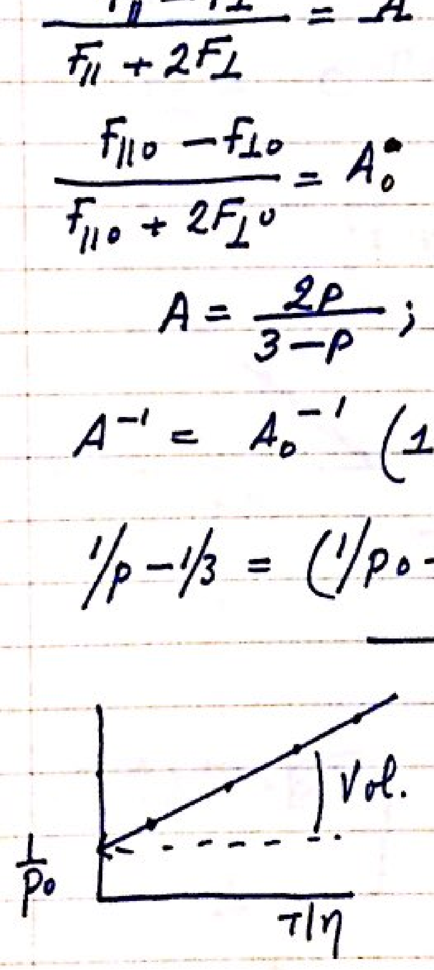
$$\frac{f_{\parallel 0} - f_{\perp 0}}{f_{\parallel 0} + 2F_{\perp 0}} = A_0 \qquad A^{-1} = 2$$

$$A = \frac{2p}{3-p}; \quad A^{-1} = \frac{3}{2p} - \frac{2}{2} = \frac{3}{2}\left(\frac{1}{p} - \frac{1}{3}\right).$$

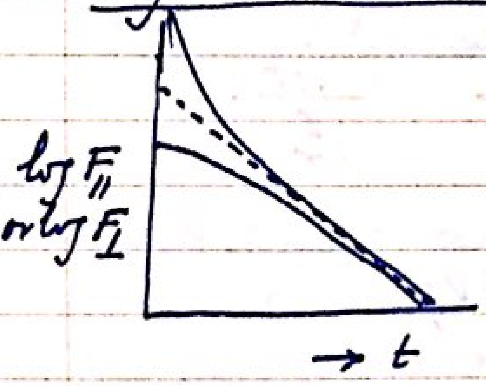
$$A^{-1} = A_0^{-1}\left(1 + 6\frac{k_r}{\Gamma}\right) = A_0^{-1}(1 + 6k_r\tau)$$

$$1/p - 1/3 = (1/p_0 - 1/3)(1 + 6k_r\tau) = (1/p_0 - 1/3)\left(1 + \frac{RT}{\eta V}\tau\right).$$

$$6k_r = \frac{1}{6}\frac{RT}{\eta V}$$



Dynamic methods of determination of k_r

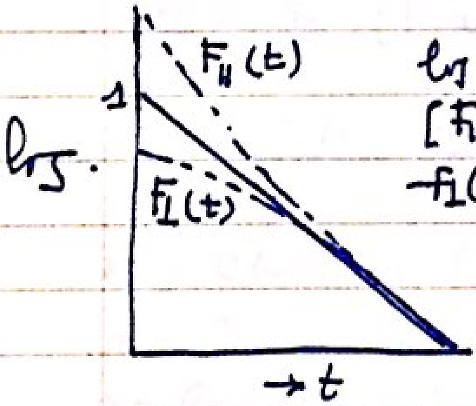


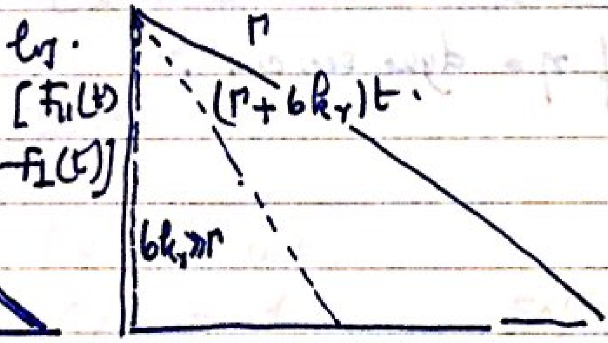
$$F_\parallel(t) = \frac{F_0}{3} e^{-\Gamma t} + \left(F_{\parallel 0} - \frac{F_0}{3}\right) e^{-(\Gamma + 6k_r)t}.$$

$$F_\perp(t) = \frac{F_0}{3} e^{-\Gamma t} + \left(F_{\perp 0} - \frac{F_0}{3}\right) e^{-(\Gamma + 6k_r)t}.$$

$$F_\parallel = e^{-\Gamma t} + \frac{2}{3} A_0 e^{-(\Gamma + 6k_r)t}$$

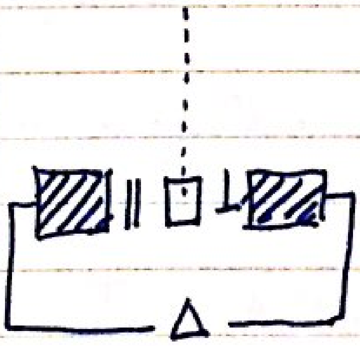
$$F_\perp = e^{-\Gamma t} - \frac{1}{3} A_0 e^{-(\Gamma + 6k_r)t}.$$

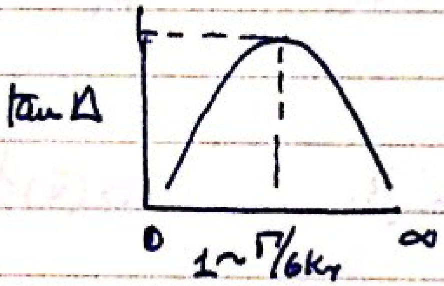




$\Gamma \sim 6k_r$

if $\Gamma \gg 6k_r$.





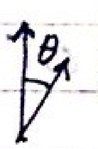
Significance of k.

$$k_r = \frac{1}{6}\cdot\frac{RT}{\eta V} = \frac{KT}{8\pi\eta a^3} = \frac{KT}{f_r}$$

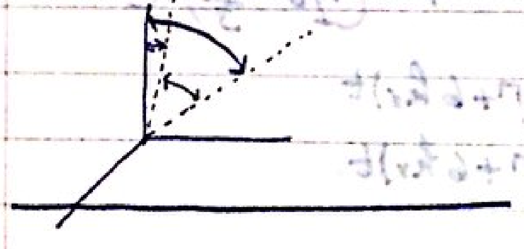
$$f = 8\pi\eta a^3.$$

$$V = \frac{4}{3}\pi a^3 \quad f = 6V\eta.$$

$$\frac{3\eta V}{RT} = \rho = \frac{1}{2k_r}$$ = rotational relaxation time.



$$\boxed{\overline{\cos\theta} = e^{-\rho/t}.}$$



μc

$$\overline{\cos^2\theta} = \frac{1}{3} + \frac{2}{3}e^{-3t/\rho}.$$

dimensions of η = dyne sec cm^{-2}.
k_r = sec^{-1}.

Differences between the methods
Static and dynamic measurements.

Lecture 19

Electronic energy transfer.
Gas phase observation of Franck and Cario

$$h\nu \longrightarrow Hg \rightarrow Th \longrightarrow h\nu'$$

'Sensitized fluorescence'.
Observations of Bowen & Mickiewicz (1942)
Energy transfer and impedance matching.

Theory of F. London (1929) and F. Perrin (1932) on resonant energy transfer. The coupled dipole oscillators (P. and Wilson). Sixth power law:

$$\frac{T(r)}{\Gamma} = \left(\frac{R_0}{r}\right)^6.$$

R_0 = Characteristic distance.

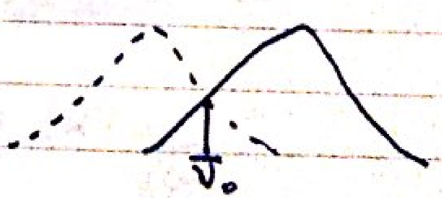
$r = R_0$ $T(r)/\Gamma = 1$ 50%.
$r = R_0/2$ $T(r)/\Gamma = 32$. or 96% transfer
$r = 2R_0$ $T(r)/\Gamma = 1/32$ 3% transfer.

Values of R_0 = App $\lambda/4$ or 1,000 Å.

Forster theory

$$J_\nu \int k(\bar{\nu})\cdot\phi(\bar{\nu})\,d\bar{\nu}. \qquad J_\nu = cm^3\,mM^{-2}.$$

$$R_0 = \left[x^2 \cdot 2490 \, \frac{\tau_0 J_\nu}{n^2 \bar{\nu}_0^2}\right]^{1/6}.$$



$f=1$. $\Delta\bar{\nu} = 1\,cm^{-1}$ $\bar{\nu}_0 = 20{,}000\,cm^{-1}$ $R_0 = 360$ Å.

Fluorescein: $J_\nu \sim 2\cdot10^{12}\,cm^3/mM^2$ $R_0 = 53$ Å.

$\varkappa^2$? 2/3 for random orientations.

Demonstration of the importance of J_ν.

" " " sixth power law.

Energy migration: Trapping – 'Exciton'

Lecture 20. – Chemical Quenching.

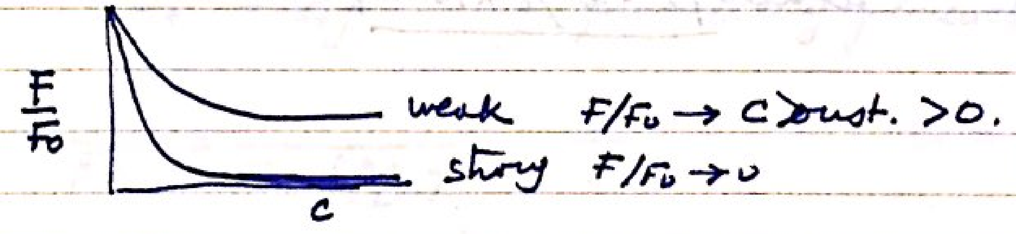
Distinction of Chemical Quenching from simple filter effects. 'Strong' and 'weak' quenching.

1. Classical collisional Quenching.

Quenching by electrolytes in solution

1. F decrease towards 0 as $[Q]\to\infty$

'Strong' ~~and 'weak Quenching~~ Quenching



2. Proportionality with T/η.

'Diffusion controlled'.

3. The rule $\tau/\tau_0 = F/F_0$ is obeyed.

—

Smoluchovsky's Theory of 'fast coagulation'

L molecules of Quencher/ml. $L = [Q].\times \frac{N}{10^3}$.

a_p, a_q = radii – D_p, D_q diff coefficients.

$$Z = 4\pi.(D_p + D_q)(a_p + a_q)\,[Q]\times\frac{N}{10^3}$$

$$Z = 4\pi\,(D_f + D_Q)(a_f + a_q)\,\frac{[Q]\cdot N}{10^3}.$$

$$D = \frac{KT}{6\pi\eta a}.$$

$$D_f + D_q = \frac{KT}{6\pi\eta}\left(\frac{1}{a_f} + \frac{1}{a_q}\right)(a_f + a_q)\,\frac{[Q]\cdot N}{10^3}.$$

$$= \frac{4\pi KT}{6\pi\eta}\left(2 + \frac{a_f}{a_q} + \frac{a_q}{a_f}\right).$$

$$a_f/a_q \simeq 3 \quad a_q/a_f = 1/3.$$

$$\frac{2}{3}\,\frac{KT}{\eta} \times 5.33 = 3.55 \times \frac{KT}{\eta} =$$

$$\frac{KT}{\eta}\,(25^\circ C) = 1.38\times10^{-16} \times 33{,}300.$$

$$= 4.5954 \times 10^{-16} = 4.59\times10^{-12}$$

$$\frac{2}{3}\ 16.31\times10^{-12} \times N \times 10^{-3}.$$

at 25º $\boxed{Z = 0.98\times10^{10}.}$

$$k = \frac{R}{N}$$

$$D = \frac{kT}{6\pi\eta a}$$

$$Z = \frac{2}{3}\frac{kT}{\eta}\left(2 + \frac{a_p}{a_q} + \frac{a_q}{a_p}\right)[Q]\cdot\frac{N}{10^3}. \simeq \underline{\cancel{k_q}}$$

for a molar solution $Q = 1$.
If $a_p/a_q \sim 2$.

$$Z = \frac{8}{3}\frac{kT}{\eta}\frac{N}{10^3} = \frac{8}{3}R\left(\frac{T}{\eta}\right) \times 10^{-3}$$

$\cancel{N' = 6\times10^{20}}$ $R = 8.31\times10^{7}$ T/η at 25° = 33,300.

$\cancel{8\times6\times8.31\times10^{27}\times3.3\times10^{4}}$

$$\frac{8\times3.3\times10^{4}\times8.31\times10^{7}\times10^{-3}}{3} \sim.$$

$1.1\times66.4\times10^{8} \simeq 10^{10}$ lt mol sec^{-1}.

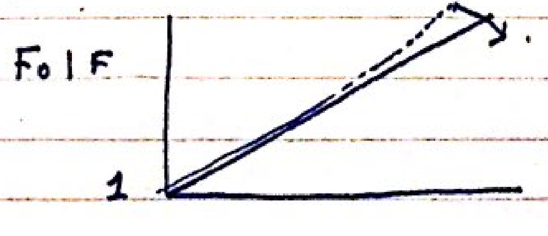
$k_+^* = Zf$ $\quad$ f = probability of quencher/encounter.

$$\frac{F}{F_0} = \frac{\Gamma}{\Gamma + k_+^*[Q].} = \frac{\tau}{\tau_0.} \qquad \tau_0 = \frac{1}{\Gamma}$$

$$\frac{F_0}{F} = \frac{\tau_0}{\tau} = 1 + k_+^*[Q]\,\tau_0. \quad = 1 + K[Q].$$

Stern and Volmer. (1919). K = S.V. constant
If $\tau_0 \simeq 10^{-8}$. since $k_+^* \leq 10^{10}$
$k_+^*\tau_0 \leq 200$. lt.M^{-1}. $\quad$ (if $f \sim \frac{1}{2}$)
$[Q]_{\frac{1}{2}}$ is Q at which $F_0/F = 2$.
then $[Q_{\frac{1}{2}}] \geq \frac{1}{200}$ M/lt or 5×10^{-3} M.

Oxygen Quenching



Quenching by complex formation.

Quinones, Purines, aromatics

1. 'Strong' or 'weak' quenching
2. Temperature effects are variable, often $d/dT < 0$
3. $F_0/F \neq \tau_0/\tau$ often $\tau_0 = \tau$ (τau does not change upon quenching).

$$A + Q \overset{K}{\rightleftharpoons} AQ$$

$$K_{eq} = \frac{[AQ]}{[A][Q]} \qquad K_{dis} = \frac{[A]\cdot[Q]}{[AQ]}$$

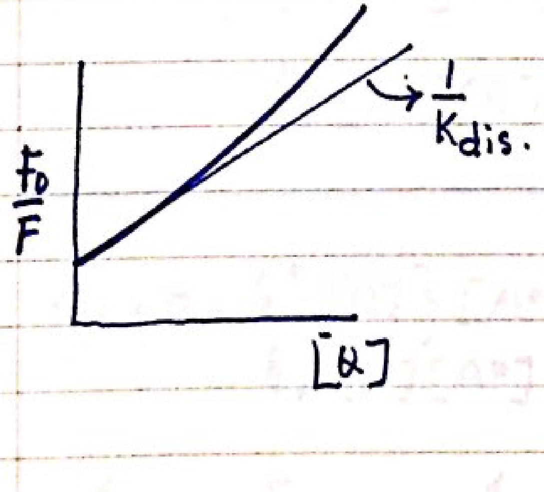
$$K_{dis} = \frac{\alpha}{1-\alpha}[Q]$$

$$[Q] = [Q]_0 - [AQ] \sim [Q_0]$$

$$A + h\nu \rightarrow A^* \longrightarrow A + h\nu'$$

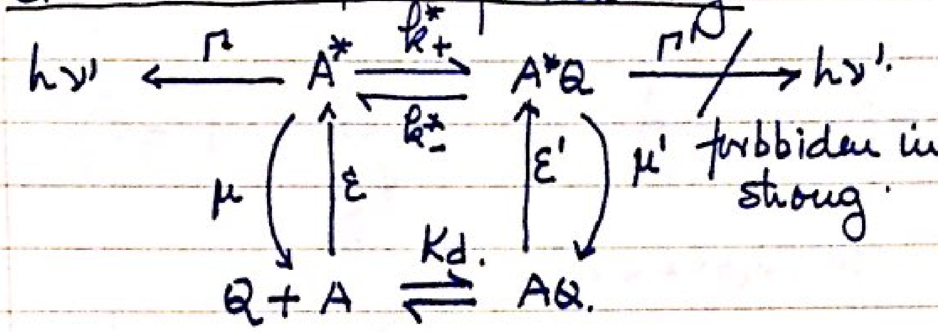
$$AQ + h\nu \rightarrow AQ^* \not\longrightarrow$$

$$\frac{F}{F_0} = \frac{[A]}{[A]+[AQ]} \quad \text{or} \quad \frac{F_0}{F} = 1 + \frac{[AQ]}{[A]} = 1 + \frac{[Q]}{K_{dis}}.$$



but $\tau = \tau_0$ at all values of F/F_0

General descripti~~on~~ of Quenching.



$\frac{d[A^*]}{dt}$; $\quad d\,\frac{[A^*Q]}{dt}$.

$$\frac{d[A^*]}{dt} = \varepsilon[A] - (\mu + \Gamma + k_+^*[Q])[A^*] + k_-^*[A^*Q].$$

$$\frac{d[A^*Q]}{dt} = \varepsilon'[AQ] - (\mu' + \Gamma' + k_-^*)[A^*Q] + k_+^*[A^*][Q].$$

Excitation at isosbestic point of A and AQ $\varepsilon = \varepsilon'$.
$k_-^* \ll \mu'$. For strong quenching

$\frac{dA^*}{dt} = 0$.
$$k_-^*[A^*Q] + \varepsilon[A] = (\mu + \Gamma + k_+^*\,k[Q])[A^*].$$

$$k_+^*[A^*][Q] + \varepsilon[AQ] = [\mu' + \Gamma' + k_-^*][A^*Q].$$

$$\{\mu + \Gamma + k_+^*[Q]\}[A^*] - k_-^*[A^*Q] = \varepsilon[A].$$
$$-k_+^*[Q][A^*] + \{\mu' + \Gamma' + k_-^*)[A^*Q] = \varepsilon'[AQ].$$

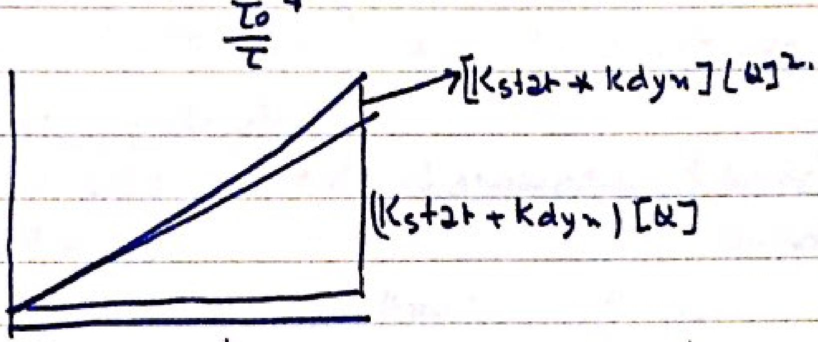
$$\frac{1}{\mu + \Gamma} = \tau_0 \qquad \frac{1}{\mu' + \Gamma'} = \tau_0'.$$

$$\left(\frac{1}{\tau_0} + k_+^*[Q]\right)[A^*] - k_-^*[A^*Q] = \varepsilon[A].$$
$$-k_+^*[Q][A^*] + \left(\frac{1}{\tau_0'} + k_-^*\right)[A^*Q] = \varepsilon'[AQ].$$

Vaughan & Weber
Biochemistry $\underline{9}$, 464. (1970)

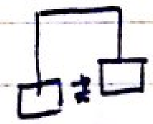
If $q_0' = 0$ and $k_-^* \tau_0 \ll 1$.

$$\frac{F_0}{F} = \left(1 + k_+^*[Q]\right)\left(1 + \frac{[Q]}{K_{dis}}\right) = \left(1 + (K_{stat} + k_{dyn})[Q] + K_{stat}k_{dyn}[Q]^2\right).$$



necessity of measurement of both ~~parame~~ F and τ.

Case of FAD fluorescence



$$\frac{F_0}{F} \sim 11.$$

$\tau_0 = 4.5.$
$\tau = 2.5.$

$$\frac{F_0}{F} = \frac{1}{\alpha}\left(1 + k_+^*\tau_0\right).$$

$$\frac{F}{F_0} = \alpha\frac{\Gamma}{\Gamma + k_+^*}$$

$$\Gamma = \frac{1}{\tau_{FMN}} \qquad \frac{1}{\Gamma + k_+^*} = \tau_{FAD}.$$

$$\frac{F_0}{F} = \cancel{\alpha}\,\frac{1}{\alpha}\cdot\frac{\tau_{FAD}}{\tau_{FMN}}$$

$$\alpha = \frac{F}{F_0}\cdot\frac{\tau_{FMN}}{\tau_{FAD}} = \frac{1}{\underline{11}} \times \frac{4.5}{2.5} = \frac{4.5}{27.5} \sim 17\%$$

Intrinsic Causes of Quenching.

21 Absorption Spectroscopy of Proteins

Chromophores involved.

$>C=O$
$\quad|$
$\ NH$ (190 nm) app 7,000 cm^2/mole.

Longer wavelengths:

220, 230. $\pi \rightarrow \pi^*$ of aromatics (B bands).
248 – 280 " " (L bands).

	L_1 Bands	K_{max}.
Phalanine	$\lambda_{max} = 248$	150
Tyrosine	$= 275$	1,250.
Tryptophan	280	5,400.
Cystine	— none	~ 200 at 250.

— Uses of ab. spectrophotometers in denaturation. Differential measurement
— method of Laskowski and Herskovitz. —

Fluorescence = Tryptophan 20% (yield) 350 nm.
Tyrosine 20% 303 "
Phalanine 2%.

Effects of incorporation in proteins — Difference with absorption. Environment = Relaxation.

Effects of O_2 quenching and I^- quenching
Observations of Lehrer & of Lakowicz & Weber.
Polarization of the fluorescence —
Fluorescence lifetimes —
Fluorescence Spectrum.

The effect of prosthetic groups.
NADH, FAD, Heme, Pyridoxal.

<u>Lect 23rd.</u>

Fluorescence pol. spectrum of protein
Evidence for and against E. Transfr.

P_{305}/P_{270}	
1.33	Indole
1.45	Tryp.
1.68	N.Gly.Trp.
H 2.18	HSA (1.Trp).
2.5	50% PG.
2.1	BSA (2 Trp)
2.5	50% PE.
2.4	Lysozyme.
3	50% PG

<u>Quenching of fluorescence.</u>

Distinction of O. and I. residues by I^- quenching
Electrostatic effects. Exps. of Lehrer with copolymers.
Quenching by O_2. Relative and absolute efficiencies of O_2 and I^-. Effect of denaturation.
'Diffusion' of O_2 through protein.
Energies involved in residue interaction
Fluctuations of structure allowing O_2 diffusion:

$$Z = A e^{-E/RT}$$

$Z \geqslant 10^9$ $A = 10^{12} - 10^{13}$.

$$Z = 10^{12.5} 10^{-E/1.38}.$$

$$\log Z = 12.5 - \frac{E}{1.38} \quad \text{or} \quad E = (12.5 - \log Z) 1.38.$$

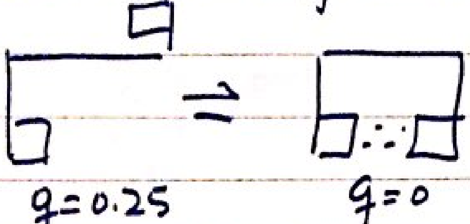
$Z \geqslant 10^9$ $E \leqslant 3.5 \times 1.38$ $(= 4.83)$ ~ 5kcal.

$Z \geqslant 10^{10}$ $E \leqslant 2.76$ (3kcal).

Prosthetic groups.

Natural P.G.

Folded Structure of FAD.



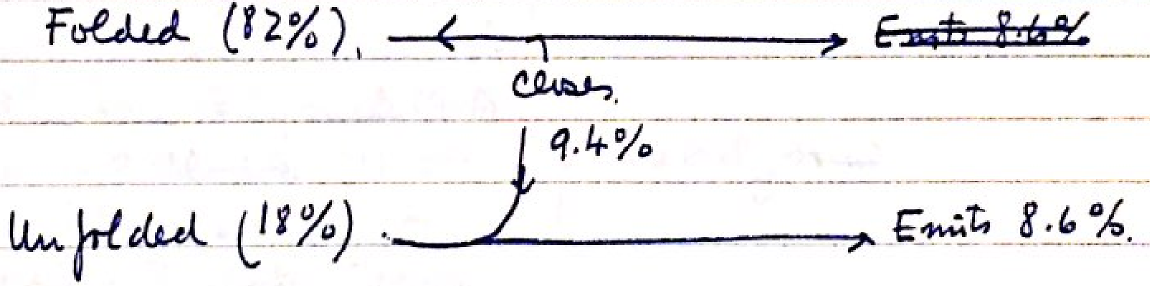
$\tau_{FMN} = 4.5 ns.$

$\tau_{FAD} = 2.4$ $\quad \tau_{FMN} = 4.5$

$\frac{\tau_{FAD}}{\tau_{FMN}} = 0.\cancel{185}.53$

$q_r = 0.098 \quad q = q\alpha \times \frac{\tau_{FAD}}{\tau_{FMN}}$; $\alpha = 0.185.$

$k_+^* = 1.5 \times 10^8 \, sec^{-1}$



At 20°C

$$\left.\begin{aligned} \Delta H_o &= -4.3 \\ \Delta F_o &= -1 \\ T\Delta S_o &= -32. \end{aligned}\right\} \quad \Delta H_o = \Delta F_o + T\Delta S_o.$$

Effect of pressure: The volume decreases.

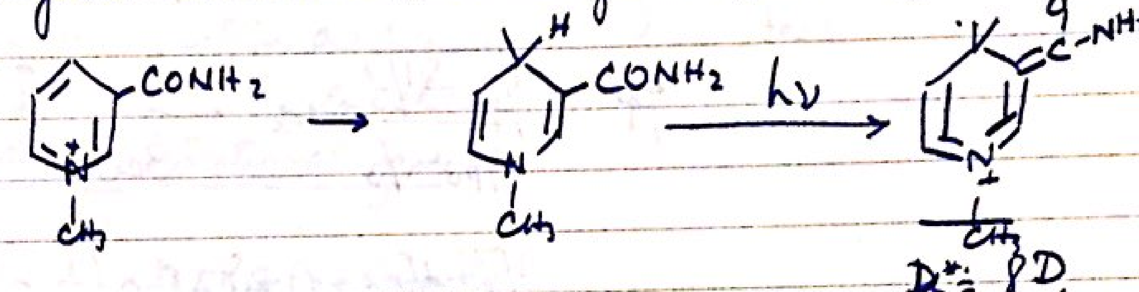
Flavoproteins

Mostly nonfluorescent – Either ads. of closed form. or quenching by residues. SH, aromatics, histidine.

Lipoamide dehydrogenase: An exception.

NADH

Conformation in solution. Abs. E. Transfer.
Change in yield with solvent: – Change in spectrum (460–420m)



$\mu^* = 8D.$

Effects of combination with proteins.
Exaltation of the fluorescence in ternary complexes.
Polarization of the fluorescence.
Energy Transfer in Proteins

'Artificial' prosthetic groups.
Conjugation of proteins with:
Fluorescein isocyanate (4–6)
Dansyl chloride. (12–15)
SH-fluorescent reagents.
} Covalently bound.
Pyrene Butyrate (120).

Non-covalent

ANS., bis ANS.

Study of the environment – Determination of rotational rate. Description of the effects in spherical molecules. The effect of shape and hydration.

$$Vol = \underline{M.W} \times 0.75 + 0.10\,MW = M.W \times 0.85$$

$$\rho = \frac{3\eta V}{RT} = \frac{3 \times 10^{-2}}{2.5 \times 10^{10}} \times V_{molar} \sim \frac{3}{2.5} V \times 10^{-12}.$$

$$\rho \simeq V \cdot 10^{-12} \qquad V = 10^5 \quad \rho = 10^{-7} s.$$

Partial Rotation of ligands attached to protein

Significance of p_0 in Perrin's plots.

Molec. Dynamic Simulation. Estimation of 20°-30° rot.

$$~~1/p~~ \quad \frac{1/p - 1/3}{1/p_0 - 1/3} \; ~~24~~ = \frac{2}{3\cos^2\theta - 1} = \frac{}{2.64907.}$$

$$\overset{20^\circ}{} \quad 1/p - 1/3 = 1.2128 \times \left(\frac{1}{p_0} - \frac{1}{3}\right) \underset{1.9889.}{\downarrow}$$

$p_0 = 0.45 \rightarrow 0.38$

$p(20^\circ) =$ Easy to detect.

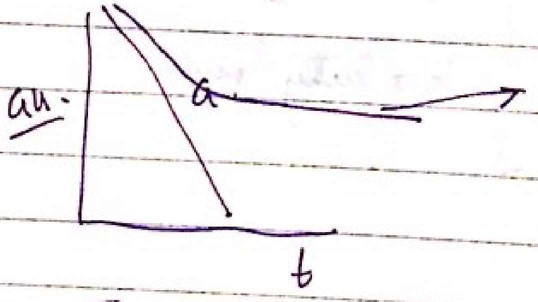
30° → 0.29. ~~1.60 x~~

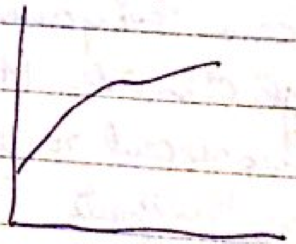
Experiment with BPTI

Internal Dynamics of Proteins. –

Anisotropic Rotations

Characterization. DPPF → Pulse fluorometry

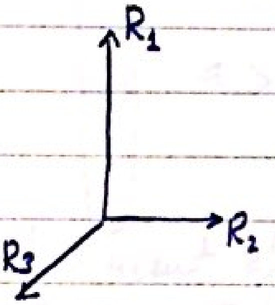




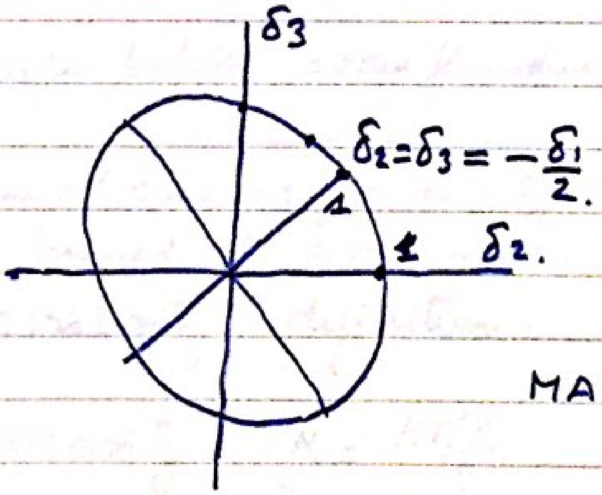
Stokes Einstein Relation

Are there partial rotations?
Rigidity & Flexibility of macromolecules. Dependence upon time.
Observations on protein conjugates.
What is the effect of molecular shape:

<u>Classification of rotational types.</u>

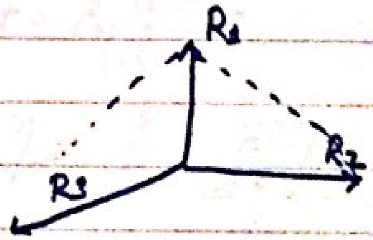


$$\frac{1}{3}(R_1+R_2+R_3)=\bar{R}$$
$$R_1=\bar{R}+\bar{\delta}_1$$
$$R_2=\bar{R}+\bar{\delta}_2$$
$$R_3=\bar{R}+\bar{\delta}_3$$
$$\delta_1+\delta_2+\delta_3=0.$$
$$\delta_1^2+\delta_2^2+\delta_3^2=v. \quad = \text{variance.}$$
$$\delta_1=-(\delta_2+\delta_3).$$
$$\delta_1^2=\delta_2^2+\delta_3^2+2\delta_2\delta_3$$
$$\boxed{\delta_2^2+\delta_3^2+2\delta_2\delta_3=v.} \quad \text{eq}$$

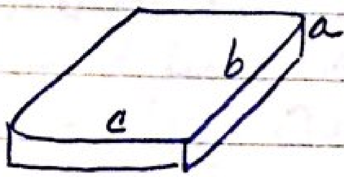


$\delta_3=0$
$\delta_1=-\delta_2.$

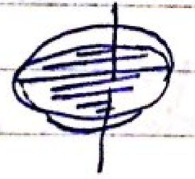
MAM.



Rotations of rods and plates.



$a \ll b \sim c$. oblate ellipsoid of revolution



$a \gg b \sim c$ needle, prolate ellipsoid.

Moment of inertia, of a plate and needle.

Plate $I_a \sim I_b \sim I_c$ = not distinguishable from a sphere.

Needle $I_a \ll I_b \sim I_c$ = yes ——

Effect of partial rotation.

The case of γ-globulin. –

Energy transfer between added fluorophores – Calculation of distances.

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cture – Determination of 'microscopic viscosity' by probes.

25. – Effects of the immediate environment –

macroscopic viscosity = definition. $F = A\left(\frac{dv}{dz}\right)\eta$.

microscopic viscosity = $R = KT/f_r$. $\eta = \frac{F}{A} \Big/ \left(\frac{dv}{dz}\right)$

$D = KT/f_t$.

force per unit area per unit velocity grad.

$f_r = 8\pi a^3 \cdot \eta$.

$f_t = 8\pi a \cdot \eta$.

η = macroscopic viscosity.

$V = \frac{\pi r^4}{8 l \eta} \Delta p$.

$f_r = \alpha V \cdot \eta_m$.

$f_t = \alpha V^{1/3} \cdot \eta_m$.

η_m refers only to the immediate neighbourhood of the molecule. Therefore one can understand why it has to change if there are different bonds between the molecule and its environment.

Examples. Cells and pure liquids.
Molecules that make H bonds and molecules that do not.

The examination of microscopic viscosity by means of probes.
1. Studies in micelles and membranes.
Viscosities and their significance.

$$\rho = \frac{3\eta V.}{RT}$$

$$\rho(\eta = 10^{-2}) = V \times 10^{-12}.$$

$$\rho_\eta = V \times \eta \times 10^{-10}.$$

$$V = 10^5 \quad \eta = 1-10 \quad \rho_\eta = 10^{-5} - 10^{-4}$$

$$\rho_\eta = 10-100\ \mu sec.$$

Turnover # of enzyme = 10^4 (or less) that is TN is limiting not ρ.

$\rho = \rho_0 e^{-E/RT}$ where E = energy of activation. In that case a change in viscosity will change the rate of reaction.

Measurement by O_2 diffusion and perylene rotation

The Philosophy of probe.

Transformations $\Delta F_T + \Delta F_I - \Delta F_{II}$.

$\Delta F_I - \Delta F_{II}$ ideally close to zero.
importance of selection rules.
Equilibria disturbed by probe.

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

Representation of the Fluorescence Spectrum:

This can be displayed as the relative Energy ~~contained~~ per ~~in a certain~~ wavelength interval $E(\lambda)$ or wavenumber interval ~~$\Delta\bar{\nu}$~~ $E(\bar{\nu})$. or as the relative number of quanta. $n(\lambda)$ or $n(\bar{\nu})$ respectively. From the relation between λ an $\bar{\nu}$ we have.

$$\frac{\Delta\lambda}{\Delta\nu} = \frac{\nu}{\lambda}.$$

$$\Delta\lambda = -\frac{1}{\bar{\nu}^2}\Delta\bar{\nu} = -\lambda^2\Delta\bar{\nu}$$

$$\Delta\bar{\nu} = -\frac{1}{\lambda^2}\Delta\lambda = -\bar{\nu}^2\Delta\lambda.$$

It follows that

$$E(\lambda) = E(\nu)$$

$$\frac{E(\lambda)}{E(\bar{\nu})} = \frac{\nu}{\lambda}; \qquad E(\lambda) = E(\bar{\nu})\cdot\frac{\bar{\nu}}{\lambda} = E(\bar{\nu})\cdot\nu^2.$$

$$E(\nu) = E(\lambda)\cdot\lambda^2.$$

$$E(\lambda) = n(\lambda)\cdot\frac{hc}{\lambda.}$$

$$E(\bar{\nu}) = n(\bar{\nu})\,hc\,\bar{\nu}$$

$$n(\lambda) = \lambda E(\lambda).$$

$$n(\bar{\nu}) = \frac{E(\bar{\nu})}{\nu}$$

$$\Delta\lambda = \Delta\nu\cdot\lambda^2 = \Delta\nu/\nu^2$$

$$\begin{tikzcd}
E(\lambda) \arrow[r, shift left, "\lambda^2"] \arrow[d, shift right, "\lambda"'] & E(\bar{\nu}) \arrow[l, shift left, "\lambda^2"] \arrow[d, shift left, "\nu^{-1}"] \\
n(\lambda) \arrow[u, shift right, "\lambda^{-1}"'] \arrow[r, shift left, "\lambda^2"] & n(\nu) \arrow[l, shift left, "\nu^2"] \arrow[u, shift left, "\nu"]
\end{tikzcd}$$

$$\Delta\lambda = -\frac{1}{\nu^2}\Delta\nu$$

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

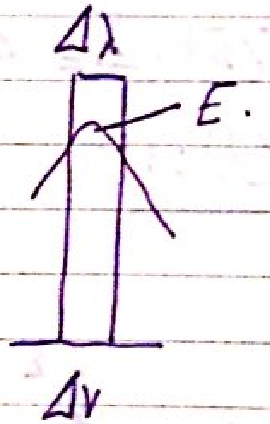
$$\Delta\lambda = -\frac{1}{\bar{\nu}^2}\Delta\bar{\nu} = -\lambda^2\Delta\nu$$

$$\Delta\nu = -\frac{1}{\lambda^2}\Delta\lambda = -\underline{\underline{\nu^2\Delta\lambda}}.$$

$$\frac{E(\cancel{\ })}{\Delta\nu} = \frac{E(\cancel{\lambda})}{\nu^2\Delta\lambda}.$$

$$E(\nu) = \frac{E}{\Delta\nu} = \frac{E}{\Delta\lambda}\frac{1}{\nu^2} = \frac{E(\lambda)}{\lambda^2}. = E(\lambda)\cdot\lambda^2$$

$$E(\nu) = E(\lambda)\cdot\lambda^2.$$



$$E(\nu) = \frac{E}{\Delta\nu}$$

$$E(\lambda) = \frac{E}{\Delta\lambda}.$$

$$E(\nu)\,\Delta\nu = E(\lambda)\,\Delta\lambda.$$

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

$$\Delta\nu = -\frac{\Delta\lambda}{\lambda^2}.$$

$$E(\nu)\,\frac{\Delta\lambda}{\lambda^2} = E(\lambda)\,\Delta\lambda$$

$$\boxed{E(\nu) = E(\lambda)\cdot\lambda^2}$$

Since $\Delta\bar{\nu} = -\frac{\Delta\lambda}{\lambda^2}$ or $\Delta\lambda = \frac{\Delta\bar{\nu}}{\bar{\nu}^2}$.

$$\bar{\nu}\cdot \underline{E(\bar{\nu})} = \lambda\cdot E(\lambda).$$

$$E(\lambda) = E(\bar{\nu})\cdot \nu^2.$$

$$E(\nu) = E(\lambda)\cdot\lambda^2$$

$$n(\lambda) = \underline{E(\lambda)}\cdot\lambda$$

$$n(\nu) = \frac{E(\nu)}{\nu}\cdot \#.$$

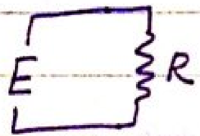
$$n\cdot\left(\frac{hc}{\lambda}\right) = E(\lambda).$$

$$\begin{tikzcd}
E(\lambda) \arrow[r, shift left, harpoon, "\lambda^2"] \arrow[d, shift right, "\lambda"'] & E(\bar{\nu}) \arrow[l, shift left, harpoon, "\nu^2"] \arrow[d, shift right, "\bar{\nu}^{-1}"'] \\
n(\lambda) \arrow[r, shift left, harpoon, "\lambda^2"] \arrow[u, shift right, "\lambda^{-1}"'] & n(\bar{\nu}) \arrow[l, shift left, harpoon, "\bar{\nu}^2"] \arrow[u, shift right, "\bar{\nu}"']
\end{tikzcd}$$

Synchronic detection of amplitude-modulated signals.
(A.A. Kharkevitch – 'Phenomenes non-linéaires et Paramétriques en Électronique'. Dunod, 1960, Paris page 194.)

Voltage is modulated according to.

$$E = E_0 \left[1 + m f(t)\right] \sin \omega_0 t .$$



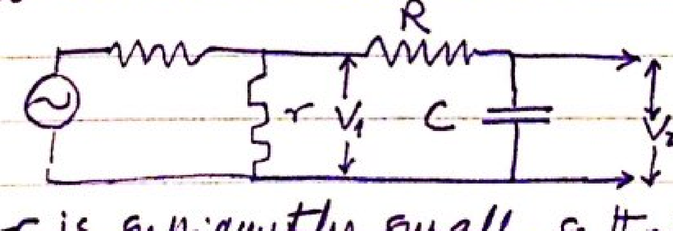
The conductance of R is supposed to vary with the same characteristic frequency ω_0. so that

$$Y = Y_0 \left(1 + n \sin \omega_0 t\right).$$

The current in the circuit will be

$$I = EY = E_0 Y_0 \Big\{ \left[1 + m f(t)\right] \sin \omega_0 t - \frac{1}{2} n \cos 2\omega_0 t \left[1 + m f(t)\right] - \frac{1}{2} n \left[1 + m f(t)\right]. \Big\}.$$

Synchronous detection possesses selectivity.
Consider the circuit below.



r is sufficiently small so that R does not perturb the synchronous detection

$$V_1 = Ir = EYr$$

$E = E_0 \sin \omega_0 t$
$Y = Y_0 \left(1 + n \sin \omega t\right)$

$$V_1 = E_0 Y_0 r \left[\sin \omega_0 t - \frac{1}{2} n \cos (\omega_0 - \omega) t - \frac{1}{2} n \cos (\omega_0 + \omega) t \right].$$

The output from RC has three components:

frequencies	amplitudes
ω_0	$E_0 Y_0 r / 1 + j\omega_0 RC$.
$\omega_0 - \omega$	$\frac{1}{2} jn \, E_0 Y_0 r / (1 + j(\omega_0 - \omega) RC)$
$\omega_0 + \omega$	$-\frac{1}{2} jn \, E_0 Y_0 r / (1 + j(\omega_0 + \omega) RC$.

if $\omega_0 \gg \frac{1}{RC}$. or $\omega_0 RC \gg 1$.

$$\frac{E_0 Y_0 r n}{2RC} \; \frac{1}{\sqrt{(\omega_0^2 - \omega^2)^2 + \frac{1}{R^2 C^2}}} =$$