Biophysical Chemistry
for Life Scientists
NTU Fall 2000
Lecture 13
December 29, 2000

Principles of Spectroscopy

Reading Assignment Eisenberg and Crothers, Chapter 12.

Interaction of Light with Matter

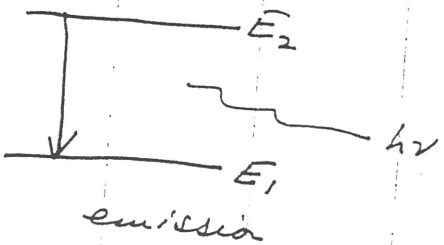
Can take many forms

(1) Absorption and emission of photons

Photons are annihilated or created

$E_2 - E_1 = h\nu$

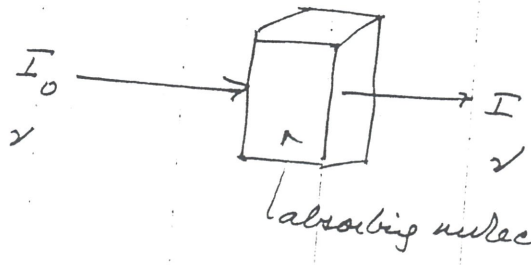
must satisfy Bohr relation



$E_2 - E_1 = h\nu$

emission could be induced or spontaneous

most absorbing materials satisfy Beer-Lambert Law



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If I_0 = incident light intensity at frequency ν

I = intensity of transmitted light at same frequency

c = concentration of absorber

ϵ = molar extinction coefficient

l = length of light path through the absorber

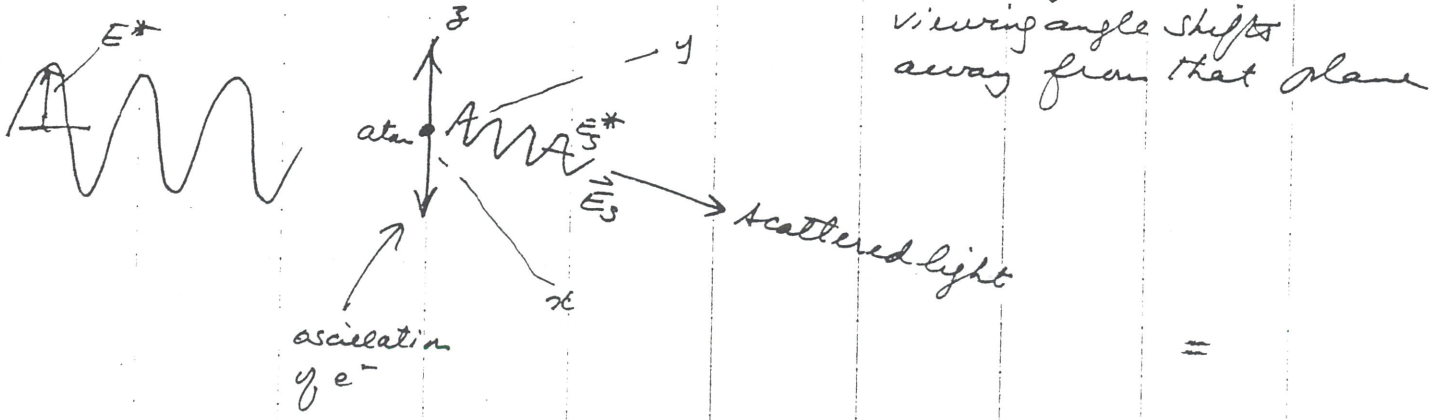
Then,

$$\log_{10} \frac{I_0}{I} = \epsilon l c$$

Quantity $\log_{10} \frac{I_0}{I} = A$ is called the absorbance or sometimes optical density (OD)

(2) Light scattering

An isolated atom scatters light because the electric field of the incident light wave forces the electrons in the atom to oscillate back & forth about their equilibrium position. By the laws of electromagnetism, when a charge changes its velocity, it emits radiation. Light is emitted uniformly in all directions in the plane $\perp$ to oscillation, but decreases in amplitude as the viewing angle shifts away from that plane



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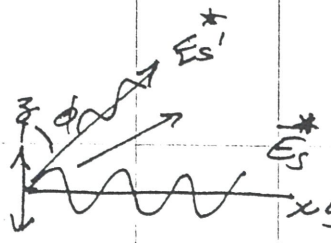


$$E_s^* = \frac{|e|a \sin\phi}{c^2 r}$$

$|e|$ = electron charge
a = accelerating of electron
c = velocity of light
r = distance from oscillator
ϕ = angle between axis of oscillation and plane containing direction of propagation of scattered light.

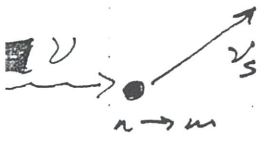
Intensity of scattered light $\propto |E_s^*|^2$

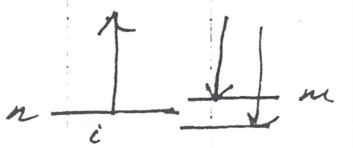
→ Scattered light not necessarily at same frequency as incident light.

a) Rayleigh scattering

Scattered light at essentially same frequency as incident light.

Frequency shift due to
(i) molecular motion → Dopplar shift
(ii) change in internal energy states of scattering molecule

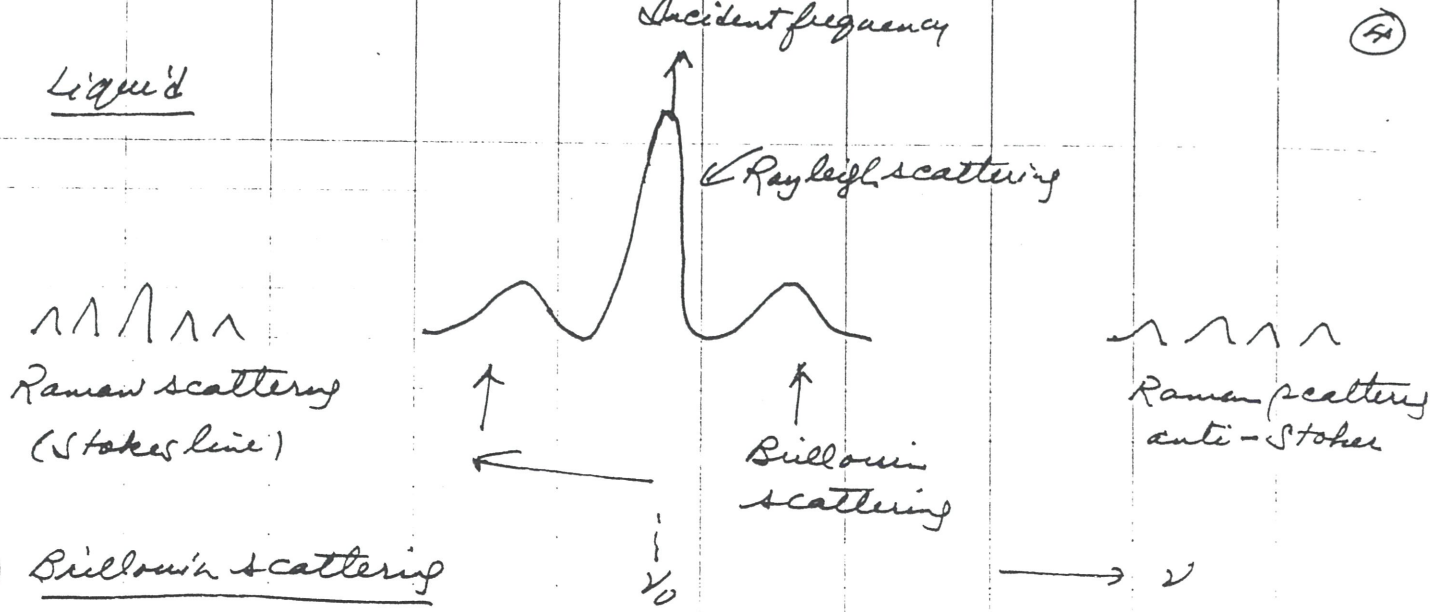




inelastic scattering =

Inelastic scattering important for gases, e.g. Rotational Raman
In liquids, random molecular motions broaden Rayleigh scattering

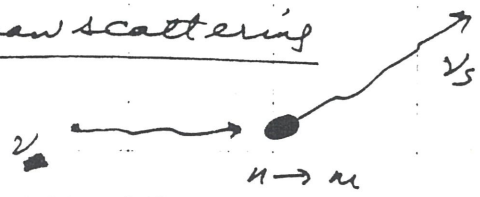
Liquid

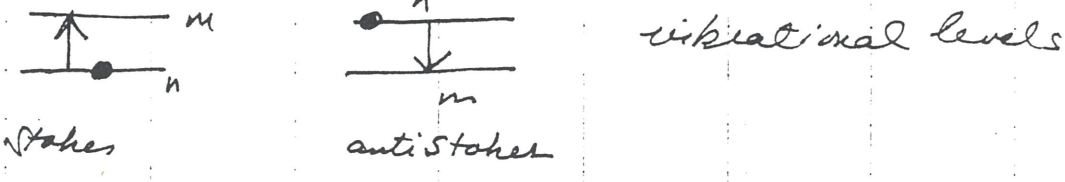


b) Brillouin scattering

In addition, in liquids, two special scattered lines, the Brillouin lines appear because of coherent molecular motion in sound waves that propagate through the fluid.

c) Raman scattering





Phenomena related to light scattering

a) Reflection — light scattered in the opposite direction of incident light

b) Refraction — light scattered in the forward direction combines with incident beam to give rise to the phenomenon of refraction. The physical effect of this recombination is to make the transmitted light appear as though it has travelled more slowly thru sample than

through a vacuum.

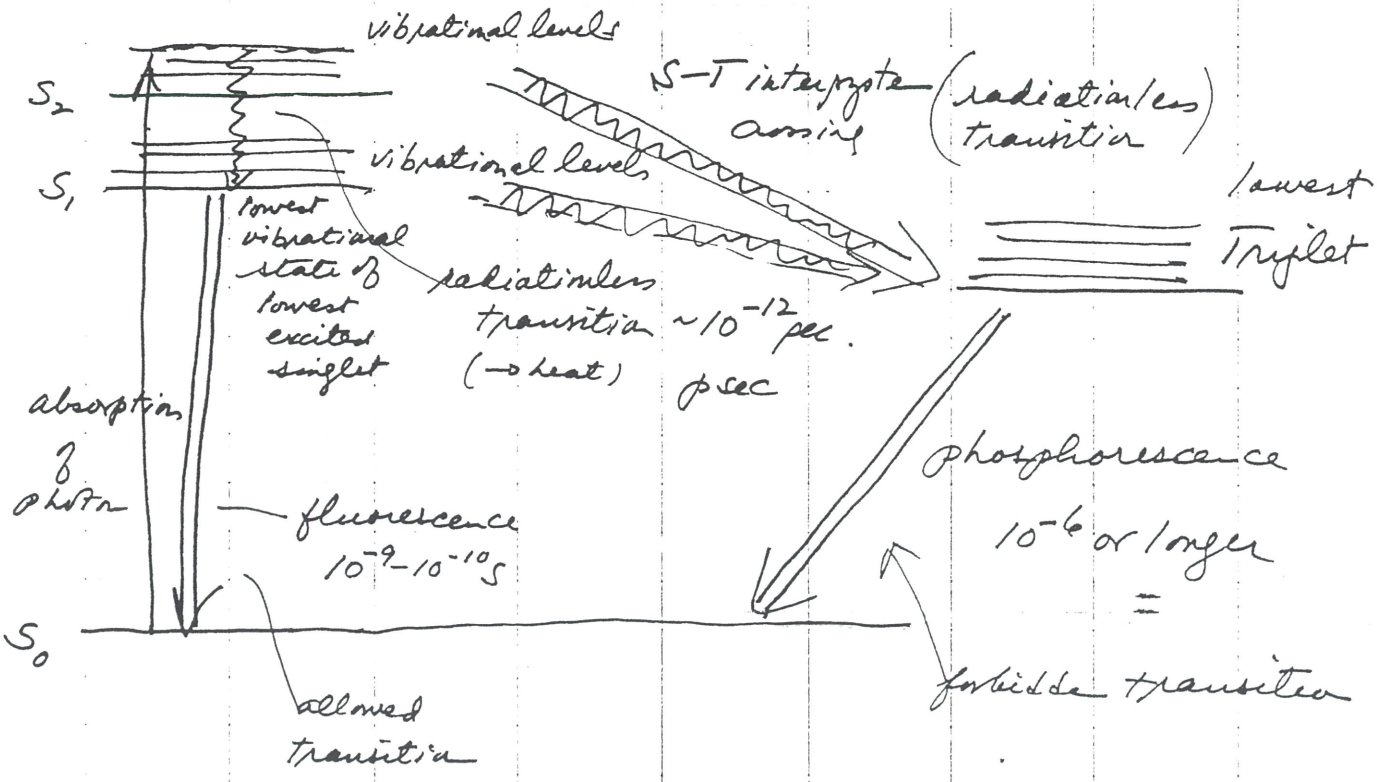
$$\text{Index of refraction} = n = \frac{\text{Velocity of light in vacuum}}{\text{Velocity of light in substance}}$$

c) Diffraction — superposition of scattered waves from individual atoms or molecules in sample

If sample is highly ordered, diffraction pattern periodicity in the distribution of atoms & molecules in the sample

can be used to deduce or infer relative positions of atoms in a sample.

Fluorescence and Phosphorescence



Optical Rotation and Circular Dichroism

a) Optical rotation

Chiral molecules can rotate the plane of polarization of plane-polarized light as light passes through sample

b) Circular dichroism

Chiral molecules will absorb right and left circularly polarized light differentially.

The Electromagnetic Spectrum and Molecular Spectroscopy

Study Table 12-2, p. 530-531

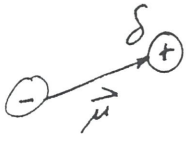
ν, s^{-1}	λ	Spectral Region	Type of Transition	Spectroscopic Method
		Radio Waves		
10^4		Longwave		
10^5	10^6 cm			
10^6	10^5 cm	Broadcast	Rotation of macromolecules	Dielectric measurements
10^7	10^4 cm		Nuclear orientations in a molecule	NQR
10^8	10^3 cm	FM	Nuclear orientations in a magnetic field	NMR
10^9	10^2 cm			
10^{10}	10 cm	Microwave	electron spin flips in a magnetic field	ESR
	1 cm		Rotation of small molecules	microwave
10^{11}		Far Infrared	Rotation of smallest molecules	
	10^3 μm			
10^{12}		Infrared		
	10^2 μm		Vibrations of solids, liquids, large molecules	Raman and infrared
10^{13}				
	10 μm			
10^{14}		Near infrared	Vibrations of small molecules	
	10^3 nm			

ν, s^{-1}	λ	Spectral Region	Type of transition	Spectroscopic method
10^{15}		Visible light < Red, Yellow, Blue	Transitions of outer electrons	Visible
	10^2 nm			
10^{16}		Ultraviolet, Near UV, Vacuum UV		Ultraviolet
	10^2 Å		Transitions of inner electrons	
10^{17}				
	10 Å	X-rays		X-ray
10^{18}		Soft x-rays		
	1 Å	Hard rays		
		γ-rays	Transitions within nuclei	Mössbauer

Absorption and Dispersion

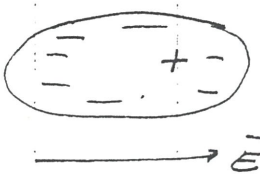
$\vec{E}^*$ of light wave has two effects on a molecule or atom

(1) it induces a dipole moment in atom or molecule



$$\vec{\mu}^{in} = \underset{\sim}{\alpha}\, \vec{E}^*$$

α = molecular polarizability



$\vec{\mu}$

(2) If the molecule has a permanent dipole moment, it can attempt to align the molecular dipoles (counteracted

by thermal motions)

Debye showed that for molecules in the gas or in a dilute solution, $\vec{\mu}$ & $\underline{\alpha}$ are related to the dielectric constant ϵ of substance.

$$\frac{(\epsilon - 1)}{(\epsilon + 2)} \frac{M}{\rho} = \frac{4\pi N_A}{3}\left(\alpha + \frac{\mu^2}{3k_B T}\right)$$

ϵ = dielectric constant

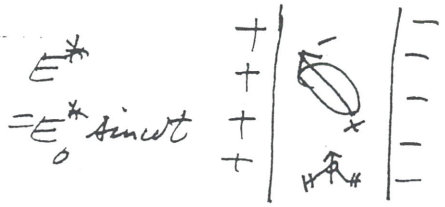
at high temperatures, $\vec{E}^*$ cannot align $\vec{\mu}$ very effectively so α dominates

Also, when the frequency of light wave becomes very high, permanent dipoles of molecules cannot followed the oscillations of $\vec{E}^*$ and therefore do not become aligned, even partially, with [redacted] $\vec{E}^*$

So $$\frac{(\epsilon_{hf} - 1)}{(\epsilon_{hf} + 2)} \frac{M}{\rho} = \frac{4\pi N_A}{3}\alpha$$

and it turns out $\epsilon_{hf} = n^2$ or square of refractive index of substance.

→ Dielectric absorption of a hemoglobin solution. Dispersion



2 components, say

Hb $\quad \nu_{rot} \sim 10^7$ Hz

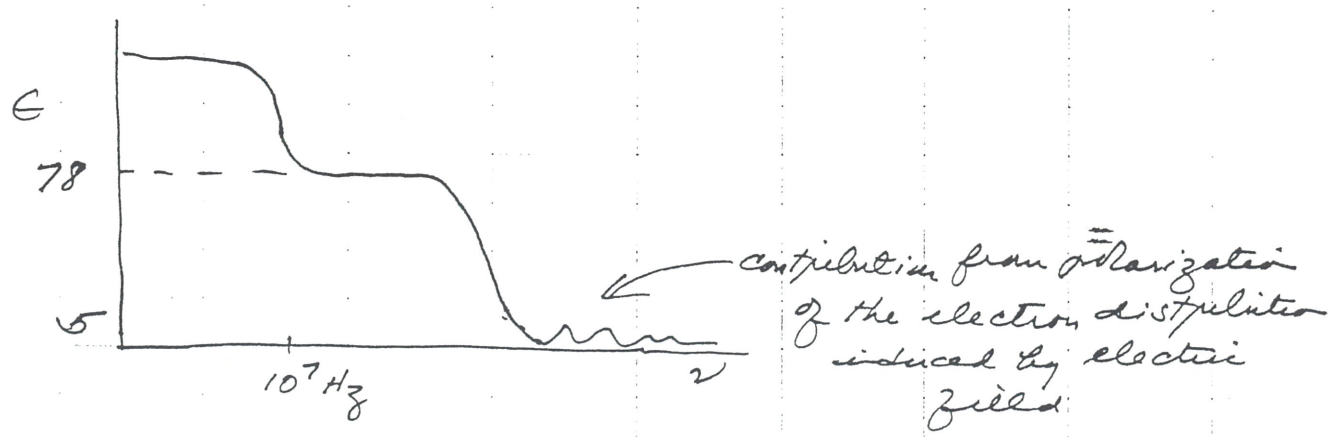
H_2O $\quad \nu_{rot} \sim 10^{11}$ Hz

At $\omega/2\pi < 10^7$ Hz, both Hb and H_2O will contribute μ^2 and to ϵ of solution.

As $\omega/2\pi$ is swept through 10^7 Hz, solution will absorb power from rf circuit. Absorption is caused by the lagging of Hb dipoles beyond the oscillations of the alternating electric field. As $\omega/2\pi$ passes through this region, the dipoles of the Hb can no longer come to alignment with the a.c. field.

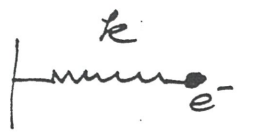
So, at frequencies above 10^7 Hz, μ^2 of Hb no longer contributes to ϵ of solution. This decline in dielectric constant is called a dispersion, and the absorption of energy associated with it is called a dielectric loss.

Similarly, as $\frac{\omega}{2\pi}$ passes through 10^{11} Hz, similar dielectric absorption and dispersion occurs as the water dipoles can no longer come into alignment with $\vec{E}$*.



Dipoles can absorb power from rf circuit when their oscillations lag 90° behind the polarizing $\vec{E}^*$

Consider



$$E^* = E_0^* \sin \omega t$$

Driving electric field : $E^* = E_0^* \sin \omega t$

Vibrational frequency of e^- : $\omega_0 = \sqrt{k/m}$

At resonance, $x = x_0 \sin \omega t + x_{90} \cos \omega t$

x = displacement of e^- from equilibrium position

$x_0 \sin \omega t$ is phase with force $(E_0^* \sin \omega t)$

$x_{90} \cos \omega t$ $90°$ out-of phase with force $\begin{cases} \text{if } x_{90} < 0 & \text{lags} \\ x_{90} > 0 & \text{leads} \end{cases}$

→ The work done on e^- in a complete cycle of the electrostatic field is zero, if the oscillation of the e^- stays in phase with the driving field $(x = x_0 \sin \omega t)$

Work done by field on charge $= \int_{cycle} F \, dx$

$$= \int_{cycle} q E^* \, dx = q \int_{cycle} E^{0*} \sin \omega t \cdot d(x_0 \sin \omega t)$$

$$= q E^{0*} x_0 \int_0^{2\pi} \sin\theta \, d \sin\theta \qquad \theta = \omega t$$

$$= q E^{0*} x_0 \left. \frac{\sin^2 \theta}{2} \right|_0^{2\pi} = 0$$

→ Work is done on e^- in a complete cycle of the electrostatic field, if the oscillation of the e^- lags 90° out-of phase with the driving field $x = -|x_{90}| \cos\omega t$

Work done by field on charge $= \int_{cycle} F\,dx$

$$= q \int E^{0*} \sin\omega t \cdot d(-|x_{90}| \cos\omega t)$$

$$= -q\, E^{0*} |x_{90}| \int_{cycle} \sin\omega t\, d\cos\omega t$$

$$= +q E^{0*} |x_{90}| \int_0^{2\pi} \sin^2\theta\, d\theta$$

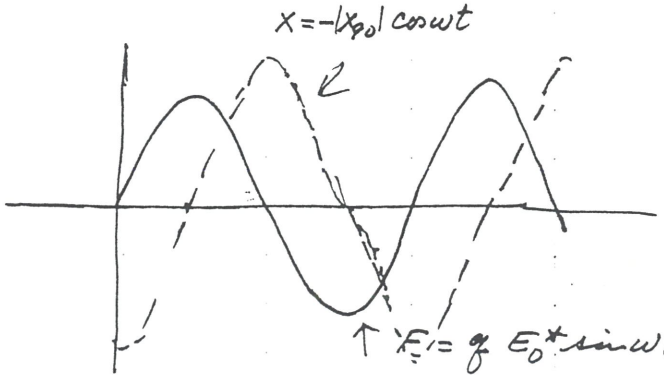
$$= \pi q E^{0*} |x_{90}| \qquad > 0$$

$\therefore e^-$ or charge absorbs power from rf circuit but $q < 0$ for e^-, so e^- gives up power to rf circuit!

Note that when oscillation of e^- (resonance) leads driving field by 90°,

work done by field on charge $= -\pi q E^{0*} |x_{90}|$

so e^- or charge does work on the rf circuit.



Condition for absorption

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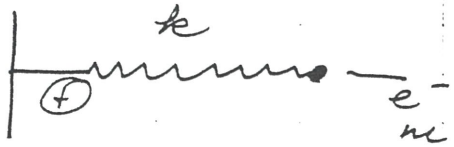
Classical Mechanical Description of Absorption and Dispersion — detailed treatment.

Problem



$\Longleftrightarrow$

$E^* = E_0^* \sin \omega t$

1. Electromagnetic wave : $E_0^* \sin \omega t$ $\qquad \omega = 2\pi\nu$

2. Natural frequency of oscillator :

$$\omega_0 = \sqrt{\frac{k}{m}}$$

3. Forces on e^-

a) Driving force of electromagnetic wave : $-|e| E_0^* \sin \omega t$

b) Restoring force between negative electron and positive charge : $-kx$

c) Damping force, which is proportional to velocity of moving electron and arises from frictional and radiative energy losses : $-\eta \frac{dx}{dt}$

Newton's equation of motion applied to e^-

$$\sum \text{Force} = m \frac{d^2x}{dt^2}$$

$$-|e| E_0^* \sin \omega t - kx - \eta \frac{dx}{dt} = m \frac{d^2x}{dt^2}$$

General solution to linear second-order differential eq.

$$x = x_0 \sin\omega t + x_{90} \cos\omega t$$
$$= x_0 \sin\omega t + \beta x_0 \cos\omega t$$

Differentiating,

$$\frac{dx}{dt} = \omega x_0 \cos\omega t - \omega\beta x_0 \sin\omega t$$

$$\frac{d^2x}{dt^2} = -\omega^2 x_0 \sin\omega t - \omega^2 \beta x_0 \cos\omega t$$

Substituting into $F = ma$, and collecting terms,

$$[-|e|E_0^* - kx_0 + \eta\omega\beta x_0 + \omega^2 x_0 m] \sin\omega t$$
$$+ [-k\beta x_0 - \eta\omega x_0 + m\omega^2 \beta x_0] \cos\omega t = 0$$

True for all values of time.

$$\therefore \quad -|e|E_0^* - kx_0 + \eta\omega\beta x_0 + m\omega^2 x_0 = 0$$

and $$-k\beta x_0 - \eta\omega x_0 + m\omega^2 \beta x_0 = 0$$

Then $$\beta = \frac{\eta\omega}{m\omega^2 - k}$$

and $$x_0 = \frac{|e|E_0^*}{m\omega^2 - k + \eta\omega\beta}$$

Since $k = m\omega_0^2$

Define $\omega' = \eta/2m$

$$\beta = \frac{2m\omega\omega'}{m\omega^2 - m\omega_0^2} = -\frac{2\omega\omega'}{\omega_0^2 - \omega^2}$$

$$x_0 = \frac{|e|E_0^*}{m\omega^2 - m\omega_0^2 + 2m\omega'\omega\beta}$$

$$= -\frac{|e|E_0^*}{m}\left[\frac{\omega_0^2 - \omega^2}{(\omega_0^2-\omega^2)^2 + 4\omega'^2\omega^2}\right]$$

Note that when periodic driving force is removed, one has a damped oscillator, in which case

$$x = x_0 e^{-\omega' t}\cos(\omega_0^2 - \omega'^2)^{1/2} t$$

where $\omega' = \eta/2m$

Polarizabilities

$$\alpha = \frac{\mu^{in}}{E^*} = \frac{-|e|x}{E^*}$$ [redacted] Static field E^*

For our problem here,

$$\mu^{in} = -|e|x_0 \sin\omega t - |e|\beta x_0 \cos\omega t$$

in phase component — out-of-phase component (90°)

Define $\alpha_0 = -\frac{|e|x_0}{E_0^*}$ in phase polarizability

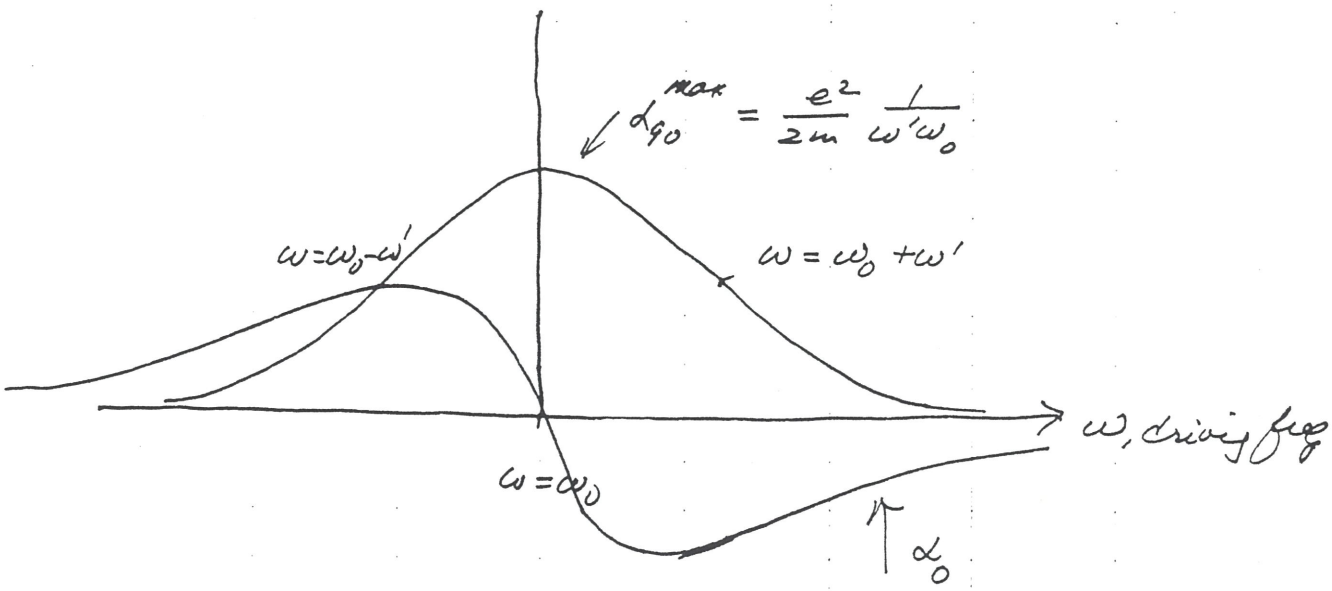
$\alpha_{90} = \frac{|e|\beta x_0}{E_0^*}$ out of phase polarizability (chose sign such that it is +)

Then $\mu^{in} = \alpha_0 E_0^* \sin\omega t - \alpha_{90} E_0^* \cos\omega t$

Substituting

$$\alpha_0 = \frac{e^2}{m} \frac{(\omega_0^2 - \omega^2)}{(\omega_0^2 - \omega^2)^2 + 4\omega'^2\omega^2}$$

$$\alpha_{90} = \frac{2e^2}{m} \frac{\omega\omega'}{(\omega_0^2 - \omega^2)^2 + 4\omega'^2\omega^2}$$



It turns out

$$\text{Extinction coefficient } \epsilon(\omega) = \frac{4\pi N_A}{2303c} \omega \alpha_{90}$$

$$n^2 \cong 1 + \frac{4\pi N A \rho}{M} \alpha_0$$

refractive index

$$I_s(\phi = 90) \propto \frac{I_0 \, 16\pi^4 \alpha_0^2 \nu^4}{r^2 c^4} =$$

Reading Assignment Eisenberg and Crothers, Chapter 13

From last lecture,

$$\alpha_0 = \frac{e^2}{m}\left[\frac{\omega_0^2 - \omega^2}{(\omega_0^2-\omega^2)^2 + 4\omega'^2\omega^2}\right]$$

contributes to dispersion

$$\alpha_{90} = \frac{2e^2}{m}\left[\frac{\omega\omega'}{(\omega_0^2-\omega^2)^2 + 4\omega'^2\omega^2}\right]$$

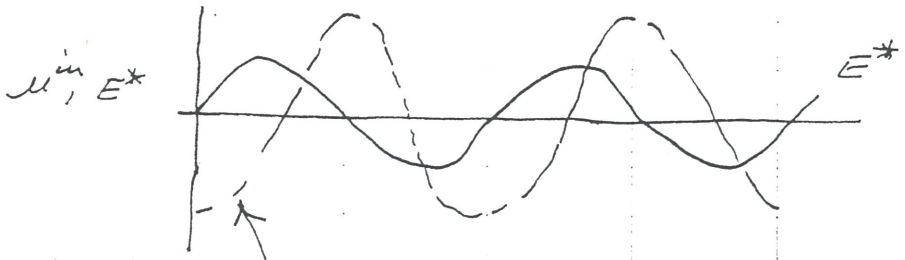
contributes to absorption

where $\mu^{in} = \alpha_0 E_0^* \sin\omega t - \alpha_{90} E^{0*} \cos\omega t$

For $\omega \sim \omega_0$ (resonance)

$\alpha_0 \cong 0$

$\alpha_{90} > 0$ $\qquad \alpha_{90}^{max} = \frac{e^2}{2m}\frac{1}{\omega'\omega_0}$



$\mu^{in} = -\alpha_{90} E_0^* \cos\omega t$ (90° lag)

For $\omega \gg \omega_0$ or $\omega \ll \omega_0$,

$(\omega_0^2 - \omega^2)^2 \gg 4\omega'^2\omega^2$

$$\alpha_0 = \frac{e^2}{m} \frac{1}{\omega_0^2 - \omega^2}$$

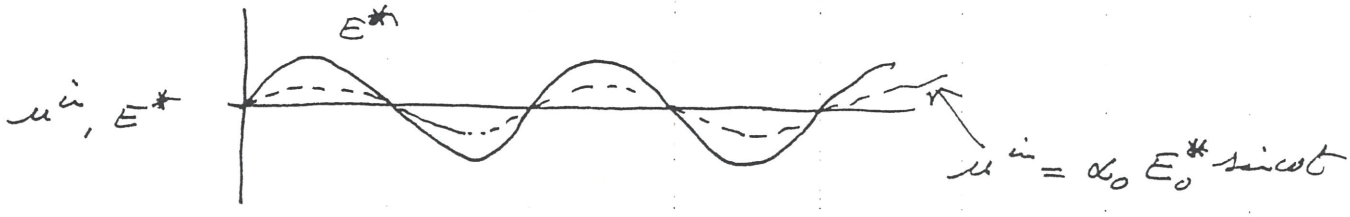
α_{90} small.

$\therefore \mu^{in} = \alpha_0 E_0^* \sin\omega t$ (in phase only or 180° out-of phase) depending on sign of α_0

at low frequencies,

$\omega_0 >> \omega$, $\alpha_0 > 0$

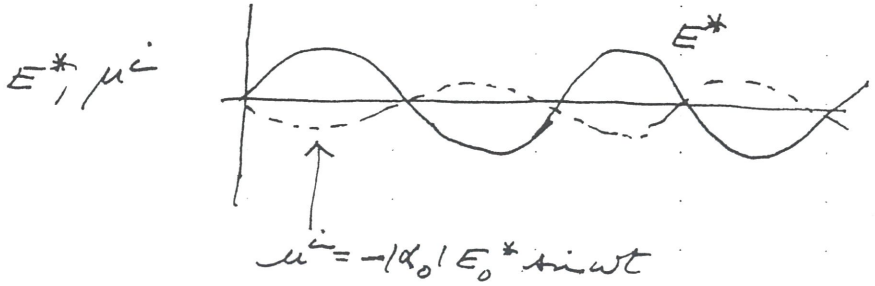
μ^{in} in phase



at high frequencies,

$\omega_0 << \omega$, $\alpha_0 < 0$

μ^{in} out-of phase by 180°



Interaction of light with molecules – Quantum mechanical treatment

must solve time-dependent Schroedinger equation

$$\mathcal{H} = \mathcal{H}^{\circ}_{\substack{\text{isolated} \\ \text{molecule}}} + V(t)$$

where $V(t)$ describes the interaction of electrons with light wave.

Assume 1 electron.

If electric interaction, $V(t) = -\vec{\mu}_{el} \cdot \vec{E}^{*}$

If magnetic interaction, $V(t) = -\vec{\mu}_{m} \cdot \vec{B}^{*}$

Discuss electric interaction, where $\vec{\mu}_{el} = -|e|\vec{r}$

Treat, for simplicity, two-level system.

——————— b W_b $\Psi_b(\vec{r},t)$

——————— a W_a $\Psi_a(\vec{r},t)$

$$\Psi_a(\vec{r},t) = \phi_a(\vec{r})\, e^{-iW_a t/\hbar}$$

$$\Psi_b(\vec{r},t) = \phi_b(\vec{r})\, e^{-iW_b t/\hbar}$$

} solution to time-dependent Schroedinger equation for $V(t) = 0$

In presence of $V(t)$,

$\Psi(\vec{r},t)$ must be a linear combination of these two

states, but the coefficients C_a and C_b are time-dependent

i.e., $\Psi(\vec{r},t) = C_a(t)\,\phi_a(\vec{r})\,e^{-iW_a t/\hbar} + C_b(t)\,\phi_b(\vec{r})\,e^{-iW_b t/\hbar}$

Substituting this solution into time-dependent Schroedinger equation, we get

$$i\hbar\left(\phi_a(\vec{r})\,e^{-iW_a t/\hbar}\frac{dC_a}{dt} + \phi_b(\vec{r})\,e^{-iW_b t/\hbar}\frac{dC_b}{dt}\right)$$

$$= V(t)\left[\phi_a(\vec{r})\,e^{-iW_a t/\hbar}C_a(t) + \phi_b(\vec{r})\,e^{-iW_b t/\hbar}C_b(t)\right]$$

multiplying above from the left by $\phi_a^*(\vec{r})\,e^{iW_a t/\hbar}$ or $\phi_b^*(\vec{r})\,e^{-iW_b t/\hbar}$, we obtain

$$i\hbar\frac{dC_a}{dt} = V_{aa}\,C_a(t) + V_{ab}\,C_b(t)\,e^{-i(W_b-W_a)t/\hbar}$$

$$i\hbar\frac{dC_b}{dt} = V_{ba}\,C_a(t)\,e^{-i(W_a-W_b)t/\hbar} + V_{bb}\,C_b(t)$$

where $V_{aa} = \left(\int \phi_a^*(\vec{r})\,\underline{V}(t)\,\phi_a(\vec{r})\,d\vec{r}\right)$

$$V_{ab} = \left(\int \phi_a^*(\vec{r})\,V(t)\,\phi_b(\vec{r})\,d\vec{r}\right)$$

$$V_{bb} = \left(\int \phi_b^*(\vec{r})\,V(t)\,\phi_b(\vec{r})\,d\vec{r}\right)$$

=

a set of coupled first-order differential equations

If we take $\underset{\sim}{V}(t) = -\vec{\mu} \cdot \vec{E}_0^* e^{i\omega t}$

where $\vec{\mu} = -|e|\,\vec{r}$

then $V_{aa} = 0$, $V_{bb} = 0$

and

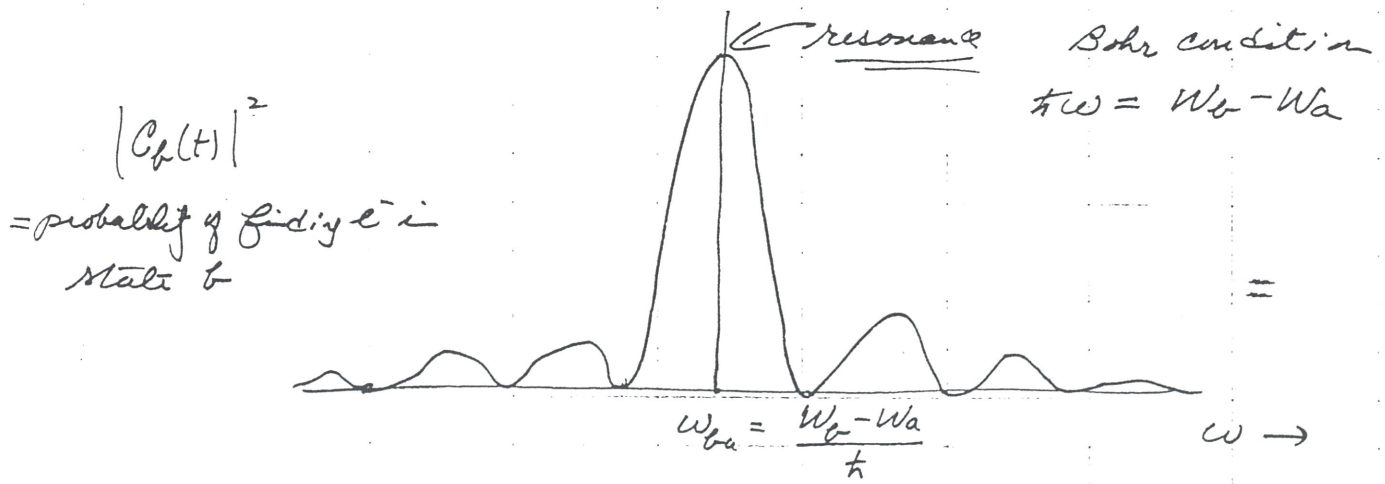
$$-i\hbar \frac{dC_a}{dt} = C_b(t)\, \vec{\mu}_{ab} \cdot \vec{E}_0^*\, e^{-i\left(\frac{W_b - W_a}{\hbar} - \omega\right)t}$$

$$-i\hbar \frac{dC_b}{dt} = C_a(t)\, \vec{\mu}_{ba} \cdot \vec{E}_0^*\, e^{-i\left(\frac{W_a - W_b}{\hbar} - \omega\right)t}$$

Solving simultaneously, with boundary condition $|C_b(0)|^2 = 0$ at $t=0$, i.e., electron is in quantum state a at $t=0$, and for small E_0^*

$$|C_b(t)|^2 = |\vec{\mu}_{ba} \cdot \vec{E}_0^*|^2 t^2 \frac{\sin^2\left[\left(\frac{W_b - W_a}{\hbar} - \omega\right) t/2\right]}{2\left[\left(\frac{W_b - W_a}{\hbar} - \omega\right) t/2\right]^2}$$

where $\vec{\mu}_{ba} = \int \phi_b^*\, \vec{\mu}\, \phi_a\, d\vec{\tau} = \vec{\mu}_{ab} = \int \phi_a^*\, \vec{\mu}\, \phi_b\, d\vec{\tau}$



Now the rate at which molecules in state a are transformed to state b by the presence of light

$$\frac{dP_b}{dt} = \frac{d}{dt}\int |C_b(t)|^2 \, d\nu$$

$$= \frac{1}{2\hbar^2} \left| \vec{\mu}_{ba} \cdot E_0^* \right|^2$$

Customary to write

$$\frac{dP_b}{dt} = B_{ab} \, I(\nu)$$

B_{ab}: initial state → final state

where B_{ab} is the transition rate per unit energy density of radiation

and $I(\nu)$ is the energy density incident on the sample at frequency ν

From E & M, $\quad I(\nu) = |E_0^*|^2 / 4\pi$

So $\quad B_{ab} = \left(\frac{2\pi}{\hbar^2}\right) |\vec{\mu}_{ab}|^2 \cos^2\theta$

where θ is the angle between $\vec{\mu}$ and $\vec{E}_0^*$

above result for oriented molecules illuminated by polarized light.

In solution, molecules reorient wrt to E_0^*, so must average $\cos^2\theta$. Since $\overline{\cos^2\theta} = 1/3$

$$B_{a\to b}\ (\text{solution}) = \left(\frac{2\pi}{3\hbar^2}\right) |\vec{\mu}_{ab}|^2$$

above derivation for stimulated absorption ($a \rightarrow b$)

Simulated B coefficient can be obtained for stimulated emission ($b \rightarrow a$), i.e., $B_{a\rightarrow b} = B_{b\rightarrow a}$ or $B_{ab} = B_{ba}$ } Einstein coeff. for stimulated absorption & emission

Now the rate at which energy is removed from light will depend on the number of $a \rightarrow b$ absorption transitions stimulated by the light, on the number of $b \rightarrow a$ emission transitions (also stimulated by the light), and on the energy of the photon ($W_b - W_a = h\nu$) $= \hbar\omega$

$$-\frac{d\,I(\nu)}{dt} = h\nu\left(N_a B_{ab} - N_b B_{ba}\right) I(\nu)$$

where N_a, N_b are the number of molecules per cm^3 in state a, and b respectively

$I(\nu)$ is the energy density of light incident a sample at frequency ν

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Beer-Lambert's Law

For optical transitions, N_b negligible.

$$\frac{dI(\nu)}{dt} \cong h\nu\, N_a\, B_{ab}\, I(\nu)$$

$$= h\nu \frac{C \cdot N_A}{1000} B_{ab}\, I(\nu)$$

$C \equiv$ conc. of absorbers (all in state a)

Now $\frac{dI(\nu)}{dl} = \frac{dI(\nu)}{dt}\left(\frac{dt}{dl}\right) = \frac{1}{c_{light}} \frac{dI(\nu)}{dt} = \frac{h\nu}{c_{light}} \frac{C N_A}{1000} B_{ab}\, I(\nu)$

(l = path length)

or $dI(\nu) = \left(\frac{h\nu\, C\, N_A}{1000\, c_{light}} B_{ab}\right) I(\nu)\, dl$

or $\epsilon(\nu) = \frac{h\nu\, N_A}{1000\, c_{light}} B_{ab}$

(ϵ = extinction coefficient)

or $B_{ab} = \frac{1000\, c_{light}}{h\nu\, N_A}\, \epsilon(\nu)$

or integrating over absorption band

$$B_{ab} = \frac{1000\, c_{light}}{h N_A} \int_{\text{absorption band}} \frac{\epsilon(\nu)}{\nu}\, d\nu$$

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