Basics of Fluorescence Spectroscopy
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Lecture – 02

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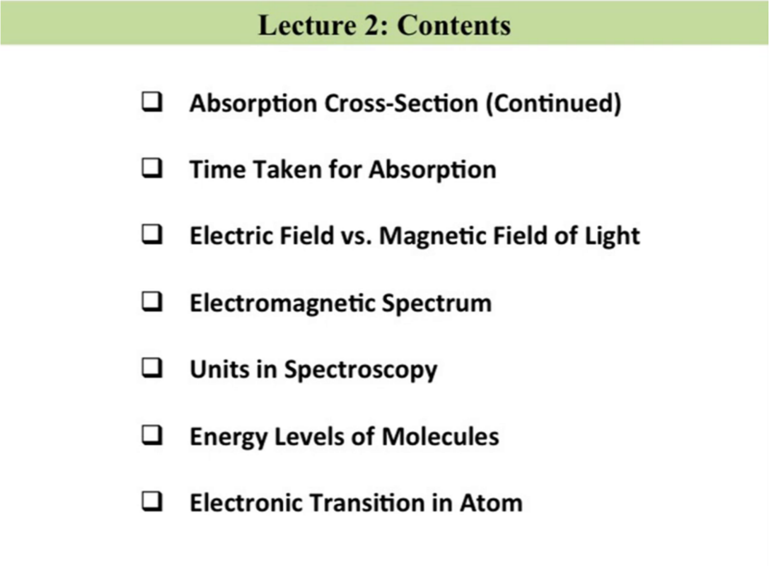

Lecture 2: Contents

- ☐ Absorption Cross-Section (Continued)
- ☐ Time Taken for Absorption
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- ☐ Units in Spectroscopy
- ☐ Energy Levels of Molecules
- ☐ Electronic Transition in Atom

Welcome to the second lecture of the course entitled Basics of Fluorescence Spectroscopy. In the last class we have discussed about some introduction about the fluorescence spectroscopy and the brief history of that and then we discussed about the light matter interaction and for us the most important light matter interaction being through the absorption of light by the matter and to discuss that absorption of light by the matter we discussed the Lambert Beer law and then we bought the concept of the absorption cross section of the molecule.

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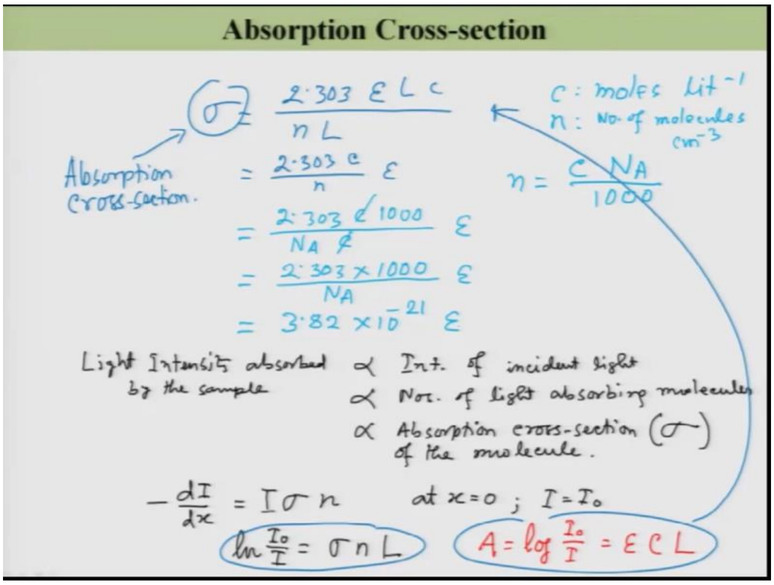

Absorption Cross-section

$$\sigma = \frac{2.303\,\varepsilon L c}{n L}$$

$$= \frac{2.303\, c}{n}\varepsilon$$

$$= \frac{2.303\,\cancel{c}\,1000}{N_A\,\cancel{c}}\varepsilon$$

$$= \frac{2.303 \times 1000}{N_A}\varepsilon$$

$$= 3.82 \times 10^{-21}\,\varepsilon$$

Absorption cross-section.

c : moles lit^{-1}

n : No. of molecules cm^{-3}

$$n = \frac{c\,N_A}{1000}$$

Light Intensity absorbed by the sample $\propto$ Int. of incident light

$\propto$ Nos. of light absorbing molecules

$\propto$ Absorption cross-section (σ) of the molecule.

$$-\frac{dI}{dx} = I\sigma n \qquad \text{at } x=0\ ;\ I = I_0$$

$$\ln\frac{I_0}{I} = \sigma n L \qquad A = \log\frac{I_0}{I} = \varepsilon C L$$

And I entered over here that sigma equal to 2.303 epsilon l C by n l which turns out to be 2.303 C by n into epsilon where this epsilon as you know is molar absorptivity or molar extinction coefficient and its unit is liter mole inverse centimeter inverse you can also change it from the centimeter inverse to millimeter inverse or length inverse, right. So, now, as you can see here the T C over here the unit of the C we generally express in terms of moles per liter.

So, here is moles per liter and here that n that n which I have defined as number of molecules per C C, right. So, number of molecules per C C. So, it means that I have to convert these 2 units accordingly. So, this n right in terms of C can be written as C into Avogadro number divided by 1000 as simply as it is. So, now, if I plug in this value of n over here, so what I will going to get is 2.303 and this C is over here and then I will going to have a 1000 factor over here and divided by divided by n a coming from here and then C 2 epsilon. So, this C C will cancel out over here and I was going to have 2.303 in 2000 divided by Avogadro number into epsilon if you calculate this; this will come as 3.82 into 10 to the power minus 21. So, this is going to 3.82 into 10 to the power minus 21 to epsilon.

So, what we will got is that absorption cross section is nothing, but that proportionality constant was used for the Lambert Beer law and this; obviously, has to be related with the epsilon value. So, as epsilon depends on the wavelength abduction cross section also has to depends on the wavelength let us see this; the concept of absorption cross section here.

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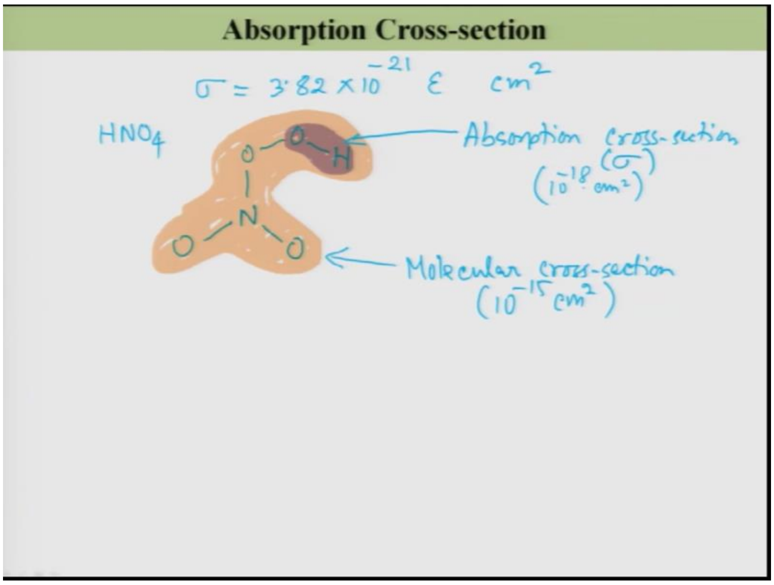

Absorption Cross-section

$$\sigma = 3.82 \times 10^{-21} \, \varepsilon \ \text{cm}^2$$

So, what we got we got sigma which is absorption cross section is equal to 3.82, 10 to the power minus 21 into epsilon and; obviously, the unit is important that is centimeter square because I have expressed epsilon in terms of liter mole inverse centimeter inverse. So, I got this emitter centimeter inverse now let me draw a one molecule let us say I, let me draw this H in O 4 plus this molecule. So, here if I draw this frame of this molecule looks like this. So, now, the molecular cross section molecular cross section is the whole area of this molecule for example, if I may now highlight over here. So, this whole region is my molecular cross section. Now if we use a light whose wave number is about thirty 400 centimeter inverse and we know that will going to excite my O H bond. So, vibrational excitation so that particular light will be going to interact only with this O H bond. So, the absorption cross section for this 3400 wave number light is not the whole molecular cross section.

So, in this case for O H bond excitation this is my absorption cross section for that particular frequency of light particular wave number of light. So, here; obviously, I can write that this is my molecular cross section and this one is my absorption cross section which is known as sigma. So, the if the molecular cross section let us say for this particular molecule is 10 to the power minus 15 centimeter square and this absorption cross section for this the small region right it must be smaller than this and if you somehow calculate it this will going to be 10 to the power minus 18 centimeter square.

It gives me a idea that once write n number of photons will appear. So, these n number of photons of that particular frequency will collide with this molecule, but only small fraction that fraction will determined by this 10 to the power minus 15 the 10 to the minus 18 by 10 to the power minus 15. So, only that fraction of the photon will be used to excite the molecules I mean to excite the O H bond from ground vibrational level to the excited state.

So depending on the wavelength; obviously, the value of this absorption cross section will change. So, later on I will give you that assignment where you will be able to calculate this absorption cross section of a molecule and you will going to understand the significance of absorption cross section and how it is related to the molar extinction coefficient. So, now, let us move to another important phenomena related to the absorption and my question is that how much time it will take for an electronic absorption right. So, let us talk about this electronic for specifically over here because we are dealing with the fluorescence in this particular course.

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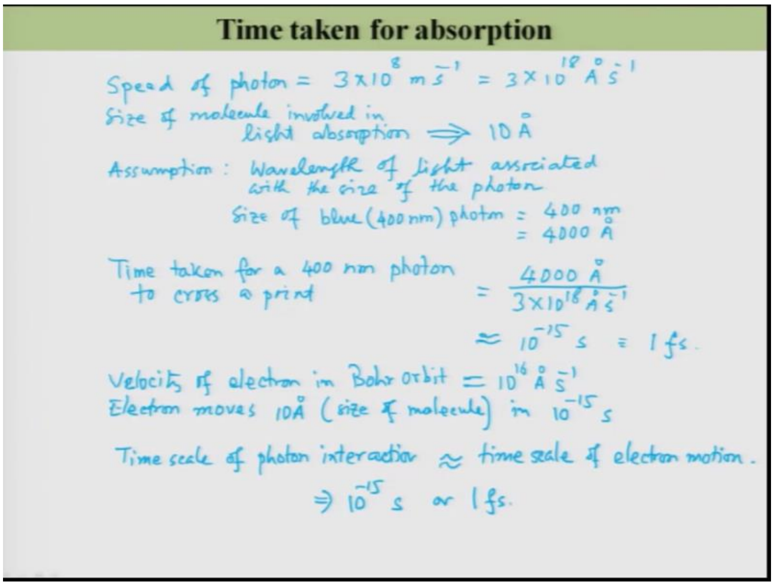

Time taken for absorption

Speed of photon = $3 \times 10^{8}\ m\,s^{-1} = 3 \times 10^{18}\ \mathring{A}\,s^{-1}$

Size of molecule involved in light absorption ⇒ $10\ \mathring{A}$

Assumption : Wavelength of light associated with the size of the photon

Size of blue (400 nm) photon = 400 nm = $4000\ \mathring{A}$

Time taken for a 400 nm photon to cross a point $= \dfrac{4000\ \mathring{A}}{3 \times 10^{18}\ \mathring{A}\,s^{-1}} \approx 10^{-15}\ s = 1\ fs.$

Velocity of electron in Bohr orbit $= 10^{16}\ \mathring{A}\,s^{-1}$

Electron moves $10\ \mathring{A}$ (size of molecule) in $10^{-15}\ s$

Time scale of photon interaction ≈ time scale of electron motion.

$\Rightarrow 10^{-15}\ s$ or 1 fs.

So, let me write few fact that first speed of photon is equal to 3 into 10 to the power eight meter per second right I can write this as 3 into 10 to the power 18 angstrom per second right and let me also assume one thing very simple assumption that is that size of molecule involved in light absorption let us say this is about 10 angstrom its very valid assumption right and now I will going to make another assumption which where I would like to take the size of the photon right size of the photon is associated with the wavelength of the light. So, light has said 2 different kind of property has connected by the de Broglie hypothesis.

So, first is light is wave and light is photon right. So, when I say is 400 nanometer light; that means, the wavelength of the light is 400 nanometer, but it also gives the photon right it also behaves as a particle. So, now, my assumption here is that the size of the photon is associated with the wavelength of the light. So, I write here the wavelength of light associated with the size of the photon right it means the size of a blue photon which is resolving this 400 nanometer is 400 nanometer. So, for example, size of blue; that means, 400 nanometer photon is equal to 400 nanometer or lets in the different unit is 4000 angstrom right.

So, now I can easily calculate because I know the speed I know the size. So, the time taken for a 400 nanometer photon to cross a point right I can easily calculate. So, time taken for a 400 nanometer photon to cross a point is simply given by 4000 because that is the; my size divided by the speed right. So, 4000 angstrom divided by 3 into 10 to the power 18 angstrom per second right. So, if I calculate roughly this will be 10 to the power minus 15 second this 10 to the power minus 15 second means 1 femtosecond. So, this is 1 femtosecond. So, if you are going to excite your molecule with this 400 nanometer light. So, that 400 nanometer light will be present to interact with the part of this molecule only for one femtosecond. So, if the absorption has to be takes place then that absorption of the light has to be takes place within that time frame right and also the velocity of electron in Bohr orbit right we all know that this is 10 to the power 16 angstrom per second. So, sorry velocity of electron in Bohr orbit is equal to 10 to the power 16 angstrom per second.

But I have already determined the size of the molecule is about 10 angstrom. So, electron moves 10 angstrom right that is my size of molecule in 10 to the power minus 15 second. So, what we got here that the timescale of photon interaction and electron motion in the molecules they are same right. So, it means that time scale of a photon interaction is similar to time scale of electron motion which is 10 to the power minus 15 second or one femtosecond and this is the time scale for the absorption process. So, now, a light like electromagnetic radiation consists of electric field and magnetic fields are actually coming and interacting with the matter is my molecule for this particular case I am taking. So, now, this that is nothing, but the light matter interaction right, but what I already said in the last class that the electrometric radiation consists of both electric and magnetic field.

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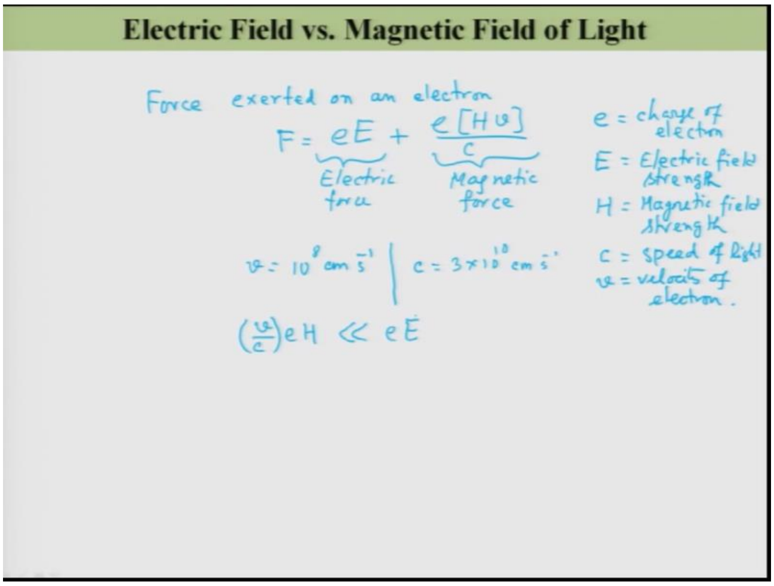

Electric Field vs. Magnetic Field of Light

Force exerted on an electron

$$F = \underbrace{eE}_{\text{Electric force}} + \underbrace{\frac{e[Hv]}{c}}_{\text{Magnetic force}}$$

e = charge of electron

E = Electric field strength

H = Magnetic field strength

c = speed of light

v = velocity of electron.

$v = 10^{8}\ \text{cm}\ s^{-1}$ | $c = 3 \times 10^{10}\ \text{cm}\ s^{-1}$

$$\left(\frac{v}{c}\right) eH \ll eE$$

So, which are the electric or magnetic or both the fields are responsible for this light matter interaction or one is more responsible than the other so that we can easily check. So, what I have to look here is the force exerted on an electron by the light right. So, if I write force exerted on an electron by the light, so, this F is equal to e E plus e H v divided by C. So, this is this is from the physics right. So, this is the electric force and this part is my magnetic force. So, here e is charge of electron and this capital e is electric field strength H is magnetic field strength and this C C speed of light and v is the velocity of electron. So, what we know the value of v and value of C and with this we can estimate right the contribution of these 2 term. So, v is equal to 10 to the power 8 centimeter per second whereas, C C is equal to 10 to the power 10. So, 3 into 10 to the power 10 centimeter per second so v by C e H is much much less than e into E. So, what we can see is that effect of magnetic force is negligible compared to the effect of electrical.

So, for everyday discussion will not going to include the magnetic field because the magnetic field contribution is much smaller at least for just one percent or less than one percent than the electric field right. So, we will going to discuss only the electric field or we will going to consider only the electric field when we will discuss the in the light matter interaction in the field of electronic transitions. So, now, let me show you the energy levels of the molecules.

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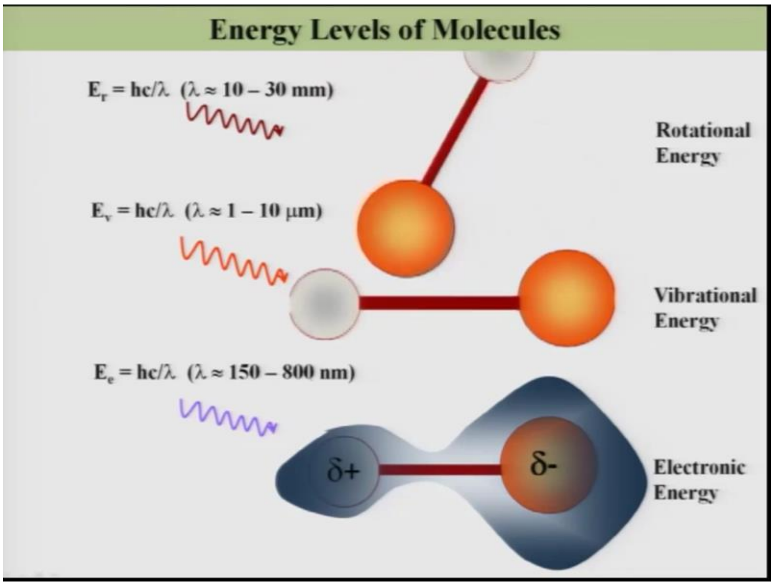

Energy Levels of Molecules

$E_r = hc/\lambda$ ($\lambda \approx 10 - 30$ mm)

Rotational Energy

$E_v = hc/\lambda$ ($\lambda \approx 1 - 10$ μm)

Vibrational Energy

$E_e = hc/\lambda$ ($\lambda \approx 150 - 800$ nm)

Electronic Energy

I have a molecule I have simply showing this molecule as a diatomic molecule. Now if I give the energy which corresponds to the wavelength of 10 to 30 millimeter, this is in the region of microwave right is the microwave region. So, then this energy can initiate the molecule to rotate at a different speed. So, this is the rotation of the molecule. So, I am going to change the rotational energy level right from ground state to the excited state and other states right. So, now, if I change the wavelength from 10 to 30 millimeter to 1 to 10 micrometer which falls in the IR region of this electromagnetic rate electromagnetic spectrum right, so what is electromagnetic spectrum. I will come in the next slide.

So, in this case this light is responsible to create the vibrational excitation within this molecule. However, if you tune your light to 100 UV or visible region like 150 to 800 nanometer then this light will going to excite the molecule from ground electronic level to the excited electronic level. So, which obviously, going to change the distribution of the electronic cloud within this molecule as we shown over here, this is the electronic distribution once you excited the light the distribution changes and it may create the dipole within this molecule.

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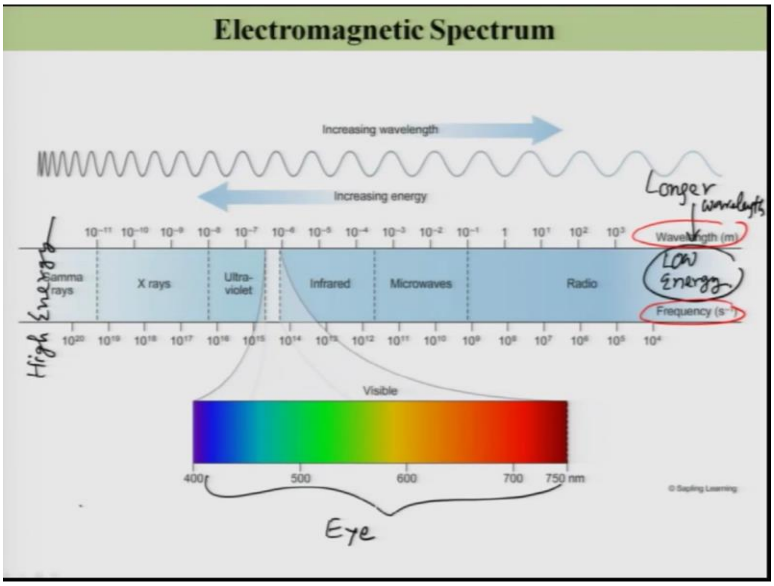

Electromagnetic Spectrum

So, this is the electromagnetic spectrum we all know this in this electromagnetic spectrum as you can see here the left side is high energy side over here and you have this gamma ray and you have this gamma ray over here is a high energy and this side is my low energy. Low energy means longer wavelength right, low energy means longer wavelength. So, as you can see over here; here the length is shorter and in this case the wavelength is longer. So, we start with the gamma; gamma rays where the frequency. So, is 10 to the power twenty and then if we come to 10 to the power 16 this falls in the UV region and then some somewhere between 10 to the power 15 to 10 to the power 14 we got this our visible region. So, our eye is only sensitive to this much region eye can only detect this light other light by our eye you cannot see.

But there are some other instruments some other devices which can be used to see these kind of light like either x-ray or infrared or microwave and so on and so forth. So, this is electromagnetic spectrum and as we can see here I have many different units over here is written frequency here it is written a wavelength right so; obviously, they are interconvertible; they are interconvertible.

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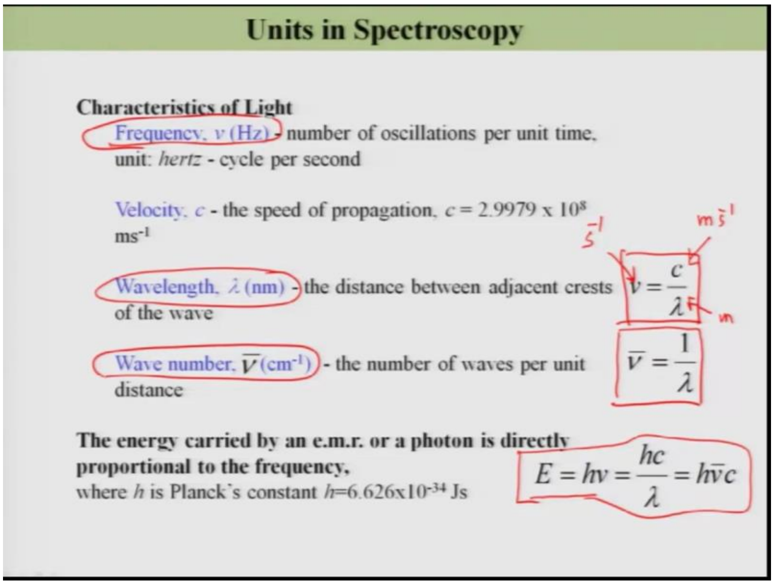

Units in Spectroscopy

Characteristics of Light

Frequency, ν (Hz) - number of oscillations per unit time, unit: *hertz* - cycle per second

Velocity, c - the speed of propagation, $c = 2.9979 \times 10^8$ ms^{-1}

Wavelength, λ (nm) - the distance between adjacent crests of the wave

Wave number, $\bar{\nu}$ (cm^{-1}) - the number of waves per unit distance

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

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

The energy carried by an e.m.r. or a photon is directly proportional to the frequency,

where h is Planck's constant h=6.626x10^{-34} Js

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

So, here this is very important what I want to tell you that this unit in spectroscopy. So, the characteristics of light can be represented not only in frequency not only in wavelength, but also in terms of other parameter and these parameters are interconvertible. So, what is frequency? Frequency is number of oscillations per unit time and unit is hertz the cycle per second right and what is wavelength; wavelength is the distance between adjacent crests of the wave right. So, this is my wavelength and this frequency and wavelength are interconvertible over like this way nu equal to C by lambda. So, try to remember this fundamental relationship nu equal to C by lambda what is the unit of nu the unit of nu is second inverse unit of C is meter per second and this can be represented as meter. So, you see it is second inverse so easily.

So, now this another thing which was not there in that electromagnetic spectrum, but we can also get this important unit which is defined as the number of waves per unit distance right. So, it must be related directly inversely proportional to the wavelength. So, nu bar this is denoted as nu bar and nu bar is one over lambda right and we also know that the energy carried by any electromagnetic radiation that or the photon is directly proportional to its frequency. So, E equal to H nu, so I can write E equal to H nu or E equal to H C by lambda because nu equal to C by lambda or equal to H nu bar C. So, please remember these relationships which will be used more frequently at the later part of this course.

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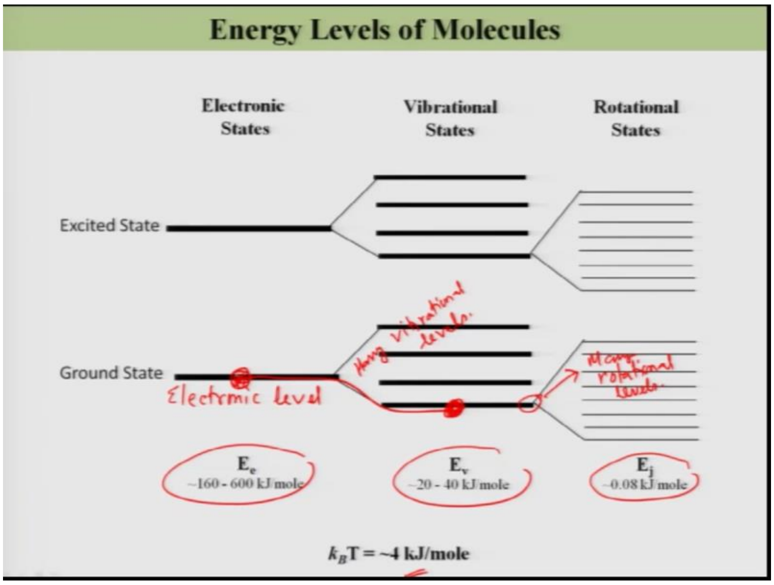

Energy Levels of Molecules

Electronic States

Vibrational States

Rotational States

Excited State

Ground State

Many vibrational levels.

Electronic level

Many rotational levels.

E_e ~160 - 600 kJ/mole

E_v ~20 - 40 kJ/mole

E_j ~0.08 kJ/mole

k_BT = ~4 kJ/mole

Now, let me show you the different energy levels I already showed you the rotational level right where the small amount of energy is required to excite from one rotational level to the another rotational level vibrational states well serve showed you and then electronic states. So, in this case right if these are the 2 electronic level of this molecule each electronic level could have several vibrational level as shown over here. So, this is just electronic level and this is each electronic level consists of many vibrational levels and each vibrational level is consist of many rotational levels right. So, when I and you see the typical energy difference between the 2 levels are like this for the rotational level these for the vibrational level and this is for the electronic level and at room temperature the energy available is this much.

So, according to Boltzmann distribution, so we have these rotational levels maybe populated the higher rotational level maybe may be populated, but the system will be in the lowered electronic level as well as in the lower vibrational level the system and the normal condition if I do not heat up the system normal condition the system will be in the lower electronic level over here not only here, but over here I do not talk about this part because this energy difference is too small.

So, I will be going to have my system over here. So, the lowest vibrational level of the ground electronic states the lowest vibration level of the ground electronic states. So, I will be going to see my system in this particular state.

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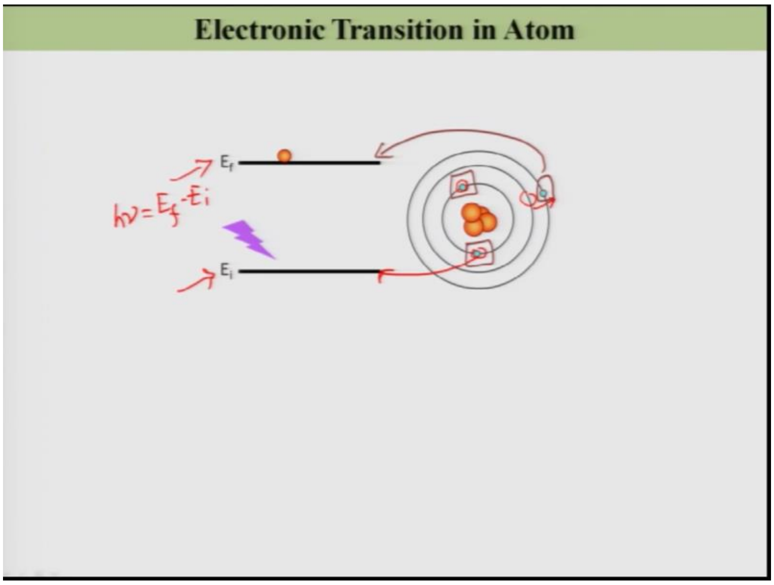

Electronic Transition in Atom

So, now, let me take a very simple example of an atom right and to let me consider that it has 2 state which you are shown over here E I is one state over here and this E F is another state over here. Now if I give the suitable energy which in terms of H nu which is equal to E F minus E I then this system can be promoted from the E I level to E F level because now you have given the required amount of energy to change the energy state of this particular atom from E I to E F level. Now it means for atom one electron goes from one level one orbital to another orbital.

So, as shown over here; here you see this electron is moving from one orbital to another orbital. So, in this case what I have showed you that for the simple atom the meaning the meaning of these 2 level means at this position at this position this is my electronic arrangement and after excitation these electronic arrangements refer to this energy state. So, please remember this the these particular electronic arrangement the electron one is over to here and one is here these refer to this state and when the electron moves one is to here and 1 is here that refer to this particular state right. So, let me finish the second lecture over here.

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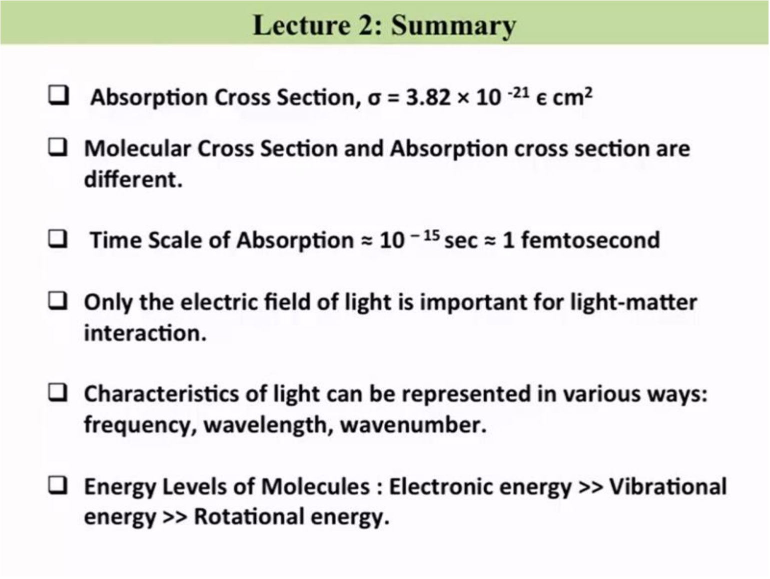

Lecture 2: Summary

- ☐ **Absorption Cross Section, $\sigma = 3.82 \times 10^{-21}\, \epsilon\ \mathrm{cm}^2$**
- ☐ **Molecular Cross Section and Absorption cross section are different.**
- ☐ **Time Scale of Absorption ≈ 10^{-15} sec ≈ 1 femtosecond**
- ☐ **Only the electric field of light is important for light-matter interaction.**
- ☐ **Characteristics of light can be represented in various ways: frequency, wavelength, wavenumber.**
- ☐ **Energy Levels of Molecules : Electronic energy >> Vibrational energy >> Rotational energy.**

And next in the next class, we will be going to discuss about the electronic excitation in molecules.

Thank you.