Basics of Fluorescence Spectroscopy
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Lecture – 03

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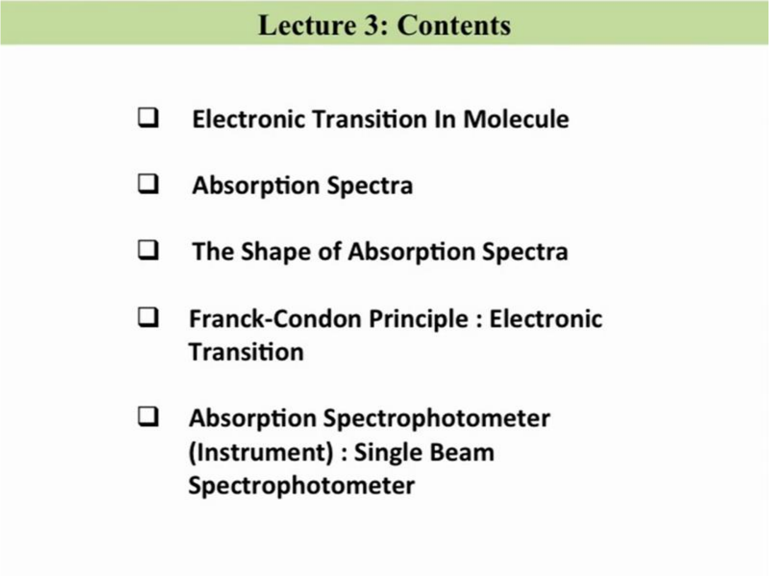

Lecture 3: Contents

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Welcome to the third lecture of the course entitled Basics of Fluorescence Spectroscopy. So, in the last 2 class, we have discuss we are discussing the basics of fluorescence spectroscopy itself; that means, the introduction brief history and the light metal interaction how will going to excite the atoms or molecules by absorbing light and so on and so forth.

So, in the last class we finished with the excitation of atoms by electromagnetic radiation from one electronic level to another electronic level. So, today I will continue with this, in case of the molecule. So, as I told you earlier, the molecules will going to have different electronic energy levels and each electronic energy levels discuss having a different vibrational levels.

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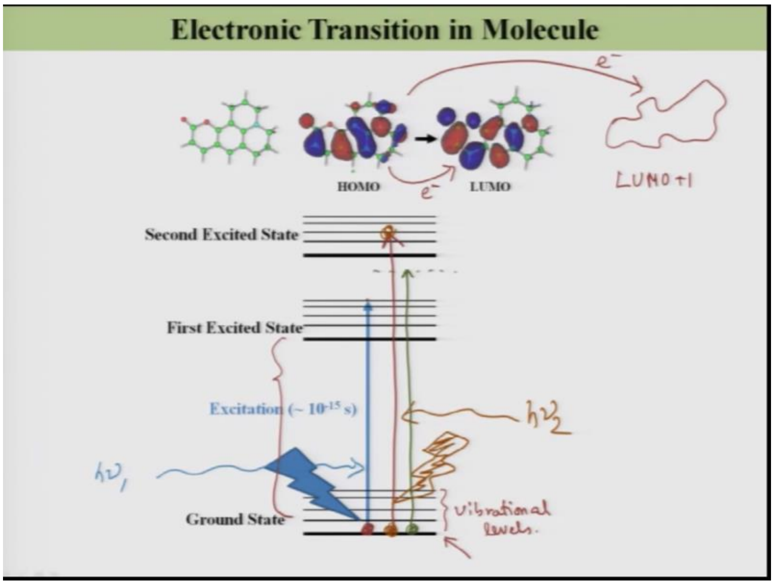

Electronic Transition in Molecule

So, here I have showed, here I have showed the grounds electronic level of a molecule and where these are different vibrational levels a. So, and as I told you that according to the Boltzmann distribution and in the room temperature the energy state of this molecule is the lowest electronic level as well as the lowest vibrational level of the lowest electronic level. So, the molecule will see it over here as shown over this yellow ball.

Now, if I provide a suitable light whose energy is actually related with the energy difference between the ground and the excited electronic level then this light can be absorbed. Actually 2 condition has to be satisfied for the light absorption - first the light energy has to be same as the difference in the energy between the excited state and the ground state of the molecule like in this case the incoming photon energy must be equal to the difference in the energy of this excited state and the ground state. Secondly, the transition moment integral should be non-zero.

So, in this particular course I am not going to discuss in detail about this transition moment integral as of the basic nature of this course and obviously, this a needs a little bit of knowledge on the quantum mechanics. Now here you see here according to this particular diagram I have excited this molecule from b equal to 0 of ground electronic level to b equal to 1, 2, 3, 0, 1, 2, 3, 4, b equal to 4 of the excited electronic level, it could be here, also it could be here also depending on something which you I will come after may be 5-10 minutes, but anyway. So, let I have excited this molecule from here to here right I have excited the molecule from here to here it means that then and also note here that I have written the excitation 10 to the power minus 15 seconds which we already have discussed in the last class that the time taken for absorption is about one femtosecond.

In this let me give you one example to comprehend this time what is one femtosecond, what is one femtosecond and how I will going to realize that word femtosecond that femtosecond? The number of femtosecond in one second that is what 10 to the power 15 10 to the power 15 femtoseconds consists in one second is that number is same as number of second present in 30 million years number of second present in 30 million years. So, you see that how small it is just this is to comprehend that number femtosecond anyway. So, let us come to our discussion. So, here we have excited this molecule right from or grounds to the excited state.

Now, if I draw if I show you the structure of the molecule this is the structure of the molecule and these are the molecular orbital like in previously what I have showed you that for atom there are different electronic orbitals of these atoms and the electron moves from one orbital to the another orbital that is for the atom, but for the molecule I have to talk about the molecular orbital. So, this is the different molecular orbital, this is HOMO highest occupied molecular orbital that is 2 electron is present over here and this is LUMO lowest unoccupied molecular orbital; that means, is (Refer Time: 05:26) inter orbital right.

Whenever I excite a system from ground state to the these excited state it means I took one electron from this HOMO and put it the LUMO, it could be HOMO to LUMO it could be HOMO minus 1 to LUMO plus 1 depending on the molecule to molecule depending on this transition whether some transitions are allowed some are not that depends on the transition moment integral, but I am not as I said I will not going to discuss all those finer details over here just to give you the overall idea about this floor basics fluorescence spectroscopy anyway. So, let us consider that this is ground state this is excited state; that means, one electron is taken from here and we will put it in the LUMO right. So, this is correspondingly this transition.

Now, I will going to have this LUMO plus 1 also and let us consider this LUMO plus one some looks something like this way this electronic cloud right in this molecular frame. Now if you put this electron from HOMO to LUMO plus 1 is also possible right HOMO to LUMO plus 1; that means, you are going to excite for this particular system from here to over here this electronic state. So, this is about this electronic transition in the molecule this is about the electronic transition in the molecule.

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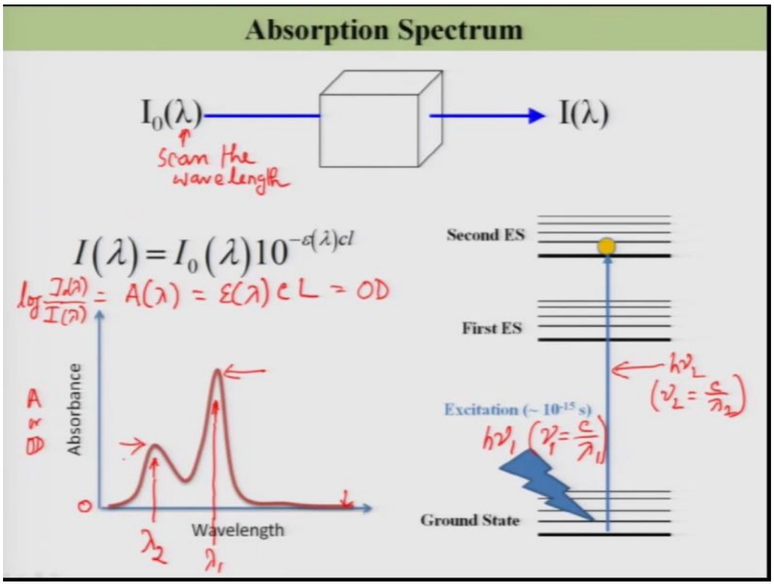

Absorption Spectrum

$$I(\lambda) = I_0(\lambda)10^{-\varepsilon(\lambda)cl}$$

$$\log\frac{I_0(\lambda)}{I(\lambda)} = A(\lambda) = \varepsilon(\lambda)cL = OD$$

Now, I want to show you that how will be going to measure the absorption spectra of a molecule, yes.

Student: (Refer Time: 07:03) HOMO to LUMO plus one is not allowed then (Refer Time: 07:07)

Yeah. So, it is a good question. Some transitions are allowed some are not as we know right. So, if one transition is not allowed let us say for example, this question is that if the HOMO to LUMO plus 1 transition is not allowed then I will not going to see it in the experimentally. So, then the next transition will be HOMO to LUMO plus 2 probably depends on the molecule if it is allowed then I will be able to see it means that when I use a light of a particular frequency let me tell you that frequency is nu 1 let us say for example, so; that means, different color to make it clear for you. So, in this particular case I have used a frequency nu 1. So, I promote the system from ground state to the first excited electronic state and when I used a frequency nu 2 then I promote this system from here to here right from here to here.

So, if you use a frequency in between in between from between nu one and nu 2 whose energy is. So, that you will go from here to here then that particular photon will not going to be absorbed by this molecule right. So, simply if I scan the frequency or if I scan the wavelength for this molecule I will going to see a different picks right. So, that is what we measure this absorption spectrum of the molecule. So, basically what will going to do I have to scan this wavelength? So, this is important I have to scan the wavelength right. So, the I 0 is coming it interacted with the sample and then it is passing through the sample depending on these electronic levels and the relationship between this energy of the incident radiation lambda that is corresponding to some energy h nu.

So, some lights will going to be absorbed by the system some light will not right and; obviously, this I is a function of lambda is equal to I 0 10 to the power epsilon which is also a function of lambda C L; that means, this is nothing, but a which is a function of lambda is equal to epsilon which is a function of lambda C and small l or capital L or C L and this is equal to log I 0 by I this both are; obviously, the function of lambda, just it is it not. So, the when I excite this when I scan this thing what I will get I will get somewhere there is a lot of absorption of the light. So, the value of I is much smaller than I 0, let us say over here the value of I is smaller than I 0 here is much much smaller than I 0 and in this case where it is 0 right here it is 0 then in this case the I and I 0 are same. So, log 1 equal to 0. So, I got this 0 over here.

So, the scan of wavelength and plotting it and when plotting the absorption a or OD, this also known as O D. So, a or optical density OD is nothing, but my absorption spectra now you see here the 2 different clearly I can see 2 different transition one transition is over here another transition is over here right if the corresponding wavelength is lambda one if this corresponding wavelength is lambda 2 then let us see this molecular energy diagram over here. So, when I shine this light this electron moves from one state to the other state right that must be corresponding to this lambda 1. So, here this light is h nu one and what is the relationship between nu one and lambda 1 nu is equal to C by lambda. So, nu 1 equal to C by lambda 1, so this particular transition is corresponding to this particular maxima in my absorption spectra now when I change the wavelength of the light to the shorter wavelength right here wavelength is larger here wavelength is shorter; shorter wavelength means the higher energy as you can see over here. So, I excite this system from here to here right. So, in this particular case this is h nu 2 where nu 2 is equal to C by lambda 2. So, I got this lambda 2 intensity. So, I got this lambda 2 intensity A. So, lambda 2 maxima in my absorption spectra is it not, in this case, the absorbance is more in this case absorbance is less it is related to this epsilon R and in consequence related to the absorption cross section of this molecule for these 2 different type of light.

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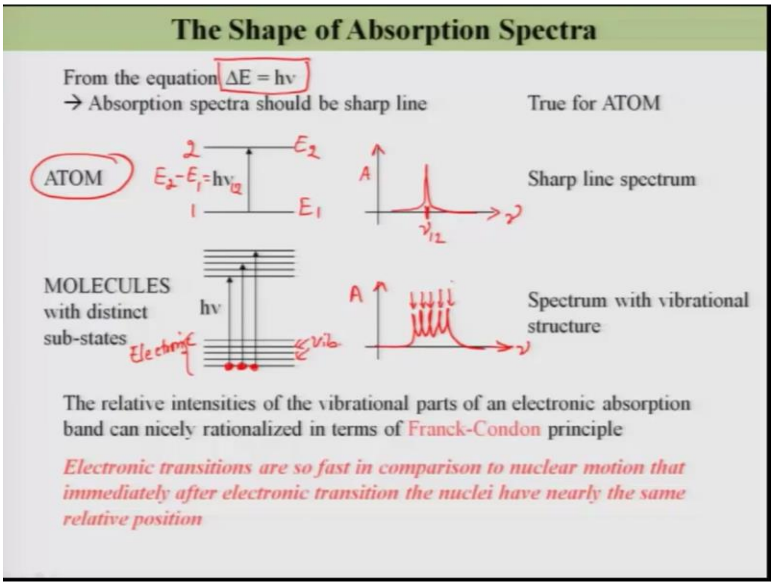

The Shape of Absorption Spectra

From the equation $\boxed{\Delta E = h\nu}$

→ Absorption spectra should be sharp line

True for ATOM

ATOM

Sharp line spectrum

MOLECULES with distinct sub-states

Spectrum with vibrational structure

The relative intensities of the vibrational parts of an electronic absorption band can nicely rationalized in terms of Franck-Condon principle

Electronic transitions are so fast in comparison to nuclear motion that immediately after electronic transition the nuclei have nearly the same relative position

So, now, with in connection with this let me discuss the shape of that absorption spectra right this is important. So, from this whatever I discussed you have seen that if the energy is first of all the transition has to be allowed which I have not discussed please note that. So, far allowed transitions which is defined by this transition moment integral let us say this transition moment integral according to that the transition is allowed then it has to satisfy that delta E is equal to h nu then only the transition will allowed again I have taken, this simple atomic system and here if you have this 2 level the level one and level 2 that there is no other level right there is no other level.

So, if this E 1 this is the energy E 1, E 2, sorry and this E 1 if E 2 minus E 1 equal to h nu then the system will go from level 1 to level 2 and in this case if you plot here what we want to plot let me let me plot nu or lambda whatever I want I can plot and in this case if I plot absorbance right A. So, what I will get I will get a sharp line like this right and this particular value is let me say let me write this 1 2 this value will be nu 1 2 is it not. But as molecules as I said that it consists of many levels although for the electronic these are the electronic this is and these are the different different vibrational although initially it stayed over here the molecules stayed over here, but it can go at any level and it can go any level if it is allowed transitions again I am telling that. So, it can go to the any level; that means, I will going to have a multiple transitions multiple lines just for this simple example I will going to have 1, 2, 3, 4, 5, total 5 lines over here. So, the spectrum will looks something like these.

Let me redraw this will looks something like this right again I am plotting this against nu and I am plotting this absorbance over here. So, shows the spectrum a in this case a spectrum is a sharp line, but in this case the spectrum with the vibrational structures.

Now, this relating intensity of these different lines right they are not same in actually. So, the relative intensity of this vibrational part of an electronic absorption band can easily be rationalized by the Franck Condon principle. So, what is Franck Condon principle Franck Condon principle is based on that what we have already calculated the time taken for absorption is 10 to the power minus 15 second very very small time right. So, the principal says the electronic transitions are. So, fast in comparison to the nuclear motion nuclear motions are slow relatively right it takes about the picosecond more than that for a nuclear motion that the immediately after electronic transition the nuclei have nearly the same relative position. So, nuclei does not move right during the electronic transitions.

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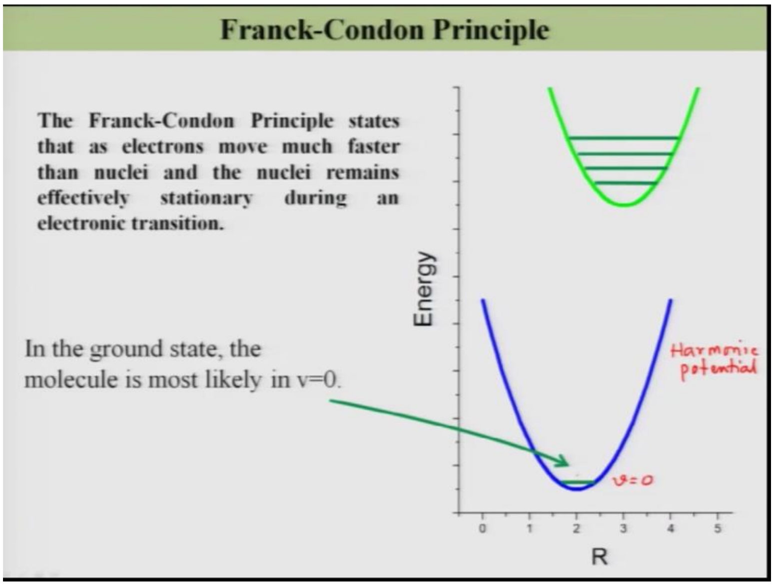

Franck-Condon Principle

The Franck-Condon Principle states that as electrons move much faster than nuclei and the nuclei remains effectively stationary during an electronic transition.

In the ground state, the molecule is most likely in v=0.

So, I have this particular diagram to explain you this Franck Condon principle right here this axis is my nuclear axis nuclear separation and I have taken a very simple harmonic potential over here. So, this is my harmonic potential just simple vibrational of diatomic molecule that is it. So, now, these are the different vibrational levels in this particular state and these state, these are 2 electronic levels of this molecule so obviously, as I said that in the ground state the molecule is most likely to stay in the v equal to 0 state like v equal to 0 level over here now when there is a transition from these state to the upper state right district to the upper state.

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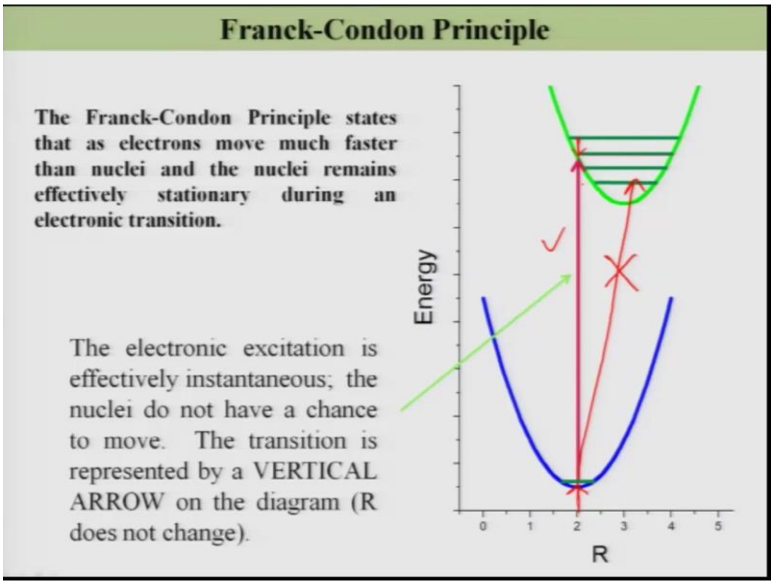

Franck-Condon Principle

The Franck-Condon Principle states that as electrons move much faster than nuclei and the nuclei remains effectively stationary during an electronic transition.

The electronic excitation is effectively instantaneous; the nuclei do not have a chance to move. The transition is represented by a VERTICAL ARROW on the diagram (R does not change).

As you as you can see over here right the nuclear position it is approximately is too over here that is remains same when you excite over here that R value does not change this R value does not change when you move from this level to the other level R value does not change right this is the Franck Condon principle right. So, I cannot draw this lines like this way it means the R value is changing during the transition, but Franck Condon principle says that the electronic transitions are ultra fast in nature is so fast that during transition the nuclei will remain as it is nuclei I will remain as it is, so, this is wrong. So, this transition is represented by a vertical arrow right vertical arrow not a slanted one not a slanted one.

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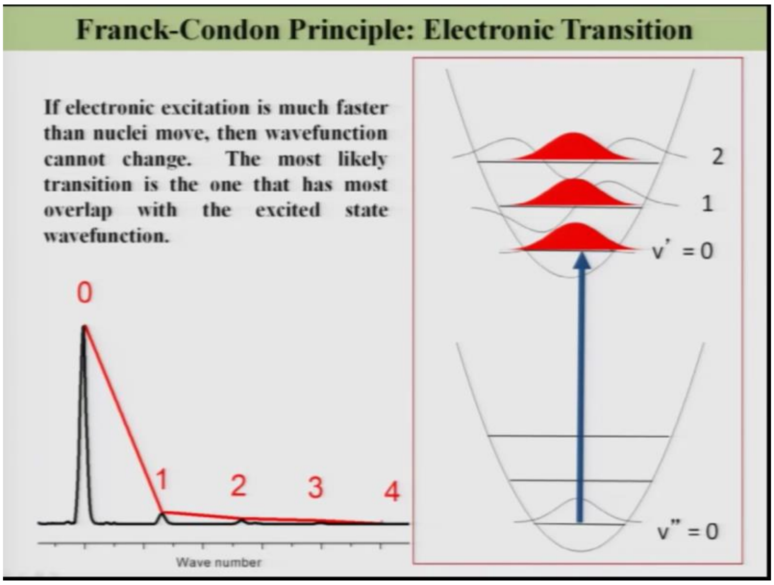

Franck-Condon Principle: Electronic Transition

If electronic excitation is much faster than nuclei move, then wavefunction cannot change. The most likely transition is the one that has most overlap with the excited state wavefunction.

So, this is not very difficult to understand. So, to make you understand I have drawn this 2 harmonic potential again this is in the ground state denoted as ground state this is the excited state. So, these are this wave function as you know these harmonic potential wave functions of v equal to 0 level looks like this and as I have only the molecules or system present inter v equal to 0 levels. So, I have just drawn only one a wave function over here, but in the excited state the system can go anywhere right anywhere. So, it can go from v equal to v prime double prime equal to 0 to v prime equal to 0 or 1 or 2 and so on and so forth. So, once excitation so this can go to here or here, so this a wave function I have just plotted over this 3 v value v prime equal to 0 v prime equal to 1 and v prime equal to 2.

So, now, you look at over here as the nuclei will not going to change its position during the transition right during the transition; that means, is wave function will remain same because these wave functions are nothing, but the nuclear wave function right this is. So, the wave function will remain same and the most likely transition will be the one that has most overlap with the excited state because then they have to be same they has to be same. So, from here to here when the system will go from here to here it looks that it reach that similar wave functions right whatever you are present in the ground state. So, the intensity of this transition will be much more compared to these or these so obviously, in this case what you can see is that the 0 0 transition which is denoted by here 0 0 transition.

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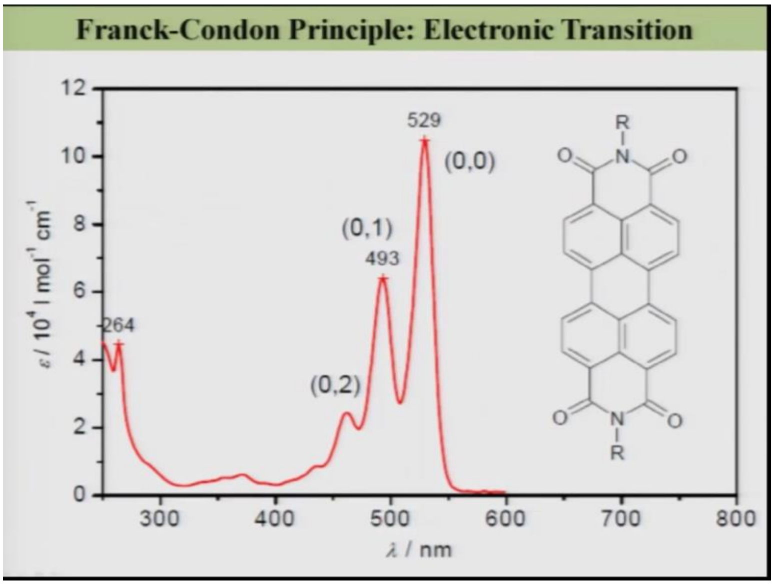

Franck-Condon Principle: Electronic Transition

It is strongly intense, but a; the 0 1 and 0 2 transitions are weak compared to the 0 0 transition. So, this is the typical measure absorption spectra of this molecule here you can see the clear observance of these vibrational features and this one this one this 5 twenty nine nanometer band is 0 0 and this is most intense band right and then the next band comes as 0 1 band then 0 2 band and so on and we have another over here which corresponds to the a s 0 2 a s 2; that means, ground to the second electronic excited state and so on.

So in this case the 0 0 transition was most allowed our transition because the position of this well this harmonic potential and the position of these harmonic potential they are almost similar; however, if they are shifted there is no guarantee that these 2 are exactly same.

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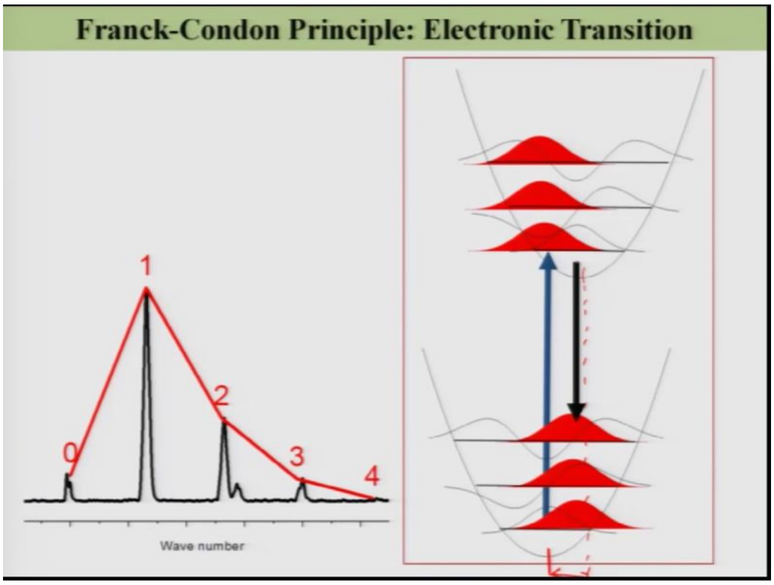

Franck-Condon Principle: Electronic Transition

If they are shifted like they are same here, but these they could be shifted also at this position now the minimum position here and this minimum position here they are not same right they are not same it is shifted. So, in this case right when I excite the system these particular excitation will see a different overlap compared to the earlier right what I have shown in this case you see this overlap is most intense over here because ultimately this is this wave function I have to make a square right. So, this has a most overlap in this case. So, the 0 1 transition is most intense transition. So, this is that relaxation which I will come later, right now I am not talking about this relaxation part.

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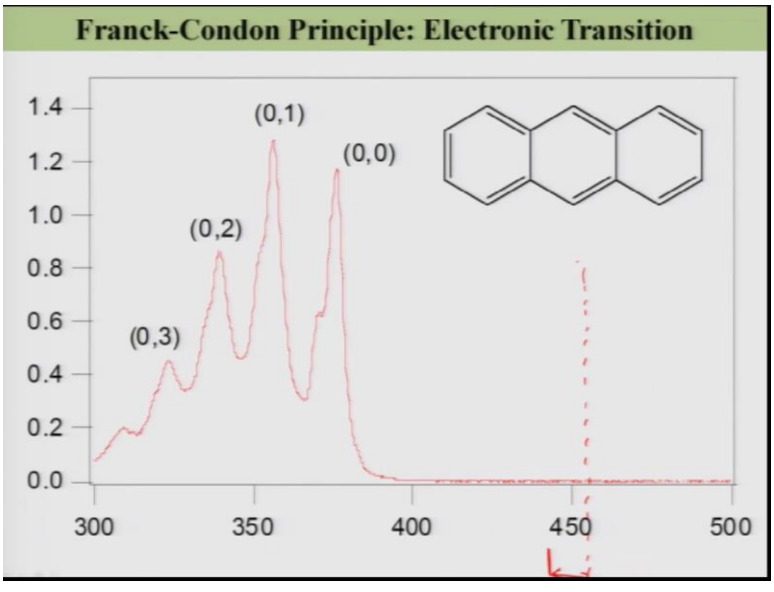

Franck-Condon Principle: Electronic Transition

So, this is the measured absorption spectra of another molecule this is the anthracene and as you can see here the 0 0 transition is less intense than 0 one transition which is a similar case like this and which is similar case like this.

So, now, let me show you a the somehow what is the instrumentation of this spectrophotometer well the spectrophotometer is an instrument which is used to measure which is used to measure the absorption spectra.

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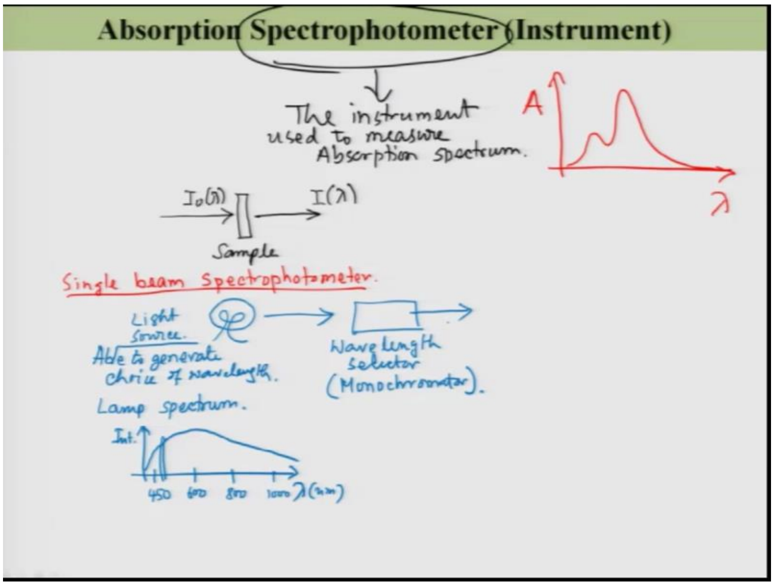

Absorption Spectrophotometer (Instrument)

The instrument used to measure Absorption spectrum.

Single beam Spectrophotometer.

Light source.

Able to generate choice of wavelength.

Wavelength selector (Monochromator).

Lamp spectrum.

And I have already explained you what is the absorption spectra absorption spectra is a plot of absorbance versus wavelength. So, it I showed you that some typical absorption looks something like this way; that means, I have to scan the wavelength and I have to measure the; I have to measure the a measuring a means measuring I right. So, so basically what I have to do I have to I have this I 0 I have my sample over here this is my sample after passing through come I right do not forget that this is wavelength dependent; that means, I have to somehow scan this wavelength.

So, this is a function of wavelength this is also a function of wavelength. So, I have to measure this I 0 and I how I will going to measure this I 0 and I. So, a first let me discuss the single beam spectrophotometer over here. So, this spectrophotometer again, again, again this term is the instrument used to measure absorption spectrum right. So, there are 2 varieties of this instrument the first one is beam the single beam spectrophotometer. So, in this instrument what we have; obviously, you have to generate the light of different wavelengths. So, I have a light source over here, which could be a simple tungsten filament lamp incandescent lamp which you see every day life depending on the choice of the wavelength right.

If I want the visible light from 450 nanometer to 800 900 100 nanometer then some normal lamp is good enough the tungsten lamp is good enough if you want the v then you need this special type of lamp because the transition able does not emit the UV light right forget about that. So, I need a lamp which can generate that choice of wavelength which can generate the choice of wavelength. So, light source able to generate choice of wavelength then I need something which is avail to select your desired wavelength right. Let us say this particular lamp can generate a wavelength of a wide region so for 450 nanometer to 1600 nanometer or something like that so this is the lamp spectra lamp spectrum, let us say it is like this wavelength versus intensity simply light intensity lets it is like this here it is my 450 nanometer here is my 600 nanometer here is my 800 nanometer here is my 1000 nanometer and so on so, but what you need a particular lambda.

So, you need some device which is which can used as a wavelength selector. So, I have a device I just name it as wavelength selector actually is known as monochromator that you have seen if you used to computer disk and you shine in white light like normal sunlight then you see; right so; that means, the white light is converted into different color that probably also you have seen in your high school. So, that prism right prism can convert the white light into is called corresponding colors. So, this is the similar type of device and there is a way that at your desire you can choose your color of light you said that in this device that I want 500 nanometer these device will only give you this 500 nanometer light. So, at part your choice this device will select your wavelength.

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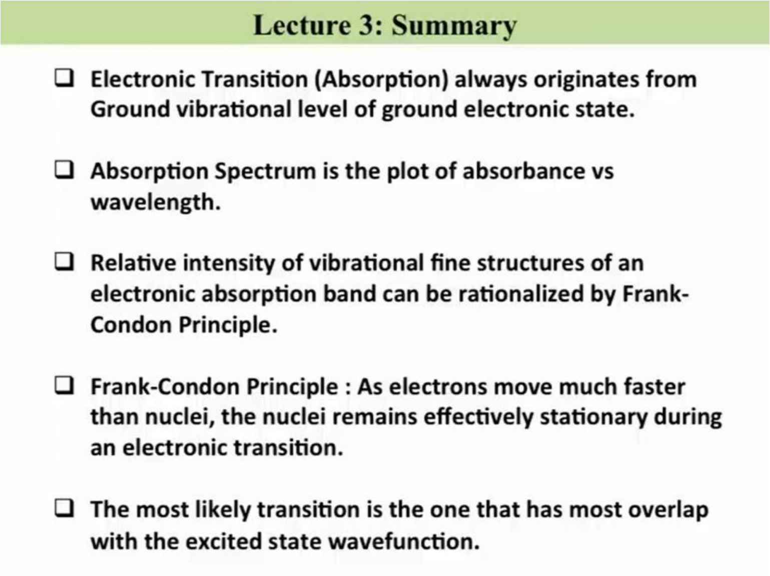

Lecture 3: Summary

- **Electronic Transition (Absorption) always originates from Ground vibrational level of ground electronic state.**
- **Absorption Spectrum is the plot of absorbance vs wavelength.**
- **Relative intensity of vibrational fine structures of an electronic absorption band can be rationalized by Frank-Condon Principle.**
- **Frank-Condon Principle : As electrons move much faster than nuclei, the nuclei remains effectively stationary during an electronic transition.**
- **The most likely transition is the one that has most overlap with the excited state wavefunction.**

So time is up. So, we will continue our discussion in the next class.

Thank you.