Optical Spectroscopy and Microscopy

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Module No # 05
Lecture No # 25
Fundamentals of optical measurements and instrumentation

Hello and welcome to this lecture series on optical spectroscopy and microscopy so far we had described the process of emission and resulting from absorption of the light by the matter. And then we looked at some of the properties of this emission process itself. Now I am going to take you or I am going to present to you in this class in this lecture a way of abstraction the energy representation in this molecule that is convenient and will help us to follow various different processes and possibly make predictions about the nature of the emission and that will be see okay.

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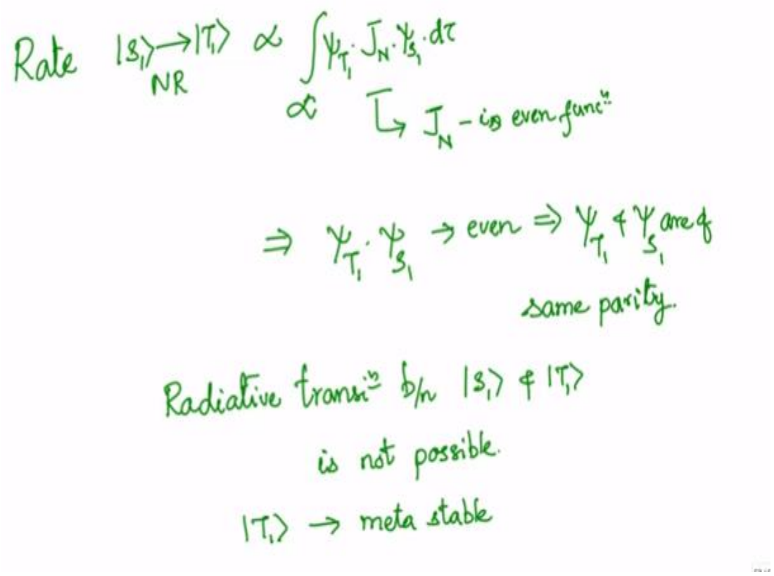

$$\text{Rate } |S_1\rangle \xrightarrow[NR]{} |T_1\rangle \propto \int \psi_{T_1} \cdot J_N \cdot \psi_{S_1} \cdot d\tau$$

$$\propto \quad \hookrightarrow J_N - \text{is even func}^{n}$$

$$\Rightarrow \psi_{T_1} \cdot \psi_{S_1} \rightarrow \text{even} \Rightarrow \psi_{T_1} \,\&\, \psi_{S_1} \text{ are of same parity.}$$

Radiative transi^{n} b/n $|S_1\rangle$ & $|T_1\rangle$ is not possible.

$|T_1\rangle \rightarrow$ meta stable

Nature here means what will be color of the light and relative to the excitation light and so on and so forth alright.

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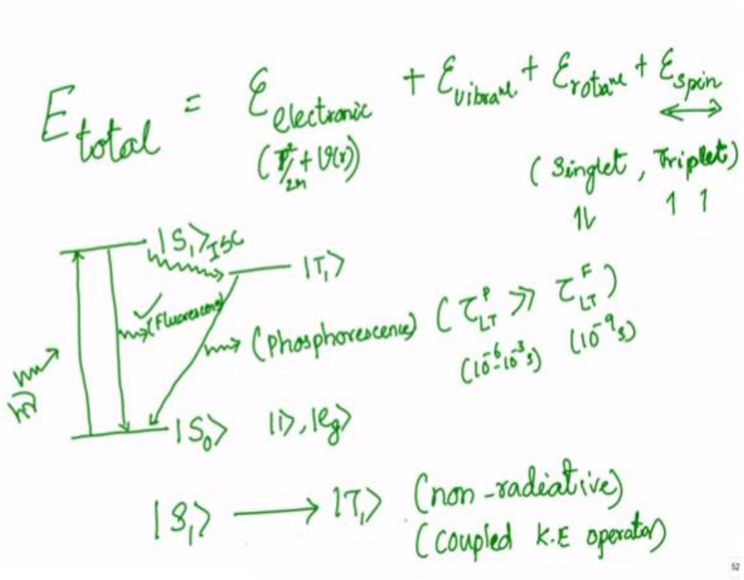

So now we have been drawing these; the energy level diagrams something like this with a line representing the energy of the electron mix state that we are actually talking about.

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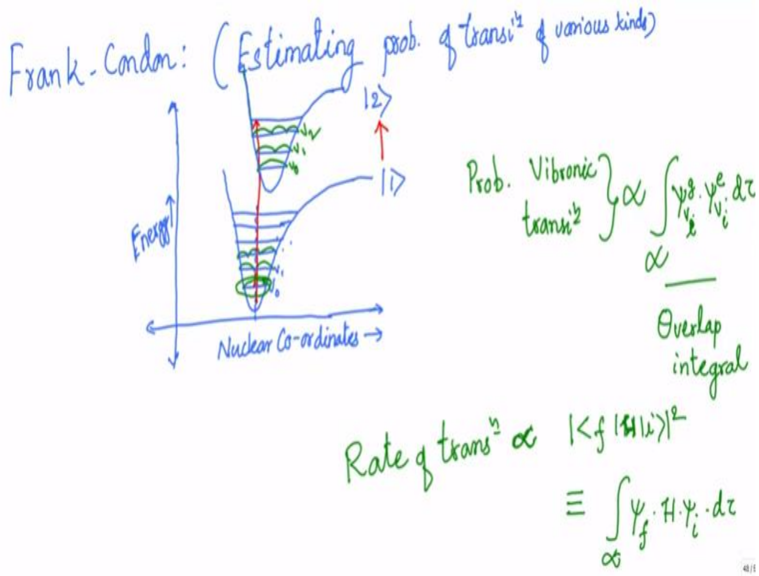

Or sometimes we have also been drawings where we have like this like in a Franck Condon things where we are talking about vibrational including the vibrational energy like this. You can imagine to if I were to precisely represents all the form of energy associated with the transitions that I am actually talking about in an energy level diagram and then trying to compute from or the possible combinations of the transition that we can actually see it will be quite intricate and pretty laborious process.

And it can be tedious to and pretty confusing and then in a diagram that is similar to this. So in order to make it easy and abstract.

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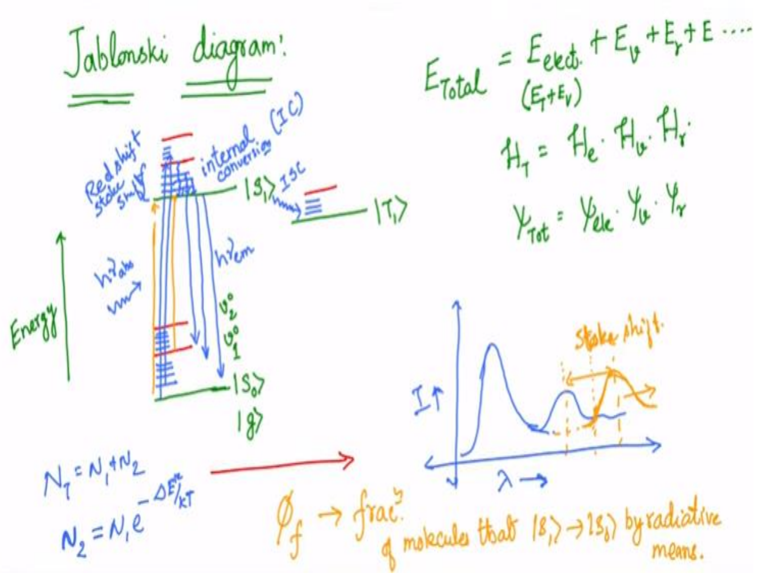

So we use in the field we use set of diagrams called as Jablonski diagrams this named after a polish scientist where we lead go the nuclear coordinate axis alright. So we do not make any explicit assumptions about which part of the I mean at which inter nuclear distance we are actually drawing this energy level diagram. Since thanks to Franck Condon principle we know that they are verticals. So let us the idea here is that let us concentrate only on the energy level themselves without having to worry about the nuclear coordinates here.

So in that scheme of things then we would represent an energy state with a straight line and the energy axis is still here. So clearly this we would have in here various lines representing different energy levels right you remember our E total right consist of E electronic which by itself in turn consist of E translational and E potential and then we have the E vibrational then we have E rotational and E is been so on and so forth okay.

So now corresponding the Hamiltonians would be H total times Hv times Hr and so forth and then the wave function is also similar to that. The assumptions here there can be treated independently alright that is the Born Oppenheimer approximation. So for each of this I mean will be talking about here dominantly the electronic and vibrational and rotational dominantly electronic and vibrational but little bit of rotational to and very rarely the different spin states except I will except from me mentioning how do we actually represents in the diagram the processes will be concentrating or the transition among the electronic virbronic vibrational as well as electronic.

So it is a called vibronic transitions so in such a scenario the electronic energies are larger corresponding to the visible light radiation range and we represent them by this sort of lengthy line followed by vibrational energy okay and okay we need to have should have drawn it vibrational energy actually and in between the vibrational and electronic or in between the vibrational themselves or the rotational energies okay.

So like that okay so now this is for the ground state okay so we could write it as and then singlet ground state where we can write it as S0 if you are going to talk about triplet state transition all that stuff otherwise it is simple to write this ground state I can get G and this is referred I mean that the first straight line is our vibrational state 1 of the ground electronic state okay. And this would be V20 and so on and so forth.

And here again the rotational states would corresponds to the ground vibrational as well as the electronic states and then we have in between this lines. Now this first singlet exciting state right that is the electronic state that is higher up you remember the corresponding to the potential energy diagram that is up. So that is drawn somehow like this which is another straight line because in this diagrams the states are represented by the arrow and the length of the arrow I mean thickness of the length necessarily I mean do not have any specific meaning though generally the longer the arrow the energy gaps between the corresponding states are larger okay.

So similar structure exist in the singlet excited state 2 so we could go ahead and draw out that it is a okay. So now where do we how do we represent the triplet state even though this axis perse does not necessarily mean the nuclear coordinates and here are any coordinates here we tend to use this separation in this axis to represents a different system of energy right. Remember I told about the inter system crossing and the process so there we talked about system of energy right.

So here singlet energy states and then the triplet energy states and so on and then since there is no ground triplets state we start with first state itself I mean it is for the triplets is T1. So the triplet state would fall somewhere lower than this but then to distinguish so we do this here. If you remember when I was actually talking about phosphorescence and then drawing out the picture I had drawn out in space and it was intentional is the is just not is just that it is this is more complete way of representing where you are taking into the rotational and vibrational states.

We will see in a minute why it is complete then we also have a corresponding, rotational and vibrational energy levels in the triplet state. Now in order for us to estimate where the absorption is going to happen we need to know where the most likely place the molecule will start. Now the electronic energy states are higher in energy followed by the vibrational which i said is lesser and the correspond to infrared region and the rotational ones are even less at the microwave region and if you actually estimate the population of various energy states higher energy states using Maxwell Boltzmann distribution right.

We have this expression we have seen this before which is N total is given by N1 + N2 and then the number of molecules in a state 2 is given by NI E to the power - delta E by Kt where delta E is the E12 / K2 where delta E12 is the energy separation between the levels E1 and E2 and N2 and N1 are the number of molecules in the state 2 and 1 okay. The ratio of N2 to N1 is given by this Boltzmann function I mean distribution function and it is scaled by Boltzmann constant K and the absolute temperature T.

So if you increase the temperature the populations to go up okay of the excited state. Now directly from this as you can see when the energy separation goes down you will see the populations of the higher energy states I mean the higher energy states starts to populate more. For general so as a result if you see the most of them would start from ground the electronic states that first year and very rarely you do see some amount of occupancy in the vibrational state of depends on the temperature.

And then of course in the rotational energies there are the high energy states are quite like nicely populated. So the transition the molecule can start from anywhere and then can make this straight transition to reach any of this places okay and the probability of reaching that place is given by our overlap integral please recollect it is given defined by the Franck Condon factor and overlap integral which is the integral of the wave function vibrational wave functions. So between the I mean start and the final states.

And so you would also see some of them starting from here may up here or may end up little up and so on and so forth. Depending on this you are going to see spectrum is basically a plot in air absorption intensity as a function of wave length. Now you would since they are distributed slightly at a different energy and then you would see different probability of excitation between this transitions.

You would tend to see transition going something around the resonance they do not drop to 0 but the around the resonance and just like (()) (15:07) they do not drop suddenly to 0 but they drop down little slowly even more slowly because of this molecular spread and all that stuff okay. So if you look at it is slightly coarser manner and then you can think of the energy statement in the spectrum being an integrated foam that happens more often in solution phase we will come to that why it is the case in the minute with the characteristics peak functions here.

So there is intensity or the rate of transition per se now the interesting point is now we would like to know where in here the fluorescence actually happens. If we are if often we in the lab you study solution we measure the fluorescence in biological systems in a solution phase when we do that in a solution phase what happens is that the you remember our kinetic energy of operator the kinetic energy operator has a energy corresponding to thermal energy and these transition infrared transitions and the rotational energy transitions since just the way they are populated here they are close to resonance of thermal energy.

As a result they can actually start from any of these high energy states and then come back to the ground vibrational and rotational state of the electronic excited state okay. So by releasing this energy in the form of heat to the solvent molecules that come and collide. So they transfer this energy so we call this as internal conversion the energy in terms of the I mean the energy of the system mean that is existing in term of the vibrational and rotational form has been transferred to the solvent molecule to increase its kinetic energy right.

This is often called it as IC and this is pretty similar to the kinetic energy term that we have actually worked out for phosphorescence. And similarly the molecule we have seen before can also undergo a transition excited to plus state we call that as inter system crossing and from here I mean from here then they come back to the ground state. Now let us see if they are coming down to the ground vibrational and rotational energy of the electronic excited state and from there when they are making the transition this can be radiative.

This is a I mean this energies are so much close to the resonance of the thermal energy the rate constant the you get here is very high as a result these processed are takes over I mean the conversion are very hardly I mean hardly ever you see a molecule in a solution phase starting from the higher energy vibrational rotational states. But then they all start from the ground electronic I mean ground vibrational rotational state of the electronically excited state.

Now here it as various different possibilities again determined by the Franck Condon factor now if you look at it and what you first thing you realize is that this amount of energy right is lost alright. So the light energy that you used to excite the molecule that is that the molecule observed right and is going to be higher than the light energy that you get out through emission okay or so to speak since the energy is coming down the wave length of the photon that are what you are getting out is going to be longer or to say it is this corresponds to a red shift in the color of the light radiation we are talking about.

Because they lose this energy and it is also called as the stoke shift now if you plot in the same graph the fluorescence naturally that is going to occur at the following the last the lowest energy absorption peak right. Because that is a this cannot be any lower because that is called as the this can be any lower. So, will come to that in a minute so and there is also a peculiar function right. Because you see the molecule as not moved much and then you can actually see that the probability of the transitions that they are happening from the ground vibrational rotational state of the electronic state to the ground state would tend to mimic that of the original transition up there.

Or in other words there is an I mean there is an principle called as a cautious principle or effect which says that if were to place a mirror at the excitation peak corresponding to this band origin or around this band origin. In the fluorescence would be symmetric about this peak okay remember this usually it you will see only one peak the reason being in a solution phase the reason being everybody collapses to this S1 state and the from the S1 state they come to I mean there is only one way you I mean one only place where you can come to which is the ground state.

And that energy corresponds to lower energy and then the radar part of the spectrum and the clearly you can see this corresponds to our stoke shift okay. And it is because of the stoke shift that the fluorescence per se becomes very useful and it has a tremendous instrumental I mean tremendous values in terms of and improvement insensitive in terms of when it comes to instrument making a spectrometer okay.

So if you want to detect so because of this stoke shift what happens is that if you want to detect these transitions then you want to separate the photons that are getting scattered or transmitted and then the photons that are actually emitted then the stoke shift is a very meaningful very useful way of actually separating out because they color is different but interestingly what you can actually do is that we can do this thought experiment where also you can do it in lab where the property of this fluorescence emission okay.

Unlike any other scattering process right when the photon is coming from the scattering process is a peculiar one which is you take this incident light radiation okay. If it really is an electronic absorption and then the emission then as I move this wave length across this peak right the fluorescence emission should not shift in the wavelength why? Because when I am actually moving my incident light radiation across along this wavelength what I am actually doing is I am increasing or decreasing the incident light radiations energy.

Which means either I am actually going to this or all the I mean energy something like this and higher when I go left or and lower when I go right so which mean all the time you are actually going to the higher electronically excited state from you are starting from there once you reach there the molecule as no history of how it reached there once it reaches there it reach stays there for nanosecond before it comes down spontaneously there is no coupling between the excitation and emission as the result the spectrum of the emission is a pretty unique for a given molecule and it is determined by the molecular properties of that molecule rather than anything else.

Unlike a scattering process wherein the scattered; photon will track the incident light photon to a good degree okay. So this is a very good way of determining whether you actually watching the fluorescence or some scattering and typically it happens in a lab when you take a solution and then put it into a spectrometer you not only see the fluorescence what you are actually seeing is that you are seeing all the photons that are coming into the detector and you will have photons that are coming because of the fluorescence and also you will have photons that are coming because of a scattering from the solution.

Because the if you are taking an aqueous solution down in a molecule present there is water and there is a good amount of Raman scattering that can happen in water and then we I mean and then the this scattered photons you will come in and they will track and the one way you know if it is a fluorescence or scattering is that by changing the excitation wavelength you watch if the peak of the emission or peak of your detection shifts.

If it shifts you know it is clearly Raman and of course if you know the molecule that you are studying then it is since it is a finger print of its molecular structure of the fluorescence. You can actually look at the fluorescence peak and then the nature of this fluorescence emission will identify what the molecule is and that is really the where the specificity comes from. Now second not only that the fluorescence originates from there but and it does not have I mean originates from here S1 and it does not have history about how it came to the excited state.

But the you can guarantee the fraction of the time that the molecule will go into a triplet state versus the ground when direct say S1 to S0 transition is guaranteed to that is we know the rate of transitions right we know the rate of transmissions are determined at the molecular level right a given a molecule I can go ahead and write down the matrix element between F1 and S0 and F1 and FT1. Now what it means is that if I have an ensemble of molecule then it is a probabilistic thing for a given molecule to go into this way or that way.

Or in other way since the probability of this transitions are fixed the branching ratio or to speak if I take a molecule and leave it here the number of times that it is going to go down route S1 to S0 versus S1 to T1 right that we call it as branching ratio because it branches in 2 different ways is fixed. So you can directly tell you tell if a given a molecule whether I mean what fraction of the time it actually comes down to the ground state by emitting a fluorescence photon.

And that we term as quantum efficiency of fluorescence or in general if we can generalize and then talk about quantum efficiency of any process for that matter in here what we are interested in this quantum efficiency of fluorescence it is typically are presented as Phi F. Now this tells you fraction of molecules okay I will formally define it in a next class but let us see notionally this is the idea here fraction of molecules that undergo the transition S1 to S0 by radiative means.

There are various ways one can actually determine this VF but the key factor is that it is also the molecular function right I mean that is the physical intuition that you can actually I was trying to motivate you and then talking about these are given by the rate process determined by the right processes and then once the molecule comes to the excited state it has to come down to the ground state by one way or the other.

So if you add up all the probabilities that should I mean add up all the probabilities that should add up to 1 and you know by mean you can normalize to that and estimate the branching ratio. So thus now we can actually see few properties we have already seen quite a few properties of fluorescence but let us say state and this is the point where we can actually state what are all the properties that can actually determine the fluorescence intensity.

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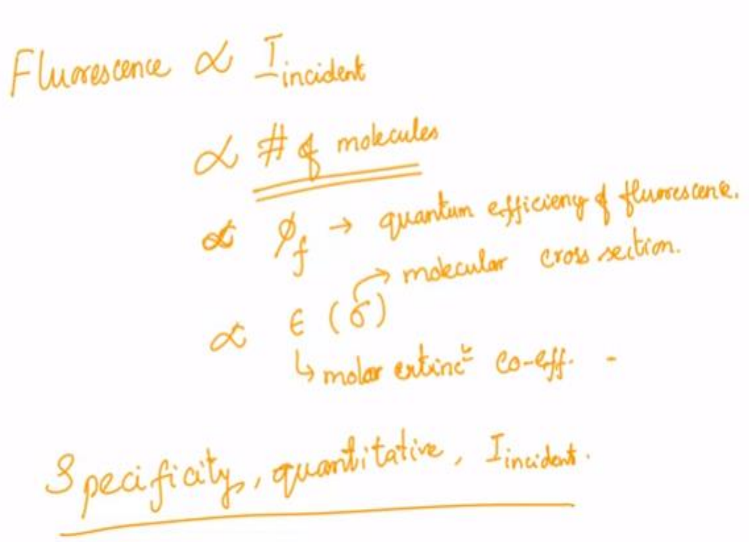

Fluorescence $\propto I_{incident}$

$\propto \underline{\underline{\# \text{ of molecules}}}$

$\propto \phi_f \rightarrow$ quantum efficiency of fluorescence.

→ molecular cross section.

$\propto \epsilon \; (\sigma)$

↳ molar extinc^{n} co-eff.

$\underline{\text{Specificity, quantitative, } I_{incident}.}$

So florescence is directly proportional to intensity of the incident rays incident light right we have more intensity more number of molecules go to the excited state. So clearly you have larger number written back in the sense the probability of transition is fixed. So if we increase the number of the exited state the number that comes back down through florescence have to go up. So it is directly proportional to the intensity of the incident photons it is directly proportional to the number of molecules themselves right.

See this is a molecular property so if I increase the number the number of molecules now it should scale the florescence scale up and it does a scale up and of course it is proportional to a quantum efficiency of florescence higher the florescence a quantum efficiency larger is your florescence. So the and then you can increase the incident light no matter however you increase it the molecule as certain probability of interacting with it and then going to be exciting state and that we know is determine by molar extension coefficient epsilon or in this course or in in general we call it as sigma the cross section.

So this is sorry molar extension coefficient or molecular cross section and this I had told you that the quantum efficiency. Thus you can see why I mean from here we can actually see the florescence can be extremely useful one it is very specific I mean that the spectrum of florescence is specific for a given molecule. So it is a it is really the spectra signature is really an identity for a molecule so it is highly specific one specificity.

Second it is very quantitative because you see here it is directly proportional to the number of molecules. So it directly reports the number of florescence molecule that you are actually probing okay. So and then you can modulate that by modulating the incident intensity of the light so because of all of this properties are it is a very it turns out to be the very useful in for studying many of the biological systems or for that matter any soft condensed matter properties.

In a next class we will deviate a little bit from florescence and go into generation of light itself and what are all the sources that we know off and actually not sources that we know I mean how can we generate lights of a particular with a particular property and this is become handy because when you want to eventually if you want to actually move on to the fact of using these kind of light sources to build up a microscope or a spectrometer photometer.

And if you need to know that and we need to know how do we actually even can I mean what is the working principles of the light sources that are being used here. So we were going to look into that the light sources and their governing principle okay I will see in the next class.