Optical Spectroscopy and Microscopy

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Module No # 05
Lecture No # 22
Fundamentals of optical measurements and instrumentation

Hello and welcome to the course on spectroscopy and microscopy optical spectroscopy and microscopy lecture series. So far we have seen the description of light matter interaction and then we went ahead and looked at a property of the fluorescence submission of anisotropy. And then said what is the maximum anisotropy that you can actually get when you freeze the entire solution just like that and then come in with the polarized light and then excited see what is the maximum that you can get.

And if you start with solution random I mean a solution where the probability of if you find in the molecule in there is no orientation dependence then we said that we can go ahead and estimate that and that turns out to be 0.4 alright. There we made an important assumption right the important assumption being that the transition dipolar moment or the absorption transition dipole moment okay is oriented same as that of the emission transition dipole moment.

Again the transition dipole moment is basically the orientation of the electromagnetic interaction with that of the states of the electronic states we write it as it comes directly from our TDBT.

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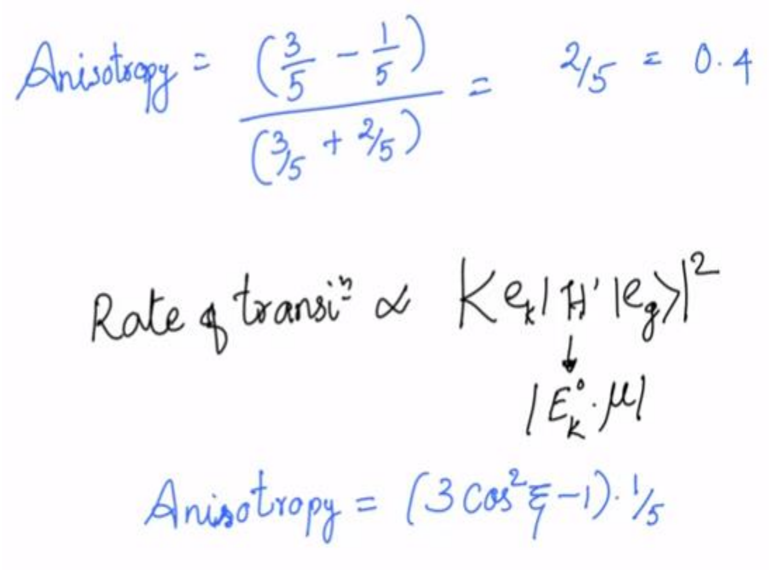

$$\text{Anisotropy} = \frac{\left(\frac{3}{5} - \frac{1}{5}\right)}{\left(3/5 + 2/5\right)} = 2/5 = 0.4$$

$$\text{Rate of transi}^{n} \propto \left|\langle e_k | H' | e_g \rangle\right|^2$$

$$\downarrow$$

$$|E_k^{\circ} \cdot \mu|$$

$$\text{Anisotropy} = (3\cos^2\xi - 1)\cdot 1/5$$

So the we write the rate of transition as modulus I think ek (()) 02:39) eg square okay. Or in general we can actually we written as perturbation I think square and the; what we did was we went ahead and replaced this; a perturbation Hamiltonian with the electric field vector and then if you treating the light in terms of semi electromagnetic radiation. However I mean a symptoms of a wave but you can also do that in a (()) (03:22) where we talked about this containing product ek naught times a dipole moment because of the changes in the charge distribution that the molecular encounter because of the transition alright.

So now so this is basically what gives rise to this is what gives rise to your orientation dependence and this dependence need not be having same orientation for absorption and emission. If the transition so the dipole moment created for absorption and the emission are not oriented in the same direction then the anisotropy that we have estimated previously undergo I mean has to be accounted for because what we have is that in going from can we have the in going from the expression where we multiply the probability of excitation with the fraction of the molecule and this is essentially selection due to excitation right.

Preferential selection due to excitation preferential excitation due to the orientation dependence that is the considering only the excitation part alright. So when we when from here to here where we are actually estimating the fluorescent intensity what we are actually doing is that we are assuming that the suppose the molecule. Now in this left hand side graph the molecule that is oriented along this red line that as I have drawn before where to emit fluorescent photon that emission probability is also of the same direction.

But the not be the case I mean it can actually change suppose if we were to assume that I am going to take a different color so if I do that emission dipole we to be of some angle okay let us call it as zeta where okay. So now if there is an angle like that of zeta associated with this emission right this is absorption and Mu emission now clearly this will be 1, 2 okay. So now what is the going to happen is we are going to so the emitter light but then you are actually putting your polarizer along your dark this green line or the blue line.

So this we are going to call it as I perpendicular or I mean to change back to that this we are going to call it as I perpendicular or I parallel right. So now I parallel right so now the now we have to estimate the account for the fact that the fluorescent photons that are being emitted from theta plus zeta from the polarization of from the x axis what is the probability that will make it through either this or either the I parallel polarizer or the I perpendicular polarizer.

So if we assume the zeta to be 0 then the probability that the photon will excite the molecule would be exactly equivalent to the probability that the florescent photon emitted at that angle will be deducted by the I parallel and that is why we are just simply multiplied we just took that Cos square term and we multiplied by another Cos square so we got that Cos to the power 4 and the same way we have done that and for the perpendicular we took that sin component I mean it Cos square is because of the excitation and then the sin square is because of the emission because you are looking at the 1 you are looking at the polarizer that is perpendicular to the excited emission.

However this will not be the same when we have the angle zeta that had mentioned then we have to modify this sin square and Cos square terms if you do that and then arrange and do all of that. What we will see is that the anisotropy goes as vector turned out to be 1 over 4 right that expression it goes as 3 Cos square zeta -1 times 2/5. So it is going to go down from 0.4 alright it is going to go down to the point where if the zeta is actually 90 degrees right with the absorption and emission dipoles are 90 degrees to each other or this is 1 / 5.

So now you can see when the angle zeta when it is the angle between the emission and the excitation dipole moment when it is going to 0 then what you have is 3-1 2/5 the 0.4 but for as the angle increases right the Cos square term comes down from 1 as a result this number decreases the product 3 times the Cos square zeta decreases thereby reducing the isotropy to the point where when it is 90 degrees right the excitation and emission are 90 degrees what you have is Cos 90 and this whole term becoming 0 and you end up having negative anisotropy so to speak all right at interesting notion.

But I do not think we will get into that at all here but just keep in mind when there is a difference between this 2 transitions dipole moments there are some interesting effects that can occur that it can lead to okay. All right now good so the second part I start we started of talking about anisotropy and describing this stating that the solution that we are going to consider is that in a glassy state what is that glassy state mean really.

The glassy state really means if you are able to measure this isotropy before any rotational movement had actually taken place okay. So if you were able to have a very high time resolution and you are able to actually look at very high time resolution compared to the rotational motion of molecules if you are following them then if you can actually follow the measure the fluorescence before the rotational motion what you get is this value of 0.4.

So as you go in a sorry excite the molecule and then you watch as a function of time then what is going to happen is that this anisotropy is going to evolve as a function of time it starts because they do not that the preference does not necessarily stay there I mean just they orient in all direction and then they become finally become equal to 0 alright. So this is dk of such anisotropy can give you information about how the individual molecule how fast the individual molecules are actually rotating.

And this is a exactly how the anisotropy is used as a biophysical tool to follow the rotational motion and thing major things that contribute for rotational motion how are they changing in responds to various perturbations from outside it could be reacting with another molecule as a result its increasing its size then the ability to rotate I mean the speed at which it rotate will be changed or it can actually bind to a different molecule where in the ability to rotate itself it is restricted okay.

So any of this you can any of such things you can actually follow it using this wonderful method or very simple but it gives it extremely powerful alright. So that is about anisotropy now let us look back at the fluorescent itself.

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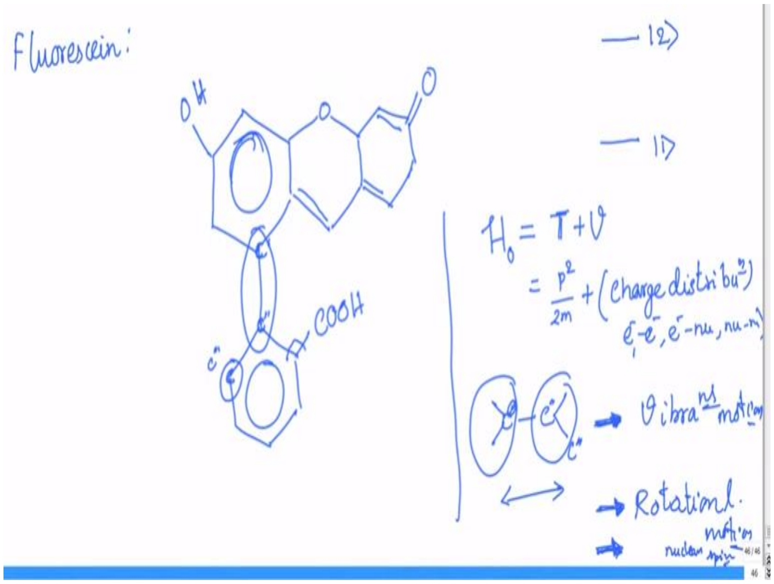

$$H_0 = T + V = \frac{p^2}{2m} + (\text{Charge distribu}^n)$$
$$(e^--e^-, e^--nu, nu-nu)$$

Now all this while what I have been talking about is fluoresces that we have stated originating from at 2 level atom right. Now in reality the fluorophores are pretty complex and they are necessarily atoms for example if you were to draw a structure of a fluorescein right a fluorescein is a very common molecule and it has structure somewhat similar to this I mean it has a structure something like this.

As you can see this has multiple atoms and each connecting to each other through multiple bonds as a result when we talk about at the energy levels here we have a quite a complex set of energies that we need to talk about this is just an example but there are when typically we are talking about a molecule they are a multi atomic multi nuclear molecules. So naturally when you are looking at multi atomic multi nuclear molecules there are energies apart from the electronic energies that comes into play.

So what are this energies it is a first list them out and before listing them even let us look at what you mean by an electronic energy right. We got this electronic energy states by considering a Hamiltonian and the Hamiltonian H naught is the unperturbed Hamiltonian we said and these are the solutions of the (()) (14:49) equation taking the Hamiltonian the unperturbed Hamiltonian. When you are constructing an unperturbed Hamiltonian H naught what you are using it is an addition of linear sum of the kinetic energy operator and the potential energy operator.

Kinetic energy operator captures the translation motion right this motion of the center of mass of the system which has we can actually write it as p square by 2m the momentum by the mass and the potential energy operator takes into account that charge distribution okay. Now what do we mean by that the energy associated with the charge distribution is essentially the columbic force that we are talking about and there are variety of potential energy that come into picture because that is multiple atoms multiple nuclear electrons that we are dealing with.

So you are talking about things like electron-electron repulsion electron nuclear or proton attraction nuclear-nuclear repulsion and so on alright. So as the number of atoms increase this combination right this is for the atom number 1 1 mean the electron 1 of the atom 1 and the electron 2 of the atom 1 so on and so forth you can enumerate and you can see there are a complex set of potential energy terms that I mean that we need to put into picture and that is how we estimate the electronic energy states available for this molecule alright.

So that is good wonderful we know what goes into estimating the electronic energy states but apart from this what we have not accounted for or energies because of the relative motion of the nuclei between among themselves. Like for example it could be a vibrational motion where if you are taking up any part of the molecule let us say for example across a bond something like this then we are having the carbon atom here and then another carbon atom here right.

So now I am going call that C prime C double prime so what you have is this 2 entities right entity number 1 and entity number 2 can have vibrational motions which are along this access where they can compress and expand to say 1 and like this there are multitudes of I mean multiple possible vibrational modes and they can have complex things little more complex in when we include the third atom let us C prime and C double prime and then another carbon here so let us call that as C triple prime.

So now with respect to C double prime now the C triple prime and C prime can actually move with respect to this like bending motions and stuff like that. So there are different modes of vibration that is possible and all of these modes of vibration where the different parts of the molecules are undergoing an oscillatory motion with respect to the center of sum center inside the molecule and the center of mass itself is not we are not movement of that itself is not considering that we are considering this translation motion.

We call that as vibrational motion like this you can also think of a rotational motion where as long as there is a degree of freedom for rotation again the same degree of freedom has to exist for vibration you can think of a degree of freedom for rotation and if the molecule will rotate about some internal reference frame it could typically they involve at inter atomic bonds and you have this as rotational motion.

And apart if you want to be complete than apart from this you can also think of having to worry about or having to account for energies associated with spin right I mean there are I mean we are taking we are listing out the motion so that the idea here is that there is a corresponding Hamiltonian which is giving which we would deal with to account for a corresponding energy that we are dealing with. So in that spirit you also need to think about spin I mean nuclear spin the energy due to nuclear spin okay.

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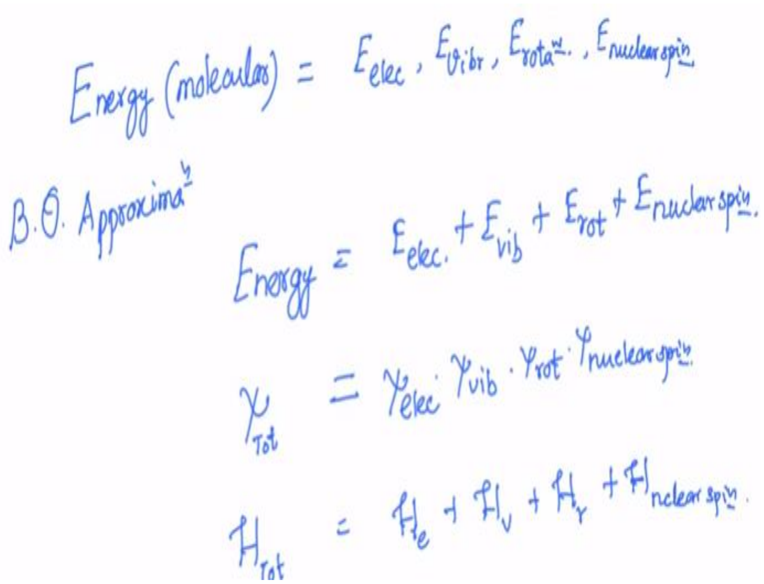

$$\text{Energy (molecular)} = E_{elec}, E_{vibr}, E_{rotat^{n}}, E_{nuclear\,spin}$$

B.O. Approxima^{n}

$$\text{Energy} = E_{elec.} + E_{vib} + E_{rot} + E_{nuclear\,spin}.$$

$$\psi_{Tot} = \psi_{elec} \cdot \psi_{vib} \cdot \psi_{rot} \cdot \psi_{nuclear\,spin}.$$

$$H_{Tot} = H_{e} + H_{v} + H_{r} + H_{nclear\,spin}.$$

Now in general when we write then the energy molecular energy here when we are talking about as multiple components the E electronics, E vibrational, E rotational and E nuclear spin. So to account for all of them simultaneously and estimate the energies of the system becomes hot so max bond and Franck Oppenheimer came up with the approximation of Oppenheimer approximation wherein they said if you write down the way function.

I mean you can actually treat this energies independently essentially they said you can treat the changes in this energy or you can treat these energies independently you treat these motions in independently alright. And what it amounts to is that we can actually write this energy the molecular energy total energy as E electronics + E vibrational + E rotational and + E nuclear spin okay.

So this would mean when you are writing the wave function or the state vector that we talked about of (()) (22:40) actually write as E electronic because they are since they are independent you can actually write them as a product of a simple product of the corresponding ray functions the electronic means I electronic psy vibrational and psy rotational of course and nuclear spin. See that is an approximation right I mean Borne and Oppenheimer said that you can actually consider them independently when you say this you can consider them independently which means they can linearly submit I mean that is the idea here.

So if you do that then the psy total can be written something like this which would allow us to write the; I mean since the energies are linearly additive then corresponding the Hamiltonian's alright can also be written down nicely as a sum okay. This as an important bearing when it comes to spectroscopy and determining what happens to the transitions in complex molecules such as fluorescence okay.

Now when we look at fluorescence or these complex molecules the way they the Borne Oppenheimer approximation comes into picture is in estimating or helping us to estimate how to deal with the transitions okay. So Borne and Oppenheimer said that you can actually separate them like this.

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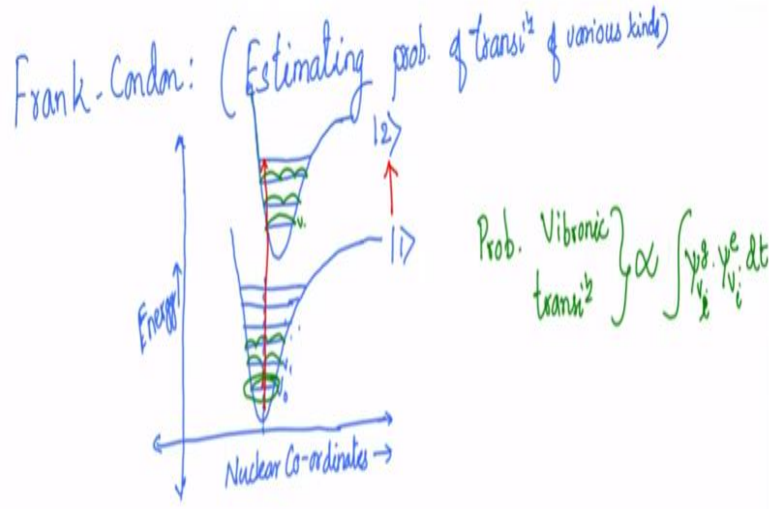

Frank-Condon: (Estimating prob. of transi^n of various kinds)

Prob. Vibronic transi^n $\Big\} \propto \int \psi^{g}_{v_i} \cdot \psi^{e}_{v_i} \, dt$

Now Franck are another set of scientist right Franck and Condon okay so they formulated this principle though independently but initially Franck put forward this explaining the photochemical reactions but then later generalized to released by Condon for known as Franck Condon principle for estimating the probability of transitions various transitions okay that can happen in a molecule like fluorescence and complex molecule right and transitions of various kinds right.

Various kinds here I mean the electronic vibrational rotational and nuclear spin so now what they state is that if you were to actually draw out the energy as a function of the nuclear coordinates okay. Now I am going to state this because I mean I am going to derive this because elementary quantum mechanical course would actually would try to do would have would do this. So for our practical purposes you can actually take it as a statement.

So if you were to draw the energy as a function of nuclear coordinates okay what we mean by nuclear coordinates here? It could be simple spatial location if you are talking in terms of vibrational motion so it take to nuclei in the complex molecule like our C prime and C double prime and I am going to represent the distance between them in space and that would be my one of the nuclear coordinates or I can take C prime C double prime and triple prime.

And when I am talking about the blending notion then I can I need to represent the distance between the C prime and C triple prime. So or so is the generic term for representing or to capturing the motions intra molecular motions in a system and so when we are actually talking about vibrational and electronic energies is very convenient to have this. Now what we are if you plot that we know that the potential energy diagram the energy diagram of course it is negative energy so we what we see is that it has a characteristics shape and because of the vibrational motions so we are talking about a energy here alright.

So when you quantize this these lines represents the various vibrational states V0, V1 so on and so forth basically if you were to so that is the mean position of the bond and when you have to stretch or compress what happens to energy that is what we are plotting out here and it turns out that these are happening in quantize steps and a harmonic oscillator analysis in a quantum mechanical class could have given you this kind of an graph.

This is good now a same this same analysis can be done for a molecule that is existing in a ground electronic state as well as a molecule that is existing in a excited state electronically excited state. Now you can represent both of them in the same graph so when you do that what you so to be general so we can actually think of representing like this drawing out this lines for the electronically excited state right I mean the square and this is our state 1 and the state 2.

Now the question that we are asking is when you say that the molecule is going from the ground state to the excited state that is this transition I am going to change the color to represent the transition so that we can capture this transition. What is happened what exactly is happening to the vibrational states okay. So Franck and Condon’s they said in this diagram in this energy level diagrams the transitions electronic transitions are perpendicular to the nuclear coordinate axis or in other words the electronic transition happens so fast that they really do not give time for the nucleus to move around much.

If you were to represent that in this diagram that would mean the transition from the ground state to the excited state are going parallel to the energy axis or straight perpendicular to the nuclear coordinate axis okay. Now what is it tell about the probability of such transition so now if you look at the wave function corresponding to this vibrational levels so they take these peculiar shapes okay so we can actually draw out the wave functions.

So they would have a structure something like this actually rather than even a wave function we can actually ask the amplitude square of the wave function which represents the probability of you finding a molecule with the in that interatomic distance that we are actually talking about and then the energy of V0 vibrational V0. So here you see that you will see that molecules with vibrational energy V0 will most likely exist in the equilibrium position while with energy V1 would have a distribution something like this and so on and hence so forth.

So we know this happening okay so now we can draw out this probability distributions for the ground as well as for the excited state. Now since it is going to be perpendicular my drawing is not quite the lines are not straight but imagine if it were to be perpendicular alright. So let us try to redraw that so I am going to consider only few states here so if you try to redraw that and then take represent the transition.

So a vertical line would go something like that the ground state so on and hence so forth. Now what you can actually do is what they say is that in order to estimate the probability all you have to do is you take the ground vibrational probability description this is highly likely to be found here and then that if you excite electronically they can reach to this states given by since the however when the molecule comes to this state the chance that the molecule will find you will find the molecule at this inter nuclear distance with an electronically excited state with the ground with the vibrational energy V0 is low.

This transition probability that is starting from V0 of the ground state to V0 of the excited state is going to be low and the way I have drawn you are going to be highly likely to see the molecule when it goes to the excited state to reach V3 okay. Or in other words they say the probability of a vibronic right vibrational and electronic so vibronic transition is directly proportional to the overlap between the ground and the excited.

So in general it is let call it excited vibrational states okay now if the overlap is 5 this integral is going to be high so you have a higher probability of this seeing this vibronic transition. So since there is a higher probability of seeing this vibronic transition the intensity corresponding to the energy will be high in our spectrum alright. So this thus in combination I mean Franck Condon principle in combination with our transition dipole moment allows us to predict which of the intensities will be you will be able to see and which you will not be able to see in general.

So in the next class what we will see is I mean you can actually go ahead and then you starting from Born Oppenheimer approximation how we can actually show if you make the assumption that the transitions are purely I mean when there is electronic transition can say that the nuclear do not move there is no nuclear motion that is happening they are not coupling the nuclear transition nuclear change in the nuclear position then you can actually derive Franck Condon principle from that it is a direct consequence of Born Oppenheimer approximation.

I think we will see that as an addendum to the course an addendum in the addendum lectures but what we need to know is the not necessarily the details of how we get the Franck Condon principle but the principle itself that is and the fact that the principle allow you to predict the intensity of the fluorescent given transition fluorescent transition or an absorption transition. So and as a result what it will allow us to do is we allow us to write in a more general form at different notation or a different way of following various thing that can happen in this absorption and emission phenomena in general okay. We will see that in the next lecture alright.