Basics of Fluorescence Spectroscopy
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Lecture – 08

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Welcome to the 8th Lecture in this series. In the last class what we were discussing about some properties of fluorescence.

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So, I discussed about Kasha's rule, here we said that wherever you going to excite the molecule the emission will come from the lowest vibrational level of the first (Refer Time: 00:43) state. That means the second one will come automatically that emission will not going to depends on the excitation wavelength. So, the second one is that no excitation wavelength dependence of fluorescence spectra.

The third one what we have seen is that emission will always be at the longer wavelength than the excitation or absorption. So, emission is at longer wavelength than absorption, here obviously I am referring to the maxima. The point number 4; the property number 4 is that excitation spectra is same as the absorption spectra. So, excitation spectrum is same as the absorption spectrum. And the property number 5 is the absorption or excitation spectra have a mirror image relationship with the emission spectra. Or I can write the emission spectra spectrum is mirror image of the absorption or excitation; is they are same, right, spectrum. So, and this emission is at longer wavelength than the absorption this is nothing but my stokes shift. So, here is my stokes shift. So, these are the 5 properties of the fluorescence.

And now today what I will going to discuss is 4 different parameters of fluorescence. So, fluorescence parameters: so the number one is being to be the fluorescence intensity. So, here is my fluorescence intensity. Generally we are denoted by either I or F whatever you want you can use it: so either I or a general notation of this. But before continuing this let me tell you clearly that these set of properties of the fluorescence are general property and there are several exception on these some molecules they do not emit from the lowest vibrational level of the first simulated system instead it is emitting from the second electronic state and so on and so forth.

So, there are some many many exceptions, but these are the general rules. What I said that 5 different properties of the fluorescence. So, the first parameter is fluorescence intensity as I said and it is denoted by I or F. So, this fluorescence intensity is nothing but the intensity in the fluorescence spectra or emission spectra; that I have showed you. So, here is my intensity versus wavelength plot and this is my emission spectra; let us say for a particular molecule in the particular solvent. So, you can refer either to the intensity of the maxima. So, I can write intensity at lambda max, I can write it like this way or I can integrate the whole part and I can say that the intensity of the area; I can also say like that.

So, the area is also intensity, so this is the area. So, this is one of the property, some molecule shows a strong intense emission, some molecule shows a weak emissions so on and so forth. However, you automatically you can understand that if the number of molecule increase the fluorescence intensity increase. So, this fluorescence intensity is a function of the concentration; so this is the function of concentration. If the excitation lighting use to excite the sample is very high; that means the photon flux is very high. So, depending on that absorption cross section more molecule you are going to absorb this light right photons. So, more molecule will go into the excited state. So, in that case more fluorescence will come from the sample. That means, this fluorescence intensity is also a function of excitation light intensity; excitation light intensity.

And obviously, this is also depends on the molecule to molecule. So, the molecular property is also involved in this fluorescence intensity. So, also invert the molecular property. So, you see one, two, three, that means this fluorescence intensity is not a very useful parameter to deal with, because it varies with concentration it varies with excitation light intensity and obviously this is a property of the molecular itself and so. So people generally do not use this fluorescence intensity to characterize the fluorescence property of a molecule instead people use this fluorescence quantum yield, generally denoted by this symbol phi f.

So, this fluorescence quantum yield is defined as rate of decomposition of the excited state by emission of radiation divided by rate of absorption or rate of creation of the excited state. So, this phi f is defined by rate of decomposition of the excited state which involves emission of photon divided by rate of creation of the excited state which is nothing but the rate of absorption. So, this is rate of absorption. So, phi f is defined like this.

Let me explain it by taking a simplified Jablonski diagram. In the last class I showed you the Jablonski diagram, so now let me show a much more simplified version of this Jablonski diagram. So, I have this ground electronic state over here this is my S 0 state and this is my first electronic excited state it is this is my S 1. I have some sort of vibrational structures over here.

Now, I excite this system from here to here then the system will relax back to the ground state, so this is my absorption of radiation. And eventually the system will come back

from the high vibrational level of the first singlet excited state to the lowest vibrational level of the first singlet excited state those are the vibrational relaxation. I can give some rate constant to it; let us say I am giving these as k v r let constant of vibrational relaxation, then the system will come from this state to the ground state by emission of photon. That means, this corresponds to the k r or there may be some other pathway that the system will come from the excited state to the ground state without emission of radiation that is the mind or non-radiative pathway. So, I will have the other channel like this way to which is k n r.

After excitation I am now going to have these three different rate constants over here k v r, k r and k n r. Now, only this decomposition channel of the excited state; I mean these decomposition channel of the excited state involve the emission of radiation; only this one right other 2 are not. So, this one is and this one they are not; only this one is the (Refer Time: 12:50) channel which involves the emission of radiation right. So now, if I want to write this phi f, so this phi f is what the rate of decomposition of the excited state which involves emission of photon. This will simply be k r. So, I simply write here k r divided by the rate of creation of the excited state that is rate of absorption. That means, this one plus this one plus this one this is my total rate right of the absorption. So, I just write k r plus k n r plus k v r. So, this is the fluorescence quantum yield of this particular compound.

Now, if you increase the concentration what will happen? Again this k r plus k n r plus k v r they will not change because they are rate constant, so it will not going to depends on the concentration of the molecule. So, it is also independent of the concentration of your system. So, this phi f a fluorescence quantum yield is independent on the concentration of the sample, it is independent on the excitation light intensity, but and it only reflect the property of the molecule. So, over the fluorescence intensity fluorescence quantum yield is a better parameter to define the fluorescence. So, I will going to show you later that how we will going to measure or estimate the fluorescence quantum yield for a particular compound.

Now, let us move to the property parameter number three which is fluorescence lifetime.

Generally, denoted by this symbol tau, it is denoted by the symbol tau f. So, if you ask what is the definition of fluorescence lifetime is the average time the molecules spend in the excited state is the fluorescence lifetime. Let me explain this by taking again this simplified Jablonski diagram over here. So, let us say this is my ground electronic level this is my excited electronic level and by absorption of light you have created the population over here, this allows vibrational level you have created the population over here right. And that population has been created instantaneously because absorption is just a very very fast process like less than a femtosecond process.

So, instantaneously you created the population at this excited state. And let us say the concentration of this excited state population is n star. And obviously, this excited state will not going to stay there forever. So, it will come back to the ground state. And I talked about the 2 different type of deactivation: one is radiative another is non-radiative and let us write this as the radiative one is like k r as usual and then non-radiative one as k n r; k r and k n r. So, let us write it properly separate rate constant and k n r is non-radiative decay. This is fluorescence we know; this is my fluorescence.

But remember that both of these process depopulate the excited state right both of these process. So, if I now write how the excited state population is changing with time; that means, minus d and star d t. That is how the excited state population is changing with time. So, minus d n star d t that will be equal to k r into n star k n r into n star. So, this is equal to

k r n star plus k n r n star. So, what I have is equal to minus d n star d t equal to n star k r plus k n r, or you can write minus d n star by n star equal to k r plus k n r d t. Let us integrate it, after integration and putting the boundary condition of at t equal to 0 the excited state population is maxima. So, n star is equal to n star 0.

So, immediately what we are going to get is n star at time t equal to n star 0 into e to the power minus t by tau F where tau F is equal to 1 over k r plus k n r. So, here you see this equation define how the excited state population in which time scale is deactivated. Suppose you have started with 100 molecules in the excited state, this equation tells me or suggesting me that it is not the case that these 100 molecule will come back from the excited state to the ground state after a certain time; suddenly, is not like that. Because, if I plot this I can plot it n star versus t right let us plot it; n star versus t. So, at time t equal to 0 over, here is my 0 time right at time t equal to 0 this n star t equal to n star 0. So, we will start with n star 0 value. So, this is my n star 0 when t is very very high, infinity then this is e to the power minus very high value; so that means, this n star will be 0. So, let us say for very high value of t over here this value is almost 0

So, I start from here I will end over here. In between let us say- when in between it follow this exponential behaviour so the function will looks like this. It means that initially at time t equal to 0 there is this much of population, as time goes the population is decreasing here at this time, at this time the population is now this much, at this time population is now this much right and so on and so forth.

When this t is equal to tau F what we will get. So, when t is equal to tau F, because this tau F is the time constant, inverse of the rate constant you see this tau F is the time constant that is inverse of this rate constant. So, I just wanted to see that when t equal to tau F what will happen. So, when t equal to tau F what I will going to have over here n star t is equal to n star 0 e to the power minus 1. So, this is 1 over e; that means, the population we will decay about 37 percent. So, 37 percent is remaining right, so population has been decayed about 63 percent. So, this n star is 63 percent of the original population decreased, is not it.

So, at time t equal to tau let us say somewhere over here this is my tau f. So, here 63 percent population has been decreased. So, I should write decreased otherwise; so decreased. That means, when t equal to tau F some fraction of the population is coming back from the excited state to the ground state. And majority is coming before t equal to

tau F, this is before t equal to tau F and this part is after t equal to tau F or when t is greater than tau F and where here is t is less than tau F, is not it. So, the population is coming from the excited state to the ground state not of a sudden. So, this is important.

So, continuously the population is coming from the excited state to the ground state. And the average time the molecules spend in the excited state is denoted by this tau F; the fluorescence lifetime. As you can see that here it is the inverse of this rate constant of the deactivation it does not depends on the concentration itself. So, this is independent on concentration, it is also independent on the excitation wavelength, because in case you will use the strong excitation power you will going to send more molecule in excited state. So, it does not depend on the concentration so it will not going to depend on the excitation light intensity.

So, this fluorescence lifetime is also a very important parameter of fluorescence which does not depend on the concentration or the excitation light intensity.

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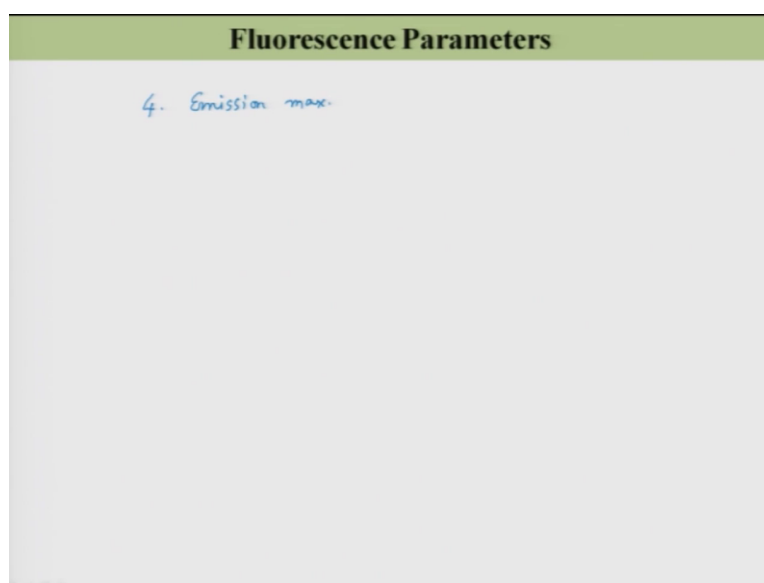
The fourth parameter is very simple one so let me quickly discuss this before we finish this today's lecture. So, this fourth parameter is the emission maxima. So the molecules right: every molecule has this characteristic emission maxima, some molecule emission, maxima is in the orange colour, some molecular emission maxima is the blue, some are green and so on and so forth. So, by knowing the emission maxima we can also comment on this

molecular property and that is why the emission maxima is one of the important parameter of the fluorescence.

So, before I end this here. So, let me tell you that this fluorescence quantum yield; I missed it when I will discuss in this fluorescence quantum yield. The value of this fluorescence quantum yield could be anything between 0 and 1.When this k r plus k n r value is much much smaller than this k r right then this is nothing but k r by k , so this is equal to 1. When these values are much more compared to k r then small quantity divided by very large quantity that means the value is close to 0.

So, depending on the molecule; depending on what, depending on the photo physics of the molecule which I will probably discuss later. That means, how much is the radiative rate constant how much is the non-radiative rate constant. So, the fluorescence quantum yield varies a lot. So, for example, rhodamine 6G in ethanol the fluorescence quantum yield is 0 point nine 4 very high. So, it tells me that the non-radiative rate constant for this molecule is very very low, but radiative rate constant is very high.

For example, coumarin 153 in ethanol, this value is about 0.54. So, it is 50-50 kind of thing. Whereas, for the ANS 1 8 aniline or naphthalene sulphonate in short ANS in water this value is about 0.003. So, it means the radiative rate constant is very high so most of the energy from the excited state is dissipating in terms of heat not in terms of light. So, that is why the fluorescence quantum yield is very low in this case.

So, we will finish here, and we will see on the next day.

Thank you very much.

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Lecture 8: Summary

❑ Fluorescence parameters
  ▪ Fluorescence intensity
  ▪ Fluorescence quantum yield
  ▪ Fluorescence lifetime
  ▪ Fluorescence maximum

❑ The dependence of the fluorescence intensity on the concentration of molecule in the solution and on the intensity of excitation light make it a useless parameters.

❑ The other three of the foretold parameters are independent of the concentration of molecule in solution and intensity of the excitation light, hence much more useful parameters

❑ Lifetime of any energy state is the average time spend by the molecule in that energy state