Bio-Physical Chemistry
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Lecture - 38
Electronic Spectroscopy Absorption and Fluorescence

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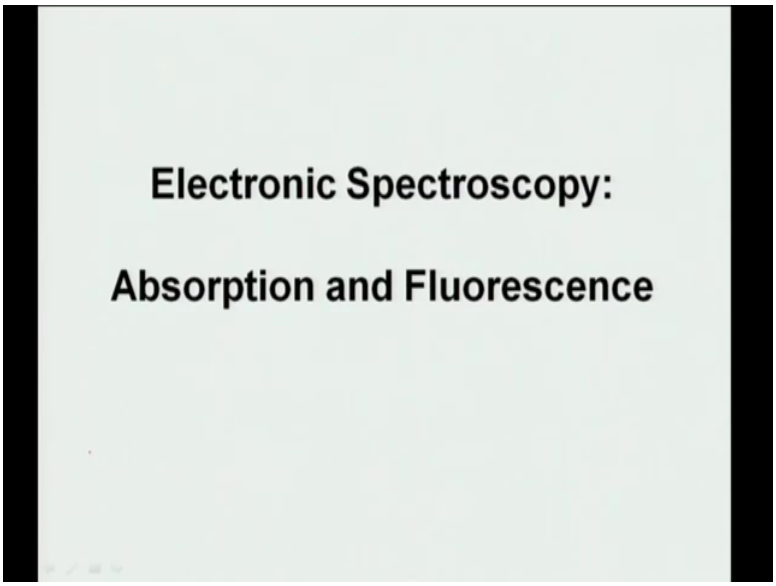

So, last class we had started talking about absorption spectroscopy right and we end where I think we ended the class by showing you this you know that cell where you have this wall and all those things right.

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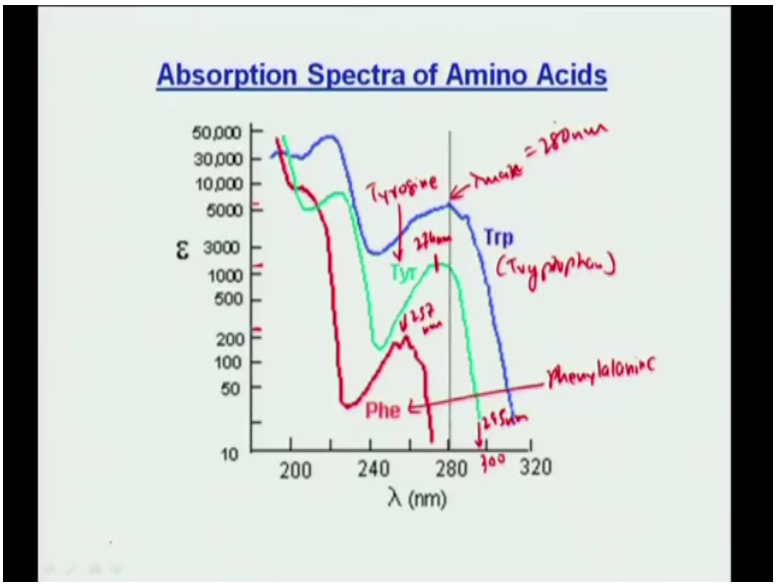

So, let us look at that again. So, this was the absorption spectra of amino acids that is what we were talking about. So, on the y axis you have the extension coefficient that is plotted against the wavelength right.

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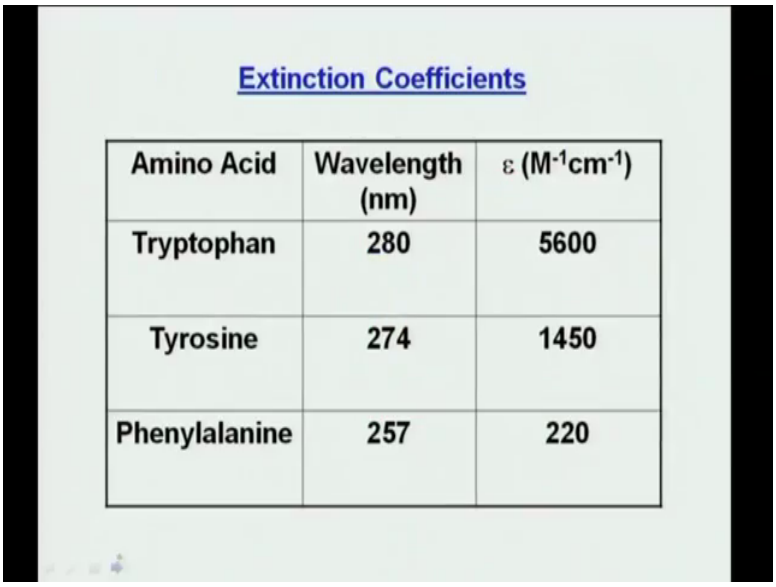

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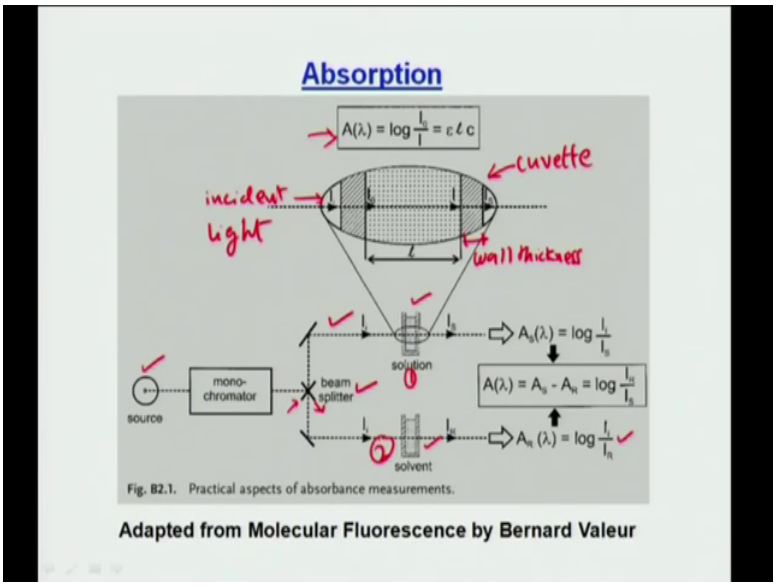

So, this is what we were talking about and what we said was that you know based on your absorbance which is this one A lambda absorbance this is equal to log of I 0 by I which is equal to epsilon times c times l right now, but this I 0 and I they are little bit different in the sense that I 0 is the intensity of light which is striking the sample solution right not the cuvette. I is the intensity which is coming out of the or which is being transmitted by the sample solution right so that means before it leaves the cuvette.

So what I mean is as I was saying see if this is a zoomed in portion of your cuvette. So, this is your sample cuvette and you have zoomed into a portion of the sample cuvette right. So, here you see this is your thickness. This is your cuvette wall thickness ok. Now when we talk about the path length that path length essentially should not be including should not be including the

cuvette wall right why? Because that cuvette what wall does not give rise to anything; it is nothing there.

So the path length should essentially be that path length throughout which you have the solution that is why you see the l varies from where are the l ranges from where the l ranges from or the l is within the inner walls of the cuvette's that is essentially what your l is right. But then when you stick your cuvette in an absorption spectrometer when you stick it there, you have to have the walls right and because of this the incident light which is this one this is the incident light. Where is this light coming from? This light is coming from the lamps we discussed yesterday.

This incident light II is the one which is striking the outer wall but by the time it reaches the sample solution at the inner wall it is not necessary that I is to be equal to I 0. There can be some scattering there can be some other extraneous absorption and all these things and hence typically what always happens is I 0 is less than I of i the incident light. So, the same thing happens on the other side. The I S which is the one which leaves the sample or leaves the cuvette is not the same as I which is the one which is coming out of the sample right.

So how do you take these things into consideration? I mean how do you make adjustments for this or how do you correct for this? So, what people have devised is people have devised something known as a double beam spectrophotometer double beam. So, in double beam what it refers to it is means two beams right essentially double beam.

So, what they do is you can see you have the source. This is the source you know the lamps you have then you have a monochromator because I showed you that the lamps have these series of wavelengths right the series of wavelengths and the monochromator, what it does is; it selects the wave length for you it disposes it and then it selects the wavelengths for you right.

And then after the light is coming out of that yeah after dispersion, then you have a beam splitter you see this is this is known as a beam splitter. So, beam splitter means what it sounds beam splitter means it splits the beam that is it. So, you have a so you have a mirror like this

ok. So, you essentially have a mirror like this right which would pass one half like this and the other half comes to the other side ok. So, that is why you will see it is a combination of two that is one which goes in this direction, the other one which comes in this direction. So, one gets reflected to the left the other one gets reflect to the right ok.

So now you started with one beam coming out of the monochromator, you have spreaded into two. So, that is why it is called a double beam spectrophotometer. Now what is the use of this? Now think about this. So, suppose in this one say in this one where I S S stands for your sample ok. And here when you are talking about this one; it is I R where R is your reference. Now what I mean by that? That means, suppose you have taken a compound in a certain solvent which is a sample the reference is typically the one which does not have the

Student: (Refer Time: 05:03)

compound; that means, it is only the solvent for you right.

So what you do is you can see here on this side. So, look at the solution side ok. So, look at this. So, look at this side what do you have is you have I I and then you have I S because you cannot measure I 0 and I. You cannot measure that you can only measure the you only know the one which is incident the one which is coming out right.

So, then what do you do is this one goes to a detector and then you plot A or you calculate A based on log of I I by I S. So, incident by what the sample sends through. Now you do the same thing. What do you do is now see you do not know what the incident light is right; you do not know what the incident light is because you do not know what incident light is being or it is hitting the sample because again of the simple reason that you have this wall.

So, now, what you do is the other half the other half goes to the goes to the reference which is a solvent only no sample, there you get log of I I over I R right. So, the first one was I incident over I sample the next one was the next one is I incident over I reference no sample.

Now, what would happen is what would you do if you do A S minus A R. What would happen? What are you taking care of? Look at that. Do you have I I anymore? You do not

have I it is already cancelled right. So, this is what you call you measure the absolute absorption spectrum; that means, by doing this by doing this dual beam what you have done is you have taken I I in both the cases, you have done a subtraction and that has cancelled out.

So what you are left with is the absolute or true absorption spectrum which is referred to as I R over I of S ok. So, I R obviously would be higher because you do you do not have the sample. So, it is going to have high intensity and I S where you have the sample the intensity would be lower and you get the true absorbance ok.

Now how is experiment typically done? Please also remember this. This is not the only thing the thing is if you having two beams that means; you are using two cuvette's say this is cuvette 1; this is this cuvette 2 right.

Now no matter how much carefully you manufacture the cuvette obviously you do not manufacture, but the company manufactures it for you; there would always be subtle differences in the cuvette's always be subtle differences. So, what people would do then is the first thing they would do is they would do a baseline. Base line means in both cases; that means, in both the cuvette's without putting sample what would they put? They would put I R; that means, R reference. So, what would this take care of? This would take care of what; whatever difference you have in terms of the cuvette path length and cuvette dimensions and all those things.

The next one so, this is your base line correction. So, the next one what you do is now you put in you take the I R in one of the cuvette's I mean in one of the sample (Refer Time: 07:59) out and put in is like you have done it. And then that is why when you will do your absorption spectroscopy, you would see there is something known as a baseline correction. This is exactly what you are doing and the reason being that no matter how much you try, all cuvette's essentially

Student: (Refer Time: 08:14)

are not the same there are bound to be some differences. The difference might be small, might be big depending upon how carefully the manufacturer manufactured it. So, this is the principle behind a double beam spectrophotometer right.

Now you know you think about this if you would do it with a single beam, you would not be having this luxury. That is why double beams are so very frequently is nowadays.

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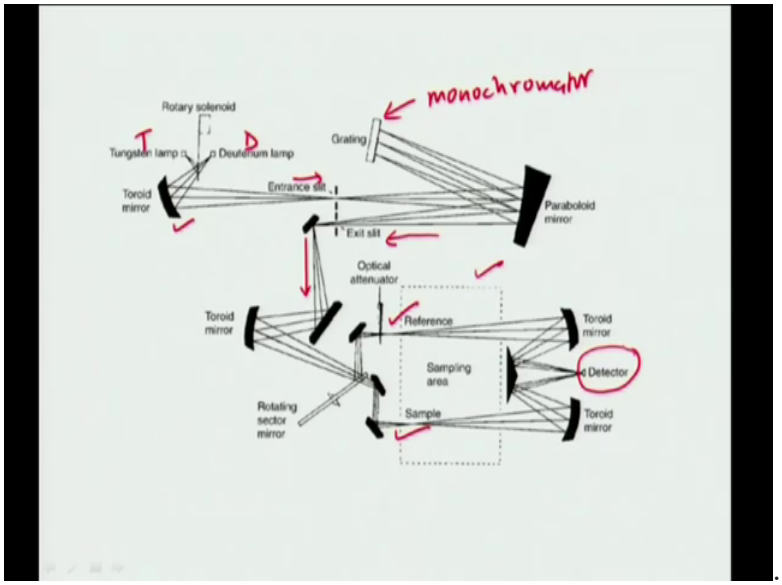

So, this is just a block diagram of your absorption spectrophotometer. So, what happens is you can see the two lamps. This is the Tungsten lamp. The Tungsten lamp and this is the Deuterium lamp. These are the two lamps out here; so, these by these two square things right. After that just let us not go into specific just try to understand the scheme the general scheme. Once a light comes out the light has to be directed towards the sample.

So, what does that, what is it done by? It is done by a mirror color Toroidal mirror. Do not worry about a toroid is the mirror is so chosen that you have the maximum amount of reflectance or the maximum amount of light which is guided towards the sample itself; that is what you pick out.

Now after that what happens is you have an entrance slit a slit which allows it to go through. You have a mirror out here, then you have a grating. So, this is a part of your monochromator. This is a part of your monochromator; remember the monochromator we had in the previous slide. So, this is a part of the monochromator. It is a monochromator might be having one grating or two gratings depending upon that would be a single grating or double grating. So, this grating what would it would do is whatever light it is coming in, it would help disperse it.

Once it is dispersed, now you can see what happens is again the light comes back the parabolic mirror. This mirror which reflected the light onto the grating again collects it. Once it collects it comes through the exit slit like this. So, one was the entrance through which you went towards the parabolic mirror went to the grating, came back to the parabolic mirror. The parabolic mirror refocused, it came through the exit slit. Now once you have the exit slit, then look at this your sample is here right. This is your; this is your sample area. Again you have to guide the beam or the light towards the sample area.

So, what you have is after this exit slit, you have another mirror. So, this is a direction. So, consider this is a toroid mirror. You have all of other optics, but finally, see what happens you have a reference beam and you have a sample beam. So, two beams are coming out ok.

And then again have out here and then you have a detector. So, either you have either you have two detectors two detectors means; one for the reference and one for the sample. That means, you have two beams; or you can have one detector and then do something so that you can get the proper absorbance right.

So, this is typically how the inside how the inside of an absorption spectrophotometer looks; a double beam wall right. You know depending upon who manufactures it; components would differ, the type of mirrors would obvious different, the way you know design the monochromators would differ, but the principle still remains the same, you are going to have this thing in general ok.

So that is you know a typically all what I had to tell you about absorbance. Just one thing I need to point out which I forgot last time before I go on to Fluorescence is this.

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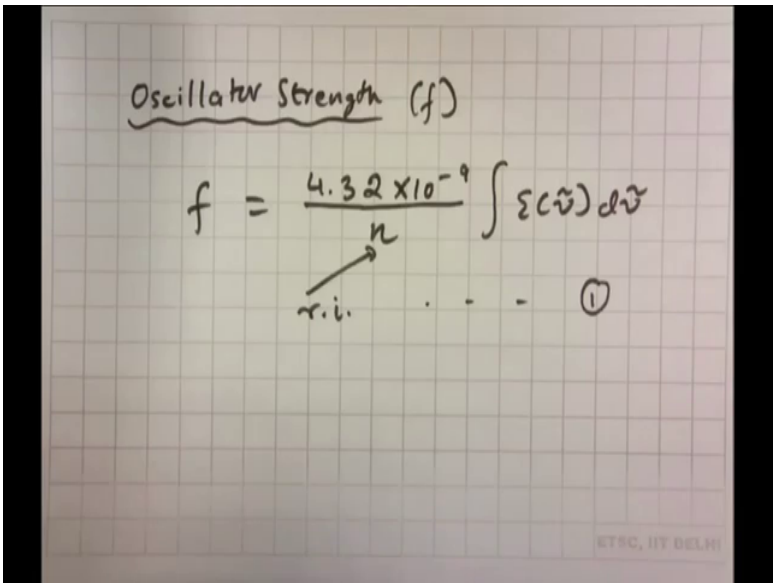

So, there is something known as oscillator strength. So, there is something known as oscillator strength. Now where did this you know name oscillator come from? So, think about this. See what was the intrinsic meaning behind an extinction coefficient? The intrinsic meaning behind an extinction coefficient is; what is the probability of that molecule absorbing light at that

wavelength ok that was the meaning of the extinction coefficient it is so intrinsic to the molecule, it is a fundamental property of the molecule.

Now how does it happen? When a electromagnetic light comes in that is your energy density comes in; it is varying as a function of time either cosine or sin. Now what it does is when it hits your sample it also sets the sample in motion. And it does not set the sample in motion; what does it set motion; what does it set motion into? It sets the electrons into motion right because that is the ones which are easily moved around.

Now, the electrons would also respond to this oscillating nature of light. So, what would happened is; these electrons would oscillate because electrons would oscillate there would be changes in charge distribution all these things. So, remember this would bring about a change in your transition in a change in your moment, giving rise to transition. So, we talked about the transition moment.

So the bottom line is; that whenever your light comes in it is sends your electrons in motion. Now depending upon their frequency; if the frequency is high then the electrons would also oscillate very with a rapid frequency, if the frequency is low; then the electrons will be oscillating less frequently.

But, so what it means is the mere extent of this oscillation of electrons will determine to what extent it absorbs at that wavelength. And hence this oscillator strength should be related, this oscillator strength should be related to your molar extinction coefficient in some way or the other. They should there has to be a relation because both of these are speaking about the same thing; one is talking about the probability of the transition, the other one is talking about the oscillations of the electrons which finally, lead to the transition. So, those you know typical this should be related.

So, what happens is; so this is I just give you the relation. The oscillator strength it is symbolized by this thing f. So, f the oscillator strength is equal to I will just give you the value

just remember it 4.32 times 10 to the power minus 9 over n; n is a refractive index remember this is a refractive index times this times this ok.

So, what it means is. So, again n is a refractive index of your solution ok. And what do you have in your integral? What do you have in your integral is here just look at this you have what is epsilon? Epsilon is extinction coefficient epsilon is extinction coeffient, but what units are you expressing it in? Nu bar you are expressing it in nu bar; that means, centimeter inverse

Student: Yes sir.

Right times d nu bar. So, this essentially is your whole absorption spectrum if you keep in mind this essentially is a area under your whole absorption spectrum.

Now this is how an oscillator strength is related to epsilon okay just by this equation. Just keep this in mind because both of these are talking about the same thing right The reason we choose nu bar well; there are other reasons for it, but it is essentially because nu bar is proportional to energy right. And you are supplying it with some energy density and according to that your electrons are oscillating with that you know whatever frequency you are supplying ok.

Now again this is based on the electromagnetic nature of light; this is based on classical physics right. What about when we are talking about quantum mechanics? When we talk about quantum mechanics we did an integral; what was that integral? That integral was the transition moment integral remember; we talked about the transition moment.

So that was purely from quantum mechanics right. We talked about wave functions and all those things. We talked about over lap the Planck quantum over lap and all those things. So, see if this oscillator strength is relate to extinction coefficient which talks about probability of transition; your transition moment also talks about your probability of transition, then again both of these things should be related right. Because again both of things are talking about the

same thing right. Why should they are not be relate? Only one thing is one is classical the other one is quantum; that is it that is the only difference.

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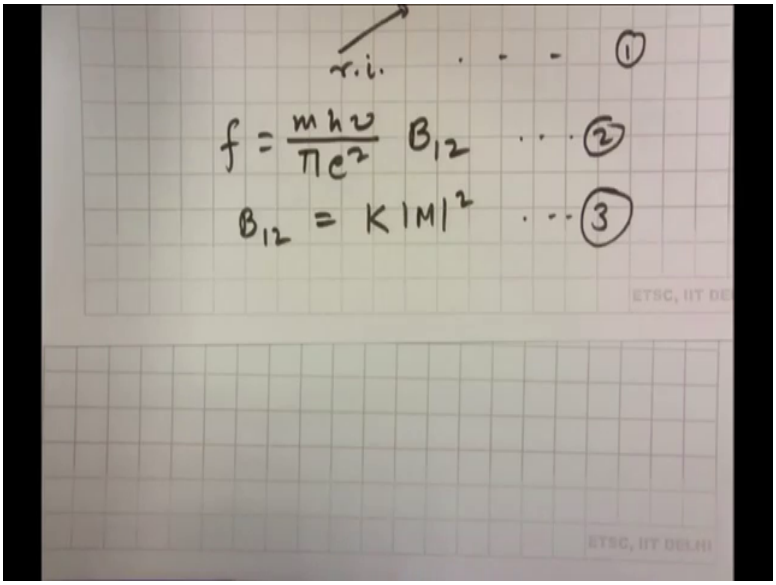

So, again so, there is a relation for this. So, you just for the sake of knowing it; f is related to I will give you m h nu over pi e squared times B 1 2. Let this be 2 where m where; m is the mass of the electron m is the mass of the electron, nu is the corresponding frequency and e you know as electronic charge right. Do you remember what B 1 2 is?

Student: (Refer Time: 16:42).

Where did we encounter B 1 2?

Student: (Refer Time: 16:46) Einstein's.

Einstein's.

Student: Yes sir.

Coefficient right? Einstein coefficient of absorption this is the Einstein's coefficient absorption B 1 2 ok. So, this is what it should be related to because we are talking about adoption transition. Now B 1 2 B 1 2 is related by K times M squared where; K is a constant do not worry about the constant is. What is M? The M was a transition moment we talked about. So, B is proportional to the square of the transition moment integral of the transition moment, f has a relation with B, B is proportional to M squared, that means f is also proportional to what?

Student: M squared.

M squared? So, this is a relation between the oscillator strength and the transition moment ok. So, again it just says is that the probability of the electrons oscillating the probability of the electrons oscillating determines the transition. And because it is a transition I better have a relation between these oscillator strength and the transition moment integral; that is what we looked at just now ok. Do not worry about what the constants are and all these things. We have we do not have time to go into the those details in this course at least. But this is the general expression or the general feeling you should be having about these two things one is the oscillator strength and the other one is a transition moment both of these talk about the same thing good. So that was the last bit of information I want to tell you about absorption.

So, now, if you have excited the molecule; that means, the molecule is I mean the molecule is excited as absorbed light, the electrons have gone to the excited state but it cannot stay there forever? Right, they have to come down; that means, they have to get deactivated or de excited. So you go there, it stays there for some time depending upon a host of other factors

which we will talk about and then after sometime, it will come down to the ground state again; that means, it will relax or it will de excite from the excited state ok.

So, what are the processes involved? Obviously, one of the process is involved is fluorescence right.

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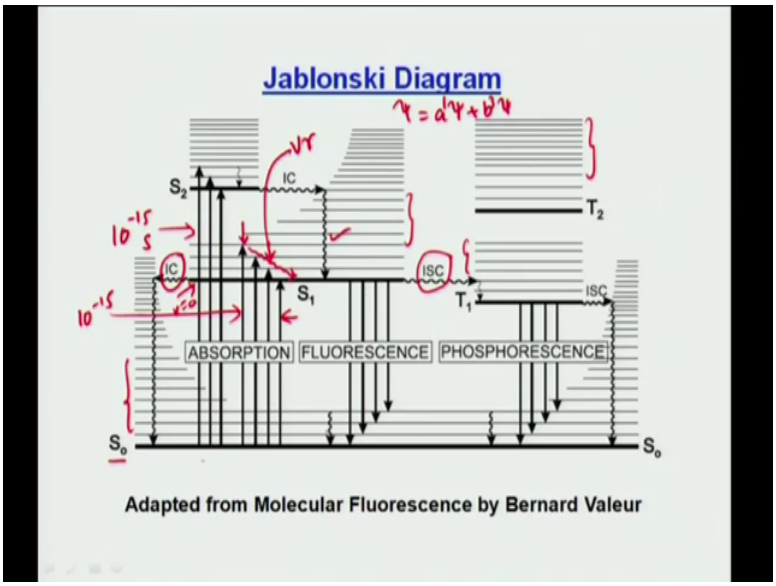

So, but let us look at a very important diagram I am sure you have heard about this is called a Jablonski diagram. Now look it has too many levels energy levels, but do not be intimidated by that all of these things you have seen before. First look at it step by step what S 0? S 0 is a singlet ground state for you okay. What is S 1? S 1 is the excited singlet state for you right. What is T 1?

Student: Triplet state.

T 1 is a triplet state good. Now you look at all these levels you look at all these levels associated with S 1 what are these levels? These are your.

Student: Vibrational levels.

Vibrational levels. Remember, when we were drawing the diagram we had those vibrational levels right. So, similarly any electronic state like S 0, S 1, T 1 would be having it is corresponding vibrational levels. So, whether you look at S 0, whether you look at S 1, whether you look at T 1, you would be having those levels anyway out there ok. So, that is why what do you have is in S 1 you also have these levels, in T 1 you have these levels right, in T 2 you have these levels ok.

Now what are S 2 and T 2? So, if S 1 is the first singlet excited state; S 2 is what? The second excited state; T 2 is what? The second excited triplet state right, ok. Now you have done an absorption. So, right so, this is your absorption; this is your absorption right. So, absorption takes place very fast very fast what is the fastest time scale or what is the approximate time scale takes place in? The absorption takes place in about.

Student: (Refer-Time: 20:30).

10 to the power minus.

Student: 15.

10 to the power minus 15 seconds; so, 10 to the power minus 15 seconds that is what we are looking at absorption 10 to the power minus 15 seconds good. So, now, remember when we have made this transition the nuclei have not responded. Only after making this transition do the nuclei start responding to this change process good.

Now suppose it is so let us say suppose let us look at these bunch of arrows see you have gone to a S 1. Now you can see as we discussed last time you are not going to have only one

transition right? Because you are if you are you are having this transition for where V is equal to 0 state of S 0 because the electron density goes like this you are going to have a band of transitions right throughout that wave function right.

So, that is why you have these series of transitions to different you know vibrational level. This vibronic transitions we have talked about this intensity before. But the deal is this guys the deal is; that you look at this say you look at this arrow, this arrow takes the molecule to the highest vibrational excited state in S 0.

Student: hm.

Based on this diagram right because this is representative diagram ok. Now let me tell you this; there are two things that can happen either the molecule from here comes back straight to S 0 comes back straight to 0 by some way or the other or instead the from here it slowly first decays to the first vibration state of S 1 itself and then comes down.

So, let me tell you this again. Consider the fact that you have gone to the highest vibrational excited state of S 1 right. So, that means, you are already in a higher vibrational energy in S 1. There are two things that can happen. It can the molecule can relax straight from here this higher level to the ground state S 0 or it can slowly lose it is energy not slowly, it can lose it is energy go to V is equal to 0 of S 1 and then come down; there are two ways of doing right.

Now this line this wiggly line. I just drew is referred to as.

Student: (Refer-Time: 22:55).

V r which is referred to as vibrational relaxation ok, you will see it in the next slide. This vibrational relaxation, it is called vibrational relaxation simple because the relaxation is happening between what states?

Student: Vibrational states.

Vibrational states right. What is the time scale guys? The time scale of this relaxation is 10 to the power minus

Student: (Refer-Time: 23:14).

Minus?

Student: (Refer-Time: 23:16).

10 to the power minus 12 seconds. 10 to the power minus 12 seconds is pretty fast ok. It is faster it is faster than the time the molecule will take to relax from this state to the ground state. So, you can imagine what will happen is which one will proceed now? Because V r is much faster than this process so, you will be having a vibrational relaxation first and then it would come down to the ground state. Because it is timescale is just faster than the time it needs to come down to the ground state straightaway. So, this is called a vibrational relaxation.

If you would ever you know look out these processes you would see that there is a another process known as IVR which is called intra molecular vibrational relaxation. And people who are specially doing gas phase spectroscopy are very interested in this ok. Now that is essentially we are looking at right.

But remember in gas phase, I told you do not have too much of broadening as compared to condensed phase. Because condensed phase you have too many molecules too many interactions your gas phase it do not have that. So, that is why people can potentially look at those, but here it is very limited.

Now, so, one was vibration relaxation right. So; that means, you are having relaxation in the same vibrational levels or rather in the vibrational levels of the same electronic state. I am not talking about relaxation in two different electronic states. Also second is vibrational relaxation is a non radiative path length; that means, when a relaxation is happening it is not giving out

any photons; what is it doing? It is giving out energy; it is giving you this energy as the thermal energy. Where did we use this do you remember? Where did we use this relaxation before? Come on, one of you will have to tell me.

Student: Kinematics.

Student: It is kinematics in (Refer Time: 25:02).

Ok good, which part? That itself was huge, which part? What was the last thing we discussed in kinetics?

Student: (Refer Time: 25:13).

Ok the second last thing? It was not the last thing before optical trigger.

Student: Temperature jump.

Temperature jump, we discussed temperature. What did we see in temperature jump? You heat the solvent; the solvent goes out to a vibrational excited state, it relaxes it loses the energy. And that energy is picked up by what? The protein molecules this is essentially what is happening.

So, that excess energy gets picked up by whatever molecules you have in the surroundings right. So, anyway in this case it gives out the excess energy and goes to V is equal to 0 of that electronic state S 1 ok. Now look at this process; this I C, what is the full form of I C?

Student: Internal (Refer-Time: 25:54).

Internal conversion. Where does internal conversion occurred between which two states?

Student: S 2

Between two electronic states right either; S 1 is 0 or either.

Student: (Refer-Time: 26:08).

S 2 S 1. But look at this if you look at the IC, the internal conversion it is again a non radiative pathway again a non radiative pathway. See whatever wiggly lines you have these all referred to non radiative pathways that is the importance of the wiggly lines. Now this internal conversion if you look at this, please keep this in mind this internal conversion when you are having this conversion from S 1 to S 0; you are not you are not having the conversion from S 1 to V is equal to 0 level of S 0 where are you having the conversion to.

Student: (Refer-Time: 26:45).

You are having a conversion to a higher vibration level of S 0 itself isn't it? See how would the conversion happen? The conversion would only happen very easily if you would have isoenergetic energy levels.

That means, if you have these two if you have these two; the molecule the electron can be here the electron can be here it does not matter, because these are isoenergetic there is no energetic bias. It is not you know it is like a Boltzmann population right this is; obviously, they are of a same energy so they have equal probability of being here or here.

Now what will happen is; you think about this see this is S 1 the V is equal to 0 state of S 1 right so this is the one. So, this is this is the V is equal to 0 state of S 1. And this is S 1 this is overlapping with a high vibration state of S 0. The moment this one relaxes to S, the high vibration state S 0 you know that this V r is very fast. So, the moment happens it will come down. That is what you see to the lowest vibration state V 0.

Now this is how an internal conversion happens. So, it is always remember, it is always between isoenergetic levels; isoenergetic levels means levels having the same.

Student: Energy.

Energy. It is just not jumping from one energy level to the other this picture you have to have in mind ok. So that means, whenever I C happens, it would be like this. Similarly, you can also have I C from S 2 to S 1 right; again there you see the lowest vibrations to S 2 is overlapping or is equienergetic with a higher vibration state of S 1. It goes there and then it again comes down to V is equal to 0 of S 1 ok. So, that was your internal conversion.

But please remember the internal conversion and I come to this in the next slide where it is written out there. The internal conversion is essentially happening between two states having the same what?

Student: Multiplicity.

Same multiplicity; that is very important. Internal conversion; that means, internal conversion between two states having the same spin states same multiplicity right ok. Now what about the SC, what is ISC?

Student: Inter system (Refer Time: 28:48).

It is inter system crossing as the name suggests it is inter system; that means you are now moving from one system to the other. So, what are the two systems here? The one is say S 1 to.

Student: T 1

T 1. Again you see in this ISC what is happening is it is again an isoenergetic transition right or isoenergetic conversion. That means the V is equal to 0 level of S 1 is overlapping or is equienergetic or isoenergetic with a higher vibrational state of

Student: T 1.

T 1; it goes there then it comes down ok. So, this is inter system crossing. Now the question is inter system crossing allowed or not?

Student: Yes.

Just tell me, is inter system crossing transition allowed or not?

Student: No.

Student: No.

Why is this why is it forbidden?

Student: Del S then it form some inter change (Refer Time: 29:36) del S not equal to 0.

So there is a change in spin.

Student: (Refer Time: 29:38).

Multiplicity. Now if you having a change; obviously, it is forbidden and why do you see it?

I mean you should not be seeing it right? Theoretically you should not be seeing it.

Student: (Refer Time: 29:50).

Why it has happen.

Student: There will be mixing between them.

Mixing. So, there is mixing there is mixing between the triplet and the single state. That means, whatever triplet state you see or whatever singlet state you see that psi is equal to a coefficient and psi.

Student: Singlet.

Singlet plus a coefficient times psi triplet. So, hence what I can write out here is; I can write that psi because of this inter system crossing say is; a plus b psi 3. There is a and b are the coefficients; a is the coefficient of the wave function correspond to the singlet state, b is the coefficient of the wave function corresponding to the triplet state. That means, that wave function is a mix of two states singlet and triplet.

Now to what extent this mixing happens; would finally, determined to what extent you have the inter system crossing. But there is something which increases the mixing there is a term there is a term which refers to this mixing or increases the mixing; what is that? The term ends it is two words the last one is coupling, what is the first word?

Student: (Refer Time: 31:10) Scopic.

Ok just go just go beyond.

Student: (Refer Time: 31:17).

The first one is spin; what is the other one?

Student: Orbital (Refer-Time: 31:21).

Yeah. So, it is spin orbit coupling. I told you everything almost right. It was no longer a question I was giving you the answer.

To my own question anyway. So it is a spin orbit coupling it is the spin orbit coupling which determines this intersystem crossing ok. So, you have to have spin orbit coupling right. Guys do not write I have this I will even give you a handouts. So, do not worry about it just listen.

Now because this is a fundamental picture of fluorescence this you always have to keep in mind if you are doing fluorescence right. Whatever intensity of fluorescence whatever emission of fluorescence you see is governed by these processes as simple as that.

Now two things whatever we have talked about I S C, I C, V r right these are all non radiative processes; that means, they do not come with emission of photons right. So what are the two radiative processes that are left for us? One is fluorescence the other one is.

Student: Phosphorescence.

Phosphorescence. So fluorescence; obviously, is from S 1 to S 0 almost always only there are a few cases where you can have fluorescence from S 2 to S 0 there are very few cases. So, one is fluorescence and then the other one which is from T 1 to S 0 right. Remember which is against spin forbidden, but it gives out light it is referred to as phosphorescence ok.

So; that means, in the Jablonski diagram you have a combination of two types of processes. One process that gives out photons the other process that does not give out photons; that means, you do not see. But how would you see? It is like this; suppose you are putting in 100 photons; that means, a molecule is absorbing 100 photons right.

If all the; that means, if you are pumping in 100 photons and if you have a detector it is an ideal case you also look at 100 photons; that means, each and every photon that it absorbed it give out one fluorescence photon.

So; that means, it is quantum yield it something known as a quantum yield is 1. So, for every observing photon it has one emitting photon right; I am talking about fluorescence now let not go to phosphorescence ok.

However, the moment you see that the number of photons emitted is far less it cannot be greater than the number of photon absorbed. It is far less than the number of photons absorbed; what does it mean? That means, the other photons which you could not see must have gone through what?

Student: Non.

The non radiative channels. So; that means, you have a combination between the radiative processes and the non radiative processes and that finally, determines to what extent you are going to see emission. See the only thing you see is emission remember. But by seeing the extent of emission you know how much of radiative non radiative process you have; essentially how much of non radiative process you have. That is why this parameter called quantum yield is of such a huge importance in fluorescence right; that is essentially what it says. It the yield means it is like if you are doing a synthetic reaction you always talk about the yield right.

In this case you are also doing a reaction, but a photochemical thing. So, it is essentially a photo physical you are not doing any chemistry. So, that means, you heated with photons, it goes to the higher state, it comes down to the ground state right. And whatever number of photons it yields in terms of fluorescence will give you the quantum yield. So, this is typically how a Jablonski diagram or you know the features of a Jablonski diagram right. Is there anything that I missed? Let me check out. I do not think so, but anyway we will see soon.

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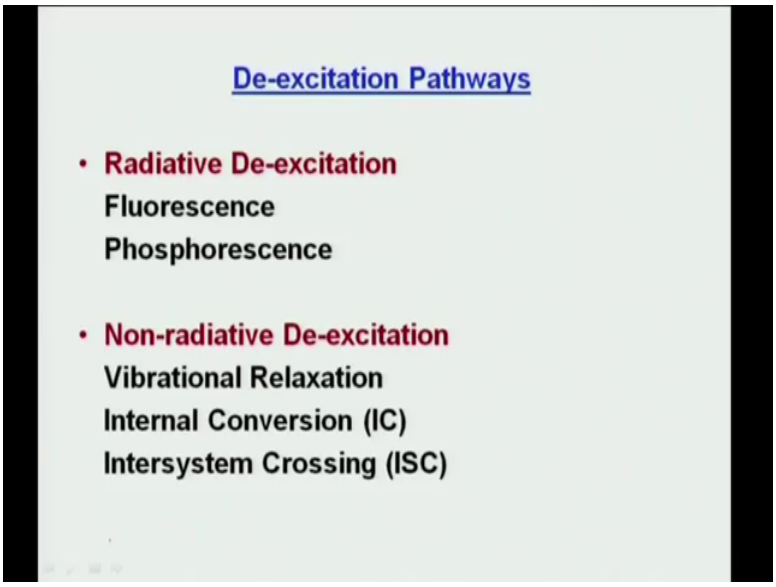

So, as I said what are the deactivation pathways? One is a radiative de excitation it involves; fluorescence and phosphorescence. The other one is a non radiative de excitation which involves; vibrational relaxation interval conversion and inter system crossing. And I said that vibrational relaxation is very fast about 10 to the power minus 12 seconds right.

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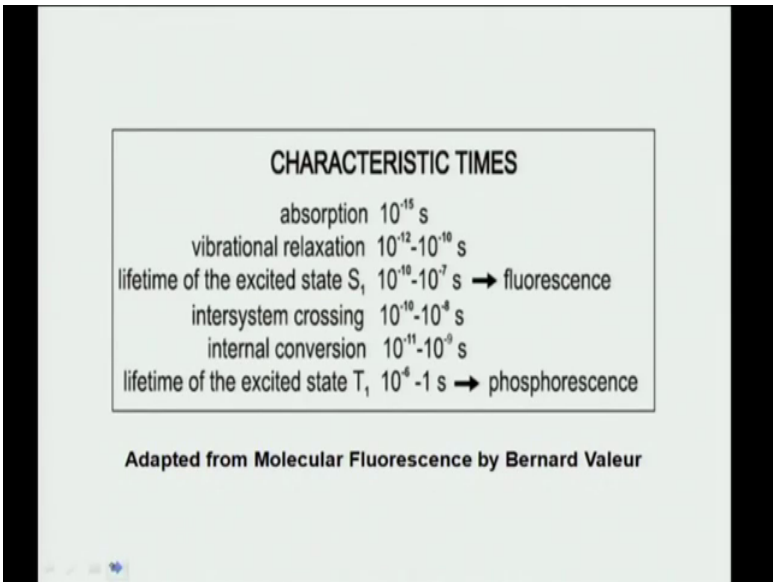

So, let us look at the characteristics times now; absorption about 10 to the power minus 15 seconds, vibrational relaxation you can say 10 to the power minus 12 minus 13 seconds, fluorescence that is lifetime of the excited state can vary from 10 to the power minus 10 to 10 to the power 7 seconds. So, you can see now; even before fluorescence happens; that means, it comes down your vibrational relaxation is much faster. So, remember I said that it always has to go to the V is equal to 0 state.

Similarly inter system crossing which is 10 to the power 10 to the power minus 6 seconds. Internal conversion;nternal conversion is non radiative it is from one electronic state to the other electronic state of same multiplicity it is still 10 to the power minus 11 to 10 to the power minus 9 seconds. So, your vibrational relaxation is still faster than what?

Student: (Refer Time: 35:51).

Internal conversion right. And then you have the lifetime of the excited state which is a triplet the first fluorescence is it typically much delayed right. See fluorescence is from is the order of nanoseconds, first fluorescence is in the order of microseconds where; 10 to the power minus 6 is micro.

Student: Sir I S C is much faster than I C.

I S C.

Student: Much faster than I C.

Where is it so? It states 10 to the power 8 to 10 to the power minus 10 minus 10 to the power minus 8 right. I C is 10 to the power 11 to 10 to the power minus 9.

Student: (Refer Time: 36:20).

Which is faster?

Student: I C.

Where? You look at the range.

Student: (Refer Time: 36:28) is faster.

Ok good; you have not crossed systems right? Anyway so yeah, where was I?

Student: (Refer Time: 36:41).

Why is phosphorescence so delayed?

Student: (Refer Time: 36:48).

Again it is been forbidden it takes time. So, that is why so, delayed. So, that means you can understand only after fluorescence is over will phosphorescence happen right? Good.

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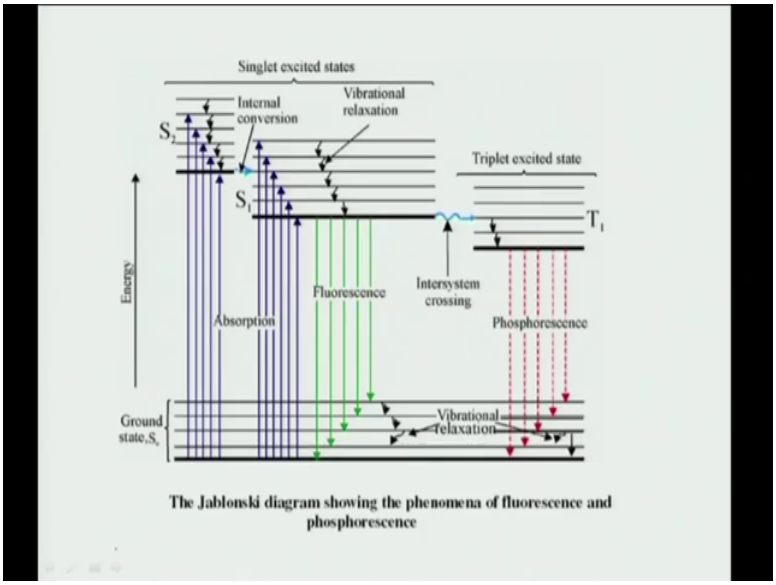

So, this is again a very similar diagram the only thing different which is shown out here is the vibrational relaxation. You can see it is not showing the vibrational relaxation to the V is equal to 0 state in that specific electronic state right ok.

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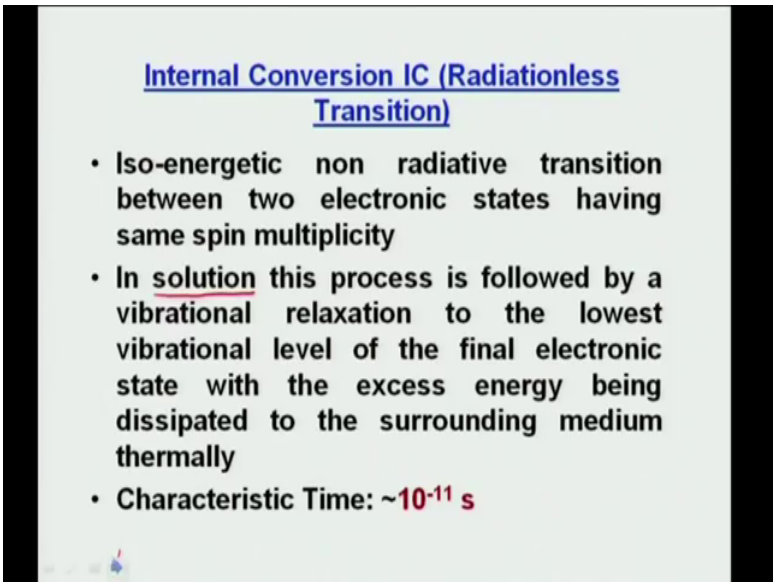

So, we talked about internal conversion. So, as I said this is an iso-energetic non radiative transition between two electronic states having same spin multiplicity. In solution this process is followed by a vibrational relaxation to the lowest; vibrational level or the final electronic state with the excess energy being dissipated to the surrounding medium thermally.

Ok now please notice this; in solution that means, essentially in condensed phase. See vibrational relaxation will depend upon a lot of things, but; obviously, one of the immediate things it will depend upon is it is interaction with the surrounding solvent.

When you go to gas phase again then you will realize that you do not have any solvent molecules. And hence probably in that case you would be able to pick out some vibrational

structure which you do not see in the solution phase ok. Specially from the higher vibrational states.

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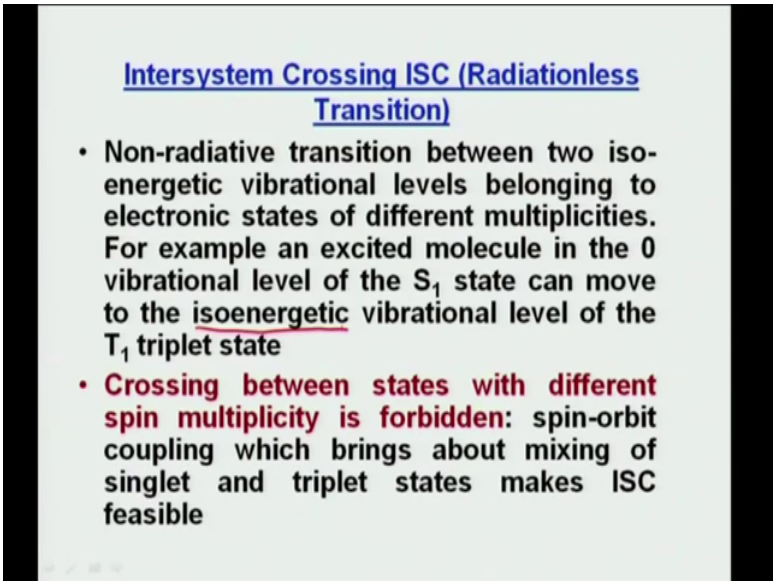

And we talked about the characteristic time.

So, the inter system crossing it is a non-radiative transition between two iso-energetic vibrational levels belonging to electronic states or different spin multiplicities. For example, an excited molecule in this 0 vibrational level of S 1 state can move to the iso-energetic vibrational level of the T 1 triplet state.

So, this what is again a key word iso-energetic vibrational level. The crossing between states with different spin multiplicities forbidden, but still you observe it. Because there is something

known as spin orbit coupling which brings about mixing of the singlet and triplet states. As I wrote that equation down it makes I S C or inter system crossing feasible.

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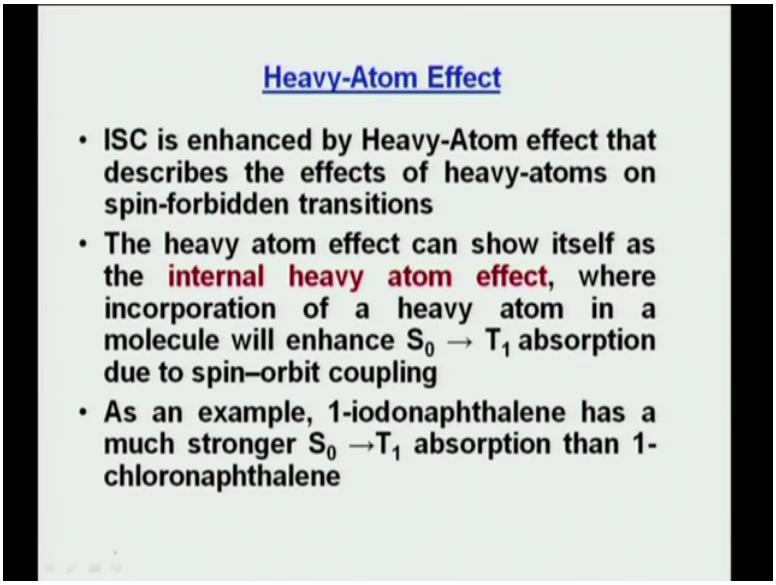

Now what are the things that enhance or increase spin orbit coupling? There is one thing which is known as heavy atom effect. In a heavy atom effect at as is suggests is being brought out by an atom which is heavy; that is why the name. So, what happens here is inter system crossing is enhanced by heavy atom effect that describes the effects of heavy atoms on spin forbidden transitions.

The heavy atom effect can show itself as the internal heavy atom effect, where the incorporation of a heavy atom in a molecule will enhance the S 0 to T 1 absorption due to spin orbit coupling. So, that means, it goes to goes from S 0 to S 1 and then it goes to T 1 that is what this S 0 T 1 absorption ok.

Why is it called internal? It is called internal because it is present in the molecule itself. That means, it is a part of the molecule you have not you have not done anything externally to it is already a part of the molecule. And I will give examples very soon in the next table.

So, as an example one Iodonaphthalene has a much stronger S 0 to T 1 absorption than one Chloronaphthalene. So, both are Naphthalene derivatives right? One has Chlorine the other one has Iodine; which one is heavier?

Student: Iodine.

Iodine is heavier. So, where would you see a better T 1 population? In case of Iodonaphthalene right.

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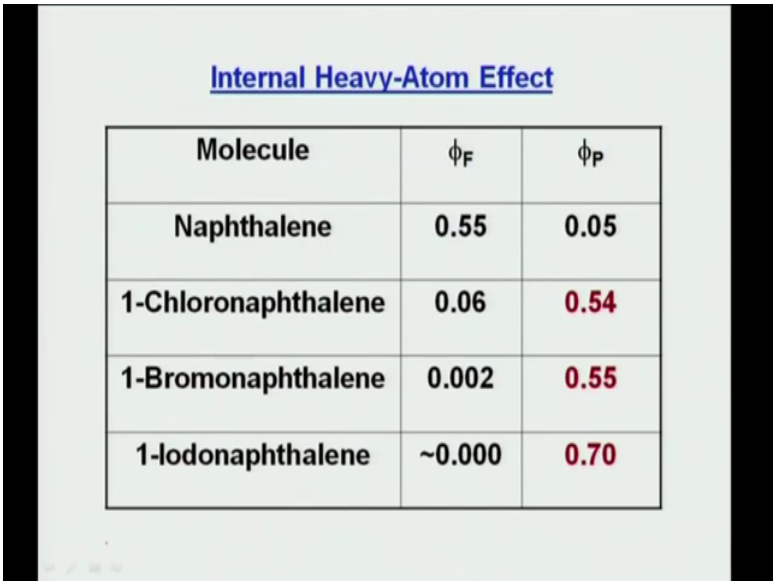

So, that is why it is this table is very informative. It is referred to as internal heavy atom effect. What it says is; you have the molecule then the first column is your phi F which is the fluorescence quantum yield I will come that come to that later I have kind of given you an idea. And the next; obviously, is the Phosphorescence quantum yield. Now see what happens you take Naphthalene; the fluorescence quantum yield is what? 0.55; the Phosphorescence quantum yield is really low 0.05 right.

Now you go to 1- Chloronaphthalene; in 1- Chloronaphthalene what happens to the fluorescence quantum yield? It immediately decrease to 0.0. Accordingly the phi P which is the Phosphorescence is increased, why? Because Chlorine now is heavy, then heavier than Hydrogen. It is induced at heavy atom effect and it is enhanced inter system crossing by spin orbit coupling.

Now; obviously, you guys would be smart you go to Bromo; if you go to Bromonaphthalene what would happen? You see the phi F is really low 0.002 almost non existent and then phi P is 0.55. And you go to Iodonaphthalene, what happens? It is almost not there is almost no fluorescence. And essentially whatever you see is in terms of a delayed emission which is Phosphorescence in this case ok, so this is your internal heavy atom effect. Because you see internal being that it is a part of the molecule itself. So, then if one is internal heavy atom effect, then; obviously, the other one would be?

Student: External.

External? Right.

Student: External.

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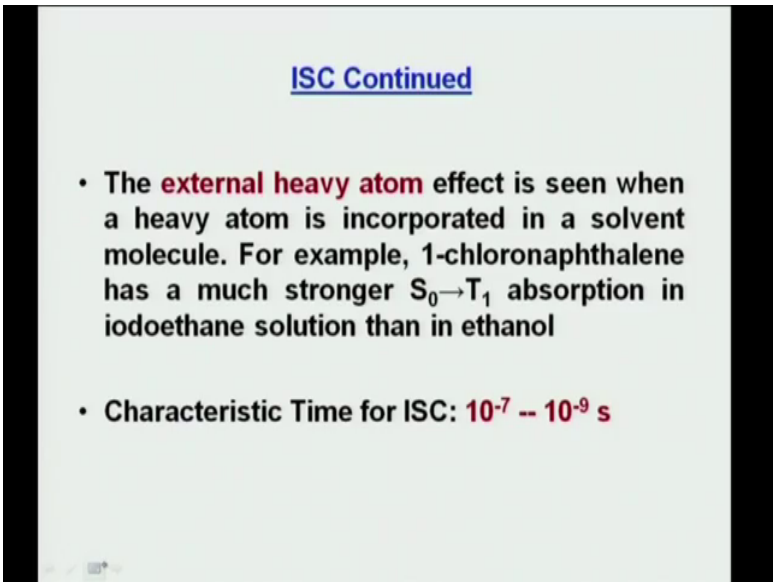

So, then the external heavy atom effect is seen when a heavy atom is incorporated in a solvent molecule. Means if you take 1-Chloronaphthalene, it has a much stronger S 0 to T 1 absorption in Iodoethane as compared to.

Student: Ethanol.

Ethanol because in Iodoethane you have that.

Student: (Refer-Time: 41:58).

Iodine moiety. So, this is external because it is not part of the molecule, but it is a part of the medium which is a solvent that is why it is external right.

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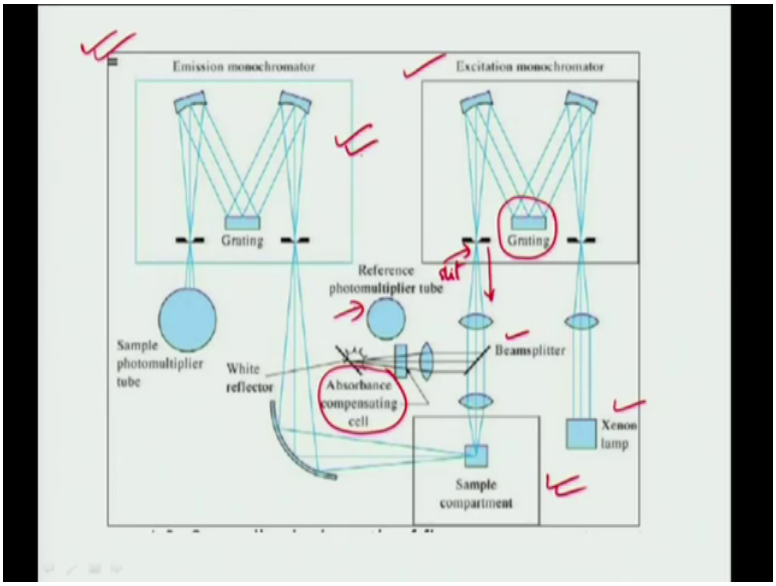

So, this is the characteristic timescale for inter system crossing ok. Now so this is an you know like the layout you saw for an absorption spectrometer it is this the layout for a fluorescence spectrometer. But let us just not go there; let us do a quick derivation.

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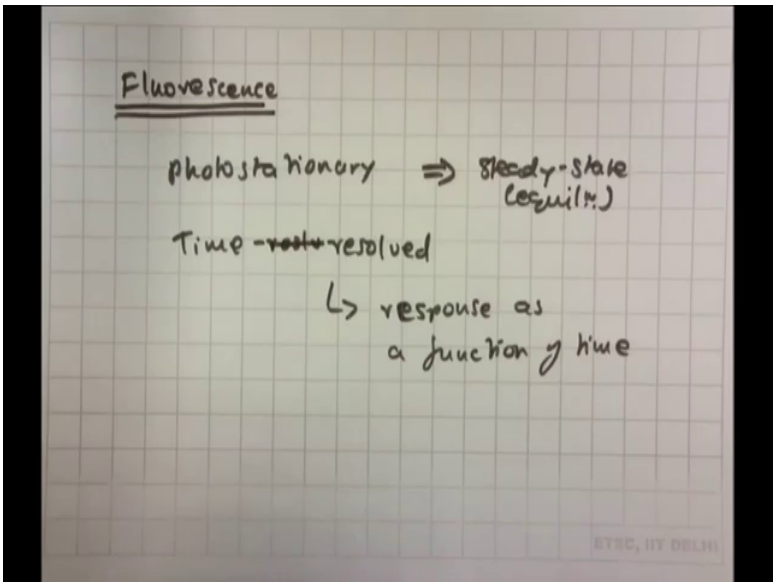

So, let us talk about fluorescence a little bit. So, fluorescence can be observed under two conditions; one is photo stationary and the other one is sorry this is time resolved. So, this is your steady state measurement which is equilibrium and the other one is your response as a function of time.

So, this one is response as a function of time. Now let us first consider the time resolved case; just for the sake of discussion let us first consider time resolved case. So, in the time resolved case. So, this what is happening right.

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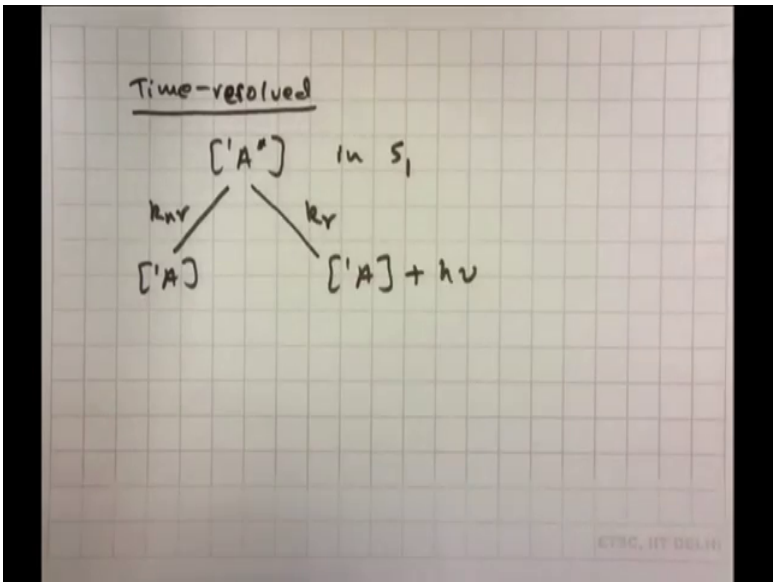

So, what you have is you have done an excitation; that means, the sample has absorbed and a set of molecules have gone to the excited state. So that means, you have if the molecule is A 1 stands for singlet. So, you have 1 A star concentration of molecules in S 1 right (Refer Time: 44:09). Now this one can come down by generally two types of processes; what are the two types of processes? One is radiative and the other one is.

Student: Non radiative.

Nonradiative, right. So, then I can have this I will have this where we having this one would come to 1 A and this would be say I will say plus h nu so this would be k r k radiative. And I write h nu because it is radiative that means, it is giving out photons h nu. The other one again

it is 1 A, but this is non radiative so I write k n r. And I am not writing h nu anymore because, it is non radiative.

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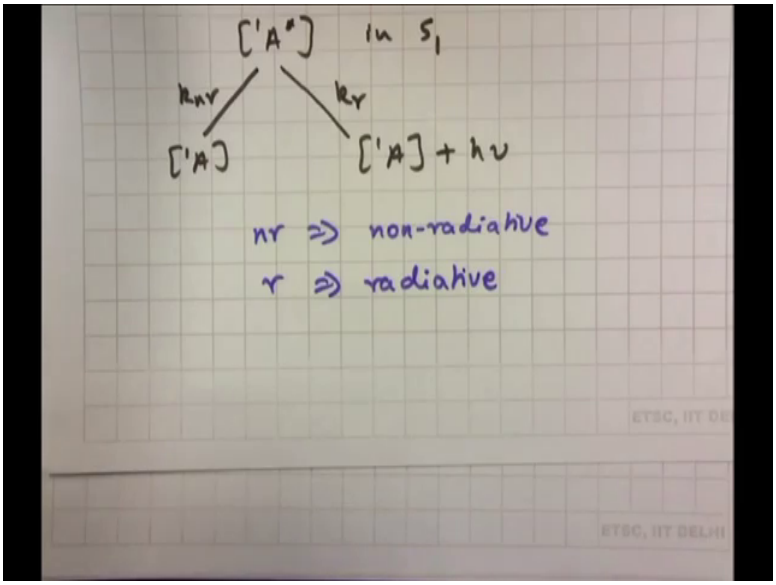

So, n r stands for non radiative; that means, n r so, n r stands for non radiative and r stands for radiative and; obviously, k are respective rate constants that goes without saying.

Now if you have to try to devise an equation for the rate of it is decay. So, this is how it is done. The way it is done is if you remember your T jump temperature induced laser induced temperature jump rather. So, what happens is; I said one thing that you have to do the temperature jump very fast boom like this and then the protein slowly relaxes. So, here also you understand you have to do this excitation very fast ok. And then that means, it must be much faster than the fluorescence lifetime or the right the time it takes to come down. And then you follow it is progress as a function of time ok.

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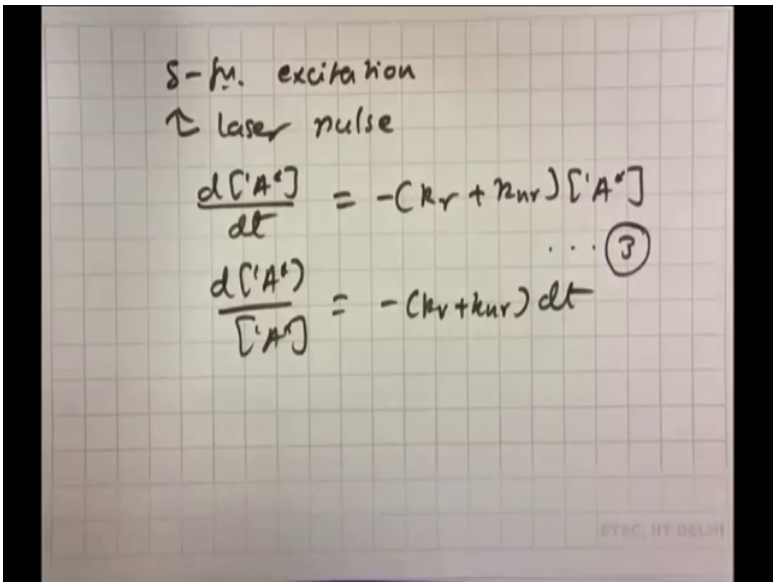

So, what do you do is? You do a delta function excitation; that means, this is done with a laser pulse this is done with the laser pulse. And do your excitation which is very fast right that is what it means by delta. That means, time width is very small it is there the laser pulse is there for a very small amount of time. It excites it and then you observe the decay of the excited state right.

So, then what will happen is what I can now essentially write is; d of A star over d of t should be equal to what? Should be equal to k r plus k n r 1 A star right. So, let us this be 3 ok. So, now what I can do is this is your kinetics regular kinetics. So, I have d of 1 A star over 1 A star is equal to minus k r plus k n r d of t ok.

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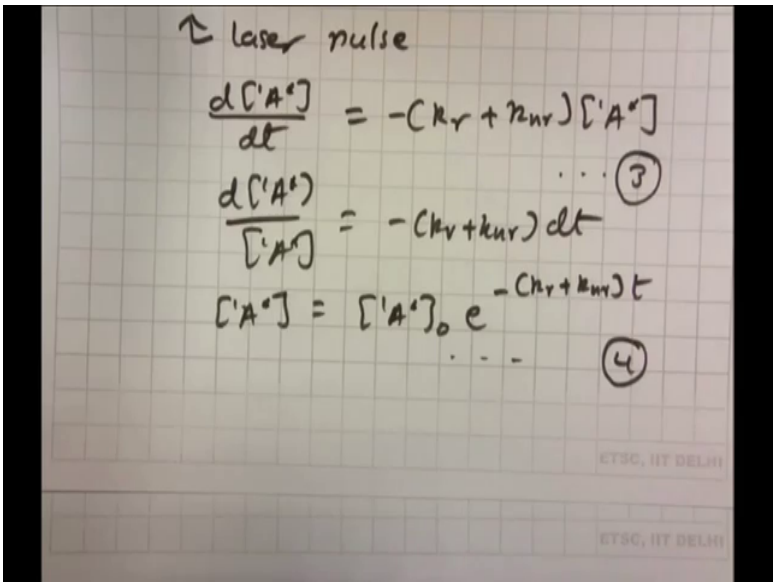

So, then what I can write is 1 A star is equal to after integrating, 1 A star is 0. That means, this is the concentration of molecules, you have in the excited state at t is equal to 0 that is the moment you have done the excitation; times e to the power minus k r plus k n r t. This is equation number 4 right. This is just an exponential decay right. It is exponential decay because that is how you set it up right.

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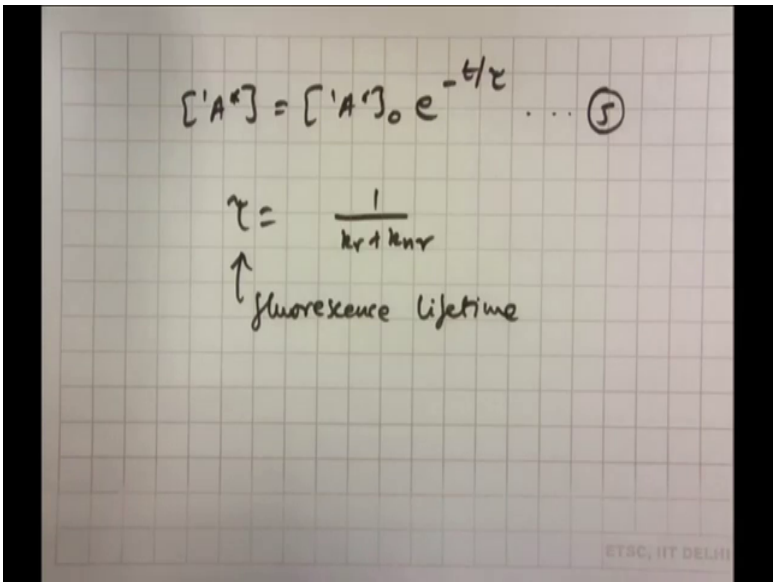

Now, what I can do now is; I can rewrite this equation as 1 A star is equal to 1 A star 0 e to the power minus t by tau minus t by tau. Let this be 5 where tau is equal to 1 by what is tau equal to 1 by k r plus.

Student: k n r.

k n r. Remember when we did laser induced temperature jump it was what? k 1 plus k minus 1 right? Here it is k r plus k n r n n ok. So, this tau which is the fluorescence lifetime; so this is referred to as your fluorescence lifetime; it depends upon two things. What does it depend upon? It depends upon the rate constant of the radiative process plus the rate constant of the

Student: Non radiative.

Non radiative process. So that means, when you are looking at a fluorescence when you are looking at the fluorescence you are; obviously, looking at fluorescence, but you are looking at a process which is dictated by two different processes. One is the radiative and the other one is a non radiative ok.

That is why I said that when you are looking when these things comes down it does not come down just by one process, it comes down by two processes broadly speaking. And to what extent one process would be more or less than the other would depend upon the type of the molecule the environment or a host of other factors. You know that is what makes fluorescence so interesting.

Now, but the problem is if you are doing a lifetime right; if you are doing a lifetime remember; this is what you get tau. So, tau is essentially the time it takes for the molecule to reach 1 by e of it is original value ok. So, that means, you have 1 by A star 0 to start with a t is equal to 0.

So, this tau it is a exponential relaxation right. It tells this tau is the time it takes to reach 1 by e of the original value which was 1 A star 0; you know that is how your exponential lifetime is defined right. Now the problem is this guys because we know the tau has both k r and k n r; if I only make a measurement of tau, I would not be able to get these right. Because I have one measurement, but I have two variables which is k r and k n r.

Now the same thing happened in laser induced temperature jump what did we have? We had k 1 plus k minus 1, but both of these were coming together so we could not get that. So, what did we need in that case? For the for the temperature jump, we needed equilibrium right because we had the k equilibrium. So that means, we had two equations two unknowns what would we do here. Obviously, one is tau the other one which is discussed with you is the other one is the quantum yield.

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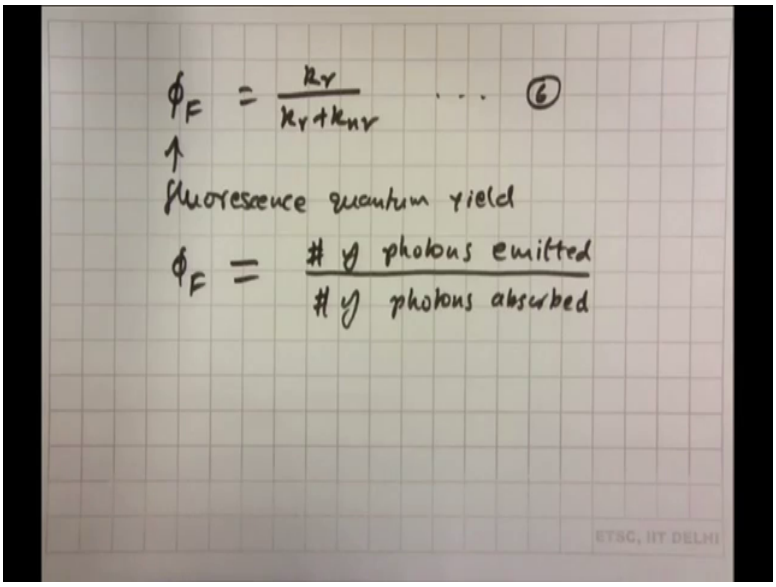

The quantum yield is the fluorescence quantum yield phi F is given by k r over k r plus k n r. So, this is equation number 6; this is referred to as the fluorescence quantum yield. This is referred to as a fluorescence quantum yield and once you have this fluorescence quantum yield. We have two equations one is tau equal to 1 by k r plus k n r and phi F is equal to k r over k r plus k n r right. You can use these two equations to get the two unknowns ok.

Quantum yield calculation or measurement can be done separately not an issue people do it on a regular basis right. So, essentially then the definition of quantum yield phi of F is equal to the number of photons emitted the number of photons emitted over the number of photons absorbed; this is the definition of quantum yield.

So, essentially it is a ratio right; it is a ratio of what? The number of photons which actually come out as light in this case fluorescence over the number of photons, it originally absorbed.

So; that means, if you have 100 photons if it gives out 100 photons in the form of light your phi F would be what?

Student: 1.

1, if it gives out 50 photons accordingly these phi F would be? 0.5 like that good ok.

Now this was your time resolved. So, time resolved means you have excited the molecule right. And then you are looking at it is progress; that means, it is decay from the excited state to the ground state as a function of time right. So, that is why they are like time resolved spectrophotometers right which do this job routinely for you. You have excite and then it comes down and you fit it to a certain routine a certain form some functional form and you extract your parameters tau and all these things.

Now we would not be discussing that at much length. However, I would just you know like to divert your attention to something which we do regularly at least all of you do regularly possibly on a regular basis is steady state fluorescence right.

That means, it is like an Absorption Spectrometer; now you are having a fluorescence spectrometer. And you are sticking your sample into to look at what the fluorescence spectrum is like you had an absorption spectrum for tryptophan, tyrosine all these things these should also be having their corresponding fluorescence spectrum.

So, just give me a little more time we will be done with this. So, going back to the slides; so, this is a typical this is a typical you know diagram or layout optical layout of a fluorescence spectrometer.

Now see what see how similar it is absorption right? And what are the things it has? You have to have a an a source which excites your samples because if the sample does not absorbed it will not go to the excited state no fluorescence.

So, you have a xenon lamp right. Now you have a xenon lamp in this case, but in case of absorbance you do not have a Xenon lamp it is not that you cannot have a Xenon lamp people can have xenon lamps. Only that in that case you had two lamps deuterium, tungsten it is using a more common right.

See after that what it does this because you have to spread this out it goes into a compartment which has the excitation monochromator. So, again you disperse your Xenon lamp beam into it is into it is component wavelengths. I will show you the Xenon lamp spectrum. After that it comes out after that it comes out like this and you can see out here.

So, this is the grating I was telling you this is the part of the monochromator this is a grating when it gets dispersed you can see out here. So, this is your slit; that means, there is an opening out here which allows which by which you can determine; how much of light you allowed to pass through to hit the sample.

So, if you would be doing if any of you would have done study state fluorescence; you would see that in the control panel or in the software you determine something known as slit width. Whether it is 5 nanometer or 1 nanometer or 10 nanometer this essentially what it is.

So, you can imagine one thing this is your beam of light having you know the total output coming in. If you open it you will be having the maximum density. Now you slowly close what will happen? The amount of light that comes through and hits the sample decrease.

Now the reason there is a reason you do it. One of the main reasons is some of the samples are very conducive to photo bleaching; that means, if you have too much of sample coming in or too much of light coming in, then that fluorescent molecule can actually get damaged right irreversibly damaged. So, you would want to stop that.

There are also other things you know sometimes what happens is that your detector what are we detected is has a certain response where it is linear to the number of photons that is coming

in. If you assign too much light then; that means, there would be too much fluorescence and would cross that linear region right.

So, anyway there are host of other things. But the bottom line is using the slits what you can do is you can control the amount of light that is coming in and hitting your sample right. Then; obviously, you have a sample compartment out here right; this is your sample compartment. Now which I will not discuss with you, but I will just quickly tell you is you see there is a beam splitter here. Remember the beam splitter we had in the absorption spectrometer. In this case what it does is; here one is coming to the sample a part of this light is going to and absorbance competing cell so compensating cell; so, this one.

This is called a reference sample it goes to reference photomultiplier tube. And the reason is this; to very to be very sure if you are doing a fluorescence experiment say for 10 hours; that is the time you need, you started with a certain intensity of light before say at 5 minutes you started with a certain density.

Now when you go to 500 minutes there is no guarantee that the intensity you started with would be exactly the same as the intensity you have after 500 minutes. So, what you would see is; if you are doing time consuming experiment, then because the intensity of that lamp has decayed as a function of time during that time interval because it is a very long experiment. The fluorescence intensity would get would depend upon the excitation intensity. So, whatever decrease or increase you would be having would not be related to the sample would, but would actually be an artifact of what the light that is sent through. So, then what do you do is this reference photomultiplier you know what it does? What it does is always normalizes with respect to your beam that is coming in.

So, you take your actual sample and you divide it by the reference photo multiply intensity. That means that one actually tells you or actually normalizes whatever intensity you have and you get the corrected or true not true the corrected fluorescence spectrum. So, that is what you should always use if you are doing it for a very very long time ok.

Specially if you are doing like a thermal melt for a protein doing using fluorescence, it takes time you better do this. That is how you will get the absolute I mean best signal or spectra ok.

So, after the sample compartment it comes out you have reflected out here. Now this sample compartment again goes into an emission monochromator. So, the sample is emitting; it emits light over a range of wavelengths like it observes over range of wave lengths that emits over range of wavelengths. So, to look at what wavelengths are we have to disperse the wave lengths you disperse it with the help of a grating. Now after that it comes to the pmt and you record your spectrum ok.

So this again is just a typical layout of your florescence spectrometer. See there is nothing big about it yeah; obviously, putting these things together the company does it for us. So, we do it right we do not have to worry about this. But if you look at each and every component you would understand that trying to figure out why each and every component is there it is not a big deal. Because you know what why each and every component is there; you know it is not something we can never think of. We know why a monochromator is? We know why a slit is there? We know now at least we know to a certain extent why the reference photo multiplicative is there?

So, all these things we can logically think. If you would ever think about this while you are doing an experiment; you would see that experiment for you becomes a lot better. Because you understand it a lot better now, you have a personal feel about that experiment. And that is why it is so essential for you guys to know what is there inside the box you are using without just treating it as a box ok.

So, that is why I am taking this been to show you the optical layout which is which means it is not it is I mean it is nothing out of the ordinary; just some very ordinary components you put together right. Obviously, expertise comes in terms of you know manufacturing these things and all right and then you put together in terms of photometer.

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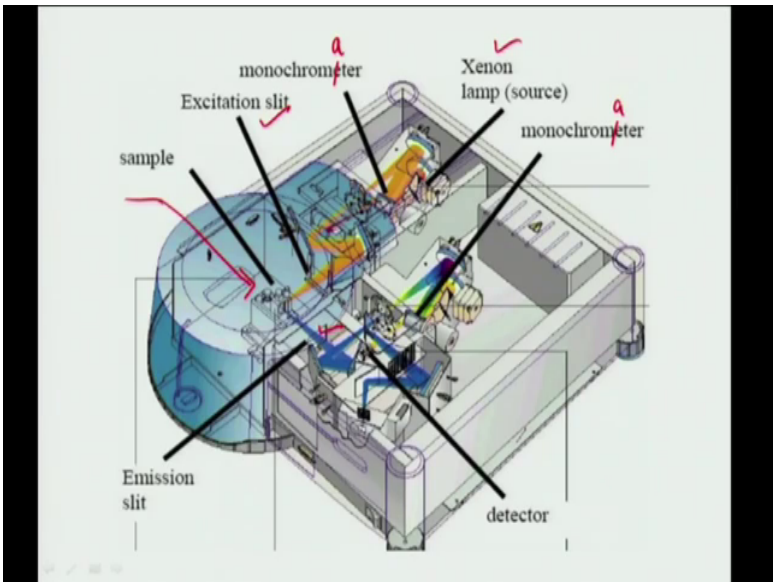

And here, this is a Photometer that people typically use out here. So, you can see this is you know this we have taken the top of a Photometer right. This is no longer a block diagram right; this is a Photometer for you. Now see what happens; is this is a Xenon lamp source right you can see this is a lamp out here right, then this there a lamp.

So, this monochromator so it should be a out here. So monochromator comes here heres a monochromator out here right. From the monochromator you can see the this brown thing kind of gives you the path yellowish brown thing gives you the path of the excitation light. So, it is from the lamp it goes here, there a mirrors, monochromatic comes through. You can see this excitation slit and this is your sample compartment; can you see? This is your sample compartment right. This is sample compartment ok.

Now after the sample compartment what happens is? The sample emits light. So, this is your fluorescence light this is you are looking at if fluorescence comes out, you can see you have a detector out here right. You have another monochromator which is the emission monochromator I showed you out there. Then you have the detector and it detects the light field ok.

So, just think about this based on the block diagram, you understood what is there inside there is nothing big. It is just components put together so that you can collect the spectrum ok.

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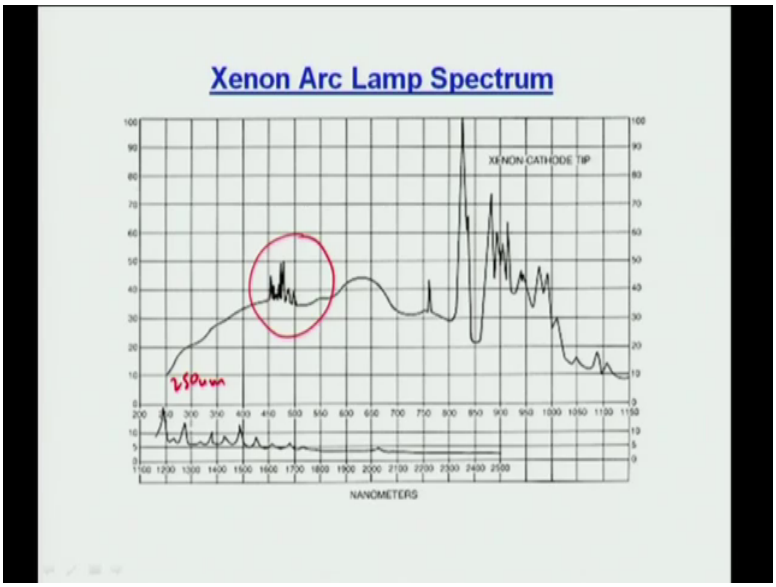

And finally, this is your Xenon Arc Lamp Spectrum. Why did we use that? You can see this is how the spectrum goes; it goes from about this is 250 nanometers on the x axis and it goes to pretty high as you know 1.1 or 1 point 2 micron. Now you can understand why if I would be using a UV visible spectrophotometer and I would not be taking a xenon lamp. Can you tell

me now why? I would rather use a Deuterium lamp than a xenon lamp, why? When I am doing a UV visible experiment UV, I would rather try to start from 200 right. Now at 200 if I am going to look at an absorbance your proteins typically have a backbone absorbance at about 222, let me tell you that.

So, if I would be trying to look at a protein absorbance at 222, would I ever get that with a xenon lamp? Why not? Because you look at the intensity of the xenon lamp. What happens? At 250 it is gone after that there is no nothing else from the Xenon lamp. But if you would use a deuterium lamp, the deuterium lamp you see would kind of go very high up. So, that is why you would use the deuterium lamp rather than a xenon lamp in an absorption xpectrophotometer. But it is not that people do not use it if you want to go to 250 and above no problem you use that xenon lamp ok.

So, and if some of you have ever done this fluorescence experiment; you would see that there is an instrument calibration that goes on. The instrument that photometer it calibrates itself according to this region of the xenon lamp.

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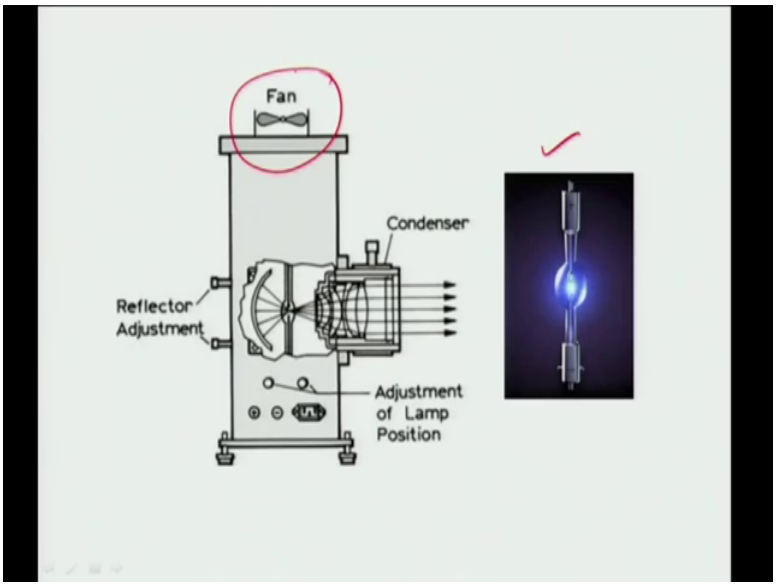

And this is how a xenon lamp typically looks ok. Now this is a personal replace from my side; do not take the cover off and look at xenon lamp; because that might be the last time that you look at anything right. The reason is these are depending upon the power, depending on the wattage. It is a very high luminosity, this is a very high intensities. Even before you realize it will damage your eyes you would never want to do that right. And these also have very high voltages going across them.

So, you can see what happens is; that is why it is not just exposed like that it is housed in a typical housing. What the housing has is you forget this condensers reflect adjustments and all these things. You look at this the fan like when you guys get heated up, you need a fan to cools you so down the same thing happens with the xenon lamp right. So, it is not only we that we that need fans it is also these instruments that need fans.

So xenon lamp because it is giving out so much of light that housing typically gets very warm. So, if you would ever look at the photometer at the place where you have the xenon lamp you will see there is a fan running all the time. It cools it if you do not have that fan running, you would never if you see that the fan is not running never put your instrument on; because it will damage your instrument.

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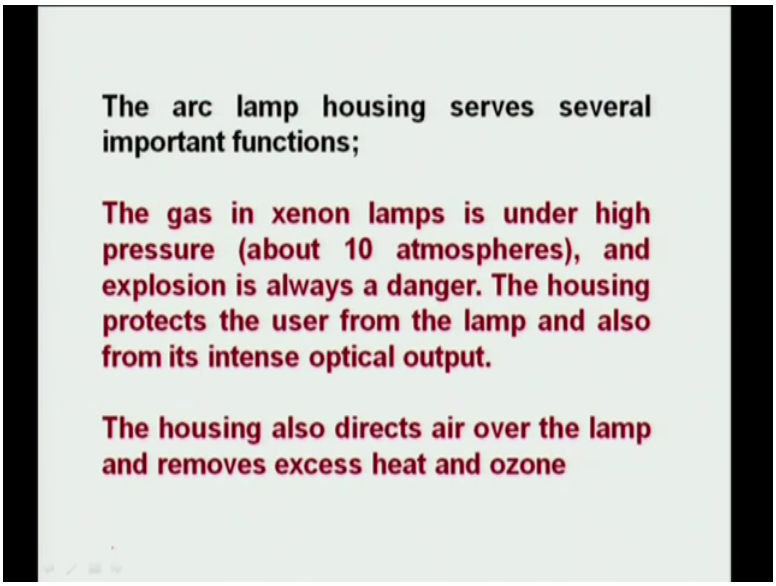

Well these are the functions of the arc lamp as I just said I will just complete it. The gas in xenon lamps is under high pressure about 10 atmospheres and explosion is always a danger. The housing protects the user from the lamp and also from it is intense optical output. The housing also directs air over the lamp and removes excess heat and ozone it also removes ozone ok.

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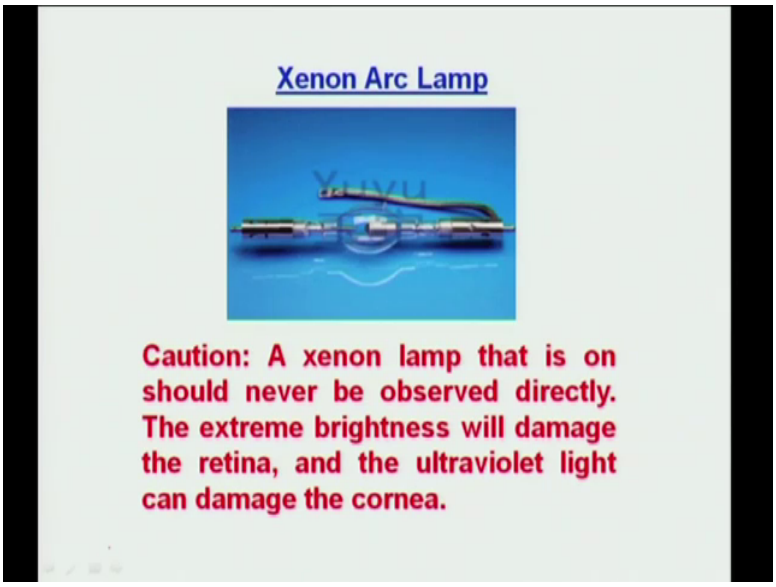

So, again this is the xenon arc lamp and the caution with which we end the classes a xenon lamp that is on should never be observed directly. The extreme brightness will damage the retina, and the ultraviolet light can even damage your cornea ok.

So, you have to be careful see you just do not go and open up an instrument. At least first you know what I should be doing, what I should not be doing. There are many you know each and every instrument comes along with a manual; it is called a manual right.

It tells you how you should be doing things how you should not be doing things. Instead of how you should be doing things, sometimes take a look at how we should not be doing things right; that will sometimes help you really. That is a short thing that is small much smaller than

what you should be doing, but that is where you should start or at least that is a first thing you should keep in mind before; trying to do what things you should be doing ok.