Bio-Physical Chemistry
Dr. Pramit Chowdhury
Department of Chemistry
Indian Institute of Technology, Delhi

Lecture - 37
Spectroscopy: The Franck Condon Principle (Contd.)

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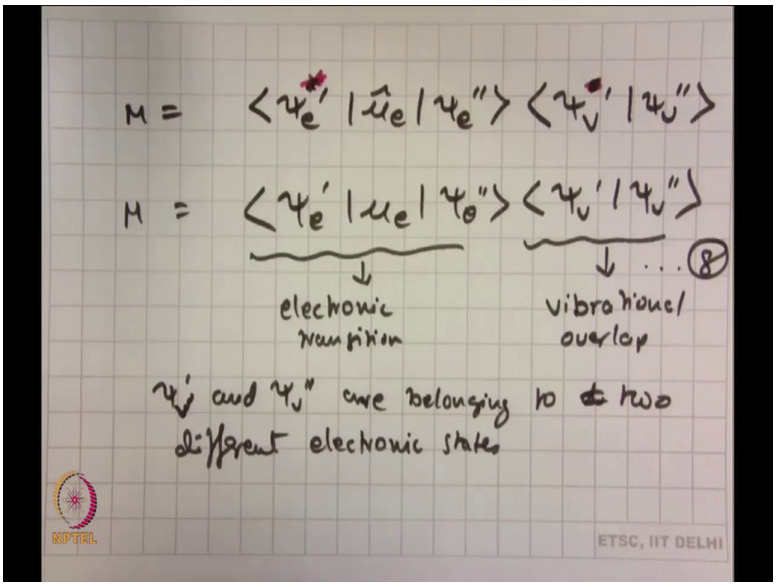

So, we will continue with the discussion on Franck Condon principle. So, if you would remember what we did last class was that we had derived this relation for a transition moment right. And you can see that this transition moment had this expression final expression.

So, there were it could be grouped into two; the first one which has the electronic dipole moment operator, where e stands for electronic right. So it has in the direct notation the psi

the electronic prime ok, which is excited state electronic. Then the mu e the electronic dipole moment operator then psi electronic double prime which is your ground electronics state right.

So, this if you would do this integral, this would be based on your electronic transition only ok. There is no vibration involved now, only pure electronic right. But as we have seen as we have seen that your electronic motion is also to a certain extent governed by the nuclear coordinates right, because it cannot separate it out.

In time scale you can separate it out, but then the electronic coordinates will always or the electronic motion will always have a parametric dependence on the nuclear coordinates. Hence you will be having this vibrational overlap coming into. So, the next one was your vibrational overlap or the Franck Condon overlap.

So, this tells you which to vibrational states one from the ground state and one from the excited state are overlapping most and that is where the most intense vibration or the most intense transition would be ok. Because that is, where you have the largest overlap in terms of the wave functions right.

And we also said this that if you would take the square of this you would get the Franck Condon factor. And the Franck Condon factor is the one which finally, determines the intensity of the transition happening or you are looking at or transition that is happening. That means, the intensity is proportion to the Franck Condon factor which is the overlap squared ok.

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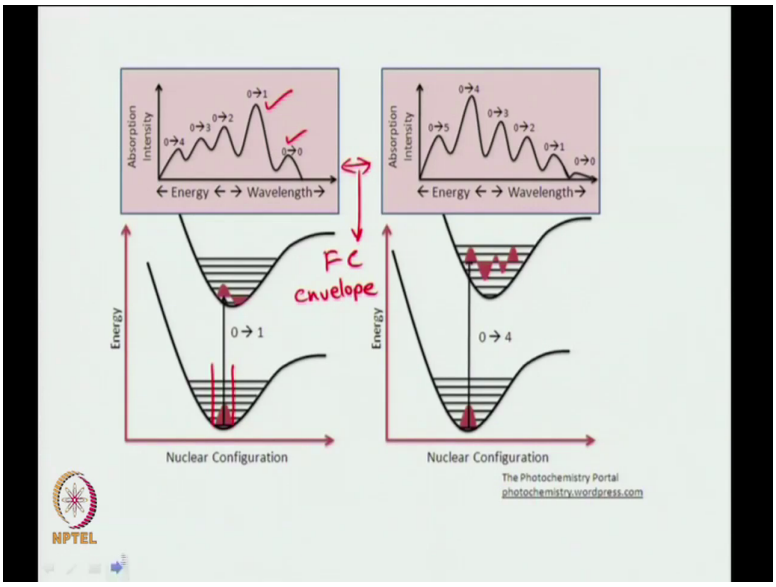

So, coming back to the slides now; so, this is what we you know looked at. Last slide we talked about this Franck Condon envelop right. And what we said was that you do not have only one transition, instead what you have is you have a series of transitions. Series of transition results because of the fact that it is not that the electron density is only there at one position, you have an electron density throughout, let us it is kind of a delocalized thing.

And hence because of this, so because of this you can be having transitions from here, you can be having transitions from here. So that means, the transition can occur throughout the band wherever you have the wave function out there ok, this would give rise to your Franck Condon overlap.

So, that is how you see. So, in the first case what you would see is that, in this case it is 0 to 1 is the transition having the maximum intensity. In the next case, the 0 to 4 is the transition

having the maximum intensity. The difference between these two be in the ladder case; that means, in the second case you have a much larger difference in what? The inter nuclear distance in the ground state and the excited state now that is typically how it comes about ok.

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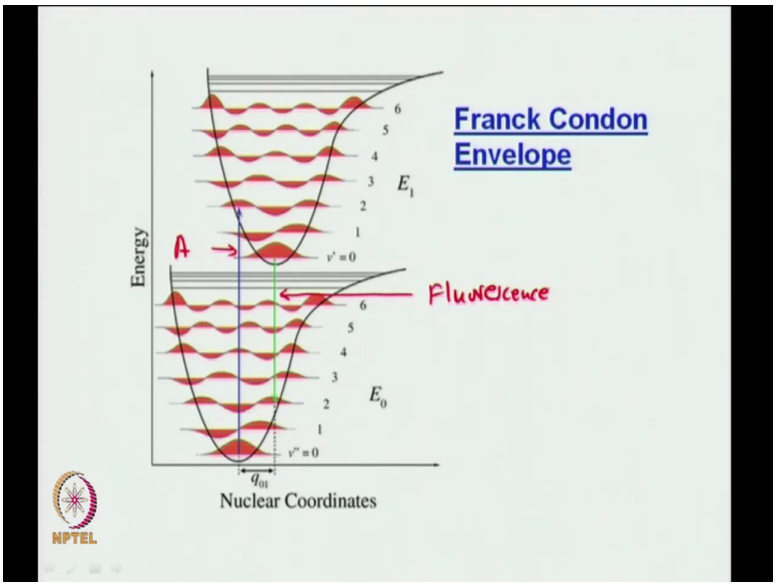

So, moving on again we are looking at the Franck Condon envelope. So, if we take a look at this, I am talking about this at length, because if you would up any absorption spectrum, so mostly of organic molecules you would always see these structures coming in ok. And what are these structures? Due to these structures are essentially your vibronic transitions, that means, your transitions where vibration is coupled to you electronic motion or electronic transition, you would typically see those.

Now, obviously to what extent you see those will also depend upon the conditions, that means, the solvent conditions and everything right. But again looking at this Franck Condon

envelope, so between the ground state and the excited state where the ground state is E 0 the excited state is E 1. Then here you see that the way this one is drawn, that means, the way the higher potential energy was shifted. What is happened is the which one would be the more most intense transition for the absorption in this case, which one do you think would be the one, intense one?

Student: 0 to 2.

0 to 2 right. So, the most intense form would be 0 to 2 right. Now, this was your absorption right. So, this is your absorption, A absorption right. Now, what happens is if it is going to go up its also going to come down right, it can come down either by emitting light, that means, emitting photons or by not emitting photons. Say if it emits photons, it would be called radiative transition.

If it does not emit photons it would be called radiation less, that means, it does not radiate any photons, it does not give out any light ok. So, does not matter whichever way it comes down. If we look at it the coming down would be very similar to the way it went up. That means, if it went up vertically the coming down will also be vertical because the coming down is again the moment of electrons right.

So, again the Franck Condon transition is not only vertical in one direction is also vertical in the other direction. Because what are you playing with your essentially dealing with moment of electrons right. So, essentially now what you can see out here is based on this, if this green one, if this green one is well say it is florescence, I will come back to florescence later.

If this green one is florescence, so even for florescence now tell me which one would be the most intense transition? So, here it is occurring from v prime is equal to 0 in the excited state and coming down to a higher vibrational level in the ground state. Now, which one is it? It is again 0 to 2.

Student: 0 to 2.

Is not it? Ok, so that means, while going up you had 0 to 2 as the most intense; while coming down you again have 0 to 2 as the most intense. And this you do not have to think about it is just evident from the way the picture is being shown or the picture is drawn that is where you have the maximum overlap ok.

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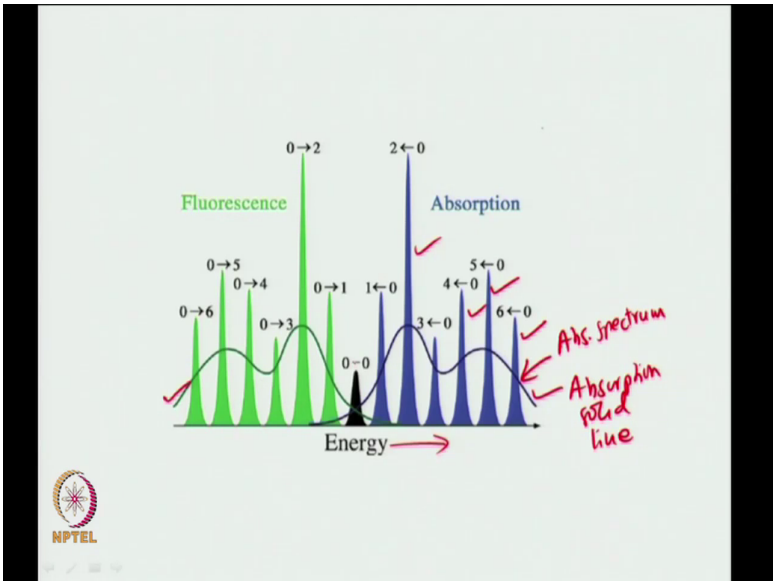

So, then if you would draw this Franck Condon overlap, this is how you would it would look. So, let me tell you the blue one is the energy, the green one is florescence right. So, energy absorption is always higher energy than florescence. Remember there is something known as Stroke shift we will come to that later, but anyway, say if this is energy, so on the higher energy side, that means, to the right side, you have the absorption bands, to the left side you have the florescent bands right.

So, what you can see out here is in the absorption as we discussed the 0 to 2 is the most intense one; similarly, from florescence 0-2 again is the most intense one, but we have to have a overlap that means we have to have a progression because the other transition is also available and that is how the other intensities go ok. So, these are now, so you are just plotting the respective transitions.

Now, remember one thing. This we will not be able to cover in details, but try to appreciate the fact that if you would be having this in a solvent, I am not talking about the gas phase, I am talking about the solvent say water or say any other solvent, what will happen is your solid molecules will always collide right.

So, because of this collisions and because of this interactions what will what it will do is, it will not keep your spectrum as narrow, it will actually broaden it because you have so many interactions with the surroundings solvent molecules ok. This is called a type of broadening coming in ok.

So, because of this broadening, what you will see is when you finally see the spectrum, you would not be able to see these individually like these ok, you would not be able to see these individually you know. Instead what you would see is you would see these possibly depending upon how much of interaction how much of broadening you have, they would be merging with each other ok.

And how do you visualize that? It is very simple. You look at this line. You look at this green line and this blue line out here. What is the solid line, can you tell me what is this solid line on the right side, that means, along with the absorption? First, of all see the way the solid, the way the solid line goes is see the solid line, see if you are talking about this solid line this is the one which is the absorption one right, the absorption solid line, it is the one you are going with where does it max, it max at 0 to 2 right. Then the next maximum whatever 0 to 5 or whatever ok.

So, essentially guys this is your absorption spectrum. See if you would take an absorption spectrum of this sample in any given solvent say, this is how the absorption spectrum would look like. And what you can see in the absorption spectrum is that you do not any longer see those vibrational structures.

The reason being that they have merged together because of too much of broadening right, too much of interactions that is why, but it is not; it is not like this that you will never see those, it actually also depends upon the rigidity of your molecule, how rigid your molecule is how not flexible your molecule is ok, but anyway.

So, if this is the absorption spectrum, so this is the absorption spectrum essentially, so this is your absorption spectrum. Similarly, if you go to the florescence, you also look at your florescence spectrum ok. Now, just to make a passing comment, just to make a passing comment, two things, one is this 0 to 0 transition which you see here, the 0 to 0 transition which you see here ok. This is the transition we should be, this is the transition we should be equi energetic for both florescence and absorption right. You are going from 0 to 0 that means in absorption you are going from 0 to 0 in terms of florescence you are also going down from 0 to 0. So, they coincide, they follow the same place ok.

Number 2, number 2 is you look at the florescence, in the case of the florescence, the florescence spectrum and the absorption spectrum if you forget everything else; it if I place a mirror like this in the middle, if I place a mirror like this in the middle. If I place a mirror, would not you say that your florescence spectrum is actually a mirror image of absorption spectrum?

Student: (Refer Time: 10:13).

Right, you place a mirror and you will get the mirror image right. So, this is called mirror image symmetry or mirror image rule ok. Now, this I will discuss this later, but just to let you know because I we have this picture in front of us, this arises because; this arises because

whatever vibrational levels are involved in the absorption, the same vibrational levels are involved in.

Student: Fluorescence.

Florescence that is essentially why it comes ok. So, in most cases, in most cases, specially in cases where you would see the molecules are very rigid, there were not too much of change in the equilibrium bond length, this mirror image symmetry is typically maintained ok.

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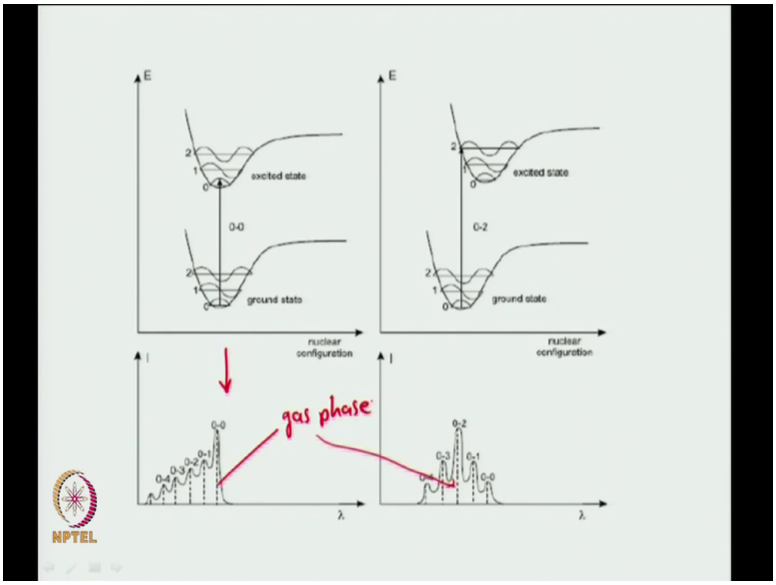

Now, this is another example see the another example is again for the same Franck Condon overlap. So, you look at the one which is on the left and the one which is on the right. On the left you see there is almost no change in nuclear configuration; that means, r equilibrium is the same. And hence if you come down you see its a 0 to 0 transition is the one which is the

maximum ok. Similarly, if you go to the right where your r equilibrium has changed, then what happens is here 0 to 0 is no longer the maximum, but 0 to 2 has becomes the maximum ok.

Now, this is what you would ask, what you would ask is ok, I have this the solid lines. Now, what are the broken lines? Can someone tell me what are the broken lines are? Always that the broken lines are the one which correspond to the peak right. So, 0 0 where wherever we have the peak we have the broken line; then 0 1, 0 2 ok. What are these?

Student: Wavelength (Refer Time: 11:42).

Well, this is a wavelength, it is on the wavelength scale that is understood right. So, remember, 0 to 0, has the lowest energy say it will be having the highest wavelength. Any other transition 0 to 1, 0 to 2, 0 to 3 would be having a higher energy, and hence would move to the lower wavelength side ok. In the next slide what you we will see is instead of lambda, we will be plotting it in terms of centimeter inverse, and then it would just swap right.

But anyway coming back to the broken lines, you know where the broken lines are coming from? If you would take this molecule ok, the solid lines, the solid lines are coming from this molecule being in condensed phase say in a certain solvent depending upon what it is an organic solvent or water.

Now, what happens if I take the molecule out of the gas phase, what will happen? If I take the molecule to the gas phase, what are you getting rid of you are getting rid of the solvent molecules right. But by getting rid of the solvent molecules what are you getting rid of?

Student: (Refer Time: 12:52).

You are getting rid of interactions that means you are getting rid of a lot of broadening. Say if you take this molecule and look at the vibration transitions or the vibronic transition of the gas phase, the broken lines are the ones which are the spectroscopic transition, you would be seeing in the gas phase of the same molecule ok.

So, the broken lines, these broken lines are those collected in the gas phase for the same molecule. So, this essentially tells you when you go from a gas phase where you have no solvent molecules none of these broadening when you come to the solvent state, you see how broad your spectrum becomes.

I will give you a simple example this you guys know ok. It is not from florescence, it is from vibrational transition; it is not from electronic, it is a vibrational transition. Now, think about the OH stretching band of water in the IR, where does it what is the range of OH stretching band?

Student: 3600.

3600 to?

So, 34, 3300. Why is it so broad, have you ever thought about it?

Student: Hydrogen bonding interactions.

Right, hydrogen bonding interactions. So, you have different types of hydrogen bonding out there, and hence that gives rise to many different vibration transition, and hence increases the width of your spectrum ok. The same thing is happening here. There hydrogen bonding is happening with a solvent water or whatever solvent you have here in the gas phase, you do not have that, and hence it becomes so narrow ok. But it does not mean that it is a single line right, it still has a certain width, please remember that ok.

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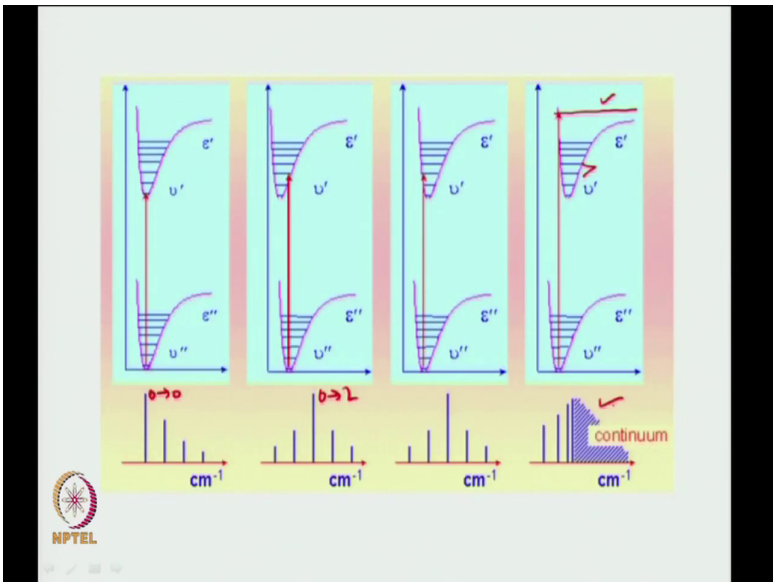

So, again you go from left to right, see what happens the left one there is no change, so it is 0 to 0 transition. So, this is the 0 to 0, 0 transition is the maximum. Now, see what happened is I have changed the scale. Initially the scale was lambda, now the scale is what?

Student: Centimeter.

Centimeter inverse. So, because it is proportion to energy the 0 to 0 one is the one which should be having the least energy, that means, the least centimeter inverse ok. Because delta e is equal is equal to what, h c nu bar right, and delta e is proportion to your nu bar.

Now, when you go to the next one, now you can see, so here what 0 to I guess 2 right. Then in this case, it is in this case is also 0 to 2. Interesting case is the last one ok, the first you kind of have seen you kind of would realize what is going on, tell me what is happening in the last

one? Tell me what is the difference between the first two and the last one? Do not look at the spectrum down, just look at the two potential energy curves, and tell me what is the difference?

Student: (Refer Time: 15:26).

See, where this arrow ends up? Where does this arrow end up? This arrow ends up here in the last one. See if I take this, if I draw vibrational level out here, the vibrational level go like this ok. In all the other cases, in all the other cases, the arrows were not that high up right, the arrows were already there, the arrows were always there where you had these blue lines streaks going through. So, what is the difference between the red vibrational level I draw drew and the blue ones in the same potential energy curve, that is what is the difference between these between these ones and this one, what do you think is the difference?

Student: (Refer Time: 16:04).

Well, I would not be saying no vibration level but go ahead just.

Student: Closely spaced.

Well, closely spaced, still a little more.

Student: Dissociation.

Dissociation. Thank you. See when you have when you have these vibrational levels these are bound within your potential energy right. See, why does quantum mechanics arises, where you know where do these you know quantum numbers arise from, typically boundary conditions, nothing else. The moment you impose boundary conditions, you have quantization right that is what quantization is all about. Now, when you go to this, this one, this is place is it any longer bound.

On the other side because you see your potential energy is already ended out here, that means, its looks like this and your vibrational level goes like this. That means, this one is already beyond your bound state, it is no longer a bound state. If it is no longer a bound state, what is it mean? If it is no longer a bound state if it is no longer a bound state, what does it mean? That means, the molecule has a probability dissociate out there because it is not bound anymore. So, the molecule will go and dissociate.

The moment the molecule dissociate what will happen is now think about this, if you are going to a non bound state, what happens to the quantization, do you have quantization anymore, no right? Because there is no bounds anymore no quantization. So, the spectrum becomes what, continuous.

Student: Continuous.

Now, come to this spectrum down, and do you understand where the continuum comes from ok. So, the continuum comes from the fact see that is the one where it is most intense, that means, if you are doing the vibration transition to that place to that place, the molecule would be having the probability of dissociating.

Student: Dissociating.

So, this is the transition which ends up dissociating your molecule right. See the fact is very simple. The fact is that if you think about this about it like this. You do a transition right and you have a change in bound order say. So, you have such a huge change in bound order, that means, there is a such a huge change in your equilibrium, that means, the nuclear moved so far apart that they almost feel no attractions right, and hence its very for them to come apart.

So, hence they dissociate, so that is typically what happens when you do a photo dissociation. That means, you go to the excited state surface where your things are not bound, you have a dissociated potential energy surface and it dissociates ok. Here I am not showing a dissociated potential energy surface. What I am telling you is that on the same excited potential energy

surface, the vibration level excited at is typically the one which is not bound by your potential energy, and hence it is a continuum.

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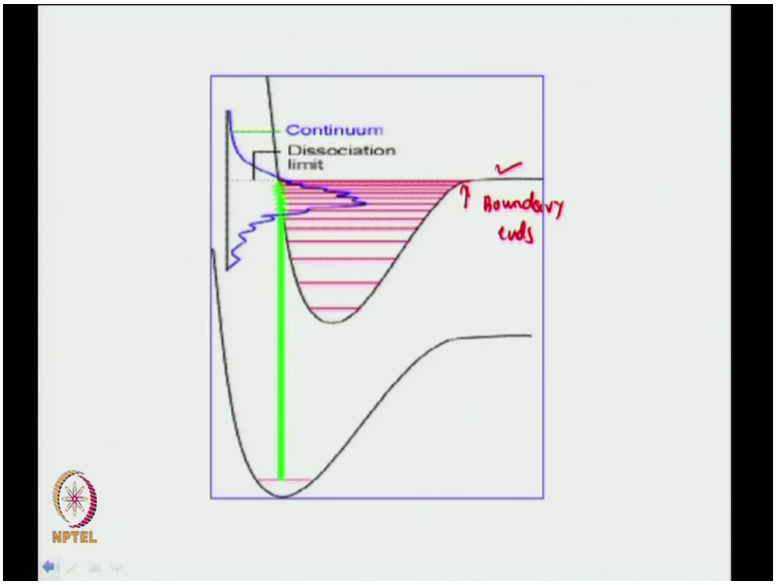

So, that is why this continuum results. To tell this to a little more clearly you go on to this part ok. So, this is exactly what the thing you are seeing out here. So, here see the green line goes here right, you can see already remember in an harmonic oscillator as you go up what happens to the spacing, they decrease anyway right. When you come here; when you come here, your boundary ends. So, any vibration level, on top of that would actually be not bound. And, if we exciting to that level what will happen is it will easily dissociates, so that is what its referred as a dissociation limit.

So, you can understand if you are doing a transition out here, up till here it is bound; up till here it is bound any transition beyond that will induce dissociation of photo dissociation in the

molecule ok. Now, try to connect this to what we have done just in a simple way, remember we talked about this optical triggering by flash photolysis. Do you remember that?

Right, that means, we have we have breaking the bond between the CO, that means, this carbon and the iron. So, this essentially what was happening, so that means, you would do, you would hit it with light in the certain way they should absorb right. And that light would be such that would absorb it would go to a state where that bond would break, that means, you are into a dissociative, dissociation region and the bond snaps ok.

So, this is another way of looking at it ok. So, this was about your Franck Condon overlap you know whether you are looking and a absorption spectrum that is what we have mostly talked about, whether you are looking at a florescence spectrum. And I as I said in many cases florescence spectrum is a mirror image of absorption spectrum ok. This is how the transitions happen. If you are looking at one side like this, the other side typically follows. So, you can just parallelly draw those transitions, and see which one is the maximum ok.

Now, let us talk about this optical phenomenon known as absorption ok, this technique. So, the basic equation in absorption is what the Lambert-Beer's law alright that is what you use, good. So, let us look at that Lambert-Beer's law real quick.

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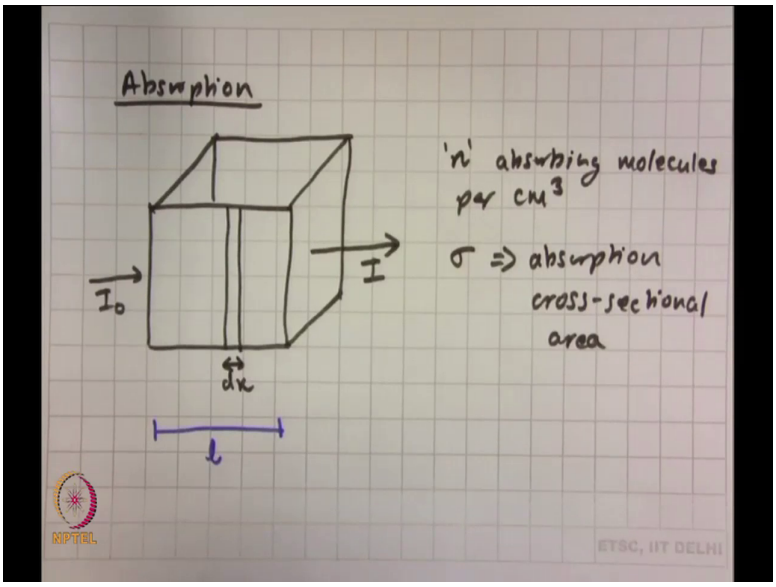

So, what I do is, I will actually derive Lambert-Beer's law for you real quick. So, it is I have a section of my solvent, that means, my sample which has the absorbent molecule in a solvent ok. And what I do is, I take a section like this and I say that the incident light on this is I 0 has having the intensity I 0, and the light that is coming out of transmitted is say I ok.

Now, what I do is in this I take a very small section, I take a very small section. I say this is d x very small section d of x. And what I also give you is the length the length the length of this is l the. So, total length is l, the total length of the absorbing medium right. So, what is I 0? I 0 is the intensity of the incident light, and what is I? I is the intensity of the transmitted light, I am not writing those things now ok, this you guys know.

Now, let us assume that we have n absorbing molecules per centimeter cube, that means, per unit volume, where the volume is in centimeter cube ok. What I also say is that let the absorb,

let sigma this is called as sigma be the absorption cross sectional area ok. Let this be the absorption cross sectional area. Now, tell me when you are going to have absorption right, so suppose you are hitting into the I 0, then the I comes out, so that means, if it is going to absorb there would be a difference between I 0. And I and that is going to tell you how much it is absorbed right.

So, suppose if I am looking at this fragment, if the if I am looking at this infinitesimal element d x, then the corresponding intensity change say its d I ok. So, what would d I by d x be proportional to? That means, the amount of change in intensity I have over that unit interval over that interval d x should be proportional to what first tell me? There would be three factors I am telling you. Let me give you one hint is the number of molecules right, obviously, because the more molecules we have more will absorbing. What is the next one?

Student: (Refer Time: 24:06).

Intensity, very good, the intensity with which you are hit. What is the other one? It is written out there.

Student: (Refer Time: 24:14) cross section.

Cross sectional area. It is cross sectional area right. Because if you have a this molecule which is like this, and if a molecule is bigger, then obviously, this one would interact more and would be absorbing much more.

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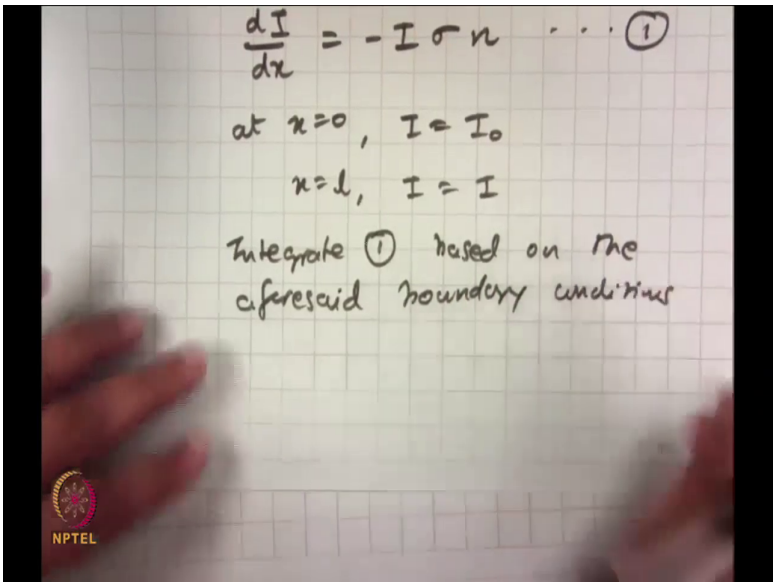

So, then based on this what you can write is d I over d of x is equal to minus I sigma n, and let this be 1 simple right. And that is what I just said it is a negative sign because you know that its being absorbed the d I would decrease means intensity is going to decrease ok. So, now, what I do is, I impose boundary conditions, that means, I say that at x equal to 0, that means, at x equal to 0, I equal to I 0.

Now, what is x is equal to 0 mean? x is equal to 0 means this. See I have taken d x. So, x is equal to 0 mean means would be this one, x equal to 0, so that means, x is equal to 0, where it has not yet enter the sample I have the intensity I 0, that means nothing has absorbed good.

Now, what I also say is at x equal to l, what is l? l is the other site, because l was the total length. So, then this I would be I right this much is coming out. So, then what I can write is if

I would integrate one based on the aforesaid boundary conditions, based on the aforesaid boundary conditions this is what I am going to have.

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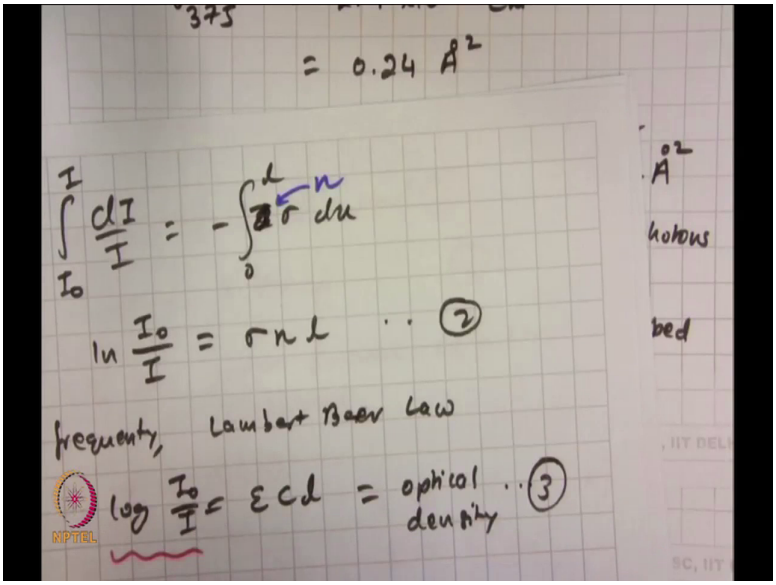

What I am going to have is I am going to have d I over I is equal to minus I sigma d of x ok. Integrate say you are going from x is equal to 0 to x is equal to l you go from I 0 to I ok. So, there I can write is.

Student: Sir, there will be n.

Ah?

Student: y there will be n.

Right, sorry, this should be n right, thank you. So, here n would be coming in. So, l and I 0 by I should be equal to what from here? Now, see I have changed the order a little bit. So, this should be equal to what, I should be having sigma, I should be having n and then I should be having l. So, this is equation 2.

See this is a form of a Lambert-Beer's equation ok. So, this is a form of your Lambert-Beer's law right. The only thing is this is not what you use frequently. What you use frequently rather is what you use frequently is the frequently the Lambert-Beer law is expressed as log of I 0 by I is equal to epsilon c times l right.

And this is equal to your optical density, and this is equation 3. And this is equation 4 ok. So, the bottom one is the one you use frequently. Now, obviously, these two are coming from the same principle. So, these two would be comparable, these two would definitely be comparable right.

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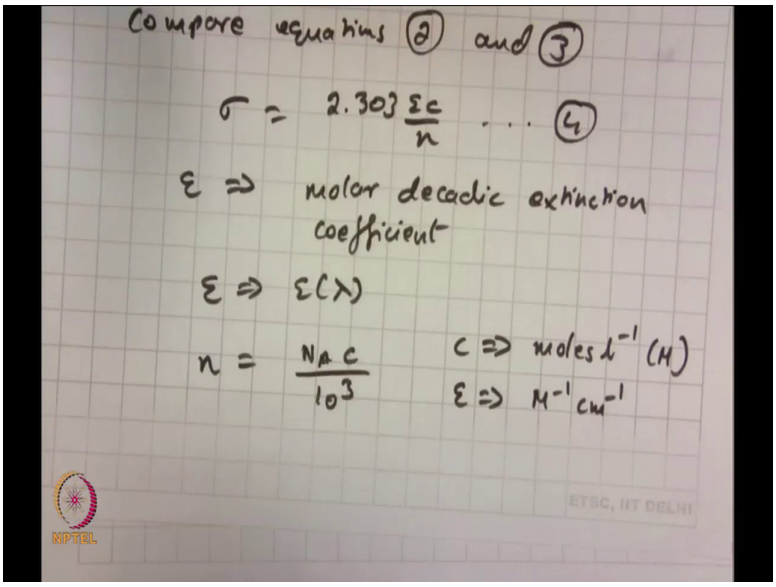

So, you compare these. So, what you do is, you compare equations 2 and 3. And this is what you end up with; what you end up with is, sigma is equal to 2.303 epsilon c over n. This is 4. That means, your molecular cross section area or absorption cross section area rather not molecule absorption cross section area is equal to 2.303 times epsilon c times c over n. 2.303 comes from that conversion factor ln to log to l n like that.

Epsilon is referred to as the molar decadic extinction coefficient ok. Please remember one thing this epsilon this epsilon is intrinsic to that respective molecule, and this epsilon also depends upon lambda it is not the same for all lambdas ok. You will see that soon. So, I can say epsilon is essentially a function of lambda, please keep that in mind, it is always a function of lambda ok.

Now, what I do is from equation 4, what I can do is, I want to replace n what I want to bring in. What is n? n is the number of molecules per centimeter cube that is what I have said. Now, suppose I want to find the number of molecules, how would I do that? So, for example, if I want to do n, what was concentration c? c is moles over litre.

Student: (Refer Time: 30:00).

Ok, c is always c is always moles over litre ok. If I am going to get the number of molecules of what would I do? I would multiply that by Avogadro's number.

Student: (Refer Time: 30:10).

N a ok. So, I would be having N a times c, but this is the number of molecules per what? Per litre, but what was my volume unit in this case?

Student: Centimeter cube.

Centimeter cube. So, what I have is N a times c over 10 to the power 3, and please remember c is given in moles per litre ok. So, epsilon comes in mole inverse centimeter inverse. So, this is m, and epsilon is mole inverse centimeter inverse. You can understand one thing, one thing is this log of I 0 by I should it be having any units? It should be unit less right?

Student: Yes.

Where it is a ratio of two similar things, it should be unit less no unit out there ok. So, that is how you get this. What I did not mention was because your volume is in centimeter cube, your l has to be in centimeter right, so that means, I can also write your l is in centimeter ok.

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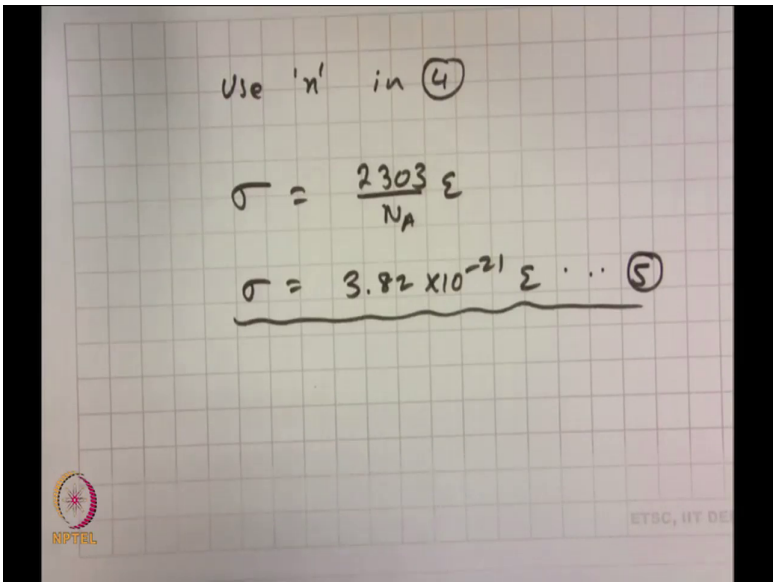

So, finally based on this if you use this value of n in 4 that means, use n in 4 what do you get is sigma will be equal to 2303 over N A times epsilon. So, what you are looking at is a relation between something fundamentally you always know which is the extension coefficient or the absorption coefficient from beers law.

Student: (Refer Time: 32:07).

To something which you probably has not realized before, which is the cross sectional area of absorption of that molecule that is something which is very fundamental. And you tell you the truth if you are doing spectroscopy, hard core spectroscopists would not talk in terms of extinction coefficient, they would rather talk in terms of cross sectional area.

Because you see when you did the derivation you never talked about extinction coefficient; what did you talked about? You talked about the area of this molecule interacting with the incident radiation ok. So, this is something you know much more fundamental in terms of a spectroscopists point of view ok, good.

So, if you do the math, then you will see this sigma would come out to be about 3.82 times 10 to the power minus 21 times epsilon, let this be 5. So, there is a relation and I will give you an example by taking a molecule, ok.

Student: (Refer Time: 33:11).

So, let me repeat again what a cross sectional area is when light is coming in that means, photons are coming in photons have to interact with their molecule right. If the photon is going to interact with a molecule, the molecule has to present a certain cross sectional area for interaction. This is the cross sectional area you are talking about.

Wherever the cross sectional area of interaction is high, your absorption would be high; wherever the cross sectional area of interaction is low, your absorption would be low and that would lead to your corresponding extinction coefficient, so that is why it all starts from the cross sectional area, ok. Now, let us see whether we can give an example and try to realize where you know what we are talking about, see thing about this molecule anthracene.

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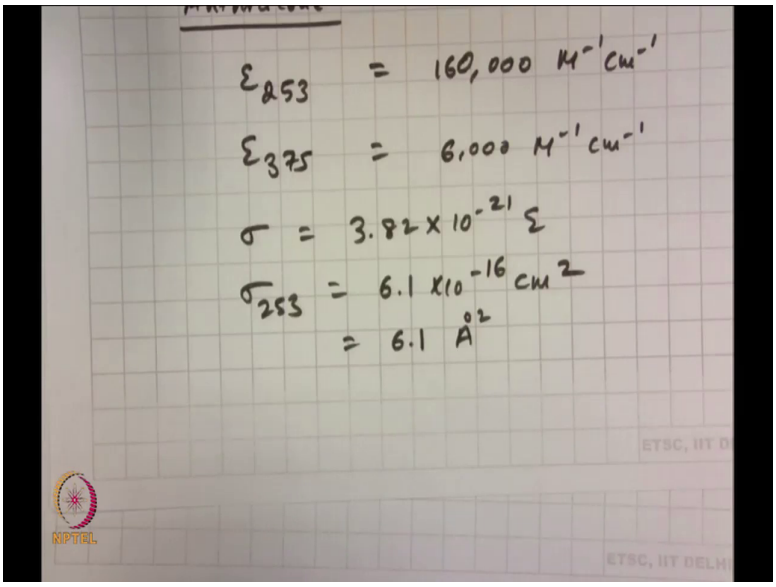

This molecule anthracene, for the molecule anthracene what you are told is that the epsilon value at 253 that means, 253 nanometers ok, so that means, the extension coefficient at 253 nanometers is given by this number; its 1.6 times 10 to the power 5, ok.

Similarly, it also absorbs at another wavelength where the extension coefficient is given by this is 375; this is equal to 6000 mole inverse centimeter inverse. Now, see the difference; in one case the extinction coefficient is very high, in the other case the extinction coefficient is very low. So, you can so you can immediately absorb or understand that if you would shine light of wavelength 253 that would be absorbed much more by the molecule than shining light at what 375 nanometers, ok. And that is what I meant by saying that this epsilon depends upon your lambda, it is a function of lambda.

It will become more clear, when I show you the absorption spectrum of tryptophan, but just hold on. Now, if this is the case, let us calculate sigma we know what sigma is right; we know that sigma is right, we know that sigma is 3.82 times 10 to the power minus 21 right times epsilon, this is from 5. So, let me give you the value say for sigma 253, the sigma 253, if you do the math, it is about it comes to 6.1 times 10 to the power minus 16 centimeter squared, ok.

Student: (Refer Time: 35:46).

And please remember if sigma is a cross sectional area, what is the unit of sigma? Centimeter squared; volume was centimeter cube, length was centimeter, then sigma has to be centimeter squared, I should have mention this before I forgot. So, centimeter squared 10 to the power minus 16 right, so I can write it as 6.1 angstrom squared ok, 6.1 angstrom squared. So that means the cross sectional area, the absorption cross sectional area at 253 for anthracene is given by this value 6.1 angstrom squared.

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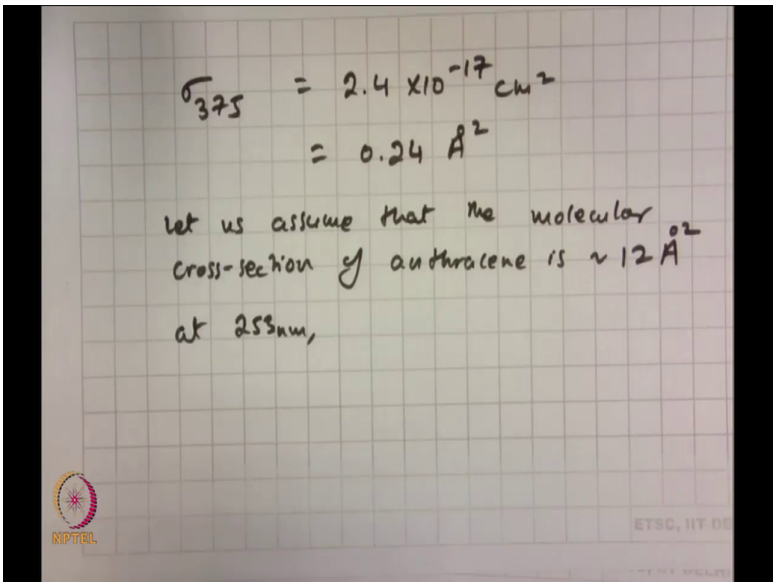

Now, similarly if we do the same thing for sigma 375, you will be ending up with the value which is 2.4 times 10 to the power minus 17 centimeter squared which is 0.24 angstrom squared ok, this is the cross sectional area at 375 absorption cross sectional area, ok.

Now, let us assume this let us assume that the molecular cross section of anthracene is 12 angstrom squared. Let us assume that the molecular cross section of anthracene is 12 angstrom squared ok; so that means, this is the cross sectional area presented by the molecule, it is not necessarily the absorption cross sectional area. Every molecule would come with an area, and this is the area talking about ok. Please, make the difference between this and the cross sectional area ok, so this is the area of the molecule.

Now, based on this value and based on the values you just got, based on the values you just got, you think about this at sigma 253 that means, for sigma or at 253 nanometer. If you

shining this light, if you shining this light, what was the absorption cross sectional area in that case 6.1 angstrom squared, what is it for the full molecule?

Student: (Refer Time: 38:05).

12, so what it is saying is; what it is saying is whatever incident photons you have, whatever incident photons, because 6 is half that of 12; so that means, at least 50 percent of the photons would be absorbed by the molecule at any given time. Why? Because your absorption cross section area at that wavelength is just half of your total molecular cross sectional area.

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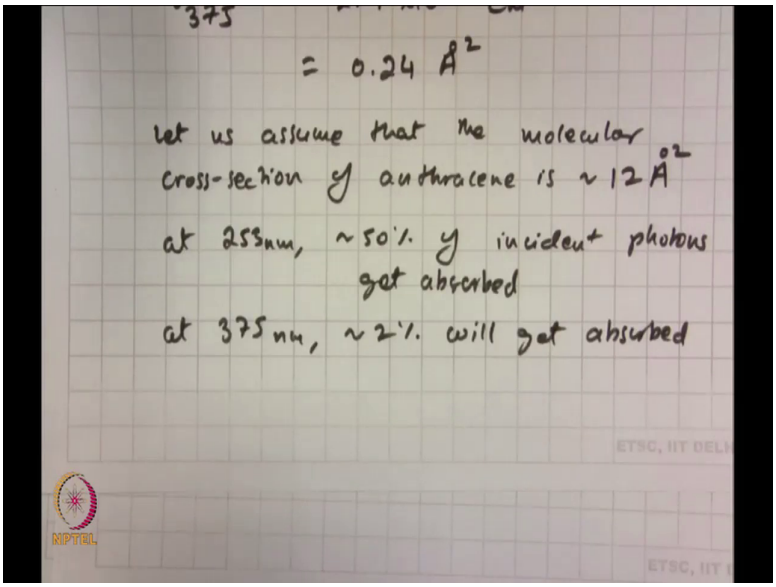

So that means, at 250 nanometer almost 50 percent of incident photons get absorbed, then you can realize that at 375 nanometers only about 2 percent will get absorbed, only about 2

percent will get absorbed, ok. So, this is an example where hopefully, I could you know convey to you the importance of cross section area.

Again I tell you, if you would suppose synthesize the dye right, suppose you would synthesize the dye or synthesize the compound, and you say that I have found this compound to absorb very strongly at certain wavelength. When I going to do this, the first thing someone would ask you is what is the absorption cross sectional area; you would not actually ask you what is the extension coefficient, well it is the same as we just realized, they just differ by a constant right.

But what they would ask you is what is the absorption cross section area, because that is much more fundamental that means, that is the one which goes into the equation when you derive Lambert Beer's law, you do not start with extension coefficient, because you do not know what it is, ok.

But just remember what does extension coefficient tell you? What it tells you is if you have a high extension coefficient that means, it will absorb strongly. If you have a see what was your absorbance? So your absorbance that means, your optical density was given by this equation right, let me show you that this is what it was given to you by right.

So, this is what we derived; this log of I 0 by I is your optical density essentially absorbance. This is equal to epsilon C times l; C is the concentration, molar concentration; l is in centimeter. If these two are constant, if these two are constant that means, you are taking the same concentration of any two compounds and taking the same putting into the same cuvette that means, having the same path length right, then what would the absorption depend upon?

Student: (Refer Time: 40:46).

Extension coefficient. So, what extension coefficient tells you is the probability or the intrinsic probability that a molecule has towards absorbing a certain radiation, it is it is related to probability ok, now think about this. What is the other thing you have found which is related to probability, here you see extension coefficients related to the probability of absorption.

What was the other thing, remember we talked about intensity what was that the probability of absorption, the intensity of absorption what was that? The Franck Condon novel app remember?

Student: (Refer Time: 41:32).

Franck, [FL] do not forget Franck. So, anyway that means these two have to be related; so these two are conveying essentially several information ok, try to relate these two things. We are coming from two different points, but you finally merge with the same thing, because you are talking about the same transition, your talking about the same phenomenon right, ok.

So, this was about you know the derivation of Lambert Beer's law, please keep this concept of molecular absorption rather absorption cross section area in mind, because this is very important. So, let us you know go back and some something a little more interesting, where you which actually do in practical life ok.

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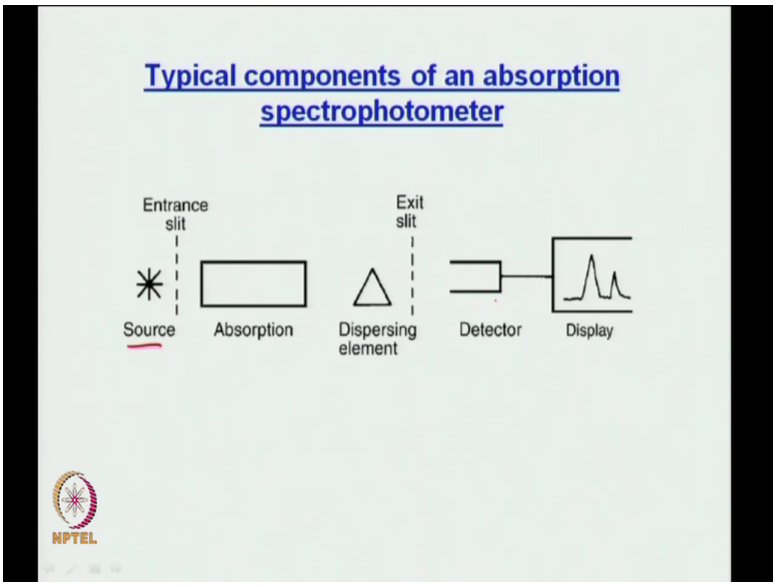

If you are doing an absorption, this is the typical component of an absorption spectrophotometer ok. The typical component of the absorption spectrophotometer has two things or few things, the first one is a source; source means you have to shine light, because it you remember the rho nu that energy density, you have to have that energy density, so that energy density comes from the source of the first one right.

Then you have an entrance slit, where do not worry about that then you would be having your sample which would absorb ok; after absorbing, it would whatever light is absorbed it tries to disperse. So, it would disperse using this is the prism you see and finally, it goes to the detector where do you finally get your absorption spectrum, ok.

So, these are the main components its simple you have a source, you have a sample, you have a detector and between you have some optical components, this is what you need ok. It is

essentially what you are looking at you are looking at the change in transmission. So, what is transmission if absorbance is log of I 0 by I, what is transmission?

Student: (Refer Time: 43:19).

Log of I by I 0 right, good. So, now.

Student: (Refer Time: 43:25).

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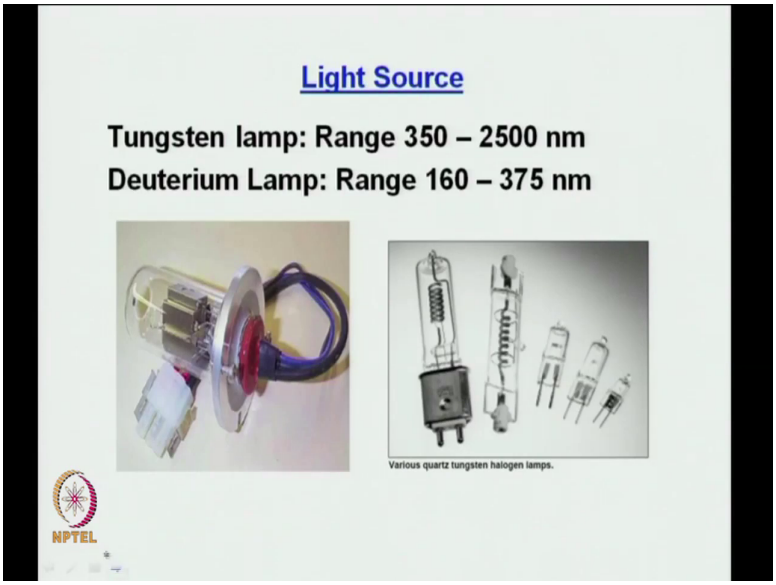

This is about a light source, there are in common UV visible spectrum photometers, there are two light sources you use ok, generally. One is it is called a UV visible spectrometer right, so that means you would be having a UV part you would be having a visible part. The one which is responsible for the UV light source is a deuterium lamp ok; if you would ever look into an

absorption spectrometer, do not be afraid I will encourage you to look into it, ok. You would see these two lamps; the first lamp is a deuterium lamp. So, this is how deuterium lamp looks, so this typically how it would look.

Now, since I have given you the picture you can take your laptop out there right in front of the computer and compare it with the lamp you have inside, it should match; if it does not match, come back and let me know ok, then I will know something new. Anyway, on the right side is your tungsten-halogen lamp ok.

So, the deuterium lamp look at the range of the deuterium lamp what is the range? It is from 160 to 375 nanometers, it cannot go beyond that that means, to go beyond 375 what you would need? You would need another source. So, for that source you use the tungsten lamp, you look at their range the range is from 350 to 2500 nanometers, huge range right ok.

So, generally when you do absorption the UV visible, you go from say 200 to 800 ok; you do not go beyond that. Because beyond that it is called near IR and you would need another detector, but it can be done if there are an IR spectrophotometers available, ok.

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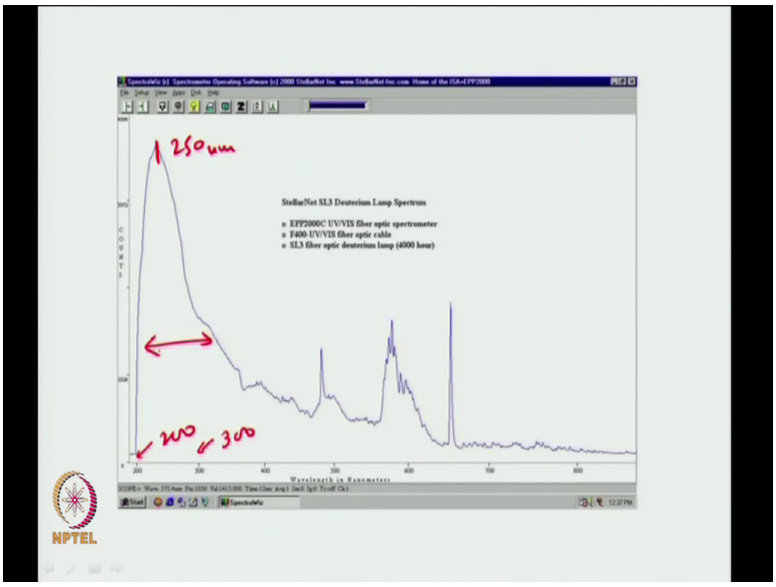

Now, this is typically the light coming out from a deuterium lamp, see where it peeks; so this is 200, so this portion is 200 ok, this is 300 and so on. You can see what is the maximum? The maximum is somewhere here right I think it is close to about what? 250 or so, ok. And then as you see as it goes towards 300 and down its intensity decreases, so this is the kind of useful range you have for the deuterium lamp ok.

So, this is the see this is the spectrum of the light that is coming out of your deuterium lamp, I high I would like to encourage you to look up this process, why do you have this spectrum or you know what gives rise to this spectrum. Can you tell me what gives rise to this spectrum, what happens to deuterium that gives rise to the spectrum?

Student: (Refer Time: 46:06).

Student: (Refer Time: 46:09).

Pi to pi transition.

Student: (Refer Time: 46:12).

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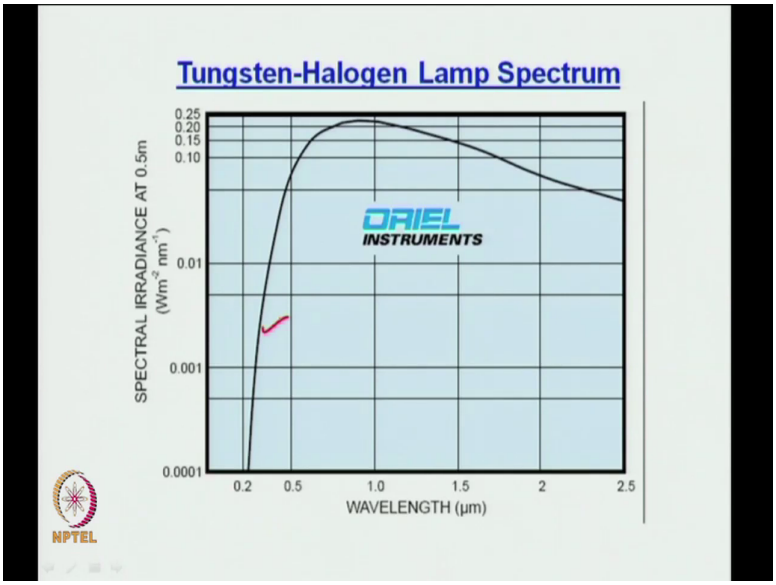


Go slow, go slow your too fast from me. Look this up, look it up its interesting; now look at this, this is a tungsten-halogen lamp spectrum. A tungsten-halogen lamp spectrum, it is not only a tungsten it is also a tungsten-halogen ok, please keep that in mind halogen is present there for a certain reason.

You look at the spectrum, the wavelength is in micrometers 0.2 micrometers is 200 nanometers right, it goes from 0.2 to 2.5. And you can see this is its spectral radius that means that is what its lamp profile is ok; and this is why you can use this for your absorption studies, right. Now, guys you know tungsten lamp right; why do you need to have a tungsten-halogen can anyone tell me?

Student: Because there are so much, so many photons are coming out.

Student: So, we have to coat with halogens say to control the number of photons coming out.

Well you are right, but not to control the number of photons. See you are absolutely right, what it does is have you seen these normal filament bulbs we use?

Student: Yes.

Right, you would see that when they go back, they have these black deposits from the sides right. See we have this black deposits what it means is these black deposits black right, it would absorb some of the radiations that is coming out and hence its intensity you know power decreases as a function of time. So, what this what does halogen do, it does not control the number of photons, what it does is?

Student: It increases the number of photons.

Right, no it not the photons; what it does is, it takes the black one and brings it back over to the that means, whatever you have a tungsten deposited out there, it oxidizes tungsten and brings it back to the filament, ok. It does not allow it to deposit out there, because of the certain reactions with the halogen that is why you need to have halogen out there ok; you just cannot do with the tungsten.

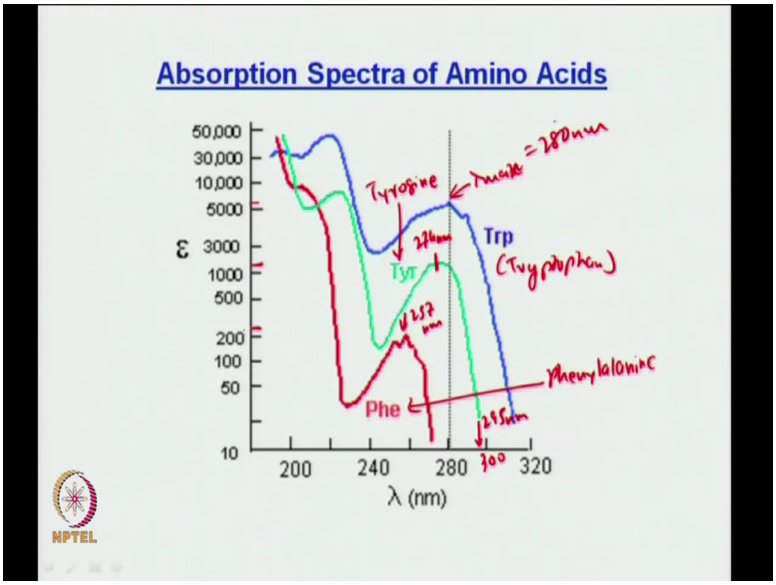
So, if you are ever buying a spectrometer and you see its only giving you a tungsten lamp, then the first thing you do is do not go for that you have to have a tungsten-halogen.

Otherwise within depending upon the usage within a very short time you will see a spectrometer is gone bad that means, a visible part is not working.

So, now you can realize it is a simple thing whenever you use it, you use it as a box, but these are things that go inside. But see if you are going from the UV to the visible region right, you need both the lamps working in UV region is not it? That means, they have to work together. If one of the lamps go bad, then actually you cannot do your absorption spectrum. Deuterium lamps are typically much more costly than tungsten-halogen lamps, far more costly.

Student: (Refer Time: 48:51).

(Refer Slide Time: 48:52)



Right, as I told you I would be showing you the absorption spectrum of these amino acids. So, look at the absorption spectrum the blue one is that of tryptophan right, so this is tryptophan,

this is tyrosine and this red one is phenylalanine. Now, guys look at this very carefully what do you what is what do you have on the x-axis?

Student: (Refer Time: 49:30).

x-axis you have.

Student: (Refer Time: 49:32).

Wavelength, lambda. What do you have in the y-axis?

Student: (Refer Time: 49:36).

On the y-axis you have what?

Student: (Refer Time: 49:39).

You have the extension coefficient right. So, this is typically if you would ever look up an absorption spectrum, you know this is typically how the plot would come. You see when you get the absorption spectrum in your you know UV visible instrument, you do not get these things what do you get you get 0.1, 0.2, 0.3 whatever right, but listen is very easy for you to convert it to epsilon right how?

Because you know absorbance that is o d is equal to epsilon c l; you know c, you know l, you know o d, you get epsilon and essentially then you plot epsilon against lambda. What does this tell you? see c n l are just something which you are putting in that means, you know what the concentration, you know what the length is. Epsilon is some the property of which is intrinsic to the molecular as we was just discussing, because that is relate to what? The cross sectional area. So, it is always good for you to know what the epsilon value is at any given wavelength.

Now, you can see what is happened is you look at the blue one which is tryptophan. (Refer Time: 50:34) and the tryptophan your epsilon is actually varying, remember I said that epsilon is a function of wavelength, now this is what I meant, your epsilon is not uniform throughout ok; it is a function of wavelength. Now, here if you are looking at 240 nanometers and above; 240 nanometers and above, so the peak the lambda m u the lambda max absorption of tryptophan maximum is above 280 nanometers.

So, what does this mean? What it means is that if you are exceeding it 280 nanometers tryptophan would be having the maximum absorbance ok. But look at this when you are going to excide at 280 nanometers, if your protein along with tryptophan has tyrosine that will also absorb, because look at tyrosine.

What is the lambda max of tyrosine? The lambda max of tyrosine is about I think 274 nanometers ok. But if it is at 274 nanometers at 280, you can see it still has enough absorbance that means, if you are exciting tryptophan and the protein has tyrosine, the tyrosine has also get excited to a huge extent just based on based on your absorption spectrum ok.

Now, you come to phenylalanine. The phenylalanine we generally do not use, so phenylalanine you can see it is about so I think this; this is about 257 for phenylalanine ok; so phenylalanine is for blue shifted ok. So, the two most important ones we consider in proteins is one is tryptophan, the other one is tyrosine ok. Now, suppose you would or I would like you to selectively excite tryptophan with minimum interference from tyrosine, where would you excite?

Student: (Refer Time: 52:15).

Do not look at me, look at the spectra and tell me, you can see out here. So, this is whatever this is so this is 300 right; so the I think yeah, if this is 300, this is 300, you can see here tryptophan is still enough absorbance right. So, generally you will see when people are doing florescence studies, if they are going to look at tryptophan, where they excite is they excite at the wavelength referred it is known as.

Student: (Refer Time: 52:44).

295 nanometers ok. You can see when you excite at 295 nanometers the tyrosine absorbance has already decreased to large extent, but tryptophan is not. So, hence what you are doing is, you are select you are trying so selectively excite tryptophan, but still you have some contribution from tyrosine, but that is much less as compared to what you would have had. If you would have excited at 280 nanometers, so that is one of the reason why when people are selectively looking at tryptophan, they tried to excite at 295 nanometers, ok.

See this is nothing new, it is not a huge thing, it is very simple you have to look at the absorption spectrum that is it, that is why absorption spectrum is so, so important for you. If you are going to do ant florescence studies or any study specially with florescent, what you do is you start with an absorption spectrum period right, there is no alternative to that ok.

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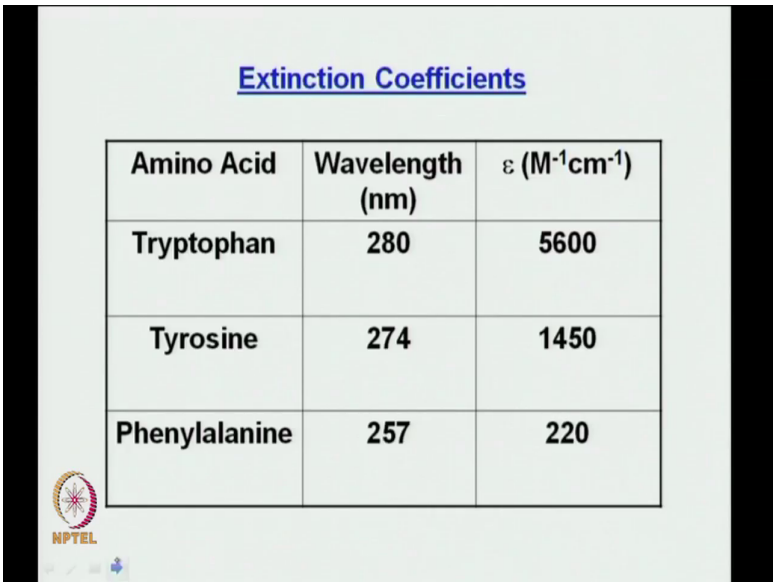

And I will just give you the extinction coefficient, so these are the extinction coefficients, so look at tryptophan at 280 it is 5600, at tyrosine at 274 its 1450, phenylalanine at 257 its 220. So, now you can understand why we do not use phenylalanine right, because phenylalanine the absorption coefficient is very small hence the cross sectional area is also very small.

Tryptophan has the highest, that is why tryptophan is one which is also most used right, that is why we so frequently used tryptophan for a studies ok, so that is what it was showed to you in the previous graph. You can see this is tryptophan this corresponds to 5600 out here, see this is tyrosine this corresponds to about 1450 out here right, and this phenylalanine this corresponds to about 220 out here ok, it is just a evident from your absorption spectrum.

So, guys this is how you would read in absorption spectrum ok. If you are given any absorption spectrum, these are the things you are supposed to actually interpret. And

remember if you are going look at a florescence, suppose you know tryptophan florescence, I am telling you that is why you know if and of if you have if you do not know already.

So, if you are going to excite tryptophan then you cannot excite at any arbitrary wavelength, you have to excite at that wavelength where it absorbs, because it is not. If it is not going to absorb, it would not be having any emission; so that is why if you are going to start with a new compound which are synthesized, again the first thing what you would do you take the absorption spectrum and excite within the absorption spectrum to see whether it has florescence or not and then other things come later clear, ok.

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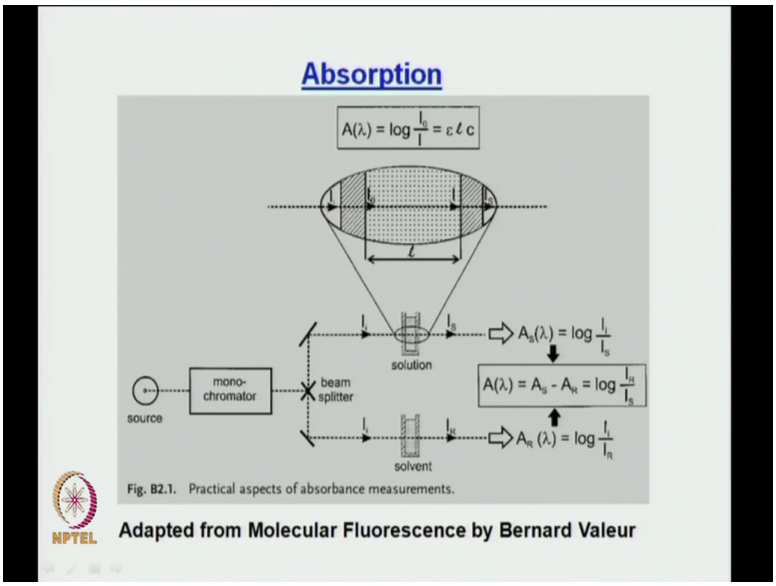

So, there is one another very important, I will just leave you this, because I have already run out of time. I just leave you at this I will start on this in the next class. So, if I am going to start from this, just I am going to tell you this, you look at this you look at this you know

ellipse subtle thing. What do you have is, you are looking you are zooming into a portion of a cuvette, cuvette means your sample cuvette where you have placed here absorbing solution ok.

Now, what are these shaded portions? So, anyways these dotted portions are your solutions, the shaded portions are what the shaded portions are? Your walls; that means, your thickness of your wall cuvette wall, your cuvette walls are not, I mean the cuvette walls have some thickness right.

Student: (Refer Time: 56:04).

That means, the bottom line is if you are having an incident light of intensity I, I i coming in, there is no guarantee that the one which is hitting the solvent or the hitting the sample is actually I i, why? Because before that it has to pass through that thick wall right and hence some kind of absorption some kind of scattering can happen, ok. So, these things you have to take care of.

Similarly, when you go there, when you go to I which is getting transmitted right, this I and this I s are can be different. So, what you will see is if you are going to an UV visible spectroscopy, specially the UV one, you have to use a certain cuvette which does not absorb the UV radiation. That is why what cuvette do you use?

Student: Quartz.

You use a quartz cuvette, because it has minimal absorption in that region. However, if you go to visible, you can easily use a glass cuvette, because it does not absorb, but glass cuvette will tremendously absorb.

Student: (Refer Time: 54:03).

In the UV that is why we will never use that ok. So, anyway this is where we will start from in the next class ok, and after that we move on to florescence.