Modern Instrumental Methods of Analysis

Prof. J.R. Mudakavi

Department of Chemical Engineering

Indian Institute of Science, Bangalore

Lecture No. # 11

Fluorescence and Phosphorescence Spectrophotometry 1 Theoretical Aspects

Welcome to the next session of our course. In this course, we will discuss about fluorescence and phosphorescence spectrophotometry. Basically fluorescence and phosphorescence spectrophotometry are variants of absorption spectrophotometry; that is molecular absorption spectrophotometry.

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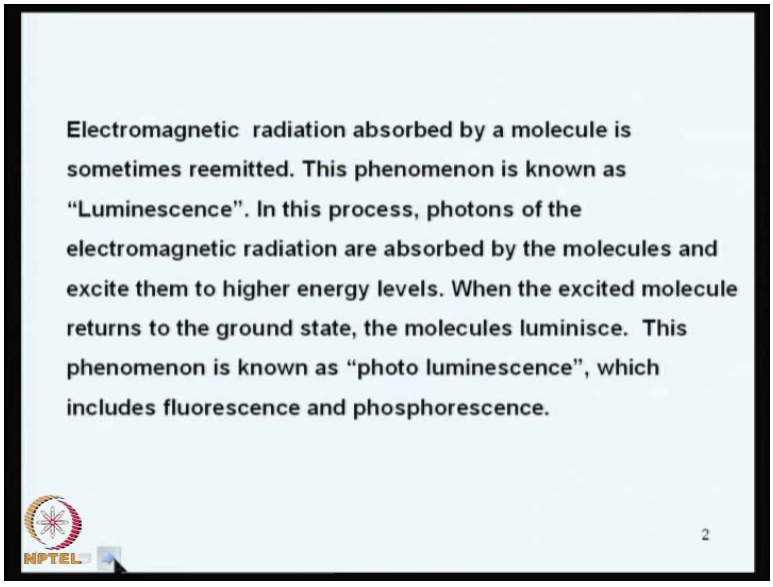

We have discussed already in the previous classes that electromagnetic radiation is absorbed by the molecules. The measurement of the emitted radiation with respect to the incident radiation is known as "absorption".

Now, in fluorescence what happens is electromagnetic radiation absorbed by a molecule is sometimes reemitted. This phenomenon is known as "Luminescence". In this process, photons of the electromagnetic radiations are absorbed by the molecules and excite them to higher energy levels and that is to electronic energy levels. When the excited

molecules return to the ground state, the molecules luminisce, this phenomenon is known as “photo luminescence”, which includes fluorescence as well as phosphorescence.

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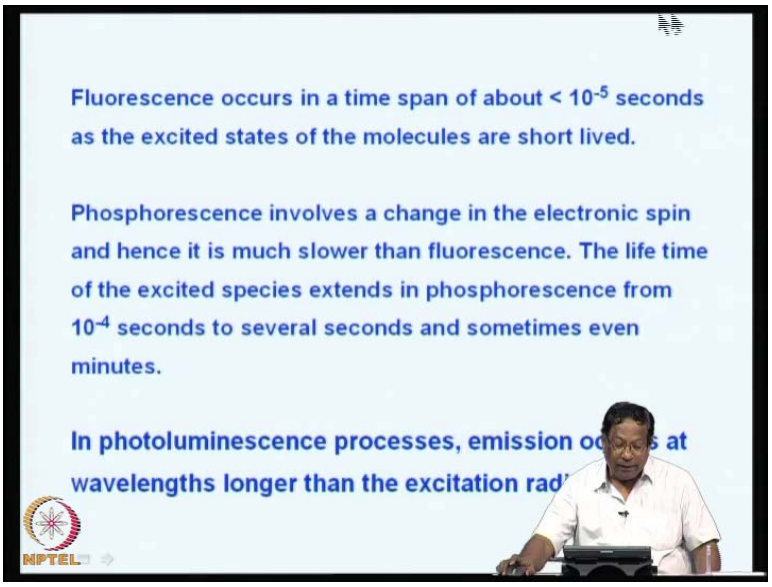

Sometimes, there is a chemical reaction accompanying the excitation and that is known as, “chemi-luminescence”. So, we have 3 types of photoluminescence processes: one is fluorescence, and another is phosphorescence, and another is an interaction of matter with a chemical reaction and that is known as chemiluminescence. We will discuss about all these things in this class.

In general, fluorescence occurs in a time span of about 10 raise to minus 5 seconds. That means, when the radiation is falling on the matter, the absorption occurs in about 10 raise to minus 15 seconds and fluorescence occurs in a time span of about 10 raise to minus 5 seconds. That means the excited states of the molecules are very short lived.

Phosphorescence, in comparison, it involves a change in the electronic spin and therefore, it must be much slower than the fluorescence. The lifetime of the excited species in phosphorescence from varies from 10 raise to minus 4 seconds to several seconds, sometimes even minutes. In photoluminescence processes, that are fluorescence as well as phosphorescence, the emission occurs at wavelengths longer than the excitation wavelength. That means you take it around 300, 350, the excitation would be always higher than the incident radiation. This is the very important concept of fluorescence and phosphorescence.

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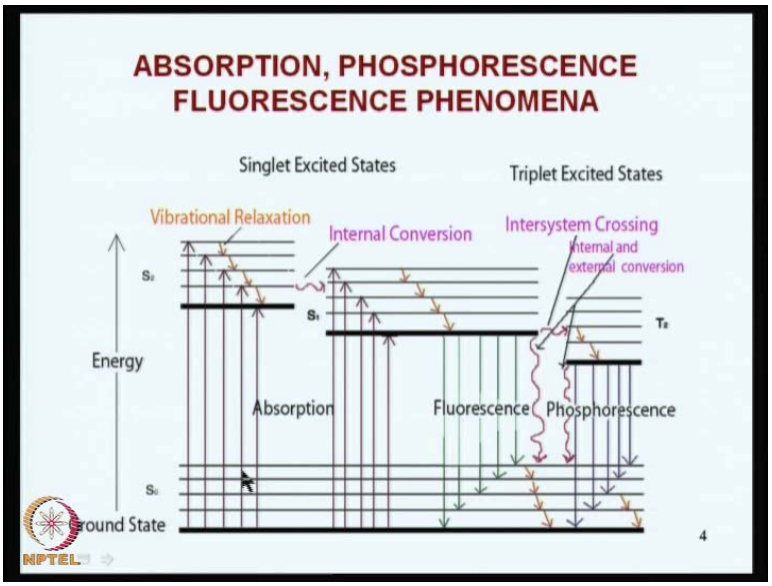

Now, in this figure, we have seen in our second lecture, the excitation processes I had shown earlier. Now, you can see here, this is an absorption phosphorescence and fluorescence phenomenon. The first one, I have shown is absorbance here. The excitation occurs from S 0 to S 2, different levels of excitation levels and the abscissa is energy. The excitation occurs from the ground state, S 0 state, to S 1, S 2 etc. Then we have singlet excited state and triplet excited states.

Now, you can imagine, a substance getting excited to higher energy level, it is like standing on the ground floor and you are looking at staircase and you are kicking a ball to the first floor. It need not land exactly on the first floor, but it can go to higher steps or lower steps. So, you can imagine those higher steps and lower steps as the vibrational energy levels. So, first floor is the excitation energy level. The steps along with that are vibrational energy levels and each vibrational energy level is further associated with number of smaller steps that is known as rotational energy levels.

When you excite an electron from the ground state to the next higher electronic state, it need not go to the ground state of the vibrational energy level of the excited state. It can go to any of the vibrational levels of the excited states and then what happens? The electron will lose its extra energy and comes to the ground state of the excited molecular state. From there it comes back to the ground state and if there is no change in the absorbance, no change in the wavelength, then we call it absorption. If there is a change;

if it is accompanied by an emission of radiation then it is known as fluorescence. That is what we are showing here in the middle part of the spectrum (Refer Slide Time: 06:05).

Here, you can see that it has gone to the fourth state, third, second, first etc. With these excited states, they will lose their energy and then comes to the ground state of the excited molecule. Then from there it comes back to the ground state. Again, it need not come to the ground state 0. It can come to any of the vibrational levels in the ground state and then sometimes what happens is there is another metastable state. That is known as… and so the electron, from excited state S 1 it will come to t 2. That is slightly lower than the molecular excited state, so it crosses over and there it stabilizes. So, from there, again it is usual desorption and emitted by radiation and this is known as "phosphorescence". So, all these 3 processes could occur simultaneously or separately.

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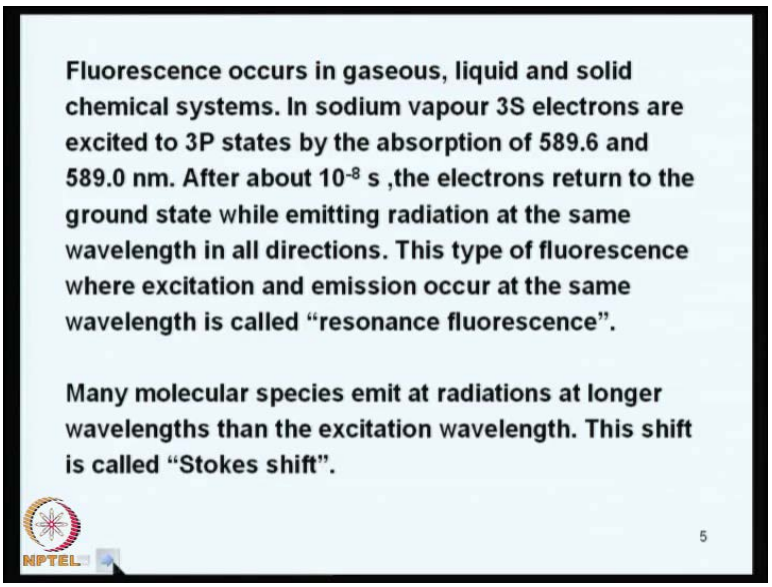

Now, that means a molecule, which absorbs radiation need not fluoresce, but a molecule which fluoresces definitely must absorb. A molecule, which absorbs, which gives you fluorescence need not give you phosphorescence. But, a molecule which gives you phosphorescence must fluoresce and therefore, it must again be absorbed. So, an absorption chromophore is always essential for fluorescence and phosphorescence, as well as chemiluminescence processes also.

Now, fluorescence occurs in gaseous state, liquid state or in solid chemical systems also. For example, you would have seen in sodium vapor, 3 S electrons are excited 3 P states

of the order of about 589.6 and 589 nanometers. You would have seen sodium vapor lamps all over the town and you will see only yellow color in that, in your day to day observation and street lights. But, even that yellow color is due to the absorption of sodium atoms, which will take the radiation to the next higher energy level that is electronic excitation state. That is again doublet, corresponding to 589.6 as well as 589 nanometers.

So, after about 10 rise to minus 8 seconds, the electrons return to the ground state while emitting radiation at the same wavelength. That means it absorbs the radiation and then goes to the next higher energy level and then emits, but the emitted radiation is also of the same excitation radiation. That means there is no fluorescence occurring, but the emission is also occurring and absorption is also occurring. This is known as “resonance fluorescence” and that means any (( )) (Refer Slide Time: 09:26) occurring at the same wavelength of absorption is known as resonance fluorescence.

Many molecular species emit radiations at longer wavelengths than the excitation wavelength. This I have already told you and this shift is called as “Stokes shift”.

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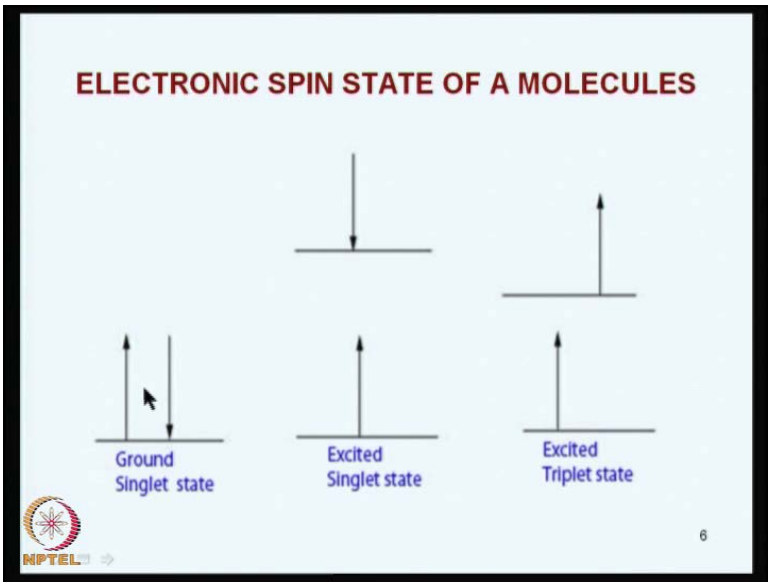

So, what happens when the electron gets excited? You can see this figure here. This is the ground state, singlet state and then electron is excited, and the spins are as they are. Here, in the ground state, they are paired and the singlet state also, you can see that this

electron is spin is the same way, as well as, the other electron even in the excited state, it has the same spin. Now, this is known as Singlet state.

Another possibility is when the electron is excited to the next higher energy level; here you can see the spin of the second electron is opposite. So, you will see that this is an excited state, but change its spins so this is known as “Triplet state”.

In fluorescence, we get the singlet state excitation. That means, spins of the excited electrons do not change, whereas, in phosphorescence, the spins of the electrons, in the excited state changes. So, this is the basic difference between fluorescence and phosphorescence.

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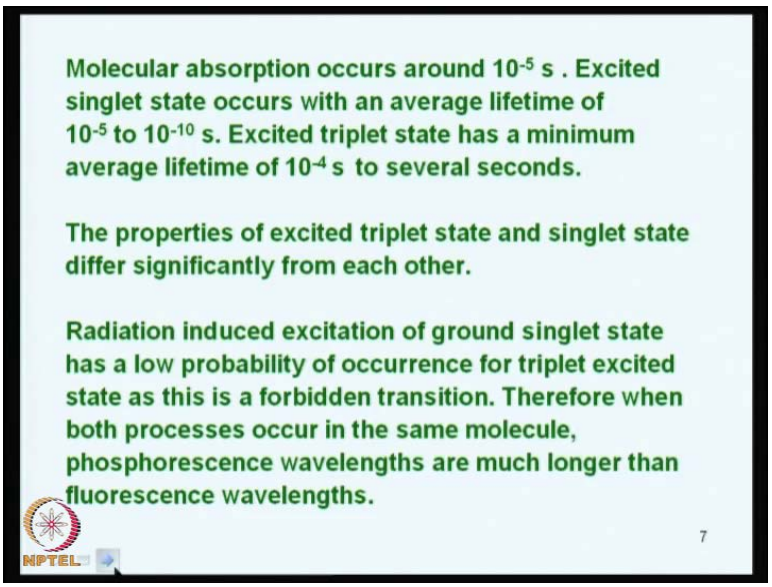

As I have already told you, molecular absorption occurs in about 10 raise to minus 5 seconds and excited singlet state occurs with an average lifetime of 10 raise to minus 5 to 10 raise to minus 10 seconds. Excited triplet state has a minimum average lifetime of 10 raise to minus 4 seconds to several seconds. You would have seen in old time pieces, you would have seen that green color time pieces, the green color, which will shine at night. So, that is all phenomenon of phosphorescence.

The properties of the excited triplet state, as well as singlet state, differ significantly from each other. Now, you can see here that the radiation induced excitation of the ground singlet state has low probability of occurrence, for the triplet excited state, because in

general, triplet excited states are forbidden transitions. That means the probability of a triplet transition occurring is much less compare to singlet state excitation. That means electrons do not get changed in there spin. Therefore, when both processes occur in the same molecule, phosphorescence wavelength should be much longer than the fluorescence wavelength.

So, you could, if you are able to see both of them; for fluorescence as well as phosphorescence, the first higher energy level emission would be fluorescence and still lower energy level would be phosphorescence.

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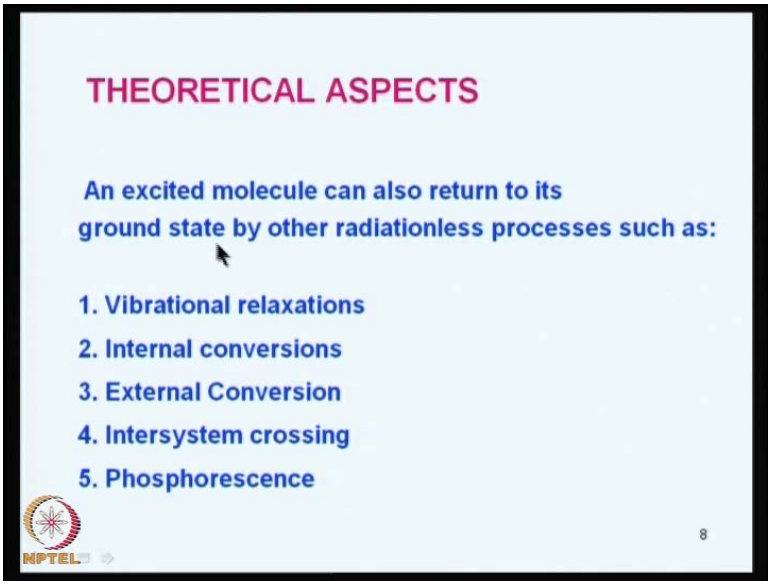

Now, let us look at some of the theoretical aspects of fluorescence. An excited molecule can also return to its ground state by several other radiationless processes, such as, vibrational relaxation. It can, as I had told you that it can go to any of the higher energy state in the vibrational levels, and it will lose its energy, and then again it will fall or fall back. Then it can go for internal conversion and then external conversion, another possibility. Then there is intersystem crossing and of course, phosphorescence.

Why I have included phosphorescence in this is because it will reduce the probability of fluorescence, because we are discussing fluorescence in this case. So, phosphorescence would be and would act as a deterrent for fluorescence and fluorescence would act as a deterrent for phosphorescence. So, we had to consider both these aspects.

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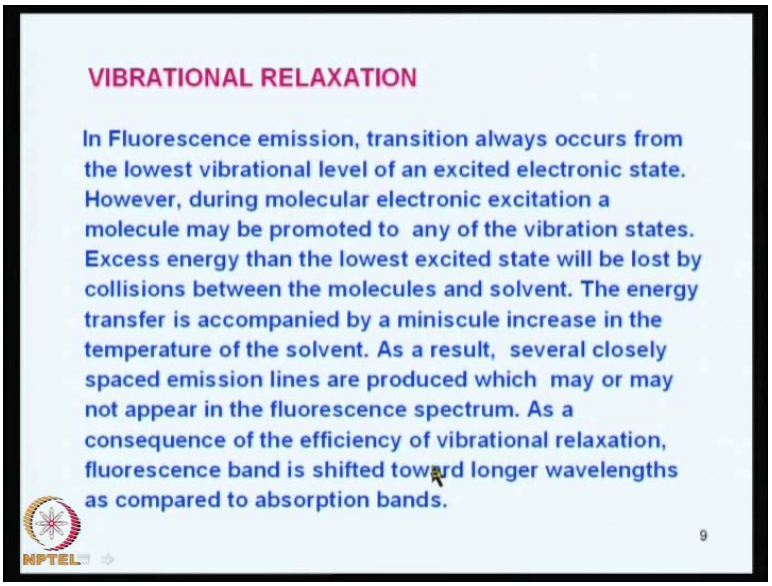

In general, what you see is in fluorescence emission, transition always occurs from the lowest vibrational level of an excited electronic state. However, during molecular excitation, a molecule maybe promoted to any of the vibrational states. Excess energy than the lowest state, would be lost and, how this energy would be lost? It has to be by interaction with the solvent molecules. So, it will pass part of its energy to the solvent molecules and then lose its excess energy, comes to the ground state vibrational excited level. From there, it will come down to the ground state. So, transfer is always accompanied by a miniscule increase in the temperature, because there is interaction of solvent molecules along with the fluorescence compound.

This interaction, change of energy or exchange of energy, must be occurring and it must take place in some form or the other; it cannot be destroyed. So, this occurs as a slight increase in the temperature of the solvent. As a result, several closely spaced emission lines are produced, which may or may not appear in the fluorescence spectrum, because the amount of energy change involved in fluorescence in vibrational changes are much lower than the excitation to electronic levels. So, what happens is as a consequence of that efficiency of the vibrational relaxation, the fluorescence band is shifted towards longer wave. It appears as a fluorescence band, instead of a single fluorescence line. So, you would get a spectrum like this and with a Lambda max Gaussian curve, you can imagine and it is compared and it is comparable to absorption band.

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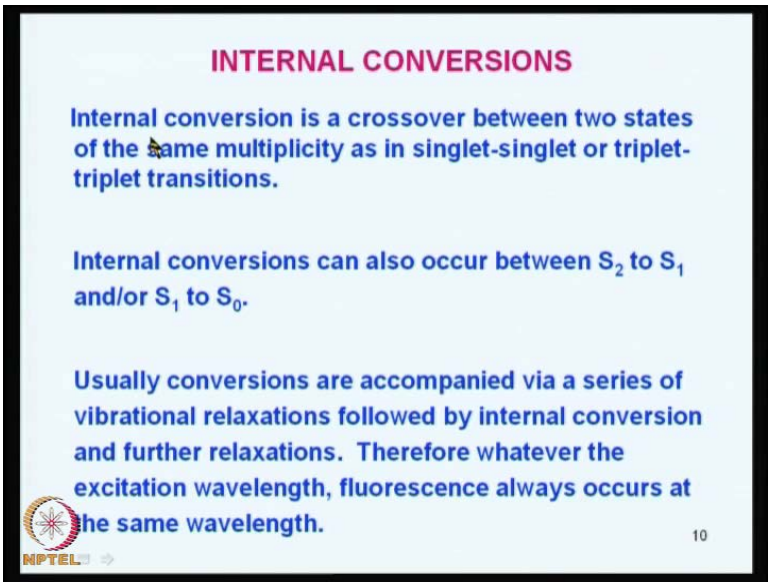

Now, you can see that another possibility that you have discussed is, “Internal Conversions”. What is this internal conversion? It is actually a cross over between two states of the same multiplicity, which means no change in the spin of the electrons as in singlet-singlet or triplet-triplet transitions.

So, if a singlet state goes to singlet state, there is no change in multiplicity, and if a triplet state goes to triplet, again there is no change in the multiplicity, whereas, a singlet system going to triplet state would involve a change in the multiplicity. So, internal conversions can also occur between different excited levels. For example, S 2 to S 1 and then S 1 to S 0, like that different energy levels they can occur.

Usually, such conversions are accompanied by a series of vibrational relaxations, as in as, shown earlier followed by internal conversion and further relaxation, which means slightly more energy is lost in internal conversions, therefore, whatever the excitation wavelength, fluorescence always occurs at the same wavelength; that is longer wavelengths.

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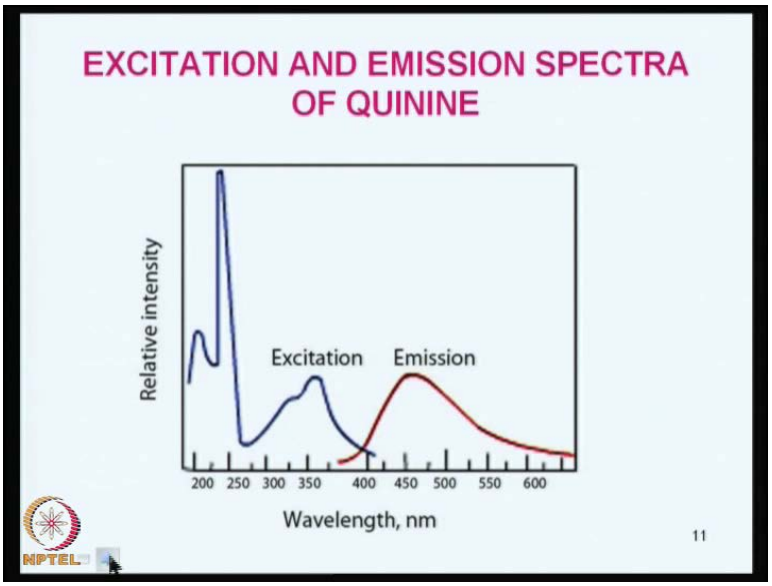

Now, here you can see the excitation and emissions spectra of quinine. So, you can see that there is a relative intensity is plotted versus wavelength, and you are able to see the figure. So, in this figure the excitation is there, one at 250 and there is one more around 310 or something, and another is around 360. This is excitation spectrum; that means if you take a quinine substance pass the radiation around this wavelength, containing this lambda max and then it will start fluorescing.

It will look appear different color. Here, (Refer Slide Time: 18:25) I am showing you a figure like this. This is rhodamine 6 g. You can see that the color is somewhat reddish, and this is very common. What I would like to show you is if you measure the light somewhere here, like this, you would see that the radiation falling on the paper is greenish, whereas, the radiation near the glass, between, near this point, between this light as well as the substance is somewhat pinkish.

This is actually a phenomenon of fluorescence. See, the radiation that is falling on the sample is generating pink fluorescence, whereas, what is being transmitted is blue. So, see the difference, one is excitation and another is emission wavelength.

So, naturally the substance would be looking pinkish, but when the radiation is passing through what comes out is a bluish light. Obviously, bluish light has got a longer wavelength and that is fluorescence. Similar phenomenon occurs here in the quinine, if we choose and go for this excitation, you would see a color, emitted color, having a

lambda max of 450. That means, you are not seeing any of these absorption peaks, excitation peaks and even though you have producing this radiation. What comes out is only this wavelength; you can see that this has got a longer wavelength. What you have supplied is 210, 250 and then 350, what is coming out is a spectrum with about 450 to 460 nanometers. This is emissions spectrum.

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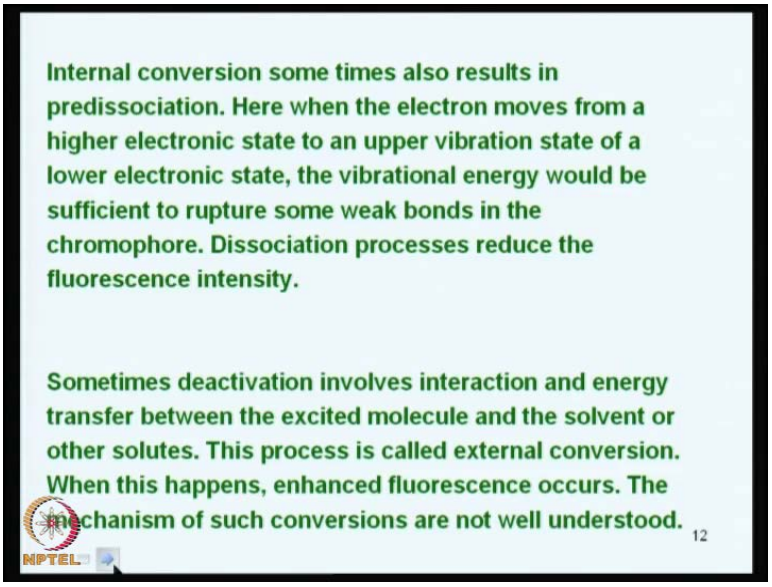

Internal conversion, sometimes also results in predissociation, which means the substance may dissociate and fluorescence will come down. So, here when the electron moves from higher electronic state to an upper vibrational state of a lower electronic energy level, the vibrational energy would be sufficient to rupture some weak bonds in the molecule chromophore. So, dissociation processes, obviously, they will reduce the fluorescence intensity. If there is no dissociation, the bonds would be there, fluorescence would be there and you would be observing some amount of radiation. But, if the chromophore itself is getting destroyed, obviously fluorescence also would be reduced to that extent.

Sometimes, what happens is deactivation involves interaction and energy transfer between excited molecule and the solvent or other solutes also. This process is called “external conversion”. In internal conversion, there is interaction only between the molecule and the solvent, and in external conversion there is flourophore that is fluorescent compound, solvent molecules as well as other solutes present in the sample.

So, this is known as external conversion. Now, even external conversion would lead to decrease in the fluorescence. That is why in any of the fluorescence methods what we normally measure; we should always make sure that there are no external solutes in the sample and that is what you do as interference studies. So, what happens if there is some other substance which will absorb fluorescence apart from the solvent molecules? So, when this happens, enhanced fluorescence also occurs sometimes, because the external solutes can also increase the fluorescence, because they may also have a chromophore. So, however the mechanisms of such conversions are not very well understood.

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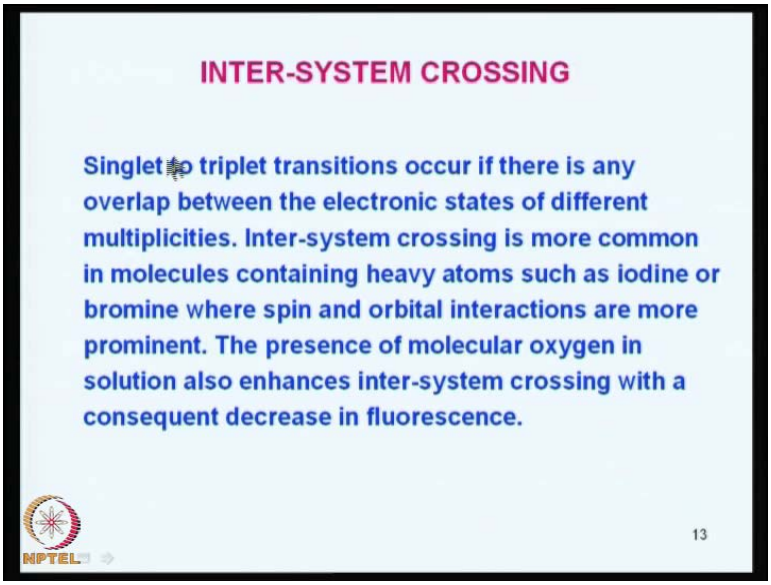

The inter-system crossing, as we are discussing, it is basically a question of singlet to triplet transitions, and they occur, if there is any overlap between the electronic states of different multiplicities. That means singlet to singlet is there, singlet to triplet also is there, if the triplet state is having a slightly lower level like this. The higher level is singlet, lower level is triplet, and so if there is a possibility for the molecule to come down to this level, and then this is again a stable triplet state, then the interactions can occur.

So, inter-system crossing is more common in molecules containing heavy atoms. For example, in the chromophore, if there is iodine molecule, then intersystem crossing possibility is much more. Sometimes, there may be a bromine compound and if the parent compound is fluorescing. If there are a bromine substitute and then that means

there would be higher fluorescence occurring and this is known as inter-system crossing, because they provide is triplet system that helps in the cross over.

So, the molecule will go to higher energy state, lose its for vibrational energy level, come to the ground state excited level and then immediately it crosses over to lower triplet state. So, this triplet state is the one, which will lose its excess energy and come down to the ground state.

So, the presence of iodine, bromine and some other halogens etc, heavy atoms, basically, they aid in this inter-system crossing and sometimes because spin and orbital interactions are more prominent, in such cases. Then the presence of molecular oxygen also enhances inter-system crossing with a decrease in fluorescence.

Any molecular, any solutions, for example, if you take, there will always be some amount of dissolved oxygen. So, this dissolved oxygen that is molecular oxygen, it enhances inter-system crossing that it brings it to the lower triplet state, meta stable state we call it, and then that obviously has to decrease result in the fluorescence but not phosphorescence, so inter-system crossing is one thing.

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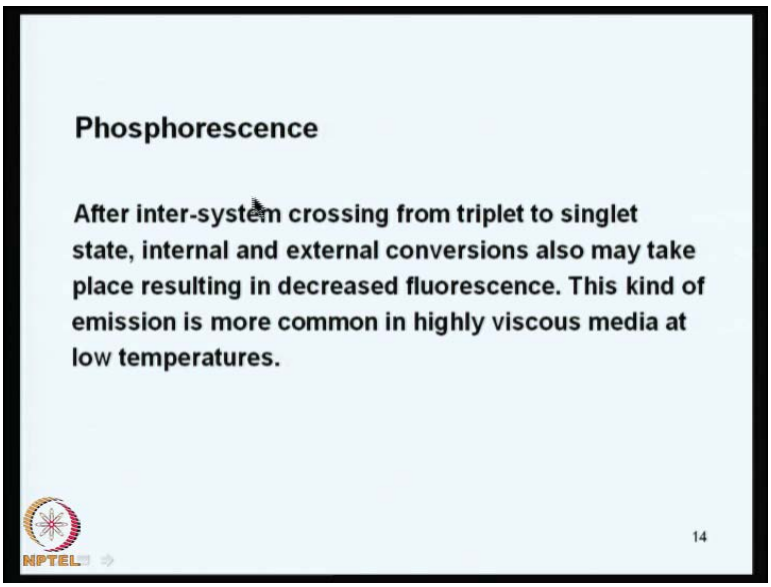

Phosphorescence, as such, after the inter-system crossing from triplet to singlet state, internal and external conversions in the excited states also can take place, resulting in the decreased fluorescence. This kind of emission is more common in highly viscous media

or at low temperatures. So, actually most of the phosphorescence measurements are done at very low temperatures because they are more stable for measurement.

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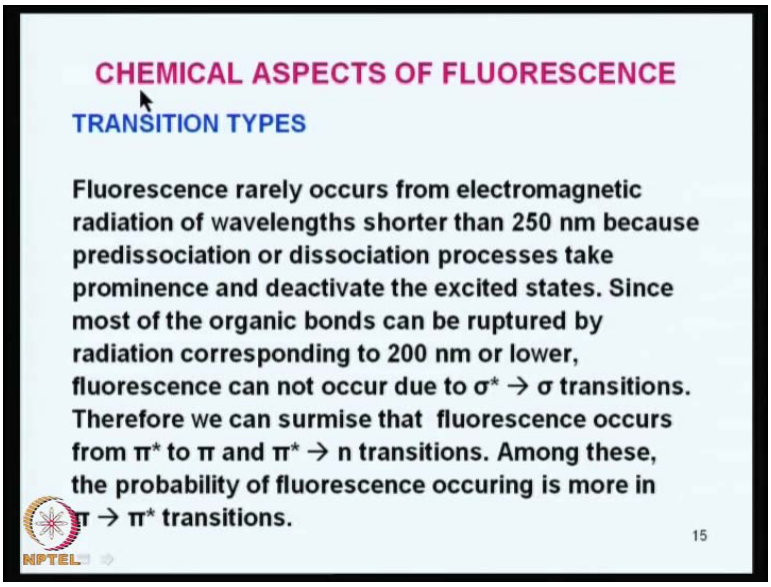

So, then there are different types of chemical aspects. Let us discuss now, the different types of transition types. Now, what happens? Look at fluorescence, fluorescence rarely occurs from electromagnetic radiation of very low wavelengths.

For example, less than 250 nanometer wavelength does not result in excitation, because the energy of the electromagnetic radiation below 250 is so high that it instead of exciting, it ruptures the bonds, so it destroys the molecular structure. So, you would rarely see fluorescence occurring from wavelength of less than 200 nanometers or lower. What happens is this radiation of 200 nanometers or lower, they promote sigma to sigma star transitions, and sigma star to fluorescence does not occur, because bonds are being broken, so chemical is being destroyed.

Therefore, we do not like excitation, using very low wavelengths. Now, what are the other possibilities? We can surmise that sometimes the pie star to pie and pie star to N transitions. Among these, the probability of fluorescence occurring is more in N to pie star transitions than pie to pie star transitions. Why, again because pie to pie star transitions, are having higher energy, which again can destroy part of the chromophore or fluorophore.

So, the most of the radiations responsible for fluorescence, correspond to slightly longer wavelength, ultraviolet radiations, sometimes visible also. But, in general, they are all long wave length ultraviolet rays corresponding to N to pie star transitions.

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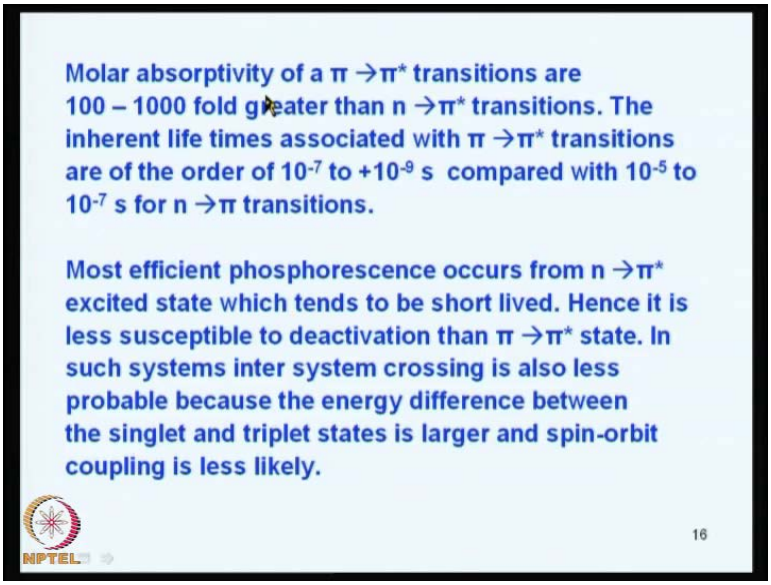

So, molar absorptivity, if you take a look at it, they of pie to pie star transitions are of the about 100 to 1000 fold greater than N to pie star. So, the energy of the transition, energy of pie to pie star is also so much higher. Therefore, the inherent lifetime associated with pie to pie star transitions are of the order of about 10 rise to minus 7 to 10 rise to minus 9 seconds, as compare to 10 rise to minus 5,10 rise to minus 7 for N to pie star transitions. That is why we say most of the time fluorescence occurs from N to pie star transition, because the lifetime of the fluorescence; N to pie star transitions, are of the order of about 10 rise to minus 5 to 10 rise to minus 7 seconds. The fluorescence probability, also its lifetime, is also of the almost of the same order.

Now, most efficient phosphorescence should also occur from N to pie star excited state, but that should be very short lived. The excited state end to pie star should be short lived and when it comes down to triplet state, the triplet state should have longer life. So, the pie to pie star, it is N to pie star transitions are less susceptible to deactivation from pie to pie star transitions. In such systems, inter system crossing also is less, because the energy difference between singlet and triplet states is much larger and spin orbit and coupling is

also not possible in such cases. Now, I have combined here some of the observations of chemical nature.

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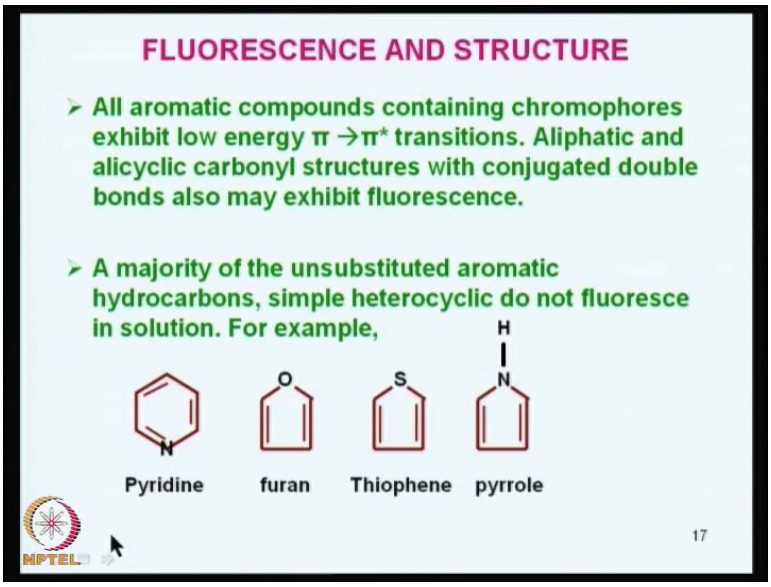

For example, you can see that all aromatic compounds containing chromophores exhibit low energy pie to pie star transitions. Aliphatic and alicyclic carbonyl structures, we have seen such structures, in our discussion on spectrophotometry, such structures with conjugated double bonds also exhibit fluorescence. So, a majority of the unsubstituted aromatic compound; what do they do? Simple hetero-cyclics, they do not fluoresce in solution, but such systems such as pyridine and then furan, thiophene, pyrrole, such structures I have put them here on the screen and you can see that they do not fluoresce.

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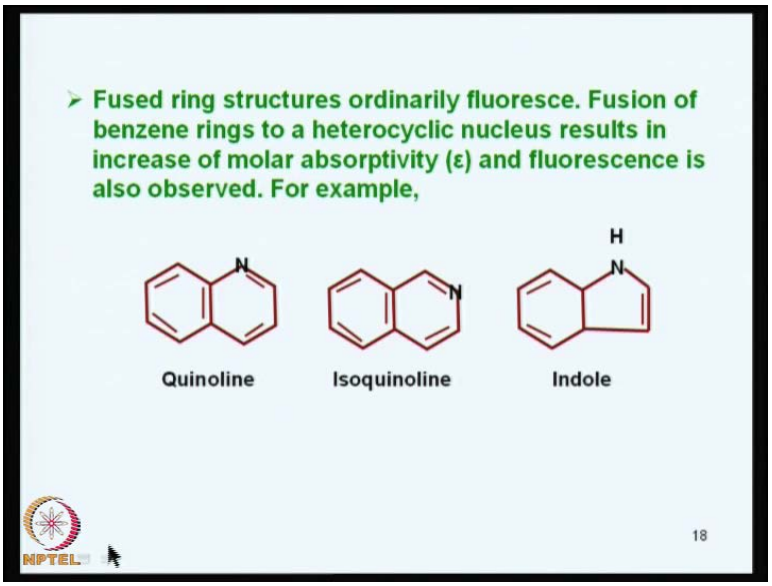

Now, fused ring structures, another observation. Suppose, I have quinoline with 2 benzene rings with one of the benzene rings containing nitrogen and that is quinoline. Such substances ordinarily fluoresces; isoquinoline and indole, indole, which is 1 6 membered ring and another is 5 membered ring with nitrogen. So, these substances fluoresce. That means, the planar structure, if it is bigger, that means fluorescence is much more; single aromatic structure, like benzene, toluene etc, they do not fluoresce on their own. Whereas, you put 2 aromatic rings and fused them together, it gives a much bigger rigid plain in which the electrons are organized and such systems fluoresce more.

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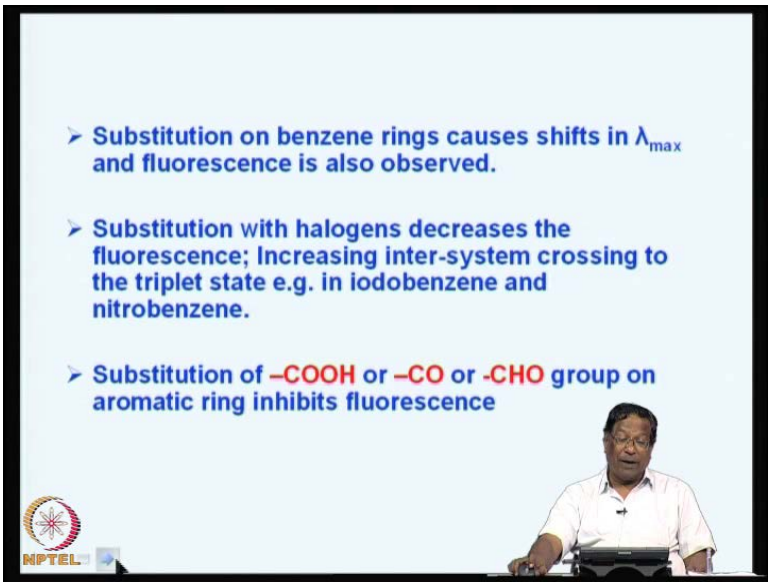

And another observation that I have put together is substitution on benzene rings. It shifts to the lambda max and fluorescence is observed. You put any substitute it like benzylalcohol and other things they started giving you fluorescence. Substitutions with halogens, what happens? Halogens are bigger molecules. There is steric hindrance, so fluorescence comes down; fluorescence decreases, increasing the inter-system crossing. We have already discussed that iodine and such substances, if I put on aromatic substances, inter-system crossing is much more. Therefore, fluorescence decreases; so in iodobenzene and nitrobenzene, such substances are examples.

Suppose, I substitute; take a benzene ring and substitute by C O H group converted into acid; benzoic acid or C O group or C H O group, aldehydic group on the aromatic ring. Again this inhibits fluorescence so that means the rigidity is destroyed. Liability of the electrons becomes more.

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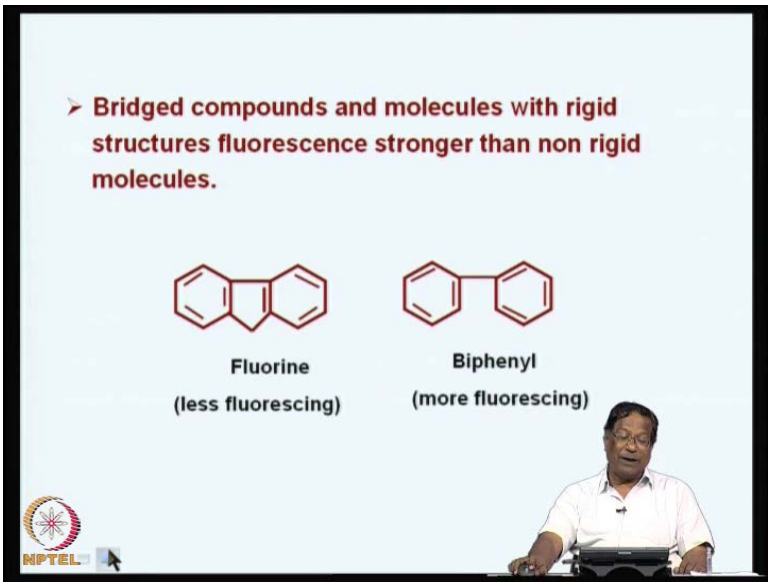

Suppose, I take two aromatic rings and I bridged them together with another aromatic ring; rigidity increases. If the rigidity increases, according to our observation, two fused benzene rings such as benzene and quinoline, two fused rings with benzene and pyridine that is quinoline that gives you fluorescence. Suppose, I bridge one more or bring one more ring, I make the substance more rigid and such rigid compounds will give you enhanced fluorescence.

I have put here one benzene ring, second benzene ring, and third is a pyrrole ring and here I get fluorine that is less fluorescing and 3 rings. But, if I just bridge them with a single bond here, as I shown here in biphenyl that is more fluorescing, so the rigidity of the molecule is also a very important concept in fluorescence

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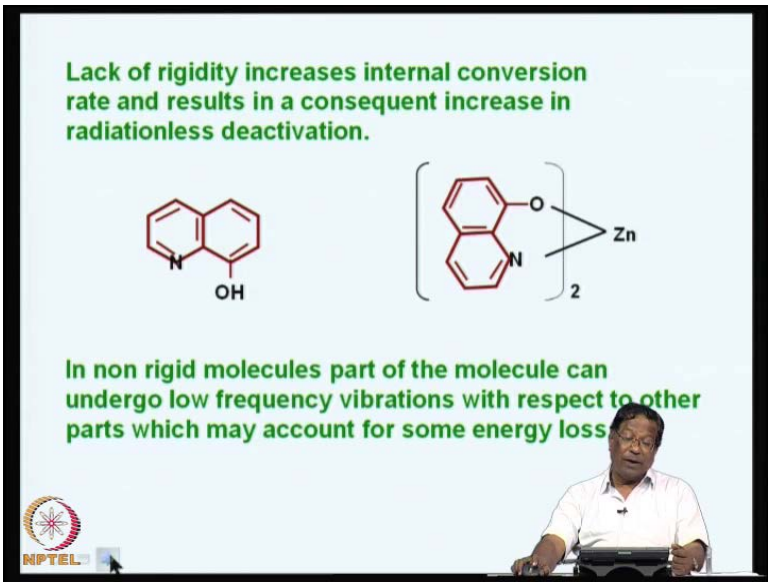

Therefore, whenever we want to find out a fluorescence reagent for a substance, we should always look for many aromatic rings, which are joint by bridging; should not have C O O H groups, acidic, the C H O, carbon monoxide C O group, C H O group etcetera But, they should rather be bridge compounds, so molecular rate should be high, rigidity should be more. Such compounds would be good candidates for fluorescence development.

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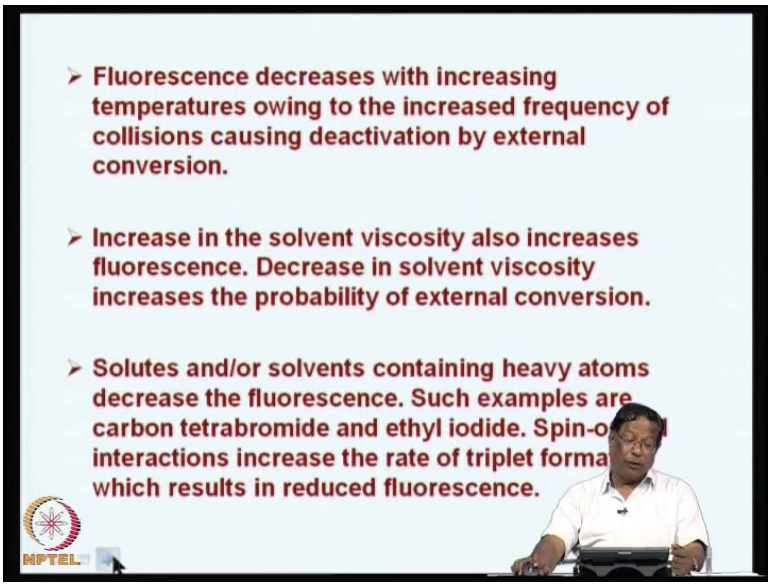

Now, another observation is fluorescence decreases with increasing temperature. Now, why this should happen is because in increased frequency of collisions. If the collisions are more, the inter-system crossings is more and inter collision, so the energy would be transferred much faster and there is no chance for fluorescence to occur, that means deactivation will occur. You take a fluorescence substance like this and then you heat it and when you heat this substance, it will lose its fluorescence, whereas, if you do not heat it, bring it down to room temperature, then the fluorescence would be much higher.

So, temperature is a deterrent for fluorescence and increase in the solvent viscosity as we have discussed earlier it increases the fluorescence. Why, because decrease in solvent viscosity increases the probability of external conversion. So, we should always try to increase the solvent viscosity by using polyvinyl alcohol, glycerol and some other substances. Basically, it is to introduce in homogeneities in a system, where the fluorescence can be measured. Now, sometimes this observation, look at the last one (Refer Slide Time: 37:40) solutes and or solvents containing heavy items, decrease the fluorescence.

This we have already discussed. Such examples are carbon tetrabromide and ethyl iodide. Suppose, you want to reduce the fluorescence of a compound then, what you should be adding? You should adding carbon tetrabromide and then you can add ethyl iodide. They will reduce the fluorescence. So, you can control the fluorescence of a

substance to any level you want by adding such substances, which will increase or decrease the viscosity. So, spin orbital interactions normally increase the rate of the triplet formation, which results it again reduced fluorescence.

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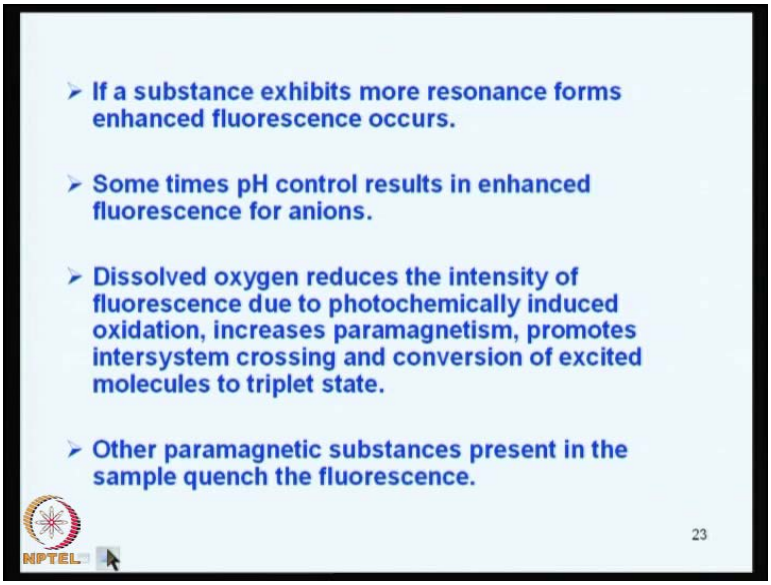

If a substance exhibits more reasonance forms then what happens? Fluorescence would increase or decrease? The electrons will be more mobile, so the structure would be more stable, that means, fluorescence should increase. If the structure gets destroyed then fluorescence should decrease. But, if the substance has got more resonance structures, there would be equilibrium among the two. It would be stable, this energy would be lower and fluorescence would be enhanced.

Sometimes, P H control, you increased the P H control or decrease the P H, which also results in enhanced fluorescence, because the anions, generally whenever we are talking of the fluorophore, we talk of anions. It is not the cation. Cation would be a substance containing calcium, sodium, potassium etc but, the anion is a larger molecule that is a fluorophore. So, fluorophore concentration can be increased in alkaline conditions.

I carry and take the system in acidic medium, add a little bit of sodium hydroxide or potassium hydroxide, the anionic concentration will decrease; sorry, it will increase. Cationic concentration will decrease. So, anionic concentration, when it increases, there is addition of fluorescence and that means enhancement will be there. Next point is dissolved oxygen. This we already discussed. It reduces the intensity of fluorescence,

due to photochemical induced oxygen. This we have not discussed. Earlier, we had simply stated that the dissolved oxygen reduces the fluorescence. How does it do is because it induces oxidation.

So, obviously oxidation will reduce the fluorophore concentration and once the concentration decreases, the paramagnetism increases and it promotes inter-system crossing and conversion of excited molecules to triplet state is more. So, oxygen, the mechanism of decrease of fluorescence due to molecular oxygen is always due to the increasing paramagnetism, promotion of intersystem crossing, as well as conversion of the excited molecule to a triplet state, and other paramagnetic substances also, if they are present, they would act likewise that is the quench the fluorescence.

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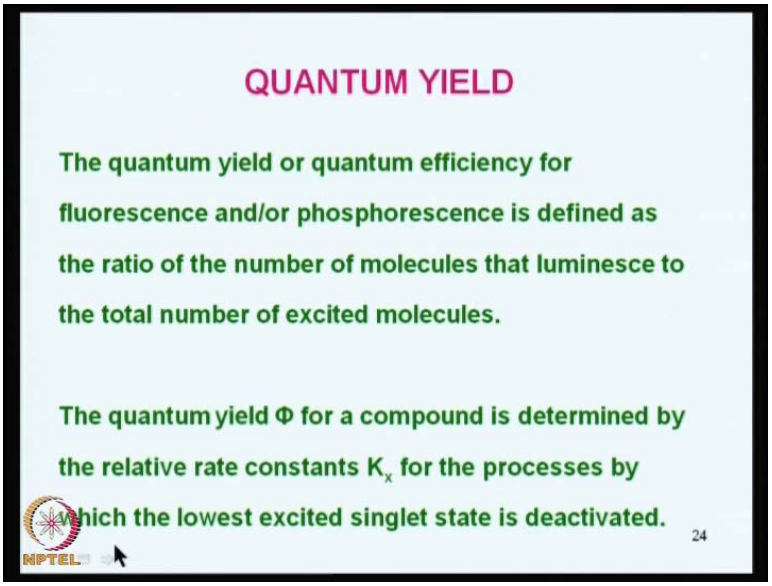

Now let us look at some of the theoretical aspects of spectrophotometer fluorescence. Now, you would see that fluorescence in a substance like this would be occurring all over the substance. That means it would be emitted in all directions. It will just because I put a lamp here and showed you this fluorescence, it does not mean that it is occurring only here, because the initial radiation is coming from this end and I am able to observe the figure this side, so you are able to see it. But, suppose, I put it here, again it will be and it will be occurring at a 360 degree angle.

So, the efficiency of fluorescence is an important concept. How do you define the efficiency is by quantum yield or quantum efficiency. So, the quantum efficiency for

fluorescence or phosphorescence, for that matter, we define it as the ratio of the number of molecules in the luminesce compare to the total number of excited molecules; how many are the excited molecule luminesce compare to total excited molecules.

The quantum yield, phi, for a compound is determined by the relative rate constants K x Here, I am defining it is K x. So, x will refer to various processes for which the lowest excited singlet state is deactivated; it has to get deactivated.

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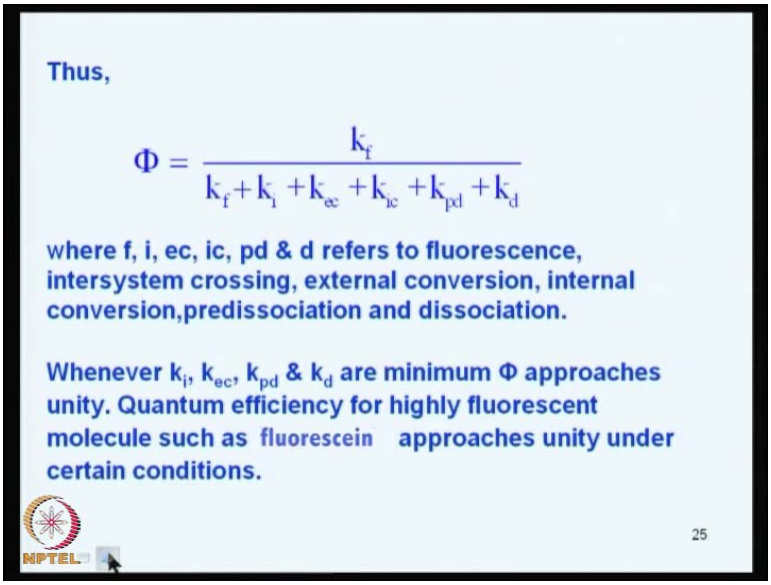

We can define it like this. Phi is equal to k f, that is formation of the excited spices to the so many other processes, where the excited molecule concentration decreases. What are the processes? One is formation, another is this inter-system crossing. Here, I put it as k i, that is the rate of the inter-system crossing, and then external conversion, k e c, and then there is pre-dissociation. This also, we had discussed and then k d, that is dissociation. So, all these, the ratio of all this formation constants compared to all other formation and dissociation constants, we will give as the quantum efficiency.

Whenever, k i, k e c, k p d, that is pre-dissociation and then dissociation, they are minimum, that means when the denominator is minimum, the numerator is more, that means fluorescence will be more. So, when k i, k e c, k p d and k d, are almost near zero, this equation reduces to k f by k f and that is one. So, the maximum fluorescence efficiency approach is always 1, but, you will never get it. It is a theoretical possibility, because almost you cannot have any system where there is no fluorescence. I mean

where there is no inter-system crossing (Refer Slide Time: 45:01) e c, pre-dissociation, some amount of heat transfer; all these things will always be there.

So, quantum efficiency for highly fluorescence substance, such as fluorescein approaches unity under certain circumstances but, you would never get 100 times change, that is ratio of 1. So, how do you quantify the fluorescence? Whenever, you want to develop any analytical method, you must be able to quantify the absorbed phenomena in terms of the concentration of the substances what you want to measure. So, suppose a substance is fluorescing, you can measure the concentration of the fluorescing compound in relation to its fluorescence emission.

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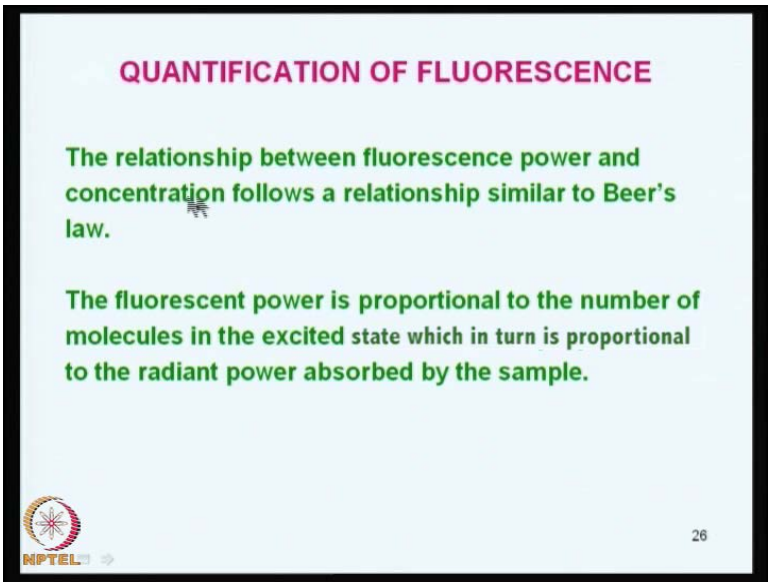

You have to find a relation between fluorescence as well as concentration. We have done it in spectrophotometry by deriving the Beer Lambert's law. Now, the relationship between fluorescence power and concentration, we can assume that it follows a relationship similar to Beer Lambert's law. The fluorescence, we can state it like this that fluorescence power is proportional to the number of molecules in the excited state, which in turn is proportional to the radiant power absorbed by the sample. The sample has to absorbed the radiation; it has to give out fluorescence, the fluorescence that comes out should be is proportional to the number of molecules in the excited state, which is proportional to the radiant power.

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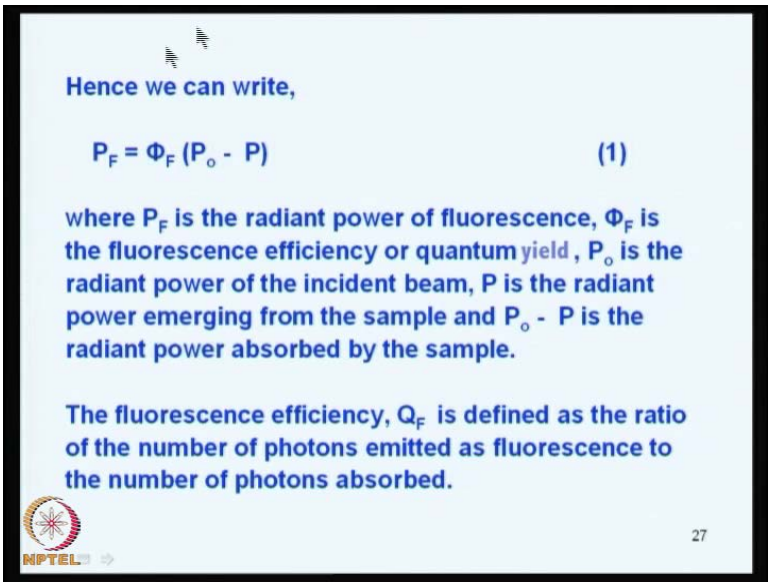

So, the decrease in the radiant power is what we are looking for and a relationship with it to related to the concentration. So, we can write P F is equal to Q F in to P 0 minus P. What is P F? P F is the radiant power of the fluorescence, and Q F is the fluorescence efficiency or quantum yield and P 0 is the radiant power of the incident beam. P is the radiant power emergent from the sample and P naught minus P, the difference is the radiant power absorbed by the sample.

So, the fluorescence efficiency Q F is defined as the ratio of the number of photons emitted as fluorescence that is more understandable, because, photons can be measured. Whatever, is the photon corresponding to emission, can be measured. So, we are going to correlate the fluorescence intensity to the photons and that is measured as fluorescence to the number of photons absorbed. We have a sample, allow part of the radiation to fall on them, part of the photons will be absorbed and part of the photons will be emitted as fluorescence. So, we are going to measure the fluorescence.

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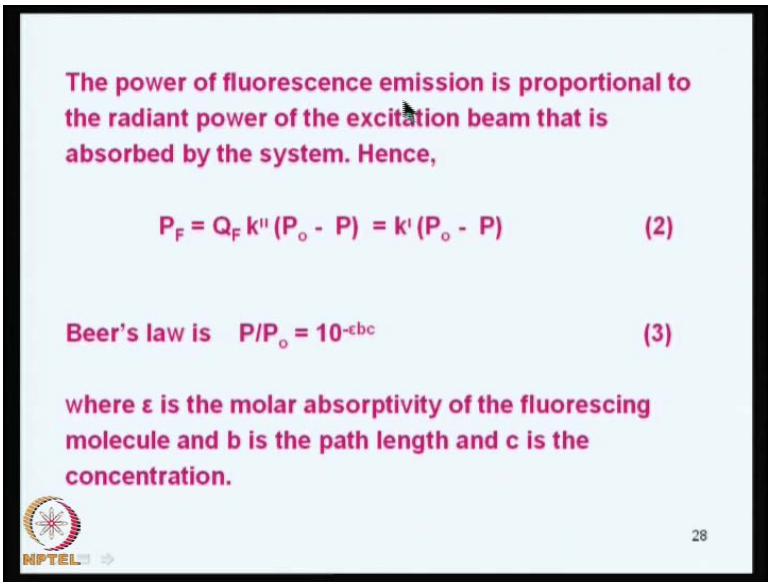

Now, you can see the power of fluorescence emission is proportional to the radiant power of the excitation beam that is absorbed by the system. We can write P F, that is power of fluorescence is equal to Q F, that is quantum efficiency, and a constant K double dash, P naught minus P, that is amount of radiation that is absorbed. So, you can combine both of these constants, fluorescence efficiency, as well as second constant, and make it to a single constant K dash. So, P F is equal to K dash into P naught minus P that is P naught minus P is the absorbance and that is measured. P F is the fluorescence. So, what is Beer Lambert's law? P by P naught is equal to 10 raise to minus epsilon b c, where epsilon is the molar absorptivity of the fluorescing molecule and b is the path length and c is the concentration.

You can see that equation 2 and 3 are almost comparable. The same thing that P F, we if express it in as the ratio, we should always get end up with a similar relationship, where epsilon is a constant, corresponding to fluorescence intensity, not the molar absorptivity, as well as path length and another is concentration.

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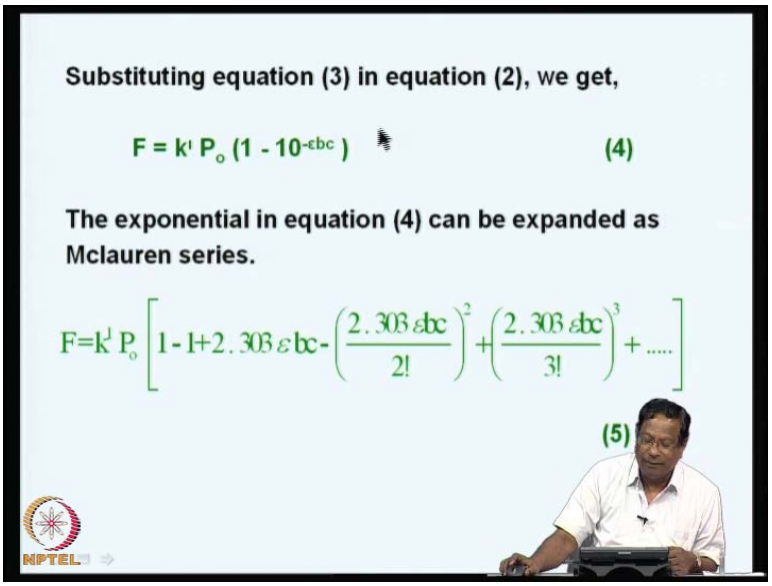

We can look for this equation and when we write that equation in this form, F is equal to K dash into P naught into 1 minus 10 to the power of epsilon b c. Now, the 10 rise to minus epsilon b C, can be expanded as a series; Mclauren series. So, if you write it like this, k dash into P naught into1 minus 1 plus 2.303 epsilon b c divided by 1 factorial minus 2.303 epsilon b c by 2 factorial whole square plus 2.303 epsilon b c whole divided by 3 factorial cube; like that the series will expand.

And once we have this expansion in place, we can see than this 1 and this 1 will cancel. What we have left with is k dash P naught 2.303 epsilon b c, if we assume that the squares if they are less than one, then they keep on decreasing and they will have not much significance in the fluorescence process.

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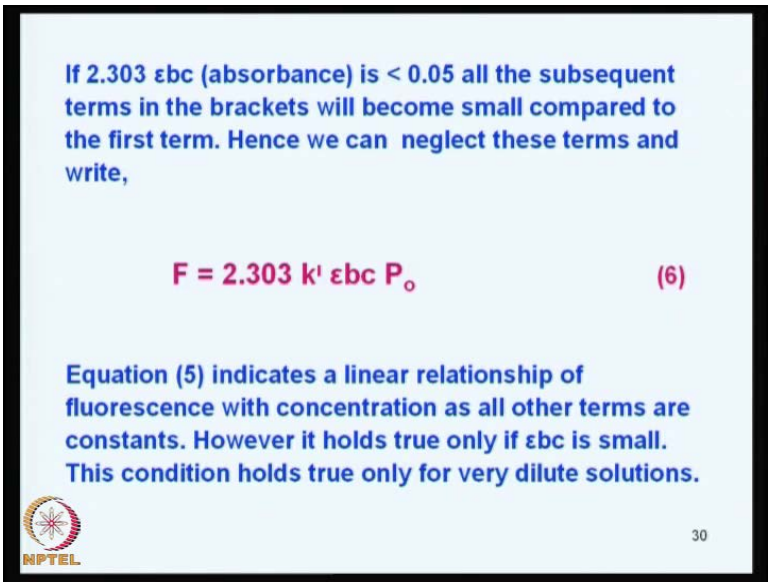

That means, we can neglect them and write an expression something like this. If 2.303 is less than 0.05, then we can neglect the other terms, and simply write it as 2.303 K dash epsilon b c multiplied by P naught. That is incidental and this is nothing but, Beer Lambert law. Put all these constants, 2.303 K dash and epsilon into another constant, and remaining is epsilon b c. So, this equation indicates a linear relationship of fluorescence with concentration. All other things are constant, so only concentration is varying. So, obviously if you measure fluorescence with respect to concentration you should end up with a linear relationship.

So, it holds true only if epsilon b c is small. You see the whole exercise of doing this expression and then expanding it into a Mclaurene series is to make sure that the epsilon b c is small and so that we can neglect the squares and cubes in higher orders. So, this condition holds true only for very dilute solution. If the solutions are more concentrated we cannot apply this rule. So, epsilon b c value should always be lower than 0.05 and that is one of the precondition.

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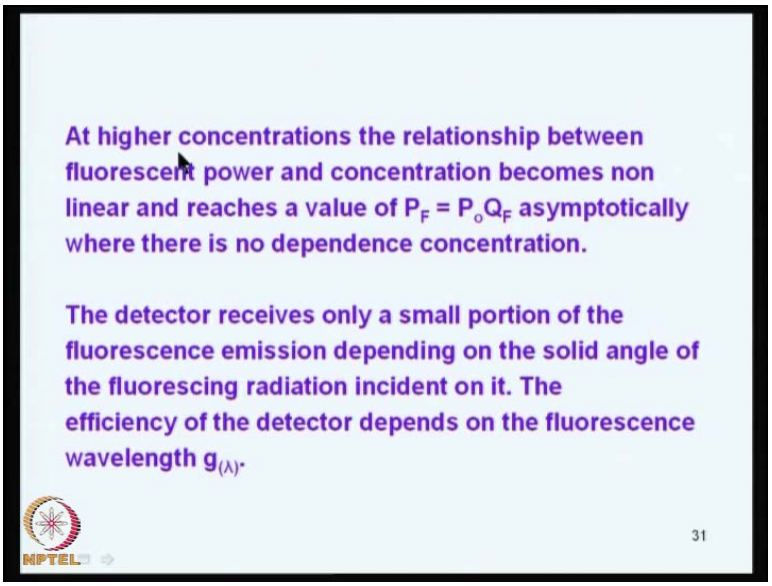

That means fluorescence Beer Lambert's law, it holds good only for very dilute solutions. Fluorescence linearity holds true for very dilute solution. This is advantageous, because if you want to measure the flow of the river, all you have to do is take a substance like this fluorescing compound, put it in the river, go downstream, take 2 kilometers, take another sample and measures (( )). It will be much diluted. Anyway, so this is how the river flows are always monitored by measuring the fluorescence.

So, at higher concentrations what happens? The relationship between fluorescing power and concentration becomes non-linear, that means it reaches the value of P naught into Q F asymptotically, where there is no dependence on concentration. So, fluorescence should not be measured at very high concentrations.

The detector, basically in a spectrofluorometer, receives only a small portion like what I had showed you here. It is if the detector is coming like this (Refer Slide Time: 53:48), if I place a detector on this side, near outside, near this side, then it will measure only this portion that is coming out, but not that is appearing fluorescence this side and other sides. So, only a small portion of the detector or detector experiences only a small portion of the fluorescence. So, I have to make a correction for that factor also. The Beer lambert's law there should be modified further by introducing another concept K, which corresponds to the solid angle through which the detector is being receiving the fluorescence radiation.

The equation becomes slightly much more modified and the fluorescence emission depending upon the solid angle of the fluorescing radiation incident on that. The efficiency of the detector again depends upon the fluorescence wavelength, so whatever comes out of the system we measure.

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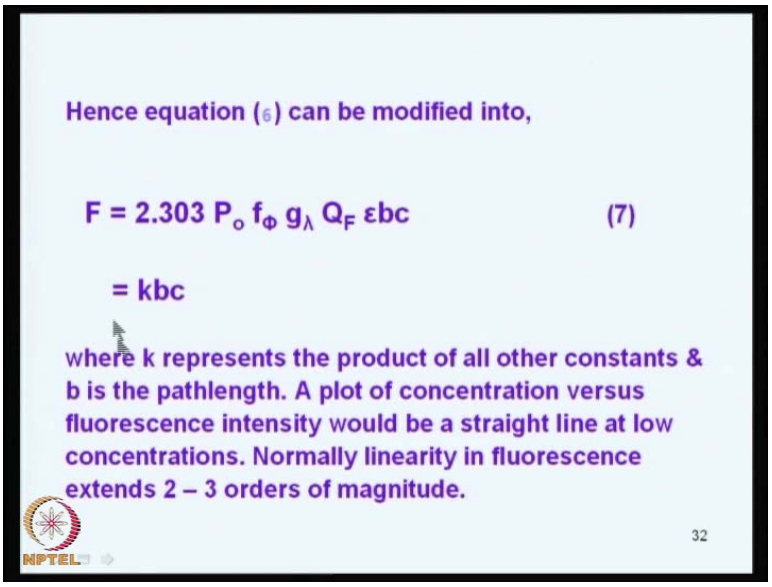

So, equation 5 can be written modified into equation 2.303 P 0, this is one of our previous constant, which is incident power, f phi, that is fluorescence, g lambda that is emission wavelength, and then Q F is efficiency. This has to be put into linear relationship such as k b c, where k represents the product of all other constants, put all of them into one constant. Then remaining thing is b that is path length, which can be varied as other thing is c, the concentration. So, path length, as long as you use the same cell like this (Refer Slide Time: 55:39). This path length, one centimeter path length, if I measure everything in this, the fluorescence should be linear, so long as this path length is constant, the other instrumental factors remain constant.

A plot of concentration versus fluorescence intensity would be a straight line at low concentration normally; linearity in fluorescence extends 2 to 3 orders of magnitude compare to spectrophotometry.

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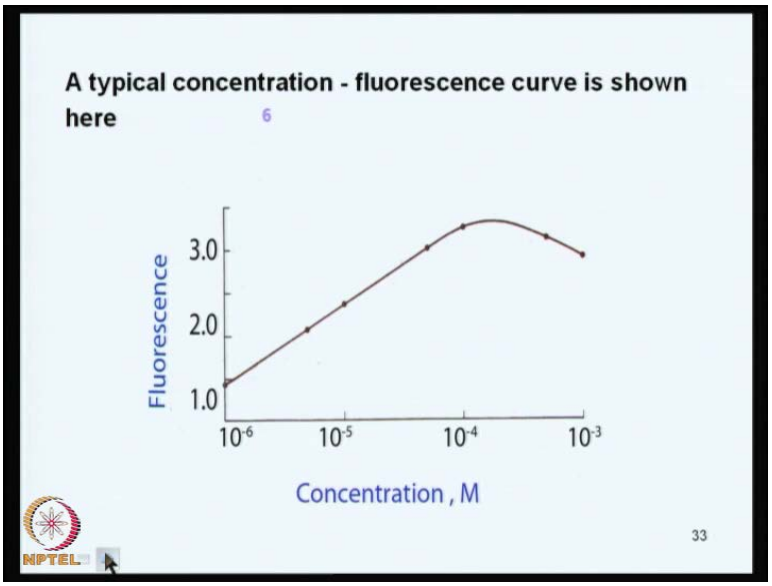

This is a very nice concept to determine the substances at very low concentrations that is if spectrophotometry produces. You are able to determine a substance spectrophotometrically at ppm, parts per million levels, obviously three orders down the line, which means nanogram level of substances, can be determined using spectophoto using fluorescence. I am showing you a typical concentration fluorescence curve, here you can see there a linear relationship over and then it will fall off.

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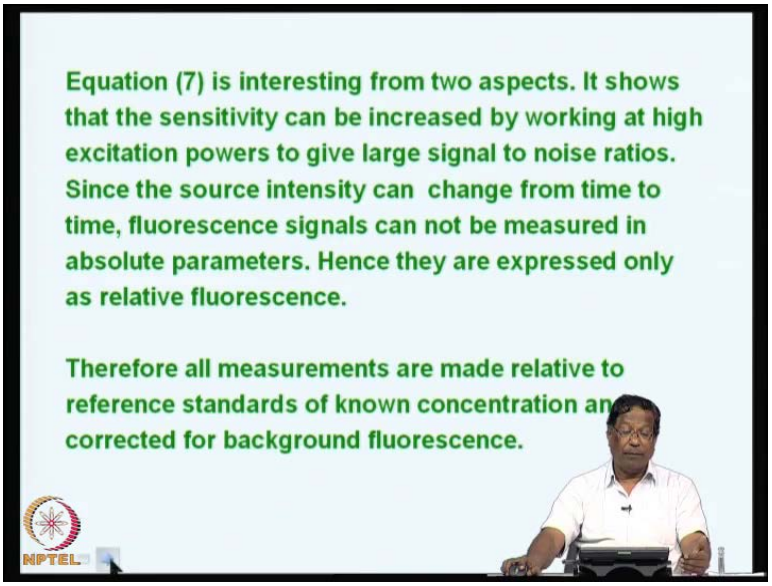

This equation has got interesting from two aspects but, we will continue our discussion in what way it differs from the previous absorbance curves. In what way, this figure is much more important as well as it differs from the absorbance. So, two aspects are there and then we will discuss about what are those two aspects in which fluorescence spectra are different and then we will discuss about the optics and instrumentation in the next class.