Modern Instrumental Methods of Analysis

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Lecture No. # 12

Fluorescence and phosphorescence Spectrophotometry 2 Instrumentation

Welcome to the next lecture on fluorescence. This is a second part of that. Before that I would like to spend some time again, on the principles on which we had discussed in the last class that is regarding the fluorescence spectrophotometer theoretical aspects. What we did in the last class is essentially to understand what is the fluorescence phenomenon, and phosphorescence phenomenon and photoluminescence phenomenon. What we said was that the electromagnetic radiation is absorbed by a molecule is sometimes remitted.

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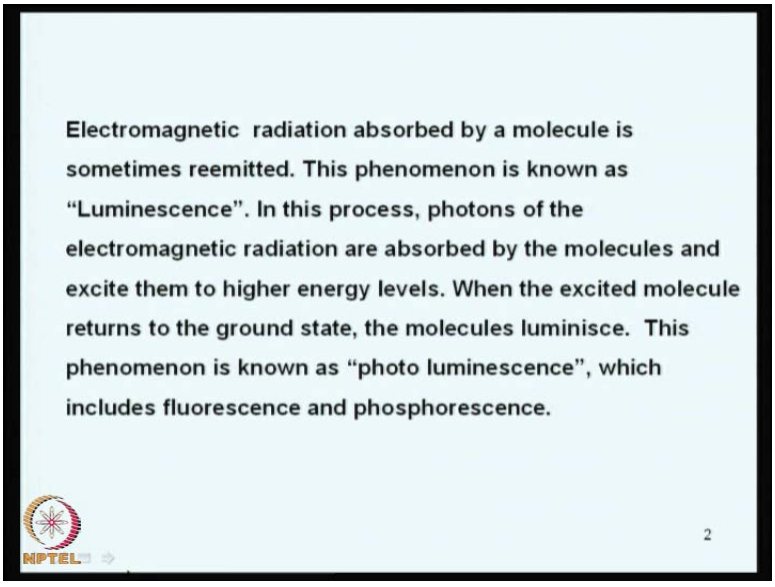

This phenomenon is known as "Luminescence". In this process, photons of the electromagnetic radiations are absorbed by the molecules and excite them to higher energy levels. When the excited molecule comes down to the ground state, the molecules luminisce. This phenomenon is known as "photo luminescence". This includes fluorescence as well as phosphorescence method.

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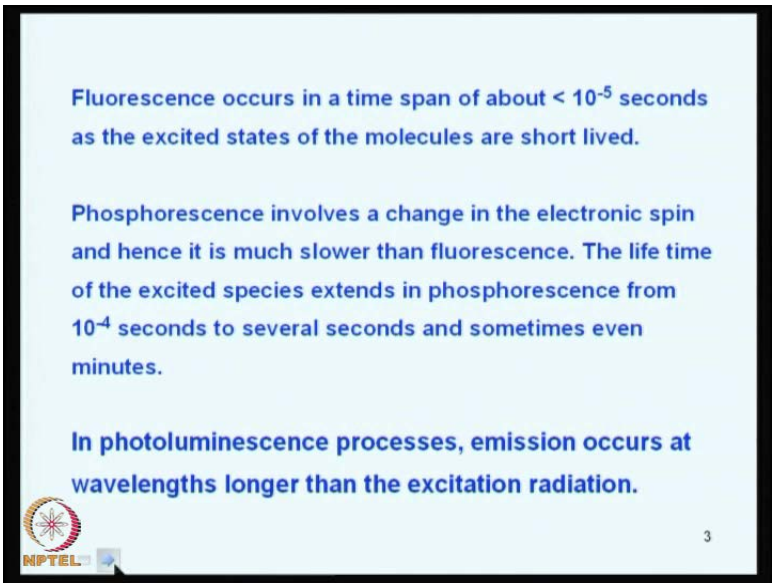

Fluorescence occurs in a time span of about 10 raise to minus 5 seconds as the excited states of the molecules are short lived. Phosphorescence involves a change in the electronic spin. Hence, it is much slower than the fluorescence. The lifetime of the excited species extends in fluorescence from 10 raise to minus 4 seconds to several seconds, sometimes even minutes. In photo luminescence process, emission occurs at wavelengths longer than the excitation wavelength.

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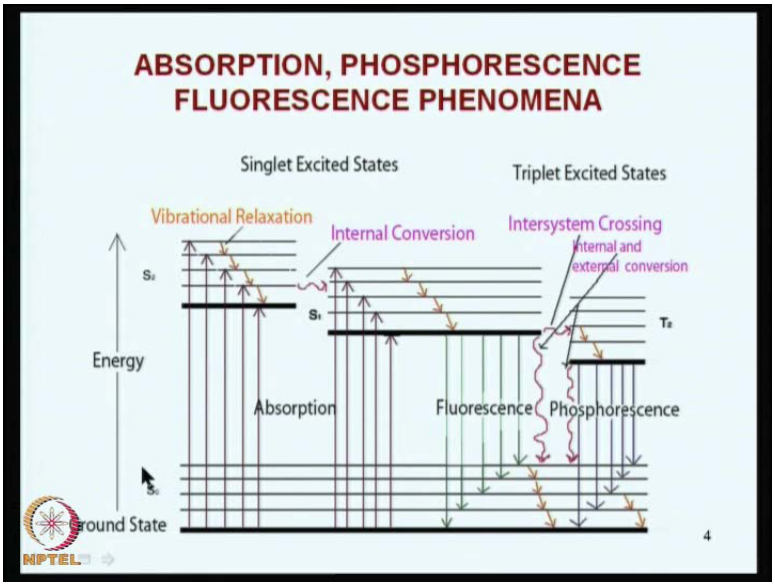

Now, this is the figure we had discussed earlier. The first one is absorption, the second one is fluorescence and the third one is phosphorescence. this is on the right hand corner. Here, you can see an absorbance; it comes down to the ground state and of the excited level and then returns to the ground state of the unexcited level. Here, it loses the extra energy in different vibrational states of the excited state, and then comes down to the ground state, but they may return to any of the vibrational energy levels of the ground state. So, there could be some amount of gradation in the spectra; fine structure.

In phosphorescence, apart from the excited energy state, there is a separate metastable state. So, from the first excited state, they all come down to excited metastable state, from there it returns to the ground state; excited molecules. This process takes more time.

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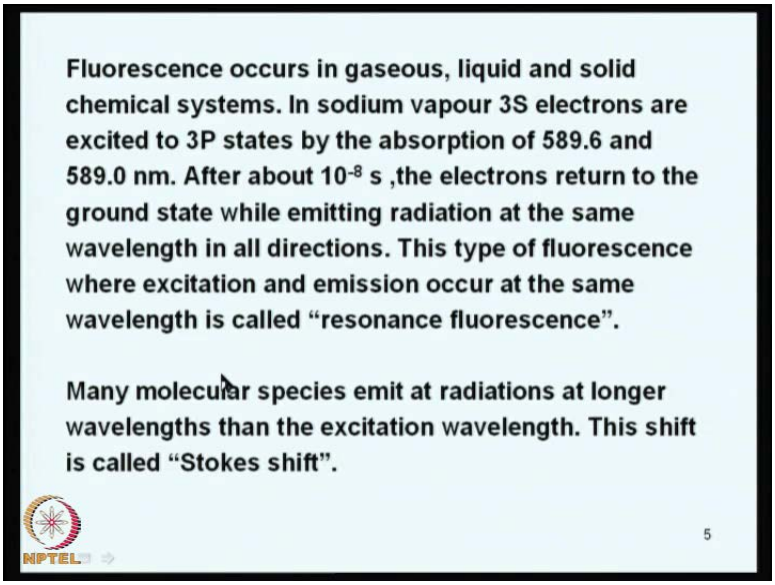

The third one and then we have discussed that fluorescence occurs in gaseous, liquid and solid chemical systems. In sodium vapor, 3 S electrons are excited to 3 P states, by the absorption of 589.6 and 589 nanometers. After about 10 rise to minus 8 seconds, it returns to the ground state. If the emission occurs at the same wavelength then, we call it as "resonance fluorescence". Many molecular species emit radiations at wavelengths longer than the excitation wavelength. This shift is called as "Stokes Shift" and what happens in the electronic excitation.

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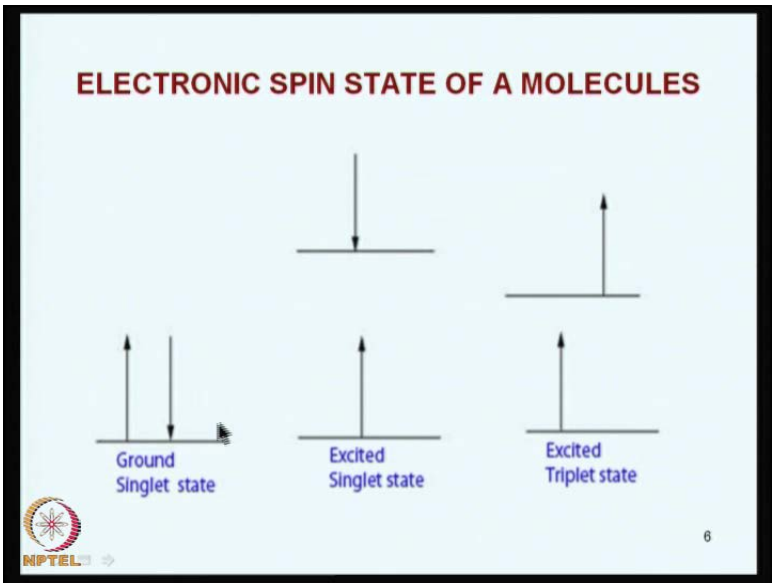

One is in the ground state, it goes to the excited state and here the spins are the same as in the ground state. One electron is pointing up and another is electron is pointing down here. Even in the excited state, there is same spin but, in another possibility the spin also may change in the excited state. If there is a spin, there is this is known as triplet state, otherwise it is known as singlet state.

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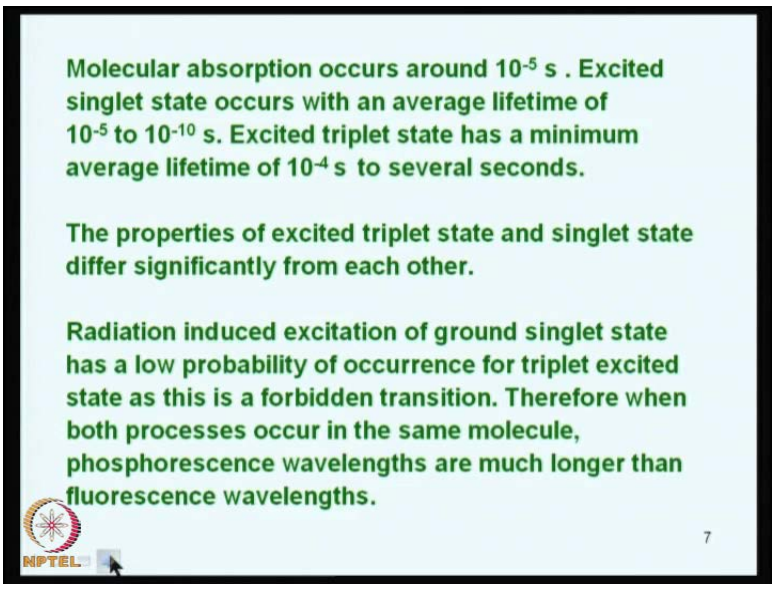

The molecular absorption occurs at around 10 raise to minus 5 seconds and the properties of the excited triplet state and singlet state differ definitely, significantly from

each other. So, the radiation induced excitation of ground singlet state has low probability of occurrence for the triplet excited state, because triplet excited state is usually a forbidden transitions. Therefore, when both processes occur in the same molecule, phosphorescence wavelengths are much longer than the fluorescence wavelengths.

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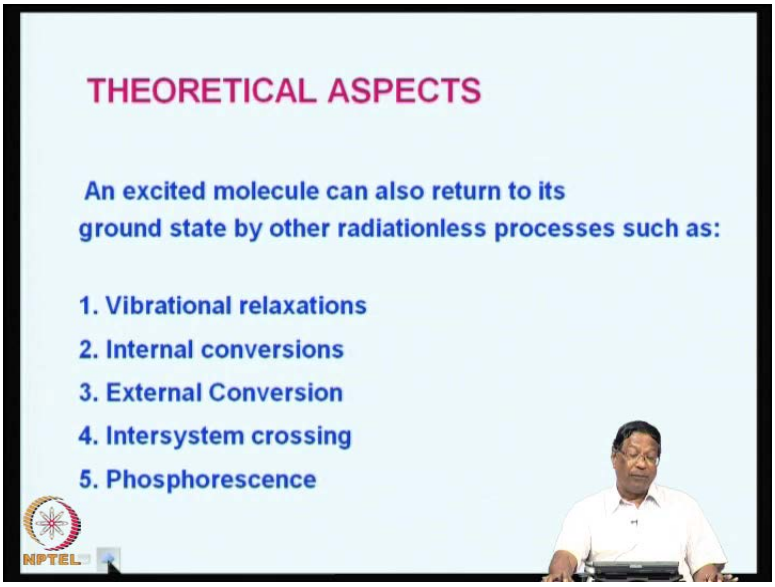

The theoretical aspects, we have discussed that is an excited molecule can also return to the ground state by other radiation less processes, such as vibrational relaxation, internal conversion, external conversion, inter-system crossing and phosphorescence techniques.

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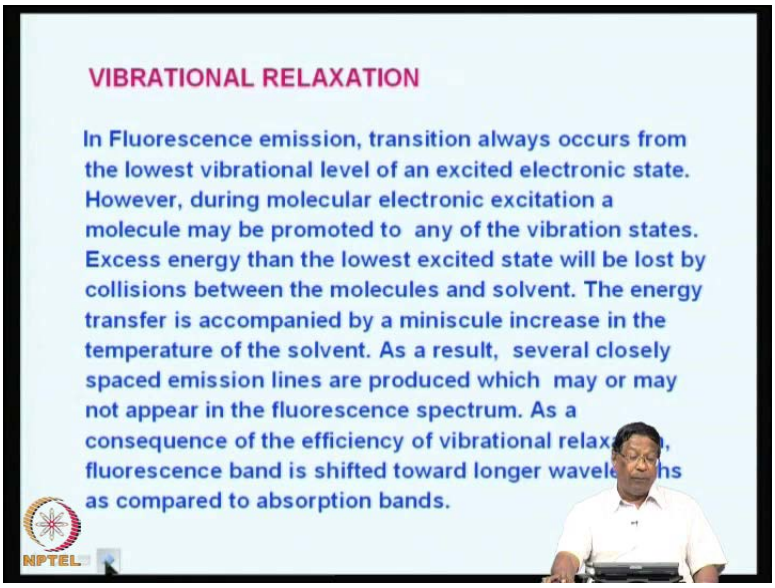

Then we have discussed about vibrational relaxation and then we have discussed about the internal conversions.

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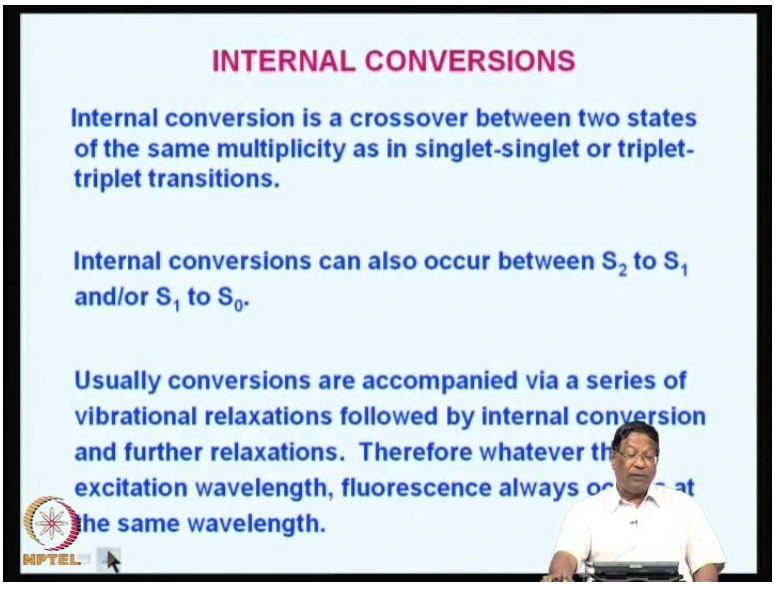

That is between S 2 to S 1 and S 1 to the S 0. Usually, conversions are accompanied via series of vibrational relaxations, as we have seen in the figure earlier. So, whatever, excitation wavelength is fluorescence always occurs at the same wavelength.

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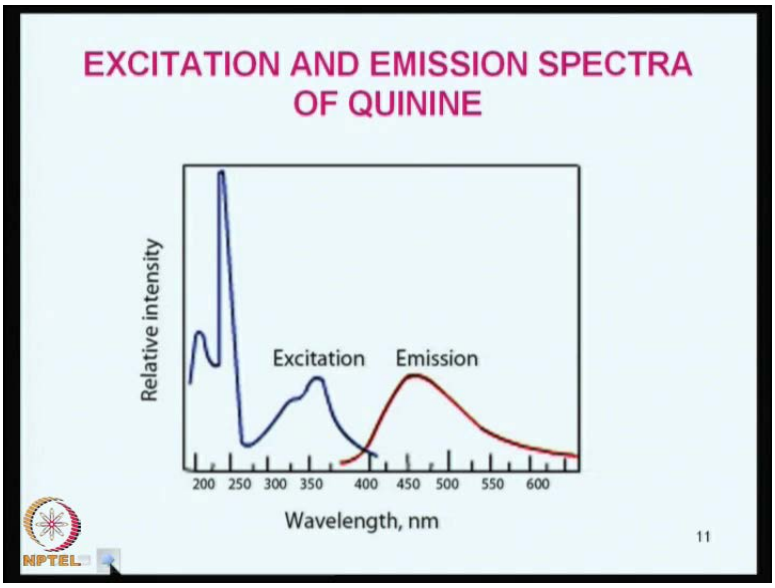

Now, this is an excitation and emission spectra of quinine and you can see that excitation spectrum is only up to 350 nanometers up to 400. Whereas, emission spectrum starts from here and the lambda max for the excitation occurs around 450 to 460 nanometers. That means it reaffirms our supposition that the excitation and emission are slightly different spectra, but they almost look same. As you see, in this figure here, but the emission spectra lambda max occurs around 450 and this is at 350.

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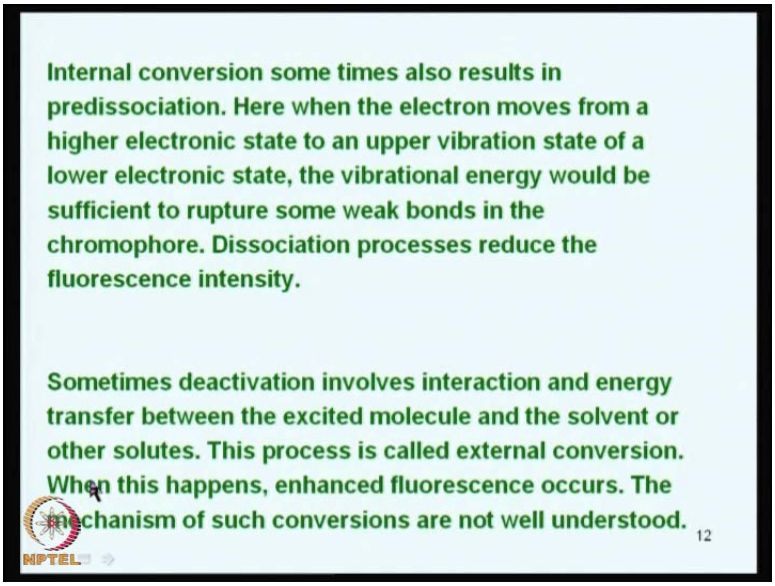

Then we have discussed about mechanisms on how the electron returns to the ground state by predissociation. When the electron moves from higher electronic state to an upper vibrational state of a lower electronic state, vibrational energy sometimes would be sufficient to rupture some weak bonds in the chromophore. So, dissociation processes obviously reduce the fluorescence intensity.

Therefore, high energy system should not be used for fluorescence because they could rupture the bonds. Sometimes deactivation involves interaction and energy transfer between the excited molecule and the solvent and other molecules, other solutes. This process is known as external conversion. Sometimes this can lead to enhance the fluorescence also, but the mechanism of such conversions is not well understood.

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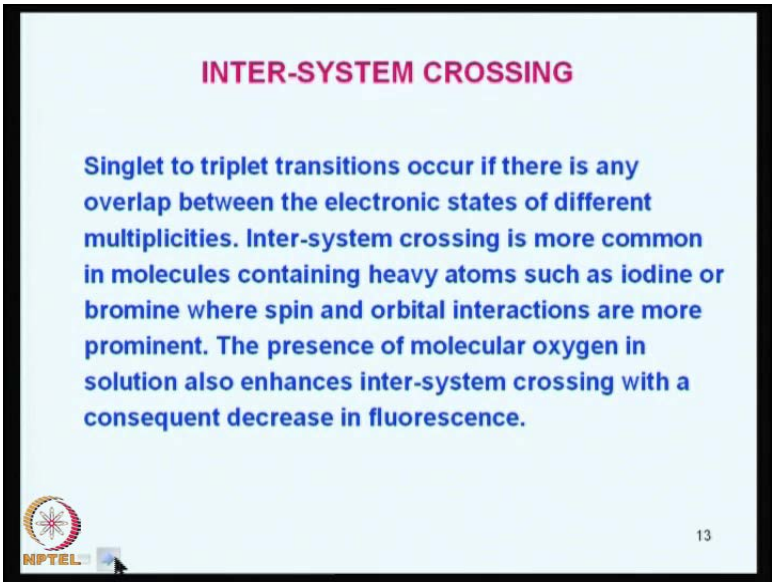

You would see that these reactions are extremely complex and lot of research is required in this area. Then we have discussed about inter-system crossings if there is a triplet transition possible. There could be an overlap between the electronic states of different multiplicities. So, in molecules containing heavy atoms such as iodine or bromine, where spin and orbital interactions are more prominent. The presence of molecular oxygen in solution also enhances inter-system crossing with a consequent decrease in fluorescence. Then we have discussed about phosphorescence and here we have already said that crossing from triplet to singlet state, internal and external conversions also may take place, resulting in decreased fluorescence.

This kind of emission is more common in highly viscous media at low temperatures. Some of the chemical aspects, we have discussed earlier that means we have we cannot use sigma to sigma star transitions, because they are highly energetic and they could rupture the bonds. Instead of exciting the electrons, the electrons are separated from chemical bonding.

Therefore, we can surmise that fluorescence can occur not from sigma to sigma star transitions, but from pi star to pi and pi star to n transitions. Among these the probability of fluorescence occurring is more in n to pi star transition.

So, molar absorptivity of pi to pi star transitions in general, are about 100 to 1000 folds greater then n to pi star transitions. The inherent lifetimes associated with such transitions are of the order of about 10 raise to minus 7 to 10 raise to minus 9 seconds.

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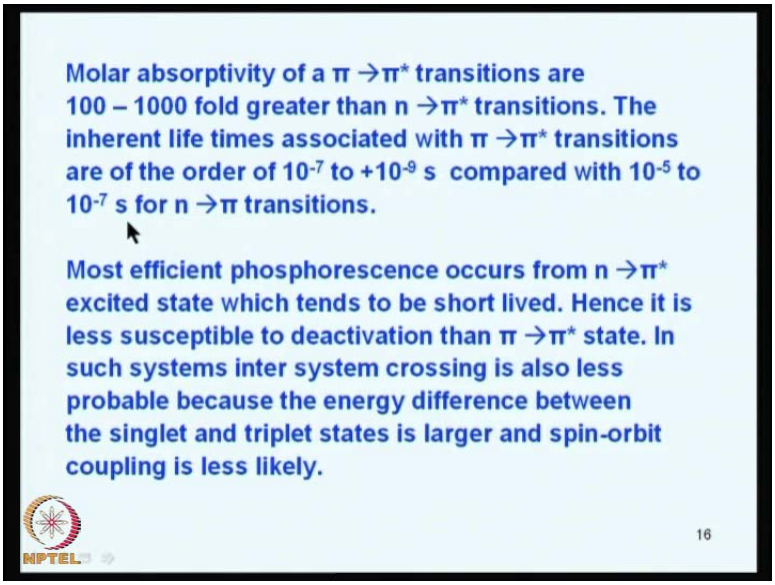

Compared with 10 raise to minus 5 to 10 raise minus 7 seconds, for n to pi star transitions, so obviously n to pi star transitions would lead to fluorescence rather than pi to pi star transition. So, most efficient fluorescence can occur from n to pi star excited state, which tends to be short lived. In such systems inter-system crossing also would be very less. Afterwards, we have discussed some of the important features of fluorescence.

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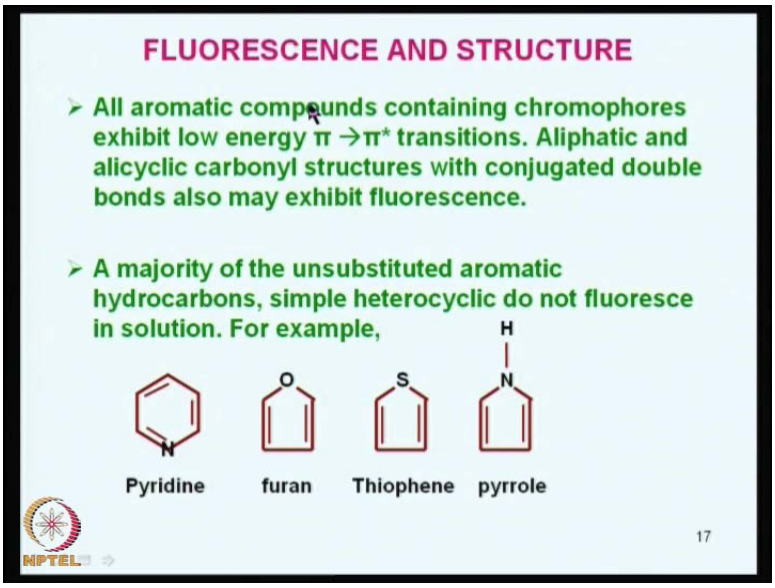

And here we have observed some of the features, which are characteristics of fluorescence. So, all aromatic compounds containing chromophores usually exhibit low energy pi to pi transition. This we know already because we have already discussed with even when we are discussing spectrophotometry. So, aliphatic and alicyclic carbonyl structures with conjugated double bonds also may exhibit fluorescence. Then we would like to show that in such compounds, a majority of the unsubstituted hydrocarbons do not fluoresce on their own, because most of the substances have got pi to pi bonds. So, pi to pi transitions may not lead to fluorescence.

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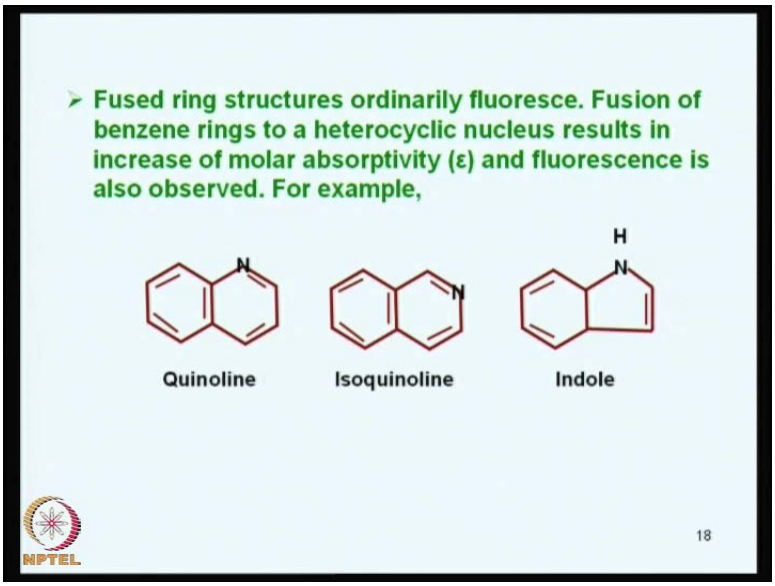

And then we have discussed that compounds with multiple structures like quinoline like this one, isoquinoline and indole. These have got rigid structure rigid planer structures and they could lead to fluorescence. Another possibility is substitution of benzene rings, which will increase the lambda max of the fluorescence. This is also seen quite often. Sometimes substitution with halogens decreases the fluorescence.

Increase in intersystem crossing to that triplet state. For example, in iodobenzene and nitrobenzene, this would decrease the fluorescence and suppose we introduce groups such as COOH, carbon CO group or aldehyde group like CHO, on an aromatic ring. They would withdraw the electrons from the pi electronic system and make the fluorescence process so much difficult. That means they inhibit the fluorescence.

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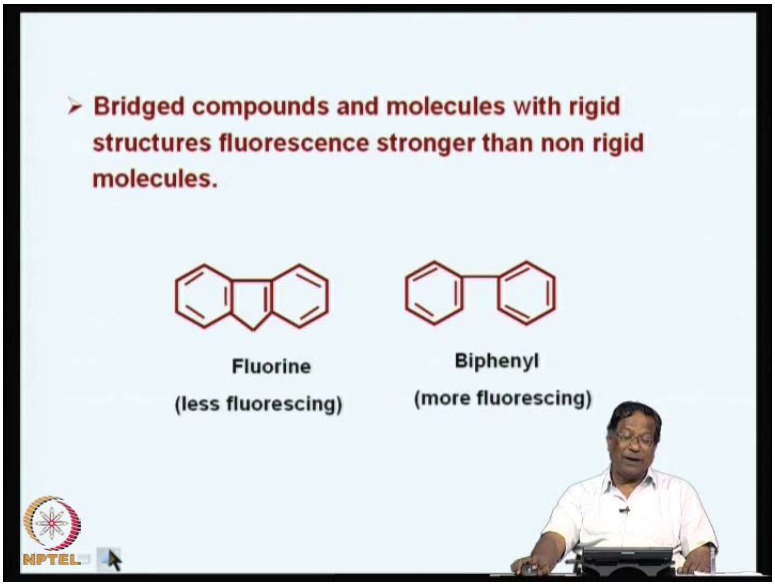

Bridged compounds like this, what we are having flourine and biphenyl, they show fluorescence stronger than non rigid molecule. This is another aspect we have discussed. So, lack of rigidity sometimes increases the internal conversion rate and results in consequent increase in the radiation less deactivation.

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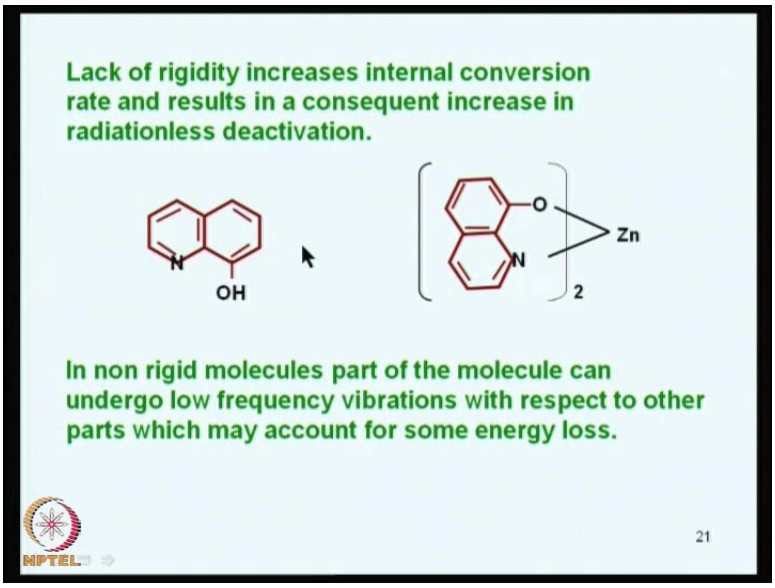

In non-rigid molecules, part of the molecule can undergo low frequency vibrations with respect to other parts, which may account for some energy loss. Here, I am showing you a quinoline with it is complex with zinc and this is 2 molecules of 1 is to 2 stoichiometry. This compound fluoresces, and then fluorescence decreases with increase in temperatures. This we have discussed and solvent viscosity increases because of the in homogeneity.

Solutes and solvents containing heavy atoms decrease fluorescence is another observation and that examples I have given are carbon tetrabromide and ethyl iodite. Here, spin orbital interactions increase the rate of triplet formation. Then we have discussed that if a substance exhibits more resonance forms, more fluorescence could occur. That means high or more of these structures, lower is the energy and still lower energies are required to excite the electrons to the next high energy level, because more number of resonance structures will always decrease the system energy.

Sometimes, P H control is also very important that results in the fluorescence for anions. Suppose, you take a substance, a multi ringed substance into basic medium and anion is produced by dissociation and that could happen that could lead to fluorescence of one of these species and that is mostly anions. Dissolved oxygen reduces the intensity and other paramagnetic substances also present in the sample could decrease the fluorescence. So, with these observations what we can do basically is to try to quantify the fluorescence

phenomenon with respect the concentration if you want to apply the phenomenon for the determination of substances.

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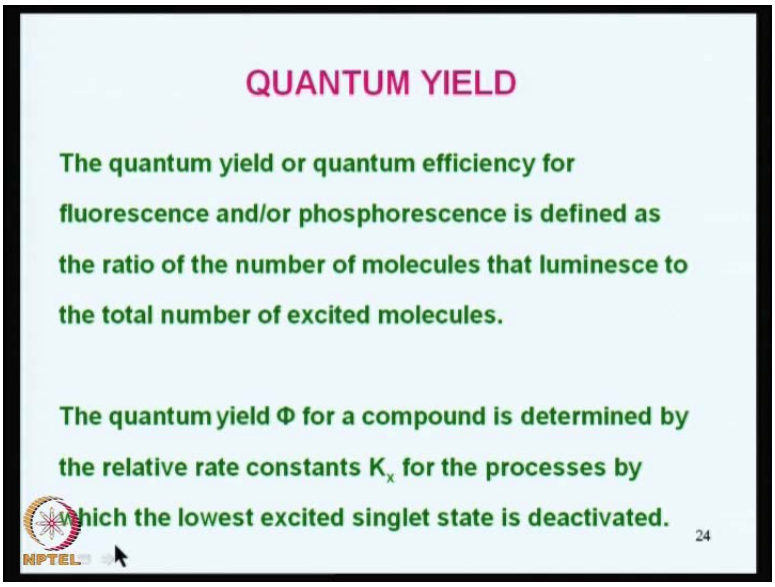

For these we need to define some of the concept that is quantum yield to have defined and quantum yield or quantum efficiency for fluorescence or phosphorescence is defined as the ratio of number of molecules that luminesce to the total number of excited molecules. Quantum field, phi, for a compound is determined by the relative rate constant K x, for the process by which the lowest singlet excited state is deactivated.

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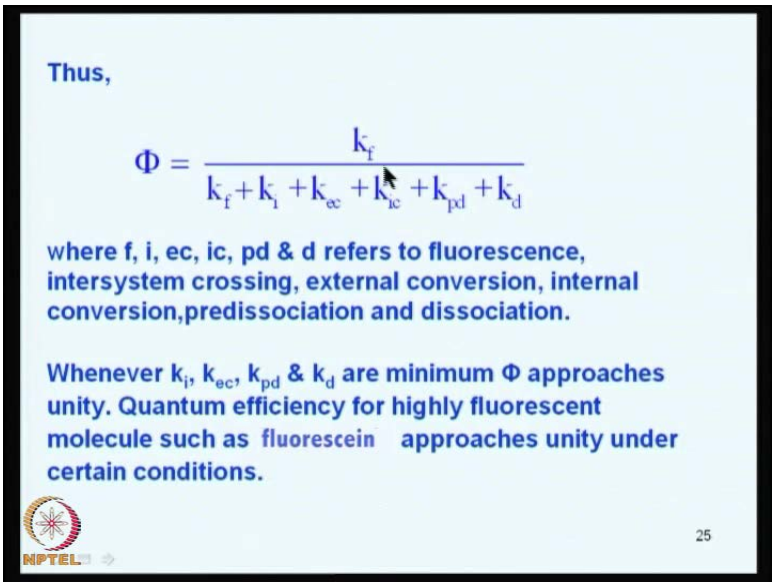

We can write it like this. Phi is the formation constant k f divided by formation constant and the intersystem crossing, and this k e c refers to external conversion, and then internal conversion, another constant is pre dissociation and dissociation. So, all the substances, all the terms in the denominator would lead to reduced fluorescence. So, if the terms k f, k ic, k e c and k p d and k d, if there are reduced then the quantum efficiency would approach unity.

So, the relationship between fluorescence power and concentration follows basically a similar relationship likely Beer Lambert law that we have tried to derive in the previous class and it is supposed to be proportional to the number of molecules excited, which intern is proportional to the radiant power that is hitting the molecule and subsequently that is absorbed and then we have written couple of equations involving P f.

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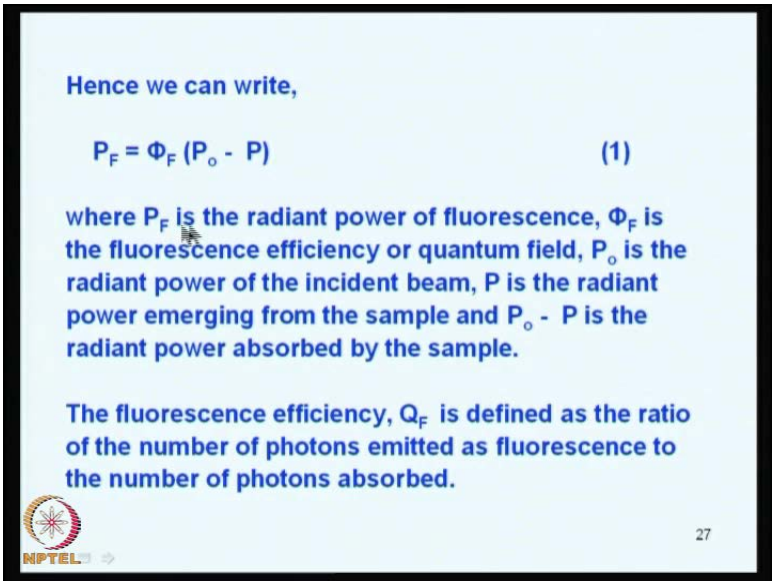

That is radiant power of the fluorescence is a function of the Q F that is fluorescence efficiency or quantum yield, and that corresponds to multiplied by P naught minus P, where P naught minus P is the radiant power absorbed by the sample. The fluorescence efficiency is defined as the ratio of the number of photons. This we have discussed earlier. We can write using the equations the power of fluorescence emission is proportional to the radiant power of the excitation beam that is absorbed by the system.

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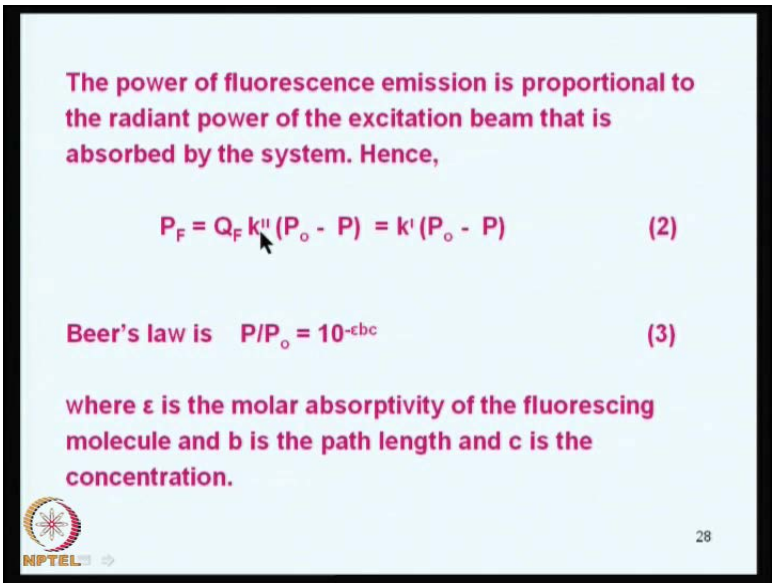

So, you can write a incorporate couple of constants k double dash, so fluorescence efficiency multiplied by a constant multiplied by the difference in the radiation that is P naught minus P. Since, this is also Q F, which is also a constant for a given system, we can convert it into another constant k dash into P naught minus P. But, Beer Lambert law it is nothing but, P naught minus P, P by P naught is equal to 10 to the power of minus epsilon b c, where epsilon is the molar absorptivity of the fluorescing compound and b is the path length and c is the concentration.

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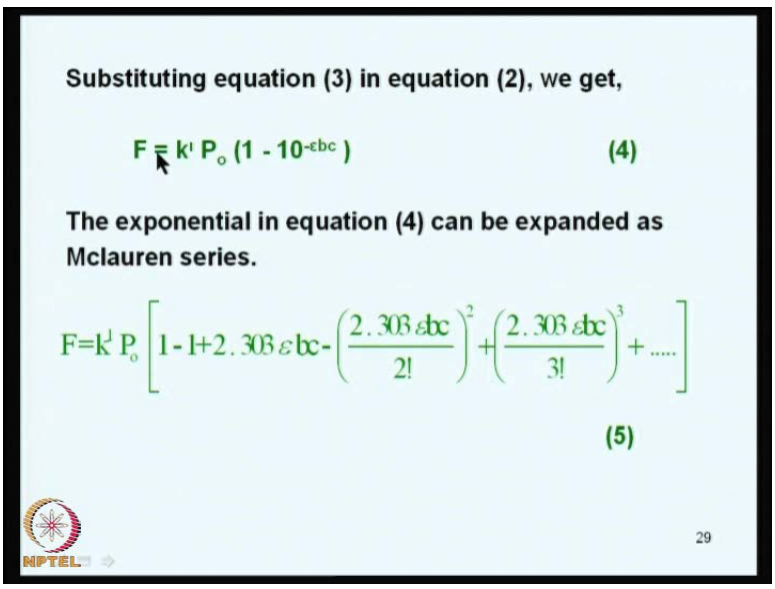

So, substituting all these in this equation, we get fluorescence intensity is proportional to a constant multiplied by P naught and 1 minus 10 to the power of minus epsilon b c. The exponential in this equation is nothing but, a McLauren series. We can expand it and while converting it into natural numbers logarithms, 2.303 is a factor that emerges, then this one and this one, the expansion term, one and one cancel. Now, what we have is 2.303 epsilon b c and all other terms. Now, if the epsilon ratio, if the quantities, fluorescence quantities are very low then the square terms and cubic terms can be neglected. If we do that we will have k dash P naught into 2.303 epsilon b c. so, one and one will get cancelled the other terms are being neglected.

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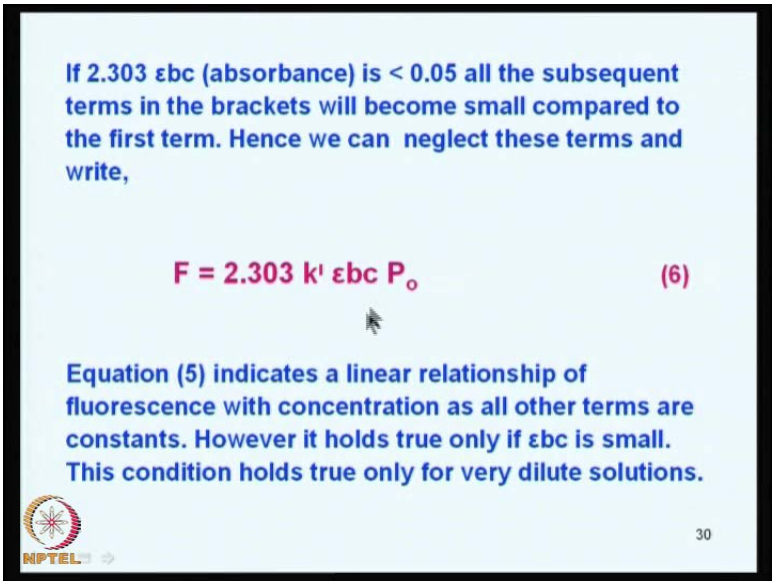

So, when we do this we have something like this; 2.303 k dash into epsilon b c into P naught, where P naught is the incident energy and this equation holds well only if epsilon b c is very small. That means it holds well for only dilute not for concentrated solutions.

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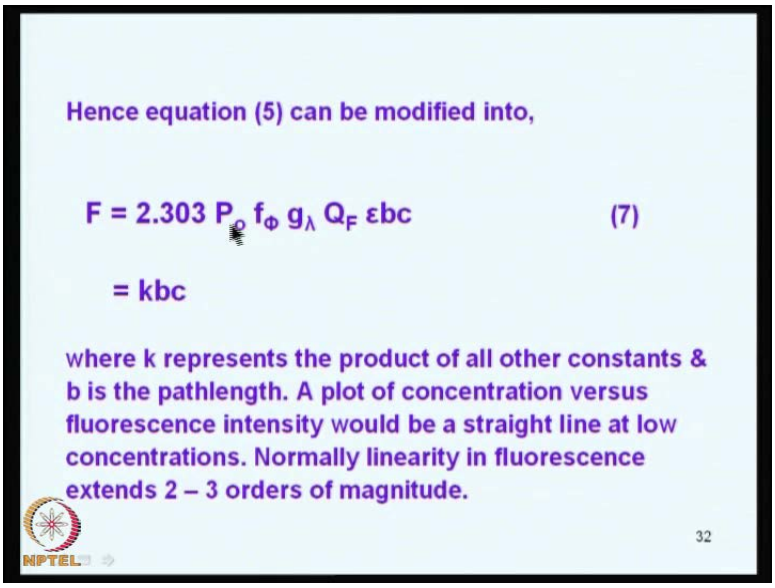

We can again remodify that equation and 2.303 P naught into fluorescence efficiency multiplied by g lambda into Q F into epsilon b c. That should be all, then put all the constant into one another separate constant known as k b c, where k represents the products of all other constants, and beta is b is the path length, and a plot of absorbance fluorescence versus concentration should be a straight line at low concentrations. Normally, linearity is high in fluorescence and it covers the order of about 2 to 3 magnitude of concentration.

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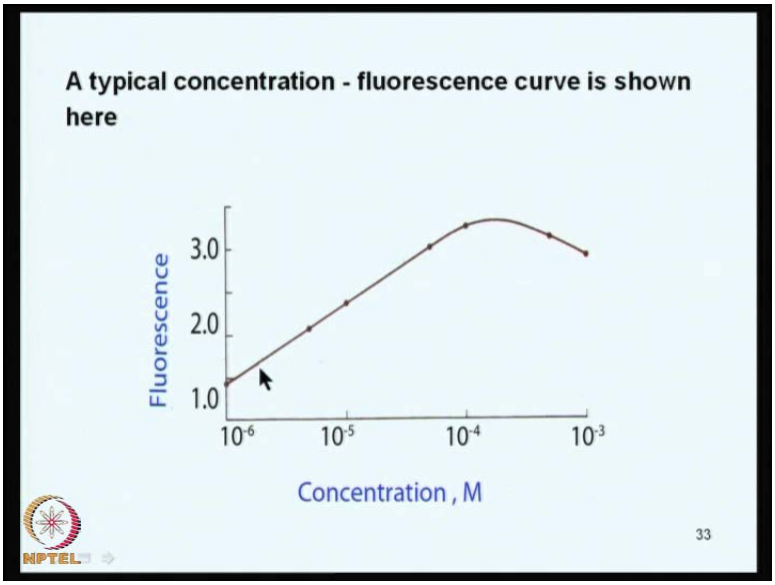

So, this equation if you plot, it would look something like this, and there will be some sort of a reversal concentration at fluorescence at higher concentration.

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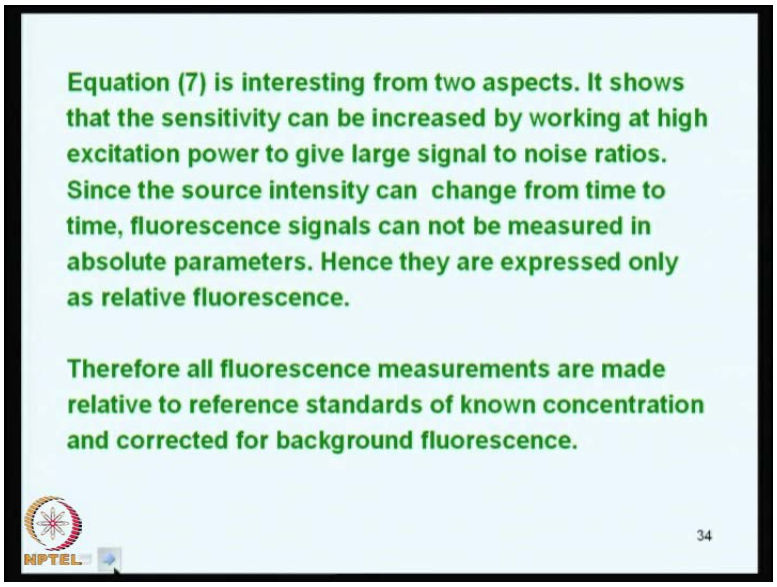

Now, that equation 7, if you do not remember we can go back this equation. F is equal to 2.303 P naught into f phi into g lambda Q F into epsilon b c, this equation is interesting from two aspects. One is it shows that the sensitivity can be increased by working at high excitation power, because it is proportional to P naught. So, if we use higher P naught the fluorescence also is directly proportional so it increases the fluorescence. For this reason, we try to use higher intensity of the source and then we get lo large signal to noise ratio.

Since, the source intensity can change from time to time, fluorescence signal cannot be operated, measured in absolute parameters, because the source intensity itself if it is changing, there is no specific point for which we can refer. That means they are always expressed only as relative fluorescence. This instrument with our source we got so much of reading that is what we are essentially aiming at.

So, these are made, so how do we go for internal comparisons? One thing is we can take a reference standard and specify the conditions for the reference standard and under the same set of parameters like slit widths, source intensity etcetera you can measure and report your fluorescence measurements.

Of course, they must be corrected for background fluorescence also. Another interesting feature of this equation is the freedom to adjust (( )) (Refer Slide Time: 24:35). Remember, we discussed about the intensity, source intensity P naught; if I can use higher intensity my fluorescence range increases. So, if I use more slit that means I am permitting more of the incident light to fall on the sample. So, this also could lead to higher fluorescence. So, the slit widths, it is in spectrofluorometry are much more important than in the spectrophotometry. I use higher slit widths, I increase the source intensity, I use better source at better intensity, and I can get higher fluorescence. So, both these aspects are covered by the expression number 7, which we discussed in the previous slides.

So, it also depends upon the beam geometry. Suppose, I use a different angle like instead of a small beam passing through, I can use a bigger beam and then more light will pass through. So this is how I can look at the slit widths and the beam geometry. Therefore all slit widths are critical factors in fluorescence, instead of the actual cell dimensions. Even though we use the cell dimensions as standard cells, they are not so important in the measurement of fluorescence, but slit widths are the critical factors and solid angle to which, on which we focus the spectrophoto on the cell containing the sample is more important. So, this aspect we have to remember.

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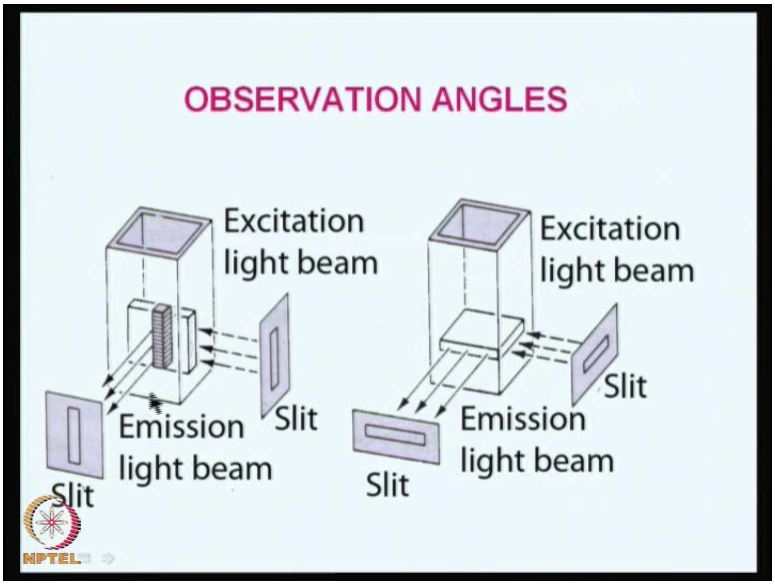

You can see in this figure that we have a slit, emission, excitation beam. The excitation beam passes from this slit onto the sample and then I am measuring the fluorescence at right angles through another slit. So, this is the observation angle. Suppose, I measure on this angle, somewhere here, that means I will be measuring fluorescence as well as absorbance. That is not correct, when we want to measure fluorescence we should always measure at right angles. Not necessarily at right angles, but at a solid angle. Now, you can look at this figure also.

If I arrange this slit, horizontally like this, instead of like this, if I arrange it horizontally like this, then floures well any incident light will fall on a solid block and it comes out again measured at right angles through another slit, which is also arranged horizontally. This gives me a freedom to use a very small sample as big as or as small as the slit widths what we are employing. This is the beauty of spectrofluorometry. That means solution volumes are not so important in spectrophotofluorescence measurements.

What we need is the solid angle coming from the slit and the geometry of measurement. So, we can work with very small amounts of the order of about few micro liters using spectrofluorimetry. Now, you can discuss this further that is if the analyte is too concentrated. Then what happens is the fluorescence versus concentration curve reaches a maximum and falls off. Now, this curve I have shown you earlier this one (Refer Slide Time: 28:51) it comes back, and then the fluorescence curve falls off, and this behavior can be attributed to the attenuation of the signal exciting radiation, as it passes through the cell and results in a further decrease in the exciting power.

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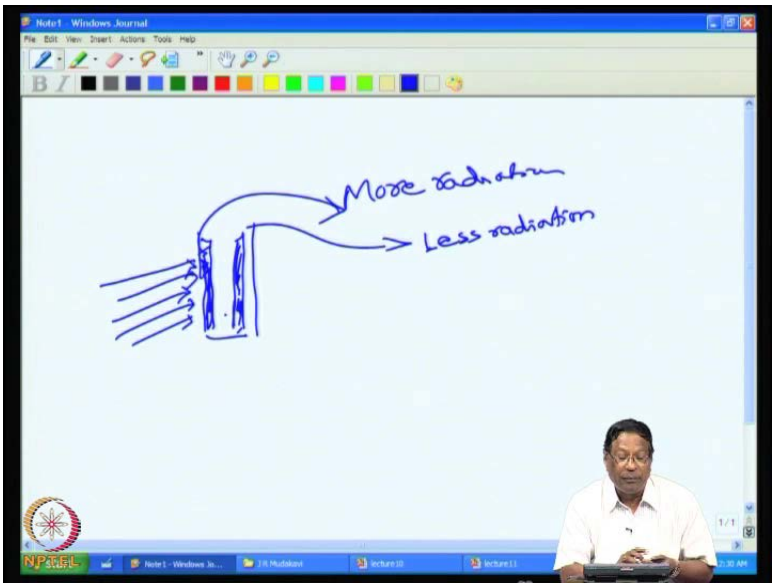

Suppose, you have a figure like this, fluorescence intensity is coming out like this. The portion here would receive much more radiation than another portion, somewhere here. This receives more radiation and this receives less radiation, subsequently if the cell is bigger. So, if the analyte is too concentrated like I was explaining, the fluorescence versus concentration reaches a maximum and falls off, and the portion of the sample that is near the receiving end of the incoming radiation, will receive more incident power than the sections, which are farther away.

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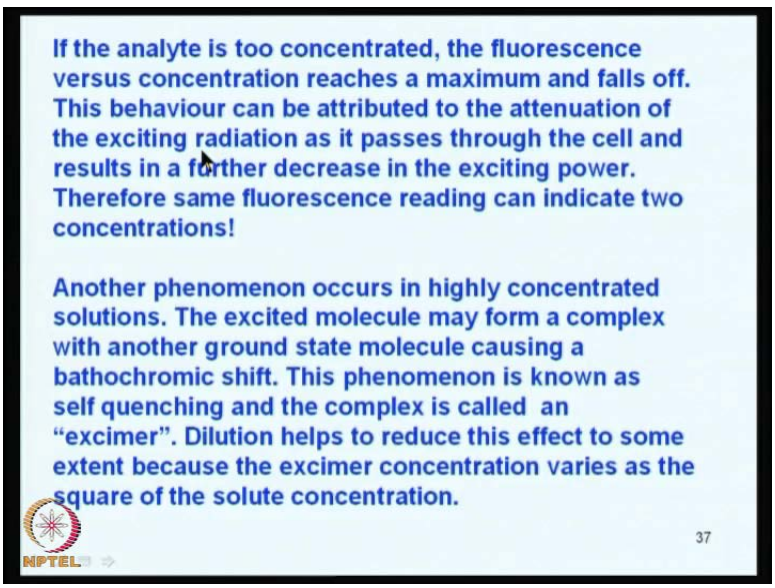

So, this behavior can be attributed to the attenuation of the exciting radiation itself, as it passes through the cell and results in a further decrease in the exciting power. Therefore, the same fluorescence reading can indicate two concentrations. One is it can indicate one concentration as it falls. But, as subsequently, the total fluorescence if you measure it would be lower than that. So, depending upon the thickness of the fluorescence section it can indicate too concentrations.

Therefore, another phenomenon that occurs in highly concentrated solutions is that the excited molecule may form a complex with another ground state molecule; not with the excited state. This can cause a bathochromic shift that means towards longer wavelength region. This phenomenon is known as self quenching and the complex is called an "excimer" and on dilution we can reduce this effect by dilution to some extent. Because the excimer concentration varies as the square of the solute concentrations.

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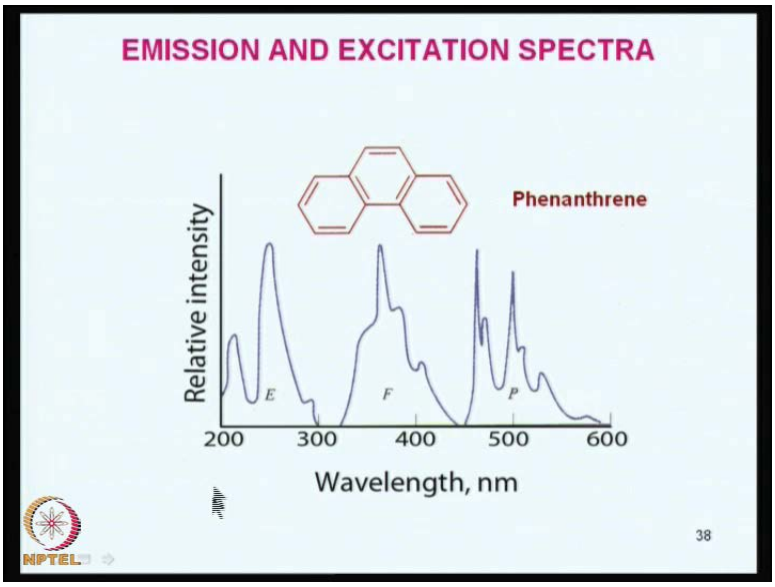

So, how does an excitation and emission spectra look? Here, I have shown a figure and the explanations are here.

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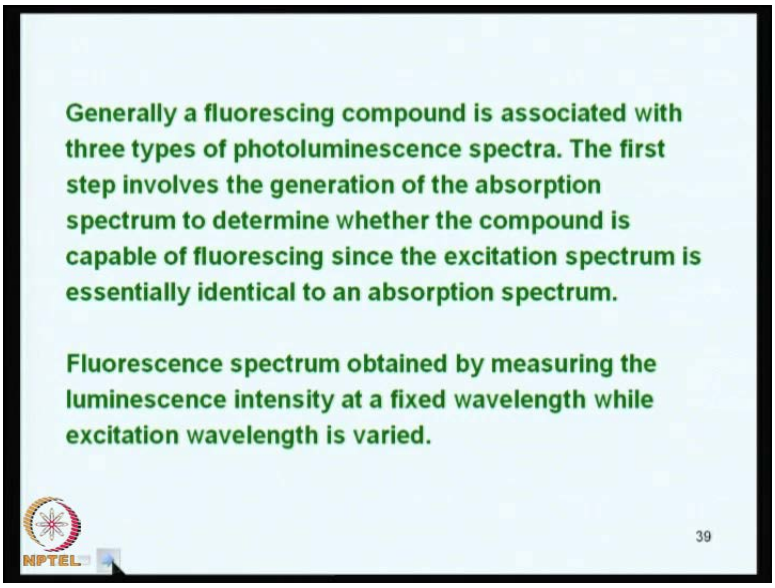

Generally, a fluorescence compound is associated with 3 types of photoluminescence spectra. The first step involves the generation of the absorption spectrum to determine whether the compound is capable of absorbing and only if a substance is absorbing then it can be fluorescing. If it does not absorb then there is no question of fluorescence. So, a substance should have an absorbing structure and then other spectra would be a fluorescence spectrum that is excitation and another could be the emission spectra that are fluorescence.

So, fluorescence spectrum is obtained by measuring the luminescence intensity at the fixed wavelength, where the excitation occurs. Now, you can see here. Again, I am going back to figure, figure e (Refer Slide Time: 33:05) this is a phenanthrene; a phenanthrene compound, it has got an absorption spectrum and then it has got an excitation spectrum, and then it has also got an emission spectrum. Among these the most of the organic compounds would be having absorption spectrum, but that is no guarantee that a substance usually an absorbing compound fluoresces, there is no guarantee.

But, a fluorescing compound can definitely will have absorbing spectrum, so a fluorescing compound like this phenanthrene or any other compound for that matter would exhibit 3 spectrums. One is the absorptions spectrum that is on the left part and then excitation spectrum and the fluorescence spectrum. So, how do you get a fluorescence spectrum? It is obtained by measuring the luminescence intensity at a fixed

wavelength, while the excitation wavelength is varied. For example, in this figure (Refer Slide Time: 34:24), I can get this spectrum by measuring the fluorescence using 500 nanometers fixed and you may vary it between 300 to 400 in this range, so I get the excitation spectrum.

If I had to determine this spectrum; emission spectrum or to fix this, fluorescence spectrum somewhere between 350 and 300, here is the fluorescence maximum, here and then I have to vary the wavelength of the detector from 500 to 600. Then I will get a spectrum like this. So, one when you are measuring excitation, you have to fix the emission wavelength and when you are measuring the emission spectrum, you have to fix the excitation wavelength. Obviously, it will be good, if you could get the highest sensitivity. Therefore, for fluorescence, we should always use highest excitation wavelength. We have to simultaneously make sure that we do not use too energetic radiations below 200 nanometers.

For example, sigma to sigma star transition, we have already discussed, that they could lead to rupture of the bonds, instead of fluorescence. Now, generally we have discussed this earlier that is once excitation wavelength is determined, fluorescence and phosphorescence spectra can be recorded by measuring the emission intensity as a function of the wavelength. Fluorescence and phosphorescence bands are also found at longer wavelengths, as pointed out earlier, I had discussed this. The wavelength difference between the two, we can calculate the energy corresponding to the wavelength difference simply by using Einstein equation which is e is equal to m c square and H C by lambda energy. If we know the lambda we can determine the energy difference.

So, the energy difference can be calculated only if we know the difference between the singlet and triplet state. If we can and how do we know that? By measuring the wavelengths, so if you know the wavelengths, you can calculate the corresponding energy levels and a phosphorescing compound would show the difference between the singlet and triplet state energy levels, if you can simply measure the wavelengths. That is a very beautiful concept to determine the energy of the triplet state.

In a true spectrofluorometers, excitations spectrum as well as the fluorescence emission can be obtained. So, excitation spectrum with suitable corrections for source output intensity and detector response as a function of the wavelength and absolute excitation

spectrum can be obtained. What do you mean by this is the fixed wavelength you are using for measuring the fluorescence, and the excitation while the excitation wavelength is scanned. With suitable corrections for the source output energy and the detector response, as a function of wavelength, an absolute excitation spectrum, we can obtain, which closely should resemble the absorption spectrum otherwise there is neither fluorescence nor phosphorescence.

So, the emission spectrum, again I have put it here that by fixing the optimum excitation wavelength. I am again specifying and highlighting this aspect because quite often fluorescence spectrums are lost; if you do not use the proper excitation wavelengths. Both emission and excitations spectra are approximately mirror images of each other, because the vibrational energy differences for the ground and excited electronic states are also roughly the same. That is why the excitation as well as absorption spectrum have to be similar.

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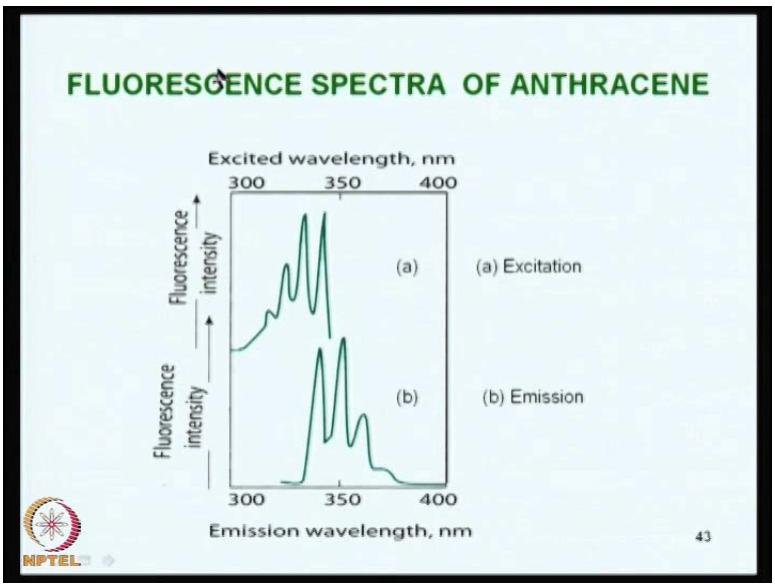

You can look at the figure here. Fluorescence spectra of the anthracene, and the first part on the top is excitation. Here, you can see 1, 2, 3, 4 figures, 4 peaks and that is excitation and at the bottom you can see this is the mirror image of this and this is the second peak in the excitation. in the emission, second peak in the emission is the mirror image of the third peak in the excitation.

This one third peak in the emission is the mirror image of the first peak in the excitation, and fourth one in emission is the mirror image of the first peak. This corresponds to this and second one corresponds to this, longer wavelength, this one is this, and this one is this. So, if you can take a look at both the emission and excitation spectrum, you will be surprised that quite often they are the mirror images of each other. It is a fantastic concept, but it is a fact. Now, let us look at the instrumentation of the spectro photofluorometers.

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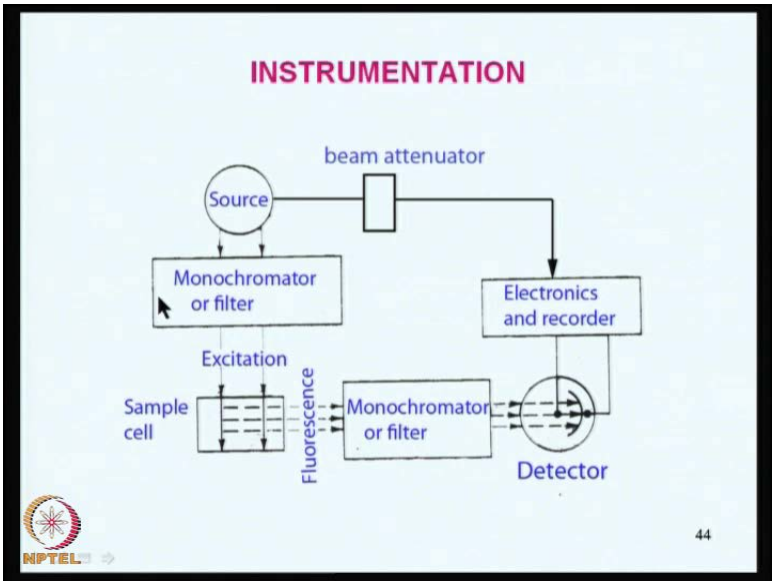

What you need is for the instrumentation is a source of radiation and then I would need a monochromator or a filter to choose the excitation wavelength. That excitation wavelength, I must focus on the sample cell and I have to measure fluorescence at the right angle of the incident radiation. That means, if the incident radiation is coming vertically, I must have a provision to make the measurement horizontally. How do I measure? Here, I need the emission wavelength that means I have to put one more monochromator or filter and that wavelength would be different from the excitation monochromator.

So, in all spectrophotometers, this is the standard feature that is from the source you need one monochromator for the excitation and after the excitation you need one more monochromator or a filter to take out the emission spectrum and that radiation is allowed to fall on the detector. You can have a beam attenuator here from the source; you can

split it and then convert it into using electronics make it fall on this then you can take the ratio of the incident radiation to that of the flouresced radiation.

This is a very simple optics of a fluorescence spectrophotometer. In general, what are the important sources now that we have discussed? It should have a source and then it should have a monochromator for excitation, it should have the sample cell and a sample compartment in which you can insert the sample like what we showed in the first class of this discussion on fluorescence. Then we need the emission monochromator that comes out after the fluorescence. We have to measure it at right angles, whatever comes out at the right angles; I need one more monochromator or a filter followed by the detector.

So, these are the essential components of a spectrophotometer. We will discuss these things one by one. Now, first of all, let us discuss the radiation sources.

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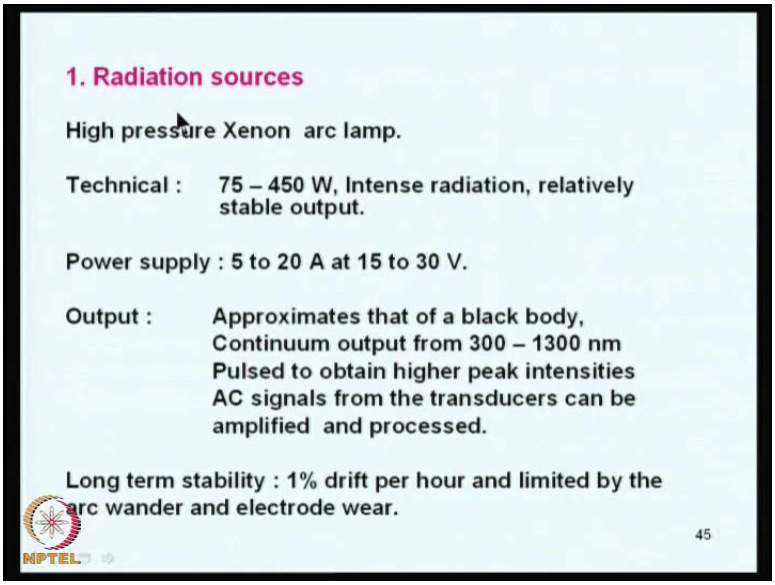

So, first is best that is high pressure Xenon lamp arc lamp; high pressure xenon arc lamp is the most preferred choice for high quality work. What are the technicals? It should be a lamp of about 75 to 450 volts. It gives intense radiation and relatively stable output. Sometimes, there could be a drift and I have written here at the bottom, long term stability, you would see about 1 percent drift per hour and that is limited by the arc wander and electrode wear.

If you run it for longtime, you would need a stabilizer and also the electrode will wear out. Then you need to bring the mirror and to maintain the same lamp intensity, because the lamp becomes very hot. You have to have a cooling system around the lamp to get the optimum output and the power supply has to be between 5 to 20 angstroms at 15 to 30 volts. Now, the output is approximate that of a black body radiation. Now, this black body radiation is essentially a continuous continuum radiation with an output from 300 to 1300 nanometers, and it is pulsed to obtain higher peak intensities. A C signals from the transducers also can be amplified and processed.

The most preferred one is high pressure xenon arc lamp and if you do not have that accuracy required, and then you can go for a xenon flash lamp. Here, the discharge is produced by high energy flash using a charged capacitor discharge, through a lamp filled with xenon. So, what you need is a point 8 mm capillary flash lamp that produces an image of 2 mm wide and 18 mm height. This is also very useful for microcell and for continuous flow measurements.

This xenon flash lamp is typically low cost and having a compact source. Then another source is blue light emitting LED. Basically, LED'S are very small lights with about 0.1 to 0.3 volts and if you take a blue light emitting LED, they emit radiation between 450 to 475 nanometers, but you need to coat the LEDs with flourophores inside the bulb and then mixtures of phosphors can provide excitation wavelengths up to 375 nanometer. This is good enough for most of the organic compounds.

Another source is low pressure mercury vapor lamp; here mercury vapor lamp is very common, very cheap, and you can just buy it across the shelf in the city wherever you are living and it has to be coated with a phosphor to emit a nearly continuous spectrum. You would have seen lot of mercury vapor lamps in your town or somewhere around. Nowadays, people are using C F L; this is essentially the same composition, but it will be a single bulb single lamp etcetera coated with a phosphor. They may also be used with a clear bulb; you need not have to coat it with phosphor. Now, when you coated with phosphor you get a continuous spectrum that means you can choose the excitation wavelength from 350 to 475 or something like that.

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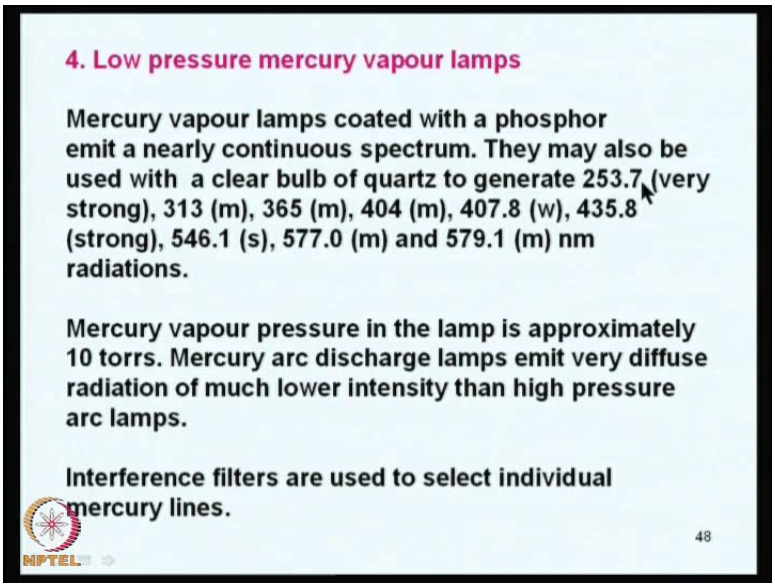

If you use a clear quartz window, then you get intense emission lines corresponding to 253.7 nanometer, and that is a very strong radiation of 253, and 313 nanometer is the medium strength wavelength; you will get one more line spectrum and at 365 is one more that is also medium like 404,407 and 435.8 nanometer this is very strong one and 546.1 is strong and 577.0 is medium and 579.1 mm nanometer radiations. What does it mean is it gives you a radiation comprising a group of wavelength, which correspond to 253.7, 313, 365, 404, 407.8, 435.8, 546.1, 577 and 579.1, that is if you use a clear mercury lamp.

Suppose, you use a phosphor coated mercury lamp; it will give you a diffused wavelength and the concentration and the strength of these lines would be much lower in the phosphor coated lamp. Whereas, if you use a clear lamp, they would give you the radiation corresponding to this, as a very high absorbance or intensity, so mercury vapor pressure lamp would have approximately 10 torse of vapor pressure in the lamps, not more. So, mercury arc discharge lamps also emit much diffused radiation of much lower intensity than with high pressure arc lamps.

If you want to use line sources, you would be using only 1 or 2 emission excitation wavelengths. So, in most of the regular instrument, 253.7 is a very normal excitation wavelength, so if you provide a filter removing all other wavelength you would be getting a very highest strength 253.7 nanometers, and suppose you want 313, you need to

use another filter. So, like the different filters are available where you can choose the excitation wavelength. So, in general what we do is we use interference filters to select individual mercury lens that is very important.

Unlike, xenon arc lamps, which will give you high intensity continuous wavelength for excitation; mercury lamps will give you high intensities specific lines, but very few lines and this could be used in low end instruments. In high end instruments, mercury lamp I mean xenon arc lamp and in low end instruments mercury lamp is preferred. Now, you can also use lasers, for example, Nd yag laser that gives you fixed wavelength laser of very high intensity, but also used in fluorescence detectors, therefore, why because they give you only one wavelength and any of the fluorescing compound, which can get excited at that particular wavelength only can be used for fluorescence.

So, these have got somewhat limited applicability compare to xenon arc lamp, and LED and mercury lamps. My order of preference would be first preference is xenon arc lamp if I have the spectrofluoremetry measurements to be made and high level measurements that is corrected spectra, excitation wavelength, I want to choose emission wavelength I want to choose etcetera, all these things. First preference would be xenon arc lamp followed by mercury lamp, followed by other, if I do not have both of them, then I would rather go for simple LED and if LED is also not available, I would like to go for Nd yag laser. Now, let us look at the optics and usually we have two types of instruments, one is filter photometers filter spectrofluorometers, sorry, it has to be filter spectrofluorometers or filter spectrophotometers spectrofluorometers.

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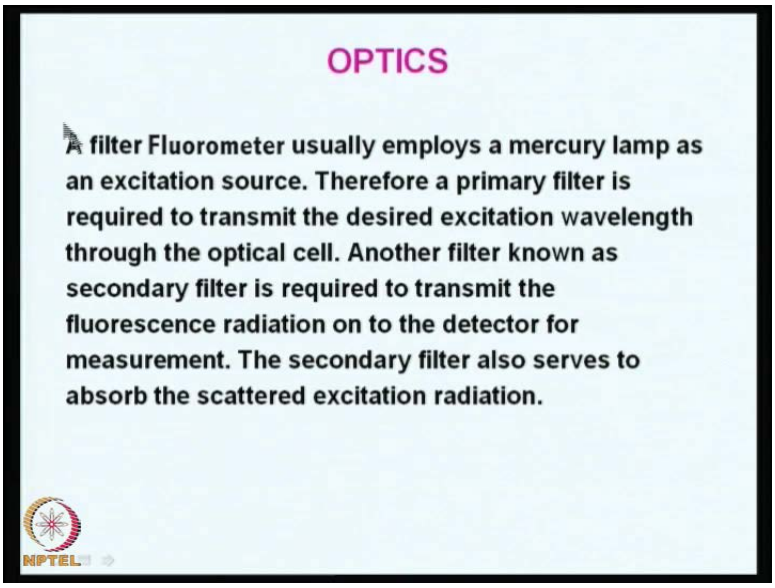

A filter fluorometers usually employs a mercury lamp as an excitation source, primary filter is required to transmit the desired excitation wavelength through the optical cell. Another filter is known as the secondary filter that is what we discuss to measure the fluorescence radiation onto the detector for measurement. The secondary filter also serves to absorb the scattered radiation, excitation radiation. So, we will continue our discussion on filter photometers as well as spectrofluorometers in the next class that is we will concentrate more on the optics in the next class; optics and detectors etcetera. So, I would stop here so that you could try to understand what are the basic things involved in the optics of a spectrofluorometer or filter fluorometer for that matter.