Atomic and Molecular Absorption Spectrometry for Pollution Monitoring

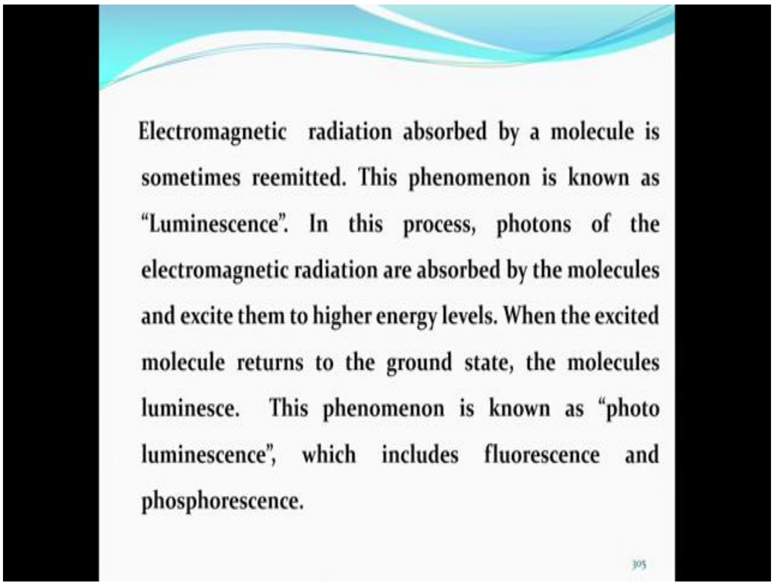
Dr. J R Mudakavi
Department of Chemical Engineering
Indian Institute of Science, Bangalore

Lecture – 21
Florescence Spectrophotometry – I

Greetings to you: let us continue our discussion on the accessories of spectrophotometer that includes fluorescence and phosphorescence spectrophotometry. Yesterday I have given a small brief introduction to fluorescence and phosphorescence phenomenon.

Basically, fluorescence phenomenon is approximately equivalent to spectrophotometry, but phosphorescence there are no it phosphorescence measurements cannot be accessed through a an accessory as a spectrophotometer; you have to have a dedicated instrument for phosphorescence, but for fluorescence there is an accessories available. But even though accessories available nowadays, I have yesterday informed you that the fluorescence phenomenon studies and requirements in instrumentation and other things have a (Refer Time: 01:23) the development of fluorescence spectrometry as a science of its own therefore, even though accessories available you can also choose a dedicated fluorescence spectrophotometer so.

(Refer Slide Time: 01:42)

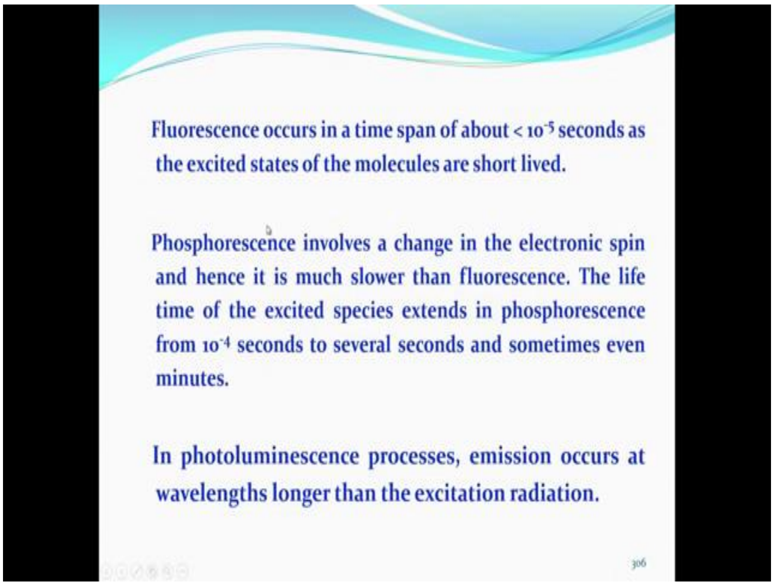


Electromagnetic radiation absorbed by a molecule is sometimes reemitted. This phenomenon is known as "Luminescence". In this process, photons of the electromagnetic radiation are absorbed by the molecules and excite them to higher energy levels. When the excited molecule returns to the ground state, the molecules luminesce. This phenomenon is known as "photo luminescence", which includes fluorescence and phosphorescence.

Let us see; what is fluorescence. Now I hope you remember that our discussion about electromagnetic radiation how it is observed by molecule and the absorption phenomenon is accompanied by the reduction in the intensity of the incident light that is absorption. And some sometimes what happens is the if the particle if the solution is not clear, then there will be scattering turbidy turbidance flow metric phenomenon and all those things reflection, reflection refraction no all these things will be taking place and one of them is fluorescence.

So, inflorescence what happens is electromagnetic radiation absorbed by a molecule in a given solution is sometimes re emitted at a reemitted. If the this is known as luminescence, in this process photons of the electromagnetic radiation are absorbed by the molecules and then excite them to higher energy level, and when the excited molecule returns to the ground state the molecules luminous; this phenomenon is known as photo luminescence also, which includes both fluorescence and phosphorescence. So, we can define electromagnetic radiation as a absorbed by a molecule either through photo luminescence method technique or through simple you can call it luminescence also.

(Refer Slide Time: 03:29)



Fluorescence occurs in a time span of about < 10^{-5} seconds as the excited states of the molecules are short lived.

Phosphorescence involves a change in the electronic spin and hence it is much slower than fluorescence. The life time of the excited species extends in phosphorescence from 10^{-4} seconds to several seconds and sometimes even minutes.

In photoluminescence processes, emission occurs at wavelengths longer than the excitation radiation.

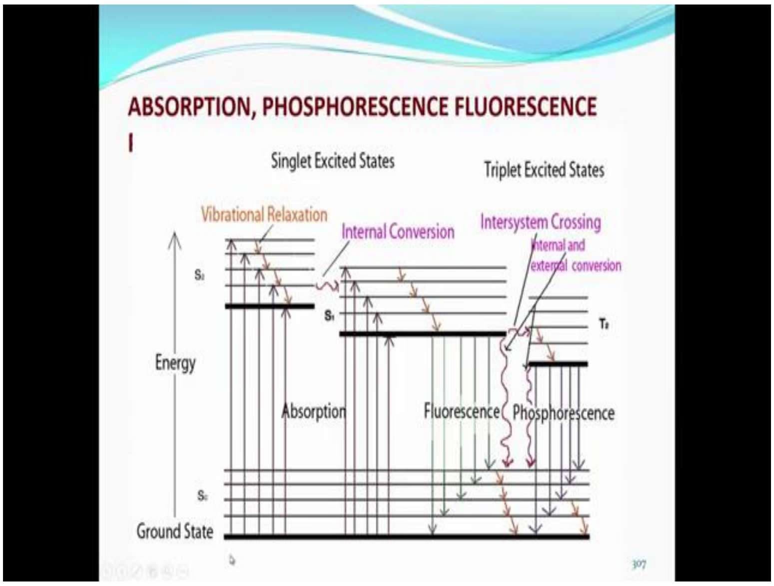
Now, fluorescence occurs in a time span of about 10 raise to minus 5 seconds as the excited molecules are short lived. In general absorption takes place in about 10 raise to minus 15 seconds.

Compare to that fluorescence is quite a slow process which take play takes place in about 10 raise to minus 5 seconds and phosphorescence takes much more time, it may extend even up to a few minutes. So, phosphorescence involves a change in the electronic spin. And hence it is a physical phenomenon it has to be much slower than the fluorescence. The lifetime of the excited species in phosphorescence also extends from 10 raise to minus several seconds minus 4 seconds to several seconds sometimes even minutes. So, this is the main difference between the between fluorescence and phosphorescence phenomena. In photo luminescence process emission occurs at the wavelength longer than the excitation radiation.

Now here you have to understand that whenever we talk of fluorescence: we talk of two wavelengths a electromagnetic radiation of two different energies and two different wavelengths, because when the molecules absorbs part of the radiation and if the radiation that is coming out is of different wavelength, then the incident light we call it as x a as the excitation wavelength and the radiation that comes out is called as emission, so emission radiation.

So, obviously if part of the energy is absorbed by the molecules, the energy of the out coming radiation would be definitely much lower than the incident light. So, if the energy is lower the wavelength should be longer it is a simple phenomena which you had discussed number of times.

(Refer Slide Time: 05:48)



ABSORPTION, PHOSPHORESCENCE FLUORESCENCE I

Now, this is the schematic presentation of the absorption phenomena; absorption, fluorescence and phosphorescence. So, here what happens is most of the molecules are in the ground state, and the many of the ground state energy states are occupied by the electrons in S 0 state, here I have the excited state S 2, S 1 and T 2 I have I will come back to you why I call them S 2, S 1 and T 2 and here I have to take you to the fundamentals of absorption phenomenon, and whenever the energy is supplied in the to the molecule in a spectrophotometer what happens is, the electrons absorbs radiation and go to the next higher energy level.

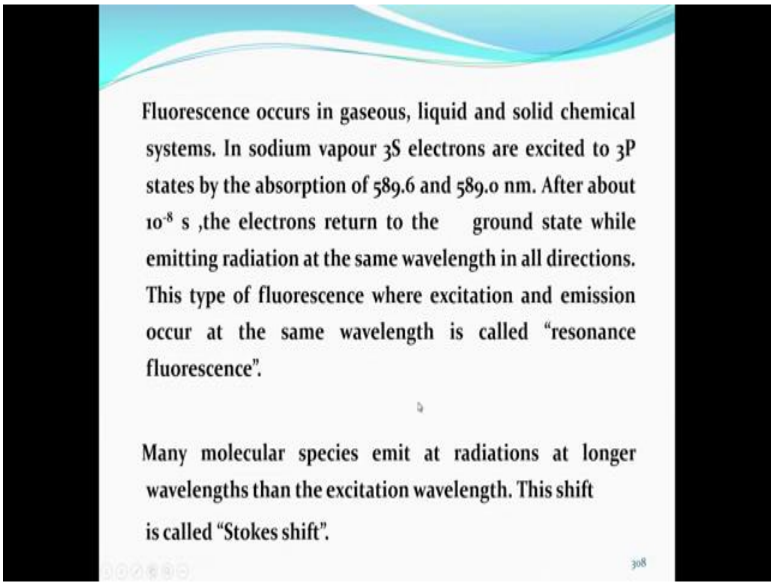
And as I have told you earlier the energy absorbed is released through several vibrational and rotational energy levels and comes back to the ground state of the excited energy level, which I have shown as a bold line here. From here the return will be to the ground state any one of these types, but mostly populated on the bold line which I have drawn at the bottom near S 0. So, in the absorption phenomena you can see that the energy is simply absorbed and the output is having energy less than the incident energy. Now hear what happens in this case this is the internal conversion, and if the electrons lose their extra energy to come back to S 1 and then.

From here obviously, the wavelength this much energy is absorbed and lost and the corresponding energy when it is emitted will result in an electromagnetic radiation that is having a longer wavelength. So, the electrons molecules can come back to the ground state at different energy levels also, in the most populated energy level is the one which will show you and the fluorescence peak. So, fluorescence phenomena are determined like this.

Now you can see I have also drawn one more system, where the bold line in the third system third type is I have designated as T 2, and internal and external conversions what happens is the from here the energy there is one more stable energy level which is not exactly an excited energy level that is electronic energy level, but slightly lower and, but having a definite energy corresponding energy. So, the molecules will not only come to the S 1 in this case as in the fluorescence, but they proceed down to T 2 and then the emission occurs at different wavelengths just like fluorescence.

So; obviously, this is known as a meta stable state and also there is there are lot of inter system crossings and it may need it may lose the energy absorbed molecules may lose the energy too other solvent molecules and their own molecules by collision and other things, but still basically the fluorescence phosphorescence and absorbance phenomena are represented like this and it is easy to understand for us how these processes take place.

(Refer Slide Time: 09:55)



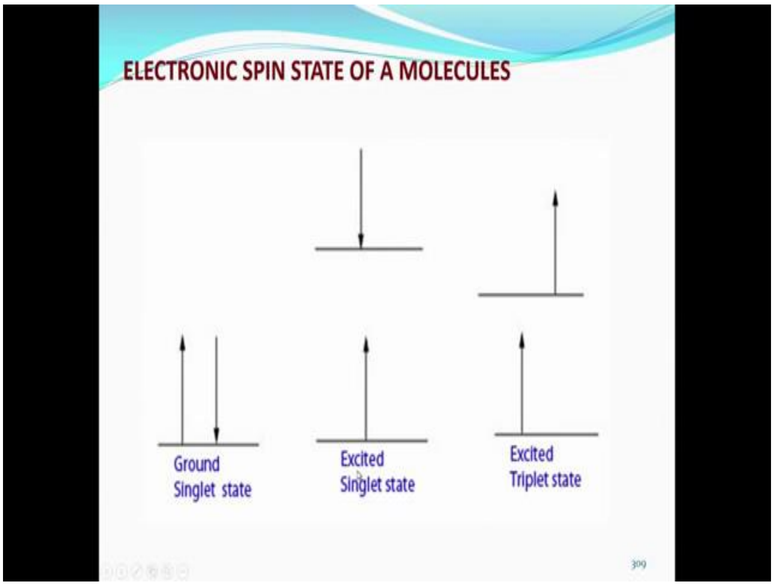
Fluorescence occurs in gaseous, liquid and solid chemical systems. In sodium vapour 3S electrons are excited to 3P states by the absorption of 589.6 and 589.0 nm. After about 10^{-8} s ,the electrons return to the ground state while emitting radiation at the same wavelength in all directions. This type of fluorescence where excitation and emission occur at the same wavelength is called "resonance fluorescence".

Many molecular species emit at radiations at longer wavelengths than the excitation wavelength. This shift is called "Stokes shift".

So, fluorescence occurs what in what kind of substances, we can see the fluorescence; we can see fluorescence in gaseous substances, liquid substances and solid chemicals also. For example, if you remember your street lights containing sodium the sodium lights will be emitting radiation around 589 and 589.6 Nano meters. Here the sodium vapor absorbs 3S, 3S electrons of the sodium vapor; vapor means basically element.

So, the electrons get excited to 3 p state that is the energy state and after about 10 raise to minus 8 seconds the electrons return to the ground state while emitting radiation at the same wavelength in all directions. This type of fluorescence where excitation and emission occur at the same wavelength is called resonance fluorescence, and many molecular species emit at radiations at longer wavelengths then the excitation wavelength this is called as stokes shift.

(Refer Slide Time: 11:25)



ELECTRONIC SPIN STATE OF A MOLECULES

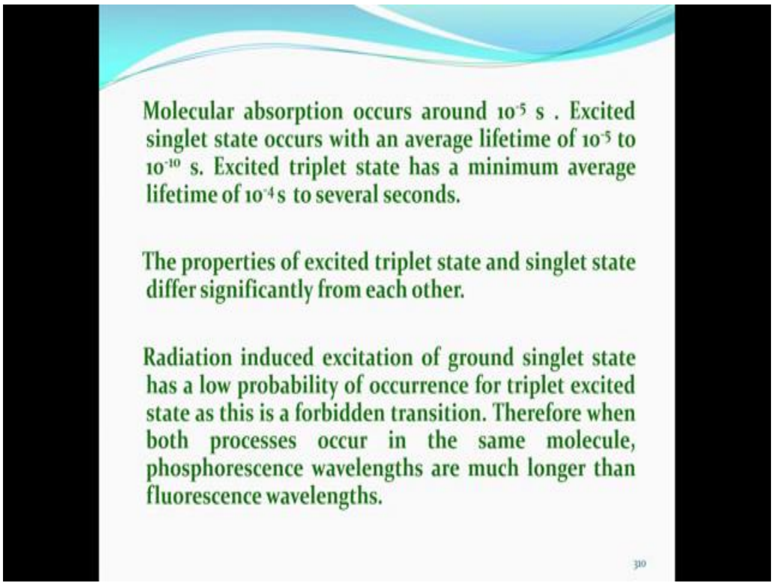
Ground
Singlet state

Excited
Singlet state

Excited
Triplet state

So, longer wavelength means stokes shift, and this is the on schematic representation electrons are paired here, and then it one electron is excited to next higher energy level this is known as excited singlet state, and then if the spins are opposite then it is known as singlet if the spins are parallel in the excited state as well as in the ground state then it is known as triplet state. So, this is a bit un natural, but there for a triplet state would be having a an energy still lower than that.

(Refer Slide Time: 12:03)



Molecular absorption occurs around 10^{-5} s . Excited singlet state occurs with an average lifetime of 10^{-5} to 10^{-10} s. Excited triplet state has a minimum average lifetime of 10^{-4} s to several seconds.

The properties of excited triplet state and singlet state differ significantly from each other.

Radiation induced excitation of ground singlet state has a low probability of occurrence for triplet excited state as this is a forbidden transition. Therefore when both processes occur in the same molecule, phosphorescence wavelengths are much longer than fluorescence wavelengths.

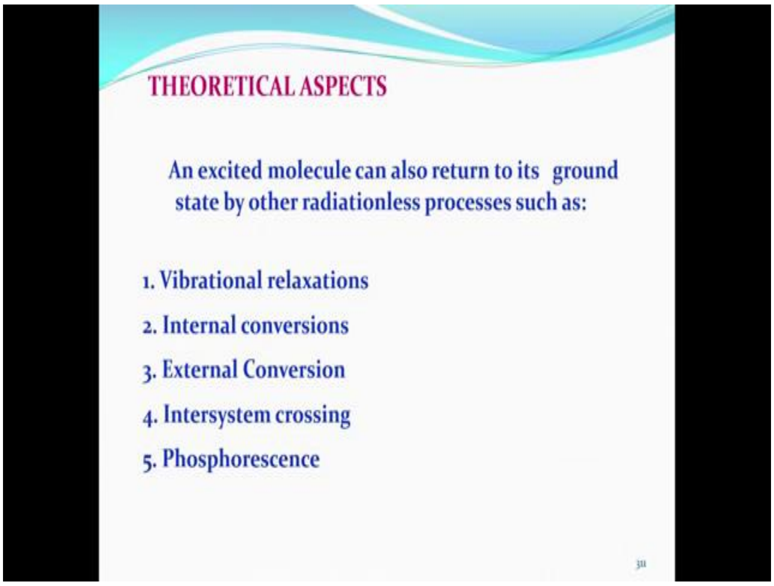
So, molecular absorption as I explained to you occurs around 10 raise to minus 5 seconds excited singlet (Refer Time: 12:14) state occurs with an average lifetime of 10 raise to minus 5 to minus 10, quite often around 10 raise to minus 8. So, this has a minimum average lifetime of about 10 raise to minus 4 seconds minimum.

So, otherwise you may not see any fluorescence. So, the properties of the excited singlet state and triplet state defer significantly from each other this is understood easily understood. So, radiation induced excitation of the ground singlet state has a low probability of occurrence for triplet state, as this is a forbidden transition; most of the triplet states are forbidden transition. So, the even though there is a theoretical possibility for the excitation to occur, the intensity of the triplet state the population the intensity of the triplet state spectrum or the population of the triplet state by the electrons is somewhat lower early.

Therefore, when both processes occur in the same molecule fluorescence and phosphorescence, phosphorescence wavelengths are much more longer than fluorescence wavelengths for example, in typical systems you may see about 10 to 20 nanometers as excitation and fluorescence difference, that is excitation maybe at emission wavelength will be about 30 Nano meter longer than the excitation wavelength 20,30 maximum 50 like that, but it would not be much higher than that, but in phosphorescence because it is in forbidden transition, concentrations are lower concentration of electron in the triplet state is lower.

So it will occur at much longer wavelengths; this phenomenon you should understand and we will see other typical phenomenon in this case also.

(Refer Slide Time: 14:26)



THEORETICAL ASPECTS

An excited molecule can also return to its ground state by other radiationless processes such as:

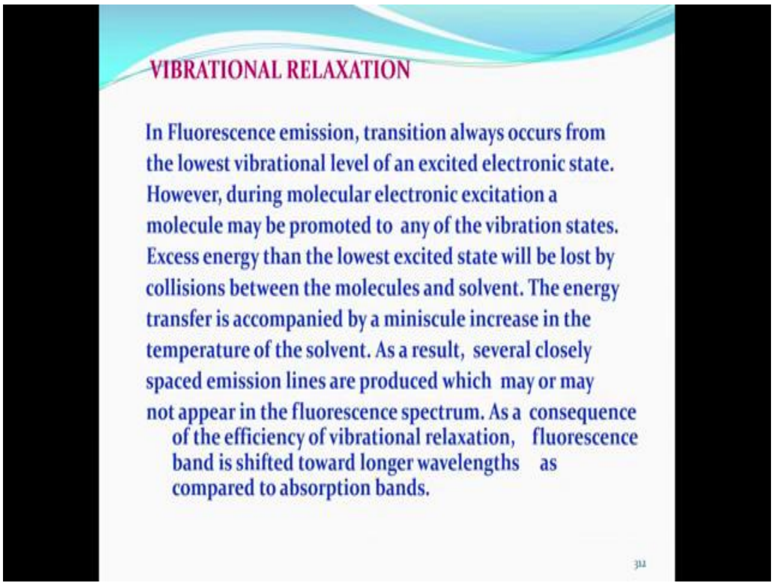
1. Vibrational relaxations
2. Internal conversions
3. External Conversion
4. Intersystem crossing
5. Phosphorescence

So, what are the theoretical aspects of the fluorescence molecule florescence molecule? An excited molecule can also return to its ground state by other radiation less processes; that means, apart from emitting a radiation it will lose part of its extra energy by these methods, one is vibrational relaxations I think all of you remember that every excited electron electronic state is accompanied associated with the several number of vibrational and rotational energy levels.

So, I have shown you in earlier also in one of the slides, that vibrational relaxations do occur in the schematic diagram and then sometimes what happens is internal conversions; this also I have shown you in the same figure and sometimes external conversion is possible, and then inter system crossing from S 2 to T 2 it is a straight forward transition, this is known as inter system crossings and of course, another phenomenon is phosphorescence. So, the excited molecule can lose its energy by different phenomenon; still emit a radiation of longer wavelength. So, it is not just one phenomena that is involved in fluorescence, but it is several other phenomenon that were involved in the loss of energy of the excited molecules.

So, look at it. So, we will take a look at each of these phenomena in a little more detail. So, my next slide will show you that in fluorescence emission transition always occurs from the lowest energy state (Refer Time: 16:25) vibrational energy level to the excited energy state. However, during electronic molecular transition excitation a molecule may be promoted to any of the vibrational states.

(Refer Slide Time: 16:28)



VIBRATIONAL RELAXATION

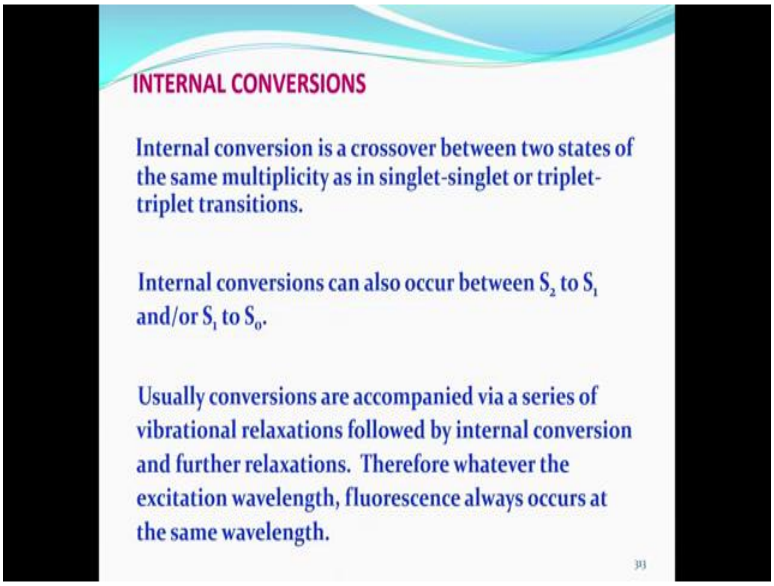
In Fluorescence emission, transition always occurs from the lowest vibrational level of an excited electronic state. However, during molecular electronic excitation a molecule may be promoted to any of the vibration states. Excess energy than the lowest excited state will be lost by collisions between the molecules and solvent. The energy transfer is accompanied by a miniscule 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. As a consequence of the efficiency of vibrational relaxation, fluorescence band is shifted toward longer wavelengths as compared to absorption bands.

312

So, excess energy than the lowest excited energy will be lost by collisions between the molecules and the solvent, that is excited molecules and the solvent. The energy transfer is; obviously, accompanied by a minuscule increase in the temperature of the solvent and this phenomenon is also observed in spectrophotometry. There also excess energy absorbed energy is released as heat, but here the, but the energy is so small that it almost goes unnoticed; but that is the only mechanism that happens in absorbance, but in florescence it is one of the processes part of the energy is lost through a absorption and is a temperature increase will be there as a result, several closely spaced emission lines are produced which may or may not appear in the fluorescence spectrum.

As a consequence what happens is the efficiency of the vibrational relaxation and decreases. So, the fluorescence band gets shifted to longer wavelength.

(Refer Slide Time: 18:00)



INTERNAL CONVERSIONS

Internal conversion is a crossover between two states of the same multiplicity as in singlet-singlet or triplet-triplet transitions.

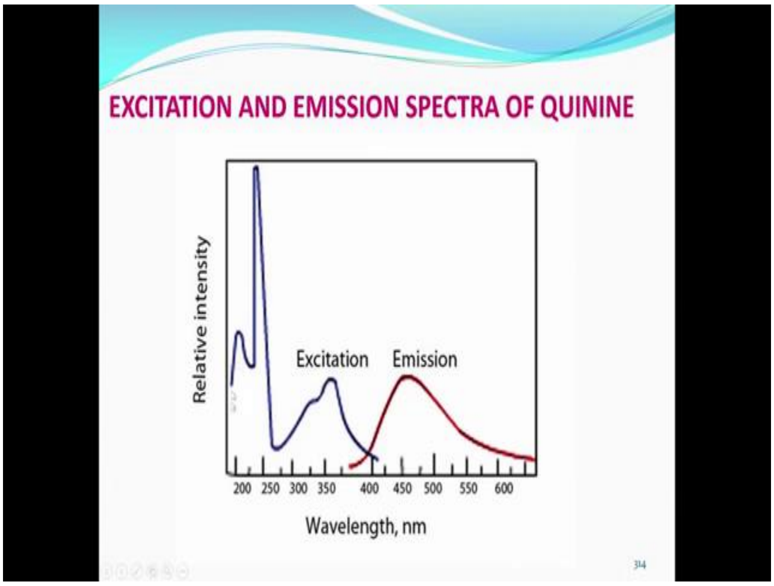
Internal conversions can also occur between S_2 to S_1 and/or S_1 to S_0.

Usually conversions are accompanied via a series of vibrational relaxations followed by internal conversion and further relaxations. Therefore whatever the excitation wavelength, fluorescence always occurs at the same wavelength.

So, another phenomenon that you should understand is internal conversions; internal conversions is a crossover between two states of the same multiplicity, as in the singlet state that is one singlet state to another singlet state transition is possible or triplet triplet transition is also possible.

So, they can occur inter system crossing can also occur between S 2 to S 1 or S 1 to S 2 S 0. So, usually these conversions are accompanied by series of vibrational relaxation as we have already seen followed by (Refer Time: 18:41) over; that means, there will be a quantum jump from the lowest vibrational energy to the triplet state therefore, whatever is the emission wavelength fluorescence always will occur only at the longer wavelength. So, you need if you are scanning a substance which is having a fluorescence property, you to look for the emission spectrum or mission peak you need not go to lower wavelength, but you only go to the higher wavelength. So, this is how an excitation and emission spectra occurs.

(Refer Slide Time: 19:17)

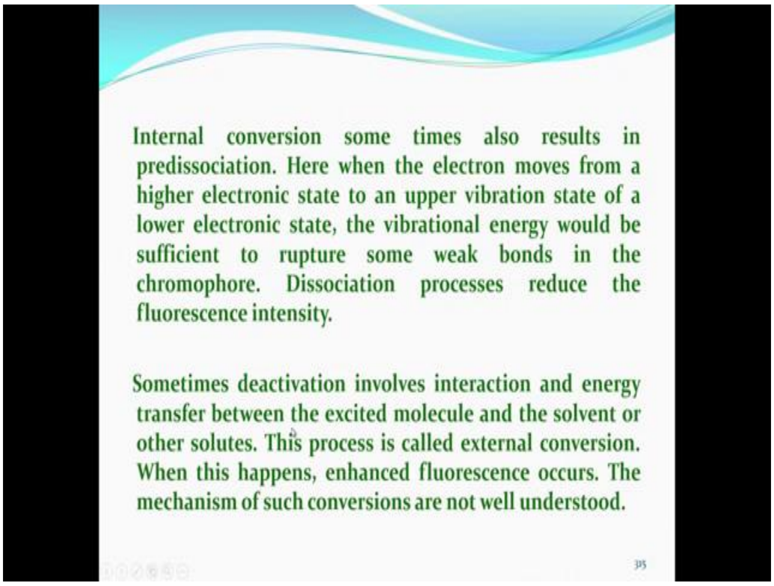


EXCITATION AND EMISSION SPECTRA OF QUININE

This is for the molecule known as quinine, and here you can see I have plotted relative intensity verses wavelength, and this the blue line is the excitation wavelength that is the radiation that is following on the molecule enter the peak wavelength is approximately somewhere around 240 or something like that. And then there are other wavelengths there is one here one here, but that is not very important; because if you look at the emission wavelength starts from about 350 this is very special case and somewhere around 375 the excitation is around 200 that means, you should use a UV lamp for this for exciting the quinine molecule, and if it is starts if the peak is somewhere around 450 the emission you need one more monochromator to go to emission to this emission wavelength maxima, that is around 450 may be slightly around 460 or so.

But still we can see that the emission spectrum is having a single peak, and it is much smoother than this, but it is not always. So, you should remember that even the xi emitted radiation spectrum can be quite complex depending upon the structure of the molecule and the amount and the intensity of this radiation that where is subjecting it to.

(Refer Slide Time: 21:00)



Internal conversion some times also results in predissociation. Here when the electron moves from a higher electronic state to an upper vibration state of a lower electronic state, the vibrational energy would be sufficient to rupture some weak bonds in the chromophore. Dissociation processes reduce the fluorescence intensity.

Sometimes deactivation involves interaction and energy transfer between the excited molecule and the solvent or other solutes. This process is called external conversion. When this happens, enhanced fluorescence occurs. The mechanism of such conversions are not well understood.

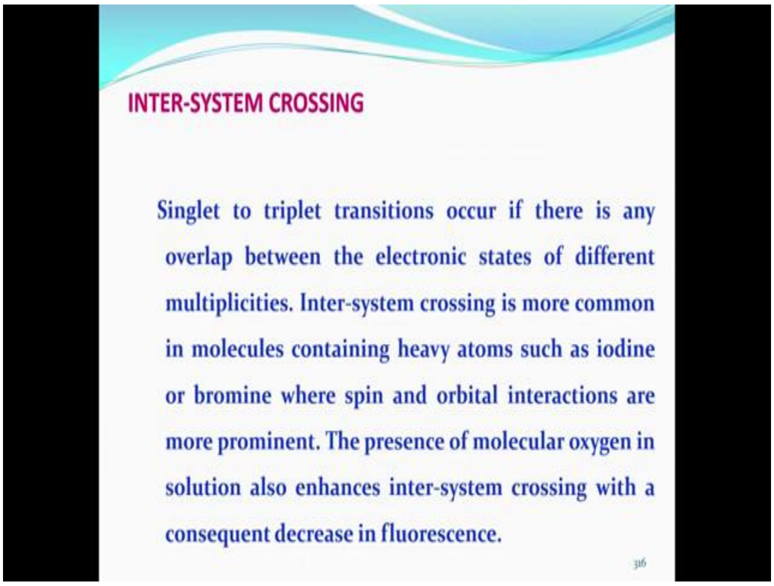
So, internal conversions sometimes also results in pre dissociation; say any energy transfer some times can cause the dissociation also. So, the electro here the electron moves from a higher electronic state to an upper vibrational state of a lower electronic state; that means we are talking of two electronic states higher and upper, but different vibrational energies of the corresponding energies. So, the vibrational energy would be quite sufficient to rupture some weak bonds in the chromophore. So, dissociation processes normally reduce the fluorescence intensity because part of the molecule is destroyed anywhere.

So, the concentration of the substance becomes very less. So, we do not want dissociation processes. So, how do these dissociation processes takes place? Basically, that is by supplying the higher energy. So, instead of 250 in the previous scale in the previous slide, instead of 250 if I do this excitation around 350 or 375 then I can reduce the dissociation of the molecule and get a higher intensity of the emission spectrum. So, that is one of the decisions you will have to take whenever you want to do research on molecules regarding the fluorescence phenomenon.

So, sometimes this deactivation what we talked of so far also involves interaction and energy transfer between the excited molecule and the solvent molecules. Solvent molecules can be aqueous solution the water molecules or it may be some other solute it may be organic solvents like butane like butanol, benzene, toluene, xylene etcetera. So, the solute molecules solvent molecules also can contribute to this deactivation. So, whenever a substance loses its energy by some interaction with some other molecules not of its own molecule, then it is known as external conversion. So, this is important because it means that different solvent molecules, different if I take butanol fluorescence intensity will be different compared to methanol or benzene or toluene or something like that.

So, the medium becomes very important in the measurement of fluorescence also. So, the mechanisms of such conversion are really not well understood even now.

(Refer Slide Time: 23:56)



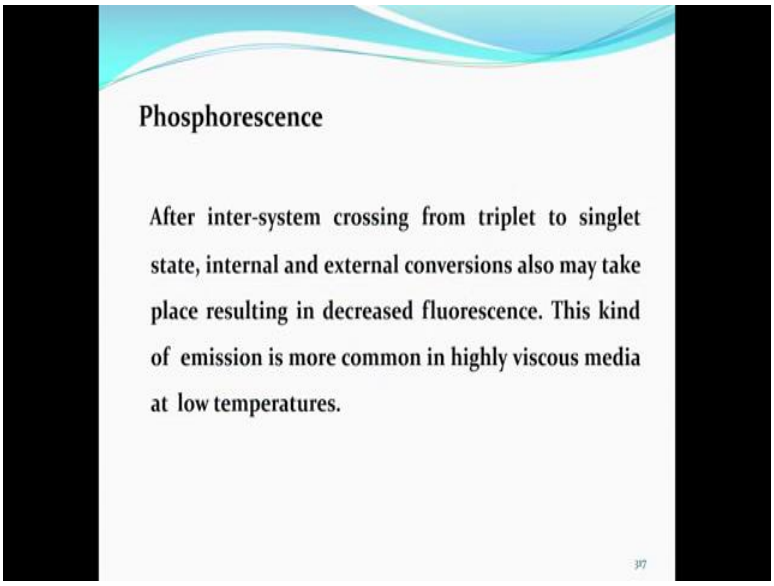
INTER-SYSTEM CROSSING

Singlet to triplet transitions occur if there is any overlap between the electronic states of different multiplicities. Inter-system crossing is more common 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.

So, now I come to this inter system crossing. So, this is another way of loss of energy singlet to triplet transitions occur is there is any overlap of the electronic states of different multiplicities. You remember I have taught you multiplicities of the spectral lines in the initial stages, when I was teaching you about the electronic structure of the atoms and molecules. So, you may refer to them again and inter system crossing is basically more common in molecules containing heavy atoms, such as iodine, bromine etcetera where the spin and orbital interactions are very prominent.

So, the florescence you can check the fluorescence of any substance involving iodine, normally it will show you a fluorescence even if you replace iodine with bromine, but the orbital interactions would be different because the structure electronic structure of iodine and bromine are different, iodine is having more electrons higher number of higher molecular weight and atomic weight compared to bromine, so the orbital interactions will be much more and more complex also. So, the presence of molecular oxygen is another factor that also enhances inter system crossing with a consequent decrease in florescence. So, the more complicated interactions the more decrease will be the florescence intensity.

(Refer Slide Time: 25:39)



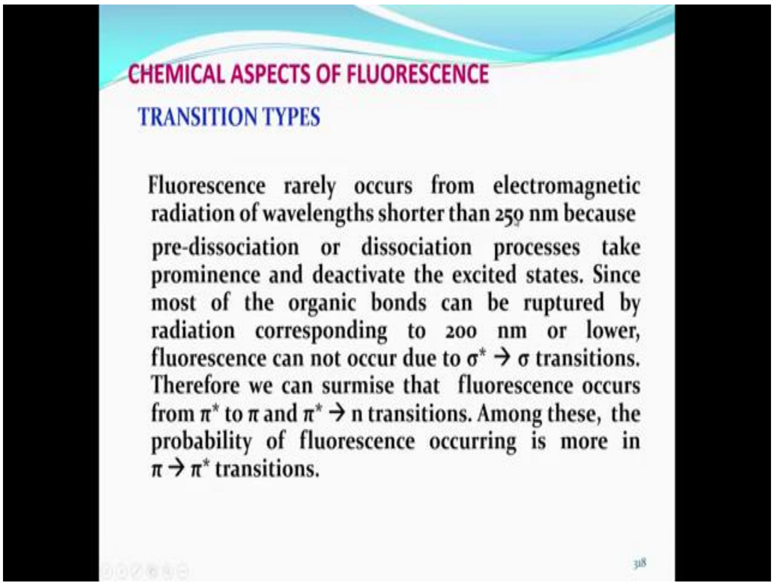
Phosphorescence

After inter-system 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.

So, now I come to the phenomenon of phosphorescence, and after inter system crossing from triplet to singlet state, the internal and external conversions also may take place resulting in decreased florescence; this kind of emission is more common in highly viscous medium at low temperatures.

So, this I have explained to you yesterday or day before yesterday where we can stabilize a system using polyvinyl alcohol, glycerol, glycerin and starch (Refer Time: 26:22) maltose etcetera and wherever such substances are there the emission gets reduced; that means, florescence gets reduced drastically. So, one has to be very careful if you want to apply the system to for monitoring of a substance, where to you should know whether there are such substances like polyvinyl alcohol and some other chemicals which are having an influence of reducing the florescence phenomenon. So, we come to the basic basics of florescence.

(Refer Slide Time: 27:09)



CHEMICAL ASPECTS OF FLUORESCENCE

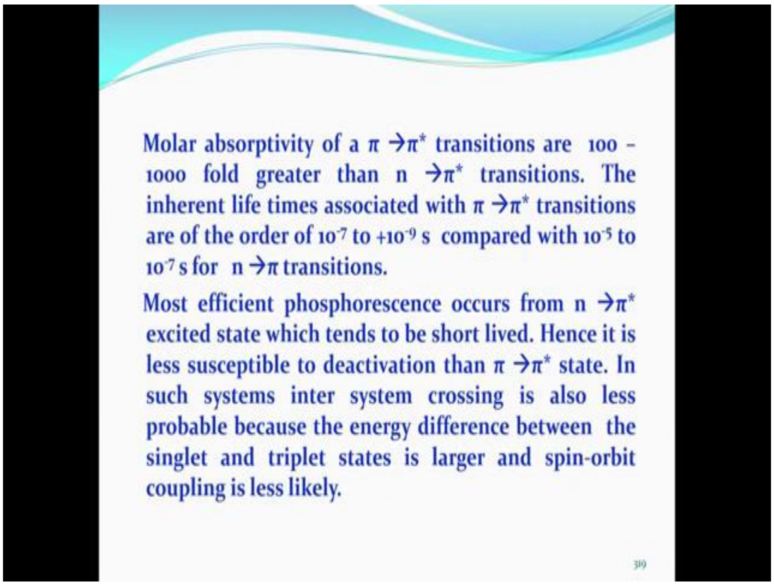
TRANSITION TYPES

Fluorescence rarely occurs from electromagnetic radiation of wavelengths shorter than 250 nm because pre-dissociation or dissociation processes take prominence and deactivate the excited states. Since most of the organic bonds can be ruptured by radiation corresponding to 200 nm or lower, fluorescence can not occur due to $\sigma^* \rightarrow \sigma$ transitions. Therefore we can surmise that fluorescence occurs from π^* to π and $\pi^* \rightarrow$ n transitions. Among these, the probability of fluorescence occurring is more in $\pi \rightarrow \pi^*$ transitions.

And these are the transition types it rarely occurs from emission radiation of more than 250 Nano meter because below 250 Nano meters or even 180 Nano meters the property of the electromagnetic radiation is to disassociate and destroy the molecules molecular bonds etcetera. So obviously, it stands to reason that we at very short wavelengths florescence phenomena does not occur. So, they even if it occurs, it will be over shadowed by the florescence by decomposition rather than florescence system to occur. So, the disassociation process is normally overtakes the pro they deactivate the excited states. Since most of the organic bonds are ruptured below 200 again florescence cannot occur due to sigma star to sigma transition. See I am talking about sigma star to sigma transitions, in absorbance we used talk of sigma to sigma star transitions, now in florescence we are talking from higher energy level to lower energy level.

So, sigma star to sigma transitions are normally not expected to produce any kind of florescence therefore, we can summarize that florescence normally occurs from pi star to pi yes definitely, and pi star to n transition still possible among these the probability of florescence is more with respect to pi star to pi transitions.

(Refer Slide Time: 28:53)



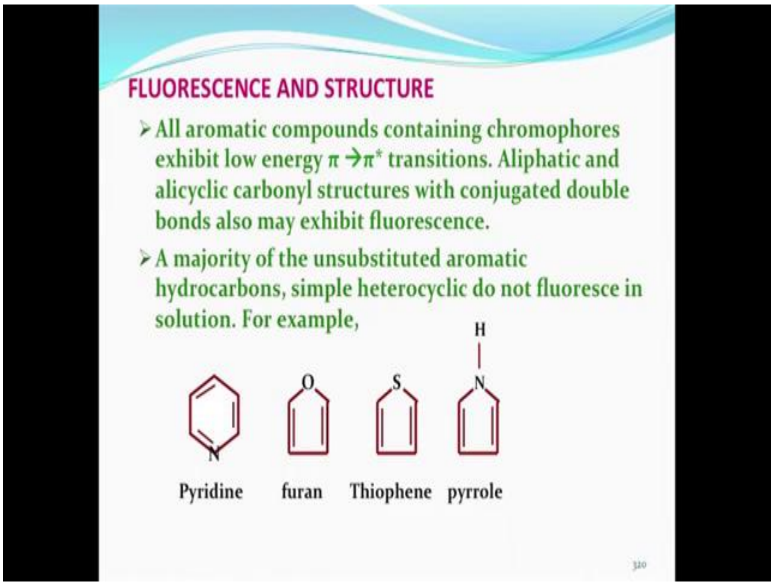
Molar absorptivity of a $\pi \rightarrow \pi^*$ transitions are 100 - 1000 fold greater than n $\rightarrow \pi^*$ transitions. The inherent life times associated with $\pi \rightarrow \pi^*$ transitions are of the order of 10^{-7} to $+10^{-9}$ s compared with 10^{-5} to 10^{-7} s for n $\rightarrow \pi$ transitions.

Most efficient phosphorescence occurs from n $\rightarrow \pi^*$ excited state which tends to be short lived. Hence it is less susceptible to deactivation than $\pi \rightarrow \pi^*$ state. In such systems inter system crossing is also less probable because the energy difference between the singlet and triplet states is larger and spin-orbit coupling is less likely.

So, molar absorptivity of pi star pi to pi star transitions are approximately 100 to 1000 (Refer Time: 29:01) greater than n to pi star transitions. The inherent lifetime associated to pi to pi star transition are also of the order of about 10 raise to minus 7 to 10 raise to minus 9 seconds of course,. So, compared with 10 raise to minus 5 to 10 raise to minus 7 seconds for n to pi star transitions. So, which is better among the two 10 raise to minus 7 to 10 raise to minus 9 seconds is a longer wavelength longer time limit for florescence to occur.

So obviously, pi star to pi transitions are most comfortable to work with and they will be more stable also 100 times at least 10 raise to minus 7 to 10 raise to minus 9 means 100 times more stable. So, the most efficient for florescence now we are talking of first florescence, this occurs from n to pi star excited state which tends t be very short lived therefore, it is less acceptable to deactivation, then pi to pi star transitions state. In such systems what happens is inter systems crossing is also very less because the energy difference between the singlet and triplet state is quite large, and spin orbit coupling is likely. So, spin orbit coupling lightly means loss of fluorescence.

(Refer Slide Time: 30:37)



FLUORESCENCE AND STRUCTURE

- All aromatic compounds containing chromophores exhibit low energy $\pi \rightarrow \pi^*$ transitions. Aliphatic and alicyclic carbonyl structures with conjugated double bonds also may exhibit fluorescence.
- A majority of the unsubstituted aromatic hydrocarbons, simple heterocyclic do not fluoresce in solution. For example,

So, is there a correlation between florescence and structures structure of the molecule? In general with the discussion so far we had we can conclude that n to pi star transitions are not suitable, because they sigma to sigma star transitions are not suitable for florescence, but pi to pi star transitions are more suitable therefore, if a system like molecule is having number of bonds which are having pi to pi number of pi bonds, then florescence can occur we do not say it will occur, but it can occur. So, aliphatic alicyclic carbonyl structure with conjugated double bonds; you remember we had discussed about such things, when we were doing the prediction of the absorption wavelength depending upon the molecular structure in the absorbance.

So, aliphatic and alicyclic carbono carbonyl structure with conjugate double bonds, wherever there is conjugation we normally expect florescence of course, absorption will always be there, but florescence also may occur. So, a majority of the un-substituted aromatic hydrocarbons, simple heterocyclic systems they do not fluoresce insolation. So, even though there are double bonds pi bonds. So, examples include the in the slide I have shown you that is pyridine, furan, they are conjugated double bonds here, there is a conjugated double bond in pyridine, that is nitrogen, is here and this is thiophine, pyrrole and all these things do not fluoresce in the aqueous in aqueous solutions.