Course on Analytical chemistry
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Lecture 30
Module 6
Spectrochemical Methods - 3 (Continued)

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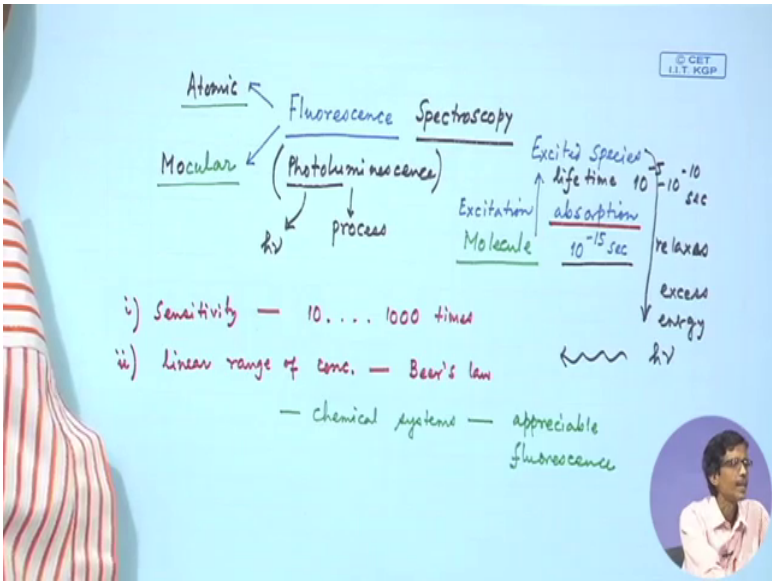

Hello, and welcome to this part of class where we are talking about the spectrometric techniques or the spectrometric methods, now we will just move from IR to another technique which is Fluorescence technique. So how we use this particular spectroscopy and we all know that when a particular absorption spectrometry or infrared spectrometry can be used for spectrochemical analysis not only in terms of the unknown analyte concentration but analysis for structure determination.

So fluorescence in the same way is also helpful for getting many informations and when we talk in terms of the corresponding atoms it can be atomic fluorescence spectroscopy, so this atomic fluorescence spectroscopy can also be useful for identifying the presence of the elements and all these things and geologists take the advantage of this atomic fluorescence spectroscopy but in a broader respect that if we have a molecule and whether we can apply this particular technique that means the fluorescence technique which is a part of the photoluminescence so is a part of the photoluminescence technique of the photoluminescence process what we can say.

So if the process is a photoluminescence process that means photons will be absorbed so in terms of the photon absorption and we can excite the corresponding material, the molecule or the species. So if we have a molecule so we get the molecular fluorescence spectroscopy so that will be termed as the molecular fluorescence spectroscopy.

And this particular fluorescence process can be useful for atomic species or the molecular species where this atomic species or the molecular species that means we want to characterise something, some molecule and one thing what we have seen so far that electronic absorption spectroscopic technique that this particular molecule when we go for absorption any coloured material or any coloured metal ions or any organic molecule which is going for absorption but in this particular we will be utilizing something by due to this absorption we can have the excitation.

So we get the species in the excited state so excited species. So now we talk about the corresponding excitation and this particular excited species if it gives rise to the luminescence pattern that means that depending upon its corresponding lifetime whether it is stable in this particular excited state that means this absorption most of the time it takes place within 10 to the power minus 15 second and if this particular excited species can have some lifetime so we talk in terms of because the absorption is taking place within this timescale or this timeframe and this exited species can have some life time in the range of say 10 to power minus 5 to 10 to the power minus 10 second.

So what happens basically that after excitation this species is there, then it relaxes so this relaxation we can just monitor where the excess energy due to the promotion from the ground level to the excited level which we all know that E1 level to the E2 level the excess energy will be emitted and that emission we basically we just monitor or measure by this particular spectroscopy. And some other technique we also know but we do not have any scope or time for analysis using the fluorescence as well as the fast fluorescence technique. The fluorescence technique we have some much more life time and it is much more complicated and which is not very common for this particular molecular species which is showing fluorescence. In case of fluorescence also there are few specialised molecules available and the characteristics of those molecules will see which can show this particular energy release after excitation.

So we have the relaxation so this will be relaxes so the exited species when it relaxes it gives the corresponding emission of energy and we get this particular one because why we use this

fluorescence spectroscopic technique over this absorption spectroscopy technique because it has a high sensitivity which is sometimes a order of say 10 to 1000 times sensitive compared to the electronic absorption spectroscopy.

So if this is sensitive so it is a better process than the absorption spectroscopy technique and which will be very much sensitive to some of those molecules which have fluorescence active and it has also another advantage that it has large linear range of concentration, linear range of concentration where we can use the corresponding Beer's law compared to electronic absorption spectroscopy where this range for the linearity is not very high but there are only very few or the limited number of chemical compounds or the chemical species or chemical systems will be available to show appreciable otherwise we cannot measure this particular fluorescence.

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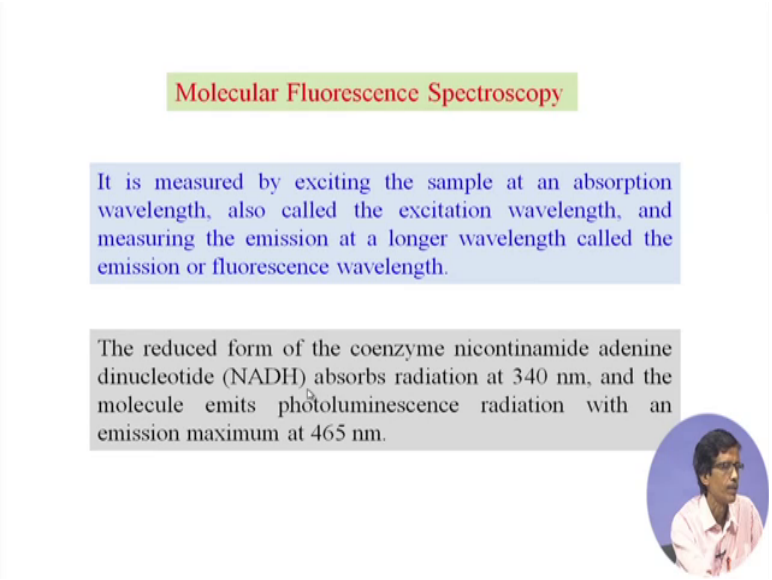

So when it shows some appreciable amount of fluorescence then that only can be useful for our measurement and what we see that how we can measure this and how we go for this excitation for molecular fluorescence spectroscopy is that what you find that when we excite the sample at an absorption wavelength so we know the lambda max for absorption and that lambda max for absorption is very much useful and we should also know very precisely the corresponding excitation wavelength because we can measure the excitation wavelength at the same time and when it coming down to the ground state we will be measuring the corresponding emission at longer wavelength.

Why it is longer that we will discuss so if it is taking place at some amount of wavelength is 340 nanometre and emission can be absorbed at 465, 450 or 480 nanometre that means at longer wavelength range after this excitation such as this example what we see that nicontinamide adenine dinucleotide which is also useful for biochemical studies also so this particular analyte for our analytical chemistry class and use of fluorescent spectroscopy is very much useful because we are handling some (())(08:50).

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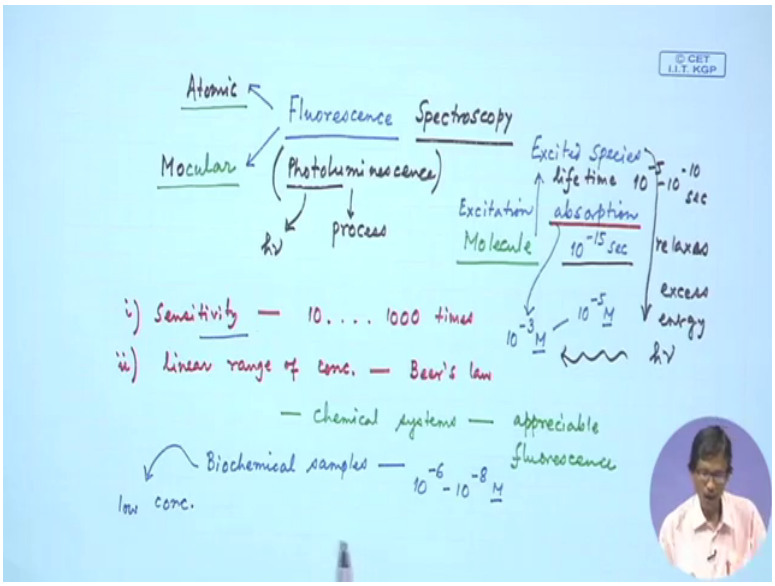

So why we are taking the example of a (())(08:54) because we are taking or we may have talking in terms of the corresponding sensitivity because this electronic absorption spectrometric method what we have seen that we can have a concentration range of 10 to the power minus 3 molar to 10 to the power minus 5 molar concentration.

We cannot go down to that particular concentration but when we talk in terms of your biochemical samples if these biochemical samples we want to measure we want to monitor whose concentration is less in the range of 10 to the power minus 6 to 10 to the power minus 8 molar since the sensitivity is high of 1 to 3 order of magnitude so we can go down from here to again 3 order of magnitude lower concentration range. So those chemical systems we can handle at a very low concentration so that is the major advantage of handling all these things.

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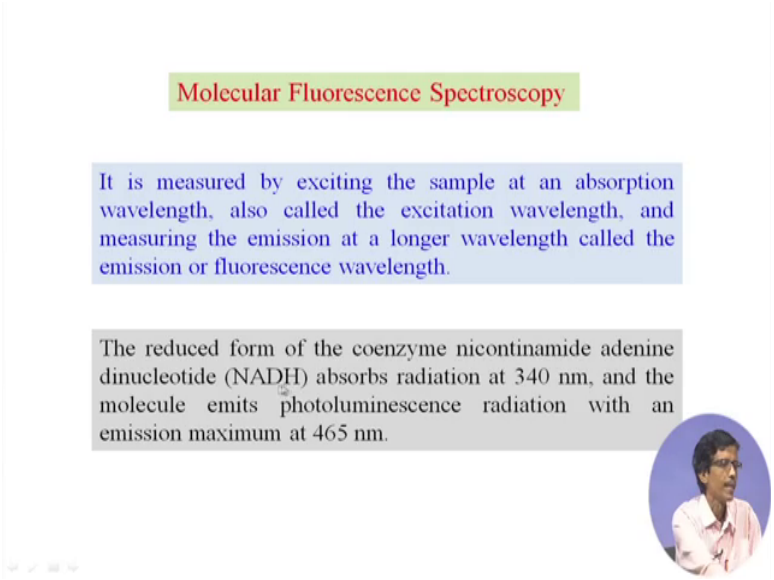

So if the species is giving rise to the fluorescence that like of our nicotinamide adenine dinucleotide we will be able to excite it at 340 nanometre that means lambda excitation, lambda ex and we then measure the corresponding emission at 465 nanometre.

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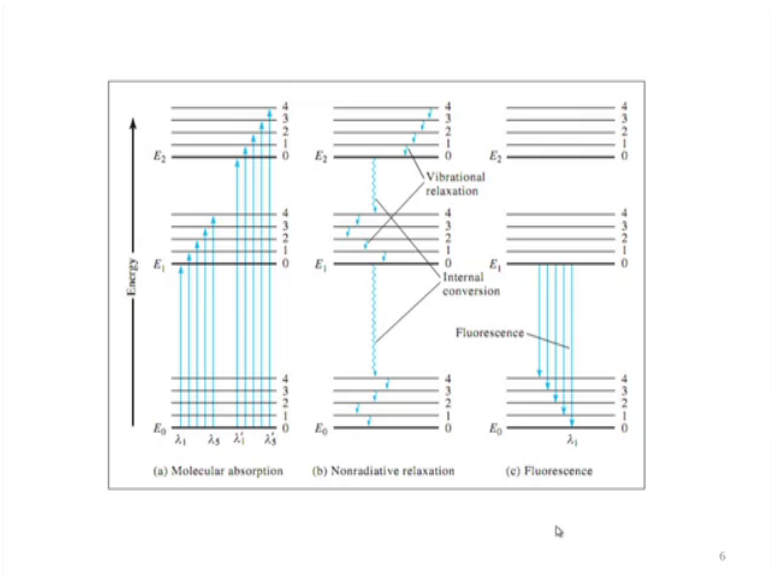

So this is the corresponding advantage of doing this and both these two will be characteristics and the instrumental set up and all these things will see how we can monitor or how we can measure the excitation wavelength as well as the lambda em that means the emission wavelength.

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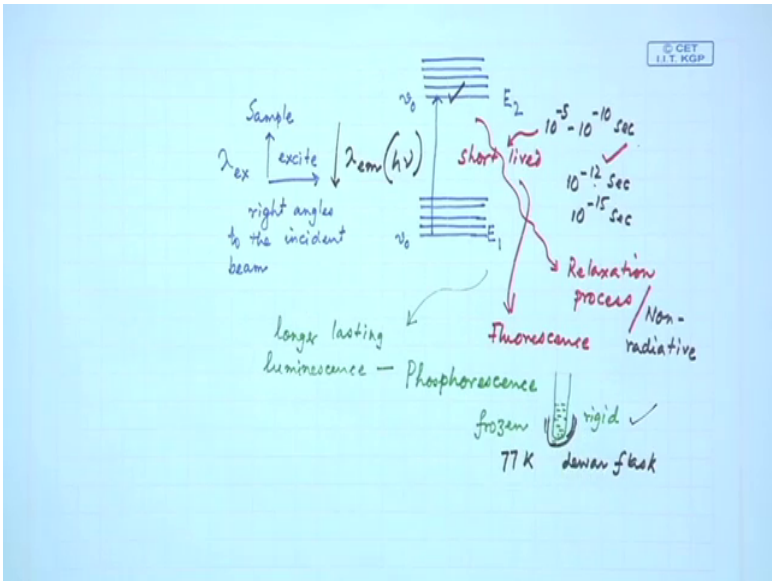

So if these are the different levels we will be considering at how these different levels will be useful and we will be talking about the corresponding values that means you have the sample and which we talk in terms of the two electronic energy levels.

That means E1 and E2 and already we have seen that it can have different vibrational energy levels while talking about the infrared spectroscopy, so you can have the different vibrational energy levels similarly here also in the upper excited states you can have the different vibrational levels V0, V1,V2,V3 and V4. So when we go for this particular excitation, so sample we just simply excite and we should know the lambda excitation and if this lambda excitation is there which is realised for transferring the species from V0 of E1 level to V0 of E2 level then we get the corresponding lambda excitation.

And when we measure that means the emission, emission will be measuring at basically right angles to the incident beam at right angle to the incident beam. So if we get that for the right angle so this measurement can give you something where we have the corresponding emission pattern, so which is lambda em so what we get there that means if we have this particular species that means how long this species when it is coming to this excited level how long it will be there so we have seen that a temperature sorry a time range of 10 to the power minus 5 to 10 to the power minus 10 second the lifetime.

So in this particular lifetime basically because if you have the higher lifetime because the absorption what we have seen that it is 10 to the power minus 5 second. So in between what is there so in between that means 10 to the power minus 12 second or 10 to the power 11

seconds all this range something is happening. So if we have this that means this is not a very long lived species so this particular lifetime of this species in the excited level, so we consider this as a short lived one that means we are not able to go down from 10 to the power minus 5 to 10 to power minus 1 or 10 to power minus 2 second.

So if this are short lived the process is known as fluorescence. So this is the case where we see at least we always expect that we should have the species that gives rise to a lifetime of this but if the lifetime is longer than this particular process so we consider that is a longer wave time or the longer lifetime, so we consider them as the longer lasting process. So longer lasting luminescence also so what is that. So when we go for this longer lasting luminescence we call it or consider it as phosphorescence.

So the phosphorescence process will have a longer lifetime and sometime the corresponding treatment for the sample the sample holder and all these things are much more complicated sometimes you can have to cool down the sample to a the things would be very rigid why it is rigid that we will discuss now and it can be in a frozen condition such that we can increase the rigidity of the material by putting the sample at a very low temperature such that you have some arrangement where you can have the temperature jacket that means the dewar flask.

So thermal jacket we can have, we can put it in dewar flask so that will give rise to the corresponding frozen condition and we measure the phosphorous and activity for that. Why we require this rigidity or the rigid sample for this is because what we look here for there that once we excite it, so it can go for different relaxation process. So this relaxation process what we get and we will be in this particular range.

So the absorption is taking place in this range then the corresponding relaxation can take place from this 10 to the power minus 12 or 10 to the power 13 or 10 to power minus 11 second. So that is basically giving rise to something where we see that this particular process is it is there the relaxation process if it is a non-radiating one, so if this is a non-radiating one will not be able to get the corresponding emission spectrum in terms of the corresponding emission of h Nu.

So the excess energy will be detect, excess energy will be lost in some other process which is known as the non-radiative relaxation process.

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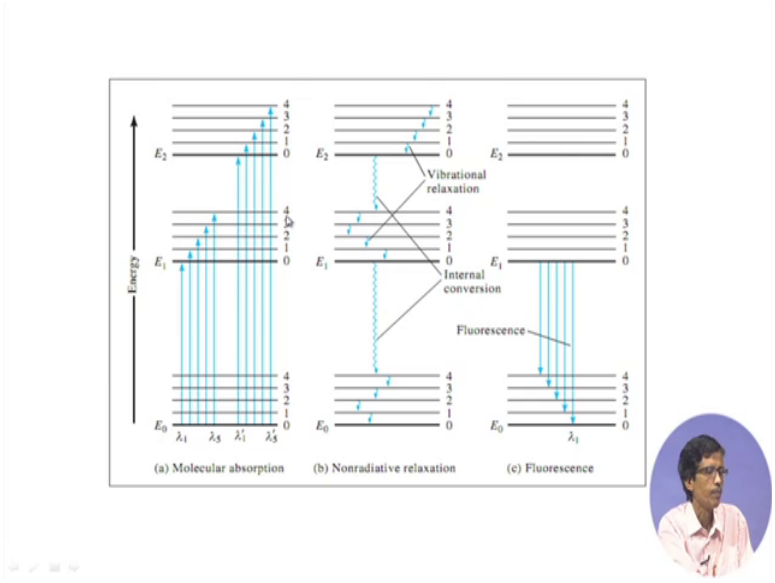

So we require something what we see from this plot again that we already seen that in case of electronic absorption or the molecular absorptions spectroscopy that is being used for the absorption from E0 level to E1 or sometimes to the E2 level and these are the corresponding vibrational level V0, V1, V2, V3 and V4 as we have seen these levels are important for IR spectroscopic analysis.

So what you see from starting from your lambda 1 to lambda 5 you see the movement from E0 level to E1 level similarly from movement from E0 level to E2 level is the lambda 1 prime to lambda 5 prime. So what are these differences basically one is lambda 1 another is lambda 2, another is lambda3. These are nothing but the movement of the corresponding species from V0 state of E0 is 0 this on the right hand side is the V0.

The vibrational quantum level is a the lowest vibrational quantum level than the first excited vibrational quantum level then the second, third and fourth excited levels. So when it is absorbing so this energy gap is more that means this V0 to V1, this V0 to V2, V3, V4 these will be more. So we can have very closely spaced lines that means lambda 1 to lambda 5 that is why the electronic absorptions were very broad, is spanning the range of lambda 1 to lambda 5.

Then once it promote the species to this E1 level what will happen that promotion of these species in E1 level as well as E2 level will give rise to something that means if we promote by lambda 5 that means we are promoting the species to V4 level that means the V4 level is there which is higher energy than V0 level of E1 but there are something where we consider

it as the corresponding species which is moving from here to the other case that means this particular V4 to V3, V3 to V2 then V2 to V1 and V1 to V0.

So these small steps jumps we call it as the vibrational relaxation. So when we have this vibrational relaxation we will get something that means all these species will come if we even if we excite it to the V4 level through vibrational relaxation it will come to the V0 level, so that particular level is very important so when all these species that means species which has reached to this level or when another mode that means the vibrational relaxation which is through this internal conversion so vibrational relaxation as well as internal conversion.

So internal conversional from E2 level to E1 is meaningful but internal conversion from E1 level to another E0 level of V4 is meaningless because it will not be able to emit any such radiation that is why it is due to the collision. So inter molecular collision affect can give rise to this internal conversion and we will not get any emission of radiation.

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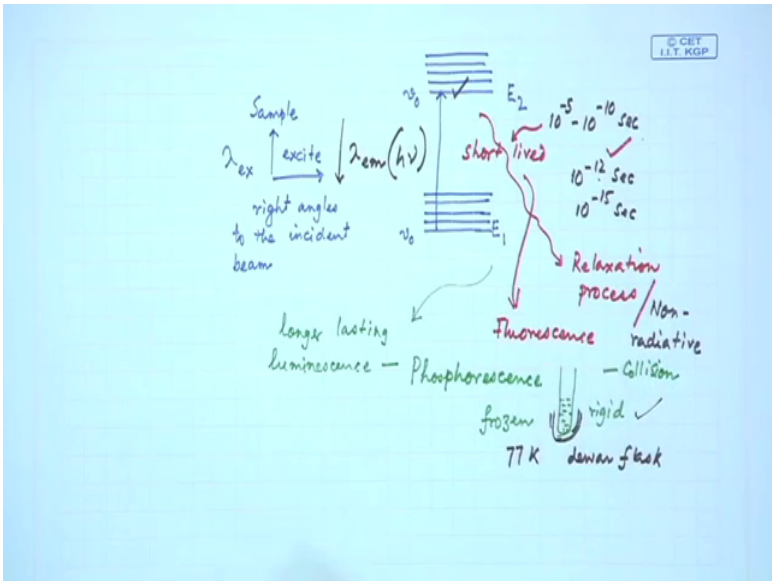

So this particular thing that means when we have this no-radiative process of relaxation and which is taking place through collision, so through this collision what we get that this particular collision that means the molecules which are interacting with each other then give rise to this particular collision process and this collision process will not give rise to any radiative transition or any radiation emission.

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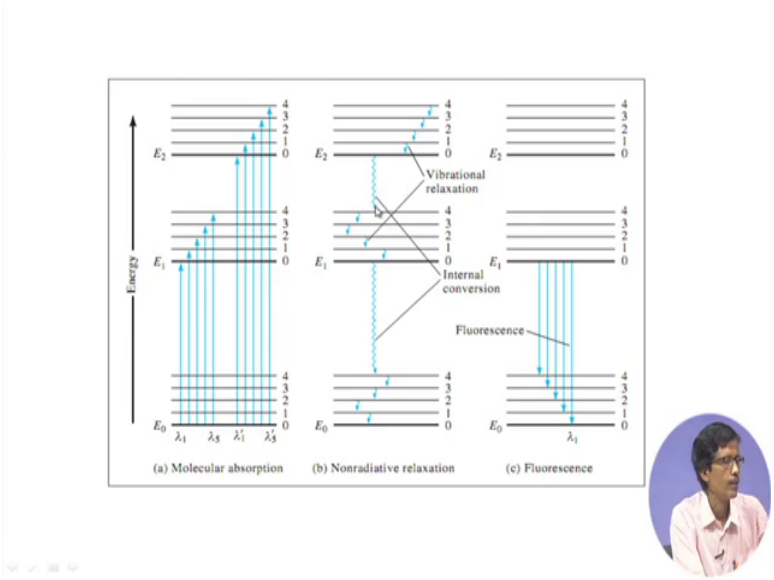

So this curly lines so curly lines over here and this particular curly over here is also meaningless because this two at the level does internal conversion and this two process will not give rise to your fluorescence emission, but what we see over here is that when all these things all these molecules from this upper excited vibrational levels is coming down to the ground vibrational level of E1then if they are allowed to emit the radiation to any of these levels of the E0 state that means it can emit the radiation to the V4 level or the V3 level or the V1 level or the V0 level, what we get? We get the corresponding species as the corresponding species is giving you the fluorescent spectrum.

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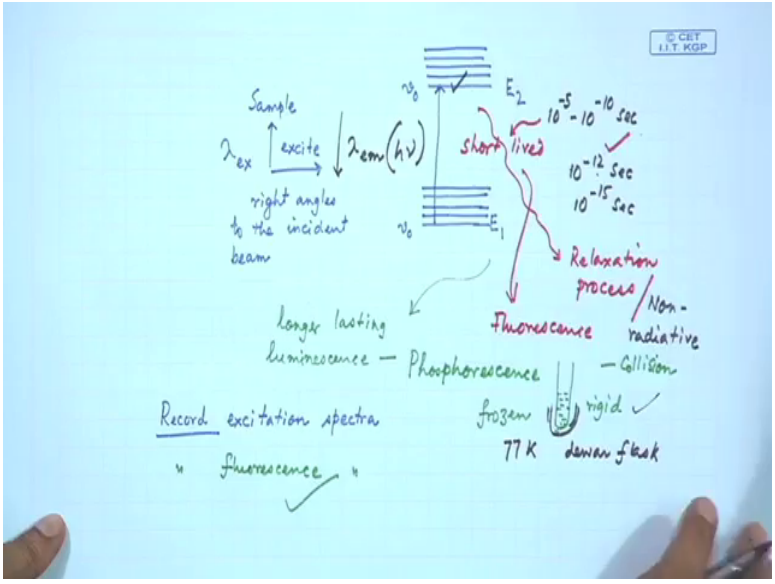

So we get the fluorescent spectrum, so what we get that means this particular one for this excitation so we have the corresponding excitation spectra, so we can measure or record the excitation, so we can record the excitation spectra because this particular excitation is characteristic for this molecular species so it can be a molecule or any other molecular species which is showing absorption then we can also record the fluorescence spectrum.

And which is our important thing that how this particular thing can give rise to the corresponding fluorescence.

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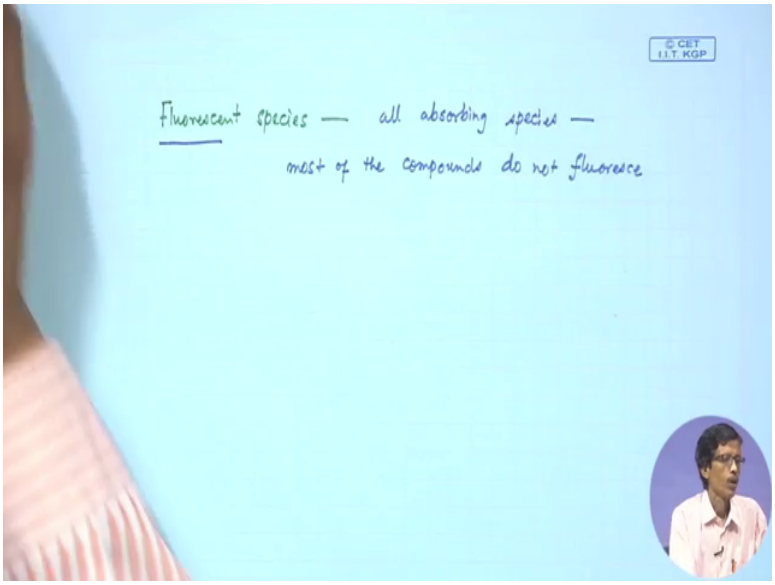

So we must have therefore some species which we call as a fluorescent species, so fluorescent species is responsible for our emission pattern and that fluorescence species is coming from all absorbing species or absorbing molecules, so all absorbing species have therefore the potential to fluoresces basically but not many compounds are there but most of the compounds do not fluoresce.

So this particular technique is not very much applicable to all other compounds so we do something where we can measure this particular fluorescence and it can be characteristic for your excitation as well as the corresponding emission pattern.

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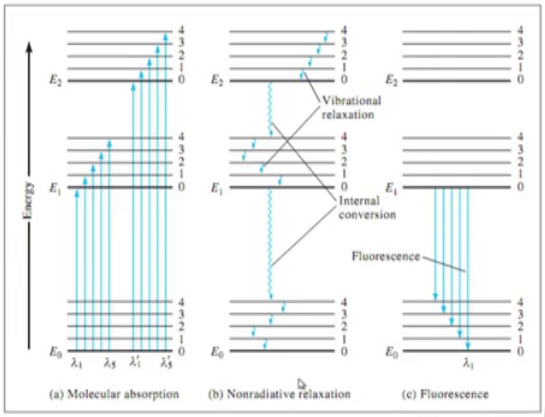
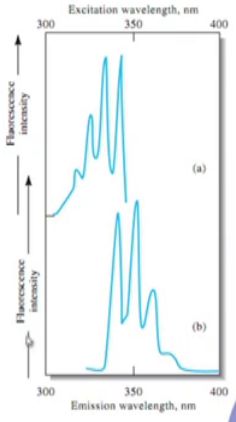
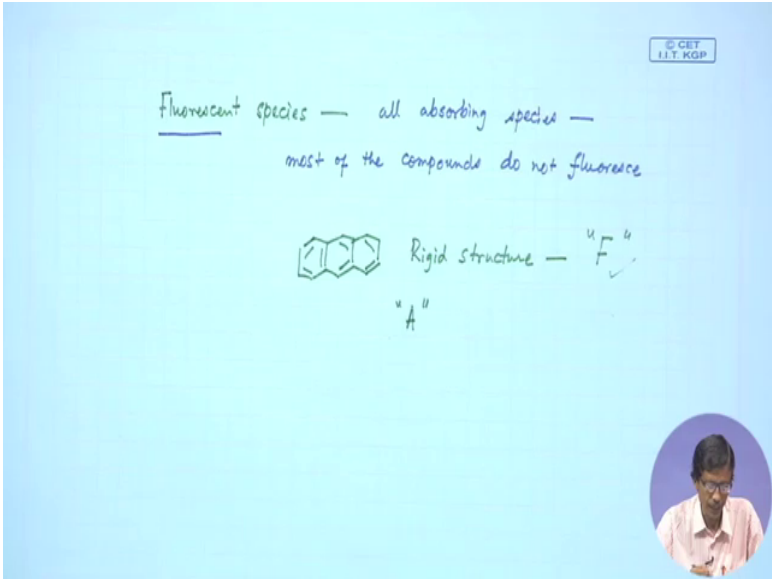


Fluorescence spectra for 1 ppm anthracene in alcohol: (a) excitation spectrum; (b) emission spectrum.





So when we get this fluorescence in this particular form that means when we emit this from E1 to E2 level what we get that we can measure these two as your corresponding level for this emission pattern so you have this so we just basically plot this from 300 to 400 nanometre and this one is a very characteristics one which is a fluorescent spectra of a level you see how low concentration we can handle. One ppm of anthracene because anthracene is a very rigid molecule we all know anthracene is nothing but a very fixed molecule of this type when we have side by side three benzene rings attached to it.

So it has a very rigid structure that is why it gives us the corresponding fluorescence intensity. And at the same time it should also absorb that means it should give the absorbance also and due to its rigid structure and less inter molecular collision, so the non-radiating pathways or the non-radiating relaxation will be less and we will get some appreciable intensity from its fluorescence. And we can go down to a one ppm which is we all know that is the parts per million, million means 10 to the power minus 6 range of concentration. So that particular range of concentration is very much useful to identify this (())(25:56) alcohol.

So the left this one that means the spectrum a is your excitation spectrum and the spectrum b is your emission spectrum and you see that in this two cases these are basically the corresponding mirror images so when we get because some of these lines because this particular one the last one is basically matching. So is basically the mirror image so the exciting wavelength what is being absorbed by these anthracene molecule for its absorption is getting back is getting back basically for the different types of relaxation what we get.

And if it is having some feature which is a very characteristic feature of this four characteristics peaks which is all below 350 nanometre but the emission spectrum is such that it is not all below 350 it is around 350. So 1, 2 peaks are at 350 or less than 350 and two are above 350 so what we get is that we get something which is in the longer wavelength region.

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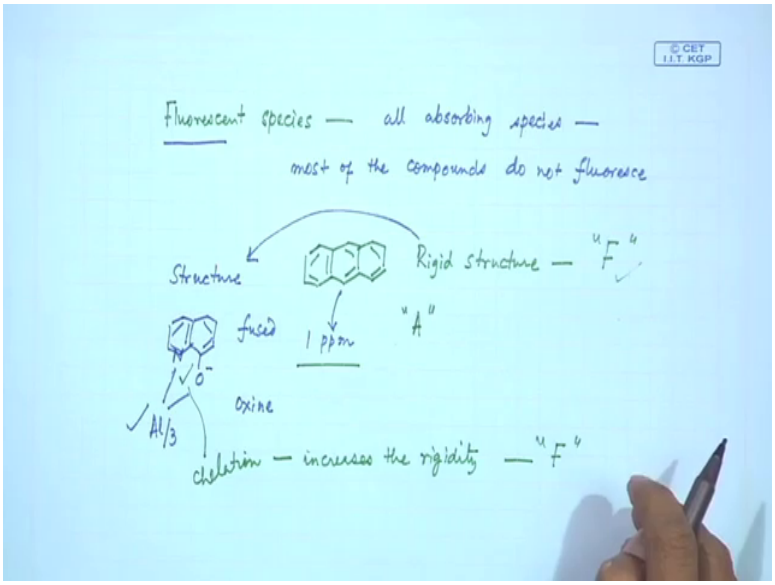

So analytically this should be very much useful for detection to a very low concentration. So this anthracene detection to a very low concentration of one ppm is very much useful and what we see that the rigidity so this rigidity is also related to the structure.

So if we take some other example of some reagent or analyte or ligand, so which is nothing but oxine molecule which is 8-hydroxyquinoline when this deprotonated this O minus so it can bound to gene, it can bound to aluminium 3 plus and three of these ligands can be attached to this oxine, so this aluminium oxine complex. So you have already two such one is (())(28:05) another is phenol ring so (())(28:07)and phenol ring both of them are fused together and this fused structure is something so this oxine can be a very good sample for giving rise to the fluorescence pattern but the analysis of aluminium.

Say if we want to detect the presence of aluminium to a very low concentration such as that of PPM level because most of the time if we go down to 10 to the power minus 6 or 10 to the power minus 7 or 10 to the power 8 molar concentration we can talk in terms of the PPM or PPB concentration. So any water sample containing aluminium 3 plus can be analysed in this fashion because we can increase the rigidity of the structure by getting one more chelating ring.

So already we have two ring one is the (())(28:57) ring another is the phenol ring but also this particular (())(29:02) so that chelation increases the rigidity that is why it give rise to very good fluorescence to us.

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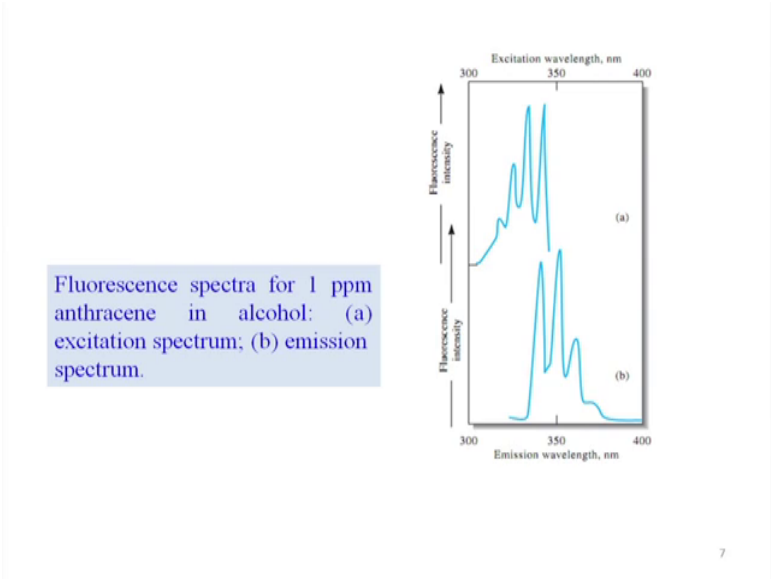

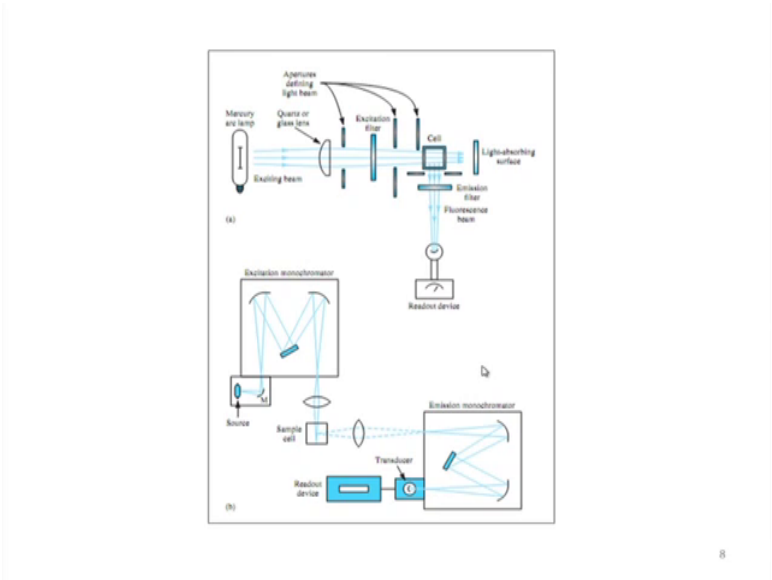

So this particular fluorescence therefore is very important to measure and we get this through a particular instrumental setup just in our previous class we have seen that how we use this particular thing that means we have mercury arc lamp that means the excitation we can have and this particular one that means that means the excitation so you have the source this is in the more simplified one, so the simplified one can be more advanced instrument for that which is a emission using the emission monochromator.

So you have the source and as I told you that you have the corresponding thing that means what we have that means the corresponding light what is impinging on the sample through this lens or the mirror and what we will be measuring is in the right angle of that. So in the right angle what we have that emissions so this is the excitation monochromator, so this is the

basic difference between our absorption spectrometer or FTIR spectrometer that we have two monochromator one is the excitation monochromator and another is the emission monochromator.

So in the excitation monochromator we can measure or we can monitor if the detector is available that corresponding excitation wavelength but it can also fall on the sample and the sample can be excited and it can emit the corresponding radiation at right angle and the transducer is there to manage the corresponding emission thing and that emission can be therefore goes to the detector and ultimately to the read out device. So the very basic difference is that the measurement at the right angle and we should also try to have the sample which will have the corresponding rigidity and we can also increase the rigidity of those samples to get an efficient fluorescence pattern.

Thank you very much.