Basics of Fluorescence Spectroscopy
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Lecture – 05

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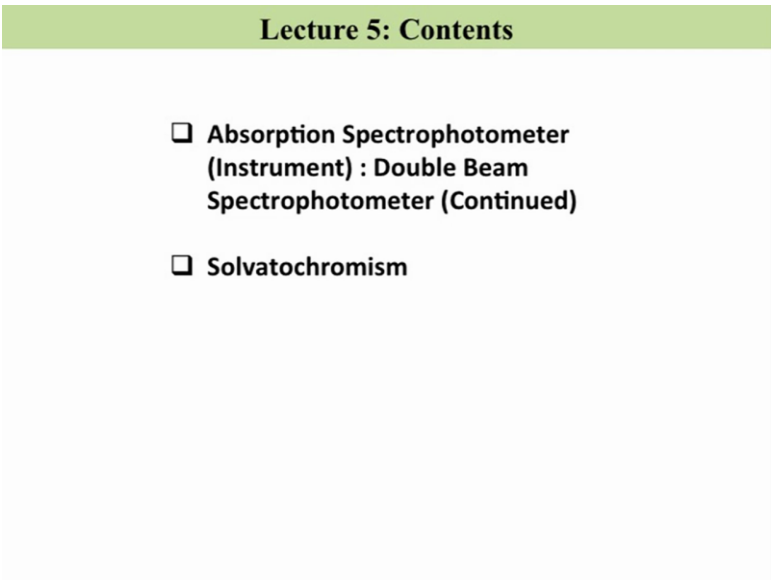

So welcome to the 5th lecture of the course Basics of Fluorescence Spectroscopy. Until lecture 4, we discussed about the several topics on this absorption and we ended with that double beam spectrophotometer and here as you see I was discussing about this double beam spectrophotometer.

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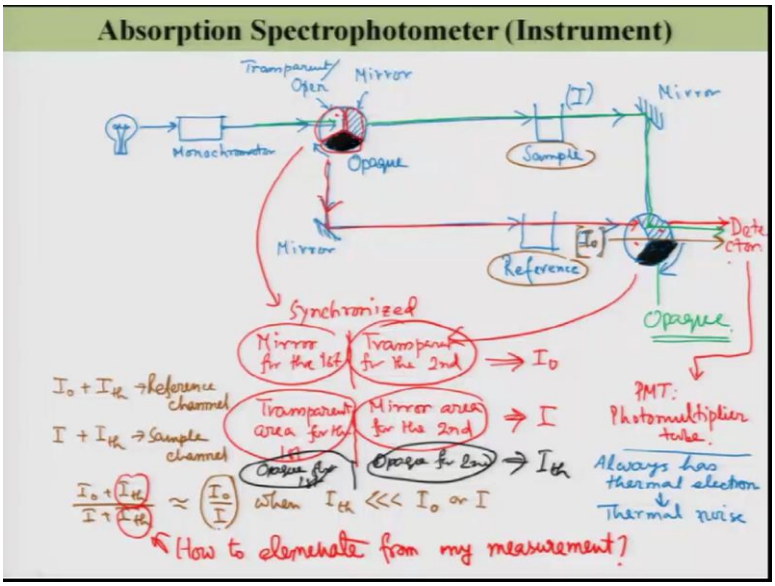

And in this case, we discussed about the role of this transparent part this is my transfer and part over here the role of this mirror part over here, but I have not discussed at this role of this opaque part. So, we can discuss this role of this opaque part.

Let us say and because these 2 rotating mirror system are synchronized in such a way that when the; it is mirror part of the first one this is transparent part for the second one and you can readily determine readily determine this I 0 value. So, here I will going to measure this I 0 and in the otherwise when it is transparent part for the first one a mirror part of the second one, I can measure this I this detector is being used for such a spectrophotometer is generally PMT. So, the detector is generally PMT the full form of this is photo multiplier tube.

So, the principle of this photo multiplier tube is nothing, but the photoelectric effect. So, photon falls right on the photocathode we generate the photo electron these photo electrons are being multiplied by the dynodes under negative potential and these generated electrons is converted to either current or voltage against some register and which is used to measure the intensity of the incoming light. So, if the light intensity is more that photon is more. So, number of photo generate electron will be more. So, the current will be more and the potential and the voltage output voltage will be more from the PMT, but these type of devices actually generate electrons even in absence of light these are called the thermal electrons right these are called the thermal electrons; that

means, there is always there is always some current coming out or voltage against some resistance.

So, there is always some current coming out from the PMT. So, these current is always present right is always present whether you have light or not this is this signal from the PMT is always present and these are called the thermal noise thermal noise of the PMT. So, PMT always has thermal electron giving you the thermal noise now if you can cool it down let us say you put all the whole system in the liquid nitrogen 77 Kelvin. So, the number of thermal electrons will be much much smaller and they will be not much count from the PMT, but that is expensive.

So, what we can do we can instead measuring that actual value of I right when you are measuring this I remember when you are measuring this I let me choose another interesting color over here may be this one when you will measure when you are measuring this I 0 or I at this PMT right. So, here is your I 0, you are measuring it at this PMT when you are measuring the intensity coming out from the thermal electron is also added on it let me assume that thermal electron intensity is I t right I t or I t h. So, here I 0 is because of this incoming light plus I th is because of this thermal electron. So, that is might the total intensity.

So, in the reference channel this is my reference channel. So, in this channel right this is my reference channel here is my reference. So, this is my reference channel in the sample channel the intensity is I plus that thermal electron will all be there. So, this is my sample channel if I th is much much smaller than I 0 or I then I 0 plus I th divided by I plus I th proper it will be almost equal to I 0 by I provided I th when I th is much much much smaller than I 0 or I.

But if it is not then this ratio will change if this ratio will change if this ratio means this ratio will change the log of this ratio will change the absorbance will change. So, there will be a lot of problem in the measurement or absorbance. So, somehow I have to subtract this I th. So, I have to get rid of this I have to get rid of this term how to eliminate from my measurement that is my question and that is why I have this opaque term. So, as I was telling this is synchronized in such a way that mirror for the first one means transparent for the second one transparent for the first one means.

Mirror for the second one opaque for the first one means opaque for the second one right; that means, I am going to measure some intensity right I am going to measure some intensity here I will let me write opaque for first is opaque for second right. So, in this case what we will going to measure I t h. So, we will going to measure I th.

Now you know; what is the value of this I th. So, you can do I 0 minus I t 0 by I minus I th and you will get the exact value of the ratio; that means, the exact value of I 0 by I you will get. So, the absorbance we will going to calculate in this way will be very very accurate right. So, these are all about the several spectrophotometer about the instrumentation what I wanted to discuss with you. Now obviously, we have to move on and before moving to the our fluorescence spectroscopic part in more detail let me show you one interesting application of this absorptions spectroscopy which will be required in while will discuss the solvatochromism in fluorescence too.

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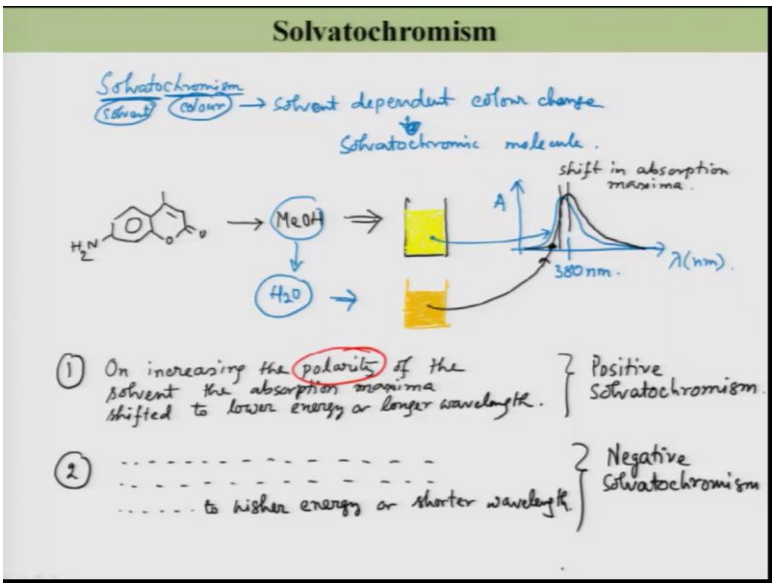

So, this is important for us also to understand this. So, we have several molecule in our world, but some of them actually show interesting property interesting property in terms of solvatochromism right. So, here solvatochromism means.

So, this chromosome means color right and this solvato means solvent. So, this shows solvent dependent coloration. So, if you put this molecule in water it will give you one color when you will put it in alcohol it will give you another color when you will put it in ether it will give another color and so on and so forth. So, this some molecule shows

such kind of solvent dependent color solvent dependent color change right and those molecule are known as solvatochromic molecule right solvent dependent color change and we will call these molecules are solvato.

Chromic molecule now what is the origin of this color of the molecule right I said that I dissolve this molecule in water or whatever solvent methanol or ethanol and I say the color of the solution is yellow why right suppose you use this molecule right. So, you use this molecule here, here when you dissolve this molecule in methanol you will going to see a yellow color.

So, like yellow coloration why because these molecule absorb to the blue color. So, these molecules absorbed in the blue color right. So, if I just roughly draw this absorption spectra of this molecule the absorption spectra is something like this the absorption maxima is around 350 nanometer which is down to you know blue color, round if blue color. So, that is why it is maybe it is 380 nanometer, correct. So, this is my absorbance this is my wavelength nanometer.

So, now, if you change the solvent from methanol to some other solvent let us say you change from methanol to water right and you see a; now I am changing this methanol to water and you see a small change in the coloration, but there is a change in the coloration. So, the color changes from bright yellow to some dark yellow something like this do not go exactly by color this is just I am trying to tell you the phenomena all right. So, like this; that means, this color was corresponding to this absorption spectra right this color corresponding to these absorption spectra that these color correspond to some other. So, absorption spectra will change little bit that is why this color will change between this. So, in this case the absorption spectra will be little different.

It will be going to absorb in the longer wavelength little longer wavelength than what was in the methanol. So, you will see small change in the absorption spectra right like this small change in the absorption spectra. So, you see the absorption maxima are changed from this position to this position right. So, there is a small shift in absorption maximum right. So, there is a shift in the absorption maxima and this shift in the absorptions maxima can be further studied to understand why such kind of change in color takes place for typical solvatochromic.

Molecules right and it has been noticed that there are 2 different types of shift in the color a change in the color the first is first kind is that when we change or we increase the polarity there is always a rate shift in the absorption maxima right. So, first one is on increasing the polarity of the solvent the absorption maxima shifted to lower energy or lower energy means longer wavelength right or longer wavelength right this is known as positive solvatochromism this is the one the case one positive solvatochromism and the other one is just opposite the second one is just opposite the first one that is on increasing polarity of the solvent the absorption maxima shifted to higher energy or the shorter wavelength right. So, everything is same like this, this, this, this, this, this, this same to higher energy higher energy or shorter wavelength this is known as negative solvatochromism.

So, let us see what is the origin of this before that let me tell you what is this thing what is this polarity polarity is the property of the molecule by which it is a cumulative property cumulative result of many many different interaction ultimately these more polarity means it will be able to stabilize a particular iron or a dipole more easily compared to a solvent with the less polarity. So, it tells me that how efficiently the solvent can stabilize a iron and a iron or a dipole. So, the more polar means it will be able to stabilize more efficiently less polar means it will not be able to stabilize that efficiently compared to the more polar solvent.

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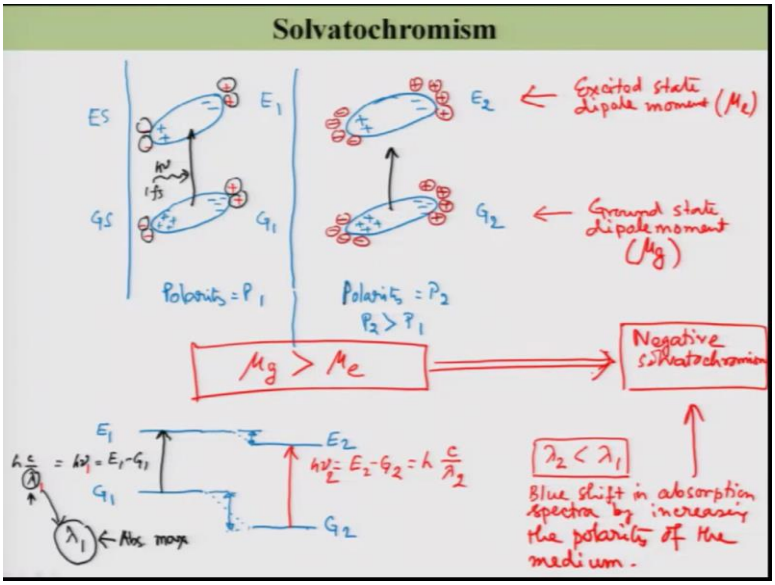

So, with this definition of this polarity let us see these cases let me take a molecule represented as this ellipsoid right and let me draw few positive charge over here and few negative charges over here signifying that this molecule is has a dipole moment in the ground state. So, this is the ground state of the molecule and; obviously, the excited state right I will have this excited state of the molecule excited state right. So, if you remember we discussed that the nature of the molecular orbital called HOMO, LUMO they are deferent.

So, in some case the electron cloud may be shifted to one end of the molecule signifying the dipole moment is becoming higher right there could be a charge transfer during the excitation. So, it is not necessarily that the dipole moment of the ground state is always exactly same as in the excited state and more often which is see that there is a change in the dipole moment of the molecule upon excitation right. So, let me take the first case over here where the dipole moment of this molecule is being reduced of an excitation right. So, dipole moment is being reduced. So, I just put 2 positive charge over here 2 negative charges over here let me write that this is the ground state having energy for yes when it is present in a solvent of polarity P 1 right let us say I put this molecule in a solvent of polarity P 1 so the energy, of this ground state after stabilization right after stabilization become G 1 and this become E 1. So, I have this is the solvent let me change the color. So, these are the solvent actually all right this is a solvent.

Solvating this dipole, so this solvent of the polarity P 1 I have denoted like this way. So, it solvated this species somehow and when you excite from ground state to the excited state right this is your excitation. So, you have excited by and it takes a very short time right. So, one from the second right I said the last day these one from the second is. So, short time that the solvent will not be able to reorganize right. So, the position of this solvent molecule these will remain as it is, but there will be some electronic rearrangement in the molecule the deployment become smaller than the ground state in the excited state, and that corresponds to a energy E 1 right. Now let us put that this molecule in solvent with polarity P 2 let us do that over here. So, polarity polarity P 2 and I consider that polarity P 2 that P 2 is greater than P 1 condition is P 2 is greater than P 1 so the same molecule; the ground state.

I will draw like this way 1 2 3 4; positive charge 4 negative charge its signifying that is dipole moment is high in the ground state and yes now the polarity of this solvent P 2 is

greater than P 1 this molecule will be solvated more efficiently in the ground state. So, instead of drawing 2 negative charge over here and 2 positive charge over here let me draw the fourth of them. So, then this is my solvent. So, I have 1, 2, 3, 4; that means, I just want to signify that more stabilization in this case. So, 1, 2, 3, 4, now I will going to excite it and we going to excite the excitation is very fast the molecule will not going to get time to reorient the solvent molecule I mean so, but the dipole moment will change, this is electronic rearrangement plus plus minus minus and all these solvents will be in the similar fashion right.

Now let me designate this energy as let me designate this energy as G 2 and E 2. So, one thing I can do I can simply plot the relative energy G 1, E 1, G 2, E 2 right. So, if I plot G 1 over here. So, if I plot this is my G 1 E 1 will be a higher energy; obviously, base in the excited state electronic energy I am putting that much of energy. So, here will be my E 1. So, the absorption spectra will be similar to this transition is it not this transition. So, the h nu would be equal to E 1 minus G 1 right. So, this is equal to h c by lambda. So, you will get this as a absorption maxima for this polarity P 1 is it not. So, let us name this lambda as lambda 1. So, this is your absorption maximum for in this polarity P 1 solvent now once you use a high polarity.

P 2 which is more than P 1 then the ground state energy is G 2. So, because the dipole moment is more and it has been stabilized much more efficiently because of this P 2. So, now, if I may draw the G 2 level over here compared to G 1. So, the G 2 will be some over here. So, it stabilized right stabilized. So, this is my stabilization energy this much is my stabilization energy. Now similar wise likewise the E 2 is also stabilized, but here you see the dipole moment is small. So, the stabilization will not be that much, so the gap over here and the gap over here will not be the same.

So, here stabilization will be smaller than in the ground state. So, now, whenever there is one; we will going to measure the absorption spectra in this case what we will going to see is this much of transition. So, this transition h nu equal to. So, in this case this is the energy of E to is not it. So, here h nu equal to E 2 minus G 2, alright so, let us say it is c by lambda 2 let me write nu 2 let me write here nu 1. So, here you see this lambda 2 is less than lambda one that means there is a blue shift in absorption spectra by increasing the polarity of the medium right which is referred as, which is referred to as negative solvatochromism. So, this is negative solvatochromism.

So, now, what we have seen here that the absorption maxima is shifted to higher energy. Shorter wavelength and that is the negative solvatochromism. This is because the ground state dipole moment is more than exited state. So, the key factor here is here you see here ground state dipole moment let us write it as mu G and here is my excited state dipole moment mu E. In this case mu G is greater than mu E, so in this case in the big box, let me write mu G is greater than mu E and that leads to this negative solvatochromism.

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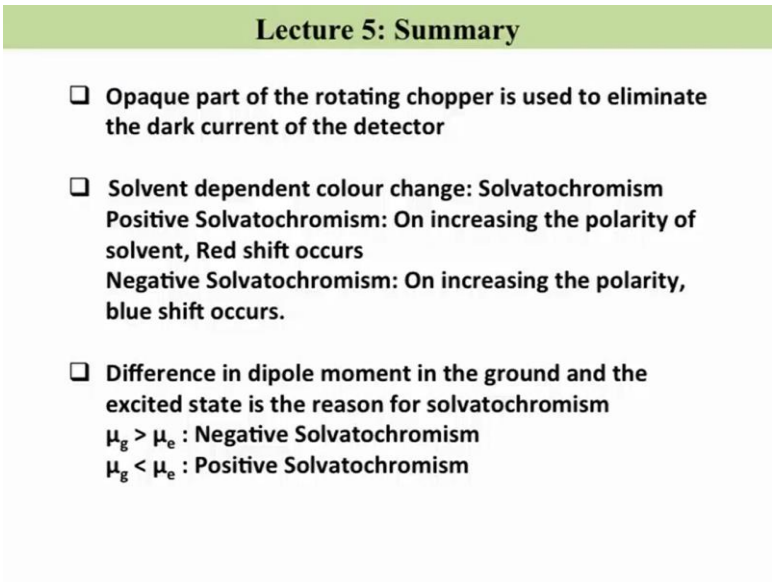

What happen when the mu G is less than mu E; that we will be going to discuss in the next class.

Thank you very much.