Basics of Fluorescence Spectroscopy
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Lecture – 12

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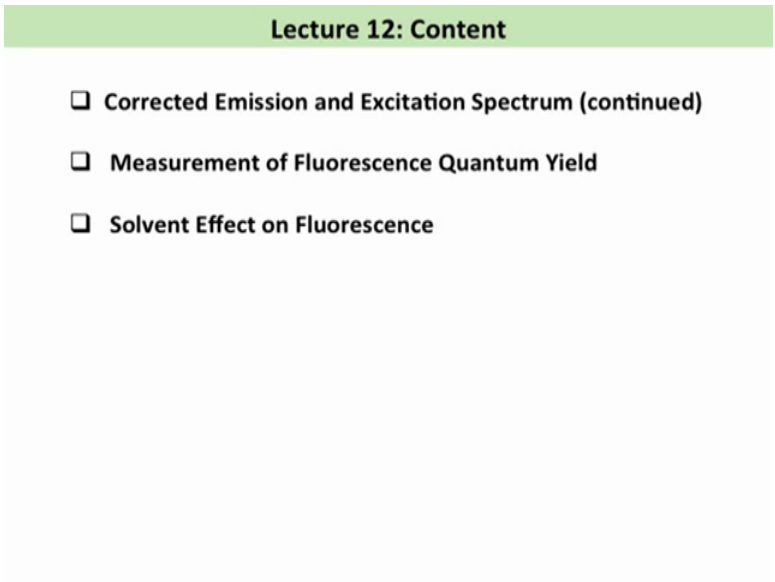

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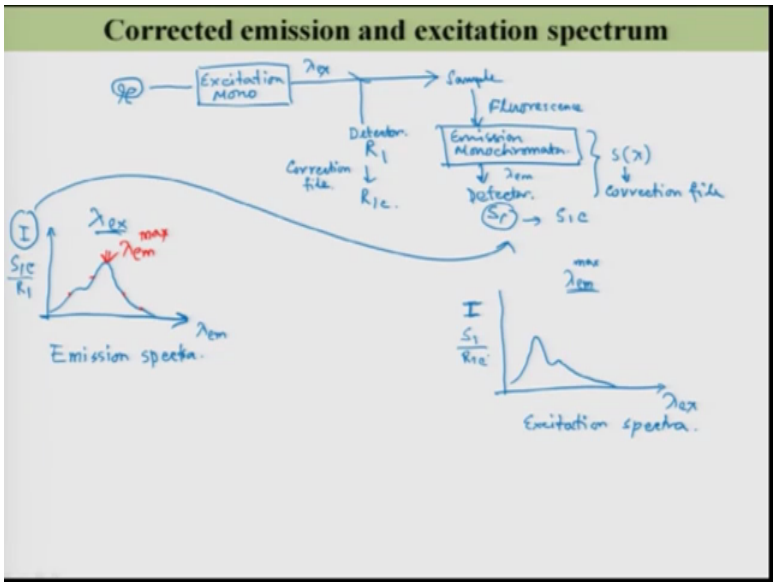

Welcome to the 12th lecture of the series. So, till last lecture, we are discussing about how to measure the correct emission and excitation spectra. And in that regard, what we

have learned is that if this is my fluorimeter, so here is my lamp; and that lamp output is selected; the desired wavelength excitation wavelength is selected out of this excitation monochromator. And that light before directly exciting your sample over here is taken small amount and detected in another detector, where this detector is named as R 1, and then the sample fluorescence. So, here is your fluorescence. And I have emission monochromator is also over here because you need to disperse the fluorescence to measure the intensity of each and every wavelengths out of it. So, here is your emission monochromator.

And then the selected wavelength out of this emission monochromator is directed to the detector. So, this is the S 1 detector. And we have discussed about the total response of this emission monochromator and the detector together is let us say s lambda there is the sensitivity and so for that you will going to have the correction file for that. So, this s one that signal is S 1 C that means, the corrected. Similarly, you will going to have this correction file for this R 1 detector, is not it. So, in this case, after the correction file for this you will get R 1 C.

Having said it let us see that while you want to measure these emission spectra which signal if you get because here that lambda excitation is being selected here lambda excitation. And here is your lambda emission; here is your lambda emission right. The emission spectra means the plot of intensity is a function of wavelength for a given value of lambda excitation. So, for a given value of lambda excitation that say this is my given value of lambda excitation lambda e x if I plot intensity I versus lambda emission then whatever you will get is your emission spectra.

So, these intensity obviously I need that S 1 to be corrected right here this intensity is being detected via at this S 2. This intensity is being detected this S 1. So, I will write this S 1. Now, this S 1 depending on the wavelength the response of this emission monochromator and detector will change, so that has to be collected, so it should be S 1 c. And now the fluorescence intensity depends on the excitation light intensity as well as the concentration; if concentration remains same then it depends only on the excitation light intensity. So, I have to normalize with excitation light intensity because in this case the excitation light is fixed is only if this lambda excitation a particular wavelength. So, whatever the signal I will get in the R 1 detector that is I do not need to correct it because

it is absolute value. I do not have to correct it, I do not have to compare with anything, this is only one wavelength. So, simply this is S 1 C by R 1.

So, remember that if you just plot s one versus lambda emission, you will not going to get the correct emission spectra, but in this case you will get the correct emission spectra. Similarly, for this excite; so this is emission spectra. What about excitation spectra? Excitation spectra are for a given value of lambda emission. So, you keep your observed lambda mission at a particular wavelength let us say over here let us say you fix your lambda emission over here lambda emission max. So, for a given value of lambda emission, it could be anything here, here, here, here, here, but I have chosen the maxima one.

So, for a given value of lambda emission right if I now scan the excitation wavelength then if I plot the intensity of that particular lambda emission is a function of excitation wavelength then as I showed that in the last day that it should resemble with the absorption spectra of the sample provided the absorbance is low. And I 0 is remain same for all these lambda excitation wavelengths. So, to fix that I 0 same for this all the lambda excitation wavelengths what I have to do I have to divide it by R 1 because that will going to give me the spectra of the lamp output.

So, in this case, for a given value of lambda emission, let us see lambda emission max, if I plot the intensity versus lambda excitation then what I will going to get is my absorption spectra. So, let me draw these absorption spectra is something like this way. So, this is my absorption excitation spectra, so which should resemble with the absorption spectra under those conditions which I mentioned. So, this is my; so which signal I should plot over here which I should plot over here, this is for a particular lambda emission. So, it should be S 1 right, and I do not need to correct it because I do not have to compare with the other wavelengths, so only S 1. So, S 1, but I must divided by the R 1 C, S 1 by R 1 C, and that needs to be corrected, so that is it for this measurement of the corrected emission and excitation spectrum.

And now let us move to another important measurement which is the measurement of fluorescence quantum yield. So, I have mentioned that fluorescence quantum yield is another important parameter of the fluorescence where like intensity one parameter, emission maxima another parameter, quantum yield is the parameter and another

parameter is lifetime which I have not discussed yet, but I will going to discuss later. So, what is the difference between fluorescence quantum yield and intensity? Fluorescence quantum yield is just only the property of the molecule; it does not depend on the concentration of your species, concentration of your sample and the excitation light intensity. So, this is a unique property of a molecule.

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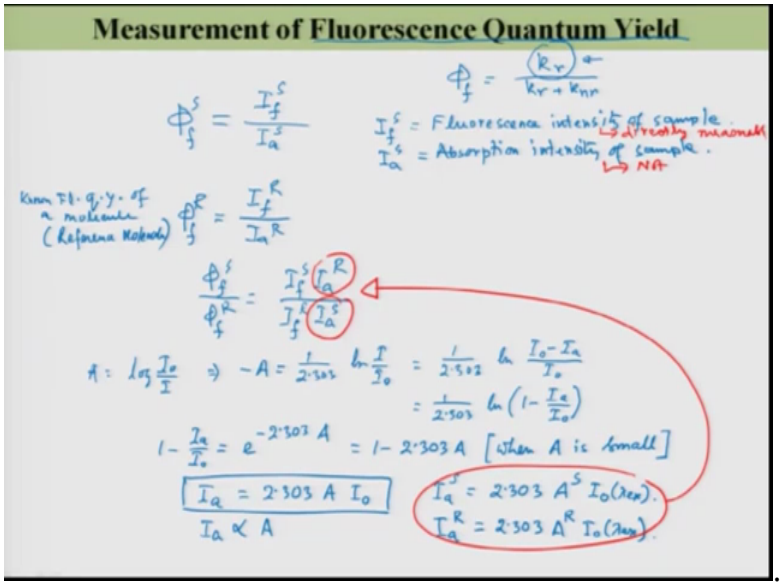

So, this fluorescence quantum yield is defined as k r by k r plus k n r that means, the process right the deactivation process which emits photon divided by total rate of absorption. So, this is the total rate of deactivation that means the total rate of absorption and this k r is the radiative process that means is going to give you the photon out of it. So, how will going to measure it. So, let us say that you have a sample and its fluorescence quantum yield is phi f s. So, I want to measure it. So, I can simply write the fluorescence intensity coming out of the sample. So, I will just write it as I intensity of fluorescence of your sample divided by the intensity absorption intensity of the sample, so I a S.

Now this fluorescence intensity of a sample is a measurable quantity right you can just put your sample in the fluorimeter and you can simply measure what is the intensity is coming out of the of the sample, but this absorption intensity of the sample, this absorption intensity of the sample is not a directly measurable quantity. So, let me write over here I f S is fluorescence intensity of sample fluorescence intensity of sample and I

a S is the absorption intensity of the sample, alright. So, just to tell you this is directly measurable quantity this guy, but it is not.

So, then it is a problem right. Instead of that, if I have another molecule, which I called ref reference and for that particular molecule if the fluorescence quantum yield is known beforehand. So, let me take another molecule, which is phi reference and this phi f r, this is the fluorescence quantum yield of a known molecule, which is referred as the reference molecule. So, here I will write fluorescence quantum yield of a known fluorescence quantum yield of a molecule right of a molecule referred as reference molecule. So, in this case, this is nothing but I f R by I a R. So, I can simply to write that phi f s by phi f R equal to I f s into I a R divided by I f R to I a s.

Now, I know A equal to log I 0 by I, so that will going to give me minus A equal to 1 over 2.303 l n I by I 0. So, this I can write as equal to 1 over 2.303 ln, this I is equal to I 0 minus I a, I 0 minus I a by I 0, this is 0. So, this is equal to 1 over 2.303 ln 1 minus I a over I 0. So, 1 minus I a over I 0 equal to e to the power minus 2.303 A, A is my observance. So, this is equal to 1 minus 2.303 A under the condition when absorbance is low. So, let me write here when A is small. So, what I will get over here after rearranging this thing, after rearranging this thing what I will get. So, this will canceled out. So, I a equal to 2.303 A I 0. So, I can write this I a is proportional to A; so I a is proportional to A. Just look at this equation.

So, if this I 0 is fixed right if the I 0 is fixed. Fixed means that when we will going to measure the intensity of the fluorescence for your sample and when we will measure the intensity of fluorescence of your reference, in both the cases the I 0 has to be fixed the intensity of the excitation light has to be fixed. In that case this I a can be replaced by this a. So, what I can write, I can write I a of my sample is equal to 2.303 A of my sample into I 0 this is my excitation light intensity for a particular lamp excitation. So, do not forget that. So, this is for the particular lambda excitation, so for this lambda excitation. Similarly, I can write I a of R that is the difference is 2.303 absorbance of this reference is not it, I 0 that lambda excitation. So, now, I will replace these two in this equation. So, these, these two, I will going to replace in this equation I r I r I a s, I will going to replace it.

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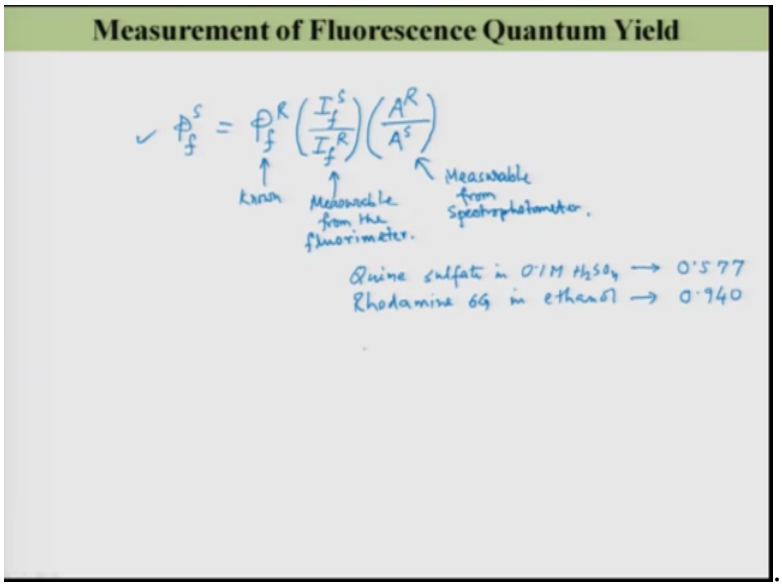

So, if I do so then what I will going to get is phi f of your sample is equal to phi f of reference intensity of fluorescence of the sample divided by the intensity of fluorescence of the reference into observance of the difference, where at that a lambda excitation, silent over here divided by absorbance of the sample. So, you see in this equation this quantity is known quantity, known measurable from the fluorimeter. And this is measurable from the spectrophotometer. So, what you will going to get is the fluorescence quantum yield of the sample. So, what reference molecule people generally used I mean which are well known. So, for example, one can use quinine sulfate in 0.1 molar sulfuric acid. So, for this quinine sulfate in 0.1 molar sulfuric acid, this phi f s is known by this is 0.577. So, you can also use rhodamine 6 G in ethanol, this is known 0.940. So, there are several such kind of known molecules are there in the literature which you can use depending on your requirement.

What is a requirement, requirement is your sample, and this reference molecule should absorb at your lambda excitation. If you have taken a reference molecule which does not absorb where your sample absorbs then you will not be able to use that molecule for your reference molecule, another important point here to tell you that if the quantum yield of your sample is not very much comparable with the reference then there will be lot of error in your measurement.

So, suppose you know the sample quantum yield of your sample is like a 0.01 and you are taking a rhodamine 6 G as a standard over here that is a reference sample 0.94. So, there will be too much of mismatch right because this rely on the intensity ratio, here you see the; this is the intensity ratio of the fluorescence. So, this intensity ratio has to be measured very correctly otherwise the error on this measurement will be very high. So, one has to use a singular quantum yield standard if it is if your sample estimated quantum yield is 0.01 then you should use your references 0.1 or 0.05 or like that. You should not use 0.9. If the quantum yield of your sample is about 0.5, 0.6 then you use a reference whose quantum yield is about that right 0.5, 0.6, so that is just another thing to mention.

Another thing is that you may ask that what if that I am the first person to measure the fluorescence quantum yield that means, no reference is known right that is also possible. And we usually use a integrating sphere for this measurement which I decided not to discuss in this class because of the time constraint, but it is possible, they can check in the literature later on. So, but this method this comparative method of the measurement of fluorescence quantum yield is well accepted all over the world and popular everyday using this method. So, this is good enough for this basic course.

So, now let us move to another topic. So, here what I have written here is solvent effect of fluorescence. Earlier what we have seen is the solvent effect on absorption spectra and we have discussed about two different cases, where the ground state dipole moment is more than the excited dipole moment. We see that on increasing the solvent polarity there is a blue shift in the absorption maxima of the particular molecule. Whereas, when for other molecule where the excited state dipole moment is more than the ground state dipole moment and we have estimated that there will be a red shift in the absorption maxima as if going to increase the polarity of the solvent and based on that we have also discussed this e t n scale of the polarity.

And now it is time to look at that what is the effect of this polarity on the emission spectra. We talked about the absorption spectra, now we have to talk about the emission spectra. So, for these emission spectra, I will just simply start with this molecule which has a dipole, so that means, the ground state dipole moment is mu g. And if you excited the excite state dipole moment could be will be mu e. So, I will just simply draw that molecule as like here over here.

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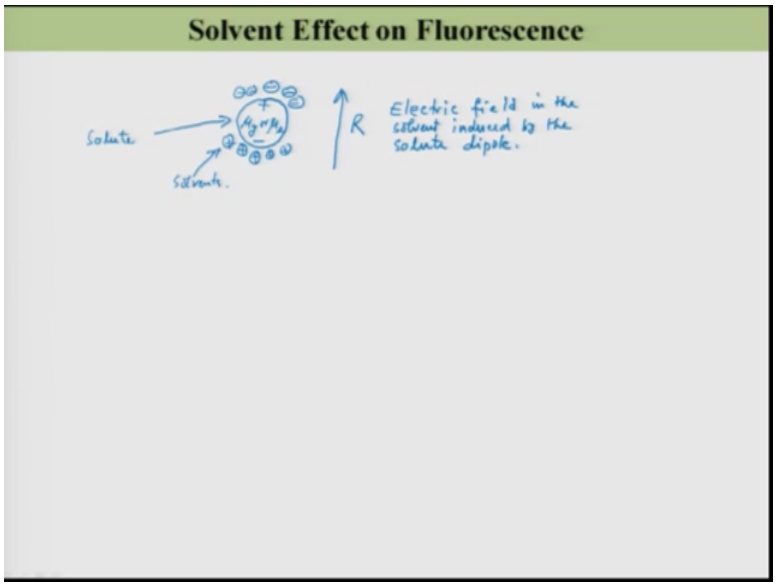

This is my molecule and this molecule has a permanent dipole over here. It could be mu g or mu e depending on how that the molecule is present in the ground state or in the excited state. So, whenever you will put this molecule in a solvent, what will happen this solvent molecule will going to sulfate these species. So, as I showed in earlier that these are the solvent molecule will try to stabilize this solute dipole. And negative charge will be over here for the stabilization. And what we will going to see is so this is my solute let me write clearly, this is my solute these are my solvents.

So, now because of the arrangement of the solvents around the solute, a reaction field will be generated, reaction field will be generated like this way. So, I name this reaction field as R. So, this R is nothing but the electric field in the solvent induced by the dipole induced by the solute dipole. So, this is electric field. Electric field in the solvent induced by the solute dipole and so that means this reaction field right this R will depend on the magnitude of the solute dipole movement mu, and also the radius of the solute and how easily that solute can induce can polarize the solvent molecule. So, I will be going to have 3 factors, which will define this reaction field R, and we will continue this on the next lecture.

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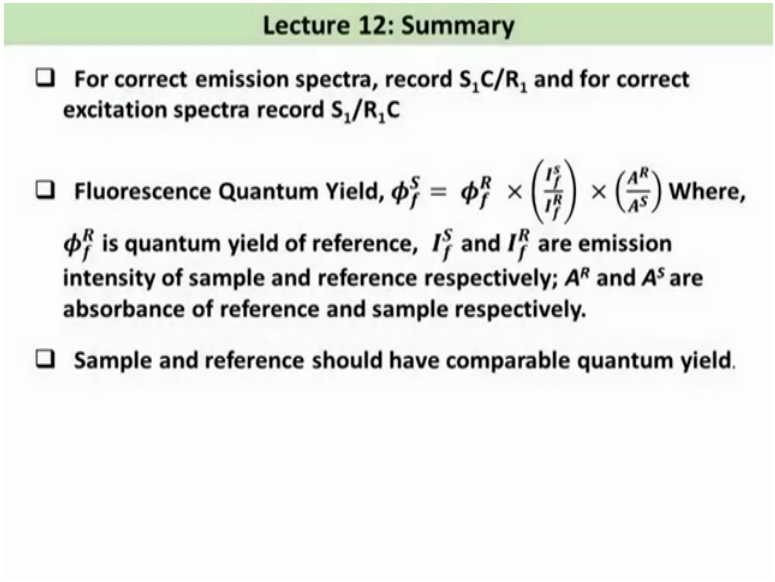

Thank you.