**Ultrafast Processes in Chemistry**

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**Lecture No. 04**
**How to Record Absorption and Emission Spectra**

Hi, so today we have come to our lab, and we will see how absorption and emission spectra are actually recorded, unfortunately will not be able to open up the machines for you, but we will try to give you an idea nevertheless. So first we show you the absorption and emission spectrum photometers and then we will record spectra for you and explain along the way.

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This is an absorption spectrophotometer. You might remember from the previous lecture, that it consists of a lamp, a source of light, a dispersing element and a slit, which combined is called the monochromator and then you have the sample and the detector.

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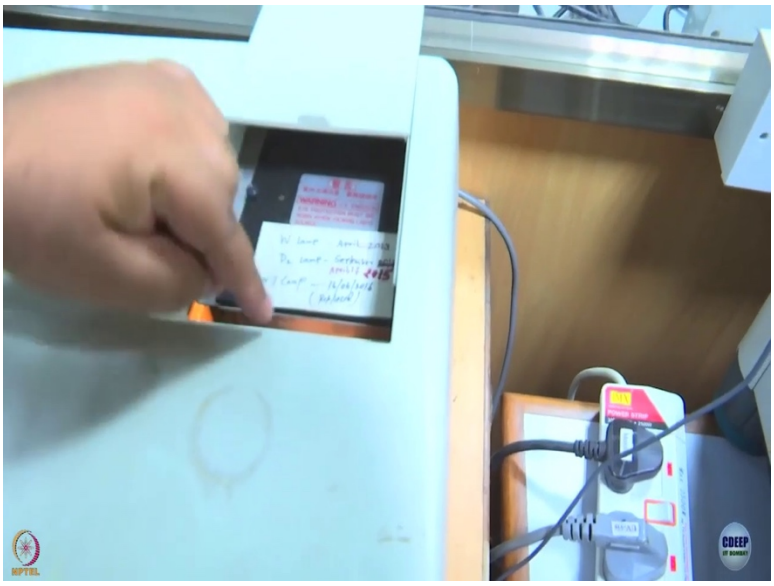

This is where the lamps are in this particular spectrophotometer if you can read the handwriting there are 2 lamps, W means tungsten lamp and the deuterium lamp. Tungsten provides visible wavelength and deuterium and provides ultraviolet wavelength. Now, you might be wondering what is the need of 2 lamps in the first place, we need 2 lamps because tungsten does not give ultraviolet and deuterium lamp is not good for visible source.

But then we have to combine the outputs of the lamp that is done by using a mirror a flipping mirror, which when we work in the visible range reflects the output of the deuterium lamp and cuts out the output of the deuterium lamp. And when we work in the ultraviolet wavelength, it does exactly opposite by swinging to a different position. So, the light from the lamp comes out, goes through a beam splitter. Well, first there is a monochromator so the color gets selected. Then there is a beam splitter. We split it into 2.

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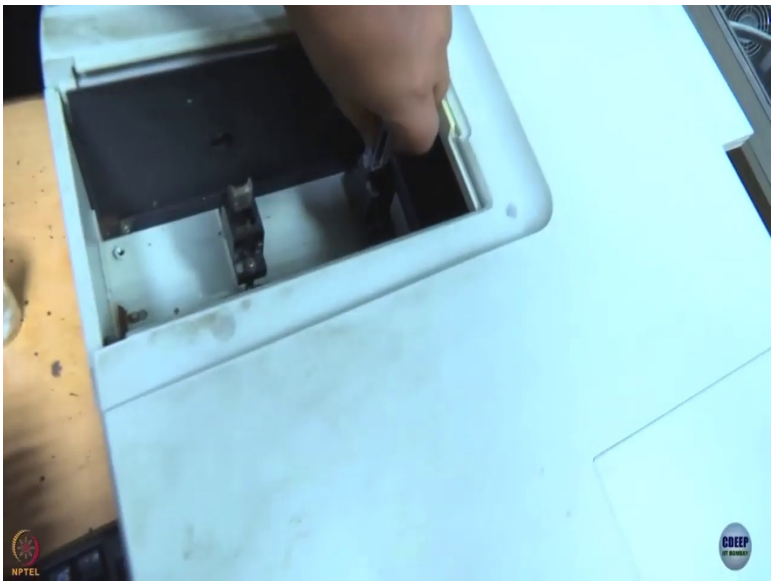

And now as you see, there are 2 places where you could keep your samples. This is called the sample compartment. This is called the reference compartment. And light comes out through these 2 holes and goes through these 2 and finally, there are 2 detectors there these 2 detectors record I0 and It respectively, and work out the absorbance. So, since we are using 2 detectors, it is important that we correct for their responses.

And that is done by using what is called a baseline correction. So what I have done now is I have put the solvent in on both the sides sample and reference in principle, I should get a horizontal flat line. If I record the spectrum, because I0 is equal to It, and log I0 by It, you know what it is, but as you will see, when we try to record the spectrum will not get a straight line. Let us take on the spectrum. Let us do baseline correction.

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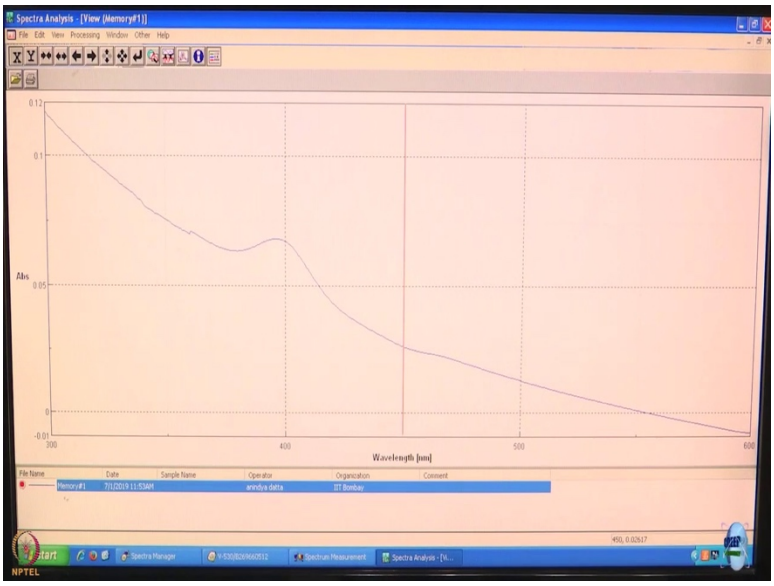


We are recording the baseline. Now, first thing to note is that it is not a horizontal straight line that is because the responses of the 2 photo detectors are not exactly the same. Secondly, as you see, the spectrum is being built point by point. That is because the way it works is the grating inside the monochromator rotates to a particular position then stops and then as long as the stationary a measurement is done, another thing you might have noted is a small thing that comes at around 350 nanometers.

So, that is where beyond 350 nanometer at longer wavelengths, we use the output of the tungsten lamp and below 350 nanometer we use the output of the deuterium lamp. So, what happens there is that the rotating mirror changes its position and reflects either the output of deuterium or output of tungsten lamp depending on the range. So here we have recorded the baseline. Now it is stored in the system and we define it as the 0 line. So, in all subsequent measurements that we do here, this baseline is going to be subtracted from whatever we get, and you will get a corrected absorption spectrum.

Last point to note here is what the graph looks like as we discussed in the previous lecture, a spectrum is always some measure of intensity plotted against some measure of wavelength x axis this here, some measure of energy x axis here is wavelength which is a measure of energy, y axis here is absorbance that we discussed earlier. And if you might remember absorbance does not have any unit at all now, coming back, this is the baseline. So, this ideally should have been flat. It is

not because of the difference in the response of the 2 detectors. Now, having saved this as baseline, we are going to record and absorption spectrum and see what it looks like.

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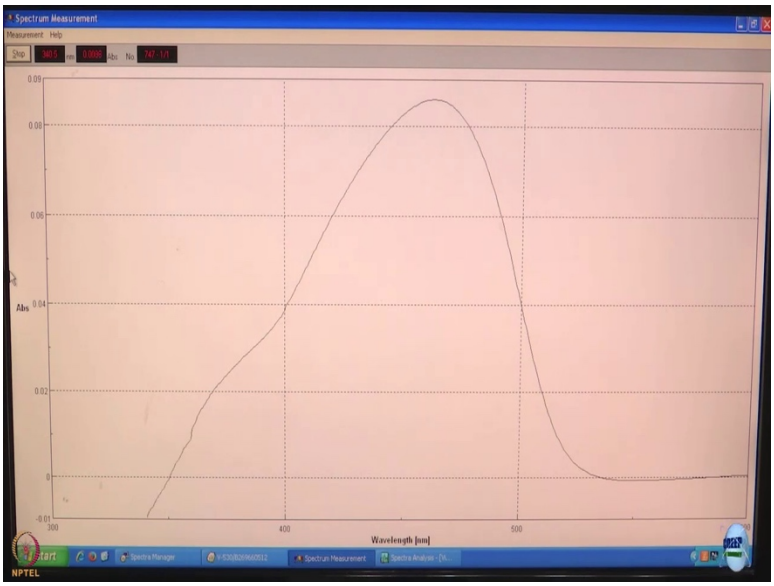


We are recording the spectrum now. And once again, you might note that the spectrum is really being built point by point. Initially, you might think that it is going down actually; it is not the values are very very small. And now the actual absorption band shows up. That is the maximum for our sample. It occurs at about 430 nanometers. So, and if you note the y axis, it is about .08 which means our sample is very, very dilute, you can do an easy calculation assuming the epsilon to have a value of 20,000. You can calculate what kind of concentration it is using Lambert Beer's law we are slow absorbance equal to epsilon CL.

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Next we show you how to record an emission spectrum, this is an emission spectrophotometer So, what it has inside is it has a lamp but this time it is a Xenon lamp which is much more powerful and has a emission over the entire UV visible infrared wavelength range. This is approximately where the lamp is, this is where the monochromator is and this is where you keep the sample. So, the light comes from this direction. It is focused at the center of this cuvette holder. Well this glass vial in which we typically keep our sample a glass of quartz vial is called the cuvette.

And as we said the last day, emission is then at 90 degrees, so the detector escape somewhere on the other side. And did not forget, we have 2 monochromator is here and excitation monochromator on this side and an emission monochromator on this side. To start with, we are going to keep our sample in here, keep the excitation monochromator at a particular position and very the emission monochromator. That way, we are going to record and emission spectrum. Later on, we will do just the opposite and record and excitation spectrum.

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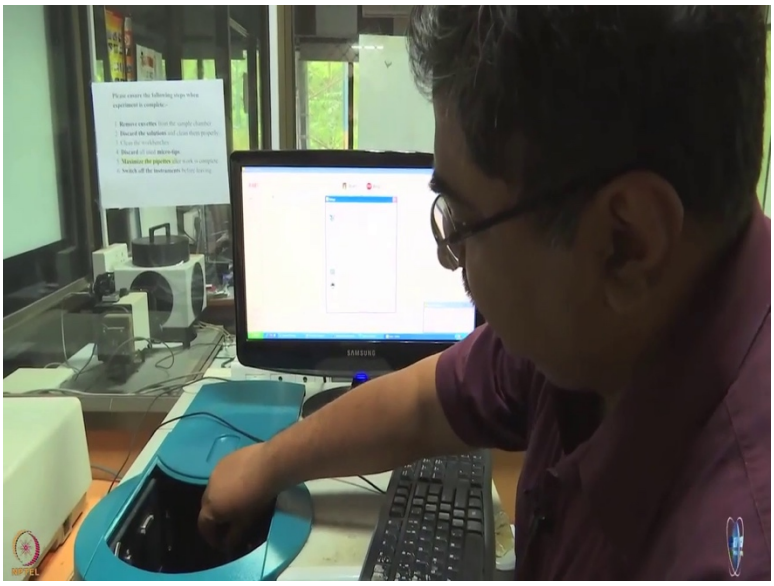

This is our sample right now I am going to put it inside the cuvette holder and record the spectrum.

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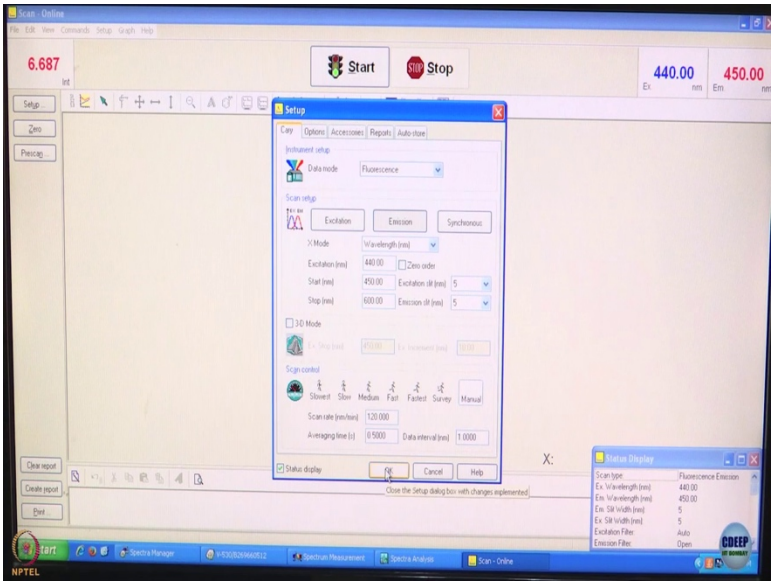


Before we begin, please have a look at the setup here. The way we have set this up we already know that the absorption is around 400, 430 nanometer. So we are exciting the sample at 440 nanometer and we are going to record the emission from 450 nanometer to 600 nanometers, remember, emission usually occurs at longer wavelength unless it is an up converting material. Now, the other important parameters to note are excitation emission slates and you might see that they are written in nanometer. So, what this slit means is the bandwidth of the light that goes through at this wavelength.

So, essentially we are saying that we are exciting with 450 nanometer plus minus 2.5 nanometers. And when we record then also we record at plus minus 2.5 nanometer kind of bandwidth. And for now, we are going to record at 120, nanometer per minute and we are using an averaging time of half a second. We are going to change this and we will see how that affects our spectrum. The last one is data interval we have kept at one nanometer this is fairly common for UV visible range, but if required, you can change it.

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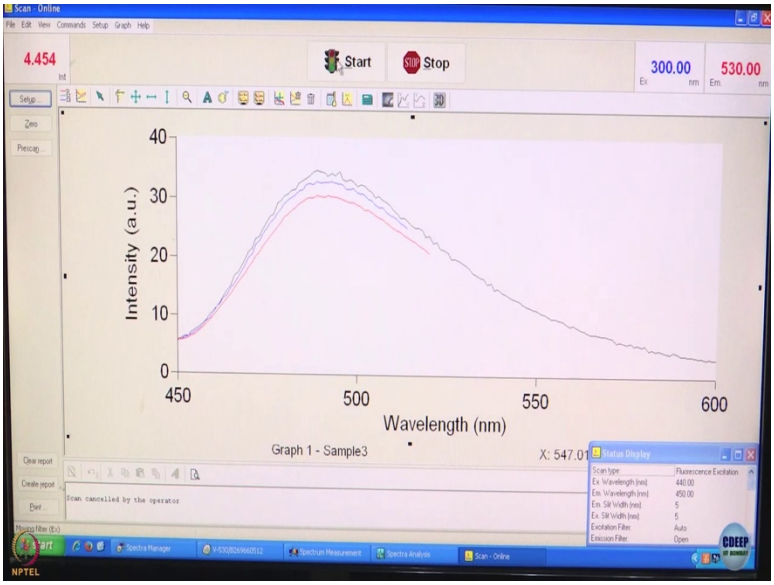


So this is our setup, I am now going to record an emission spectrum. So you can see the spectrum building right here, initially the intensity is very less. So zoom in a little bit now you can see the spectrum forming. And once again, it is forming point by point. Each point corresponds to a particular position of the emission monochromator and as you might see, the quality of spectrum is not very good, because I am doing it very quickly.

The integration time that we are using is only about half a second per point. So after this, we are going to go back to the setup, and we will change it and we will try to see whether there is any improvement in the quality of the spectrum but while we record the spectrum, note, the x axis once again is in wavelength nanometer y axis now is intensity in arbitrary unit. Now, it is arbitrary unit because the value that you get here depends on many things, it depends on what kind which you use, what is the lamp industry that particular day and so on and so forth, what is the absorbance of the sample.

So, this value asset does have it does not have too much of a meaning. However, it can be converted to a meaningful quantity by doing a comparative study and working out the quantum yield. This is something we will not discuss in this course, whoever is interested we go back to our discussion in the molecular spectroscopy course, and also refer to the previous module where we introduced it in a small way. Let us go back to the setup. Now, what we will do is we will keep everything the same, but we are going to change averaging time to 1.5 seconds.

But before we do that, let me show you the spectrum once again, let us remember when the range is from 0 to 40 y axis we captured the entire spectrum and it is rather jagged. Let us remember the shape of the spectrum. Now, let me increase this to 1.5 second, everything else is same excitation wavelength the same excitation slit width emission slit width data interval everything is same, the only thing that has changed is averaging time, which means the number of times you record at one particular point and number of times you have average instead of staying on a point for half a second.

Now we are staying for one and a half seconds. Let us see how the spectrum changes if we do that started recording, you might see the small line coming up. So what we see is it is more or less following the spectrum that we had recorded earlier. But the question is, we have made it slower. So does the quality become any better or not? We will come back to that question once we have reached the maximum, but perhaps minor point to note here is that you see, we are recording a vision spectrum for the same sample a few seconds after we are recorded the first one and you can see that they are not exactly overlapped .

This is why you have to do several experiments and take an average because there are many things that affect the absolute intensity that you record. So, now see, if you look at the rise, it is a little less jacket than what it was for the initial spectrum and even in the maximum position, it is not really as noisy as it was. So, what we have here is that we have a sample that is not very highly emissive. In such a case, you have to use a longer integration time.

In fact, even 1.5 second is obviously not enough for this measurement. You have to use something that is longer if you want to get a really good spectrum. Let us do that now, we play a game of patience and change this to 5 so, it says there is going to take 12.35 minutes to complete. Let us see whether investing this much of time has any effect on the spectrum. We have started recording you can see something creeping up here, but it is going to take some time.

So, let us take a very short break and come back and show you the spectrum and it has been recorded. So, we have given it several minutes and this is a spectrum that we have got so far. So, now, you see will not record the entire spectrum because the point we are trying to make can be made with this data. So, if you go from the first spectrum to second spectrum there is improvement if you go from the second spectrum to third spectrum when we increase the integration time from 1.5 seconds to 5 seconds, there is still some improvement.

So, if you keep on increasing the integration time some improvement or the other is going to come. But the question is when will you stop, there is always an optimal point beyond which there is no need to go. Because, if you see, the absorbance is also going down with every successive measurement, which might indicate that your sample is going bad due to prolonged exposure to light. More often than not, that is what happens with organic samples especially.

So, one needs to be careful and judicious I decide upon this integration time. Maximizing or minimizing is not the solution, you have to optimize it and find the best possible time for your measurement. So, we stop this measurement here. And now I will show you another kind of measurement that can be done on an emission spectrophotometer as have said there are 2 more promoters 1 here and 1 there 1 excitation 1 emission in the experiments that we have done so far we have recorded then emission spectrum meaning the excitation spectrum has been held in a particular position and the emission spectrum has been scanned, let us do just the opposite now.

So, here we see the emission maximum is around 500 down a little less than 500 nanometer or so, let us set the emission spectrum, I want to record an excitation scan now, let us set the emission spectrum to say 530 nanometers. And let us do a scan from say 300 nanometer to say 500

nanometer and since we did not want to spend so much of time, I will change the averaging time to 1 second. So, that is what it is and; let us see what the spectrum looks like.

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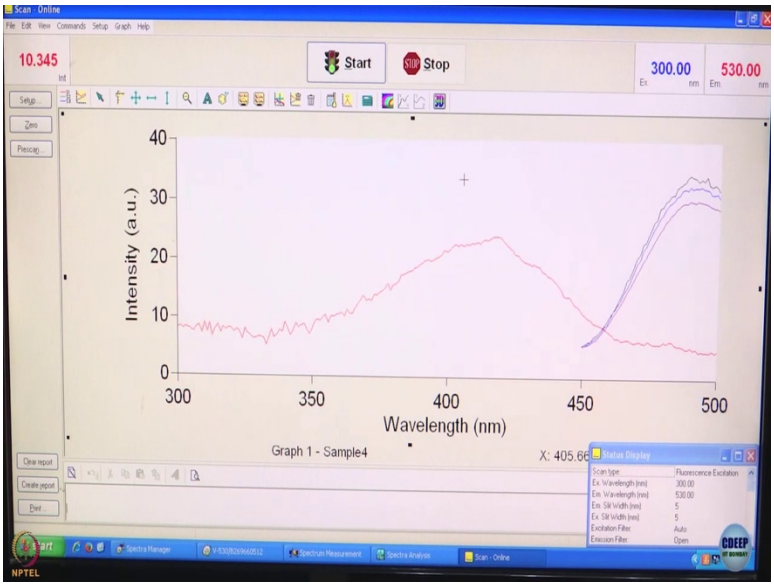


Now, we record the excitation scan, you can see the spectrum building up right here. So, the reason why you want to do an excitation scan is this you know what we are doing in this experiment is that we have kept our emission wavelength fixed at some point and we are changing the excitation wavelength. Now, we want to remember that we have discussed in class that intensity of emission is emission quantum yield multiplied by intensity of light absorbed.

So, everything else beings same if we change the excitation wavelength, what is changing is intensity of light absorbed because that is related to absorbance which is a function of wavelength. So, even though we have kept the intensity of emission, we have kept the emission wavelengths same intensity of emission is going to change and it is going to change because now you are scanning the range over which the molecule absorbs and wherever it absorbs more, we are going to get a greater emission intensity, because intensity absorption is accordingly higher.

And for an absolutely pure sample or for a sample in which the ground state is absolutely heterogeneous, we are going to get a situation where the excitation spectrum should exactly match the absorption spectrum. Because, for dilute samples especially, you can simplify the expression one minus 10 to the power minus A and you will see that emission intensity is going to depend

linearly on absorbance. So, for dilute samples in which the ground state is absolutely non heterogeneous absolutely homogeneous, we expect that this excitation spectrum that we are recording is going to be super imposable with absorption spectrum, if they are normalized to the same height.

Now, sometimes that is not the case. In this case for example, we see we are getting an excitation maximum at around 410, nanometer whereas, the absorption maximum was at 430, nanometer. What does it mean? It can mean 2 things. First, your sample might be impure. And second, your sample might not be made up of 1 thing; you might have a heterogeneous ground state. Maybe there is a keto–enol tautomerism in the ground state and that is what is deflected. If the keto form and the enol form are not equally emissive.

If the quantum is a different then it will be reflected in a mismatch of absorption excitation spectrum, this is a very, very important point to remember. Because mismatch of absorption spectrum with excitation spectrum and means something very bad that even impurity or something very good that you have an interesting system at hand where the ground state is heterogeneous. So, please remember, when you want to do emission spectroscopy, please did not just go and record the emission spectrum.

The correct way of doing it is record the absorption spectrum first, then record the emission spectrum. Finally, record the excitation spectrum and see if it overlays with the absorption spectrum. If it does, then there is nothing to worry if it does not, then either you have something to worry about, or you have an interesting story at hand. That is what we wanted to show you in how actual steady state absorption and emission spectra are recorded. Thank you.