INDIAN INSTITUTE OF TECHNOLOGY BOMBAY

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NPTEL

MOLECULAR SPECTROSCOPY: A PHYSICAL CHEMIST'S PERSPECTIVE

PROF. ANINDYA DATTA
DEPARTMENT OF CHEMISTRY,
IIT BOMBAY

LECTURE No. – 1
Frequency Domain Spectroscopy: An Introduction

Today we are going to talk about frequency domain spectroscopy. As you know spectrum essentially means a plot of say intensity against some measure of energy, it can be energy in electron volt, it can be energy in kilo calorie, it can be wavelength, it can be wave number something, and these lines tells you which transitions take place to what extent, and which transitions do not.

Why some transitions take place, and some transactions cannot, we'll come to that question shortly, not today, in a couple of days. But today what we want to discuss is how this spectra recorded? Even before we do that, let us say something else, since we are talking about frequency domain spectroscopy, what is implicit here is that there is something called time domain spectroscopy as well, frequency and time are inter convertible, in the next class we are going to talk about what is called time domain spectroscopy, and we will revisit this issue when we do that discussion.

Today we are more interested in recording intensity of transition in some way at different energies directly this is called frequency domain or energy domain spectroscopy, okay. So first of all as you know very well, there are two kinds of spectroscopy, I mean everything can be classified in many different ways, so if I ask you what are the two kinds of spectroscopy you can give me multiple answers, but what I mean is you can talk about absorption spectroscopy, and if absorption is one kind of spectroscopy, what would the other kind be? Emission spectroscopy naturally, what do you do in absorption spectroscopy? Light is impinged on your sample and you try to understand which energies are absorbed and which energies are transmitted, okay, what does that give you in your, say visible region? What does that give you, which property does absorption give you? Yeah, cannot hear you, louder. Well, easier answer, what colour is my shirt? So that was not a good colour anyway, what colour is his shirt? The body of the shirt, forget

about the sleeves, yeah, why is it that colour? Because other frequencies are absorbed and that frequency is reflected, so reflective sample, similarly if you look at the solution that is red, what does that mean? When light has gone through, blue wavelength has been absorbed and red has been transmitted, okay, so that gives you colour to put it in very simple terms.

What does emission spectroscopy give you? Which property? Well, also colour, but different kind of colour, those of you who have gone to a disco for example, would know that if you wear a white shirt, that shirt looks blue and it glows, doesn't it? Don't tell me nobody has ever been to a disco, right, why is it that the white shirt looks blue? Because they have a little bit of UV light there and the fabric of your shirt has pigments which absorb UV and give out blue light that is why it's blue and it's glowing, that is emission, okay.

Now there are two parameters are of paramount importance when you talk about absorption and emission. When you want to measure absorption the parameter that you use is absorbance which is given by log of I0/IT, where I0 is the intensity of the incident light and IT is the intensity of transmitted light, and these leads to another quantity, what is that called? Another intrinsic quantity which is characteristic of the material and the colour, so this leads to epsilon, the molar absorption coefficient and that is what tells you how probable the transition is, alright.

We'll have more things to say about this molar extinction coefficient, and how you can arrive at A, theoretical description of molar extinction coefficient little later in this course, but today we will just learn how to record it.

And similarly when you talk about emission, what is the quantity of paramount importance? Intensity of emitted radiation of course, but then the point is this if you just increase the intensity of the incident light, then intensity of emitted light will also go up, so that is not so unique, what would be a unique measure of strength of emission of your sample, it's called quantum yield, but in this case what we call it is emission quantum yield, in your course in photo chemistry you are going to talk about reaction quantum yield, so emission quantum yield is defined as intensity of emission divided by intensity of absorption, actually I have jumped a couple of steps, what is essentially means is, number of photons emitted per unit time divided by number of photons absorbed by unit time which boils down to this same thing Iem/Iabs, okay.

So now let us look at these two quantities one by one and then we will go over to the actual measurement part of it. Now when we talk about absorption spectroscopy, as we discussed what you have is, you have light of intensity say I0, incident on your sample, the light travels through the sample and the light that goes out has intensity let's say IT, what is the relationship between IT and I0? You know Lambert Beer's _7:17_ and this is how it comes, let us say the length of the sample is L. Let us consider an infinity similarly small length DL somewhere inside this sample, and let's say that the intensity of light just before this DL is I, and just after this DL it is attenuated by an amount DI, I – DI, empirically Lambert Beer arrived at two different conclusions, once said that this DI depends on your concentration, the other one said that it depends on length, and of course it depends on what I is as well, so essentially it's –DI is proportional to I, molar concentration C and this length DL, so if I write this proportionality as an equation what I get is –DI = A multiplied by I, multiplied by C, multiplied by DL, what is A? Constant to proportionally, like proportionality constant nothing else.

So of course if I have it like that, it is not very difficult for you to formulate this equation DI/I = I'll write –DI/I = A multiplied by C, multiplied by DL, and wherever you have an equation like this you know very well what to do, what should we do in the next step? We should integrate, and what would be the limits on the left hand side and right hand side? On the left hand side it should go from I0 to IT, and on the right hand side it should go from 0 to L, it's elementary, and I think many of you already know this, so what I can do is I can get rid of this minus sign, and I can instead write IT to I0, and here it will be 0 to L, what does that give you? It gives you LN, I0/IT = A.C.L, but then nobody likes to work with LN, it is much simpler for us to work with log, to the base 10, what do I need to do to go from LN, natural logarithm to log to the base 10? Multiply by 2.303, right, so this gives me log of I0/IT = now I'll write epsilon CL, this left hand side is called absorbance A, this is your definition of absorbance and this is your Lambert Beer's law.

Now just few things to remember so that we don't go wrong when we do an actual calculation, maybe not in this course, it's too easy to ask in this course, but whenever you do an actual spectrophotometric measurement, let's not forget certain things. What is the unit of C? Yes molar, right, mole per liter, what is the unit of L, centimeter or decimeter? Centimeter not decimeter, is it little strange I mean if I formulated it I would say this instance is mole per decimeter cube, L should be in decimeter, but then it has been define this way because do you ever measure anything in decimeter, tell me, when was the last time you reported some length in decimeter, but centimeter, meter these we are more conversion way, okay, so it's a matter of convenience, matter of practice, nothing else.

And then what is the unit of the left hand side? Please remember, absorbance has not unit, this might sound trivial, see absorbance doesn't have a unit, what is the unit of epsilon? Yes, this is molar and this is centimeter, so it would be per molar, per centimeter, but you can write it in different ways isn't it? Because molar you can expand into mole per decimeter cube, you can convert that decimeter to centimeter and then if you do that then of course there'll be a factor of 10 to the power something that will come, okay, but that's not very difficult to do.

Now let's move over to emission, as I said if you increase, okay, before move over to emission one more point that I forgot to make, what did we say absorbance is? A = log I0/IT and that is equal to epsilon CL. Now this linearity, this is linear equation right, absorbance is proportional to C, if I work with the constant L, but this linearity does not really hold if you go to very high concentrations. So please remember this only holds for reasonably dilute solutions.

And one more thing suppose A = 1, what is IT/I0? A = 1, what is IT/I0? 10 to the power, 10 to the power -1, right, actually if A = X then it is 10 to the power –X you can write it like that, 10 to the power -1, so it's 0.1, see even if absorbance is 1 that means only 10% of the light that falls on the sample is getting through, okay, now if absorbance is 5 then you can understand IT/I0 is 10 to the power -5, what does that mean? It essentially means that the sample is opaque at that, almost opaque at that wavelength, so whenever you go to such high absorbance values, your signal is going to be small and noise takes over and you get a very, very noisiest spectrum, so generally we want to make sure that we perform our absorption experiments for sufficiently low absorbance. Since I work principally in emission then more complications come in, I prefer to

work within absorbance of 0.1, no more, sometimes even 0.1 is too much, but absorbance of one and all for spectroscopy are not all that good, more than that you cannot even measure.

But suppose I have a solution whose absorbance I'm getting as 2, I still want to determine concentration, how can I do it? You can do an accurate dilution, the word accurate is important here, any dilution will not do, if you use well, if you use a micropipette or something like that and takes a 200 microliter of the solution diluted to 2ml and then measure absorbance do a calculation that is the perfectly valid approach, what else could I do? So this is chemistry approach of course, what would the physicist approach be? Do not forget the equation, remember all of it, the answer lies there.

Yes, use a shorter path length, you are taking an, so typical absorbance measurements are performed by taking a solution if you are working with the solution inside A, quartz vessel called a cuvette, so you can have cuvettes of different path length, instead of using a path length, instead of using a path length of 1, you can use a path length of 0.1, so these ways you can still circumvent the problem of high absorbance.

Now talking about emission you said that as you increase the intensity of incident light, intensity of emitted light also goes up, so what is the better way of quantifying emission rather than taking just the emitted intensity. So in that case what we do is we take well, as I said emission quantum yield usually denoted as Phi EM and that is your intensity of emitted light divided by intensity of light that is absorbed, please see what I have written here, I've not written I0, Iabs, what is Iabs? Iabs you can say suppose intensity of light that is incident on the sample is I0, for now let me neglect reflection, let us say that this light is part of it is absorbed and part of it is transmitted, of course the complete description would be part of it is reflected, but let us say we are working with a nonreflecting sample, okay, this is Iabs like the part of the light that is absorbed, intensity that's absorbed.

So now if I want to work further with Phi EM, I have to find a little more tangible expression for Iabs, let's see if you can do that. What is IT? IT is I0 – Iabs, right, now you already know one quantity that utilizes IT and I0, don't you? What is that? What did we get absorbance spectroscopy? Absorbance = log I0/IT, I can write this as log I0 - Iabs, right, rest of the algebra is very simple, isn't it? Raise both sides to the power, I mean 10 to the power A will be equal to I0 divided by I0-Iabs, take reciprocal of that, what do you get? You get I0 – Iabs divided by Iabs = 10 to the power –A, so what is Iabs from here? If I simplify this what do I get? This step is, yes, yes, right, what is the expression for Iabs that I get from here? So I get I0 multiplied by 1-10 to the power –A. Now see if I take this expression and plug it in here, then what do I get? I get Iem divided by I0, 1 – 10 to the power –A, alright. If you have a question please ask. Have you all clear about whatever we've discussed? Then can I get rid of this part?

Now we come to the question, how do you measure quantum yield experimentally? Well, ideally you should try and capture all the photons and do an absolute measurement, but we don't usually do that, sometimes we have to we have no other way, but usually what we do is we use a reference, a reference whose quantum yield is well known, let us say quantum yield of this reference is Phi EM R. And let us say quantum yield of the sample is Phi EM S.

Now if I take a ratio of this two, what do I get? If I do a measurement under absolutely identical conditions, please remember, identical condition is essential for the next step, identical condition means I0 is the same and well temperature is same you do the measurements back to back without changing any setting on your spectrophotometers, in that case can I not write something like this? Phi EM sample divided by phi EM reference will be, I can write Iem of the sample divided by 1 – 10 to the power - absorbance of the sample divided by Iem of the reference multiplied by 1 – 10 to the power – A of reference, generally this is how we determine the quantum yield, emission quantum yield of an unknown sample by taking its ratio with the emission intensity of a reference whose quantum yield is well known, okay.

So what's most important here is you must maintain identical conditions for recording the spectra of sample and reference, clear, question? Question? Understood? Fine, so now what does this mean? It means if you want to determine the quantum yield then it is not enough to perform an emission measurement, you must first perform an absorption measurement and then only proceed two measurement of emission, okay, so this is something that we cannot forget if you want to do actual experiments. Many of you for your chemistry projects or even lab are going to record fluorescence spectra and too many times people just go to the sonometer and start recording spectra that is not the way to do it. Please record absorption first, then only proceed to measure fluorescence, okay.

Prof. Sridhar Iyer

NPTEL Principal Investigator
&
Head CDEEP, IIT Bombay

Tushar R. Deshpande
Sr. Project Technical Assistant

Amin B. Shaikh
Sr. Project Technical Assistant

Vijay A. Kedare
Project Technical Assistant

Ravi. D Paswan
Project Attendant

Teaching Assistants

Souradip Das Gupta

Hemen Gogoi

Bharati Sakpal
Project Manager

Bharati Sarang
Project Research Associate

Nisha Thakur
Sr. Project Technical Assistant

Vinayak Raut
Project Assistant