# Basics of Fluorescence Spectroscopy
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## Lecture – 34

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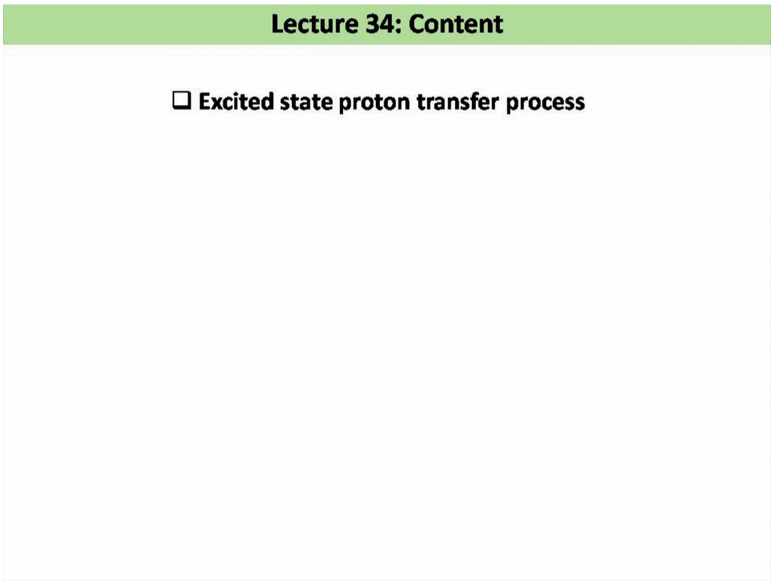

**Lecture 34: Content**

☐ Excited state proton transfer process

Welcome to lecture number 34. Today we will going to discuss the excited state proton transfer. To start with let me tell you the normal proton transfer in the ground state. So, if you have a molecule which is denoted by HA, that H which is proton can be donated either to the solvent or to some other molecule to make it A minus and H plus.

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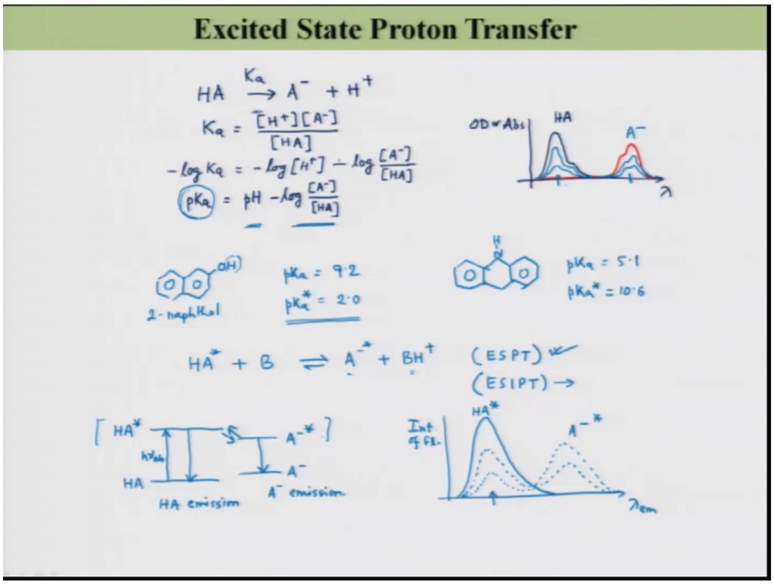

Excited State Proton Transfer

$$HA \xrightarrow{K_a} A^- + H^+$$

$$K_a = \frac{[H^+][A^-]}{[HA]}$$

$$-\log K_a = -\log [H^+] - \log \frac{[A^-]}{[HA]}$$

$$\boxed{pK_a} = \underline{pH} - \underline{\log \frac{[A^-]}{[HA]}}$$

$pK_a = 9.2$

$\underline{pK_a^* = 2.0}$

$pK_a = 5.1$

$pK_a^* = 10.6$

$$HA^* + B \rightleftharpoons A^{-*} + BH^+$$

(ESPT) ←

(ESIPT) →

Let me write this then it will be a much more clear. So, we have a molecule H A and that can be dissociated to A minus plus H plus and if I denote this equilibrium constant is K a then and the then then this K a can be written as concentration of H plus, concentration of A minus divided by this we know right concentration of H A that is it; so taking a log at both the side and again 1 minus sign. So, this will be minus log K and in this part will be minus log horizon and concentration which is nothing, but the p H and then minus log A minus by H A concentration. So, taking log I can write log minus log K a, is equal to minus log horizon and concentration plus minus log concentration of A minus divided by concentration of H A.

So, note that these concentration of A minus and concentration of H A is at the equilibrium, this is not the original concentration. So, this is nothing, but p K a equal to p H minus log concentration of A minus divided by concentration of H A. Now if I consider that these H A is associated with some chromophore right or in the other hand this H A has a specific absorption spectra, and A minus has a different absorption spectra then it is straightforward to measure the concentration of A minus and H A by the spectra of automatic method and one will get.

For example if this is the absorption spectra of H A, this is the absorption spectra of H A now if you increase the p H of the medium, a more and more H A will be converted to A minus. So, here I am plotting the absorbance or OD whatever you want this is the same thing and here is my wave length.

So, once you increase the p H of the medium then more and more H A will form A minus, and if A minus is having a completely different type of absorption let us say like this way the A minus absorption is something like this, then what we will going to see that formation of A minus at the expense of H A. So, H A will decrease and A minus will increase, individually if you know this epsilon values then from there you will be able to calculate what is the concentration.

So, initially if this was H A, then these H A will decrease and this is some for formation of this A minus. If you increase the p H more then it will be like this and so on and here this 2 H A and A minus are well separated like they are not overlapping with each other, if they are overlapping with each other you have to do a little bit of more calculations to get the relative concentration of H A and A minus as the function of p H. And once you know that you will get this far and you already know the p H and you will be able to calculate the p K a.

The lower the value of p K a the higher is the acidity of that substance, suppose this H A has p K of 6 that means, is a weak acid. If the p K the p K of H A is around nine; that means, it is not (Refer Time: 05:33) I mean it cannot donate the proton to water right if the p K of this H A is let us say one; that means, is strong acid right. For some molecule that is what our discussion is all about, for some molecule it has been seen that the molecule has a very low acidity in normal condition, but once you excite the molecule by proton radiation right the excite state of the molecule become very strongly acidic in nature. So, it can readily donate the proton.

So, for example, this molecule if you take, what is this molecule this is 2 napthol, this is the p K of these molecule is about 9.2 obviously, these proton is not level in the ground state. So, acidity is very low right.

However if you excite this molecule using 270 nanometre 280 nanometre light right and 280 nanometre if you shine, 280 nanometre u v light then the p K of this molecule drastically reduce to 2.0. So, I say that this p K a star because it is excite state phenomena it is under the proton radiation; that means, the molecule is now at the excite state not in the ground state. So, in that case the p K a star is 2, p K a star 2 means is highly acidic, but this highly acidic nature of this molecule is only present when the molecule is in the excited state. The molecule when it is in the in its ground state, then the molecule is not highly acidic in the nature right.

For example it could be completely different itself for example, let us see this molecule this molecule is (Refer Time: 07:41) right. So, in this molecule the p K a in the ground state is 5.1. So, is the moderately acidic molecule right; however, in the excited state if you shine light right in the excited state the p K a transfer to be 10.6; that means, under proton excitation these molecules become a strong base it has 5.1 in the ground state, but in the excited state it is 10.6.

So, you see the change of this acidity of this molecule under proton excitation. The case right where you see such kind of situation that p K a star is much lower compared to the p K a, then it is possible that if you have a base is sitting next to this molecule H A in the ground state, the proton could not be transferred because it is not favourable from H A.

Now, let me write this H A plus B. So, I am going to have A minus plus B, H plus. So, formation of this conjugate base or the conjugate acid of this base B right this is not favourable in the ground state, but because a p K a is very high right for this H A, but once you excite this molecule you put this molecule H A in the excited state immediately this takes place. So, leaving this molecule in the excited state that means, the proton transfer from H A to B is taking place in such kind of cases only in the excited state and this is known as excited state proton transfer in short form people call this as ESPT.

Now, if it is within the same molecule like one part of the molecule is proton donor and the other part of the molecule is proton acceptor, then it is known as excited state intra molecular proton transfer and denoted as ESIPT, but we will not going to discuss this one in this course we will only going to discuss this one.

Now, let me draw this energy diagram of this excited state proton transfer. So, in this case what we can see is that this is my H A and upon excitation it form H A star. So, this is my H nu absorption. If this proton can be donated to somebody then something else, but otherwise it will come back to the ground state and it will going to give you the H A emission in presence of the acceptor of the proton right then only it will going to give to somebody, if you just take this H A in the gaseous state alone and you are exciting I mean it will not be able to donate the proton to anybody. So, it will not going to donate something has to be that we accept it right. Typically if you dissolve it in water then water will be H three o plus then it is a it will form the congregate acid of the water.

So, in that case then H A will H A star will be able to donate its proton to form the A minus, and if I now draw these A minus it could be anything like this is my A minus energy state, here is ground state, here is A minus star right. So, this proton can be donated from it is H A star to A minus star and then correspondingly e minus emission will be observed. So, what we will get here is the A minus emission that is what you will going to see in the experiment.

So, in the experiment what we will see? In this case you are not going to record the absorption spectra because absorption is a ground state phenomena here we will going to record the fluorescence or emission spectra, because the emission is the excited state phenomena. So, we will going to record the emission spectra and let me draw a typical emission spectra of such kind of molecule, what you will going to see is when there is no one to accept the proton or the p K star value is very high right it is not possible to donate the proton, then you will only going to see the emission spectra coming out from the H A.

So, this is my intensity of fluorescence and here is your lambda emission. So, this is specifically for H A molecule right, but if somebody is there then this fluorescence intensity will decrease and another band will come, which is by the A minus star. So, this is this will decrease and another band will come because of A minus.

If you have more accepted present for this proton then obviously, this band will increase and decrease. It is clear from this diagram that the relative concentration of H A star and A minus is somehow related to this intensity, because this is my A minus right in the excited state right I should write here star. So, this is A minus star. So, fluorescence intensity is more that means, the number of A minus star is more, fluorescence intensity less means number of A minus star in that solution right is less, H A this fluorescence intensity of this band is more means number of H A star is more and vice versa.

So, if I just simply write these equilibrium this part let me write it.

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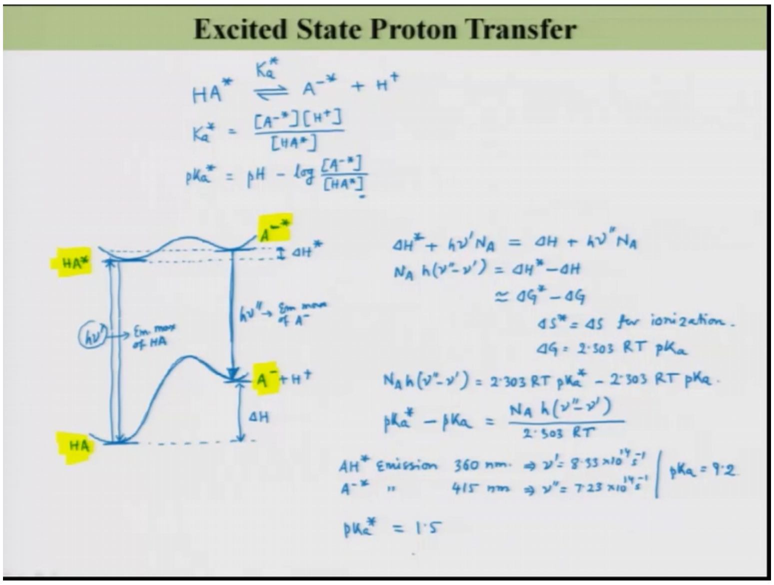

## Excited State Proton Transfer

$$HA^* \overset{K_a^*}{\rightleftharpoons} A^{-*} + H^+$$

$$K_a^* = \frac{[A^{-*}][H^+]}{[HA^*]}$$

$$pK_a^* = pH - \log \frac{[A^{-*}]}{[HA^*]}$$

$$\Delta H^* + h\nu' N_A = \Delta H + h\nu'' N_A$$

$$N_A h(\nu'' - \nu') = \Delta H^* - \Delta H$$

$$\simeq \Delta G^* - \Delta G$$

$\Delta S^* = \Delta S$ for ionization.

$$\Delta G = 2.303\ RT\ pK_a$$

$$N_A h(\nu'' - \nu') = 2.303\ RT\ pK_a^* - 2.303\ RT\ pK_a.$$

$$pK_a^* - pK_a = \frac{N_A h(\nu'' - \nu')}{2.303\ RT}$$

$AH^*$ Emission 360 nm. $\Rightarrow \nu' = 8.33 \times 10^{14} s^{-1}$ | $pK_a = 9.2$

$A^{-*}$ " 415 nm $\Rightarrow \nu'' = 7.23 \times 10^{14} s^{-1}$

$$pK_a^* = 1.5$$

So, here my equation is H A star is converting to A minus star plus H plus right. You see this equation and this equation is very similar equation or this is only in the ground state, but that one is in the excited state. So, I can simply write this equilibrium constant Ka, but as it is in the excited state. So, it is I will write the K a star.

So, I can simply write that K a star is equal to concentration of A minus star, to concentration of H plus divided by concentration of H A star again to tell you that these concentrations are equilibrium concentrations. So, if I take a negative logarithm on each side. So, I will get p K a star equal to p H right here obviously, you know just take a origin concentration, minus log of A minus star divided by H A star.

Now, you can tell that I know this emission intensity as I showed here. So, this has to be related with the concentration. Concentration is more emission velocity more. So, concentration of A minus is less emission velocity is less. So, I can immediately put this this this intensity ratio as the ratio I wrote over here, but there will be problem because fluorescence intensity does depends on the concentration, but it also depends on its own radioactive and non-radioactive pathway right. There is no guaranty that the nature of the radioactive and non-radioactive pathway for H A and A minus their exactly same.

In other words the quantum yield of H A and A minus are exactly same if not I cannot directly compare the intensity with the concentration. So, I cannot write it was much easier for me in case of absorption because earlier I know that absorption spectra, but if you know the quantum yield then probably it is you have to convert this concentration to the by taking into account the appropriate values of quantum yield, for the H A and a minus.

However, we can do another simpler thing to get this value of p K a star right which is being done or followed universally. For example, if I now may draw this this excited state proton transfer by such kind of a diagram let us see this one. So, here is my ground state right and this is your H A, and this is your A minus plus H plus and this is let us say delta H the energy difference between these two. The ground state you see this barrier is very high right. So, naturally the p K value is really high right it is not a very good proton donor.

The excited state is something like this and so, here is my state this is my H A star right this is my H A star and let us see if I have used H nu prime where this H nu prime our nu prime is the maxima of the frequency for this absorption right. So, you can excite the system from here to here and this one this energy difference let us say delta H star right then from here to here this will be our emission, because if this A minus will form then A minus from the excited state will come to the A minus ground state, and then it will take the proton back to found the H A. So, this is my full that diagram of this proton transfer. So, this one is A minus star.

So, here is my H A star this is H A here is A minus star and here is A minus right these are the four thing I have. Now from these diagram I can easily write two things, one is H A no sorry the delta H star plus H nu prime right and that also has to be multiplied by the Avogadro number. So, I can write let me write here delta H star plus H nu prime multiplied by Avogadro number right, that should be equal to if this emission is H nu double prime then Avogadro number H nu double prime that is from energy from here to here plus here to here this is equal to here to here plus here to here.

So, to make it more understandable let me draw this line also right. So, these will be equal to delta H plus H nu double prime into Avogadro number, I can write this. So, now, let me rearrange N A H nu double prime minus nu prime is equal to delta H star minus delta H. Now this delta H can be written as delta g if I consider the change in entropy in both the case are same.

So, this can be written as delta G star minus delta G, under the assumption that delta S star is equal to delta S right for ionization. And this our earlier assumption right is just whether the ionization is taking place in the ground state or in the excited state there are change the delta S the change in entropy it should be similar right it should not be very different. So, ionization right and I know what that delta G is equal to 2.303 R T p K a right. So, here I plugging this thing. So, N AH nu double prime minus nu prime is equal to 2.303 R T p K star because it is in the excited state minus 2.303 R T p K a because it is in the ground state.

So, I can simply write that p K a star minus p K a is equal to Avogadro number prime constant nu double bar minus nu bar. This nu double bar is emission frequency of the A minus and this nu bar is my absorption frequency of this H A right I am exciting over here in this case excitation and emission is same, I am not just considering here H nu bar absorption or excitation is same under this condition. If they are not same then probably you can excite to some other value, but. So, strictly speaking here I have only one level. So, if you excite some other a higher absorption level then this is actually the emission right is actually the emission for the simplicity for the simple model, I assume it to be the exactly same as the absorption and emission, but if you consider stock ship then you better to write this as a emission right.

So, H nu prime is a emission maxima of H A and this is emission maxima of A minus right. So, then this will be equal to the Avogadro number H nu double prime by nu prime by 2.303 R T that is it. So, I if I know the p K a the ground state, then I can readily calculate the p K s star in the excited state. Provided I know what is the emission maxima of this H A, what is the emission maxima of this A minus and these are other things are the constant term. Let say for example, I should give you example the for this two napthol that I showed that A H star emission is at 360 nanometre right and A minus star emission is at 415 nanometre.

So, from here I can calculate nu bar is equal to 8.33 to 10 to the power 14 second inverse is inter convertible and from here nu double prime is equal to 7.23 into 10 to the power 14 second inverse. If I know already that p K a of this molecule is 9.2 in the ground state right. So, p K a is 9.2, then p K a star can be calculated from this equation right and if you calculate you will get is around 1.5 right. So, immediately you will get this value ok.

So, now we should discuss about the rate of this proton transfer. This is that the p K a star right whether in the excited state the proton is more level or not and how to find out that is what I have done now right, but I should tell you what how we will find out the rate of such kind of reaction yes its proto transfer once excited.

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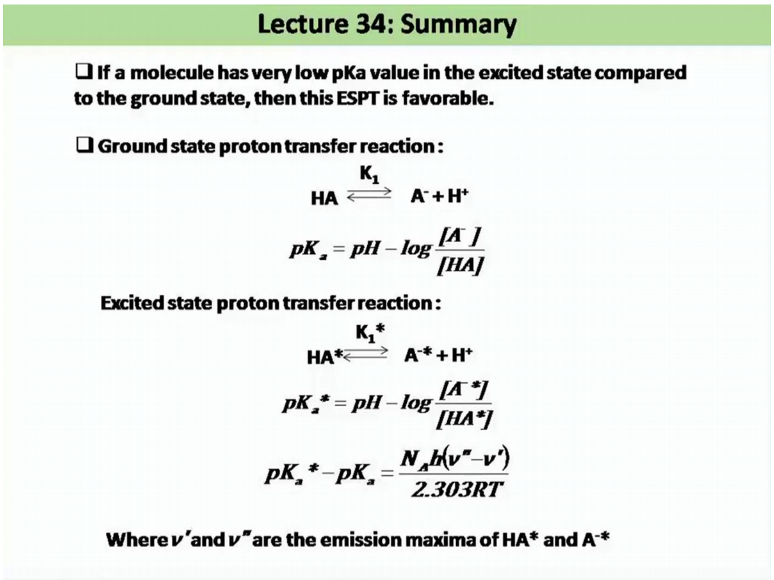

### Lecture 34: Summary

❑ If a molecule has very low pKa value in the excited state compared to the ground state, then this ESPT is favorable.

❑ Ground state proton transfer reaction:

$$HA \overset{K_1}{\rightleftharpoons} A^- + H^+$$

$$pK_a = pH - \log\frac{[A^-]}{[HA]}$$

Excited state proton transfer reaction:

$$HA^* \overset{K_1^*}{\rightleftharpoons} A^{-*} + H^+$$

$$pK_a^* = pH - \log\frac{[A^{-*}]}{[HA^*]}$$

$$pK_a^* - pK_a = \frac{N_A h(\nu'' - \nu')}{2.303RT}$$

Where $\nu'$ and $\nu''$ are the emission maxima of HA* and $A^{-*}$

That means instantaneously you are creating the excited state. So, just of a sudden you are creating a very strong acid and then it will going to donate its proton right, and is it possible to follow this reaction with time or not. That we will going to see in our next class.

Thank you very much.