Circular Dichroism and Mossbauer Spectroscopy for Chemists
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Lecture – 48
Mossbauer Spectroscopy: Applications II

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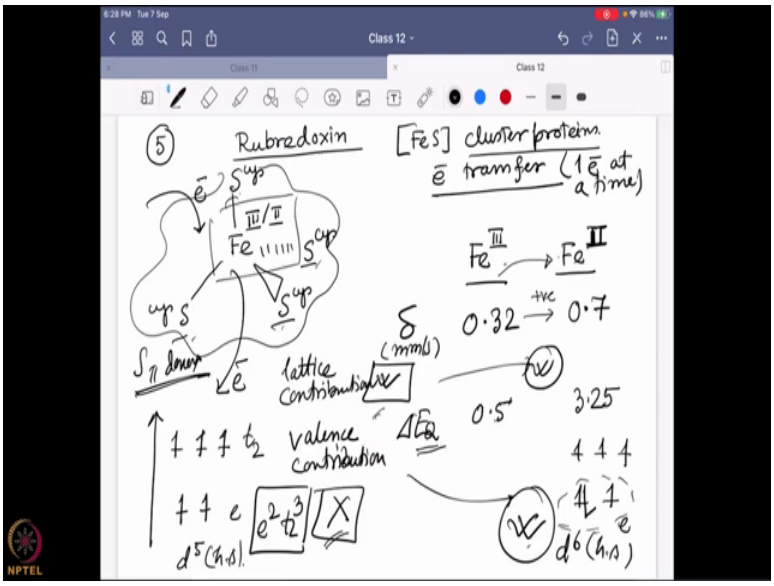

So, if not we are going to move toward the next example, the next example is a biological example of a protein called rubredoxin. Rubredoxin is a protein where there is a one iron centre and it is tetrahedrally ligated with four sulphurs coming from cysteine groups. So that is actually part of a protein where it has four sulphurs coming from cysteine, side chains and this cysteine creates a binding properties for this iron.

And over there the iron can go between two oxidation state, iron Fe^{+3} to Fe^{+2}. It is one of the most basic iron sulphur cluster proteins which is the most, simplest iron sulphur cluster proteins. And it is widely used in biology for electron transfer. And generally, these proteins transfer electron one electron at a time that means this whole system can transfer one electron at a time.

So, some electron comes over here from outside, it gives electron to someone else. And it goes from iron III to II first step, next step it goes to Fe^{+2} to Fe^{+3} back again so, it shutters between Fe^{+3} to Fe^{+2}. So, it is very much important to understand us that what is the oxidation state of this rubredoxin proteins inside the biology? So that we can understand in which direction the electron is moving?

And what are the different experiments or reactions this rubredoxin proteins are participating? So, as we just said this iron can have two different oxidation state, Fe^{+3} the oxidized state, Fe^{+2} the reduced state. And our goal is to find out can we predict how the system will look like for the boss perspective? Now, what is the coordination geometry over here iron? Tetrahedral, previously we are talking about only the octahedral so it is a tetrahedral system.

So, e at the bottom, t_2 at the top, Fe^{+3} is a d^5 system and this is all bound with sulphurs and sulphurs are π donors so, they are all going to be high spin system, it is very rarely you see a tetrahedral geometry a low spin system. So, five electrons so, 1, 2, 3, 4, 5. So now, looking into this system, d^5 system are you going to expect any valence contribution? Because it is at e_2 t_2^3 system so, it is not going to show any valence contribution.

And lattice contribution, it is tetrahedral geometry, tetrahedral geometry is not as symmetric as it is for an octahedral or square panel or spherical. So, lattice contribution will be there for tetrahedral coordination geometry. So, lattice contribution will be present valence contribution will be absent. So, what will be happening? So, let us write down the δ value of Fe^{+3} and compare that with Fe^{+2} system so, Fe^{+3} versus Fe^{+2}.

So, Fe^{+3} has a δ value around 0.32 millimetre per second these are all in the unit of millimetre per second. And ΔE_Q value around 0.5 millimetre per second. Now, if I reduce this system to Fe^{+2}, what do I expect? What I am going to expect? Is the following, is a d^6 high spin system 1, 2, 3, 4, 5, 6. Now, I have a asymmetry key over here so, I am going to see some balance contribution in this case, lattice contribution is anyways present over here.

So, what do you expect if Fe^{+3} to Fe^{+2} ? I am reducing the oxidation state. That means I am increasing the d-electron density that means I am going to increase the shielding effect. The d orbitals is going to hamper the interaction between the 3s orbital and the nucleus. The s electron density will be less inside the nucleus compared to the Fe^{+3} $|\Psi_o^2|$ will be less, it is multiplied with the negative term of $\frac{\delta R}{R}$.

So, it is going to move towards the positive side. And that exactly what happens? It moves to 0.7 millimetre per second. So, you can see the positive shift over here. What happens to ΔE_Q? Previously there is no valence contribution. But only lattice now, both valence and lattice contribution will be active. So, the splitting should be higher and that exactly what we found, it goes to higher value 3.25. So, it clearly shows that totally different system for rubredoxin between Fe^{+3} and Fe^{+2}.

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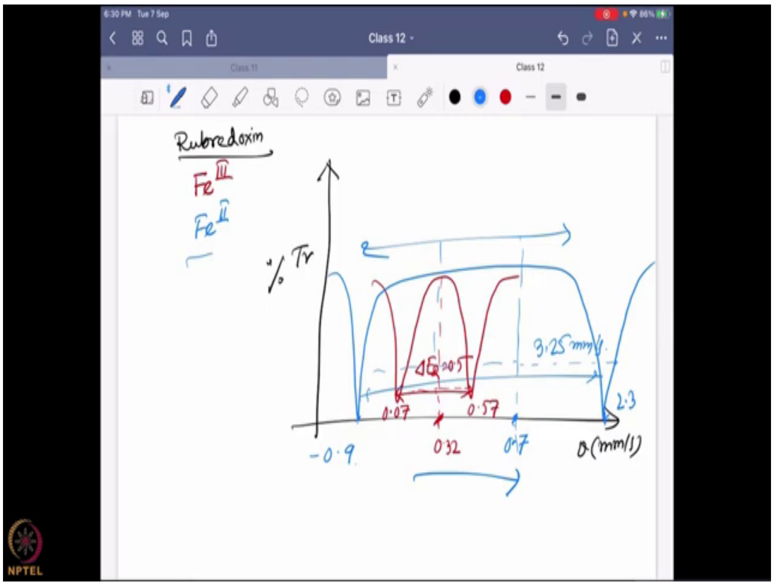

So, if I want to plot that together for rubredoxin, what we are going to get? So, say I am putting iron III in red and iron II in blue, what do you expect? In the same graph if I want to put this percentage of transmittance, it is velocity millimetre per second. So, for iron III what we expect? 0.32 No splitting. So, it is going to be somewhere, the more negative side so, first draw a line this should be 0.32 and what is the splitting? 0.5, so, either side 0.25.

So, I should see the signal like this. So, this will be 0.57 and this is another point where 0.25 so, it will be 0.07. So, you can see the difference is 0.5= ΔE_Q and the average value is 0.32. Now, what happens to iron II, iron II is going to show a value of 0.7 the δ value, splitting is 3.25. So, 0.7 is going to be the average somewhere around here and 3.25 let us say 3.2.

So, 1.6 on either side. So, this is 0.7 so, 1.6 on either side so, what will be happening? 1.6 + 0.7 is how much 2.3? So, 1 will be around 2.3 and the other is be 1.6 on the other side. So, it will be around –0.9 so, it will be far negative, something like this. So, I am not drawn it properly in the scale but if we draw it properly the average will be around 0.7, the difference will be 3.25 millimetre per second so, you can clearly see how difference the signal will be.

Not only the average value will be shifting with respect to the reduction but also the splitting, will also be increasing. So, by that you can easily see what is the oxidation state of the iron in the rubredoxin?

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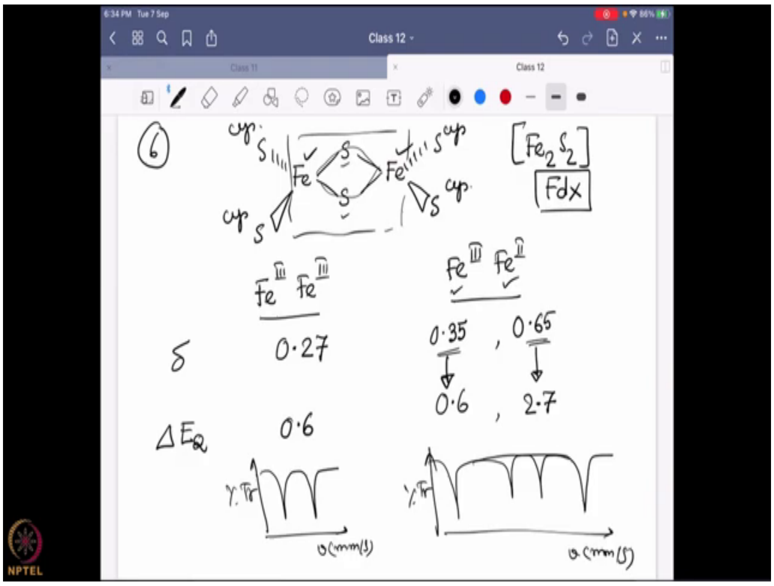

Next, we will take an example another iron sulphur cluster, where there are two irons which are breached by inorganic sulphites, like this kind of diamond geometry. So, it is known as the Fe_2S_2 cluster. And the rest of the iron coordination sides are filled up by protein bound cysteinyl sulphurs. So, this is the core and that is why it is known as Fe_2S_2 cluster or Fe_2S_2 ferredoxin in short from Fdx.

And over there, the two centres over there and we found in the oxidation side it is iron III-iron III, intuitive for this condition it is iron III-iron II. So, it does not go to all the possible oxidation state, this reduction can still transfer one electron at a time. And the oxidation state it shuttles between is +3, +3 in the oxidized state to +3, +2 in the reduced state. Now, the question is, how that delta and ΔE_Q value looks like?

We have already discussed, how the values will differ? It is very much similar to the rubredoxin. So, iron plus III, plus III it shows 0.27 and ΔE_Q value 0.6 and over there both iron shows similar value, nothing here. So, it clearly shows how good the interaction is happening between this iron through this bridging sulphur? So, they are quite well balanced with respect to the electronic distribution.

So that is why we are seeing only one kind of iron. What happens when we reduce? Now, it is one iron III, one iron II so, we see two different signals. Iron III one is coming at 0.35, iron II come around 0.65. So, they are different in the δ values, 0.35 belongs to iron III, 0.65 belongs to iron II. Again, higher oxidation state means to the negative side, lower oxidation state means to the iron side.

ΔE_Q value it remain 0.6 corresponding to the peat at 0.35 and this peat 0.65 it goes to 2.7 very similar to what we have discussed in the earlier system. So, iron II splitted further because it now have both valence and lattice contribution. Whereas in iron III case, it is only the lattice contribution. So that is why you can see the difference. So, what do you expect in the preliminary system?

We expect only one kind of signal in oxidized state, in the reduced state you will see two sets of signals like this. Which clearly distinguish between the oxidation states of the iron in this complex proteins which are very difficult to understand in other experiments. And Mossbauer is very critical because in Mossbauer only iron is active, all the other systems are actually blank or does not contribute to the signal.

So, it is very easy to do that with respect to the Mossbauer spectroscopy. That is how it is going to look like for this [Fe_2 S_2] Fdx.

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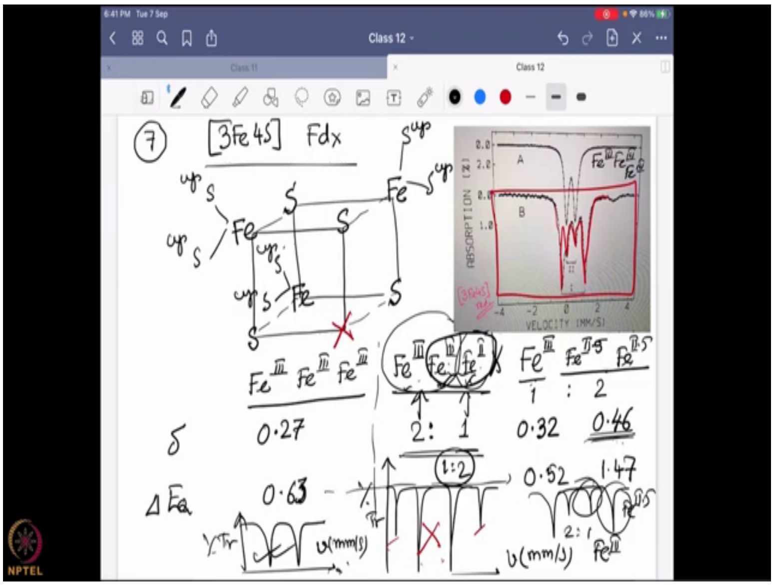

So, one more example we will take and that is the example of [3Fe 4S] or [3Fe 4S] Fdx. What is the structure of [3Fe 4S]Fdx? It looks like a cube and alternatively you have iron and inorganic sulphurs bound over there. And one of them actually does not have an iron over there so, over here iron is actually missing, otherwise it will be a complete [4Fe 4S] cluster.

So, over here it is a [3Fe 4S] cluster, one of the iron is missing. And over there you can see the irons are still missing some of the coordination geometry which are actually filled up again by the cysteinyl sulphurs. And through this cysteinyl sulphur bonding this cluster is covalently connected with a protein and this step also transfer electrons. And what it has been found? That the oxidation state it is iron III, iron III, iron III.

So, what is the δ value expect? Very similar, compared to the previous one 0.27, ΔE_Q value is going to be 0.63. Now, what will be the reduction state? Reduction state is going to be one electron reduction. So, you expect it will be iron III, iron III, iron II. Now, over here, what do we expect? In iron III, iron III, iron III it is very straightforward, you see one signal at a time. Now, what happens? If it goes to iron III, iron III, iron II.

So, you are going to see a signal belongs to iron III and one signal belongs to iron II and what should be the ratio? The ratio should be 2 is to 1, two signal belongs to iron III, one signal belongs to iron II. And iron II is going to be on the positive side and should be further splitted. And iron III should be on the negative side and less splitted. So, the data we are expecting is the following, at iron II will be more splitted and iron III will be less splittted.

But almost twice in size that is how the signal it should look like with respect to the 2 : 1 ratio we are expecting. Or now, let me show you how the signal actually looks like or let me do it over here, how the signal actually looks like? So that is how the iron II, iron III system comes out. The top one is actually the iron III, iron III, iron III, one signal only one splitting. So that is fine, the rest of them should be two signals.

So, we see two signals over here, over here, we see for this one, two signals. But in the opposite way, we expected the middle one should be higher in intensity because that should belong to III and the side ones should be lower in intensity that belongs to iron II. But over here see, it is actually kind of opposite, the outside one is actually higher intensity than the middle one.

So, instead of 2 : 1 it is showing something 1 : 2, how I can explain it? But at the same time I am taking only one electron that can be explained because it is actually not forming iron III, iron III, iron II. It is actually going through a mixed balance system, one iron III and one iron II actually in interacts between them. And it forms iron 2.5, iron 2.5 by interaction between this iron III, iron II. It creates two iron 2.5 systems, the other one remains iron III.

Now, you can imagine a iron III will be remaining in one equivalent iron 2.5 is two equivalent, iron III is higher oxidation state. So, it is going to have a value, δ value to the negative side 2.5 is going to have a value on the positive side 0.46 and also look into the value 0.46. Previously, we found the values around 0.65 or 0.7 for the iron II and for iron III, it is around 0.27 or 0.3 and this is giving me a value in between around 0.45.

So, it is also showing from the value it is not 3 not 2 it is somewhere in between so, it is iron 2.5. And it also affects a similar way to the ΔE_Q it goes to 0.52 and 1.47 and that is why instead of this particular signal we saw that signal in the different way. So, the outside ones are actually higher in number, middle one is actually lower in intensity and we get a 2:1 value like that.

So, this one equivalent is belongs to iron III and this two equivalent belongs to iron 2.5. So, this is actually a very unique example of Mossbauer spectroscopy. So, it not only give you the exact oxidation state it also shows that mixed balancing which is very difficult to understand from other spectroscopic in general. But it cleared and significantly differentiate with respect to the other states and give you an idea, what is actually happening over here?

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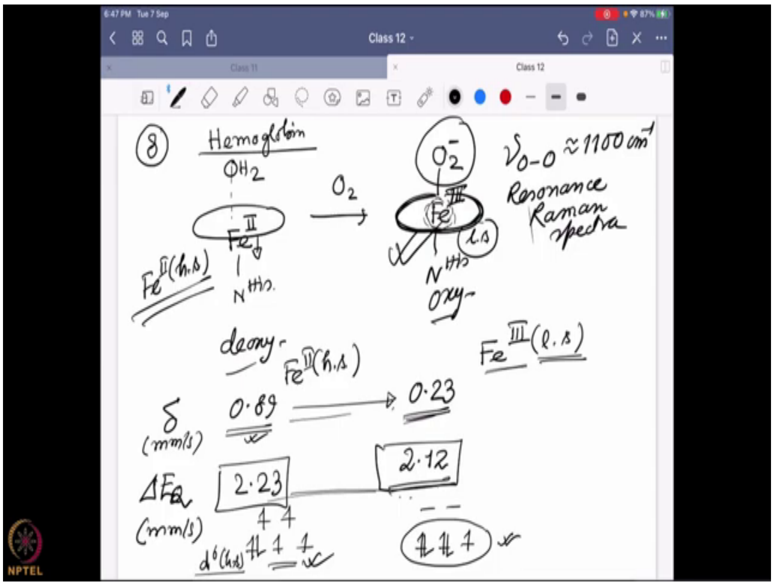

Now, the next example, next example is an example where most of you have gone through, it is about the haemoglobin. We already know in a haemoglobin, there is iron system binds with a porphyrin, generally a Fe^{+2} oxidation state, connected with a histidine like this. Very loosely connected to a water molecule which is we known as a deoxy form before it binds to the oxygen. And once the oxygen comes over here, it binds to that oxygen and it comes to this porphyrin ring plane.

Before that it lies a little bit lower side than the porphyrin ring. And this one it is suggested it is a Fe^{2+} high spin system and there is a lot of controversy, what is the oxidation state? What is the spin state of this oxy? Now, let us take a look if Mossbauer spectroscopy can resolve what is happening here? So, we take the Mossbauer spectroscopy of the deoxy and oxy form. The δ value is found for the deoxy form is 0.89 millimetre per second.

The ΔE_Q it is find around 2.23 millimetre per second. So that is fine it belongs to a iron II, high spin system. So that is but this value is so that is why, it is so much highly positively shifted δ isomer shift value because of the oxidation state also the spin state. In the oxy state once they measured it they found that delta value shifted to very low value 0.23 millimetre per second and ΔE_Q value is 2.12.

So, what is happening? So, first of all what is this huge shift, positive to negative side? So, it shifts this actually belongs to both the change in the oxidation state and the spin state. So, over here the suggestion was that it is actually going from iron II high spin, in the deoxy form to iron III low spin in the oxy form. So, the low spin if it is happening it will shrink down a little bit the overall iron centre and it will fit in the porphyrin.

So, it is actually favour in that. And not only that it is going to iron III state and that is why the change of oxidation state change of spin state and that is why that much of shift because it is removing the d electron density. Now, whether it is a low spin or iron plus III, it is also supported by this ΔE_Q value. You can see ΔE_Q value is not shifted that much, why? Because previously we are saying, it is actually a high spin system, d^6 high spin system.

And that is why we are creating not only lattice contribution but also valence contribution over here. It is going to give you valence contribution and also lattice contribution obviously because of asymmetric coordination. And if it also goes to a low spin system, an iron plus III state then it is also going to contribute valence contribution and also lattice contribution by here. And that is why both of them are going to have very similar ΔE_Q value.

So, the delta value changes isomer shift but the ΔE_Q value remains almost same and it can be explained if it remains in iron III low spin value. So, if it is becoming iron III low spin from iron II low spin that means someone has to take one electron out. Who is taking that electron? So, the only possibility is that this oxygen is taking one electron and becoming a superoxide ion. So, if it forms a superoxide iron or not how we can prove?

So, people have drawn Resonance Raman spectroscopy. And try to find out what is this the stretching frequency of the oxygen-oxygen bond in this system? And they found it is around 1100 cm^{-1}. Which actually falls in the range of superoxide if it is peroxide it is around 850 cm^{-1} if it is oxygen it is 1500. So, it is 1100 which shows it is superoxide.

So, with the help of the Mossbauer and Resonance Raman it has been almost univocally find out that in the haemoglobin, when the oxy form is formed it is actually a iron III low spin system and oxygen is remaining in the superoxide. So, there is an electron exchange between them. However, the electron exchange is quite reversible that is why once the haemoglobin comes to the cell, it interacts with the myoglobin.

The myoglobin binds oxygen and the haemoglobin of the iron readily gets the electron back from the oxygen the superoxide atom and releases oxygen and it goes back to iron II state. So, Mossbauer spectroscopy was a huge help to find out that really you can figure it out what is actually happening in the haemoglobin during the oxygen interaction?