Circular Dichroism and Mossbauer and Spectroscopy for Chemists
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Lecture – 31
Applications of CD Spectroscopy - III

Hello and welcome to this new segment of CD spectroscopy and Mossbauer Spectroscopy for Chemist. My name is Arnab Dutta and I am an associate professor in the department of chemistry at IIT Bombay. So, in the previous few segments we have covered, why do we care about CD spectroscopy? And we find out one of the important aspect of it is the symmetry. And one of the important and significant aspects of symmetry is chirality.

And chirality defines a lot of things not only in biology but also in the chemical industries. And over there we found that the biology actually triggers the molecular recognition by chirality.

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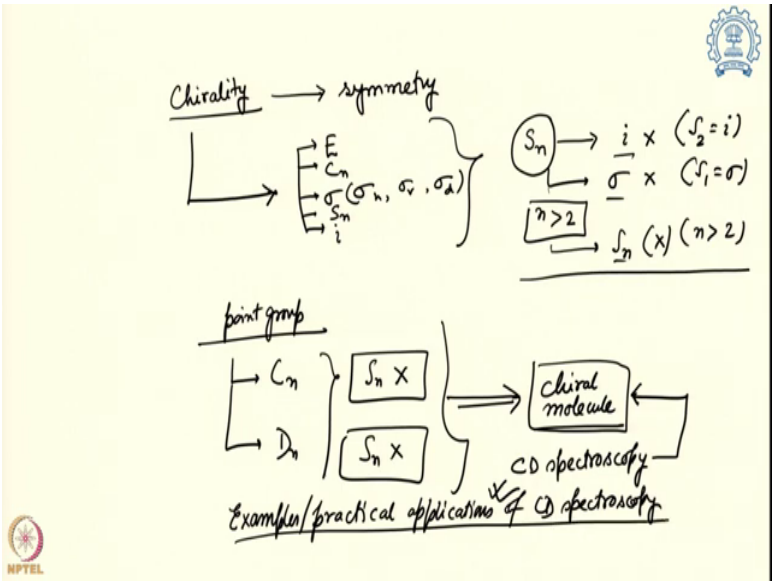

And over there we have figured it out that the chirality is an important aspect of symmetry. And that is why presence of chirality can be described by different symmetry elements that is actually present over here and those are known as five different symmetry elements operation E, operation C_n, operation σ of different kind of σ, σ_h , σ_v and σ_d . We also have improper axis of rotation and centre of symmetry i.

And all those things combined we find that if we do not possess an S_n axis of rotation then we can say our molecule is chiral which we actually define in different ways that we do not have a center of symmetry because S_2 belongs to i or if a molecule does not have any σ plane because it is nothing but S_1 or in higher form of S_n axis where n is greater than 2. So, if you neither have each of those, we can say that the molecule is chiral.

So, any chiral molecule cannot have either of this. So, then we figure it out, if we go to the point group definition and figure it out what is the point group of the molecule? Then you figure it out if my molecule belongs to C_n or D_n point group. This two point group does not contain any S_n symmetry element. So, obviously, these two point group will belong to chiral molecule.

And later on, we figured out, how CD spectroscopy can be used to monitor chirality of a molecule? And over there we look into a circularly polarized light that is actually tilted at a particular angle which actually create the ellipticity and optical rotation altogether. And we get a new segment of signals in CD spectroscopy which is only active if the molecule is chiral and with respect to that we can monitor chiral molecules.

So that is why CD spectroscopy is a unique feature in our hand, a unique tool in our hand by which we can follow the chirality of a molecule. Now, with all those things in our hand now, we would like to have some examples or practical applications of CD spectroscopy. And over here we will take four examples, one after another.

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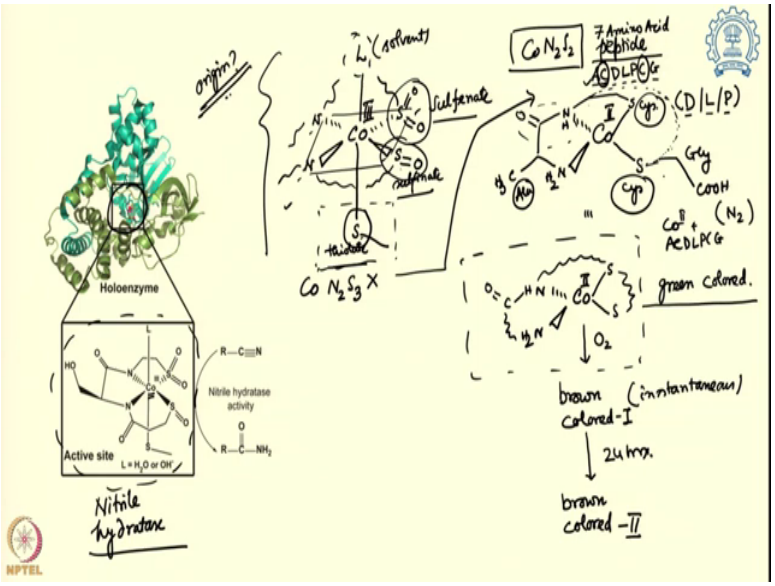

So, let us go to the first example. Over here, I am showing you the structure of an enzyme. The name of the enzyme is nitrile hydratase and this enzyme nitrile hydratase is cobalt based enzyme the active site is shown over here which is blown up over here. And you can see there is a cobalt system bound to that which is actually bound in octahedral geometry. So, let me draw that over here.

So, over here you have a cobalt molecule which is actually, octahedral geometry. The square plane is made out of two nitrogen and two sulfur based system but not any sulfur one of them is SO_2 based molecule and one of them is S=O bond molecule and which is connected. And which are connected to other systems and one of the axial ligand is a thiolate based system thiolate. This is known as sulfinate. This is known as sulfenate.

So, these are the three different ways we can found the sulfur is bind to this molecule and we also have another axial L which is typically a solvent molecule. And those are the different nitrogens and sulfurs are coming from the active site which is coming from the site chains of the proteins and also even from the backbones of amides. So that is I am showing you with this wiggly bond.

So, over here you can clearly see that it is actually forming a nice octahedral geometry. Now, this question comes to our mind why in this molecule I can have three different sulfur oxidation states present which actually coordinates through the cobalt. And what later we found is very much important for the activity of the cobalt and cobalt typically is in cobalt three state.

In this particular geometry cobalt three goes to a low spin state and it is very much stabilized. Now, the question is how this particular unique geometry is formed? So, what is the origin of it? And we know that is actually, biosynthesized and to understand, what is the possible biosynthetic pathway? Researchers have developed a model complex of this. In this model complex they have put all these different groups around it in it's original form except this axial thiolate.

So, you can see over here we can say it is the cobalt bind to two nitrogen, three sulfur and one X which is a variable molecule. Over here, we actually convert that to a CoN_2S_2 geometry, how we did that? So, we start with a cobalt molecule and put it with a simple seven amino

acid ligand, a seven amino acid peptide we have gone through the different nomenclature of the amino acids and with single letter it is A C D L P C G.

So, it is alanine, cysteine, aspartic acid, leucine, proline, cysteine and glycine. And over here, what we found that? This peptide bind the cobalt in a tetrahedral geometry in the beginning. So, although shown over there, it actually is a tetrahedral geometry. So, let me draw in the tetrahedral form. So, how that is coming is the following way? So, in the tetrahedral geometry means this wedge bond is above the plane.

The broken of wedge bond is below the plane and the solid lines on the plane. So, their dihedral planes are perpendicular to each other. Previously over, in this case, they are in the same plane. So now, how it is bound? Let me draw that it is the free amine group of the alanine chain. Then it is the CH_3 group there comes the carbonyl and amide bond. So, this is the amide bond between A C that means alanine and cysteine then comes the cysteine group.

And there comes the sulfur and over here there is a CH_2SH group and then this part come back and creates the other cysteine part. So, this is one of the cysteine. This is one of the cysteine, so, these are these two cysteines and then there is another glycine part which is going to showcase you the free carboxylic acid group. So, this is the alanine part, this is the first cysteine.

Then in this particular chain we have this D L P group that means aspartic acid, leucine and proline and that actually means to help to make this turn and you have the other cysteine and the glycine. So that is how this whole amino acid look like. So, from now on, I am going to make it a little bit simpler for that and which I drawing it like this. So, these are the two sulfurs.

This is the amine and this is the amide group and this wiggly bond means they are connected with this amino acid chain. And when you first started the reaction the cobalt is in +2 oxidation state. So, how the reaction is done we take a Co^{+2} solution put that in this amino acid solution. The cobalt is typically pink in colour in aqueous solution. The ligand itself is colourless when they bind to each other they showcase a very nice green coloured solution.

And the reaction is done under anaerobic condition. So, this reaction when it is done between the reaction between the Co^{II} plus this amino acid it is done under nitrogen condition, no oxygen and it forms is nice green coloured sample. Then when we add oxygen to it at once, it turned brown colour instantaneously. However, we found that this brown colour solution remain brown but there is a slight change in the colour over 24-hours.

So, I am still writing is brown coloured but write brown colour II and this is brown coloured I. So, there are two different brown colour solution but they are slightly different in colour. And we wanted to know what is actually happening over there? What is happening around this cobalt sample? So that we can understand what is actually going on? So, for that first we take the optical spectroscopy and over there we found the following.

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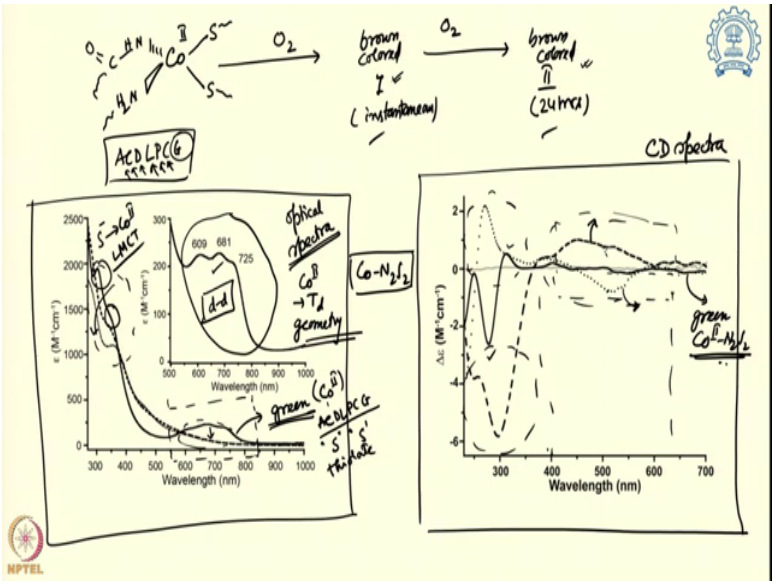

So, this is the optical spectroscopy of those molecule and over here the solid line is the green coloured solution, the initial green colour solution which is Co^{II} and the peptide connected system, where the two cysteines are bound with thiolate groups. How do we know? So, over there you can see it is a green coloured solution, so, obviously it has a particular band in the visible region and which is showcase over here.

This particular three bands coming over here which is showing that the Co^{II} is really in a tetrahedral geometry and at the same time it has this CoN_2S_2 configuration where the thiolates are putting a lot of electron density back to the cobalt. And that is shown up over here this cobalt d-d based transition in tetrahedral geometry and along with that this particular bands we are seeing over here this is the thiolate to Co^{II} to LMCT band.

So now, from the optical spectroscopy, we are quite sure that yes, we have a tetrahedral Co^{II} molecule with N_2 S_2 geometry. Now then when you find the oxygen and find the first brown colour here is the two spectra you can see the dotted line and the dashed line. And you can see over here this particular signal is gone this LMCT bands are gone. So, the d transition bands are gone. And there is some changes over here also in this particular region.

However, the dotted line and the dashed line you can see is very much similar, not too much difference. So, although they are different but there is not much change in the visible region so that way not for change in the colour but they are quite different. So that is what we try to find. And over here, what is the problem we are facing? That the optical spectra cannot differentiate between brown solutions I and brown colour solution II.

And again, brown colour solution I and brown colour solution II the difference is, it is forming instantaneously. And it is a very much slow production after 24-hours and both of them once it is exposed to oxygen. So, we decided to look into this particular system through CD spectroscopy and this is the CD spectra I am showing over here. So, why CD spectroscopy the logic behind that is over here we are using a peptide as a ligand, A C D L P C G.

And over here other than the glycine all other are chiral amino acid. So, obviously they are going to have some chirality. In addition to that it is forming a 2-dimensional structure when it is binding to the cobalt in 3D space. So, obviously that is going to have some chirality because you are making the system out of a bunch of L-amino acids. Now, the question is what it is actually happening there?

And between this brown colour solution I and brown colour solution II are we having any difference over the overall structure. So that we are going to find out from the CD spectroscopy and over here that we are showing so, the solid line is again over here is the green coloured solution that we know it is from $Co^{II}N_2S_2$ geometry. So, you can see not much change over here in the feasible region.

But there are certain bands over here which shows that amino acid is there and binding to it. But very interestingly, you find that although an optical band over here in this region between

500 to 700 is quite band or not that is specific for the optical spectra. This particular region becomes very much active when you are talking about the CD spectra you can see in the visible region it is quite stark change.

So one thing the first one is actually, coming out over here and there is another dotted line coming over here. So, this is the CD spectra represented by brown colour I and brown colour II and you can see clearly they are different. So, what it is showing? That both the complexes are actually, bind to this amino acid still now. Why? Because we are seeing this band in the visible region and in the visible region, the peptide itself does not have any absorbance.

So that cannot show any optically active or chiral band in the visible region. It is happening because cobalt is bound to this optically active system. So, some of the cobalt bands typically the cobalt d-d and cobalt based LMCT bands becomes now optically active because of the presence of this chiral amino acid environment around it. And that is actually showing me the signals and the signals are actually different.

So that shows that although I am using the same amino acid signature the two brown colour solution is showing different spectra and not only that they are actually difference in the magnitude. And also, the phase one is in the positive one is in the negative. So, it shows that a certain change happening in the coordination geometry which is also obvious from the below 300 region which is showing that the amino acid is undergoing a lot of change around it.

So that is what is actually happening over here? So then we try to go forward, what is possibly happening?

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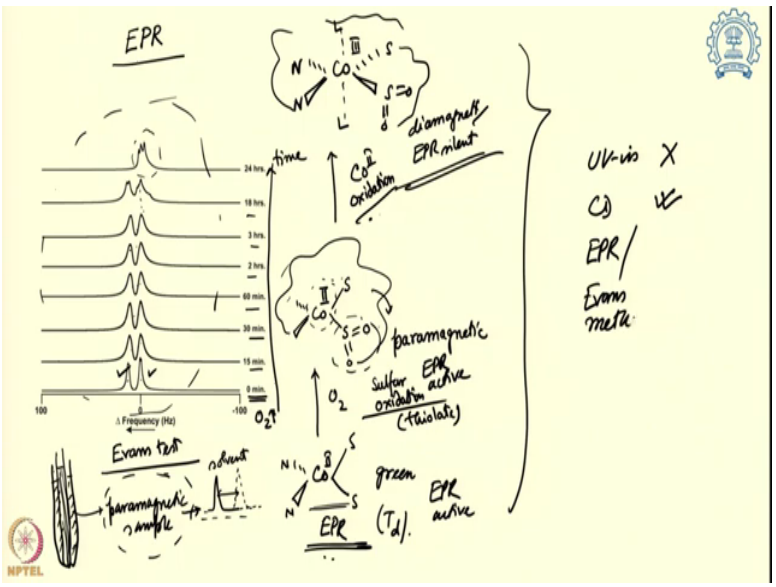

For that we actually, take EPR as our another spectroscopy evidence to find out, what is actually happening? So, over here what we did? We take this $Co^{II}N_2S_2$ system which is obviously having a EPR active system because the state tetrahedral geometry, Co^{II} state which is probably close to a cobalt S = 3/2 system then when it find that oxygen and we are creating the first brown colour solution.

Over there, what we actually did? We did an experiment, called Evans test where we actually put our sample and try to figure it out how many solvent signals we get? So that is actually done by taking an NMR tube and in this NMR tube we actually put concentrically two tubes together, one just having our sample and the other one having a blank solution. So, the same solvent one is with sample, one is without sample.

Now, with the sample, if we have a paramagnetic sample, I am going to expect a well shifted solvent signal because of the presence of the paramagnetic moment. It will create a local signal of magnetic field which will be shifting the solvent signal, whereas the outs inside the solvent has no other paramagnetic sample. So that will not show any signature. So that will come in original position.

So, you should see two different signals and the difference between those two will depend on the amount of paramagnetism and amount of the sample present in the search. So that is what is actually happening over there. In the system, how much paramagnetic sample I have? And how much is the paramagnetic moment I have? So, those two come together you can see,

there are two signals at 0 minute after oxygen is actually come into this green coloured solution and it turns brown.

So, the first thought we have that over here the sulfur is getting actually oxidized first rather than cobalt and that is becoming an SO_2 kind of system which is nothing but S=O system and the other one remain as it is. And the cobalt remains in Co^{+2} because it is getting oxidized the coordination geometry will have some change. The overall orientation of the peptide around it will showcase some change which already been shown in the CD spectroscopy.

So, from the black to this dotted line there is a change because we are facing this change in the amino acid side chain. So, this peptide bond is actually changing it is geometry that is why you are seeing but the cobalt still remain in Co^{II}. So that is why we see two different signals and then we try to follow that up with different time. And then what we see that this two bands now started to merge up and at one point time after 24-hours.

They merge it to one signature which says at this point of time I have a diamagnetic sample. So, this is paramagnetic still now and over here it becomes diamagnetic. How I can have a diamagnetic cobalt if it becomes Co^{III} and Co^{III} to become diamagnetic it has to go to a octahedral geometry which is coming with the ligand. These are the nitrogen and these are the sulfurs we already have.

So, over here also from tetrahedral Co^{II} it is now coming to Co^{III} system which is diamagnetic in nature and that is why I am seeing this change in the evam test. So, they become EPR silent. So, this is EPR active, this is also EPR active but this is EPR silent system and with that thing coming into the picture we say we are over here the first oxidation is on the sulfur that means the thiolate is actually getting oxidized the side chain.

And the next step, the Co^{II} is getting oxidized and becomes Co^{III} and when it is going to Co^{III}, it is changing it is coordination geometry coming to octahedral system and this is again showcased by the change in the CD spectra. So, this dotted line and dash line are different because there is a change between Co^{II} to Co^{III} and also change in the primary coordination geometry which is shown over here.

So, with that we can see UV rays cannot give us any idea but we see some changes CD is giving us idea that there is a change in the coordination geometry which is supported by the EPR and Evans method by which we can follow paramagnetism that is showing that I am undergoing a change of thiolate first and cobalt later. So, next we want to have a better proof of that this is actually, really happening, first is the sulfur then is the cobalt.

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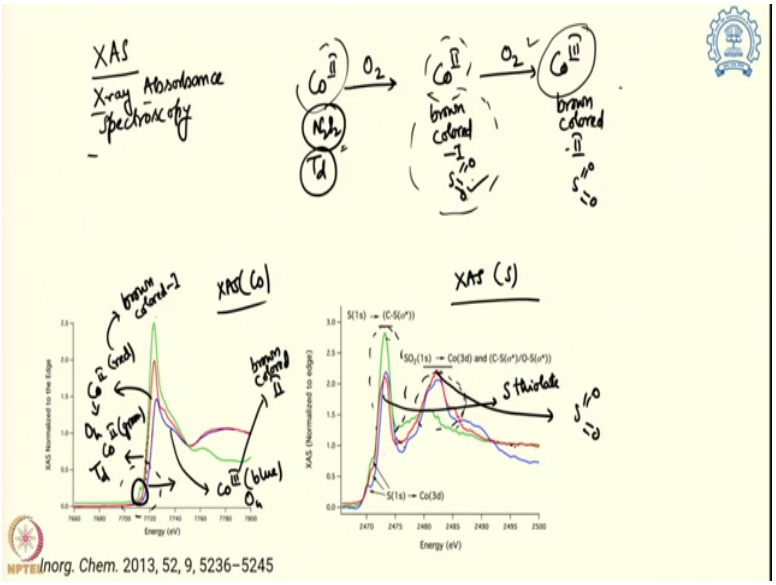

For that we have done another spectroscopy called XAS X-ray absorbance spectroscopy. And over here, you can see this is a band where over in this particular region you look closely. You can see the green coloured system is on the left hand side that shows that this oxidation state of this green colour solution is actually lower, where the blue one is on the higher side. So, the blue one is obviously a Co^{III} the green one obviously a Co^{II}.

Now, look into the red one, almost at the same region as the green. So, the red one is also a Co^{II} system. However, you see there is a small hinge over here on the green one which signifies that your Co^{II} signal or the green one is actually having a non-centro symmetric geometry. That means it does not have a centre of symmetry which is showing that this Co^{II} over here we are seeing in the green signal is actually tetrahedral geometry.

Whereas the red one is Co^{II} but octahedral geometry that is why it is having no signature over here and the blue one is obviously not there, so, it is Co^{III} and octahedral. So, this particular system says yes, my oxidation in the first step does not occur to the cobalt it occurred at the last moment because this red colour is the brown I solution and the blue trace is the brown coloured II solution.

And over here we found this Co^{II} remains Co^{II} and the Co^{III} happens only in the second step. This is the XAS spectra of sulfur, previously it is XAS spectra of cobalt I should have mentioned this is a sulfur. And over here we again see the same thing the green one is the $Co^{II}N_2S_2$ geometry in tetrahedral mode. And over here you can see only one particular band over here which shows that this is actually a cobalt thiolate system.

The red one you can see there are two bands coming up a new band shows up over here that is because one of them is thiolate this is and the other one signifies sulfinate band and this remains same even in the blue region when we actually looking into the brown colour II solutions. So, the green one is the original solution of $Co^{II}N_2S_2$, the red one is again similar to here is the brown colour I solution and the blue one is brown colour II solution.

And over there we found this is already sulfinate over here and it remains as it is over here. So that means again, our hypothesis is correct that the Co^{II} sample, when you put it there, the first oxidation happens on the sulfur. The second oxidation happens on the cobalt. And this is giving us an idea, how, in the real system, the biogenesis of this particular enzyme would happen.

First, they start with all thiolate system and slowly it is reacting with oxygen and forms one of the sulfinate.

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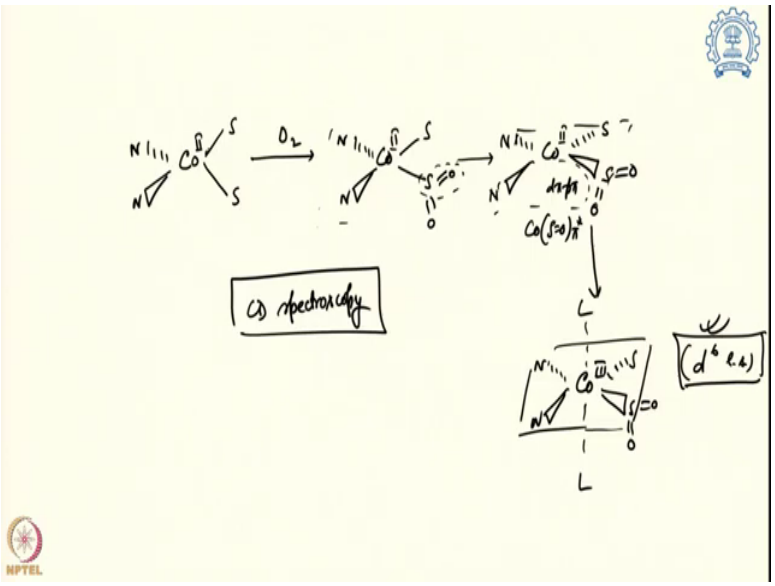

The sulfinate because of this S=O it actually helps the cobalt to move to the octahedral geometry. So, let me just draw that first we start with thiolate when it get oxidized, one of them get oxidized to sulfinate and we found oxidation only with only one sulfur, not both of them get oxidized. And this S-O will like to be come into the same plane as a square planar.

So that the Co^{II} and S=O Π^* bond can do interaction, d-Π p-Π interaction between cobalt and, S=O Π^* which is happening over here. The other one remain as thiolate and this one this interaction ensures that it comes to the square planar geometry and once it comes to the square planar geometry then it is driven to lose one more electron and become Co^{III}.

Because in square planar is geometry it will prefer to go to a octahedral one with this square planar geometry base and over here that will ensure that your cobalt is getting oxidized because at low spin d^6 it will be stabilized. So that is what is happening over here in the cobalt system? And this is actually, first the hint we actually got from the CD spectroscopy so which clearly shows what it is actually happening in the geometry around the peptide?

And that we found over here and that is what we got over here. So, this is a very nice example to showcase how we can use CD spectroscopy to figure it out? The change in the primary coordination geometry via this d-d or LMCT bands which is connected to the central metal. Specifically in the presence of chiral ligand scaffold and if you have a amino acid, peptides, proteins this is creating this chiral ligand and scaffold.

You can also have the other versions of organic ligand. So, if you have an organic ligand or amino acid or peptide bound metal complex. And you try to follow, what is the reaction happening? You can use CD spectroscopy to follow that reaction and what is happening around the scaffold of this metal cluster? So, this is one of the most important examples of use of CD spectroscopy for the application of following metal complex reactivity.

When it is having a chiral environment with respect to the ligand and this chiral ligand is actually inducing chirality with this otherwise, achiral metal based bands. So that is what we have gone through for in the next segment. We will cover a few more examples where we will further go the applications of CD spectroscopy. So, thank you for joining us in this particular segment.