Circular Dichroism and Mossbauer and Spectroscopy for Chemists
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Lecture – 32
Applications of CD Spectroscopy-IV

Welcome to this next segment of this course, CD spectroscopy and Mossbauer Spectroscopy for Chemist. My name is Arnab Dutta and I am an associate professor in department of chemistry at IIT Bombay. So, today we are going to discuss the applications of CD spectroscopy.

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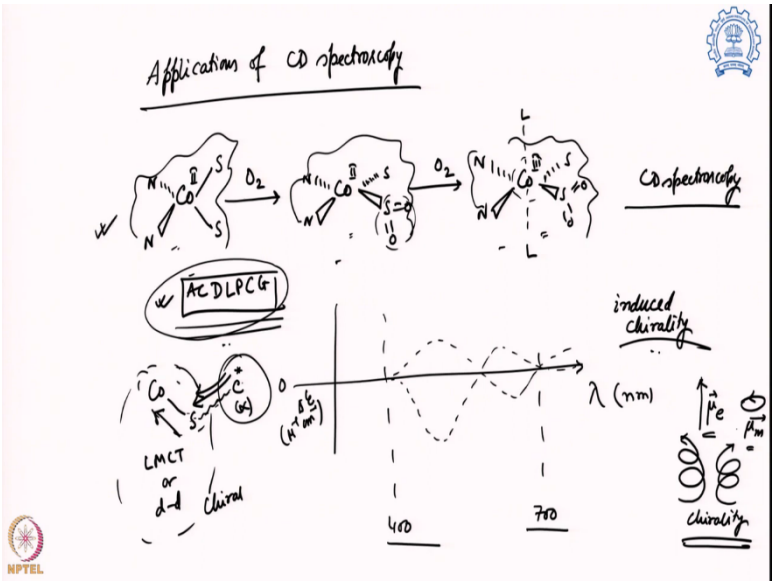

So, in the previous segment we started this discussion of applications of CD spectroscopy. And over there we show, how a change in the cobalt oxidation geometry from Co^{II} in presence of oxygen. It goes to Co^{II} but this sulfur become sulfinized and slowly over time it come to square plane and then it goes to form Co^{III} system, these are the solvent ligands.

And over there, this coordination change we actually, figure it out with CD spectroscopy, where we found different signature of CD spectra for each of these particular complexes. Because this all the different geometries actually give us different configuration of this amino acid ligand which is bound to this cobalt which is a 7 amino acid based ligand, A C D L P C G which actually got to different coordination geometry.

And that is actually, affected by the change in the sulfur oxidation or cobalt oxidation over here and showcase the change in CD spectra. So, one thing I want to mention over here that when you looking into the series spectra, we are mostly looking into the wavelength region of 400 to 600 nm or even say 700 nm in the visible region. And this is the Δε axis this is a 0 line.

So, sometime we saw features like this and sometime we saw features like this. So, there are some changes we are seeing. And over here the changes are happening between 400 to 700 nm where the amino acid itself does not have any particular band unless your system has a optical band, it cannot be optically active or CD active. So, first you have to have a band over there.

And amino acid itself does not have a band but the metal cluster when it binds to this Co-S it might have a particular LMCT band, ligand to metal charge transfer or d-d transition band which is actually coming up. And those are becoming chiral, those particular bands. Now, over here you can see this cobalt system over here is a tetrahedral this is more of a square planar and these two octahedral geometry which are typically not belong to a system which can be chiral.

However, they are still chiral. Why? So, because this chirality is generated by the amino acid it is bound to is the cysteine amino acid is bound to and which is actually of α carbon which is chiral. And that actually, induces some chirality into this LMCT or d-d transition band that is showcased over here. So that is why we call them induced chirality. Because the metal center itself may not be chiral.

But the presence of chiral ligand induces some chirality over there. And why does it get induced? We have gone through that earlier a molecule is chiral and it can show optical activity that means signature in the CD spectroscopy band when the transition is not only active electrically or dipole moment wise also magnetically or rotation wise. So that means both of them has to be active.

So, it is a dipole moment wise and also it has to do with the rotation wise. So, I should write μ_e and μ_m and both of them are active at same time. Then you see this helical system which actually induces the chirality. So, this is actually happening mostly on the α carbon but some

of it also transferred to this cobalt band through this bonding happening between this cobalt ligand interaction.

So that actually induces some part of this electrical and magnetic moment that means this helical moment to come towards the cobalt which actually makes this band optically active. And that is what we have shown with this cobalt based sample example.

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Let us look at another one example where we are here, showing you a cobalt sample with a salen ligand. What is the salen ligand? This is salicylaldehyde a very common aldehyde. This salicylaldehyde reacts very easily with an amine. And this CHO group becomes an imine and from this particular ligand which is known as the salen ligand. And if I introduce cobalt over there in half equivalent amount it creates this particular complex Co-salen complex.

And over here, the Co-salen when I prepared I prepared this complex with different kind of amines which is actually, nothing but benzylamine, phenylalanine, tyramine or tyrosine. This has actually an amine group present over here at the end of this wiggly bond. And over here you can see the tyrosine and phenylalanine are amino acid. So, I want to see if this chiral amino acid can induce some chirality to this cobalt band which is originally not chiral at all.

But can it have some individuality and we can showcase that with the reactivity that we are seeing. So, to understand that we are going to do that but the first question is why do I require to understand this Co-salen complex? That is because this Co-salen complexes that I have

shown over here is actually, I am drawing this salen bond like this in a cartoon, this is a salen one and this complex is quite robust quite stable.

And that is why we wanted to see if this catalyst or this complex is robust, complex can do some reaction with proton and produce hydrogen or not. And if it is doing that if the presence of amino acids in the periphery can help with some proton binding or not, this is known as the proton relay so that is our goal with making this complex. So, let us take a look, what are the reactions we have done?

So, first, take the salicylaldehyde react with these different amines some of them are actually, derived from the amino acids, react with that produce the salen ligand produce the cobalt complex and then we did electrochemistry.

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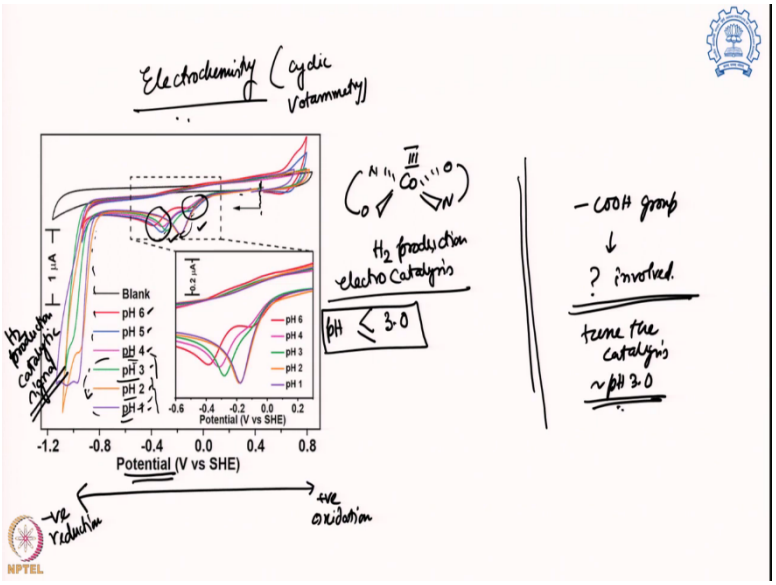

So, what is electrochemistry? This is electrochemistry known as cyclic voltammetry, where in this particular axis, we change the potential applied potential on that particular catalyst and further we go to the negative side, this is the reduction side. Further, we move to the positive side that is the oxidation side. So, you can see we put that complex at different pH condition and then try to move the potential from this particular position towards negative region and then coming back.

And over there we see particular signature over there this is the material that the cobalt material we have Co-salen complex. That is actually, showing some signature peaks over here which is believed to be the change in the cobalt state. So, it is behaved that it is $Co^{III/II}$

and $Co^{II/I}$ change possibly. And over here you can see the both the changes are actually combining at a condition especially at the lower pH region.

So, before that all of them has two signature, so that means two different signature that means the cobalt is actually, having a little bit different symmetry. And then over here we see a huge signal coming out over here that is the catalytic signal which signifies that now you are seeing a hydrogen production. And over here you can see this signature started coming out only after pH 3 not before that upto pH 4, there is not much signature but after that only this catalysis happens.

So that means it is active for hydrogen production below pH 3. So, I should say hydrogen production, catalysis in this electrochemical setup. So, we write it electrocatalysis only less than 3 of pH only then it will trigger so, you have to come below pH 3. The question is why we have to go to below pH 3 to do that? So that means something is there which is actually, getting changed around pH 3.

So, when you look into this catalyst structure one more time over here, we see there is only one particular group which can indulge this when we have this tyrosine and phenylalanine that is having this carboxylic acid group. And that has a pK_a around 3 to 4 and that possibly has done something to do with the catalysis. So, this carboxylic acid group is it involved in some way.

So that it can control or tune the catalysis around pH 3. So that is the one of the hypothesis we have. Now, we need a proof for that.

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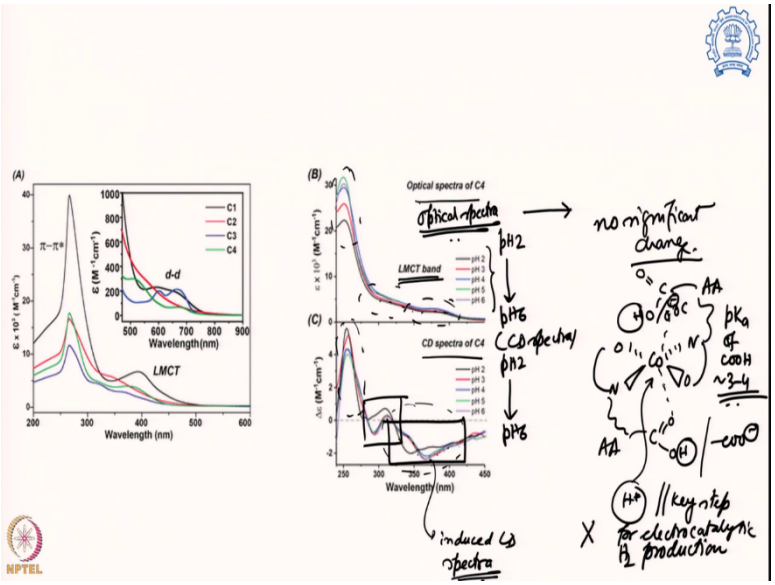

To prove it, we again go back to the optical spectroscopy. So, these are the optical spectroscopy of different complexes, where we have the tyrosine, benzylamine, tyramine and tyrosine. And over here this is the tyrosine complex we take the optical spectral different pH from pH 2 to pH 6. And you can see there are some bands this is the Π-Π* band coming from this ligand based system, there is two hump one over there one over there.

And those are coming from the LMCT band. Now, over here when you go to pH 2 to 6, the optical spectra, does not show any significant change. However, when we do the same experiment with the same solution at the same condition with CD spectroscopy and we move from pH 2 to pH 6. Look carefully what we found? We have some signature bands one is over here and 250 nm this is actually, coming from the ligand itself.

However, there are some signature over here and 350 to 400 region which is actually, the induced CD spectra which is actually coming because of the amino acids is pushing some chirality towards the cobalt center which is itself is chiral. But some of it is bands become optically active and it showcase it is bands in the CD spectroscopy. And over here very carefully, see as we go beyond pH 3.

There is a clear shift in the bands over here, particularly in this region and this region which shows that some changes is happening around the band over here which is actually triggered by the change in the system around it. And when you are drawing the system around it, we have this cobalt, we have this salen ligand and over there through the salen ligand we have

this amino acids bound to it phenylalanine or tyrosine which also have a carboxylic acid group.

And this carboxylic acid group can lose one proton and becomes carboxylate and once it forms carboxylate it can directly bound with the metal from the axial position. And if that is actually happening then it will stop the catalysis. And where does this carboxylic acid to carboxylate interaction happens that happens around the pK_a of COOH which is around the region of 3 to 4. So, does that is actually triggering the change why the CD spectra is changing?

And that is if it is true if the carboxylate is present in deprotonate form, it will bind to this cobalt and it will not allow any proton to come and bind to this cobalt center which is an key step for electrocatalytic hydrogen production. And that is not going to happen and hydrogen production will be stopped if carboxylate is there and binding to this signature.

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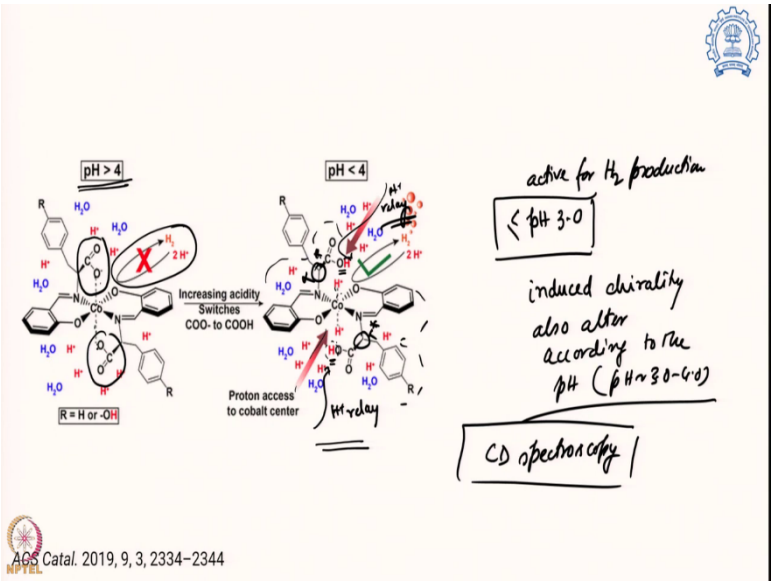

And that is probably actually happening so, beyond pH 4 that means, if where pH is beyond 4, this is carboxylate form and it is binding directly to the cobalt and it is stopping the protons to come and bind to it and no catalysis is happening. But if we go below pH 4 this is getting protonated. So, it leaves the cobalt center so that the proton can come and bind at the same time, the carboxylate OH group can help to relay the proton which will help to increase the catalysis rate.

And that is why this kind of Co-salen complexes are active for hydrogen production only at below pH 3 or so. So that is why the catalyst is happening and we know that this is actually, happening the carboxylate binding to this is changing and this is the α carbon which is the chiral carbon and their interaction with the cobalt will change depending whether the carboxylate is bound or not.

So, the induced chirality will also modify or also alter according to the pH of the solution, especially around 3 or 4 and that is also we found in the CD spectra that over there. This change is happening around that region which showcase, yes, this kind of carboxylate-carboxylic acid change is happening. The cobalt coordination geometry is changing the induced chirality is changing and giving us an idea like what is actually, happening over here.

And that is what happens for this particular complex and over here again, CD spectroscopy give us an insight, what is probably happening around the complex? When we have a chiral, the active ligand bound to this metal so that gives us an idea whether it can be electrochemically active or not.

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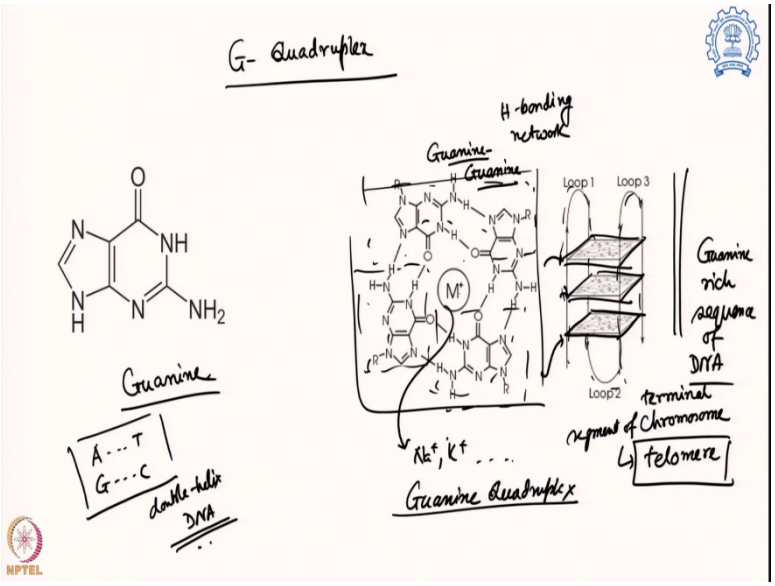

The next application, we will go with G quadruplex. So, this is the structure of guanine one of the basic nuclear base that can be found in the biology which forms this A T G C, adenine, thyronine, guanine and cytosine among them one is one of them and it forms very nicely interaction with cytosine. So, we know A T and G C they form very nice hydrogen bonding which is actually the template interaction for formation of the double helix DNA.

However, we also found that this kind of guanine can interact with another guanine. So, it can form a guanine-guanine interaction and especially, it form guanine-guanine interaction through hydrogen bonding network. And that typically form this kind of tetrad kind of structure where four guanine group is coordinated to each other and in the middle there is a presence of a metal and generally Na, K, Ca.

They actually help to stabilize this kind of interaction between four guanine which is known as guanine quadruplex that is four guanine together and over there we are showing you, this is the four guanine, how it looks like in a plane? So, this is the structure it is shown over here and you can have multiple of them which is connected and they kind of create this kind of loop and this is mostly happen when you have a guanine rich sequence of DNA or RNA.

And over here it is pretty much mostly found in the tail end of the chromosome on the terminal segment which is also known as telomere and this particular signature is found over there. Now, what is the significance of that? Scientists are still working on that. What is the significance of this guanine quadruplex? One thing is for sure they actually stabilize the telomeres segment.

And the telomere segment is very important because that is the part which is actually, affected by the surrounding environment most. And over here, the presence of this telomere it is going to interact with this environment and can be eaten up. But if you have this kind of quadruplex G quadruplex formation, it stabilizes it and stabilizes the telomere portion and also the chromosome of the system.

So that is why having a guanidine environment is very much important but we need a system. We need a spectroscopy by which I can know whether my system has a G quadruplex or guanidine quadruplex there or not.

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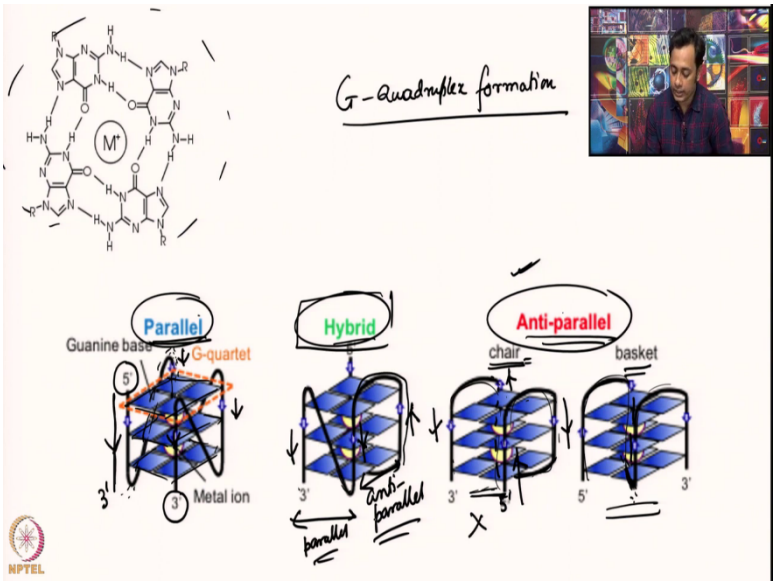

For that we first try to understand how many different kind of signature is possible. So, there are three different signatures possible, parallel, hybrid and anti-parallel. So, what is parallel and anti-parallel? So, first when you have this kind of G quadruplex formation you can see, this is the background of the nucleic acid coming and then the guanidines are coming together and forming this very nice planar system stabilized by some metals in most of the cases.

And forming the sheet of G quadruplex, however, we want to know, what is the sequence? What is the order of the sequence when they are going? So, we have two ends are written by 5' and 3'. So, over there look into that how the 5' and 3' is connected. It is going bottom once and then this segment again and then is coming down again. So, all the time 5' and 3', it is actually going down, going down, going down.

So that means, all the different portion the guanines are coming all of them are seeing the DNA backbone it is 5' to 3'in the same direction, so that is which is known as the parallel. If it is in the opposite direction, for example, take is over here, so, this is the 5' to 3'at this end, whereas over here 5' to 3'in the other end, this is also other end, this is on the other end.

So, you can see the next to each other they are actually, opposite in nature. In one end, it is going bottomers, 5' to 3' other end is going upwards 5' to 3' so that is known as the anti-parallel. And you can see the difference between parallel, anti-parallel. You have to have a hinge to ensure that the backbone bends and have the similar parallel signature.

Whereas in the anti-parallel you do not have to change anything it just flow with the flow. And over here the anti-parallel can have two different signature, one is called the basket and the chair. In the chair from what happens? This backbone over here you can see it actually, goes over there and connect. So, it is not covering the base of the system, whereas over here is going over there and then it is connecting coming and coming through the bottom.

So, the bottom part is actually covered by this DNA backbone. So that is why it is called the basket there is a basement over there of this nuclear basis. Whereas over here there is no basement, so, it is called the chair **(()) (23:53)** the lower part is actually empty. So, this is anti-parallel and over here you can see there is no hinge at all it just flow on it is own. So that is why one side is 5' other side is 3' anti-parallel.

Then there is a possibility of hybrid system where you have both of them one side you can see it is coming at the same so, this is up, this is down so, this is the anti-parallel portion over here. And then there is a hinge, it is goes bottom again, so, this segment is parallel and this is anti-parallel, so, you can have a mixture of that and that is known as the hybrid. So, when you have a G quadruplex, we not only want to know like is there is present or not.

But what is the signature? And how I can differentiate between parallel, anti-parallel and hybrid by CD spectroscopy?

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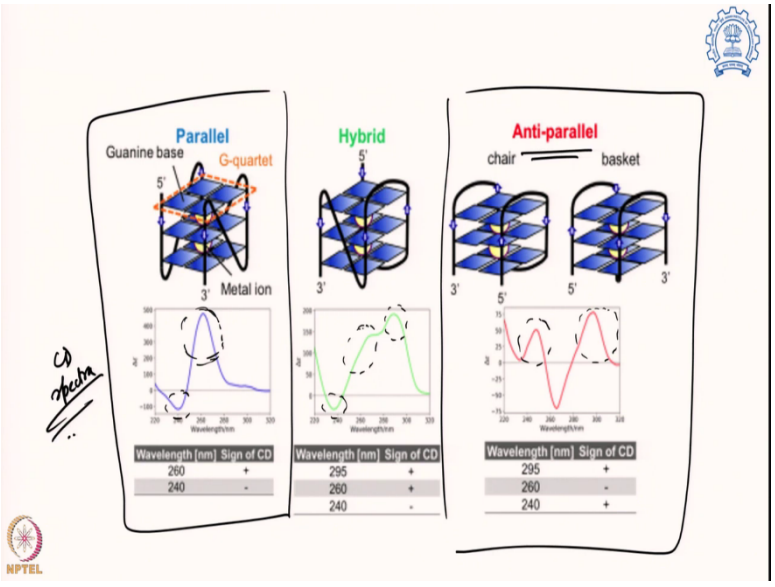

So, this is the CD spectra of all these systems, this is the parallel one and you can see a very strong signature around 260 nm on the positive side, a negative one at 240 nm and only one

band coming over here. And the anti-parallel one at 240 nm, you have a positive signature and there is another signature around 290 nm over here. So, it is, it will be shifted for the anti-parallel one.

So, what is happening in the hybrid? You are going to see a mixture of it. You are going to see some bands are actually in the negative region and a little bit band over here, 260 nm which is showing they have a parallel component. And then there is this band over here around 290 nm which showcase that I actually having a anti-parallel component.

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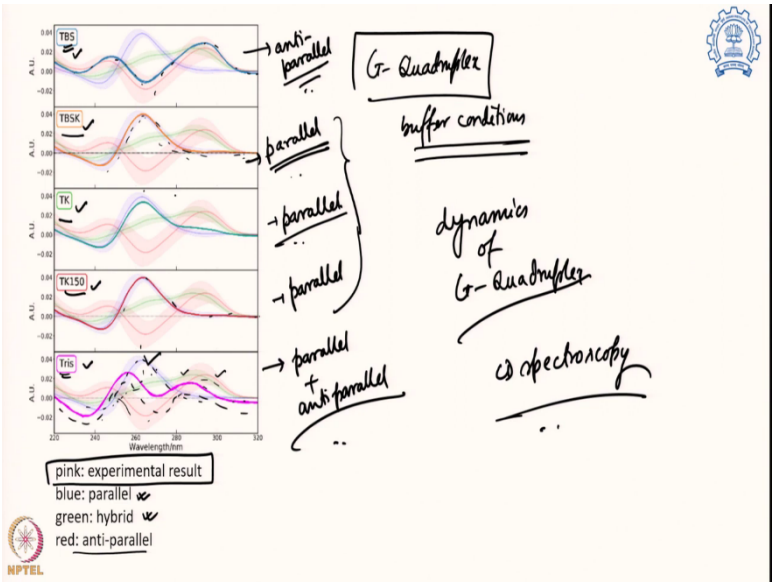

And then we can actually look into the different signature of a G quadruplex and different conditions. So, these are all different conditions and these are the different buffer conditions and over here you can see each of them has four signature, one is the pink colour sample this one. This is the original signature, what I am getting blue one if I am going to get parallel signature, so only one positive band around 260 and negative band around 240 for all of them.

And then hybrid system is given by the green one and the anti-parallel is given by the red one. And then we put the same G quadruplex sample at different buffer condition and figure it out what is actually happening? So, over here in the TBS buffer, what I am seeing? This is my graph over here and you can see it is very much similar to matching with the red one that means anti-parallel.

So, at this condition it is forming an anti-parallel symmetry over here now this particular signature. What we are seeing is matching with the parallel one? So, TBSK system, it become a parallel system. It remains parallel even in this TK geometry so, this parallel G quadruplex is found in both of them. Then the TK150 again similar it is parallel. So, all of them is remaining parallel in TBSK, TK and TK150.

Then you put the tris geometry then you start seeing some change it is not totally parallel, not totally anti-parallel, not even totally hybrid, so, it is somewhere in between. So, you can see it is slowly trying to come from a totally parallel to anti-parallel system it is somewhere in between. So, it is a mixture of parallel plus anti-parallel. So, for there you can follow the dynamics of G quadruplex very nicely with CD spectroscopy.

And that we have done over here and we can easily follow it up. What is actually happening over here? Whether, it is a parallel anti-parallel or a mixture of both or even a hybrid system. So, with that we can say that the CD spectroscopy very can be very important for us where we can follow, what is actually happening to the G quadruplex structure? In the presence of different amounts and different nature of the buffer solution and that is found over here.

So, over here today we will be concluding this section of the CD applications where we have gone through how we can use CD spectroscopy to find out whether, a metal complex is bound to a chiral ligand or not and what is the changes happening during the catalysis or during the different molecular reactivity? And not only that we can also follow up the changes happening in the DNA structure, especially G quadruple structure.

When we actually expose that to different kinds of buffer and what kind of geometry it is forming? So that is why CD spectroscopy is a very powerful tool to understand, what is happening in the molecular level? How much minimal changes are happening? And follow the chiral signature with this particular spectroscopy. So, with that we would like to stop it over here. Thank you. Thank you very much.