Inorganic Chemistry of Life Principles & Properties
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Lecture – 13
Techniques used inorganic chemistry life- continuation

Welcome you back to the next to the course on Inorganic Chemistry of Life. In the previous class we have looked at aspects related to spectral methods such as method on mass spectrometry method, and fluorescence spectroscopy and we have been looking at the NMR spectroscopy. I said I will show some examples to study both the dynamics of this as well as the paramagnetic relaxation things to. Yes.

So, what we looked at in the previous case was that the spectrum when you have a diamagnetic that means no paramagnetic species that will come the proton NMR spectra will come in 0 to 10, 0 to 15 ppm.

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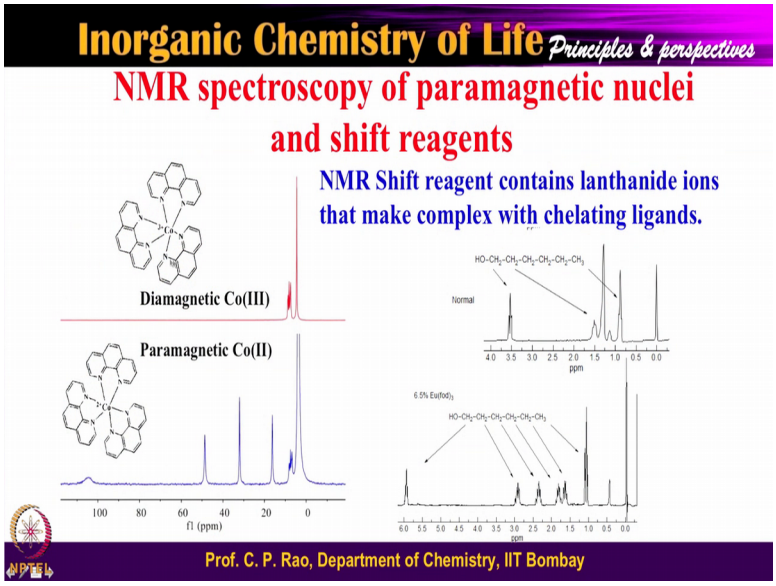

Once you have a paramagnetic central that they will shift to any extent depending upon the contact shift. So, these are referred as the contact shifts. One example showed on the left side is that a cobalt 3 plus which is a d 6 system and this d 6 system having all electrons being paired becomes diamagnetic.

And it comes as if like if you take a just phenanthroline moiety NMR or you take its cobalt 3 complex it will be like and the other hand instead of the cobalt 3 plus you take just cobalt 2 plus all that you have is just 1 electron difference.

So, 1 electron now it is a d 7 system, and the d 7 system resulting in a paramagnetic centre the cobalt centre cobalt 2 plus center. So, the coordination is the same kind of thing, but that will shift the proton resonances to even 100 ppm. So, going from very small region of this within 10 ppm to about 100, 110 ppm as you can see all of these. This kind of thing is a boon in disguise because the paramagnetic contact will shift these ones to a greater extent, ok.

On the right side same thing as shown example of the hexanol, and hexanol has got all these CH 2 groups, the 5 CH 2 group, 1 CH 3 and 1 OH. The OH is seen the CH 3 seen, but all the CH 2 have come within the small region. But now if you had some paramagnetic reagent like you some kind of any Europium, Europium species that europium species will bind to this and to different extents at the of course, the at the alcohol positions and that will bring a contact shift and the methylene, first methylene, second methylene, third methylene etcetera. The maximum will be in the first methylene less will be in the second methylene, third methylene, etcetera etcetera you can see this kind of a contact shift will resolve all the CH 2 groups that are buried under this particular envelope to this kind of thing.

So, its is it not a great boon to have a paramagnetism while the organic chemistry the paramagnetic we call as an impurity, and for inorganic chemist by inorganic chemist biological inorganic chemist that impurity what they think is actually a boon in this.

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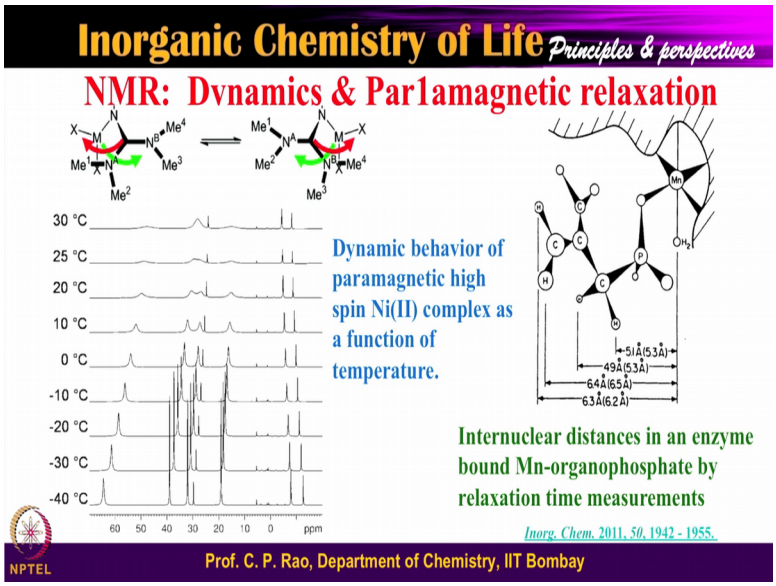

So, in metalloproteins metalloenzymes you have a lot of transition metal ions in this, many of those transition metal ions could be paramagnetic and then they when they bind to the protein one can study the their shifts too.

It is not only that you can also study some kind of a dynamics. So, dynamics is very important, ok. So, the dynamics is very important because the proteins have always have various variations in their conformation and dynamics when the substrate binds, when the substrate is converted to product and the product is released all of these steps the protein does not sit idle or sit stationary will be kind of changes. So, such changes can also be followed.

So, is a small example is shown over there where this particular ligand which is binding through this nitrogens can shift to the other nitrogen this particular nitrogen is here. So, on the right side you have that particular. So, this is a kind of a binding changes in that, so this kind of a species moving into this kind of species. So, these are in dynamic equilibrium and this dynamic equilibrium is controlled by the temperature, ok.

Is the lowering the temperature the dynamics control will be more control not so much of change, and if you allow it to a room temperature or higher temperature then this exchange will be very fast you can also call this is an exchange between one kind of species to another kind of species. The species present on the left side to that species

present in the right side. What is the difference? The difference is bound centers to the metal that itself is bound.

Now, you can see there is a host spectral here minus 40, minus 30, minus 20, minus 10, 0 etcetera, so that means, you are going from down to up you are increasing the temperature from minus 30 to almost room temperature a little greater than the room temperature, ok. So, therefore, here you have more or less the arrested kind of a thing this exchange less and exchange is exchange arrest is less so that means, freedom is more much more freedom, much more freedom, then they will be changing very fast.

So, exchanging slow to exchange in fast; so, from these you can this these peaks which are several here, and they may come closer and they become border and they merge and that what is called the coalescence behaviour. So, from this coalescence behaviour of these protons shifts you can measure the barrier that is present and going from the species on the left side to the species on the right side.

So, there is always an energy barrier that is present and this energy barrier can be crossover by increasing the temperature. So, lower the temperature the species can be seen separately, the increase the temperature this species interconversion is fast and you cannot resolve that. So, that means, dynamics of the protein can be very well studied by NMR spectroscopy.

In the previous slide we are talked about the paramagnetism contact shifts. So, therefore, you can study in here you have dynamics. So, the paramagnetism not only shifts the contacts also paramagnetic relaxation time 0 also. And you know the NMR spectra governed by two types of relaxations ok; So, NMR relaxation, longitudinal and transverse.

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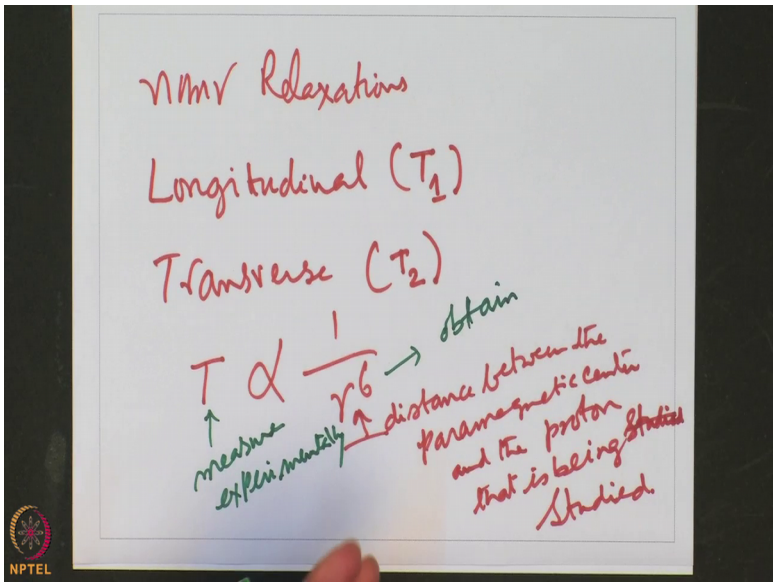

So, I will not go into details these is called T 1, is called T 2. So, these are again influenced by the protein conformation and the dynamics too ok, and the other thing is that they influence different centres or the if it is a proton spectra different centres or the proton based on their distance, ok. So, these relaxation times the T is proportional to 1 by r to the power of 6.

So, r is the distance between the paramagnetic centre and the proton that you are looking at that is being studied, ok. So, that is being studied ok. So, therefore, this phenomena can also be explored and exploited and that is what is example is showing here. This is a framework is a protein framework and there is a manganese ion and this magnesium is bound to protein on one side and the other side is this bound to this particular phosphate moiety having this common (Refer Time: 09:08).

You see this is a paramagnetic centre and if you see this proton is by this much distance, and this proton is by this much distance, and this proton is by this much distance and this proton is by this much distance. So, by measuring the relaxation times you can get the distance.

As I told you the relaxation times and the distances are related by the T is equal to T is proportional to 1 by r power 6. So, this you can measure experimentally at this you can obtain, ok. So, therefore, in the if you measure the relaxation time, so this protein in presence of this manganese and this one then you can get how this is oriented ok, from

the distances you can fit into your model and that minimize the model and that model will give you the where it is binding how it is binding with respect to the proteins.

Similarly, you can also study some protons of the protein 2 in terms of the relaxation times. So, therefore, the relaxation times will give distance and that will fix a protein conformation. So, you can study the protein conformation also indirectly here by using the relaxation measurements the of these that particular proton under the influence of the paramagnetic ion.

So, paramagnetic ion proton, this proton is under the observation. So, one proton is shown over there, one proton is shown over there, another proton is shown over there, another proton is shown over there, you can get their distances.

Using these distances you can uniquely build the whole structure so that means, using the paramagnetic NMR, contact shifted NMR, paramagnetic relaxations, dynamic NMR. You can study almost all kinds of phenomena between the protein and the substrate binding, the protein a the when the substrate is converted in the product all these kinds of dynamics that is happening can be studied in that two.

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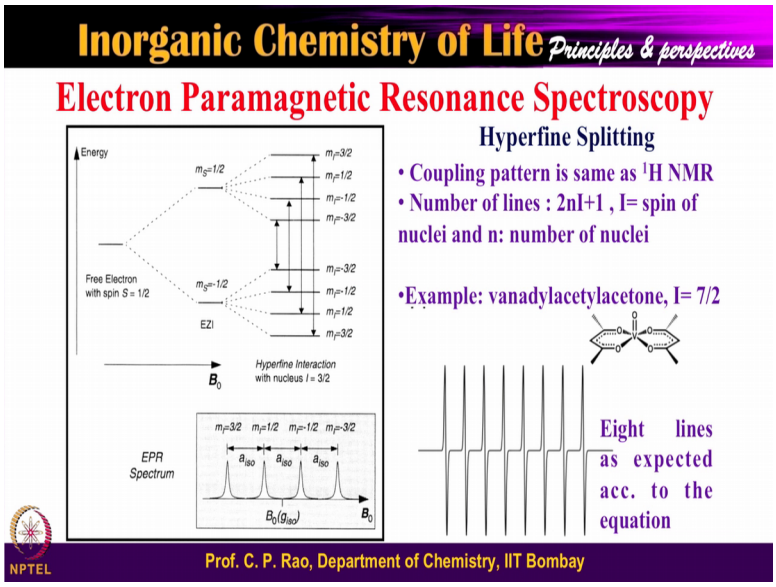

Now, let us slightly switch the gears to another technique. So, right now what we are saying nuclear magnetic resonance where the nuclear spin is changed. Now, instead, instead of the nucleus suppose you take electron. So, electron is also having the spin of

the positive spin of the negative just like the nuclear spin plus half minus half you are also have electron having a plus half and minus half.

So, therefore, we can also look at the resonance by the or transition by the electron spin going from one type of spin to the other is not only in proton ok. So,. So, in case of the electron you can see that the in the absence of any magnetic field you have the same energy and when you apply the magnetic field these two break up, ok.

And now the energy gap between this state and this particular state of the spin is quite large as compared to that the quite different as compared to that of the proton because in the proton case there is a mass involved the electron case the mass is not involved or mass is very small. As you know the mass is almost 1800 to 2000 smaller in case of electron as compared to the proton.

So, therefore, since the mass term comes into picture is breaks will be different. So, therefore, these are found these transactions are found not in the megahertz region, but they are found in the gigahertz region. So, therefore, in NMR, in the NMR spectra found in a megahertz, this is mega and in a EPR these are found in gigahertz ok. So, this is giga, gigahertz, so gigahertz megahertz.

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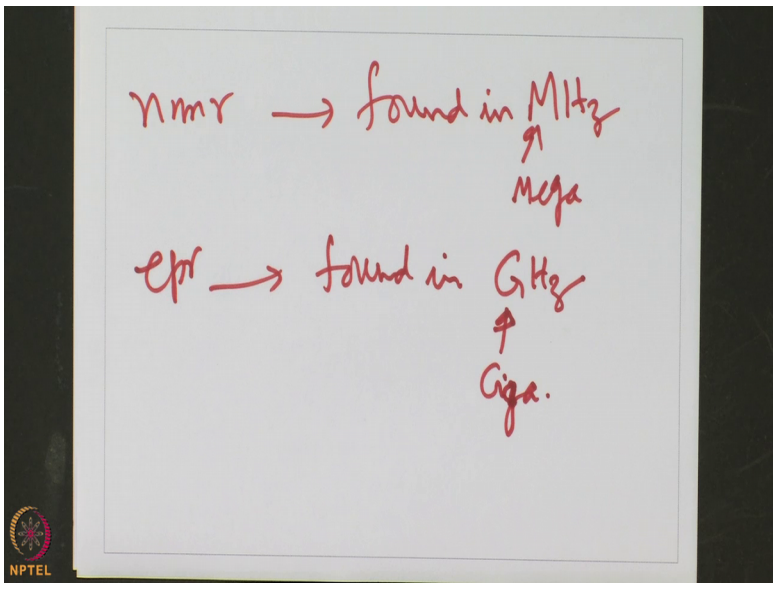

So, therefore, that energy difference is definitely there. And this can further coupled with the nuclear spin if you have and this in this particular case 3 over 2 nuclear spin and

further gets spitted and both the lower and electronic state higher electronic state both get. Now, you can see the transitions going from this one's, ok. I am not talking about the all the transition rules etcetera etcetera.

So,. So, big that will become too much more into the physical chemistry aspects, but just appreciate that these 4 transitions are possible, and these 4 transitions are shown over there here 1 2 3 4. So obviously, their gap differences different means which means energy is different; that means, they come at a different values. So, instead of measuring in the frequency mode you measure in the field strength in Gauss.

So, one would basically measure in that Gauss. So, therefore, if the x axis in this is note the frequency like in the NMR which is converted into the ppm here it is the Gauss. So, you keep changing slowly the magnetization or magnetic field strength and the different transitions can be understood, ok. The nuclear splitting under the influence of field and then the nuclear sorry electrons the two spins of the electrons are split in the magnetic field and then sub further a (Refer Time: 15:21), and each of these nuclear spin the electron spin is further with a nuclear spin and further split.

So, therefore, you get, so this kind of thing is this splitting is a hyperfine and this kind of connectivity is called super hyper fine ok. So, that means, you can find both the nuclear spin connectivity as well as the electron spin things too ok. So, therefore, the number of lines will depend upon 2 nI plus 1, the I is the spin of the nuclei n is the number of such nuclei, ok. So, therefore, how many of such nuclei’s are.

Let us take one example to understand this you have a vanadylacetylacetone. So, V double bond O this is called vanadyl and acetyl acetonate. This one acetyl acetonate another acetyl acetonate, each of the acetyl acetonate is 1 minus, the two of them are 2 minus, oxo is 2 minus totally 4 minus and vanadyl 4 plus and therefore, it is a neutral.

So, this is this acetyl acetonate vanadyl actually, and the vanadyl has got a nuclear spin off 7 over 2. And how many vanadiums are there? Only 1. So, that means, if you apply in this particular formula 2 nI plus 1, 2 into 1 into 7 by 2 plus 1. So, if you take the case of this one in the 2 nI plus 1 in a vanadylacetylacetone complex for the I for vanadium is 7 over 2.

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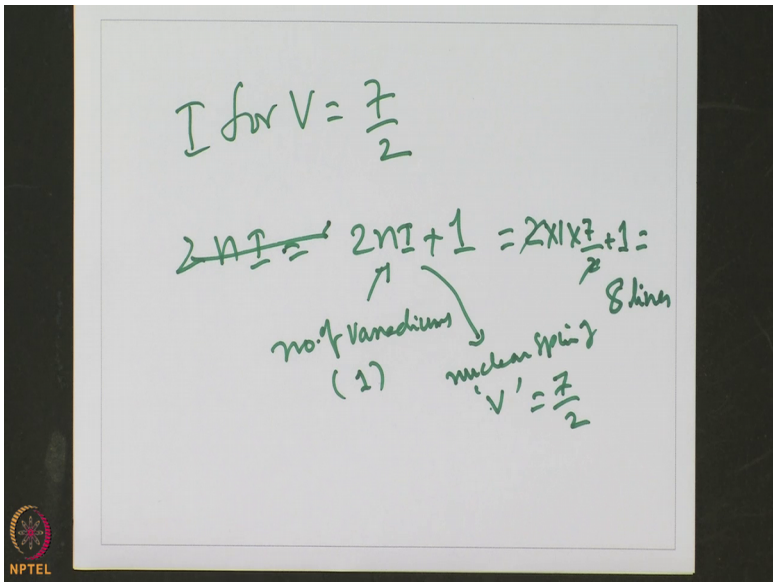

So, if you take 2 nI plus 1, 2 nI plus 1, 2 nI plus 1. So, n is number of vanadiums in this, this it so 1 and I is nuclear spin of vanadium and that is 7 over 2. So, therefore, that is equal to two into 1 into 7 over 2 plus 1 that will be 8 lines.

So, you should get 8 lines spectrum in this and you can see that the 8 line spectrum is shown over there 1 2 3 4 5 6 7 8, 8 line spectrum. So, this means this is a nuclear hyperfine coupling is being seen in this example, ok.

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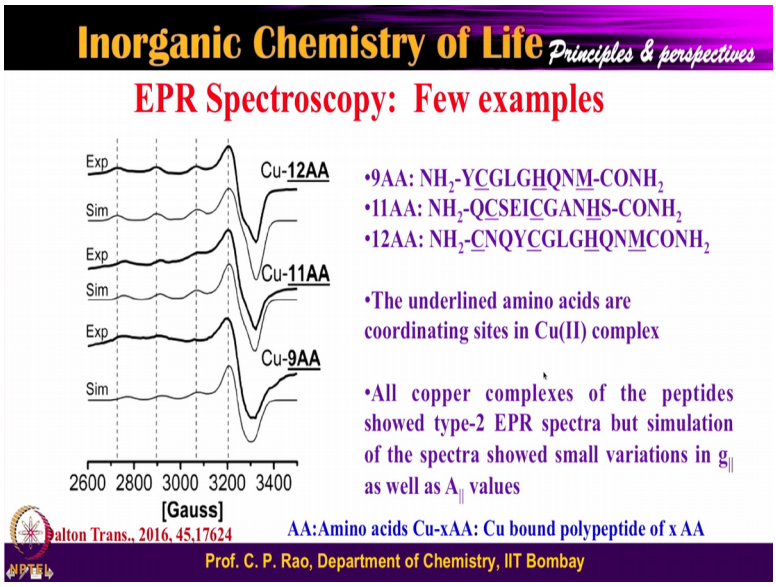

So, now let us continue with the EPR aspects of it. Now, in the EPR aspects of it let us look at inorganic complex which is a copper complex of a peptide, ok.

So, let us look at the copper complex of the peptides. So, in this we have a 9 peptide a eleven amino acid peptide, a 11 amino acid peptide, these are the EPR spectra measured, exp refers to experimental, sim refers to simulated. You can experimentally measure you can also stimulate using the parameters then all that you see the 11 and 12 and whereas, 9, so there as some little hyperfine kind of a coupling that you see different, but otherwise very similar kind of things, ok.

And this can be understood from, see these are the this is a sequence what is binding in this is the 9 asistente, histidine and a methionine. In case 11 a asistente, a histidine, another aistente and a histidine and in 12 asistente, asistente, a histidine and a methionine, ok.

So, you have all of these are bound to the copper center of course, copper you know all these things is copper 2 plus, no point in looking at copper 1 plus you know why? Why copper 1 plus cannot be studied? Copper 1 plus is a d 10 system, copper 2 plus is a d 9 system, so d 9 system. So, d 9 system will have an unpaired electron d 10 system will not have an unpaired electron therefore, copper 1 cannot be studied at all ok.

So, that is the kind of a thing. In fact, in some enzymes where copper 2 plus is there you look at the EPR spectrum which is coming from the copper 2 plus very nice then you add some reducing agent and the copper 2 plus becomes copper 1 plus and then it turns into from d 9 system to d 10 system and becomes EPR in act; so EPR active to EPR inactive 2. In this case all of these are EPR active and that is because copper 2.

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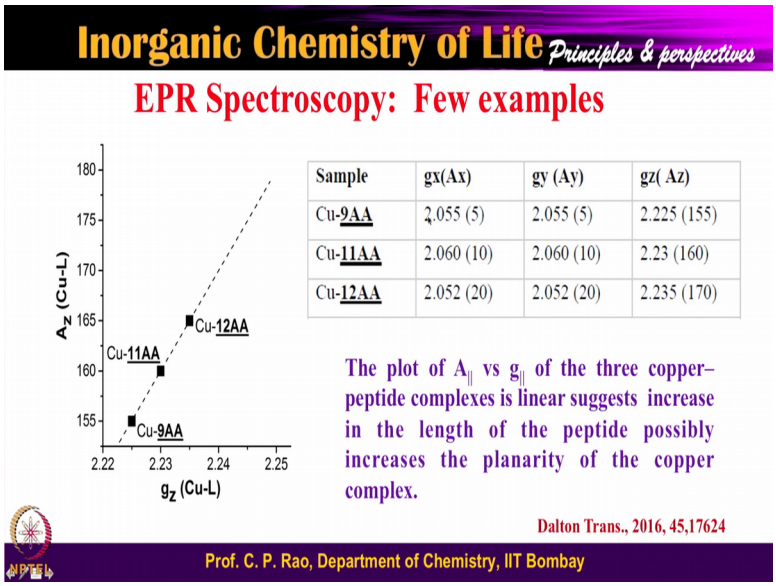

Now, this is being analyzed better in this particular in the next slide and this is the slide. So, there are 3 copper complex as you can see and from the EPR spectra you can get the there are the shift that is found which is found in the form of the Gauss as a as a position ok, you can get that kind of thing is also referred as a g value in many cases. So, the g value is plotted in terms of the Gauss and the coupling ones it just for a while if you look at these ones and the distance between these ones is the is the coupling values.

So, as you can see from here to here, here to here, here to here similarly here. So, these the coupling values which is referred as the A values. So, the A is hyperfine coupling and this is the position of the p the g. So, the g a to g correlation you see so nicely connected by a straight line. So, these all not important, you do not go through all these things all that you need to look at it is this particular thing.

So, therefore, the positioning, the EPR peak position versus the connectivity between this thing in terms of the hyperfine coupling values are co-relatable from one to the other and that is what we can make in this. And as I told you that you can also study not only copper many other paramagnetic things, like manganese can be studied and iron can be studied. So, molybdenum can be studied some of them in their oxidation states where they shown the paramagnetism for all of them one can study and of course, interpretation of the EPR is somewhat difficult compared to the other spectroscopy that I have talked to you earlier, ok.

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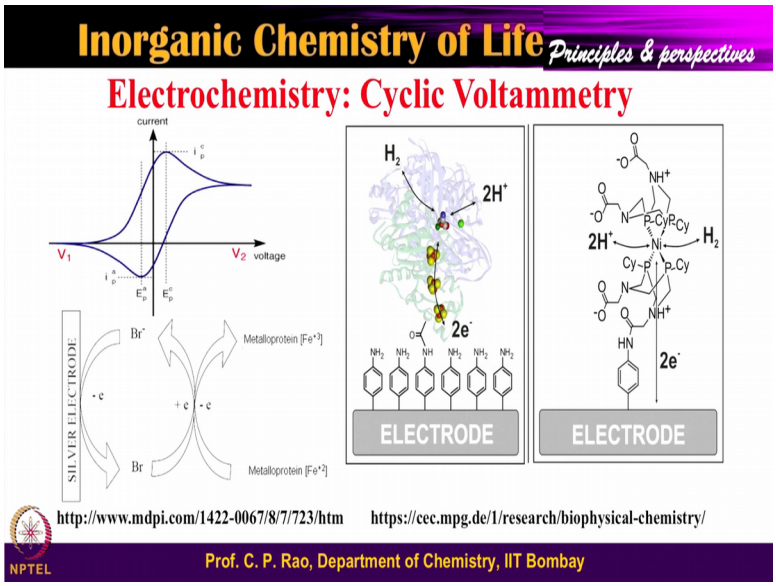

So, let us look at another technique another techniques is the electrochemistry. So, the electrochemistry this we are talking about the cyclic voltammetry. So, electrochemistry is basically you and electron to the system or molecule, you remove an electron to the system or a molecule what happens to it? How do one measure in terms of (Refer Time: 22:40)? So, this is what is basically studied.

So, one of the ways is that you apply a potential or vary the potential and measure the current. So, if you take apply a potential, vary the potential and measure the current therefore, the current versus the versus the voltage is called voltammetry, current versus the voltage is called the voltammetry yeah sorry voltage versus the current is the voltammetry, voltage versus current.

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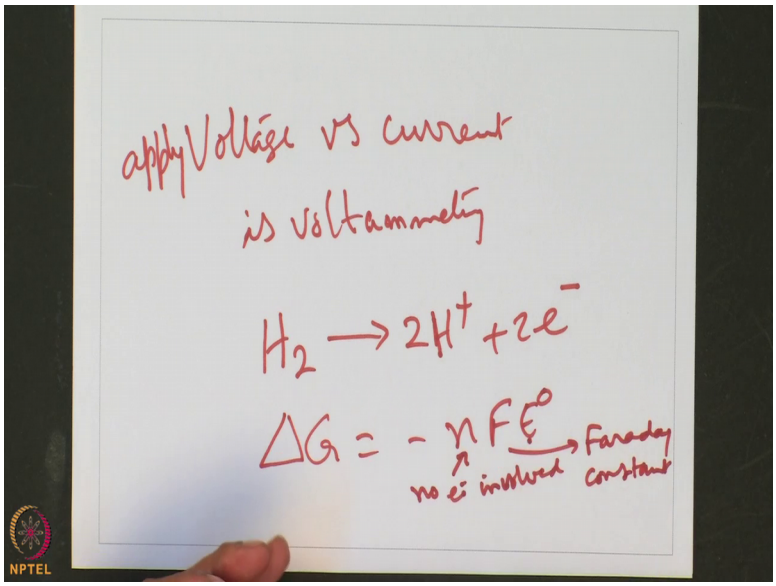

So, you apply voltage, voltage versus current is voltammetry. Of course so, what you do in this you apply keep applying the potential and measure the measure the current. So, in this case why it became cyclic? Just follow me as I take this cursor, let say I took a solution containing a compound which is electro active I start from this particular voltage and I keep increasing the voltage towards this direction. So, it goes along with this, ok. And there is a sudden increase in the current this and at these stage I reverse the potential.

So, if I reverse the potential it does not go through the same it comes from the another. So, it comes through this. So, it comes through the whole thing and then goes back. So, I start from V 1 go to V 2, and reverse the switch the direction of the potential and then come back. Potential has a sign a positive potential negative potential. So, going from this to this increasing means going towards the positive direction and going from here to here is you going from this potential and decreasing it is a negative sign.

So, you have a positive sign you have a negative sign, ok. So, so you can reduce you can preoxidized kind of a species and that is also. So, it becomes a cyclic. So, therefore, it is a cyclic voltammetry going from left to the right and then reversing the potential and coming back and this is where you can make the whole difference in this particular methodology you can make.

For example, you take a electrode here it is shown example a silver silver electrode and take a solution of the protein a metalloprotein and a metalloprotein containing the iron center iron metalloprotein, iron with a 2 plus and going to iron 3 plus. So, that is what oxidation. And this one the Br going to Br minus is what reduction.

So, this oxidation of this is connected with this reduction and this reduction is connected with the electrode. So, from electrode you go from this Br minus to Br, Br minus to Br is oxidation and this oxidation will give away the electron and this electron will be picked by the bro Br and this way or it will be picked up by this to return back and in the process it will activate the metalloprotein ion 2 plus 2 ion 3 plus. So, therefore, it is coupled with this. So, everything is coupled with electrode ok.

So, at the electrode you are releasing the electron and this electron is being taken up by this, and as a result of this process is coupled with this and going from ion 2 plus 2 ion 3 plus oxidation. So, you similarly you can do reduction as well. So, you can do oxidation of the protein, you can do reduction of the protein, everything is possible by changing the potential here.

So, and you know that in case of the metalloproteins and metalloenzymes (Refer Time: 26:49) in disguise you have metal ions, metal ions having different oxidation states therefore, you can take them protein from one to the other. And the protein also has a confirmation therefore, the potential is also affected by the conformation and the protein as well; So, the medium the protein conformation and the redox going between the positive to the negative all of these kind of things, ok.

Let us take one example here. So, this is an electrode the electrode is modified and the modified by this kind of thing and this particular thing is connected with the protein. So, this whole background kind of a thing soil etcetera is a protein. In that protein there are centers there is 1, there is another 2 3 3 4 etcetera and these are all electron transfer centers and primarily these are all iron sulphur centers and then here you have actual reaction centre where the reaction occurs from H 2 going to H plus, H 2 going to H plus plus 2 electrons is what? Oxidation.

So, H 2 going to H plus plus electron to H 2 going to 2 H plus plus 2 electrons, ok, so this is an oxidation process; This oxidation process is can be identified you can be

studied and the electronic is transfer from where from the electro to these to these, from there to here, from there to here, from there to here at the end the reaction site is this one.

So, it is at this side hydrogen goes to proton and, but to get you need this process. So, this entire process is controlled by the iron sulphur proteins having different redox potentials. So, having different redox potentials is a boon in disguise, that is why nature has identified its own or managed its proteins to have centers of iron sulphur with different potentials. I will explain this more details when I come to the iron sulphur proteins story in this.

Simple example is shown over there. Simple example is an electrode modified with this moiety which is connected to this particular moied. So, this amine having the the phosphorous centers bound to the nickel centre and another ligand. So, this whole thing you can do redox and this particular molecules can convert H plus 2, H 2 and then H 2 to H plus. So, they can do both reversible in this, so H 2 getting in H plus 2, so redox kind of thing.

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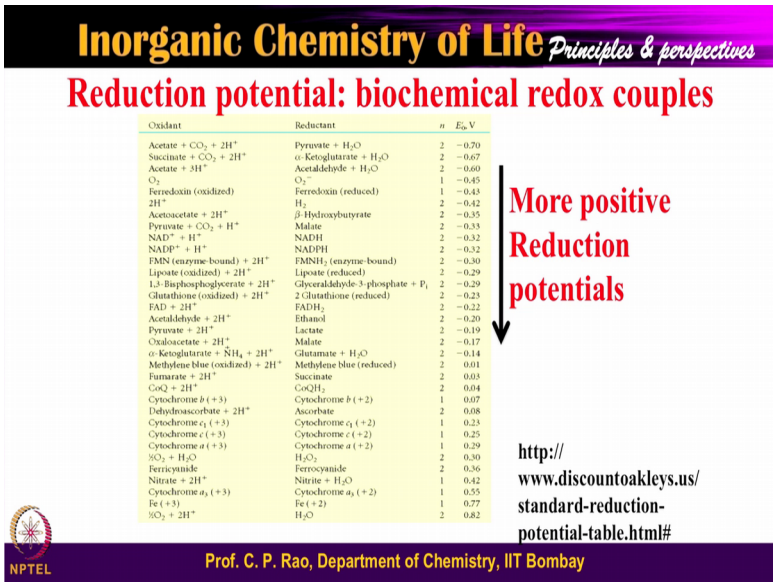

So, therefore, one can basically look at all these things. Look at different kind of redox system here you have as you go from top to the bottom you are having the reduction potentials are becoming less negative or rather more positive; that means, more reduction is favorable. How do you understand this? You can understand this by delta G is minus nFE or E naught.

So, if the potential is minus this minus into minus is plus if the potential is plus plus into minus is minus. So, more reduction potential positive means it will be minus more feasible. So, n is number of electrons involved in the reaction and this is Faraday constant. So, the more positive potential will make delta G more negative; that means, reduction potential more positive means reduction is more favored. So, as you go from these to these more more reduction is favored. If you go in the reverse reduction more and more oxidation is favoured in this.

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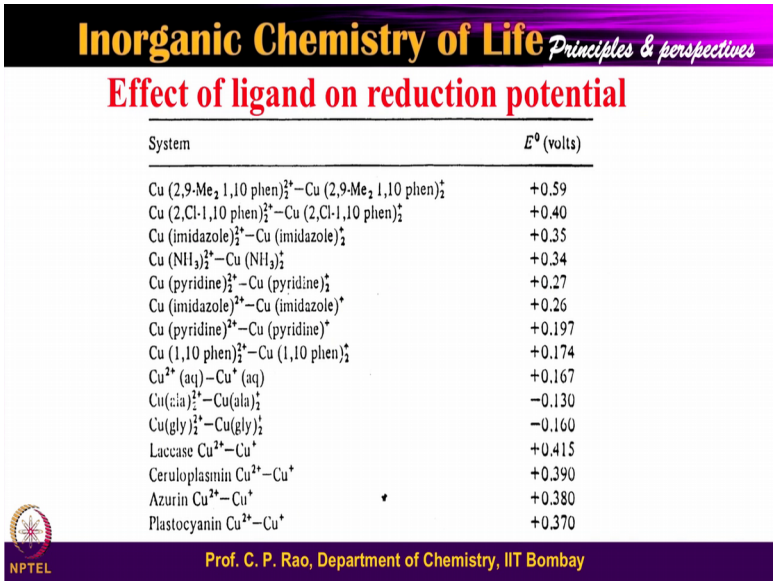

Let us see this in the context of an example of a copper proteins. You can see here these are all small molecules up to here, and if you go down here this is a laccase, this is an enzyme copper, ceruloplasmin enzyme, azurin enzyme, plastocyanin is an enzyme. So, why are we studying all these? We are studying all these to find out what kind of a centres are present in the proteins.

So, therefore, we are taking a small molecular copper complexes here. This copper complex between 2 plus, 1 plus potential is plus 0.59. The next complex with the chlorine is a plus 0.40, similarly you go to imidazole kind of thing plus 0.26, you go to the pyridine plus 0.197. So, your potential is smoothly changing from plus 0.6 to over this.

And if you look at the so what is happening? As you go down further and further the nitrogen kind of a bindings are reducing, oxygen bindings are increasing. You go to the

copper alanine you are mainly they lot of carboxylic oxygen are being bound therefore, the potential is basically converted from positive to the negative. So, therefore, redox potentials or dependent on what is directly bound to the metal centre they are also important or they are also dependent on the protein conformation as well all those parameters will affect, ok.

So, going from nitrogen is to the oxygen is to the minus containing oxygen carboxylic etcetera the potential is now you look at the peptides and the proteins the laccase plus 0.4, ceruloplasmin plus 0.39. So, these are all roughly very similar. What it means? Basically in this proteins you have a lot of nitrogen or imidazole binding is involved in all of these therefore, the redox potentials are very similar to those this particular derivatives, ok.

So, what we have a scene? We have continued with the NMR in this lecture, then we have continued with the EPR which is an extension, then we have looked at the electrochemistry and started with a redox potentials in the terms of the cyclic voltammetry. Then we looked at we try to compare these redox potentials going from a positive to the negative as a function of change in the in this binding centres are changing, because as you go from nitrogen centres to the oxygen centres, to the negatively charged oxygen centres the redox potential changes from positive to less positive to negative, ok.

So, what does that mean? That means, that the reduction is less favored the oxidation is more favoured. When you have more positive potential more reduction is favoured in that. Now, if you compare these things with the enzymes, so the enzyme, the laccase and the ceruloplasmin, the azurin and the plastocyanin and they are all plus 0.4 around that ok. So, that kind of a potential is certainly will coming within this range or this. So, therefore, one can, one can explain that in this proteins the protein centres are having a lot of imidazole binding, nitrogen ligand binding may be even some sulphur ligands binding as well in all these aspects.

Now, you can see that that. So, we can use a variety of spectroscopy techniques talk to you about the NMR, then we are talk to EPR, then I have talked to you redox potential; If you more techniques I will talk in the next class and then with that we will finish the techniques part of it.

Thank you very much.