Biological Inorganic Chemistry
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Lecture -13
EPR

Hello, good morning everybody. So, we are with the physical methods; what are the different physical methods we can try. So, under module-3 we talk today only on first on EPR, which is nothing but your electron paramagnetic resonance. Like the resonance technique what we have seen in our previous two classes, that resonance Raman technique; so, this is electron paramagnetic resonance. So, how this paramagnetic resonance can help us in understanding the metal ion environment in the biological systems.

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So the different concepts, what we try to cover in this particular class that why we need EPR spectroscopy. So, whenever we have a biological sample in hand and we suspects that there is some metal ion and if the metal ion present is of the categories like iron or copper or manganese or nickel, we try to expect that it can have certain kind of paramagnetism in it, that means unpaired electron is there. So if you have unpaired electron, then we can use the EPR spectroscopy nicely. Then if the sample changes from any metal ion sample, or the metal ion complex sample to a metalloprotein sample; we can still use the EPR spectroscopy for its characterization and its identification.

And the next four things, what we can talk a little bit about the basically the keyword based thing; that means what we call as the Zeeman interaction. What anisotropy can help us in understanding, because this term is very much familiar with the magnetic properties also, then hyperfine interaction; because we will talk about the hyperfine splitting and the zero-field interactions.

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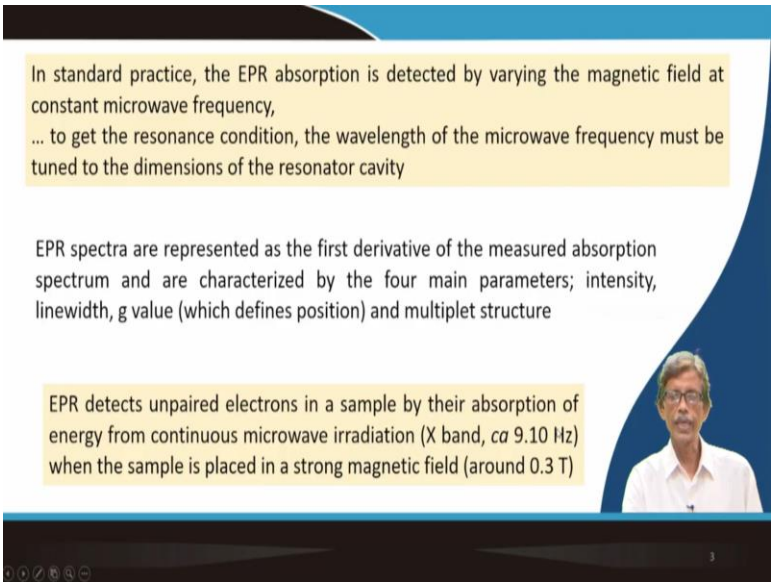

So, what we see that in standard practice, the EPR absorption; how the EPR absorption we can see like your electronic absorption. We have seen earlier that the radiation in the UV visible region, if it hits or strikes the sample in a solution, we see the electronic absorption. But in this particular case, it can be detected by varying the magnetic field at constant microwave frequency.

So if we vary the magnetic field at certain point, you find that the microwave frequency will be absorbed by your sample. And how we can define that where this position of microwave frequency absorption, what is the energy, all these things we should know. So, for a particular resonance conditions, again the resonance condition; because it is resonating from one state to the other. The wavelength of the microwave frequency in megahertz region basically, must be tuned to the dimensions of the resonator cavity. So, we have the microwave source, the (()) (3:24) we have; and within that (()) (3:25) we have the sample, which is the cavity like your NMR.

We put that particular in EPR tubes also. So you can have the powder sample we can put or the solution samples we can put in that EPR cavity. Then what we get that EPR spectra are presented as the first derivative of the measured absorption spectrum. So, if it is absorbing at a particular microwave frequency; but it is not, that will scan basically the corresponding magnetic field; and characterized by four main parameters like any other spectroscopic technique. So, why we use the different spectroscopic techniques? Because we want to know the intensity of absorption. That means the concentration or the population of one particular state to the other, if we can change.

Then line width, how big the line width is that is very much characteristic for the system; then the g values, which defines the position. So, at a particular value of g is equal to 2.0023 or something like that, we will be able to know that it is related to some ligand based ion, or the radical, or it is metal ion based. And then the multiplet structure that means some other kind of splitting on that particular g value. So, what is that particular band we will be using? That means what microwave irradiation or micro frequency we can use. So there are different types of EPR spectrometers that are available nowadays; but the most basic one and the most simplest one is your X-band EPR spectrometer.

So, that is why we write in this fashion, that means the microwave irradiation is taking place at X-band region, which is 9.10; not megahertz it is gigahertz, sorry. It is gigahertz, 9.10 gigahertz frequency; so that 9.10 gigahertz or 9.1 gigahertz frequency is sufficient to see that particular absorption. So, when the sample is placed in a strong magnetic field and a small width the magnetic field is we can vary. So, it is unlike your NMR spectrometer that will see in our next class that what range of a magnetic field we will be using for recording your NMR spectrum. So, here it is less basically is only 0.3 Tesla.

So, these are the conditions, the microwave frequency, what range we will be handling; because there are other bands of EPR spectrometers that are available. And what (micro) the corresponding magnetic field we can use to study this particular absorption.

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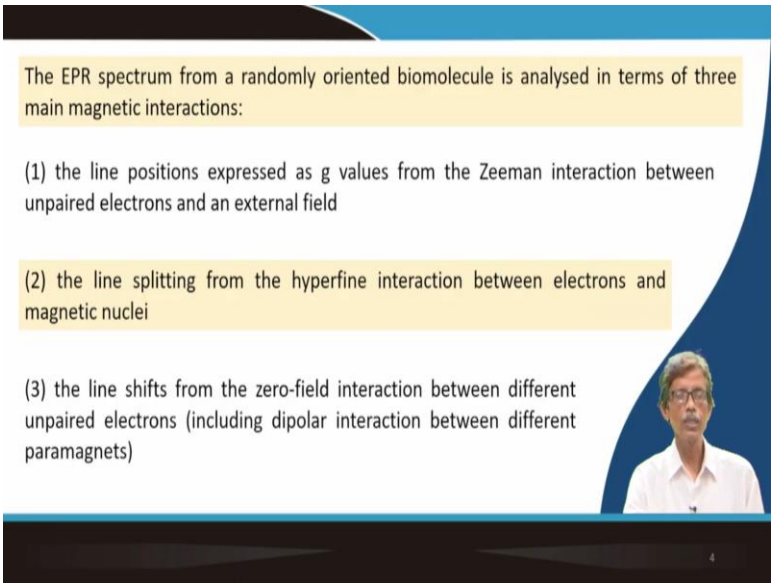

So, when we have the different samples like the biomolecules, we straightway to go to the biomolecule sample; and we expect that the biomolecule sample has unpaired electron, like copper, proteins or copper based enzymes. We all know that the copper has one unpaired electron, if it is mononuclear site, which is the 3-D anion system. So, whether we will be able to detect that particular center in terms of its corresponding sensitivity; that means the EPR sensitivity of the copper site. So, we can have three different magnetic interactions because everything we are doing under a field of magnet.

So, we can have the corresponding line positions; so line positions, just now I told you that you can have the corresponding g value. So, g is equal to 2.0023, or g is equal to 4.3; so you can have the g value positions at two different positions. So, how these positions are coming? So they are basically due to the Zeeman interaction between the unpaired electrons and the applied external field. What magnetic field we are using? We have two huge magnets and inside that cavity, we put the sample. So, anywhere you can go, that is why every time I am giving this example for Wikipedia sites.

So, if you go to the Wikipedia, say to you, it will have this typical figure for the setup, how EPR spectrometer looks like; and what is the particular magnetic field, what magnetic field you can use, and where we can put the sample and how you record it. Then, the lines splitting from the hyperfine interaction between the electronic and magnetic nuclei. So, if you have the electrons,

unpaired electron that is giving you the signal; but we all know the spinning nuclei also can have some magnetic effect. So, if these two are interacting, that is why the electron spin, giving you the EPR, and the nuclear spin giving you the NMR.

But here, if the electron spin is part out due to the presence of the magnetic moment of the nucleus, will have some interaction and we get the corresponding hyperfine interaction. Then the line shift from the zero-field interaction; so the line can shift also due to a zero-field interaction.

So, when no external field is applied, but inside the sample, you can have a magnetic field between the different unpaired electrons, including dipole interaction between different paramagnetics. So, you have the sample, you have the unpaired electron, and you have the corresponding nucleus spin also. And if those interactions that are taking place, we can have many information related to these. So, these are all finer details of all these informations, what we can have in our hand.

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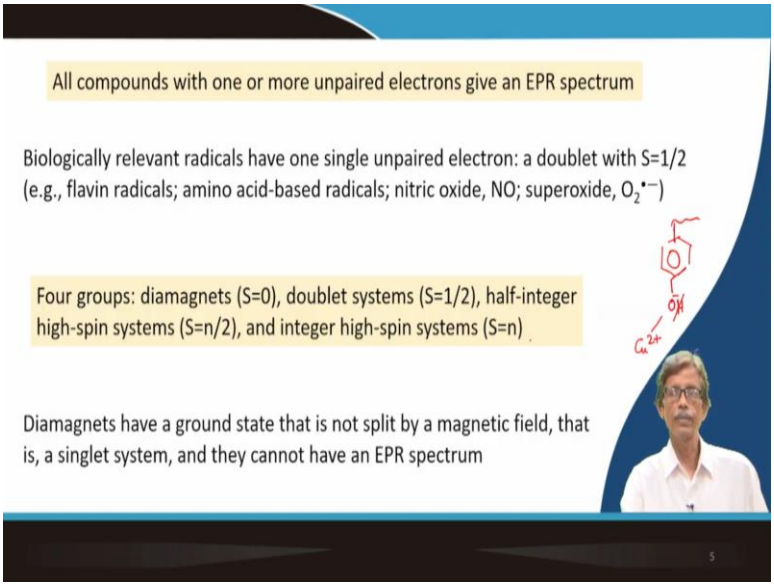

So, what we get now that all compounds with one or more unpaired electrons. So, the basic assumption of all of these things that any compound you can have having unpaired electron can give an EPR spectrum. So, how it looks like we have seen that electronic spectrum, the electronic absorption spectrum we have seen. Last time we were talking about the corresponding methyl red solution. If you put in the corresponding UV visible spectrometer or

spectrophotometer, we find the corresponding broad absorption band. So is it like that or it will be something else?

So, any compound, any simple compounds, any copper salt; say a copper component available. Say copper acetate or copper sulfate or copper nitrate, whether this can also give you the corresponding EPR spectrum or not? And what sort of EPR spectrum we can get out of that particular group sample of copper nitrate, or corporate acetate or corporate sulphate. So, once we have that particular information from the measurement of the typical salt, the metal ion salt. What we can do? We can compare that particular EPR spectrum with that is obtain from the biomolecules containing, say copper.

So, the EPR spectrum of the copper enzyme, or the copper biomolecule can be correlated or can be compared with that of the EPR spectrum what we can get from the corresponding copper salts or the copper solutions. So, powder the dried powder we can put inside the tube, and put in the cavity, the EPR cavity. Also the solution and another technique also we can follow that you can have the solution. We are not isolating all of these things, sometime in-situ some solution we have studied; and you have measured such as the protein is also there in the solution. So, that you directly put inside the EPR tube; but within the cavity, you can have some arrangement that the nitrogen liquid, nitrogen Dewar you can put, or we can fix.

So, within that liquid nitrogen Dewar, you can put the liquid nitrogen; and you freeze the sample, such that it can form a glass at a temperature of liquid nitrogen. Relevant temperature all we know is 77 Kelvin; so at 77 Kelvin, you get the frozen sample like that of your powder sample. So, that frozen sample can also give you the corresponding spectrum. So, what we will be looking for like that of your copper; similarly, other relevant radicals can have a single unpaired electron. So, like a doublet with S is equal to half state; how we call it the doublet? S is equal to half. That means one unpaired electron; but the spin multiplicity, which is twice S capital S, twice S capital S plus 1; so 2 into half plus 1, so it is a 2.

The magnitude is 2, so that it is a doublet; so state is doublet. So, if you have a doublet state like any unpaired electron, like a flavin molecule, we all know the flavin is a biological molecule. So, flavin radicals if it has an unpaired electron, it will be a radical; then amino acid-based radicals, like tyrosine. We all know the tyrosine and that tyrosine is what? Tyrosine is nothing but your,

so, tyrosine is that you have the amino acid backbone; so you just consider that you have the backbone, then you have OH. So, you have the pendant tyrosine radical, tyrosine anion.

So, you have the H is if it is go for the deprotonation; so it will be O minus, such that O minus can be coordinated to the metal ion centers, such as your copper two plus. It is coordinating to your copper two plus center, but it can so happen that sometime how these amino acid radical is coming. That you have this particular one, O is O minus; and this O minus the phenolate ion. We consider that phenolate ion is bound to copper two plus. Now, if we consider that I will then again, further we go for the oxidation. So, if this particular system can be oxidized, you can have two possibilities; one is that you can oxidize the metal ion center, or you can oxidize the phenolate ion, the O minus.

So, when the O minus bound to copper 2 plus, it can have higher tendency. There are examples in galactose oxidase or other places, that instead of oxidizing the copper 2 plus center, you can oxidize the phenolate ion. So, that phenolate ion this O minus can be converted to O dot, which is your radical; so, that is the typical example of your amino acid based radicals. So these amino acid based radicals can also be detected by this particular spectroscopic technique. So that is why the confusion will not be there, whether you are able to oxidize the copper center, or whether you are able to oxidize the phenolate center.

Because when you go for another electro-chemical technique; we will consider in our next to next class, that electro-chemical measurement, the cyclic voltammetric studies. So, cyclic voltammetric can only detect the corresponding potential value, where you are finding some oxidation; but where from the oxidation is taking place that we do not know. So, these amino acid-based radicals or the nitric oxide; the nitric oxide itself has an unpaired electron, unlike carbon monoxide. So, when the nitric oxide is bound to that particular metal ion center, say iron center in haemoglobin or myoglobin, we find that some interaction can take place with that nitric oxide radical.

That means extra unpaired electron on it with that of your iron center, having more number of unpaired electron. Then superoxide also, so superoxide anion can also can be detected by means of these EPR signals. So, these are all very much important, among with all these four groups of species. So, you can have a diamagnetic system, where S is the small S, capital S is equal to 0 or small s is also equal to 0. Then the doublet system, just now we are talking about having one

unpaired electron, then half-integer high-spin system. That means half three half or four half; sorry, five half and all this.

Then integer high-spin system, where S is equal to 8; so, S is equal to 1, S is equal to 2 like nickel 2 plus and all this. So, how these systems can be identified? So if you have a diamagnetic system, so diamagnets have a ground state that is not split by a magnetic field. You have a singlet system. So, if you have a singlet system that cannot have an EPR spectrum; so we consider that this is EPR silent. So, you do not get any other response from that particular system, where the system is diamagnetic in nature, like that of your zinc 2 plus.

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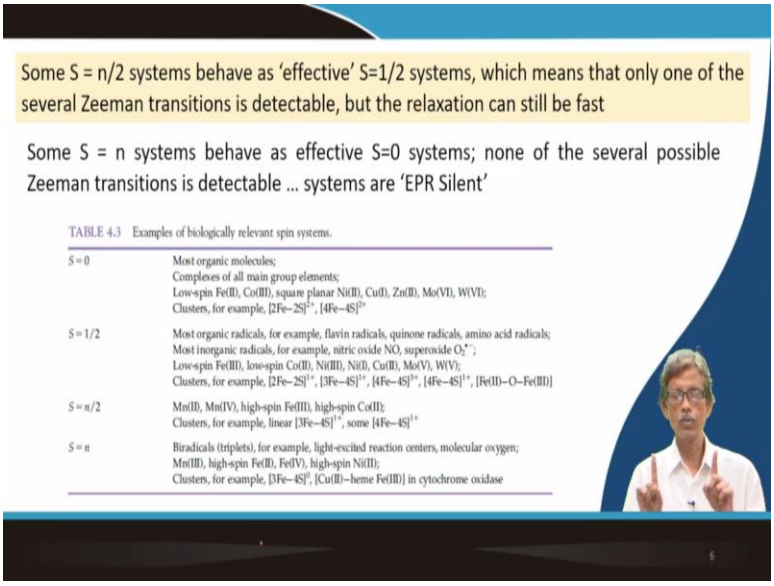

We know zinc 2 plus is the 3-D chain system, and their zinc 2 plus cannot have any EPR signal. So some S is equal to n by 2 systems; so n by 2, it can be 3 by 2, it can be 5 by 2, or it can be 7 by 2 system. When you have more number of unpaired systems close by, but effectively it can behave as S is equal to half system. That means it is showing some response, which is equivalent to that of the presence of one unpaired electron. That means the other unpaired electrons are highly delocalized. So this particular situation arises in case of iron sulphur proteins, the ferredoxin protein.

We have seen that the simplest possible ferredoxin is 2-iron, 2-sulphur FE2S2 ferredoxin system. So, if the individual iron centers, unpaired electrons from the individual iron centers are considered, we do not find that system is like that of your S is equal to half system; but it will be

having more number of unpaired electrons. But if the delocalization or the interaction, the magnetic interaction, or the magnetic coupling is so strong, that only it can show the corresponding response for one half and there is one unpaired electron.

That means only one of the several Zeeman transitions is detectable in that particular case; but the relaxations can still be fast. If the relaxation is fast, you can have many number of these Zeeman transitions, but whether we are able to detect it or not. There are some n, S is equal to n system, behave as an effective S is equal to 0 system; none of the several possible demand transitions are detectable, and system will be calling as EPR silent. So, you have unpaired electrons, you have paramagnetic centers; but they are so strongly coupled like copper-copper system.

If it is a bi-nuclear system and two unpaired electrons in each copper system; if they are strongly coupled and if the ground state is x equal to 0, and the excited state, maybe x is equal to 1. You will not find any EPR signal out of that particular system. So what we get, there are different biologically relevant spin systems, where you can have S is equal to half system. S is equal to one half, S is equal to n half and S is equal to n. So, the first category you can have most radical organic molecules, where you do not have the corresponding paramagnetism. But, in the second case, if you see that the 2-iron, 2-sulphur system you see, when you can have the corresponding reduced form; that means 2FE-2S 1 plus, or 3Fe-4S 1 plus; so, it is a reduced form.

So, in the reduced form also like that of your nitric oxide and superoxide, you have single electron paramagnetism. Then n half is equal to that manganese-II, manganese-IV high spin iron two, and high spin cobalt-II. We have more number of unpaired electrons; and the corresponding clusters are also there, where you can have more number of unpaired electrons. Then S is equal to n means S is equal to 1, S is equal to 2, S is equal to 3, something like that; where you can have the examples of light excited reaction centers.

That means the corresponding oxygen evolving center in photo system two; and also in cytochrome oxidase also, where you have more number of unpaired electrons. Not only copper-copper interaction, but you can have one copper is interacting with one iron center, in cytochrome C oxidase; the situation will be much more complicated.

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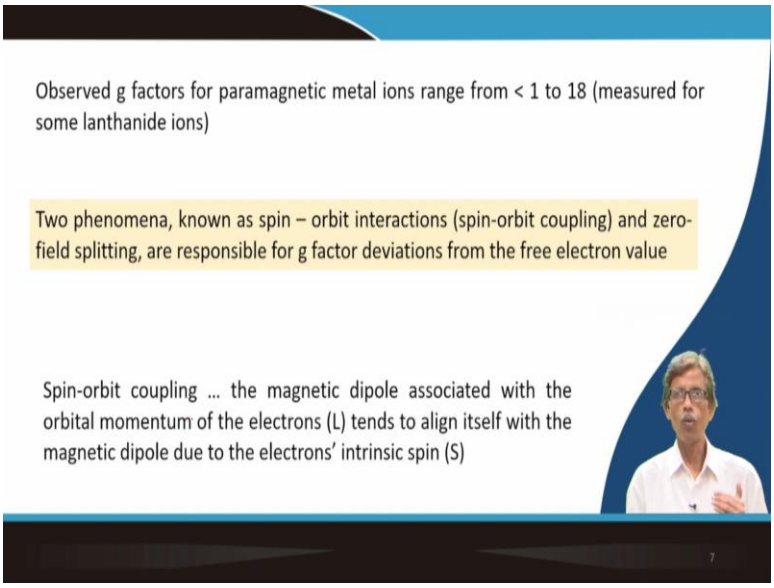

So, we observed the g factor, but how many unpaired electrons we will be able to detect for a characteristic type of EPR spectrum? So, it can be greater than 1, or it can be 1 also and up to 11. So for lanthanide ions, and even for more than one lanthanide center if it is present; we can have a huge magnetic moment. Then, if we see that the spinning motion and the orbital motion of an electron responsible for generating the magnetic moment. But if they are coupled together, we find that the interaction will be calling as the spin-orbit coupling and the zero-field splitting. So, these are the two things how these can be dealt with. So it is a very complicated thing for the theoretical part of the EPR spectroscopy.

But what we should know for handling the biological samples, the metal ions in the biological origin? What we can do or how we can determine all these things. So how we can tackle the spin-orbit coupling, even if it is there? How it happens? The definition wise, it is a magnetic dipole associated with the orbital momentum, due to the orbital motion of the electron, which is capital L, small l the quantum number we know, when you add up all L, L1, L2, L3; we get the capital L. When it is trying to align with the electrons intrinsic spin, which is also the capital S. So, far we are considering about the capital S only. Now, if you bring the L, how the magnetic moment due to capital L, will part of the corresponding spin of S. So, your property or your structure of these particular spectrum will be changing.

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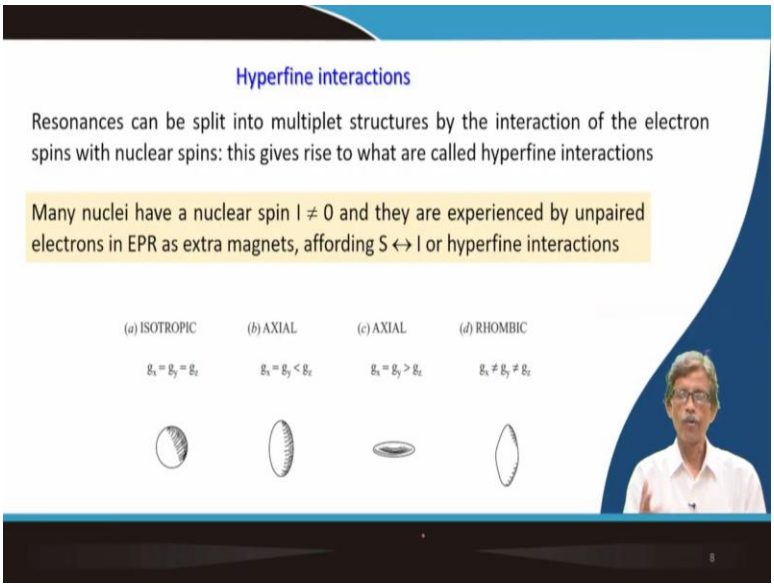

That is why we get the hyperfine interaction out of the corresponding magnetic moment from the nucleus, like the copper nucleus. So, resonances of all these resonance signals, you can split into multiplet structures by the interaction of the electron spins with the nuclear spins. So, if you allow the interaction of the election spin with that of the nuclear spin, we get what? We get the hyperfine interactions. So, in a simplest language, if your electron spin is interacting with the nuclear spin, you will be getting the hyperfine interaction. So, if you do not have any nuclear spin, then capital I is equal to 0; so they are experienced an unpaired electron in EPR as extra magnets; so not equal to 0, affording S-I interaction.

So, if you S-I interacts in capital S, capital I interaction, when I is not equal to 0; that means you have nuclear spin, you will have the hyperfine interaction. Then we will find that if you consider that the typical isotropic signal, that means one signal; and EPS spectroscopy will be recording it as a derivative spectrum. So that derivative spectrum what we get is due to that isotropic signal. When the arrangement, the electronic arrangement around the unpaired center is gx is equal to gy is equal to gz. But if it is not like that if you elongate one particular axis like gz for the axial system; so gz is not equal to gx is equal to gy, we will get one axial thing.

Then if you compress that particular gz axis, you will get another oblate type of axial orientation; and rhombic is that you have distortions in all three directions x. y, and z. So, you have rhombic signals.

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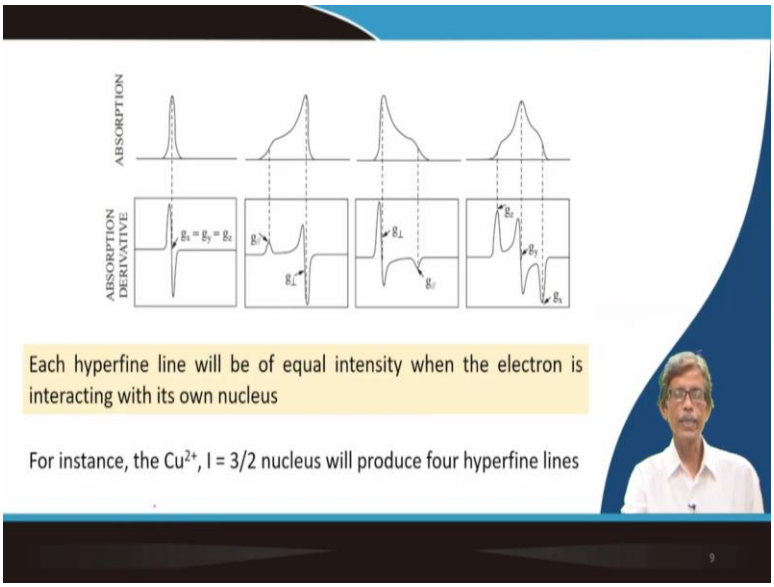

So, how it look like? So, when you have a rhombic signal, you see this one, the fourth one. So, this is absorption and the derivative one; so derivative one when you get, you will see the three different values gx, gy and gz. So, you will three signals, but the derivative signal will have a different shape; because gx is different to that of your gy, and gy is different from that of your gz. So, isotropic signal and two axial signal one is of g1, g-g parallel, this is g parallel; in the second category, g parallel is greater than g perpendicular. And the third category is g perpendicular greater than g parallel. So, these are the most possible types of electronic spectra; sorry, EPR spectrum, you can get out of these measurements.

Then each hyperfine line will be of equal intensity, when the electron is interacting with its own nucleus, such that unpaired electron on copper 2 plus, if it tries to interact with the nucleus of the copper center; what happens? What is the nucleus spin of the copper? copper 63, that we should know, which is I is equal to 3 by 2 or 3 half. So, when it is interacting with that particular 3 half system, you would definitely get something where you can have four hyperfine lines; so, the value will be found out from twice I plus 1. So, this twice I plus 1 value is your hyperfine lines. If it is interacting with the copper nucleus; and that also signifies that you definitely have a corresponding copper center, so that copper center can be identified.

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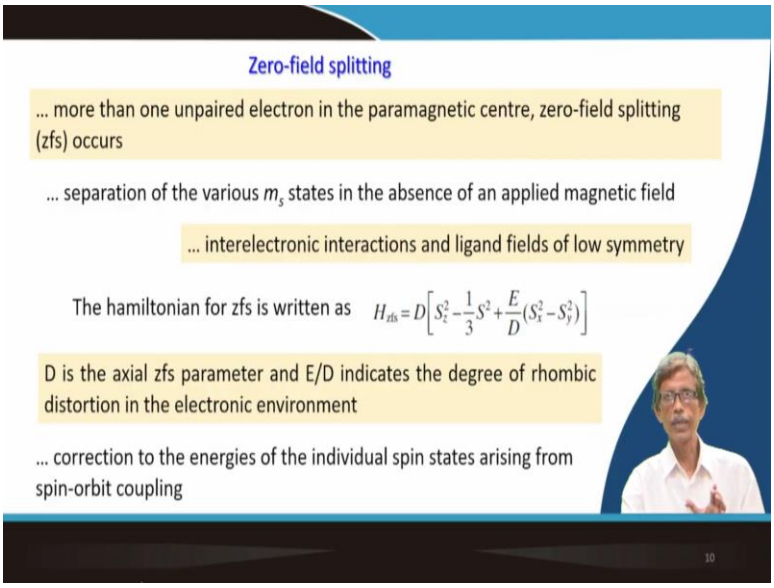

So, the next level is that another splitting is due to the zero-field splitting, when you have more than one unpaired electrons. And the zero-field splitting or ZFS is taking place there or occurring there. And the separation of various mS states, we know S; we then when the magnetic field is there, we know they are splitting in mS levels, or mS states. So, when you do not have the applied magnetic field, the internal magnet or internal magnetic field can split these levels, when you do not have any external magnetic field. That means the field is 0, but it can still can have some spitting.

So, interelectronic interaction and the ligand field of low symmetric can basically give rise to that sort of splitting. Mathematically people express, we can express it also, the Hamiltonian for the corresponding splitting. And we can write, because this formula is important to derive the some parameter, like the parameter, like D and E values. So, it will have only the E value, D value and S values, and H of zfs. So, the Hamiltonian for zero-field splitting can be written in terms of capital S, capital E and capital D. So, D is the axial zfs parameter and E by D indicate the amount of rhombic distortions; that means distortions in three directions.

If you have distortions in x-direction, y-direction and z-direction and all three are not same. So, the rhombicity of that particular distortion can be indicated by E by D value, not simply the D value. So, these parameters can be extracted out because the corresponding theoretical fitting or the simulation of the spectrum is available with the use of these parameters. That means D-

parameter or E-parameters, all of these; you can feed to get a simulator EPR spectrum. And the simulation for that particular one can extract out those parameters; and that will also tell you this particular copper center can have these parameters.

And we can find out about their corresponding environment in solution, without knowing the typical X-ray structure for that particular center. So, it is basically a part of correction to the energy levels of the individual spin states, due to spin-orbit coupling. So, once you have the spin-orbit coupling, you have some corrections to the spectrum. Then further if you have the zero-field splitting will go for the further corrections, further level of corrections.

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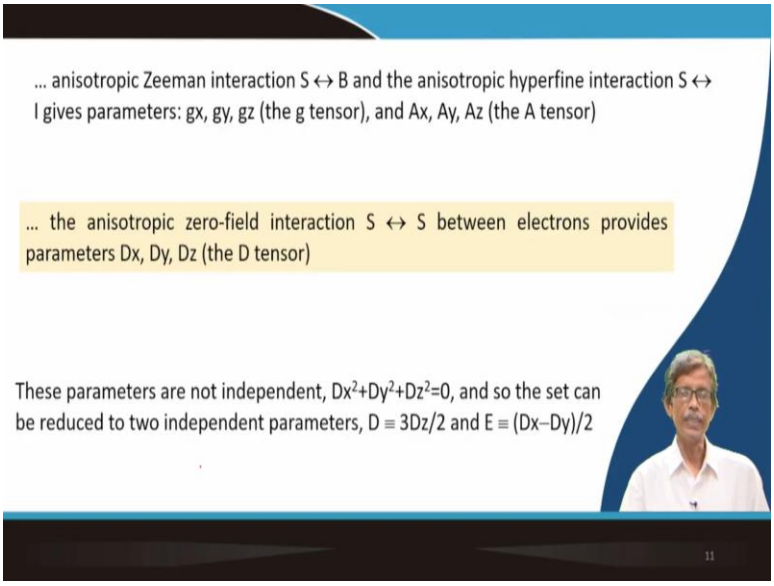

So, the anisotropic Zeeman interactions for S with B that means the zero-spin of the metal ion center with that of the external field, giving us gx, gy and gz. That means the g tensor as well as the A tensor; A tensors are nothing but your hyperfine tensor values, and the corresponding magnitudes. But the anisotropic zero-field interaction, S-S interaction; that means the field for the system also, and the field for the unpaired electron, can give you the D tensor. So, you have g tensor, you have A tensor, and you have the D tensor.

So, these values are very much useful, and these parameters are not independent particularly the Dx, Dy, and Dz values. They are related square of these three, summation of squared of these things will be equal to 0. So, you can have two independent parameters. Therefore, the D and E values, capital D and capital E values can be useful to us.

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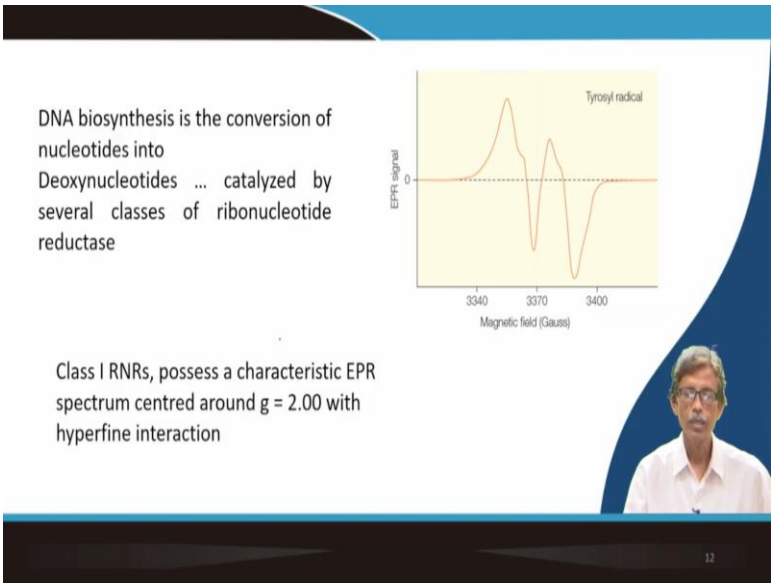

So, lastly I will talk about the example, what example you can find out, what EPR spectrum you can find out? In a biological world also; we can talk about the DNA biosynthesis. And which is nothing but the conversion of nucleotide into the deoxynucleotides. That means one OH will be converted to HC-HC; OH will be converted to CH. So, deoxynucleotide from the sugar part that ribose sugar will convert it to your deoxyribose sugar. And which is catalyzed by several ribonucleotide reductases; that is why the ribose sugar, ribonucleotide and the reductases.

So, if you go for the reduction reaction with that of this particular system, like that of your class one ribonucleotide reductases, that can be characterized again by your EPR spectrum. And which is again centered around g is equal to 2 or 2.0023, like your standard reference material DPPH we call. Whenever we measure the EPS spectrum, will give the DPPH diphenyl-picrylhydrazyl; which is a very stable radical system, which is stable in room temperature, which is stable at the room solid state, and which is also stable in solution also. So with that hyperfine interaction, we find that particular one; what we find is that particular spectrum.

So, this is your corresponding tyrosine radical; it is not a copper center thing. It is a tyrosine radical and that tyrosine radical is further splitted. So, you have the magnetic field, you have the EPR signal, the signal intensity and you record it. So, you see the range we are considering; so you have the corresponding field values in Gauss, and that field values will be converted to the

g-value. And then we can find out what are the different A values, capital A values for this particular interaction; that means the hyperfine interaction.

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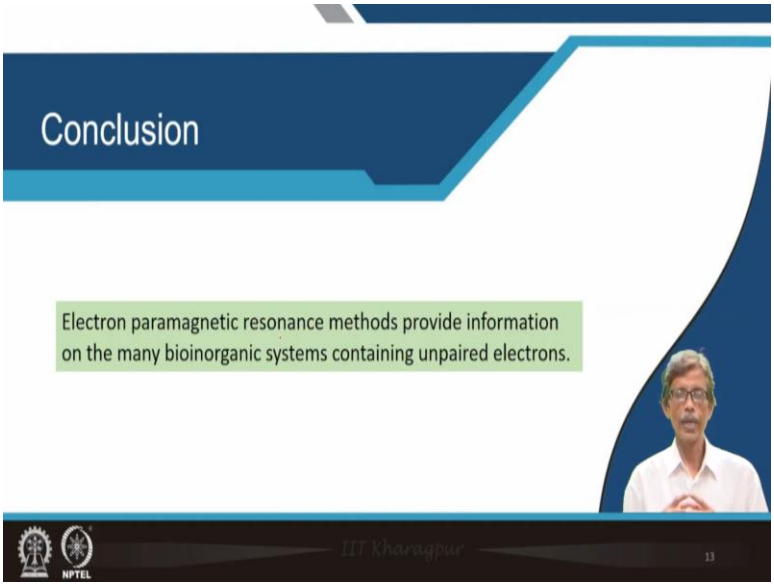

So, finally what have see? We have seen from this particular study that electron paramagnetic resonance. So, this is a particular spectrometric method, provide useful and very good informations about many biological systems; particularly of metal ion origin, or particularly of radical origin. So, if you have a tyrosine radical, or if you have any other radical system like that, of your DPPH radical, we will be able to detect that in solid state as well in the solution state. So, any unpaired electron, so what you can do quickly, you go for the measuring the magnetic moment. Once you measure the magnetic moment, you go for running the corresponding EPR spectra.

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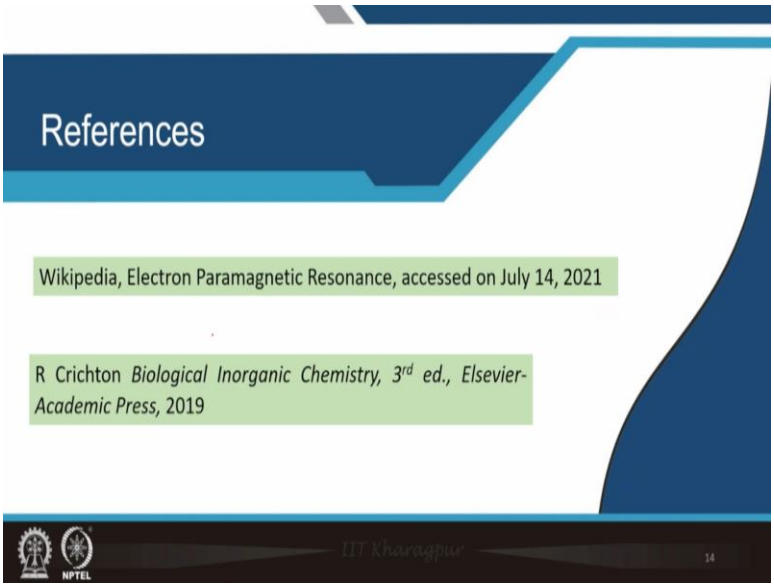

Then finally the references, again, the go visit the Wikipedia page of Electron Paramagnetic Resonance page also, and with that you get the corresponding cross-references. And you did a little bit from there first, and then you go to the corresponding book, what I am giving every day to you. And that reference will also be helpful for your understanding. So, thank you very much.