Biological Inorganic Chemistry
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Lecture - 12
CD and Raman Spectroscopy

Hello, good morning once again, so we are continuing the different instrumental techniques. So, in this particular class, which is under that again physical methods, we will talk about the two techniques particularly, the circular dichroism technique and the Raman spectroscopy technique.

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So, if we consider these two techniques, and if we try to put on these in a fashion that what are the concept; because they are very basic concept basically. What does it mean like that of your electronic absorption spectroscopy? Because, it is not possible to discuss everything in detail. But you should know the technique that this technique will be useful for the determination of this particular parameter of this particular information you can gather it. Then two Raman spectroscopy techniques, mostly we should always be very much careful about what is known as the conventional Raman spectroscopy; and what is known as the resonance technique or the resonance Raman spectroscopy.

Because afterwards we will consider three of them together and we will consider that resonance technique. Sometimes we ask the what are the resonance techniques you know? So this is one such example, which is your resonance Raman spectroscopy. Then two other techniques are

NMR methods and the EPR methods, which is nuclear magnetic resonance; and another is electron paramagnetic resonance.

So we should know about what is resonance; so this particular terminology because these you can use as the keywords, you go to Wikipedia page or internet anywhere you can go or book also; you try to search it in that fashion that I should know about the resonance. What are the resonance techniques you can have whether you are talking about the Raman spectroscopy or NMR spectroscopy or EPR spectroscopy?

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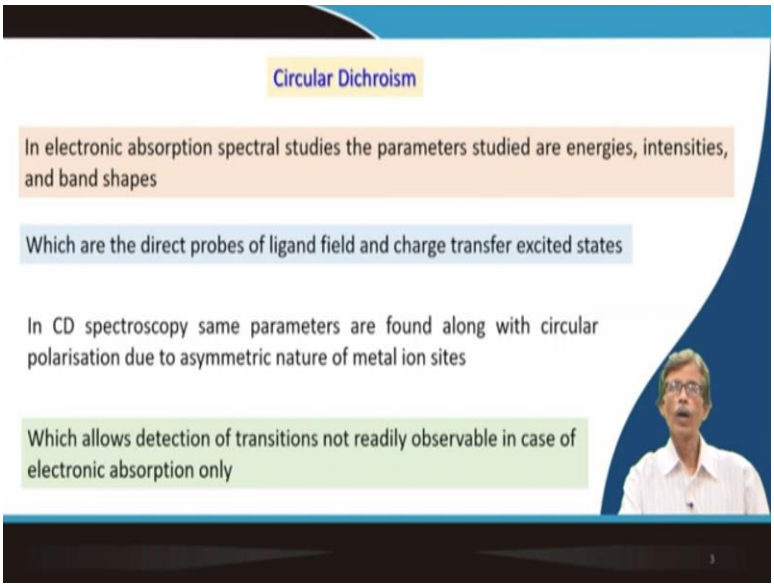

So, what is that circular dichroism? How it is related to the typical electronic absorption Spectral studies? Just now we have seen that why you have taken the example of electronic absorption. That we know that you have the sample and sample you placed in a cuvette; and you exposed it to the corresponding electronic radiation in the range of say 380 to 780 nanometer. And we will find at some point if you scan it, there will be absorption. So, when it is matching for your electronic transitions from the E1 level to E2, you will have the absorption; and that we can see in the corresponding spectrum.

So that spectrum can give you the color and many other informations, if we consider that that particular transition is due to the d-d transitions, centered on the metal ion. Because we are always very much interested to know about the corresponding thing about the corresponding transition metal ions, like manganese, like cobalt, like nickel, like copper. So if we consider these

transition metal ions, all we know is that the transition metal ions; when it is dissolved in water medium or in aqueous solution, it is colored. So, you have to catch that particular color in terms of your lambda max value as well as in terms of your epsilon max values.

So, what do we get from there is the parameters, the energies lambda max in nothing but your energy; that means the delta e value. So this delta e value all we know is equal to H-Nu and H-Nu, when Nu is converted to your Lambda, you get the corresponding lambda value. So, how much they are intense also, the intensities that means your epsilon max values or the absorbance values. So, the molar absorptivity is basically tell you about the intensity of that particular electronic transition.

Because when you try to determine a very low concentration, you have a protein, very huge molecular weight, several thousand molecular weight. But only you have one metal ion is present in it. But not that metal ion, but your protein is also very much expensive in nature; because you have to isolate it from some biological origin. So, it will have some time taken thing, and it will have some, also some chemicals also required for that; so, we try to avoid that to increase the concentration. But, how to detect the very low concentration of iron present in that particular protein sample that we have to see, and finally the band shapes.

We are writing something as the typical Gaussian shape of the spectrum; but it is tilted towards one side, it can be tilled towards the right side, or is a typical bell shaped one. So, the shape can also have some good information to give us. So, these are basically direct and a lighter analytical thing or direct probes of ligand field and charge transfer excited states. So, if you have a ligand field for the d-d transitions, and we find that d-d transition is there; so the ligand field is only you can consider.

But from the molecular orbital point of view, if you consider that the ligand orbitals are also taking part into the situation; such that your for your carbon monoxide or cyanide ions also, you have to consider about the CT transitions, the charge transfer transitions. So, the charge transfer transitions in the excited state also you have to find out for that particular situation. So, what we usually find? So in CD spectroscopy, same parameters are found along with circular polarization. So if the light, your light is circularly polarized, due to asymmetric nature of the metal ions sites in it.

So that we find that the polarization circularly polarized light you use, and due to asymmetric nature of these metal ion sites, you have to use the corresponding spectroscopy, which is your CD spectroscopy. Which, will allow you to detect the same type of transitions, the electronic transitions; which are readily observable in case of electronic absorption only, but having some special features. So what are those special features that we nee now. Why you are using CD? Why not the simple absorption spectroscopy or electronic spectroscopy?

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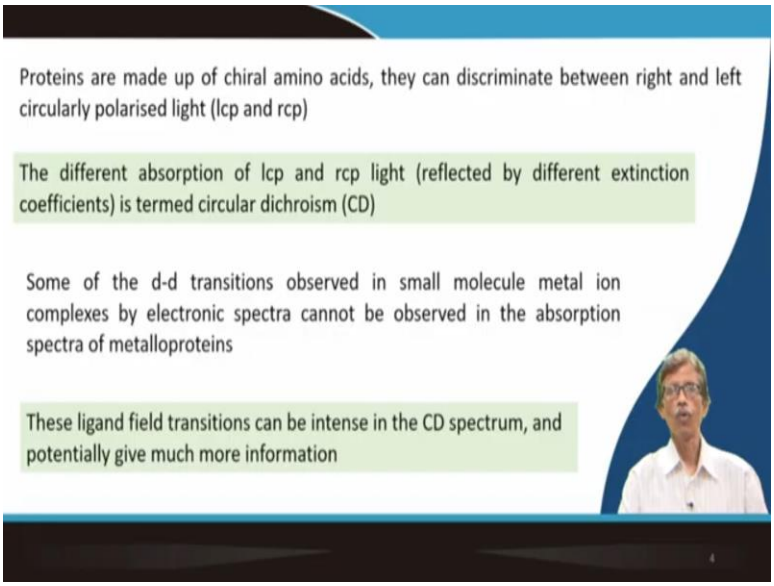

So, what you find there that we have the proteins, and all we know that the proteins are made of chiral amino acids. So, that is why you require a circularly polarized light; so the optical activity, probably you know little bit. That is why the optical activity that ORD, we need optical rotatory dispersion we call. Similarly, the circular dichroism also can come into the picture, if you have a chiral amino acid or the metal ion center can also be chiral, depending upon the helicity of the ligand, which is binding to your central metal ion center. So, you have two circularly polarized light, one is the right circularly polarized one or lcp; another sorry rcp, and another is the left circularly polarized light or lcp.

So, now will find that how these two circularly polarized light can be useful during this particular electronic absorption process. That is very simple one, very much close to that of your absorption spectroscopy. Now, we find that these two lights basically lcp and rcp lights will absorb differently. When we find that, we have the simple light, which is passing through the

sample of say methyl red, we see one typical band. But if you are now having a different sample little bit complicated sample having chiral amino acid or a chiral metal ion center.

Now, instead of passing this light, as the simple light, you pass the light as circularly polarized light; and try to draw the same absorption spectrum. You will find two different types of spectrum due to this particularly different types of lights; because you have helicity, different level of helicity. So it will absorb differently with the lcp, and will absorb differently with rcp. So, some of these d-d transitions, what we find for this particular electronic transition; say for iron centered one, or for the nickel centered one is small molecule metal ion complexes. We find by electronic spectra, which cannot be observed in the absorption spectra of the metalloproteins.

Because as I told you that the d-d transitions are weaker; and these d-d transitions are unlike your charge transfer transitions. So, when you have only as one iron center in which protein structure, your concentration of that iron center is less. So, the signature for that iron center is very difficult to detect through the conventional spectroscopy or the electronic spectroscopy. Either you have to increase the concentration of that iron, which is very difficult to do or you have to go for some other technique, where you can increase the sensitivity of the instrument; sensitivity of the detection technique for this metalloproteins; that is why you have to go for CD spectrum then.

And if we clearly monitor that it is possible to find out the ligand field transitions, which can be intense in intensity; and can potentially give many more informations, which we do not able to gather from simple electronic spectroscopy.

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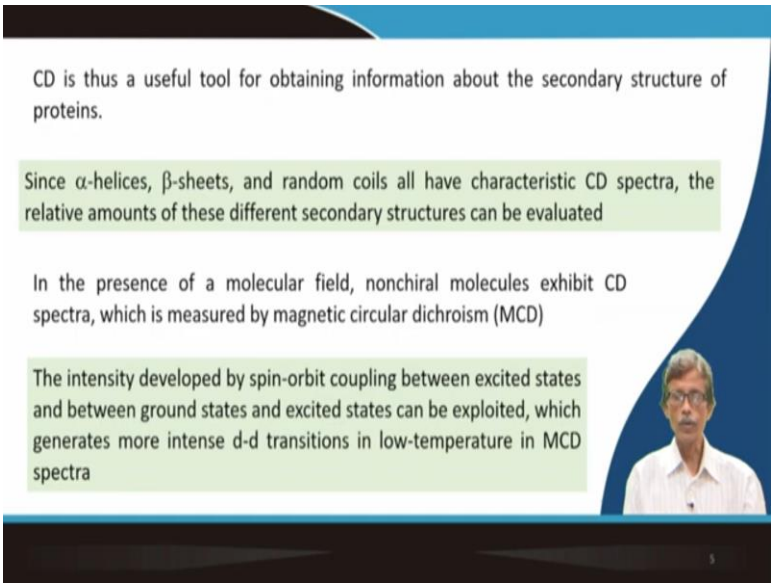

So, CD is thus in our hand and can be useful tool for obtaining many information about the secondary structure of the proteins. So, when we write this particular statement, you can also write it in your notebook and try to understand, we are talking something, which is little bit complicated from your simple electronic spectroscopy; where you can use simply by replacing our eyes by colorimeter, and from colorimeter to a spectrophotometer. But now from spectrophotometer we are making our life little bit complicated, by moving from spectrophotometer to a CD spectrometer.

Why? Because your sample is also getting complicated, is not that simple iron you are measuring. The concentration of iron you are measuring, or the iron in amine environment, iron in EDT environment, or iron in any other ligand such as 8 hydroxyquinoline you are measuring. But you are measuring this particular thing in a complicated very huge ligand environment of biological origin, such as your protein from metalloproteins or metalloenzymes. So, you have to go for little bit complicated fashion, because we have this within the secondary structure. So, what are the primary structures of the protein?

You can recall back, in my previous classes, I have discussed nicely; what is your primary structure of the protein and what is your secondary structure of the protein. And there are also, we have discussed again by showing the figures also, what are alpha-helix, and what are your beta-sheet structures. So you have the alpha-helices, beta-sheets structure and random coils, so,

coiling also you can have. And these three things together, if we consider that I want to determine the alpha helix; the concentration of the alpha-helix also sometime, concentration of the beta-sheets that how much beta-sheets are there. And the amount of random coiling present in that particular structure.

So, the relative amount of these different secondary structures can be evaluated. So, when you have these component, you get the secondary structure in the protein environment through the coordination to the metal ion. So, you have a molecule and magnetic field or the molecular field; sometimes we find that one step ahead to from CD. Then, if you have the non chiral molecules, they can also; because we are talking about the chiral molecules. But, if you have the non chiral molecules that can exhibit CD spectra; but now you can have other technique, where we can consider it as a magnetic circular dichroism technique.

So, you can have some much more complicated one, where the intensity developed by typical spin-orbit coupling is a magnetic phenomena. We know that we get the spin magnetic moment through the spinning of the electron, and we get the orbital magnetic moment through the orbiting of the electron around the nucleus. But they always give the magnetic momentum the mu value, the mu Bohr magneton we write. And when they are coupling together in the one state and the excited state; between the excited states, you have two excited states. And they are coupled together between these two excited states, or between the ground state, and the excited states; can be used when you study MCD.

So, they go for the intense d-d transitions in lower temperature. So you have to cool down the system, cool down the sample such that you can increase the intensity of the d-d transition; so that is the ultimate goal of the thing. Since your metal ion concentration is less, your metal ion responsible for d-d transition is also less. So, you go for some technique where you can increase the intensity of that d-d transition; so one such is your MCD technique.

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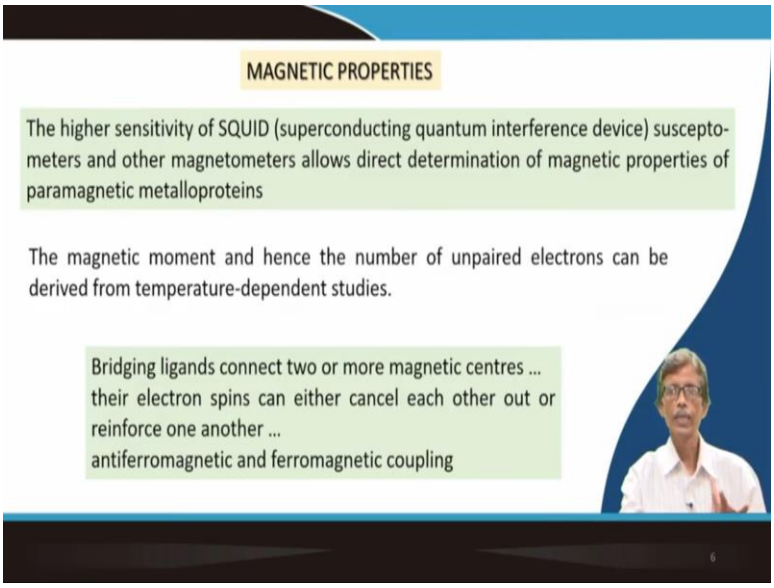

Then we can talk about since we are talking about the magnetic thing. Then when the magnetic properties, all we know that how we can measure the magnetic moment. So, these magnetic moment measurement is also very useful one. But again more sophisticated instruments you require; so highly sensitive SQUID you require. So, which is scope superconducting quantum interference device, which is nothing but your determination of the magnetic moment of say once sample, paramagnetic sample within a huge envelope of protein structure, say several thousand molecular weight but one iron center.

How to detect the magnetic moment of that sample? So definitely you need a highly sensitive super conducting quantum interference device, compared to your typical gouy balance, or other kind of susceptibility balances, or magnetometers. Because most of the time, we will find that your metalloproteins are paramagnetic in nature; so we should know about the magnetic moment of that particular metalloprotein. So, that in the characteristic property or characteristic parameter, which can tag we can tag that with that particular sample.

So, the number of unpaired electrons, whether you have a copper center in copper protein, how many copper centers are there? Can be determined if you go for the temperature dependant studies. So this temperature dependence studies can tell us that your magnetic moment is changing, because the magnetic property is temperature dependent all the time. The chi-G value

and the chi-m value, and finally the mu effective or the mu Bohr magneton value is dependent on the temperature.

Now, one more important thing now we are talking about here, what we discussed in my previous class, just immediate previous class that the cysteine residues, two such cysteine residues are available, which are bringing their to hold these two are metal ion center, one is your iron, another is your nickel; so it is coming like this.

So you have the bridging from this sulphur, and bridging from this another sulphur. So, if you have bridging ligand and which are connecting or clipping, we call it is clip. So, which are clipping or connecting two magnetic centers that means two metal ion centers. Then you have the election spins within these two metal ion centers; do not go for this complicated iron and nickel center; think about simple metal ion salt like, copper acetate.

We all know that the copper acetate is a dimer; so this salt is available in any lab laboratory, in school level laboratories it is available. But if you go for the measuring the magnetic moment, you find some unusual result, as the magnetic moment is less; not single electron paramagnetism it will show, but less than that magnitude. So, something is happening there, what is happening? So, some interaction in taking place, because you have two copper centers nearby; close by you have two copper centers, which are clipped by acetate. So, four such acetate clips, are there. That is why your formula of the copper acetate is not CuOSe whole 2 copper, which is the bivalent one Co2 plus.

Charge is balanced by 2 acetate anion is not that, it is whole of 2 again. That means you have 2 copper centers and 4 acetate ions and 2 water molecules at the two terminal positions. So, like that of your cysteine residues, you can have the corresponding acetate anion simple acetate anions are there.

If it is available, it can be used for coupling between these two electron spins. Either they can cancel or you can add up; so you can have two types of coupling. One is anti ferromagnetic coupling, another is ferromagnetic coupling; so that we will also discuss, even if you simply studying the copper protein. So, the magnetic properties of these copper proteins are also useful to understand to know about the interaction. And at least you should know that no these two metal ions are nearby. That is why they are interacting; that means they are very close.

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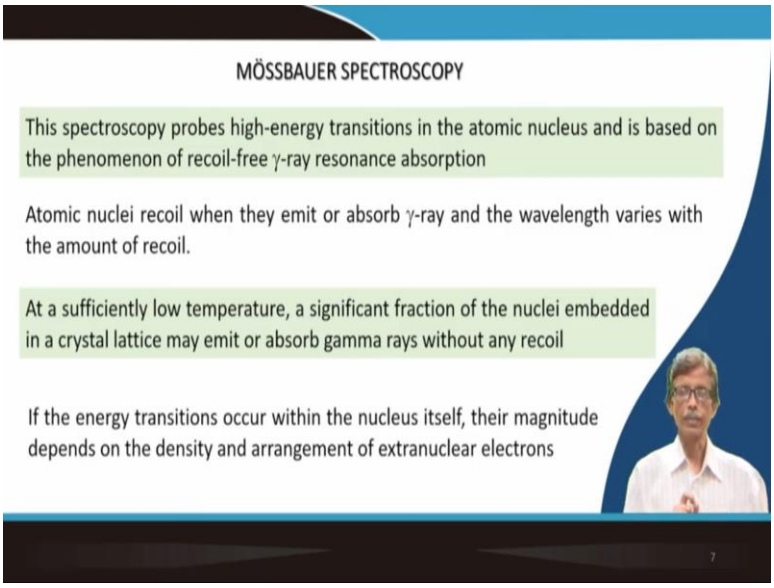

So, the catalytic cycle, whatever we are talking about, is related in that presence or positioning of these two metal ion centers. One more technique, which has basically talked here is the Mossbauer spectroscopy. So, Mossbauer spectroscopy mostly people talk in terms of the iron center. So any iron materials, solid state material we can identify; or any such iron in proteins; because the material, which is there as iron ion in the biology. So, iron proteins or iron enzymes, or iron biomolecules are huge. That is why you can have a huge book, a complete book you can have; where iron in biology can be written.

So, all these iron samples in biological origin can be studied by this particular spectroscopy; so is a different type of one. So, that is why I am tempted to give you the example of these; at least you should know the same basic principle of Mossbauer spectroscopy. Is nothing but the high energy transitions, so is the nuclear level transitions. When you can have a gamma ray, so if it absorbs the gamma ray. The source is also something related to that of 57 iron center, which is providing you the gamma ray; but your sample can absorb that particular gamma ray, and show some feature, show some absorption.

And from that particular absorption, you can find out that this particular sample, which is iron; but it is Mossbauer sensitive. Since, it is giving Mossbauer spectral pattern or Mossbauer spectroscopy signatures. So that particular nuclei that means atomic nuclear which iron; it can recoil when they emit or absorb X-gamma ray; and the wavelength varies with the amount of

recoil. So if your source is a different iron center iron 57, but your iron protein or iron metalloenzyme is there, so it is supplying this and it is absorbed, but recoil is, no recoil is there.

So, recoil free X-ray resonance absorption can take place, gamma ray absorption can take place; and we can have some corresponding signature for your Mossbauer spectroscopic identification. So, it is the low temperature identification, and some of these nuclei is embedded in the crystal lattice may emit or absorb gamma rays without any recoil. So, if the recoil is not there, we know that it can absorb some gamma rays. But when the energy transitions occur within the nucleus itself, that means the nuclear energy levels, like electronic energy levels we can have the nuclear energy states; one is ground state, another is excited state.

So, you can if you can promote the nuclear level from the ground state to the excited state through the absorption of gamma-rays, what we find? The magnitude, basically the magnitude of absorption depends on the density and arrangement of the extra nuclear electrons. That means if your surrounding around the iron is changing, it can have effect on the nuclear transitions; and different level of that particular recoil free gamma resonance absorption can take place. And you can have the corresponding Mossbauer spectroscopy signature also, which is different.

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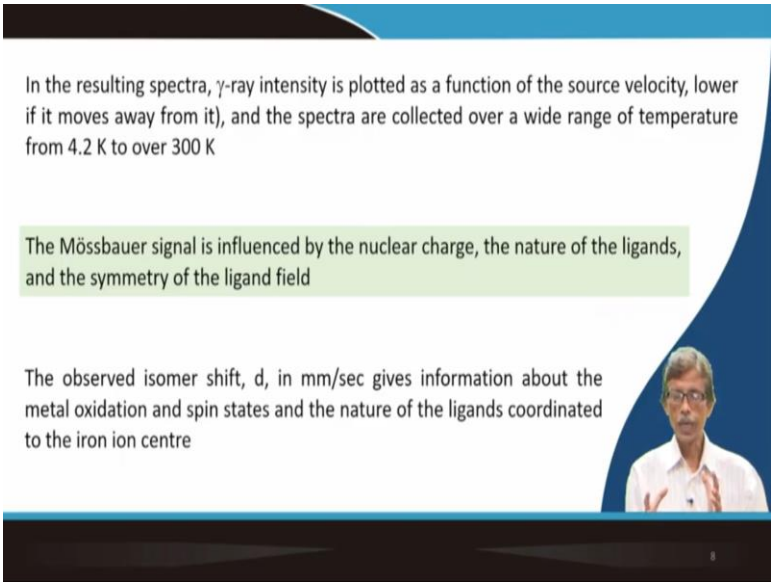

So, what do we get? We get a spectra, and where the gamma ray intensity is plotted, as a function of source velocity; so, now you see the recoil velocity. So, something is moving, so is the moving thing is there for the spectrometer within the spectrometer; all arrangements are there

is a definitely a complicated arrangement. So, lower if it moves away from it, so that is why you find the Doppler Effect. And the spectra are collected over a wide range of temperature from 4.2 Kelvin, which is your liquid helium temperature to 77 K, which is your liquid nitrogen temperature, to room temperature which is your 300 Kelvin.

So, this signal what we get is influenced by the nuclear charge. This nuclear charge is fine for the type of iron center is present; if it is 57, we know the nuclear charge; then the corresponding nature of the ligand, which is our ultimate goal. Why we are going for Mossbauer spectroscopic measurements? So the nature of the ligand we can find out and the symmetry of the ligand. Suppose your iron center is five coordinated. So, whether we should go for a square pyramidal environment, or a trigonal bipyramidal environment; or a more distorted one, which is neither square pyramid, not trigonal bipyramid.

So, this particular symmetry of the ligand field surrounding this particular iron center can give you good informations, which can be identified by these spectroscopic technique. So, Mossbauer spectroscopy that is why is a very useful one. And the observed isomer shift, the parameters what we can derive in d in millimeter per second, which is the movement, which is the velocity; gives also the metal iron oxidation state: the spin state, the nature of the ligands coordinate to the iron ion center. So, you see many informations what you are gathering from there is important.

Suppose you have iron site, and if I ask you I want to identify. First, you know, that iron has been identified in myoglobin say. Then what you should know about that particular iron? So, the qualitative identification of iron is that it is iron, which is present in your myoglobin, which is present in your hemoglobin, which is present in your haemprotein. And which is also present your hydrogenase; just now we have seen in our previous class. So what you find now that not only your nature of the iron is important, but the corresponding oxidation state is also important. Whether your iron is the ferrous iron or a ferric iron; that is also a typical question always we ask.

Then the spin state, whether you have a high spin state or low spin state. So, slowly we are making our life complicated, but in one step by measuring the Mossbauer spectroscopic signatures; you can find out all these informations along with those ligands, which are coordinated to the iron center. That means if it is myoglobin center, what you find that you have

4 nitrogen centers, then the globin center, then what you know? We know that when myoglobin is taking up oxygen giving you oxymyoglobin. So, one oxygen will come from the top.

So, you have a octahedral environment of two types of nitrogen all together five; four from porphyrin ring, one from the global nitrogen, and one oxygen from the O2 molecule. When oxygen is not there, it will be occupied by water molecule. Now, it is important to know what is the oxidation state? Because the electron transfer can take place. Because initially people were puzzled to identify the oxidation state of iron in hemoglobin and myoglobin, then the spin state also. Because we know the O2 molecule, which is in air also, which we are taking during our breathing process, O2 is a triplet one having two unpaired electron.

So, two up spin, one up spin oxygen molecule having two unpaired spin. So, the spin state for the O2 molecule is important. Because when it is coordinating to your iron site, we should know what the oxidation state, as well as the spin state is changing, and then the environment. What type of nitrogen you have? Where your oxygen is coming? So, all these informations can be found out, if you nicely analyze the Mossbauer spectroscopic data.

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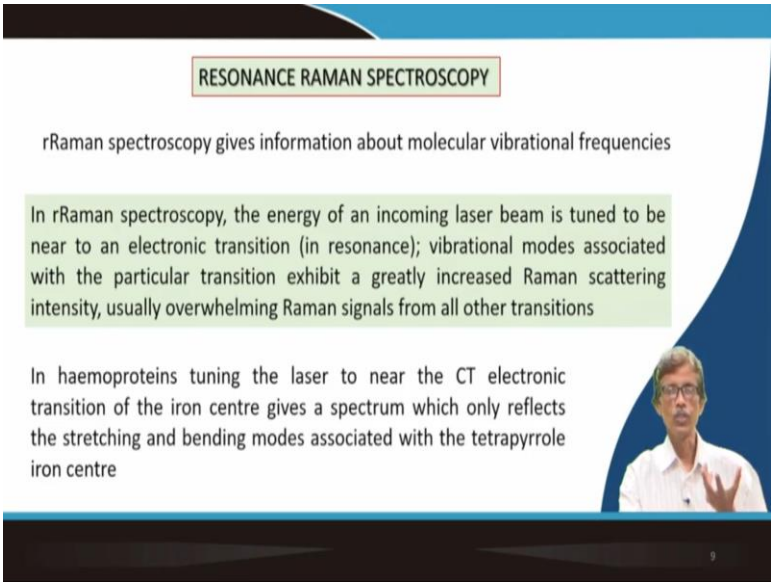

Then quickly will see the resonance Raman spectroscopy. In resonance Raman spectroscopy give the informations about the molecular vibrational techniques, which is very much similar to that of your IR spectroscopy. But, which is a resonance, that is why small r is attached over here. So, vibrational frequency, we have a bond say carbon monoxide is attached to your site. What

we have seen just now in your hydrogenases metal ion site, carbon monoxide is there. So, CO stretching frequency, the bond stretching frequency when it is stretched; when we are stretching the carbon and oxygen is elongated bond, and the compressed bond.

So, the bond stretching frequency, you have a characteristic stretching frequency in FT IR. Similarly, the Raman signatures are also there and which in little bit sophisticated one. That is why every time, whatever we see that we need much more sophisticated analytical technique to identify these metal ion sites in biology. So, your biological life is much more complicated and you should be competent enough even spectroscopically also to identify those sites nicely through spectroscopy, through X-ray structure determination, or through any other technique.

So, we find that when you apply the resonance Raman technique in haemproteins, you have the laser there; because it is not that IR radiation is only fine. So, charge transfer electronic transition can take place and that particular charge transfer electronic transition, you excite the sample. So that is why you can have a resonance and that resonance can be useful to identify the stretching and bending modes, associated with tetrapyrrole iron center, which is your backbone of your haemproteins. Whether you have the myoglobin and haemoglobin, or whether you have the cytochromes.

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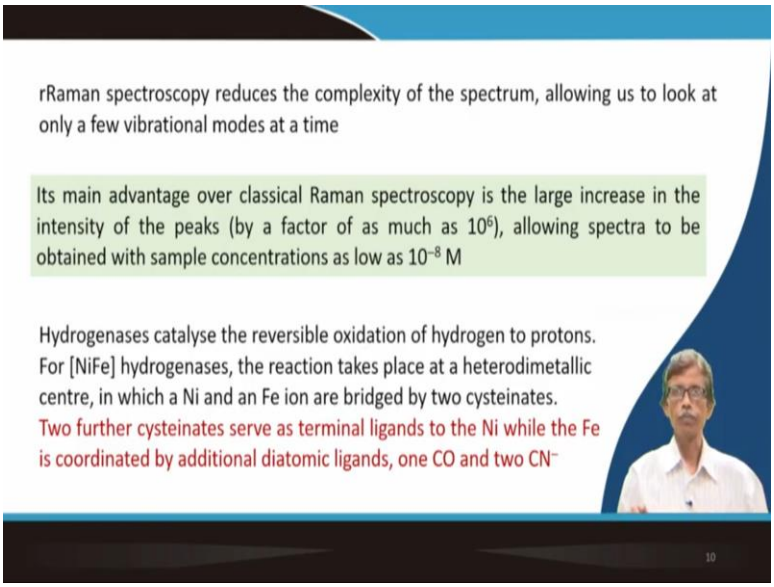

So, resonance Raman spectroscopy reduces the complexity of the spectrum, allows us to look at only a few vibrational modes at a time; because the intensity is enhanced, is resonance. That is

why all these resonance techniques are useful. The resonance is taking place, so intensity of the weak signals are enhanced many times; and that enhancement we are nicely picked up and we can find out the corresponding absorptions. So, its main advantage over classical Raman spectroscopy, where small r is not written, only Raman spectroscopy technique. Because you can increase large increases in the intensity of the peaks of the order of say, 10 to the power 6; you see the magnitude of increased.

So, 10 to the power 6 times you can increase the intensity. So you can go down to a concentration, which is 10 to the power 6 times lower. So you can handle a concentration of 10 to the power minus 6 molar concentration very quickly by this particular technique. And that is why it allows the samples concentration to go down to 10 to the power minus 5 molar concentrations. No other standard techniques can be so useful to find out or handle this particular low concentration, which we can use for your metalloproteins or metalloenzymes. So, what we find why it is useful? So read it nicely, understand it nicely.

I will not read all these things nicely from there. But what we are looking for? We are looking for the binding of Co and CN minus in hydrogenases and during the catalytic cycle also, in the very beginning we have seen that you have X between iron and nickel site; you have iron site, you have nickel site, you have X in between. So, whether we can able to identify that X also through this particular technique? So, all these structures have been explained over here. What we have seen in the figure, this is there in language also is given to you.

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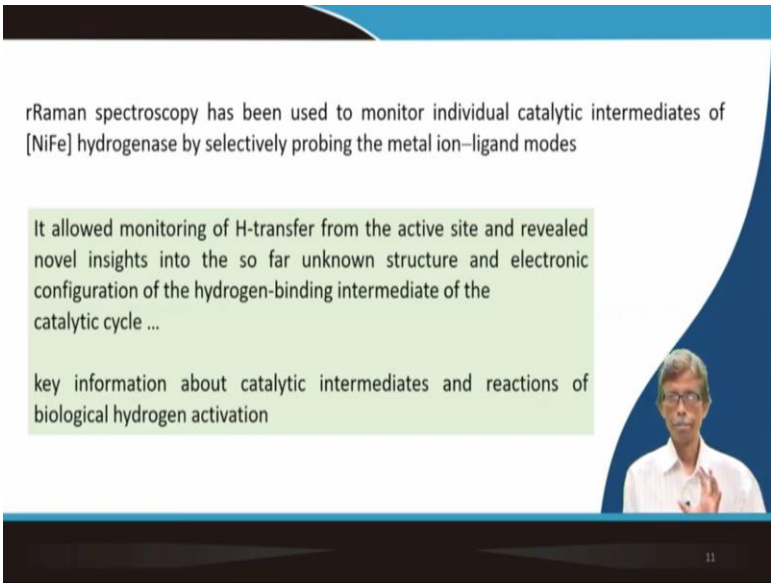

Now, when you find that the in resonance Raman spectroscopy, which we monitor the catalytic intermediate; whether your hydride is bound to these two metal ion centers or not, that we can find out during your catalytic process. So, that is why the technique is very important that allows us to for the hydrogen atom transfer from the active site and revealed the novel insights into the structure and its function, even the catalytic cycle.

So, is the key and useful information about the catalytic intermediates and reactions of biological hydrogen activation. We know about the active site, but what is happening during the catalytic process; that we can find out from your, this particular r Raman technique and resonance Raman technique.

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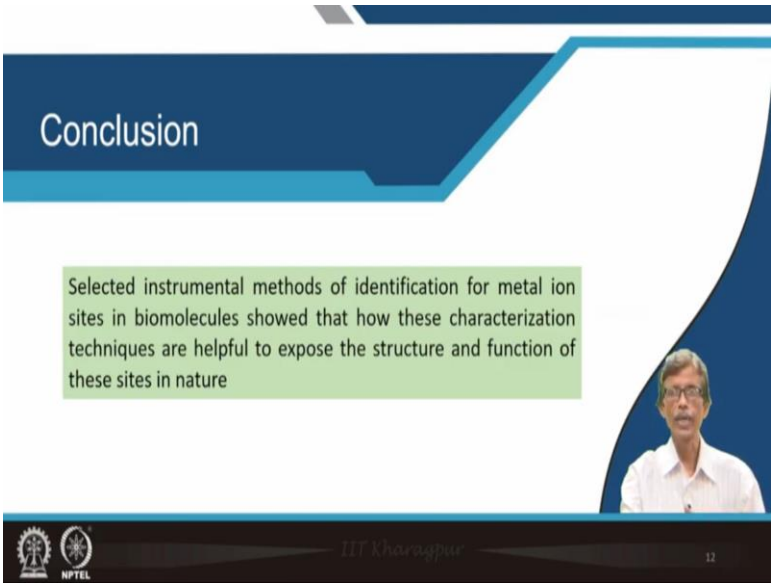

So, let us conclude here, now so the different types of instrumental methods, for finding the nature of the metal ion sites in the different biomolecules. And their characterization is very useful, which can expose the structure and function of these active sites in nature. So, the natural sites we can identify, if you are master enough in all these instrumental techniques.

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So, again the references, then Instrumental Chemistry also, you can have Wikipedia page for that and take out all the different techniques. You go for the CD, you go for the resonance Raman and

find it out there from nicely and the book, definitely the book also. Thank you very much for your attention and your presence.