Inorganic Chemistry of Life Principles & Properties
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Lecture - 14
Techniques used inorganic chemistry life - continuation

Welcome to the next lecture on Inorganic Chemistry of Life. In the previous lecture we have looked at some techniques of this spectroscopy and electrochemistry is NMR spectroscopy nuclear magnetic resonance, electron spin resonance spectroscopy and then also electrochemistry. All of these are extremely useful techniques for studying the reaction dynamics, product formation all these kinds of things or the enzyme related once protein related things as well.

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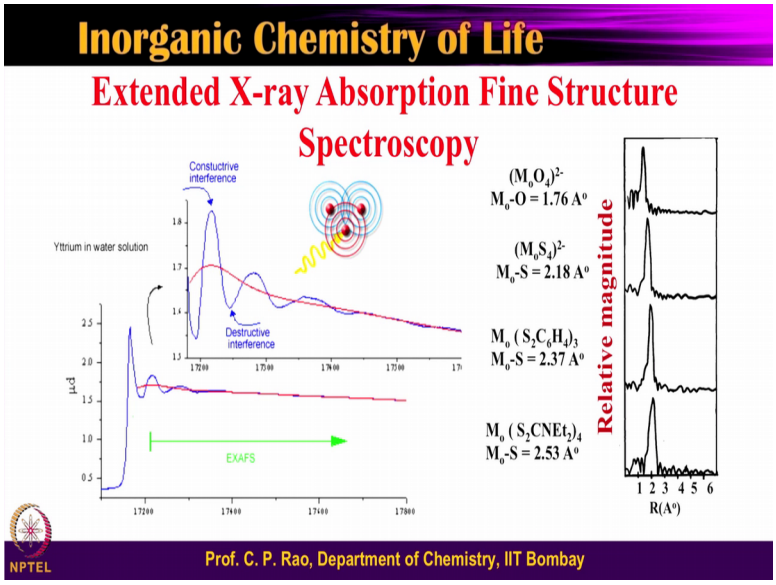

Now, let us look at another technique, next to that is called the extended x ray absorption fine structure spectroscopy. It is a short form is generally referred as EXAFS: E X A F S.

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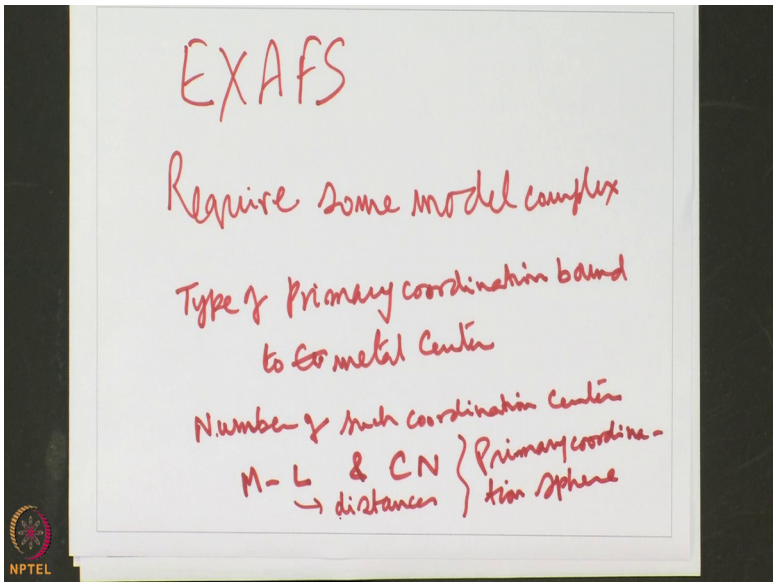

So, extended x ray absorption fine structure spectroscopy what is this used for, why is this used. This is used basically to get in a metalloprotein or a metalloenzyme, there is a metal ion, there are side chains are attached either through o or n or s are combination of these that is called primary coordination.

So, in order to get the primary coordinated atoms to the metal centre, one can use this particular spectroscopy, which is called extended x ray absorption fine structures spectroscopy. It is at the fine structure coming from the x ray absorption method and that is what we look at and the we will come to the principal just in a while and therefore, this is very useful.

Another aspect why people use EXAFS why not crystal structure? We have already talked to you few lecture prior to this x ray crystallography we talked about different resolutions, you can get the entire protein to a resolution to a very high resolution as well. But when you have such a thing where do you want to use this one. For x ray diffraction you need a single crystal for EXAFS data, you do not require single crystal, the sample can be in the form of a solution, it can be form of a powder it can be any form uniform there is no necessity of having a single crystal to be used.

So therefore, in such cases we use extended x ray absorption fine structure; however, the information is very very much limited. That means, you get primarily the primary coordination sphere, but not entire protein structures, where as crystallography you get

the entire protein structure that is one if its, but in some cases we required that to know the metal bound region etcetera

Second thing is that it is not you know simple to use this to resolve the fine structure that you got, if you want to interpret in terms of the metal ligand distances and their angles, we need some model complexes or which are very close to that. So therefore, one needs to study the EXAFS studies even on the model complexes too. So, require some model complexes. So, in you basically use these model complexes study because in model complexes already - crystal structures are known therefore, from that you can try to get the parameters and use those parameters to solve the primary coordination structure in the metalloproteins and metalloenzyme.

Initially it has been you used quite a lot for the molybdenum case and later on there are several cases even for ion enzymes as well take this let us look at the principle. Let us take this is the metal center and there are some ligands attached to that or primary coordination centers. So, when you incident x radiation obviously, we know that the x radition can now got even the core electrons, the electron is knocked out electron will move out of this.

So, electron beam of the electron moving out. So, when it moves out, it will start interacting with the electron cloud of the neighbour atoms, which is called the ligating centers. So therefore, this wave of the electron wave that coming out of this will interact with the electron cloud of this ones therefore, you have some kind of an interference between the incoming or outgoing electron and the neighbor atom electron densities.

So, that interference can be constructive can be destructive. So, when the interference is destructive, you have no peak when interference is constructive you will get a peak. So, this is what you are saying a peak that you are saying. So, that is how one can explain and of course, this is from the distance from the centre of the metal ion. So, you can get. So, you get peak of this kind and this kind this can be can be taken in water any kind of a solutions lot of not a problem, and can we converted into the distance versus the relative magnitude relative intensity kind of a plots ok.

So, to convert those things you require certain parameters and those coefficients of parameters are obtained by studying for the model molecules. So, for the model molecules, you can get all those coefficients and those coefficients you can use it. Now

here on the right side you have a case or an example, where the molybdenum is bound to here tetraoxo tetrathiol and dithio benzene kind of a complex or dithiol (Refer Time: 06:49) kind of complex. So, you have all these are their centers. So, these are all small molecules.

So, from the small molecules; obviously, you can get corresponding the metal to the ligand distance you can get from this ok. So, this is metal oxygen and this metal sulphur. So, this a h sulphide kind of thing, then it is a tilade sulphur you know that the metal oxygen when it is single bond versus metal oxide double bond you will have a difference between these two. Metal oxygen single bond metal thio single bond, there will be again the lengthening other thing and from a sulphido bond to the metal to the tilade bond to the metal again you will have a increased.

So, therefore, this bond distances will differ and from the intensity, you can try to quantify how many such atoms are present. So therefore, by analyzing this entire data so, the by analysis, you can get the type of a primary coordination type of the primary coordination bound to metal centre bound to metal centre o you can also get the number of such condition centers.

So, essentially you can get coordination number and the metric data from the metal to the ligand distances; so M L and coordination number. So, these can be obtained M L distances and the coordination number. So, this is what. So, what it constitutes. So, constitutes the primary coordination sphere. So, one can resolve the primary coordination sphere using EXAFS technique.

So, that is to make it simple I am not taking him to more examples etcetera and it can be a study. All that you required is sample can be in any form, it can be liquid it can solution it can be anything or it can be you know put on something else, anything can be studied it can be water solution etcetera, but you require some model molecules. So, some model molecules will help you to get this coefficients and the coefficients will help you to solve this solve this particular spectrum into a useful; useful means distance relation distance reintensity this one.

So, you have to convert from this to a distance. So, this is coming from the interference, this is upon after conversion by using certain kind of a transformation certain kind of a

fourier transformation you will get this. So, this is actual spectrum transform this is the raw data. So, raw information from this you can get this.

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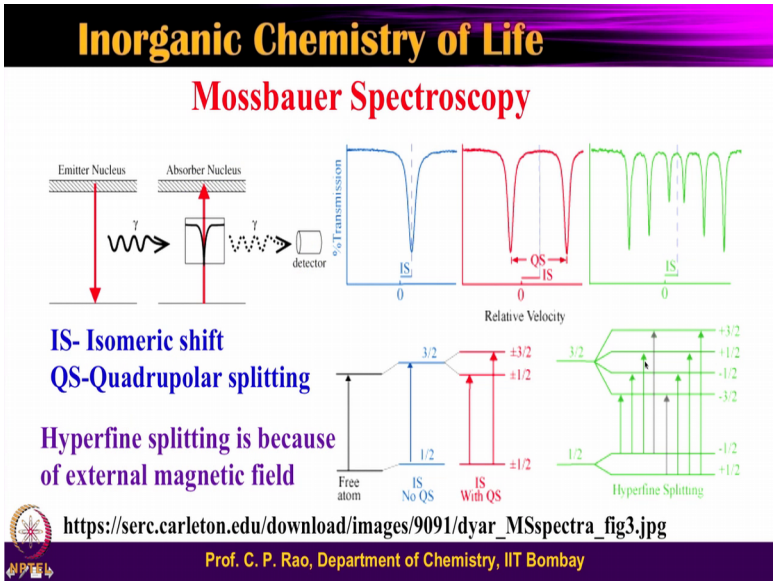

So, let us now look at another technique, which is a nuclear technique. So, nuclear transitions, we have looked at the nuclear spin transition earlier electron spin transition and now we looked at the nuclear transition ok.

So, the nuclear transitions are basically in the gamma radiation. So, they are very high in energy nuclear transitions are gamma radiation so very high energy. So, these form a nuclear transition.

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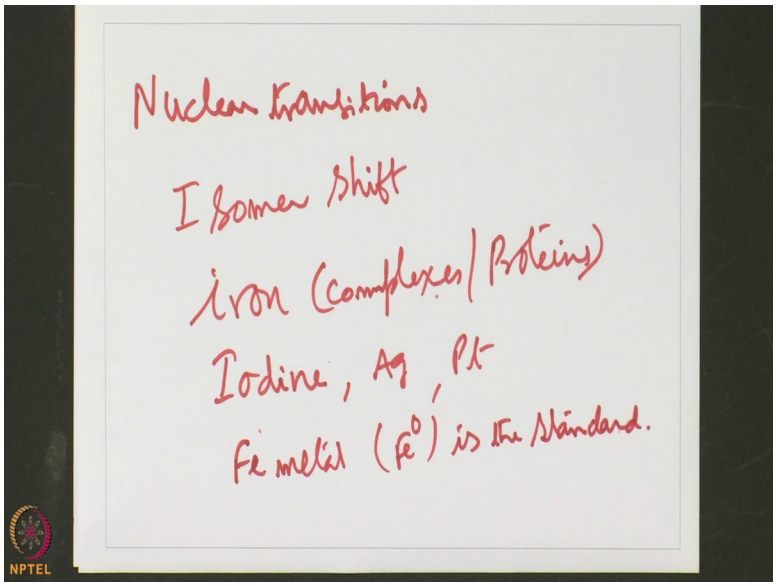

To understand this background for this, you need to understand the just like electro distribution nucleon distribution there is something called nuclear shell model just like we have the electron shell model more shell model. So, similarly you have so that will talk about the placement of protons placement of neutrons etcetera, but that will be outside the scope of this particular course. So, I am not going into the detail of this.

Rather I will try to take you to the utility of this how one can use it, what is the simple basic principle associated with it. In NMR what are you looked at? We look at the chemical shift. So, in the in the Mossbauer what you will see equal into that chemical shift is called the Isomers shift. So, this is removed referred is a isomer shift. So, what is this isomer shift and how does it come? Isomer shift suppose you take these the nuclei should have a I value first of all in order to study the Mossbauer and most commonly studied is the iron.

So, iron and iron complexes iron proteins etcetera iron containing proteins all of these are well studied. You can study many other nuclei, but not so much of important, iodine can also be studied. So, you can study for iodine and you have many other things like silver, platinum, any cases you can study the corresponding. So, isomer shift is when you take simple iron metal verses your iron complex, what will be the difference between this two and that what you study essentially to get the isomers shift.

You need to prepare a gamma radiation, that is by called which is called the emitter nucleus and this is your compound where it will absorb; so absorber. So, this gamma radiation is absorbed ok. So, as a result that you get the corresponding peak is coming in. So, this peak is shown as the percent of transmission versus that of the isomer shift; isomer shift with respect to the standard.

So, in case of iron compounds iron 0 is a standard. So, in case iron complexes, F e metal or F e 0 is the standard. So, with respect to that you can define the isomers shift with respect that you can define the isomer shift now. So, there is other things which are. So, in case of these complexes, these complexes will have some kind of a symmetric asymmetric kind of geometry. So, such kind of geometries will further split these particular bands into more than 1.

So, you can see there are two peaks are coming out of the one that is what is called quadrupolar coupling quadrupolar shift that comes into these ones, because the nuclei nuclei having the quadrupolar system. So therefore, quadra quadrupolar polar nuclei means greater than 1 I is equal to greater than 1.

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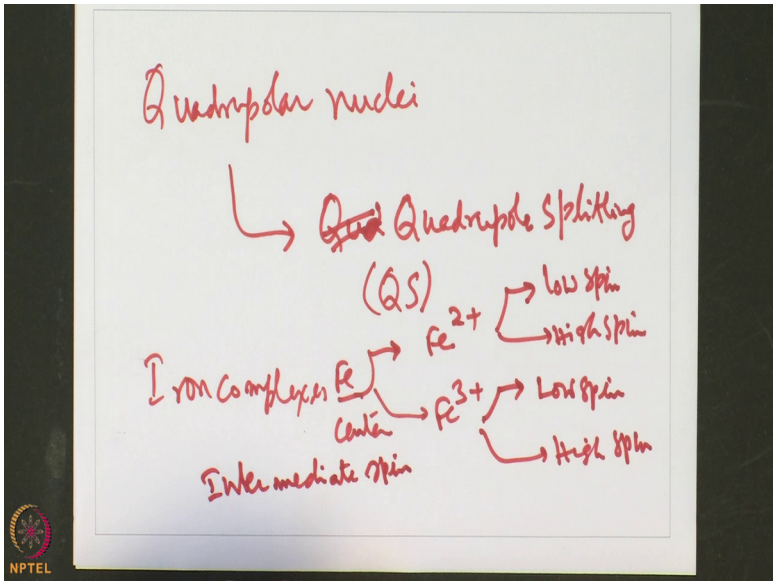

So, this is quadrupolar nuclei will couple with this transition and give the splitting and that gives the quadrupolar splitting.

So, what you finding in this is quadrupolar splitting and this is referred simply s QS how does one do this one basically? So, you have a gamma radiation coming out from the emitter and you have the a sample, sample is put on it on a rails therefore, you can move the sample either towards the radiation away from the radiation, to adjust, to get the resonance, to get the transition. So therefore, if this is a source and if this is a source which is giving gamma radiation, if this is sample the sample is put on top of rails and then you try to move the sample towards and away towards and away. So, with this particular thing you can get the transition. So, where at a particular stage you get a transition and that is what the value of isomer shift is.

So, the isomer shift will come and this basic shift is compared with respect the iron 0 kind of thing ok. So, here with no isomer shift. So, you have the plus half and plus 3 or 2 and when you have the quadrupolar splitting, then you can get further split of these ones. So, you have the transition going 1 and then going two and these 2 transitions are same over their ok.

So, therefore, similarly various cases here, even if this half splits further into plus half minus half and 3 over 2 shifts splits into plus 3 over 2 plus half, minus half minus 3 over 2. So, you have a transitions taking place therefore, you get correspondingly all these bands. So, these ones can be seen more there. So, and this is also influenced by the kind of a geometry asymmetry you have with rest of the metal centre. Because metal center is a coordinated metal center, primary coordinated metal center therefore, the will be dependent on these ones ok.

So, let us get bit more how do we utilize this data information etcetera. So, for example, you know iron complexes. So, they in the case of iron complexes the iron centre the iron centre will have the iron centre. So, will have iron 2 plus or iron 3 plus and each one of these can be either in low spin or high spin and similarly iron 3 plus can also be in low spin and high spin and in case a irons even get intermediate spin too.

So, I would like to inform you the one of the most important techniques, that can differentiate iron 2 plus, whether it is in a ferrous whether it is a ferric and whether it is in a high spin whether low spin. So, ferrous ferric two forms, high spin low spin two forms, 2 into 2 4 different forms. In fact, in addition the iron case you also get some

intermediate spins too. So, more than four forms all of these can be very well identify when you study Mossbauer spectroscopy.

No other method will give a full resolution of this low spin high spin versus the if the iron 2 plus iron 3 plus even if you study the magnetic plot even you studied the EPR all of this cannot give full unambiguous way of identifying, whether the iron center is in ferrous form or ferric form 2 plus form or 3 plus form, whether the iron center is in the low spin, whether the iron center is in the high spin. The only technique that you have is the Mossbauer spectroscopy. So, from the isomer shift you can talk about this the whether it is in the iron 2 plus iron 3 plus and from the quadrupolar splitting you can get whether it is a they in the low spin, whether in the high spin all of these can be identified.

So, they iron twos will have a different kind of a lines and 3 will have a different kind things then low spin will have a different high spin will have different for all of these the geometry of the primary coordination sphere will also play a role. So, I think I will leave that Mossbauer technique at that particular stage and then go to one another technique.

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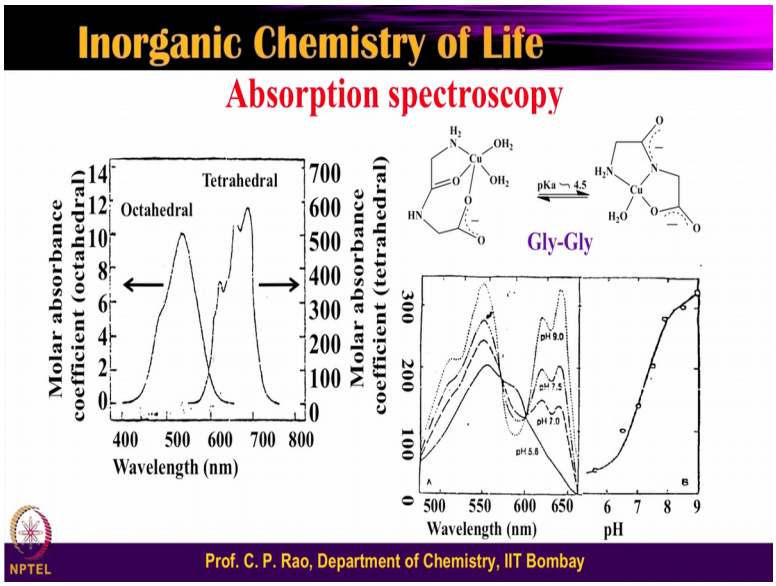

The which is can be used very easily very easily one can use it very simply, in almost every research labs you will have absorption spectrometer therefore, studying the absorption spectroscopy of the protein, protein ligand interactions, protein substrate interaction, substrate converting into the product all of these can be studied by absorption spectroscopy.

So, absorption spectroscopy in this the ground state electrons or excited to the excited state by the absorption of energy primarily, either in the ultraviolet region or in the visible region depending upon the type of electron or nature of the electron that you are talking about ok. So, that is very well known, most of the transitions simple organic molecules or in the u v region or slightly shifted when you have a lot of conjugation into the visible region is that.

In case inorganic chemistry or inorganic complexes or inorganic proteins, one of those metalloproteins we want to look at we need to understand the following things. Now let us take two of the geometries through the most popular geometries or the complexes say octahedral and tetrahedral and octahedral and tetrahedral without going into more details, I would like to tell you the following things.

You know the intensity or absorption of these is dependent on the epsilon is absorbance of these. So, absorbance of light to what extent it absorbs depends on epsilon depends on c and l, c is concentration and l is path length and this is the absorption coefficient.

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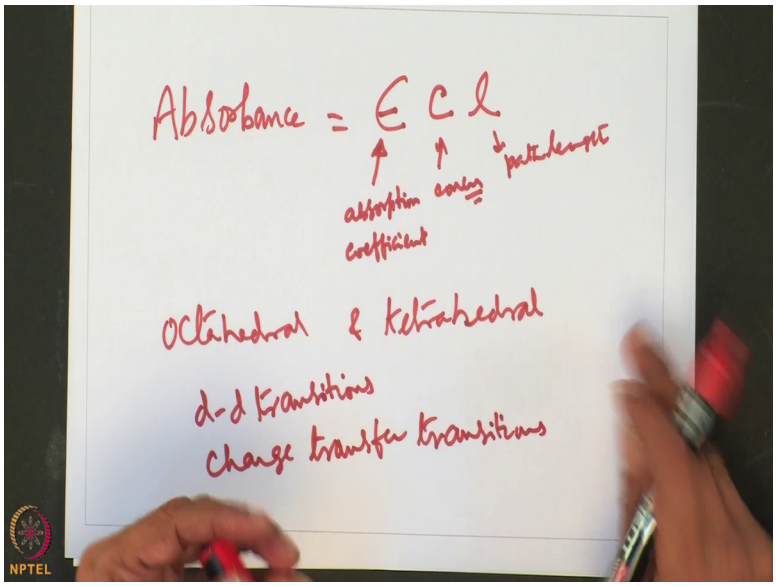

This depends on the power of absorption of the particular group in that particular molecule. How good, how strong, how weak, it can absorb the light of that particular wavelength ok.

So, this is the basically absorption coefficient and for every group at any given epsilon, it will never be absorbing in the same level some of them will be absorbing very strongly, some other groups will be absorbing weekly, some other groups may not absorb it all at that particular wavelength; that means, their epsilon will differ. If by keeping the concentration constant path length constant you can basically look at the absorption levels or absorbance or molar absorption coefficients.

So, this kind of a molar absorption coefficient can be compared. Now let us come most common geometries or octahedral geometry and tetrahedral geometry. For reasons that I am not explaining here, in case of the in both the cases we are talking about the d-d transitions or we can talk about the other transitions as well charge transfer transformations also. So, these are the transition that we look at.

Now, you look at this particular plot it shows the what is plotted here is the molar absorbance or epsilon molar, on this side this is meant for the octahedral and on this side its meant for the tetrahedral. So, what do you seen this? The tetrahedral ones molar absorption is 100 to 700, and for the octahedral complexes something like 0 to 12 or 14 or something and let say 0 to 10 and 0 to 600.

So, therefore, there is a increase of 50 to 60 fold absorption level are increased by tetrahedral complexes. Now if you take a protein let say you take a take a metalloprotein and remove the MATLAB so, it will be a apoprotein.

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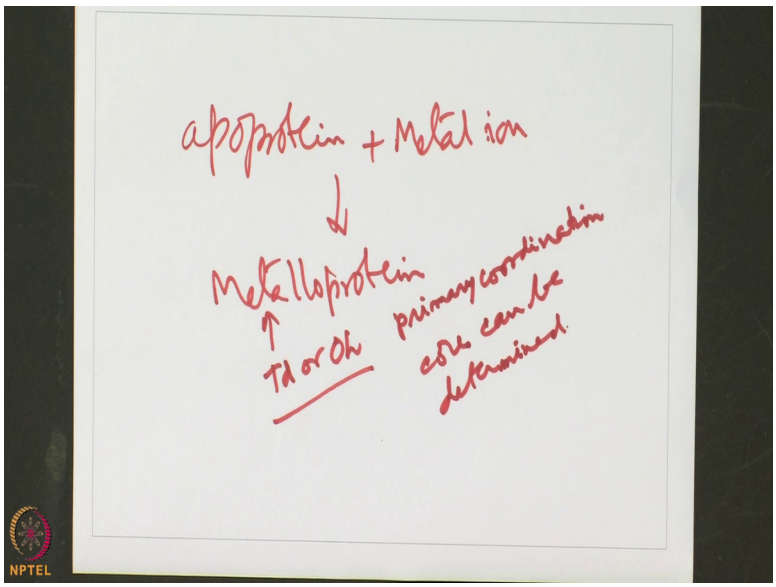

So, you take an apoprotein and you start adding the take apoprotein and add metal ion. So, you start forming metalloprotein.

So, you can measure the absorption spectrum as a function of addition the concentration metal ion etcetera. So, you will get more and more and from that we can calculate the absorbance levels and epsilon values everything is possible for that. From that epsilon you can get whether the epsilon range is in the very small value, molar epsilon or is a large value from that you can identify the ion that you added; so metalloprotein whether this is tetrahedral or octahedral.

So, that core the primary coordination core can be can be determined. So, that is very interesting. So, you take the metalloprotein remove the metal ion start, adding the metal ion to that you can titrate that too. So, it is a boon in disguise they have a different absorption capabilities, the capability with the lower with the higher and reasons are there which are which will be explained in spectroscopy course and based on their inorganic chemistry courses, but I will not going to the details of that because some of the transitions are allowed some of the translations of forbidden all those kinds of things come into picture, which I am not explaining you all that I wanted you to see that appreciate that this d-d transitions. The d-d transitions are very strong in tetrahedral at least 50 to 64 or even 100 fold stronger in tetrahedral and they are very weak in octahedral reasons I am not explaining for you.

Now, take some example case here over here and you take Gly-Gly di gly is a peptide you take the di gly and add the copper salt. So, as you keep adding more and more copper salt copper to have something, you can study the absorption spectra. Now you can also make of course, a complex with it and change the pK p H of the solution too what will happen? When you go from here the initially form complex which is in the form of carbonyl and carboxylic.

Now, when you increase the p H to somewhat greater then the amine is binding the nitrogen here that amine nitrogen is binding carboxylic binding. So, the binding modes will change because this p H will change the p K characteristics of the corresponding groups therefore, some of these groups become. So, this is as it is this nitrogen will go into binding form. So, this nitrogen form will into binding form carbonyl will go out of this.

So that means, a different p H's if you do a p H titration and then plot the absorption spectra you can see you can see that between the 600 and 650, and you also have at 550 one of these both the d-d transitions as well as the charge transfer transition are changing. Now if you take this intensities epsilon molar extinction coefficient and plot as a function of p H, you get a sigma ideal carbonyl sigma ideal carbonyl. If you take a derivative of this, you will get the peak position if you derivatized this one or take somewhere center point of it the center point will give the p K for this particular protein.

If you more than 1 p K will be 2 curves will be say molar curve more than 2 it will be 3 sigma molar curve. So, it will be like this.

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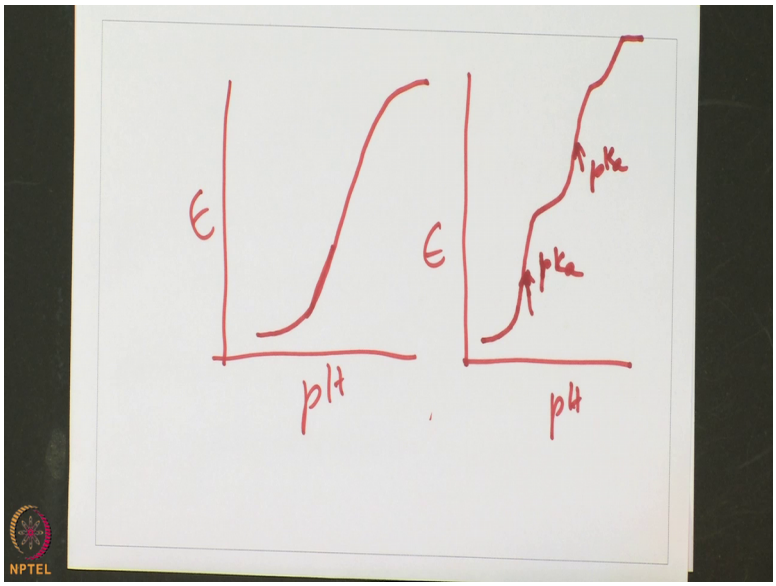

So, therefore, you can get any number of p K is that are present. So, that for you have p H versus epsilon and then if it goes and in some cases here you have only one and then p H versus epsilon then you may have etcetera etcetera. So, you will have 1 p K here p K 1 p K 2 etcetera.

So, you can have that two. So, different p K is a protein can be established by using this one and you can establish the octahedral tetrahedral. So therefore, quite a well things now suppose you doing a reaction with the substrate substrate may displays some other things a geometry will change. Therefore, the absorption spectrum will change now the substrate binds again the geometry will be refilled, again you can study that as well.

So, therefore, there are various methods of spectroscopy, various methods of microscopy crystallography x ray diffraction EXAFS so, many techniques can be used to establish the protein dynamics, protein conformation, protein substrate interactions, substrate conversion to product all of these kind of things. So in fact, what I would like to say is let us recapitulate what all I have talked about is till now I have a talked about the how one can one visualize the metalloprotein.

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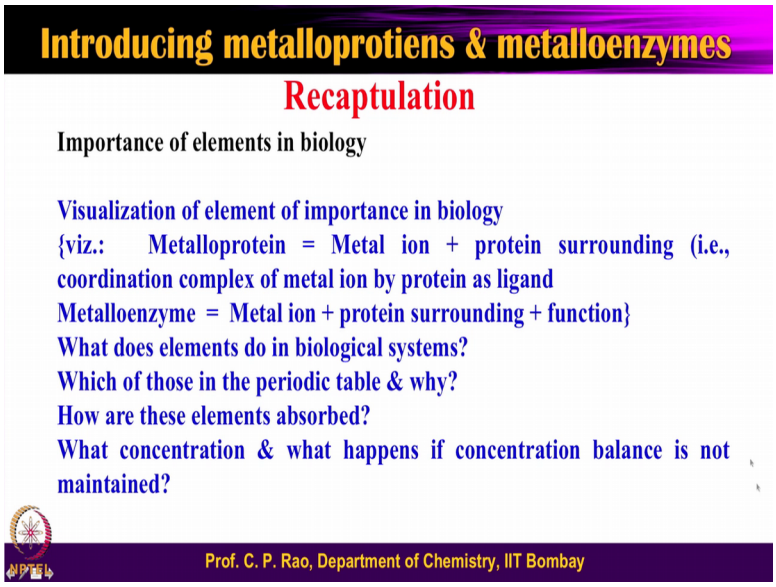

A metal ion plus protein surrounding is like a coordination complex. Metalloenzyme metal ion plus protein surrounding plus function; so ions are explained to you in the beginning, what does the elements do in biological system, which of these elements in periodic table and how are these elements absorbed, what concentration what will happen when the concentration is changed explain to you this is deficient and excess syndromes all of this.

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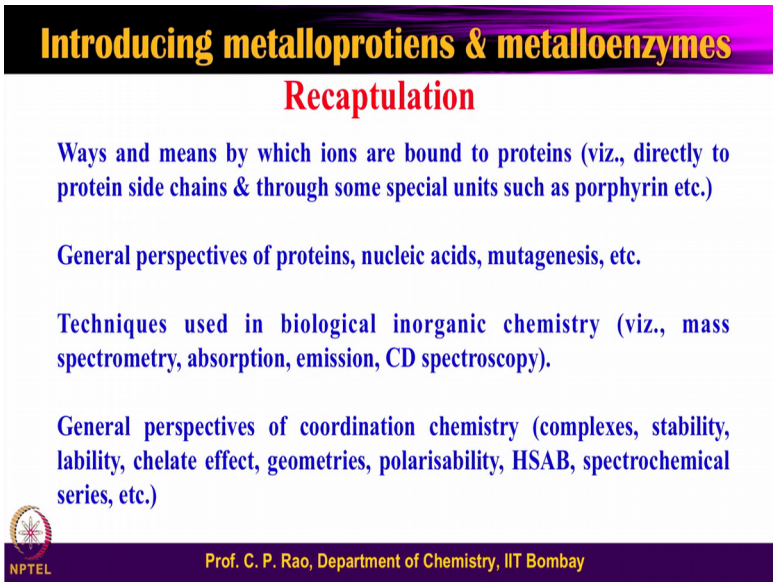

So, what are the various ways by which these ions which ions are bound, you can be bound directly to the protein side chain, they can bound through special units like prophyrins etcetera and then intern their bowl, general then after that I explained to you general perspectives of proteins nucleic acids mutagenesis and lately in the last 3 lectures as so. So, I have tried to cover the techniques of the biological inorganic chemistry like spectroscopy, microscopy, crystallography etcetera prior to that I have also explain to you coordination chemistry principles.

So, this will bring to a kind of a conclusion of the overall integrated introductory approach to this particular course of introducing metallo introduction metallo metal ions and the inorganic chemistry of life. In that inorganic chemistry life these are all very introductory and these introductory things useful both for the bachelor student as well as masters students, who will be continuing the course further.

Thank you very much.