Inorganic Chemistry of Life Principles & Properties
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Lecture – 12
Techniques used inorganic chemistry life – continuation

Good morning, welcome you all for the next lecture on Inorganic Chemistry of Life. Let us just have a brief recap on the previous aspects that I have covered. Just in the previous lecture I begin to the things which are used in the biological inorganic chemistry, bio organic chemistry.

List of the same can be seen over there as I have already shown in the previous lecture, of this few aspects I have already completed. The aspects such as the protein isolation purification, something on the CD spectroscopy, something on the gel electrophoresis, and a single crystal X-ray diffraction, I have also completed things related to the microscopy etcetera.

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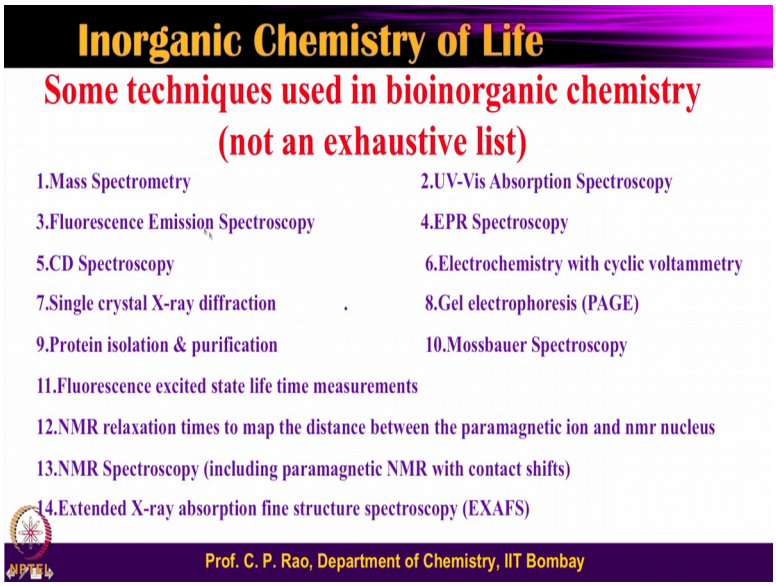

So, in this lecture and the lecture followed by this, I will try to sought of go through the major spectroscopy techniques that I will be that are generally used in the biological inorganic chemistry or in other words in organic chemistry of life because you need to evaluate the reactions that happen in the biological system where the proteins are one of

the major portion or cellular material. So, for this you require both the spectroscopy and microscopy methods.

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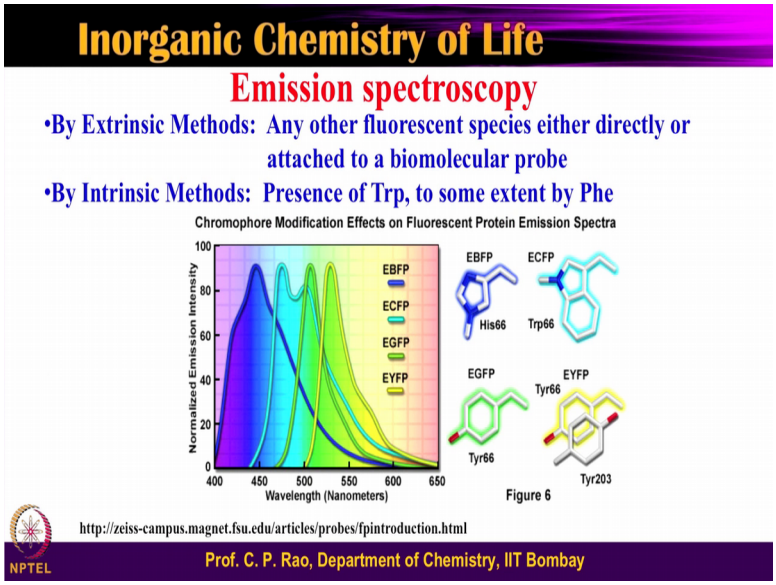

Let us look at one of the method here see we have methods on absorption spectroscopy, we have methods on emission spectroscopy, we have methods on nuclear spin, we have methods on electron spin and we also have a methods in the nuclear transitions. So, variety of these methods are there as you can see on the previous ones, the nuclear transition is basically the Mossbauer spectroscopy and NMR and EPR refers to the electron spin and nuclear spin methods, ok.

So, let us start with one on the emission spectroscopy, ok. So, what is emissions spectroscopy? In an emission spectroscopy you have to have a species which is excited state which is an electronically excited state. So, you know absorption spectroscopy will have the electron transition from the ground electronic state to an excited electronic stage.

Now, emission spectroscopy you look at what happens an excited electron when it returns back to the ground state and that is what. So, the kind of a emission that occurs that you are looking at in the form of radiation of course, there are other ways by which the excited state can be quenched which is called radiation less processes. But we are concerned with the radiation process this radiation processes is referred as the emission

spectroscopy and the one which is which is very commonly used is the fluorescence spectroscopy, ok.

So, in case of biological systems the fluorescence spectroscopy can be used primarily for those species which can be easily electronically excited. For example, those amino acid residues having a the side chains of aromatic type. We know we have already seen the aromatic like side chains you have in the histidine, you have in the fenylalanin, you have in the tyrosine, you have in the tryptophan etcetera etcetera. So, we have looked at all of these. So that means, all of these are very well suited candidates for studying their fluorescence spectroscopy.

Secondly, why they are associated because they are a part of the protein, if there is some change in the protein or dynamics in the protein, then obviously, that change in the protein conformational change in the protein or dynamic change in the protein would reflect on the emission properties of these particular residuals. Therefore, monitoring their intrinsic you know fluorescence emission is an important thing.

So, could be fenylalanin, it could be tryptophan, could be you know tyrosine all of these kind of things. Occasionally people will also do something called extrinsic one, in the extrinsic one what you do is you attach certain kind of a fluoroforce or fluorescence moieties and then study their fluorescence as a function of whatever changes that you bring change in concentration change in the pH change, in the temperature change, in the viscosity change in the whatever kind of a changes that occur in the biological system or when they go from a fluid part to the to a to a tissue pod etcetera all of these can be.

So that means, emission for measuring the emission spectroscopy you require fluorescent moieties, these fluorescence moieties could be intrinsic, like those present in a fenylalanin, tryptophan tyrosine etcetera or these can be an extrinsic fluorescent species which are attached directly to the protein for studying the protein changes.

That can be attached one at one one end of the protein, or they can be attached somewhere in the middle of the protein or in any portion of the protein; that means, protein is modified with a fluorophore and such a kind of things is referred as the intrinsic fluorophore. And those of tryptophan fenylalanin in histidine all of these are referred as the intrinsic fluoroforce, ok.

So, let us look at one example a chromophore modification that affects on the fluorescent protein, ok, so the emission spectra. So, you can look at one of the case is the emission blue, B stand for blue here and the fluorescent protein and then in the second case here the cyan color emission cyan fluorescent protein than emission green, G stands for green fluorescent protein and emission Y, Y stands for yellow.

So, emission Y there is yellow fluorescent protein. So, we have different modification the blue one gives the gives the emission here the cyan one gives the emission over there, the green one gives the emission over here, the yellow one gives the emission over there. So, you can see the emission wavelength is shifted; that means, emission colour is also shifted.

So, this can be helpful where to study the protein diet dynamics protein conformation etcetera by looking at the changes either in the intensity or in the position of the emission or both in some cases see that you can see in all of these, ok.

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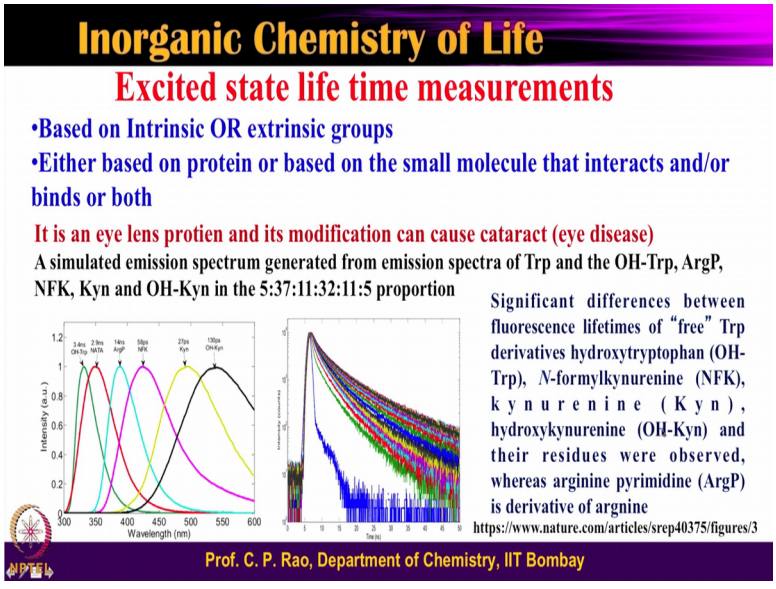

And let us look at another example, but this example is not just simply based on the on the emission, but it is based on the excited state life time. As I mentioned to you the emission spectroscopy comes out because there is electronic transition from the ground to the excited electronic state and this ground to the excited electron state will make the species in the excited state. And now the excited state will not remain in the excited, but that will return back to the ground state.

So, return back to the ground state is a time taken in this. So, that is what the time taken for this that you study, excited state lifetime measurements. Generally these lifetimes are in microseconds in nanoseconds, femtoseconds, all these kinds of things, (Refer Time: 08:05) power 6 minus 6 minus 9 minus 12 etcetera minus 15, etcetera. So, the fluorescence they are there. In the fast fluorescence of course, you know that they are in the milliseconds to even a fractional second to seconds as well.

So, therefore, we are going to we are looking at how what is the rate what is the time, rate and time are related to each other rate and time are inversely related to each other ok. So, one by time is the rate one by rate is the time. So, therefore, does not matter if I use the word rate of excited state decay or if I say lifetime of the excited state. So, the excited state lifetime means how long the species sits in the excited state, ok.

So, this is again the life time is dependent on various parameters the parameters of the protein dynamics itself, protein conformation itself and viscosity of the medium many other parameters, pH are the medium various things. So, all of these will influence the excited state. In some cases it will stay longer in the excited state, in some other cases this still shorter in the excited state.

Therefore, excited state lifetimes becomes an important parameter for us to monitor if you want to study the dynamics or the protein, or not only dynamics suppose you have a small molecule which reacts with enzyme. In case of enzymes what are the small molecules which reacts? In case the enzymes it is the substrates binding to the binding to the enzymes.

So, when the substrate binds to the enzymes there are some changes can occur in this conformation. When the substrate is converted to the product again there could be some changes in the conformation when the product is released there can be some changes in the conformation the protein. So, all of these conformational changes or in the other word some kind of a dynamics can be studied by excited by following the excited state lifetime measurements. These are dependent on the various parameters like viscosity, dynamics, conformational changes, pH various other factors concentrations all of these like the ones, so very nice.

So, we have enzymes you want to understand whether the whether the substrate is bound or not you can study. So, you want to study whether the whether the enzyme the

substrate enzyme has acted on the substrate has converted the substrate into the product or not that can also be studied in various things.

So, here I have taken an example slightly different in nature. This example is coming from a protein called eye lens protein. You know that we have several proteins that function in the eye lens and some of these proteins get modified as a function of age, time, as a human grows that you know you must have heard some of your elder saying that I am not able to have a good vision I am not able to see very well, that is because they have certain kind of a layer they called as which is nothing, but a cataract.

So, the contract is nothing but for a chemist it is nothing, but some kind of a modification of the protein ok. So, what can modify the protein? Various side chains can modify in the protein. Some of these can be even glycosylations. So, all of these will modify, when they modify some of the proteins may join together may aggregate.

So, protein is modified because the side chain is modified, the modified protein is in turn is added up together to form a kind of a polymeric species, so you for the kind of layer. So, individual protein molecule is making connecting together just is like suppose you have these lens proteins or like a rod like molecules that you have. So, and you have another rod like molecule and you have another rod like molecule you have another rod like molecule etcetera. So, these are individual lens proteins, ok.

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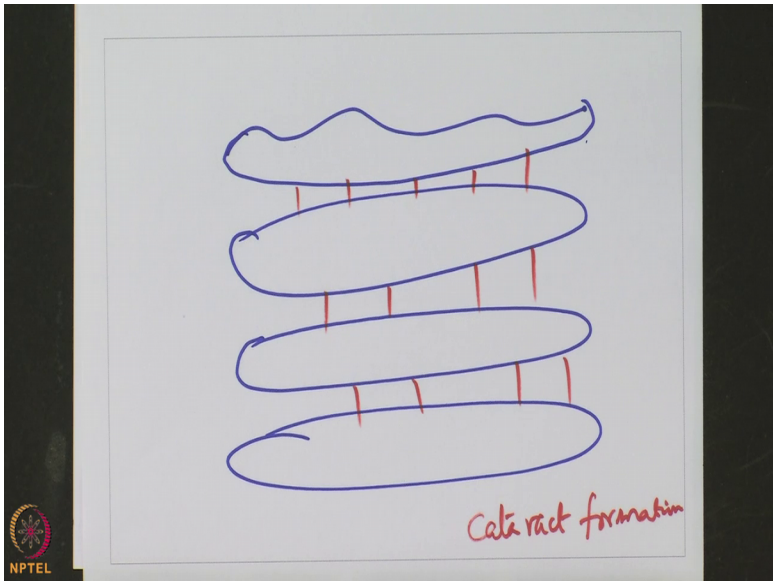

So, this these lens proteins due to the modifications they get connected to that, so they get connected to that. So, they are modified in the various ways and this modifications can be explained.

Now, this forms like a individual rods are joined together now form a kind of a layer which we call is a cataract formation. So, this is what is the disease. And we as a chemist can identify such kind things. So, you can identify such things because the when it is modified any of the thing is modified you can see that the modified portions their dynamics is different therefore, lifetimes are different. When they are connected together the lifetime will also change.

So, here we have shown some example in some modifications are brought here. As you can see in this particular lens protein some modifications simples tryptophan, hydroxytryptophan or ARGP variety of this modification. It is not necessary what this modifications are at this time for us to know all that we know is that these amino acid side chains are modified.

Some of these modifications will lead to the aggregation phenomenon. So, the protein to protein, rod to rod, rod to rod can join like the way that I have shown you here and these are all joined together and to form a kind of a layer. So, such kind of things can be studied. So, when the modifications can be studied because the shelf in their emission they also change in their emission pattern in terms of the lifetime.

So, all of these things are referred as the lifetime decay curves and these decay curves are generally fitted to exponentials it can be 1 exponential, 2 exponential, 3 exponentials that is monofunctional, bifunctional, trifunctional etcetera. So, these are some of the things that we can. So, we can resolve these and get the lifetime measurements> And the lifetimes will vary as a composition of what kind of a changes have taken place to what extent they have taken a place in this.

So, I think this much of a message is a good enough, so that means, what I would like to say is using their emission spectroscopy, using the excited state lifetime measurements, you can you can study very clearly the binding of the substrate to the protein or enzyme, the reactivity of the substrate to convert to the product and the changes that occur in all these. And all of these kind of a parameters or changes in the pH changes in the

dynamics of these or temperature everything can be monitored, so very well. So, that is one of the very vital technique.

Now, let us smoothly switch over to another technique another technique is mass spectrometry. What is a mass spectrometry? Mass spectrometry is based on the mass. So, mass of a species, mass of a an ion, mass of a molecule when it is converted into an ion ok.

So, therefore, you convert the molecule into an ion and you measure that, so from that you can get the mass of this, ok. So, the molecules or converted into their ions and these ions are measured. So, more details of mass spectrometry I am not giving here. The instrumental detail but I would give details of how it can utilized.

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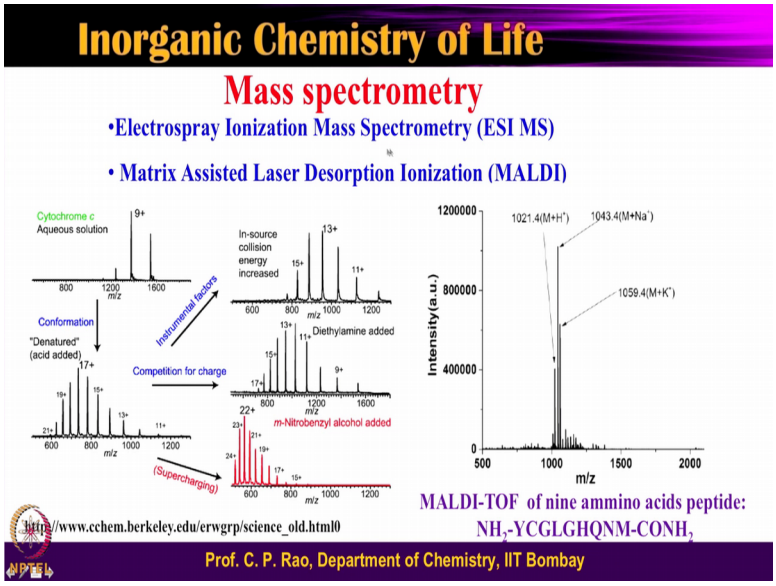

So, there are a large number of mass spectrometry methods are their of which I am talking to you about two other techniques one is ESI, electro spray ionization mass spectrometry and other one is called MALDI. What is MALDI? Matrix Assisted Laser Desorption Ionization, Matrix Assisted Laser Desorption Ionization Spectroscopy.

Generally for smaller size peptities molecules you can use ESI MS. For bigger size proteins and huge molecular weight proteins they are all studied by MALDI spectroscopy and both of these will not bring much of a you know breakage in the molecule, but just makes a soft ionization these are all soft ionization processes in that.

So, in this you have a electrospray which is solvated electron which gets added to your molecule and convert into a species, ionizable species and that is pieces you are measuring.

In the other you have you mix your compound a molecule or protein with a matrix protein is a matrix which and then shine with a laser light and the laser light is absorbed by the matrix and then transfers this energy to the to the molecule. And then as a result of that molecule gets ionized molecule gets dissolved and ionized, that is what you study in the MALDI.

So, here is some example. As I told you and you know very well in mass spectrometry you basically have an piece of information of importance is that what you measure is m by Z, ok. So, you measure m by Z, ok.

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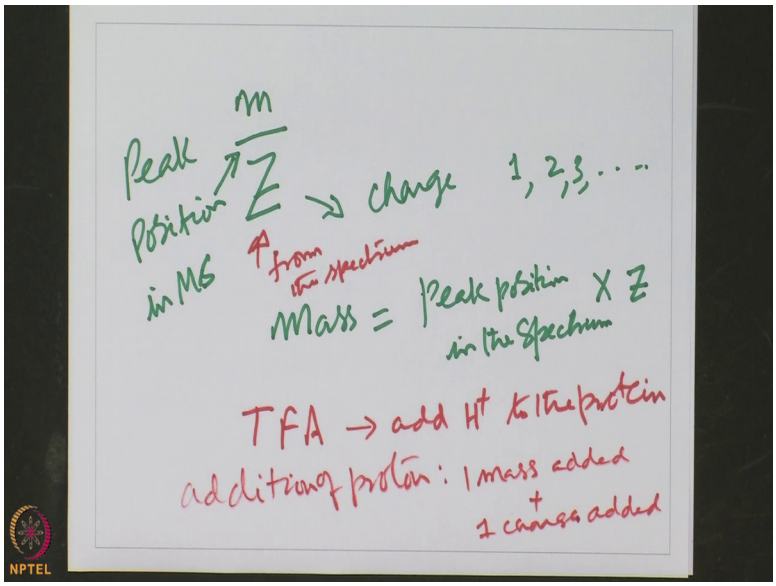

So, Z is the charge. So, the charge can be 1, it can be 2, it can be 3, it can be any number. So, if it is a negative charge minus 1 minus 1 minus 3 if it is a positive charge plus 1 plus 2 plus 3 etcetera. Ao that means, the peak position is peak position in mass spectrometry mMS spectrometry is referred as the m by Z.

So, in that the Z it refers to the charge. So, if you want to get the a mass is equal to the peak position in the spectrum multiplied by Z value. So, this will give actual mass. So, this will give actual mass. So, this you will find in the spectrum, from spectrum and you

can also find the charging. So, therefore, you can get the mass of the species. So, this is an important thing.

Now, here there is some example is taken over here, ok. So, it is not very important what this example is, but this happens to be the cytochrome c. Cytochrome c just take as it is and put it into the electrospray ionization mass spectrometry you get one peak for the 9 charges. So, whatever the values you get multiplied by 9 will be the molecular weight of that particular thing.

Now, as I said that the Z can be played with. So, Z means can be charges can be added it will removed this can happen by adding a charge. For example, so, you add the charge by adding some kind of a acid to that, ok. So, you add acid, acid (Refer Time: 19:51) is for example, trifluoro acidic acid, ok. So, trifluoro acidic acid which is TFA this will add charge H plus to the protein. So, this will add H plus to the protein. So, therefore, that is that can be really understood.

And now you see spectrum here computer this you get more number of species. So, species with 9 plus and 11 plus, 13 plus, 15 plus, 17 plus etcetera, and then you can also charge a lot. So, then you can see much more charge like even 22 plus, 25 plus etcetera all of these kind of things are there. So, one of the that all of these kind of things are there.

So, one of the think that we learn from this is simple protein may not be able to solve the structure solve the mass of this of this particular protein, then you add some acid that will the basically the nature and to this denatured protein you can add more and more H plus and then more and more H plus will add to Z. So, that value increasing. So, Z value you can become 1, can become 2, can become 3, can become 4, here we have even you know 11, 13, 15, here you have 22, 23 etcetera 24 all these kind of things.

So, from this you can solve resolve and get the protein mass in that you can also make some changes in the in the instrumental parameters can get a little bit more you know irons to 11, 13, 15, 11, 11 plus charge, 12 plus charge, 13 plus charge, 14 plus charge, 15 plus charge. So, what we understand from this? We understand from this when you have a protein, protein has side chains, you are seen carboxylic groups, you have seen phenolic groups, you seen amine groups, you have seen amide moieties, you have many many kind of a group are there.

So, these groups you can either add a proton imidazole is also you can add a proton you can remove a proton. So, when you add a proton you are adding 1 mass plus 1 charge. So, addition of proton, so, addition of proton addition of proton is 1 mass added plus 1 charge added. So, that is how you need to calculate and get the corresponding thing.

So, now you know how many charges you added, how many protons you added etcetera from that you can make out. So, so this is possible because the there a lot of side chains in the protein which can absorb or take and protons. So, it can be protonate. So, can be protonated by some extent by more extent to is one of the technique which is very commonly used for the ESI MS aspect.

The other technique is the MALDI assisted laser absorbed desorption ionization. You can see the one of the example is shown for a small peptide, 1 2 3 4 5 6 7 8 9 peptide 9 amino acid peptide as you can see that the corresponding peaks are obtained more here. In this case also you can get resolve the peaks into different charge which I will show you on the next slide, but you can see that this particular thing basically one can study the study the mass out of these ones, ok.

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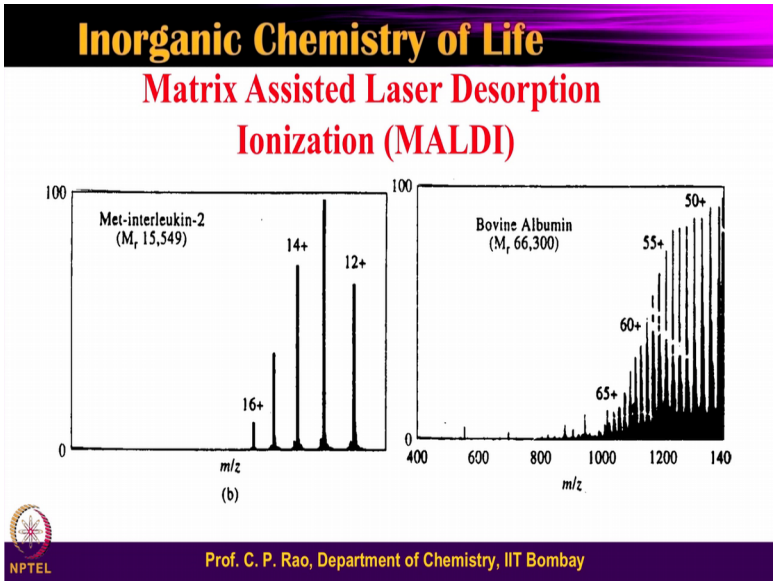

In the next slide here you can see the MALDI for two proteins this is one on the left side one on the right side. The one on the left side is met interleukin is a protein interleukin 2, a protein who molecular weight is 15,549; that means, around 15.5 5 kD, kilo Dalton we

referred generally as a kilo Dalton, ok. So, protein molecular weights are generally in kilo Daltons. Protein molecular weights in kilo Daltons these are referred as kD, o, kD.

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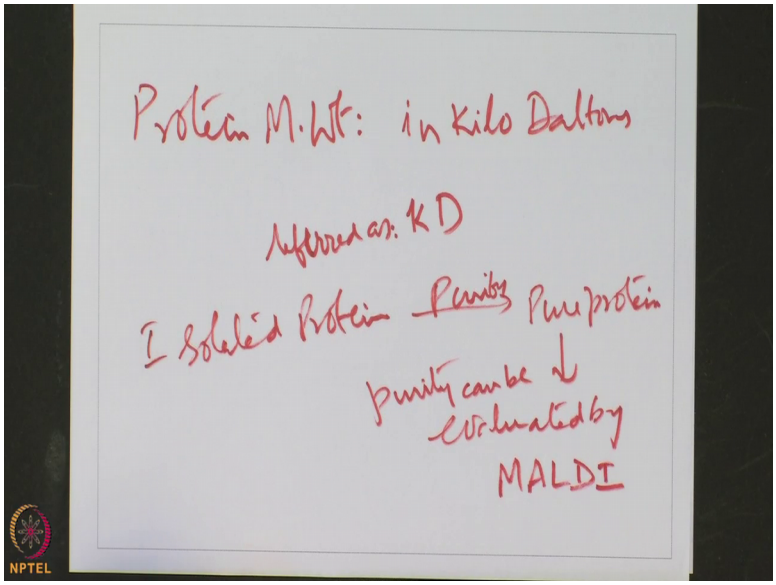

So, you will have a 5 kD, 10 kD, 50 kD, 100 kD, 200 kD, 1000 kD there are proteins of all these. Now, here you can see that interleukin 15,000, we have a peak for 12 charges, 13 charges, 14 charges using more than 2 or 3 of these you can solve the mass for this one. And here bovine albumin it is a huge protein because it is a tetramer. So, bovines albumin is tetramer it can also can be called as a bovine serum albumin and its molecular weight is 66,300, 66,300 which is called 66.3 kilo Dalton and you can see, that means, more number of residues more number of side chains. So, therefore, more charges if possible.

Here you will have about 150 to 170 amino acids, here you will have 650 to 700 amino acids, so obviously, the thing is increased by at least 4 times. So, molecular weight of this 50,000, 60,000 about 4 times you can see the charges 14, 15, 16, you can see here 55, 60, 65. So, everything is increased by 4 times the number of residuals are increased by 4 times the mass is increased by 4 times. The charge species these are all Z values, Z value 50 plus, 55 plus, 60 plus, 65 plus. So, using this you can solve the mass of this.

Now, let me tell you which I have not shown in the slide when you do in the earlier slide I have show that the people isolate the proteins from cells from other materials etcetera. When you isolate you purifier to at certain stages of initial stage of purification the

protein what you want is there also some other proteins their how do we know that so easily. So, you can use the MALDI technique, because the mass of those impure proteins impurity proteins is different from the mass of the of the actual protein. So, therefore, from them the MALDI you can find out the protein impurity into.

So, isolated proteins purify then you get a pure protein this can be evaluated by purity can be evaluated by MALDI. So, you can find if there are some impurities what kind of impurities everything you can find excellent kind of a technique that you have.

So, let us see the next part of if is the mass spectrometry, then we go to NMR spectroscopy.

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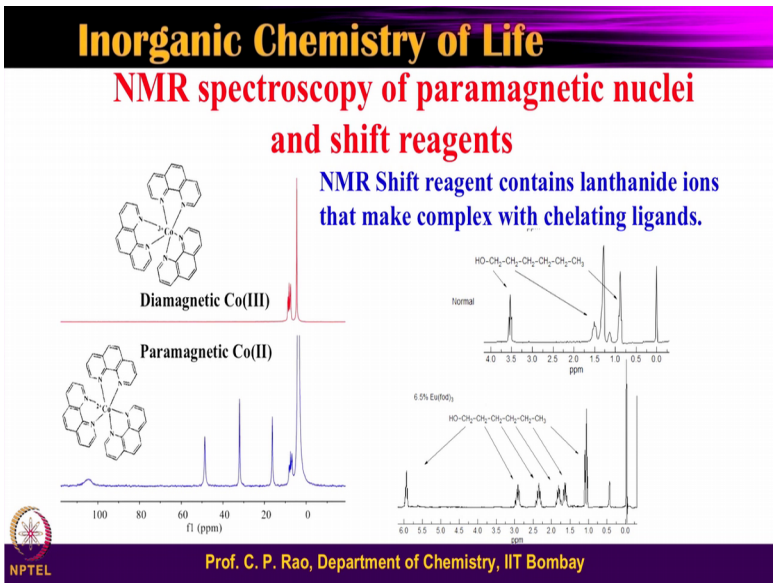

So, let us look at the NMR spectroscopy. We are very familiar with NMR spectroscopy what is the NMR spectroscopy? Nuclear magnetic resonance that is nuclear spin of the nucleus having the spin of plus half nucleus spins or it can take plus half and minus half 2, ok.

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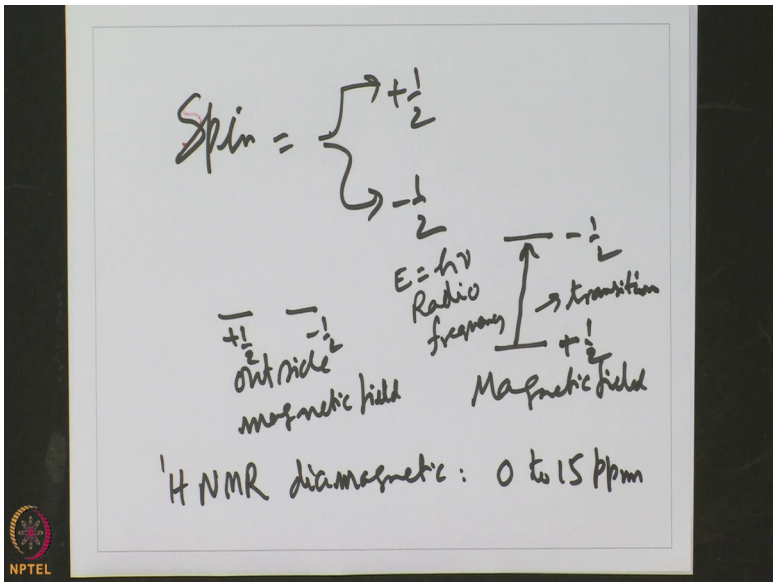

So, therefore, these two under magnetic field these two having same energy outside the magnetic field say let say plus half and minus half and you apply a magnetic field.

So, these are in the magnetic field, these are split into the plus half into minus half ok. So, this species which your present to the other this is the transition. So, in other words you cause a trans nuclear spin transition going from one kind of a spin to the others spin that will result in the nuclear spin transition, kind of a technique and this requires energy and this is in the radio frequency this is the h nu is equal to. So, by supplying that kind of energy you can cause the transition.

Then generally in the NMR, in the diamagnetic means organic molecules if you take their proton NMR spectrum runs aware between let say proton 1 H NMR of diamagnetic goes from 0 to 15 ppm ok. Whereas, when you have some species such as when you have some species such as a paramagnetic species when you have and these the shifts magnetic the NMR spectral the shift in the under the presence of paramagnetic species, under the presence of the paramagnetic species.

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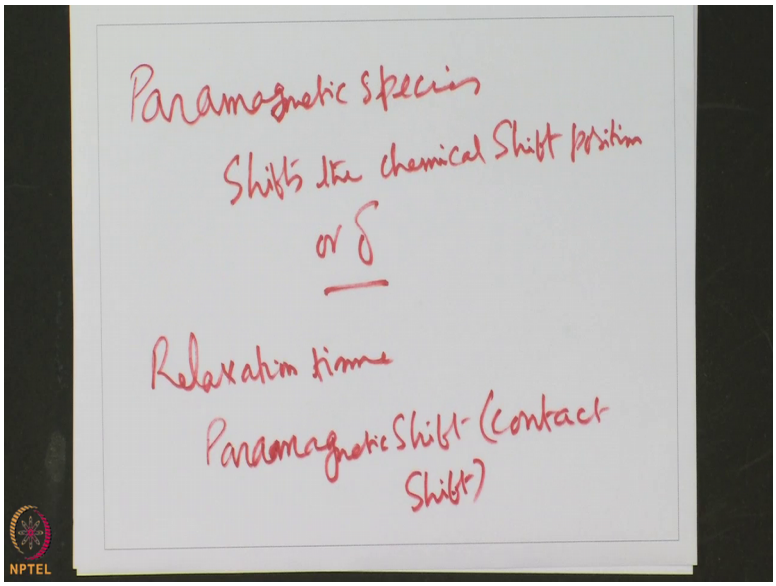

So, the paramagnetic species shifts the chemical shift position who known as delta, ok. So, the delta can be changed, the other parameters that can also shift is the relaxation time, and in metalloenzyme you know there are lot of metal ions are involved transition metal ions and these transition metal ions are basically have some of them will have the paramagnetism. So, therefore, one can easily make out structures by using this such kind of a paramagnetic species on this.

Here we have taken one the phenanthroline the complex or the cobalt 3 plus with the cobalt 3 plus here is d 6 where all electrons are paired and this is diamagnetic. So, in this diamagnetic kind of a system then you have a you have no shifts happening in this and therefore, you get the spectrum very small (Refer Time: 31:08) 0 to 10 or 0 to 15, but instead of cobalt 3 you take cobalt 2. So, the cobalt 2 is paramagnetic.

Now, you see the same set of peaks have spread to almost 100, 110 ppm, from going from 0 to 10 ppm is going from 0 to 100 and 10 ppm and that is what is called the paramagnetic shift. So, this is called the paramagnetic shift or the contact shift. So, this is a bovines in disguise you know because in metallo proteins, the metal ions is connected to different side chains residuals of the protein and therefore, those residuals where it is connected directly and if the metal ion is paramagnetic they can be shifted here too, ok.

Just an example is shown over there. You take some hexanol and add some shift reagent which is a European compound. The simple hexanol everything is within 5 ppm and

hexanol with the European complex that you see it goes much down feel. And each of the CH 2 group can be separated out, one CH 2, other CH 2, other CH 2, other CH 2, other CH 2, other CH 2, ok. So, in a fact what I would like to say is that the NMR generally organic chemistry thing is a curse, but for a bio inorganic chemistry biological inorganic chemistry is a boon and the paramagnetism will shift the spectrum, and as a result of that we can get the different things resolved.

And in the next class I will demonstrate one example a these and then explain you much better.

Thank you very much.