Advanced NMR Techniques in Solution and Solid-State

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Module-45
Introduction to 2D NMR

Lecture-45

Welcome all of you. We have been discussing in last couple of classes about product operators, when using that we know how to analyze a given pulse sequence. Of course, I discussed and I said the product operator formalism is developed based on density operator theory and we wanted to know how the density operator evolves as a function of time. That is we called as rho of t and rho of 0 is the initial density operator. If I know that, then I can find out rho of t by a simple expression e to the power of - i H of t rho of 0 t to the power of i H of t where H is Hamiltonian, rho 0 is the initial density operator.

And then we worked a lots of examples; and also the norms for working out how the magnetization evolves during free precession, how they evolved during chemical shift and J coupling. And we took the simple example of, let us say one pulse acquisition, spin echo in the homonuclear case, spin echo in the heteronuclear case and I showed in the spin echo case example where by a pictorial diagram, how, what we understood can be explained in a simple terms using product operators.

And for that, we also introduced what are called the rotations operator, product operator rotations, what happens to that when we apply a pi by 2 pulse on the X axis or Y axis or Z axis. What is the product IX will do, what IY will do etcetera; and I said IX is nothing but the rotation of X axis, IY is the rotation about Y axis, etcetera. And I also said there are 3 diagrams about what happened to the product operator, when you rotate about a particular axis X axis, Y axis or Z axis.

And the diagram tells you what will be the next product operator when it undergoes rotation about a particular axis. From the diagram, we could understand and write, in case, let us say, if there is a rotation around Z axis during free precession, then we know, we discuss about the general formula, always when you want implement it using the diagram, it is like cosine of

the old operator, plus sign as the new operator, is the general way we have to write the outcome of that rotation.

And I also said one more important thing, the time ordering of the pulse sequence will remain same, we have to go from left to right. Let us say first RF pulse then the delay, another pulse another delay, whatever it is, the sequence has to be followed. But let us say during the delay in the block period of a certain delay there is free precession, chemical shift evolution and J coupling evolution would be there. I said you can take in any order, it is immaterial.

And when you are applying the RF pulse, you can assume that chemical shift or J couplings are switched off temporarily. And then subsequently, you can think of doing the free precession time and how chemical shift evolves and J coupling evolves independently, the order does not matter whatever the order you want you can work out. And I also said when you apply a hard pulse let us say if you have 2 spin system, you can treat them as cascade of 2 spins one applying on spin 1, other applying on spin 2, you can individually work it out.

That also we discussed; we took several examples. And finally, we understood how using these product operators, we can analyze a given pulse sequence. At the end, we also understood, of course, first we started with the single pulse where there is no coupling and we ignored relaxation during the pulses and the delay and afterwards and later also during the free precession time.

But when we took the example of 2 couple spins, we brought in the coupling constant. Then we introduced product operator for individual spins. For example, spin 1; I 1x, I 1y, I 1z then similarly for another spin 2, I 2x, I 2y, I 2z. When J coupling is taken into account, we have to take the product of each of them and we worked out there are 4 into 4, 16 product operators when we have 2 coupled spins.

And we also understand one important thing in such cases, the J coupling from the in-phase magnetization because of the J coupling goes to anti-phase magnetization. And this is the one which is responsible for the transfer of polarization from one spin to other spin. This is what we especially, understood. And in the last example of INEPT we also saw how the polarization transfer takes place in the heteronuclear case.

What happens if apply 180 pulse one spin, let us say I spin or another spin S spin or what happens you apply 180 pulse on both the spins during the echo sequence and we came to know which will evolve whether chemicals shift will evolve or coupling will evolve in each of the sequences. So, this is what we understood. And with this, we could understand many of the 1D pulse sequences and we understood lot of phenomenon interpreted NMR spectra everything. But now, from today, we will jump into a new concept, new idea, new discussion of a new topic called two dimensional NMR. That is what we are going to discuss from today.

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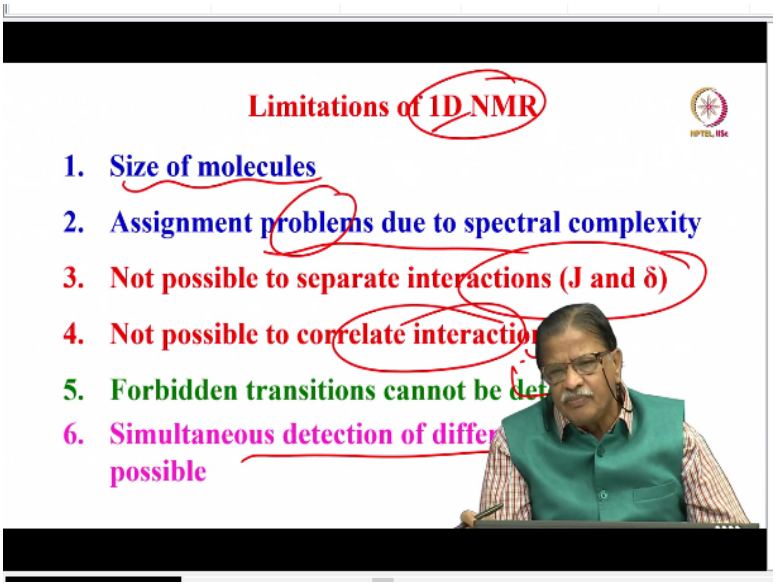

Now, in the two dimensional NMR when I am discussing what is that 2D will do and what is the advantage of two dimensional NMR over one dimensional NMR? That is what you have to understand. And first of all the limitations of one dimensional NMR is the size of the molecule, if you go to a bigger molecule like, let us say, a big protein consisting of several amino acids or a big polypeptide consisting of several amino acids; in which case the number of transitions that you are going to see in the proton NMR spectrum, in a given range of 0 to 10 PPM, will be enormous.

It is because of various interactions present, chemical shifts of individual protons and many of them are interacting with others, there is a J coupling interaction. As a consequence there are multiplicity of the transitions; the spectrum will be enormously complex to analyze. So, size of the molecule as it increases, in ID there is a limitation. Similarly assignment problems due to spectral complexity.

As I said, when there are N number of spins presented, let us say, when I have several chemically inequivalent protons, if you wanted to take the NMR spectrum of that, in a 0 to 10 PPM range, you will have lots of peaks, the spectral complexity. So many peaks are overlapped, it is very complex to analyze and assigning each proton or a particular proton is going to be a challenging task.

And at the same time, if I want to separate the interaction of chemical shift and the J coupling from one dimension NMR, it is not possible. Of course, we can do select to decoupling and everything and find out which is coupled to which, etcetera. But I want to separate both interactions independently. I wanted only J coupling information and I want to get only chemical shift information separated out in one dimension NMR, is not easy. So, we need to go for 2D for that.

And of course, if I want to do the correlation of one information with respect to other. Let us say in a given molecule there is 1 proton; it is coupled to another proton. And I want to know whether which is coupled proton x, y or z, I have to do N number of experiments of decoupling, hit at one of the proton and see the change in the spectrum in the ID NMR spectrum, it is a very cumbersome experiment. You have to do N number of decoupling experiments to identify these things and you cannot correlate which is coupled to which like that. So, such types of things are not possible in 1D NMR, in a straightforward way. So, we need to go for a higher dimension for that. And forbidding transitions, for example we have been discussing, of course, we will discuss after this 2D, the multiple quantum in detail.

The transitions other than delta = + or –1, change in the magnetic quantum number between 2 energy states. If it is other than + or –1, they are all forbidden transitions in NMR and cannot be detected. That is what I was telling. But there is a way we can detect indirectly, for that again from 1D NMR it is not possible, we have to go to higher dimensions.

And then simultaneous detection of different nuclei. If I am doing 1D NMR I can detect only proton at any given instant of time or I can detect only carbon 13. Ok another RF channel if it is there, that I can do some selective decoupling or broadband decoupling, but I cannot detect both nuclei or 2 or 3 nuclei simultaneously, it is not possible. So, in which case, we need to go for higher dimension.

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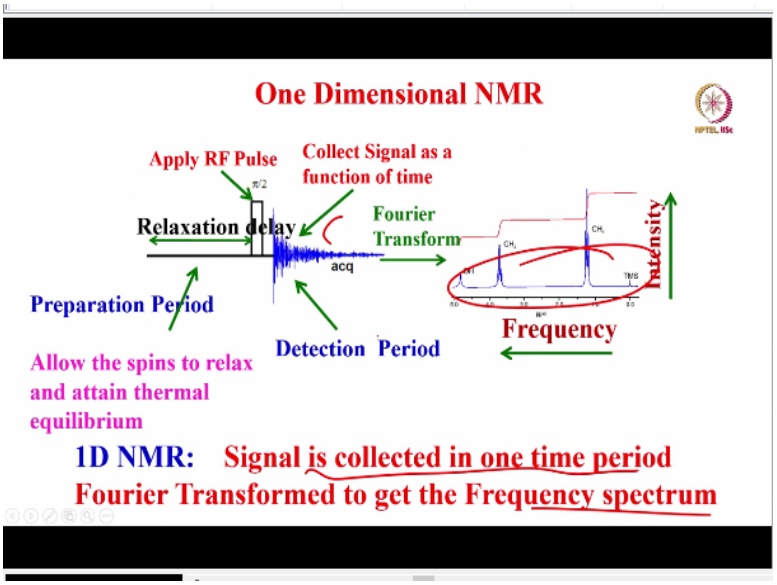

So, these are the limitations of 1D. How 2D will overcome some of these problems we look at it as we go ahead? First to start with the simple 1D NMR which we have been discussing since several weeks. 1D NMR one dimensional simple one plus experiment, apply 90 degree pulse with a given delay for a dead time delay. Start acquiring the signal this is called free induction decay. That is what we knew. We have been discussing, I do not need to go in detail. And I am going to collect the signal as a function of time here. And then I will do the Fourier transformation, then I am going to get the frequency domain spectrum like this; some spectrum, it need not be same FID, but some others spectrum I am showing. So, what essentially it means? Time domain signal will be converted to frequency domain.

This what we discussed when I discussed Fourier series, Fourier transform, etcetera. And in this one dimensional spectrum, there is chemical shift along this axis or frequency and intensity on this axis. But still, it is called one dimensional spectrum, why I am going to tell you later. And basically, before applying any pulse, we need to ensure that spins attain thermal equilibrium.

That is called a relaxation delay, relaxation time we have to give and this period is called a preparation period. So, I will allow the spins to relax and attain thermal equilibrium before application of the RF pulse. That is called a relaxation delay. And then this is the time period under which I am going to collect the signal is called detection period. This is a detection period. So, this is what I am going to detect.

1D NMR basically from this diagram what you will understand is, 1D NMR is something where the signal is collected in one time domain. Here signal is collected only in one time domain and you do the Fourier transformation to get the frequency domain spectrum. One dimension Fourier transform and we get frequency domain signal. That is a simple 1D NMR.

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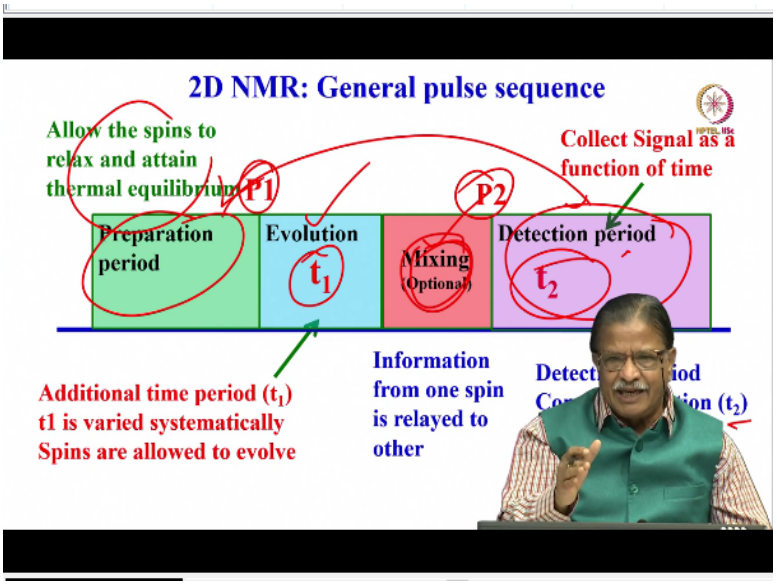

What is it two dimensional NMR? The general pulse sequence for a two dimensional NMR is given like this, we have the preparation period like we had in 1D, and we have a detection period where you collect the FID like in 1D. That is these 2 are common both for 1D and 2D. In-between we have mixed, we are brought in 2 things, 2 periods, one is called the evolution period other is call the mixing period.

So, this is a constant time detection period, we collect the FID in a conventional way like we do in 1D NMR. And this is mixing period, which is optional time depending upon the type of experiment. That as we go ahead with a particular 2D experiment we will discuss. And the evolution time is a time which is incremented. That is we want to see as time is incremented t1 period incremented systematically, we will understand how the magnetization evolves as a function of time. So, this is called evolution period. So, now we have 2 time periods. Remember. Here as usual, you allow the spins to attain thermal equilibrium; then we apply radiofrequency pulse P1, then allow the spins the additional time period, which is varied systematically and we allow the spins to evolve under different Hamiltonian, depending on what you want to detect. And then mixing period is optional. Here what happens information from one spin, you relayed to other spin, there is polarization transfer taking place or some information gets transferred.

And the last period is the detection period; then we apply another pulse, it is a detection pulse and then start collecting the signal in a conventional one dimensional way, this is called detection period t2 and this is basically is a two dimensional general pulse sequence. Now, over the years, 100s and 100s of pulse sequences have been designed to extract a particular information in a two dimensional NMR.

So, there are a number of two dimensional NMR pulse sequences. But overall, we can understand that any 2D pulse sequence, generally has broadly these types four time periods, preparation period, evolution period, mixing period and detection period.

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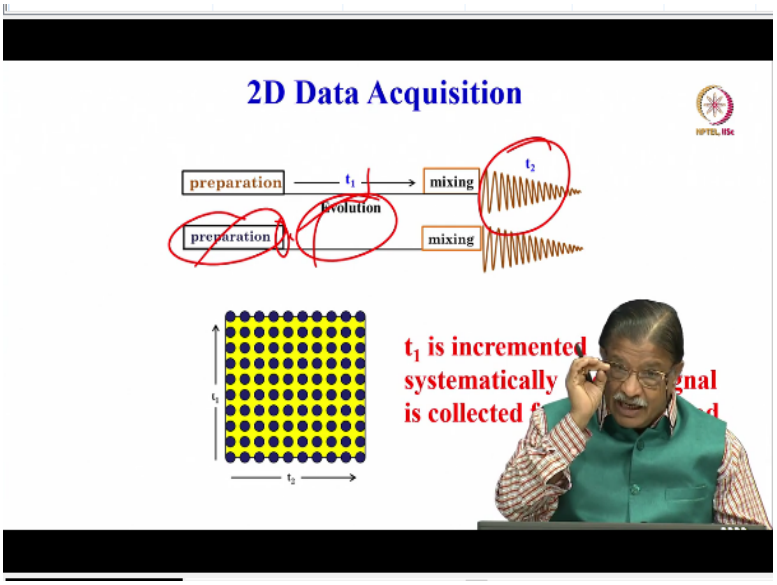

Now, how do we acquire a 2D data then? In the 1D it is simple, apply radiofrequency pulse, collect the signal. And how do you acquire two dimensional data in 2 time periods, t1 and t2. What we do is start like this. Assume will start here, there is a preparation period. And there is an evolution period here. What we will do is initially, we make the evolution time as 0, there is no delay at all. Immediately after preparation period and this mixing period start collecting the signal. Then I am going to give a delay, little delay and then t1 is incremented, another delay, another delay like that I keep on incrementing the delay, you see every time I increment the delay and now t1 period is increasing. And then finally, we are collecting this part, we are collecting the signal in the t2 dimension. So essentially, we are incrementing the t1 as a function of time, t1 is systematically incremented and for each t1 period, we collect the signal in the t2 period. T2 period is constant. Now what is happening in t1 period? We will understand that. So, this is a way 2D data is acquired.

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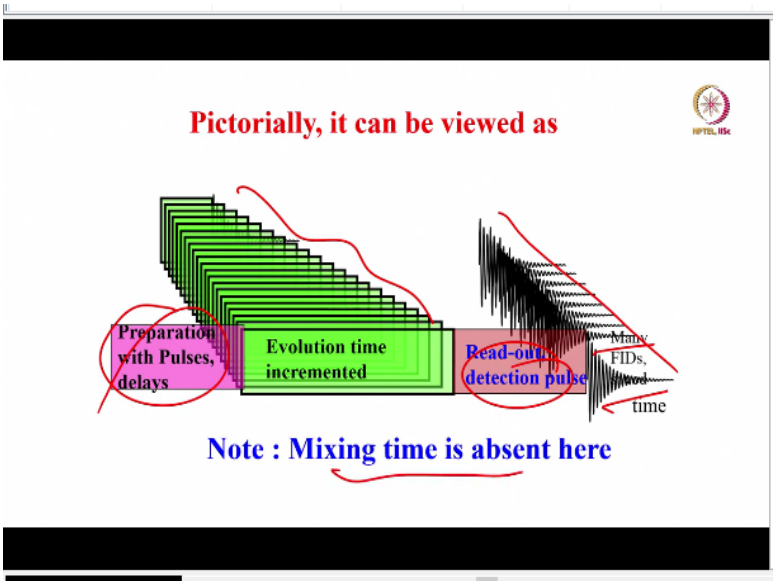

But now pictorially if you look at it, it looks like this. This is the preparation period, pulses are delay whatever you want, keep it constant for spins to attain thermal equilibrium. The evolution time as you are incrementing the time, you see it looks as if how your FID period is getting incremented, the time period is getting incremented. And this is the read out pulse, detection pulse whatever you apply and FID is collected in the time domain here.

This is a constant FID, constant period t2, here mixing time is absent. But sometimes you can say the second pulse you apply for detection is also a mixing pulse.

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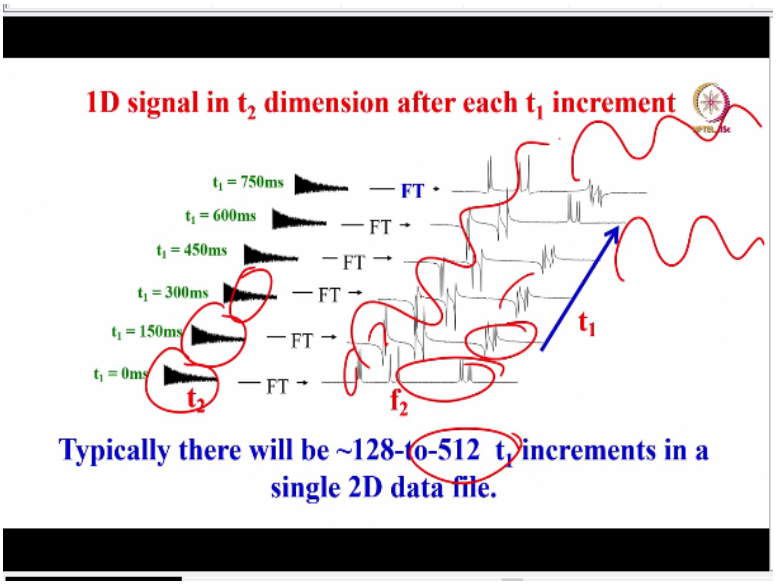

So, now 1D signal in the t2 dimension after each t1 looks like this. This is the 1D FID, for each t1 in the t2 period. Now, in the t1 period what is happening is as we are collecting the signal, let us say I do the Fourier transformation in each given period, I get a spectrum for

one delay and I get the spectrum for another delay, as I keep on incrementing the t1 I get the spectrum like this.

Now, you can see that intensity is positive, mixed phase goes negative, goes negative again come back. So, we creates an oscillatory function, undergoes oscillation in intensity. See this is an important thing; intensity is oscillating and behaving like a free induction decay. So, what we are trying to do is we are incrementing the time you are artificially or creating a pseudo FID. You are indirectly creating FID.

And how many such points will have? Typically 128 to 512, if you have enormous memory; you can increment the t1 points even more. That is up to your choice. So, basically increment t1 period; for every t1 period you collect this time domain data in the t2 period and as a function of every t1 point, you measure the intensity and follow the path. It appears as if you are creating a pseudo FID.

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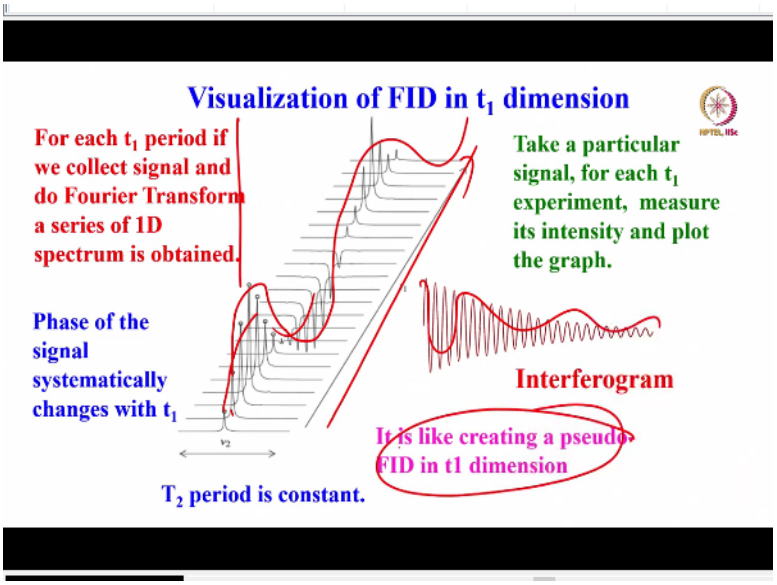

So, now, if you visualize the FID in the t1 dimension it look like that, for each t1 period if you collect the signal and do the Fourier transformation, you get the series of FID like this. That is what we saw in the previous slide. So, now take the top point of each of them, see it goes like this, is it not an oscillatory function? it is like free induction decay and as t1 becomes enormously large there will be a decay in the signal. So, there will be exponential decay here also, enormous decay in the signal.

So, it behaves like a FID; an exponential decay. So, here t2 period is constant, but only t1 is incremented here you see, in this direction. So, now, if you see the phase of the signal systematically it undergoes change as a function of t1. And now, take a particular signal for each given experiment measure its intensity and plot the graph and then you are going to get an interferogram like this here. You will get an interferogram.

This is another time domain signal you have created, analogous to what we get in t2 dimension. That is a constant period, it is a variable period. In this t1, period is varied, but created an interferogram like this. This is how FID is created in the t1 dimension. So, it is like creating a pseudo FID in the t1 dimension. Please remember that.

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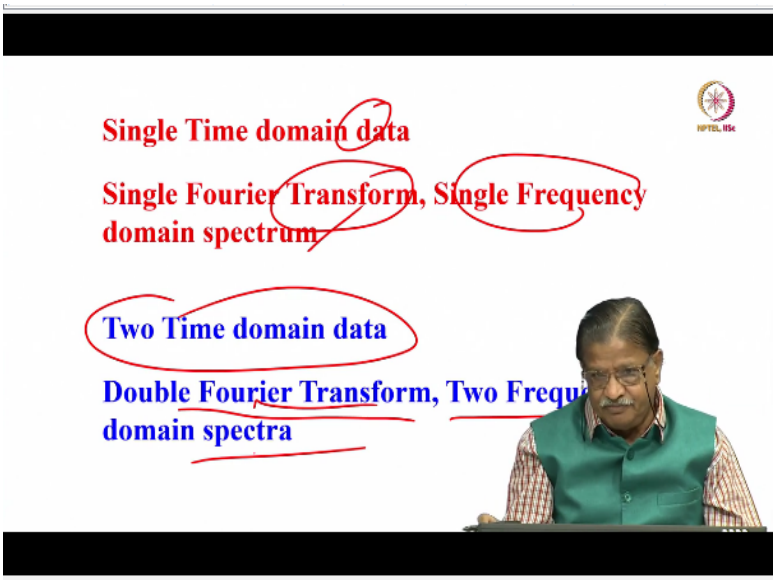

So, now we can come to a general conclusion. In the one dimensional NMR when a single time domain data, I did a single Fourier transformation and I get a single frequency domain spectrum. That is correct. Now, in the two dimensional data we have two time periods, we have to do double Fourier transformation, because we have two time periods you get two frequency domain spectra. So, two dimensional data you have two time domain data, two time periods or two time domain data, otherwise. And you do the double Fourier transformation, you get 2 frequency domain spectra.

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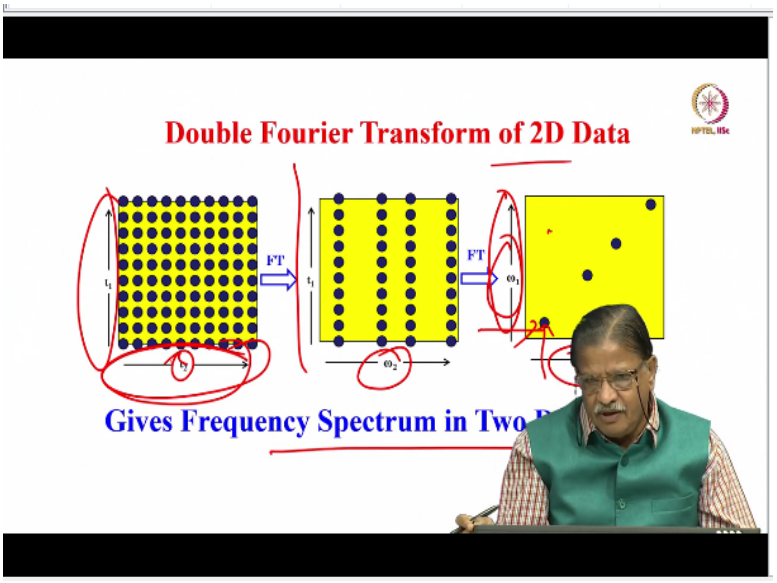

So, this how double Fourier transformation of a 2D data looks like. For example, I have collected let us say, data in both t1 and t2 like this, , t2 is constant, I keep on incrementing t1; I am incrementing t1 and collecting the data like this. I have done the experiment, collected the time domain data both in the t2 period and t1 period, I will do the Fourier transformation. Now the Fourier transformation, in which dimension I am doing? in the constant period t2.

So, now in the t2 time domain period, after doing Fourier transformation, it became omega 2, frequency now in this dimension. But still in this dimension it is a time period becauseI have not done Fourier transformation. Now, again, I will do the Fourier transformation in this dimension also. Now I have frequency domain here, the omega 2 dimension, frequency in the omega 1 dimension.

The spectrum now looks like this. The time domain data became frequency domain data in two dimensions now, you have the frequency, you can measure in this dimension, we can also measure the frequency in this dimension. So, depending upon where you get the peak and what type of experiment you do, a two dimensional data after double Fourier transformation, you have a frequency domain spectrum that contains a lot of information. So, it gives frequency spectrum in two dimensions by doing double Fourier transformation, this is how it works.

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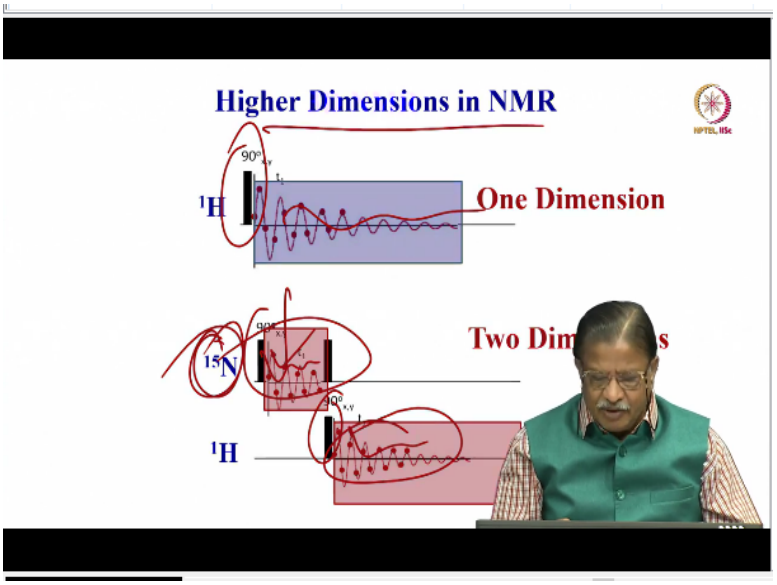

You understand. Now about higher dimension: can we go even higher? We discussed about 2D. Can I have a three dimensional data, four dimension, five dimension, N dimension? why not, it is possible, but only thing within the limit of complete decay of the signal before it completely relaxes, you have to acquire all the data. So, there is a time limitation as far as the relaxation is concerned also.

But you can have N dimensional data; we will consider how we represent the 2D and 1D, 3D etcetera; in the higher dimensional NMR. First apply an 90 degree pulse, collect a signal, it is 1D. This is a one time domain. Now, I can have two dimensional data, it can be different nuclei, it can be heteronuclear, it may be even homonuclear also no problem. You can apply selective pulse, hard pulses whatever it is on one channel, you can apply another pulse on other channel.

And then you can collect the t1 in the X nuclei, you can collect this signal in the proton channel. So, you can collect the signal in the t1 dimension, in the X nuclei channel or in the t2 dimension; the proton channel either of them is possible. So, we will collect time domain data in two channels for 2 nuclei. That is what I said, what is restricted in the 1D, it is possible here. Using 2D data you can selectively detect 2 nuclei or even 3, if you go to 3 nuclei, if you go to 3D. It is possible; this is how we collect a 2D data for 2 different nuclei. Now, this is two dimensions.

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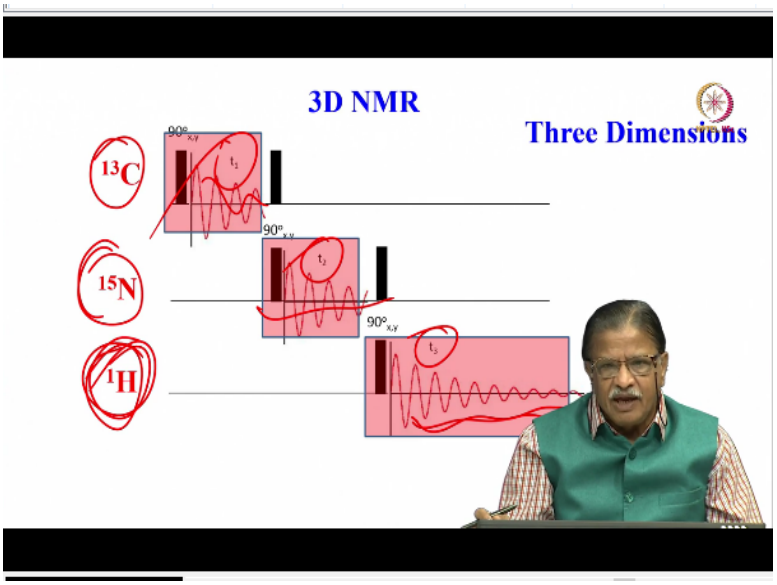

What is 3D NMR? Can I go to three dimensional? Of course yes, then you can have 3 different nuclei carbon 13, nitrogen 15 and proton. You apply a pulse on proton, carbon 13; collect the FID, this is one period. Then on nitrogen you apply couple of pulses and do something for nitrogen spins and collect signal here that is t2; then the last period t3 is here, t1, t2 and t3. This may be on the proton channel to collect the signal.

Finally, you have to collect the signal in one of the channels. Proton channel is collected here because it is highly sensitive. So, we have 3 different nuclei and 3 different time periods, it is 3D NMR. You have to do Fourier transformation in each time period, you get frequency in the three dimensional NMR spectrum. So, this is what it is. Three dimensions are highlighted here, 3 time periods and three dimensional NMR.

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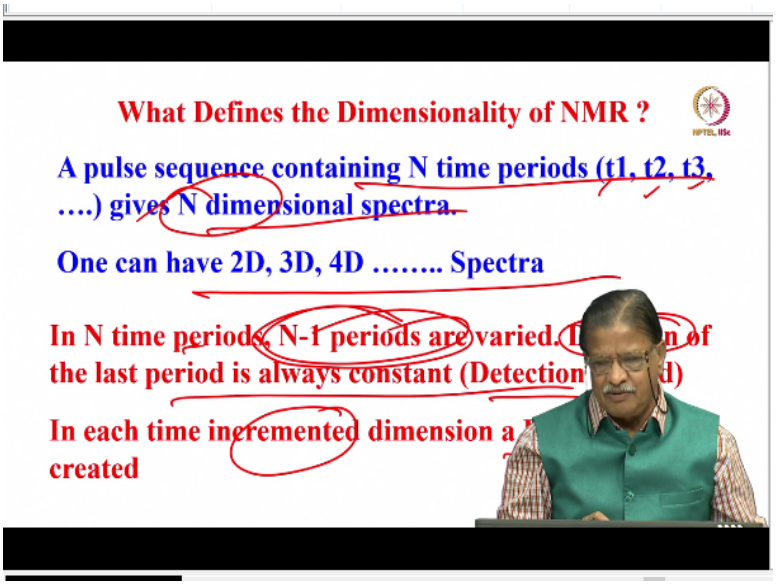

So, now question is what defines the dimensionality of NMR? A pulse sequence containing N time period gives N dimensional NMR spectrum. Remember a pulse sequence containing N time periods like t1, t2, t3 previously we saw for 3D three time periods t1, t2, t3 are there. You can have more t4, t5, t6, no problem. Depending upon the number of time periods you get that many dimensional NMR spectra.

So, for three time periods you get 3D spectrum, for 4 time periods you get 4D spectrum. So, you can have 2D, 3D, 4D, ND spectrum is possible. So, another important point we should remember is if I have N time periods, N – 1 time periods are varied, generally. Of course, if you want you can keep one of them constant or that is special experiment; you can have N number of experiments. But generally, in N time periods N – 1 periods are varied and the detection or the last period is always a constant. That is called detection period. You understand, in the N period, N – 1 periods are varied; last period is the period in which you detect the signal, that is a constant period called a detection period. In each time period, the time is incremented in that dimension and you create pseudo FID like we observed in the 2D.

And each of them you have to increment like in t1 and then create pseudo FIDs and that many number of time domain signal you get, that many Fourier transforms that many number of frequency domain spectrum you get.

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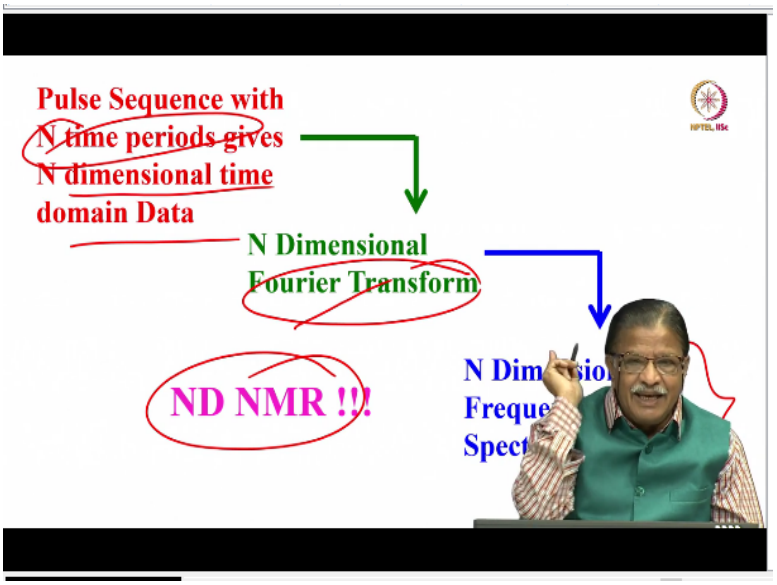

So, we can have generalized like this, you have a pulse sequence with the N time periods, I design a pulse sequence, homo or heteronuclear does not matter; N time periods are there, one is fixed and the N – 1 I vary and create N dimensional time domain data. If pulse

sequences with N time periods gives N dimensional time domain data and do N dimensional Fourier transformation; you get N dimensional frequency domain spectrum.

Please understand, you get N dimensional frequency domain spectrum when you do N dimensional Fourier transformation. It is important to understand. So, then we call this as ND NMR. N dimensional time domain data, N dimensional Fourier transformation, gives N dimensional frequency spectrum called ND NMR.

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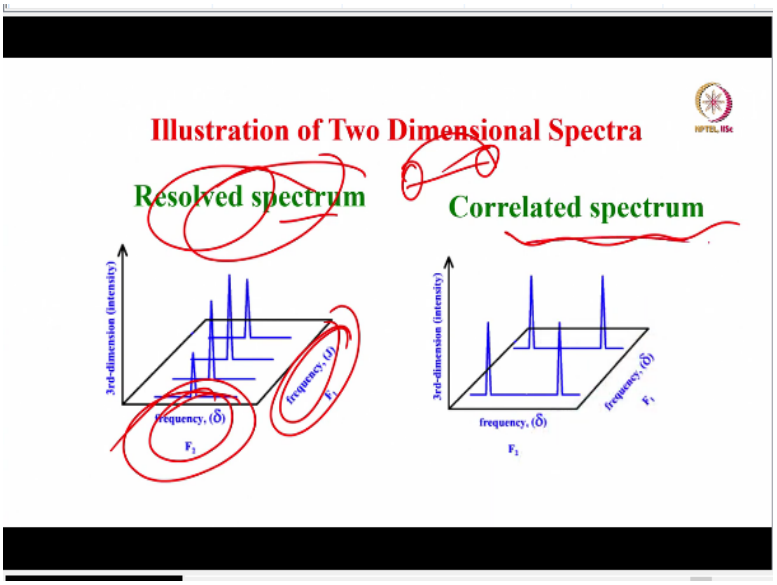

Now, illustration of a two dimensional spectrum. How do we illustrate the two dimensional spectrum? Basically, there are various ways of defining, various ways of having a 2D spectrum. Now, over the years NMR spectroscopy has advanced so much you have N number of experiments to derive information and each time you can have a different information in different dimension.

But basically broadly speaking, I will say there are 2 types of experiments we can think of. One is the resolved spectrum, where in two informations are resolved in two dimensions; or separated in two dimensions. In one dimension you can have a frequency, other dimension you can have a J coupling. That means, you can have a chemical shift here, you can have J coupling here, J coupling and chemical shift are present in the 1D spectra, both will be present. They are inseparable, but in this two dimensional NMR you can separate out both of them in two dimensions. That is called resolved spectrum, you are resolving 2 information, two parameters of NMR.

Otherwise, we also have correlated spectrum. In the sense I can correlate the information from one part of the molecule to other part. For example, there is a big molecule I have proton here and I have a proton here. I can correlate how it is coupled to this, I can correlate the information from one of these spins with other spin is called correlated spectrum. So, you can have a resolve spectrum and you can have a correlated spectrum; both are possible.

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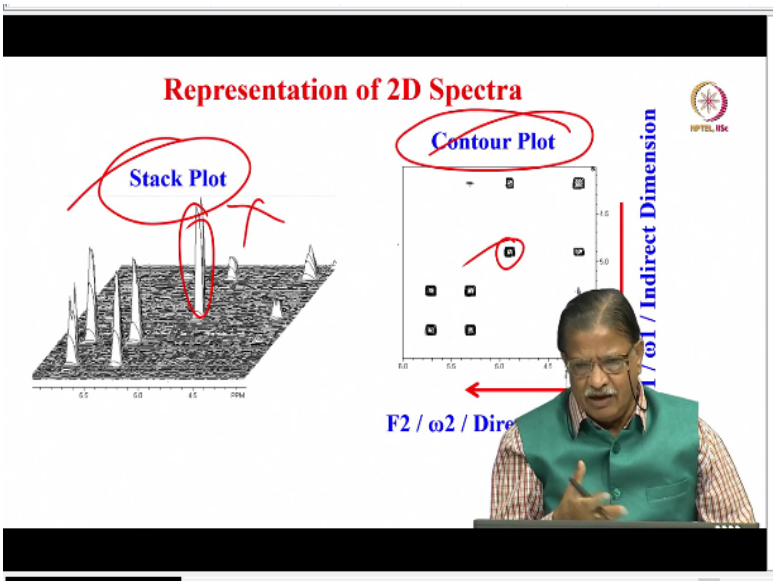

So, how do you represent the time domain data? In the very, very good old days, long back, probably even when I was young; they used to have a stack plot where it used to be plotted like this. Now, it is no more done. It is completely done away with. The representation of the 2D data is only a contour. The each peak is represented the form of a contour here. This is a spectrum actually, it is a peak, it is represented in the form of a contour plot. So, this type of stack plot is almost done away with, and nobody use that nowadays**.** Always contour plot.

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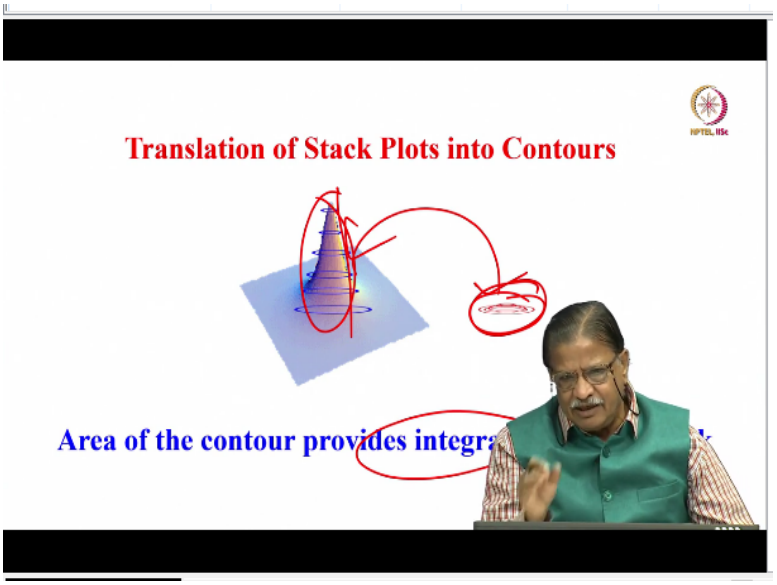

Then you may ask me a question, how does contour plot is represented when there is a peak like this? How do we call it a contour, how it is represented, how do we translate peak into contour? It is simple. Let us say I am going to a mountain, I go to the bottom of the mountain, go one round, take the area and put a circle and I go further up, go around, get the area put a circle, go further up take the area. Like this, each time I keep on going at different heights taking area, put the circle, go around, take the area and then put a circle. So, that means essentially this contour area gives you the area of the peak. That is the way the contour is represented or the peak is represented as contours. So, this what happens, you have different areas, go up and up and up. Every time you go up here like this, go up here, measure this area, put a circle here and go up measure the area put corresponding to that another contour, another circle, another circle like that, keep on stacking one over the other here.

The same stack plot is now translated into the contour here. This is a translation of stack plot into contour plots. So, a if I get the area of the contour in the 2D spectrum, it means I am getting the integral area of the peak. That is what, it is just translation of the integral area of this peak into the area of the contour; it is represented instead of peak, as a contour here. That is all. That is how that peak is translated into contours.

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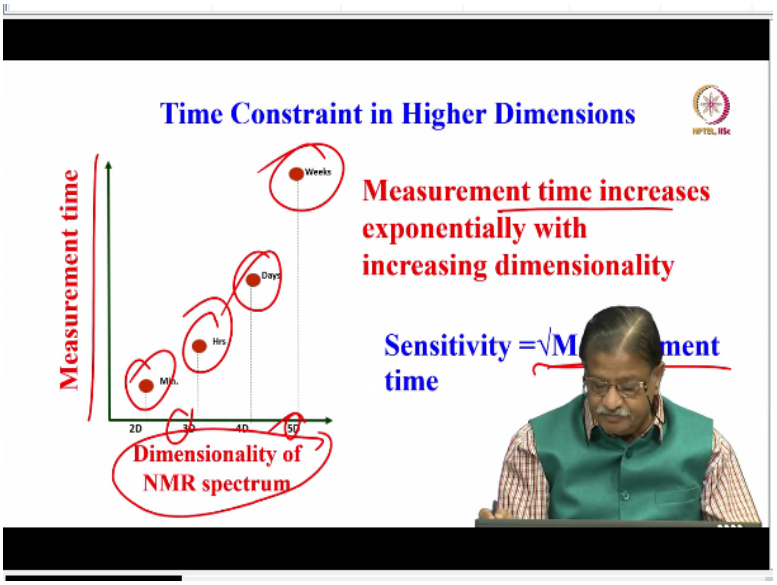

Now, let us discuss about the time constraint in higher dimensions. If I am trying to go to higher and higher dimensions, is it easy? Is it possible for us to go to all higher dimensions whenever we want or whatever the molecule we have. No, there is always a time constraint. For example, here is a plot about measurement time and the dimension of the NMR.

If I go to 1D NMR normally if I want to the proton NMR for a small molecule or even a reasonably big molecule, which do not have abnormally large long relaxation times, you can get the NMR spectrum, a proton spectrum of such molecule in less than 5 minutes; nowadays, very fast. You go to two dimensional NMR, how much time it takes? You have to acquire the data in two dimensions, you have fixed time and variable the time every time when you acquire the data. It is time consuming. Two dimensional data definitely takes more time than 1D NMR, time domain data, how much time it will take? Now in the present day methodology rapidity is there in the acquisition, processing, everything; you can say it is of the order few minutes; make 30 minutes to a half an hour to 1 hour, like that. No problem. That is also still faster. You go to 3D NMR it can go up to hours. Go to 4D NMR, it will go to days. If you want to take 5D NMR you may have to spend weeks to acquire the data, it is not easy. You have to consume enormous amount of the instrument time, the measurement time increases exponentially with increasing in dimensionality. Further, the sensitivity also goes by root of measurement time, that you should understand. We discussed in the very first course about signal to noise ratio ,everything, how many times you have acquire the signal to get the S/N, it is the root of N, all those things we will discussed. So, it also goes by the measurement time.

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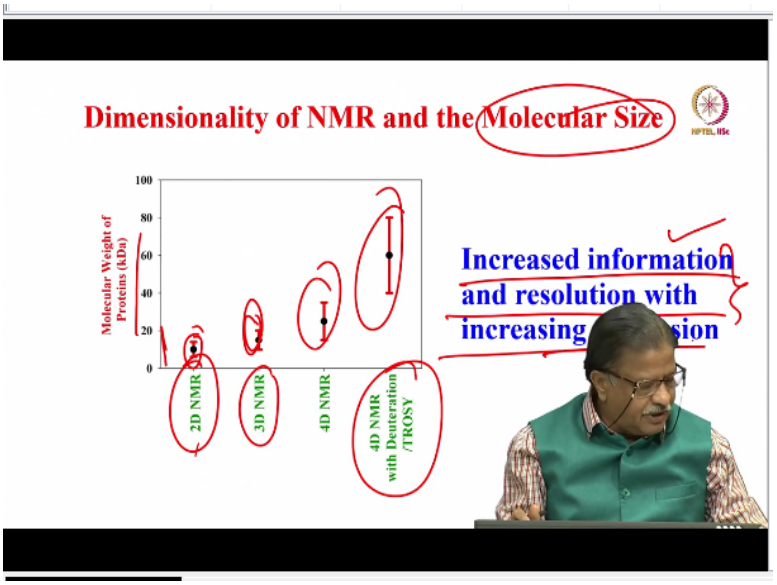

And now dimensionality of an NMR also depends upon the size of the molecule you are looking at. It is not that you take a simple molecule like a water and you want to go to take the higher dimensional NMR, I want to go to higher frequency. These are all not needed. You have to judiciously decide which type of spectrometer you require, what frequency and what is the type of molecule, whether you want to 1D NMR, 2D NMR, 3D NMR that depends upon the spectral complexity or depends upon the size of the molecule.

If I take just the benzene and you would take 100 Megahertz, 50 Megahertz, 60 Megahertz is sufficient, you get a single peak. You do not have to go to 800 megahertz; I do not have to do 2D NMR. So, dimensionality and the frequency which you want to choose for doing the experiment, all depends upon the molecular size and spectral complexity. If I want to take a 2D NMR, generally, you will have to go when the molecular size become higher and higher, molecular weight becomes more.

See you can go up to 0 to 20 kilo Dalton and we are taking the example of proteins here. Then up to 0 to 20 let us say, you can go to 2D NMR, reasonably okay. Around that 20 to 30 if you go even 3D will do, 4D you can go up to 40 kilodalton like that. Higher if you go up to more than 40 up to 80 to 100 kilo Dalton, you need 4D NMR, and even higher experiments other special experiments you require.

So, dimensionality increases with the size of the molecule. There is increased information and the resolution is with increased dimensionality. As you go to higher dimension, you have increased information and you can have a better resolution in the sense you are resolving the

information or spreading the information in different dimensions; and as you increase the dimension these two parameters benefits you a lot.

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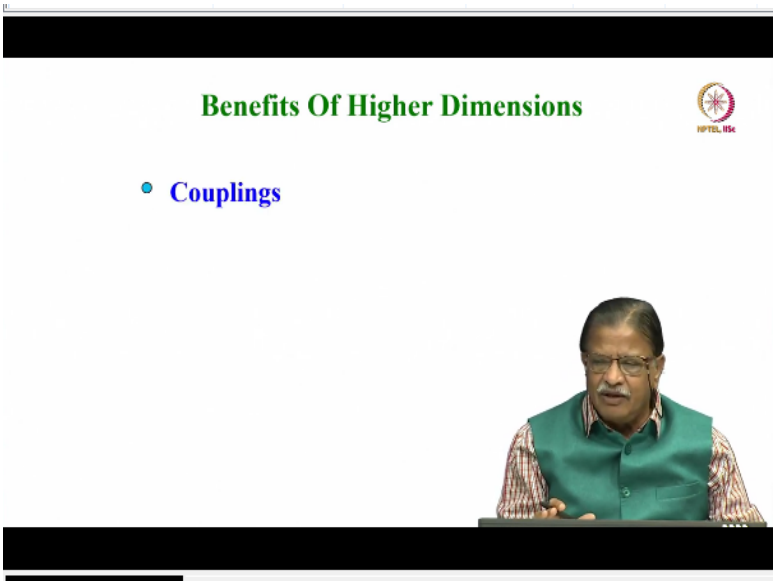

I will go to the benefits of higher dimension and then it will take more time to explain. Now the time is up. What I am going to do is, I will stop here and we will come back and continue with this firm next class. But today what I wanted to tell you is I started introducing the 2D NMR, it is another dimension of NMR which was introduced way back in 1971 after the first Jeener's experiment and Professor Richard Ernst is the one who pioneered this type of technique and got Nobel Prize.

He is the one, an authority of this 2D NMR, he was responsible for the development of this technique, an introduction of this type of techniques. And nowadays, there are N number of such type of multi dimensional experiments; both homonuclear and heteronuclear, designed by various workers in the field. And what is happening in one dimensional NMR, I said you apply a pulse and collect the signal in one time domain, you do the Fourier transformation, you get one dimensional spectrum.

In a two dimensional on the other hand I said there are four time periods, preparation period, evolution period, mixing period and detection period. Of course, mixing period is always optional that depends. Sometimes even in two pulse experiment, the second pulse itself can be treated as a mixing pulse. That is okay. So, four time periods are there, preparation period is to allow the spin to attain thermal equilibrium.

Evolution period is the one where we increment the time systematically, every time increment the signal as a function of time we collect the signal in t2 dimension. Now as a function of time you create a pseudo free induction decay in the evolution period. We have time domain data, two time periods. Do the double Fourier transformation we get two frequency domain spectrum, and always if you have N dimensional spectrum, you have N time periods, N - N periods are usually varied. One period is kept constant. So N time periods, N dimension Fourier transformation you could ND spectrum and I said it also depends upon the time. As you go to higher and higher dimension, enormous amount of time is going to be consumed. I said two dimensional NMR you will get spectrum in few minutes, 3D NMR few hours, 4D NMR few days. And if you go to 5D NMR, etcetera, you go to several weeks of acquisition of the data. The instrument time will be consumed enormously.

And also I said depends on the molecule size. 1D NMR for a small molecule you can take spectrum in 5 minutes; or if you go to 2D NMR you can go up to molecular weight of 20 kilodaltons. And for 3D NMR slightly more you can go up to 40 and above 40 to 50 or 60 will go to 4D NMR. If you go to even higher molecular size of protein, you go for 50 kilodaltons. you may have over 4D NMR with another special experiment. So increased information you get with increased resolution, as you go to increased dimensionality. So, this what we understood today; I am going to stop here, I will come back and continue with the 2D NMR in the next class. Thank you.