One and Two-Dimensional NMR Spectroscopy for Chemists
Prof. N. Suryaprakash
NMR Research Centre
Indian Institute of Science – Bangalore

Lecture – 51
Two-Dimensional NMR

Welcome back. In the last two classes we started discussing about two dimensional and multi-dimensional NMR, in particular two dimensional NMR; where I discussed the limitations of one dimensional NMR spectra, the need for going into higher dimensions, and I also discussed about the dimensionality time constraints, the size of the molecule which can be studied by different types of multi-dimensional NMR experiments and varieties of other things were discussed. Lot of information was given to you.

And now we have to appropriately choose the particular dimension of the experiment; the dimensionality choosing is very important for a molecule. Now how do you choose a dimensionality required for a molecule?

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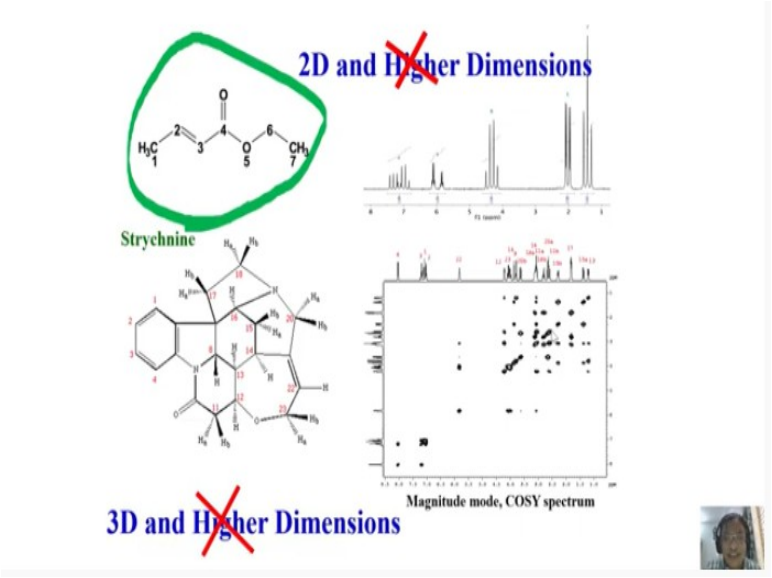

Okay, we will go for that; how do you require to choose the dimensionality and see that is very appropriate. You cannot take a simple molecule, say for example a molecule of this size which is having 1, 2, 3, 4, 5 different types of protons present, and then you just cannot say just because the molecule is available and the spectrometer is available for you, you will go and do the 800 megahertz spectrum.

I will record 800-megahertz spectrum and I will record two dimensional, I will record 3D NMR, etc. What is the need for that? We should understand. You have to go to higher dimensions only when the lower dimension cannot give the required information. It depends upon the size of the molecule, number of chemically inequivalent protons. For example, your molecule may be big it may have a number of protons, that are chemically equivalent. For example, you can take kekulene or for example you take annulene. Lot of protons are there but many of them are equivalent in which we have only one or two types of chemically inequivalent protons. So, then what is the point in going to higher dimensions? So higher dimensions choosing is very important. You have to first look at the type of spectrum you get in 1D NMR and afterwards see whether you can extract the information in the straightforward manner, with the easy analysis, and then you have to choose the appropriate dimension, okay.

Let us look at a molecule like this. You take 1D NMR, very simple spectrum, you have only well resolved peaks and well resolved multiplicity pattern. Very easily you can analyse. This will be a triplet because of this, this will be a quartet because of this, etc. Just by looking at it hardly it takes maximum two minutes to analyse the spectrum; worst come to that you might want to analyse and measure the separations and get the chemical shifts along with the coupling information, then also it won't require more than five minutes for you.

So, 5 minutes for recording this spectrum if you take; In 10 minutes this molecule can be completely analysed taking the 1D NMR spectrum. Then the question is why do you require 2D for this? Why do you require higher dimension? For this molecule there is no need of two dimension or higher dimensions; only one dimension, simple one-dimension proton spectrum is sufficient. If you want to get the carbon coupling, carbon information also one dimensional carbon will do; simple you see; using DEPT you can identify the carbons attached to different protons, very simple; it will not take more than 5 to 10 minutes for analysis. So, you can safely discard the higher dimension requirement for the molecule of this size.

Now let us go to a molecule of this size; it is strychnine. This has large number of protons as you can see, number of protons chemically inequivalent have been marked here. Take a proton spectrum of this molecule; one dimensional, in a reasonably high spectrometry

sequence you can take in 500 or 600 , you get very beautiful spectrum; you see it is a fantastic spectrum well resolved, very easily you can make the assignment.

I will tell you even for this struchnine molecule, you do not require more than one dimension. Frankly here you took let us 5 minutes to analyse, here you will take fifteen minutes or half an hour to analyse. Okay, take 1 or 2 hours if you want to completely assign and extract the coupling information, everything if you do; it will not take more than 2 to 3 hours maximum. The complete analysis including recording of the spectrum will not take more than 2 hours or 3 hours; even then you do not require 2D and higher dimension.

But okay to save the time, and to make our analyses faster and easier, we can go for 2D for this. This is reasonably acceptable because reasonably big size of the molecule; you can go for 2D; where you see well resolved peaks area there you can try to understand everything. It is okay reasonably good molecule, reasonably medium size molecule for which you can go to 2D; but you do not require 3D and higher dimensions for this molecule. You should not go to 3D and higher dimension; what you get out of that?

Of course even for small molecule sometimes you go to different type of experiment depending on the particular information which you want to derive; for that you can design an experiment of your own; you can go to two dimension, three dimension etc. But for the routine analysis two dimensional experiment is more than sufficient even for this Strychnine molecule. You do not require higher dimensions; then the question is when do I require higher dimensions?

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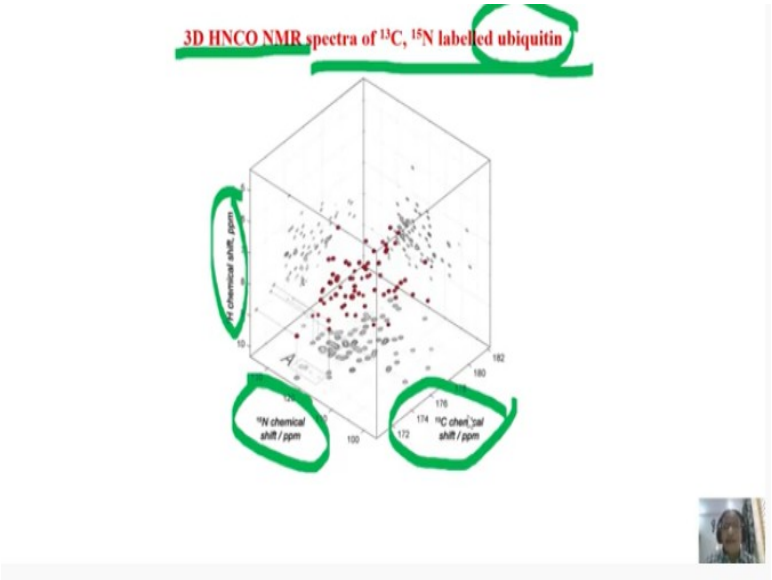

This is where you have to go. Take for example carbon 13 and nitrogen 15 label uniformly label ubiquitin, it is reasonably a big molecule. I do not know maybe it has more than 50 amino acids and each amino acid you can consider as a particular coupled spin system. All protons; right from NH proton to methyl if you go, there are so many protons in between and many of them are coupled among themselves. As a consequence, the spectrum is really complex, I understand; even 1d or even 2D is little difficult to analyse the spectrum of the molecule of this size; okay?

So you have to resort to three dimensions. It is a typical spectrum of an experiment called 3D HNCO of this ubiquitin molecule, it is just to show you how the 3D spectra are represented. Okay, here three dimensions; means in one dimension we have proton channel, other dimension you have nitrogen, and in third dimension we have carbon 13. In three dimensions, the 3 different nuclei can be chosen. And the analyse of this 3D spectrum is not pretty straight forward, like 2D; you have to take planes in different directions. You can take a plane along this axis; you can take a plane like this; or you can take a plane like this. Accordingly, you can break this 3D spectrum into several 2D spectra, and then make the analysis. Okay, it is not fairly a difficult job. It can be done, but what I want to say is for a molecule of this size, you definitely require higher dimensions. This is how you have to choose.

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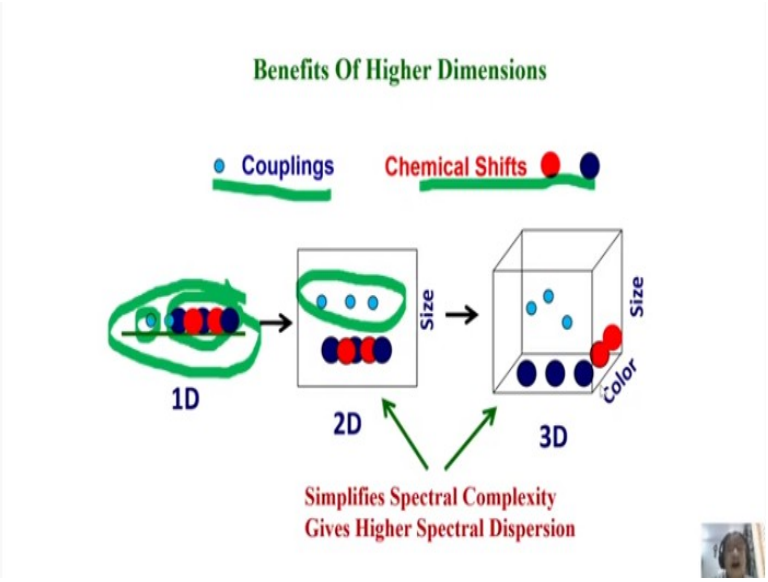

Now what are the benefits of higher dimension? One thing of course you saw here, the analysis becomes simpler; why? we will see that. Let us consider a simple example, we have a molecule, where we have different chemical shifts. Let us say it could be homonuclear; there are several chemically inequivalent protons are there, we have several chemical shifts it could be heteronuclei, it does not matter. Or we have couplings, each proton is coupled to other protons or other NMR active nuclei; number of couplings could be present. In which case if I take a 1D spectrum, let us say proton-proton coupling, proton-carbon coupling everything are overlapped like this. All peaks are there; we have couplings; we have different chemical shifts; which are identified by different colours and different sizes; the small spherical balls represent couplings; bigger spherical balls here represent different chemical shifts, Homonuclear. It could be Heteronuclear; but I have taken, let us say, the Homonuclear case. No problems, does not matter, I do not have to specify the type of 2D of the experiment. I have taken a 2D experiment something which I know I can resolve this. What I am going to do is first I can separate out all the couplings in one dimension. On the basis of the size, all smaller coupling values, the smaller balls, the smaller size spherical balls are pulled out in this dimension.

Now the complexity coming because of this reduced quite a bit already. You have some of it shifted up in one dimension. Now I can do one more thing why not I separate out different colours of the balls. Here i can put black colour in this direction, and red colours in this direction, at the same time I can pull out the different sizes of the small ball sizes in this dimension.

Now I took three dimensions and 3 information have been put in three different dimensions. What did we do? By doing this we have reduced spectral complexity. The dispersion became much, much better. The higher dispersion came and instead of so many peaks congested in a small area, it was clumsy for us to analyse, now we have simplified the complexity. So because of this, the spread of the peaks, we have got better and better dispersion, and your analysis becomes much better. You understand the benefits of higher dimension; first and foremost you can simplify, spread the information content in different dimensions, where you can extract information the way you want. Design the experiment and in an orchestrated way to can extract the coupling information; the chemical shift information or any other spectral information of your choice, got it. This is the biggest benefit of higher dimensions; okay.

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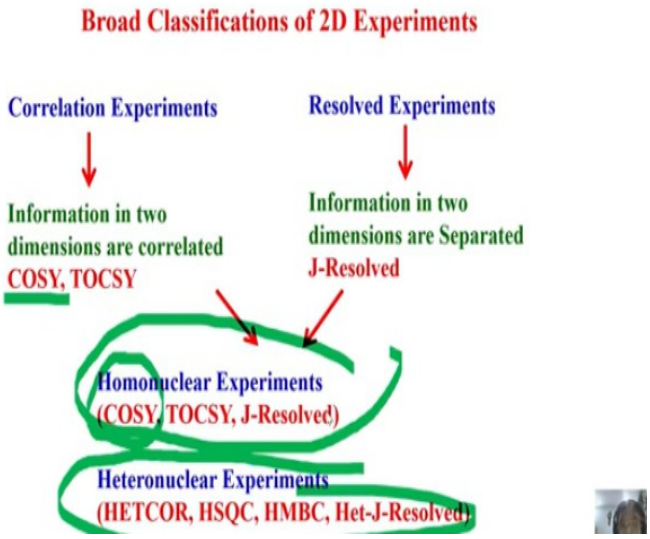

You understand now; I have already told you, how we have to choose the dimensionality and what higher dimensions will benefit you. Okay, now the question is I said you go to higher dimension; but i did but I did not tell you which type of experiment you have to choose. You may ask me a question, I have a reasonably medium size molecule, molecular weight of 500 or 1000 like that, now I do not know what experiment have to choose. I know the spectrum is complex like strychnine, which I showed you. But you might say slightly complex than strychnine, but I how to analyse, tell me what experiment I have to choose. That is another thing, choosing the proper experiment is very important in deriving the information. So, now I am going to tell you what are the types of 2D experiments which can give you information? What type of information I can get from that? Basically I will classify the 2D experiments or in general multi-dimensional experiments; you can take it on multi-dimensional experiment.

But my focus is on 2D today, and for the remaining t 1 or 2 classes I focus on two dimensions so we say it is for 2D. But in general, you can classify these for any multi-dimensional experiment like this, Okay. A broad classification of 2D experiments are like this; one is correlation type of experiments, where information in two dimensions are corelated. What type of information can be corelated? it can be chemical shifts of Homonuclei.

For example, I have a molecule, reasonably big molecule, which has showed you recently; 5 different chemical shifts. The different chemically inequivalent protons are there; not big, okay, molecule with different types of equivalent inequivalent protons are present. Now when I wanted to analyse the spectrum, what I was looking for coupling patterns; so that I know which is the coupled neighbour for each proton. I had a CH2 group attached to the CH3 group, I know CH2 will split CH3 into triplet; I also know CH3 will split CH2 into a quartet, based on that I could say this CH3 attached to CH2.

Similarly, for other protons I can do; understand. Now I want to see the carbon assignment; I go to carbon and take the carbon couple spectra, DEPT spectra and identify the carbons. So, reasonably with one or two small tricks I can make the assignment of that; but if I give you a big molecule like we saw in the protein; so many amino acids are present so many different types of protons are present. Now in a crowded spectrum, I want to know which proton is coupled to which peak. which is the peak corresponding to this; let us say, I have a bunch of peaks here; this proton maybe experiencing coupling with different types of other nuclei coupled to it through covalent bond. Now how do I know what is the coupling strengths? which is coupled to it? which is just immediate couple partner? which is coupled one that is two bonds away? which protons is coupled that is three bonds away?

I have to do the experiment. For this in the olden days we used to do a typical experiment called decoupling, which I already told you heteronuclear decoupling; same way we were doing Homonuclear decoupling; that also I explained. If I have a coupled spin system like this, so what I have to do is, I will irradiate at this frequency, and see how the multiplicity is getting changed? If it becomes a singlet, then I know this is coupled to this. That was a four linen pattern; now it becomes a two-line pattern because of this; then I say this is coupled to this; and this doublet is coming because of another proton which is coupled to this. So, like this we had to be n number of experiments; all selective decoupling experiments to identify the coupled partners. So that sequentially I can go CH3 is coupled to CH2; this is CH2 is

coupled with this; this CH2 is coupled with this; like that finally after doing number of such experiments I know the complete molecular structure or conformation; and I will get the functional groups present, etc.

How they are present in the molecule CH3, CH2, CH, and I can start writing the structure. In the molecule like ubiquitin or some other big protein molecule how do you do? It is not that simple as I said for a small molecule. For that we you have a correlation type experiment which is simple, where I can correlate the chemical shift of one proton which is coupled to it. I have a two-dimensional spectrum; in this dimension that is I had a proton here, which is coupled to another proton. This coupling information I will spread in two dimensions I know if there is a peak here this is coupled to this. So, this type of coupling information where the two protons are scalar coupled, mediated through covalent bond can be correlated.

We can correlate two protons which are coupled to each other or more including the long range coupling. If one proton is experiencing many couplings, all of them can be understood; which is coupled to which. Sequentially, we can start going one by one and make the assignment; that is called chemical shifts correlation. We can correlate the chemical shift of homonuclear spins which are coupled between or coupled among themselves, with many protons. The same thing can be done in this experiment called TOCSY, where the whole spin system can be identified, and this is Homonuclear.

Of course we can do another type of experiment called resolved type, in the sense when I looked at the complexity of the spectrum, I said we have chemical shifts in addition the multiplicity is there because of J coupling; this multiplicity comes because of one proton coupled to many other protons which are bonded to it, which has interactions with neighbours. As a consequence, one proton splits into multiplicity.

So many multiples will be there it could be doublet, triplet, quartet, and all these multiplicity patterns which I discussed could be there. Now how do you extract the coupling? Will it not simplify my analysis, if I pull out the coupling information in one dimension and retain only the chemical shifts in another dimension. Remember these two parameters are invariably present in your spectrum; they come as a pair you cannot avoid that. But is there any way I can resolve it?

This is called separation type experiment. Here I correlate one information with respect to another through covalent bond. Chemical shift to chemical shift correlation. Here I can resolve them; I can separate out the coupling parameters. I can get chemical shift in this dimension and pull out the J coupling in this dimension. Then what happens? I have greatly simplified the spectrum. These are called resolved type experiments; simple example is called J resolved where I retain proton chemical shift here, I can get only J couplings here. I separate out two information in two dimensions.

So, this is another class experiment called resolved type experiments okay. Now the question is you may ask me what happens if multi nuclei? if several NMR active nuclei are present in the molecule; should I have to restrict this only to Homonuclear? Can I do Heteronuclear experiment like correlation and resolved type experiments of heteronuclei? sure can it be done? Sure, both experiments can be done for Homonuclear spins and also for Heteronuclear spins; very simple.

For example, Homonuclear correlation experiments, like this, COSY, TOSCY Homonuclear resolved experiments are J- resolved. Similarly, Heteronuclear experiment are Heteronuclear correlation HETCOR, HSQC, HMBC, Het-J-Resolved; these are all such examples. As we go ahead, we understand some of the sequences; of course each and every sequence you can understand how we get information. Mathematically you can understand by spin physics, you can trace the magnetization pathway, how it is moving. Right from before application of a first pulse, when the spins are in thermal equilibrium till the end, till we detect the signal, what has happened to the magnetization, what has happened to the spins can be monitored. This is what all about designing of the pulse sequences; and designing of the experiments. This is what is called manipulation of spin dynamics. We can do that to get the different experiments. Let us not go to understand the spin physics of each of these pulse sequences that itself is a big semester course.

So since this is basically focused on application and utility in chemistry; we will give one of the pulse sequence ;I will tell you how it works or in a simple way without going deeper into mathematics, because I do not want to frighten you; and we will see how we get information. And then similarly we take couple of examples of varieties of this spectra; and then try to analyse and see what we can get. This is what we are going to do today.

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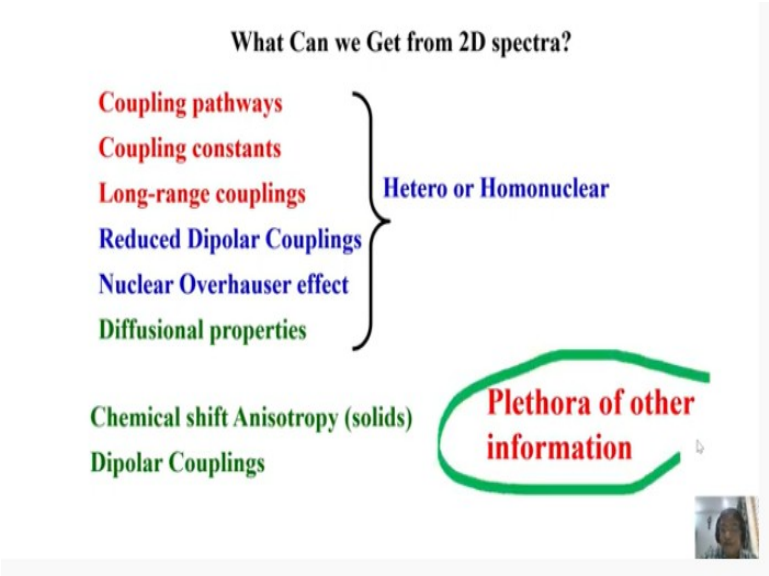

The question that you can ask me is, okay I know I have a molecule; I know the size of the molecule; and I know what type of 2D experiment; what dimension of NMR experiment I require do; and from the previous slide you also know what type of 2D experiment you have to do. But what are the other information that I can get from 2D spectra? which you may not know; see in the correlation experiment you correlate chemical shifts, J-resolved experiment you resolve 2 parameters; separate out in two dimensions; but can we get any other information from 2D spectra which you can utilize later.

Of course, there are number of information available that you can extract from the 2D spectra. One; you can extract the coupling pathways, pathway of the two spins that are coupled; you can extract the coupling constants by resolved type experiment; you can get the coupling homo or Heteronuclear in this dimension, you can get chemical shift; you can get long range couplings; that is very interesting. Let us say I have 1, 2, 3, 4, 5 or 6 protons are coupled. This proton has a long range coupling to this, which is 3 bonds away; this is one bond, this is two bond, this is separated by 3 bonds; and this proton is coupled to this, does not matter homo or Heteronuclear. I am looking for say Heteronuclear case; long range homo nuclear couplings are very small. So, for Heteronuclear case; I will consider it is the carbon, it is coupled to proton here 3 bonds away.

Can I get this coupling? only this coupling, supressing these two. It is possible, you can get only this or you can get the only one bond coupling. Like that you can design experiment; get a specific information you can get. Only long-range couplings or you can get only one bond

couplings like that. You can get the information on reduced dipolar couplings; it is another information which you get, when you partially align the molecule.

I said in the very first class remember, I said in the NMR can be studied in solid state, solution state and also I mentioned in the liquid crystalline phase. Where you can partially align the molecule; where partially aligned dipolar couplings, chemical shifts isotropy, etc will get reflected in a spectrum. That is what I said. So these are all called reduced dipolar couplings, called RDC's which are extremely useful in getting the structures of bio molecules. Many times in the biological applications, the biomolecular structure case studies these RDC's are used as constraints.

We get this value to get the geometry information, that is also possible. We can get NOE. What is NOE? We discussed nuclear Overhauser effect, so we can get the spatial proximity information, that is possible. We can get diffusion information, if you have a combinatorial mixture of different molecules, with all diffusing at different rates, I can find out the diffusion coefficients of these molecules and identify the number of components present in the mixture.

That is a fantastic application, that we can obtain. If we go to solid state, we can get chemicals shift anisotropy, we can get dipolar couplings. Remember this information are not obtainable from the solution state spectra. You know why? I already told you because of the motional averaging this information get completely nullified. You will not get it in the solution state. However, they are present in the solid state.

We can get this type of information when I do solid state NMR, that is this dipolar coupling. Again further, we can get homonuclear dipolar coupling only; we can get only heteronuclear dipolar couplings; that is also possible. So, you understand this is only a tip of an iceberg I am giving you, okay? You can get a lot more information from varieties of two-dimensional spectra, that is possible. Or you go to higher dimension, you get much more information so I would say there is a plethora of information we can extract from varieties of 2D spectra. okay.

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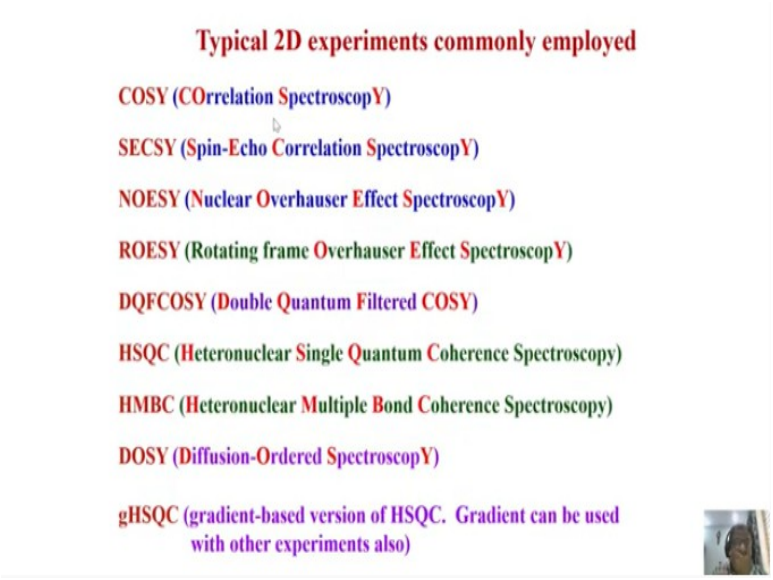

And these are the typical 2D experiments commonly employed. If you go to the literature, let me tell you at present you can get several hundreds of 2D and 3D NMR experiments possible. Several hundreds of people, many pioneers in the area of NMR spectroscopy who have been working or been doing research in this area, have designed number of experiments. They are very much useful for us, we should thank them for contributing so much.

Now there are number of such experiments available, just go to the literature or see any books of NMR, at least several hundreds, I can right now say about 200- 300 types of experiments are available, or even more. So, but commonly employed some of these things in a day to day analysis. Of course, you can do corelated spectroscopy called COSY, where you can corelate one information with other information like chemical shift, homo- and hetero-nuclear; you can do spin echo correlation, NOESY, nuclear Overhauser effect we can do to get the spatial information. ROESY the same NOESY information we can obtain in the rotating frame, called rotating frame Overhauser effect. We can use double quantum filtered COSY; this COSY has certain limitations. All these things are improvements, you know somebody develops a pulse sequence, one of the pioneers in the area developed a pulse sequence for some information. And then some other people who are working in the area they will keep improving it day by day. So, if there are certain constraints or if there are some limitations, or there are some issues related to one type of experiment, the improved versions will be made available later. Exactly, this COSY has some limitations. Now as a consequence, DQFCOSY came. This can partially address many of the issues which were there in the COSY experiment.

Similarly, HSQC is an experiment where you can correlate chemical shift between two hetero nuclei, like proton-carbon or proton-nitrogen, etc. This is done in a different way, this is done in an indirect way which is called inverse detection. We do not directly observe the carbon, I will tell you when we go ahead. This is by using what is called polarization transfer technique, which I told you in one of the classes, so that we can enhance the signal intensity. The same thing you can do for heteronuclear multiple bond correlation; and not direct one bond like we said long range coupling. We can say this proton is coupled to carbon, two bonds away or three bonds away, directly I can extract that information. I can get diffusion properties by DOSY experiment. Many of them I can simplify the experiments by using gradients, instead of phase cycling. I did not explain much about phase cycling; do not worry. That is not immediate necessity now. But just I wanted to tell you number of hundreds and hundreds of such experiments are available for routine use; but the proper choice of what you want to do you can get. You understand.

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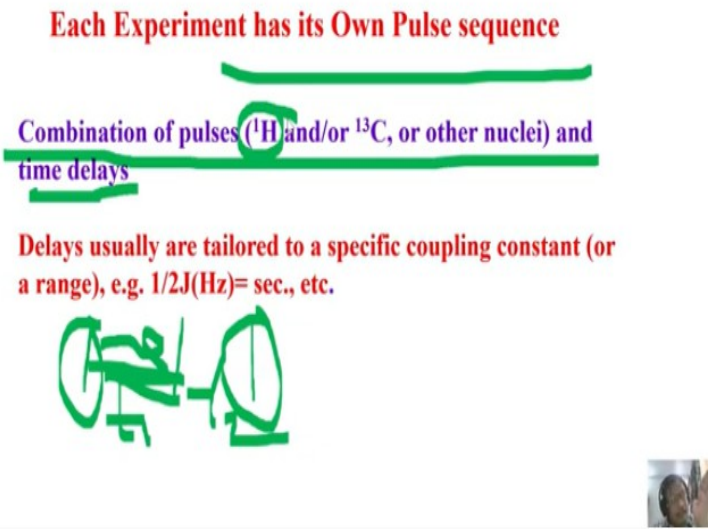

Now how do you design a pulse sequence is the next question. Okay, then are many pulse sequence are there; many information is available; I know what experiment to choose; I know what information generally different types of experiment give. But my question now is how do we design different types of experiment. Of course, for this course that is not necessary thing; it is not the basis of this course. In this course I am trying to tell you more from the application side or utility point of view.

So, there is no need to worry about it; but just to give you a broad idea how the pulse sequences are designed. Each pulse sequence is combination of various pulses with delays.

Simple example you take COSY, COSY has two pulse sequences 90-degree pulse t1 period which is systematically varied and then another 90-degree pulse; and then start collecting the data. So, you have a 90-degree pulse t1 period another 90-degree pulse and then start collecting this area and this is systematically varied; this is t1 and this is t2.

Now this is a delay, so this is the 90 degree pulse, it need not be 90 it can be different angle in some experiment. Take for example simple DEPT experiment the last pulse was of different angle, we knew we can do DEPT 45; DEPT 90; DEPT 135. Accordingly, we get different information is it not? Similarly it depends upon the type of pulses; and the pulse whether you apply proton or carbon; and what is the delay you are going to give.

Remember delay is very important in DEPT and INEPT experiments we found out after the magnetization was brought back there was anti phase magnetization; we knew if we start doing the decoupling it will get nullified. So we gave a delay and made sure these components further evolve and then we started doing decoupling. See the small one third of that tau delay was very useful there.

Similarly, these pulses, the pulse angle you apply; the 90-degree, 180 degree or 45 whatever the flip angle; the angle through which you flip the magnetization; tilt the magnetization is called flip angle. A flip angle of the pulse and the delay matters a lot. That will tell you more about your pulse sequences; okay; and we can design based on what happened to magnetization at different delays and different pulse sequences; like we saw by the vector diagram for any APT and INEPT. Of course every pulse sequence we cannot do by vector diagram.

There is a way to understand by mathematics called product operators; that is a big chapter by itself. That is not the part of this course, so I will not tell you about these things. We well see in one of the classes next time, may be in the next course we can discuss that. Delay can be chosen by J coupling. Supposing J coupling is 10 hertz; j coupling is in hertz; the time is inverse of that, so in seconds. so 1/10 ; 1/20, calculate what is the time in seconds, you know that.

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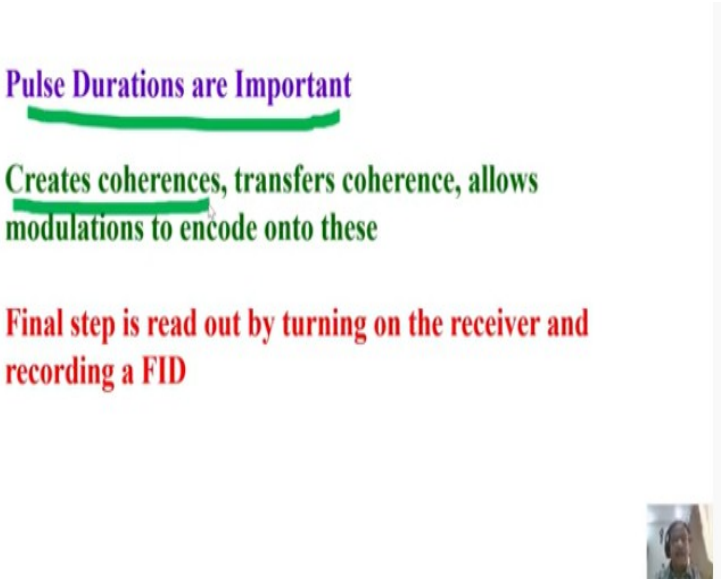

So, like that we can design the pulse sequence; pulse durations are important because during this duration many things happen; pulse delays are important because during this time, we can create coherences. You know what is a coherence, I told you in the first or second class itself bring the magnetization from z axis to xy plane all the spin packets; all the magnetization vectors are immediately at the given time will be brought to coherence, in one phase. That is the phase coherence is created, then afterwards, of course there is a phase decoherence that is a different question.

So, for creating coherences; for transferring of coherence; one spin magnetization can be transferred to other spin, and we can modulate all those things; encode it to a different spin; all those things can be done. So, this delay, pulse delays, pulse width, pulse flip angles all matters a lot; and the phase of the pulse in which direction you apply matters a lot; and which direction you apply in which direction you detect that matters also.

So finally all these things are taken into account for designing the pulse sequence of our choice; the experiment of our choice, and the final step is reading out the signal by detection pulse is called read pulse; and then collect the free induction decay. That is the last detection period of the multi-dimensional experiment. You understood what I am trying to say? So, the designing of the pulse sequence requires little bit of understanding of spin dynamics, so that we should know what is happening to the magnetization during the application of different pulses of different widths; on different nuclei with the different delays; how they are evolving. If you understand that you know what is the final outcome. That is the way we design the pulse sequence.

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So, in each 2D experiment relationship between 2 or 3 spins are established by transfer of magnetization between the spins. How we do the transfer the magnetization and everything is another type of discussion we have to do. What I will do is I will stop. In this class I have told you more about Two dimensional NMR, continued from last two classes and I discussed about varieties of pulse sequences available; what pulse sequence we can give, what type of 2D information from the spectrum we can derive in a particular type of 2D experiment; and how do you choose the 2D experiment; what should be the size of the molecule for a particular 2D or 3D experiment; and how much utility is there, everything we have discussed today. We will come back in the next class and discuss more about these things and literally we have to start analyzing couple of 2D spectra so that you get more familiarity.