Advanced NMR Techniques in Solution and Solid-State
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Module-46
2D NMR Concepts 2D Experiments
Lecture - 46

Welcome back all of you. From the last class, we started discussing about 2D NMR. In the last class I introduced 2D NMR were I explicitly mentioned 2D NMR is different from 1D NMR in the sense we have 2 time periods. General sequence for any 2D NMR I said consists of a preparation period, evolution period, mixing period and detection period, and the detection period time is always constant where you acquire data in a conventional way like you acquire for 1D spectrum.

In the evolution period, t1 is incremented systematically; for every t1 you are going to create an FID you are going to have a time domain data, we can create as a function of incremented t1 a pseudo free induction decay. You create a time domain data during the evolution period. And you have 2 dimensional time data, one the detection period, which is always constant. Pseudo free induction decay is created in the evolution period. Do the double Fourier transformation you are going to get 2 frequency domain spectra; double frequency spectrum.

So, this is what we discuss about the 2D NMR. And then, of course, it is not restricted to 2D you can go to N dimensions, we can get 3D, 4D, etcetera. But what defines dimensionality? N time periods; N periods has N dimensionality, N different time periods should be there, in that N time periods one of them is a constant, last time period where we acquire the data; and the remaining N- 1 time periods you are going to vary systematically and create a pseudo FIDt in each of them. So, for N time periods, we do N dimensional Fourier transformation we are going to get a N dimensional frequency spectrum; that is ND NMR. And then now the question is how do you choose which dimensionality you require? It depends on the molecule size and of course, as you go to higher and higher dimensions, I told you it takes enormous amount of the instrument time. I said 1D NMR for a reasonably small molecules even reasonably big molecules, in the present-day spectrometers, so much of sensitivity gain because of high magnetic field, etc and various techniques you can get the spectrum in less than 5 minutes. On the other hand, if you go to 2D NMR for the same molecule you may take several minutes, maybe 30 minutes, 40 minutes, 50 minutes like that. If you go to 3D NMR it might take several hours. If you got 4D NMR it may take days or if you go to 5D NMR it may take weeks. So, the dimensionality as you increase the, time we required to acquire signal also keeps increasing.

And of course, it depends upon the molecular size. Dimensionality also depends upon molecular size. For example, for small molecular weight of you know, let us say 300 or 400 etcetera just 1D NMR is sufficient; you can simply analyze such molecules, we saw the analysis the 1D spectra in the previous classes, you know 1 or 2 examples we took, we analyzed the 1D spectrum.

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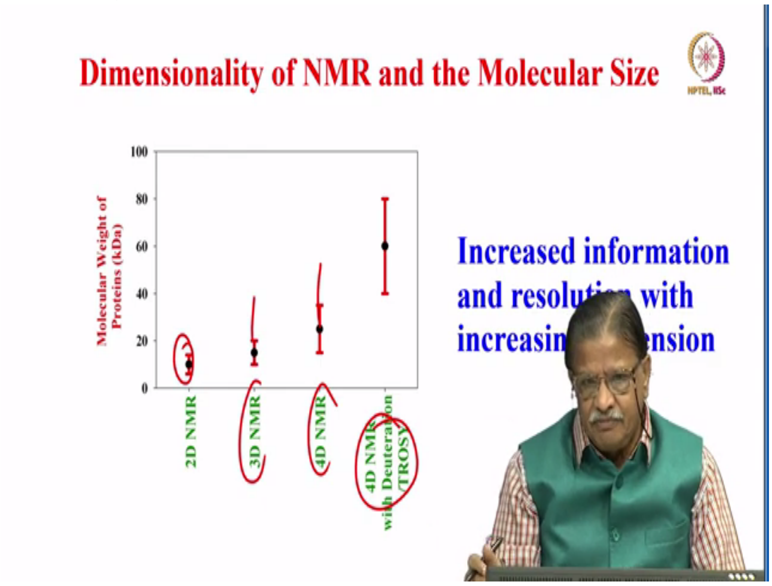

Dimensionality of NMR and the Molecular Size

Increased information
and resolu with
increasin ension

If you go to 2D NMR, molecular size, as an example of the protein, it could be up to 20 kilo Dalton. For 3D NMR slightly you can extend It; 4D NMR of course can go up to 40 or 50 KD. Beyond that, if you need 4D NMR you require special experiments also. So, as you keep on increasing the dimension, you will get the increased information content. And at the same time, you can also get the resolution, because you are spreading the information in different dimensions; that is what we discussed.

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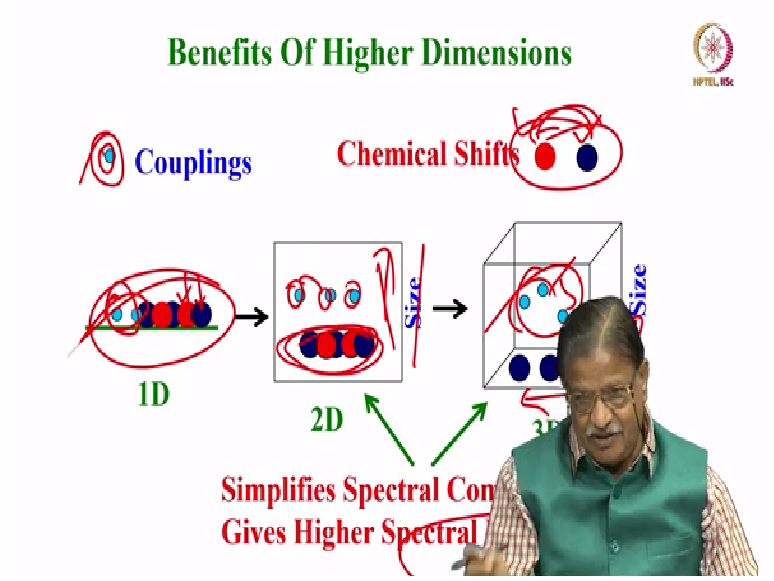

Benefits Of Higher Dimensions

Simplifies Spectral Con

Gives Higher Spectral

Now, I want to tell you one thing today. Fine, what is the benefit of going to higher dimensions? The benefit of going to higher dimension is, I can show you like this, let us say we have couplings; it is already present, I also have chemical shifts for 2 different nuclei, I have given different colours, blue and red; and couplings are very small interactions; small balls are written there.

Now, let us see the 1D spectrum, it is not of different nuclei, let us say different chemically inequivalent spins you can call it, because 1D spectrum of different nuclei cannot get on the same 1D spectrum. So, we call it different chemical inequivalent nuclei of the same molecule. Now we have different protons, and we have J couplings; everything is overlapped. It is a complex spectrum.

If you want to analyze this 1D spectrum, it is fairly complex. You need to spend a lot of time. What on the other hand, we can do is, go to 2D. I can do 2D experiment in such a way I can resolve this on the basis of the sizes, let us say, I can have J coupling which are smaller in strength in this dimension, all these type you know black and red balls what is written here are of the same size. So, on the basis the size, I can divide this information in 2 dimensions.

So, I can put all the J coupling which are called small interaction strengths, which are as small ball sizes that can be here. This is one way. But still, there is a complexity here, because different chemically inequivalent protons, different spins are overlapped here. Still, resolution is poor, although you have got already resolution, some resolution by going to two dimensions. But to increase this further, we can go for one more dimension, we can go to 3D

NMR. What we do is see, in this dimension we change the colour of the balls here; that is the J coupling here; we have one chemical shift of protons here one type of protons, another type of protons are here. Now, this black balls, red balls and blue everything are separated in 3 dimensions. Look at these it was all clumsy, everything was overlapped. And it is complex spectrum, it was very challenging to analyze 2D helped us a bit, in resolving the information getting better resolution. If we go to 3D, even better. you are going to get better resolution. That is what happens as you go to higher and higher dimension, you get benefits of resolution, benefits of information content spread in different dimensions. Easily you can extract the information. So, it simplifies the spectral complexity as you go to higher and higher dimension, because of higher dispersion. Dispersion will be there, it spreads the information content in different dimensions.

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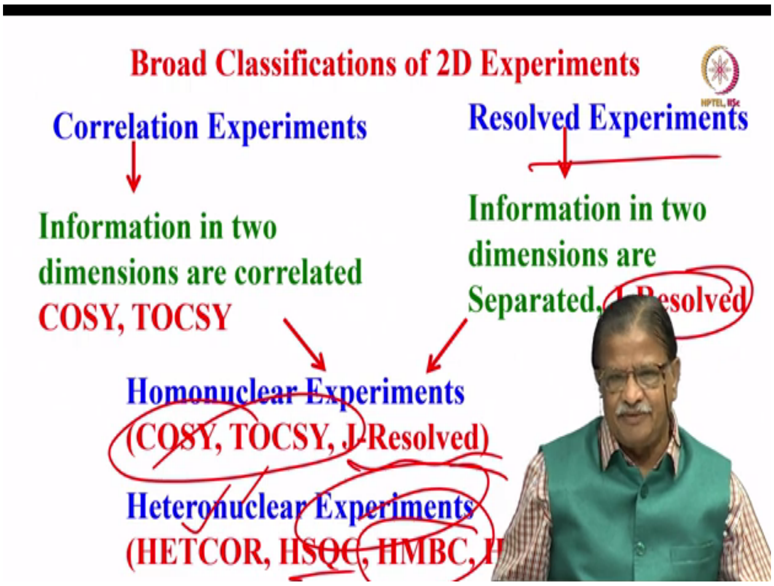

Broad Classifications of 2D Experiments

Correlation Experiments

Information in two dimensions are correlated

COSY, TOCSY

Resolved Experiments

Information in two dimensions are Separated, J-Resolved

Homonuclear Experiments

(COSY, TOCSY, J-Resolved)

Heteronuclear Experiments

(HETCOR, HSQC, HMBC, H

With this now, if I have to do 2D NMR experiments, or any 2D or 3D experiment. We will restrict all our discussion 2D only, we do not touch upon 3D in this course. We will say broad classification of 2D experiments. In my view, my way of looking at it, can be into 2 things one I call them as correlation experiments. This correlation experiment is the one which has information spread in 2 dimensions or two dimensional, information can be correlated.

I can have one here, one information and I have another information in other dimension. But I can correlate the information from this dimension to this dimension. Either I can correlate chemical shift, it will be chemical shifts of homonuclei; or chemical shifts of heteronuclei. I can correlate; if I know the chemical shift one of the protons here, I want to know if the other that proton is coupled to it, I can get that. I can get the chemical shift information of that proton here, in this dimension.

If I know I have carbon 13 here, in this dimension, let us say I have a proton here, I can get the proton chemical shift here and carbon chemical shift here, in this dimension, then I know which carbon is attached to which proton. So, I can have this correlation information that is one type of experiment. Basically, we can have some experiment like TOCSY, COSY, 1 or 2 examples we can touch upon in this course. Remember, I am going to touch upon briefly. This was discussed in depth extensively in the previous course. For the benefit of those who did not take the course I am just touching upon when I come to COSY with 1 or 2 simple examples.

Other thing is resolved experiment. The resolved experiment is another type of experiment where the information content is spread into 2 dimensions. That is the resolution. Here you are correlating, you are resolving, separating the two information. For example, J-resolved information, J-resolved experiment. In J-resolved you have chemical shift in one dimension and J coupling in another dimension; that is also possible. You can do that that is one type of experiments. But now both these can be homonuclear or hetronuclear; also could be both resolved experiments or correlational experiments. You can design in which both the dimensions can be homonuclear, let us say, proton chemical shift here proton chemical shift here that is one, homonuclear. Or proton couplings here proton chemical shift here, proton, proton couplings here; they are homonuclear experiments. You can also have heteronuclear experiments for example, you can have carbon chemical shifts here, proton chemical shifts here; nitrogen chemical shifts here carbon chemical shifts here, or proton chemical shifts here and carbon chemical shifts here, carbon proton couplings here; all those things experiments can be designed.

These are all common experiments; maybe 1 or 2 we can touch upon in there as quickly as possible we go. For example, for homonuclear experiment, it is called COSY, TOCSY, these are correlation experiments, J-resolved is he resolved experiment; heteronuclear experiments are heteronuclear correlation, HSQC, heteronuclear single quantum correlation Varieties of experiments are there, mind you this only tip of an iceberg.

If you go to the literature of NMR, several 100s of such experiments have been designed by various stalwarts of NMR, various experts, they are designed varieties of experiments, which benefits all of us. And we are not going to touch upon everything, because it is going to be a big ocean, only couple of 1 or 2 simple experiments. We will see how it works, what information we can extract from that? That we will work out in this class.

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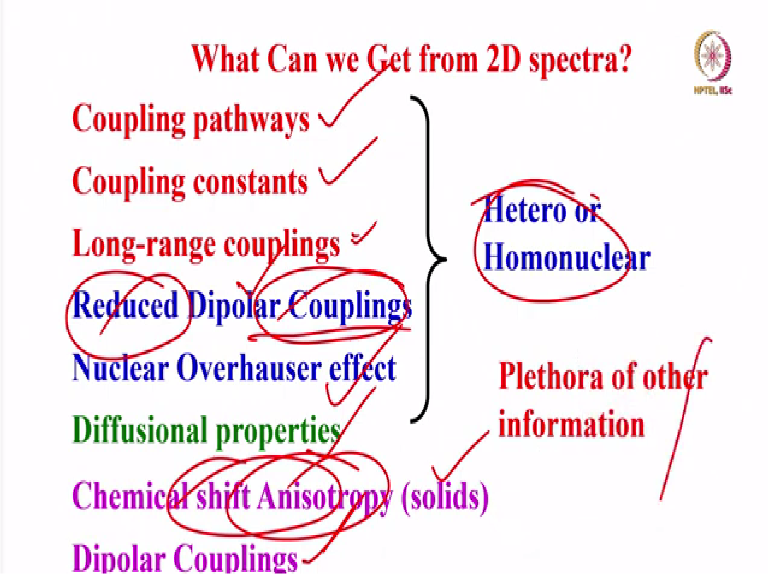

What Can we Get from 2D spectra?

- Coupling pathways
- Coupling constants
- Long-range couplings
- Reduced Dipolar Couplings
- Nuclear Overhauser effect
- Diffusional properties
- Chemical shift Anisotropy (solids)
- Dipolar Couplings

Hetero or Homonuclear

Plethora of other information

So, now you can ask me, what are the other things we can get in the 2D NMR? I can correlate this one, I can correlate chemical shift homonuclear, I can correlate heteronuclear chemical shift, I will resolve the 2 parameters, chemical shifts or J coupling into orthogonal dimensions. What else we can get from a NMR 2D data? If I get 2D spectrum, many things you can get, I can get the coupling pathways, I can get the coupling constant, and long range coupling constant. I can get to reduce dipolar couplings all these I can measure. I did not introduce dipolar coupling maybe when I go to solid state NMR, magic angle spinning and cross polarization I will discuss something about dipolar coupling, but reduce dipolar coupling is another thing where you can have a partially averaged system, oriented system where dipolar couplings are partially averaged. And you get like dipolar couplings, you can measure. That is the reduced dipolar coupling. Remember when I we discussed when I gave the NMR parameters internal interactions parameters, I said about dipolar coupling also is one of the important parameters which gets averaged out in the solution state. I said that it dependent upon the distance between two interactive spin;s it is special interaction, it is through space, not through bond.

That is what I said. We can get information about the NOE, you can get the information or the diffusion and you can get information of the relaxations varieties of things. In all these things you can have homonuclear information or heteronuclear information; imagine varieties of combinations you can think of. And if you go to solid state all 2D experiment what you can do in liquid state, similarly, there are varieties of 2D experiment designed for solid state also, depending upon the type of interaction parameters that are present in solids, that is also possible. For example, chemical shift anisotropy; I told you, in NMR all the parameters are anisotropic, chemical shift anisotropy. J coupling anisotropy, dipolar coupling is there and quadrupolar coupling, all these are anisotropic.

The chemical shift anisotropy, what happens in the solution state? gets averaged out. But if we go to the solid state, it is present, we can extract that information that gives us some information. If we get chemical shift anisotropy from the NMR spectrum, that gives information about the charge distribution at the site of the nucleus, I can measure the dipolar couplings. The advantage is, if I get the dipolar couplings I can get the distance information. See so many information content is there; all these can be extracted by variety of 2D experiments. The 2D experiment is really very, very useful. The voluminous amount of information is there, a lot of experiments are available, you can derive all those things, but designing varieties of experiments. Remember all these things what I said is only keep an iceberg; there are many things we can derive; many information. So, there are a plethora of experiments, plethora of information content that you can determine from the 2D NMR spectrum.

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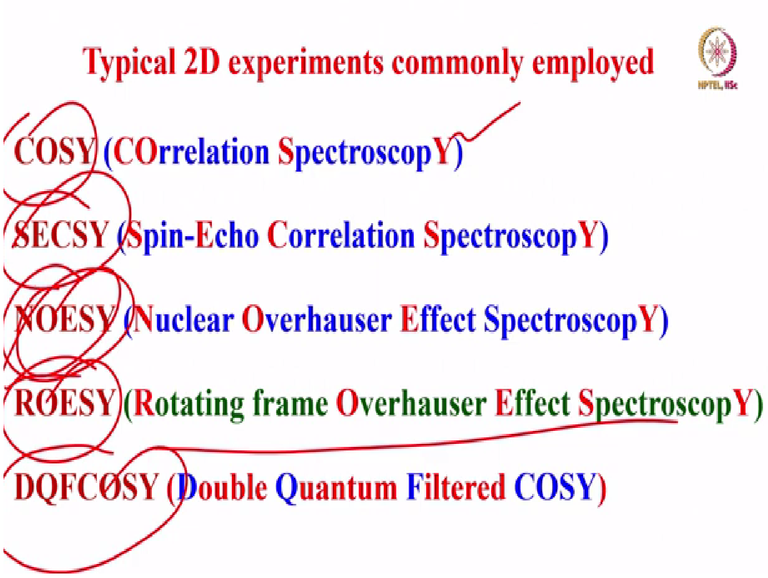

Typical 2D experiments commonly employed

COSY (COrrelation SpectroscopY)

SECSY (Spin-Echo Correlation SpectroscopY)

NOESY (Nuclear Overhauser Effect SpectroscopY)

ROESY (Rotating frame Overhauser Effect SpectroscopY)

DQFCOSY (Double Quantum Filtered COSY)

The NMR spectroscopies are very, very crazy people in the sense, they always design an experiment give a name for it. That is the way, depending upon the pulse sequence. You can design a pulse sequence, based on the information you want to derive by understanding the spin dynamics; that is what most of the stalwarts of NMR spectroscopy have done all across the globe. They have been doing that and then give an acronym for it; a name for it.

For example, COSY means, Correlated Spectroscopy; it correlates information. It can be homonuclear COSY or heteronuclear COSY, in case of homonuclear COSY we correlate to chemical shift information between 2 coupled protons, that is called COSY. SECSY another experiment called Spin Echo Correlated Spectroscopy. NOESY, Nuclear Overhauser Effect Spectroscopy; that is where you get the information about 2 nuclei which are close in space where NOE transfer is going between two nuclei, because of irradiation of one. We discussed about it. You Irradiate one of the protons and see the change in intensity of other one. So, NOE information can be obtained by NOESY. There is also called ROESY, ROESY is Rotating frame Overhauser Effect Spectroscopy you can have the DQFCOSY it is Double Quantum Filtered COSY these are as I mentioned, only less than 1% of NMR experiments that are available in the literature. So, many are there several 100s of such pulse sequences are there. They have been given acronyms. Just to give you the idea to see what type of experiments, how the acronyms are given, I mentioned these things COSY, SECSY, NOESY etcetera.

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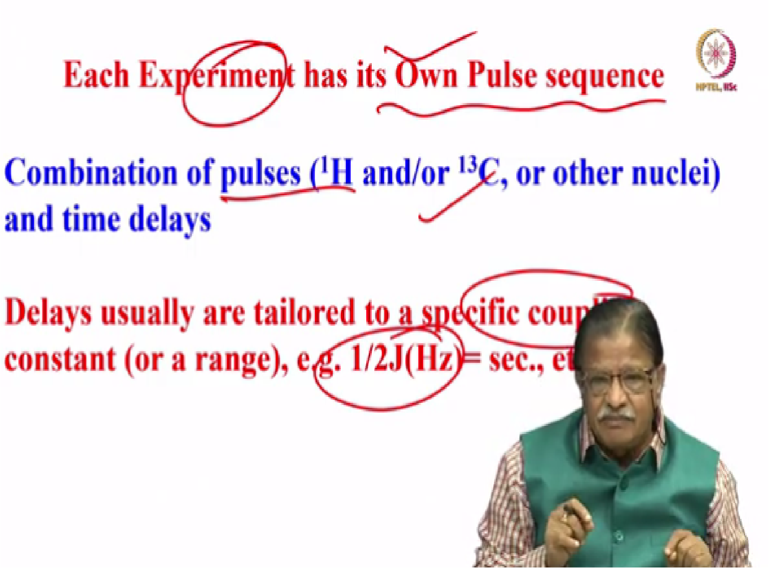

Each Experiment has its Own Pulse sequence

Combination of pulses (^{1}H and/or ^{13}C, or other nuclei) and time delays

Delays usually are tailored to a specific coup
constant (or a range), e.g. 1/2J(Hz)= sec., et

Further going ahead, we can have HSQC experiment Heteronuclear Single Quantum Correlation Spectroscopy, HSQC here you are going to correlate 2 different heteronuclei based on one bond coupling between them; and also HMBC Heteronuclear Multiple Bond Correlation, that is you are correlating proton or carbon which are separated by 2 or 3 bonds away. Similarly, all these experiments can have gradient versions. We discussed lot about gradients. Remember pulse field gradient for selection of the coherent transfer pathway etcetera. There are varieties of such experiments possible by using gradients. So, each of these pulse sequence people are modified accordingly for the information retrieval, every time when they are modified, you would get additional information or prove that sequence is better by either getting the better signal to noise ratio, or better resolution, etcetera. Like that people out designed number of experiments.

You can also have a DOSY, DOSY is the Diffusion Ordered Spectroscopy, where you can get the diffusion information in one dimension. If there is a mixture of molecules, you can separate them out based on the diffusion coefficients in the solution state; that is also possible. So, imagine the types of experiment are there. We can even touch upon DOSY if there is a time, I do not know how much time is left, we still have a lot to cover .a lot of things about solid state NMR 2D, MQT, if possible pureshift, varieties of things are there.

I do not know whether we have time if there is time, we will touch upon that. Now, what these pulse sequences do? What does each experiment do? Each of these experiments has its own pulse sequence. When I say COSY, immediately it comes to my mind. It has 2 pulse sequence 90 t1, 90 t2, it comes to my mind. Similarly I can think of TOCSY that is one way; heterochemical correlation that is one thing; the HSQC one pulse sequence. All these experiments has their own pulse sequences. As an NMR spectroscopist, if I simply a read out that name HSQC, COSY, HMBC etcetera, the type of experiment, the pulse sequence and information content that information that is retrievable from such experiment suddenly comes to my mind. So, each experiment has its own pulse sequence. And combination of pulses sequence are there; sometimes couple the experiments to derive information. For eg, HSQC-TOCSY I can make, I can combine HSQC sequence with TOCSY; make it 3D experiment HSQC, TOCSY. Like that you can have combination of the pulses; combination of experiments. And again it could be both homonuclear or heteronuclear, You can apply pulse on proton apply pulse on carbon; and you can have vary the delays in these different experiments to get different information, and change the magnetization in different axis, and bring them into contact in different mixing periods so that different type of information is transferred between the spins; varieties of all such experiment can be designed.

So, each of these experiments has its own pulse sequence. And usually when we have delays, especially we discussed about INEPT where we can have the delays tailored to a specific coupling constant or over a small range like 1 over 2J. Remember we discussed the in phase become anti phase we observed that at exactly 1 over 2J, 2 protons are coupled or 2 spins are coupled both hetronuclear we observed that when you bring the magnetization into the XY, they start fanning out, at exactly 1 over 2J, we saw that both with vectorial diagram and also by product operators, we found out it is going to create anti phase magnetization. Like that using those information, we can design the particular experiment. So that in this experiment for example, I tailor it such that it could be implemented in a polarization transfer experiment to transfer information from one spin to other spin. So, varieties of such information is available in each of this pulse sequence, which has its own pulse sequence.

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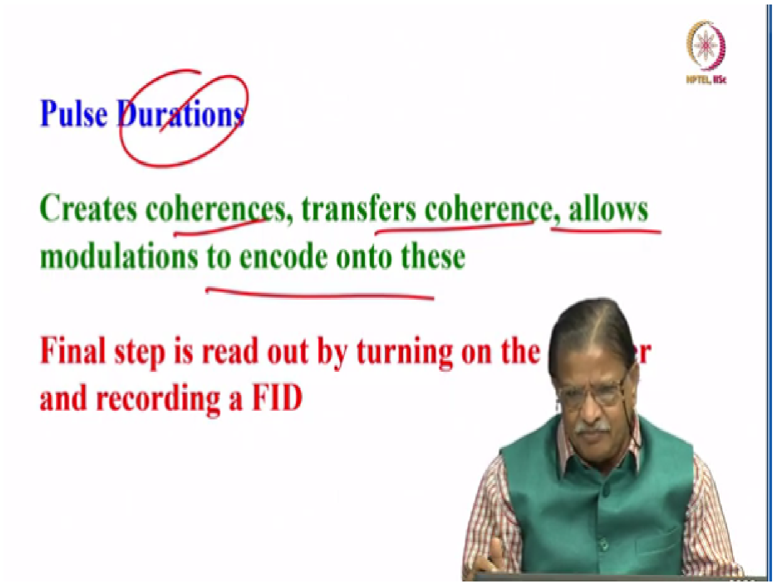

Pulse Durations

Creates coherences, transfers coherence, allows modulations to encode onto these

Final step is read out by turning on the r
and recording a FID

Now, the pulse durations, that is also very important; that creates coherences sometimes, it transfers coherences, from one spin to another spin; it allows the modification of one and encode this information into other spin, that also possible. And you do all those things, up to the last time period, where you are applying the final detection pulse to detect the signal, and start collecting the data in the detection period, where you collect the real FID; record FID in the time domain t2. Since we are discussing only 2D in the t2 period. So, this is what always happens, pulse duration is very important that decides variety of information which you want to derive.

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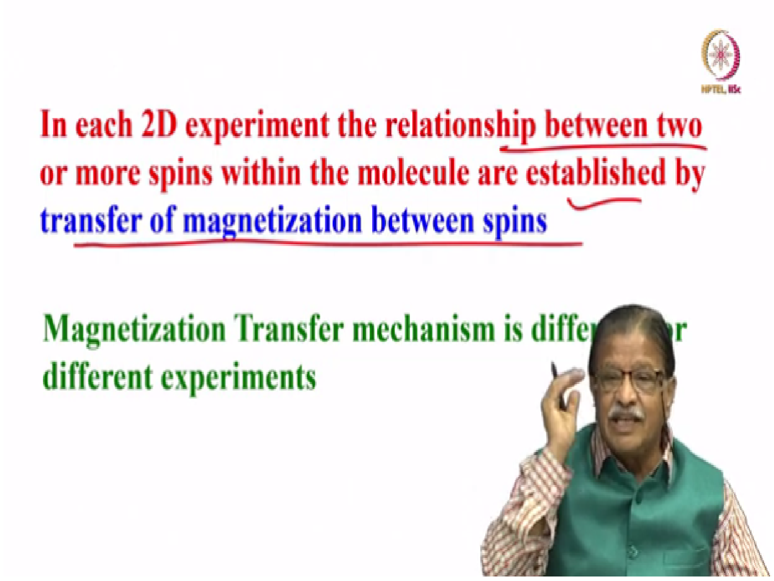

In each 2D experiment the relationship between two or more spins within the molecule are established by transfer of magnetization between spins

Magnetization Transfer mechanism is diffe r different experiments

Another important thing is, in each 2D experiment, the relationship between two or more spins within the molecule are established with transfer of magnetization between the spins. If I want to establish a relation between two spins, let us say, in any given molecule, I have 1 proton here, another proton next to it. I know there is a J coupling between them, there is an interaction through covalent bond. If I want to find out whether this is coupled to this, I can do a correlation experiment where there is a transfer of magnetization between 1 spin to another spin; that gives me a peak in the 2D spectrum. So, all these things what happened in each 2D experiment, if you are depending upon the information which you are trying to derive, the relationship between the 2 spins or with even more spins within the molecule are stablished only with the transfer of magnetization.

Of course, that is another thing; very important thing, the transfer of magnetization one thing with gives to the relationship between two spins. Next, if you go to the magnetization transfer, the mechanism in which it transfers, mechanism in which the magnetization gets transferred is different in different experiments.

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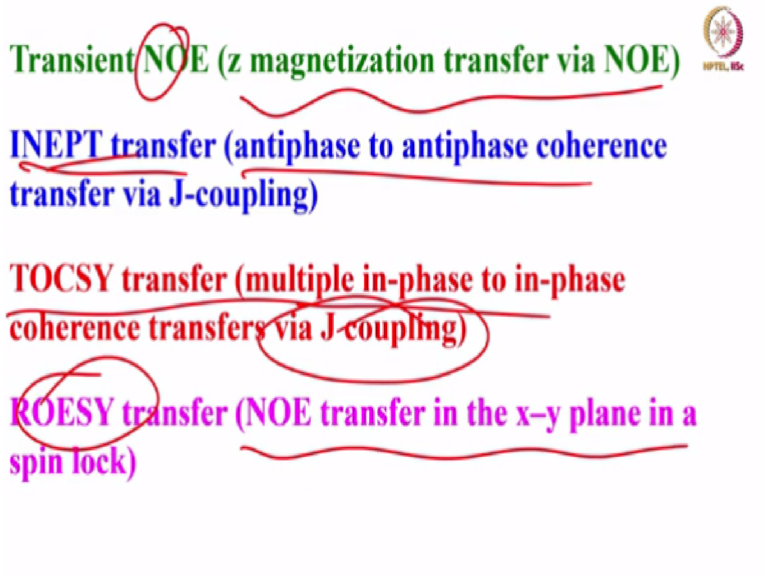

Transient NOE (z magnetization transfer via NOE)

INEPT transfer (antiphase to antiphase coherence transfer via J-coupling)

TOCSY transfer (multiple in-phase to in-phase coherence transfers via J-coupling)

ROESY transfer (NOE transfer in the x–y plane in a spin lock)

Just to tell you we can do an experiment called transient NOE, we discussed about steady state NOE also; we can have an experiment on transient NOE where z magnetization transfer takes place via NOE. You can have an INEPT, remember we discussed INEPT where anti phase magnetization transfer from one spin to another spin; anti phase to antiphase coherence transfer.

J coupling is important, that is what we discussed. J coupling is the one which creates anti phase coherence and that is responsible for anti phase to anti phase coherence transfer. It is possible only through J coupling. So, in experiment like INEPT the magnetization transfer or polarization transfer takes place from anti phase magnetisation, anti phase coherence to anti phase coherence. TOCSY another experiment which we will discuss, it is multiple in phase to in phase coherence that is another very interesting thing.

Again, it happens via J coupling; coherence transfers take place via J coupling; it is in phase to in phase transfer. ROESY is rotating overhauser effort spectroscopy where the NOE transfer takes place in the xy plane; that is during what is called a spin lock. Maybe I will explain spin lock when I come to solid state where we discuss a lot about cross polarization etcetera. I will discuss what is spin lock etcetera at that time.

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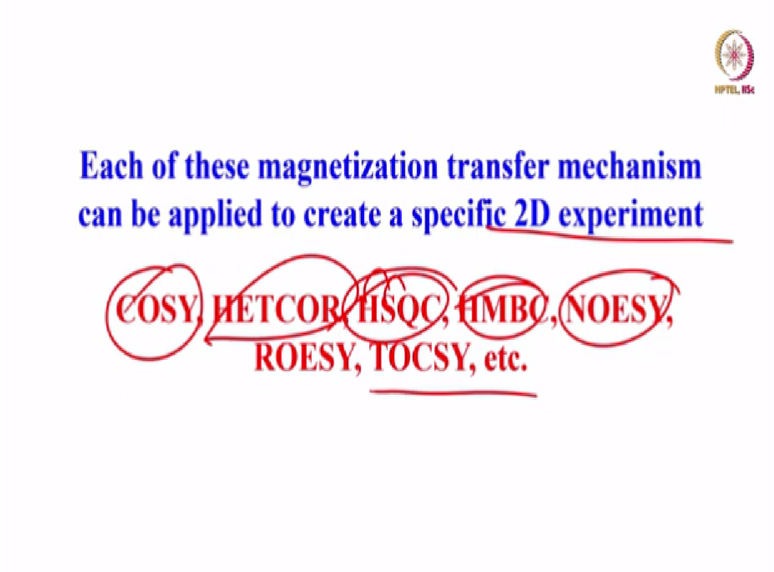

Each of these magnetization transfer mechanism can be applied to create a specific 2D experiment

COSY, HETCOR, HSQC, HMBC, NOESY, ROESY, TOCSY, etc.

And each of this magnetization transfer mechanism can be applied to create a specific 2D experiment. Remember, the magnetization transfer mechanism if I know I can create a specific 2D experiment; for example, COSY correlation information, where the magnetization transfer takes place to correlate 2 protons which are coupled between themselves or 2 or more protons coupled among themselves. HETCOR, it is the correlation of chemical shifts between 2 or more hetronuclear spins.

HSQC single quantum coherence where it is inverse experiment. I will discuss about inverse experiment when we discuss single quantum correlation, multiple bond correlation, multiple bond coherence, NOESY, ROESY, TOCSY. All these experiments are designed, and in each of them the way the magnetization transfer takes place is specific to each experiment. That is how different pulse sequences are designed in NMR. So, remember, each pulse sequence is designed where the mechanism of the magnetization transfer is specific to each of these experiments.

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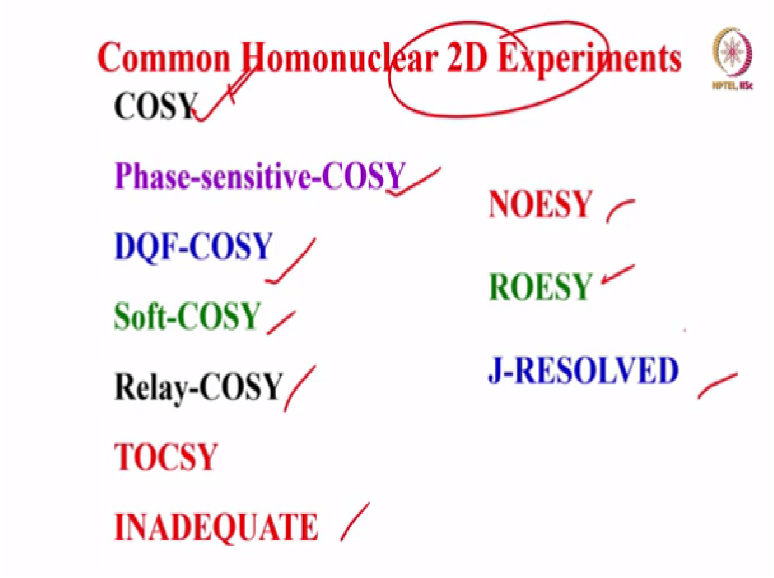

Common Homonuclear 2D Experiments

COSY

Phase-sensitive-COSY

DQF-COSY

Soft-COSY

Relay-COSY

TOCSY

INADEQUATE

NOESY

ROESY

J-RESOLVED

Now, common 2 dimensional experiment which everybody uses for example, most of the time very sophisticated 3D experiments or very sophisticated 2D experiments specially designed experiment, that is the research of NMR spectroscopist to get particular information. Commonly a chemist or biologist if they want to just to characterize their molecules, they do not need to go to very high, sophisticated latest state of the experiments for deriving information. Sometimes simple experiment like COSY, phase sensitive COSY, DQF COSY, soft COSY Relay COSY, TOCSY, INADEQUATE, NOESY, ROSEY, J-resolved these common experiments are suffice to get the results. You do not have to go to complex 2D spectrum or 2D experiment. So, common homonuclear 2D experiments where I am talking about homonuclear are, these things; COSY, TOCSY, NOESY, ROESY, inadequate etcetera.

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Heteronuclear 2D Experiments

HETCOR

COLOC

HETCOR-RELAY (HEHAHA)

HETERONUCLEAR J -RESOLVED

Heteronuclear 2D experiments. Similarly, like homonuclear you can also have heteronuclear where heteronuclear correlation is there. More or less nowadays nobody uses because inverse detection has come HSQ,C you use COLOC is long range correlation experiments homonuclear relay experiment level TOCSY is also called for HOHAHA homonuclear Hartman Hahn. Same way hetronuclear relay is called HEHAHA, heteronuclear Hartman Hahn experiment. Heteronuclear J-resolved experiment is also there.

These are common heteronuclear experiments. As I said, these type of experiments take enormous amount of time. Nowadays, people do not use this type of experiments at all.

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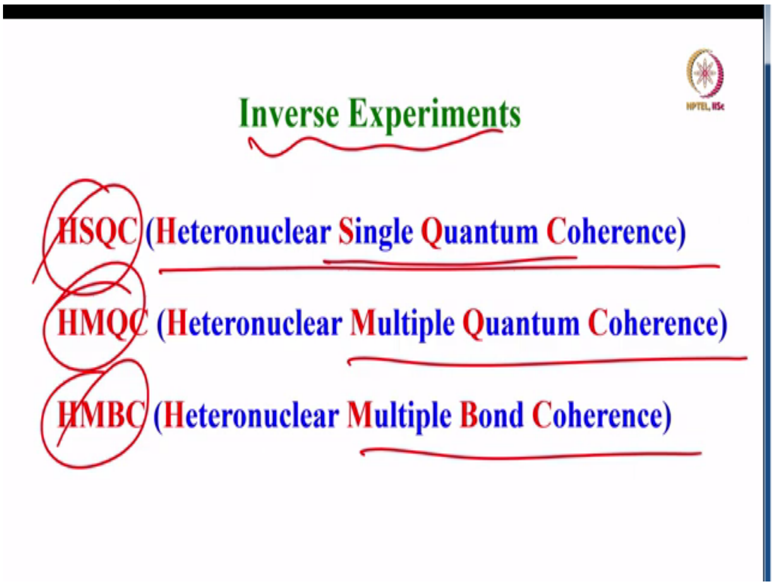

Inverse Experiments

HSQC (Heteronuclear Single Quantum Coherence)

HMQC (Heteronuclear Multiple Quantum Coherence)

HMBC (Heteronuclear Multiple Bond Coherence)

And for that new experiments have been designed, they are called inverse experiments. An example is HSQC it is an experiment. It is heteronuclear a single quantum coherence where you can correlate the chemical shift information between heteronuclei. For example, carbon to proton, which are directly bonded, one bond correlation experiment, how it works we will discuss later. It is called heteronuclear single quantum coherence experiment.

HMQC heteronuclear multiple quantum coherence; HMBC heteronuclear multiple bond coherence; so all these experiments are inverse experiments. What is an inverse experiment? When you go further, we will discuss. Later I will come to HSQC, etcetera. We will have an in depth discussion about these types of experiments later, especially HSQC and HMBC. How do we get the information, we will discuss.

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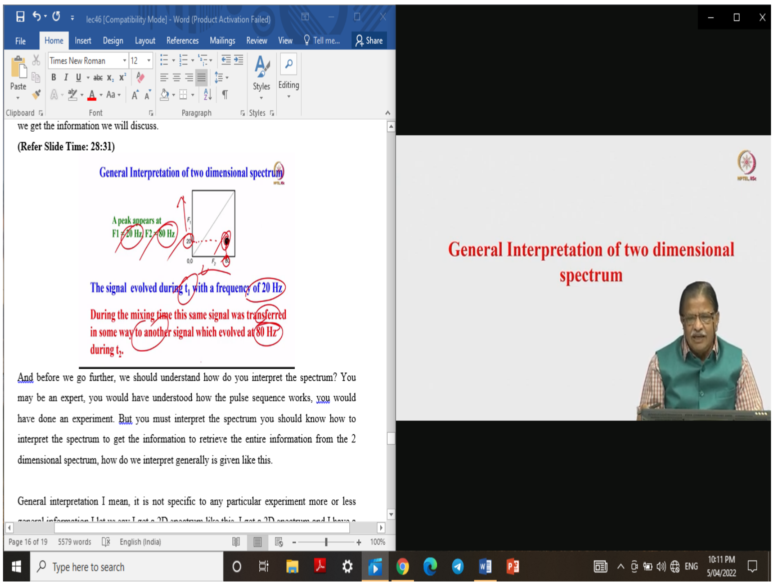

we get the information we will discuss.

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General Interpretation of two dimensional spectrum

A peak appears at F1 = 20 Hz, F2 = 80 Hz

The signal evolved during t_1 with a frequency of 20 Hz

During the mixing time this same signal was transferred in some way to another signal which evolved at 80 Hz during t_2.

And before we go further, we should understand how do you interpret the spectrum? You may be an expert, you would have understood how the pulse sequence works, you would have done an experiment. But you must interpret the spectrum you should know how to interpret the spectrum to get the information to retrieve the entire information from the 2 dimensional spectrum, how do we interpret generally is given like this.

General interpretation I mean, it is not specific to any particular experiment more or less

General Interpretation of two dimensional spectrum

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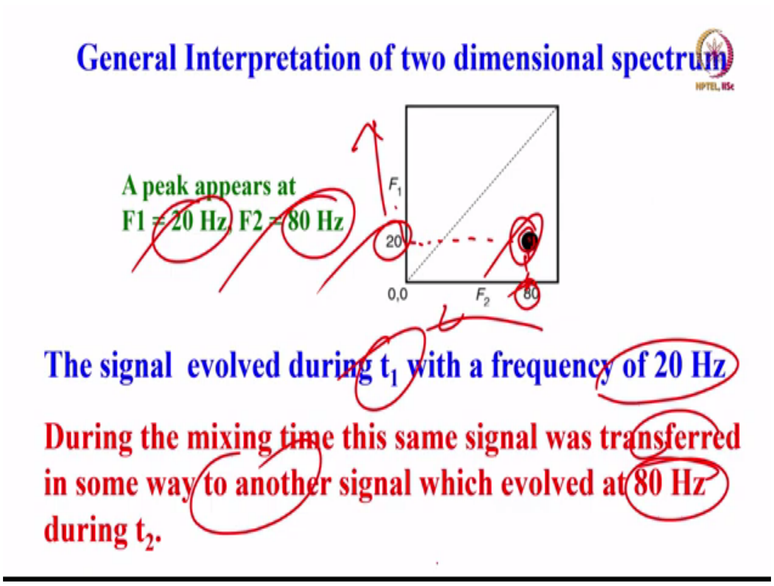

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The signal evolved during t_1 with a frequency of 20 Hz

During the mixing time this same signal was transferred in some way to another signal which evolved at 80 Hz during t_2.

General interpretation; I mean, it is not specific to any particular experiment more or less general information. Let us say I get a 2D spectrum like this, I get a 2D spectrum, and I have a peak here; 2 dimensional spectrum. This is F2 dimension this is F1 dimension. And if you look at it, I get a peak here, and I go here and correlated here, it comes at 80 hertz here, this is in the F2 dimension and in the F2 dimension draw horizontal line, here it comes at 20 hertz.

So, I see a peak in the F1 dimension correspond to 20 Hertz; I see a peak the same peak in the F2 dimension, I read it as 80 hertz. So, this is one peak in the 2D spectrum I read in the F1 dimension is coming at 20 hertz, I read the same thing in the F2 dimension coming at 80 hertz. So, the signal, what you understand is, that is evolved during the t1 period with a frequency of 80 hertz; that was in t1 period it was at 80 hertz; during the mixing period something happened for it. The signal was transferred some way by some mechanism by some polarization transfer method to another spin, or this itself evolved at 80 hert,z during t2. The signal what happened which evolved at 20 hertz in t1 period, during the mixing time what happened to the same signal, it was transferred in some way to another signal, which appears at 80 Hertz. The same signal transferred to another one which evolved 20 hertz in t1, it appeared at 80 hertz in t2. So, that how you have to interpret. The peak which appeared here which is at 20 hertz in a F1 and 80 hertz in the F2 means, during t1 period it was evolving at 20 hertz. In between during the mixing time something happened, it started giving this magnetization to another spin and then evolved at 80 hertz. That is the interpretation; you have to understand that.

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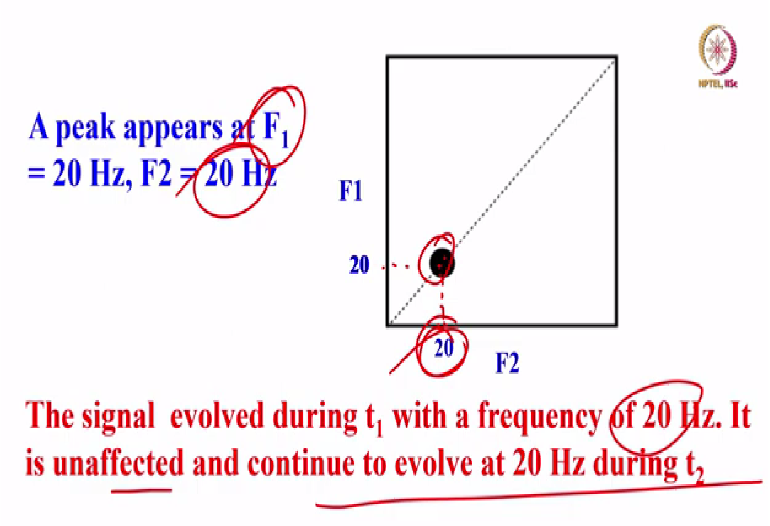
A peak appears at F_1 = 20 Hz, F2 = 20 Hz

The signal evolved during t_1 with a frequency of 20 Hz. It is unaffected and continue to evolve at 20 Hz during t_2

Let us say you go to the other one. Now, there is another interpretation. Now, I have a peak here, draw a line here in t2 dimension you see it is at 20 Hertz; the same peak here you see at 20 Hertz. It means I have a peak which appears in the F1 dimension at 20 hertz, in the F2 dimension also it appears at 20 Hertz. That means, the signal evolved during t1 with 20

Hertz, it remains unaffected, it did not change at al. It did not give energy or magnetization another spin. It remained unaffected and continue to evolve at 20 Hertz in the t2 period; that is the interpretation.

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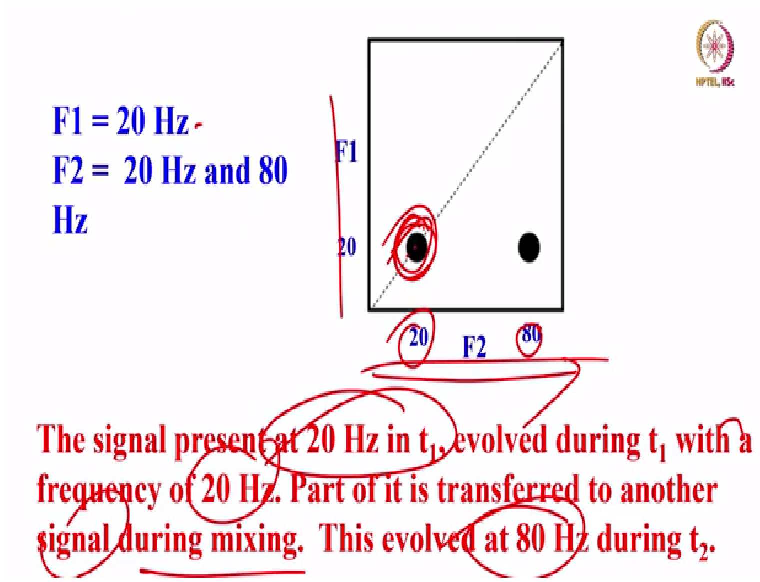

F1 = 20 Hz

F2 = 20 Hz and 80 Hz

The signal present at 20 Hz in t_1, evolved during t_1 with a frequency of 20 Hz. Part of it is transferred to another signal during mixing. This evolved at 80 Hz during t_2.

We will go to the next one. Now, I have a peak at 20 hertz in the t1 period, I will have a peak at 20 hertz and at 80 hertz both, in t2 period. In t2 period now you see I have 2 peaks, in this dimension this and in this dimension I only have 1 peak. So, in F1, dimension I have 1 peak at 20 hertz,in F2 dimension I have 2 peaks at 20 hertz and 80 hertz. How do you understand this? The signal at 20 hertz in t1 evolved during t1 with a frequency of 20 Hertz; remain unaffected, but evolved at the same frequency. what it means is, it transferred part of its magnetization to another signal, another spin during mixing time which is at 80 Hertz understand. The signal which was evolving at 20 Hertz in t1, it continued to evolve at 20 hertz during t2. In addition to that during the mixing period, gave part of its magnetization to another spin which evolved at 80 hertz during t2. This is what you have to understand. You have to interpret the 2D spectra like this.

So now, what I am going to do is next we will understand the particular 2D experiment. Since the time is up, I am going to stop. But you understood in this class, we discussed varieties of pulse sequence are possible, we understood. We have broad classification of experiments; homonuclear correlation experiments, and resolved experiments; again both can be homonuclear and heteronuclear.

And then we understood each pulse sequence has its own way of magnetization transfer during mixing, its own way to correlate to get the information. So, somehow 1 spin gives information to another spin in some way, some mechanism of transfer is there. And we understood how it happens for example in INEPT, where anti phase to anti phase transfer takes place, and in the case of the TOCSY in phase to in phase takes place in xy plane. All those things we understood. In case of NOESY we have Z magnetization transfer, all those things we discussed.

Each of these has been given names, there are umpteen number of pulse sequences available, umpteen experiments; each experiment is designed to derive a particular information. So, what are these things, how you design it, what is important is the pulses and the delays. You have to tune the delay, tailor it for particular information you derive. Otherwise you understand from product operators, we know how in a given sequence when you apply pulse delays, how the magnetization evolves, understand the spin dynamics and design experiment to get the information that you want to derive; that is possible. So, we saw varieties of common experiments, like, COSY, TOCSY etcetera or heteronuclear experiment like heteronuclear correlation, HECTOR, HSQC etcetera, like we also have inverse experiments which is nothing but HSQC, HMBC, HMQC etcetera. And we also understood how to interpret the 2D spectrum.

If I have a peak let us say at some frequency in F1 and some frequency in F2 ,what does it mean? what is that frequency of evolution in the t1 period? What is its evolution frequency t2 period, during the mixing period whether it has given coherence to other spin or not, you can understand this by looking at 2D spectrum. That is how we interpret the 2D spectrum. So, with this we will go to special experiments, try to understand few of these things in the next class.

So, I am going to stop it here. We will come back and continue with different types of experiments, starting with the basic experiment like COSY, from the next class. Thank you very much.