One and Two Dimensional NMR Spectroscopy for Chemists
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Lecture - 52
Two Dimensional COSY

Welcome back. Today, we will continue with our discussion on two dimensional NMR. I hope you are all with me. In the last couple of classes I extensively discussed the need for a 2D NMR, varieties of pulse sequences available; and information derivable from these pulse sequences and the proper choice of an experiment for you depending upon the size of the molecule, what type of experiment we have to choose, depending upon what type of information you want to derive, all those things I said.

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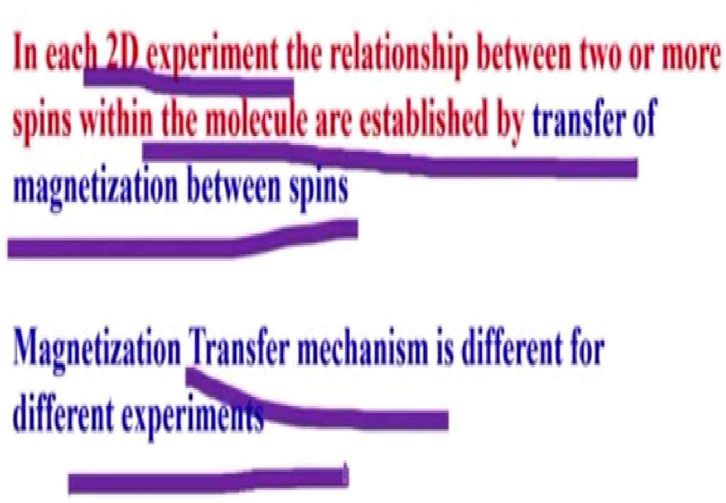

In each 2D experiment the relationship between two or more spins within the molecule are established by transfer of magnetization between spins

Magnetization Transfer mechanism is different for different experiments

And I also said, each pulse sequence has certain delays, certain pulse widths, certain flip angle, depending upon the direction in which you apply the pulse, depending upon the flip angle of the pulse, depending upon the delay you have given. By using spin physics you can trace the path of the magnetization. You can understand what is happening to the nuclear spin. You can understand the behavior of the nuclear spins by knowing spin physics.

Every time you cannot represent by a vector diagram, which we did simple for APT and INEPT. We have to adapt a higher level mathematics called product of vectors, which is not going to be discussed here, because it is an advance topic by itself and I do not want to frighten you bygoing into too much of mathematics. This course is aimed at showing you the utility of the 2D NMR and giving you the conceptual understanding of many of the NMR, one dimensional, 2 dimensional NMR, concepts, experiments, and their utility.

So I do not want to discuss about those things, but I told you how we can the design pulse sequence. One another interesting thing which I want to tell you is, in each 2D experiment, the relation between one or two spins within the molecule are established by what is called magnetization transfer, because the spins have to interact, there must be transfer of spin polarization or spin magnetization, otherwise there is no interaction; because of the interaction, we have to have transfer of spin magnetization and it happens in any of the 2D experiment. This magnetization transfer mechanism is different for different experiments. If it were to be same, then the only one experiment would have been there. So this is the beauty of NMR, where you can understand what is happening to the magnetization in different ways. What happens to magnetization, x axis, y axis, z axis, whether there is in-phase magnetization, whether there is anti-phase magnetization. All those things you can understand by understanding the magnetization; how it behaves with different pulses, different delays, and which direction it is applied. So we can understand the transfer of magnetization, mechanism of transfer.

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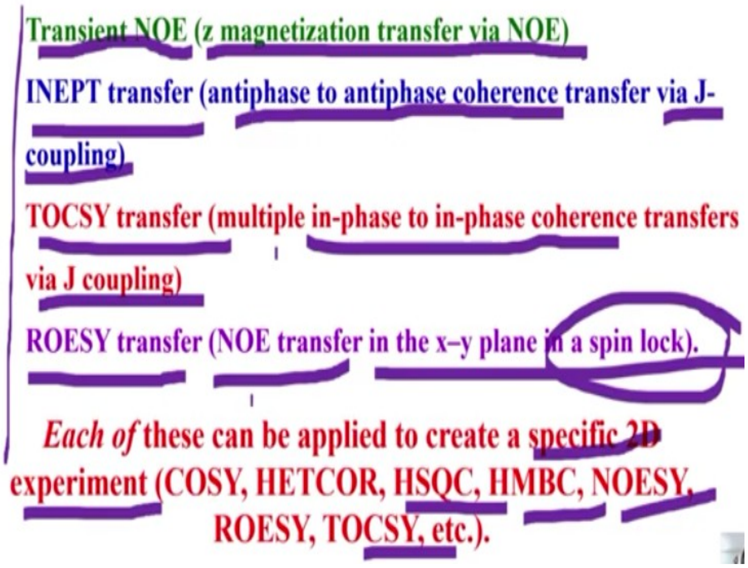

Transient NOE (z magnetization transfer via NOE)

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).

Each of these can be applied to create a specific 2D experiment (COSY, HETCOR, HSQC, HMBC, NOESY, ROESY, TOCSY, etc.).

So in most of the experiments, there are various ways, I will highlight only some 2-3 experiments, which you may need it. We do transient NOE. In this case, z magnetization is transferred via NOE. The magnetization is along z axis. This is transferred between two spins, which are in close spatial proximity by NOE. That is one way. In the z direction itself, z magnetization itself will be transferred.

INEPT transfer; INEPT, we have discussed, which polarization from abundant spins is transferred to less abundant spin. Here, antiphase to antiphase coherence transfer takes place. We saw what is antiphase. We observed that in the INEPT and APT case. Carbon 13 signals, one was -3, other was +5, they were in antiphase direction. They were opposite to each other. That can be done in INEPT via J-coupling. That is antiphase to antiphase coherence transfer there.

You can go to TOCSY, total correlation in another experiment, which we discussed. Here, multiple in-phase to in-phase coherence transfers takes place. That is another interesting thing, again through J-coupling. This is through space, this is through J, this is through J. There is a ROESY experiment. Again NOE transfer takes places in the xy plane. Here it is the z axis, here xy plane, in a spin lock.

Transient NOE, steady state NOE, driven NOE, rotating frame NOE, varieties of experiments are possible. But I just wanted to give one or two information. In all the experiments, there is a mode, by which magnetization gets transferred. There is a pathway. There is a way in which magnetization is transferred between two interacting spins. This is essential. Otherwise, you will not get the required information.

This is what we understand by spin physics and then design the experiment. You can exchange the magnetization, or transfer the polarization between antiphase to antiphase, in-phase to in-phase in the xy plane, in the rotating frame, or z magnetization, varieties of things we can do; these are the things. And based on these things, number of experiments could be created and have been created and we have specific experiments, like COSY, heteronuclear correlation, heteronuclear single quantum correlation, heteronuclear multiple bond correlation, NOESY, ROESY, TOCSY, etc.

All these things have been designed. If you do understand the spin physics, the way the magnetization behaves in the pulse sequence, in all these things you will find out, something different. I just mentioned only couple of them. There may be several ways the magnetization gets transferred.

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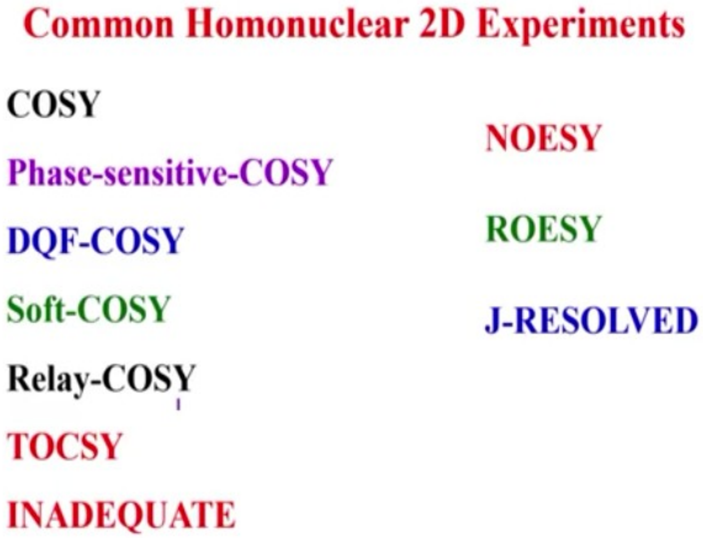

Common Homonuclear 2D Experiments

COSY
Phase-sensitive-COSY
DQF-COSY
Soft-COSY
Relay-COSY
TOCSY
INADEQUATE

NOESY
ROESY
J-RESOLVED

Now with this, I want to tell you one thing, the common homonuclear 2D experiments, which invariably people use for the analysis; not all, but many of them. You can use COSY, phase-sensitive COSY, double quantum filtered COSY, soft COSY, relay COSY, TOCSY, inadequate, these are all the experiments. As we go ahead one by one, we will try to understand or whatever possible, we will try to do, possibly may not have time to see everything. And also we have NOESY, J-resolved, we can understand. ROESY, I do not think I have time. I may not be able to discuss these things in this class, because NOESY itself, we have taken 2 classes, still you have not been able to complete. 2 or 3 classes we took, still lot more we have to do and I abruptly stopped. You may have to come back again to continue further. That is a different question. So, whatever is possible to cover in the remaining classes, I will try to do with specific examples, so that you get a feel for what is going on.

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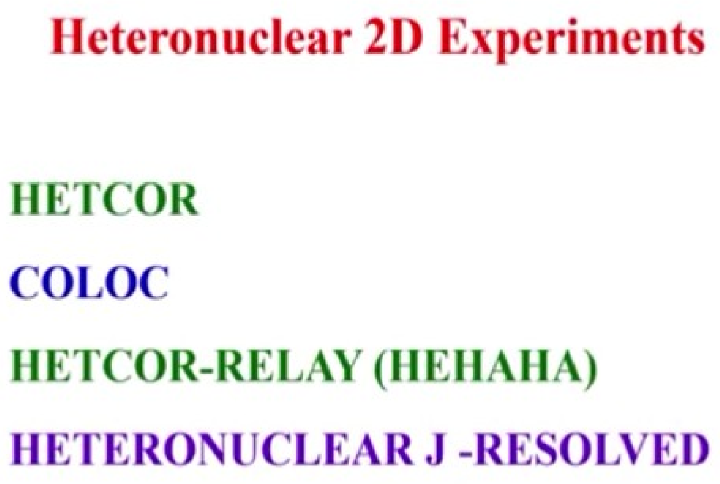

Heteronuclear 2D Experiments

HETCOR

COLOC

HETCOR-RELAY (HEHAHA)

HETERONUCLEAR J -RESOLVED

Similarly, heteronuclear 2D experiments, commonly used are HETCOR or COLOC. These are all outdated. Nowadays, nobody is going to use this type of experiment. To enhance the sensitivity and the speed, we use inverse detection. There is a relay experiment called HEHAHA, heteronuclear Hartmann–Hahn. In the homonuclear case, we have TOCSY. It is called HOHAHA, that means homonuclear Hartmann–Hahn. This is HEHAHA, heteronuclear Hartmann–Hahn. So heteronuclear J-resolved, there are number of such experiments possible. We can do that.

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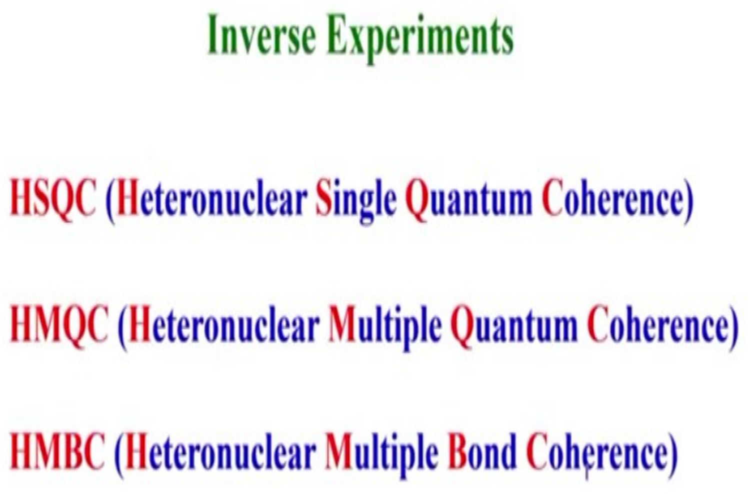

Inverse Experiments

HSQC (Heteronuclear Single Quantum Coherence)

HMQC (Heteronuclear Multiple Quantum Coherence)

HMBC (Heteronuclear Multiple Bond Coherence)

So there are inverse experiments, which are very, very powerful and very popular. Nobody uses this HETCOR, COLOC, I told you, nowadays. These types of experiments are not used, because they are very time consuming experiments. Of course, information you get is similar with these experiments, but these are faster experiments. It saves lot of time. They make use of polarization transfer. What it does, take the magnetization from abundant spins, give it to carbon or nitrogen, low abundant spin; and again take it back to proton. In this process, you will know what is happening to the dilute spin. You can indirectly detect dilute spin by taking the magnetization from abundant spin, give it to rare spin or dilute spin, taking again back to abundant spin. These types of experiments are called inverse experiments, where you detect the abundant spin. What is the advantage? Faster experiment, if I detect carbon, as I told you, we discussed about sensitivity. It is very, very less sensitive compared to proton, nearly 5700 to 5800; okay 6000 you take, no problem. 6000 times less sensitive compared to proton. Whereas if I detect proton, it is easy, but indirectly I can get the information about carbon or dilute spin. That is the advantage. So these are all inverse experiments. Now it is commonly used, nobody uses HETCOR, COLOC such experiments, but they were designed and these are all the extension of that, improved versions of that for speed of the experiment and to enhance the sensitivity.

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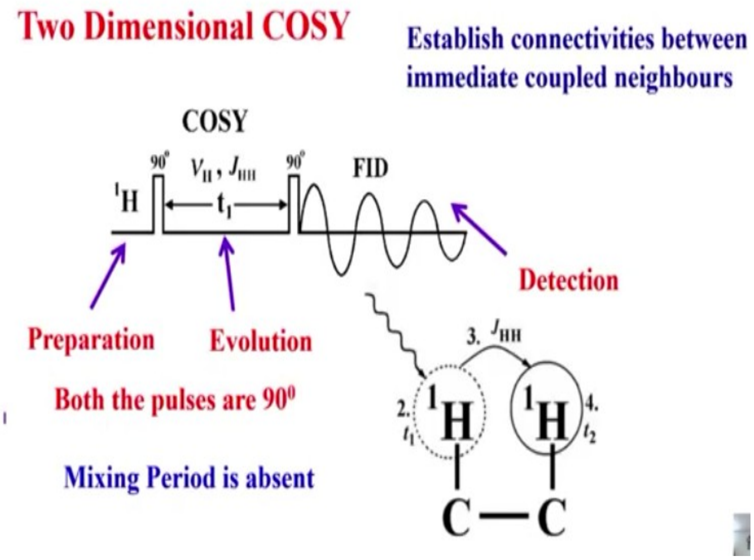

Two Dimensional COSY

Establish connectivities between immediate coupled neighbours

Both the pulses are 90^0

Mixing Period is absent

Now we will start with a simple experiment called two dimensional COSY experiment. This is where you start with. This is the simplest of the possible 2D experiment. Number of 2D experiments are there. This is simplest to understand, simplest to interpret the spectrum. There is no need to break your head at all. This has four time periods as I mentioned for any routine or general 2D experiment. This is called preparation period. This is evolution period.

There is a mixing period here. I said there are four periods, preparation, evolution, mixing, and detection. This is a detection period. Where is mixing period? It is absent in this type of experiment or you can consider this microsecond pulse; 10 or 5 microsecond 90 degree pulse you apply, it will create the artificial mixing period, no problem, does not matter or in reality, there is no extra time delay given. You can say mixing period is absent or call this delay itself as a mixing period, no problem. What is happening in the preparation period? Preparing the spin system, what do you mean by preparing the spin system? In this example, in this type of experiment, you want to make sure nuclear spins attain thermal equilibrium. Put the sample in a magnetic field, wait for sometime. After some few seconds, spins start aligning in the direction of the field or the direction opposite to the magnetic based on population distribution, they get aligned, very simple and we prepared the sample. Of course, this can be different. You can prepare that in a different way or different experiment, no need, but in this example I am taking preparation first at the thermal equilibrium. Next is the evolution. Look at the evolution period. Here we are varying the delay.

Indirectly, I am creating a pseudo free induction decay oscillating damped signal I am going to collect as a function of t1 when I increase the t1 as a function of time, keep on increasing the delay, then I keep on getting the FID, I collect the signal and I am going to generate a pseudo free induction decay in the indirect dimension, which is also damping like exponential decay, similar to this.

So in the evolution period, we allow the spins to evolve and then we start detecting the signal. What happens here and here, I will explain to you later; and here we are going to detect the signal and this is the simplest of the 2D pulse sequence, but in this case both the pulses what we apply here and here are 90 degree pulses. These are 90 degree pulses and there is no need to have this as 90. You can have COSY 45, varieties of angles you can have.

You can have a small angle, small flip angle COSY, varieties of things you can do. But in the first COSY experimental designed these 2 are 90 degrees and this is what happens. I will apply a pulse, increment t1, collect the data points here, collect data here, t1 data, t2 data. I have two-time domain data. I will do the Fourier transformation and I will get two frequency domain signals, fine. What do I get out of this experiment?

The thing is this experiment establishes the connectivity between immediate coupled neighbours, beautiful. That is what I said. If I have a molecule with CH3CH2, there is another CH and another CH2. Now consider this CH3. This is CH3, CH2 and CH3. I do not know, whether this CH3 is connected to CH2 or this CH3. Whether this is next to it or CH2 is next to it, I do not know. Of course, I know from the multiplicity pattern. Supposing, it is complex spectrum, I do not know. What will I do?

This gives me connectivity information between immediate couple neighbours. What does it mean? See it must be a couple neighbour. So J-coupling is necessary. Without J-coupling, COSY does not work. You need J-coupling for COSY type experiment. Some coupling information is required. So when one proton is coupled, for example I take CH3 her;e and then CH2 here and another CH3 here. Now I get correlation of this to this, this to this.

I can say from the spectrum this is its immediate neighbour and this is its immediate neighbour. Like that, I can start getting immediate connectivity information of the neighbours. This is a very beautiful information. It is like getting multiplicity analysis, which we do in the 1D, so that to know, which is coupled to which. Like I said, one dimensional periods, that we have to selective decoupling to get the connectivity information, to see which proton is coupled to which. I said we need to do 100s of such experiments to get that information; do not need to do it now. One two dimensional experiment gives me complete information; all connectivity information, of all the protons, which is coupled to which. So it is easy, does it not simplify our life? very easy to analyze. This is the most beautiful and the first experiment of the 2D.

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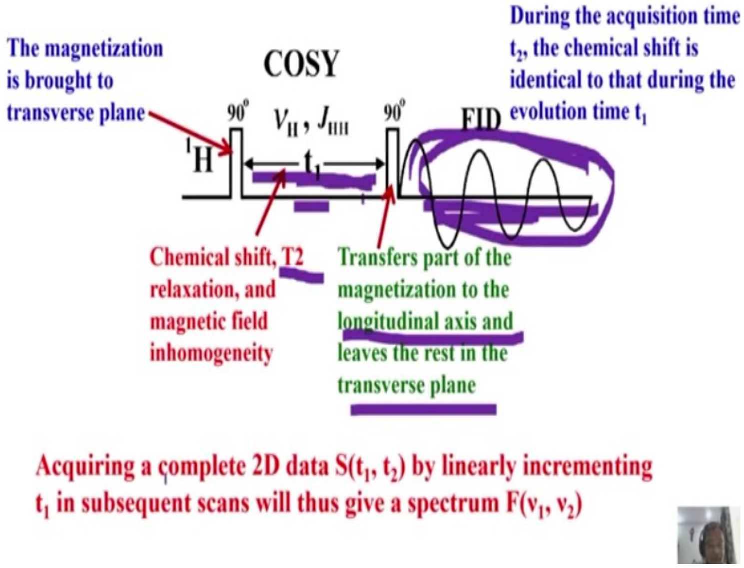

COSY

The magnetization is brought to transverse plane

During the acquisition time t_2, the chemical shift is identical to that during the evolution time t_1

Chemical shift, T2 relaxation, and magnetic field inhomogeneity

Transfers part of the magnetization to the longitudinal axis and leaves the rest in the transverse plane

Acquiring a complete 2D data $S(t_1, t_2)$ by linearly incrementing t_1 in subsequent scans will thus give a spectrum $F(\nu_1, \nu_2)$

Now we will understand, what is happening in this COSY experiment. The magnetization in this case is brought back to transverse plane. This is all we know. This is a 90 pulse, if you apply, magnetization, which is along z axis, will come to xy plane, transverse plane, that is fine. Now here, what happens here? the spins, which are brought to this transverse plane, they start evolving; and instantaneously, they will be in phase; and after some time, the spin packets start moving in different frequencies, fast and slow moving components. But during this time what happens, they start evolving as a function of chemical shift, as a function of t2 relaxation and if there is a magnetic field inhomogeneity, all these factors contribute here. All these things will make magnetization evolve here, start evolving in this period.

Then what we do is, we apply a second 90 degree pulse. What this does? It transfers part of this magnetization to the longitudinal axis, because this is already some are in transverse plane; part of it transferred to longitudinal axis; and part of it will be left in the transverse plane. That is what it does. Second 90 degree pulse, transfers part of the magnetization to the longitudinal axis and leaves the rest in the transverse plane; and finally during this period, we acquire the signal.

Here the chemical shift should be identical to that during this evolution period. It has evolved and whatever the chemical shift was there for different spins here, is also retained here, is identical. So now what we do? Increment the t1 point and acquire complete 2D data set in two dimensions. Vary this, fix this constant period, collect signal; keep on varying, like you take full time domain data, complete time domain data by linearly incrementing, t1.

Of course, this incrementing you do linearly, you can also randomly vary the t1 in some experiments. We can also do that experiment not t1, but in some mixing times, we can randomly vary the mixing time and all those things. But in this case the t1 period is linearly incremented in subsequent t1 points, so that you get a full time domain 2D data, time domain signal is mentioned, here it as S(t1, t2).

Signal is S in both the dimension is called t1 and t2. They are represented as S (t1,t2), that means you have got the signal in two dimensions. Now you do the double Fourier transformation, which I showed earlier, then you are going to get the frequency domain spectrum in both the dimensions. That is all you are going to get. This is what the experiment does.

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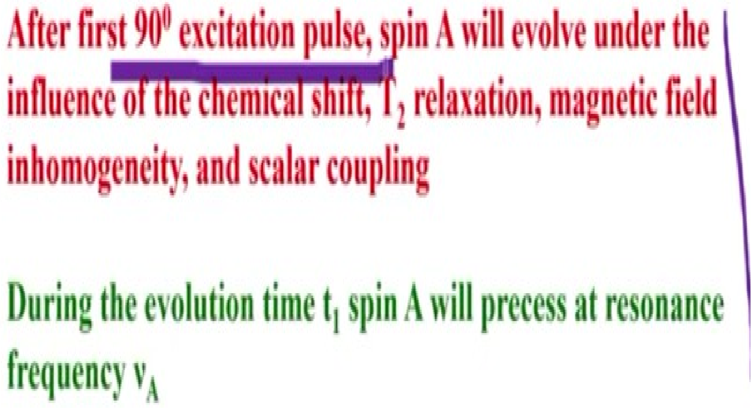

After first 90^0 excitation pulse, spin A will evolve under the influence of the chemical shift, T_2 relaxation, magnetic field inhomogeneity, and scalar coupling

During the evolution time t_1 spin A will precess at resonance frequency v_A

So now let us take the example of two spins, which are coupled. How we get the peaks? This is what happens, but how one spin has correlation to other spin? This gives correlation information. What is correlating? and how it does. I give you hand waving argument here. Please, there is a detailed mathematics to understand this, you have to go deeper into that to get completely what is happening. Just it is only a hand waving argument I am giving for you, to get a feeling for it.

This is not the final, so please remember. It is a hand waving argument or some points, which I am giving you to get the concepts clear for you. If you want the detail understand of what is happening, you must go to mathematics. Let us not do that and I will give you conceptually what is happening. After first 90 degree pulse what happens, let us say, I have two spins coupled A and X; weakly coupled. A will be a doublet; X will be a doublet.

They are coupled; each of them is doubled, because there is a J-coupling. As I told you, J-coupling is a requirement for COSY. If spins are not J-coupled, you will not be able to do COSY experiment. So let us say, spin A evolves under the influence of chemical shift, T2 relaxation, magnetic field inhomogeneity and scalar coupling, all, which I told you here. It evolves under various parameters, chemical shift, relaxation, magnetic field homogeneity. Of course, coupling, which I did not mention here, everything will be present. The spin A starts evolving under the influence of all these parameters, all the four contribute to this. During the evolution time t1, spin A will be precessing at the resonance frequency of νA, because remember the chemical shifts of νA and νX are different. So νA has its own resonating frequency or precession of frequency; νX has its own precession of frequency, they are different.

But since I am taking only A and X as two spins, A spin will precess, I denote as νA. Precessional frequency of A is ν and I call it νA in t1 period.

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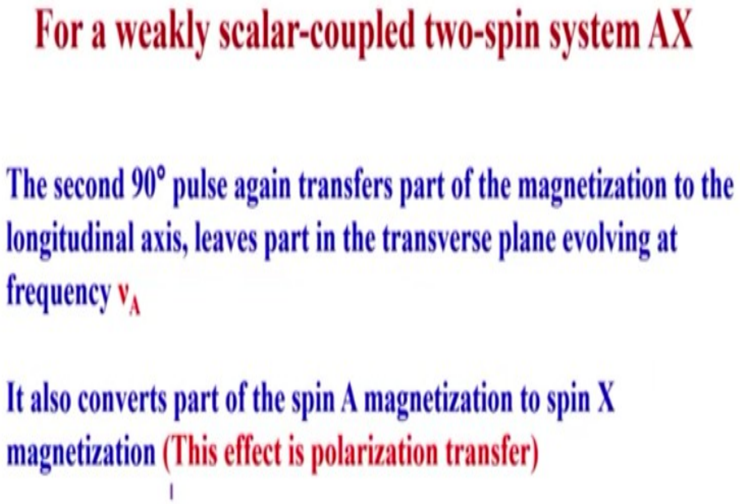

For a weakly scalar-coupled two-spin system AX

The second 90° pulse again transfers part of the magnetization to the longitudinal axis, leaves part in the transverse plane evolving at frequency ν_A

It also converts part of the spin A magnetization to spin X magnetization (This effect is polarization transfer)

Now what happens with the second 90 degree pulse? It again transfers part of the magnetization to the longitudinal axis, which I said earlier and leaves part in the transverse plane, evolving at the same frequency νA, remember. Already magnetization is a transverse plane, spin A is evolving under the influence of chemical shift, J-coupling, magnetic field, inhomogeneity, scalar coupling; everything.

Second 90 degree pulse transverses part of it back to the z axis; this longitudinal axis and leaves part of the magnetization in the transverse plane. What is happening? Their precession frequency is still νA. There are evolving at νA frequency only; that is not changed, but it also converts part of spin A magnetization to spin X magnetization. That is the polarization transfer effect. This is called polarization transfer, effect of polarization transfer.

What happens is, the spin A in addition to precessing about its own frequency, during the period of evolution, it gives part of its magnetization to spin X. That is a very interesting thing. It gives part of its magnetization to spin X by what is called polarization transfer.

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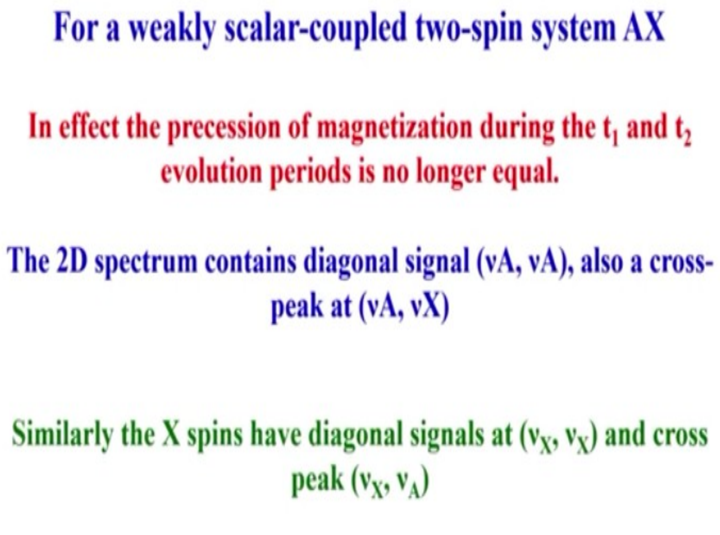

For a weakly scalar-coupled two-spin system AX

In effect the precession of magnetization during the t_1 and t_2 evolution periods is no longer equal.

The 2D spectrum contains diagonal signal (νA, νA), also a cross-peak at (νA, νX)

Similarly the X spins have diagonal signals at (ν_X, ν_X) and cross peak (ν_X, ν_A)

Now in effect what happens is the precession frequency of the spins during t1 and t2 evolution period is no longer the same. What happens, in the t1 and t2 period, the precession frequency of spins are not same, because it is not remaining as individual νA and νX. Individually νA was processing frequency nu A, which is part of the magnetization to νX also and part will go back to z axis.

The effect is you cannot say during both the time periods, the precessional frequencies are same. They are not same; they are different. So the 2D spectrum, then what happens, because it gives the magnetization in the t1 period to X also, in the detection dimension what happens, X will have its own precession of frequency plus the frequency obtained because of spin A. So it will be appearing as two frequencies, one at νA and one at νX.

What happens to precession frequency of νA, that magnetization is given to nu X. So we get peaks at the precession frequency of νA, which I in t1 period is νA; remains same in t2 period also, that is one signal. We also get a peak whose precessional frequency is νA in t1 period, which has given its magnetization to X, whose precession frequency is νX in the t2 period. So in the t2 period, we have peaks, whose precessional frequency in evolution periods is not different, as a consequence these frequencies are different.

We get frequencies depending upon whether it has the same frequency or given the magnetization to X spin. Now what do you mean; whose precession frequency has not changed, which remains same, part of the magnetization is went back to z axis, which remains same. It has given magnetization X spin, that will appear only as νA and νA. They are called diagonal peaks.

The frequencies, which remain same in both in t1 and t2 dimensions, both in evolution period and detection period, the frequency remains same; that comes as what is called a diagonal peak in COSY. In COSY, the diagonal peak comes where the precessional frequency of the spin both in t1 period and t2 period remains same. On the other hand, the precessional frequency of νA, which is transferred to νX appearing as νA and νX also together at νX in the t2 dimension, that comes as what is called the cross peak.

The cross peak comes because of polarization transfer of A spin to the X spin. This happens because of J-coupling. So when there is a J-coupling, there is a transfer of polarization; as a consequence, the precession frequency of X is also seen at the precession frequency of A. They appear as νA and νX, both the frequencies. They are called cross peaks. It is called a cross peak. When the frequency of νA remains same in both the dimensions, it is a diagonal peak and the precession frequency of A is νA and also νX, this is a cross peak.

This is for A spin, what about the X spin? Identical, same explanation. When the precession frequency of X remains same in t1 and t2 dimensions, it gives a diagonal peak at νX frequency. If νX also has given its magnetization to νA, similar argument, then in the 2D spectrum has the νX frequency, you can also see the effect of it at the νA precession frequency.

In the 2D spectrum, you get diagonal peaks, whose frequency remains same in both the dimensions, whose frequencies are different in both the dimensions, come as cross peaks, which is true for A spin; it is also true for X spin. And I took the example of two weakly coupled two spin system, to understand this. I suppose you have got the point now.

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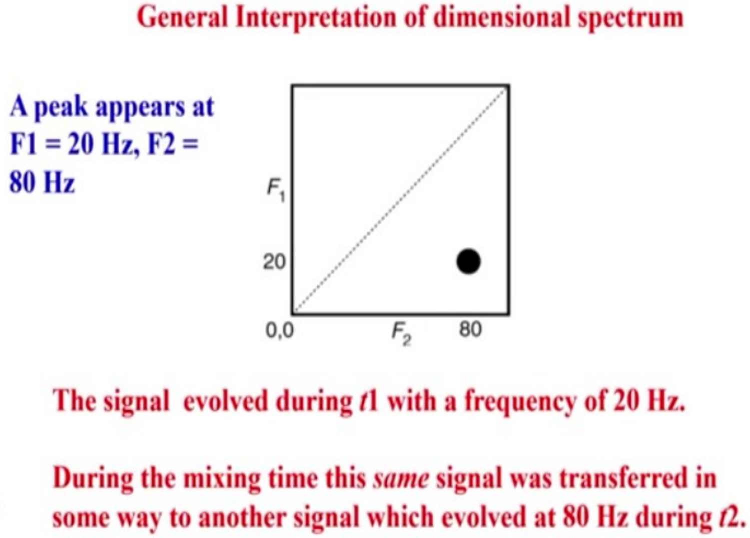

General Interpretation of dimensional spectrum

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.

Now let us try to understand the general interpretation of a two dimensional spectrum. It could be COSY, NOESY, ROESY anything. With our understanding let us see, what is the peak. I was telling you about frequency νA in both dimensions, νA. νX, νX, νA, νX,νX, all four possible combinations we discussed. Now let us say, I have one 2D experiment, take COSY, no problem, any experiment. For understanding purpose, we will take COSY.

This is an experiment of 2D, which we have done and I see a cross peak here. Is the peak diagonal? This is not on diagonal. There is no diagonal peak, that means, if you want to interpret, if you go in this dimension; at F1, this precessional frequency is 20 Hz, in the F2 its precessional frequency is 80 Hz. So what do you understand from this? This signal has evolved in the t1 dimension at a frequency of 20 Hz, but in the t2 dimension, it has evolved at 80 Hz. I suppose you are understanding me. Because of the mixing, the magnetization of this is transferred to this, it is evolving at 20 Hz in the given dimension. As a consequence the peak which is evolving at 20 Hz, precessing at 20 Hz, let us say, in t1 dimension has given its magnetization to coupled spin; and as a result, it is giving a peak at 80 Hz in the t2 dimension. This is what you have to understand.

So this spin has given magnetization to another spin and this spin has its own frequency retained in the t1 dimension, it is always 20 Hz. Whereas in the t2 dimension, it is 80 Hz. This is how you have to interpret. You have to do like this. So if I want to see what is the precession frequency of this peak or this spin or this magnetization, I will say in this F1 dimension, it is 20 Hz and in the F2 dimension it is 80 Hz. That is the way you have to interpret a 2D spectrum, any 2D spectrum.

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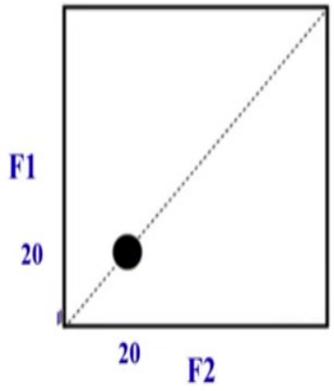
A peak appears at F1 = 20 Hz, F2 = 20 Hz



The signal evolved during $t1$ with a frequency of 20 Hz. It is unaffected and continue to evolve at 20 Hz during $t2$.

Now let us say, I have a peak here. Remember, now the peak is on the diagonal. What do you understand from this? There was a peak, which was precessing at 20 Hz in the F1 dimension and this frequency is unaffected and this spin has not given magnetization to any other spin. So it continues to remain at 20 Hz precession frequency even in the F2 dimension. That means, it has no coupled partner to give its magnetization to other spin. As a consequence, this peak comes as a diagonal peak. When the diagonal peak is there, take a line here, take a line here, both the frequencies are same. You understand? Now this peak is coming at 20 Hz in the F2, 20 Hz in F1. So this frequency remains same in both the dimensions; and it has not given energy to any other peak in this dimension or in this dimensions. As a consequence, it has no coupled partner. So it is a diagonal peak, that is what you have to understand.

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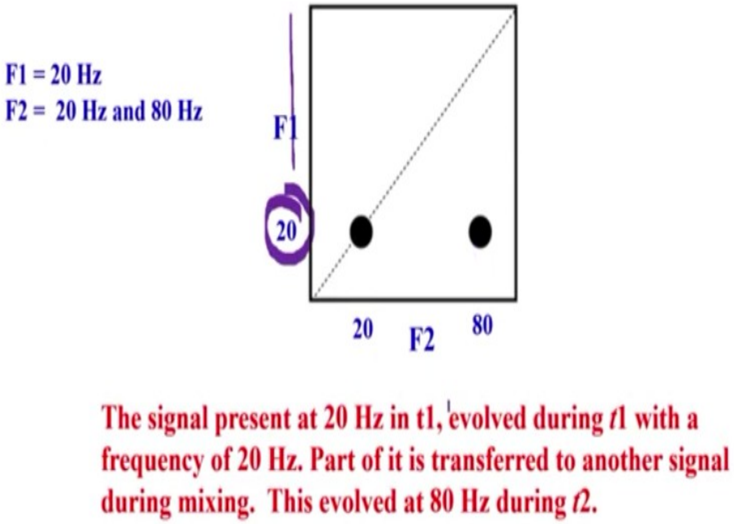

F1 = 20 Hz
F2 = 20 Hz and 80 Hz

The signal present at 20 Hz in t1, evolved during *t1* with a frequency of 20 Hz. Part of it is transferred to another signal during mixing. This evolved at 80 Hz during *t2*.

Now let us think a situation like this. I have two peaks. There was a spin, which was precessing at 20 Hz in F1 dimension. During the process of mixing, it will give its magnetization to another one, which is precessing at 80 Hz in the t2 dimension. You understand now, you have to see this has a frequency of 20 Hz in the F1 dimension. It has a frequency of 20 Hz and 80 Hz both in the F2 dimension, how? Look at it.

In this dimension, you have only one peak, which correspond to 20 Hz, but this is t2 dimension. The t2 dimension has one peak here at 20 Hz; there is another peak here at 80 Hz. That means, this spin, which was precessing at 20 Hz in the t1 dimension has given its magnetization to another spin, which has a precession frequency at 80 Hz. As a consequence, you can see it has retained its frequency and also given part of its magnetization to another spin, which has evolved at 80 Hz in t2.

I hope I made this point clear for you. Let me repeat. There is a spin which is precessing at 20 Hz in the t1 dimension. In the evolution period or mixing period, what happens during the process of this, it will give part of its magnetization to another spin, which is at 80 Hz in t2 dimension. At the same time, it has retained its frequency also in the t2 dimension. So if you analyze this spectrum in the F1 dimension, it has its 20 Hz. In the F2 dimension, it has retained its frequency also gives magnetization to another spin, whose frequency is 80 Hz.

What do you understand from this? This spin is retaining its own frequency in both the dimensions giving the a diagonal peak and also it is giving a cross peak for the spin, which is coupled through scalar coupling. Now I will tell you this is coupled to this. I have to find out this diagonal peak for this. We will see in the next COSY spectra.

But for interpretation, you understand this spin has given the magnetization to another spin, which is resonating or precessing at 80 Hz in the t2 dimension. This is how you have to interpret any 2D spectrum. I have taken an example of one simple thing. This spectra, beautiful spectra taken from Keeler's book. You can see that. Now I know how to understand and how to interpret the spectrum. I suppose, you understood everything.

Now in this class, you understood in depth about 2D sequence, especially number of possible 2D experiments, what we can get out of it and especially I took the example of a COSY two pulse sequence, how the magnetization evolves, what happens to magnetization, how one spin precessing at a particular frequency in the t1 period, transfers its magnetization to another spin during the evolution period and mixing period and how the peaks will appear, both diagonal peak and cross peak.

Diagonal peak is the one, where the spin retains its precession frequency in both the dimensions. Cross peak is the one, in which the spin gives part of its magnetization to another spin, which it is coupled to it. As a consequence, it comes with a different frequency in the t2 dimension. So that is a cross peak. So we took several examples and saw how to interpret 2D spectrum with only one or two peaks present and this interpretation is invariant for any type of 2D experiments.

With this, I will stop here. We will come back and start with the analysis of the 2D spectra in the next class.