One and Two-Dimensional NMR Spectroscopy for Chemists

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Lecture – 53
COSY and Examples

Welcome back all of you. In the last two classes or even three classes I would say, we discussed a lot about multi-dimensional NMR, focusing mainly on two dimensional techniques and I explained to you the time dependence; choosing the dimensionality; what is the advantage of different two dimensional experiments; what type of experiment you have to choose; and what is the polarization transfer mechanism for some of the pulse sequence like INEPT, TOCSY, ROESY etc., and number of pulse sequences which are commonly used both for homonuclear and heteronuclear were discussed.

I took the special example of a COSY which is simple two pulse sequence where I discussed and explained how the magnetization between two coupled spins, especially when I took the example of A and X; how it gets transferred from A to X or X to A. I said in the evolution period t1 the precessional frequency of spin A when it remains same, in the detection period also it gives rise to the diagonal peak, whose frequency remains same (νA , νA) both in t1 and t2 dimensions.

However, if it gives magnetization to the coupled spin also; as a consequence then in the frequency domain spectrum we are going to get a peak, which is different. In addition to diagonal you may I also get a peak in the frequency spectrum that is a cross peak. This is what we discussed and took an example and showed how we get cross peaks and how we get diagonal peaks.

So, please the remember only the diagonal peaks in the COSY come when there is no coupling. As consequence, if there were N protons let us say, none of them are coupled amongst themselves, in which case you get only peaks on the diagonal and nothing else. And what do you get in the one-dimensional spectrum? it is exactly same. As a consequence, the diagonal spectrum of the COSY corresponds to one dimensional spectrum; simple. Please remember that diagonal peaks in the COSY spectrum corresponds to a simple one-dimensional spectrum.

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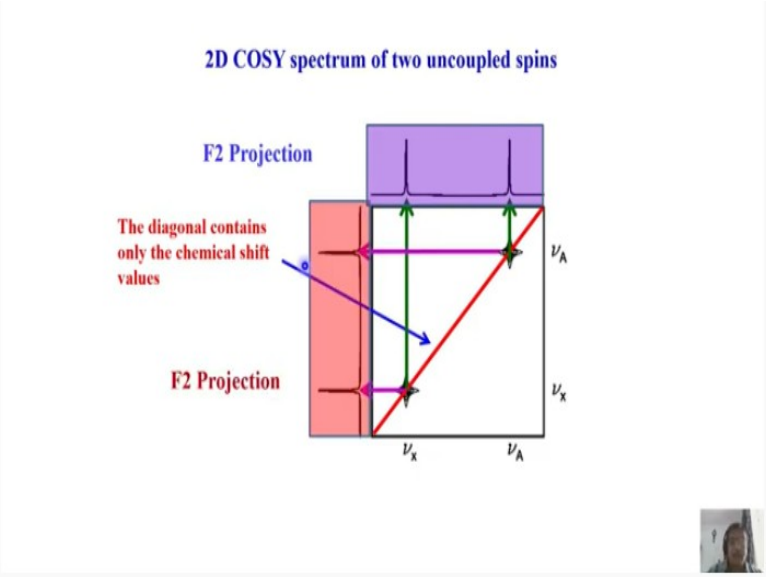

2D COSY spectrum of two uncoupled spins

The diagonal contains only the chemical shift values

Now, let us take an example of 2D COSY spectrum of uncoupled spins; two uncoupled spins I am taking. Let us say one is precessing at the frequency νA; other is processing at the frequency in νX. Now, when we take the COSY spectrum of this molecule, we are going to get spectrum like this. Remember, I said these two spins are uncoupled. As a consequence, this νA has the same precessional frequency both in the t1 dimension and in t2 dimension.

Similarly, the spin X has the same precessional frequency both in t1 and t2 dimensions. So, then what is the type of peak you are going to get? you see; you are going to get peaks only on the diagonals. Remember as I said in the last class, J coupling is one of the requisites for COSY; only then the polarization transfer takes place between two coupled spins. That is a mechanism of transfer through J coupling.

Now, J coupling is not there. So, this continues to precess and it will not transfer its magnetization to X in the t1 period. Similarly, X will not transfer magnetization to A. So, there is no cross peak at all; what you are seeing is only diagonal peaks. So, this is a 2D Spectrum in the contour form, okay. Now we can do one more thing, you can get a spectrum; this is a diagonal; contains only the chemical shift information.

Now I can plot the projection of the spectrum. This is the F2 projection; a spectrum if it the projection is taken and plotted on the top; this peak comes like here, this peak comes here. This looks like a one-dimensional plot. Of course, it is not one-dimensional spectrum, but the plot looks like one dimensional spectrum, not correspond to this one dimensional spectrum, but it is a projection. Of course, if there are peaks here; that will also come okay.

Now, you can see it take the same projection along this side also. I am sorry this should be F1 projection it is a typo it has to be F1 projection. The projection along this axis is F2 projection. This is how normally 2D COSY spectrum is plotted. Get the spectrum; plot it in both the dimensions both the frequency dimensions; and you can start looking at the peaks. These are the diagonal peaks.

If there is a coupling if the spin reaches magnetization or coupled spin you get cross peaks which are symmetric with respect to the diagonal on either side. Because remember why it is symmetric A peak gives magnetization to X, X will also simultaneous give to A. So with respect to the diagonal, because chemical shift range is same in both the dimensions; the cross peaks in the COSY are always symmetric. Okay, this is the spectrum corresponding to two uncoupled spins.

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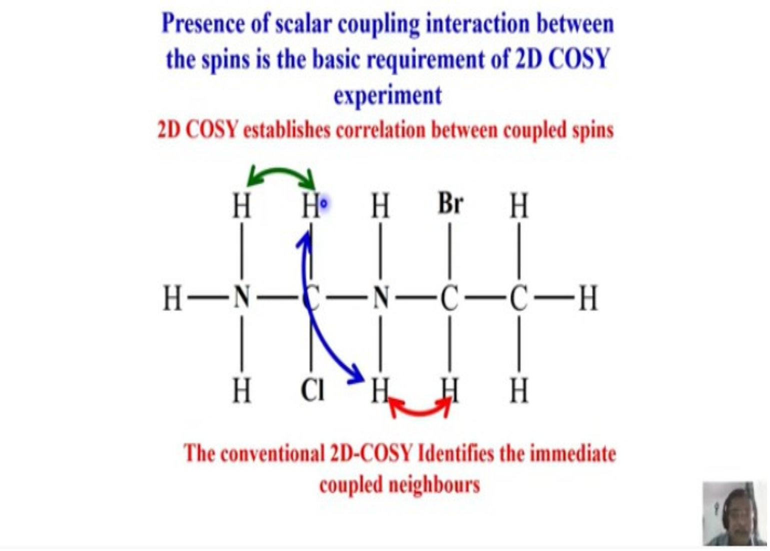

Presence of scalar coupling interaction between the spins is the basic requirement of 2D COSY experiment

2D COSY establishes correlation between coupled spins

The conventional 2D-COSY Identifies the immediate coupled neighbours

Okay, now let us consider the example of what type of information COSY gives. The presence of scalar coupling interaction between the spins is a basic requirement, as I said, if I take a molecule like this and start looking at COSY spectrum, what I am going to get? This is coupled to this through J coupling and the chemical shift of this comes on the diagonal, the chemical shift of this comes on the diagonal and because there is a J coupling and these two also gives rise to the cross peaks.

Similarly, this and this gives rise to cross peak; and this and this gives rise to the cross peak. It can go like this; it can go on and on like this. So, what I am trying to say is, so long as there is a coupling, you get cross peaks among all the coupled spins. Of course, depending upon the strength of coupling, you may also get long range coupling in COSY; you can see this is coupled to this also, you must see this cross peak also.

Let us say this proton, in t1 period it is precessing at certain frequency; and gives part of its magnetization to this. It can also give part of the magnetization to this; if there is a long range coupling. It is not restricted only to immediate neighbours. Of course, probability of this is with immediate neighbours because that coupling strength is larger. But does not mean it cannot give its magnetization to the protons, which is coupled to that are 2 or 3 bonds away.

It can also give rise to long range coupling and the cross peak to that one. So, this is the thing; although I have written the 2D COSY conventionally identifies immediate coupled neighbours. Please remember, you can also get cross peaks to the long range coupled protons, please remember this point okay.

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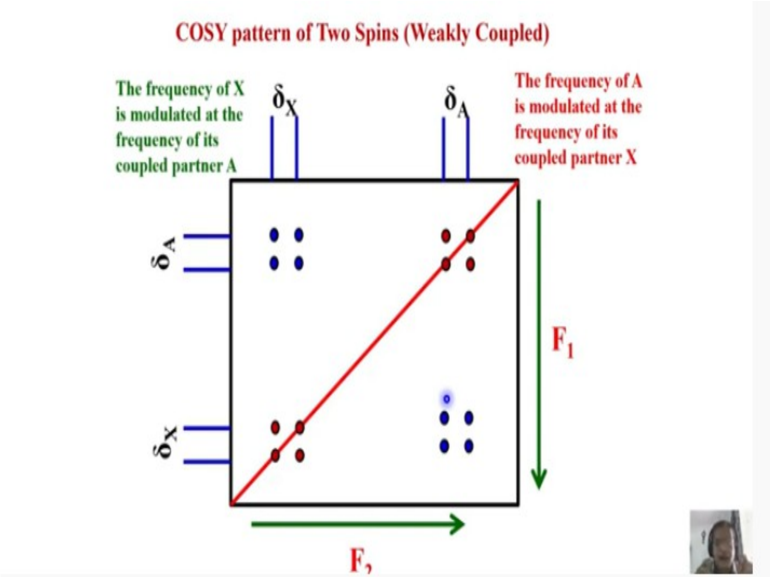

COSY pattern of Two Spins (Weakly Coupled)

The frequency of X is modulated at the frequency of its coupled partner A

The frequency of A is modulated at the frequency of its coupled partner X

Now, I will take the COSY pattern of two spins which are weakly coupled, in the previous example I took uncoupled. Now, what is the pattern you are going to get if the two spins are coupled? already we discussed and especially weakly coupled case, it must be treated like two A and X spins. So, you will get a doublet A; and a doublet for X; this we have already analysed N number of times. So, I do not have to repeat it.

So, let us say this is the AX spectra. I am going to plot the projection, the projection is nothing but what you see on the diagonal here. This is the F2 projection This is the F1 projection; and I call this as chemical shift of A; this is the chemical shift of X. The same spectrum I am getting in this dimension also, because I am correlating only chemicals shifts; identical spectra I am getting in both the dimensions. Okay, this is the diagonal.

Now in the COSY spectrum what are the peaks you are going to get? First identify the dimensions F1 and F2; t1 and t2; omega1 and omega2; whatever you want, you can identify. Now, first, I am going to identify the peaks on the diagonal. See I am going to identify 1, 2, 3 and 4. The 4 peaks are on the diagonal, corresponding to chemical shifts or the frequencies of two doublets; for all the four peaks.

There are certain peaks here and here; we will see that, we will come to that later. And what does it mean? the delta A is processing in both t1 and t2 dimension with the same frequency. So, it gives rise to the peak at its own frequency; that is diagonal peak. So this is nothing but νA, Mu A in the F1 dimensions also. In F1 dimension it is the frequency νA and in F2 dimension also the frequency is νA. So it appears at its own frequency; it is a diagonal peak.

Now similarly X also appears at its own frequency; because the frequency of X the precessional frequency remains same in both in t1 and t2 dimensions; fine. Now, if you look at this, there are cross peaks between them; You see these are doublets and we know this is coupled to this; and there is a polarization transfer between this spin and this spin, okay. When it happens, when the spin gives part of its magnetization to this in the t1 dimension; during the evolution time or this gives to this because of coupling, I said we get cross peaks; true. But why there are extra peaks here in addition to diagonal? These are also cross peaks. They are in both A and X; this is called auto correlated peaks. They correlate within themselves. A spin, the doublet pattern corelates within itself, it is not correlation with another spin. In the doublet pattern, the two frequencies will correlate within itself; they are called auto corelated peaks. So, you can see these auto correlated peaks both for A and both for X; both are auto correlated peaks. Is it clear for you now.

But remember, this does not tell me about which spin is coupled to which? this is simply within the multiplet there is a correlation, that is all. It does not give any additional information. Simply remember they are auto correlated; these are auto correlated peaks. A correlate within itself; X correlate within itself. This is what I said, A appears at its own frequency, X appears at its own frequency; both give auto correlate peaks.

Now the frequency of A is modulated at the frequency of its coupled partner X; I told you. A gets modulated because of X; because it gives part of polarization to X during evolution. Similarly, X gives part of its polarization to A. The frequency of A gets modulated at the frequency of its coupled partner. So what will happen to A? It is going to give cross peaks, at the frequency of A in the F1 dimension and at the frequency of X in the F2 dimension. Please understand this is a frequency of X in the F2 dimension, this is the frequency of A in the F1 dimension. So, because it gets modulated due to the frequency of the coupled partner, A will give rise to frequency at X at its coupled partner. Now what happens to X, X also gets modulated because the interactions are mutual in NMR. So, X also gett modulated at the frequency of A, and give rise to cross peak at its coupled partner A.

Now you understand A not only gives frequency at its own frequency it gives rise to auto correlated peaks because of the splitting; frequency of A gets modulated with the frequency of X because it is an coupled partner and give rise to cross peaks or peaks at X. Similarly, X appears at its own frequency; not only that it gives rise to auto correlated peaks for its doublets. In addition to that X gets modulated because of A, these two interactions are mutual and it gives rise the cross peak at A. That is what it is; so when the frequency of A gets modulated you got peak here; when the X frequency gets modulated you got cross peak here; simple logic.

If the three protons that are coupled among themselves, at the frequency of A, the M and X get modulated; let us say I have three protons, A M and X. At frequency of A, M and X also get modulated, so you got cross peaks at M and cross peaks also at X.

If you look at M, M gets modulated with the frequency of A and X; so it gives cross peaks at A and cross peaks at M. Similarly, X has coupled partners, A and M and it gets modulates at the frequencies of A and M, and gives rise to cross peaks at A and M. So, this is simple logic; whenever a coupled spins have their frequency modulated at their respected coupled partners, there you are going to see the cross peaks.

Please understand. So whenever one of the coupled spin gets its frequency modulated with its coupled partner, at that frequency you get a cross peak. So, this is what happens; so these are called cross peaks, okay.

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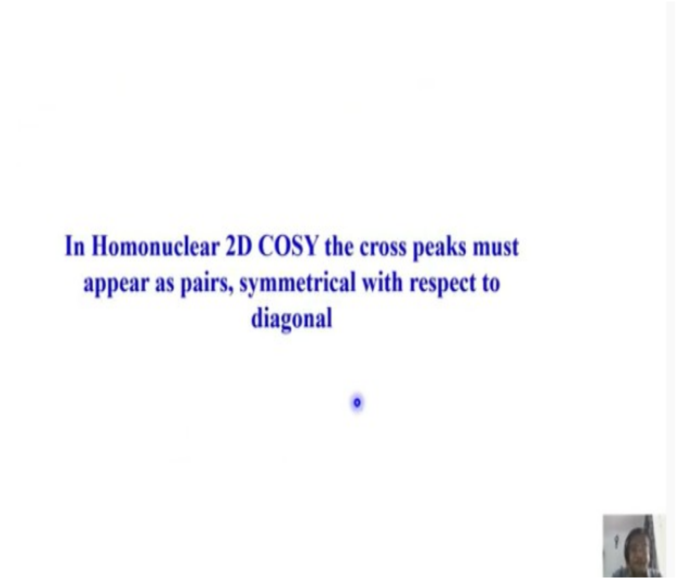

In Homonuclear 2D COSY the cross peaks must appear as pairs, symmetrical with respect to diagonal

Generally, not generally always, remember in homonuclear 2D COSY the cross peaks must appear as pairs; symmetrical with respect to the diagonal. The cross peaks always appear as pairs remember you saw you know. This and this always appear as pair. If A gives cross peak to X, X also has to give cross peak at A; and it is symmetric with respect to the diagonal. You see it has to be perfectly symmetric; this is one of the patterns for COSY. Please remember that; it has to symmetric with respect to diagonal.

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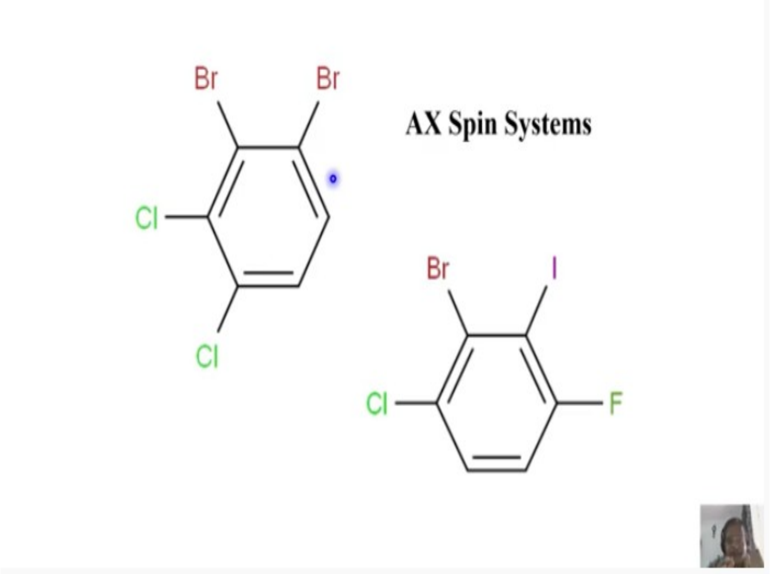

AX Spin Systems

So, now with this what we will do is; let us see I have mixture of two AX spin systems. I have chosen this molecule deliberately. This and this gives rise to coupling, it forms a coupled spin system, I assume the chemical shift between these two protons are sufficiently large compared to scalar coupling. So, I can consider this as a weakly coupled spin system AX. I chose another molecule similarly, I know I will say these two protons also form weakly coupled AX spin system. Now I take the mixture of both the molecules and I want to do COSY.

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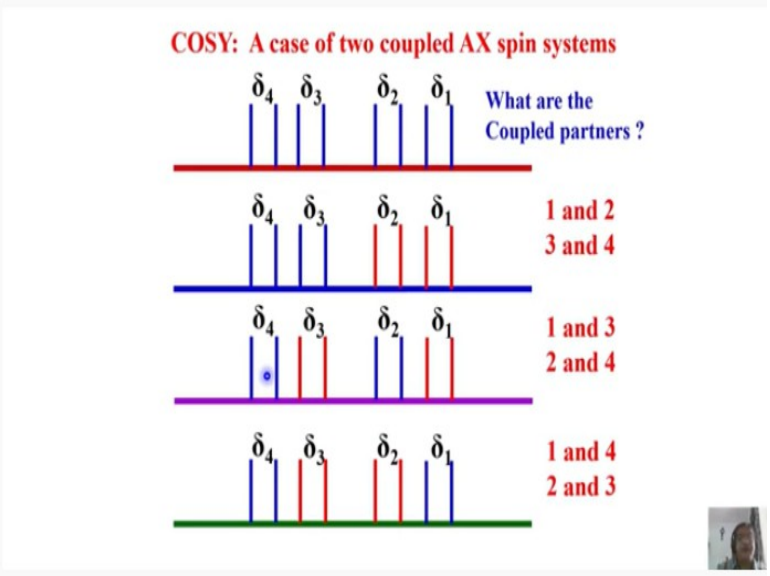

COSY: A case of two coupled AX spin systems

What are the Coupled partners ?

1 and 2
3 and 4

1 and 3
2 and 4

1 and 4
2 and 3

How does the 1D NMR spectrum look, first of all. This looks like this, why? each AX spin system gives rise to 4 lines, 2 lines at A and 2 lines at X; this we have been discussing for quite some time. So there are two such AX spin systems so you should get 8 lines. And what are these? chemical shifts of 1, 2, 3 and 4; all the 4 protons are there. I have 4 chemical shifts and each of them is a doublet because each one is coupled to its coupled partner.

So, because of that there are 2 sets of 2 doublets. Now the question for me is what are the coupled partners? I do not know. I have just taken the 1D spectrum, this what I get. Now I want to know in this spin systems, I want to identify which are these 2 doublets and which are these 2 doublets. Now if I am asked a question, whether this and this form a pair of doublets; whether these two form pair of a doublets; whether this and this form of pair of doublet, or this and this form pair of doublet? I do not know. There are several possibilities here.

I am getting a conventional spectrum. Now I want to identify the coupled partners. Let us say two reds are coupled partners and two blues are coupled partners. Then I know these are all chemical shifts of A and X, A and X. What about the coupled partners? Are 1, 2 and 3,4 you do not know; these two could be coupled partners 1, 3 and 2 4. Also, these two could be coupled partners 1 4 and 2 3. so various possibilities are there. Now if I had asked to find out which are the coupled partners; what you will do?

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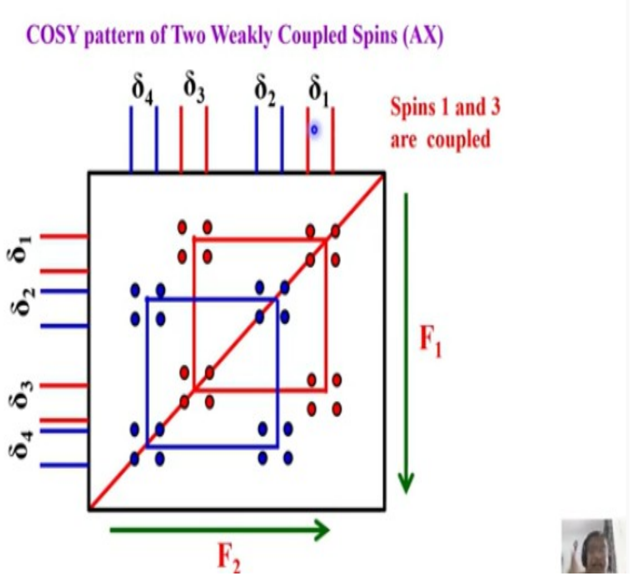

COSY pattern of Two Weakly Coupled Spins (AX)

Spins 1 and 3 are coupled

Simply run a COSY experiment. Now what is the type of pattern I am going get for a COSY experiment tells me which are the coupled partners. Please understand; simply do 90-t1-90 COSY experiment. That is all I do, a two-dimension COSY. Now my first suspicion is possibly 1 and 2 are coupled partners; I do not know or 3 and 4 coupled partners; if this is the situation what type of COSY spectrum I get.

Look at this these are all diagonal peaks and autocorrelated peaks. Now if these two are the coupled partners 1, 2 now I will get cross peaks between 1 and 2; and similarly if 3 and 4 coupled partners, I will get cross peaks between 3 and 4. See what another interesting thing you should know, it is symmetric with respect to the diagonal. To identify the cross peaks all what you have to do is sit at the centre of this peak, doublet; come vertically down, you will come to the centre of this doublet; go horizontally you will be at diagonal; again go vertically up, complete the square. It forms a beautiful square, because the cross peaks in the COSY are symmetric with respect to diagonal. Then I know, the diagonal peak if you see this and this, these two are the protons that are coupled between them. So, the cross peak tells me there is a cross peak between this and this; here and here; that means this proton and this proton are coupled. There is J coupling between these two. So, this is one of the AX coupled spin system. It is one of the coupled partners, clear.

Now let us look at other one. So, I completed this square. Started from the central came down went up and competed this square; this undoubtedly tells me spin 1 and spin 2 are coupled. Go to next one, you should get a pattern similarly for 3 and 4; because this is the chemical shift of 3 and this is the chemical shift of 4. Look at the spectrum, come down, go horizontally, go up and complete the square. So, this diagonal peak and this diagonal peak, sorry, this this proton and this proton are coupled. And that is why we are getting the cross peaks for these two.

So, delta 3 gives diagonal peak and also cross peaks with its coupled part 4 here. Similarly, 4 gives a diagonal peak and also gives cross peak with its partner delta 3; so they are symmetric; perfect. Now I know what are the coupled partners? This is assuming these two are coupled partners, this should be the type of COSY spectrum we get.

Now what happened to the second possibility. I thought this could be coupled partners 2 red and 2 blues are there; may be these 2 red doublets are coupled; these 2 blue doublets are coupled. Are they coupled spins? we do not know. Now if that is the situation how my 2D COSY spectrum comes? Again write down the diagonal peaks. Now because these two are coupled partners, the cross peaks appear between 1 and 3; perfect. you see from the centre of this delta 1 come vertically down, go horizontally up, and complete the square. That shows delta 1 and delta 3 which are diagonal peaks, they are coupled. As a consequence, they give cross peaks between them clear. So, if these two are coupled delta 1 and delta 3, these are cross peaks this pattern you should get in COSY; and if delta 2 and delta 4 are coupled, again you get cross peak like this between 2 and 4. Now this the pattern. Look at the pattern for this COSY spectrum when these two are coupled. And COSY pattern we saw when these two are coupled. Entirely different; but it gives you fantastic information about coupled patterns. You can find out which is coupled to which.

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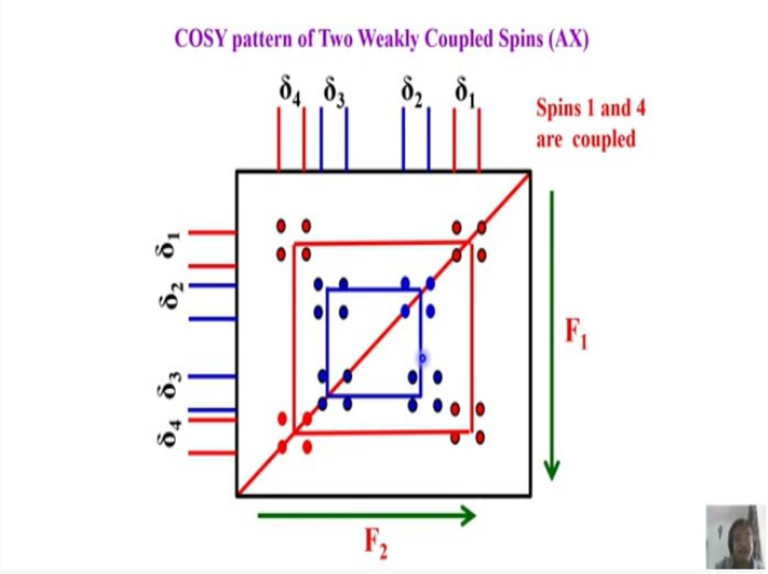

COSY pattern of Two Weakly Coupled Spins (AX)

Fine now what is the last possibility I thought. One possibility 1 and 4 are coupled; and 2 and 3 are coupled. Now tell me how do we get the cross-peak pattern. Again, I write down the diagonal peaks. Now I am assuming 1 and 4 are coupled; 1 should be raised to cross peak to 4, it should be here. Similarly, 4 should give rise to cross peak, it is here. So now start from here; come down; go horizontally; go up and complete the square. Now you identified these two coupled partners. Similarly now go to 2 and 3, 2 and 3 gives are the coupled partners, like this 2 gives cross peak here. 3 gives cross peak to 2. And you can complete the square. See the patterns depending upon which proton is coupled to which. There are three possibilities and we saw three types of coupling patterns. Cross peak patterns are different in the COSY. So then you can identify easily coupled partners. This is a hypothetical example. I took two coupled spin systems, I wrote a stick plot here to make you understand.

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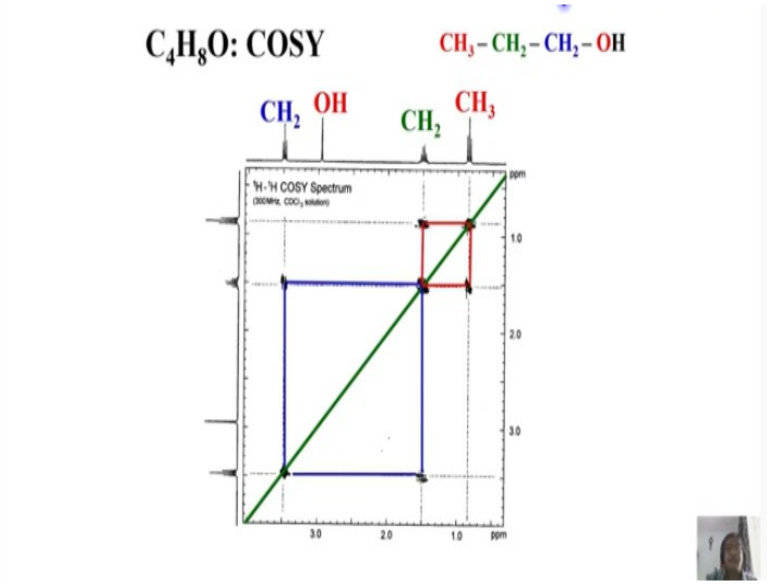

C_4H_8O: COSY

$CH_3-CH_2-CH_2-OH$

But in reality, you can understand yourself, and let us start with a simple example. A realistic example of a COSY spectrum like this. The 1D spectrum of this molecule is given as a projection here. Now this is a CH3 proton I know, it always comes at high field. Of course, it is a simple spectrum I can interpret. Assuming I do not know anything about analysis, I simply ran a COSY.

Now start with one thing; At least some thing we will have to start with; we will start with this peak. I know this peak is coupled to this; because you start from the diagonal, come vertically down, go horizontally, and complete the square. That shows this diagonal peak and diagonal peak, corresponding to these two protons, are coupled. Now we identified the coupled partners; that must be this CH3 and this is CH2, fine; because they are triplet and a quartet.

But you can see some more molecule, this CH2 can also experience coupling with this. That is why we have pentet pattern. We know that you know. Whether it is pentet or sextet pattern we are seeing. So, we will continue further. We identified two protons. Now from the CH2 which we identified continue further, and you can form another square. Come here; hit a cross peak; go horizontally and complete the square. You see another peak. So that means this proton and this proton are also coupled. And this proton corresponds to CH2. That means this CH2 is coupled to this, and also to this. Very easy to assign. You understood, how to assign the COSY spectrum. And for OH there is no cross peak; because OH is not coupled to anything. So I am not seeing any cross peak. In principle there should have been a diagonal peak, it is missing; okay.

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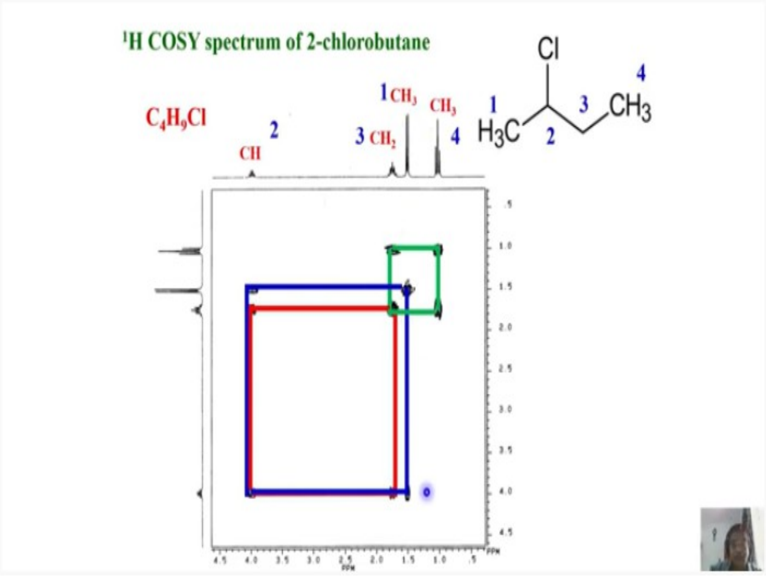

^{1}H COSY spectrum of 2-chlorobutane

C_4H_9Cl

So now let us look at this molecule 2 chlorobutane. I know the formula; I know the spectrum. Of course, again you start interpretation, start with one of the peaks which you know. I think this must be CH3, we all agree right. It is coming at high field, it is a triplet because of the CH2. Let us start with assumption; start with the peak; or we can start with this CH3. This is doublet because of this, that is also fine. We do not have to start with this, we can start with this also.

So with that CH3 now come vertically down, go horizontally, then you hit upon a peak, you will complete the square. If you hit upon this proton; then this must be CH, because the CH3 was a doublet because it is coupled to CH. And the CH is a quartet because it is coupled to CH3. You identified that now. What is next? you can also see that, this CH, if you carefully see, has two cross peaks. One here, okay; in addition to that it has another cross peak. You trace this completely, and you will see it is going to hit upon this diagonal peak.

This is CH2, this CH2 or this CH proton is coupled to the CH2 has given a cross peak. Now this CH2, again complete the square, this CH2 is coupled to this CH3. See you can complete the square. From this go horizontally, vertically, complete the square. So very easy is systematically, stepwise manner you can go, started with CH3 which I knew; I completed this; I hit upon CH3, I know that, and I also came to know the CH is coupled to CH2, from CH2 I can find out it is also coupled to CH3. So entire coupling network I could assign from one spectrum. That is the beauty of COSY.

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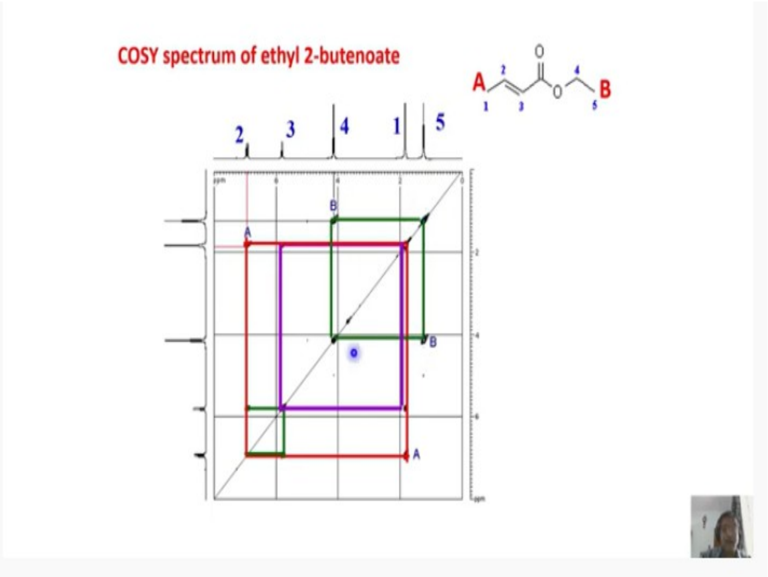
COSY spectrum of ethyl 2-butenoate

Let us quickly go to another molecule; ethyl 2-butenoate. Now there are 5 groups of chemically inequal protons; 1 and 2 here. 1, 2 and 3 here; identified as A group and B group, fine. Now let us look at one of the peaks which you think is easily assignable; Let us look at this; I take CH3 proton; here it is numbered 5. If you carefully see, it is a triplet, it is not expanded the triplet, no doubt about it is a triplet. we will start with the known one, from this CH3, come down you hit upon a peak, again complete the square; and you see this peak. This form a perfect square. This CH3 is coupled to this one, that is CH2. And there is no other coupling possible. only one independent square, you cannot find any more coupling to CH2 or CH3. No cross peaks anywhere, clear. That shows this form a coupled spin system. Then what is left. Next, we consider this one, this terminal CH3; start with this, come down, and then complete a square. So, A is coupled to 2; fine but at the same time you see 1 has two cross peaks. It also has a long range correlation to this proton, you understand? See now 1 is coupled to 2; 2 also coupled to 3; and 1 and 3 are coupled. You understand the point. Now very clearly 1 is coupled to 2 that square is completed. At the same time you see cross peak between these two; then what is leftover. This is 3.

So, this is a diagonal peak. There is a cross peak between this peak and this peak; so this must be proton 3. Also you can see there is a cross peak between 3 and 1. You can see there is a cross peak here, here and here. So complete the square. Now there is a cross peak between 3 and 1. So, 1 has a coupling with both 2 and 3. It forms one coupled spin system. Interestingly we saw a long range correlation in this COSY spectrum. And this is another spin system where we got only correlation to two coupled partners. I hope you understood how we can interpret the COSY spectrum.

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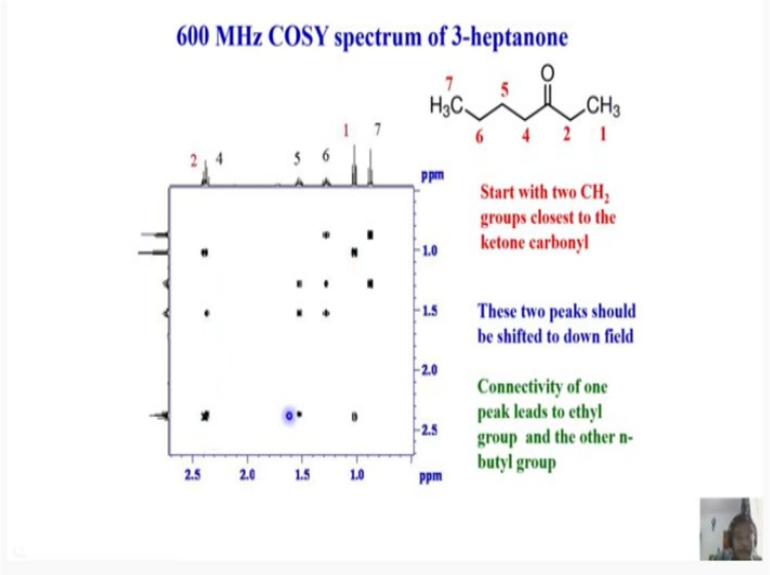

600 MHz COSY spectrum of 3-heptanone

Start with two CH_2 groups closest to the ketone carbonyl

These two peaks should be shifted to down field

Connectivity of one peak leads to ethyl group and the other n-butyl group

This is the last example I will take, so that you will become more familiar with COSY. There is no point in continuing further like this. There are N number of, or infinite number of molecules are there, we can keep on interpreting and analyzing several of them, till you become conversant. But I am giving you the idea. I am giving you the hint as how to go ahead in analyzing the COSY spectrum.

I think with this you can continue further. Now here. there are seven groups of chemically inequivalent protons. It is a very famous molecule you can see in more literature and in web you can see this type of spectrum. This molecule everybody takes, this as a fantastic example to analyse the COSY, TOCSY, etc. Now where do you start. Remember there are 2 CH3s here. They are coming here. Both could be attached to CH2. As a consequence, both are triplets.

But I do not know which is which. But there is one clue for me. This CH3 is coupled to the CH2, and there is a break here; because there is no proton here. This should have a long range coupling, if the coupling is not seen this forms one spin system. And this forms one spin system. So you must get cross peaks between these two; and cross peaks among all these coupled partners. That is one hint for me from looking at the molecule.

So let me start like that. Other way out is this CH2 group, which is closest to the ketone carbonyl. That is correct. These two peaks would be shifted to the downfield, because of CO either this or this that is what we have to take. No problem, the CH2 group closest to ketone carbonyl, either this one or this one, this both of them will come to downfield, and you can see it is written here both, as 2 and 4.

Now one of them will take; it will complete a square. No other coupling you can see here. Carefully if you see, this will form a square. And this has to be coupled to 1. It forms a set of coupled spins, 2 spins which are coupled, and nothing else. No other coupling is possible. But if you go carefully there is another one here; see this is slightly at different frequency. If you draw a line, this is right here, and this frequency is slightly above this, forms a line with this.

Now we will start with the, There is a confusion here; we start with the CH3 because this CH3 is identified now. We come to CH3 here, easy because we cannot make a mistake with CH3 once you know this CH3 this has to be proton 7. Start with that, come down and complete the square. What do you understand? Proton 7 has a coupling with 6; it has to be 6 clear, so you found the square. But this is not the end, continue this diagonal from this if you come down, you see one peak here, go horizontal, go up and you see another peak here.

There is some error in this writing; do not worry; these lines have shifted a bit, but this is cross peak. You can see now 6 is coupled to 5, it forms a cross peak. Now go down further, 5 is coupled to 4 that is also understood now. So very clearly you could identify all these spins which are coupled in this group. All the spins sequentially you can see, and find out this is coupled with this, this is coupled with this, this is coupled with this. You identified all the peaks; and you identified this coupled spin system as a separate independent group. Everything is clear now.

This is the way you can go ahead and analyse any COSY spectrum. However complex it may be, simply look at the diagonal and then if you happen to see a cross peak, find out the diagonal which is equidistant on either side which is giving cross peak. And that must be the peak on the diagonal. These two are coupled. Like that you can identify the coupled partners. So, what I am going to do is, I will stop today. In the next class we continue further and more about other 2D experiments.