Advanced NMR Techniques in Solution and Solid-State
Prof. N. Suryaprakash
NMR Research Centre
Indian Institute of Science – Bengaluru

Module-48
2D COSY and Its Variants
Lecture – 48

Welcome back all of you. In the last couple of classes, we started discussing about 2 dimensional NMR. As you remember I said for 2 dimensional NMR we need 2 time periods, double Fourier transformation of two time periods gives spectrum in 2 dimensions; frequency in 2 dimensions. And then general pulse sequence of 2D consists of 4 periods; preparation period, evolution period, mixing period, detection period.

Of course, I said mixing period is optional, detection period is always constant, the evolution period is varied as a function of time systematically linearly varied. And then based on that, how the polarization transfer takes place, how the spins are allowed to evolve in the t1 time period, how the spins polarization is allowed to mix among couples spins or uncoupled spins in the mixing period, that tells you the type of experiment we are planning to do.

There are umpteen number of pulses sequence that have been this designed so far. Each of these pulses sequence has its own application and it is designed to extract the specific information in an orchestrated manner; that is what it does. And these experiments can be both homonuclear and heteronuclear. And of course, broadly I said we can classify 2-dimensional NMR experiments into two types, one is resolved type experiments and the other is correlation type experiment.

In the resolved type experiments, the two information which are inherently present in solution state NMR like chemical shift and coupling constant, can be resolved or separated into two orthogonal dimensions, that is the one thing. In the correlation type experiments, you can correlate information 2 types of information; it could be chemical shift among the homonuclear spins, chemical shifts among the heteronuclear spins, etcetera. So, these are all one type of experiments.

And I said if you have N time periods, you can have N dimensional NMR spectra, but only thing is the last time period which is the detection period is always constant; and N - 1 time periods are systematically varied. And we also discussed about the time required. As we go to higher and higher dimensions, I said one dimensional NMR may take few minutes, 2D may take let us say 30 to 40 minutes, 3D may take few hours, 4D may take days and 5D may take weeks. That is what we discussed and also we discussed about the size of the molecule.

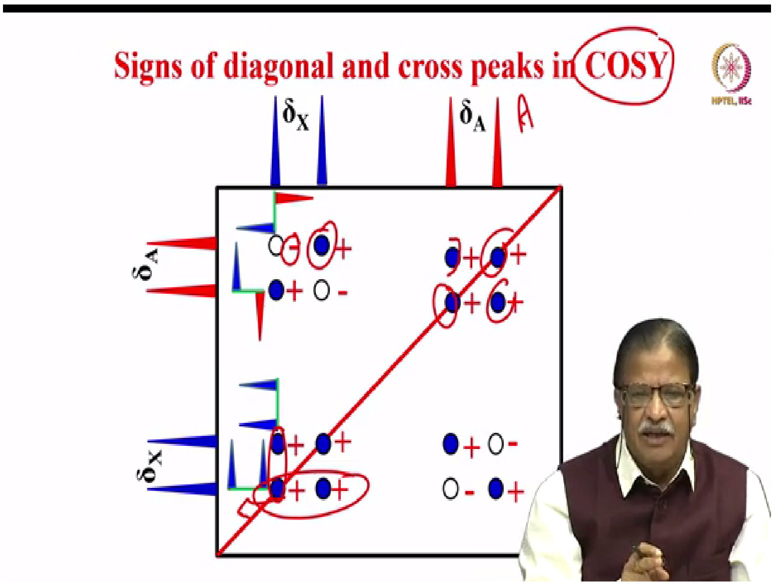
How to decide which type of experiment you want; whether it is, 1D, 2D or 3D? We can decide about those things. So, finally, we came to discuss a general experiment which is commonly used and first of its kind is called COSY, correlated spectroscopy, an homonuclear experiment. Here is a two pulse sequence preparation period, 90 degree pulse, the evolution period, another 90 degree pulse and then detection period. And the second 90 degree pulse is also a mixing pulse and we started understanding how the COSY spectrum comes? How 2-dimensional spectra in such a pulse sequence comes. I said one of the important requirement for the COSY is the spin systems must be J coupled. Here the magnetization transfer or the polarization transfer between two coupled spins takes place through the couplings. The coupling is one of the important requirement for this and we started discussing about how we get the type of spectrum, the type of the diagonal peaks and cross peaks.

I said, for example if the particular spin has certain evolution frequency, it is precessing at certain frequency in the t1 dimension, if it retains the same frequency in the t2 also, we are going to get a diagonal peak. If it gives part of its signal to another spin; the part of polarization to another spin and then in the t2 dimension it will also come at the frequency of the second spin. It starts precessing at the frequency of the second spin. So, it is going to give what is called cross peak.

We took the example of a single spin, we the peak comes on the diagonal, we took the example of two uncoupled spins and we came to know that two peaks appear on the diagonal. And the COSY spectrum is perfectly symmetric. We took two coupled spins as an example, then we came to know we will get 4 peaks on the diagonal and 4 peaks for the diagonal for A and 4 peaks for X spin, two coupled spins, weakly coupled. And also we get 2 cross peaks, one at the right side corner, bottom corner of the COSY spectrum and other on the top left hand corner of the COSY spectrum; we get cross peaks.

Each of these diagonal peaks and cross peaks are having a square pattern. Then we discussed about the phase of the peaks. I said diagonal peaks have fine structure, the 4 peaks because they split by another coupled spin. It is a J split doublet in both your F1 dimension and F2 dimension. And all the diagonals are in-phase, that is what I said, and cross peak again, this separation between the cross peak in the 2 dimensions F2 and F1 dimension pertains to J coupling. The interesting thing is the cross peak or antiphase in character. This is what we discussed and we will continue further from that point today. The signs of the diagonal and the cross peak in the COSY are given like this.

(Refer Slide Time: 06:55)



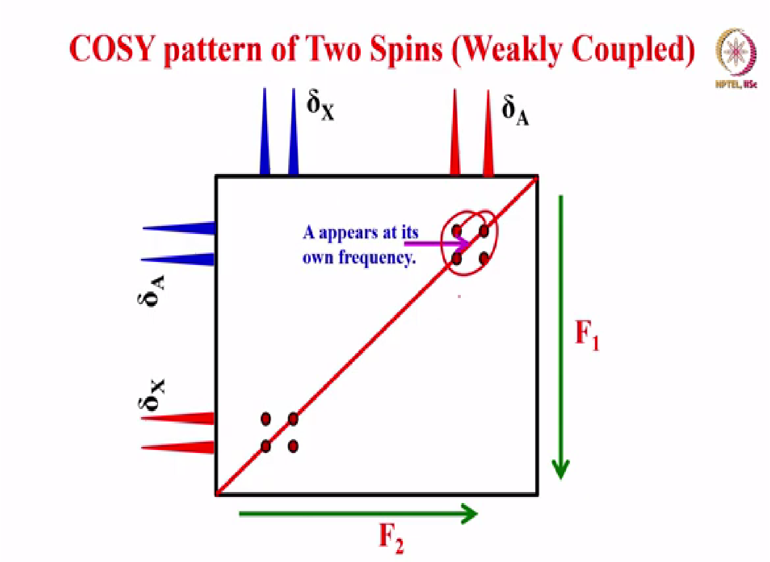
Signs of diagonal and cross peaks in COSY

Let us look at the spectrum like this, we have 2 spins coupled. When 2 spins are coupled we are going to get 4 peaks, weakly coupled spin system, let us say, this is for A and this is for X, it does not matter. We have the chemical shift of A and chemical shift X at the centre. And COSY spectrum gives a diagonal and we get 4 peaks on the diagonal for A; and 4 peaks on the diagonal for X. And we get 4 cross peaks at A and four peaks at X. The four cross peaks are on the top left corner and also you get 4 cross peaks on the top right corner here. So, this is what you are going to get now.

See this is the pattern you are going to get, for simple 2 spins which are coupled between themselves; it is a COSY spectrum. Now, this is a negative signal and other is a positive signal; so this is negative and this is positive. Cross peaks are always anti phase in character. Whereas look at this one, diagonal peaks, they are always in-phase in character. And if you take the projection or a cross section, I would say for this one the cross section both will be in-phase doublet, positive in-phase.

Whereas if you take the cross section along this also; it is in-phase doublet because again same phase. Whereas on the other hand, you take the cross section for a cross peak if you take the F2 cross section like this, you are going to see one is positive and the other is negative; it is antiphase doublet. Same way if you take the cross section along this one F1 dimension, again you see one is positive other is negative; it is antiphase doublet. So, cross peaks are always antiphase in character and the diagonal peaks are always in-phase in the 2D spectrum.

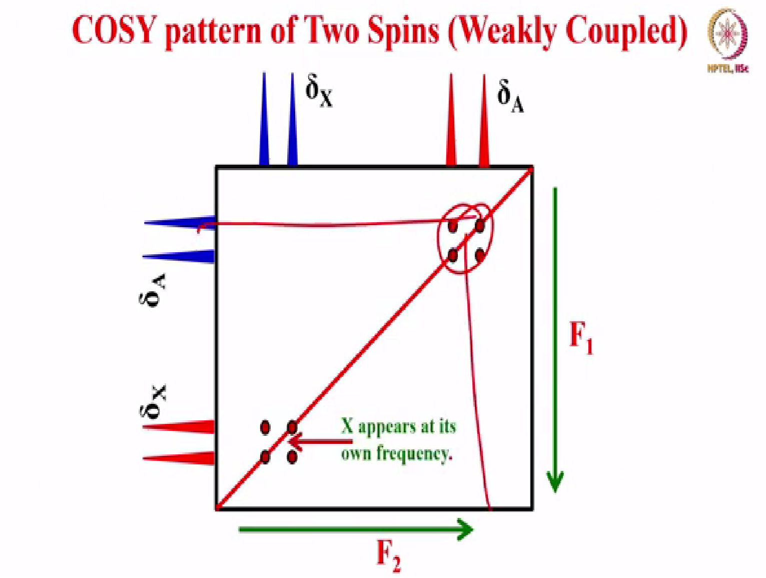
(Refer Slide Time: 08:45)



COSY pattern of Two Spins (Weakly Coupled)

And now the COSY pattern of 2 weakly coupled spin system. If we look at it, we are going to get 4 peaks. Why are we getting these 4 peaks? What are these 4 peaks for, this is because A appears at its own frequency gives rise to diagonal peak that is what we said the precession frequency of A remains unchanged both in the t1 dimension the t2 dimension that comes at the same frequency both in the t2 dimension and the t1 dimension; that is a diagonal.

(Refer Slide Time: 09:16)

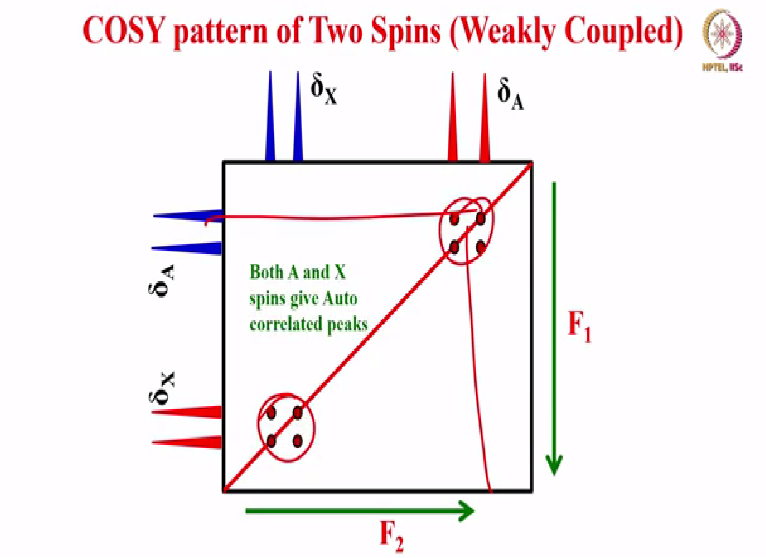


COSY pattern of Two Spins (Weakly Coupled)

X appears at its own frequency.

And similarly the X appears at its own frequency both in t1 and t2, remain same, it gives us the diagonal peak.

(Refer Slide Time: 09:24)

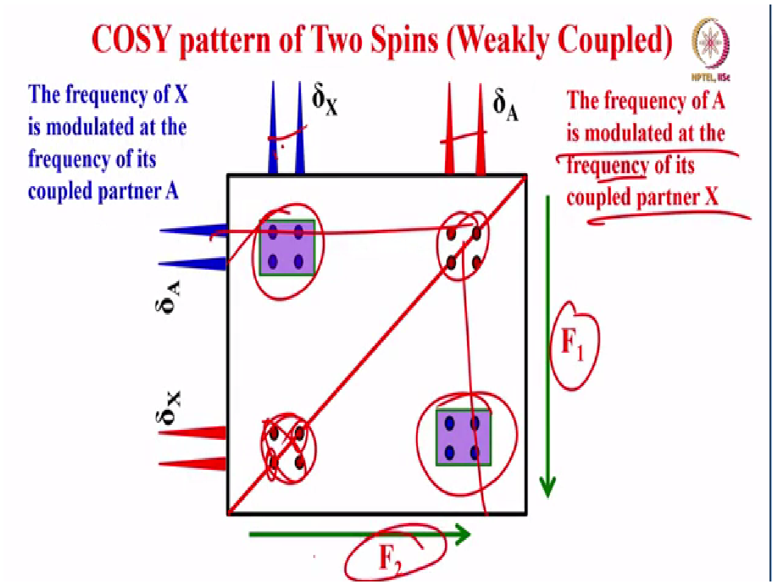


COSY pattern of Two Spins (Weakly Coupled)

Both A and X spins give Auto correlated peaks

And both A and X spin gives autocorrelation peaks; that means this peak of this doublet can correlate with another component of the doublet. For example, this is a doublet this is a doublet. Between these are two doublets, this can correlate with this, between 2 doublet peaks. These two correlate to give rise to cross peaks; these are called autocorrelation peaks.

(Refer Slide Time: 09:48)



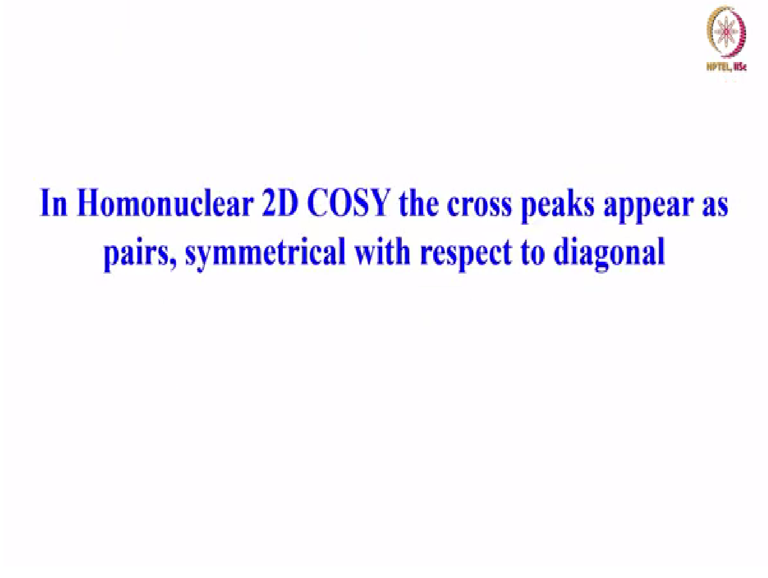
COSY pattern of Two Spins (Weakly Coupled)

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

And the frequency of A is modulating at the frequency of X, because of its coupled partner. As a consequence, you are going to get the cross peak like this. Similarly, frequency of X is modulating at the frequency of coupled partner A, and gives rise to the cross peaks here. So, these are called the cross peaks. And of course, I wanted to tell you this is a F1 dimension, and always the detection dimension is F2.

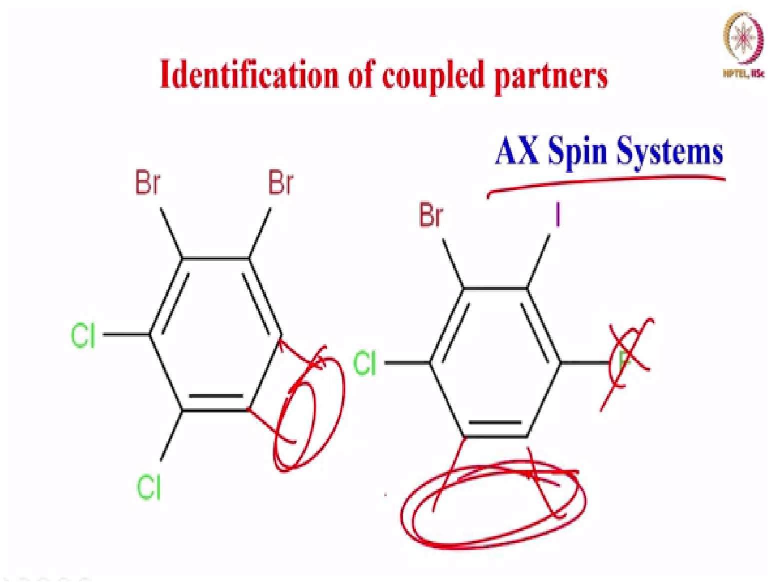
(Refer Slide Time: 10:14)



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

So, in homonuclear 2D COSY the cross peaks always appear as pairs, they come as pairs symmetric with respect to diagonal. Remember, this is the diagonal, and the cross peaks are always appearing as pairs and symmetric with respect to diagonal, there is a perfect symmetry.

(Refer Slide Time: 10:33)

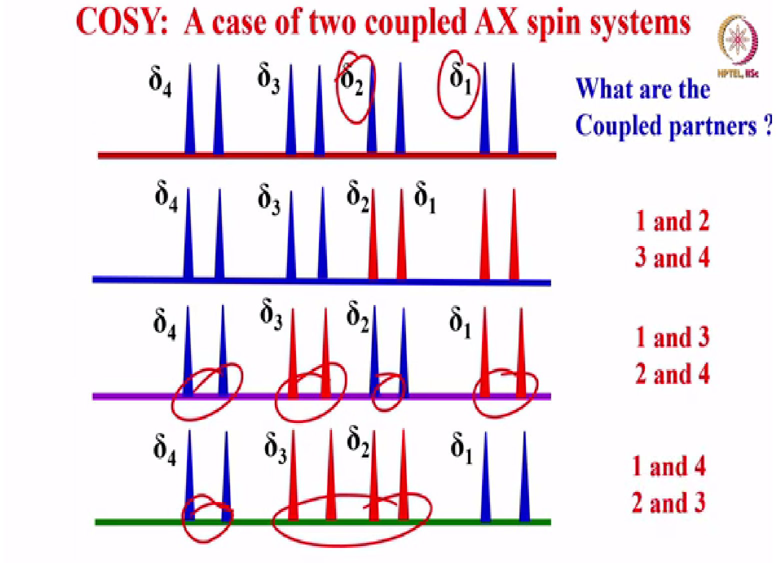


Identification of coupled partners

AX Spin Systems

Now, how to use the COSY? Simple example; as I said it identifies a couple partners. Let us take a simple example to find its utility. I will consider 2 AX spin systems, 2 spind which are coupled between themselves. I will consider for example, forget about this fluorine, I would say instead of fluorine, I put some other thing, like uncoupled spin. We have 2 protons here, 2 protons here, I will consider only proton, I ignore fluorine is not coupled or decoupled let us say, these 2 protons form an AX spin system; these 2 protons form an AX spin system.

(Refer Slide Time: 11:13)



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

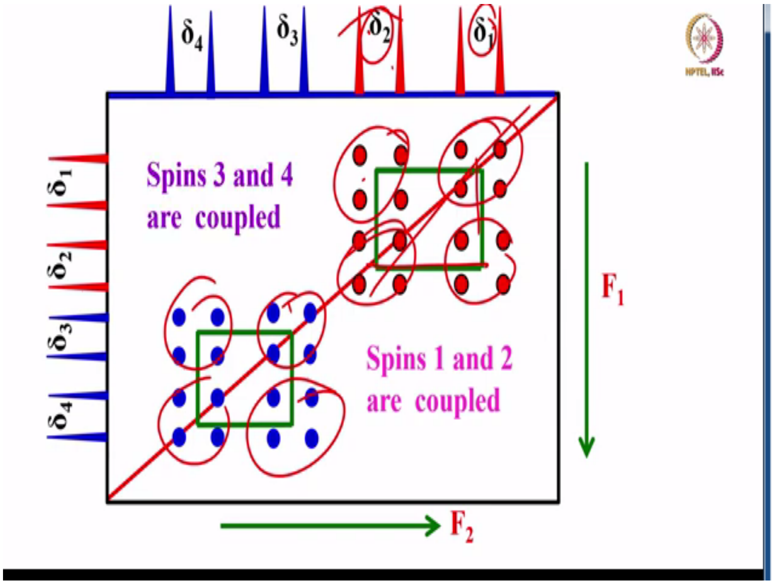
Now, let us look at the COSY spectrum for the case of 2 coupled AX spin systems. Now, each AX coupled spin system gives us to 4 peaks of identical intensities and doublet separation correspond to JAX equal to J coupling A and X; and you get doublet at the chemical shift of A and the chemical shift of X both are observed. This is what we discussed when we discussed about the Pople spin system nomenclature. For two coupled spin system you get 4 peaks.

Now, my question is which are the 2 coupled partners? how do you identify them. In the good old days of NMR what we used to is to irradiate one of them by a second radio frequency signal, with a RF of sufficient enough that it is larger than the coupling strength and see where the spectrum is getting changed. Then we will say this is coupled with this. This is a simple example, I have taken 2 coupled spin systems, 2 mixtures.

If I have a very big molecule where there are N chemically inequivalent protons, there are several of them are coupled among themselves. Then it is a complex spectrum, how many such experiments you want to do; and you have to be extremely careful and do a selective decoupling experiment to ensure that the neighbouring spins are not disturbed. So, it is a very cumbersome experiment. How in such situations, the COSY is going to help you; I will tell you now.

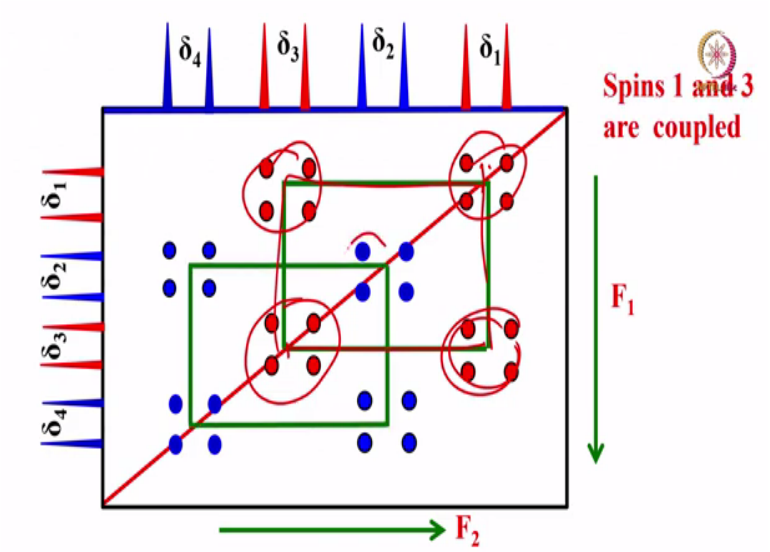
So, let us now take the COSY spectrum of this thing, to identify what are the coupled partners? One possibility, the spin 1 can be coupled to spin 2, that is possible, that is why equal intensity doublets are there. And 1 and 2 are coupled; and 3 and 4 are coupled. There is another possibility 1 and 3 are coupled, and 2 and 4 are coupled. This is another spin system, The third possibility 1 and 4 are coupled and 2 and 3 are coupled. Now, how do you know which are the 2 spins, which are coupled between themselves to identify 2 spin systems here, For that, what we are going to do is;

(Refer Slide Time: 13:26)



We simply run a COSY; as I said COSY is going to give the cross peaks between the coupled partners. Now, if 1 and 2 are coupled, we see that these are the diagonal peaks. And if I get the cross peak symmetric with respect to these diagonal peaks, that means I know spin 1 is coupled to spin 2 these are the couple partners then obviously, because of these two are diagonals, again there is a cross peak between these two, then I say spin 3 is coupled to spin 4. So, I could easily identify the coupled partners just by looking at the cross peaks. So, this is the advantage of identifying the immediate coupled neighbours in any given spin system or any given molecule. Now, this is what I can trace it. Go horizontally and vertically up complete the square, I can complete the square because there is symmetry. Similarly, I can complete the square here and I can say spins 3 and 4 are coupled spins; 1 and 2 are coupled.

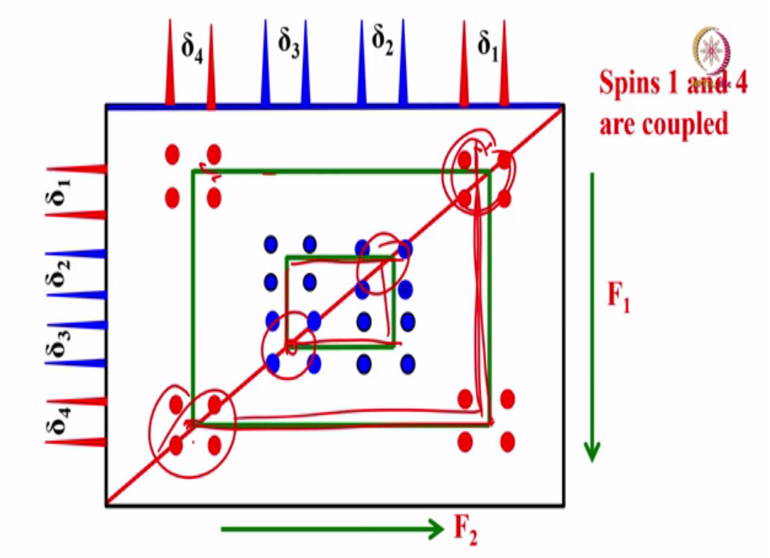
(Refer Slide Time: 14:31)



Spins 1 ~~and~~ 3 are coupled

Now on the other hand, let us say instead of 1 and 2, 1 and 3 are coupled, that is a possibility. How do you get the spectrum? Now, this is the diagonal peak here, and this is diagonal peak here I put different colours to make you understand. Now, if we go along this, and if I go horizontally along this and see some cross peaks, go vertically up hit the diagonal, complete the square, then I know we have symmetric cross peaks; that means this spin and this spin are coupled. So, 1 and 3 are coupled now, that means, obviously, 2 and 4 are also coupled because this is 2 this is 4 for each of them we get a cross peaks like this, which are symmetric. So, now I can say 2 and 4 are coupled.

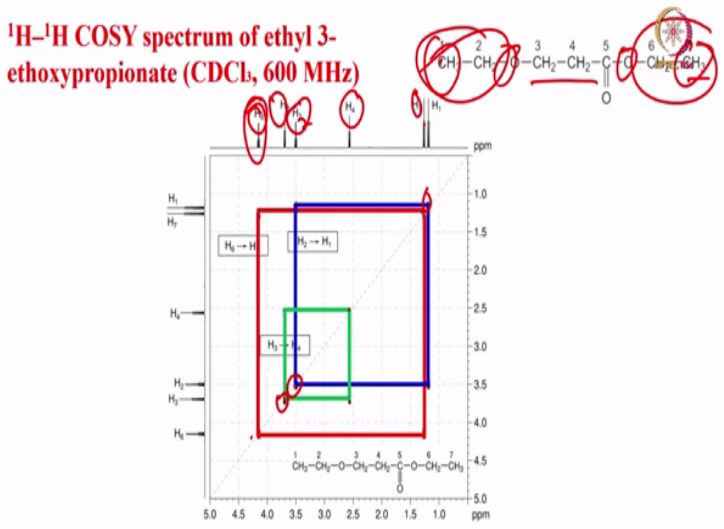
(Refer Slide Time: 15:20)


Spins 1 and 4 are coupled

Now, you think of the third possibility, if 1 and 4 are coupled, you get cross peaks like this, these are the cross peaks, so now I say 1 and 4 are coupled. On the other hand, if 2 and 3 are coupled, these 4 are diagonals. Now you go horizontally complete square like this, then I say 2 and 3 are coupled. So, you see depending upon the splitting pattern, depending upon where you get the cross peaks, just going across, from diagonal to the cross peaks; go vertically up and see which diagonals are going to be hit, I know this and this are coupled.

So, identification of the coupled partners is pretty simple and straightforward in this COSY experiment. You simply walk across throug the diagonal, go from here, go here, and there is a coupled one, another one, go like this, you can keep on going in a stepwise manner like this. So, this is what it is, 1 and 4 are coupled, 2 and 3 are coupled.

(Refer Slide Time: 16:16)

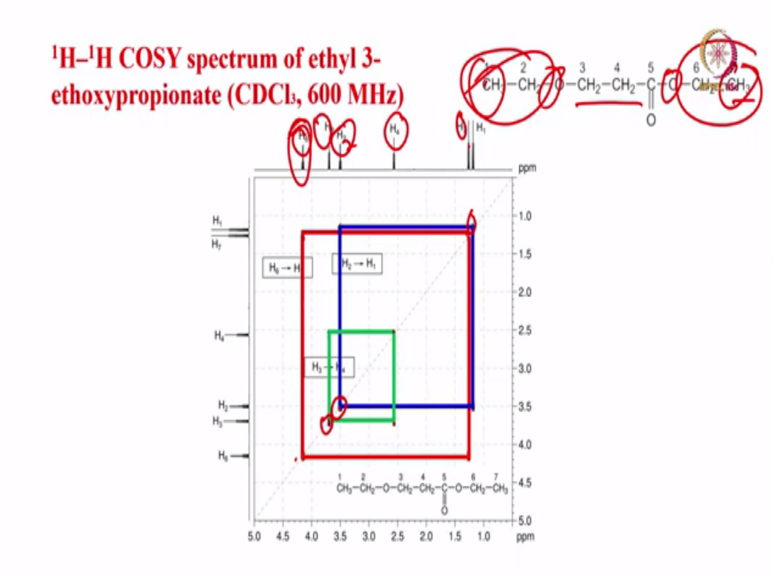

^{1}H–^{1}H COSY spectrum of ethyl 3-ethoxypropionate (CDCl$_3$, 600 MHz)

Now we will take the simple example of a molecule, the spectrum of this is a realistic spectrum, of a molecule ethyl 3 ethoxypropionate, taken in 600 mega Hertz in CDCl3, taken from the Leslie Field book. Now, let us say I want to identify which are the coupled partners, we have to start from somewhere, I should know which is the peak. We all know the high field region we get CH3 protons, there is one possibility one CH3 is here and one CH3 is here, there are 2CH3. Now identify which are the coupled partners for this CH3 this CH3 etcetera.

Now, what I will do is, I will start with one of them, left most peak which is isolated, I go from this diagonal go up, go horizontally, go vertically up and complete the square, then I know this CH3 is coupled to the 7, 7 is CH3. So, that means this is 6; these 2 are coupled partners and peaks for these 2 are identified, here and here. On the other hand, now I start with 2 some other diagonal peak here and go horizontally, go vertically up; it is going to hit this peak; this is CH3; now I complete the square.

Now, it tells me 1 and 2 are the coupled partners. Now, what is leftover? 2 and 4, because it is separated by O in between the possibility of this coupling, the long range couplings is very small or zero. As a consequence this forms another spin system. Now, we have only one diagonal here, we can go with this, start with this, go horizontally, go vertically up complete this square. Now, I know these 2 and 4 are coupled, these 3 and 4 are coupled. It is very easy to identify even for a bigger molecule like this; go horizontally then go vertically, if we hit 2 diagonal peaks, then you find out the symmetric cross peaks, then you can say these peaks which are giving diagonal; the spins which are giving diagonal peaks are coupled, J coupled.

(Refer Slide Time: 18:22)



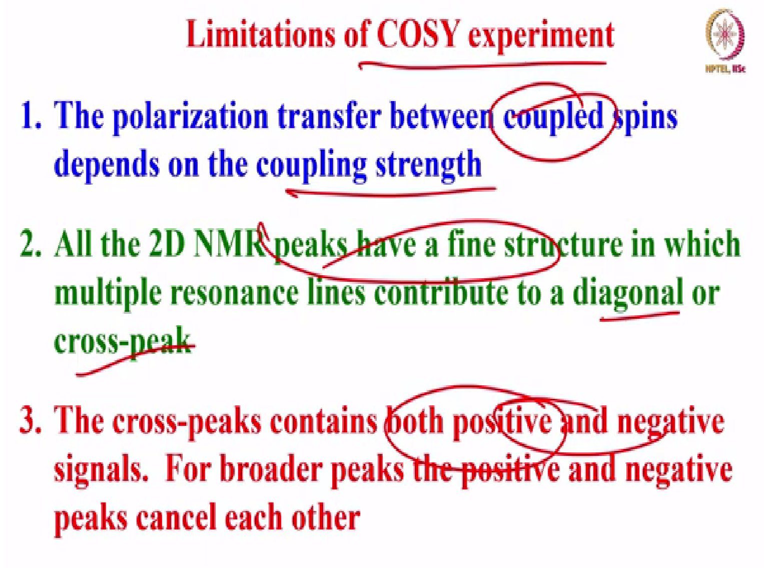
^{1}H–^{1}H COSY spectrum of ethyl 3-ethoxypropionate (CDCl$_3$, 600 MHz)

Now we take another simple example of a big molecule like this, little bigger now. We have one identified spin system here. Rest everything is in a chain. Each proton like 1 is coupled to 2, 2 is coupled to 3, 3 is coupled to 4, like that. Now, how do you identify which is which. Of course, obviously, there is one peak which is here, which is CH3 which comes at the high field. Now we start with one of the diagonal, which is also isolated peak here. Go horizontally hit a cross peak, go vertically up hit a diagonal peak, and now complete the square.

Now, I know this and this are coupled, what are these two? 7 and 8 so this is the 8 which is coming from high field then I know this must be CH2, which is coming down because it is attached to COO group. So now, you start with the other one, for example, this is another isolated peek which is downfield, I guess this is attached to bromine, because it is electro negative atom so it is coming to the downfield.

I start with diagonal, this diagonal, come horizontally hit the cross peak, go vertically up hit another cross peak and complete. So that means this 1 is coupled to 2. But if you go further, you know for 2 there is another cross peak, you can complete a square like this. That means this has to be 3, 2 is coupled to 3. Next one is immediate neighbour, then 3 should be coupled to something else, 3 must be coupled to 4. Here CH3 must be coupled to 4 here. And then you can complete square, you can say 4 is coupled to 5, I mean write here 3 is coupled to 4 like this. So, like this systematically you can walk across the cross peaks and diagonal peaks and identify which are the immediate coupled neighbours for a particular spin, for a particular group, chemically inequivalent group.

(Refer Slide Time: 20:17)



Limitations of COSY experiment

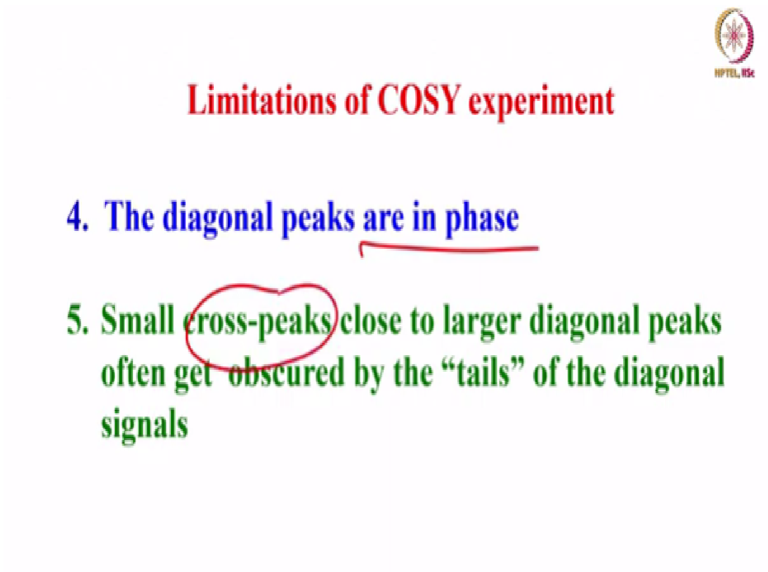
1. The polarization transfer between coupled spins depends on the coupling strength
2. All the 2D NMR peaks have a fine structure in which multiple resonance lines contribute to a diagonal or cross-peak
3. The cross-peaks contains both positive and negative signals. For broader peaks the positive and negative peaks cancel each other

So, this is how COSY is a very, very useful experiment. We can discuss lots and lots about this thing, varieties of COSY experiments have been designed where there are several variants, we will not be able to touch upon everything, may be 1 or 2 we will discuss. But there are certain limitations for the COSY experiment, it is a very old experiment, first utilized by everybody, most of the people were using COSY experiment for identifying the coupled partners.

One is J coupling is an important thing. Polarization transfer takes place between coupled spins, so coupling is important, and also depends upon the coupling strength. It can go up to 2 bonds, 3 bonds also extend upto 3 bonds, if the coupling strength is larger. And all the 2D peaks have fine structure; when there are multiple resonance peaks, then it can give rise to some overlap, many diagonal peaks and cross peaks could overlap also.

And as I said cross peaks are antiphase in character, we have positive and negative intensities. Whereas, diagonal peaks are both in-phase, both are positive peaks, and when there is a broader signal, positive or negative signals get cancels out and you may not get the multiplicity pattern at all in the COSY, especially, when you are looking at the cross peaks, because of the antiphase character. If the peaks are very broad, positive and negative peaks get nullified.

(Refer Slide Time: 21:43)



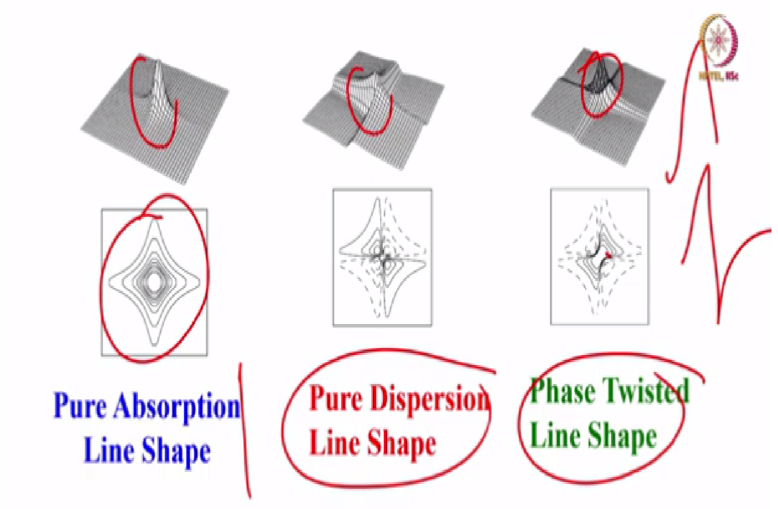
Limitations of COSY experiment

4. The diagonal peaks are in phase

5. Small cross-peaks close to larger diagonal peaks often get obscured by the “tails” of the diagonal signals

Of course, diagonal are in-phase, huge diagonal peaks will be there. Many times there are strong peaks near the diagonal peak, if there are cross peaks, it is very difficult to identify because you get tails; we call as tails in F1 dimension, F2 dimension, at the strong peak which is there on the diagonal. That will obscure the cross peaks coming close to diagonal. So, these are all some of the limitations of the COSY. The strong diagonal peaks will mask the cross peaks close to hit, because of the tails that are coming due this strong peak.

Here are certain line shapes in NMR spectrum, not only for COSY, but for all the 2D spectra.
(Refer Slide Time: 22:29)



Pure Absorption Line Shape

Pure Dispersion Line Shape

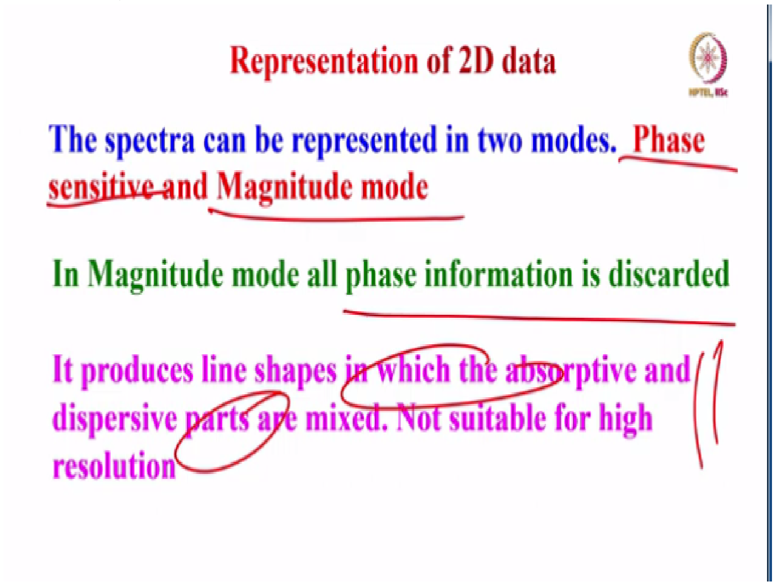
Phase Twisted Line Shape

There are certain line shapes which are well known; these are the terminologies that we use utilize. The spectrum of this pattern which is like a star. This is called pure absorption line shape, absorption both in t1 dimension and t2 dimension; it is called double absorption.

Double absorptive peak. And it is a pure dispersive peak; you see antiphase character, it is not like dispersive, absorptive is like, this dispersive is like this.

We have been discussing since a long time, and then phase twisted line shape like this, these are the things which you see, like this in the contours. These are the stack plots, these are the contours. You have different shapes like this. These are the common line shapes you come across in many or all the 2D spectra.

(Refer Slide Time: 23:14)



Representation of 2D data

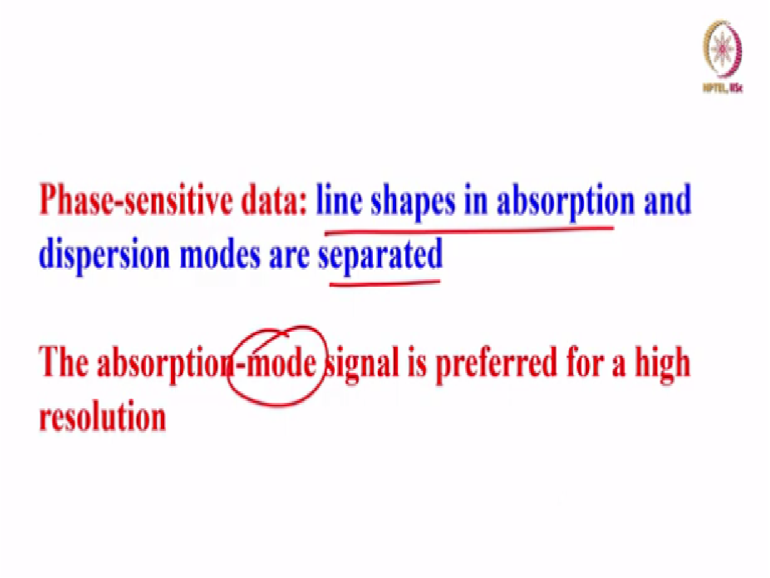
The spectra can be represented in two modes. Phase sensitive and Magnitude mode

In Magnitude mode all phase information is discarded

It produces line shapes in which the absorptive and dispersive parts are mixed. Not suitable for high resolution

So, how do you represent the 2D data? You can represent a 2D spectrum, when you want to record the spectrum, it is represented in 2 modes; one is called phase sensitive mode and the other is called the magnitude mode. In magnitude mode phase information is lost, you know how to get the magnitude. Some of the real square plus imaginary squares and then take the square root of that. In the magnitude mode there is no phase information at all. It produces lines shape in which the absorptive and dispersive parts are mixed up. So, this type of absorptive mode spectrum is not suitable for high resolution, it is not suitable to get high resolution. For representing the high resolution spectrum, I tell you as we go ahead we must get double absorptive peaks then resolution is much better, always better. So, this type magnitude mode representation of the spectrum is not suitable for high resolution.

(Refer Slide Time: 24:11)

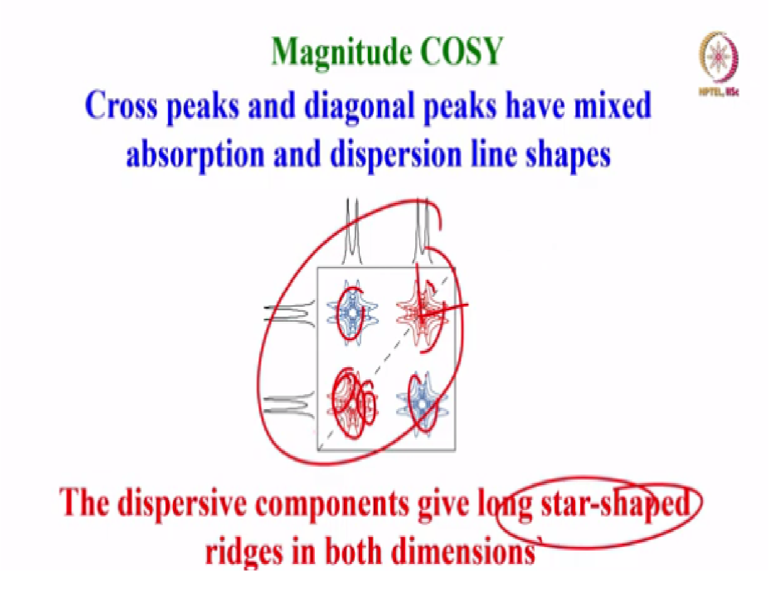


Phase-sensitive data: line shapes in absorption and dispersion modes are separated

The absorption-mode signal is preferred for a high resolution

And also in phase sensitive data, the line shapes in absorption and dispersion mode are separated out; whereas in the magnitude mode it is not possible. So, that is the reason why absorption mode is preferred. And in the case of COSY, because of the magnitude mode representation should be done always, it is not very much preferred. But of course, many ways people have overcome this method, number of variants are there.

(Refer Slide Time: 24:38)



Magnitude COSY

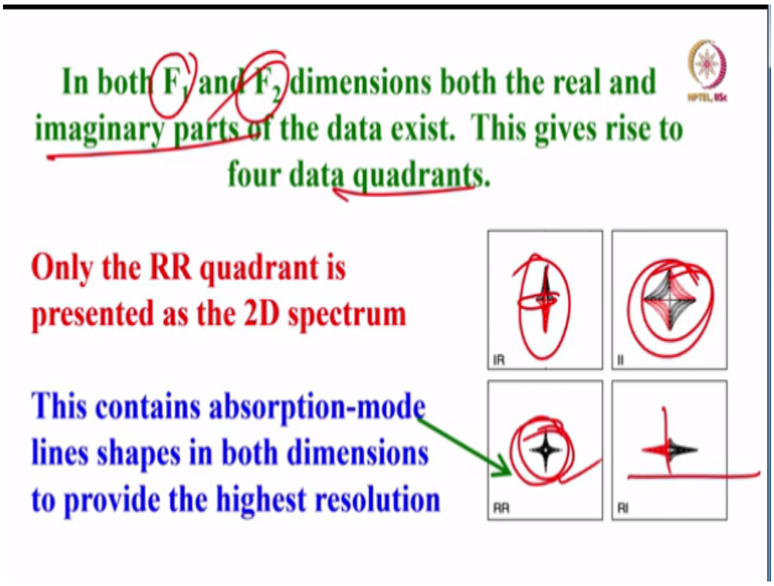
Cross peaks and diagonal peaks have mixed absorption and dispersion line shapes

The dispersive components give long star-shaped ridges in both dimensions

So, this how the magnitude COSY looks like. If they take the spectrum of any, let us say, again two coupled spin system, AX spin, we have diagonal peaks and cross peak here. And you can see the pattern like this. The cross peaks and diagonal peaks are mixed phase. The absorption and dispersion line shapes, both are there. So, dispersive component gives rise to long star-shaped ridges like this in both the dimensions. You see here in this dimension in this dimension, you hit a star shaped ridges.

So, this is what is the problem of magnitude COSY, magnitude way representation. When you have a peak very close here in high resolution, you will not be able to identify. These are the problems with the magnitude representation of the COSY.

(Refer Slide Time: 25:27)



In both F_1 and F_2 dimensions both the real and imaginary parts of the data exist. This gives rise to four data quadrants.

Only the RR quadrant is presented as the 2D spectrum

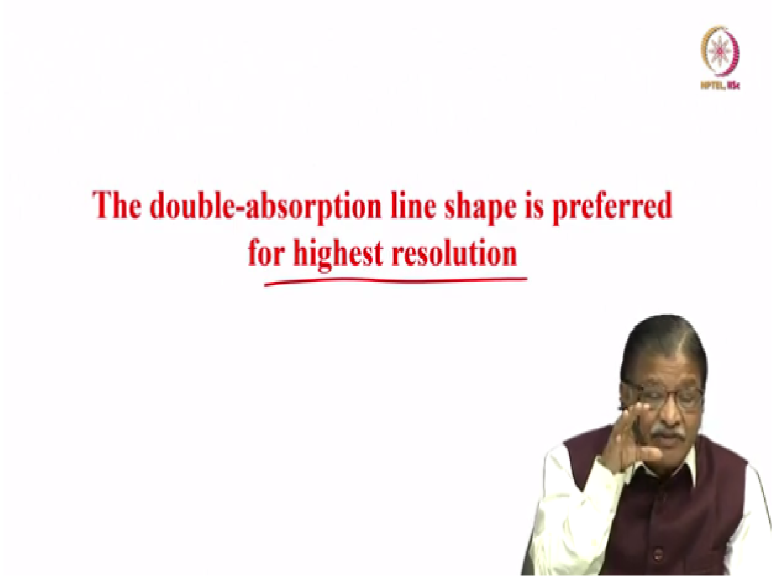
This contains absorption-mode lines shapes in both dimensions to provide the highest resolution

Another important thing, what I wanted to tell you is, when you take any 2D spectrum, when you do the double Fourier transformation, in Fourier transformation when we discussed about the Fourier series and Fourier transformation, take a simple cosine wave, we said you will have a real part and imaginary part. We saw that, similarly if we take the sine wave we saw real part and imaginary part. So, in both F1 and F2 dimensions you have real part and imaginary part, in each of them.

So, that means both the dimensions, if you consider we have both real parts and imaginary parts. There are 4 possibilities; real, real; real imaginary; imaginary real and imaginary imaginary. So, there will be 4 different quadrants you can think of; the ways we can represent a signal. For example, this is imaginary in one dimension and real in the other dimension. In this case both are imaginary; see, in this case it is the real real, imaginary real and in this both are real, real; and here real in this direction and imaginary in this direction.

So, only the important thing is real, real quadrant. This is the way you have to represent the spectrum in the 2D spectrum. This is how you have to represent the cross peak. This contains double absorption mode; and this provides highest resolution. See if you plot like this you will not get better resolution; but if you look at this, you can see the difference between this and this, it gives better resolution. So, the best representation of the 2D spectrum is always in a double absorption mode; you get better resolution.

(Refer Slide Time: 27:13)

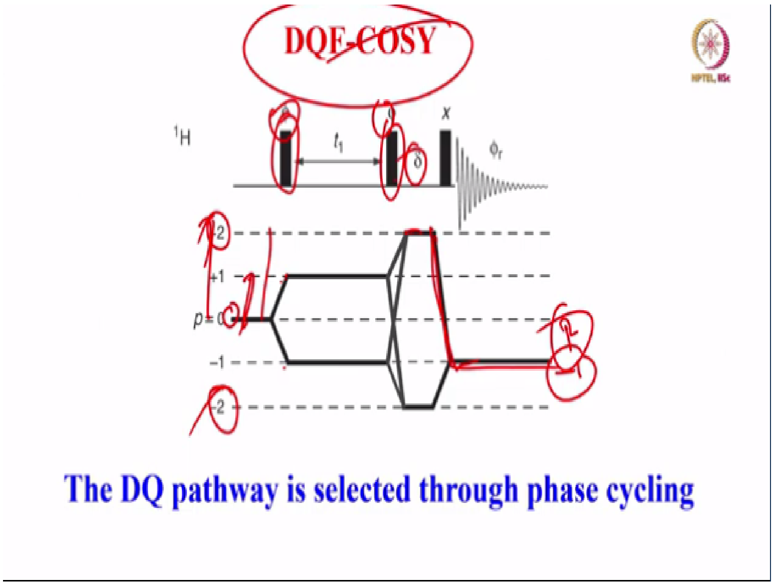


The double-absorption line shape is preferred for highest resolution

So, double absorption mode is always preferred for highest resolution. Please remember, if you have recorded 2D spectrum, double absorption mode is preferred because of highest resolution. The magnitude mode is not at all preferred, because it gives ridges and mixture of phases everything; it is not suitable for high resolution.

So, with this having said, we can go to other types of 2D experiments, before that we look at some of the variants of the COSY sequence very quickly. This is a simple 2 pulse sequence, but this has been modified over the years by several stalwarts in the NMR spectroscopy to derive information and to overcome the problems and to enhance the resolution, sensitivity etcetera. So, as a consequence several variants have been developed, we will concentrate only on 1 or 2.

(Refer Slide Time: 28:09)

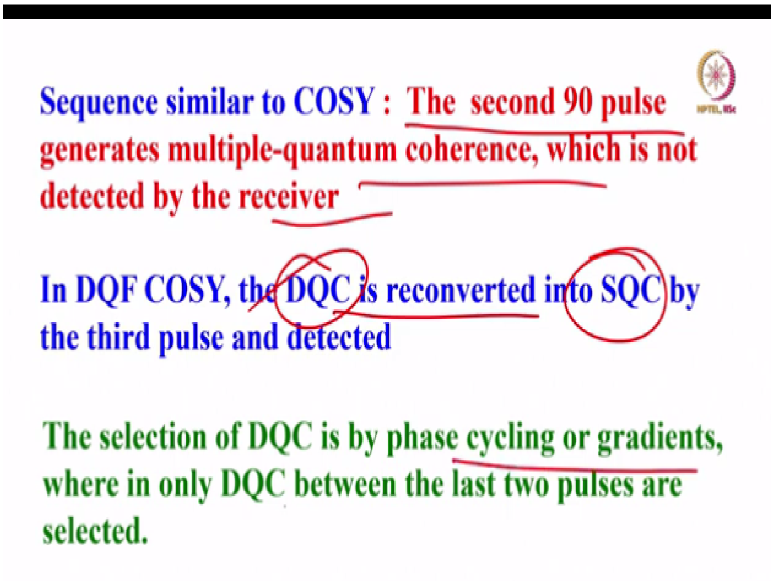


The DQ pathway is selected through phase cycling

One is important, it is called DQF COSY what is DQF? Double Quantum Filter COSY. For example, what is double quantum or single quantum; may be after 2D I will take multiple quantum spectroscopy. There we will discuss, the change in the magnetic quantum number between two energy states, let us say, plus or minus 1 is the single quantum, and plus or minus 2 is double quantum, if the spins undergo flipping from let us say alphaalpha goes betabeta, it is double quantum of energy. It is double quantum transition.

So, what we can do is? We can select a pathway in the COSY, two pulse sequence and in between there is a delay. As I told you remember when we were talking about the coherent pathways selection, first pulse always creates +1, -1 coherence and second pulse always creates multiple quantum coherences. So, it is going to create higher quantum coherence for example, p = +2, -2 like this. Whatever the pathway you choose finally, we should come to p = -1 this is what generally in all the spectrometers it is detected. We have to choose finally p = -1 pathway. But how we can do is? This can be done by phase cycling or also by gradients; both we discussed. So, what the double quantum filtered means, after this +1, -1 is created, the second pulse will create higher quantum. Let us say there is +2 here, from +2 I will come to -1, from +2 to -1 there is a way we can select the coherence transfer pathway by both the gradients; we discussed that. Now when you are going for double quantum what will happen? It is a filter from here you come here directly. So, advantage is large single quantum peaks, which are singlets, like solvent peaks and reference peak, which are all singlets, you will complete suppress. So, double quantum filtering has a lot of advantages.

(Refer Slide Time: 30:23)



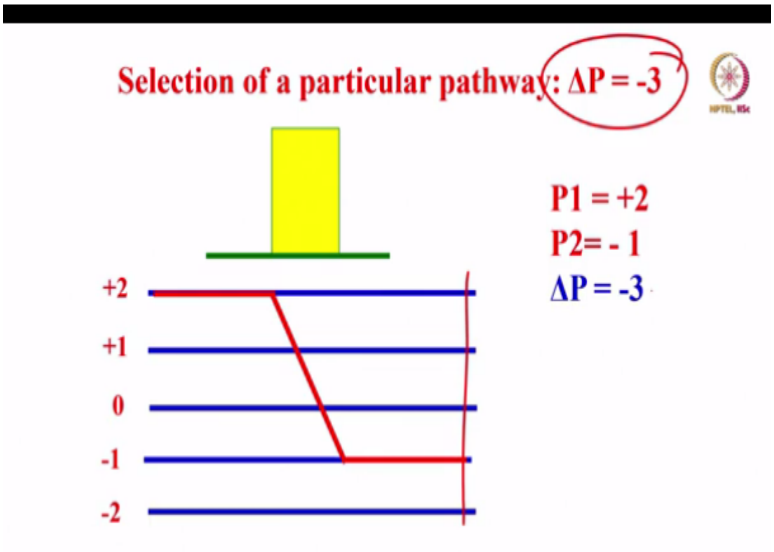
Sequence similar to COSY : The second 90 pulse generates multiple-quantum coherence, which is not detected by the receiver

In DQF COSY, the DQC is reconverted into SQC by the third pulse and detected

The selection of DQC is by phase cycling or gradients, where in only DQC between the last two pulses are selected.

And now, this sequence is similar to COSY. The second 90 pulse generate multiple quantum coherences, as I told you, which is not detected by the receiver. Even in the COSY previously we discussed 2 pulse sequence 90 t1 90 sequence. Second pulse always creates multiple quantum coherence, but our receivers will not see that, it will not detect. So, in DQF COSY what we do is? As I showed you we go to +2, let us say double quantum, select the coherence transfer pathway from DQ bring it -1. So, what we do is? We convert double quantum coherence to single quantum coherence by third pulse; and then we detect that. Select the coherence and after the third pulse we are going to detect that. The selection of DQC is by phase cycling or gradient, you select particular pathway between last 2 pulses.

(Refer Slide Time: 31:19)

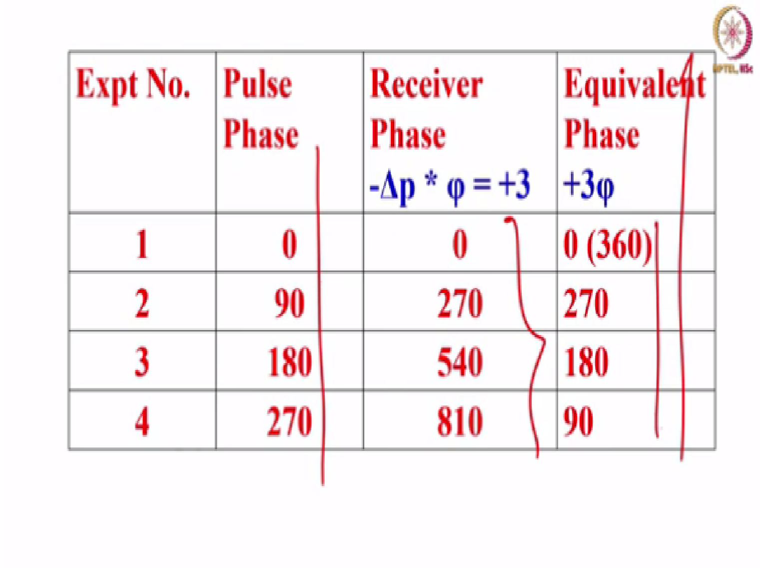


Selection of a particular pathway: $\Delta P = -3$

P1 = +2
P2= - 1
$\Delta P = -3$

And you know the phase cycling is like this. This is exactly we took as one of the example for delta P = -3 from +2 to -1. Dont not worry about the pulses here and here, I am just showing you, this we discussed from +2 to -1 here; delta P = -3, P1 = 2 and P2 = -1; and we discussed both by using gradients and also by using phase cycling, how we can select this particular coherence transfer pathway, we discussed this.

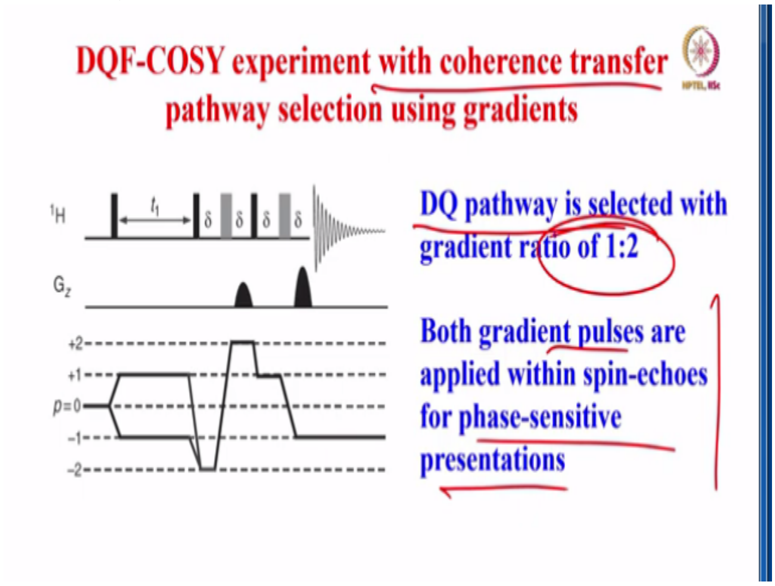
(Refer Slide Time: 31:58)



Expt No.	Pulse Phase	Receiver Phase $-\Delta p * \varphi = +3$	Equivalent Phase $+3\varphi$
1	0	0	0 (360)
2	90	270	270
3	180	540	180
4	270	810	90

And these are the phases where we worked out for the receiver. When the pulse phase is like this, the transmitter phase, this is what we arrived at as the receiver phase. And this is the equivalent phase we discussed, everything, I am just giving you this thing there are ways you can choose different pathways. It need not be one, there may be several ways of choosing the particular pathway. Accordingly you have to choose the phase cycling. So, the receiver phase goes in the opposite phase when the pathway that is chosen is like this for this phase cycling.

(Refer Slide Time: 32:32)



DQF-COSY experiment with coherence transfer pathway selection using gradients

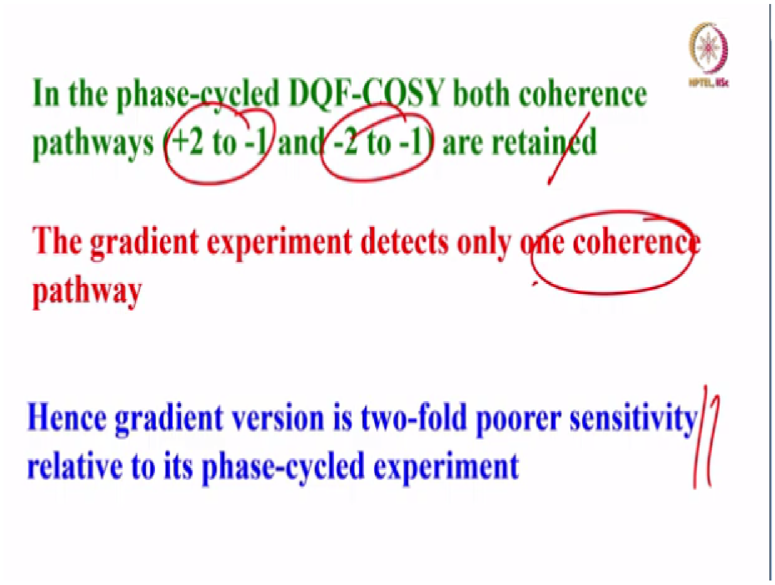
DQ pathway is selected with gradient ratio of 1:2

Both gradient pulses are applied within spin-echoes for phase-sensitive presentations

Now, the DQF COSY experiment with coherence transfer phase selection, we can also do by gradients. How do you do with gradients? We discussed, we worked out this one also again, here the gradient ratio has to be 1 is to 2; in which case it will select the double quantum pathway. You please go back to my pulse filed gradients and we discussed used the gradient ratio 1 is to 2 to select this double quantum pathway. So, both way we can do; using gradient pulses and we can use phase cycling.

But in addition to that we can spin echoes sequence also; this is to get phase sensitive representation. That is possible more details we will not discuss, but this is the idea, we already discussed how to select the phases, how to select the coherence transfer pathway using gradients.

(Refer Slide Time: 33:28)



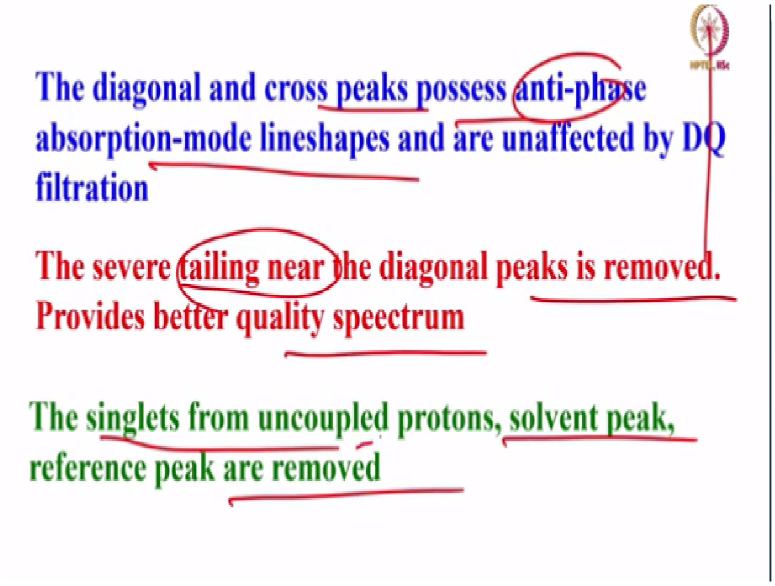
In the phase-cycled DQF-COSY both coherence pathways (+2 to -1) and (-2 to -1) are retained

The gradient experiment detects only one coherence pathway

Hence gradient version is two-fold poorer sensitivity relative to its phase-cycled experiment

So, in any way in the phase cycled DQF-COSY both coherence pathways +2 to -1 and -2 to -1 are retained; which are called P peak and N peak, both are retained. The gradient experiment detects only one coherence, +2 to -1, only one of them is retained. That means gradient version is poorer by 2 fold, because you have double the signal here, here you have only half the signal. So, a gradient version is poorer in intensity by 50%; that is the important thing to remember. Phase cycle version is having better sensitivity, because you can get both P and N type both can be chosen; both are retained there.

(Refer Slide Time: 34:16)



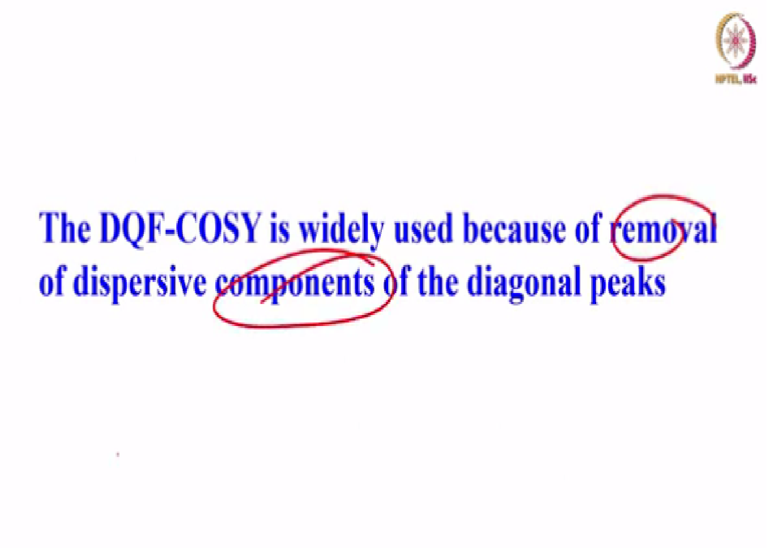
The diagonal and cross peaks possess anti-phase absorption-mode lineshapes and are unaffected by DQ filtration

The severe tailing near the diagonal peaks is removed. Provides better quality speectrum

The singlets from uncoupled protons, solvent peak, reference peak are removed

That is the advantage of using phase cycling in the DQF COSY here. Here both diagonal and cross peaks possess the antiphase absorption mode lineshapes and they are not affected by double quantum filtration at all. What did we do? We used the double quantum filtering by choosing the path from +2 to -1. And then nevertheless, we have antiphase character for both diagonal and cross peaks; there are unaffected by DQ. As a consequence, what happens? If there is a tailing near the diagonal peak, as we observed in the COSY, it is not there. The tailing are removed. So, it is going to give you a better quality spectrum. The biggest advantage of DQF-COSY is, it gives you a better quality spectrum compared to COSY spectrum. Another advantage is if you have singlets coming because of uncoupled protons, or solvent peak for example CHCl3 only one peak will be there; or the reference TMS only one peak will be there, all of them are removed. All singlets coming from any of these uncoupled protons, solvent peak or reference peak everything can be removed by double quantum filter.

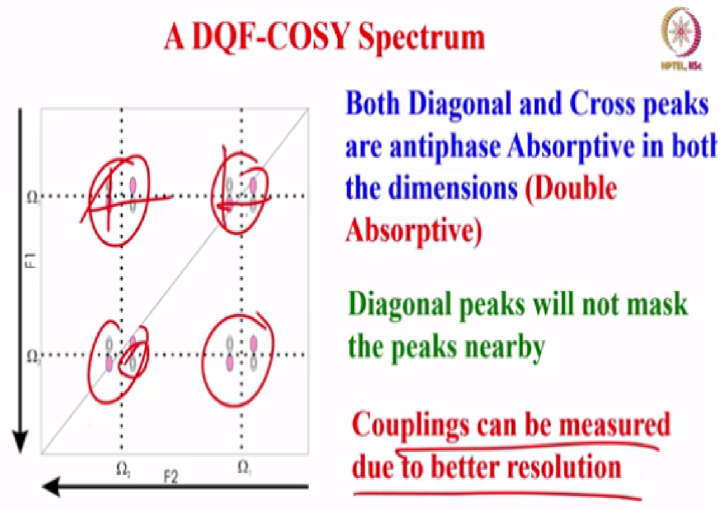
(Refer Slide Time: 35:40)



The DQF-COSY is widely used because of removal of dispersive components of the diagonal peaks

So, that is the reason why DQF COSY is widely used because of removal of dispersive components of the diagonal peak; very important please remember.

(Refer Slide Time: 35:51)



A DQF-COSY Spectrum

Both Diagonal and Cross peaks are antiphase Absorptive in both the dimensions (Double Absorptive)

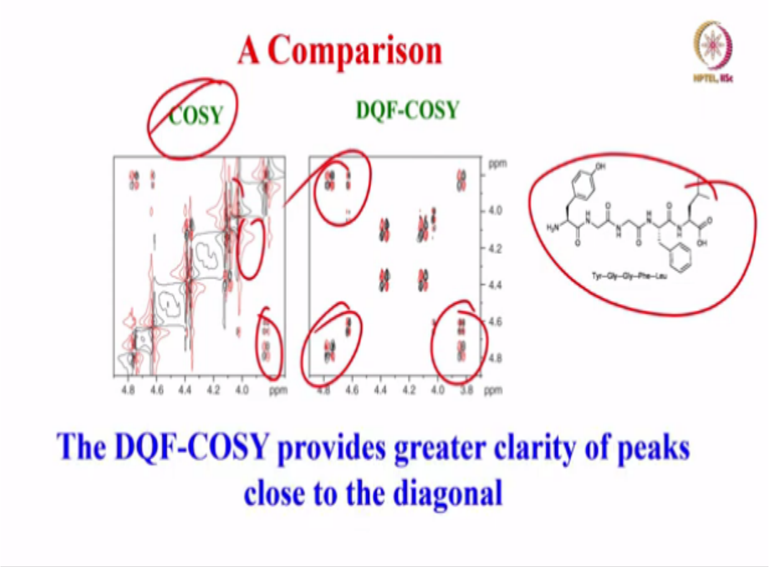
Diagonal peaks will not mask the peaks nearby

Couplings can be measured due to better resolution

I will show you a simple DQF COSY spectrum here. Look at it, these are cross peaks, these are diagonal peaks, they are different signs here, which is given in the orange colour. Otherwise closed contours and open contours are shown here. They have opposite phase one is positive and the other is negative, the signs are opposite. So, both diagonal and cross peaks are antiphase in character, in both the dimensions. In this dimension also antiphase, in this dimension also they are antiphase. Not only the cross peaks, even the diagonals also, both are antiphase; so they are double absorptive.

So, diagonal peaks will not mask the peaks nearby. So, the coupling can be measured due to better resolution. See the peaks which are coming very close to this thing or even to diagonal here or here, we can very precisely measure, because there is no tailing and resolution is much better.

(Refer Slide Time: 36:45)



A Comparison

COSY

DQF-COSY

The DQF-COSY provides greater clarity of peaks close to the diagonal

So, look at the spectrum of a simple molecule, it is a pentapeptide. Look at this COSY spectrum conversional; 90- t1-90-t2 sequence. Look at the type of spectrum here these are all the diagonal peaks, these are all cross peaks; see near the diagonals it is so clumsy, there are peaks you will not be able to identify. On the other hand, look at this DQF COSY of the same molecule. Look at the clarity of the peaks, both in the diagonal and then the cross peaks.

That is why never do the COSY experiment, if you want to get a better resolution. You always do DQF COSY experiment, it is only slight modification of the COSY; but gives you better resolution and better clarity of the peaks that are especially close to the diagonal. So, with DQF COSY I am going to stop because time is coming up. What we will do is, we will discuss another; simple 1 or 2 variants in the COSY in the next class.

We discussed today about the COSY, the types of the peaks we are going to get, both phases of the diagonal peaks and cross peaks. In conventional 90 -t1-90-t2 COSY the diagonal peaks are in-phase, cross peaks or antiphase in character. And it gives us the large tails near the diagonal peaks and because of that, the peaks near the diagonal peak gets masked, and always it has to be represented in the magnitude mode. And it is not good for high resolution.

As a consequence, the modified version we discussed is a double quantum filter COSY, where use a double quantum filter, because of the third pulse. What you are going to do, the second pulse creates multiple quantum and you filter it by selecting a particular pathway from +2 to -1 or whatever the pathway using phase cycling or gradients. And the third pulse converts the double quantum into the single quantum detection and detected.

And we observed here both diagonal peaks and cross peaks are antiphase in character, it can be represented in double absorptive mode. It gives better resolution and better clarity, especially for the peaks close to the diagonal. So that is why DQF COSY is always advantageous and preferred over the conventional 90 -t1-90-t2 COSY experiment. With this I am going to stop now, we will come back and continue further in the next class. Thank you.