Advanced NMR Techniques in Solution and Solid-State

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Module-47
2D COSY Experiment
Lecture - 47

Welcome all of you, from the last 2 classes we started discussing about 2D NMR. I introduced what is the 2D? What is dimensionality in NMR and how we get N dimensional spectra. We do N dimensional Fourier transformation and we are going to get N dimensional frequency domain spectrum. This is what we saw. And we also understood how a pulse sequence is important. Each 2D experiment is defined by given pulse sequence. There are N number of pulse sequences and there have been design N number of 2D experiments accordingly have been developed.

It could be both homonuclear and heteronuclear; if you want to correlate the information or to resolve the information and accordingly for homenuclear experiment I gave examples of COSY, ROESY, TOCSY etcetera. The resolved experiment is J resolved experiment. Same way the heteronuclear correlation experiments are there like HETCOR, HSQC, HMBC, HMQC etcetera. And in each of these experiments, if particular information is derived, there is a way in which magnetization transfer or the polarization transfer takes place from one spin to another spin during the mixing time.

Accordingly, different information can be derived. And the pulse sequence is designed based on the spin dynamics, dynamics of the spin, how spin behaves at different stages of the pulse sequence. We know, what information we are getting out of this. And this is how we can even understand looking at the pulse sequence, what is that we are going to get in this pulse sequence?

This we understood using product operators also; we know how to analyze the pulse sequence using product operators. That is how it is; we know all those things. And then I also said sometimes delays are important, pulses are important, where do you apply the pulse, which axis apply pulse, which flip angle of the pulse all those things matters a lot, in each of these experiments. And let us say you do an experiment and get the spectrum.

How do you interpret the spectrum. If I have a peak, how it correlates the information what happens to a particular peak. Let us say, I have a peak at some 20 hertz or 30 Hertz in the F1 dimension and the same peak if it has some frequency in the F2 dimension, how do you understand that. We discussed that, 1 or 2 examples we took. I said a peak which was evolving; let us say at 30 hertz in t1 dimension, If it also has only 30 Hertz in the t2 dimension then we say, it remains unaffected. Whereas on the other hand, if I have a peak, let us say at 30 Hertz in t1 dimension, also has a peak at 70 Hertz in the t2 dimension, then we can say in the t1 dimension this peak at 30 Hertz has evolved and part of it magnetization is given to another spin, and spin evolves at 70 Hertz. As a consequence, part of it remains unaffected. It gives a peak at 30 hertz. The one which has given magnetization or polarization to other spin, evolves at 70 Hertz in the t2 and you get a peak in the t2 at 70 hertz. So, there is a way we can interpret this type of 2D spectrum. Thus we discussed a general way of interpretation of the 2D spectrum. And most important thing we also understood what is the information content you get. As you go to higher and higher dimensions, higher the dimension, it consumes more and more time. You can deal with bigger and bigger samples. Sensitivity also goes by square root of measurement time. And we took a lot of examples, and I showed what happens, how much time we take for a 1D, 2D or 3D or 4D experiment. And also how it depends upon the size of the molecules. Varieties of things we discussed. That was basically the broad idea. All these things are extensively discussed in the previous course. This is for the benefit of those who did not take my previous course I am giving this. Now we will continue with the COSY sequence a little bit. It is commonly used experiment. Today we will start with a COSY experiment and understand what is a COSY, how it correlates the information.

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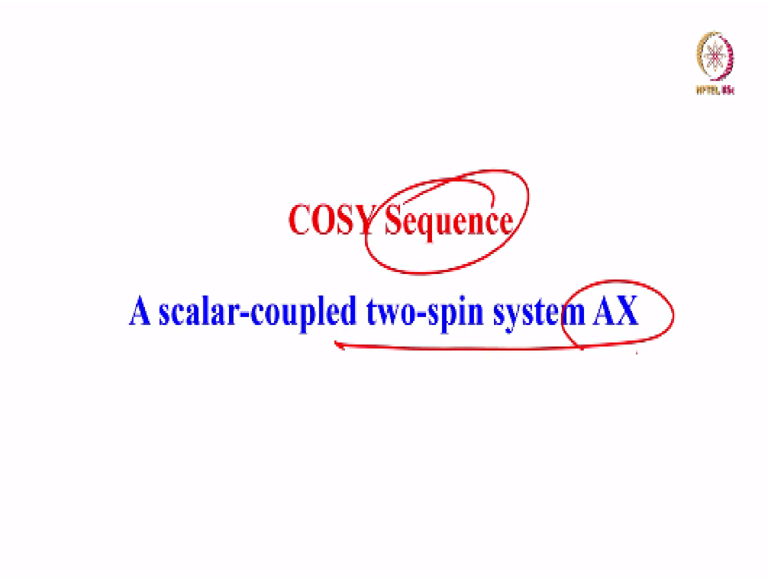

COSY Sequence

A scalar-coupled two-spin system AX

We will start with this. It is a COSY sequence and I take the example of scalar coupled 2 spin case AX. That is the example we take. Before going to that will take the uncoupled spins and see what is a coupled spin later. Right now we worry about what is a COSY sequence. It is like this.

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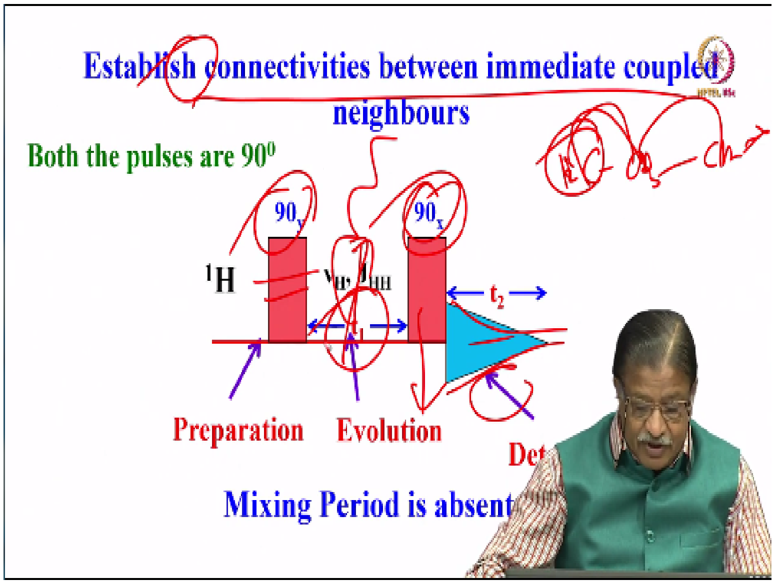

Establish connectivities between immediate coupled neighbours

Both the pulses are 90^0

Mixing Period is absent

What a COSY does? COSY the correlated spectroscopy is the expansion for it. It establishes connectivities between immediate coupled neighbours. Please understand, immediate coupled neighbours. For example, I have a molecule CH2 CH3 some CH2-X like that, then, if I take the COSY spectrum of it, it tells me CH2 sitting next to CH3 and first this CH3, first CH2 next and this CH2 is next like that. It gives the connectivity information between immediate coupled neighbours. Of course, not immediate connectivity, it can go little longer also, for that are n number of variants of this experiment. But immediately you understand that COSY gives rise to connectivity information of immediate partners, which is sitting next to which; that information you are going to get. And basically, it is a 2 pulse sequence; two 90 degree pulses. That is a basic experiment. One 90 degree here, it could be X or Y; here another the 90 degree pulse. There are two 90 degree pulses, which is defined like this. It is the width of the pulse.

And this is a t1 period in which the evolution of the magnetization takes place; this is a t1 period which is incremented systematically. You apply a 90 degree pulse. Of course, there is no mixing period here; it is absent. But you can call this also as a mixing period and then start collecting the signal. It is a detection period, here you are going to collect the free induction decay. And here the t1 is incremented, you collect the pseudo FID, and do the double Fourier transformation of it. You get the frequency domain spectrum which gives in spectrum in 2 dimensions.

That is what you are going to get, the frequency 2 dimensions in COSY. Now, for our understanding, we know the preparation period because this is the period under which we wait for the spins to attain thermal equilibrium. It comes to thermal equilibrium. And then this is the evolution period, where spins transfer their polarization, information content of one spin is transferred to the other. That is what we are going to get the information here. The polarization transfer takes place during the evolution period and then we apply a detection pulse and start collecting the signal.

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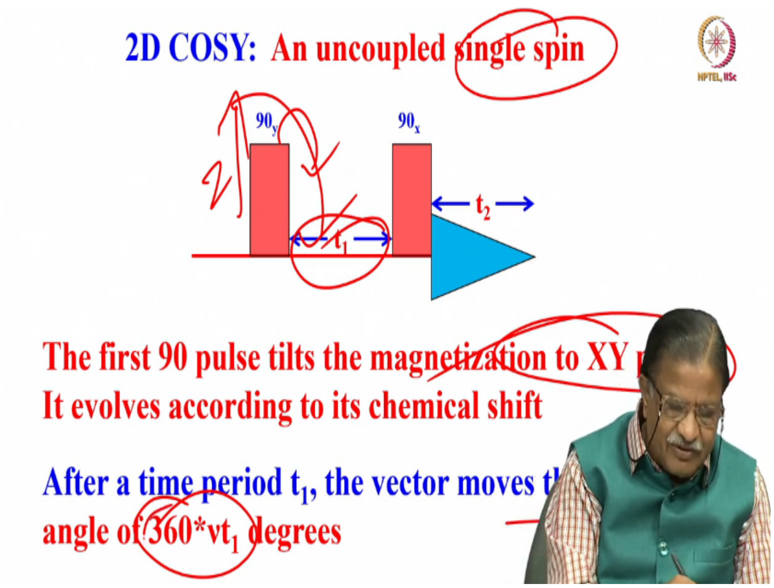

2D COSY: An uncoupled single spin

The first 90 pulse tilts the magnetization to XY p
It evolves according to its chemical shift

After a time period t_1, the vector moves t
angle of 360*νt_1 degrees

Now, before going to the coupled spin, we will understand how does the 2D COSY spectrum look in an uncoupled single spin. I have a single spin, there is no coupling at all, only one chemical shift, how does the COSY spectrum look? Now, the 2D spectrum I take. I apply first 90 degree pulse tilts the magnetization to XY plane that all of us know, we have been we have been discussing for quite some time. This is what the first 90 degree pulse does.

Bring the equilibrium magnetization along Z axis to XY plane here. Then what will happen? here it will start evolving during the free precision, according their offsets or according to the chemical shifts, they start evolving. Now we are dealing with a single spin it will start evolving according to the chemical shift. After a time, let us say t1, what will happen to the magnetization vectors which are in the XY plane, they start moving and how much they move, the angle in which they go is given with 360 star nu into t1.

Remember this we discussed this during the evolution of magnetization and the evolution of J coupling a lot. We wrote this equation and calculated how much is the rotation, how much different spin vectors are moving when they have a different chemical shift with respect to centre nu naught everything even calculated for few examples. So, after a period t1, the vector moves through an angle of 360 star nu of t1 degrees.

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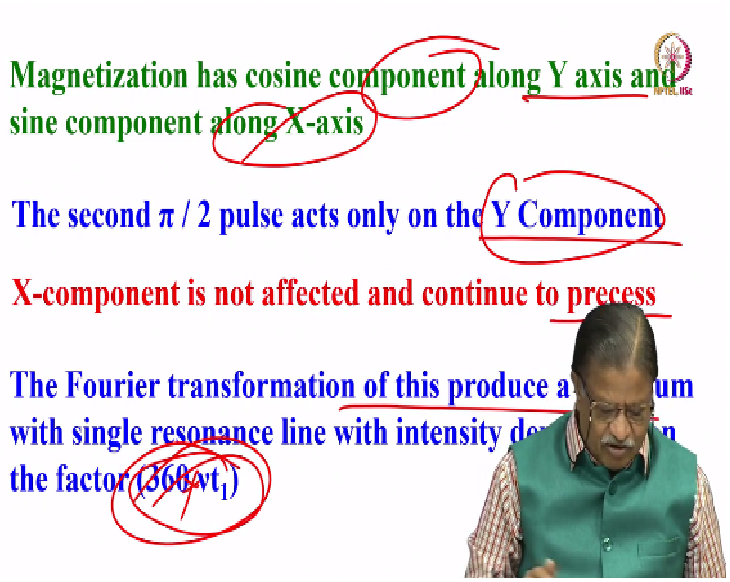

Magnetization has cosine component along Y axis and sine component along X-axis

The second π / 2 pulse acts only on the Y Component

X-component is not affected and continue to precess

The Fourier transformation of this produce a um with single resonance line with intensity de n the factor $(360\nu t_1)$

Now magnetization has both cosine component and the sine component. This what we understood even from the product operators for one pulse experiment. Apply 90 degree pulse to bring the magnetization XY plane, we understood immediately at the time t = 0 magnetization goes to - Y and then start developing both cosine and sin components. Oscillatory components will be developed both along X and Y, which we call cosine and sin components; We had discussed this a lot even during product operator formalism.

So, magnetization has cosine component along Y axis and sine component is along X axis, now second pi / 2 pulse acts only on the Y component, X component is not affected, and continue to precess. X component is not affected at all; when I apply second pulse after a certain period during the evolution period, the magnetization getting evolved and you apply after some time 90 degree pulse. Now, what is going to happen? 90 pulse acts only on the Y component. What happens X component? it continues to precess. It remains unaffected at all; that is what happens.

The Fourier transformation of this one now it is going to give me a spectrum with a single frequency. What is the intensity of the signal? that depends upon how much it has moved with respect to nu, this is 360 star nu t1; How many degrees it has moved, that defines the intensity measured; if a complete coherence is along one axis we have maximum intensity, then as it starts dephasing, and develops other X and Y components, the intensity keeps coming down.

That is what we have already seen. So, this is the factor which defines the intensity of the spectrum. Now, the Fourier transformation of this gives a single frequency, the spectrum correspond to a single frequency whose intensity is given by 360 star into nu of t1.

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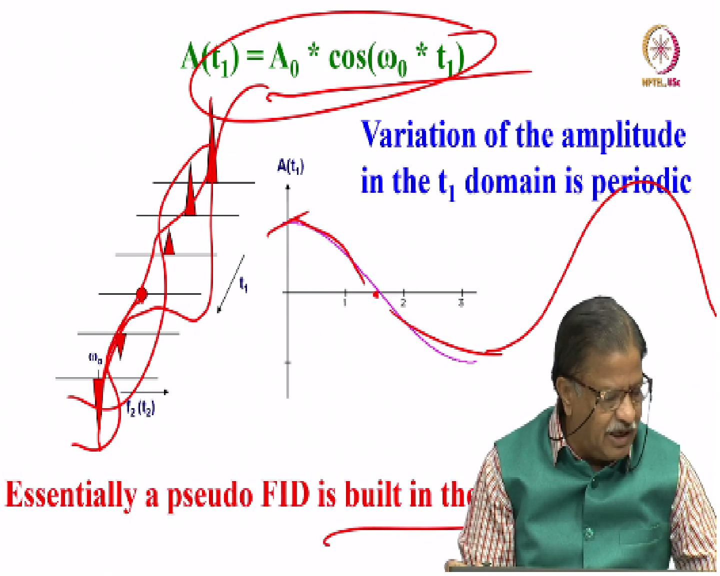

$A(t_1) = A_0 * \cos(\omega_0 * t_1)$

Variation of the amplitude in the t_1 domain is periodic

Essentially a pseudo FID is built in the

Now, how the intensity varies is given by this expression, we are taking the cosine component of that; cosine omega 0 star t1. Now initially let us say magnetization is like this, it starts going like this; monitor this red colour, it starts going like this, it forms an oscillatory thing. Initially intensities let us say is negative or you start like this, it goes like this, starts with intensity maximum, keeps on coming down, goes to 0 and then goes negative and continue like this. In fact, it undergoes oscillation, it is an oscillatory function. The amplitude varies in the time domain and it is a periodic function. If it has a definite period, keeps on isolating according to the period, this is what we discuss in the Fourier transformation of the periodic function, etcetera. Essentially a pseudo FID is built in the F1 dimension like this. This is what we discussed while introducing 2D.

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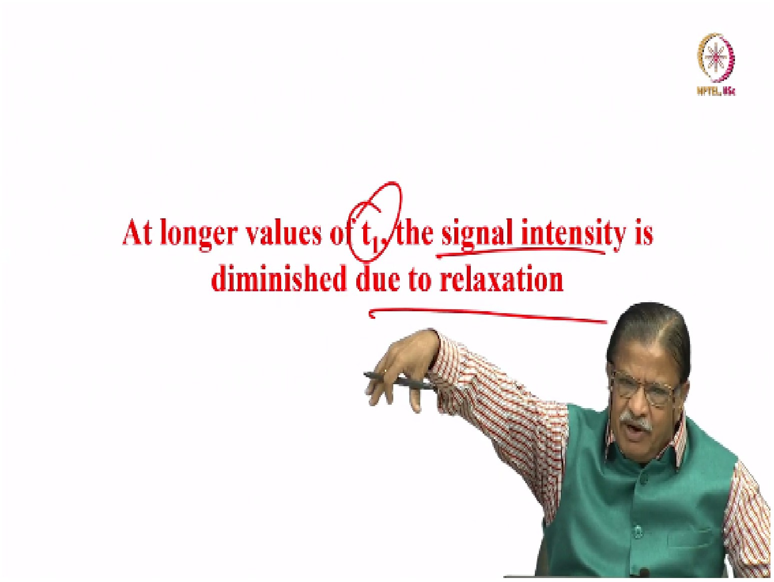

At longer values of t_1, the signal intensity is diminished due to relaxation

At longer values of t1 what is going to happen to the signal intensity? it diminishes due to relaxation; it becomes very, very small and finally, there would not be any signal; you will collect only noise. So, afterwards there is no point in collecting the signal. So, the signal keeps on decaying when you keep on increasing t1 period. So, it creates the pseudo FID, which also decaying; collect the signal.

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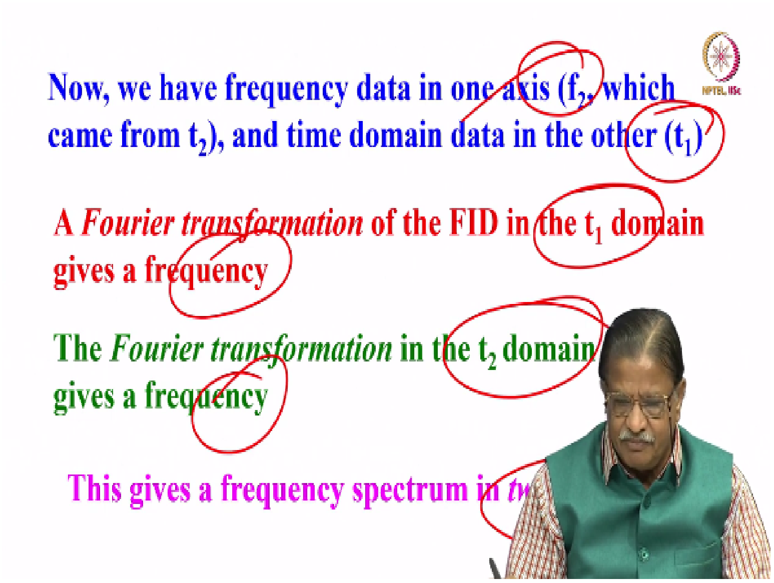

Now, we have frequency data in one axis (f_2, which came from t_2), and time domain data in the other (t_1)

A *Fourier transformation* of the FID in the t_1 domain gives a frequency

The *Fourier transformation* in the t_2 domain gives a frequency

This gives a frequency spectrum in *tw*

And now you have a regular time period and you have a signal in the t2 period; that is a detection period; always that is constant, t2 is constant; you have the FID there. Now we have a frequency data in one axis when you do the double Fourier transformation that is t2, similar to a conventional 1D NMR. And a time domain data in the t1 that is pseudo FID you have created so your have frequency in F1. So, Fourier transformation of FID in the t1 domain gives one frequency and Fourier transformation in t2 domain gives you another frequency.

So, we have 2 things I have time domain data both in t1 period and t2 period. Do the Fourier transformation of one time domain you will get one frequency domain spectrum. Do the Fourier transformation in both the dimensions you get frequency domain spectrum in both dimensions. In both dimensions you get a frequency spectrum.

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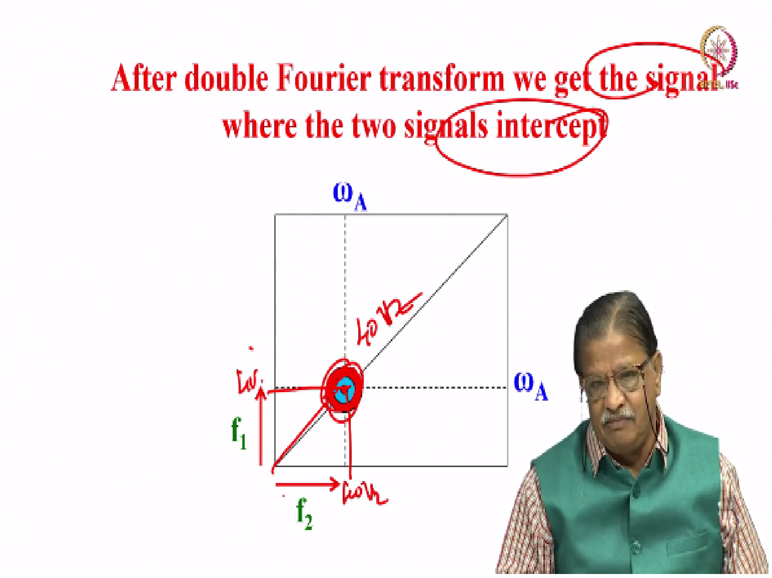

After double Fourier transform we get the signal where the two signals intercept

This is how the spectrum look. It is single spin, no coupling nothing after double Fourier transformation we get the signal where the two signals intercept. Remember, there is no coupling nothing for it to transfer the magnetization, no spin is there to interact. So, let us say this is the frequency at which it is evolved in the F1 dimension, call it 40 hertz, let us say. It is evolving at 40 Hertz during t1; it continued to remain 40 Hertz in t2 also. It did not change at all. In t1 if you go along this axis, it is 40 Hz, come along this axis it is going to be 40, it did not change at all. There is no spin to give the magnetization. Whatever the frequency at which it was evolving in the t1 dimension continue to evolve in the t2 dimension with the same frequency. So, it is going to give a single peak. If you look at it is exactly on the diagonal. The diagonal peaks are the ones where the evolution frequency in the t1 dimension and the t2 dimension both remain same.

You understand, I have a spin which is evolving at a certain frequency in the t1 dimension if that frequency remained same even the t2 dimension, in a homonuclear experiment like this COSY, you get that peak at the diagonal; on the diagonal you get that peak; that is a diagonal peak.

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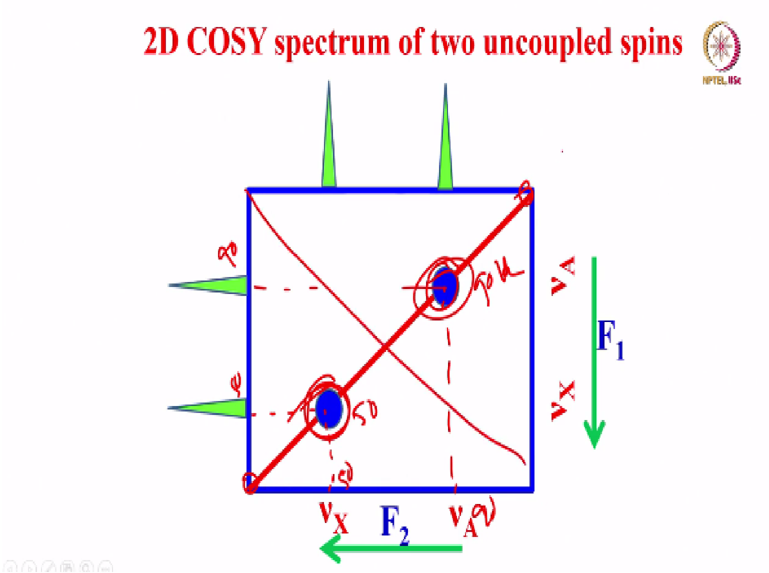

2D COSY spectrum of two uncoupled spins

So now, I take the 2D spectrum of two uncoupled spins. There is no coupling at all very nice. I have one spin here, have another spin here; there are two spins, there is no coupling. I say this is at 50 Hertz, this is at 90 Hertz. You draw a line here along this axis; draw a line along this axis this is at 50, this is at 50. Draw a line along this axis here, this is at 90, draw a line along this axis here this is at 90. So, in both the cases, these are two uncoupled spins, there is no way they can transfer the magnetization between themselves.

The peak which was evolving at 50 Hertz during t1 continue to evolve at 50 Hertz during t2. So that peak is coming on the diagonal. Similarly, the peak which was evolving at 90 Hertz during t1 continue to evolve at 90 Hertz during t2. It did not change, it did not transfer magnetization to any other spin. So, it again comes on the diagonal. So, when there are uncoupled spins in the COSY experiment, the peaks are always situated on the diagonal.

Important thing please remember, if there are uncoupled spins, the peaks are always on the diagonal. What is the diagonal? This is a principal diagonal, you have to consider starting from left corner to the right corner. Not this one, this is anti diagonal, this is a diagonal. So, this is what is important when you consider COSY spectrum of two uncoupled spins. Now, I took the example of uncoupled spins and we wanted to see how the 2D spectrum look. This is how it comes; both the peaks are on the diagonal. Now, we will go further.

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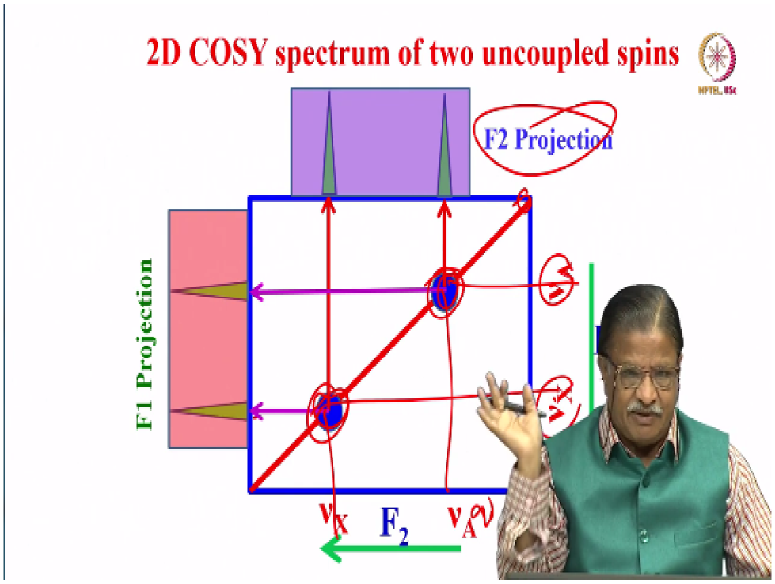

2D COSY spectrum of two uncoupled spins

I can take these 2 projections; I can plot on the F2 dimension like this, it is a projection, it is called F2 projection. Now, both the peaks are coming here this is called F2 projection. If I take the projection along this axis, this is called F1 projection. So, F1 projection gives you spectrum which is projected along this axis and F2 projection gives you the spectrum which is projected along this axis. This is how basically a spectrum of 2D COSY of two uncoupled spins.

This corresponds to nu X, this is nu X it goes like this, this correspond to nu A, this is also nu A it goes like that. So, the diagonal frequency, diagonal peak if you measure the frequency, it corresponds to chemical shift of the individual spins. For this peak this is the chemical shift, for this peak this is the chemical shift. For uncoupled spins which comes on the diagonal, the frequency on the diagonal corresponds to their chemical shifts, please understand this.

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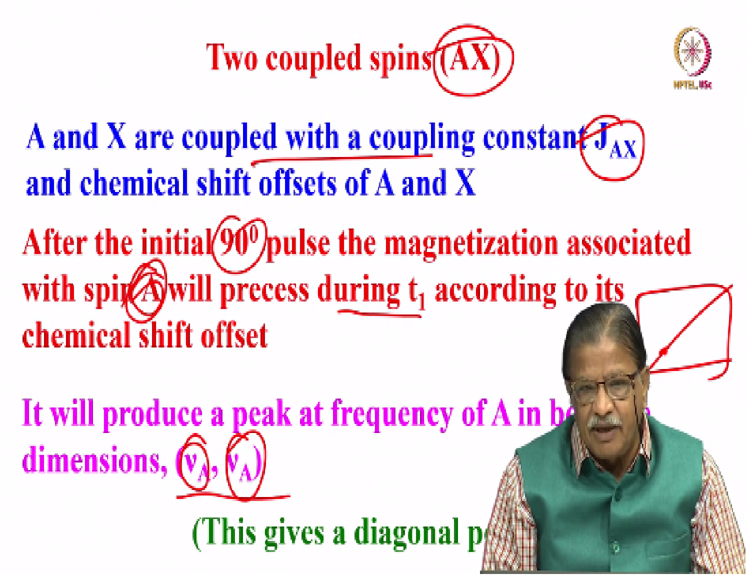

Two coupled spins (AX)

A and X are coupled with a coupling constant J_{AX} and chemical shift offsets of A and X

After the initial 90^0 pulse the magnetization associated with spin A will precess during t_1 according to its chemical shift offset

It will produce a peak at frequency of A in b dimensions, (ν_A, ν_A)

(This gives a diagonal p

Now, with this information, let us try to understand the COSY spectrum of two coupled spins A and X. Two spins are coupled, J coupling is present now, what will happen? during the evolution period t1, it evolves under chemical shift and also due to J coupling, both are present now. Now, very interesting thing is going to happen. Now we have a coupling constant JAX. After the initial 90 degree pulse the magnetization associated with spin A will precess doing t1 according to its chemical shift.

Spin A has its own chemical shift. Spin X has its own chemical shift. So, it starts precessing in the t1 period at its regular chemical shift offset. Then it will produce a peak at the frequency A in both to dimension, nu A, nu A. That means in both F1 dimension and F2 dimension it is going to have a peak, which is on the diagonal, it gives a peak here, it gives a peak here. So, what it means, the spin A is going to give rise to a peak which remains undisturbed both in the F1 dimension and F2 dimension; this is F1 dimension, this F2 dimension. So, I have a peak here, which remains same, which is on the diagonal, this gives a diagonal peak.

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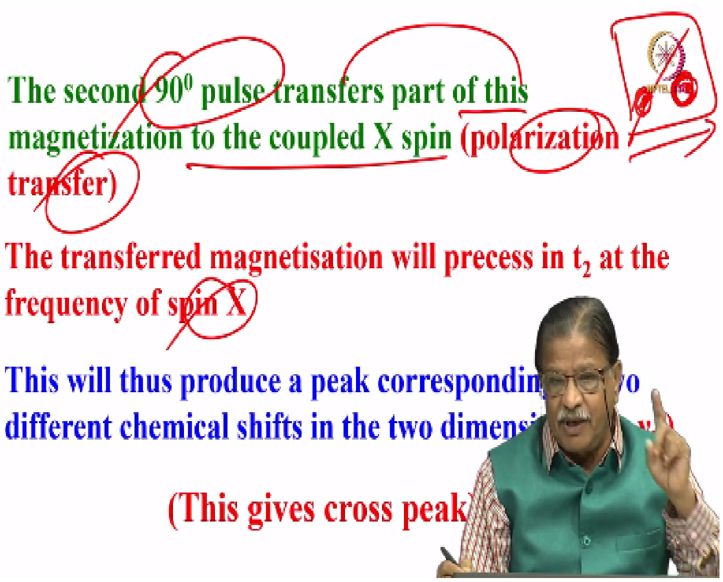

The second 90^0 pulse transfers part of this magnetization to the coupled X spin (polarization transfer)

The transferred magnetisation will precess in t_2 at the frequency of spin X

This will thus produce a peak correspondin o different chemical shifts in the two dimensi

(This gives cross peak)

Next, what the second pulse does? that we understood for spin 1 case, single spin case. With second 90 degree pulse what happens? it does something different. In the second 90 degree pulse, there was no coupled spin it continued to evolve at the same frequency, gave diagonal peak in a single spin case. Now, we have coupled spins, the second 90 degree pulse transfers part of this magnetization to the coupled X spin, A and X are coupled.

So, A not only is evolving during t1 when you apply a second 90 degree pulse, it transfers part of its magnetization to X spin. This what is mixing, here mixing is happening. In t1 period both chemical shifts are evolving and J coupling is evolving. In the 90 pulse mixing takes place and part of this magnetization gets transferred to coupled X spin. This is what is called polarization transfer; we discussed about this a lot.

The transferred magnetization now will precess in t2 dimension the frequency of spin X and the interesting thing is happening. If there is no transfer magnetization, in uncoupled case both in t1 and t2 it was precessing at the same frequency. Now during coupling it gives part of its magnetization to spin X. As a consequence in the t2 dimension this spin also precess at the frequency of the spin X.

Imagine when we were interpreting the 2D spectrum I said, if there is a peak like this one here. It was precessing at this frequency both in t1 and t2. At the same time it also had another frequency in the t2 which is here. So, the frequency of that, let us say this frequency in the t1, evolved in this frequency. At the same time it transfers part of its magnetization to other coupled spin and it evolved at this frequency in the t2 dimension. That is what it means. And this is going to give you a peak like this one on the diagonal; which is evolving at its own frequency. And another one which is evolving at the frequency of the coupled spin X. And this is what is called the cross peak. Now a peak in addition to chemical shift of A, also comes at the chemical shift of X in the t2 dimension. This is called cross peak.

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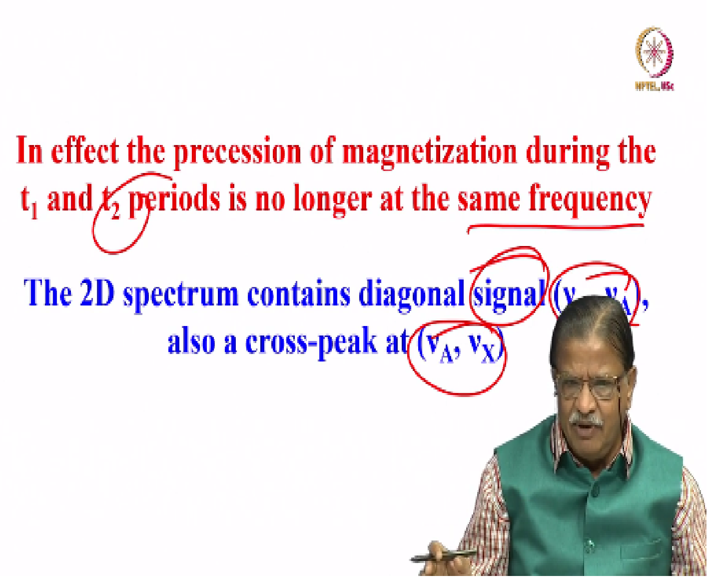

In effect the precession of magnetization during the t_1 and t_2 periods is no longer at the same frequency

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

Now, you may ask me what will happen to X spin? I was discussing about A spin. Identical behaviour. The X spin also behaves like A spin. That means it evolves, let us say, at its own chemical shift frequency in the t1 period and also in the t2 period gives rise to diagonal peak; and gives part of its magnetization to spin A and the evolves in the t2 dimension at the frequency of spin A, at the chemical shift of spin A. You understand. Now two things you have to understand. A spin was coupled to X, A will evolve in the t1 dimension at its own chemical shift frequency. During the mixing, it gives its energy or polarization to X spin. So, it evolves not only at this frequency, but also at the frequency of X, to which it is coupled to. Identically spin X evolves during t1 at its own chemical shift frequency. In addition to that, because it is giving its polarization to the coupled spin A, in the t2 dimension it evolves also at the chemical shift of the spin A.

Essentially you are going to get a spectrum like this one here, one here. This will evolve at the chemical shift here; this will evolve at the chemical shift here. And it also gives a diagonal; now we are talking about X spin it should have been X X not A A, I am sorry, sorry for the mistake it should be nu X, nu X. And also cross peaks at nu A nu X; you understand. So, this is what is happening. X spin behaves identical like A spin, A spin gave a diagonal peak at nu A nu A and cross peak at nu A nu X. The X spin gives diagonal at nu X nu X. And it is going to give cross peak at nu A and nu X. That is what happens. In effect the precession of the magnetization during the t1 and t2 periods is no longer at the same frequency. The magnetization precesses at two different frequencies, its precessional frequency in the t1 is different, its precessional frequency the t2 periods is different. So, as a consequence, 2D spectrum contains diagonal frequency of nu A, nu X and also cross peak at nu A nu X, for both A spin and X spins, please understand.

The precision frequency of the magnetization during t1 and t2 periods are no longer same. The 2D spectrum contains a diagonal signal nu A nu A for spin A, nu X nu X for spin X; and nu A nu X and nu X nu A are the cross peaks at the respective chemical shifts.

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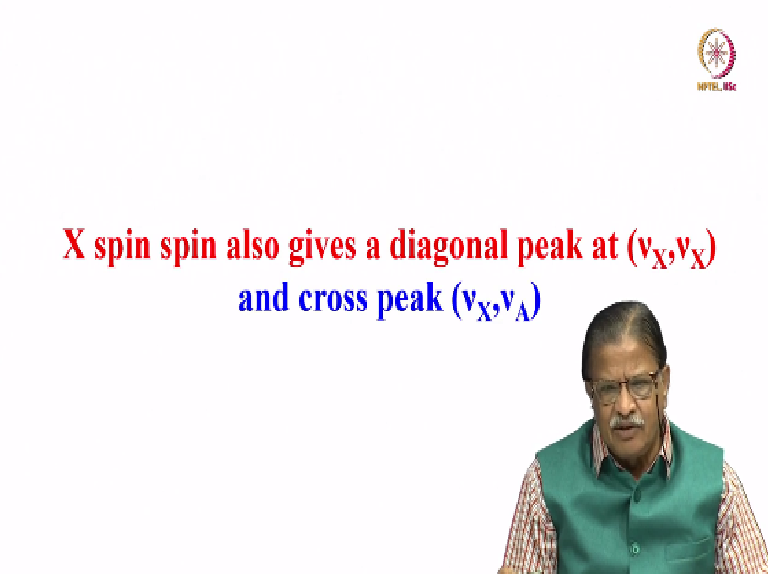

X spin spin also gives a diagonal peak at (ν_X,ν_X)

and cross peak (ν_X,ν_A)

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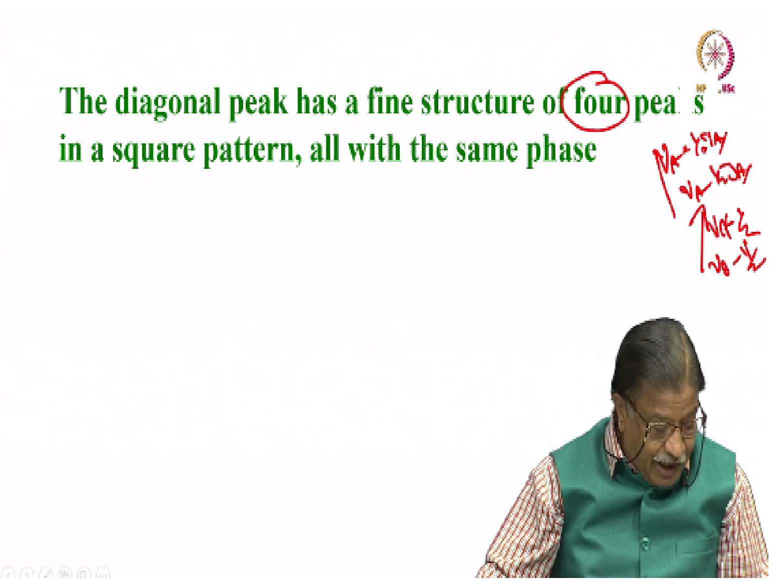

The diagonal peak has a fine structure of four peaks
in a square pattern, all with the same phase

$\nu_A + \frac{1}{2}J_{AX}$
$\nu_A - \frac{1}{2}J_{AX}$
$\nu_X + \frac{1}{2}$
$\nu_X - \frac{1}{2}$

So, X spin also gives a cross peak and then also a diagonal peak. The diagonal peak has a very interesting structure. It has a fine structure of 4 peaks, why? because it is J coupled. The diagonal peak is not just evolving at the chemical shift frequency. In fact, I did not tell that because I did not want to bring in the coupling till now. Now I am bringing in the coupling, it is not evolving at nu A it is evolving at nu A + half JAX and nu A - half JAX in the F1 dimension. Similarly in the F2 dimension it evolves at nu A + half JAX and nu A - half JAX. That means in both F1 and F2 dimensions you get doublets. So, I am going to write a diagonal peak like this, if I get a 2D spectrum, I am going to get a diagonal peak like this. I have a 2D spectrum.

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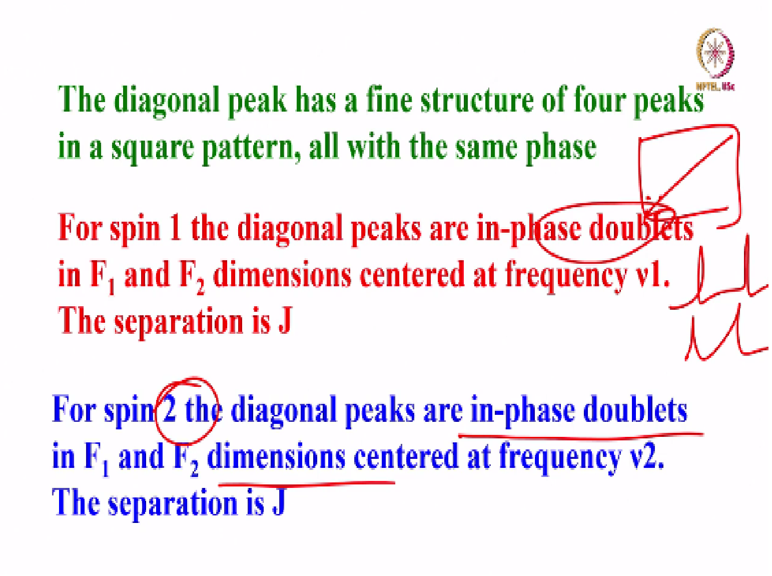

The diagonal peak has a fine structure of four peaks
in a square pattern, all with the same phase

For spin 1 the diagonal peaks are in-phase doublets
in F_1 and F_2 dimensions centered at frequency ν1.
The separation is J

For spin 2 the diagonal peaks are in-phase doublets
in F_1 and F_2 dimensions centered at frequency ν2.
The separation is J

I have a 2D spectrum here, let us say I am considering an spin A, earlier I said it is a resonating at the chemical shifts of spin A; No, what happens is, it will have one peak here, one peak here; this is nu A - half J AX here it is nu A + half J AX here. Similarly you have peak here and here. So, in reality how it comes is? It comes like a square. I will tell you it comes like a square, maybe I have the drawing, I will show you, it comes like a square. What is going to happen is, it comes like a square.

So, we will see that for this one now, for spin one the diagonal peaks are in-phase doublets, we discussed, in-phase antiphase in-phase dispersive in-phase absorptive everything. They are in phase doublets; both are in phase doublets in the t1 dimension. Similarly, in the t2 dimension or F2 dimension also it is in-phase doublet; both are separated by J. Interestingly, both are separated by J. So, for spin 2 also identically the diagonal phase are in-phase doublets both in F1 and F2 dimensions centered at the frequency nu 2.

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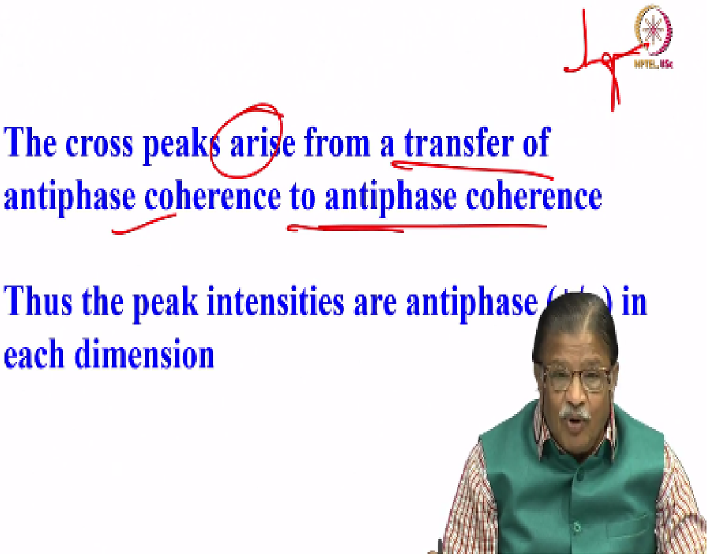

The cross peaks arise from a transfer of antiphase coherence to antiphase coherence

Thus the peak intensities are antiphase ([illegible]/[illegible]) in each dimension

So, the cross peaks also will have a similar the structure. In the sense the cross peaks arise from a transfer of antiphase coherence during the mixing; that is what we discussed. I told you, how magnetization transfer, coherence transfer takes place in INEPT experiment, with product operators also we understood. It is this J coupling that is responsible for creating the antiphase coherence and this is responsible for transfer of magnetization from one spin to another spin, that is what we said.

So, because of the transfer of magnetization the cross peaks are antiphase in character, that means, the one signal is positive other is negative of the J split doublet. This is a doublet split because of J coupling, in that one is positive other is negative. That cross peaks are antiphase in character in both the dimensions.

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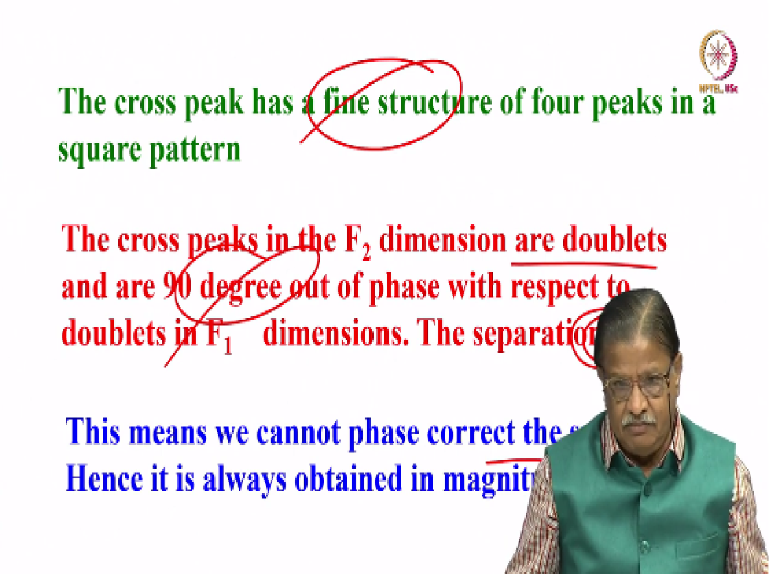

The cross peak has a fine structure of four peaks in a square pattern

The cross peaks in the F_2 dimension are doublets and are 90 degree out of phase with respect to doublets in F_1 dimensions. The separati

This means we cannot phase correct the

Hence it is always obtained in magnit

Not only in one dimension, in F1 and F2 both also. Similarly cross peaks has a fine structure, four peaks in a square pattern, cross peaks in the F2 dimension is a doublet that are 90 degree out of phase with respect to F1. Similarly, in the other dimension also it is a doublet; and out of phase. One is a positive and the other is negative peak; antiphase doublet. And the separation in each case is always a J; but you do not try to do the phase correction here. You cannot do that. I remember I told you when we were talking about in-phase, antiphase and all those things dispersive, absorptive, I said. Dispersive and absorptive you can make the phase correction. But in-phase and antiphase, you cannot do that. If you make in phase as antiphase, again, again other will become antiphase. So you cannot correct the phase of these two. So, it is not possible to correct the phase of these cross peaks. As a consequence, the spectrum of the COSY is always represented in the magnitude mode. Spectrum is always supposed to be in magnitude mode, that is the important thing.

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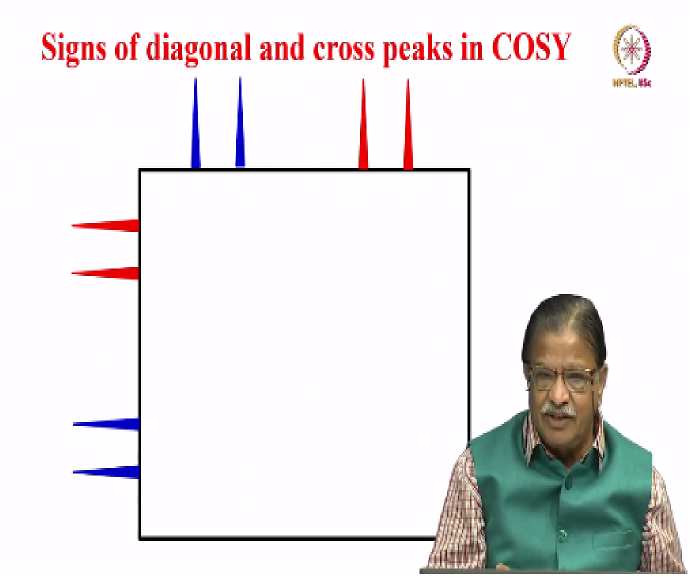

Signs of diagonal and cross peaks in COSY

So, we will diagrammatically see how the diagonal peaks and cross peak comes, etcetera. I take some more time to explain, it will take at least 5. No it may take more than 5 minutes. What I am going to do as the time is running up, I will stop here. But today what we discussed? we discussed about the simple example of a 2D COSY sequence. The COSY is a correlation sequence, it is a correlated spectroscopy. It is nothing but a simple two 90 degree pulse sequence with t1 and t2 periods, t1 is in systematically incremented.

Do the double Fourier transformation you are going to get a frequency domain spectrum, and we took the example of single spin. If it evolves at a particular frequency in the t1 dimension, it evolves at the same frequency in the t2 dimension, because it is not coupled to any other spin, there is no way it can transfer the magnetization, because of the mixing during the second pulse. So, it always gives rise to the same frequency in both F1 and F2 dimensions.

So, if we plot the 2D spectrum, the homonuclear 2D spectrum, this gives rise to a single frequency, appear at the diagonal. So, exactly on the diagonal you get a peak whose frequency is exactly the same in both F1 and F2 dimensions. And then we took the example of two uncoupled spins. It behaves same; that is what we said. The two uncoupled spins also behave the same, there is no exchange of magnetization, no transfer of magnetization between two spins 1 and 2 or spins A and X, whatever it is.

But then both of them give rise to single peak exactly on the diagonal. That means each of these uncoupled spins continue to evolve in the same frequency both in F1 dimension and in the F2 dimension. Then we are into the example of two coupled spins, there we understood in addition to individual chemical shift offset, there is also evolution to the J coupling during t1, when you apply a mixing pulse there is a transfer of magnetization from one spin to another spin. You know antiphase coherence transfer takes place between two spins.

As a consequence, what is going to happen is the spin A which was precessing at one frequency will also give its magnetization to spin X; and then it starts precessing, in addition to its own frequency, also at the frequency of the X in the t2 dimension. Same thing for the X spin. So, as a consequence, in addition to its own frequency which is on the diagonal, which whose frequency of evolution remains same, both in t1 and t2, it also gives a cross peak. The same thing happens for the X nuclei, X spin, that is what we discussed.

But we also said it is not exactly at their respective chemical shifts, because of the J coupling there is a splitting. We will have doublets in the F1 dimension, doublet in the F2 dimension, especially for the diagonal, both two doublets are in-phase, they are positive in-phase absorptive. Whereas cross peak are antiphase coherence to antiphase coherence transfer, they have antiphase in character. You cannot correct the phase of that. So, as a consequence, you represent the COSY spectrum, always in the magnitude mode.

This is what I said. And we will see the sign of the diagonal cross peaks, how the spectrum looks etcetera. In the next class and take 1 or 2 examples of it, try to analyze these spectra and go to other 2D spectra later. So, thank you, I am going to stop here. We will meet again in the next class for further discussion. Thank you.