One and Two Dimensional NMR Spectroscopy for Chemists

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Lecture – 54
Variants of COSY and TOCSY spectra

Welcome back. In the last couple of classes, we have been extensively discussing about multidimensional NMR spectroscopy with the special emphasis on two dimensional NMR. I showed you number of possible experiments possible with different acronyms like COSY, ROESY, NOESY, TOCSY etcetera. Varieties of pulse sequences can be used and I described about the advantages of different 2D experiments.

How the polarization transfer takes place between the interacting spins in all the sequences and varieties of information was shared with you. And in the last class exclusively we discussed more about the COSY sequence which is a two pulse sequence which is very important. So, we even took some examples of molecules and analyzed the spectra. We found out also the diagonal peaks correspond to one dimensional NMR spectrum.

Where there are coupled spins there is a correlation between the two chemical shifts and gives rise to cross peaks in the COSY spectrum. So through which we are able to understand and make the assignment of the coupled partners, that was a very easy thing. Now we will go further.

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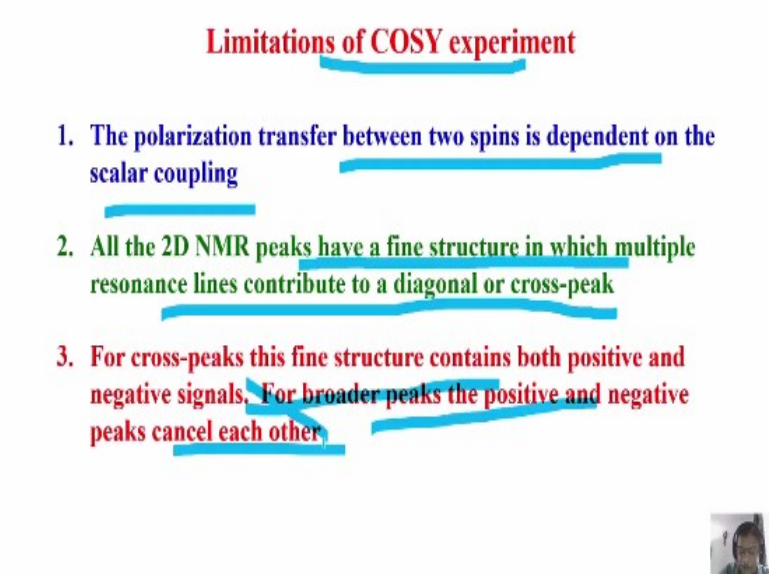

There are certain limitations for the COSY experiment. First, the polarization transfer between two spins is dependent on the scalar coupling. Sometimes it may so happen if the coupling is weak, you may not be able to transfer the magnetization. Or if a long range coupling is there with coupling of the order of negligibly small strength, you may not see the cross peak. So it depends upon the scalar coupling strength.

And the 2D peaks have a fine structure, in which multiple resonances contribute to diagonal or cross peaks we saw that. We saw autocorrelation peaks, and we also in the case of cross peaks also the multiplet patterns. The fine structures will be seen. And another thing is for cross peak the fine structure contains both positive, negative phase signals. That is a major drawback. When these frequencies are close by, it may so happen, when the coupling is very small. If this positive, negative phase of the signals, come close by, they get completely nullified. They may finally when they come so close; you may get zero signal. There may not be signal at all. And another thing for broader peaks these things will cancel out completely; for a broader signal these things will completely get nullified, when they come close by; that is one thing.

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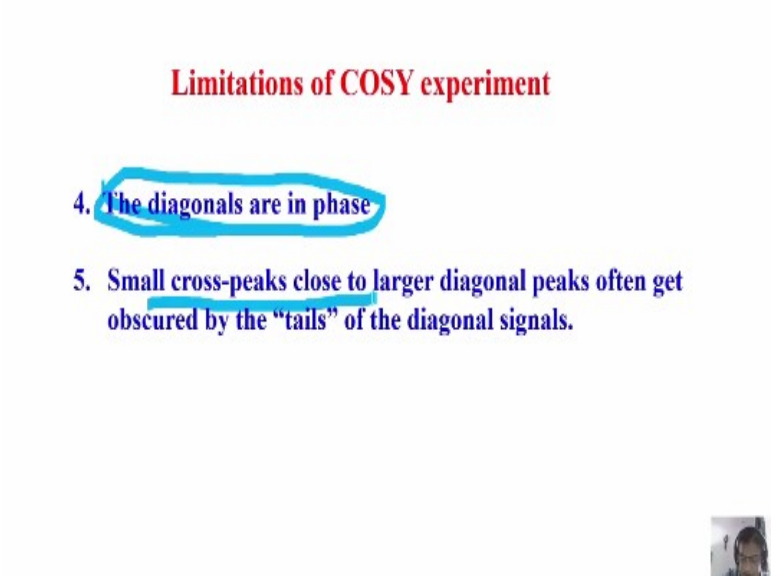

Another thing is the diagonal are always in phase gives rise to strong diagonals. So, as a consequence if you have a small cross peaks close to stronger diagonal peaks, often what we call these peaks as tails in the 2D spectrum, they obscure by the diagonal peaks. Sometimes the diagonal peaks are so strong, they will obscure the cross peaks which are close to it. So we will not be able to see cross peaks very close to diagonals. These are all some limitations of the COSY experiments.

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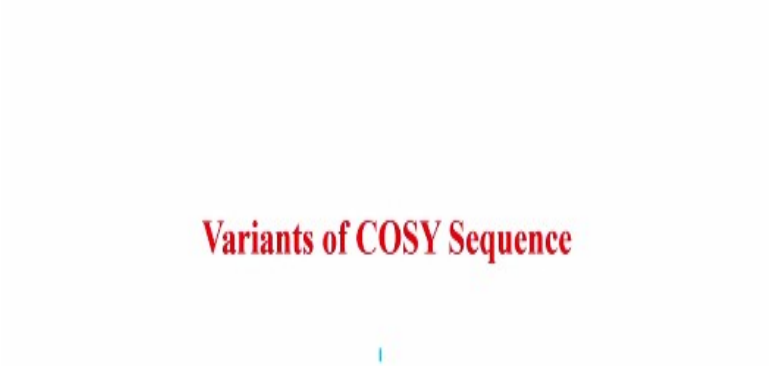

The number of variants of the COSY experiments have come out. I am not going to discuss everything because everything is an improved version. We can talk more about each and every pulse sequence. But to mention, remember we have sequences like COSY 45 and we have what is called long range COSY, RELAY COSY, we have AE COSY, PE COSY, Soft COSY, DQF COSY, etc. There are number of such variants and everything is an improved version to address some of the challenges of the basic pulse sequence. So, we will see at least one or two of these things and what is commonly employed COSY sequence for the routine analysis.

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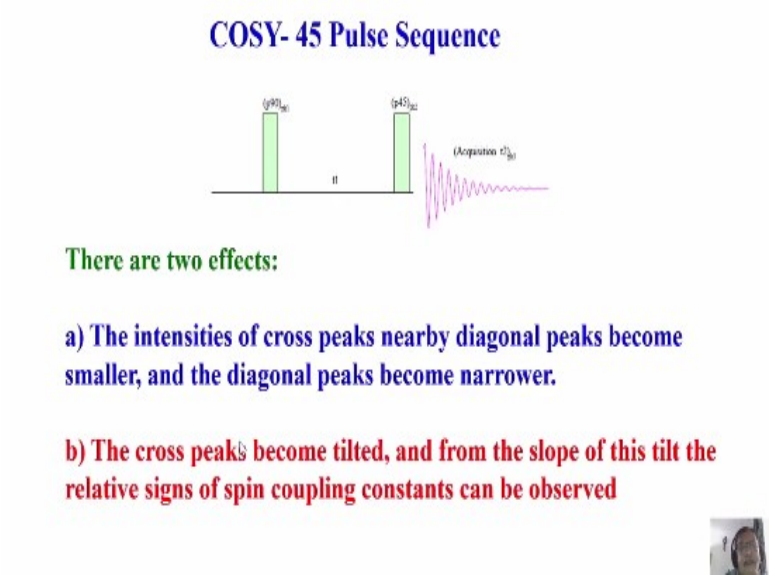

The commonly employed COSY sequence are; you can see COSY-45. This has an advantage. Intensities of the cross peaks nearby the diagonals become smaller and the

diagonals become narrower, that is one advantage. And in this case we have the cross peaks gets tilted. As a consequence, depending upon the slope of the tilt we can get signs of the couplings. It is one biggest advantage. It is like a small flip angle COSY, or soft COSY. We can get relative signs of the couplings, using COSY-45. Anyway this is only an information I will not be going to the more details.

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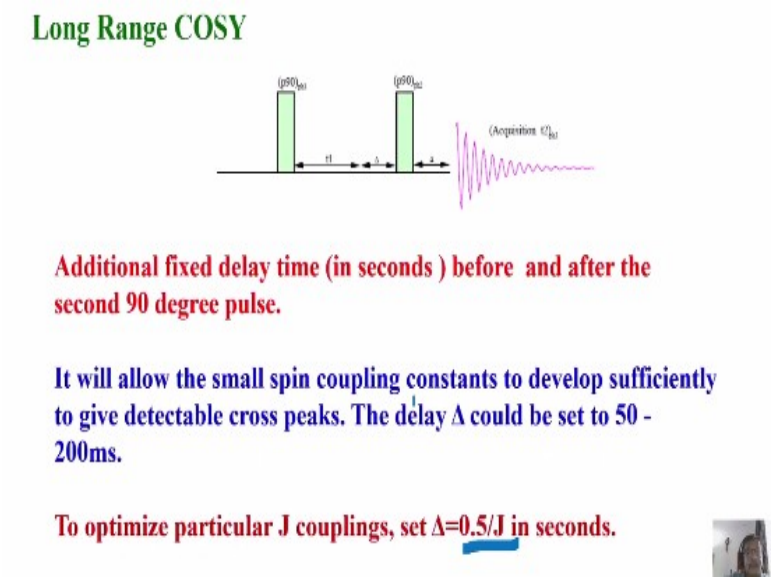

And another thing is, in the long range COSY an additional fixed delay time is incorporated just before and just after the second 90 pulse. Then what happens, it will allow the small spin-spin couplings to develop sufficiently and give detectable cross peaks. Small spin spin couplings, if they are there; they will start evolving here, and there will be evolution of such type of couplings and you are going to get detectable cross peaks.

And this delay is not very large, and it is of the order 50 to 200 milliseconds you have to use that is all. And of course, we can optimize for this particular things, if you want to get a long range, really long range coupling, if you know that value you can say 0.5 / J is what the delta you have to use.

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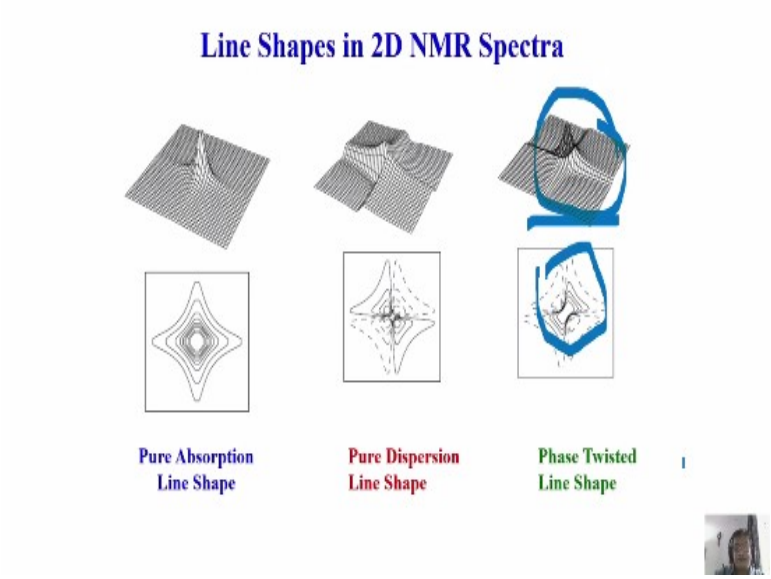

And next point we have to discuss about the lines shapes in 2D NMR. This is not only in COSY in most of the 2D sequences, when the spectrum is recorded, if you look at, for example, this stack plot, you can see on the four sides you will see a tail like thing which is moving. This type of spectrum when you take the contour plot, it looks like this. This is called a pure absorption line shape. Pure absorption line shape is always good for us. It gives you better resolution and we have to get the spectrum, record the spectrum in pure absorption mode. There are various ways of recording the spectrum; in a magnitude mode, in a pure absorption mode, pure dispersion mode, phase twisted line shape will be there, and in some case, varieties of line shapes will come. But always depending on the pulse sequence, you may have a different line shapes. Best is to use a sequence in such a way you get pure absorption line shapes in the 2D spectrum; that gives a better resolution. And sometimes you get peaks like this, that is called pure dispersion line shape. And here you can see this is what is happening. It is the twisted line shape, look at it. If you record the contours they look so bad.

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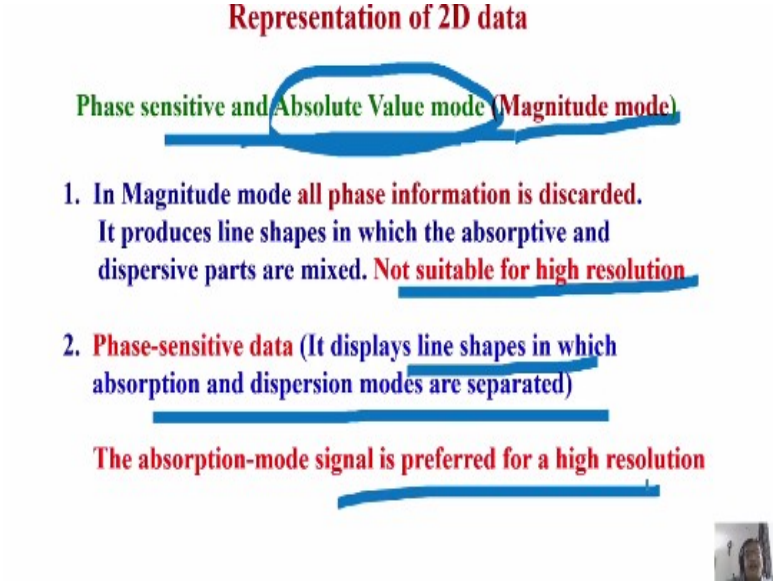

So there is a way to represent the 2D spectrum. Most of the time it is represented in the absolute value mode. Generally, phase sensitive and absolute value mode. Absolute value mode means you discard the phase information, It is a magnitude mode and no phase information is available. But the problem is a magnitude mode if you record, it is not good for high resolution. It always produces line shapes with both absorptive and dispersive shapes.

In the magnitude mode it so happens it is broadening the signal, and it is not suitable for high resolution. Phase sensitive data is better. It displays line shapes in which absorption and dispersion modes are separated. So absorption modes is always preferred for a high resolution data, high resolution spectrum.

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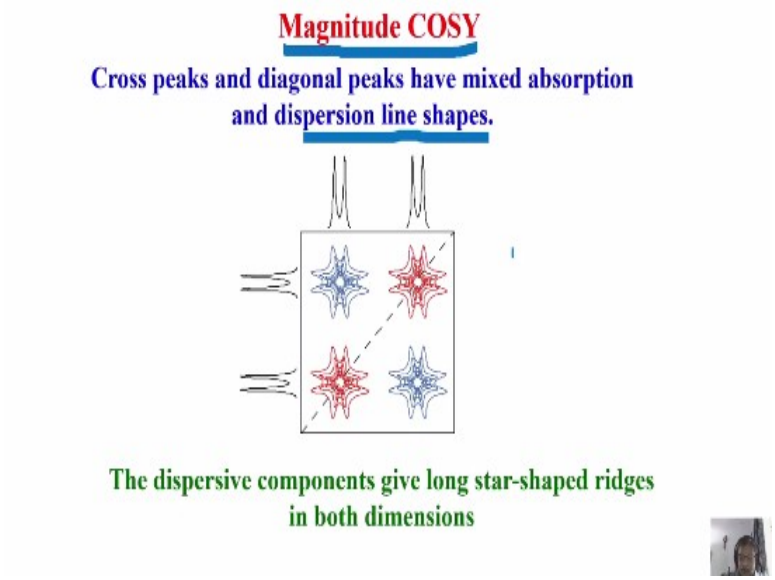

Just to compare look at the magnitude COSY spectrum, look at it. It is like a star shaped peaks, both diagonal cross peaks appears as if there is a sharp shape ridges are there in both dimensions, both cross peaks and diagonal peaks appear like this. The reason for it is it has a mixed absorption and dispersion line shapes. As a consequence, you see when the peaks are too close, you will not be able to resolve.

The magnitude COSY that is why gives sometimes broad peaks; you will not be able to get good resolution, because it is a mixture of phase, it has a mixture of absorption, dispersion lines. Of course, absorption and dispersion line shapes, I already discussed with you long back; when I discussed about the 1D.

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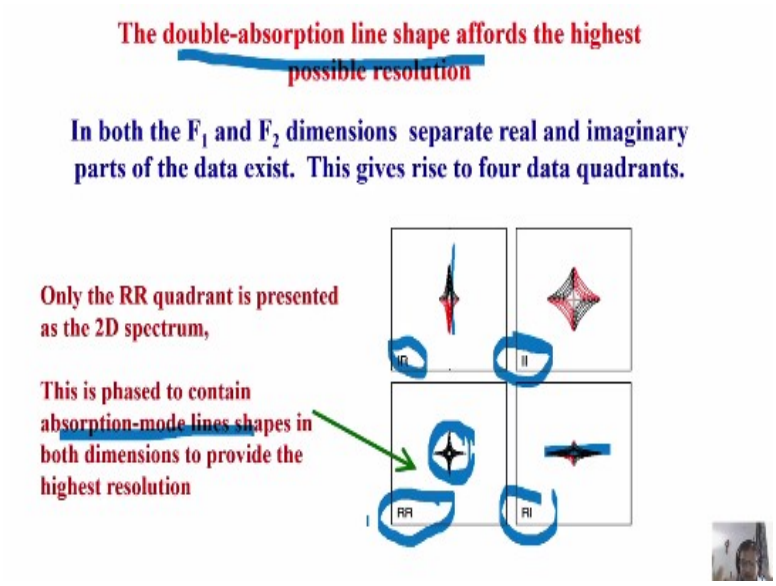

In reality, if I take double Fourier transformation spectrum; any Fourier transformation has real part and imaginary part. This is very important to understand. It has a real part and imaginary part. In these 2 dimensions, we have again both the real and imaginary parts. If a consider, there are 4 possible combinations. We can get imaginary one and real in one-dimension; imaginary, imaginary; real imaginary and real, real.

So, in the 4 quadrants you can have different types of presentations of the spectrum, and what is most important for us, you look at this peak, compared to this is really broad. Look at this one, this is having a tail like this; and this is also having like this; whereas this one is the best one. See this phase. The absorption mode line shapes in both the dimensions always gives you highest resolution.

Please look at the spectrum what is shown here. All you have to do is, you have to record the spectrum in double absorption line shaped mode; just remember this. There are various line shapes, but do not go for magnitude type spectrum. You always record the spectrum in double absorption mode, that gives a better spectrum. In all the 2D experiment you have to do that.

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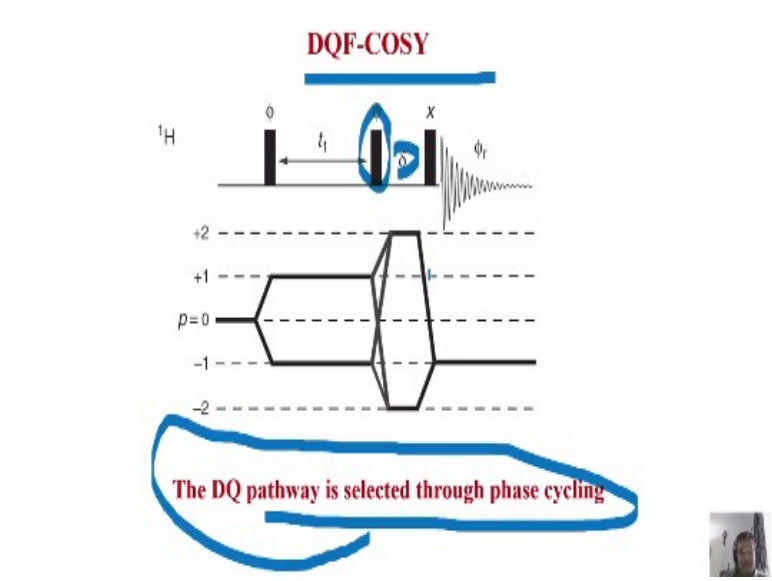

And next is another thing, what is called DQFCOSY. This is what is normally used generally. It is the better version of the regular COSY what we have 90-t1-90-t2 COSY. But only thing is we have another small angle pulse with a delay between these two; otherwise everything remains same, because it creates a multiple quantum; double quantum here and we have to choose the double quantum pathway and then it is selected through phase cycling.

How do you choose double quantum pathway is by phase cycling? I have not discussed about phase cycling and all those things till now. Of course I gave you a small idea about the phases of the pulses earlier.

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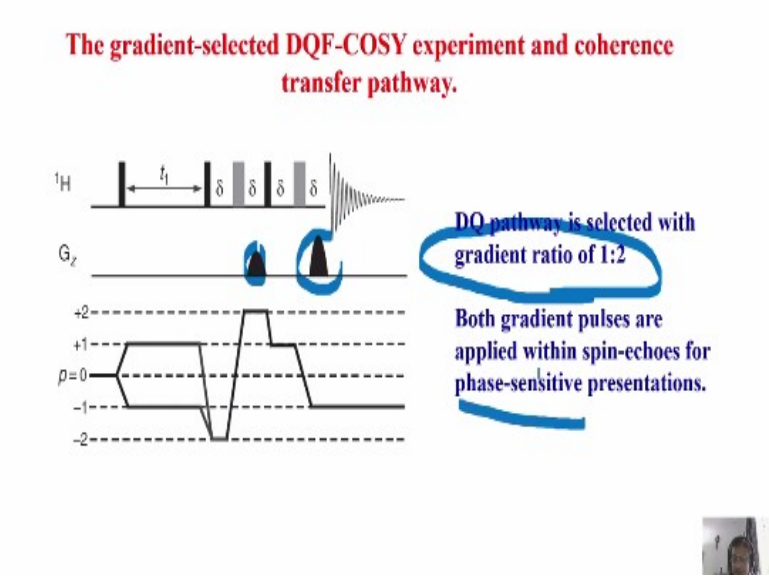

In fact, you can get the gradient version of this. We can select the double quantum pathway by using gradients. There are small gradients applied here and here; these are like spin echoes sequences in the middle of the delay here. I am going to select the gradient ratio 1 : 2, this will select the double quantum excitation and then we can get the spectrum of DQF COSY.

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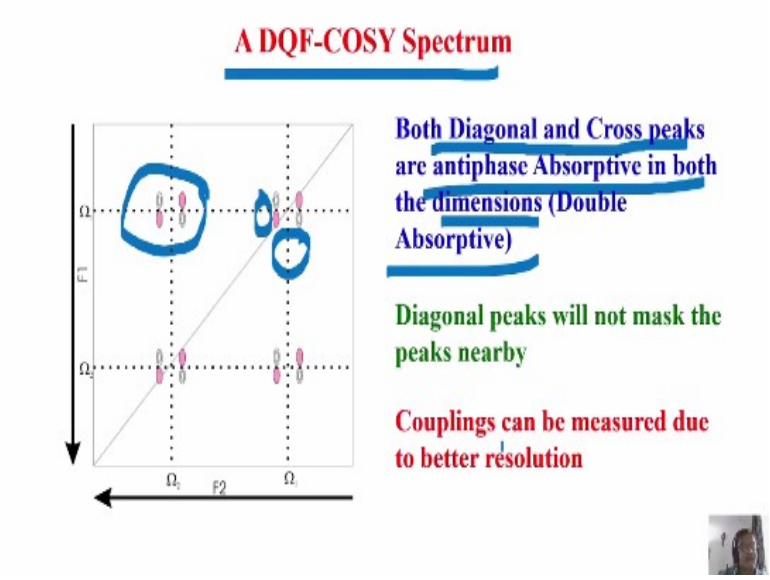

Simply I wanted to show you DQFCOSY is a much better spectrum than COSY spectrum, just please remember that. If you take DQFCOSY spectrum, and look at the spectrum fantastic spectrum; look at this one, compared to what we saw in magnitude COSY spectrum and various types of spectra with a different line shapes. In this case both diagonal and cross peaks are antiphase absorptive in both the dimensions. It is called double absorptive mode.

It is double absorptive anti phase, it is very good. It always gives very high higher resolution. In any case always double absorptive mode spectrum if you record, look at the resolution you will get in both F1 and F2 dimensions, it is double absorptive mode, and you will get beautiful and highly resolved spectrum. And even if you have a peak near the diagonal, like here or here; it is not going to be obscured like our magnitude COSY.

So diagonal peaks will not obscure the cross peaks which are close by. So the coupling can be measured with a better precision in DQFCOSY. You get coupling information like this, very beautifully you can measure it. So the DQFCOSY give you the better spectrum. I gave you example of a COSY to analyze and everything, but what you should try to do is; you should always try to do DQF COSY spectrum, do not try to do regular COSY.

Another advantage the peaks coming from reference or solvent peaks which give single peaks are all get suppressed in the DQF COSY experiment. You do not get solvent peak. Because of double quantum excitation, the single quantum peak of solvent will not come through in the DQF pulse sequence. As a consequence, this solvent and reference signals everything will be absent. You will get only well resolved double absorptive mode spectra of your samples so that is the best thing you have to use.

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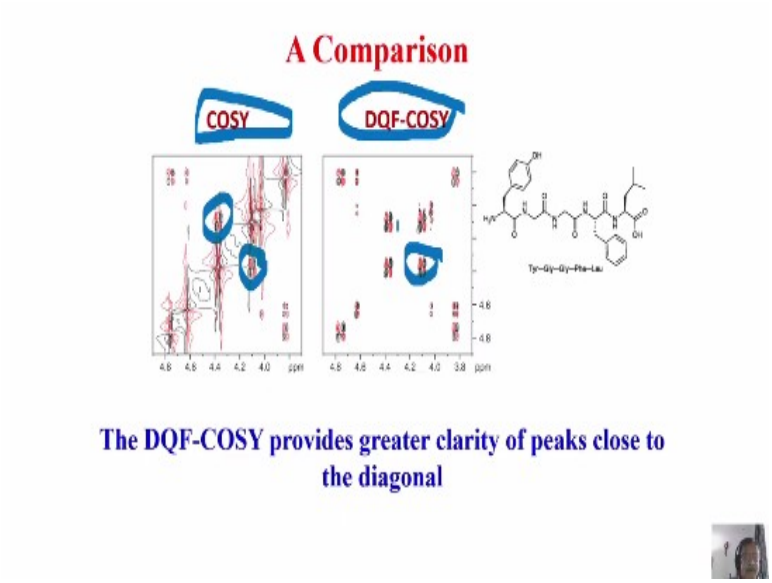

Just to compare a regular COSY and then of this molecule 1, 2, 3, 4 pentapeptide. The regular COSY is this one and this is the DQFCOSY. Lok at this point, especially at the diagonal you see the ridges on either side. If you have a spectrum like this for example you have a cross peak here. How do you identify? This is a better in case sometimes you get tails

on either side. I mean both the peak extented like this, you see the ridges, they are called tails.

So T1 and T2 tails will be sometimes so large, these peaks get obscured. And on the other hand in the DQFCOSY look at this one, a fantastic spectrum you are going to get. So always whenever you wanted to do your COSY experiment for the assignment purpose; do only DQF COSY, do not try to do conventional COSY.

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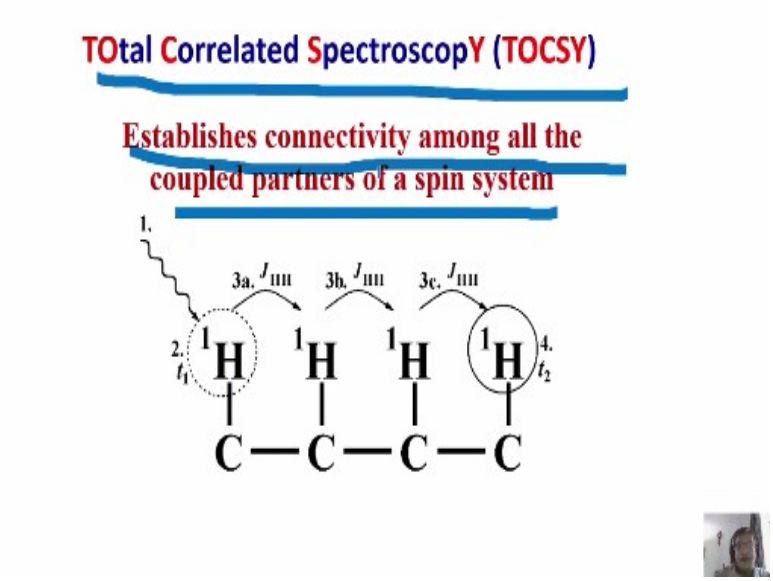

So now with this; there are lot more things we can discuss about COSY. Varieties of COSY sequences are there, how to get the signs of the couplings, using AE COSY or PE COSY or Soft COSY, etc. We can discuss a lot, but that is purely for academic interest. Right now we will not go for that, because you need to know how to analyze the spectrum of different molecules using various 2D sequences and our time is also limited.

So next I will go to what is called total correlated spectroscopy (TOCSY). It is a fantastic experiment, much better than the COSY. What is the advantage of this? It establishes connectivity among all the coupled partners of a spin system. You know what is a spin system? I have already explained to you, what is a spin system. The coupled partners form a spin system. If you take let us say there are two different spin systems, you can identify these different spin systems easily. All the spins which are coupled among themselves will be identified in one experiment. What we did in COSY, we went step by step 1 is connected to 2, 2 is connected to 3, 3 is connected to 4, etc. Like a step by step manner you went on identifying the cross peaks for the assignment purpose. But in TOCSY, in one single

experiment you can identify all the coupled partners. It is a much, much better experiment. This is what happens.

Exactly analogous to COSY, only thing is the magnetization transfer instead of going to the immediate neighbor, it keeps on transferring from one spin to other spin, other spin, among the coupled partners. So how much time it takes to transfer depends upon the time I am going to allow them to mix up that is what the TOCSY is all about.

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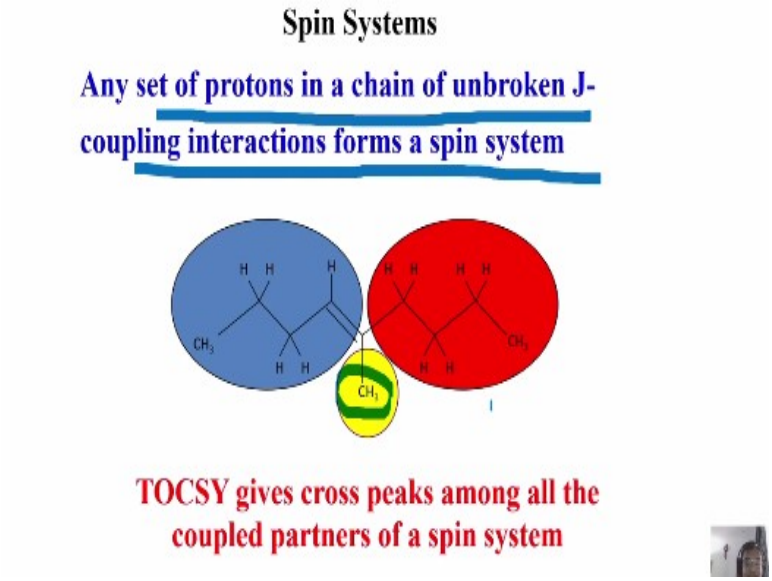

So before that let me tell you about the spin system. Of course, I discussed. Let me show you. Consider a molecule like this; any set of protons in a chain of unbroken coupling interactions forms a spin system. In the 1D NMR when I was discussing I told you about spin system. This is the exact definition of that. A chain of unbroken J coupling interactions if it is there that forms a spin system.

Now here look at it; this molecule. Among this, this and this, there is a coupling. Let us assume 1, 2, 3, 4 bond coupling between this and this is 0. Similarly 1, 2, 3, 4 bond coupling between this and this, is 0. So then among these spins, there are couplings. Only thing is there is CH3 is not coupled to these two. Let us assume that. Then these three form three different spin systems. This forms one spin system because this is coupled to this, this is coupled to this, this is coupled to this.

Similarly, here each spin is coupled among themselves, if there is unbroken chain of coupling, but this to this long range coupling may be 0, it does not matter. So long as there is

a chain of unbroken coupling that forms a spin system. So now we have three coupled spin systems. This is how we have to identify the spin systems. And TOCSY does this in one experiment; very beautiful experiment. So TOCSY gives cross peaks among all the coupled partners of a spin system.

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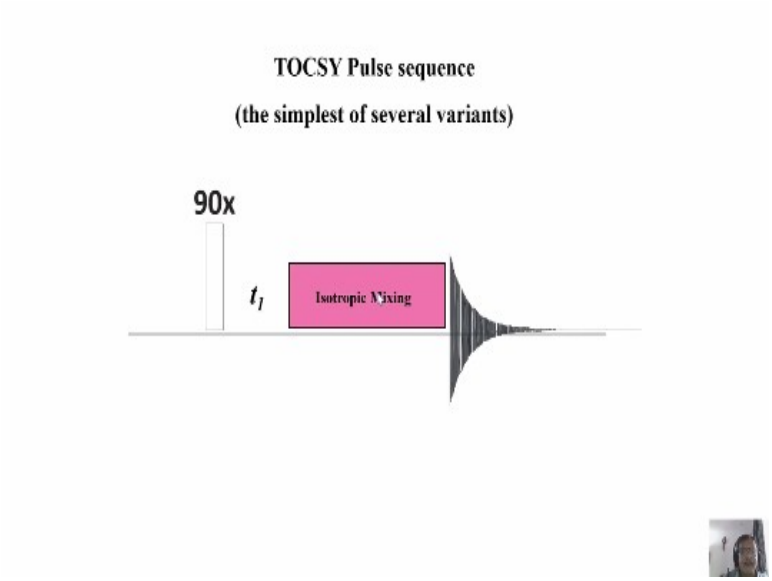

How TOCSY work? It is a simple experiment. What did we do in COSY? 90 t1 90 and then collected signal. But in this experiment, instead of a t1 pulse we have what is called isotropic mixing. What we do in isotropic mixing is, we bring the magnetization to X axis in that particular axis itself we can apply a RF pulse for a longer duration. Then what is going to happen? then we will retain the spin along that axis only for a long time.

We are locking the spin in that axis; that is why it is called spin lock pulse. In the spin lock pulse what happens is; it so happens it bring in a sort of what is called strong coupling effect among all the coupled spins. What happens; it removes J coupling, when the J coupling is completely removed, it becomes 0, and only chemical shifts are there. What you call that spin system? When all chemical shifts are within a small range the J couplings will be removed. Now it forms a strongly coupled spin system; and then magnetization transfer among the spins become much better. So what I was telling? what happens in the case of isotropic mixing is simply you remember, if you do not understand anything. It is called a spin lock pulse; you bring the magnetization from z axis by applying a 90 pulse along the particular axis, let us say x or y.

In the same axis where the magnetization is there, we apply what is called isotropic mixing; another RF pulse called spin lock pulse, where the magnetization that is the spin vectors are retained in the same axis for a long time. And it is all applied in such a way the spins lose their identity. You cannot say, let us say in the spectrum so many chemical shifts are there, with so many spins with different chemical shifts, that information is lost. Spins lose their identity and behaves like a strongly coupled spin system with the absence of chemical shifts. There are no chemical shifts at all. Then it is easy for the spins to talk to themselves and exchange magnetization among themselves. As a consequence, all the coupled spins in a given coupled system, the magnetization transfers take place. In the COSY magnetization transfer takes place only for the first immediate neighbor and then second long range, third long range coupling, like that. But in this case because of the isotropic mixing spins lose their unique identity and a sort of you are creating a strong coupling situation, and magnetization transfer takes place among all the couple spins in a easy way. That is what happens.

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But in this case how it happens let us see. We have different spin systems in the molecule. I showed an example where three different spin systems were there. Then how the magnetization is transferred. A beautiful example for this 4 X 100 meter relay. It is from one of the Olympics. Look at it there are three teams which are participating in this running, relay. This is a Ghana team, USA team and Italy team. What happens in the relay? The person who is going ahead first, and he has to transfer; pass on his baton to another person who is following him next; and he will run and he will pass on the baton for another person who will follow him; each one will pass on the baton which they are carrying in their hand. So what they will do? This Ghana runner will pass on the baton only to his teammate; next

person and he will give this next person. And he will not directly give to the last person that is not allowed; he will give only to next person; exactly the magnetization transfer goes from one spin to another spin, very easily. First it goes to the immediate coupled neighbor, then the next one, next one, next one, the magnetization transfer keeps on going. But between these teams he cannot give his baton to this person; not allowed.

So this forms one coupled spin system and this team forms one coupled spin system and this forms another coupled spin system. So he will pass on baton to his team, he will pass on baton to his team. Exactly like this analogy, in the spin system the magnetization transfer takes place among the coupled spin system; like the way it is going first immediate coupled neighbor then second neighbor then third, like that.

And depending upon the mixing time you are giving in pulse sequence which is there. There was isotropic mixing time, depending upon the mixing time you are giving; the extent of magnetization transfer takes place can be decided. You should not give too much also; then there can be back transfer of magnetization. You have to choose optimum. It does not matter now it happens.

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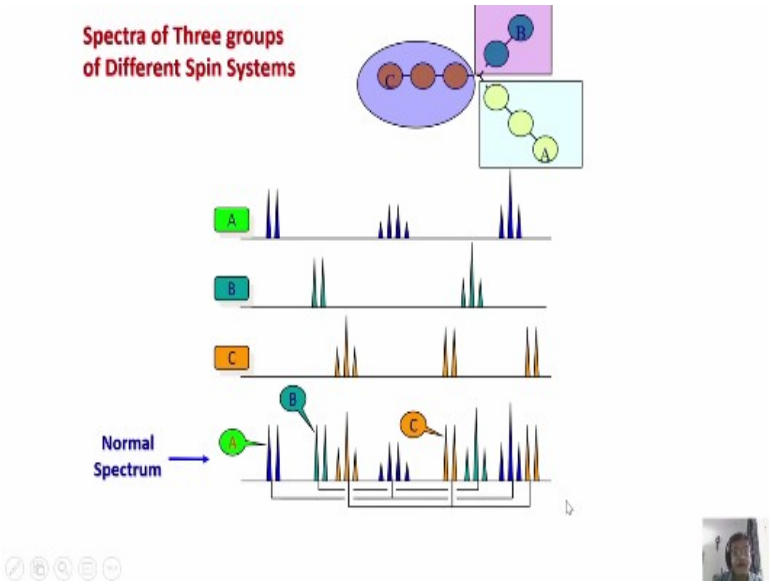

Let us now look at the spectra of three groups of three different spin systems which we saw. How does it look? These are the three spin system we will take. These are three protons coupled here, three protons here and two protons here. A hypothetical molecule; do not worry about the chemistry whether it is a CH3, CH2 we do not care, whatever may be terminal group; it has to be CH3, we do not care. We are only hypothetically showing some molecule.

In this molecule, let us say A gives rise to a triplet, a quartet and a doublet; this is what it is, one is a triplet, other is a quartet, last one is a doublet. Let us take B, B has only two types of protons, one is the doublet; other is a triplet, that is also fine. In principle, this has to be a quartet; and this has to be a CH3. We are not worried about real chemistry we are only hypothetical molecule taking just to understand TOCSY.

This is C, again three spins are coupled. Now again let us assume one is a triplet, one is a doublet, and other is a triplet; that is what we are going to get. Now in reality what type of spectrum we are going to get if I take 1D NMR? 1D NMR does not distinguish the spin systems. As far as 1D NMR is concerned, we are recording all the spectrum of all the spin systems simultaneously, which are overlapped like this.

It is a conventional 1D spectrum where you do not know which is the coupled spin system, which are the coupled partners also we do not know. But now this is the spectrum and we understood these are the different spin systems.

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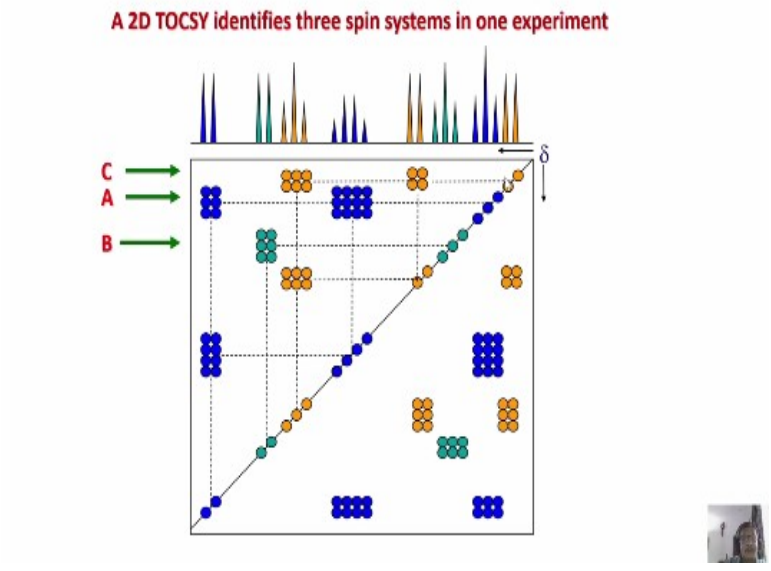

Let us go and record the TOCSY spectrum. Let us see what is going to happen? this is a beauty, look at this one. Now in the TOCSY this, this and this, see, in this TOCSY experiment this doublet, this doublet and this triplet, they form a coupled spin system. Now similar to COSY sit on the diagonal keep on going vertically completely to the end of the spectrum, same way horizontally till the end of the spectrum.

You see, you hit up a number of cross peaks, but this is a diagonal peak this is coupled to this, this is also coupled to this. So these three group form a coupled spin system. That is exactly what we saw in the previous example when I showed this with different colors. But not along this axis, it also gives along this axis. Similar to COSY experiment, TOCSY is also perfectly symmetric with respect to diagonal.

It is perfectly symmetric; you see, come along this axis, you see this, two cross peaks go along this axis you get two cross peaks, you can complete the square. You know which are coupled partners. In fact you keep on drawing vertical lines like this ;you can identify all the three spin systems. All the three coupled spins like this. This is one spin system with three spins. All can be identified there. There is a redundant information.

One vertical row itself is enough to identify all the coupled partners. Now go to the next one, next one is triplet, quartet and doublet; fantastic you can see this. Now go along this axis, you can identify this group, this group and this group. All the coupled partners of this spin system is identified; that is also very easy. Now we go to the next one; this one, we have a triplet and a doublet; look this is a triplet go along this axis you are going to hit a doublet.

There are only two peaks here; so this means this and this are the only coupled spins in the group. In this spin system, this, this and this are coupled partners. In this spin system this, this and this are the coupled partners. So you can identify this from the horizontal axis and also vertical axis. So the F1 dimension also gives the similar information, F2 dimension also gives you similar information.

From TOCSY in one experiment you can immediately identify all the spin systems. I can give you one example where this type of application is seen where you find it more useful. Go to the analysis of the big peptide or a protein. It is made up number of amino acids; each amino acid starting from the methyl group to the NH proton if you go, they form a coupled spin system.

What happens is, if you run a TOCSY and start drawing lines like this, vertical lines, if there are 20 amino acids, you will get 20 vertical lines or horizontal lines, like this. So you can identify each amino acid peaks, all the spin systems of each amino acid in a single TOCSY experiment. You understand; so the analysis becomes very simple by identification of spin

systems of each amino acid, that is the beauty of TOCSY. So, TOCSY is one of the important experiments, where all the coupled spin systems can be identified by a single experiment; see these three.

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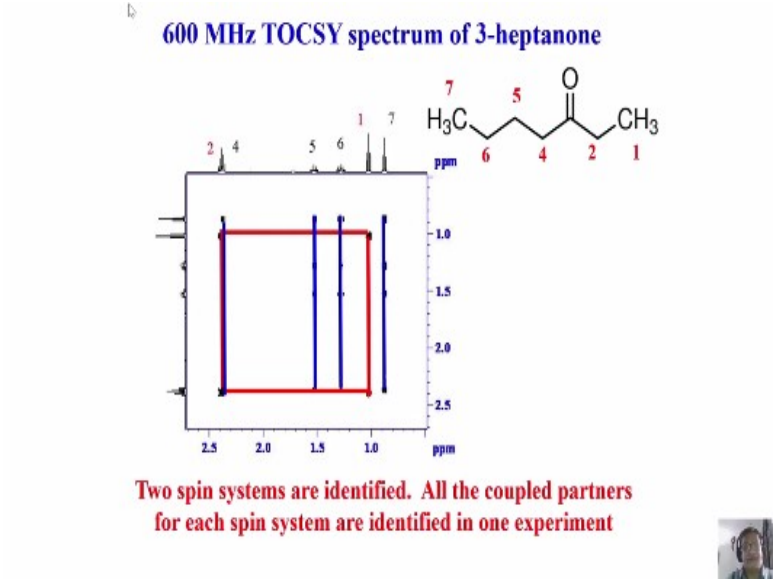

Now we will compare what is a TOCSY and what is COSY later. It is TOCSY of one molecule called 3-heptanone. Okay I think we tried to analyze this. We started analyzing using COSY, 1, 2 and then we got a cross peaks between 4 to 5, t to 6, 6 to 7 etcetera. Now I know 1 and 2 are the coupled partners. Start with 1 continue; completely come here. See you can see only see only two spins coupled to it each other.

These are the only two spins which are coupled. It has to be 1 and 2. Whereas 7 is coupled to 6, 6 is coupled to 5, 5 is coupled to 4. Sit on the diagonal of 7, keep on coming down vertically you can hit upon all the three cross peaks. This tells you this is coupled to this, this is coupled to this, and all the coupled partners can be identified in one single experiment. We go like this or go like this, in either way we are going to get the identification of all the coupled partners.

This is a best experiment so very easily you can pick up the peaks belonging to the spin system. Like this, this is one spin system, this is another you can go and take these peaks in the horizontal dimension, that is the F2 dimension or in F1 dimension; either way. This is in the F1 dimension, along F1 dimension you can do or you can do along F2 dimension; both are same. Both will give you identical information.

So two spin systems in this molecule were clearly identified. All the coupled partners for each spin system were identified by one simple experiment. Very very simple, with one experiment everything can be done. It is the biggest advantage of TOCSY.

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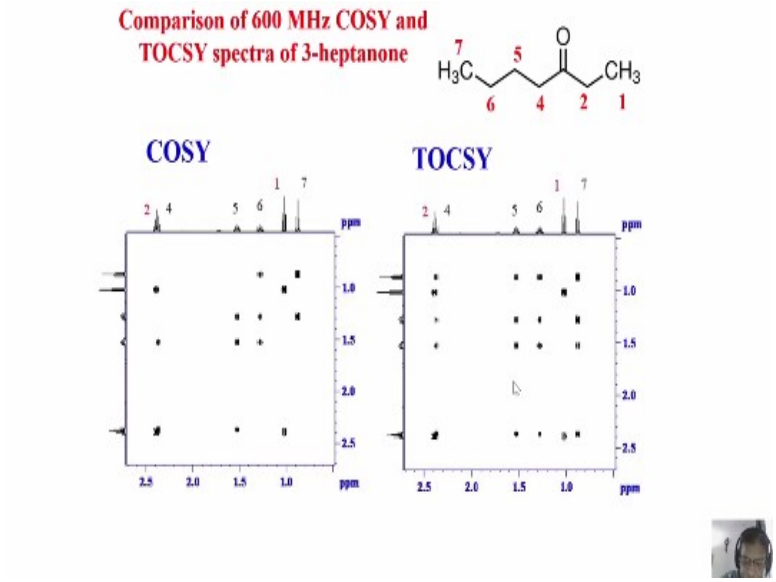

Now we will compare the same molecule TOCSY along with COSY. This is COSY, this is TOCSY. What is the difference between this and this? Remember, I said in the case of COSY first we identified proton 1; from the diagonal we came here; again we went to horizontal. Number one we are identifying, from 1 diagonal we came here, you got a peak, went here completed the square, and there was no other coupled partner, that was easy.

For these coupled protons we started with 7, we came down completed the square. From this diagonal again we completed another square. From this diagonal we completed another square, like that. We had to go step by step, to identify all the peaks in COSY. Whereas in the case of TOCSY it is different. In one shot; in one experiment all the coupled partners have been identified. This is the biggest advantage of TOCSY.

So you people have got some idea. Continuing with the COSY experiment, I told you today about some of the limitations of the COSY and for overcoming that and improving that, many challenges have been addressed by improving this sequence. There are number of variants of COSY experiments; like COSY 45, long range COSY, AE COSY, PE COSY, Soft COSY, DQF COSY, varieties of things.

But remember I always tell you have to record DQF COSY if you are doing the COSY; because that gives you double absorptive spectrum, very beautiful spectrum we saw, where resolution is much better compared to the normal COSY experiment. So the peaks near the diagonal are not suppressed. So that is the advantage. They will not get obscured. The diagonal peaks will not obscure the cross peaks.

And extension of that we went, this is called a relay COSY; multiple relay with a single relay, double relay, third relay and finally to the magnetization of all the coupled partners; which is called total correlation spectroscopy. So we show that total TOCSY is much better version, better experiment than COSY, where you can make the assignment of all the coupled partners in one experiment. So I have discussed about two types of correlation experiments, homonuclear.

Now we will go to heteronuclear correlation experiment and then take one or two examples of the inverse detection and also homo and heteronuclear J-resolved experiments. I will give some examples and then we will switch over to different topic. So, I will stop for the day.