Advanced NMR Techniques in Solution and Solid - State
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Module-49
TOCSY Heteronuclear 2D Experiments
Lecture - 49

Welcome back all of you. In the last class we discussed about COSY sequence; the phases of the diagonal peaks and cross peaks in the conventional 90-t1-90-t2 COSY. We understood diagonal picks are always in phase and cross picks are anti phase in both t1 and t2 dimensions. As a consequence, we see diagonal peaks, if they are very strong, give rise to tails and masks the peaks which are close to the diagonal and it has to be always represented in magnitude mode.

And it gives like a star like structure, especially for all the diagonal peaks and cross peaks and it is not suited for high resolution. We also understood and took the example as how to use the COSY especially for the identification of the immediate coupled neighbours. All those things we discussed, and another variant of the COSY, that double quantum filtered COSY. It is a 3 pulse sequence, first 2 pulses are there, first pulse always creates plus 1 and minus 1 coherence, second pulse always creates multiple quantum coherences. And we know we can put a multiple quantum filter, and choose the pathway from plus 2 to minus 1 by using either phase cycling or the gradients. And then we found out both diagonal peaks and cross peaks are anti phase in character here. And we can represent the spectrum in double absorptive mode. It is better for high resolution and we will not get into the spectrum, like you know the strong peak which is masking the peaks close to it and such problems are not there.

We took the example and compared one of the samples for both COSY and DQF COSY; fantastic resolution you could see for the DQF-COSY. Another advantage is you can filter out all the singlets, uncouple protons, solvent peak, reference peak, everything which is there can be filtered out, because you are going through a double quantum filter, they are all uncoupled singlets. So, as a consequence, you can filter it out. So, there are several advantages and conventionally, nowadays you need to use only DQF- COSY.

COSY is not necessary it is only for understanding purpose, better clarity, better resolution everything you can get better in DQF-COSY, than in conventional COSY. So, this is one of

the variants we discussed. Today, we will go further and see another 1 or 2 simple variants. And then quickly without going into the details, we will go to the other experiment, because we do not have much time and then with only a few more classes left, we have to complete the course.

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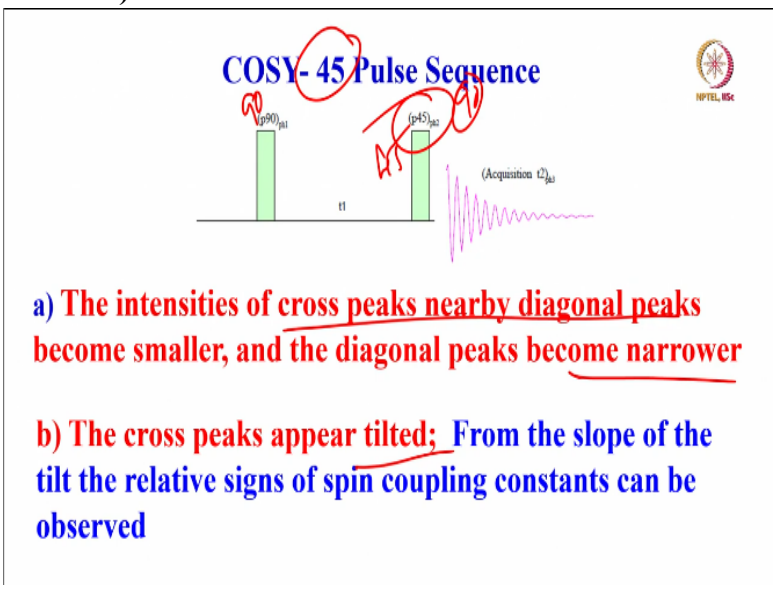

So, what I will do is I quickly show you what is COSY 45 sequence. It is in another sequence, where the second pulse is 45 degree. In the conventional COSY, this is 90, this is 90, but in the COSY 45, this is 45. Here advantages are the intensities of cross peaks near the diagonal becomes smaller and the diagonals become narrower. That is one thing; and the cross peaks appeared tilted. The relative tilting of the cross peaks gives you the information about sign of the coupling. Depending upon the slope of the displacement vectors of the cross sections, the directions in which the cross peaks are tilted, we can get the relative size of the coupling constants. This is another important thing which you do not get in the convention 1D NMR.

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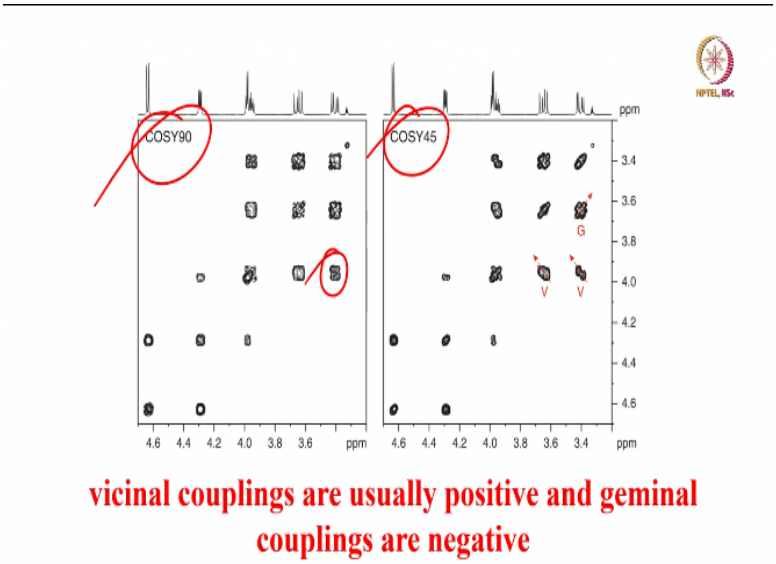

Look at it, this is a simple spectrum of some molecule you do not have to worry. This conventional COSY 90, this is COSY 45. Look here, especially this region of the cross peaks. Look at this one, some peaks are missing. Nevertheless, you see this appear tilted here, whereas this appear tilted here. It will give us some information about the sign of the coupling; the direction in which it is tilted that is, this is the slope is negative; this slope is positive. If you get some value of the coupling for this and this; and this and this, they are opposite in sign. So, we can get the relative signs of couplings. I am not talking about absolute sign, you cannot get it. But are the relative signs of the coupling can easily be obtained. So, for example, in this case, vicinal couplings are usually positive and geminal couplings are negative. So, as a consequence you can see this is geminal, this is vicinal.

So, from this, you can easily find out sign of the couplings. Of course we have the idea the geminal couplings are always negative and vicinal couplings are always positive. So, if you consider this tilt s negative sign, then this is negative. Like that we can get the relative sign of the coupling.

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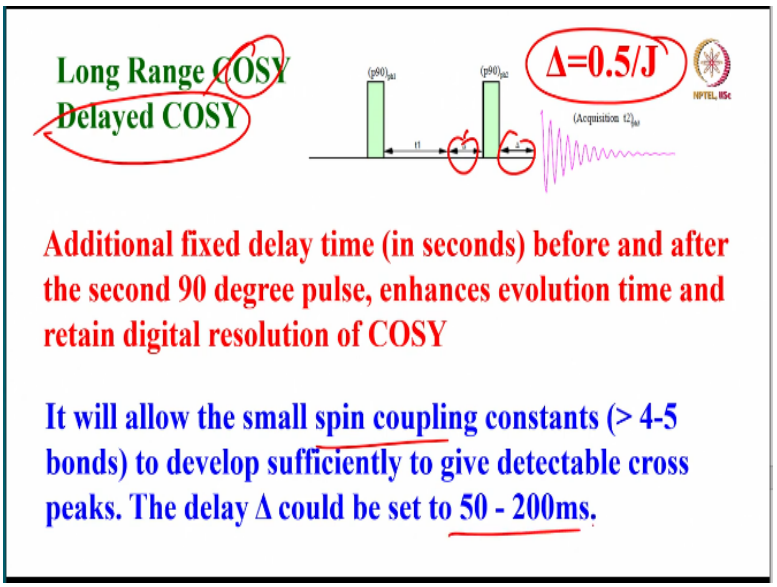

There is another experiment called long range COSY. It is also called delayed COSY, delayed correlation spectroscopy. What we have to do with the same 90 t1 90 t2 sequence, before and after the second pulse a small delay is introduced. This delay pertains to 0.5 / J. The advantage of additional fixed delay before and after enhances the evolution time while retaining the same digital resolution. That is another thing it is as good as telling you have enhance the digital resolution. What does it mean? You get better resolution, small couplings which you cannot get in the conventional COSY you can get it from this long range COSY. So, it will allow small spin coupling constants, greater than 4 bonds or 5 bonds away between 2 protons which are coupled, which was difficult to detect in a conventional COSY you can detect from the long range COSY, also called delayed COSY. So that is the biggest advantage of COSY, how do you set the delay, it depends upon 0.5 / J and you have to approximate it and take some average value.

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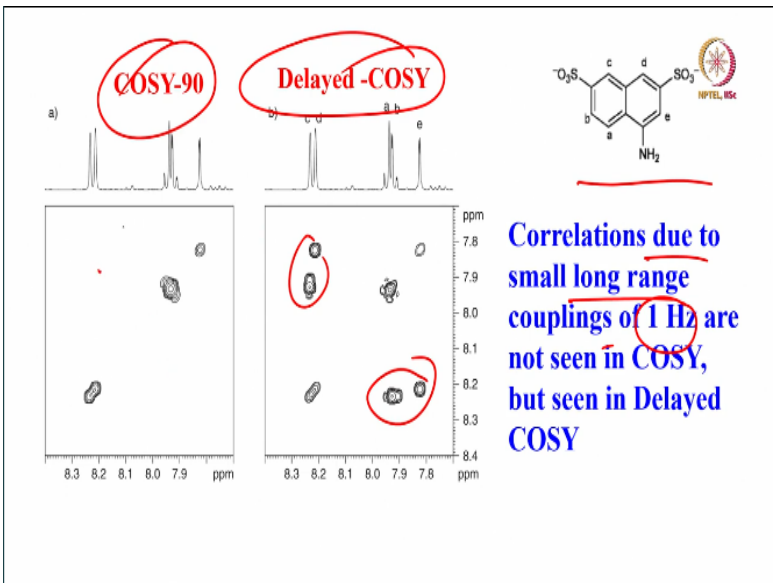

This is a simple spectrum of this molecule. Look at a COSY 90, this is a delayed COSY. Can you see additional peaks here? Which you do not see here. This is the advantage of the delayed COSY. The correlation to the small long range couplings of up to 1 hertz, around 1 hertz are not seen here in the COSY here and here. But you can see very small correlation is there between this and this, which is not seen here. But you can see that in the delayed COSY, that is an advantage.

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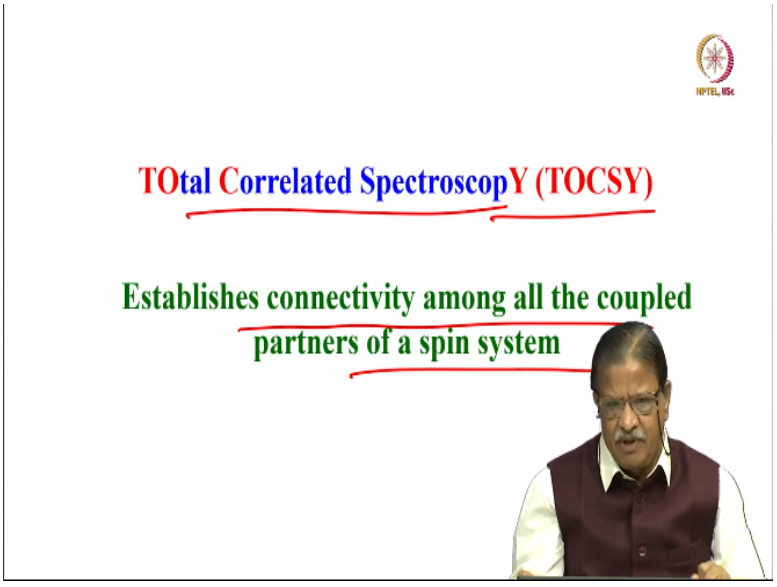

There are so many pulse sequence designed for the variants of COSY, like soft COSY, small flip angle COSY, AE COSY, exclusive COSY, varieties of things or a PE COSY, all are variants of the simple 2 pulse sequence. We do not need to go through all of these sequences, but simply remember, all these are improved versions to circumvent some of the problems which are inherently present in the 2 pulse 90 t1 90 t2 COSY sequence.

So, with this now, we will go to the next another experiment called TOCSY. TOCSY means total correlated spectroscopy. The name clearly tells you it will identify all the coupled spin systems in a single experiment. It establishes connectivity among all the coupled partners of a spin system. Remember, I told you what is a spin system. It is a coupled group of spins, where one is coupled to other is coupled. All of them are coupled among themselves. One of the coupling maybe 0 does not matter, but they should form part of the coupled spin system. Then you will see that polarization transfer takes place among all of them and establishes connectivity among all the coupled spins. COSY establishes connectivity with the immediate neighbours, whereas TOCSY establishes connectivity among all the coupled partners of a spin system.

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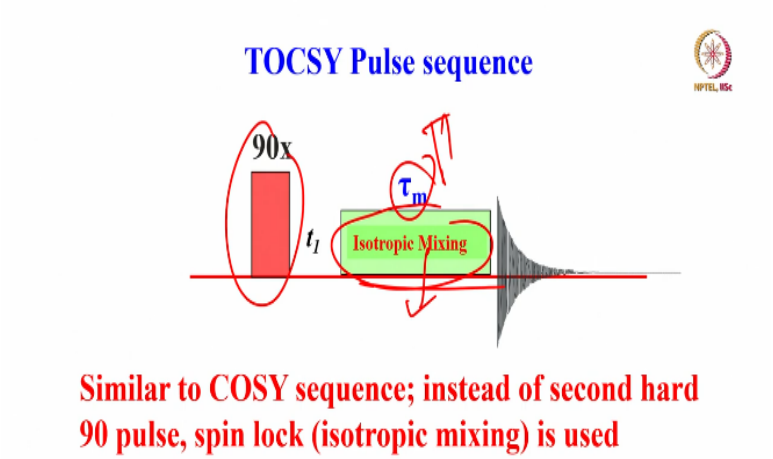

So, what is the TOCSY pulse sequence? This is a simple 90 t1 isotropic mixing Tm and FID. It is a very simple experiment and similar to COSY sequence, but only thing this instead of a second 90 pulse, you are going to have a broad isotropic mixing pulse, it is called spinlock pulse. It is used for isotropic mixing, called spin lock. Its pulse width could be out of several milliseconds.

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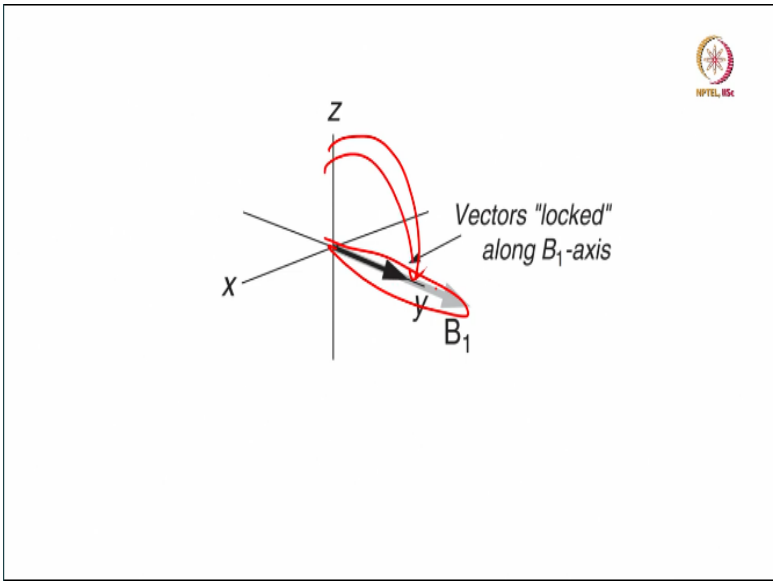

So, what is happening in this case is bring the magnetization from z axis to y axis by a 90 degree pulse. Immediately you can apply a pulse along the y axis, RF pulse, the B1 field now is along y axis where main magnetization is also there. Then what happens is the vector gets locked along the B1 axis, it is called spin locking. The spins are locked along the B1 axis; that is what happens.

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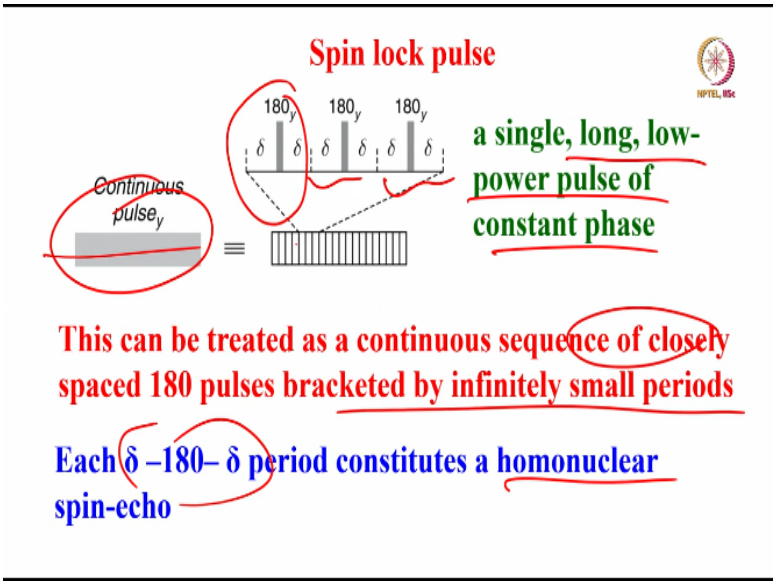

How does the spin locking works? It is a continuous pulse as I show you here, it is a sort of a wide pulse of RF with several milliseconds. Then how do you understand the working of this spin lock? You will divide this entire continuous pulse into number of spin echo sequences like this. You all know, the spin echo sequence, delta 180 pulse delta, it is a spin echo, another delta 180 delta another 180 delta. So, what you can think is, you can think this as a single long low power pulse of constant phase. You treat this as a continuous sequence of closely spaced 180 pulses bracketed by infinitely small periods. So, 180 pulse period small

periods, 180 small periods like that, you can treat it as if there are infinite number of spin echoes. In the end a continuous pulse can be treated like infinite number of spin echoes. So, each delta tau delta period constitutes homonuclear spin echo. You can treat it like that, it is not exactly like, that there are no gaps. There are no delays like that, but it can be treated. And if you take infinite like that, it can appear to be continuous.

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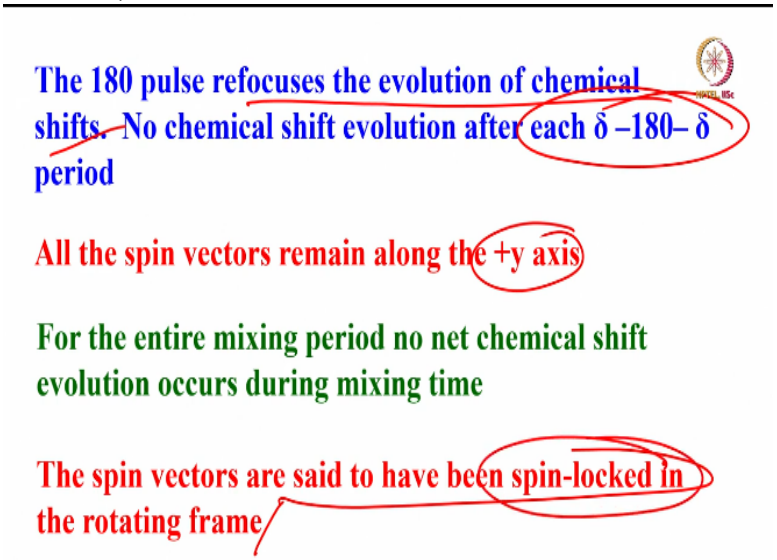

So, 180 pulse was what does it do, in the spin-echo homonuclear case? We discussed this using the product operators, it refocuses the evolution of chemical shifts; that is what 180 pulse does. And there is no chemical shift evolution during this delay. And you are going to have all the spin vectors along y axis, there is no chemical shift evolution, there is no offset, it is not going to evolve, the spins always get locked along the y axis, that is why it is called spin locking.

They remain along the Y axis; along the direction in which you are going to apply the RF pulse. So, that means this tau 180 tau sequence, now, we have treated for the entire mixing period has infinite number of such spin echoes. So, you can treat that the spin echo, spin vectors completely said to be spin locked in the rotating frame. But when you bring the magnetization to the xy plane, you are already thinking about the rotating frame; this we have discussed, what is the rotating frame everything in the previous course. So, I am not going to touch upon that. Those who are interested you can go back and look into that. So, the spins are locked along the B1 field in the rotating frame.

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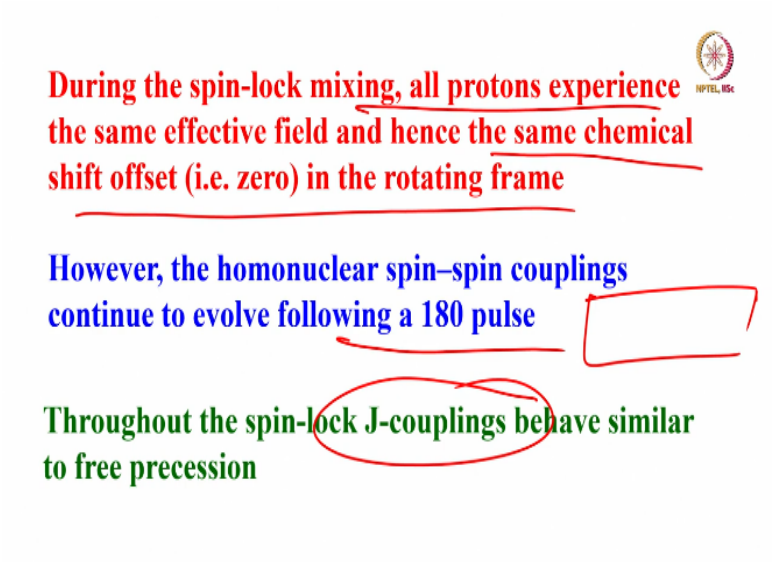

So, during the spin lock the protons experience the same effective magnetic field, all the protons experience the same effective field, irrespective of the chemical shifts. They experience the same effective field and hence the same chemical shift offset. What is that chemical offset now? It is 0 that means all protons have experienced 0 chemical shifts. There is no chemical shift at all; none of the protons will evolve in the rotating frame; that is what is going to happen.

So, what will happen to couplings? In the spin echo sequence when we discussed we clearly mentioned the homonuclear spin echo refocuses chemical shifts, but not the couplings. The couplings will continue to evolve following the 180 pulse. So, throughout the spinlock continuous period for over a several milliseconds, what is going to happen, chemical shift do not evolve, but the J couplings start evolving, as if there is a free precession. If there is a delay, there is a free precession, as if there is a rotation about Z axis, like that the J coupling starts evolving; only the chemical should do not evolve in the spinlock sequence.

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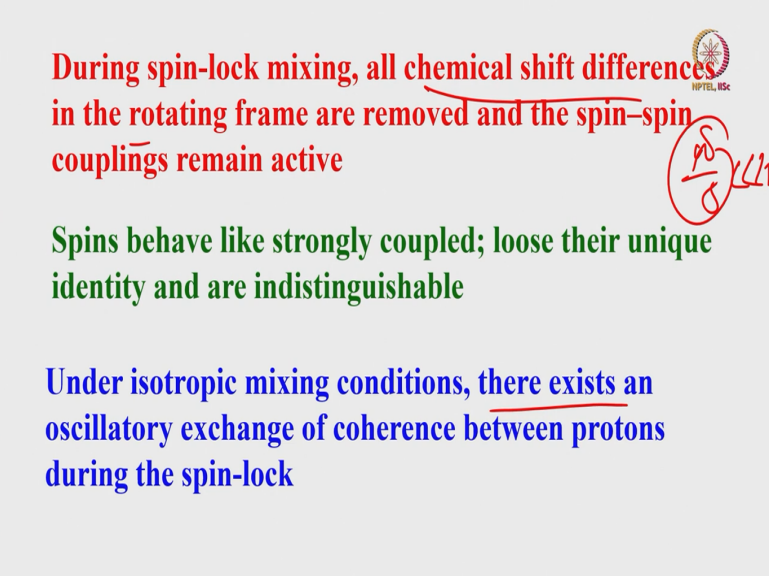

So, that means the mixing will happen. When the chemical differences are not there, it is 0 All the chemically inequivalent protons have no chemical shifts at all. That means chemical 0, but coupling is present. What does it mean? It means you are forcibly creating strong coupling effect; This is what we discuss under weak coupling and strong coupling when we discussed about spin nomenclature. When the chemical shift separation is quite large compared to the coupling, we call them weakly coupled spin system. Whereas, when the couplings are comparable to chemical shifts or chemical shifts are almost 0, much much smaller j over delta then what is going to happen. If delta / j you consider if delta is very, very small is much smaller than 1, then spin systems are strongly coupled. Now, you are forcibly creating a strongly coupled spin system under isotropic condition.

So, spinlock, what it does? it creates isotropic mixing condition and there exists an oscillatory exchange of coherence among the coupled spins, spins are exchanging energy; exchanging coherence. Although there are no chemical shifts, there is spinlock.

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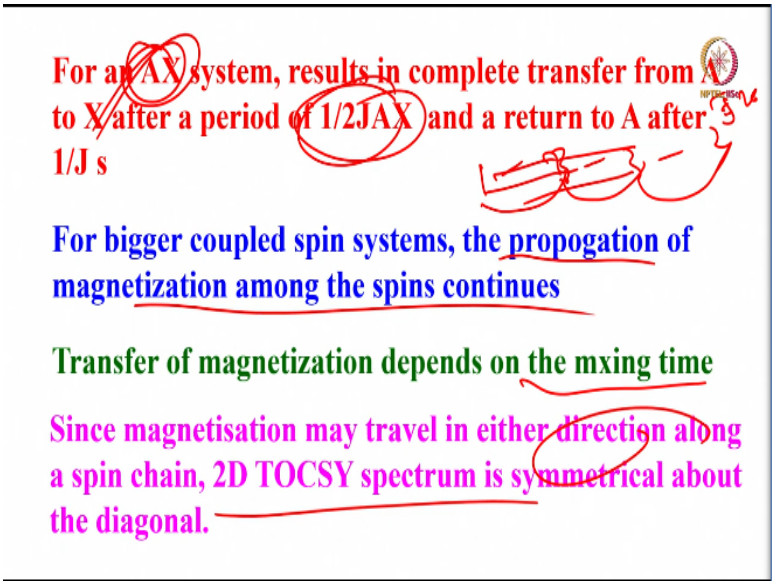

So, consider an example of AX system, 2 coupled spin system with a coupling constant of J. Then what will happen? Let us say spins are evolving for half of the coupling, J is let us say 20 hertz; at exactly 10 hertz, what will happen? assume A is giving its coherence to X, it will give complete coherence to X in half the J period. During the remaining half what will happen? It starts coming back, X will start giving to A.

So, during the 360 degree complete cycle, there is a exchange going on, it will give the energy coherence to the other coupled spin and comes back also in one full 360 degree cycle, this is what is happening in the AX spin system. If we take half JAX there is transfer of magnetization from A to X for the remaining half J it will come back. Of course, this is only for example of 2 spins. The propagation of the magnetization among the coupled spins continues with more coupled spins. It is not a simple explanation and it depends upon the mixing time. Let us say I have 1, 2, 3, 4 like that several spins a coupled, if I give a small delay the repolarization transfer is only from 1 to 2, if you give little bit more it will go from 1 to 2 and 2 to 3 and then bit more it can go to 3 to 4 like that, depending upon the mixing delay, the polarization transfer among the couples spin can be extended among all the coupled spins.

There is a limit for it if you extend beyond what will happen? it will start coming back there is no use. So that is what, an optimization you have to do. Since the magnetization may travel in either direction along the spin chain, it can go from here and come back; it can gofrom here come back. So, this transfer of coherence among the couple spins is bi directional, it can go forward and come back along the chain. So that means the coherences established among

the coupled partners is symmetrical with respect to diagonal; similar to COSY it is symmetrical with respect to diagonal.

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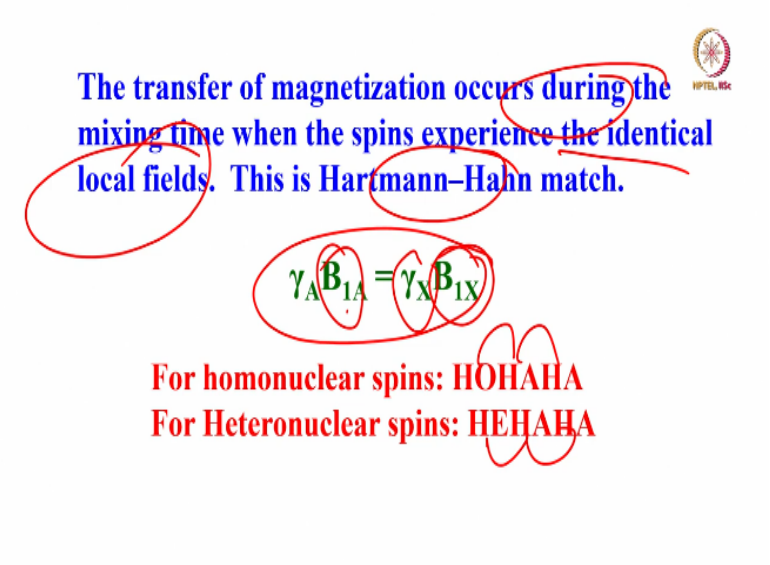

So, the transfer of magnetization occurs with the mixing time. The spins experience the identical local fields. When it will happen? the transfer of magnetization takes place when the spins experience the identical local field. They behave like identical spins, then spins will lose their identity; when there is no identity for the spins, all of them behave same. So, the energy transfer can take place; and what is this condition? This condition is called Hartmann Hahn.

Gamma A into beta 1, the power of RF power of A = gamma X into beta 1 of X, RF power of X. if this condition is matched, it is called Hartmann Hahn match. Then both the spins, I will take into example of A and X spins, both these pins have the same local field; and they have lost their identity, there is no chemical shift evolution in this spinlock period, they simply exchange the coherence. And this type of thing can happen for both homonuclear case and heteronuclear case.

The Hartmann Hahn polarization transfer, if it takes place between homonuclear spins, it is called homonuclear Hartmann on HOHAHA. If it is heteronuclear; it is called HEHAHA heteronuclear Hartman Hahn match.

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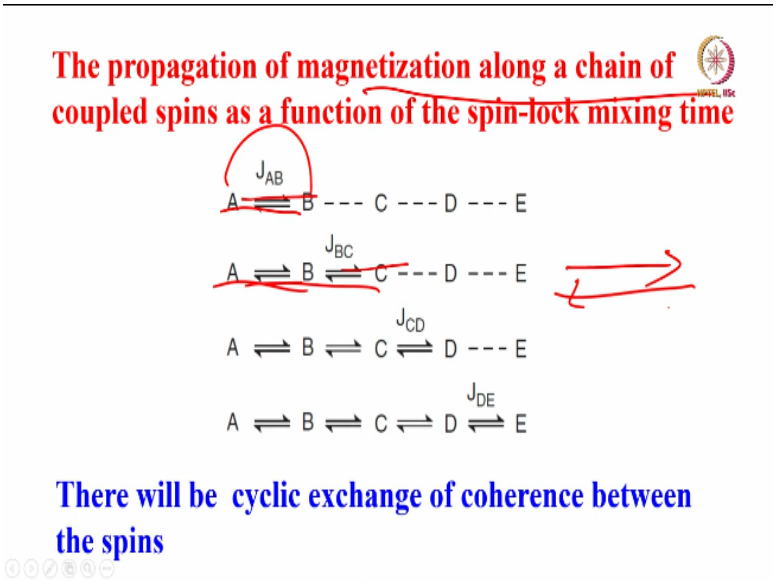

So, you can do it both for homonuclear case and heteronuclear case. The propagation of magnetization along a chain of couple spins as a function of spin lock time, you can explain like this. Let us say A is coupled to B it goes forward and also come back simultaneously, going on bi directional. So, further mixing time if it becomes longer A to B coming back similarly go to B, it comes back, B to C, it will come back.

Similarly, keep extending C to D, D to E like that. In each step, there is a forward exchange of magnetization and also backward. It gives and also takes back. So, there is a cyclic exchange of coherence between these spins.

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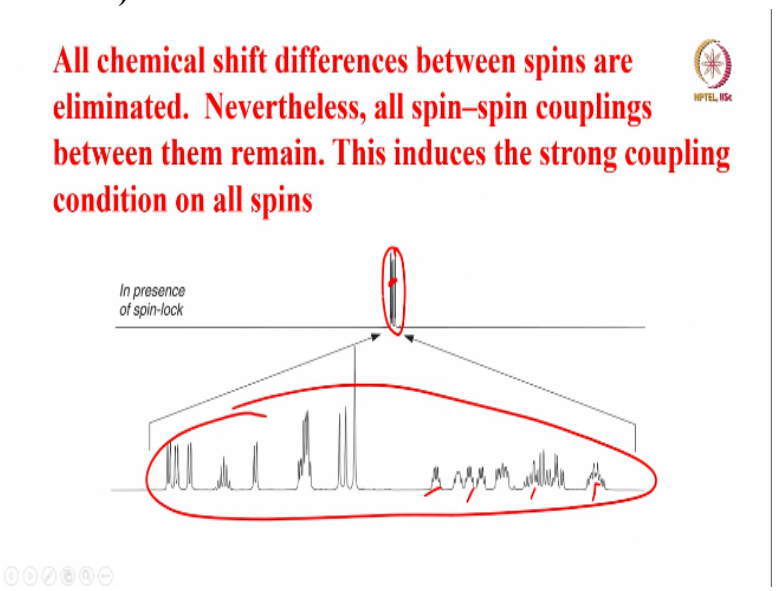

So, in short, you will do like this. Let us say this is a spectrum of some molecule, different chemical shifts are there here. Now during mixing, what is happening is, as if there is no

chemical shifts, all are like this, but they are coupled among themselves. So, you are forcibly or inducing strong coupling effect for this.

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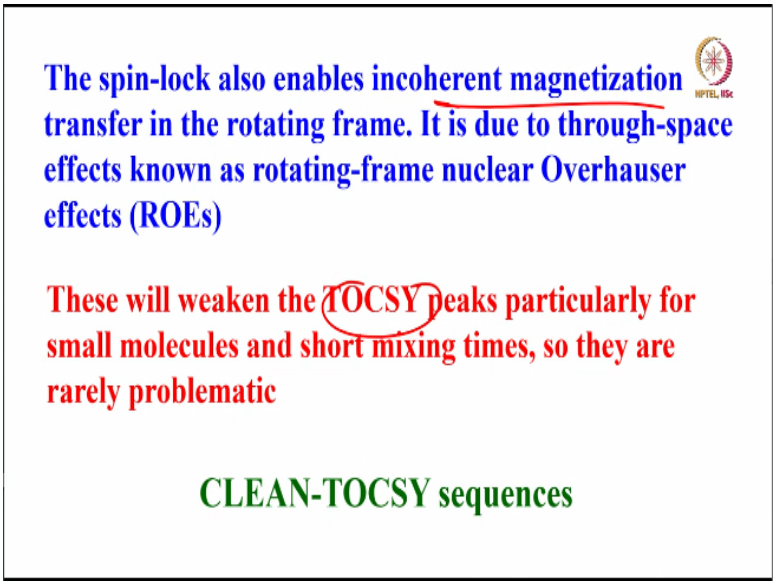

So, spinlock also enables sometimes incoherent magnetization transfer; that means it need not be through covalent bond, it can be through space also. That is a special type of experiment called nuclear overhauser effect in the rotating frame. We discussed NOE, but we have not discussed ROE. I think if there is a time I will come back and discuss at the end of this course, let me see.

But this is called rotating frame overhauser effect, where the polarization transfer takes place, not through covalent bond but through space. And those peaks can also come, then it will weaken the signal. TOCSY peaks will become particularly weak, especially in smaller molecules. They are problematic. Sometimes you get exchange peaks also; you get coupled COSY peaks also, you get in TOCSY. So, all these things are present there are various problems and this sequences over the years, number of TOCSY sequences have been improved, they are called CLEAN- TOCSY sequences; that has been improved.

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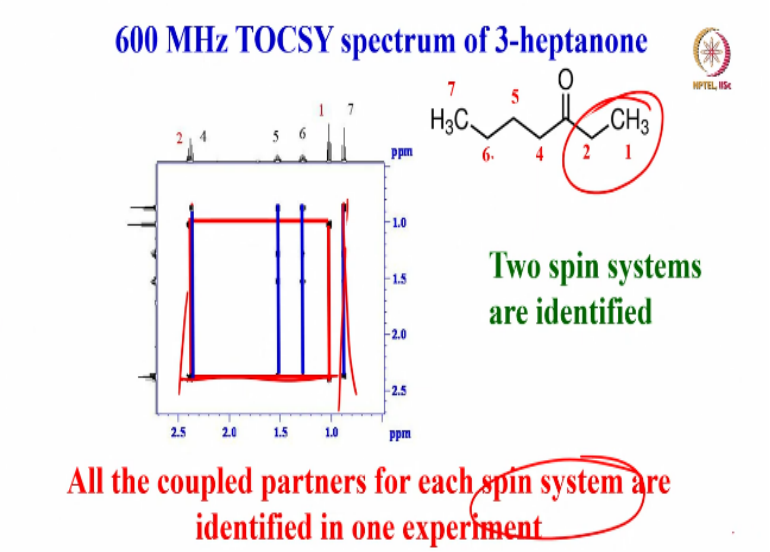

So, now, we will look at the 670 megahertz TOCSY spectrum of simple molecule 3 heptanone. And this is what it is. Now it is going identify the coupled partners in one experiment. What you have to do in COSY you have to go step by step; one is coupled to 2, 2 is coupled to 3, like this you have to keep going, like a ladder. Here, you do not have to do that, in one experiment the entire spin system is identified.

For example, here 1 and 2, it forms a spin system. See and no other thing is coupled here, you will not see any peak either in the F1 dimension or in the F2 dimension, only 2 peaks are there. These 2 are diagonals and only 1 cross peak is there. So, that means 1 and 2 are coupled; that forms a spin system. Now, go to the other one, you see 7, and this is the diagonal, it gives cross peak. This is another diagonal, it gives cross peak, is another diagonal peak one more peak is there here. So, 7 gives polarization to 6, 6 gives to 5, 5 gives to 4; see it just keeps going on like this. And finally, entire coupled spin system will gain polarization. See here to here, here to here, the polarization transfer taking place. So, all these things completely are in one vertical line, if you go horizontally like this, all spin systems can be identified. They are same, you can see, there are different chemical shifts along F2 and same along F1; they all form a spin system. So, you can go vertically or horizontally, either way, they are all coupled spin systems. So, the two spin systems can be easily identified by this method. This is what the TOCSY does. It identifies the coupled partners for each spin system in one single experiment.

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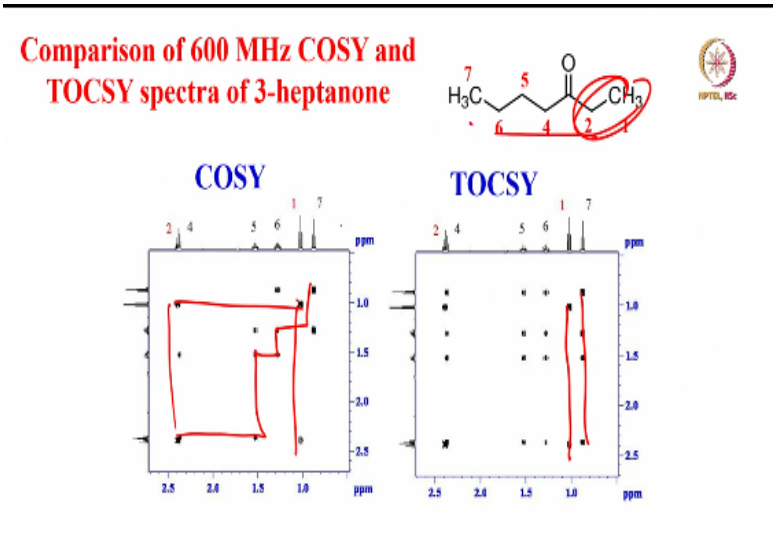

So, let us compare TOCSY and COSY. This is a COSY and this is TOCSY for the same molecule. Here just we understood it forms one and this forms one, two coupled spin systems here identified in 1 shot. Here what do we have to do, in this COSY systematically one to here, then here, here, then here, here like that it goes. Whereas it is simply identified in one shot here. Here again, you have to come here and identify. So two spin systems to identify in COSY especially in this case 1 and 2 are coupled, it is easy. But when 4 protons are coupled you have to go in a ladder like this, 4 steps, because each time identify only the immediate neighbour, whereas the TOCSY identifies in a single experiment; that is an advantage.

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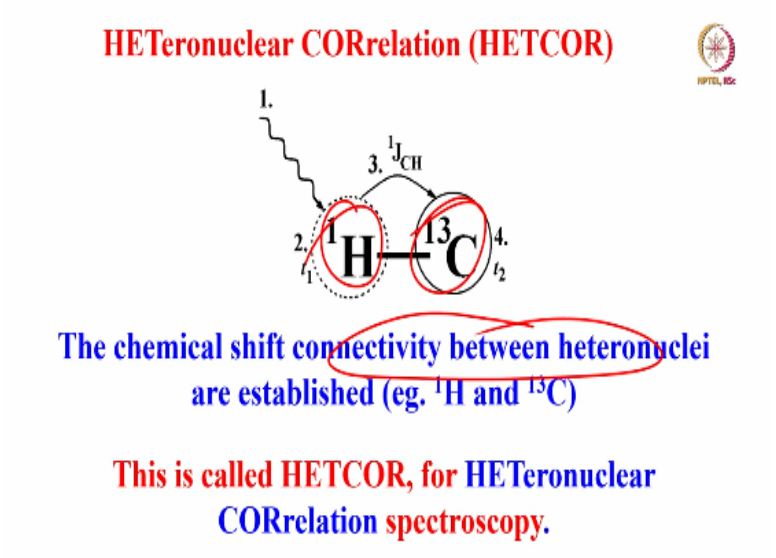

There are a number of TOCSY experiments called clean TOCSY sequences. Those who are interested can go to the literature; there is no time to cover everything. We will go to the heteronuclear correlation experiment. It is again a correlation experiment. Instead of

homonuclear we are correlating 2 different heteronuclei for example, you can correlate proton to carbon, proton to nitrogen, carbon and to any heteronuclear you can correlate.

So in this case, the chemical shift connectivity between 2 heteronuclei are established. What did you do in COSY identified 2 coupled partners, the chemical shifts were on the diagonal which you identified very easily. So, we could identify it established the chemical shifts connectivity among homonuclear spins in COSY. In this case, we are identifying or establishing chemical connectivity between two hetero nuclei. That is why it is called heteronuclear correlation experiment or called HETCOR, heteronuclear correlation spectroscopy.

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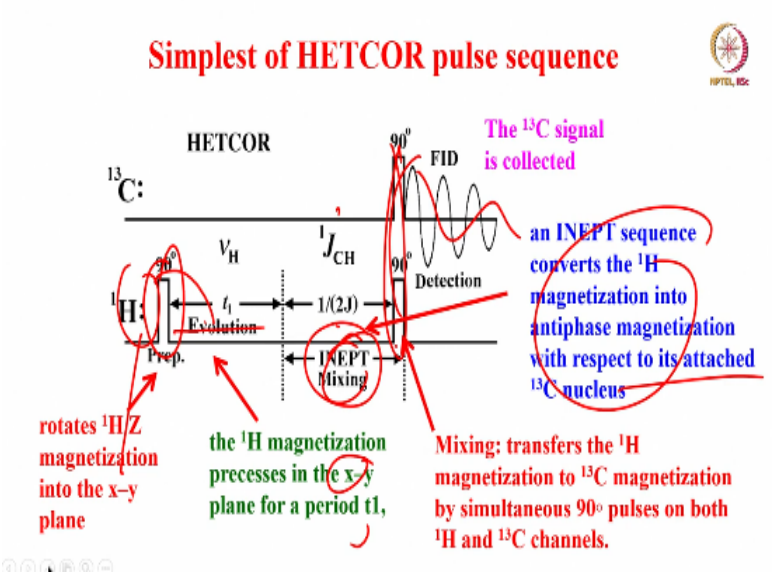

In a simplest form HECTOR looks like this, very simple experiment. A 90 degree pulse before that, there is a preparation period here, everything is done. It is in thermal equilibrium, 90 degree pulse rotates magnetization to the xy plane, here they start evolving; it evolves and precess in the xy plane for a period t1. Evolves under the influence of the chemical shifts of protons and also J coupling between carbon and proton.

Here there is a INEPT mixing, so because antiphase character, J coupling creates antiphase signals, so there is a transfer of polarization from proton to carbon, transfer takes place here, It is INEPT sequence. And then that happens and mixing transfers signal here; INEPT creates transfer of polarization from proton to carbon. And simultaneously you apply 90 degree pulse on both, the magnetization transfer is taking place and then here is the last pulse, 1H magnetization is in anti phase with respect to attached carbon 13, the INEPT sequence. And

then you transfer the magnetization to proton and start collecting the proton signal. Of course, we can have decoupled version and coupled version. Simply understand, in a short form, I want to say one thing, what we are going to do here is in a simple way, you bring the magnetization to xy plane allow it to evolve, during mixing transfer the magnetization from carbon to proton; start detecting the proton, you can also do the decoupling; this is the simple experiment for HETCOR.

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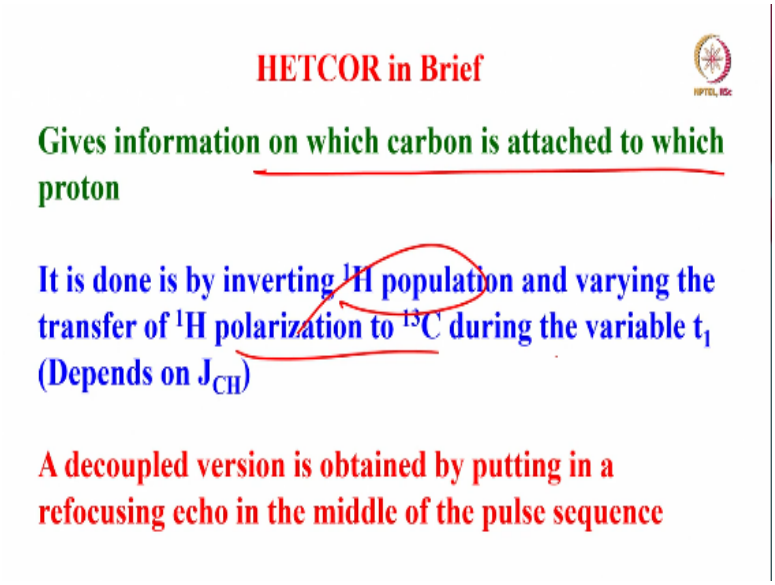

In brief, it gives information on which carbon is attached to which proton; you can also do by it is done by inverting the proton population and transfer of proton polarization to carbon 13 during t1. That is what happens in the t1 period. Although we didnt discuss in stretch. So, you can have the decoupled version, several variants are there.

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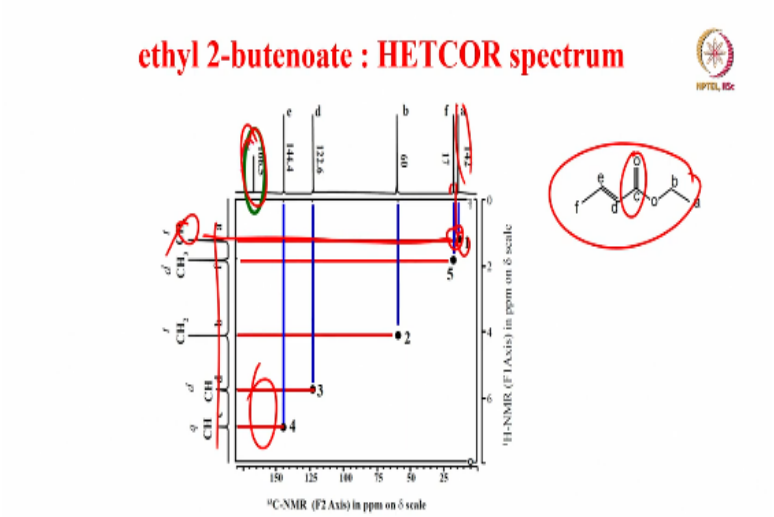

But nowadays, we do not going to do that; we will go to HSQC. That is why I do not touch up on it, I just wanted to tell you, there is a heteronuclear correlation experiment, in a different way we can do that. Look at this molecule, you have a simple molecule; in this axis it is proton; in this axis carbon 13. If you go along this axis, you get the proton chemical shift; go along this axis you get carbon 13 chemical shift.

So, it says proton chemical shift for 1 is here and its attached carbon chemical shift is here. So, simultaneously you can detect both nuclei; it correlates chemical shift of two heteronuclei. So, similarly, for this 5, 2, 3 and 4 like this, you can identify different chemical shifts of coupled heteronuclei, which are coupled. And one interesting thing is if you have peak like this there, it does not give any cross peak here; that means that is usually quaternary carbons, like this which are not protonated or non protonated carbons, Ipso carbon like that. In carbon 13 proton HETCOR spectrum, I am talking heteronuclear correlation, if there is no cross peak that means those carbons are not attached to protons.

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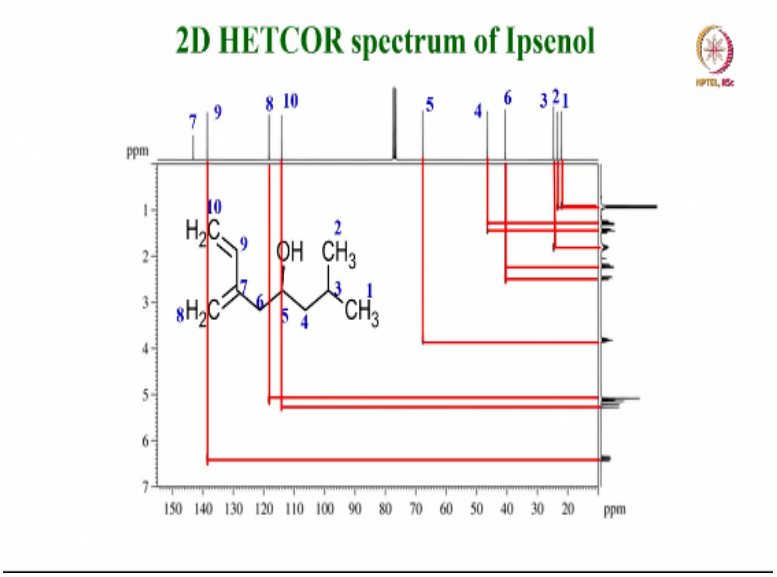

Remember this. Ofcourse, we can start seeing this for a bigger molecule. So, there is no time to go through it, we will not worry about that.

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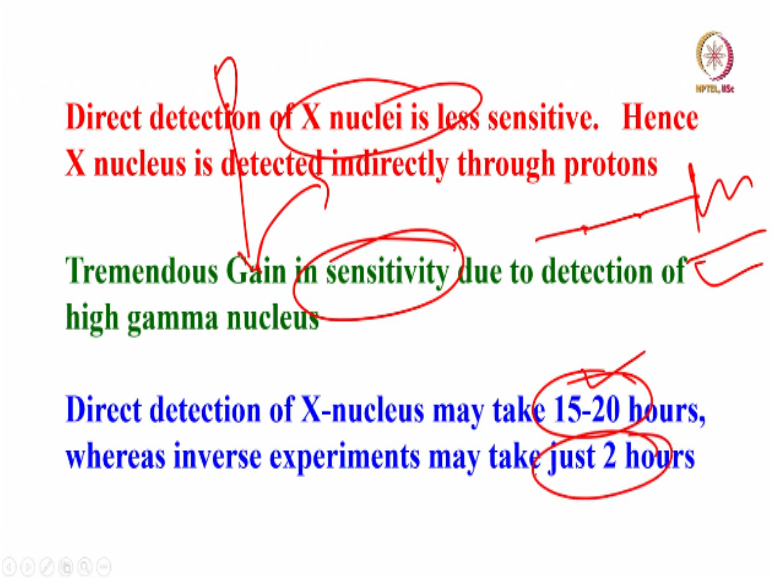

We will go to the next experiment called inverse spectroscopy. The previous slide is only just showing for a bigger molecule, how we can establish correlation of chemical shift of carbon and proton. It is for a bigger molecule. I will now introduce a new technique called inverse spectroscopy. What is inverse spectroscopy?

The direct detection of x nuclei is less sensitive. Carbon 13, as I told you, right in the first class, previous course, this course we have discussed sensitivity of carbon is nearly 64% smaller than that of proton in taking int account the natural abundance, it is 6400 times less sensitive related to proton. Hence the direct detection takes a lot of time, especially if you go to bigger molecule containing large number of dilute spins, or less sensitive nuclei carbon. On the other hand, if we do inverse detection by taking the magnetization of the proton give it to carbon and detect carbon or take it back to proton and detect in the indirect way, there will be a tremendous gain in the sensitivity, if you detect the proton. You can do that. Directly detect carbon, it takes enormous amount of time, take the proton signal give it to carbon and take it back from carbon and give to proton, then in between something has happened. So, we can see during this process there is some transfer of coherence. So, direct detection takes 15 to 20 minutes, on the other hand, the indirect detection, where you transfer the magnetization for proton to carbon takes less time.

Of course, this heteronuclear correlation experiment there are several ways you can get, from carbon to proton take back to proton, proton to carbon, take it back to proton, and so there are 3, 4 or 5 different approaches. Generally what is adopted in the inverse experiment is

Coherence is transferred from proton to carbon, then from carbon taken back to proton and proton is detected by decoupling the carbon 13.

But, simply remember, the inverse detection is very, very advantageous because direct detection of this thing takes 15 to 20 hours. Whereas inverse detection just take 1 to 2 hours enormous saving in the time, because there is a gain in the sensitivity.

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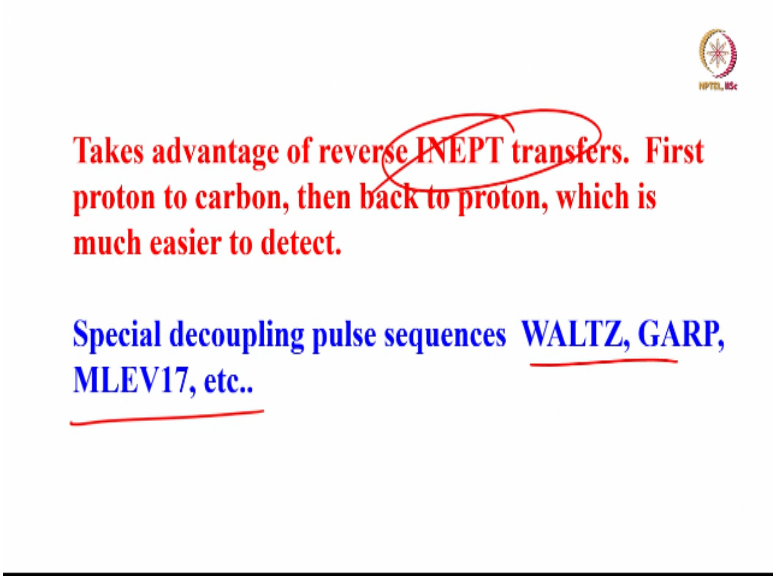

It takes advantages of reverse INEPT for transfers, first proton to carbon and then back to proton, which is easy to detect. And you can do broadband decoupling, simultaneously Varieties of pulse sequences are there for decoupling WALTZ, GARP, MLEV17 etcetera, we can discuss those things. There is broadband continuous wave decoupling; that is commonly used. I told you about heteronuclear decoupling; that can be adopted.

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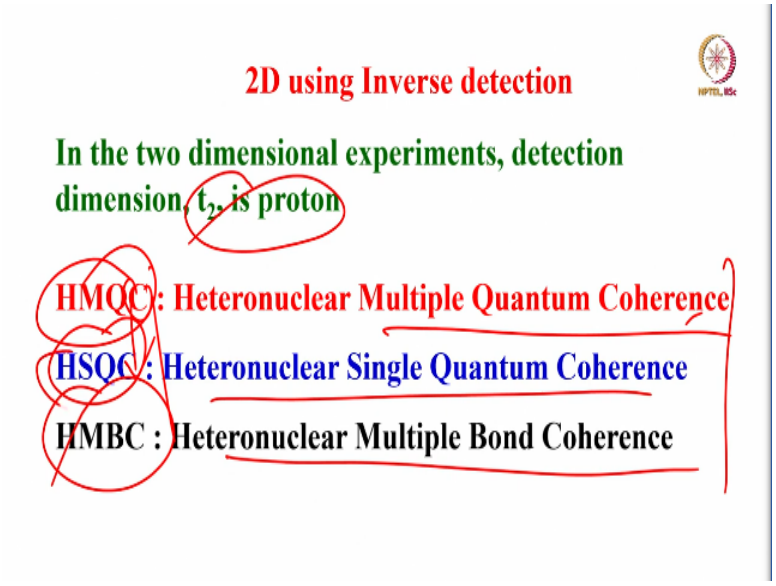

In 2D inverse detection, in two dimension experiments, the detection dimension is always proton. Why? It is advantageous to detect proton, which is highly abundant spin half and high gamma nucleus, compared to carbon 13. That is why in the inverse experiment detection dimension is proton, t2 is proton. In between you do some jugglery, take the magnetization give to dilute spin, take it back and all those things you do. But final detection is proton, while decoupling carbon or nitrogen, whatever the nuclei.

So, there are various experiments like this identified; they are HMQC, HSQC, HMBC etcetera. This is called heteronuclear multiple quantum coherence, heteronuclear single quantum coherence, heteronuclear multiple bond coherence. There are n numbers of such things are; there are varieties of modifications of experiments of these things. So, we quickly look at HSQC and HMBC today.

Of course, HMQC gives similar information like HSQC, but litttle subtle modification is there will not go to HMQC but discuss only HSQC and HMBC. We will discuss a little bit more with some examples.

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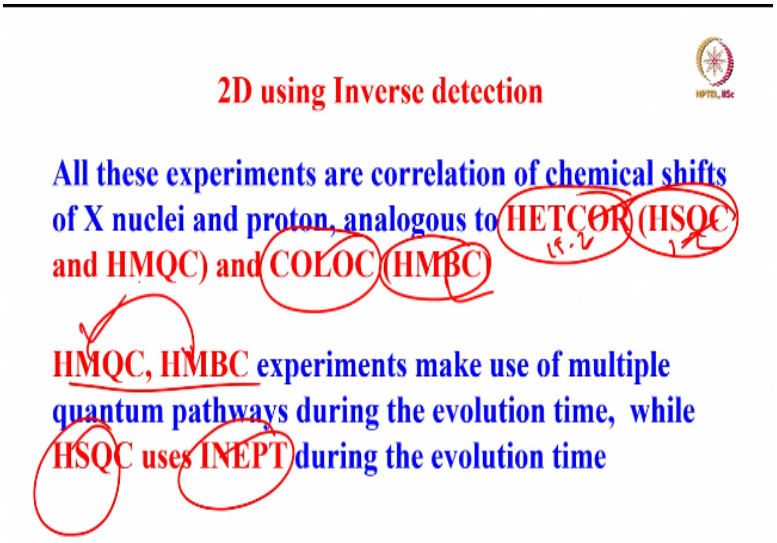

So, how does 2D inverse detection is done, inverse detection. All these are direct correlation experiments, there is a direct correlation. For example, I told HECTOR but directly can detect proton in one dimension carbon in other dimension. It is heteronuclear correlation of two different heteronuclear spins; chemical shift correlation, likes heteronuclear COSY. The same thing inverse version is called HSQC. This is faster, this will take 15 to 20 hours; this will take 1 to 2 hours.

Similarly we have HMBC, the Inverse experiment, the direct detection experiment for this is called COLOC; correlation of long range coupling; long range correlation; long range coherence can be correlated. The inverse experiments, have some of the direct experiments also, one to one analog of this you can find, like HMQC, HMBC. Both of these things make use of multiple quantum pathway. HSQC during evolution time or HSQC makes use of single quantum pathway, and it uses INEPT also, during evolution time.

Very important thing. These 2 makes as multiple quantum pathway, HSQC male use of single quantum pathway, and also it uses INEPT during evolution time.

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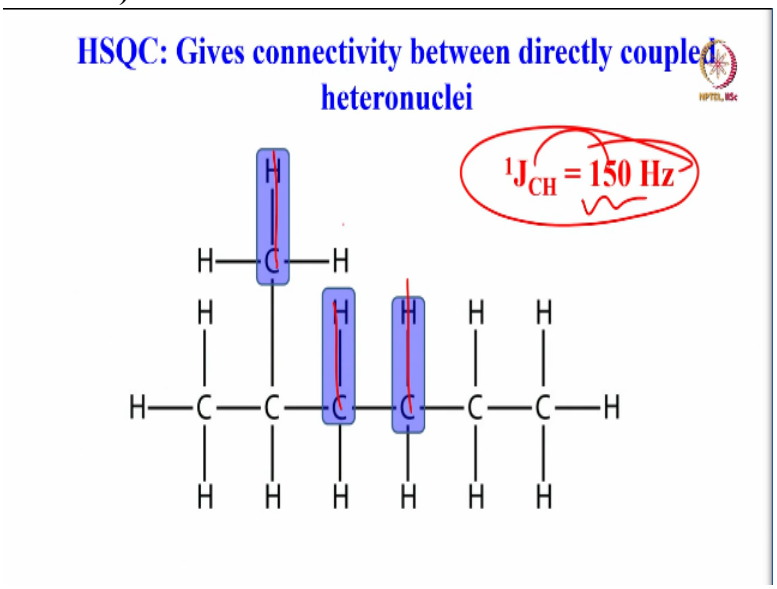

So, what HSQC gives is connectivity between directly coupled protons analogous to HECTOR. See if I have so many carbons that are inequivalent, I can say this carbon is coupled to this proton, this carbon is coupled with this, this direct correlation information it gives. So, it is based on direct coupling one bond proton carbon coupling strength, which is approximately of the order 150 Hertz. So, you can use that, and then set up the experiment. If your mixing time is set for this, it so happens you will establish the correlation among directly coupled carbons and protons, two heteronuclei.

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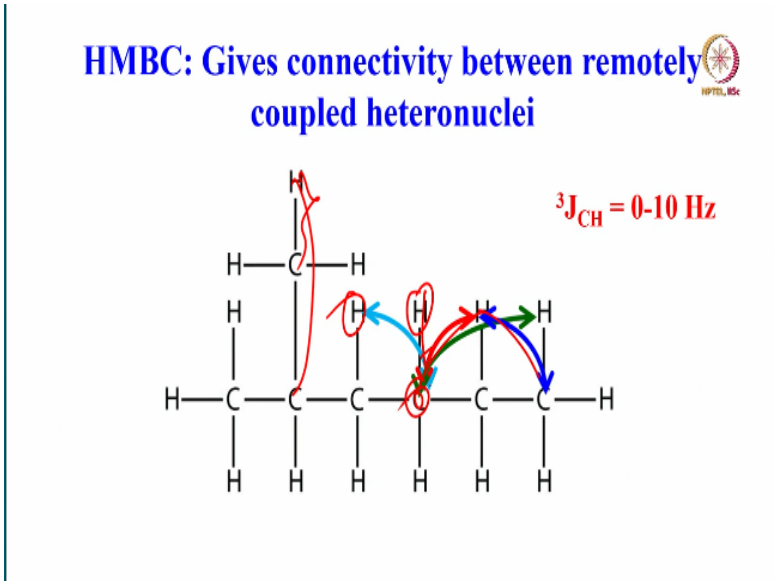

HMBC gives connectivity between remotely coupled nuclei. Same thing if I want to do, for example, let us take this carbon, correlation to this, HSQC does. For this carbon to this carbon, which are 2 bonds away, we call long range, or multiple bond correlation, HMBC does this. This is what HMBC gives. This HSQC gives, whereas this to this HMBC gives. Similarly this to this HMBC gives, HMBC gives this also, and it can go to 3 bonds or 4 bond away. For example, this it can give. So HMBC gives multiple bond correlation; HSQC gives correlation between directly bonded heteronuclei. So, with this we will come back and then discuss something more. The time is getting up, we will come back and discuss HSQC bit more. Take some examples of HSQC spectra and some HMBC and afterwards we can also go for some other experiment like J-resolved and if possible, INADEQUATE, all those experiments we can consider. So, we will come back and see quickly. For today we have discussed about correlation experiments in homonuclear case, and heteronuclear case, direct detection, like COSY, TOCSY and HETCOR. TOCSY gives the direct connectivity among all the coupled partners in a single experiment. COSY establishes connectivity, chemical shift connectivity among the coupled partners, immediate coupled partners or immediate neighbors.

Heteronuclear does a similar experiment direct detection, whereas, the directly bonded proton carbon can be identified. And this direct detection takes enormous amount of time, the improved version is there, that is called inverse reduction, where the magnetization of the proton is given the carbon and taken back the proton and proton is detected. In this process, we can establish the correlation between proton and carbon, other heteronuclei also, but this universe detection is more sensitive and is much faster.

So, there are various experiments. For inverse and direct detection, there is one to one correlation for HETCOR, its is equivalent to HSQC; COLOC is equal to HMBC, etcetera. With this I am going to stop here, we will come back and continue, take one example of HSQC analysis, etcetera in the next class. Thank you.