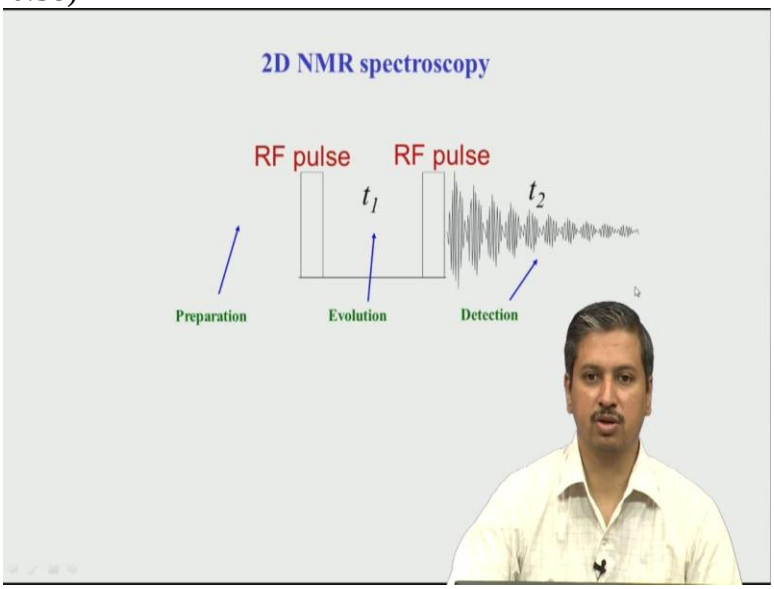
Principles and Applications of NMR spectroscopy
Professor Hanudatta S. Atreya
NMR Research Centre
Indian Institute of Science Bangalore
Module 5
Lecture No 23

So in this class we will now continue with 2D NMR principles, how the 2D NMR data is recorded and will start to now look at a few 2D spectroscopy experiments specifically. So let us start again with the basics of the principles of 2D how it is acquired is shown here.

(Refer Slide Time: 0:38)



So we saw this is a very simple schematic scheme which has basically RF pulse applied and then you evolve and during this period we (trans) we said we can transfer the polarization or a magnetization to be and then it gets detected. So again remember we saw that this is a non-selective in nature. Meaning this is not just applied to one spinning particular, it is applied to all the spins in the molecule.

Similarly this is applied to all the spins, so because of both the spin on both sides we saw that there are four combinations one is called two are called diagonal peaks and we saw two will be across peaks that is off diagonal. Now let us come to the more detail of how experiments are actually recorded so what happens is this is called a preparation period and this is just a period before the pulse. So this is similar to this is same as the relaxation delay.

So if you recall when we said for 1D NMR when you put a sample in a system in a magnet you have to wait for some time because during that period the equilibrium magnetization along Z axis has to be built. The building process takes some time that is t1 relaxation and you wait about 4 to 5 times t1. So that is called a preparation period, you are preparing the system for applying a pulse.

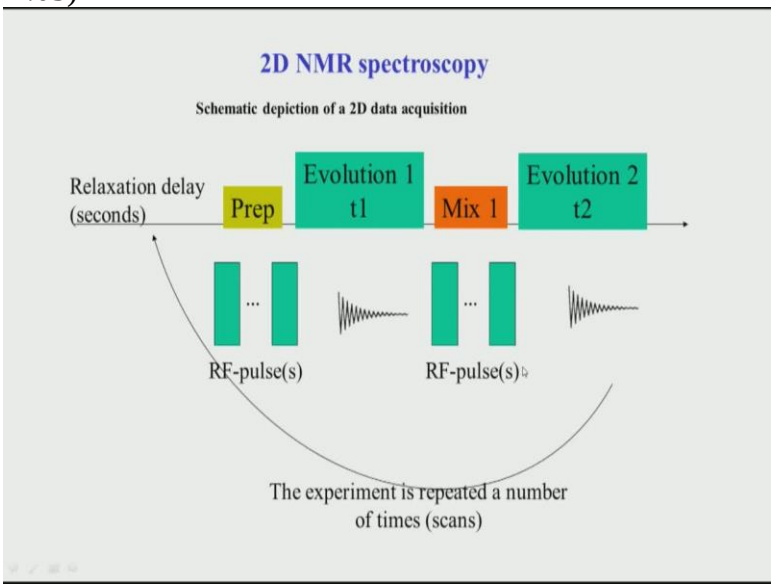
When you apply the pulse there is excitation then evolve evolution, evolution meaning the chemical shifts are now evolving or the oscillations are taking place in the X and Y plane and during this process it is also relaxing back to Z axis and also it is dephasing in the XY axis. So relaxation basically is happening during this time also the most very important thing which is going on is a transfer or the coupling of the chemical shift one chemical shift whichever is exited here to another spin to which it is interacting by J coupling.

Now after this period we now apply a second pulse which is again non selective in nature it is applicable to all the spins but now this brings into XY plane only those spins which are along Z. So that means during this transfer of magnetization it has transfer from A to B but the B when it is transferred the B is not along XY it has been along Z. So the transferred part is along Z but the non-transferred part is along A along X.

So this transferred part which is along Z has to be brought into the XY plane by applying a second pulse. So this is called a mixing pulse. Why is it call a mixing? Because we are mixing the magnetization from A to B because the transfer took place here. But transfer does not mean it can be detected because after transferring the second spin is still along Z axis. So that has to be brought into XY axis plane by applying a pulse.

And that is called mixing pulse and then after that we start detecting the spin evolution of the seconds nuclei second nucleus which has now been excited. And that is a physical detection, that is called the FID. Means signal is inducing a current or EMF in the coil similar to what we saw in 1D, so this is actually like a 1D FID.

(Refer Slide Time: 4:03)

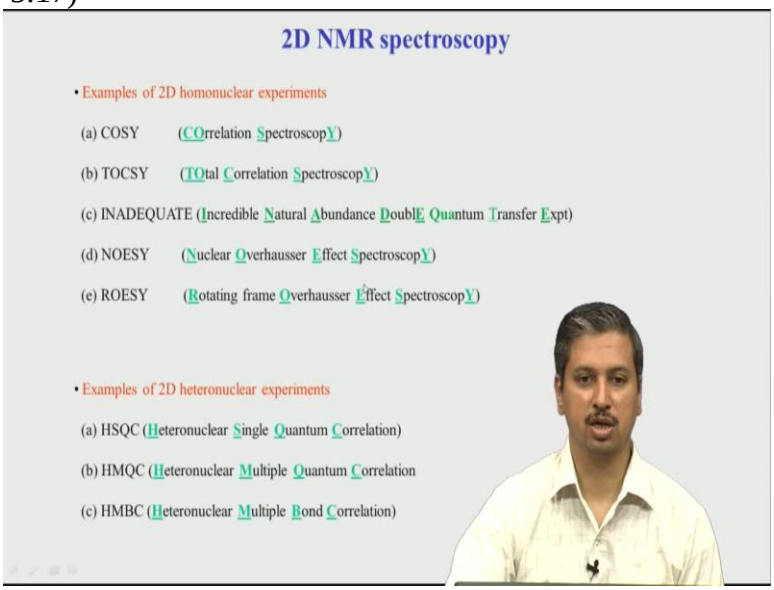


So this scheme is shown again in this picture. So we have a preparation period which before applying the pulse we apply the pulse we have an evolution period and then we have a mixing time mixing pulse and then it is an evolution again because of the second spin. So this process is repeated n number of times. So this is the preparation period which is relaxation delay. So now we are applying RF pulses. So this process is repeated n number of times so that is called scan.

So one scan means one single whole process which goes from relaxation delay to the end of the acquisition **.** Acquisition means end of this. Then you again go back to this period (bef) when during the relaxation wait for the complete relaxation delay then start applying a pulse. So this like that you can keep on doing this n number of times and that is how we call the number of scans.

So this is basically called signal averaging because we are trying to get rid of the noise by averaging out the noise. So signal is enhanced, so signal to noise is increased. So this is basically schematic.

(Refer Slide Time: 5:17)



Now now we will start moving into more specific experiment. So in this course we are not going to cover the entire 2D NMR gamet of experiments because that will take basically take a very large amount of time so I would only focus on a few key important experiments 2D NMR which is typically used by 90 percent of the chemists who use it with their organic chemistry for organic molecules also it is the experiments which are used by peptide chemist because peptides also we do work with homonuclear NMR.

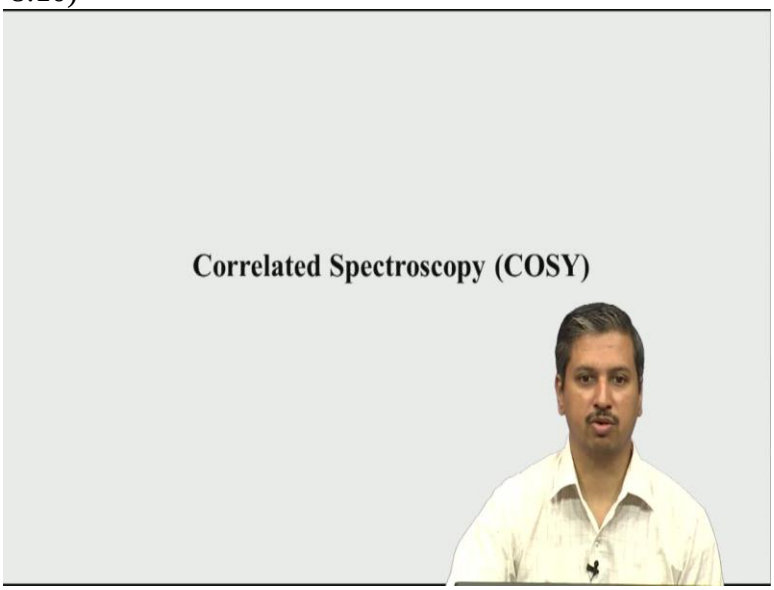
So this is called as a homonuclear NMR experiment. Homonuclear meaning remember is proton to proton correlation or carbon to carbon correlation both are the same nucleus. So we use the word homonuclear. then we will look at the hetronuclear meaning we are now using carbon and proton together and one axis will be carbon, another axis will be hydrogen or you can use both carbon and nitrogen or any combination of hetronucleus.

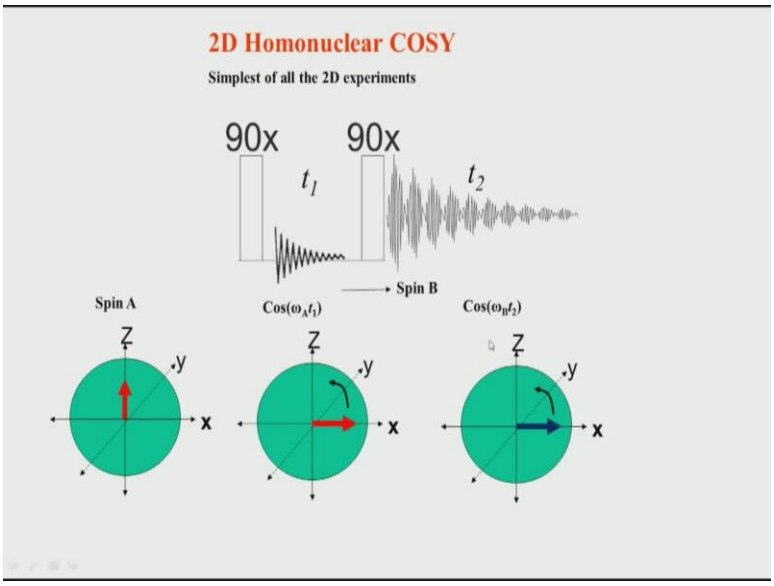
But 90 again 90 percent of the experiments are typically proton to carbon but proton to nitrogen is also very popular in the case of analyzing when we analyze biomolecules. So we look at specifically these three experiments only 2D NMR and this basically list is lot of principles of 2D NMR. And when we do the application part this is also all this will be useful because as I said mainly these are the set of experiments used by maximum number of people for their research analysis.

So now if you look at the names NMR is full of jargons full of names acronyms so that this acronyms one should know from where they come so that is what is shown here so COSY is a most famous 2D NMR experiment. In fact one of the first experiments which was published and 2D NMR started its beginning from there that is by (()) (07:19) but the ideas were already known before (()) (07:22) published so the idea of COSY the mould COSY comes from this correlation spectroscopy.

TOCSY comes from total correlation spectroscopy. So will see this as we go along. So you can see there are many interesting acronyms and these have become basically the jargon in NMR. And similarly here we use the word HSQC which says Hetronuclear Single Quantum Correlation. So the words each one of them sounds very technical so we would not go into details of why this what is quantum, single quantum and so on. Because the tech four courses more on focus on the basic aspects but we will see know we will see later on how this experiments are useful.

(Refer Slide Time: 8:10)





So let us start from the first experiment which is correlated spectroscopy or you can say correlation spectroscopy. So remember in NMR every experiment has an associated with it a pulse sequence. So we defined that pulse sequence earlier, pulse sequence is basically the blue print of how an NMR experiment works, ok. So example of what is the pulse sequence for a 1D is nothing but is a simple one pulse. And what is the pulse sequence for a 2D it will have not just one pulse it will have a pulse it will have some delay which is called evolution then it will have another pulse which is called mixing and the acquisition.

So you see there are series of pulses applied and it delays in between. So we use the word pulse sequence for the complete sequence. So now let us look at there is homonuclear COSY experiment, so this is how it works so you have a 90 degree pulse so now we will start loosing little bit more technical terms. So this pulse which I earlier labeled it as RF pulse is basically a 90 degree pulse means RF flip angle, the flip angle is 90 degree and it is applied along the X axis.

So what basically it means that that magnetization which is from Z axis before this pulse is flipped by 90 degrees and it comes to the XY plane. And then during this period it evolves so this is what we already saw so this in fact is already the scheme for COSY. So although we did not use the word COSY earlier but what was happening the illustration what was being shown were the general 2D scheme is actually was taken from this scheme for COSY.

Then you apply the second pulse which is a mixing pulse. And remember during this period there are three things going on; number one it is a chemical shift evolution isolation in the XY plane. Number two relaxation to the Z axis as well as in the XY plane and number three, transfer of magnetization to the second spin a neighboring spin, ok. So this is the simplest of all experiments in 2D, nothing can be simpler than this and this in fact the one of the first ideas how 2D was proposed.

So let us see now in a vector diagram what is going on. So in NMR is very useful to analyze this kind of pulse sequences with this kind of vector diagram. Because this gives you more clear picture of what is going on with this vectors with the magnetization remember this is an equilibrium magnetization and that is all we have to start with the experiment. We cannot, we have to come back to this position again to start the next experiment next meaning next scan.

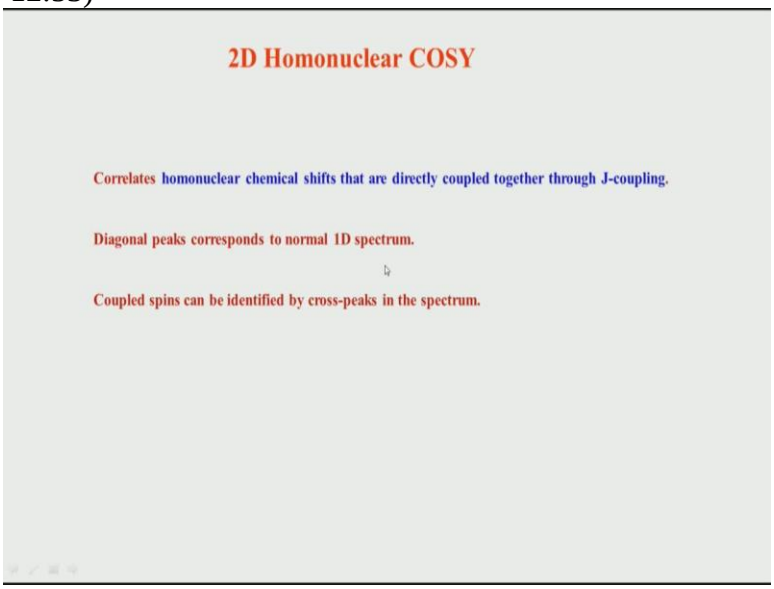
So every scan again remember in the previous slide we saw every scan you have to come back to equilibrium and for that you have to wait for 3 to 5 times the relaxation delay sorry t2 3 to 5 times the t1 relaxation and that is called relaxation delay and that period is about few seconds or it can be few tends of seconds depending on the t1 value. So for therefore we will start from here which is already assuming that it has come to equilibrium, then I apply a pulse.

So when I apply a pulse the magnetization has come by 90 degrees from Z axis. And this is what is basically because of this RF pulse. And remember I am ignoring this where it is along X or Y, I will simply use the word XY means it is in the horizontal XY plane. But technically speaking this is not really the correct way to say but for understanding purpose we can say generally that whatever will be the pulse here whether it is X or Y such still we use the word that it is in the XY access plane.

So now when it has come to the X axis let us say the Cosine omega t is the evolution part that is the how it is FID is evolving chemical shift is evolving during t1. So that is shown by this arrow black color here and it is simply turning around. So this is oscillation which is going on in the XY plane because of this chemical shift omega A but remember there is also what is happening is the dephasing in the XY plane and that is not shown here and dephasing is basically the t2 relaxation.

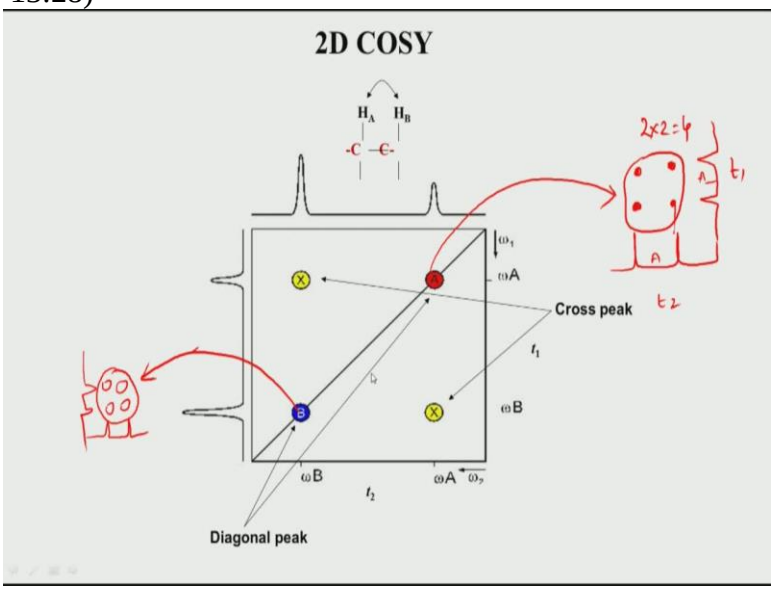
So that part I am not going to show here. All now I am going to talk about is how this frequencies are basically happening. So this is how it is happening in the XY plane. Now you transfer to second spin B and the second spin B now gets excited and that is shown with this black arrow bluish arrow. Now this bluish arrow is basically now the spin B and that is now evolving in the time. So this is again repetition sort of of the general 2D schematic.

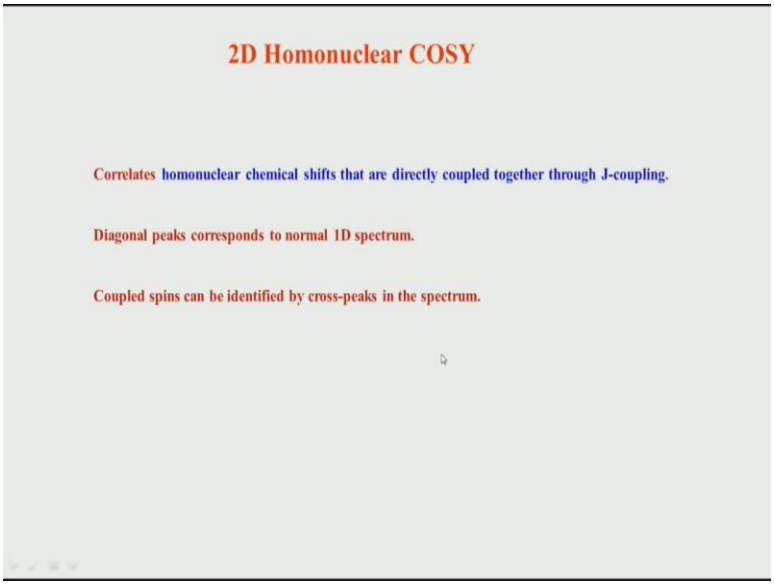
(Refer Slide Time: 12:53)



Now let us go and see what kind of spectrum we get. So now what is the conditions for a 2D COSY to happen? The 2D COSY to happen is that they should be coupled by J coupling. This is the most important thing, they should be directly coupled to protons. The if they are directly coupled together through J coupling then only you can expect to see any cross peak or peak between those two atoms.

(Refer Slide Time: 13:28)





So as we saw in the schematic picture that the diagonal peak corresponds to the 1D spectrum let us see this in the next slide here is what is shown here. So if you look at it these are nothing but the same frequencies appearing both on this axis and as well as this axis. So that this is nothing but a simple 1D information. Suppose I have recorded a 1D of this molecule I would have got two peaks at A and B right? So hydrogen atom two hydrogen atoms and A and B protons. So I would have got two peaks, ok.

Of course there would have been a J coupling see this would be a doublet, this would have been a doublet. So in a 2D also although we are not showing here there is a doublet hidden here, there is a doublet here, there will be a doublet here in both the dimension. So remember in NMR the coupling will appear in 2D NMR the coupling will appear in both axis. Why because if you go back to this picture this is also like a 1D and this is also like a 1D.

Therefore if there is coupling here splitting because of J coupling the (spe) J coupling splitting comes here also ofcourse this is 1D standard 1D spectrum but this is also like a 1D. So therefore in a 2D although we do not show like this, we are I am showing only like a circle here because I am trying to explain qualitatively but in reality you will actually get two peaks doublet here also for A if it is coupled to B and similarly in this side this B also will show two coupling doublet to A.

Similarly during in this dimension, the horizontal here also it will have a doublet and here also it will have a doublet. So what will be the case let us see this pictorially now let me explain. So if you see we have basically a doublet in the t1 part let us say we are looking at A and here also we will have a doublet again let us say we are looking at A.

So what we will get is this to this will give you one peak, this to this will give another peak, this is another peak, this is another peak. So you will see 2 into 2, 4 peaks so basically this whole thing is actually like this, ok. So inside this peak red color A you are basically having 2. Similarly if you see here if you zoom this here also you will get 4 peaks because of J coupling on this side and J coupling on this side, ok.

So what you are basically getting inside this blue peak is a B is basically for smaller peaks. So that is what is there so but ofcourse we will not consider those smaller multiplates inside the peak because for as far as a this 2D is concerned remember why are we doing 2D NMR? We are doing 2D NMR not for measuring the couplings, we are using 2D NMR for using the couplings to transfer the energy magnetization from A to B.

So our interest goal is not to measure J coupling, so for if you want to measure J coupling you can always do it from your 1D NMR. But our goal is to correlate two frequencies. So when we correlate we only then look at the center of the peaks so our focus will be at the center. So this

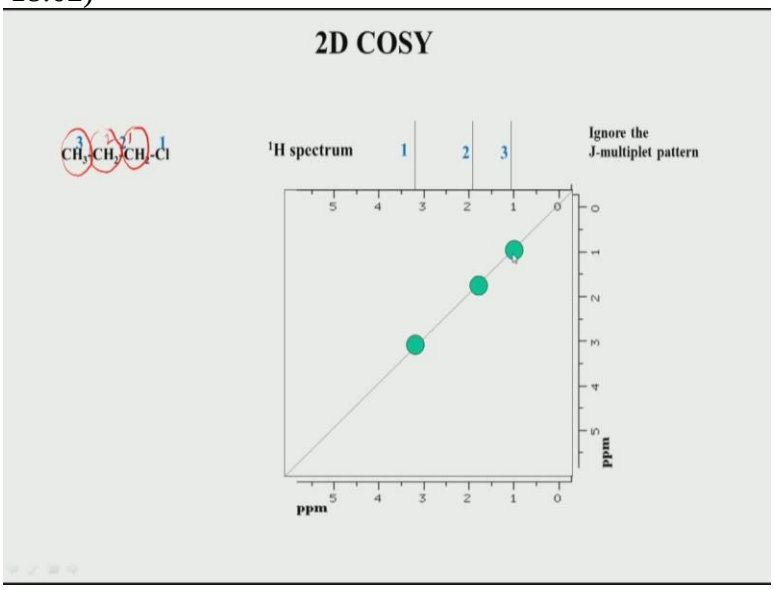
whole thing will be now our center is actual value chemical shift because remember the chemical shift of A is not this or this, it is at the center.

Similarly the chemical shift of A is again here at the center. So our center of this peak is our actual chemical shift value and that is what we are interested in and not in the coupling, ok. So similarly cross peak will also have a multiplate structure inside this but as far as the analysis is concerned we normally do not consider that we only focus on the overall position means the value of the chemical shift here at the center, ok.

So you see this is marked as X because it is a cross peak, so cross is X so we use a word peak. Sometimes in literature or in textbooks people we also use the word off diagonal, this is diagonal and this is off diagonal. So keep this in mind. Third nomenclature which is used in literature is called transpose peak so if this is a peak the transpose of this peak is here. Similarly is transpose of this peak is here, similar to what we use in the matrix algebra nomenclature.

So these are the different terminologies which you will come accorss in the books as well as when I am talking about this. So this is a diagonal peak and this is a cross peak.

(Refer Slide Time: 18:02)



So now let us just again look at schematic for a molecule. So I am not going to show you the actual spectrum here but schematically what to get if you record a 2D COSY or let us say a this molecule, ok. So we have propyl chloride so this 1 here has got displaced so this is 1, this is 2, ok

so we have 1 is this, 2 is this and 3 is this. So we are again remember we are looking only at the protons hydrogen we are not focusing on carbon. So when you record a spectrum of this 1D NMR this you will get something like this.

Why this 1 is here? Because it is downfield shifted again remember because of the inductive effect and 2 is little bit away and 3 is a last because 3 is most away from carbon is chlorine so therefore it is least deshielded so it comes here, so this is fine. Now let us ignore the J multiplet pattern. So remember again when we go to 2D NMR in this entire course we will not be focusing on the J multiplet in 2D, ok. So J multiplet as far as 1D is concerned we have already looked at it and you saw how we can use it for getting the structures but when it comes to 2D we normally we do not considering them.

So now let us come to the 2D here. So I get a 2 dimensional plane so this is a 2D NMR so I am recording 2D mole of this spectrum so you will have a 1 axis which is t1 axis that is our proton and this is also proton. So we are looking at 2D COSY which is a homonuclear correlation experiment. So that means both dimensions will be hydrogens protons or both can be carbon. So we can also have a carbon COSY keep in mind and we can also have a proton COSY, but again the most popular is proton COSY and we are going to look at only proton proton correlation.

So now if you look at this molecule again so which is coupled to which now? We see 3 if you look at this 3 hydrogen number 3 methyl is J coupled to number 2 . 2 is coupled to 1 and 2 is also coupled to 3. Because remember if one atom is coupled to another atom the other atom has to be be coupled again to the previous one. So if A atom A is couple to atom B by J coupling, atom B automatically is coupled to atom A by J coupling both are coupled to each other.

So 2 is also coupled to 3 and 2 is also coupled to 1. Similarly if you go to 1, 1 is coupled to 2. So now what do we expect to see in this kind of a spectrum? What we expect is a correlation means connection between two hydrogens which are coupled to each other. So that means I expect on this axis a correlation if you look vertically if this is 3 that means let us start from here if this is 3 I expect a correlation from 3 to only 2 and expect a correlation from 2 to both 1 and 3.

And in 1 in this line I would expect to see a correlation from 1 to 2 but I would not expect to see a 1 to 3 connection why because 1 to 3 are not connected or coupled to each other so obviously I

do not expect any correlation in chemical shift between 1 and 3, ok. Because when magnetization of 1 evolves it cannot transfer to both 2 and 3 because it is not coupled to 3.

So now let us see what happens in the peak pattern. So this is first thing is diagonal peak to remember every 2D homonuclear experiment is to it always has a diagonal peak. Why is that? You have to recollect few slides back we said in the last class the transfer efficiency cannot be 100 percent homonuclear. It is always less than 100 percent. That means part of the magnetization is retained on its own.

That means if I have a spin A and B or 1 and 2 in this case, 1 magnetization proton 1 will not transfer all its energy on magnetization to 2. It will keep almost a lot of it on itself 80 percent typically. So only 20 to 10 percent will go this side, so that means that one which does not go anywhere which is 80 percent will evolve at during t1 that is during vertical axis in omega 1 that is chemical shift of 1 and it will also evolve with chemical shift of 1 on this axis. Because it has not transferred 80 percent of it.

So therefore fourier transform their use your diagonal peak which is on both axis it is the same chemical shift. So you can look at this picture here both of them in this axis as well as this axis has the same value so we call it as a diagonal. Similarly if you look at this peak here 2 and 2 there will be always some peak here and 3 and 3 again keep in mind we are ignoring the J coupling pattern inside the peak.

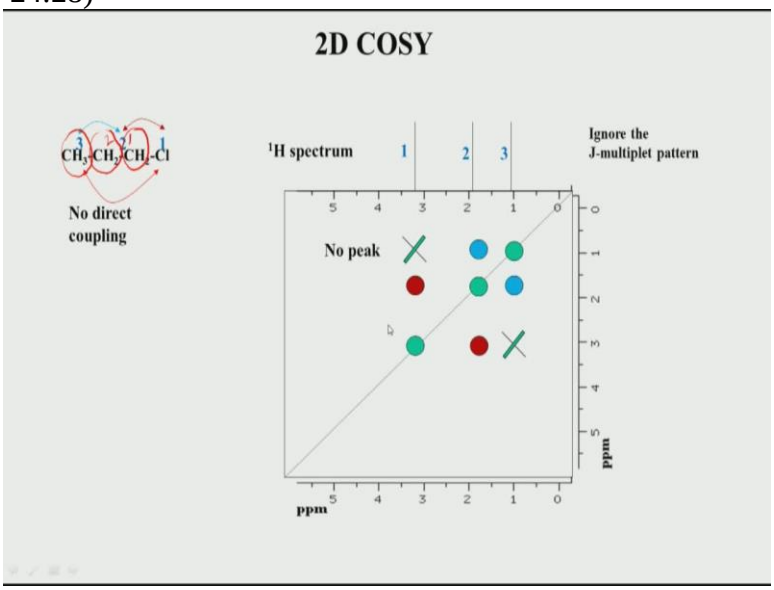
So this peak is not actually like a circle it will be like a four peaks inside this but we are going to ignore because our focus is on the chemical shift value which is the center of this, ok. So this is diagonal peak. So what is the use of diagonal peak? Basically it is set sort of useless. It has no value for us because what does it give? It does not give any information. It only says this is this, this is this and this is this on both axis.

That means what in extra information do I get? I do not get any information. So diagonal peaks in NMR are kind of a problem trouble to us more than giving us an information. So another one of the areas of research in NMR is to have a diagonal less homonuclear experiment. So there have been lot of papers published where scientist have tried to get rid of this diagonal but remember it is not very easy because this is something which behaves like is magnetization you

cannot distinguish NMR between what remains on itself and what is transferred to somebody else. Both of them look the same.

So how do I selectively suppress or remove the diagonal but I want to keep the transfer information, ok so that is called cross peak.

(Refer Slide Time: 24:28)



So will see now the cross peak so first let us see between 1 and 2. So as expected between 1 and 2 I would get a cross peak that means if you draw a line like this from here to here and here to here so these two green atoms are 1 and 2 and common to 1 and 2 there is a connection. There is a correlation between 1 and 2 which is reflected or shown in this red spot here.

So this peak the presence of this peak itself tells you that these two atoms are correlated means connected, ok. So that is very useful information you are getting here now. In a 1D NMR I would get simply 1, 2 and 3. I would not get the information whether the coupling what I see here is because of 1 to 2 coupling or is it because of 1 to 3. If I have structure in my hand yes then I can say that it is 1 to 2, but if I do not have a structure and my goal is to get a structure if I do not have information of this and I have only this spectra 1D definitely it is very difficult to say whether 1 is coupled to 2 or 1 is coupled to 3. Only if I do this 2D NMR COSY experiment then I immediately get the information that these two atoms are connected to each other.

So in case I am getting a doublet here which is not shown but in case you are getting a doublet here the doublet is only because of its coupling to 2 and not to 3. So that is the take home or important message we should see from this analysis. Similarly if you look at this side this is nothing but a transpose means a mirror image a reflection because remember both ways a transfer takes place it goes energy goes from 1 to 2 as well as 2 to 1.

So we saw that in the previous slides in previous class. So I expect a mirror image symmetry so this is an extra peak. Our interest is only on this one or this one, we do not need both but it is sometimes good to have so that we can confirm that this is really a correct peak. Now let us move on to the second pair which you see here between 2 and 3, I expect coupling between this two hydrogens and this 3 and this is also is shown here.

That is again confirms. So what will be the peak pattern of 2? It will be a quintet. Why it will be a quintet? Because there are two protons sorry in fact it will be a sextet. Why it will be a sextet? Because there are three protons on one side two on this side. So the proton two you will get actually six peaks but then that six peaks does not tell me to what is it coupled. Ofcourse in the case of this it is easy to say it has to be coupled to this side and this side.

But if you have a number of peaks the sextet does not tell me any information to actually which other two hydrogens it is coupled and that information comings from here. So this says that 2 is coupled to 3 and also 2 is coupled to 1 so this 2 is actually now a 2 is also coupled to 1 so this 2 is basically what is shown here 2 is coupled to 1 and 1 is coupled to 2 and this 2 is also coupled to 3 and 2 is coupled to 3.

But if you see these two peaks there are no peaks here why? Because 1 is not coupled to 3, ok. So this line arrow which I am showing now is a indication of between coupling between 1 and 3 but there is no coupling we do not expect any direct coupling from 1 to 3. So in a COSY you will not get any cross peak between 1 and 3 in for this molecule.

Similarly from here you will not get 3 to 1 connection. So this portion will be empty. So only peaks we will get is this red color cross peaks the green color diagonal peaks and the blue color cross peaks, ok. So this is how basically it helps you to figure out what are the coupling patterns in a (to) molecule by simply recording the experiment 2D homonuclear COSY which is one of

the simplest experiment. We will see a real spectrum in the next class where we will look at a real molecule where will see a real molecular spectrum and see how interactions are reveled between two protons and interaction between two protons are reveled in a 2D COSY experiment.

But remember again 2D COSY experiment is only giving you connection between two protons which are directly coupled to each other, ok. So in the last example we saw it is not giving any cross peak between the protons 1 and 3. But there are interesting experiments which can also give 1 to 3 connection even though they are not directly coupled and that is the very famous experiment called 2D TOCSY. So in the next class we will go to 2D TOCSY along with some more examples of COSY.