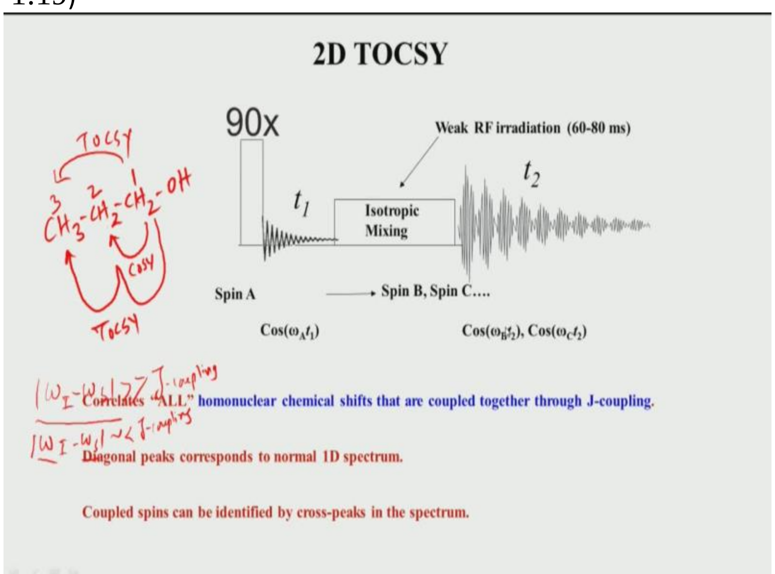
Principles and Applications of NMR spectroscopy
Professor Hanudatta S. Atreya
NMR Research Centre
Indian Institute of Science Bangalore
Module 5
Lecture No 24

In the last clast class, we saw the simplest studio NMR experiment which is 2D COSY which is an abbreviation for Correlation spectroscopy. And there we saw that you can only get the cross the cross peaks or the chemical shift correlation between two protons which are directly J-coupled to each other. But if they are not J-coupled to each other then you do not see any interaction or any peak between those two atoms two hydrogens in a 2D COSY spectrum.

So this is useful sometimes, whereas it may not be useful. Ideally you would like to know what given proton is coupled to which all other protons in the molecule, that is directly coupled. But we would also want to know what are the other protons it is indirectly coupled to all the other protons in the molecules. So that experiment information comes from another experiment known as total correlation spectroscopy 2D TOCSY which is what we will see now.

(Refer Slide Time: 1:19)



So if you look at this picture here so this is a pulse sequence of further 2D TOCSY experiment. So what happens here is that you start first from a pulse, 90 degree pulse similar to what we see in COSY you apply a 90 degree RF pulse on a a spin and all the spins in the molecule. This is non-selective in nature. So this will be applicable to all the hydrogen atoms.

Now once the hydrogen atom is or once the proton is brought to the xy-plane, this starts evolving in chemical shifts. This is what we saw in the last class. So this evolution is because of the chemical shift. Now this decays in time because of the T2 relaxation. So this is also something which we have seen. So while this process goes on, in we saw that in the case of COSY it it gets coupled to other hydrogen atoms in the molecule because of J-coupling.

In this case of 2D TOCSY what we do is, we allow for a short time here so the coupling to other hydrogen atoms during this portion is not of so much importance but what we do is after some time, we apply this box, what is shown here. It is known as isotropic mixing. now what is isotropic mixing? Isotropic mixing is nothing but weak RF irradiation applied continuously for 60 to about 80 milliseconds.

So this is basically just a simple like a pulse, it is not a pulse in the sense, it is not a very short microsecond pulse but rather it is a weak RF pulse applied continuously. So it is not a pulse but weak RF irradiation applied continuously for a duration of 60 to 80 milliseconds. Now during this period, what happens is the hydrogen atoms in one molecule starts getting coupled to hydrogen atoms of the other in the same molecule.

But remember, in the case of standard COSY the coupling takes place only because it goes from one hydrogen to the indirectly coupled hydrogen but in case of an isotopic mixing, it get coupled not only to next neighbouring hydrogen, it will also be coupled to little bit far away hydrogen, not directly because of J-coupling but indirectly because it is coupled to a intermediate hydrogen.

So let us see what in a pictorial manner, how we can understand this. So let us say we have a molecule, you can take the case of Propanol. Here let us label this as 1, 2 and 3. These are three hydrogens. So we saw that in case of COSY, this is what we will get. This is in case of COSY. But in case of TOCSY, you will get from here to here and then again from here to here. So this is in case of TOCSY.

So in a TOCSY experiment, you actually go from one hydrogen, a to a far away hydrogen that is the from 1 to 3 by because of the intermediate coupling to 2. Because one is coupled to 2 and 2 is coupled to 3, the 1 gets coupled to 3 in case of a TOCSY but not in the case of a COSY. So in the case of a COSY this is not possible.

In the case of a COSY transfer takes place only from the nearest neighbor hydrogen atoms. So there is no transfer from this hydrogen to this side to all the way up to three in that COSY. But in a TOCSY experiment, because we apply what is known as isotropic mixing what happens is because of the intermediate, the magnetisation first flows from here to here, then it gets transferred from here to here.

So this is why now we can say that the information of 1, the chemical shift of 1 starts getting correlated with the chemical shift of 3. So we will see that as we go on. But this is a special feature of TOCSY that it couples to hydrogen atoms which are not directly coupled to each other but still they are actually coupled through intermediate coupling. So suppose that these two did not exist. Ok?

So let us assume that for example, let us say this was something like a ketone group here and there was no hydrogen here, then this to this coupling would not be possible also in a TOCSY because there is a break in between. If there was suppose, break here, that means suppose there was no hydrogens here then there would be no mechanism. No way for this hydrogen to couple to this even in a TOCSY because it needs this intermediate coupling. Ok?

So, this kind of a break is possible in some molecules and that will separate this side of the molecule from the other side. We will see some examples of this as we go along. So the now the two things is; one is - what is this isotropic mixing actually? So isotropic mixing is doing what is called as it makes the spins go into something like strong coupling scenario.

So because of the time restrictions and because this is a basic course, we are not going to go into the details of what, how exactly this strong coupling happens but you have to just keep in mind that in general COSY a normal COSY, we are in what is known as a weak coupling situation and when it comes to TOCSY, we make the spins go into a strong coupling situation.

So let me briefly tell you what is this weak coupling and strong coupling. So weak coupling we define as the chemical shift of two hydrogens. let us call it Omega I - Omega S. so let us say these are the chemical shift of two hydrogen. The difference in the chemical shift is much much greater than the J-value J-coupling. If this happens we say that the two spins are weakly coupled.

But if the reverse happens, if Omega - Omega S is let us say almost similar or less than the J-coupling that means the two spins are so closeby that their interaction or their difference in the chemical shift is smaller than the J-coupling. When can this happen? For example, let us say it happens when the chemical shift is almost zero. Suppose this two these two spins I and S, suppose let us say that their difference in the chemical shift is let us say almost zero.

For example, let us say it is at 7, one is at 7 ppm, other it as as a result 7 . 0001 ppm. So on a 500 MegaHertz if you calculate 001 ppm corresponds to 0 . 5 hertz. Now 0 . 5 hertz is very small. J-coupling typically of the range of we saw that 5 to 10 hertz in general between two protons. So you see we are now if protons have very close chemical shift values and their difference is smaller than J-coupling then we say that these two are strongly coupled these two spins.

And in a strongly coupled scenario, things happen very in a different manner, compared to how it happens in a weak coupling. In the case of weak coupling, the chemical shift difference is much greater than the J-value. For example let us say that you have a peak at 7 ppm and there is another peak which 7 . 1 ppm. So difference is 0 . 1 ppm. And I can consider let us say 500 megaHertz spectrometer.

So the chemical shift difference of 0 . 1 corresponds to 50 Hertz. Now 50 Hertz is much greater than 5 or 10 Hertz which is a typical J-value between two hydrogens in general. So therefore in those two spins that is 7 and 7 . 1, now we will call it as weakly coupled. In this case, in TOCSY, what we do is normally the spins are always weakly coupled.

But by doing some technical thing like a applying a weak RF radiation, we create a situation where the chemical shift difference between the spins are lost and because of that they enter into the strong coupling scenario. So how does happen? as I said that we will not be able to go in detail for the more details about this there are excellent books that is the standard textbooks which we have recommended. You can look at that.

So the idea is that you apply a RF radiation such we use the word spinlock. So you will come across the word spinlock in the textbooks. The spin this application of weak RF is we also say we apply a spinlock. So when we apply a spinlock we essentially create a situation in which the chemical shift difference between the two peaks or two atoms goes almost close to zero and therefore, J-coupling does not go to zero and therefore if the chemical shift difference becomes very less compared to J-value, it becomes strongly coupled.

So but when the strongly coupling when strong coupling happens, this this thing starts happening that the spins start interacting with remotely coupled spins even though they are not directly coupled but because of the intermediate hydrogens. So this for example, in this case, hydrogen 1 gets shows interaction with 3, not directly but through the intermediate hydrogen atom 2.

So this a basic idea on a TOCSY and the advantage here is that you get interaction between all the hydrogens which are coupled to each other. Ok? So we will look at that now. So let us say mathematically, let us see what happens. So we have applied a pulse, 90 degree pulse to one spin A. Now that spin A starts evolving because of chemical shift here. So that is what is represented mathematically by cos Omega t.

So in this case it is pinned A so we say Omega A and the this time period here is known denoted as t1. Ok? So now during this isotropic mixing as I mentioned, you basically see that the interaction of A not only happens with B but it also happens let us say with another spin which is remotely coupled through B. This coupled not directly through A, it is coupled to B. B is coupled to A, so A gets coupled to C in a (())(11.37).

So you see we can call this kype of type of a scheme as a relayed COSY. So if you , if you know, in a relay race typically what happens is there is a first runner. He carries the baton with him, he runs for some time, transfers the baton to the next person and the next person then runs for some more distance. He gives it to the next third person and so on. So this is called a relay race.

Similarly if you are having a relay magnetisation transfer here. You first evolve A then transfer the magnetisation to B and then B does not evolve but B because of J-coupling to C gets transferred to C and so on. And the finally after all the transfer has taken place, we then evolve the chemical shifts of both B and C. So which is shown here by two chemical shifts here, so cosine Omega B t2 and Cosine Omega C t2.

So you see that what happens is that because of this coupling that because of this spin A to B and there is a transfer of magnetisation from A to B and in during this period, B to C also takes place and that is therefore you get the chemical shift of C as well in the FID. So the Final FID now contains not only the chemical shift of B but also it contains the chemical shift of C. But where is the chemical shift of C coming from because from transfer from A to B to C.

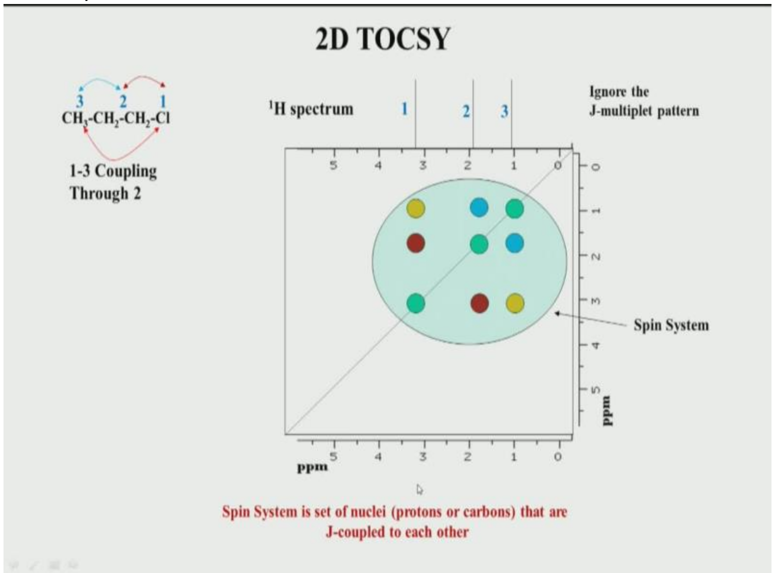
So this is what happens in a TOCSY. All the it correlates, so it correlates all the homonuclear chemical shifts that are coupled together through J-coupling but remember they may not be directly coupled to each other but in a group they are coupled in a group. So we can say this belongs to one group. Okay? So they belong to one group in which one is coupled to 2, 2 coupled to 3 and vice versa.

Ok? So this is how what is this is the information which you get in a TOCSY spectrum. We will see more schematic in real spectrum down the line. And then what is what does the spectrum look like? As we saw in the case of COSY you expect a diagonal peak. So remember here, if you look back in this picture here, the spin A does not transfer all its magnetisation to B and C and so on. It only transfers part of it.

This is what we mentioned in the last class as transfer transfer efficiency. So the transfer efficiency of magnetisation is never 100 percent in case of homonuclear. I hetero-nuclear yes we can achieve 100 percent, we will see that later but in homonuclear we will not get 100 percent. So whatever does not get transferred and remains on the spin will evolve again with spin A here during t2, right so what you have shown here is only the transfer part.

But the non transfer part if you recollect in the last class we saw that in a like in a COSY, the non transfer part also remains and that will evolve first here during t1 with Omega A t1 Cosine Omega A and then it gets transferred. It does not get transferred. It remains on A, it will again evolve with a chemical shift of A. so that is called as a diagonal peak. And that one gets which gets transferred, we use the words cross peaks. Ok?

(Refer Slide Time: 14:57)



So let us see the spectrum how we will expect for this particular molecule again the same system which we saw in the last lecture. So this is propyl chloride, so the atom hydrogen atom 1 is here, 2 is here and 3 is here. So if you record a standard 1D spectrum again for the sake of simplicity, we are ignoring the J-multiplet pattern. So if you record a standard simple 1D proton spectrum, you expect this kind of specrum, peak pattern.

Now if you go to TOCSY, where you see on the horizontal axis is hydrogen and the vertical axis is also hydrogen, so both these axis are hydrogen. So now let us draw what we exxpect for this particular molecule. So like in the COSY, we first get the digonal peak, which are nothing but the peaks coming at the same position. So for example 1, 1 ppm 2 and 2 ppm, 3 and 3 ppm for this.

So you see this is diagonal peak is nothing but a simple 1D itself on from the both axis. So this has no value for us but it is always present, you cannot avoid these type of peaks. Then let us see cross peak so like in a COSY, you expect direct crosspeaks because the direct coupling is there between 1 and 2. So even though you do not need TOCSY for this, COSY also gives the same information but TOCSY also contains COSY inside it.

So that means TOCSY is similar to COSY in that respect because it also has a same peak pattern between the neighboring hydrogens like we have it in a COSY. But what is more important in

TOCSY is further interesting information between 1 and 3 which I will show you now. So this is again between 2 and 3 which is not very surprising. This is similar to what you get in a COSY in a COSY spectrum because this is a direct coupling between 2 and 3.

But in a TOCSY what will happen is we will get an additional peak shown here, orange colour peak and where is that coming from? That is coming because of this here, 1 to 3 coupling through 2. So remember there is a through transfer. So you cannot get this direct coupling straightaway. You need an intermediate relay. You need somebody to relay the magnetisation from this side to this side. So if I remove this protons here, I would not have got these peaks.

Ok? So now you see all of these three hydrogens, three types of hydrogens are now showing all complete coupling to each other. So if I have three hydrogens, I get three into three, nine peaks. If I have four hydrogens like this, we will have sixteen peaks. So square of the number. A very simple idea because, it is just that all are coupled to every each other so you will get a square number. Ok?

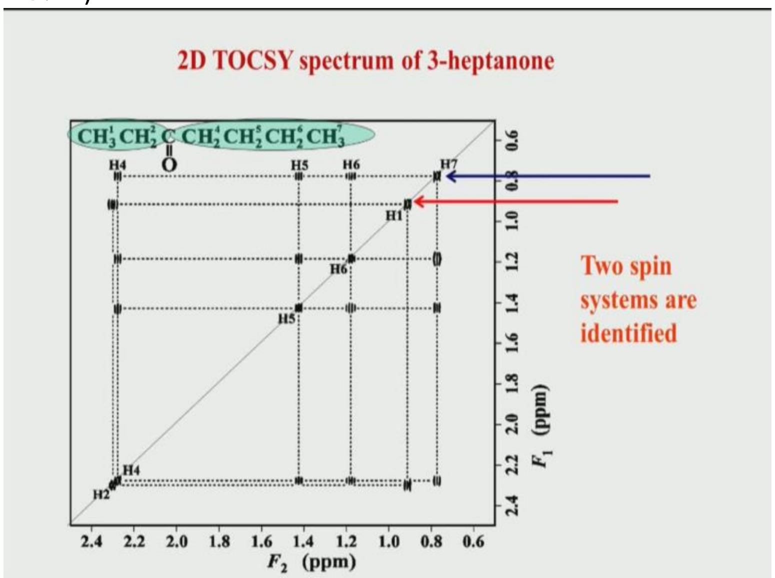
So this kind of a pattern, this kind of a atoms in this molecule like this which are coupled to each other, all of them. That is known as a spin system. So this is a very important terminology in NMR spectroscopy. It very often use everywhere. So in our case in this case how now we have come first time to this terminology. So let us look at it more carefully. So the idea of a spin system. So what is a spin system?

Spin system is basically a set of hydrogen atoms protons which are coupled to each other, there is no break in the coupling and all the three together as set. It forms a set of three hydrogens which within them they will be coupled to each other, not directly but indirectly in this case for example. But they are A is coupled to B, B is coupled to C and within them they are all coupled to each other. And that set of atoms spins, we will call it, we call it as a spin system.

So if you if you record a TOCSY for a spin system, basically all the hydrogens in that same system will show like this coupled. So if there are N hydrogen atoms in a spin system, in a TOCSY you will get N square number of peaks for that system, ok? So this is the basic defination or the meaning of spin system. So again we repeat, the spin system is a set of nuclei that is it can be either proton or carbon. So set of nuclei that are J-coupled to each other within the set.

So this is a very important point in NMR in general. We will use this nomenclature as we go to more difficult or more advanced aspects.

(Refer Slide Time: 19:24)



So now let us see for an actual experimental spectrum, this is spectrum taken from the book Neil Jacobson’s Introduction to NMR, there we have shown for this heptanone. So now we will look at this concept of spin system which comes out here.

So now if you see this structure of the molecule, what do you notice? You will notice that there is a break here. This carbonyl is kind of a break because on this side, these four hydrogens, 4, 5, 6, 7, they are all coupled to each other. In the sense 4 is coupled to 5, 5 is coupled to 6, 6 is coupled to 7 and vice versa. So in a TOCSY, I would expect 4 to 7 in interaction not directly but through the intermediate.

But not, this is not going to be the case for between 4 and 2 because remember as we discussed in the last slide, there is a there is a intermediate carbon. It does not have any hydrogens, so there no direct coupling between 2 to 4 and therefore indirect coupling is also not possible because there is no hydrogen here which will carry the magnetisation from 2 to 4. So there is a break here. Similarly but here this side, 1 and 2 will be coupled to each other.

So you see this portion of the molecule, we will now call it as one spin system and this portion of the molecule will be called as a second spin system. So that is what is shown here, if you look at this spin system set of spins, then you see there is a interaction between them. So you have H7 with H6 with H5 with H4. Similarly H4 to H5 to H6 to H7. you see there are if you count the number of pins it is a square.

So four into four, sixteen peaks you will notice in this spin system. But if you look at 1 and 2, which is shown here, with a red arrow, they are only coupled to each other so there is only four peaks, 1, 2, coming down 3 and 4. So two into two four. So you see this spin system which has only two hydrogen atoms will be now only four peaks. So this is how you know in NMR, we can distinguish or differentiate spin systems because within the spin system the TOCSY, we will have all the couplings whereas between the spin system, they will not have any coupling.

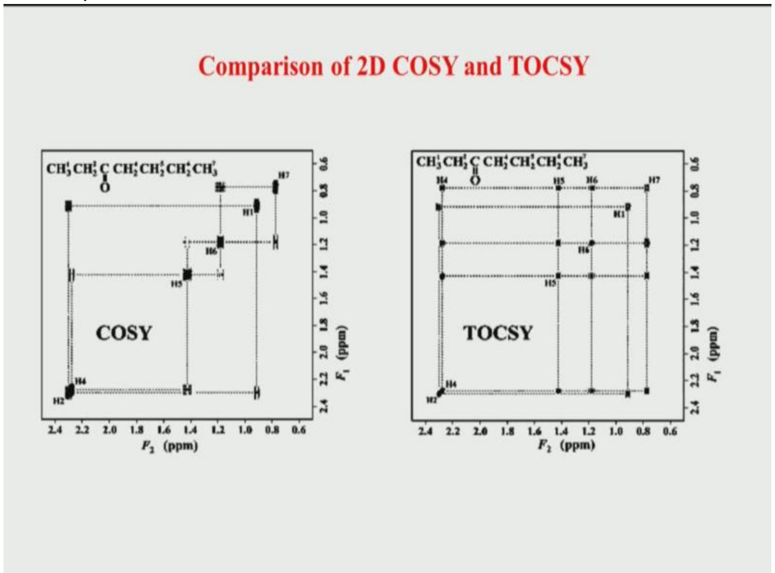
So NMR TOCSY experiment is therefore very useful because now you can identify two spin systems here in this molecule which you could not have identified from a COSY. Because in a COSY, you would have seen two to four all only one one interaction. Of course,if you are in COSY, you would not have seen this also two to four, that would have probably helped us to also figure out the presence of two spin systems in this molecule.

But TOCSY is a very clean, clear cut way to find out, because all the spins which are coupled to each other will show a clean I mean the sixteen peaks because there are four into four. This side will show two into two and that will denote a difference. So this is a very important when it comes to amino acids in proteins and peptides. There I mean each one amino acid we know we call it as a spin system.

So in amino acid you have alpha, beta, gamma, delta carbons. There within one amino acid, there is coupling to each other but there is no coupling between two amino acids in a peptide, so in a TOSCY experiment. And J-coupling is not there between hydrogen of one Amino acid to the hydrogen of a neighbouring amino acid.

So what happens is, that there is a break because of the peptide bond in case of peptides and we say that thus one amino acid is kind of separated or isolated as far as proton J-couplings are concerned from the neighboring hydrogen and we use the word spin spin coupling.

(Refer Slide Time: 23:17)



So this is an example, now let us see how can we compare, COSY and TOCSY. So you see this is the same molecule here, heptanone and you can see in a COSY, we see only the direct coupling which is what we saw, that it only give you the direct three bond or J-coupling peaks pattern.

Similarly here to here, it gives you only from 7 to 6 but in a TOCSY, you see you see all the couplings everywhere, in the sense all the peaks are coupled to all other peaks because of this through bond through transfer through protons but again (())(23.50) system is separated from the neighboring so obviously you dont get thus between the two spin systems. But within one spin set of spins you get all the correlations.

So now you can if you look at, compare these two pictures, what is this telling us? It is telling us that the COSY concept , the COSY peaks are actually all present in TOCSY. So which means COSY is a subset of TOCSY. Ok? So TOCSY is much higher more peaks present than COSY so it is a some in some applications COSY will be useful where you are not interested in looking at spin system because you are interested in looking what hydrogens are coupled to what hydrogens directly then COSY is sufficient.

But if you are interested in the forming a spin system, then we use the experiment TOCSY. So it depends on the type of application which we are interested. So typically when we go to small organic molecules com.s, we not go for TOCSY. We usually are satisfied sufficient to have a COSY for structural analysis. But if you are looking at peptides and proteins, there you have to have a full fledged TOCSY spectrum because there your job is not only to identify one amino acid, there will be several amino acids in the chain in the peptide chain or a protein chain.

Therefore there it is much more difficult system to handle and there we would need TOCSY. So this brings us to the end of TOCSY experiment so we have seen now two experiments. One is 2D COSY and 2D TOCSY. What we will do now in the next class, we will go to a new experiment known as 2D Nuclear Overhauser Effect spectroscopy, That is 2D NOSY.