One and Two dimensional NMR Spectroscopy: Concepts and Spectral Analysis
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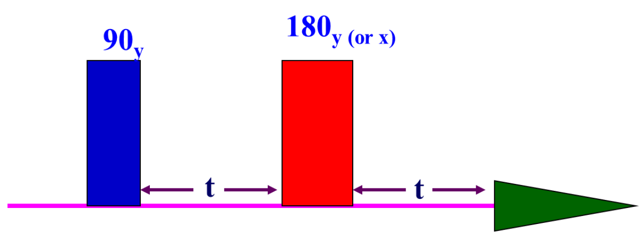
Lecture 32: Spin Echoes

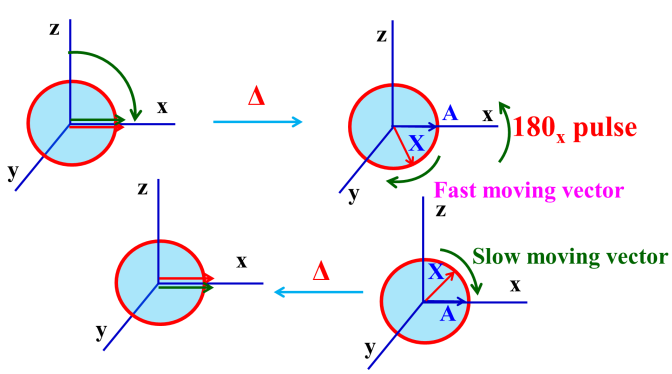
Last couple of classes, we extensively discussed about the analysis of proton spectra and the analysis of spectra of several heteronuclei. Heteronuclei what I mean is anything apart from protons, like carbon, fluorine, phosphorus, nitrogen 15, boron, selenium, silicon, platinum, varieties of nuclei we covered. And we especially we delved deeper into carbon-13 NMR where I showed is is the common nuclei after proton that is most extensively studied nuclei. Lot of examples we did and I showed you how we can even get the chemical shifts, we can calculate the chemical shift on phenyl rings, chemical shifts of the carbons especially the phenyl groups there based on the substituents, and depending upon which carbon you are looking at it; whether it is ortho to a substituent, para or meta and substituent is whether mono or di substitution, asymmetric or asymmetric, like that. So, many examples we took and also we analyzed carbon 13 spectra and we showed carbon 13 can be utilized to get the structure of the molecules. And I also said something about DEPT, distortionless enhancement polarization transfer using that we can edit the carbon spectra in the sense identify the carbons where it is attached to different types of protons. We can identify CH3 carbon, CHw carbon, CH carbon, quaternary carbons. And in the phosphorus, fluorine, NMR many examples we took where we could analyze very easily and these are all friendly nuclei spin half nuclei. We could get the homonuclear couplings of fluorine or homonuclear coupling of phosphorus or heteronuclear coupling between phosphorus, fluorine, abundant spins and dilute spins. And many examples spin half nuclei; spin one more than spin half, and how they are coupled with spin one, what is the type of pattern we get. And when you are looking at quadrupole nuclei coupled spin half or how this pattern you are going to get like N14 or nitrogen 15; or boron spectrum of $BH4^{+}$ NH4 ion many examples we took. And we fairly got confidence about the analysis with respect of different nuclei. I am sure you have got confidence. With this we will switch out a different topic, because so far I gave some concepts and analysis. I will give you one or two more concepts which is very important so that we can afterwards jump into 2D NMR. For understanding 2D NMR especially this you need to understand what is called, a J modulation spin-echoes and also little bit about polarization transfer. So, in the next one or two classes we will cover these things and then subsequently we will switch over to two dimensional NMR where we can give some concepts and then analysis of the two dimensional NMR spectra.

Today I will start with spin-echoes. Spin-echoes you please remember it is a basic pulse sequence, a fundamental pulse sequence in NMR. Very first immediately after the discovery of NMR in 1945 within 5 years Erwin Hahn introduced spin-echo, a fantastic idea. This is one of the basic sequence and it transformed NMR understanding a lot. So, spin-echo sequence is very important, and it is used in many NMR techniques. We will come to that later how we do. What is the meaning of a spin-echo? If I have a single RF pulse that is what we have been doing; apply RF pulse tilt the thermal magnetization to x-y plane and start collecting the signal. In the x-y plane the spin vector start dephasing and then when it dephases there is a decay of the signal in the x-y plane, while growing simultaneous along z axis. That is what I said in the introduction class. While decaying the signal in the time domain you collect the signal, do the Fourier transformation you get the spectrum. That decay in the time domain we call it as FID which exponentially decays that all we discussed. This is when you apply a single pulse. What happens if you apply two successive RF pulses with some time delay between them? Not you know immediately, but successively with the time delay. What will happen? Then we get what are called spin echoes. What are the spin echoes? we will try to understand now. Usually a standard spin echo has a two pulse sequence 90-tau-180-tau. This is the sequence; 90 degree, pulse delay, apply 180 pulse, a delay and then start collecting the signal.

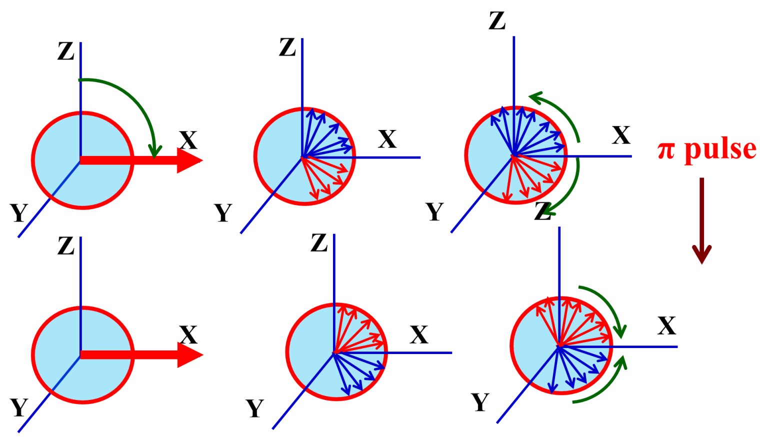


This is a standard spin echo sequence. How it works we will understand the concept later but here immediately after the 180 pulse what happens is after 90 pulses the signal start dephasing. When the magnetization comes to x-y plane, here they start dephasing. After some time you apply a 180 pulse and give some delay; equal delay this and this should be equal; and start collecting the signal. Then what is going to happen is after 180 pulse and after certain delay the spin vectors which underwent dephasing in the first period will start rephasing after this delay; something it is like a time reversal. You are reversing what happened. The phenomena what happens here the spins which dephased now will rephase. After applying a 180 pulse and exactly equal amount of time. This is called echo time, from this to this is called echo time, rephasing time.

And echo amplitude decays further with that with some time, of course I will see that later. How it works is like this first 90 degree pulse flips the nuclear spin into transverse plane. That is what we have been doing that is the basic thing of NMR. First pulse always brings the magnetization to x-y plane; the transverse plane and spin isochromats start moving faster and slower because they are dephasing. Let us say from the x-axis this is my on resonance. For those which are on resonance nothing will happen. For those which are here and here with a different offsets chemical shift offsets these things will start precessing. Some will go like this some will go like this. This is called fast moving and slow moving components. Some of the spin isochromates move faster and some moves slower. Those which move faster gain phase faster; those which move slower will lose phase fast slower. Essentially the spin isochromates start dephasing. With time entirely all the spin isochromates in the x-y plane start completely dephasing. That is important thing; apply 90 degree pulse wait for some time the spins start dephasing that is what we know. Now you apply 180 pulse. What does 180 pulse does: it turns the entire spin system and reverses the phases of the isochromats. Very interesting. What is happening now is the faster ones start moving slowly and slower ones start moving faster. Initially when we have isochromats, here some are moving faster, some are moving slower. After 180 pulse they all get interchanged here, and they start moving in the opposite directions. The ones which were here start moving slowly. the ones which were faster start moving slowly. So faster will start moving slowly the slower ones start moving faster. And exactly identical delay, that is what I said. The first time period, the first delay t and the second delay t when it becomes equal, all the spin isochromates regain the phase coherence and we get a FID. This you know what is called echo. It is like telling there are several spins vectors here; think of a situation what will happen. Think of a running race. Let us say different people are standing here. What will happen you ask all of them to start moving. Everybody start running. Different people will be at different speeds, that is fine. After some time what happens, allow them to stop wherever they are. See what would have happened, one fast runner would have moved faster another one would have moved here, some would have moved slow, some would have moved very little, like that. Depending upon the speed they are all at different places. Ask them to stop immediately. We shall ask them to turn back, reverse exactly like applying a 180 pulse. Ask them to turn back and ask them to continue running back with the same speed, because their speed will be identical and this fellow moved this much for time t and after time t when he was asked to come back for same time t, he will come back here. He will also come back here, he will come back here, he will come back here. Similarly, all the spin vectors that started from a particular place will come back to the same place. This is what is called an echo. What happens it is like different people who are running going to a running race have a different speed. After a time t ask them to stop take a reverse turn then move with the same speed. Everybody will come back to the same place. This is called an echo. So this is called spin echo. The spin isochromats will gain coherence again and this is what is called echo. Echo means you will dephase for some time; after the exactly particular time t, apply a 180 pulse and exactly after equal amount of time the spin isochromats come back and rephase. The dephased isochromats will get rephased after the identical time with a 180 pulse in between. This is called an echo. Please understand, this this is pictorially given here. We can see this; first apply a 180 pulse, then the FID start decaying like this, 180 pulse here, then what is going to happen? then they will like the runners have reversed their direction. They will again start going back like here. Whereever they they start going back. At the top of this is an echo. But in between this intensity and this intensity slightly differ, because between this time there is what is called a relaxation. Because of the relaxation intensity of the echo has come down, but it forms an echo. It gets rephased and this, of course, one of the t2 experiment. And we do this thing we measure this decay and measure the dephasing time, that is t2 relaxation in the transverse plane, the relaxation time. Okay. This is how spin echo works. It is a pictorial representation of a spin echo. Apply 90 pulse wait for time time spins start dephasing, apply 180 pulse wait for the equal amount of time, the spin starts regaining phase and forms an echo here. That is important. So this is what is happening. What is happening, to be precise, after the 90 pulse the spins start losing phase coherence, coherence means magnetization in the x-ray plane, okay. And then FID is not lost, after sometime things would have dephased and it is a common assumption that we have lost the FID. No FID is not lost but then only phase coherence is lost; there is a decoherence phase is lost, but FID is not lost. And the system will have some sort of a memory. It is a hidden memory there, it is called atomic memory. It is there, the spins will have some atomic memory and the second pulse will rephase the spin isochromates. This is what happens. So dephasing means not that FID is completely lost, the system will somehow retain its memory and that is regained by applying another pulse, and then waiting for a time t. With second time t we are going to get rephasing. What should be the flip angle of the second pulse? Of course, I said in the first example 90-t1-180-t1. That is a common spin echo sequence. That is what I showed. The common spin echo sequence is, first 90 T delay 180 delay and collect the signal after echo. But should it be always 180? need not be. It can be any angle. In fact when Hahn Erwin Hahn in 1950 did the first spin echo experiment it was 90 t 90 experiment. That is also possible. For any two pulses I said with a delay. I did not say it is 180 in the beginning. I showed it a commonly employed pulse sequences for spin echo is 90-t-180-t. And when the flip angle other than 90 and 180 are used we call this as Hahn echo. Commonly spin echo sequence uses 90-t-180-t. If other than the any of these angles is used still you can get an echo but it is called Hahn echo, because we are honoring this scientist who gave this idea. That is called Hahn echo.



Now we will understand what happens to chemical shifts what happens to coupling both in homonuclear case and heteronuclear case during spin echo sequence. And we will take the example of first two spins with different chemical shifts. I am not talking about the coupling at the moment, we come to the coupling evolution later. Right now I am assuming only two spins, the independent spins different chemical shifts and I am not worried about coupling, there is no coupling. Okay. I will take this example. Let us see for these two spins with the different chemical shifts what happens in this spin echo sequence? We have two spin isochromats one is A and the other is X. I told you spin they have different chemical shifts. Apply a 90 degree pulse bring both the spins to the X-axis or Y-axis in the x-y plane. I will always consider A spin on resonance, on resonance means when brought to x-axis, it is it is not moving. It is stuck there, okay. It is not moving. There is no offset ,it is on resonance. A spin will be always on x-axis. I brought to x-axis it will be an axis x-axis. If you bring it to y-axis, on the other hand, it will always be on y-axis. So because it is on resonance A is not moving. What about X? X is not on resonance. So what happens in the time delay Delta? The X starts moving. Look, A is on resonance. This is not moving. The X is moving, whether faster or slower I do not care, because some chemical shift difference is there hence it is moving. Okay. It is a fast moving component we have taken. If it was this side it was a slow moving, fine. At this stage what I will do is I am going to apply 180 pulse. What does 180 pulse do? It reverses okay, it completely it turns the table like this. So what will happen? the vector which is here I am applying 180 on the x-axis, it rotates along this x-axis. Rotate the axis X along this axis it completely rotates by 180 degree. Then what is going to happen? When it rotates by 180 degree, the vector which was here moved to this side, because I have rotated. Now what will happen it starts moving. The faster became slow now, because it is on the other side. It starts moving slow. It became a slow moving vector and it start moving like that. Wait for some time, the same Delta time. Then what happens? After exactly equal amount of time it will come to the same x-axis. Understand the point X is not on resonance, with a time delay X has moved from here, now you reverse it by applying a 180 pulse, it continue to move in the same direction now. And then after certain delay you will again come back to the same x-axis. Like I gave the example of spin echo where the runners go and come back at the same time after a particular period. Exactly like that it is an echo. The spin vector comes back and it comes here. What happened over the time now. A has not moved because on resonance, forget it. But there was a chemical shift difference between A and X. As a consequence, X was moving; there was offset chemical shift offset was there. That is why X was moving; faster or slow does not matter. But this 180 pulse after delay what we did is that, after the two delays the phase difference between A and X is reduced to zero. They have two different chemical shifts, in spite of it they come at the same place now. It appears as if there is no phase difference at all. That means there is no chemical shift difference between A and X. You can imagine a situation now there were two chemical shifts between A and X. After spin echo it appears as if they have the same chemical shifts. There is no chemical shift difference and this means I would say spin echo has refocused the chemical shifts. It is called chemical shift refocusing. I took the example of two homonuclear spins A and X. When you take homonuclear spins A and X, I showed with for two spins and spin echo there is a chemical shift refocusing. The difference in the chemical shift between them is removed. When the difference in the chemical shifts are removed we call it as chemical shift refocusing. All right we will go further. I took the simple example of two protons, okay. You may ask me a question, in a given sample it need not be always two protons, there could be many spins, many protons with different chemical shifts then what will happen?



Of course, true there is no difference at all. As soon as you bring all the spins in the x-axis there is coherence, here the magnetization is along this axis, called magnetization. Apply 90 pulse bring it to x-axis instantaneously there is a phase coherence at a given instant of time. As soon as you bring it to x-axis there is a phase coherence. Now with time there is a decoherence, some are fast moving vectors, some are slow moving vectors. They start moving, some move this side some move this side, with respect to this x-axis. Continue for a longer time they keep on dephasing like this after some time. But after certain delay whatever the time you give as delay you apply a π pulse after certain delay Delta. What does π pulse do? I am applying π pulse on the x-axis. You rotate along x-axis like this. Then the red spin vectors move here and the blue vectors move here. The red which were moving in this direction, they continue to move in this direction. The blue which is moving in this direction, they continue to move in this direction. What is happening? they start moving in the opposite directions, which was going like this now it will come back; one which was going like this is now started going like this. All the vectors, when you identical delay, after some time what happens? They all come back. They were moving like this they will again come back to x-axis, you understood. All the spin vectors are brought to x-axis by a 90 pulse. Instantaneously there is a phase coherence. And allow for some time there is a dephasing. All of them have different chemical shifts offsets. As a consequence some are moving like this some are moving like this. There are called fast moving and slow moving components. They keep on spanning out like this, fanning out like this. After a delay Delta apply a π pulse along x-axis, rotate along x-axis. Then you can see fast moving components are interchanged. They continue to move in the same direction. Now what will happen? exactly after a same amount of delay they all overlap, come back to the same x-axis. What happened? we created an echo. During this echo what happened now there was lot of phase differences, chemical shifts were there. Many protons many spins have different chemical shifts. But the echo finally refocused everything but to the same axis. That means chemical shifts are completely refocused. The spin echoes, in the homonuclear case will refocus chemical shifts. Remember that, this is what is happening. And every spin vector starting on x-axis will come back to the same x-axis. That is what the echo. Thats what I told you. The runners started from the same place, moved at different places for different times and afterwards when they were asked to come back with the same speed. They come back to the same place, to the same origin. Similarly started with the x-axis they started fanning out. Again came back to the same x-axis and this is spin echo. Understood the concept of spin echo. The spin echo in the homo nuclear case are refocusing the chemical shifts. These is the logic you should remember. The homo nuclear spin echo refocuses chemical shifts.

What happens if the magnetic field is not homogeneous? All these were under the assumption magnetic field is perfectly homogeneous. But inherently there are inhomogeneities in the magnetic field. It need not be homogeneous. There will be gradients of the field. The spins experience different fields. Interestingly they also get refocused. The field inhomogeneity is also refocused. That is an important point. Not only chemical shifts, the field in homogeneous if it is there, they also get refocused. Why because their resonating frequencies are different with a different fields. Again chemical shifts will be different, since the chemical shifts are refocused means ihomogeneities are also refocused. That is a logic; so in homogeneity is also refocused along with the chemical shifts. You can ask me a question and we did something in an experiment where we applied the pulse along x-axis and rotated that the spin vectors along the x-axis like this. So that we interchanged the vector directions of movement. What happens instead of X-axis, if I apply pulse on y-axis. Of course I did not discuss all those things. However, in one of the previous courses I have discussed, the pulse phase, receiver phase everything. We can apply pulse along x-axis, y-axis, plus x, minus x, plus y, minus y everything. On different axis we can apply. And not only 90 pulse, the 180 pulse also and we can ensure that depending upon the axis in which you apply a pulse we can make the vectors to rotate along the x-y plane, or in the y-z plane or the X-Z plane, either clockwise or at the clockwise. All these things are possible. You can play with spin dynamics. So now if I apply a 180 Y pulse, instead of 180 x pulse in the spin echo sequence what will happen? Let us see. Consider a situation like this. I have brought all the spin vectors to x-axis wait for some time, the spins start dephasing. Wait for some some more time, more spins are dephasing. They are fanning out. At this stage apply 180 pulse. Instead of rotating on this axis, now I am applying along y-axis here. So, I am rotating along y-axis here, not along this axis. Then what will happen here? When you are rotating, these vectors are here, these vectors are here. But when I am rotating along this axis, this will come here, this will come here. Now remember the direction of axis on which I am rotating. Here I am rotating on the y-axis; so it is like turning this table; like a pancake turning like reversing of the pancake. This is what happens. Now wait for some time delta or identical time, all the spin vectors again come back. What happened here same, you have still refocused the chemical shifts. In the previous example all the spin vectors were refocused along the plus x-axis. But now it is refocused along minus x-axis. That is all the difference. The refocusing still occurs whether you apply pulse on a plus x-axis; whether x-axis or y-axis, there is chemical shift refocusing. In one example when you applied along plus x-axis 180 pulse, there was a refocusing on a plus x-axis. If you apply 180 pulse along y-axis there was a refocusing along minus x-axis. That is all. There is no difference except the refocusing axis is different. But chemical shifts are always refocused. So please remember whether apply 180 pulse along x-axis or 180 pulse along y-axis there is a chemical shift refocusing in the homonuclear case, okay. This is a simple video which shows you how spin echo occurs. Look at it; it starts here; you apply 180 come here, apply 90 pulse bring all the spins here, they start fanning out; apply 180 pulse, it is like turning the pancake, they are rotated like this, and then go there again by fanning out. And this is where we get echo. Carefully observe this. What is happening? This 180 pulse, what it does you observe, it brings the vectors here it is not fanning out. Now 180 pulse rotates this like this, and again they start moving in the opposite directions. Please see the direction of rotation, the one which was rotating like this now they continue to rotate in the same direction and then refocus. This is the video of a spin echo. Now I also told you if there is a magnetic field inhomogeneities they get refocused. I will ask a question? What happens if there are fluctuating magnetic fields. fluctuating not main magnetic field, but fluctuating local fields. Of course that is also magnetic field, the fluctuating local fields. This is not because of this inhomogeneity of the main magnetic field, it is within the molecule. It could be diffusion, there could be different molecular motions. That can vary with time. Okay, If different molecules are there, if you take a mixture their molecular weights could be different. They may diffuse at different rates. Then the fluctuating field local fields will be different. If the molecules are undergoing diffusion or any other type of motion then what will happen? will there be a refocusing is the next question. They precess at different frequencies, before and after 180 pulse, because they are not same, because the precision frequency continuously keep changing because of diffusion or molecular motion. So as a consequence they precess at different frequencies before the 180 and after the 180 pulse. There is no question of refocusing, the decay is irreversible. In other case it is a reversible decay, that was reversible decay; the

180 pulse brought it back. There was an echo; but here this is not a reversible decay. The inhomogeneity, the fluctuating fields because of diffusion, perfusion or molecular motions is irreversible. So they are not refocused, and because of that you will lose the energy, lose the signal. Okay, signal will be lost. The intensity of the signal will be lost. So this is the idea where you used to get the diffusion coefficients. Later I will tell you that, when we come to that. But please remember now what is happening is when there are field inhomogeneities there is a refocusing, local fluctuating field because of molecular diffusion perfusion or different types of motion, the different precession frequencies will be there before and after 180 pulse. As a consequence the decay is irreversible. There is no way you can refocus it. And this what it is. This is what I just wanted to tell. We can take the example of what happens if there is a homo nuclear j coupling between the two spins. This needs bit more discussion. Since the time is getting up, it is getting over already, what I am going to do is stop here. We will take the example of what happens to the spin echo when there is j coupling later. Remember so far I said there is no j coupling. I took only chemical shifts of A and X. First A was on resonance the X was moving then I showed both will refocus after 180 pulse, whether you apply on x-axis or y-axis no problem. When we have number of spins with different chemical shifts, that is with different offsets, they all will be fanning out at different frequencies. After 180 pulse again they will refocus. I showed that chemical shifts are refocused okay. And then I said in case of the inhomogeneity that also gets refocused. If there is a local field because of spin diffusion, perfusion or molecular motions is an irreversible decay, that cannot be refocused. So I said for homo nuclear spins, the spin echo will refocus only chemical shifts. It appears as if although there are different offsets, different chemical shifts, when you apply this spin echo it appears as if there is no difference in the chemical shifts among the spins. This is what is called chemical shift refocusing which homo nuclear spin echo does. But the thing is, the spins are not havechemical shifts alone; they could be coupled. I took a hypothetical example where there is no coupling. What happened if there is a coupling? will it refocus? we do not know. I will explain that to you in the next class. Right now I just wanted to tell you how spin echo works. The spin echo is just simple 90 tau 180 tau sequence. It could be one 90 tau and it could be any angle, tau. If it is any other angle it is called Hahn echo. The first discovery of spin echo was 90 tau 90 tau sequence. Both the delays were equal and it was called time. That means spins will get rephased after exactly same amount of time. I gave an example of runners; different runners will be running at different speeds. Instantaneously asked them to stop apply 180 pulse means, reversing immediately. Theyhave to turn back then they have to run now for the same amount of time with the same speed. They all come back to the same place. I gave you this example to show that the spin echo refocus the the chemical shifts offsets. We will stop here we will take the example of what happens with a J coupling for homo nuclear, heteronuclear case, everything in the next class. Thank you very much.