One and Two dimensional NMR Spectroscopy: Concepts and Spectral Analysis
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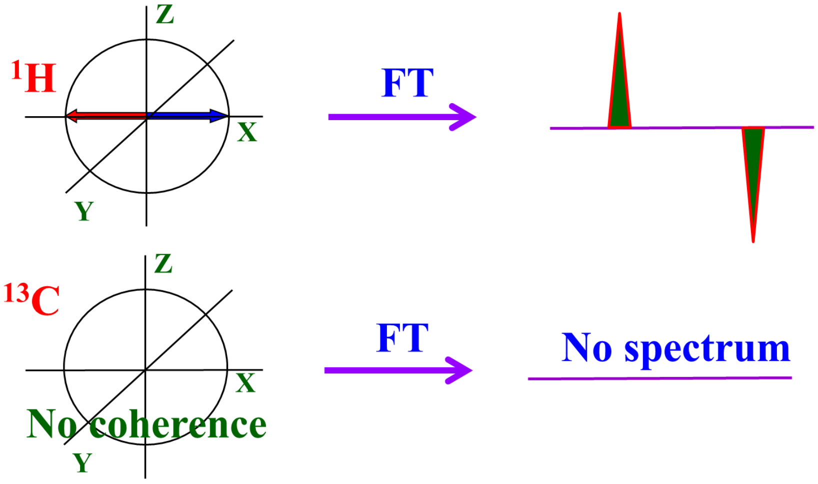
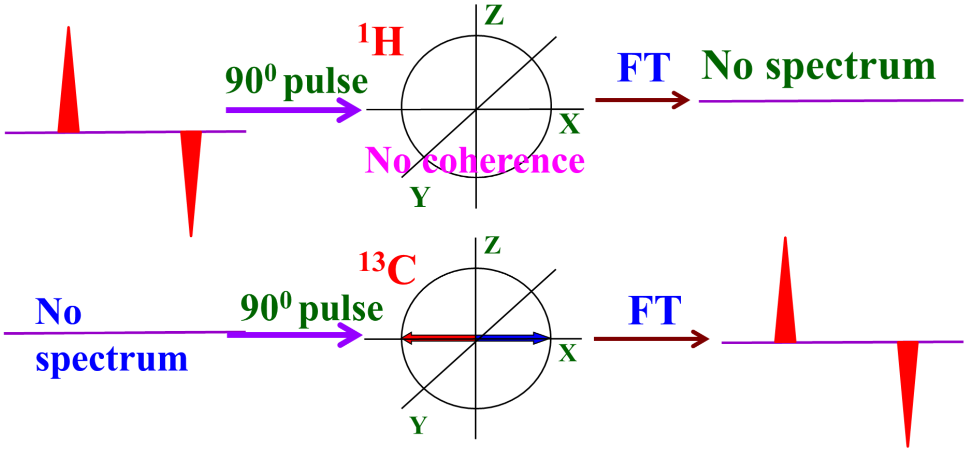
Lecture 35: Polarization transfer

Welcome all of you. Since the last class, we have been discussing about polarization transfer technique to enhance the signal intensity of dilute spins. And we took the example of homonuclear case and also heteronuclear case. And there are two ways to do the polarization transfer. One is called selective population transfer, other is selective population inversion, both are possible. That is what we saw. When we try to do the selective population transfer, we saturate one of the transitions and we saw the gain in the intensity of its coupled partner, for both the peaks there was a gain in intensity. In the case of selective population inversion also it happened, but selective population inversion has a better intensity. Same thing we saw that in the heteronuclear case. We took the example of carbon coupled to proton. Carbon has a gyromagnetic ratio of 4 times smaller than that of proton. Accordingly, we took the populations, worked out the populations such that we get the intensity of protons 4 times larger than that of the carbon. Then we saw what happens for selective population transfer and selective population inversion, both of them. And we saw in selective population inversion, we are going to get the signal intensity gain of -3 and +5. We get gain in intensity, but there is anti-phase character for that. How do you overcome this if you want to do the decoupling? Because we need to do the carbon-13 decoupling to detect the signal. If you do the decoupling by going into anti-phase character signal, the signal get nullified. Whatever the gain we get is going to be lost. To overcome this problem, an idea is to put a delay after the detection pulse for another 1/2J. Then we saw the signal which was anti-phase in character will become in-phase character. Both alpha and beta components or doublet components of the carbon 13 doublet will become in-phase. Instead of -3 and +5, they will become +3 and +5. Now, we do the decoupling, we get the gain in the signal intensity. This is a fantastic way and is called the polarization transfer technique. This is what we discussed yesterday. We will continue with further discussion today. And in the polarization transfer technique, there are two ways to do that. How do you transfer the polarization? One is by doing nuclear Overhauser effect, which I am going to discuss in one of the subsequent classes and other is what is called a coherence transfer. Coherence transfer is called INEPT. What is INEPT? We will explain that later. Now, in the sensitive enhancement technique, we will try to understand. One is, we will go for NOE, which is generally done through what is called through-space transfer. The spins need not be J coupled for it. For NOE transfer, spins need not be J coupled. What is required is a spatial proximity. As long as they are close in space, preferably less than 5 angstroms, then if you hit one of the protons or one of the spins, there is a transfer of magnetization to other spin. It could be more, or it could be less, that is a different question. But there is a transfer of magnetization without J coupling, without chemical bond. It is a direct through-space transfer. We will discuss this later. And here the efficiency depends upon $1/rij^6$, sixth power of the distance between two spins. And it is very large, because $1/rij^6$, small variation in r, because it is the power of 6, there is going to be enormous change in the intensity. So, this is the important concept. So, NOE is a distance dependent and small change in the distance will affect the NOE intensity or gain in NOE to a large extent. That will come to later.

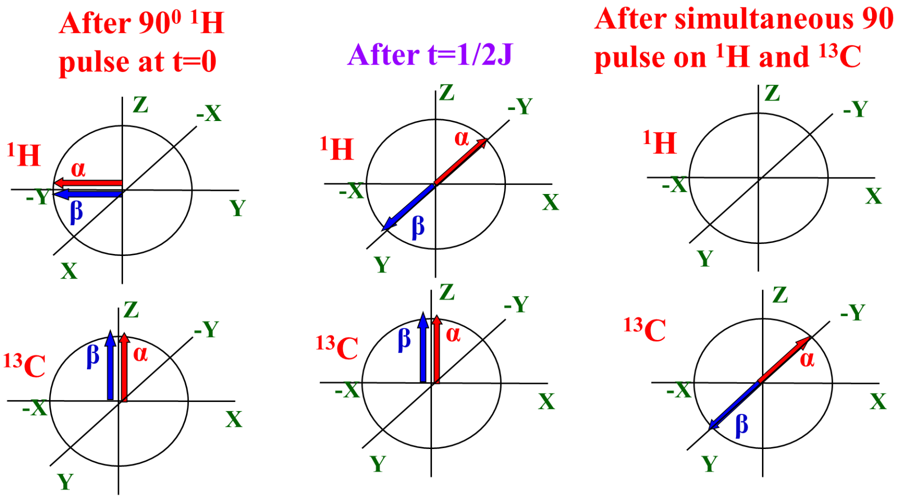
Next part is INEPT. The INEPT is what we are going to discuss today. This is called Insensitive Nuclei Enhancement by Polarization Transfer, that is called INEPT. INEPT transfer always occurs through J coupling. Remember, I told you in the NOE, no need of J coupling, patial proximity is the requirement. For INEPT, J coupling is important and it happens only through J coupling. And INEPT can be done by any two J coupled partners. It need not be only heteronuclear spins. For example, it could be two protons on adjacent carbons, that is vicinal protons, the two protons on the same carbon or adjacent carbons, that is also possible, and proton, which is directly bonded to carbon, one bond heteronuclear coupling, that is also possible, A proton and the carbon next to its own carbon, two bond heteronuclear coupling. Thus varieties of possibilities you can think of, vicinal, two bond coupling, three bond coupling, all those, the one bond heteronuclear coupling, everywhere you can do that. So, this is a very important concept, the polarization transfer. Where do you use it? We use this polarization transfer, I already discussed an experiment called DEPT, distortionless enhancement by polarization transfer. When I discussed carbon 13 spectra and I showed by doing this DEPT experiment, three experiments, 45, 90 and 135, I showed we can distinguish between CH carbon, CH2 carbon, CH3 carbons. At that time, I did not explain polarization transfer for you because I was discussing carbon 13 before, that was not introduced. Now, you have understood what is the polarization transfer. So, one of the experiments is the DEPT experiment, not only you could identify different carbons attached to different protons, there is a gain in the intensity because there is a polarization transfer in that experiment, distortionless enhancement by polarization transfer. That is why DEPT is an experiment you have to always use. Previously, we used APT, attached proton test. In APT there is no polarization transfer, it is only attached protontest. There is polarization transfer in DEPT. So, DEPT experiment also identifies different carbons attached to different protons, but here there is a gain in the intensity. Better and advantageous compared to APT. And I showed already several examples of how we can utilize DEPT when we discussed carbon 13, but only thing is, there is a polarization transfer, you please remember that we already discussed now. And also it is used in number of 2D experiment like HSQC and HMBC etc to gain the intensity of the dilute spins, enhance the signal intensity of the dilute spins if we use this one. There are two important consequences of INEPT. One is the sensitivity of dilute spins is enhanced because of polarization transfer from abundant spins to rare spins. There is a gain in sensitivity, we saw that. There is 4 times gain for proton and carbon and this is given by the ratio of gammas. If you go to nitrogen 15, it is 10 times. Now, secondly, the repetitive time for signal averaging is 5 times T1 instead of 5 times carbon. Carbon T1 is always larger than proton T1, usually. It means we can use 5 times T1 of proton, means it will save the experimental time, because the repetitive time is now 5 times proton T1 not instead of 5 times carbon 13 T1. As a consequence, there is enormous saving in the experimental time when I do ENEPT. Not only gain in intensity, there is also saving in the time.



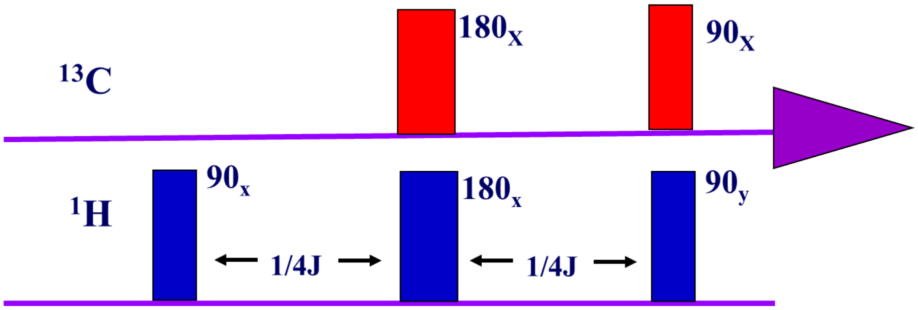
Now, how does INEPT works? We will understand that. Of course, we know polarization transfer so far we have understood. Same thing, but how we do experimentally is different, we will discuss that. All depends upon delay and application of 90 pulse, and 180 pulse that is all. We have been discussing this in the spin echoes. Exactly like spin echo, but we will see now how the polarization transfer takes place. Apply an 90 degree pulse to create coherence. Coherence means bringing the magnetization to x-axis or y-axis. Then wait for a period 1/2J. After 1/2J, the components of the doublets are opposite in phase, anti-phase doublet. So, now we will understand how it works. Apply 90 degree pulse on the proton channel and wait for a period 1/2J, there is anti-phase character for proton. That is what we have understood. So, you apply 90 pulse on whichever the axis you apply, according to the right hand component to find out where the magnetization comes. If you apply a 90 degree, let us say a pulse along this axis, magnetization is along the z-axis, it will come to this axis and then it will start moving by 90 degree for exactly 1/2J in the x-y plane. Now, this is a proton. Selectively, we are applying pulse on proton. This is a beauty of NMR. You can selectively apply the pulse on any one of the nuclear spins. What about carbon 13? We are not touching it. We are not even applying any pulse on carbon 13. That means there is no coherence in the x-y plane. As a consequence, you will not get any signal. Now, we do the Fourier transformation. Here, you are going to get a signal, anti-phase character. True. One is positive, other is negative. That is fine. Here, there is no coherence at all, absolutely no spectrum at all. This is just after 90 pulse. How the signal will be seen for proton and carbon. Apply 90 pulse, wait for 1/2J, you will have an anti-phase character signal for proton and no signal for carbon. That is a concept you have to understand. Now, another thing we will do is, we can apply 90 pulses simultaneously on both the J-coupled nuclei. For INEPT the important concept, I told you the spin should be J-coupled.



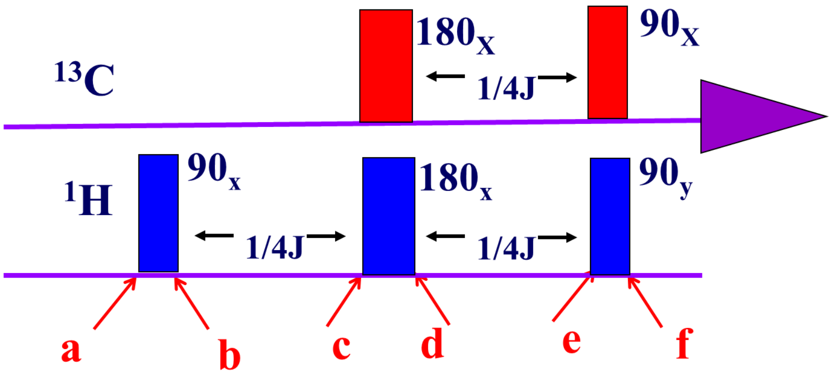
We apply 90 pulses simultaneously on both the J-coupled spins. Very interesting thing happens. When you apply 90 pulses simultaneously, the coherence will jump from one nucleus to the other. Instead of applying to only one of them, you apply simultaneously the coherence from x or one proton will jump to carbon-13 nucleus. A very interesting thing is going to happen. We simultaneously apply 90 pulses for a J-coupled spin, this is what happens. First, we had anti-phase character for proton and no spectrum for carbon-13. Apply 90 degree pulse, this is for proton and this is for carbon-13. Now, what is going to happen with 90 degree pulse when you apply? There is no coherence here, whereas carbon-13 will have anti-phase coherence. Simultaneously, I have applied 90 pulse on both, what is happening? The coherence of proton is transferred to coherence of carbon 13. Do the Fourier transformation. You have no spectrum in the started nuclei, but there is a signal in the transferred nuclei. From proton, we transfer the magnetization to carbon 13 and this becomes anti-phase character. We started with proton which is anti-phase, there was no signal for carbon 13. Apply simultaneously 90 pulse on both of them and do the Fourier transformation. Now, there is no signal in proton, but there is anti-phase signal doublet on carbon 13. This very interesting thing happens. This is what is called jump of coherence. Coherence is going to jump from one nuclear spin to other nuclear spin. With two simultaneous 90 pulses, anti-phase coherence is transferred. So, there is no coherence on the starting spin, only on the end spin, transferred spin. So, this is the simplest INEPT sequence, how does INEPT sequence works?



The simplest of which is like this, 90, 1/2J, two 90 pulses. This we have been understanding for quite some time. After a 90 degree pulse, after 1/2J, this is anti-phase character. That is how the doublet component moves apart by 90 degree, both of them. One will be move fast moving, other will be slow moving, both move by 90 degree, became anti-phase in character. That we have been understanding for quite some time. Just now I saw, I showed you already, we applied simultaneously 90 pulse on both of them. Then what is going to happen? The coherence will be transferred from proton to carbon-13. And this coherence transfer is possible only if they are J coupled. That is what I have been telling you. This is simplest of the coherence in a sequence. All I said is, you have to have anti-phase character created and apply simultaneous 90 degree pulses to transfer to the other spin. That is what we have to do. And we did this. But what happened again? Carbon 13 is anti-phase. Again there is a problem for us. See after 90 pulse, both proton vectors will be coming like this. And then carbon 13 is not touched at all. It is still on the z axis. After 1/2J, both the vectors move by 90 degree, became anti-phase in character. But here what is happening? Carbon 13 still I am not touching. I have not even applied a pulse on that. Now what I will do is, I will apply pulse on proton and also on carbon 13. Simultaneously 90 pulse on both of them. What happened? This anti-phase which was here moved here. This is a diagrammatically I am showing you, what is happening for the magnetization after 1/2J and after simultaneous application of both the 90 pulses on both proton and carbon 13. All the carbons may not be on resonance. This is I think what happens. But this should happen in such a way we have different chemical shifts for different carbons. They are all having different offsets. They may not be at the same chemical shift. Then what will happen? They will evolve as per the different chemical shifts. There will be phase distortion. They will be moving with different frequency, different phases. They will start processing in the x-y plane. If it was like I told you in the case of spin echo, quaternary carbon, there is no precession. It will be always along x axis. It is locked along that. Whereas with the J coupled one, the fast moving, slow moving will keep moving. If different carbons have different chemical shifts, they will be moving with different frequencies, different phases will be there. So, there will be phase distortion because all of them may not be having, may not be on the on-resonance because of different chemical shifts. Then how do you overcome these different chemical shifts? How do you overcome this evolution of chemical shift leading to phase distortions? We already understood this. Please remember, how do you do the refocusing of chemical shifts? Do a spin echo. When you do the spin echo, then what is going to happen? You refocus the chemical shifts. Apply a 180 pulse at the center, create a spin echo sequence. Then the chemical shift evolution can be stopped and then we will be refocusing. That is one way. So, what we do is put a 180 degree pulse in the middle of the 1/2J period. Then it will refocus chemical shifts of protons. Exactly that is what we do. So, another thing what will happen, there may be evolution of J coupling also during that period. How do you prevent that? How do you refocus J coupling? I told you already. If you have to prevent the J evolution, put 180 pulse on carbon 13 also. Simultaneously, if you put on that, then J would be refocused. So, in a sequence with chemical shift refocusing, the experimental sequence is like this. What is the experimental sequence? This was the sequence. In the case of proton, earlier 90, 180 was there. In between, you have put 180 exactly at 1/2J.



The otal delay is 1/2J. But what we did is, at the center, we have put a 180 pulse for refocusing of the chemical shifts. But now we are applying 180 pulse also to take care of J coupling, JCH, carbon-proton J coupling. Then simultaneously apply 90 pulse on both of them, transfer the coherence from proton to carbon, start collecting the signal. That is exactly what we are doing in this is ENEPT sequence.



How does the magnetization evolves during the ENEPT sequence, we can understand by a vector diagram. I will show you using the vectors, how magnetization is going to evolve during this. At different time periods of pulse sequence in the ENEPT sequence, we will lable by alphabets, A, B, C, D, E, at each stage of the pulse sequence. Then at different stages of A, B, C, D, E, what is happening to the spin vectors? We will diagrammatically visualize. We can try to understand diagrammatically. This is the pulse sequence and this beginning of the pulse is A, then 90 pulse is B, then evolution C, D, E and F. We have put six different time periods, points. This is a spin echo sequence, we apply 180. Of course, you all know what happened to the magnetization at A, nothing is being done. If we wait for a long time, the spins would have attained thermal equilibrium. That is well known. Apply 90 pulse, what will happen here? Immediately there is a phase coherence, you bring all the magnetization to one axis. You will flip by 90 degree. That is what happens. Proton is the doublet due to coupling with carbon-13 and HA and HB are the doublet components. At A, both these components of the doublet are along Z axis and is in equilibrium, because you have even disturbed, completely spins are relaxed and they are in thermal equilibrium along Z axis, both alpha and beta components of the proton. Why alpha and beta are coming? H alpha is coming because of C alpha, H beta is coming because of C beta, because of coupling. Alright, this is the diagram at point A in the pulse sequence, both the alpha and beta vectors of proton are along Z axis. We will keep it like that. Continue further. At B after 180 pulse, H alpha and H beta components of the magnetization are flipped to Y axis by 90 pulse. I am applying X pulse, so I am flipping to Y axis. All X, Y, Z are orthogonal to each other. That you know. I am just simply telling X, Y axis, it has moved to Y axis. Both alpha and beta components are there. That is the situation at point B. That is correct. Now, it is very clear for you. Both which were here, I have brought them to X axis or Y axis. What is happening now? We are allowing it to move for ¼. How much it is moving? Pi into TD into 1/JCH. You multiply by that. So, now TD is 1/J. Pi into 1/4J into JCH is pi/4. What is pi/4? 45 degrees. So, what is happening is, at C you use that equation, theta is equal to pi into TD into JCH. So, TD is 1/4J, this J and this J cancel out is 1/4. So, pi by 4 is 45 degrees. So, alpha and beta components at the point C would have moved by 45 degrees. One is a fast moving component and other is a slow moving component. This is how it is. One moves like this, other moves like this. Alpha and beta components have rotated by 45 degrees at exactly 1/4. So, this is the situation at point C. We will continue further. At point C, another thing what is happening is, immediately at that point we apply a 180 pulse on proton. We will worry about carbon-13 later. What does 180 pulse on proton will does? It will invert the populations. The alpha and beta components get inverted. What will happen? Alpha will become beta, and beta will become alpha, they get inverted. But they continue to rotate in the same direction. They have been inverted applying a pi pulse, then what will happen? We are applying a pi pulse along x. So, here you rotate like this. When you rotate like this, the vectors which are here come to this direction and they were moving like this, they continue to move in the same direction. So, at D, now we are applying simultaneously pi pulse and carbon 13 also. So, 180 pulse on proton inverted the alpha and beta components of proton. But you are also applying on carbon 13. What does this do? Remember carbon 13 is still on the z axis, we have not touched it. We have not applied pulse on carbon 13. Look at this sequence here. Now only we are applying 180 pulse. Till now we have not even touched the carbon 13 spin. Then what it will do? It will change the magnetization from plus z to minus z. They were at thermal equilibrium, carbon spins were at thermal equilibrium along plus z. Now come to minus z with 180 pulse. The population between the levels 1, 3 and also between 2 and 4 are inverted because of carbon 13. That is what we did. So, this is what happens. Before 180 pulse, this is the population difference which we saw for calculation of the polarization transfer. We just took the example. This is before 180 pulse on carbon 13. Now 180 pulse on carbon 13, what is happening? See here, these spins have been moved here and these here. So, what is happening? The inversion has taken place. Spins from this state, 2 have gone to 4. Spins which were in 1 came to 2 exactly. So, the population inversion has taken place for carbon 13 which was along z. So, H alpha component, why it was coming? It was coming because of Calpha and Hb was coming because of C beta. Now what is going to happen? H alpha which was coming of C alpha, remember before 180 pulse, the two alpha and beta components of doublet correspond to 2 alpha and beta components of carbon 13. The H alpha which was coming because of C alpha now becomes C beta or H beta. Why? Because we are applying 180 pulse on carbon. Though we are interchanging, we are inverting the population. So, H alpha is now because of C beta. Similarly, H beta is now because of C alpha. This is what we simply did the experiment by applying a 180 RF pulse. And what is the effect? The effect of this is we have changed the label of alpha and beta of proton. What was alpha became beta. What was beta earlier became alpha. The effect is we interchanged the labels of alpha and beta spin states of proton. That is what we did. What does it mean now? Earlier when you applied only a 180 pulse on the proton, we inverted the population, they continue to move in the same direction. But now simultaneously we are applying carbon 13 pulse, 180 pulse. H alpha became H beta, H beta became H alpha. That means, fast moving H alpha became slow moving H beta, slow moving H beta became fast moving H alpha. Then they continue to move in the opposite directions. They move in the opposite directions now. As a consequence, what is going to happen? The proton spin components continue to move in the opposite directions for another tau delay. What is that another tau delay? We have applied 180 pulse in the middle of 1/2J. So, another tau delay is 1/4J. So, what is going to happen now? When they start moving, continue to move in the opposite direction, they instead of getting refocused, they continue to de-phase. They continue to move in the opposite direction. If they were moving in the same direction, they would have refocused. They are moving in the opposite directions. So, what is happening for the proton? By simultaneous application of two, 180 pulse on proton and carbon, we interchanged the labels of proton. I mean, especially the proton doublets, not carbon, proton doublets. Proton also of course, 180 pulse inverted the population, but proton which was coupled to H alpha coupled to C alpha now became H beta. H beta coupled to C beta became H alpha. As a consequence, we interchanged the labels of proton spin states and they continue to move for another tau delay which is equal to 1/4J. Instead of getting refocused, they start moving in the opposite direction. They get continuously dephasing further. But you should also remember one thing. So far the carbon 13 magnetization still along z axis. We have not touched it. Only thing is we applied 180 pulse and inverted from plus z to minus z. That is all we did. Now, let us see at E what is going to happen? At E after the evolution for 1/4J, this H spin components acquire anti-phase character. They started moving opposite directions, you know. So, it will attain anti-phase character. Now, components are in the xy plane. Remember, they have acquired anti-phase character at point E. True. You know, in the previous example, you saw here, they start moving and after exact half delay, this will come here, this will come here or whatever it is. They come and become anti-phase character like that. Okay. At F what is going to happen? A pi/2 proton pulse rotates xy components. The proton pulse moves H alpha components. Now, because they are anti-phase here, pi pulse on this will rotate it. H alpha will go here, H beta comes here. This is what happens. It rotates xy components, because they are all orthogonal to each other. You know, we have been discussing this from the class 1. H alpha component goes to z, becomes z, H beta goes to minus z. The Populations between 1 and 2 get interchanged. That is what we did by applying proton 90 pulse. The population between states 1 and 2 get interchanged due to 90 pulse on proton. This is what is happening now. This is 90 pulse on proton. What happened here? You see the population between 1 and 2, wait, 2 was here came down and this 10 was here went up. At F what is going to happen finally here? The carbon 13 transitions have population difference of minus 6 and 10, the ratio of minus 3 to 5. That is what we know. When you apply simultaneously 180 pulse and also simultaneously 90 pulse, the magnetization is transferred from proton to carbon. It is minus 3 is to plus 5 is the intensity ratio. This is what we are going to get. So, we got an anti-phase character for carbon 13. Exactly what I said in the INEPT, though I explained to you, this is all we require for getting INEPT. This was added only to refocus chemical the shifts. And then finally, like we discussed by transferring the magnetization, there is a magnetization of carbon 13, 2 components, C alpha and C beta, there is the intensity of minus 3 and plus 5. This is what we saw. There is a transfer of polarization that finally is going to happen at F. The carbon 13 have anti-phase character, transferred from proton and in the ratio of minus 3 to plus 5. At F, the carbon-13 90 pulse create anti-phase transverse component that are detectable now. Here, you are also applying 90 pulse on carbon 13 for detecting and that will bring magnetization which was along Z axis to detectable along X-Y plane, X axis or Y axis. If you have a receiver here, it brings to Y axis. So, ΔC is equal to 5, ΔC is equal to -3, anti-phase character and here we can detect the signal. The component of the doublet are anti-phase with intensity -3 and 5 like in the heteronuclear polarization transfer. And of course, this is what we have to do. We have to collect the signal. But here, there are two things which you have to solve. If it is anti-phase character, we cannot do the decoupling. I told you, if we do the decoupling, whatever the intensity gained is lost. So far, we understood how the magnetization is transferred, the polarization from proton to carbon 13, by pulse sequences and in between two 180 pulses for refocusing, chemical shift, etc. we discussed. But at the same time, if you also know this is the anti-phase character, a decoupling is not possible. It is very difficult. It is possible, but you will lose the signal intensity whatever the gain you got. You have to address that issue, and another issue is there is also natural abundance carbon signal coming. That also we have to suppress. If you do not suppress it, the polarization transfer would not be 100%. We will not be seeing the 100% transfer of signal. So, we have to suppress the natural abundance carbon 13 signal and also we have to convert the anti-phase to in-phase to go through the decoupling. This is the important thing we do. So, in the next class, I will discuss how we do this. Right now, I am going to stop, but I told you what we did today. We discussed about the spin echo sequences and polarization transfer, especially polarization transfer in heteronuclear case. We understood there are two ways of doing polarization transfer, NOE and INEPT. NOE do not need a J coupling, it is a through-space direct transfer, whereas INEPT requires a chemical bond, we saw that. And then how INEPT transfer takes place from anti-phase transfer to anti-phase transfer, anti-phase signal to anti-phase signal. In proton, first we create anti-phase signal by applying a pulse and giving a delay of 1/2J, and then apply simultaneously 90 pulse both on them. The magnetization jumps from proton to carbon 13. And the carbon 13 will be anti-phase, but there will be evolution of chemical shifts also, to avoid that we apply a 180 pulse in the middle and create a spin echo sequence in the middle of TD and then you are going to see what is going to happen. We get anti-phase characters for the carbon 13. Finally, we understood diagrammatically in different stages of pulse sequences, at different stages of pulse sequence how the magnetization evolves. And we finally understood carbon 13 magnetization is achieved because of polarization transfer, and is anti-phase character with intensity -3 and +5. Also, the natural abundant signal is coming that also has to be suppressed. And anti-phase has to be made in-phase character, only then we can do the decoupling. That already we know how we can do. In the earlier example I showed you, when we are trying to do understand the spin lock. Now, the spin echo, I told you that earlier anyway. So we will discuss how do you address these two issues in the INEPT sequence in the next class. Thank you very much.