One and Two dimensional NMR Spectroscopy: Concepts and Spectral Analysis
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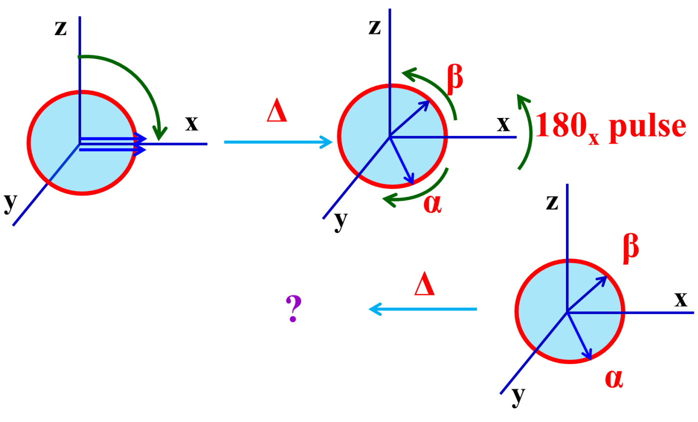
Lecture 33 Spin Echoes-II

Welcome all of you. In the last class, we started discussing about spin echoes. I told you what is a spin echo. It is obtained by the application of two pulses with a delay. It could be 90 tau 180 tau or 90 tau 90 tau. And the two delays should be exactly equal. Afterwards, you are going to get an echo. I explained to you how the spin echo works. Then what is going to happen is, the spin vectors, when you apply a first 90 pulse bring them to x axis or y axis, they will



start dephasing with the time. Then after some time, what will happen when they are dephasing? There will be fast moving and slow moving components. With the time tau, they will start moving and after certain delay, you apply a 180 pulse. Then what will happen? The spin vectors get interchanged. Fast moving becomes slow moving and the slow moving becomes fast moving. So, they get interchanged. As a consequence, after some time, all the spin vectors will assemble at the same place on the same axis. It is exactly like I gave you an analogy of runners. Several runners will be asked to run for a distance. They will be running at different speeds. After certain time, you ask them to stop where they are. Some would have moved long distance, some would have moved short distance. Whatever the distance they have moved, they are all at different distances. Like spin vectors dephased at different frequencies at different phases. And immediately ask them to turn back and run at the same speed. They will all assemble at the same place. This is exact an analogy for a spin echo. That is what happens for the spin echo case and I explained to you by taking an example of homonuclear two spins and multiple spins. I showed that whatever may be the offsets or chemical shift difference between the vectors, on doing a spin echo, the chemical shifts gets completely refocused. As a consequence, chemical shifts will not evolve. There would not be any chemical shift at all. It appears as if there is no chemical shift. They are all refocused. This is what we understood. Now, the question comes is what happens, why only chemical shift? What happened to the J coupling? That is the thing which we need to understand. Let us consider what happened to the J coupling. Of course, in AX coupled spin system, there should be J also. But I took an example of J_{AX} being 0, so that only chemical shift I wanted to consider. Now, I will consider an example with J being present, an example of two spins A and X. This is what it is, A and X two spins are coupled, and then we have doublet for A. Why the doublet comes? We have been discussing quite a bit, it is because of the spin states alpha and beta of the spin X. Similarly, X will also have the doublet. We will consider what happens to the doublet. Let us say I am going to apply a 90 pulse and bring the magnetization to X axis.

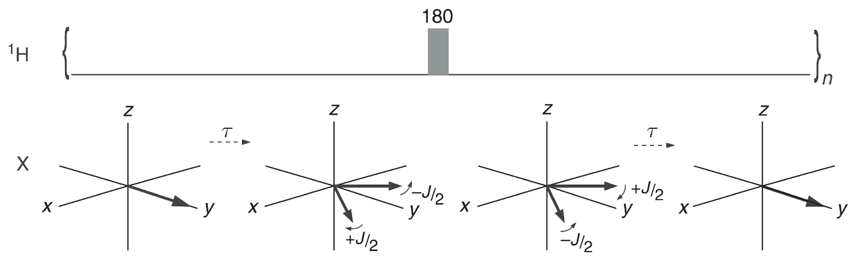
I will consider let us say spin A or X, whatever it is. Then it will have doublet. I am considering spin A. When it is coupled to spin X, it has alpha and beta components. Then we will have two vectors here, one corresponding to alpha orientation of X, other corresponding to beta orientation of X. So, we have two vectors. And with time delay what happens? These alpha and beta vectors of A start moving in either direction. So, one is a fast moving vector, other is slow moving vector with respect to this x axis. One moves towards this side, one moves towards that side. Okay. Now, you apply a 180 pulse. What is going to happen? I said everything gets reversed. You apply a 180 pulse. Now, alpha was here, alpha went there and beta came back here. In the chemical shift case what happened? They continued to move in the opposite directions and then refocus on x axis. That is what I said. But now there is a J coupling. What is the meaning of that? That means after a certain delay, the question comes will they start moving in the opposite directions? will they refocus or will they move in the different directions? Will they continue to move in the same direction or in the opposite direction? will they refocus or not, is the question? We will answer this question now.

Remember when you have vectors and will they refocus or not is the question. Remember 180 pulse also inverts the relative orientation of J coupled partner. You are considering the doublet, that is two vectors of the A spin which is coupled to X, but 180 pulse also inverts the orientation of X partner, that is to alpha and beta of X. As a consequence what happens? The doublet component of A which is coupled to X alpha will now be coupled to X beta. Remember, A will have two components A alpha and A beta. A alpha comes because of X alpha, A beta comes because of X beta orientation, but they get reversed now. So, X alpha will now be coupled to X beta and vice versa. Now alpha and beta components are interchanged. That is what I said, because of 180 pulse also acts on X spin. What is happening now? The faster moving components move slower and vice versa. Then instead of refocusing, they continue to move in the same direction. They will continue to dephase, they will not refocus. They continue to dephase. This is what is happening. They would not refocus now. Earlier what happened in the case of only chemical shift, after 180 pulse they started moving backwards, like this and then refocus along the X axis, but now because of J coupling they started moving in the opposite directions like this. So, fast and slow moving components got exchanged, they started moving in the opposite directions. So, there is no question of dephasing. In fact, it there is no question of refocusing continuous dephasing goes on. So, that means J splitting in the homonuclear case are not refocused by spin echo. Remember, homonuclear spin echo refocus only chemical shifts and not J couplings. Please remember, homonuclear spin refocuses only chemical shifts and not J coupling. This is for the homonuclear case.

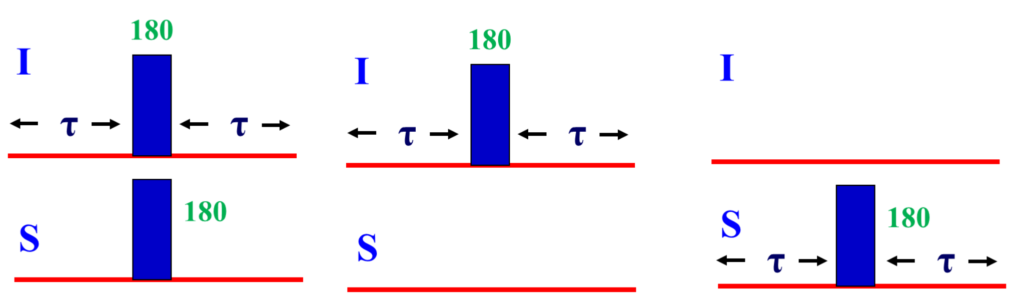
You may ask me a question what happens if I consider the hetero-nuclear spins. Not homonuclear 2 protons. Instead of that I will consider proton and carbon, heteronuclear spins. How the J evolution takes place under spin echo? Now we can think of several possibilities for the J coupled heteronuclei. What we can do is, we can apply 180 pulse only on the spin A that is possible, because you know a heteronuclei, all are at different resonating frequencies, several MHz apart. As a consequence, we can separately or individually apply RF pulse, only on spin A, only on spin X or both on spin A and spin X simultaneously. All the 3 possibilities exist. Whereas, in the case of the homonuclear you apply a 180 pulse on A, then simultaneously X pulse which is coupled it also gets reversed, because it is a homonuclear spin. But here I can separately apply 180 pulse either only on proton, only on carbon, or both of them.

Let us see when the 180 pulse is applied on either A or X only what will happen to this thing. So how the vectors will start moving? What will happen is, please remember, now I am applying the 180 pulse only on A or X. Then only that spin will experience 180 pulse, the other heteronuclear spin will not experience, because there are MHz apart. And it is not going to be touched, and it is not getting affected at all. As a consequence only particular spin on which you apply 180 pulse experiences this 180 pulse and the effect is seen only on that. So in such a case what will happen? Similar to chemical shift the J coupling will refocus. What happens after the 180 pulse is applied only on one spin, because in this case coupled spin is not touched. Only when coupled spin is touched I said alpha and beta components, for example A alpha coupled to C alpha, will go to beta

spin and they get interchanged, alpha and beta spin states will get interchanged.
That will not happen now. So similarly like chemical shift they continue to move like this and then refocus. What does it mean? If you apply 180 pulse only any one of this spins, J coupling will refocus. What happened in the case of homonuclear J was not refocusing at all, but in the case of heteronuclear whether you apply 180 pulse only on A or only on X does not matter, J will refocus. This is what happens. In fact we always say 180 pulse on a heteron

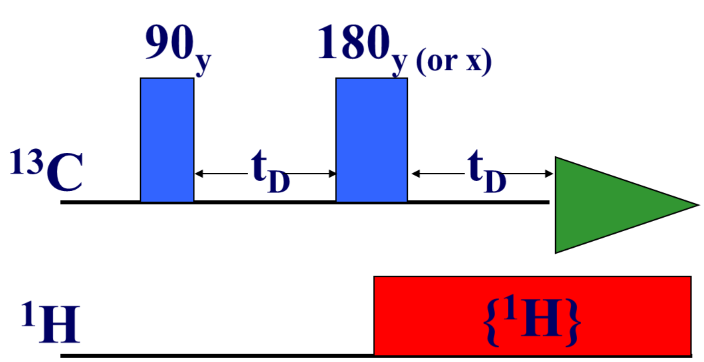


What do you mean by that J couplings will not evolve? See for example inter nuclear vector comes along y axis because of J plus half and minus half, they will start moving in the opposite directions. And after the 180 pulse what will happen they will again continue to move in the same direction, they get interchanged. But then they continue to move. Instead of plus half moving like this, it continue to move in the same direction. Only the positions gets interchanged, but the direction of motion continue to be the same. As a consequence, what happens? they refocus along same axis. It is nothing but telling they are decoupled. So, remember we always say you apply a 180 pulse at the middle of the evolution period on one of the spins A or X, in a heteronuclear system then you will have J coupling refocused, which is nothing but decoupling, that is a concept.



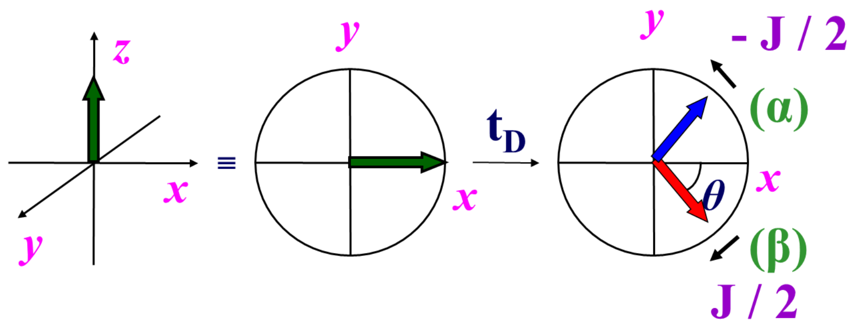
Now, you may ask me a question so far you are considering only application of 180 pulse on either A or X, this phenomena happens and J will refocus. What happens if I apply 180 pulse simultaneously on both heteronuclear spins, that is also possible. Then what will happen? it is like applying to homonuclear spins. If you apply a 180 on A spin, the

X spin states gets interchanged also. As a consequence, A which is in alpha state will experience beta and vice versa, exactly analogous to homonuclear situation. When you apply 180 pulse simultaneously on both heteronuclear spins, then vectors will start dephasing. There is no question of refocusing at all. The vectors will not refocus, you understand. Please remember the concept. In the homonuclear spin echo, the chemical shifts are refocused but not J couplings. In the heteronuclear spin echo, if 180 pulse is applied on any one of the heteronuclear spins, A or X, then what will happen J will refocus. Whereas we apply 180 pulse simultaneously on both A and X spins, then in heteronuclear J will not refocus. This is the important concept, you must remember. So, heteronuclear spin echo this is a situation. When I am going to apply 180 pulse both on I spin and S spin, where I and S are heteronuclear spins, usually I is considered as proton S as a heteronuclear spin or some other spin, the offsets are refocused, J coupling will evolve. They will not refocus. That is what I said. The 180 pulse simultaneously on both, then there is no question of refocusing of J coupling. So, only chemical shifts get refocused that is like 180 pulse like on homonuclear case. What happens if you apply 180 only on I? just now we discussed only I spin offset is refocused but J coupling is also refocused. Nothing will happen S spin chemical shift, it keeps evolving. Now apply 180 only on S spin not on I spin. What is going to happen? extend the logic now S spin offset is refocused and J coupling is also refocused, but for I spin offset I spin chemical shift nothing happens. These are the three different spin echo experiments you can do when you have heteronuclear spins involved. With this idea we understood now what will happen to spin echo cases. Spin echo is just 90 tau 180 tau sequence, and what happends depends upon whether applying on a homonuclear spin or heteronuclear spin. If it is a homonuclear spin chemical shift will refocus J coupling will not get refocused. If it is heteronuclear case, if 180 pulse is applied on one of the spins, J will refocus whereas if 180 pulses are applied simultaneously like this, what is going to happen J will continue to evolve, if applied simultaneously and both, that is it.

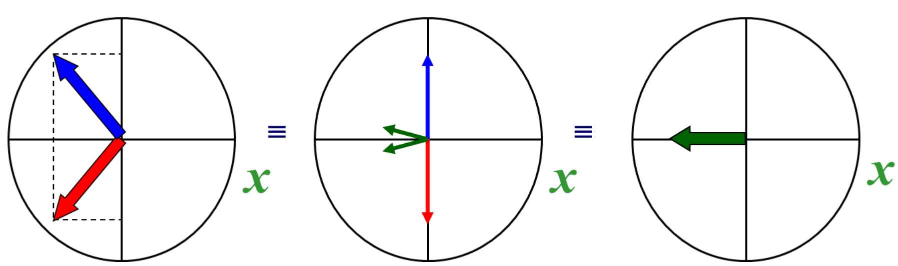


Now we will see some J modulation of spin echo which is something important concept you should know. What is the meaning of J modulated spin echo? Let us consider the 13C spin echo sequence, under decoupling. What is the spin echo sequence? It is nothing but this one; 90 td 180 td. This is a delay for echo, both are identical. The 180 pulse you can apply on Y or X axis, does not matter. And then what we do is, after this immediately we start collecting the signal by decoupling proton. We need to do the decoupling, because as I told you 13C is always recorded with the broadband decoupling, so that you get individual resonances for each chemically inequivalent carbon. So, we have to do the decoupling fine. In the first td what will happen? the coupling between carbon and proton, here I am taking carbon and proton, they evolve but during the second td, what is happening? I am applying the RF pulse; they are decoupled but chemical shifts are refocused during both the time, that we have already understood. The chemical shift get refocused but J coupling gets decoupled in the second delay. This is what is the spin echo we have to do and then decoupling; If we have to decouple this thing. But how does these different carbons evolve in the spin echo sequence. Now I am considering the heteronuclear spin echo because I am looking at carbon coupled to protons, such as, CH, CH2, CH3 and quaternary all the four. How these vectors evolve in the spin echo sequence we will try to understand that. And of course I am considering one bond coupling, forget about long range couplings we are not bothered. The evolution of magnetization vectors of the 90 degree pulse we can understand in a simpler possible way. There is an equation which I have not discussed, but in the previous courses I have discussed. After applying a pulse bring the magnetization to x-axis or y-axis and give some time delay td. The spin vectors start dephasing. They start moving differently, the faster and slow moving components will be there and depending upon the strength of the coupling they start moving. How much vectors have rotated, how much they have moved in the xy plane is given by an angle theta. And theta is given by pi into time delay into J coupling. If you now this J coupling, if you know how much time you have as delay, then I can tell you how much the spin vectors have moved in the xy plane. Very easy to calculate. All you need to know is time delay and J coupling, and I know this is a constant I know angle by which these vectors have moved, that is correct. But remember one thing since we are worried about the J coupling, the quaternary carbons are always stationary, there is no J coupling; there is no fast moving and slow moving components, and they remain stationary. They will be always along one axis. So, if you detect that you always get a positive signal there is no question about it. You bring the magnetization to x-axis from the z-axis put a receiver here you always get signal for quaternary, positive signal, fine. What happens to other carbons we are not worried only about quaternary carbons. What about other carbons and let us consider one by one. First we consider only CH carbon, the carbon coupled to single proton. What is the multiplicity pattern? It is a two spin coupled, two spin half nuclei, so carbon will be a doublet. We are not worried about proton, we are looking at carbon, it will be a doublet.

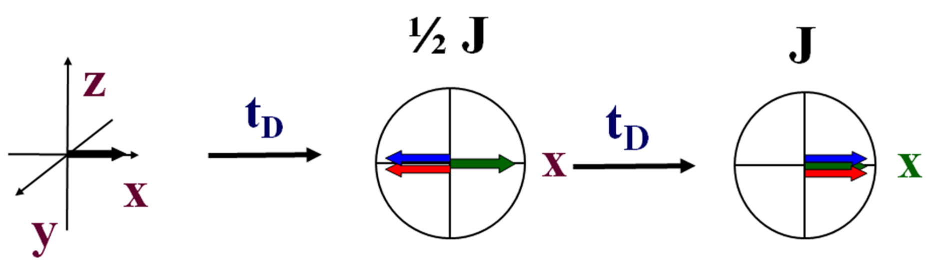
Now what we will do is after 90 degree pulse, the doublet components alpha and beta of carbon starts evolving under JCH. And for the interval TD which is given in the spin echo sequence. What is JCH we can always know, we know the JCH. This is diagrammatically what happens. The magnetization initially at thermal equilibrium, it is at thermal equilibrium, apply 90 pulse and bring it to x-axis. The alpha and beta components start rotating in opposite directions. The fast moving and slow moving components. But remember, exactly after 90 pulse, 45 degree, 180 degree, what happens we can calculate using that equation, theta is equal to pi into td into JCH. That we know, that, we can calculate. Now for different values of J how much it moves we will find out.



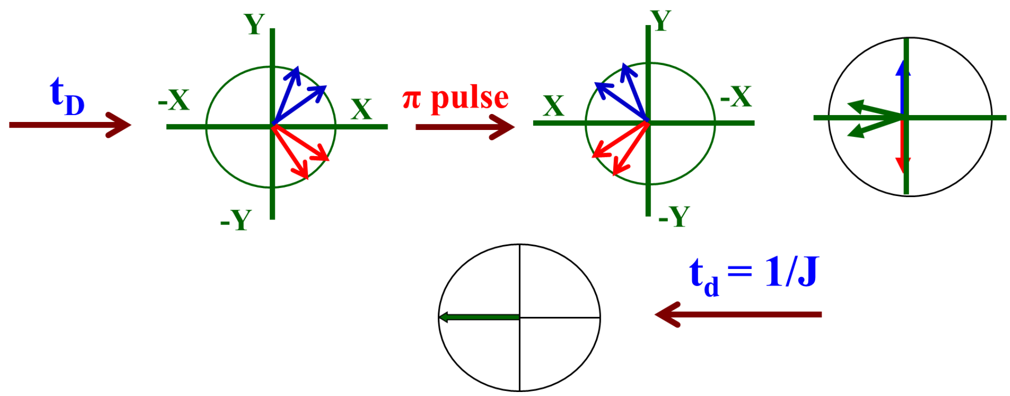
For example for td exactly to 1/4J whatever the J value I do not care, how much it has moved put TD is equal to 1/4J; this J and J cancel out, it is 1/4 of pi, it is 45 degree. What will happen is, the spin vectors both alpha and beta components would have rotated by 45 degrees, that is fine. So this how it is. At exactly 1/4J, the X and Y components have moved by 45, degree in the x-y plane I am talking. It is I am showing only the two-dimensional graph but it is from z axis it brought x-y plane you have to see in the imagine in three-dimensional space. I am in the x-y plane I am talking they moved by 45 degree from x-axis. It was there, started moving like this. Spin vectors would have rotated by 45 degree, that is what we calculated, fine. We will go further, and calculate what happens if it is 1/2J of CH. That is again put the value td is equal to 1/2 JCH, then it is become 90 degree. What would have happened the magnetization would have come along x-axis and now they start moving in the x-y plane, by 90 degree. Both of them would have moved 90 degree. From x it would have come to y; plus y and minus y components should be there. So there will be opposite in phase, there will be anti-phase magnetization. See if the doublet vector both are in the same phase like this, they are in phase doublet. If one is positive and the other is negative like this, it is called anti-phase doublet. So now after exactly 1/2 JCH the alpha and beta components would have become anti-phase. They would have acquired anti-phase character, and exactly phase difference of 180 degree. Why along y-axis? because in the x-y plane you remember 90 degree from x-axis they would have moved and Z is along this axis, so one component would have moved to plus y other would have moved to minus y. So that is what I am showing you here, and it would have acquired phase difference of 180 degree. Then if you measure the intensity, have a receiver here it is 0, whereas the quaternary it was here and you measured it from here it was full intensity. But now CH intensity at exactly equal to 1/2J because this vector alpha and beta vectors are opposite in phase by 90, each of them are anti-phase in character. It is a 180 degree phase difference, the intensity is 0, because positive negative component of the vector gets nullified. There would not be any signal, you will not get a signal, fine. Now after the time delay you apply a pi pulse. What will happen? we are given delay after exact 1/2J it would have become anti-phase. Now we apply a pi pulse. What is the duty of the pi pulse I have not discussed in this course, but in the previous course I have discussed. The pi pulse always inverts the signal; from plus z axis it will bring to minus z axis. In other words what is going to happen is it will completely reverses the alpha and beta component from plus z to minus z and from minus z to plus z and inverts the magnetization. Simultaneously you start doing the proton decoupling, this what happens. The alpha and beta components you can start resolving into vectors, and let us say, after 90 degree they get interchanged, 180 pulse invert the magnetization from here to here it became like this. Now resolve the magnetization into two components these vectors in the opposite direction gets nullified. This gets added up. So there is a magnetization along this axis. Where is that magnetization now? it is in the minus x axis. What happened now in the case of CH vector? At exactly equal to 1/2J you apply a 180 pulse and you see magnetization is along minus x axis. Both the opposing components are canceling out; the vector addition cancel this one, whereas this one gets added up. Alright that means you are going to get a singlet, that is fine. So each doublet vector now at theta is equal to 1/J we have 180 degree each doublet vector will rotate through one half like this, from here it will go to one half like this; the other one come like this and then they meet again at minus x. That is what it means. Of course components along this axis gets nullified. Only this component we are detecting, alright. What do you understand from that? In the homonuclear spin echo we discussed, this vector start dephasing; and again they come back into the same axis, both the vectors. We called it as refocusing. It means chemical shift is refocused but the signal is along minus x axis.



Instead of detecting in the plus axis, signal is in the opposite direction, minus x. What does it mean? It means if you detect the signal like this, you have a receiver here, signal is along this axis you get a negative signal. The same signal with a negative phase. The negative signal means negative phase. The quaternary was here only and it is positive. The CH is negative. That is what happens. So intensity of CH you can also calculate. I took only example of 2 or 3 time delays, like, 1/4J, 1/2J like that. But instead what will happen as a function of delay? Keep on varying the delay and calculate it at exactly equal to 1/2J signal is 0, that is what we saw. At exact 1/J it to comes back and is negative intensity. That is what we saw. At 1/J the vector component starts moving and then reassemble, refocus along negative x axis. So you are going to get negative signal for CH. So CH carbon you get a singlet. There is a chemical shift refocusing but also negative intensity singlet. And how does the intensity vary it depends upon the cosine of the angle, cosine of the delay and it is 0 for Td values equal to multiples of 1/2J and maximum for 1/2J. That is like what you saw in this graph it is 0 for 1/2J or multiples of that. It is always like this for multiples of 1/J. So J CHs generally are not identical you may ask me a question for different carbons, with proton 1 bond coupling could be different need not be same. The different molecules, one may have 140 Hz, one may have 150 Hz other may have 200 Hz, 170 Hz, like that. We always assume approximate value of 150 Hz and then start the work.



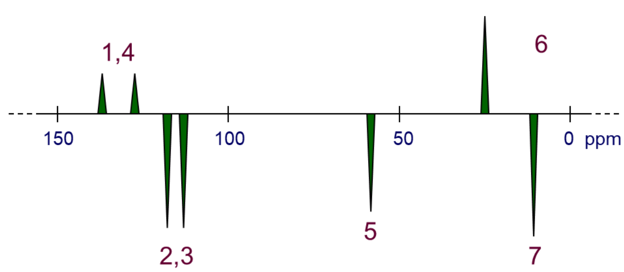
What happens to CH2 carbon? something interesting. The CH_2 carbon is a triplet. The central component will not process. It will always gets aligned along x axis. Now what will happen for a td again same apply 90 pulse bring the magnetization to z axis x axis and give a delay what will happen? exactly at 1/2J this will come back. Earlier they were anti phase like this, but now they are going to minus x axis, why because remember in the J coupling here is like this for one tablet, whereas here in the triplet this itself is J, not half like here. Here if you measure this J, the one component is here; the half the J; this is half J. Whereas here one J. That means if you have a triplet one of the component with respect to central component itself is the J value. It is almost twice of J coupling between this one doublet. We can say for the doublet it is almost half of it compared to triplet. As a consequence for 1/2J instead of CH proton where it has anti phase along y axis, they come back and refocus along this. We will now give another time delay after the 180 pulse. What is going to happen? They come back and all the three components 1:2:1, get aligned here. And we are collecting the signal here. What is that we are going to get? The CH2 carbon has a signal intensity, as a function of td when you consider, the situation of a triplet it is like this. At exactly 1/2J it is intensity. You may be surprised why it is 0 intensity previous CH case they were anti phase along y axis it was 0. But now why it is 0? We will go back and see. Here both the components of the vector doublet here and a central component is here. Again vector addition at 1/2 J is 0. As a consequence. you can see at 1/2J its intensity is 0. At 1/J all the three vectors come back to the x axis. They reassemble, they refocus. As a consequence, it is a positive intensity. So CH2 will be a positive intensity for CH2 carbon. Also you can monitor the change in the intensity.

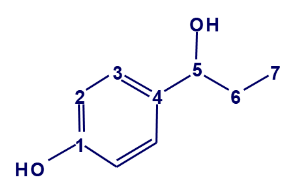


You may ask me a question, What happens to CH3 carbon? CH3 carbon, remember has four vectors from the center we have 1, 3, 3, 1. But what is going to happen is like this. So, now let us see CH3 carbon has four vectors two fast moving and two slow moving. After the td delay they will be like this. Two fast moving, two slow moving will be like this. Apply a pi pulse, then again reverse it. pi pulse invert the magnetizatioin, they invert it. Now depending upon which axis you are going to apply, I showed you already, you can apply along this axis rotate like this, we apply along this axis you can rotate like this along y axis. It is rotated again resolving into two components. The components along this axis gets nullified because of vector addition, whereas this one gets added up. After some time delay exactly td is equal to 1/J, then what will happen they will refocus and will be a negative singlet. What is happening now your receiver is here, signal you are going to get is negative, again singlet with a negative intensity. That is the behavior of CH3 is similar to CH. The intensity is maximum at 1/2J and negative maximum at 1/J. Now CH3 carbon variation if you see, exactly at 1/2J it is 0. For all the three carbons CH CH2 and CH3, at exactly 1/2J signal intensity is 0. But at 1/J, the CH3 and CH are negative, and CH2 is positive. That is important. You should remember. So I am showing this in a table like this.

Delay (t_D)	Quaternary Carbon	CH Carbon	CH_2 Carbon	CH_3 carbon
$1/2J_{CH}$	+	0	0	0
$1/J_{CH}$	+	-	+	-

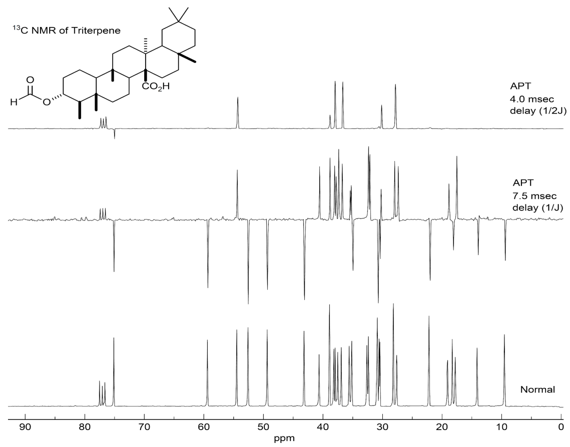
You have different time delay. When the delay is equal to 1/2J, for a quarternary carbon it will never persist and is always positive; at 1/2J all CH, CH2 and CH3 are 0. Whereas at 1/J, CH is negative CH2 is positive, and CH3 is negative. We can calculate the signal intensity as a function of J modulation is like this. As a function of J modulation if you keep on varying the td, which correspond to 1/2J or 1/J like that when it is exactly at 1/ 2J which correspond to 90 degree all the carbons will have 0 intensity. CH2 is positive, CH3 and CH are negative at 1/J. You see at 1/J it keeps going. And that is how it is and only quaternary in carbon is seen. This is important thing you remember, the quaternary carbon is unaffected. As a consequence, 1/2J quaternary is always positive, that is always seen. Only other 3 carbons are 0. So the delay depends upon 1/JCH makes CH and CH3 carbons have one particular sign, CH2 and quaternary carbons of opposite sign. Whether you make this positive or that positive is your convention. Conventionally what is done is these two are always made positive phase corrected, and these two are made negative. You can do the way you want, you can reverse it also. But convention followed is this





one. So the J coupling although it is different assumed to 150 Hz and do this and this what happens. Look at this simple example, an hypothetical example of a molecule like this. The CH2 and quaternary are positive, CH3 and CH are negative here.

This is called attach the proton test. By doing this spin echo experiment with J modulation at setting the delay which is equal to 1/JCH. You can find out depending upon the sign of the signal whether it is positive or negative you can say whether it is CH or CH3, and whether it is quaternary or CH2. That is possible. This is a realistic example of a molecule. See for this, these three are positive correspond to CH2 and this is a quaternary which is positive these are all CH3s and CHs.



And this is an example of a molecule triterpene. You see when delay is equal to 1/2J what do you expect? I showed you at 1/2J all the three carbons are 0. All the CH, CH2, CH3 are zero, but quaternary is not 0. So if you set the delay equal to 1/2J in the attached proton test only carbons which are not attached to any protons you are going to see, including CDCl3 solvent. Whereas, at delay equal to 1/J you can see that this is CH2 positive CH and CH3 are negative, and this is a convention. You compare these two, you can identify what are the quaternary carbons, you can identify what are the CH2 carbons and what are the CH3 carbons. But only thing is how do you distinguish in CH and CH3 is a bit problematic. That we have to see later. So here this is an example of an APT spectrum of menthol. Again we have a lot of CH2 and CH3 here, and all the three CH2 are here and these are CH and CH3. So basically what J modulation I wanted to cover in this class is called an attached proton test. We discussed about the heteronuclear spin echo and we I said when you apply 180 pulse only one of them, J will not refocus only chemical shift will refocus. Whereas if apply 180 pulse on both then J will refocus. All those things we discussed, we understood everything. What is happening for the homonuclear case and heteronuclear case and then in the J modulation, I said when you use a spin echo sequence with proton decoupling depending upon the delay you are going to use in the spin echo 90 tau 180 tau sequence, when delay is equal to 1/2J all the CH3 carbons have intensity 0, only quaternary will be seen. Whereas when it is exactly equal to 1/J then CH2 will be positive CH and CH3 are negative and quaternary is also positive. This way we can do the identification of different carbons attached to different number of protons. This is called attached proton test, the basic thing for this is to understand spin echo. If you know spin echo of homonuclear and heteronuclear, how it happens you can do this J modulation experiment and identify different carbons. Thank you very much. We will continue further in the next class.