

**One and Two dimensional NMR Spectroscopy: Concepts and Spectral Analysis**  
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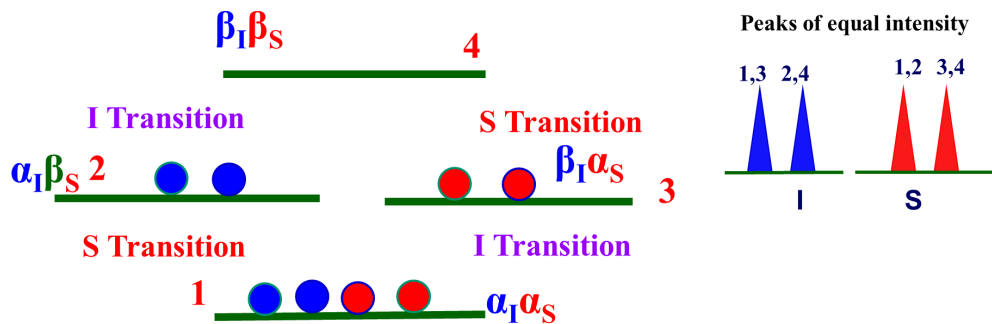
**Lecture 34: Sensitivity enhancement**

In the last two classes, we extensively discussed spin echoes. Please remember, what is a spin echo? I explained to you, spin echo is nothing but application of two pulses 90 with the delay and another pulse 180 and another delay, an identical delay and start collecting the signal, you get an echo. I explained to you what is an echo. And important concept is in the case of homonuclear and heteronuclear spin echoes, two differences we have to consider. In the homonuclear case of spin echo, chemical shifts are refocused, but not J coupling, because if you apply 180 pulse on A, the spin states of X spin also will also be affected. As a consequence, the J couplings will not be refocused, they continue to evolve. And we discussed the same thing for heteronuclear case. In heteronuclear case, there is a possibility we can do three different experiments. You can apply 180 pulse only on A spin, or only on let us say proton; only on carbon and then both, all are possible. I said if you apply a 180 pulse only on proton, that chemical shift will evolve, then what is going to happen to the J coupling? They will be refocused, because you are not touching the other spin, other nucleus at all. Only in the case of homonuclear spins the 180 will disturb both the spin states of A and X, but in this case, other is not disturbed. As a consequence, what is going to happen? J coupling gets refocused if you apply 180 pulse on any one of the spins, whether it is proton or carbon, does not matter. What happens if you apply simultaneously 180 pulse on both, then the spin states of both of them get inverted, 180 pulse inverted spin states of both of them, then H alpha which is coupled to C alpha will become H beta. Similarly, H beta which is coupled to C beta will become C alpha. As a consequence, what will happen? The J couplings will not be refocused. In the heteronuclear spin case, if we have 180 pulse on both the spins, then what is going to happen? J will not be refocused. They continue to evolve. Chemical shift on the other hand gets refocused. This is what we understood. And I said we can manipulate the delay between the 180 and 90 pulses in the spin echo sequence, especially for heteronuclear spin echo along with the decoupling of proton to detect carbon. I showed an example. If the delay is manipulated in such a way, if delay is going to  $1/2J$ , we saw that CH, CH<sub>2</sub>, CH<sub>3</sub>, all the three carbons will have zero intensity. You will not be able to detect the signal. Whereas, quaternary carbon will remain unaffected because that does not precess at all. So, at delay exactly equal to  $1/2J$ , you can detect only quaternary carbon. When you put delay equal to  $1/J$ , delta, then what is going to happen? I showed that CH and CH<sub>3</sub> carbons will be positive in intensity, whereas, CH<sub>2</sub> carbon is in negative in intensity. So, you can distinguish between these carbons. So, this is what I showed with one or two

examples. We understood about the J modulated spin echo, how we can distinguish different carbons attached to different protons. And this experiment is called attached proton test. But only thing is you cannot distinguish between CH and CH<sub>3</sub> carbons, that is a issue there. You can identify the quaternary carbon with delay at exactly equal to  $1/2J$ . Whereas, with  $1/J$ , CH and CH<sub>3</sub> both will become positive and CH negative. Depending upon how you do the phasing they are opposite in phase. But between CH and CH<sub>3</sub> to identify is some issue. We will worry about it when you come to other experiment later. With this, we will go to a new topic today altogether different topic called polarization transfer technique.

What is a polarization transfer technique? Why do we need it? We will understand. Basically, I have been telling you NMR is a highly insensitive technique. That is what I said in the first or second class itself. Basically it is an insensitive technique somehow you must enhance the sensitivity of detection by doing some tricks so that we can get better signal to noise ratio. How do you do that? There are several ways to get solution for the sensitivity issues. I am going to show couple of them here today. Solution to sensitivity problem of NMR is the first thing is to use a more quantity of the sample. Take large amount of sample. There are certain limitations for that. You cannot take too much of sample then you know there will be aggregations, etc. and then homogeneity will again get bad, you will not get a good signal. With this to some extent you can resolve the problem. What about isotopic labeling especially for abundant spins like carbon 13, nitrogen 15 you can do. But still natural insensitive issue cannot be addressed. Go to higher and higher magnetic field. I told you sensitivity depends upon the magnetic field. Higher the magnetic field, larger the separation of the energy states between alpha and beta, spin half case. As a consequence, population difference is larger I said. So, we can get better sensitivity. That is also okay. Also, I told you the sensitivity depends upon the temperature. When I discussed the Boltzmann population ratio, I explained this. It depends upon temperature. At lower temperature, we have better signal to noise ratio. So, go to very low temperature and record the spectrum. It is also not practically feasible because most of the time we do not have to do it in low temperature because there could be some dynamics in the sample which can get affected. So, although there are examples like this which is possible, each of them has its own limitations. Another way of doing is what is called playing with the spin dynamics and design a new experiment. This is something new which is in our hands. We can manipulate the spin dynamics in such a way, we can design new NMR experiment to enhance the signal intensity. How do we do that? We transfer the large population difference of proton to dilute spins. Proton has the highest sensitivity among all the stable isotopes. Take the magnetization which is larger from proton, give it to less sensitive nuclei like carbon 13, nitrogen 15 etc. thereby, enhance the signal intensity of this. You understand, proton is rich in signal. Take the signal from proton, give it a carbon and nitrogen. Then, when you detect them as a

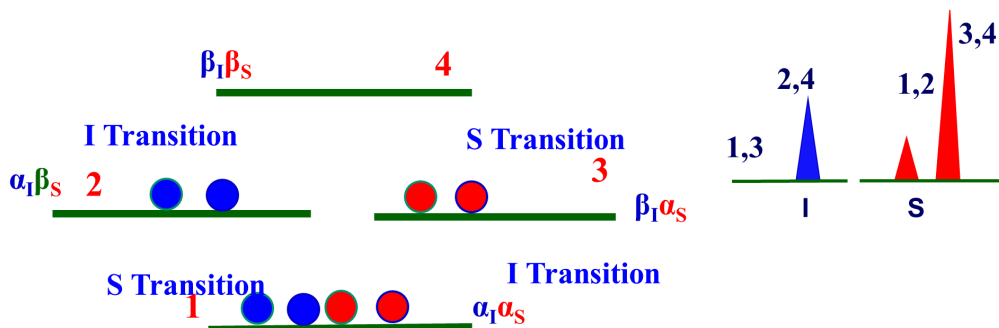
consequence, this signal intensity will go up. This is something interesting. It is like take robbing a rich and paying the poor. You take the magnetization from the rich proton, give it to poor carbon 13, nitrogen 15. How do you do? What is the condition to do? All those things we will discuss now. And this type of experiment are called population transfer experiment. I can transfer the population from abundant spins to rare spins. We can do that. This is one way we can enhance the signal intensity. How do you achieve this population transfer? There are two ways of doing it. One is called selective population transfer, and the other is called selective population inversion. There are two ways we can do. Both are possible. How each of them will work? How do we get the signal intensity more? We will see now. We will consider the example of homonuclear two weakly coupled protons. That means only two spins coupled. How many energy states are there? Four energy states we have been discussing. How do we, what is the intensity of each of the peaks in a weakly coupled case, I showed you when I discussed the Pople notation. The two weakly coupled spins, each will give doublet of equal intensity. So, you must get four peaks of equal intensity in a weakly coupled homonuclear case, and that is what happens. We will work it out by the energy level diagram. Hypothetically put some numbers to calculate the population difference. I have put four spins here, two here, two here and zero here.



Now, what we will do is to calculate the peak intensities. This I know, I told you this. See here alpha S is going to beta S. So, this is S transition. Here again alpha S is going to beta S, this is S transition. Here alpha I is going to beta I, this is i transition, this is also an I transition. Now, we know that. We will calculate the intensity of each of these peaks. What is the intensity? I told you intensity is nothing but the difference in populations. If we calculate the intensity, for example, I spin, I spin is this one, here to here, transition from 2 to 4. Here two spins I have taken, this is 0. So, 2 minus 0 is 2. Similarly, i spin 4 minus 2, again 2, 1 to 3 transition, and 2 to 4 transitions are I spin transitions. Transition from 3 to 4 is S transition. Again 2 to 2 minus 0, 2. This transition again 4 minus 2 will be 2. So, in the example I have taken, the population distribution in such a way that all the four peaks are of equal intensity because I have taken a homonuclear weakly coupled spins. So, intensities have to be equal. That is what I chose the population for calculation

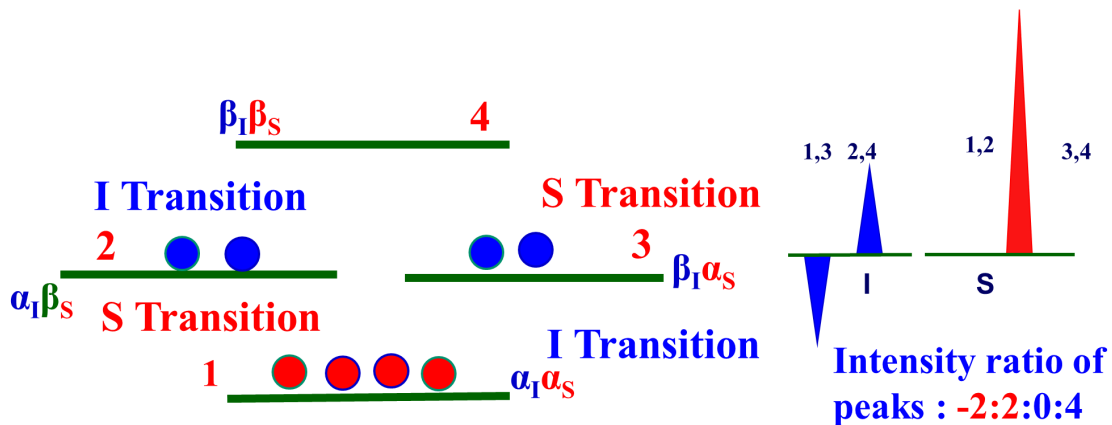
purpose. Please remember, population difference is always difference between lower energy state to higher energy state. This is what we did. We took the difference from here to here, not from here to here. Then we will say minus and all those things. Population difference is from lower energy state to higher energy state because conventionally lower energy state is highly populated. The populations have been chosen to calculate the intensity, just some numbers. Here in the betabeta state I said 0 population. Never be under the impression that there is no population in the betabeta state. There are enough of spins in all the spin states. Only for calculation purpose I have taken it 0. It does not mean there is no population in betabeta state. Please remember that there exists population only the numbers are manipulated to get equal intensity for all the four peaks. That is what I did.

Now, what we will do is, we will selectively saturate only one of the transitions of one of the nucleus. How do I do that? We use what is called a soft pulse. There are two ways, we can apply hard pulse and soft pulse in NMR which I did not discuss in this course. In the previous courses it is extensively discussed, some people can refer to it, I have discussed. Hard pulse is a sharp pulse and it can excite the entire spectrum of the range of frequencies present in particular spectrum. For example, proton 0 to 10 ppm, all protons can be simultaneously excited. If you take a soft pulse means large pulse width, I can selectively excite only one peak, two peaks or only small band of frequencies. This is called selective excitation. Selectively I can excite only one proton or one signal, one peak, we can do. Whereas, I can apply a hard pulse and excite all the signals at a time, all the protons at a time, both are possible. What in this case, we are trying to do is, selectively I am going to do saturation of only one of the transitions by using soft pulse. That is what I said, soft pulse means selectively I can do only for one of them. It is a large pulse with a larger width.



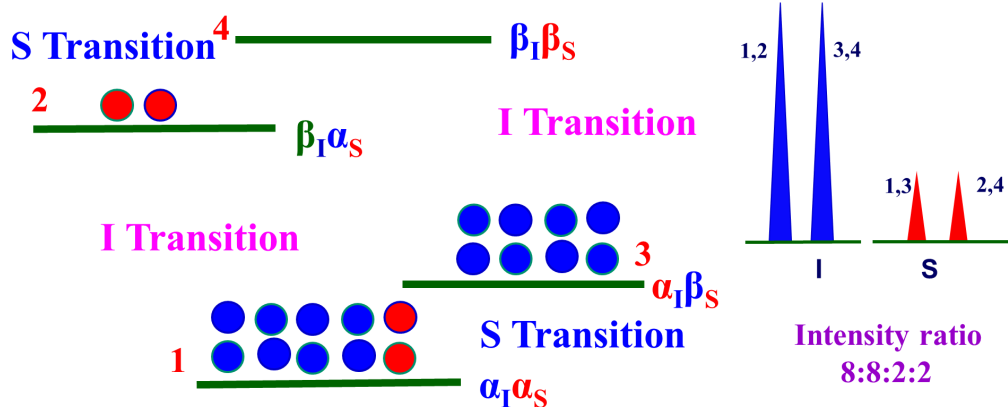
I have taken the example of spin states 1 and 3. I am saturating these two states. What do you mean by saturation? I told you already in the first class itself. Saturation means spin population of both states will become equal. That means there is no signal, you will not

get any signal. That is called saturation. So, what I will do is, I will saturate like this. I will take one spin from here and put it in the other state, from this state alpha alpha state to beta alpha state I take it. That is what I did. I saturated only one of them. Now, let us calculate the intensity between all the four transitions for AX spins. See 1, 3, there is no transition. Intensity is 0 because it is saturated. What about 2, 4? The intensity is 2. That is not affected at all. What about S spin? In the case of S spin, this transition 3 minus 0, it is 3. Whereas here 3 minus 2, it is 1. See, what happened is intensity ratio has become 0 : 2 : 1 : 3. It is very interesting. What it means is you saturate and leave it. After some time, the spin go back to thermal equilibrium. But during the process, what happened? What did we do? What does it imply? It means saturating a particular transition resulted in the change of intensity of other peaks of the coupled spins. Something interesting. I did not even disturb S spin. I only saturated I spin transition, only one of them. But I saw the change in the S spin intensity. Something interesting. So, this is a very important point you should remember. Saturating a particular transition of one spin, resulted in the change in the intensity of other coupled spin. This is what happens. What I have done by doing this? I transferred the polarization from one spin to another spin. This is what is called polarization transfer. So, by transferring this, I enhance the intensity. You may ask me a question, what is the use because I am taking homonuclear spin, they are already of same intensity. We have to worry about dilute spins. We will come to that later. So, the selective population transfer when I did, imagine what we did here is, we saturated one transition and saw the change in the intensity of its coupled partner. That is important. This is okay. Selective population saturation we did, SPT, selective population transfer.



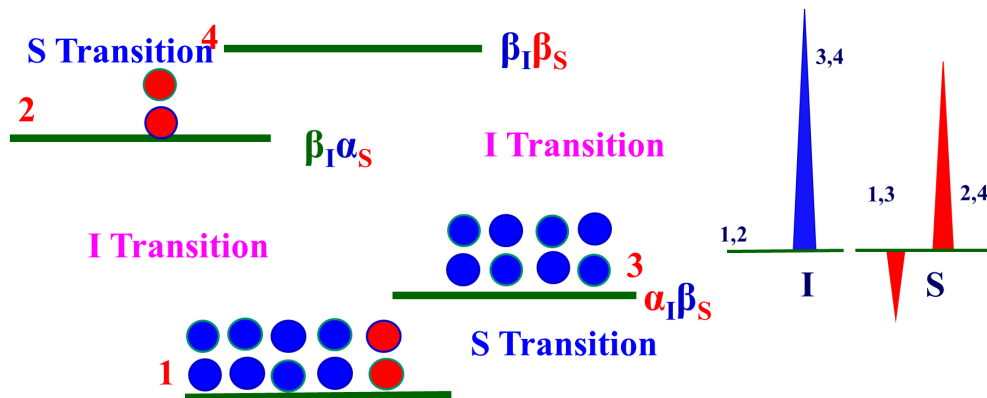
What about selective population inversion? I said that is also possible to do. Instead of saturating, can I invert it? I have been telling you. Please remember 90 pulse will bring the magnetization to x-axis or y-axis, the 180 pulse will invert the transitions. The 180 pulse results in inversion. A transition if I have to invert, that means I have to interchange the populations between alpha and beta states, that is done by a  $\pi$  pulse. So, we can selectively invert the populations of any of the particular transition. We will see what is going to happen now. Same 1-3 transition we will take. Earlier we saturated made

populations equal, 3-3. But now what we are doing? We are not saturating, but we are inverting. That means these four spins will go here, these two spins will come here. Assume I will do that by applying a soft pulse like this. Then what will happen? I did the selective inversion of transition 1-3. Let us now calculate the intensity of the peaks. Very interesting is happening. I spin transition, for example, if you take from 1-3, intensity is negative, it is 2 minus 4. Always I told you population difference is from lower energy state to higher energy state. So, population difference 2 minus 4, intensity became negative. Another I transition is, 2-4, its population difference is 2. Go to S spin transitions, here this is S spin transition, 2-2, 0. I do not get any signal. Go to other one, other S spin transition, 4-0 is 4. So, what is happening? The intensities have become -2, 2, 0 and 4. Very interesting. I saturated one transition and then got the change in intensity with its coupled partner. Identically, I will invert a particular transition and see the enormous change in the intensity of its coupled partner. This is what is called population inversion. So, selective population transfer and selective population inversion, both of them results in the change in the intensity of its coupled partner. We will understand that. So far, I was discussing homonuclear spins.



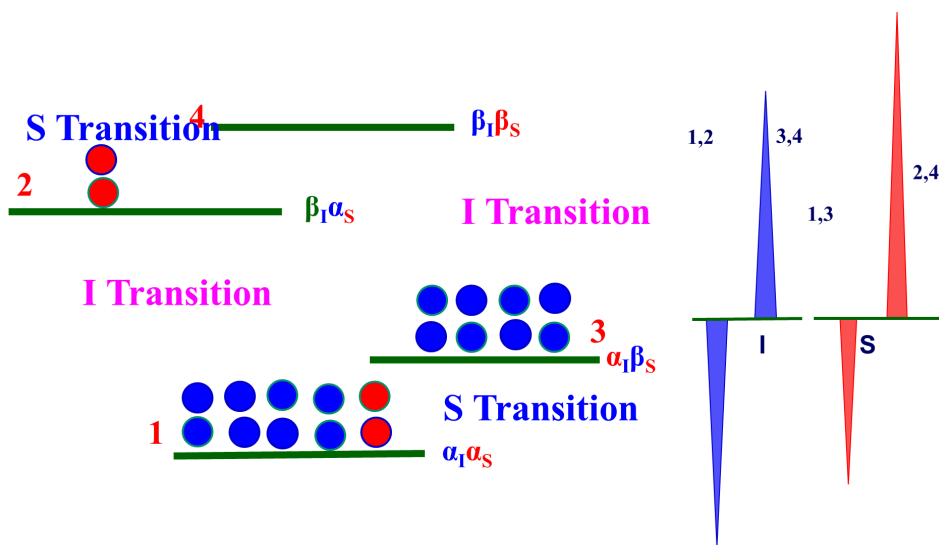
What happens if I take a heteronuclear spin system? How does it change? We will see. Remember, I consider the example of heteronuclei carbon and proton. Carbon gamma is 4 times lower than that of proton. That is what we have been telling you. Resonating frequency of carbon is 4 times smaller than that of proton. So, as a consequence, we have to redistribute the populations such that intensity should become 1: 4. In the case of AX homonuclear, all are of equal intensity. But if I take AX heteronuclear, depending upon the gamma, intensities change and are not same. If proton has intensity 8, carbon should be 2. If proton is signal intensity is 4, carbon should be 1 because 1:4 intensity, we have to maintain. Accordingly, we will redistribute the populations now. We will go there. We will see the population distribution for heteronuclear case. That is why in one of the classes I showed you, the energy level diagram of the homonuclear spin and heteronuclear coupled spins. Heteronuclear spin the energy separation is smaller because of lower gamma. Whereas, if you take homonuclear, see energy separation is very large

here. That is homonuclear spin states. This is heteronuclear spin states, lower gamma. Intentionally, I have chosen this so that you can understand it better. And I have chosen the population such that 0, 2, 8 and 10. Why did I do that? I told you, let us calculate the intensities of both I spin and S spins using the populations distribution. I is proton, S is carbon 13. Let us calculate the intensity for I spin and S spin. I spin transition is 1 - 2, intensity 10 minus 2, 8. What about other I spin is 3 - 4, 8 minus 0, again 8. They are equal intensity. That is correct. Now, go to S spin. S spin transition is 1 - 3, 10 minus 8, 2 and this is 2 minus 0, is 2. So, what is intensity ratio if I take this population? 8 : 8 : 2 : 2 or in other words, it is 4, 4, 1, 1. This is what we expect because gamma of carbon is 4 times smaller. So, intensity of carbon is 4 times smaller. That is the reason why I deliberately took the number of spins in different energy states like this. I manipulated the spins such that if I calculate, I must get the intensity ratio 1 is to 4 for carbon and proton. Proton should be 4 times larger than carbon 13. That is why I chose this. Again here in the beta beta state, I have taken 0. That does not mean there are no spins. There are enough of spins. Only these numbers have been taken for understanding purpose. We will do this same experiment what we did for proton.



What is that we will do? We will saturate one of the proton transitions. Which is the proton transition we can saturate? We can saturate 1 - 2 or 3 - 4, whatever. This is proton transition. This one 1 - 2 transition and 3 - 4 are proton transitions. I spin transitions. This transition will saturate. What do you mean by saturation? We can make both energy states equal population. We will saturate transition 1 - 2. What did I do? I made populations between 1 and 2 spin states, the Population difference as 0. That is called saturation. I have selectively saturated the transition 1 - 2. As a consequence, what is happening? Now, let us calculate the intensity for all the four peaks. 1- 2 because I have saturated, intensity is 0. Other proton transition is this one 3 - 4. You consider this is 8 spin and 0 spin. intensity is 8. That is also fine. Go to S transition. S transition is 1-3. 6 minus 8, it is -2. Other S transition 6 minus 0 is 6. The intensity ratio is 0 : 8 : -2 : 6.

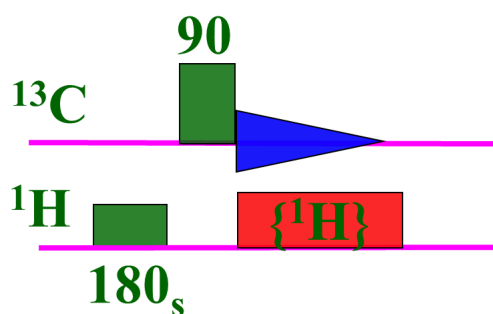
What was the earlier intensity ratio? 8 :8:2: 2. But you see now some of the signals have higher intensity. So, there is a threefold increase in intensity of one of the peaks. Instead of 2, it became 6. Three times you have enhanced the signal intensity for one of the peaks just by saturating one of the proton transition. You understand the idea? This is what we did for heteronuclear case. So, we did the saturation of one of them and there is a change in the intensity of other peaks, other coupled proton of the heteronuclear spin for at least one of the transitions, threefold increase in the intensity.



We will do selective inversion also. Why only we have to saturate? We will do selective inversion. Same transition we will take 1–2. What do you mean by selective inversion? I will take all the spins here and put it here. All the spins in this, I bring it down here. That is what is called selective inversion. I can selectively invert transition 1 - 2, I will invert it by applying a 180 pulse. This is what I did. Remember, I took the protons from state 1 to state 2 and protons from state 2 brought down to state 1. That is all I did, selective inversion. I inverted the populations. Let us recalculate the intensity now, for all the peaks. Look at it, I transition is 2 minus 10, it is -8. Another I transition 8 minus 0, it is plus 8. Look at the S transition. S transition is 1- 3 is this, 2 minus 8, it is -6. Other one 10 minus 0, it is 10. What is the ratio? Intensity ratio is now -8 :8: -6 : 10 or in other words, simply -4 :4: -4 : 5. That means, both the peaks here, it was 2 : 2 has enhanced the intensity. One of them has gone up by 5 times, one of the peak intensity has gone up by 5 times just by doing selective inversion of one of the transitions. You see the intensity now. There is a transition intensity increased for the coupled partner, one of them by 3, other is 5 times, because it was 2 became 6, 2 became 10. So, intensity became 3 and 5 times more. Of course, one is negative, other is positive, we will worry about the sign later. The phase part we will discuss later. But what I want to tell you is in the heteronuclear case by selective inversion of one of the transitions, there is enormous



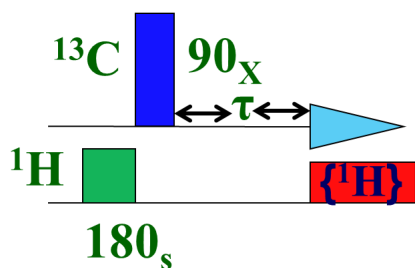
change in the intensity of the coupled partner by an order of 3 and 5. With the polarization inversion, the intensity of the carbon 13 has gone up by 4 times. For example, this is gone up by 4 times actually. Intensity of 1 -3 and 2 -4 transitions are now 3 and 5. So, instead of 2, 2 it became 3 and 5. So, it will be quite large. The  $^{13}\text{C}$  signal is proton coupled. Another important point you must remember. So far it is fine. We applied a 180 pulse, do selective inversion and enhanced the signal intensity. But how do you detect the signal? We detect the signal by doing decoupling of protons while collecting the carbon 13 signal. We apply RF pulse on protons, so that carbon proton couplings are completely broken. That is what we are going to do. And this is an experiment for that.



We apply a selective pi pulse. This is called selective pulse, 180 pulse and one of the transitions which will invert the population. And also apply 90 pulse for detection. Apply a selective pulse on proton, inverted this population, immediately apply 90 pulse and start collecting the signal. According to our understanding, this would have caused lot of change in the intensity of its coupled partner, that is, for carbon, intensities of minus 3 and 5 should be there. We will collect the signal by doing decoupling. Now, it generates two coupled peaks with the anti phase character here. This one, anti phase character is there. What is happening here is we get peaks like this, -3 and +5 intensities. If I start doing the decoupling, the vector addition will tell me that finally the intensity is going to be only 2 because we are going to nullify the gain in the intensity. Whatever intensity you have gained is lost. You understood. We did the selective population inversion of one of the proton transitions and we saw signal intensity of the coupled carbon, two peaks will have the changed intensities of -3 and +5. They are anti phase in character, one is minus 3, other is plus 5. If you add both of them, it is plus 2. When you are decoupling, you know we are breaking the coupling, both of them will be added up. When you do that, what is going to happen? 3 plus 5 and minus 3 will become plus 2, and all our efforts of selective inversion to gain the signal intensity is lost.

Then what is the point in doing this? Whatever you wanted to gain is lost by doing the decoupling. So, that does not help us. So, what do we do? We want to gain the signal

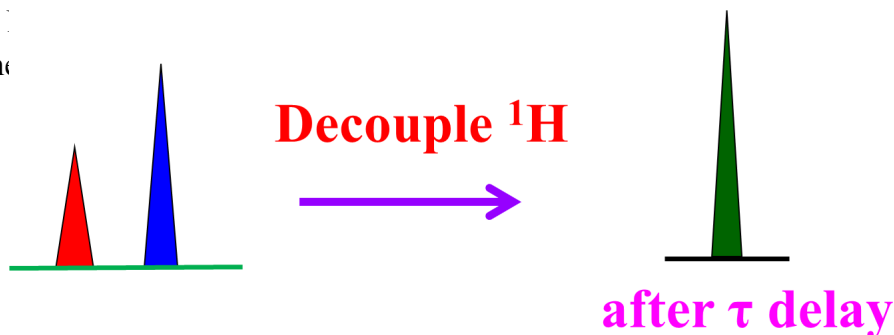
intensity. At the same time, we want to do the decoupling also. If you do not do decoupling, it is a different question. But the decoupling is needed for carbon 13. So, this experiment has to be modified somehow, so that this intensity is not going to be cancelled out. Still we get the gain and with decoupling. That is the experiment you have to do. How to overcome this problem? Very easy. You convert the anti phase peaks into in phase. One is negative, other is positive. Instead, why don't we make both positive? That is possible. How do you do that? We can do that by giving an additional delay after the detection pulse. That is very important. If I do that, then what will happen? Both the signals will become in phase. When they become in phase, they are +3 and +5, they will add up and the intensity becomes more. It will not get nullified. That is what we will do.



**After  $\pi/2$  pulse on  $^{13}\text{C}$ ,  
set the delay  $t$  to  $1/2 J_{\text{CH}}$**

See, this is the signal, this is the pulse sequence. Earlier this delay was not there. We started collecting here only. This portion was here. But now I have introduced the delay here. And what is the delay we have to put? How much is the delay? That depends upon  $1/2J_{\text{CH}}$ . What does  $1/2J$  do? I already told you. It creates anti phase vectors. Right! Already we have seen this spin in a heteronuclear spin echo and homonuclear spin echo we saw that. And exactly at  $1/2J$ , the vectors were anti phase in character. Already the anti phase character because of selective pulse we are applying on one of them. The carbon 13 coupled to it has already anti phase character -3 and +5. What we do is, we are going to give a delay, apply a pulse here, bring the magnetization vectors, both of them to xy plane. Then if you give a delay of  $1/2J$ , this will come back. That is what we saw. Again  $1/2J$  delay if you give, they will move by 90 degrees. And then they will come here. See, what will happen? This vector, both these vectors will not be here, they will go into the other axis. 90 degree they rotate. They rotate by 90 degree. See, one is here, other is here, other is here. They keep rotating and go into other axis. And then both of them will be on the same axis, after a particular delay. In principle, if this is X axis, in the xy plane they rotate by 90 degree, it should go to y axis. So, I should show this signal as this as y and this as x. I can do that. Now, because of  $1/2J$  delay, both the vectors have become positive, fantastic. Now, we will do the decoupling. It is possible to do decoupling. d up.

Now, become



Now, it became 8. Earlier intensity was 2:2. So, what happened now? It became 8. A gain in intensity by a factor of 4, this is marvellous. Gain in the intensity by a factor of 4 results in the experimental time reduction by a factor of 16,  $4^2$ . So, this is important technique called magnetization transfer or polarization transfer technique. You understood what we did now. Selectively invert one of the protons and then carbon-13 coupled peaks will become anti-phase in character. Give a delay after applying 90 degree pulse. Again the anti-phase peaks became in phase in character, and do the decoupling. You will get a singlet with enhanced intensity of nearly 4 times. So, this is what is called polarization transfer experiment. And actually, there are two types of such magnetization transfer experiments possible. One is called NOE, other is called INEPT. So, both I will discuss in the next class.

Now the time is up, I am going to stop here. So, what we discussed today, I discussed about the polarization transfer. I showed you what will happen, how do we gain the signal intensity among the coupled spins. We took the example to two spins. Homonuclear case, you can do the selective population saturation or selective population inversion. Both of them will give the signal, both in homonuclear case and heteronuclear case. In the heteronuclear case, I took the example of carbon and proton and I showed that when you do selective population inversion, coupled partner will have intensity of -3 and +5, anti phase character. Nevertheless, there is a gain in the signal intensity. But we want to do the decoupling. When you try to do the decoupling, whatever the gain we got will be lost because they are antiphase in character, it gets nullified. Instead of that, after the 90 pulse give a delay. That delay should be equal to  $1/2J$ , such that this become again inphase in character. In which case what is going to happen? you get a signal along the same axis, you do the decoupling. There is an enhancement of signal intensity by a factor of 4. Why this factor of 4 comes? Because gamma of carbon is 4 times less than that of protons, and then we got the enhancement in intensity by a factor of 4. This reduces the experimental time by 16 times. And how do we do that? There are two ways of doing polarization transfer that is NOE and INEPT, which I will discuss in the next class. Thank you very much.