**NMR Spectroscopy for Chemists and Biologists**
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Lecture No. 25
INEPT and Sensitivity Enhancement

Good morning. So welcome to today's lecture, we were discussing sensitivity enhancement. And in previous lectures we have seen that like NOE is a method to enhance this sensitivity. And if you remember, if two spins are coupled. We were perturbing one spin and because of this perturbation the signal for other spin was getting enhanced. So we will continue with that. And today we will continue with selective polarization inversion that also be discussing the last class.

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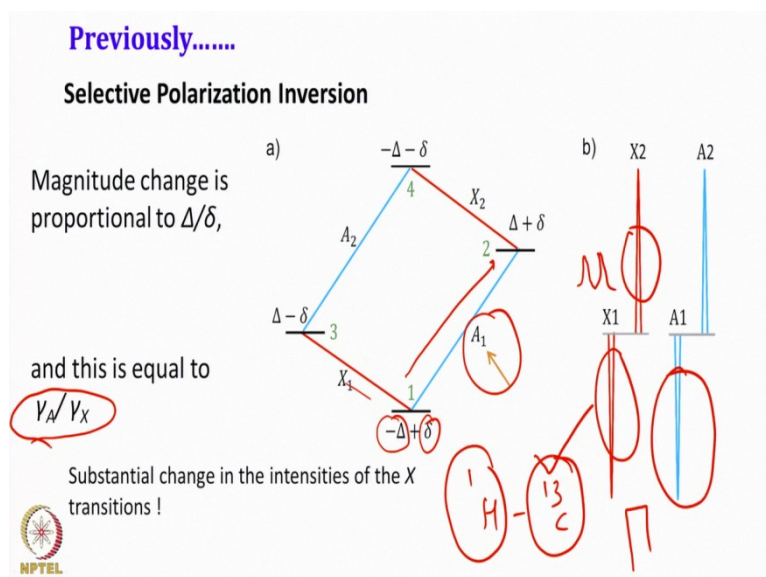
So here what we discussed that if two spins are coupled, say spin is proton and carbon 13. So energy level for these two spins are something like this Δ is for say proton and the δ is maybe for carbon 13. So two signal A_1 and A_2 for proton. X_1 and X_2 for carbon. So what we have seen that, this is insensitive nuclei which is coupled with a sensitive nuclei. And what we want to increase the signal of insensitive nuclei, which is ^{13}C.

So we have seen that if we somehow perturb or saturate or transfer the polarization of say one of the transition from the more sensitive nuclei, which is H by applying a selective pulse. So selective pulse can be say 180° pulse then population of this gets inverted and because of this population inversion the essentially signal for X gets enhanced. So if population invert of A_1,

the X that was signal which was earlier very small, say something like this. Now it becomes quite large, but one thing happens that the sign actually change.

So enhancement also we have seen that it is given by a factor which is $\dfrac{\gamma_A}{\gamma_X}$. γ_A is gyromagnetic ratio of proton and γ_X is gyromagnetic ratio of the X nuclei. So suppose, we are doing it for carbon proton. Now γ for proton is 4 times more than the carbon 13. Therefore the signal enhancement that we get in X is 4 times. This is huge or substantial enhancement in the intensity of X transition.

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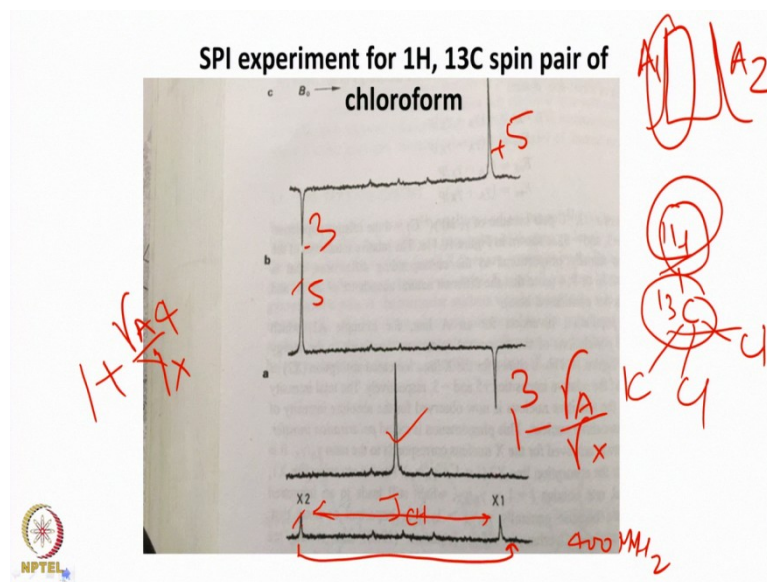
So that is what we have seen. So let us take an real example and we will see how much enhancement we are getting for. So here we are taking chloroform. Chloroform is ^{13}C -^{1}H and here we have Cl, Cl and Cl. So we have two spin system. Proton is sensitive nuclei and carbon 13 is insensitive nuclei. We are say recording a spectrum for carbon 13 on any reasonable spectrometer say 400 megahertz.

Now because both of these are coupled. So carbon 13 is spectrum for X, which is ^{13}C will be coming to here X_1 and X_2. These are the two transition for carbon 13. Since they are couple, so you are getting splitting and this is splitting you remember will be J of CH, which is generally 140 or 125 hertzs. Now if you decoupled it, so you get signal enhancement because of now both spins will merge and give you enhance signal.

But here selective polarization transfer, if you saturate the signal coming from like proton, so if you remember proton will be also splited into two, which we were calling in the previous slide A_1 and A_2. So say we perturb A_1 with selective pulse, selective 180˚ pulse. Now signal for ^{13}C will enhance and you look at enhancement factor, here for like X_2, it is quite a bit of enhance and for X_1 also it is enhance. But for X_2 it is quite a bit and this enhancement is generally given by γ_A/γ_X.

So, generally 4 times enhancement that is what we get. For say, X_2 spin or maybe 5 times actually, so 1 plus 4 that is 5 times enhancement we are getting and for this one we will $1-\gamma_A/\gamma_X$, so that is 3. So 3 and 5 we are getting enhancement, average is 4. Okay, so 4 times enhancement we are getting for ^{13}C signal and if we perturb A2 spin. So this will be giving us 5 times enhancement and 3 times. So this is +5, -3.

So essentially 4 times enhancement we get for chloroform. This is fantastic, so that is what we saw in the case 4 times enhancement for carbon 13 and if we let take it something like say nitrogen 15. So nitrogen 15 we get 10 times enhancement and that we can see because the gamma for nitrogen is actually 10 times less than proton. So that is what happens.

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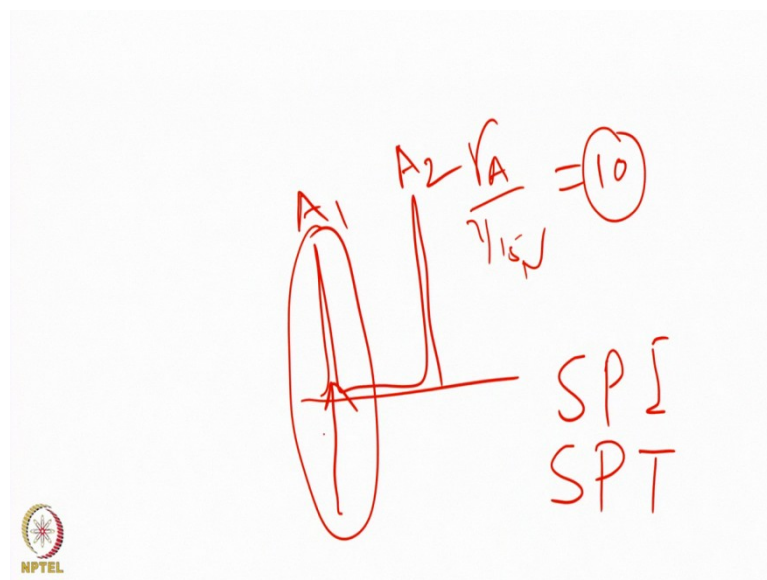
So for if we take it say gamma for proton and gamma for nitrogen and ^{15}N that is 10 times. So for nitrogen you get 10 times enhancement and this is very, very substantial. So that is what we will do in SPI selective population inversion or this is also called selective polarization transfer. In both case we are getting quite a bit of enhancement.

But the problem is that we have to select a transition and apply a transition selective pulse for inverting the population of either A_1 and A_2. So for this case, now be to get it a selective population inversion, which is many times in multiplet it becomes difficult, we get all those selective enhancement.
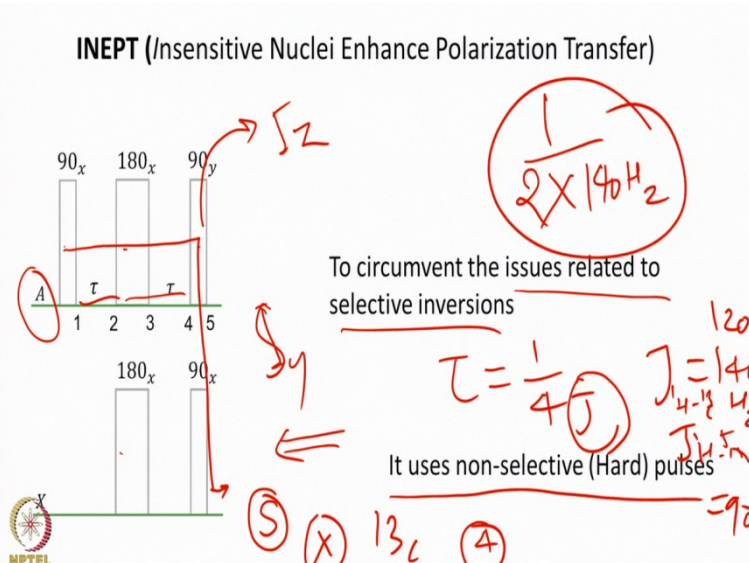
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But selecting as 180˚ pulse which is very, very specific to particular transition is difficult and when there is a crowded spectrum. Then selecting a transition which can be inverted is really difficult.

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And therefore, we discussed an another experiment, which is called INEPT, so insensitive nuclei enhancement by polarization transfer, concept is same, we are transferring the polarization from A spin to X spin. But, now we are, we do not have to be selective in inversion. So we explain this experiment, we start this with 90°pulse on A spin which is sensitive nuclei like proton. And that selectively brings the magnetisation to XY plane.

Then simultaneously pulse is applied on A spin and X spin, and in vector diagram we saw that during this τ period, they start moving a part and then you apply, then they turn it back in this direction. And again, if you apply 180° pulse on y, there actually direction changes and during this τ period, they come in $-Y$ direction, so you apply and bring A spin to Z direction of so this will be say Iz, and then you apply a 90° x pulse on carbon 13 spin, so that becomes say Sy. So that can be detected.

So now to circumvent the issue related selective inversions, this INEPT can works and they, that if you look at all here you, we are have using is hard pulse. So we have not now transitions selective pulse and we achieve essentially transferring the polarization from high gamma nuclei proton to low gamma nuclei, which is carbon. So now magnetization is starting from here, comes here and we can detect it at S nuclei or X nuclei which is ^{13}C.

And it works in similar manner, it selectively transfer the polarization from proton to carbon. So we get enhancement of 4 factor that we were saying. One important parameter here was τ and τ is a time delay, where we can select $1/4J$. So this depends upon the coupling between these two spins, carbon 13 and proton. Say typically for carbon 13 proton J value is 125 or 140 hertz, 120 to 140 hertz we can write and J_{1H15N}, it is actually 90 hertz.

So that means this τ period if we are transferring the polarization from proton to nitrogen you have to keep here τ around 2.7 milliseconds for NH transfer and for carbon transfer it is less than that. So total Tau period here should be for nitrogen transfer 5.4 millisecond and for carbon it will be $1/2J$, which is say 140 hertz that will comes around total is 1.5 milliseconds.

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INEPT has the following disadvantages

(i). Intensity anomalies

Incorrect relative intensities occur different spin multiplets and this dependents on

(a). Strength of heteronuclear J-coupling
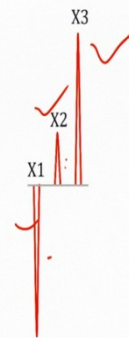(b). Multiplicity of spin-multiplets.

So INEPT has some disadvantage. Disadvantage that we had that actually it gives us a relative like incorrect relative intensity because of different spins and multiplet that we have seen. And here one important parameter that we discussed is heteronuclear J coupling. So if we are, you to select that τ times, which is suited to $1/4\,J$. Now then if the J coupling is quite strong we have enough time. If J coupling is weak then we have different time, so we have to tune our Tau and that is one of the demerit of this INEPT.

The second program is in case of multiplicity. So if multiplicity comes, then how this spectrum is going to be that is difficult. The 3 problem, the relative intensity and it depends upon heteronuclear J coupling and multiplicity of spins can cause the intensity anomaly of this spectrum that we achieve in INEPT.

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(a) Multiplicity pattern distortions occur, in systems such as A_2X and A_3X

b) Positive and negative signals in the multiplets.

Now here one can see an example. If you have an spins which is say AX, A_2X and A_3X, here inversion is going to be like this. So X_3, X_2 and X_1 intensity normally. And some of will be positive and negative signal if we record any INEPT. So we need to know which is coming from which one and this is going to be difficult. Because negative and positive signal both we are getting in same spectrum.

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INEPT sequence with regard to the multiplet distortions, the refocused INEPT has been designed

An additional spin-echo segment identical to the first one in the INEPT is added after the last 90° pulses.

This helps to refocus the positive negative components

To circumvent that, actually one can do a trick in INEPT and what we can do? We can add another say spin-echo sequence. INEPT sequence with this regard of multiplet distortions, one can design an experiment, which is called refocused INEPT, refocused INEPT means we

are adding an extra spin-echo sequence to the classical INEPT sequence. So this additional spin-echo sequence is identical to the first one and it is added after the last 90˚ pulse. And this helps us in refocusing the positive, negative signal component.

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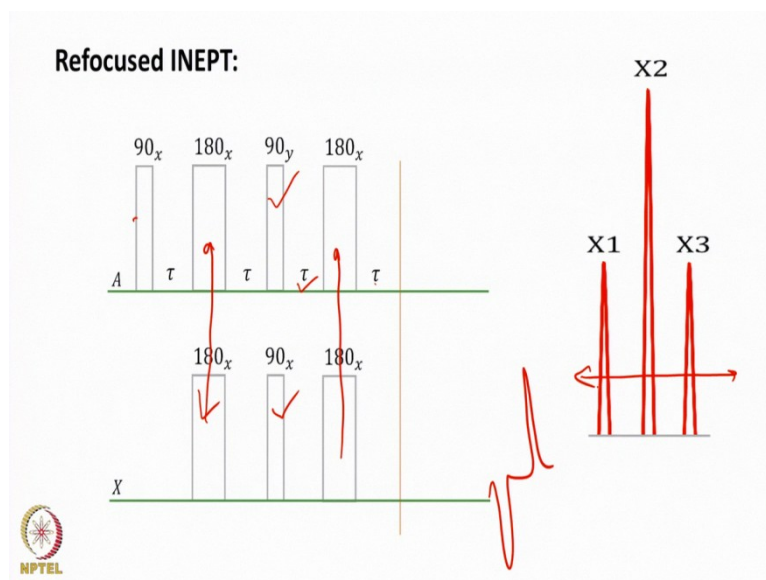
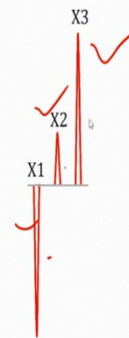So, what actually it is? If you look at here, like INEPT we are starting from a 90˚, then we are waiting for a time τ which is $1/4\,J$. Now we are simultaneously applying 180˚ pulse on A spin and X spin. We are waiting for again τ and then we are applying a 90˚ y pulse on say proton, followed by a 90˚ x pulse on carbon, so A spin and X spin. Here we saw that we are getting a positive and negative signal.

So then we are saying, let us do an spin echo added, so that means spin echo is τ, we are same τ here we are putting. Then applying again simultaneously 180˚ pulse on both spin and then we are waiting for τ. So that actually changes the phase of all three transitions and then it becomes positive.

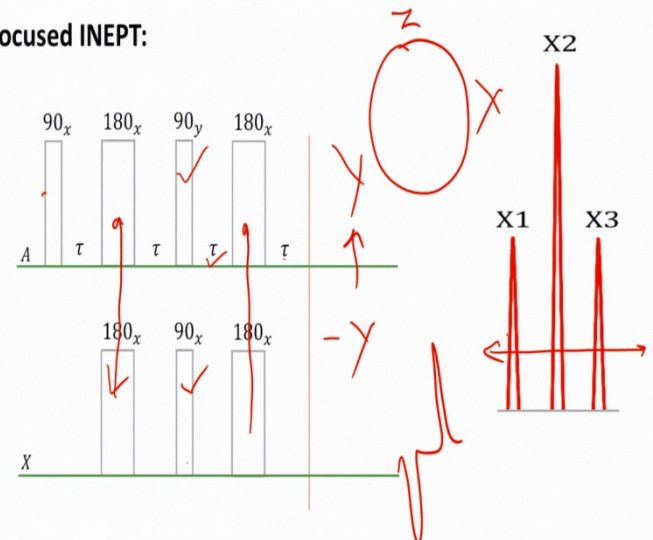So here we can see, we are getting negative, positive, negative. But if we do refocused INEPT we are getting actually positive, positive and positive. That is what is the beauty of refocused INEPT all signal becomes positive an extra thing we are doing here is applying an spin echo. So if you remember is spin echo is like a 90- τ-180 on both spin τ that refocus all the signal and gives us the positive signal. So in vector diagram let us try to understand what is happening here.

So we are starting from say Z direction for A spin. The applied are $90°x$ pulse. So if you remember last time we had drawn this schematic Z, X and Y. So we started with the direction of A spin, we applied a x pulse. So that means it went to $-Y$ direction. Now here we are

waiting for τ period, so spin starts dephasing like this, we applied 180°pulse on x, so it inverts in this direction. Then we applied another 180°pulse on x, so that changes the direction. Now we are waiting for τ, so they come together and then we apply 90°pulse on y. So now it goes to Z direction and here the magnetization on ^{13}C we applied.

So this comes to Y direction, and now we will detected that Y, that was like this. So now we are waiting for time periods, so these spins again starts to move like this. They dephase like this, we applied simultaneously 180°pulse they invert and then we applied actually another τ period, so they again come closure. So they are now perfectly aligned in the detector plane and we are detecting it and because of this detection all three become now positive. That is what we achieved by adding the extra spin echo sequence after INEPT, all signal becomes positive.

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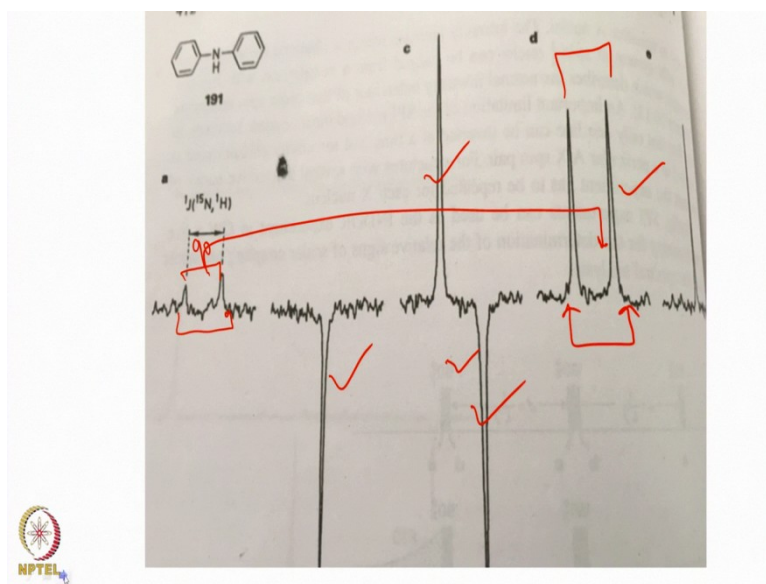
Good so, let us look at the summary of all those. So what we are saying now? This is our molecule, where we are detecting the nitrogen signal for this molecule. So nitrogen is one of the insensitive nuclei of as you know, its γ is 10 times less than proton. So if a directly detect nitrogen we are getting very less signal and this say J is 90 hertz. So it will be separated by 90 hertz.

Then we can enhance the signal by NOE and we can enhance by this. NOE signal we can get it if we do selective polarization transfer, the same signal of these two will be enhanced for one for this and one for this, if we perturb this signal for proton we are getting enhancement in two transition of nitrogen, one will be positive, another will be negative. If you do INEPT

we get this signal and if you do refocus INEPT, both signal will be positive and that is what we have here.

So by doing selective polarization transfer and INEPT, we are getting certainly enhance signal and that an enhancement is substantial. Okay, so that is what we are getting. So here is just normal signal, here selective polarization transfer because of INEPT and because of refocused INEPT all positive signal. And if you compare from here to here we are getting substantially enhance signal. So that is what is all about polarization transfer in INEPT signal.

Now I will come to another important concept of polarization transfer is something called Hartmann Hahn matching condition. So qualitatively I will explain it you today and then in the next class we will look at in more details of what actually Hartman Hahn matching condition is?

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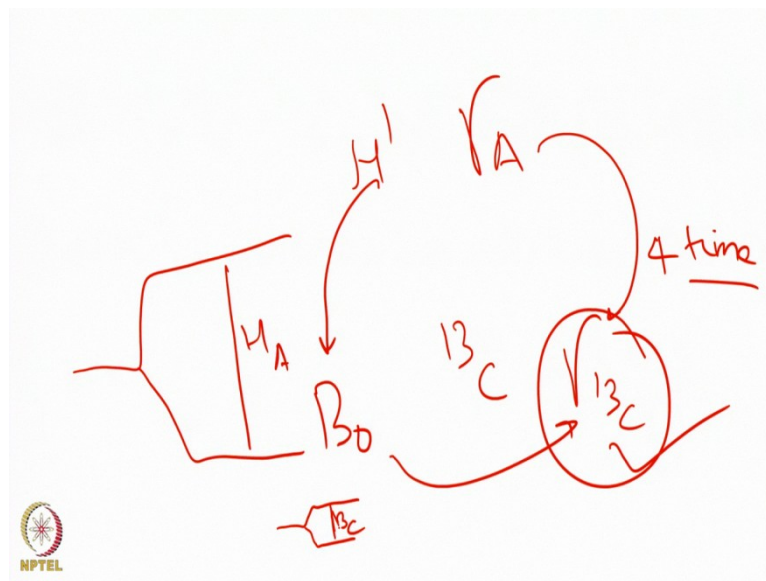
Now all the time we want to and has the signal of X nuclei range which is low from the more signal, which is it is proton. So proton has say higher signal and carbon has lower signal that is what we are saying, γ_H is more, $\gamma_{13C} < 4 \times ¿$ less. Okay, so we want do and has the signal for ^{13}C how to do that? Now, even on the same spectrometer that we recorded. So if B_0 is same the signal for proton is going to be 4 times higher than the carbon.

So we learned the trick of INEPT and refocused INEPT to start with polarization transfer, then INEPT and refocused INEPT, we enhance the signal of ^{13}C. But still we were not getting rid of say B_0, B_0 effect is there. Now suppose, we do some experiments something like this, if

the, say energy level of proton is like this, so this is a for proton and energy level for carbon is like this ^{13}C.

Now if you want to transfer this polarization from here to here, we can we do some experiments, so energy for proton is like this and carbon is like this. Can we do some trick, where we can match the energy level of both of these somehow. If you match it the polarization transfer from more abundant nuclei will happen to the less abandoned in nuclei and probably we can enhance this signal. So that means we have to match the energy gap between carbon 13 and proton to transfer this signal. How to do that?

That is next set of experiment, which he can say that we bring in the same environment by doing some trick, by doing some locking or you can like say some condition we apply, where we start matching the energy gap between these two and that is called either you put them into rotating frame of reference or we can do something call, we can match them by Hartmann Hahn matching condition. If you do that the polarization from H nuclei can be increased and that is actually call spin locking or Hartmann Hahn matching condition.

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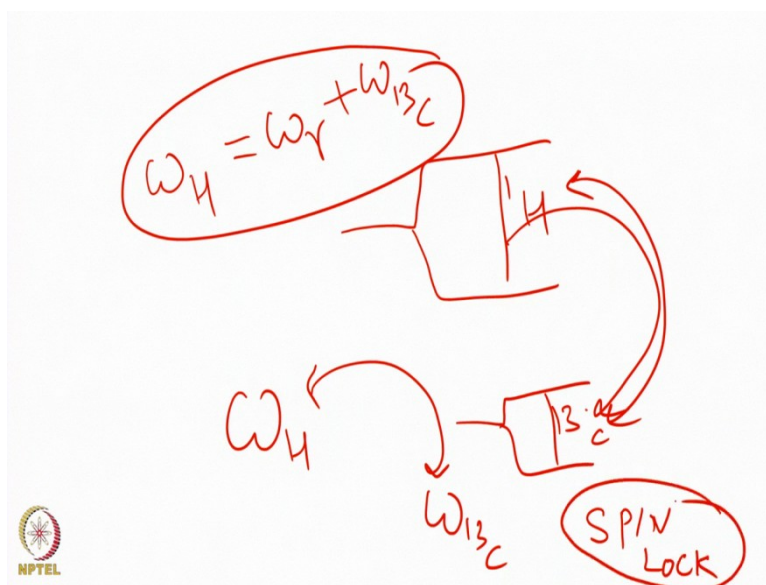
So let see what actually we have to do? And that mostly use this Hartmann Hahn matching condition is the crux in the solid-state NMR. So here, say proton signal and this is for carbon, now we want to, say this is for ^{13}C, and this is for proton, we want to match this condition. So if you say here is ω_H and here is ω_{13C} and if you want to match this condition we do something, so that we bring them in a same plane or *XY* plane and lock them there by applying some pulse.

And during that probably the with a something called zero quantum flip flop the polarization will transfer from more abundant nuclei to the less amended nuclei which is ^{13}C. If you do that magnetization will transfer. So this is called Hartmann Hahn matching condition in solid-state how it is done.

$$\omega_H = \omega_r + \omega_{13C}$$

If we match this condition than polarization from X spin transfer to X spin. H spin to X spin which is ^{13}C.

And signal can be enhanced, same concept is used in liquid state, where you bring them in XY plane and you lock them applying a weak are at RF that briefly I mention to you in ROESY, lock them in XY plane, the magnetization from H spin will transfer to the X spin and that is called actually spinlock. So locking spins in a particular direction for some time and then magnetization will transfer, it says like a simple analogy can take.

So there are 2 class of people, one classes proton and one classes ^{13}C, they are not talking to each other. But these guys has more energy than the ^{13}C, if you lock them in a room for some time after talking to each other, some energy level will be transfer from the more energy guys to less energy guys and that is how you can enhance the energy of the low-energy people. So that is called polarization transfer.

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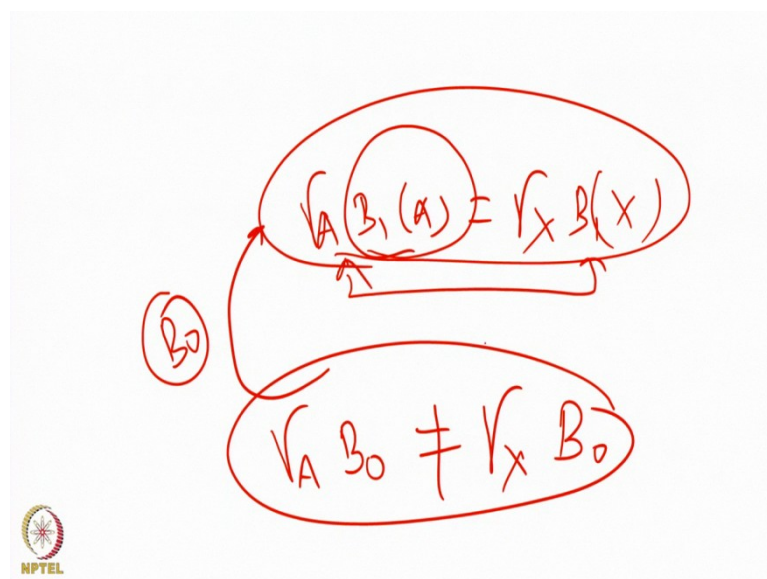
So essentially what we have to do? By applying this weak RF pulse, what essentially we are doing? Is matching this condition where we are $\gamma_A B_1(A) = \gamma_X B_1(X)$.

Okay, so now this is not happening in the Zeeman term, Zeeman term like if you remember, Zeeman is B_0 field , B_0 is main magnetic field. So earlier if you have seen $\gamma_A B_0 \neq \gamma_X B_0$ and that is how transfer is not happening.

So we are changing from here to here, we are by applying a weak RF pulse, we are trying to match the condition where γ_A which is what proton and γ_X will match. So now we are applying and weak RF pulse on proton and carbon and we try to match this condition. This is called Hartmann Hahn matching condition.

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$\gamma_{(1H)} B_1 (1H) = \gamma_{13C} B_1 (13C)$. So that means we are applying two B_1 field, one on proton, one on carbon and by matching this condition, we can transfer the polarization from proton to carbon. So that means we have to apply RF pulse on proton and an RF pulse on carbon, which actually equalizes this or which matches this condition. Then polarization from H spin will transfer to X spin and this matching condition is called Hartmann Hahn condition, so this is Hartmann Hahn condition.

So this condition is at the heart of many of the like ROESY experiment or toxic spin experiment where we bring the magnetization in XY plane and we lock them for some time by applying an RF pulse on H spin and X spin and this is also in the heart of all the polarization transfer, dipolar base polarization transfer that happens in solid-state, where we enhance the signal of X spin by transferring the polarization from H spin in solids and we detect on X spin. Because in solid, we generally detect on X spin because of the lines are sharp for X spin.

So this trick, we will discuss little more quantitatively in the next class, where we learned how to do this transfer, basically I mentioned to you, we are going to match this condition in selective polarization transfer or by spin locking and matching the Hartmann Hahn condition. So will continue from there and we discuss something called 1D ROESY experiment and 1D toxic experiment for long transfer from proton spin to coupled X spin. So I will stop at here, if you have any questions please write to us and do not hesitate to ask we will try to respond everything. Thank you very much.