

NMR Spectroscopy for Chemists and Biologists
Professor Dr. Ashutosh Kumar
Professor Ramkrishna Hosur
Department of Biosciences & Bioengineering
Indian Institute of Technology Bombay
Lecture No. 24
Selective Population Inversion

Good morning, so welcome to today's class, we were discussing polarization transfer. So in this concept, we have seen that if two spins are coupled, say spin *A* and spin *X* coupled by dipolar coupling then if we perturb one of this spins or saturate the population between two states of spin *A* the effect of that will be seen on other spin which is called nuclear overhauser effect.

(Refer Slide Time: 0:46)

Previously.....

The NOE Effect

Resonance line intensity changes caused by dipolar cross-relaxation from neighbouring spins with perturbed energy level populations.

- NOE requires a significant interaction between the magnetic dipoles of the two spins.
- Dipolar interactions drop with distance,

NPTCL ¹H-¹H NOE can only occur with a distance of <5-6Å

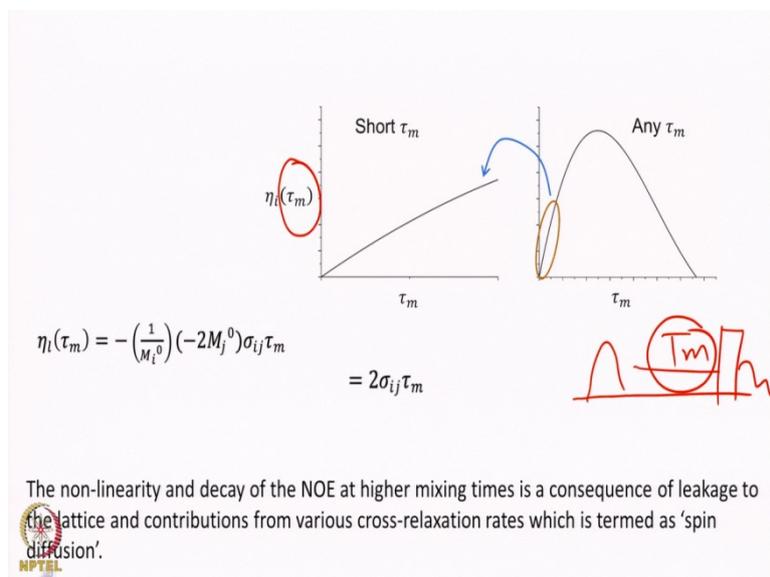
So resonance line intensity change can be caused by this dipolar relaxation from the neighbouring spin and with the perturbed energy level of one spin. So here we have seen spin *A* and spin *X*. So if you are perturbing spin *A*, effect of that perturbation will be seen on spin *X*. So for this effect to be seen they have to be interacting, interacting through space and since it is interacting through space. So there is a distance dependent phenomena.

So if say *r* is the distance between these two spins. So if distance increases the dipolar interaction between them will increase and therefore, the effect will be becoming lesser and

lesser. So because dipolar interaction drops with a distance and so this actually varies $\frac{1}{r^6}$ that

we have seen. So as we move it falls rapidly. So NOE effect can occur only up to 5 to 6 Å and after that there will be no NOE effect seen in general.

(Refer Slide Time: 1:54)

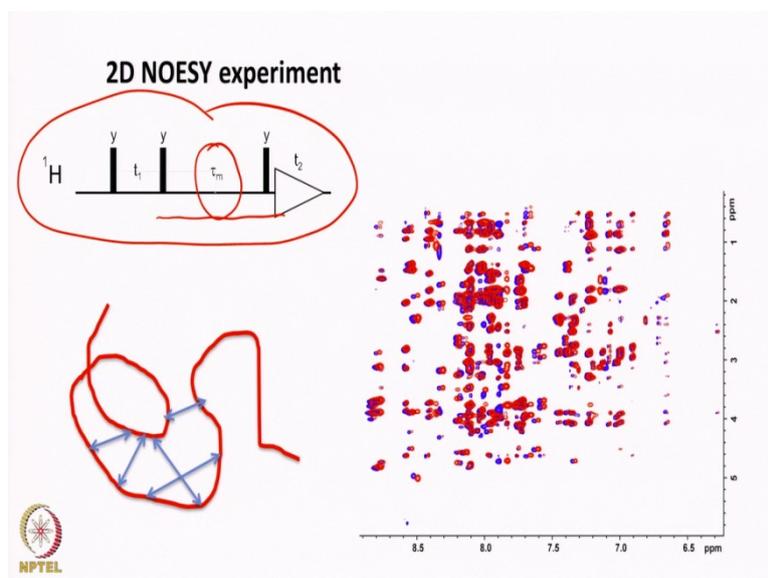


We have also seen that effect of like NOE enhancement depends upon a factor that is called mixing time. So this mixing time we have seen if we are doing a transient NOE where we apply a selective pulse on one of the spin and then we mix it for say time T_m and then we record a spectrum. So this mixing time actually dictates how much NOE effect will be seen and for short mixing time, this is linear.

So here, one can see there is a linear but if you have like longer mixing time after certain time it falls rapidly and this falls of NOE enhancement or decrease in the NOE enhancement is due to a phenomena which is called spin diffusion. That means in a simple term spin loses out its magnetization to other spin and then you do not see that much enhancement. So in short time regime like short τ_m it is a linear and that is typically used for measuring the distance in molecules, macromolecules or biomolecules.

So nonlinearity in decay of the NOE at higher mixing time is a consequence of leakage to the lattice. So spin is leaking to the surrounding, and that contribution can vary according to cross relaxation rate and this is called spin diffusion, spin diffusion means spin is diffusing to the other spin or lattice, they are just losing out.

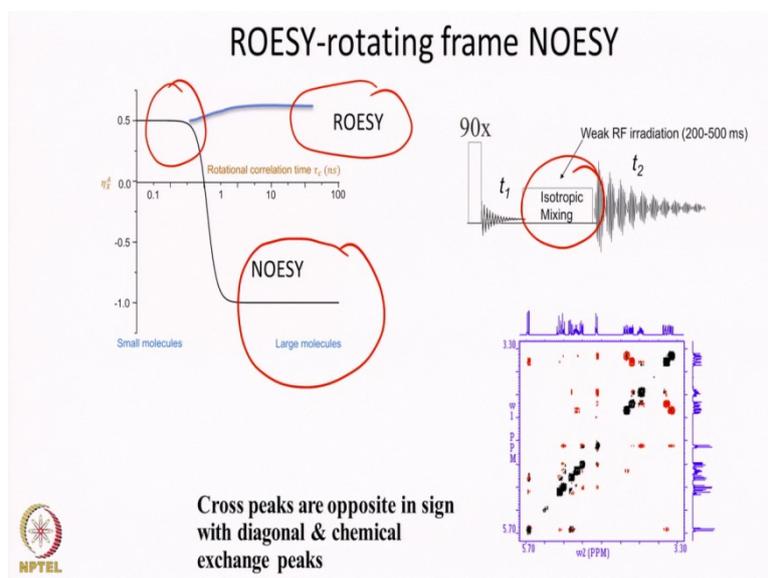
(Refer Slide Time: 3:26)



So we had seen that this NOE concept is very, very important in structural biology. So you can actually do the same concepts and you can take that in the 2-D spectrum, where you just, this is the mixing time that we discussed in the previous slide. So you can measure here in 2-D fashion. This is the pulse sequence, this we will deal in detail when we go to 2-D experiment. But at the moment just understand that much that depending upon what T_m we can mix the spins and actually this magnetization transfer or NOE effect can be seen from the neighbouring spins.

So here you can see if this spin is closer to this spin, this spin is closer to this spin and all those they will be shown in the cross peak. Now each cross peak is essentially reflecting the distance between two spins and somehow if you quantify the intensity of these peaks, we can get to know the distance between this two spins. So this NOESY concept is generally used in the social biology for measuring the inter proton distances.

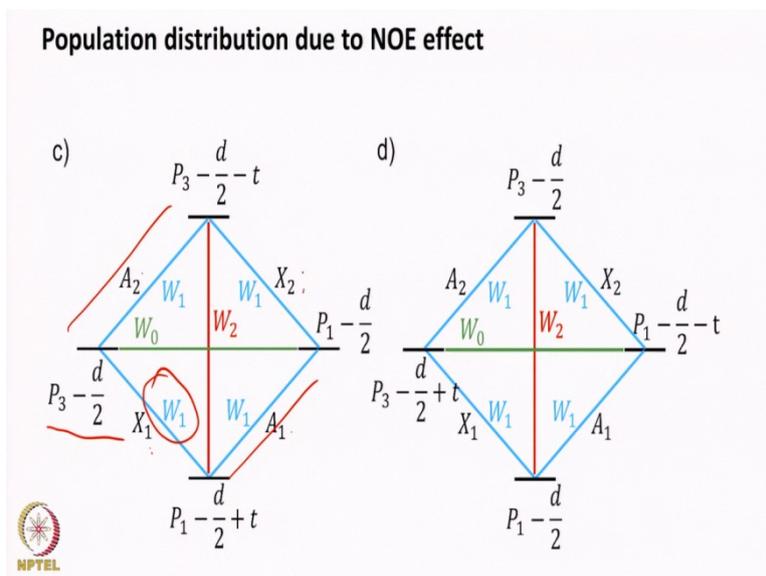
(Refer Slide Time: 4:35)



Now we have seen that earlier that for small molecules actually ROESY is a better experiment. ROESY is basically rotating frame NOESY and we had also seen that for a large molecule actually negative NOE we obtain, for small molecules, we can obtain positive NOE. So for small molecules ROESY is better experiment and ROESY you do not do anything extra, but you have a essentially a block which is called isotropic mixing block. So it is like a you are putting that there are those spins in Z direction and let them mix and but these are through a space connected.

So if that happens, you get actually distance constraints in a small molecule and that cross peak sign comes negative. So, why it comes negative? We will discuss later when we go to like you go to like NOESY and ROESY in detail. But here to remember that for bigger molecules we use NOESY for measuring the distance between two protons, for smaller molecules like small organic molecules we can use ROESY for measuring the distances and both of these has common phenomena that is actually enhancement of the signal by polarization transfer, where we perturb one of the spin and look at the effect on the others spin. Now up to this point we had done earlier.

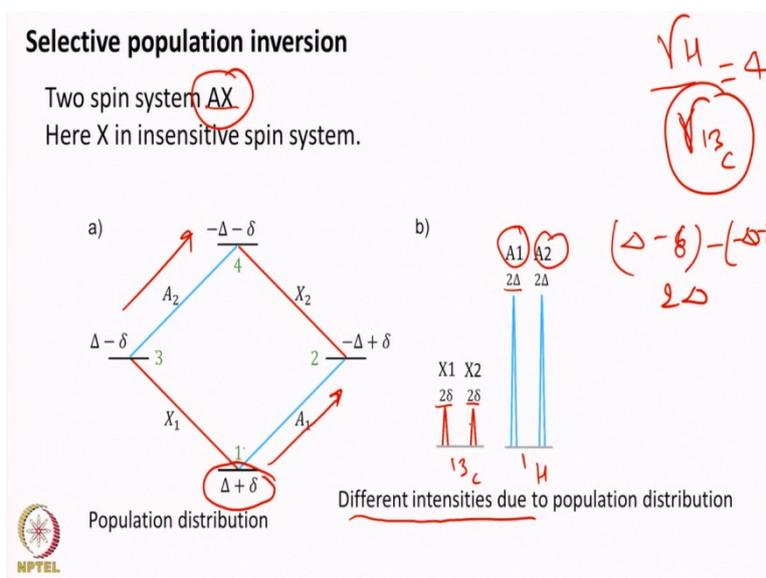
(Refer Slide Time: 6:05)



So now let us move ahead, this also actually, we had done. Population distribution happens because of NOESY effect. So we see because of relaxation population redistribute and we have seen previously that these are the population at different level. So for X transition like X has two transition X_1 and X_2 and for A you have two transition, A_1 and A_2 .

So difference between these populations is the signal that comes for A and these are the two distances that signals comes from X . So we had seen this different population distribution and how it changes because of relaxation.

(Refer Slide Time: 6:45)



So now let us move to interesting concept of selective population inversion and how we can enhance signal of one of the spins by doing this. Now here these two spins that we are considering have two γ right, they are not proton, proton but they can be carbon proton or nitrogen proton. So let us consider this as AX spin system, AX means the weakly coupled spin system like proton and carbon, proton say on 600 Megahertz, resonate at 600 Megahertz and carbon will resonate at 150 and their J is typically around 140 or 120 hertz.

So in that case, they are weakly coupled spin systems. So let us consider that and X is insensitive because it is γ , say γ of ^{13}C we know that actually it is a four time less than gamma of proton. So this is 4. So this nuclei is insensitive nuclei. So suppose we have a weakly coupled spin system AX , where A is proton and X is X nuclei like carbon nitrogen, which is insensitive.

So in that case, here is the energy level diagram for this spin system. So we have four states, state 1, state 2, 3 and 4. Now, the population of these states is suppose we have a $\Delta + \delta$, here we have $-\Delta + \delta$ and here we have $-\Delta - \delta$ forth and here we have $\Delta - \delta$. So now two transition that is happening for A spin is this one and this one, A_1 and A_2 .

So now, A_1 and A_2 and for X , it is happening X_1 and X_2 , X is our insensitive nuclei. So the intensity of each of the peak will depend upon the difference in the population. So for A_1 , the difference is from here to here. So that is 2Δ , so this minus this that is 2Δ and for A_2 this minus this. So

$$(\Delta - \delta) - (-\Delta - \delta) = 2\Delta$$

That is how A_1 and A_2 have intensity equivalent to 2Δ and they are sensitive nuclei. So they have a higher intensity. For X nuclei which is insensitive nuclei similarly, we can take from X_1 and X_2 , so this minus this, so Δ cancel out and a δ adds up, that is two delta and for again for X , it is 2δ .

So these are intensity for two insensitive nuclei that is ^{13}C , suppose and this is say proton. Now that is typically case we have for a proton carbon spectrum and this difference in the intensity is because of population distribution. So X is less sensitive nuclei therefore, signal is less and population is also less.

(Refer Slide Time: 10:07)

Two transitions for each spin

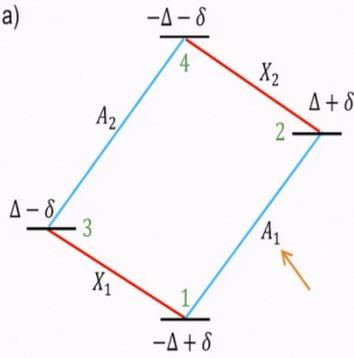
- A1: (1→2 transition), A2: (3→4 transition),
- and two transitions for the X: X1 (1→3), and X2 (2→4).
- Intensities of the A1 and A2 transitions are 2Δ
- Intensities of X transitions are 2δ each

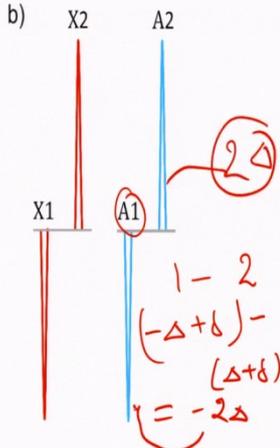


Now suppose we do a trick. So what we are doing? Now the two transition that we had is essentially A_1 and A_2 and then X we had X_1 and X_2 . So intensity for A_1 and A_2 that is what we looked is 2Δ and for intensity of for X transition is 2δ . So let us to a trick.

(Refer Slide Time: 10:32)

Apply a 180° pulse is applied to the A_1 transition

a) 

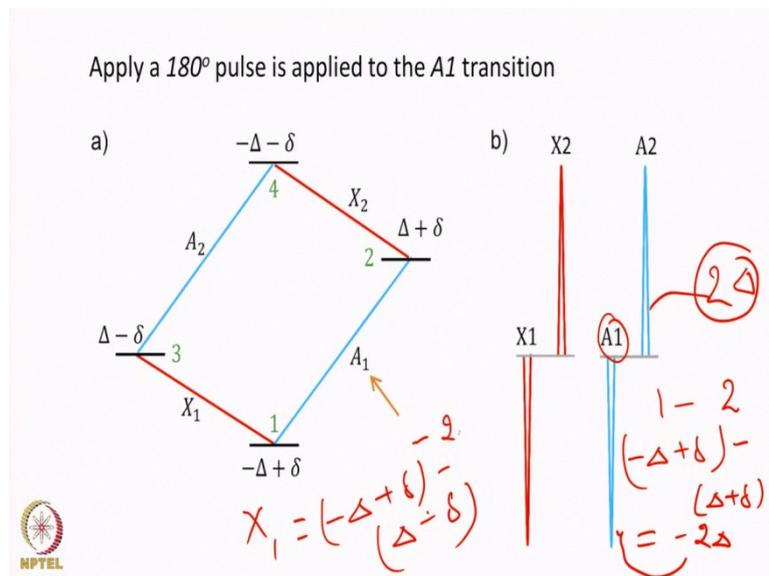
b) 



The trick is that now we selectively applying a pulse on A_1 this transition. So selectively applying a pulse 180° , so 180° pulse is for inverting the population. So we are now inverting the population in this A_1 transition. Okay, so what is the effect of happening because of this inversion falls? So now take the difference for A_1 , so this minus this is essentially A_1 , A_1 intensity will be state 1 - state 2. So that will be $(-\Delta + \delta) - (\Delta + \delta) = -2\Delta$.

So that will be -2Δ and for A_2 , it will be as usual 2Δ so this is for this and this is for this. Now, A_1 because we applied a selective 180° pulse so it inverted and A_2 remain unchanged. So because of this inversion something happens to X transition. Now that is very, very interesting.

(Refer Slide Time: 11:43)



➤ Populations of the 1 and 2 will get interchanged.

➤ And intensities of the X_1 and X_2 transitions will become $2\Delta - 2\delta$ and $2\Delta + 2\delta$, respectively

Substantial change in the intensities of the X transitions!

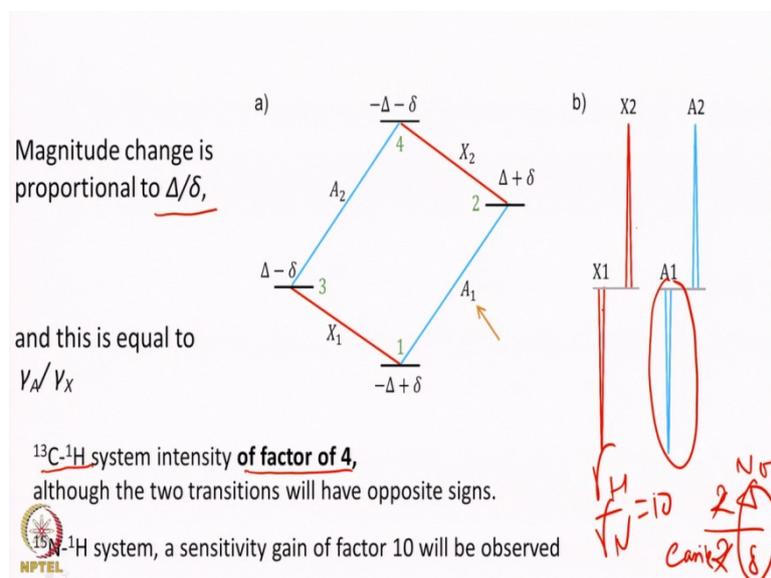
Changes in the intensities of two transitions from the equilibrium situation this will become $+2\Delta$ and -2Δ

So, what is happening to X transition? So now, as we said we applied pulse on A_1 , so population level of state 1 and state 2 changed and intensity of the X_1 and X_2 becomes now actually $2\Delta + 2\delta$. Now you can just go back and have this, so $1-3$ is for X_1 . So intensity for X_1 is $(-\Delta + \delta) - (\Delta - \delta) = -2\Delta$. So if you see this, this is actually -2Δ right. So that is what intensity we have for X transition.

That is substantial change in the X transition. Okay, so change the intensity of this transition from equilibrium state that is what we calculated is now plus $2\Delta - 2\delta$, that is a substantial change in the intensity for X spin.

So now, what we did by doing this? So earlier we had intensity for X that was 2δ . Now if you look at from the equilibrium it is a big 2Δ . So that is a significant enhancement in the signal for X spin. So by perturbing A spin we are getting enhancement in the signal for X spin and that had enhancement is substantial.

(Refer Slide Time: 13:24)



So how much enhancement we are getting? So now because of perturbation we got it as essentially 2Δ and earlier we had 2δ . So big delta and earlier we had, now and earlier we had this, right. So essentially we are getting enhancement of big delta divided by small delta, here you can see if we have perturbed A spin we are getting enhancement in X spin. So X_1 and X_2

and that enhancement is in tune of $\frac{\Delta}{\delta}$.

So that is equivalent to ratio of the gyromagnetic of this two nuclei. So γ_A/γ_X and γ_A/γ_X is 4 times so for a $^{13}\text{C}-\text{H}$ coupled system we get enhancement factor each up to 4. So although these two spins are of opposite signs, but enhancement is 4 times. So if you take for N-H

system, where $\frac{\gamma_H}{\gamma_N} = 10$. So for N-H system will get 10 times enhancement. This is huge,

perturbing A spin we are getting enhancement for X spin 4 times for C-H system and 10 times for a N-H system.

(Refer Slide Time: 14:51)

Selective inversion provided :

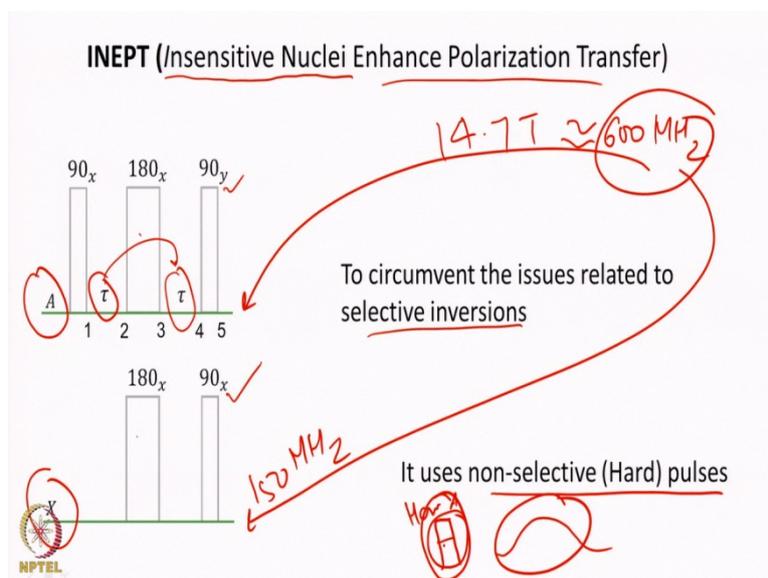
- A significant advantage for sensitivity enhancement.
- The difficulty lies in obtaining a clean selective 180° pulse;
- when the resolutions are poor or the spectra are crowded.



Okay, so selective inversion provided us as significant advantage for sensitivity and enhancement, we have perturbed one spin we are getting 4 folds or 10 folds enhancement. But there is a problem, problem is what? That like here as you look at I perturbed only one transition in A spin. So that means our inversion pulse has to be transition selective, so that it only perturb A_i spin, getting that clean pulse 180° is little difficult task.

So and especially when it is crowded spectrum, where you do not have a transition very clearly defined, like where resolution are poor in a crowded spectrum, getting a transition selective pulse, which will selectively invert one of the transition is difficult. Therefore, in those case, it can mix up and the enhancement that we are getting may not be that clean.

(Refer Slide Time: 15:54)



So to circumvent such problem actually method was developed and this method is called INEPT. What INEPT means? Insensitive nuclei enhancement by polarization transfer. So let me explain each of these terms, one by one. Insensitive nuclei, insensitive means low γ nuclei, low γ what we mean? So, generally proton has a high γ , low γ is like where population levels are not too far apart. So like carbon 13 ^{15}N phosphorus many other nuclei are low γ nuclei and they are essentially insensitive nuclei.

So we want to enhance this signal of this low gamma or insensitive nuclei by transferring the polarization from high γ nuclei, which is proton. So if we transfer the polarization from proton which is high γ nuclei to carbon 13 or nitrogen 15 which is low γ nuclei that method is called INEPT insensitive nuclei enhancement by polarization transfer that is an INEPT.

So, what actually it is? So it is a set of pulse that I am going to explain you in a moment and this is, this pulse sequence actually helps to circumvent the issue related to selective inversion because as we see if the transitions are not far apart and getting 180° selective pulse is difficult. So in those case, in INEPT case we do not need that transition selective pulse, one can use non-selective pulse and which is called hard pulse. Like symbol for hard pulse is like a square shape and soft pulse are something like this.

So hard pulse means their bandwidth is quite high and soft pulse that bandwidth is low that is how they called a hard pulse and soft pulse. So duration is short that means by and like

duration of this hard pulse can be of few microsecond, so bandwidth is going to be in 1 by microsecond, that will be in tune of say kilohertz or even megahertz. So few microsecond, this megahertz tune and the soft pulse will be larger duration like 200 milliseconds or 500 milliseconds. Therefore, actually they are bandwidth in kilohertz.

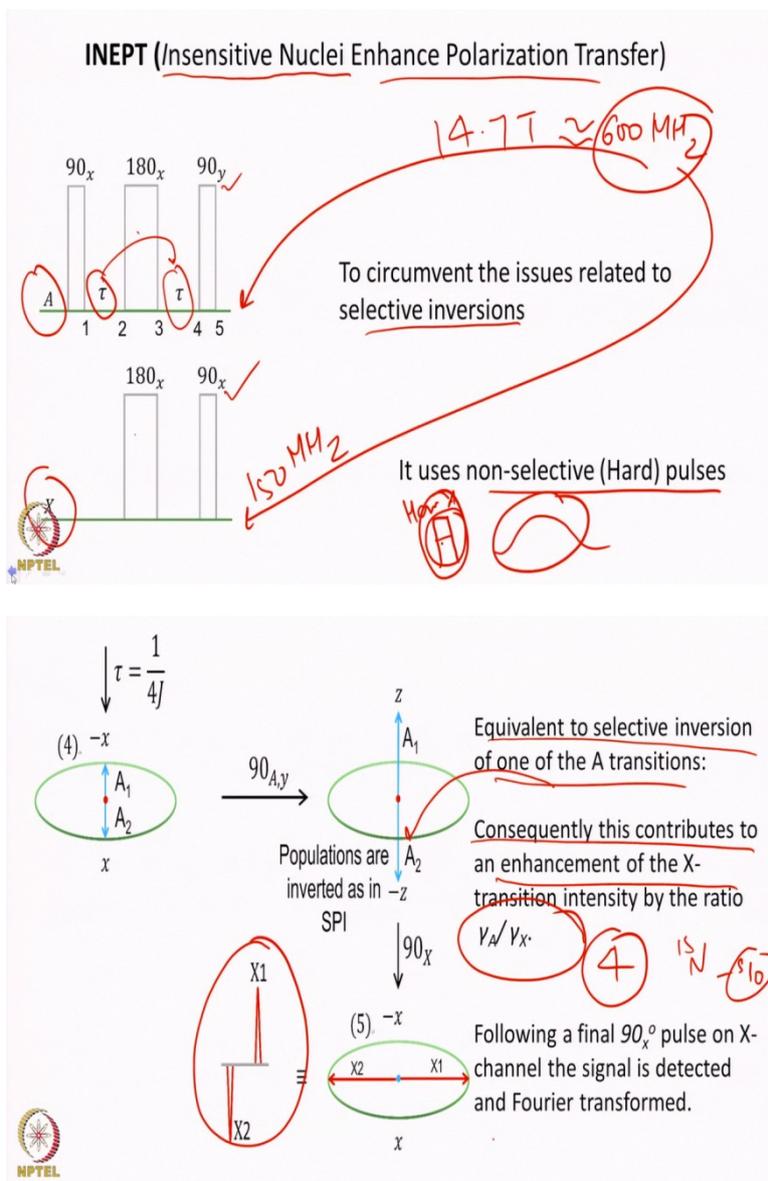
So even if you look at, so to selectively excite one of the transition in this pulse you need a bandwidth of few hertz and that means pulse has to be very long, long pulse has its own problem, one problem that can be even non-selective, like it cannot be very precisely selective that can leak out. So to circumvent such problem this method was develop, where we can use hard pulse and we do not need to selectively like use transition selective pulses.

So what this pulse sequence is let me explain you. It says that we have two spins, so here is our spin number 1 which is A spin and spin number 2 is X spin that is why these are two channels. So channels that means a frequency channel. If you are doing an experiment at say 14.7 Tesla which roughly correspond to say 600 Megahertz. So that means proton channels will be around 600 Megahertz and carbon channel will be around 150 Megahertz that is why they are two different channels. So their RF synthesizer will or work it different regime and that is how there are two channel.

So now on A channel we are applying a first 90° pulse in X direction, then we are waiting for certain time, which is τ . Then next step we are doing applying a 180° pulse on both channel. A channel and X channel. Next we are waiting for same time period, which is τ and next we are applying a 90°_y pulse on A channel, followed by a 90°_x pulse on X channel and then we are detecting, now my resonance should be in xy plane, which can be detected. So that is a simple pulse sequence for INEPT.

Starting with a 90°_x pulse, then waiting for τ period, τ period I just explain you in a minute, what actually it is, than waiting for τ period than applying a simultaneous pulse, 180°_x pulse on A spin and X spin, again waiting for time period, then apply a 90°_y pulse on A spin, followed by a 90°_x pulse on X spin and detecting it.

(Refer Slide Time: 20:43)



So what happens because of this polarization? So this is the vector diagram. Let us understand if I go back, first thing we did we applied a 90° pulse on A channel, so A means proton. So suppose, we were always discussing two transition so two vectors here, two vectors, we applied a pulse, 90° pulse on A, so that means they will go to -Y. So just a thumb of rule, now here I have drawn a circle I have written Z,X,Y first thing we are doing applying a 90° pulse on A channel $90^\circ x$ pulse.

So initially magnetization merge in Z direction, we applied an x pulse. So you apply x pulse on Z it will go to minus. So if it is moving anticlockwise, it is a minus. If it moves clockwise that is a plus. So x pulse applied on Z magnetization, it will lead to -Y direction. So that is

why we have here, these spins I , $-Y$ direction and two spins because we have, it is AX couple system, so we have two transition, one vectors source, one of the transition. So, A_1 and A_2 both now are in $-Y$ direction.

Then I said I have to wait for some period, which is τ period. Now what is this τ period that is very important. So τ is basically will be dictated by what is the coupling constant between these two spins and will see in coming classes that if you keep your τ , which is equivalent to $1/4J$, J is the coupling constant between like ^{13}C and proton. If you keep that now that will help you in transferring the magnetization from one spin to another spin when they are coupled through bond.

So that is the τ period, $\tau = 1/4J$. So if you wait for that spin and we are not doing anything. So we started like this, then we are waiting for τ period, so now in that way period spins will start moving in opposite direction from like $-Y$ axis. So here is my $-Y$ axis and both of this spins in the second case, they started moving, second case what I mean by?

So here both the spins are in $-Y$ direction and then during this τ period, they are like started from here and they are now moving like this. So that is what is shown here. So now both of these vectors are moving in opposite direction. Then we had applied 180° pulse on A and 180° pulse on x . So let us look what is happening here? So we had applied 180° pulse on A , 180° pulse will create inversions. So now these two guys are moving like this here, now they it will create inversions, so they will move like this and that is what actually it is shown.

So here 180° pulse, now they changes the direction and they will be going in this direction, they will be coming along $+Y$ direction. Simultaneously we had applied 180° pulse on x . So that actually changes the direction of the precession. So then they will start going in opposite direction. Okay, A_1 and A_2 .

Now next after this, these two pulses we are waiting for, after this pulse we are waiting again for τ period. So if you look at the vector diagram, we started like this, then during τ period they were like this and then like we inverted like this, but they were coming like this. So we applied 180° pulse on X . So they changes the direction and they again are going further.

Now after again time period $\tau = 1/4J$ they will align along X axis. So one will be $-X$ and another will be X . So now, then we are applying a y pulse. Now magnetization in X direction so we are applying a y pulse on A spin. So what we are doing? $90^\circ y$ pulse, so if you apply,

here if you apply a y pulse when magnetization is in X . So it will go to Z direction. So now my magnetization for A spin will be along $+Z$ and $-Z$.

Now my magnetization is in Z direction. For A spin it is in Z direction. So next we applied here a 90°_x pulse. So 90°_x pulse here population inversion happens, we have started both this transition in Z direction, but at this stage what we have done? We inverted one of the spin, if you look at. So then we apply a 90°_x pulse on X spin and that transfer the selective polarization enhancement happens on X spin.

So that is why we have now enhanced signal for X spin, but only caveat here is that both of these transitions will have opposite sign, but their signal has been enhanced. That is how in INEPT, you enhance this signal for X spin by application of this pulse sequence, which is nothing but couple of pulses which can be given by $90^\circ - \tau - 180^\circ - \tau - 90^\circ - 90^\circ$. So we achieved the enhancement in the signal of these spins.

So that is what we have done, so finally we get an enhancement and this enhancement is actually equivalent to selective inversion of A transition here and consequent of that enhancement we are getting in X transition and that will be essentially governed by γ_A/γ_X . So for if you are doing this experiment on carbon proton couple system, we get 4 times enhancement and for nitrogen proton couple system we get for ^{15}N , we get 10 times enhancement. So like selective population inversion we achieve enhancement, but of course will be of opposite direction.

(Refer Slide Time: 27:33)

INEPT has the following disadvantages

(i). Intensity anomalies
Incorrect relative intensities occur different spin multiplets and this depends on

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



But INEPT had some disadvantage, one disadvantage we founded that both the signal for X spin are coming in opposite direction and there can be intensity anomaly. So incorrect relative intensity can occur because of different spin multiplets. So for simplicity, we considered only two transitions, one was like this and another was like this and we found that if we invert one of these X transition, one was like this and another was like this.

But what happens if there is a spin multiplets? So then we have a problem because then again we cannot get a clean enhancement. The another thing that we looked at one of the parameter that was there $1/4 J$. So now these J is playing important role because that dictates how much τ we are keeping. So if this shift of heteronuclear or J coupling is different, then transfer of magnetization of population inversion can be different. So this dictates how much transfer it is happening.

So two things J coupling and spin multiplicity, actually dictates the transfer efficiency in case of INEPT. So to get the rid of that what we need to do, we will look at this in next class. Based on today's lecture, if you have any question, do not hesitate to ask us, we will try to resolve it. Thank you very much.