

## **One and Two Dimensional NMR Spectroscopy for Chemists**

**Prof. N Suryaprakash**

**NMR Research Centre**

**Indian Institute of Science Bangalore**

### **Lecture No: 45**

#### **Polarization Transfer Techniques**

Welcome back, so in the last class we started discussing about spin echoes which is very important in understanding the spectra editing. I wanted to see what is going to happen in the heteronuclear case, when I apply a spin echoes sequence on carbon 13; at the same time I want to do the decoupling of proton; and see what is going to happen. Of course, I was trying to explain what happens in this pulse sequence based on the vector diagram.

First thing I told you, if you please remember, I applied a 90 degree pulse brought the magnetization from the Z axis to the X axis. Now the spin vectors start moving; and after some time apply a 180 degree pulse, that is along the X axis, I can rotate it like this. Now the spin labels get interchanged; one which is moving faster start moving slow; one which is moving slow, starts moving faster.

They will now start moving and after exactly same amount of time; they refocus along the other axis. In this axis again they get refocused; this I said as chemical shift refocusing. When I took the example of CH vector, now we have only the two components because of alpha and beta components of carbon 13 due to it is coupling with proton. Now alpha component is moving like this; beta component is moving like this; one is faster, other is slower.

Now what happened to this vector? How much it has moved as a function of time delay, this we understood. This depends upon  $\pi \times tD \times JCH$ . That is the equation to say how much it has moved. Now, we found out if exactly tau delay is equal to  $J/2$ . Plug into the equation theta is equal to  $tD, J/2$ , you are going to plug it in and we find out exactly at 45 degrees, what is it going to happen, what is the angle theta?

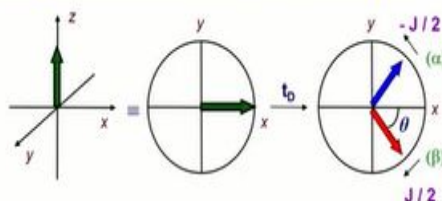
And when 90 degrees is there what happens? When 180 is there what happens, like that, keep on monitoring the signal intensity;

**(Refer Slide Time: 03:05)**

### Evolution of J coupled CH carbon

After  $90^\circ$  pulse,  $^{13}\text{C}$  magnetization ( $M_{xy}$ ) starts moving under J coupling

Each doublet component of the  $^{13}\text{C}$  vector can be labeled according to the states of the  $^1\text{H}$  it is coupled to,  $\alpha$  and  $\beta$



During  $t_D$ , each vector start evolving under  $J_{CH}$

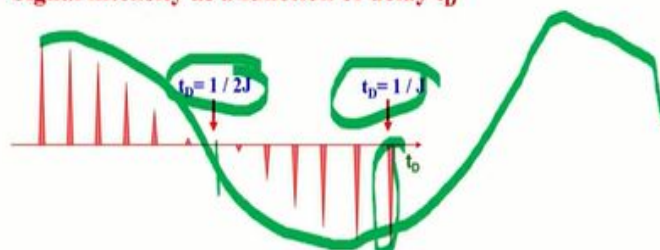
Their evolution in time is given by;  $\theta = \pi * t_D * J_{CH}$



This equation  $\pi \times t_D \times J_{CH}$  is the equation. I plugged into the equation and found out what happened to the magnetization as a function of  $t_D$ .

(Refer Slide Time: 03:14)

### Signal intensity as a function of delay $t_D$



The variation of signal intensity is the cosine of  $t_D$

It is zero for  $t_D$  values equal to multiples of  $1/2J$  and maximum/ minimum for multiples of  $1/J$

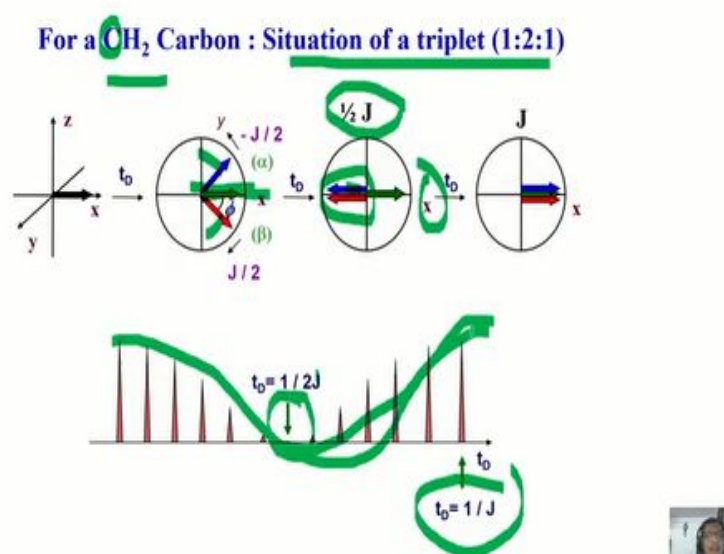
$J_{CH}$  differ: Assume  $J_{CH}$  is nearly same



And, we observed for a CH vector what is happening? When  $t_D$  is exactly equal to  $1/J$ ; we resolved it vector into 2 components, we said opposite moving components get nullified; the vector moving in the same direction gets added up and you found out what is the signal. We saw when exactly this is equal to  $1/2J$ , signal is 0, when  $t_D$  is exactly equal to  $1/J$ , see  $t_D$  is exactly  $1/J$ , signal it is negative maximum and the evolution of this magnetization as a function of  $t_D$  follows a cosine curve like this, you understand?

Now, we understood what is going to happen with this; we assumed also  $J_{CH}$  is nearly same. It is not different that is what we assumed, or we can take the average value of this.

(Refer Slide Time: 04:15)



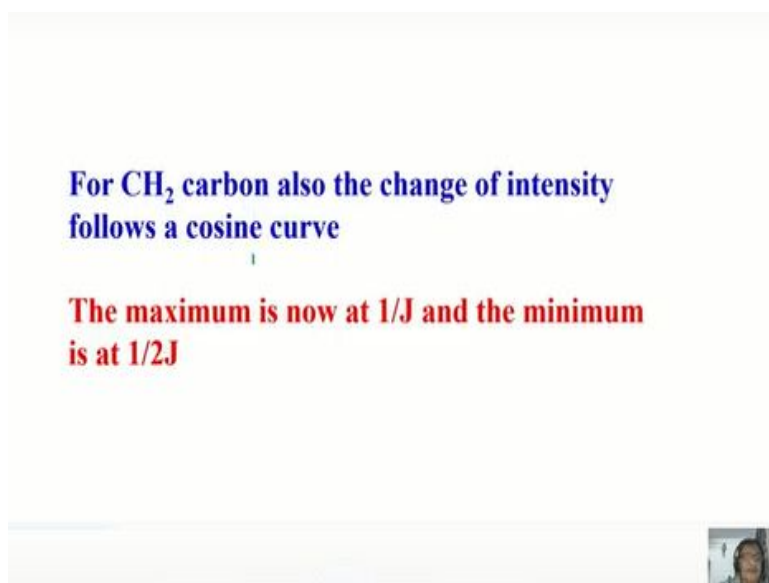
Now we will see what happened to this  $\text{CH}_2$  carbon in the same pulse sequence, echo sequence. Now what happens to carbon here? Carbon is split into a triplet because of  $\text{CH}_2$ , 2 protons are attached to it, remember. So as a consequence it is going to be triplet. So, now I resolve three components; one is the fast moving component and other is slow moving component like this; and the central component do not precess at all; It is like that, it does not move.

Remember, one is at 0, other is  $+J/2$  and other is  $-J/2$ , three components and this is a triplet, if you consider for the  $\text{CH}_2$  carbon. The carbon is a triplet and the central component do not precess, and only outer components start moving. Now exactly find out the situation, plug it into the equation, what happens to these components at  $1/J$ ,  $1/2J$  and what happens after  $J$ . If you plug it and understand the exactly after half  $J$ , these two vectors will come in the opposite direction.

Let us say my receiver is here, and they will be the opposite direction; and continue further for another delay. After  $J$  they come back to the same axis, they refocus here. Now plot the intensity for this  $\text{CH}_2$  carbon, intensity of the  $\text{CH}_2$  carbon as a function of  $\tau$  delay. The vector diagram you can understand in the same way, there is no difference at all, only thing in the  $\text{CH}_2$  case central peak do not precess and outer components start moving in the same direction.

will get different intensity plot like this. Now, when we plot the intensity of the peak as a function of  $tD$ , this follows like this again. It is not a cosine function, it is a function like this, Now what is happening here? Exactly at  $tD$  is equal to  $1/2J$ , it is 0. When  $tD$  is equal to  $1/J$  this is positive maximum. What was the earlier case? You remember what happened to CH case, it was going like this and then it was becoming maximum, negative maximum. But here it is becoming positive maximum for  $CH_2$  carbon. You see the difference when the CH carbon intensity was negative, at the same time, at the same point  $CH_2$  carbon will be a positive maximum.

**(Refer Slide Time: 07:05)**



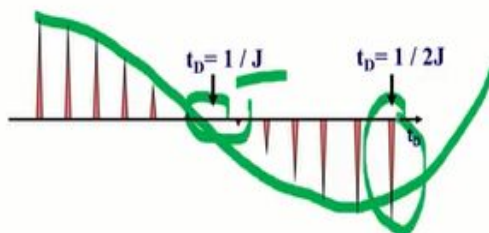
So,  $CH_2$  carbon you can monitor the change intensity. It does not follow a cosine curve, but you see the cosine curve is not going like this, a different pattern; the maximum is now at  $1/J$  and minimum is now  $1/2J$ .

**(Refer Slide Time: 07:27)**

The CH<sub>3</sub> carbon has four vectors, two fast moving and two slow moving

They behave in the same manner as CH vectors

The intensity is minimum at  $1/2J$  and negative maximum at  $1/J$



What happens to CH<sub>3</sub> carbons? No difference at all; because CH<sub>3</sub> carbons and CH carbon behaviour identical. Only thing is CH<sub>3</sub> has 4 vectors because it is a quartet; 2 positive components and 2 negative components moving in the faster and slower, fast and slow moving components. So, 2 vectors from the centre to the right; 2 vectors from to the left. That is 2 lines here, 2 lines here from the centre. Again they follow the similar path like CH.

You can trace it, both the components; how they are moving you can trace it; it is behaving exact like CH vector. So, what happens if we trace is; It follows the path like this. So what did you observe? In the case of CH also this was 0 at  $1/J$  and it was maximum of at  $1/2J$ . Now same thing you can observe for CH<sub>3</sub>. So, what did we understand from this?

(Refer Slide Time: 08:29)

Evolution of different <sup>13</sup>C signals as a function of  $t_D$  is given as

$$\text{CH: } I = \cos(\pi J \tau)$$

When  $\tau = 1/2J$ ,  $I = \cos(\pi/2)$ . Intensity is zero. For  $\tau = 1/J$ ,  $I = \cos(\pi)$ . It is negative maximum

$$\text{CH}_2: I = \frac{1}{2}[1 + \cos(2\pi J \tau)]$$

When  $\tau = 1/2J$ ,  $I=0$ , Intensity is zero. For  $\tau = 1/J$ ,  $I=1$

$$\text{CH}_3: I = \frac{1}{4}[3 \cos(\pi J \tau) + \cos(3\pi J \tau)]$$



What it means is? Of course, mathematically you can prove it, the evolution of CH follows a cosine path, CH<sub>2</sub> follows this equation, CH<sub>3</sub> follows this equation, but now when you put different values  $\pi$ , I mean different values of  $\tau$ , you can find out; when  $\tau$  is equal to  $1/2J$  what happens, and when  $\tau$  is equal to  $1/J$  what happens.

In the case of CH, when  $\tau$  is equal to  $1/2J$ ; put  $1/2J$  here, you see it becomes 0. See when it is  $1/2J$  the term equal to  $2J$ ;  $J$  cancels out, it is  $\pi/2$ ,  $\cos \pi/2$  is 0, you know that. That is why CH becomes 0; and on the other hand, when  $\tau$  is equal to  $1/J$ , this can cancel out; it is  $\cos \pi$ , so it is the negative maximum. So, these are the equations which define the intensity pattern.

I did not go to the mathematics of evolution, it is huge calculations, but remember all the three equations, all the intensities as a function of  $\tau$  for CH, CH<sub>2</sub> and CH<sub>3</sub> can be given like this; and as you plug in the values of  $\tau$ ; then you know when it is maximum and when it is minimum.

(Refer Slide Time: 09:42)

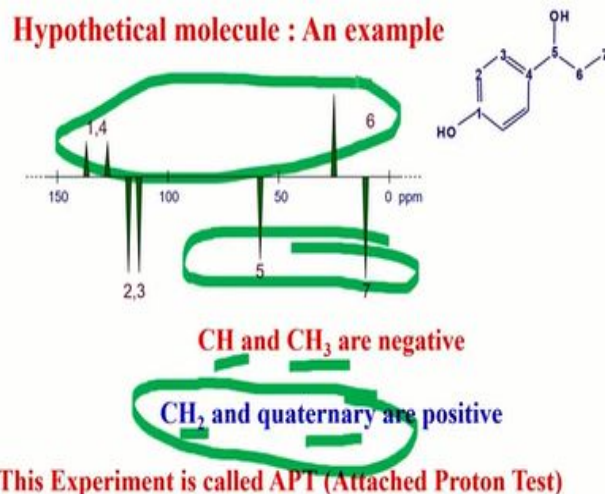
Varying delay with this spin echo sequence, one can distinguish carbons attached to different protons, depending on their signs

Carbons attached to odd number of protons have one sign.  
Carbons attached to even number of protons and quaternary have opposite sign



And, varying the delay what you understand in the spin echo sequences is; you can distinguish between two types of carbons, which are attached to odd number of protons and even number of protons, same. The reason why it is? You understood.

(Refer Slide Time: 10:00)



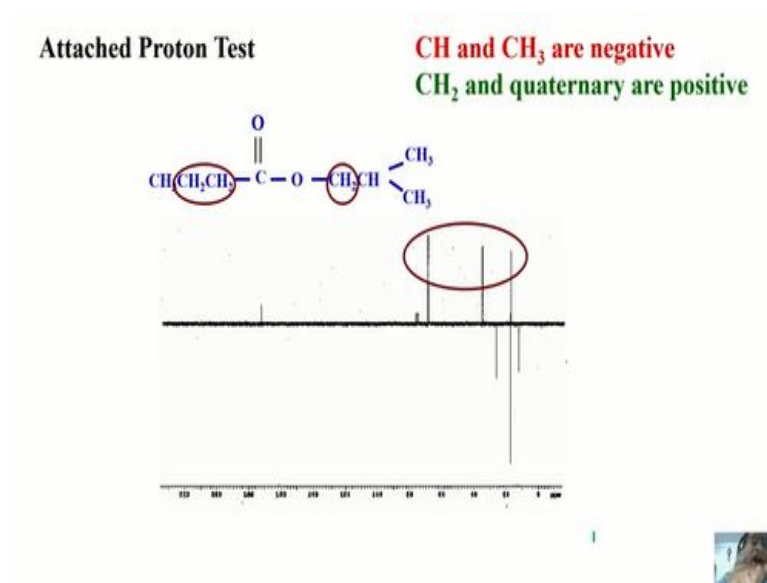
Of course J couplings are not same, as I said you have to assume they are nearly same take approximate value for it. Now in this example, what did we understand. Take an example; I do the experiment of spin echo carbon 13. Now, as I keep on varying the tau delay as a function of J, what happens is CH and CH<sub>3</sub> are negative and CH<sub>2</sub> and quaternary are always positive, at particular value of J. You can find out what happens here at  $1/J$ , this is 0,  $1/2J$  this is negative. We will consider  $1/J$  what happens here? One of them is 0, we do not consider 0 components, and we will consider only the signal which is positive or negative. Here, we consider exact at  $1/2J$  and see what happens? If you go to  $1/2J$ ; you see that CH and CH<sub>3</sub> are negative, these are negative. The CH<sub>2</sub>'s and quaternaries are positive.

What it means is? The carbons which are attached to odd number of protons have one sign; signal intensity is negative, carbons which are attached to even number of protons or quaternary have one sign, it is positive. Of course positive and negative are my convention, I can interchange this I can make this positive this negative does not matter; this depends upon how I am going to do phase correction of the spectra.

So, but the difference what you should understand is CH and CH<sub>3</sub> are opposite in the intensity compared to CH<sub>2</sub> quartet. If these are positive, these are negative. So, what it means is; you are able to edit the carbon-13 spectrum using this pulse sequence and you can identify from your spectrum, whether this carbon is CH<sub>2</sub>, or CH<sub>3</sub> or CH in a crowded spectrum, even after decoupling.

Let us say there are 30 carbons in the molecule; and you want to make the assignment, we do not want to take the coupled spectrum because it is complex. You do the decoupling; but then how do you assign? For assignment we have to do get the coupled spectrum, so that So, that you know CH<sub>3</sub>, CH<sub>2</sub>, and CH are here, doublet, quartet, triplet; but we do not want to do that and this is the way, you can do the decoupling, you can understand and find out which carbon is which. This is called attached proton test, which distinguishes the carbon based on the number of protons attached to carbon; it is called attached proton test.

**(Refer Slide Time: 12:44)**



But, there is one defect in this case. It identifies the odd and even proton attached carbons, but it does not distinguish CH and CH<sub>3</sub>. The distinguishes only odd number of protons attached to carbon and even number of protons attached to carbon; but does not distinguish whether it is CH and CH<sub>3</sub> or whether it is quaternary or CH<sub>2</sub>; that problem is always there. This will disadvantage, we have to overcome

**(Refer Slide Time: 13:11)**



## Polarization Transfer Techniques

### Enhancement of Signal Intensity



Now, we will go further; try to understand more about it; how we do when everything. We do what is called polarization transfer technique; where we can enhance the signal intensity. Here, we are only looking at the identification of different carbons; you identify the carbon; you do this spectral editing; this called spectral editing. Editing the spectrum to find out which carbon is which. But as I said, my problem of sensitivity is not solved, still carbon is less sensitive to detect compared to proton.

So, what we have to do? We have to combine this editing technique along with polarization, so that we can take the magnetization of abundant spins, give to the carbon and also do the editing, we have double advantage. You can distinguish carbons, at the same time enhance the intensity, can we do that? It is possible to do that; and that is what we will try to do by using polarization transfer technique.

**(Refer Slide Time: 14:16)**

## Solutions to sensitivity problem of NMR

- 1) Get more sample
- 2) Isotope labeling (Expensive and not practical)
- 3) Record spectrum at higher field (Practical Limitation Bo)
- 4) Record spectrum at lower temperature (not significant effect)

Play with spin dynamics and design new NMR experiments



The ways of increasing the intensity we know; they are to get more sample, isotopic labelling, go to higher magnetic field and record spectrum at low temperature, all are practically possible, and sometimes, not possible sometimes; depends upon your ability. What you can do? whether you have more sample or whether you have the facility for isotropic labelling by some chemical way, sometimes very expensive or going to very higher magnetic field, viz., if you want to get 800 mega hertz, and 1000 mega hertz spectrometer, it may not be possible to it maybe not be available, so all those limitations are there. So, let us understand in addition these things, how can we play with spin dynamics and design new NMR experiments.

(Refer Slide Time: 14:59)

## Polarization Transfer

Can we use the bigger population difference from a sensitive nuclei ( $^1\text{H}$ ) and pass it on to the less sensitive nuclei, viz.,  $^{13}\text{C}$ ,  $^{15}\text{N}$ ?

There are two ways of achieving this

Selective population transfer  
Selective population inversion

These are polarization transfer techniques



When you ask a question how do you do the polarization transfer, take the population difference from the sensitive nuclei, more sensitive nuclei like proton, and give it to carbon or

nitrogen. There are two ways of doing this, you remember, one is selective population transfer, and the other is selective population inversion. How these two techniques will enhance the signal intensity let us understand today. This is called polarization transfer technique. Please remember, there are two polarization transfer techniques; one is selective population transfer called SPT, other is selective population inversion called SPI, these techniques if you have understand, you can coupled with the editing technique later.

**(Refer Slide Time: 15:48)**

## Population Inversion

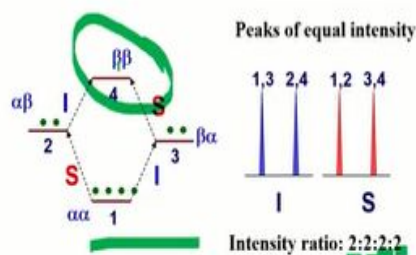
Homonuclear spins : eg. Two weakly coupled protons



Now, let us go to population inversion scheme. What is a population inversion? How does it enhance the signal intensity? Now, let us consider homo-nuclear spins; 2 weakly coupled protons. We will come to carbon 13 later, because finally our idea is to enhance carbon 13 signal intensity, not protons, any way both the protons are abundant, if I take homo-nuclear coupled. But just to give you the feel, how much is the gain in the signal to noise ratio when you take carbon and proton, related to proton and proton we will see.

**(Refer Slide Time: 16:27)**

## Two homonuclear weakly coupled protons Distribution of population in thermal equilibrium



**The population has been chosen to calculate intensity. It does not mean there is no population in  $\beta\beta$  state**



Now, I consider an example of two weakly coupled spin system. I have already discussed with you weak and strongly coupled. What are weakly coupled spin systems? And what are the strongly coupled spin systems? When two weakly coupled spins are there; and there are 2 protons weakly coupled, you have only 4 energy levels. That is what I said, 4 transitions, 4 energy levels and 4 transitions, no doubt about it, we have discussed this a lot.

All of them are equal intensity we know that; and we know the centre of this gives the chemical shift, this chemical shift, this separation gives you J coupling. I do not have to repeat again and again, I hope you are all with me; this is called AX spin system. AX spin system gives two lines for each spin, of equal intensity. Now, you will understand based on the population in thermal equilibrium.

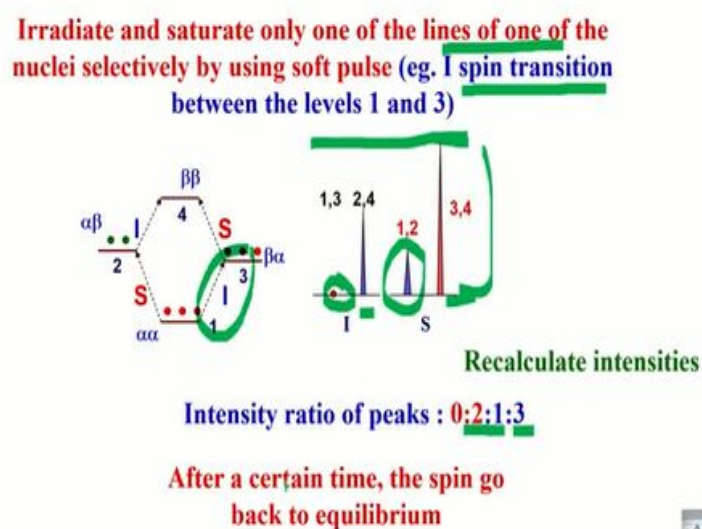
Let us have 4 energy levels you all know it is alpha-alpha, alpha-beta, beta-alpha, beta-beta, 4 energy states. I will now put spins in each energy state to ensure that population ratio is such that the intensities are always equal, because that is what two weakly coupled spin system is all about. WE should get equal intensity. For that what I do is I will take 4 spins here; just for the sake of understanding, 2 here, and 2 here.

Now what is going to happen? Look at the population differences to get the intensity of the signal. What are the allowed transitions? We have already worked out for an AX spin system, beta- beta, to alpha-beta change in the magnetic quantum number is + 1 or - 1 allowed; and this is allowed, this is allowed, this is allowed, thus there are 4 transitions allowed; and this corresponds to I peak, , this corresponds to peak of I spin, because I and S if I take, here I I is

changing from alpha to beta. Similarly here I is changing from alpha to beta. And if I take S here, here S is changing from alpha to beta and here S changing from alpha to beta. So I have labelled the spins based on their spin states alpha beta, like this. Now we calculate the intensity, I take the population difference that is what I said, the detection of the NMR signal is nothing but the difference in spin populations. Now for S transition, this is allowed. What is the difference between 4 – 2, 2, the intensity is 2, for another S transition; 2 - 0 it is 2; for I, I have put 2 and 0, this is allowed transition for I, 2-0 is 2; another I, 4-2, 2. So I have distributed spin populations, so that I must get this spectrum with equal intensity. But it is only for rough estimate; for only for understanding purpose, do not think there is no spin at all in the state, there are spin large number of spins distributed among all these spin states, finally it so happens, you get the transitions of equal intensity.

Only for understanding and calculation purpose, to make my calculation simple I have taken this. Never be under the impression there are no spins in the beta-beta state, that is wrong, That is not the case, there are spins present in the beta-beta states.

**(Refer Slide Time: 20:04)**



Now, what I am going to do is I will do a trick. I am going to irradiate and saturate only one of the lines. You know, what is irradiation? That is what I said decoupling; for decoupling we use sometimes called irradiation. I do the irradiation; when I irradiate I can saturate these spins. What I mean by saturation, I make the populations between two spin states equal. Now, I am doing the saturation of I spins, level 1 and 3.

What is the I spin here? Levels 1 and 3; look at this; levels 1 and 3 I make equal by saturation. what will happen? this was 4 spins, this was 2 spins; they become equal then there are 3 spins here and 3 spins here. So, they will distribute equally between these two, so that there is no population difference, that is called saturation. Now, when I do the saturation look at it they are equal; that is all I did nothing else, only one of the transitions I am saturating.

Now, let us see what is the intensity; you calculate this is 0. This should be zero, because I am saturating; the saturated peaks do not appear, it has 0 intensity; because there is no population difference. What about other I spin? this one, take the difference; it is 2 that has not changed, I agree. What about the S spin? S transition is here between 3 to 2; the difference is 1; and this one, S transition 3 to 4, it is 3, fantastic is it not.

What did we do? We saturated only one of the transitions, and see the effect is there on other transitions. This transition did not get affected. I did disturb the I spins energy state, I spin population, but S spin is also getting affected; see this intensity came down by half, it was 2, 2, 2, 2 all along, you know in the previous case; all were equal intensity. Now, this has become 1 and this has become 3; this one, fantastic.

When I recalculated the intensities, I find now the population distribution is such that intensity when saturate I spin is affecting S spin also. Now the intensities are 0, 2, 1 and 3. so, fantastic. Now this what happens? Let us wait for some time, I did this type of disturbance that is, saturation for a short time by applying a weak rf. I cannot apply a strong rf, because every spin, all the energy states can be disturbed, I do not want to do that.

I apply selectively only for this transition, a weak, mild RF pulse I apply; and disturb only this for saturation and keep quiet. That is what I did. After some time I removed the RF pulse what will happen? when I remove the irradiation? before it was a disturbed state; what should happen? the spins have to go back to thermal equilibrium. Exactly go back to previous case of population 4, 2, 2, 0. It has to achieve that, so that it comes to thermal equilibrium with intensity 2, 2, 2, 2; it happens after some time.

How much time it takes? we all know what is going to happen; time for relaxation and everything. This is for different type of relaxation. So, we will come to the relaxation later,

but remember I saturated and removed the RF pulse after some time, the spin system go back to thermal equilibrium.

(Refer Slide Time: 23:52)

**What is achieved ?**

**Changing the populations of the spin system, by saturating a particular transition resulted in change of intensity of other peaks of coupled spins**

**We transferred polarization from one nuclei to the other.**

**Since it is done using soft pulse, This is called selective polarization transfer, or SPT**

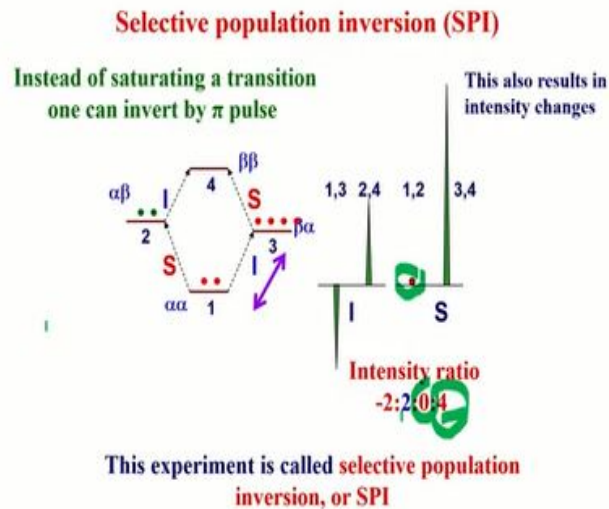


What did you achieve now? Changing the population of the spin system by doing selective population saturation, you achieved the change in the intensity of the coupled spins. Of course, they must be coupled spins. So, what did I do? I saturated one of the coupled transitions, I observed to change the intensity of other spins, this is a fantastic thing.

So, what is what we achieved ,this is called selective polarization transfer. Why did I do that? Why we call it? selectively attached saturated only one of the transitions, I did not saturate uniformly like in heteronuclear decoupling where all protons are saturated, so that you do not see coupling with carbon. That is a uniform broad band decoupling; but here only selective transition saturation. See the beauty of NMR you can play with even one single peak, single transition can be saturated.

So this is causing changing the intensity of coupled spin. This is called selective polarization transfer.

(Refer Slide Time: 25:05)



On the other hand we can also do the selective population inversion. What is population inversion? In the case of saturation, we are equating the population. Now let us do in a different thing, I apply pi pulse with particular transition, what does pi pulse do? It will invert the populations, like magnetization which was here, was brought here. this 180 pulse right?

So 180 pulse inverts the spin populations, so what it does? it is an inversion pulse, the populations instead of saturating, is inverting between these 2. The same transition I took, when I saturated; it was 3, 3; before saturation, it was 4, 2; after saturation became 3, 3. Now in the inversion the 4 which was here went here, the 2 which was here came back, just population between these two states got interchanged, that is all I did, this is called selective population inversion.

Now, let us see the intensities, recalculate the intensities, what is intensity of I spin? I spin from here to here; this is 2, this is 4 it became -2. Earlier it was 2, 1 : 1, to 2, 2 was there. Now see if I take the population difference between 1 and 3, it is -2 not to 2, 4 is this same, 2, 3 not changing, what about 1, 2? 1, 2 population is 0; this is 2, this is 4 difference is 0. So 1, 2; I am not disturbing; this will remind you, I am not disturbing things. I am only distributing this transition.

I am not doing anything for this S transition; but I made this signal 0; by doing this selective population inversion, you understand what is happening? Now consider transition 3, 4, so this is 4 this is 0, what is the population difference 4? So, what is intensity ratio? Now, -2, 2, 0, 4, fantastic; I inverted one of the transitions of the I spin; and the enhanced the signal intensity



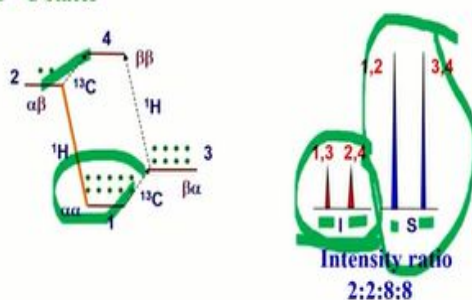
of the other coupled spin by 4, factor 4. Of course, this has come down, you will worry about it later, but what it means is, this experiment also changes the intensity.

There are two ways of enhancing the intensity of the coupled spin, one is by selective population transfer, and other is by selective population inversion. In selective population transfer we are equating the population, that is called saturation. In the selective population inversion, a particular transition populations are inverted between the energy states. That is all we have to do, okay?

**(Refer Slide Time: 28:09)**

### Selective Polarization Transfer (SPT) on a heteronuclear system

The intensity of  $^{13}\text{C}$  signal is 4 times less than that of proton.  
The population distribution is accordingly chosen between  $^1\text{H}$  and  $^{13}\text{C}$  states



Now, that was the homonuclei I wanted to show the difference for you. Now let us see what happens if you apply in a heteronuclear system. This is what our interest. Finally we want to see the enhancement of the carbon signal not proton, right? The intensity of the carbon signal is 4 times less than that of protons, remember? I give the energy level diagram of homo-hetero nuclear system, coupled spin system, two spins coupled in one of the classes, where homonuclear levels are equal heteronuclear were very well separated, two small energy states for hetero nuclear and large energy separation for homo nucleus spin. I wrote it very clearly; that is coming handy know. Please understand, that because very useful now. In the same way we write the energy level diagram now, instead of I and S, I am writing I as proton S as carbon  $^{13}\text{C}$  both were homonuclear there, I wrote I and S.

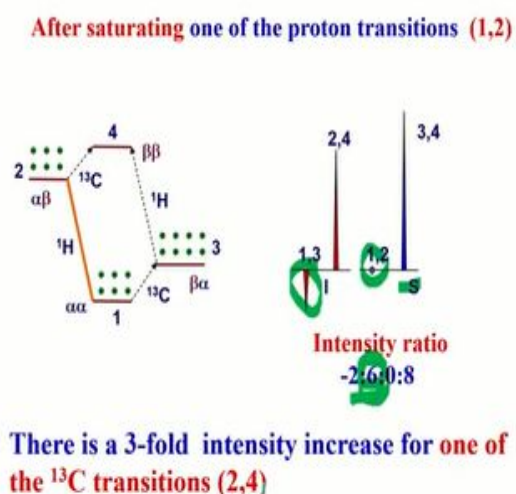
Now it is heteronuclear instead of writing I and S, I am writing this as proton and this as carbon. So, again heteronuclear 2 spins coupled, invariably has to be weakly coupled; there is no doubt about it Right. We have been discussing this quite often. So, now let us arrive at

the population distribution. As I said carbon 13 is less sensitive, 4 times less intensity compared to proton. So, I have put the populations in a different manner here.

In the case of proton it was 4, 2, 2, 0, so that everything become 2, 2 all 4 transitions of equal intensity. But now I have deliberately made this such that carbon intensity should be 4 times smaller, 4 times smaller that is because carbon gamma is 4 times smaller. Now, let us see what is intensity? I put 10 spins here, 8 here, 2 and 0 here. What is the advantage of that? See the difference now. What is this one? the proton transition, difference between 1 and 2 is intensity 8. What is proton transition 3 and 4,  $8 - 0$ , intensity is 8.

What is carbon transition?  $2 - 0$ , 2; here the carbon transition  $10 - 8$ , 2. Now you understood, why did I choose different spins here? 10, 8, 2, 0; because now compare the intensity ratio 2, 2, 8, 8 proton intensity is four times larger than carbon intensity. Because of lower gamma. Deliberately I wanted to choose this, now see how much is the gain in the signal intensity for selective population transfer, and selective population inversion? we understand now, okay?

**(Refer Slide Time: 31:11)**



Now we will see only saturation transfer; what happens? I am going to saturate one of the transitions; that is 1 and 2, proton transition I am saturating, what was the population difference 10 and 2? Now, 10 and 2 is total 12, I made it 6, 6 because I am saturating. I equalled it. So, it was 10 it was 2; I equated it 6, 6 that is all I did, now calculate the intensity of proton; this is saturated signal will not see signal, intensity 0 and this signal  $8 - 0$ , this is 8, that is not changed.

Look at the carbon now, this is 6 and 8; this is -2. What about other one? 6 and 0, 6-0 it is 6; what a marvellous change in the intensity. I saturated one of the proton signals and in the carbon signal for one of this peak, there is enormous rise the intensity; instead of 2, 2, 8, 8, it became 6, 3 times enhancement. That is what. There is 3 fold enhancement in the signal of one of the carbon peaks.

Let us see population inversion also; what happens first remember will come to the population, now you understood in the selective populations transfer in the heteronuclear case similar to homonuclear case, there is a rise in intensity; but this is a more rise in the intensity here because you are considering heteronuclear spins, and you see one of the peak will go up by 3 fold in intensity. What I will do is I will stop now, come back and continue with heteronuclear selective population inversion; then go to INEPT sequence and then DEPT.

So that how all these editing techniques will be understood, because INEPT and other things is very useful in all the 2D experiments, like HSQC and others. Whenever you want to do polarization transfer these modules are used. So, we will come back, understand this thing and continue further tomorrow.