Advanced NMR Techniques in Solution and Solid - State

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Module-23

Selective Saturation in homo and Heteronuclear Spin Systems Coupled and Decoupled INEPT

Lecture - 23

Welcome back, in the last class, we started discussing about polarisation transfer technique. I told you why we require polarisation transfer, it is to enhance the sensitivity of detection, especially for the low gamma nuclei or low nuclei like carbon 13 and nitrogen 15 which have very low natural abundance and also gamma is much, much less compared to that of proton. I said there are several ways of enhancing the sensitivity, like going to a higher magnetic field; Lowering the temperature; or labelling the low dilute spins like carbon 13 or nitrogen 15; or otherwise taking large quantitative samples. Some of them are practically feasible and some are not. Then I also said by manipulating the spin dynamics, it is possible to enhance the signal intensity. To understand that, we took two homonuclear coupled spin system and we adopted 2 strategies. One in which selectively one of the transitions of this 2 spin system, coupled spin system, was saturated; I made the population difference between one of the transitions equal, then we saw the effect of that, on the other transitions, there was a change in the intensity; that was one approach. In the other approach, what we did was, we did the population inversion of a particular transition. Instead of saturating, we inverted the population and ensured that the spins which are in the low energy level was raised to the high energy level and those which are in the high energy level was brought to low energy state. This time also we saw there is an enormous enhancement in the sensitivity of the signal. You saturate one of the particular transitions of a coupled spin, then you will see the enhancement in the signal in tensity of the other coupled spin it is quite interesting. And that was an example we took for 2 homonuclear spins. Now, today we will continue with heteronuclear spins and see how we can get the enhancement in the signal intensity; we will understand a bit more about it today.

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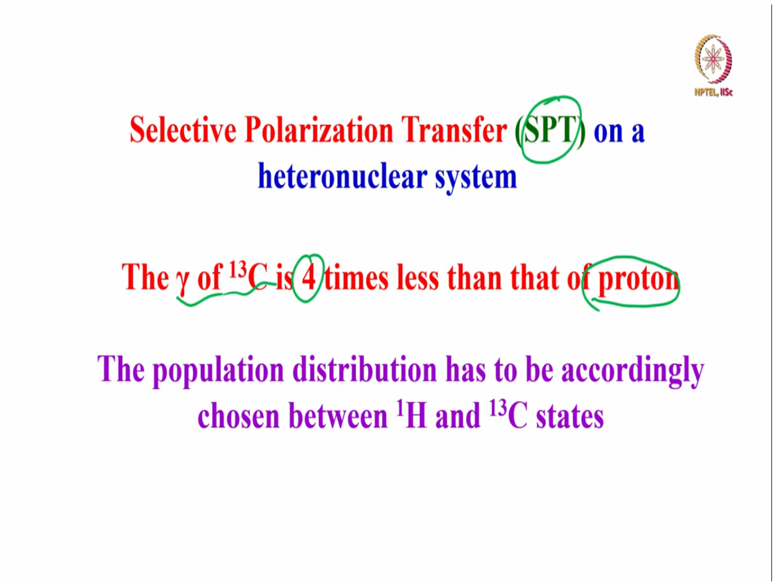

Selective Polarization Transfer (SPT) on a heteronuclear system

The γ of ^{13}C is 4 times less than that of proton

The population distribution has to be accordingly chosen between ^{1}H and ^{13}C states

So, we will start with selective polarisation transfer on a hetero-nuclear spin system SPT, I am going to do. I will take carbon 13 as an example. And polarisation transfer from proton to carbon 13. Proton of course, we all know, the gamma of the proton is 4 times larger than that of carbon 13. So as a result, what will happen is we can see that 4 times more population difference in the proton energy state,s compared to carbon 13 energy states.

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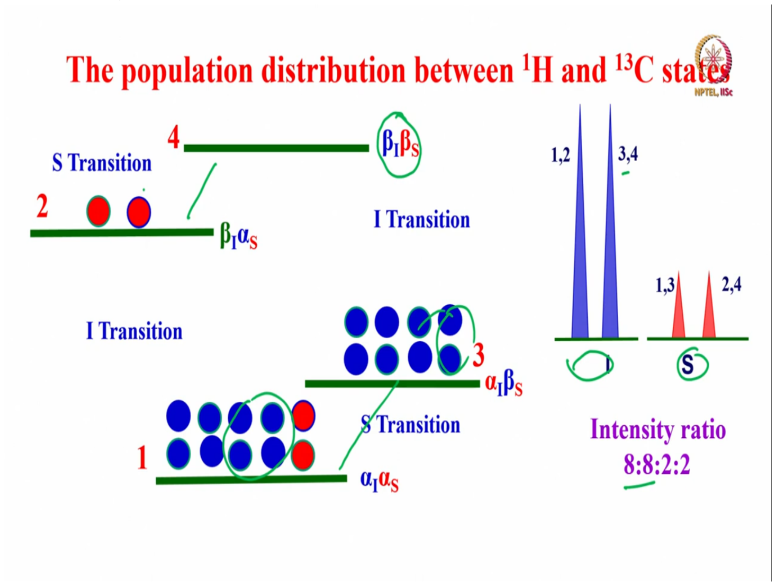

The population distribution between ^{1}H and ^{13}C states

Intensity ratio 8:8:2:2

With this idea, we will go back and see the distribution of population between 2 energy states of proton and carbon 13 each. I am going to distribute the population like this. The reason is I want to see that proton intensity is 4 times larger than the carbon 13, because of the gamma of proton is 4 times larger. So that is why I redistributed the population such that I have put 10 spins here and 8 spins here and then 2 here and 0. Again as I said, it is only for calibration purpose, do not ever be under the impression that in this state, there is no population at all, there is an enormous amount of population, only for calculation purpose I have redistributed like this. Now, with this, I calculate the energy of the transitions. Of course, you know what are proton transitions, proton transition is from 1 to 2, 8 - 2 will have 8 intensity. Similarly, you have proton transition between 3 to 4; see both are the proton transitions. Take the population difference, it is 8, 8 whereas if we look at the carbon 13 this is 2 and this difference is 2. So intensity 1 is to 4. This way I took the population like this. So the intensity of the transitions in the normal proton coupled carbon 13, 2 spin case, if I calculate the intensity, it will be 8 is to 8 is to 2 is to 2; or in other words, 4 is to 4 is to 1 is to 1.

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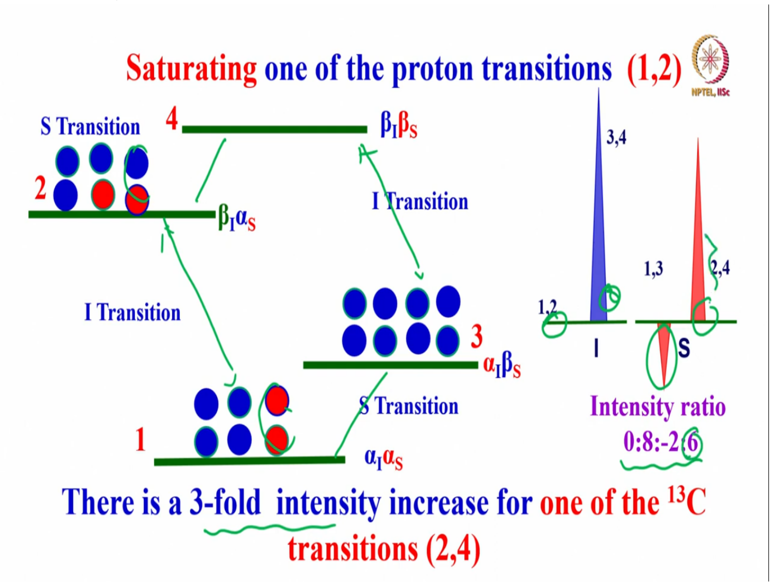

Saturating one of the proton transitions (1,2)

Intensity ratio
0:8:-2:6

There is a 3-fold intensity increase for one of the ^{13}C transitions (2,4)

Now what we will do is, like we did in the previous case we saturate one of the transitions; for example, 1 2 transitions. The transition between this and this, I transition, it is proton transition I am saturating; Once it is saturated, I make the spin population between these 2 states equal; populations are equal now. Now let us calculate the energy and population difference between the different energy states and the intensity of the peaks.

Now, 1 and 2 because I have saturated, its intensity is 0; There is no difference in the population. Now, take the 3 and 4, if you take 3 and 4 here, this is 8 - 0, this intensity is 8. Now, you go to 1 and 3, 1 and 3 of course, always I told you the population differences from lower energy state to higher energy state. So, going from 6 - 8 it is minus 2. On the other hand for 2 4, if you consider it is, 6.

So, the intensity the distribution is now, 0 is to 8 is to minus 2 is to 6. This is achieved by saturating only 1 of the proton transitions. So, there is a change in intensity that is what we understand. Now, look at this thing; one of the peak especially the transition 2 and 4 has gained nearly 3 fold in the intensity; it is 6; earlier it was 2. Remember 8 is to 8 is 2 is to 2 was intensity ratio, now, it has become 6. That means one of the transitions of the carbon 13 has become 3 times larger.

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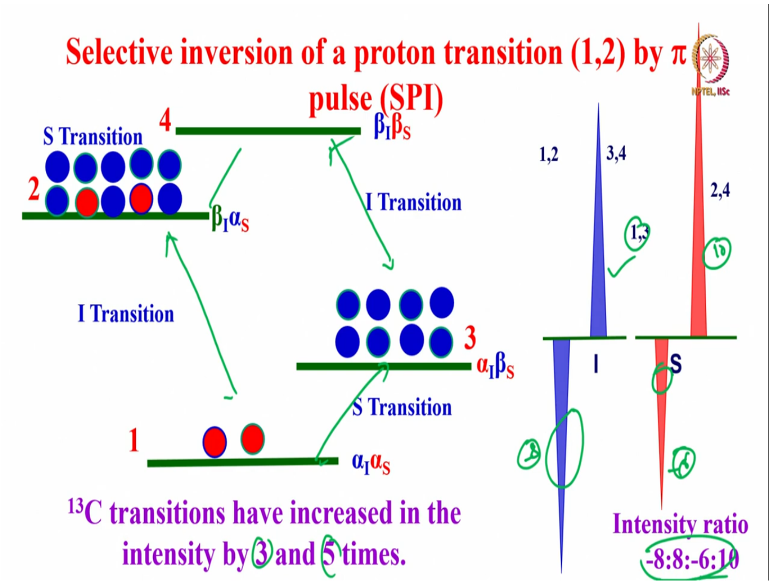

Selective inversion of a proton transition (1,2) by π pulse (SPI)

^{13}C transitions have increased in the intensity by 3 and 5 times.

Intensity ratio -8:8:-6:10

Same strategy we will adapt for selective polarisation inversion, instead of saturation. Let us see what happens if I invert the transition again, I am going to do the population inversion of transition between 1 and 2. Now, how did I do that? by applying pi pulse I inverted the population what happens when you do that, this one gets inverted, this peak gets inverted. Now, take the intensity difference between 0 and 10 it will be minus 8, this is minus 8.

Now, other I spin transition if you consider it is 3 to 4 it is 8, + 8. Look at the carbon 13 again a S spin; S spin of course, transition between 1, 3 if you consider, this one, this corresponds to 1, 3. The 1, 3 transition turns to be 2 – 8, - 6. Whereas this one, another one is, 2, 4, 2, 4 is 10. And there is enormous again in the intensity we saw for the carbon 13 spin just by doing the population inversion of one of the selected transitions of protons spin.

Now, the intensity ratio you see, it is minus 8 is to 8 is to minus 6 is to 10. And of course minus 6 is to 10, this being negative, we do not worry. We will worry about those things later. So, the carbon 13 transitions have increased in the intensity of the 2 components of the doublet, one with intensity increase by 3 times, other by 5 times. This is enormous intensity increase. So, we could transfer the polarisation from one spin to another spin to enhance intensity.

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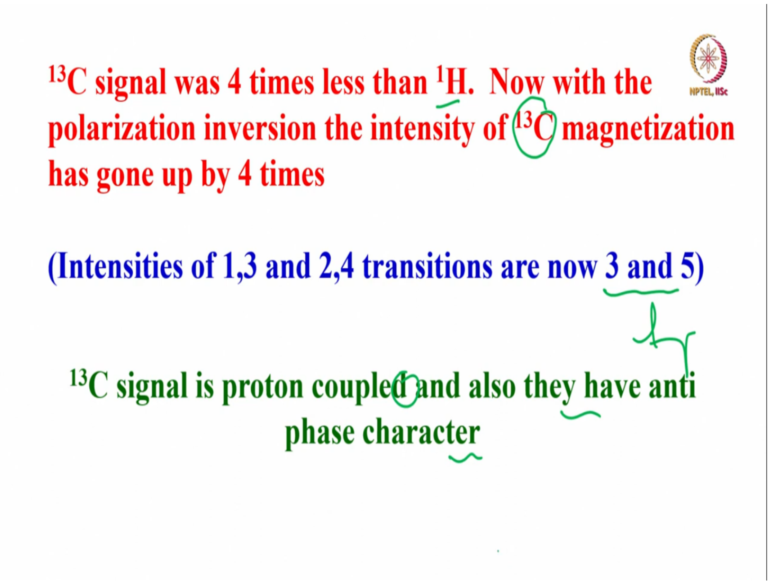

^{13}C signal was 4 times less than ^{1}H. Now with the polarization inversion the intensity of ^{13}C magnetization has gone up by 4 times

(Intensities of 1,3 and 2,4 transitions are now 3 and 5)

^{13}C signal is proton coupled and also they have anti phase character

So 13 C signal which is 4 times less than the proton. Now, with polarisation inversion intensity of the carbon 13 has gone up by four times. So, intensity of the transitions between 1, 3 and 2, 4 are now 3 and 5. It is telling that intensity has gone up nearly 4 times. The carbon 13 signal is proton coupled and also they have antiphase character. In the sense, we discuss this antiphase in phase absorptive, dispersive everything the last 1 or 2 classes. That is why I took a lot of time to explain what it is anti-phase doublet, that means one of them is absorptive other is dispersive; it will be alpha peak alpha component or beta component does not matter.

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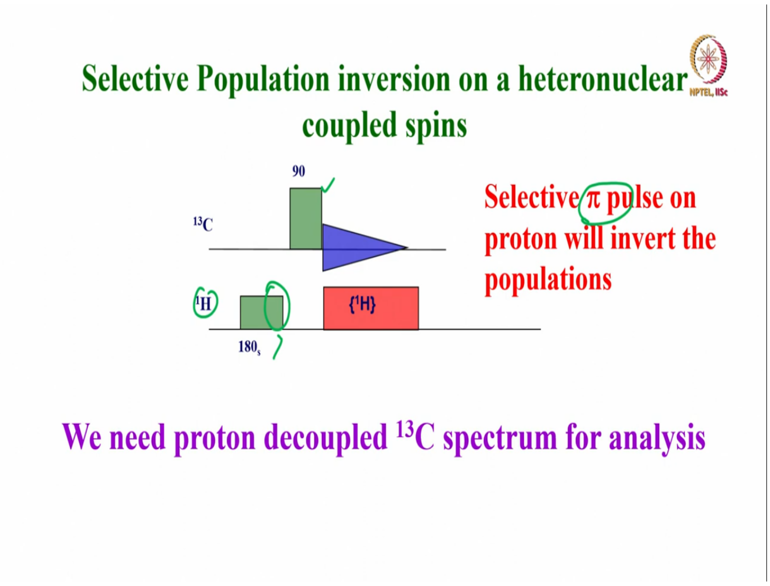

Selective Population inversion on a heteronuclear coupled spins

Selective π pulse on proton will invert the populations

We need proton decoupled ^{13}C spectrum for analysis

So, if do you select the population inversion on heteronuclear coupled spins. This is like this, this is simple pulse sequence; apply a carbon pulse, which is 180 pulse and then we apply a 90 degree hard pulse. And then this is a selective pulse; this is a proton; proton is a selective pulse. We apply for one of the transitions; it is a soft pulse, and this is pi pulse to invert only one of the populations and then you see the simple experiment we do and we are going to collect the signal.

But remember when I am detecting the carbon 13, I told you in the carbon 13 NMR. When I was analysing we normally do the broadband decoupling of the carbon 13; and detect the signal so that all couplings of the coupled carbon to all protons are broken. And you get a single peak for each chemically inequivalent carbon.

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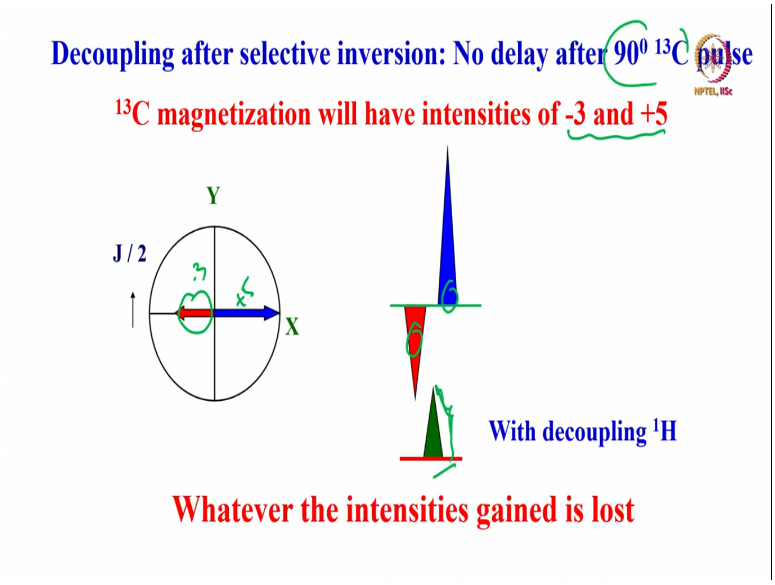

Decoupling after selective inversion: No delay after 90^0 ^{13}C pulse

^{13}C magnetization will have intensities of -3 and +5

With decoupling ^1H

Whatever the intensities gained is lost

So that is what we have to do. But if I do this decoupling immediately after the selective inversion without giving any delay after the 90 degree carbon 13 pulse, the magnetization intensities as we saw 1 is - 10 and other is plus 5, it is for carbon 13 doublet. So, that means, if we look at the vectorial diagram, this is their antiphase character, one is minus 3, this is plus 5, this is the intensity of carbon 13 doublet.

Now, if I start doing this one is negative intensity other is positive intensity, when you do the decoupling, in fact, what is going to happen you will get only 2 intensity. Minus 3 and plus 5, if you do the arithmetic you will find out it is only plus 2 with decoupling. In fact, whatever the intensity we gained, we have lost now, because of the antiphase character and minus 3 and 5. So, there is no point in doing this type of decoupling.

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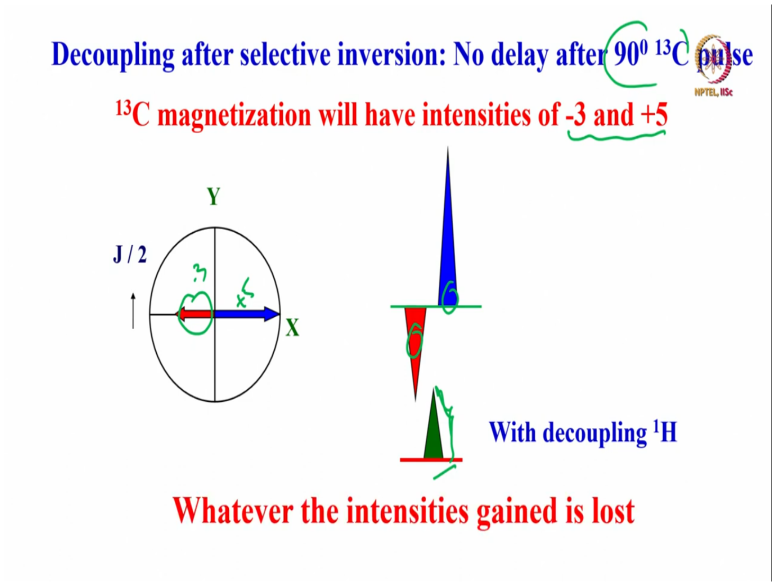

Decoupling after selective inversion: No delay after 90^0 ^{13}C pulse

^{13}C magnetization will have intensities of -3 and +5

Whatever the intensities gained is lost

So, then how do we overcome this intensity problem? There is one trick we can do. After this pulse sequence instead of collecting the signal immediately, let us give some additional time delay. This delay is 1 over 2 times J. We know the J coupling, calculate what is 1 over 2 J in seconds or in milliseconds or whatever it is; calculate the time and then give the delay and this time delay is exactly equal to 1 over 2 J.

So, I will do this after applying this pulse, apply a pi pulse on carbon 13 and then give a time delay of 1 over 2 J; and start collecting the signal, while doing the decoupling of protons simultaneously. See, I am going to decoupling and collecting the FID of carbon 13. Now, you see what is going to happen? instead of this, now, after the time delay, we are going to see the signal like this.

After 1 over 2 J what is going to happen is the components of this multiplet, this doublet component which are antiphase character, they will start coming back in the same direction. They will refocus here. Now instead of subtracting they add up.

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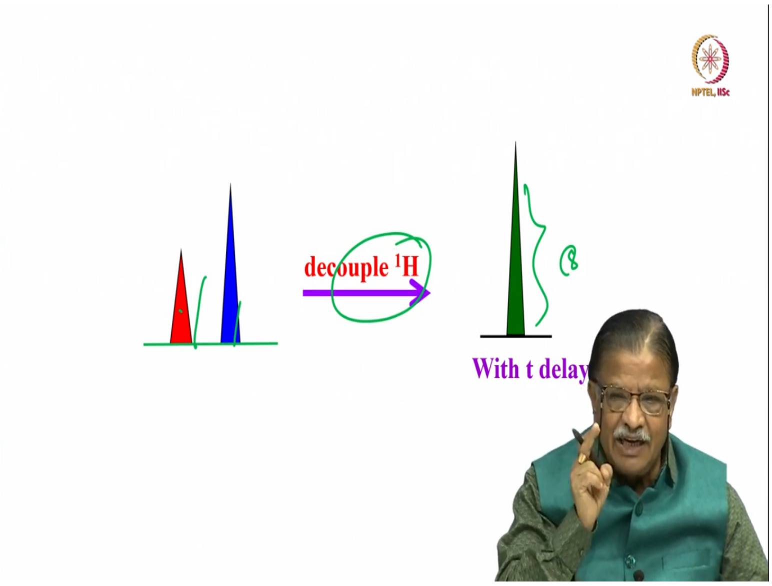

Now, if we do the decoupling, see because both are in phase doublets now, intensity is different, does not matter, but both are in phase doublet. Now I am going to do the decoupling; what is what happen? Now there is no nullification and intensity. Now, these 2 peak will collapse. And I am going to get a single peak with intensity 8; 3 + 5 value added up now, this is a trick. You added up immediately after the carbon 13 hard pulse.

If you start collecting the signal with the antiphase character the signal intensity gets reduced and, the effect of doing the polarisation transfer is nullified and other hand, give a time delay which is equal to 1 over 2 J.

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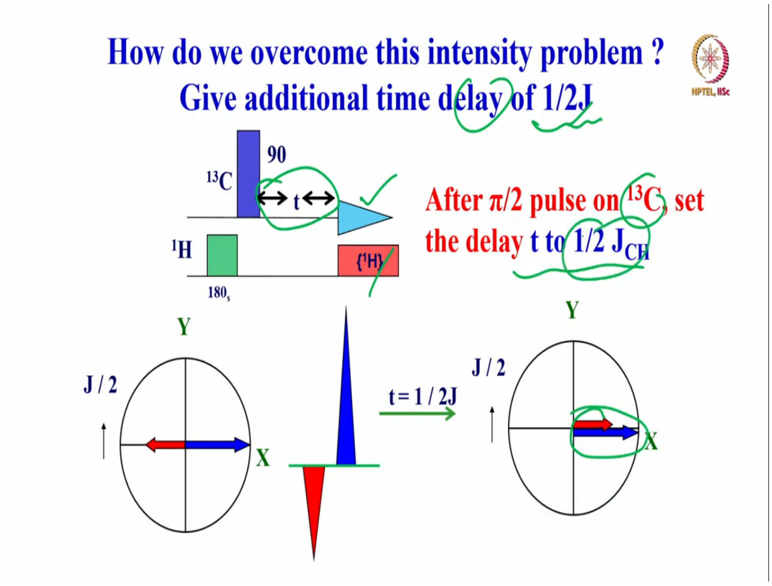

How do we overcome this intensity problem ?
Give additional time delay of 1/2J

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

1 over 2 J CH; Then what is going to happen is this anti-phase character become in phase character and do the decoupling get the single intensity.

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Insensitive Nuclei Enhancement by Polarization Transfer (INEPT)

This is a polarisation transfer technique and is always adapted in one of the techniques, very important called INEPT, INEPT experiment is called insensitive nuclear enhancement by polarisation transfer.

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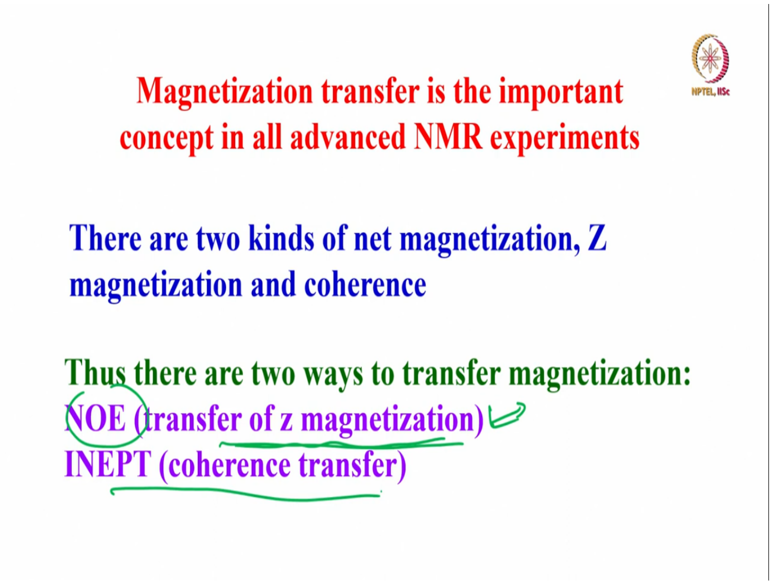

Magnetization transfer is the important concept in all advanced NMR experiments

There are two kinds of net magnetization, Z magnetization and coherence

Thus there are two ways to transfer magnetization:
NOE (transfer of z magnetization)
INEPT (coherence transfer)

This is routinely applied in a number of pulse sequences in all the advanced NMR experiments. And there are 2 kinds of net magnetization transfer; how do we transfer the magnetization. The magnetization of course, you know, the one magnetization is Z magnetization, which is in thermal equilibrium; other is a coherence, coherence you know, you bring the magnetization to the xy plane, it is coherence; and there are 2 types of magnetization. So, there are 2 ways of transferring the magnetization between the spins.

One is what is called NOE, it is transfer of magnetization Z magnetization between 2 spins. That is one way, we will not discuss that right now. We will see when there is a time, we will come back to NOE we will discuss; We have discussed the NOE in my previous course, but if there is a time I will touch upon it. Till this time in this course also otherwise, remember, NOE is the transfer of z magnetization, whereas INEPT is a coherence transfer, where the transfer of magnetization takes place in the xy plane.

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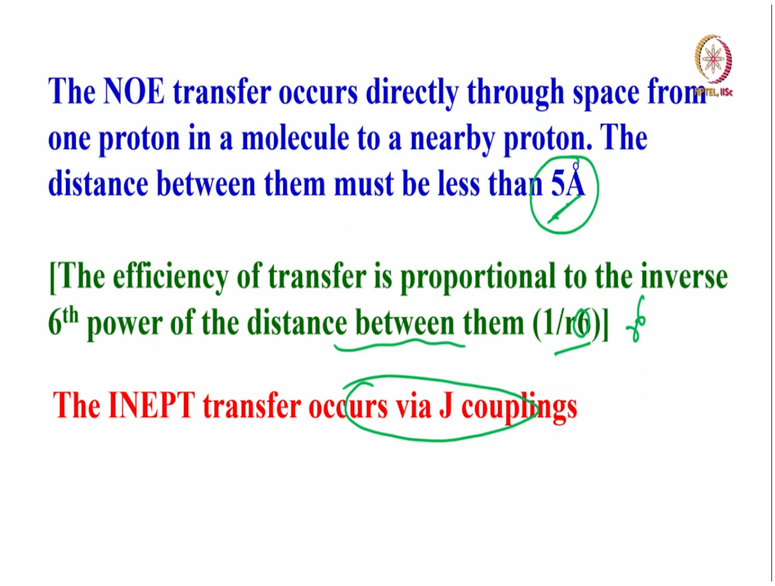

The NOE transfer occurs directly through space from one proton in a molecule to a nearby proton. The distance between them must be less than 5Å

[The efficiency of transfer is proportional to the inverse 6th power of the distance between them (1/r6)] -6

The INEPT transfer occurs via J couplings

So with this knowledge, now, what do we understand, NOE has a spatial proximity dependence, it depends upon how close the 2 nuclear spins are, if they are preferably within less than 5 armstrom there will be efficient transfer; efficient interaction and the polarisation transfer takes place, that is z magnetization, if they are very close in space. The efficiency of transfer is proportional to 6th power of the distance between them.

That means if the distance starts increasing, remember r to the power of 6. It is not r6, r to the power of 6 it is quite large. You know it is such a large value, small variation if it starts increasing the distance drastically, the efficiency of transfer comes down. It is 1 over r to the power of 6 dependence. And on the other hand in INEPT, what happens, the transfer occurs by using what is called J couplings. It is a covalent bond mediated coupling through that polarisation transfer takes place.

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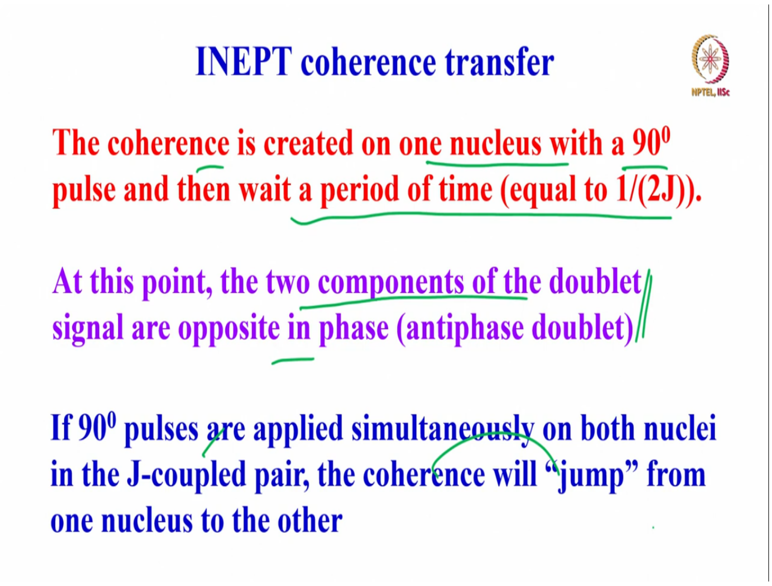

INEPT coherence transfer

The coherence is created on one nucleus with a 90^0 pulse and then wait a period of time (equal to 1/(2J)).

At this point, the two components of the doublet signal are opposite in phase (antiphase doublet)

If 90^0 pulses are applied simultaneously on both nuclei in the J-coupled pair, the coherence will "jump" from one nucleus to the other

Now, we understand what is the INEPT coherence transfer; and how does the INEPT coherence transfer work. Now, what we will do is we create the coherence of one of the nucleus with 90 degree pulse, and wait for a period of one over 2J; that is what we observed in the previous pulse sequence. Now at this point, 2 components of the doublet signals which are opposite in phase that we observed. And then if 90 degree pulses are applied simultaneously on both the nuclei in the J-coupled pair, the coherence will jump from one nucleus to other, it is very important point. See, there was an antiphase doublet actually, now what we can do is we will adapt, I will show you the pulse sequence, you apply the 90 degree pulses simultaneously on both proton and carbon, which are J coupled, then coherence transfer takes place between one to other.

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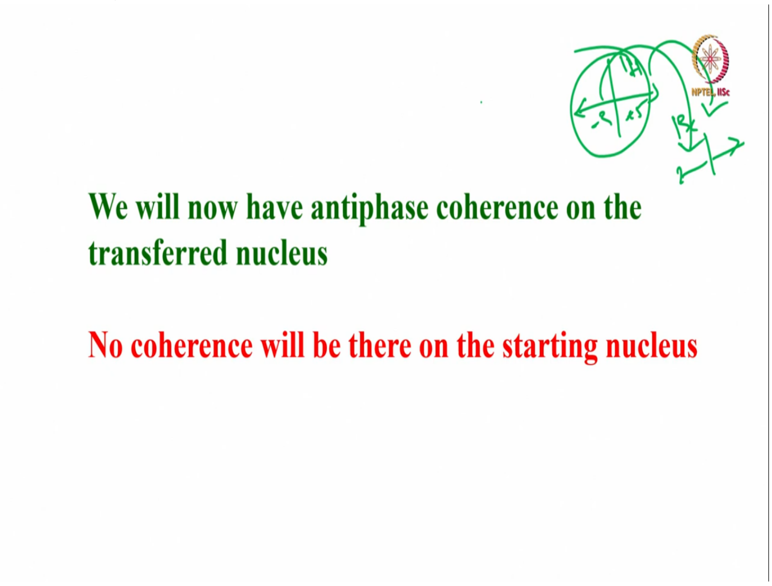

We will now have antiphase coherence on the transferred nucleus

No coherence will be there on the starting nucleus

So we will now have antiphase coherence on the transferred nucleus. Initially there is anti-phase coherence between, let us say minus 3 and plus 5, what we observed, let us say between protons nucleus, this should be transferred to antiphase character of carbon 13, the carbon 13 spins are now going to be antiphase. And this coherence completely gets transferred to this spin; and there would not be any coherence left on the starting nucleus, that is proton.

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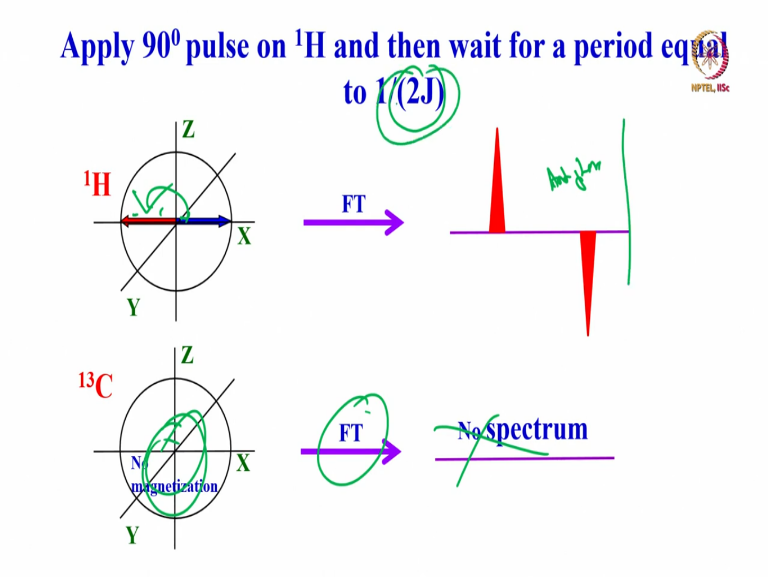

Apply 90^0 pulse on ^1H and then wait for a period equal to 1/(2J)

So, now what we will do is apply a 90 degree pulse on the proton, and then wait for a period equal to 1 over 2J. We apply proton 90 degree pulse bring the magnetization to xy plane. Now the 2 components of the J split multiplet, that is the doublet start moving and they attain an antiphase character like this, after 1 over 2J. This period is very precise, you cannot use any random value, if it is exactly 1 over 2J, you will see them in antiphase character.

So, there is no net magnetization, because they are antiphase; of course, in the carbon 13 there is no magnetization. We are not we are not doing anything with that, we are only focusing our attention on proton channel. So, now I will do the Fourier transformation of this; remember I applied pulse only on proton, not on carbon 13. For carbon 13 there is no transverse magnetization at all.

As a consequence, you do the Fourier transformation, what do you get? nothing, you do not get any signal. Whereas on the other hand, you are going to get the signal in proton. But remember we discussed a lot about this is antiphase doublet. This is the antiphase doublet, we discuss a lot about it and how the magnetization starts precessing in the XY plane. It depends on where you have the receiver. We discussed a lot in the last 2 classes. Now, it is going to be antiphase doublet; that is in the case of proton. So no spectrum for carbon 13; and on the other hand for the proton if you wait for 1 over 2 J, you get antiphase, charactered doublet.

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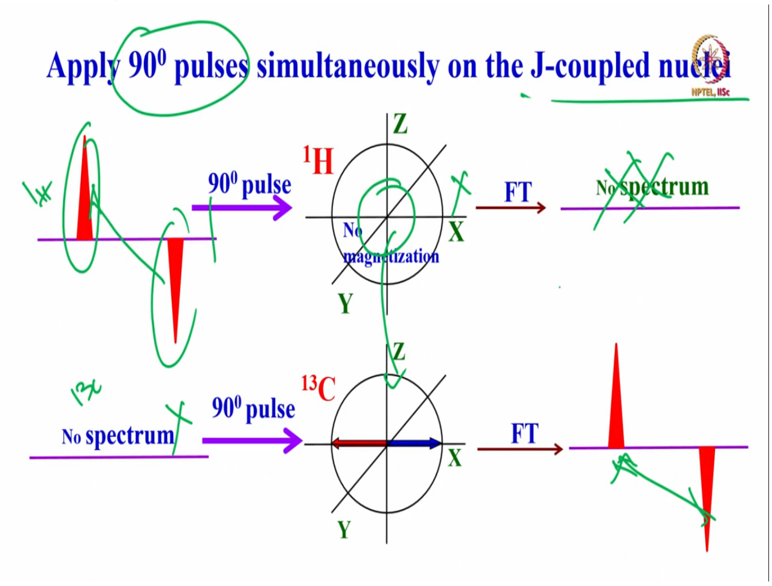

Apply 90^0 pulses simultaneously on the J-coupled nuclei

Now I am going to do one thing, I will adopt a trick. I will apply 90 degree pulses simultaneously on J-coupled nuclear, remember it is simultaneously applied. So earlier, we had this position, we are continuing from the place where we left previously; we had an antiphase doublet; the alpha component is positive adsorptive and beta component is negative adsorptive and there is no spectrum for the carbon 13; this is carbon 13 and this is proton.

Now I apply 90 degree pulse simultaneously, what is going to happen? There would not be any magnetization for the proton, and the same magnetization gets transferred to carbon 13. Very interesting thing is going to happen. In fact, we can see this when we do the product operator analysis. Now I am going to do the Fourier transformation, what will happen? There would not be any signal here; and this will become antiphase character.

What did we do? we transferred the magnetization of proton to carbon 13. The antiphase doublet of the proton, has now given its magnetization to carbon 13. The carbon 13 in turn became antiphase doublet; and there is no signal for proton, proton there is no spectrum at all here.

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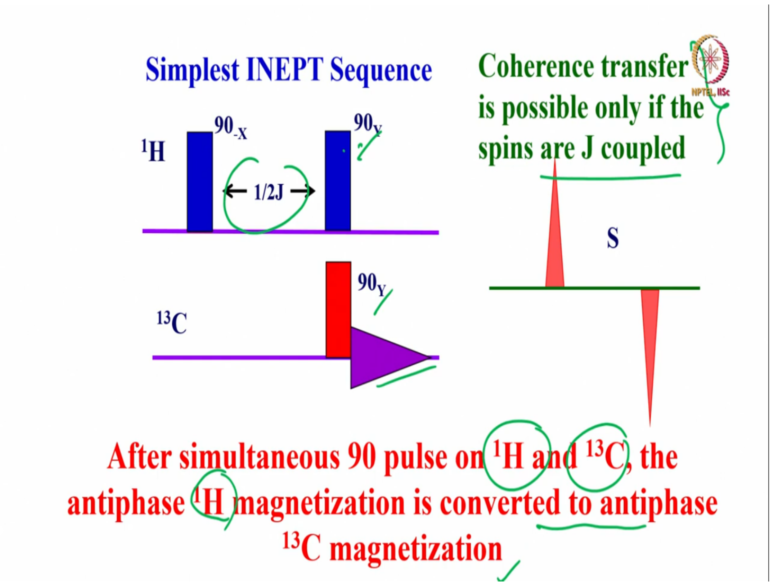

Simplest INEPT Sequence

Coherence transfer is possible only if the spins are J coupled

After simultaneous 90 pulse on ^1H and ^{13}C, the antiphase ^1H magnetization is converted to antiphase ^{13}C magnetization

This is what we did by simultaneously applying two 90 degree pulses on both the channels; on both the spins. Now we will look at a simplest INEPT sequence like this. In the simplest INEPT sequences, we can have a 90 degree pulse, give 1 over 2J delay, and then apply 90 degree pulses simultaneously one on proton and one on carbon 13; start collecting the signal. And of course, we have to do the decoupling, we will come to that later.

Now the coherence transfer is possible, only if the spins are J coupled. If there is no J coupling, it is not possible. This is because we saw that it is antiphase character of the doublet which is responsible for the transfer of magnetization; that means, it comes only because of the J coupled doublet. So, if there is no J coupling, there is no transfer of magnetization. The coherence transfer takes place only when 2 spins are J coupled. So, now the S spin has an antiphase character like this.

So, after simultaneous 90 degree pulse on proton and carbon 13, this is what we observed, the one antiphase proton magnetization get converted to antiphase carbon 13 magnetization. This is simplest INEPT sequence.

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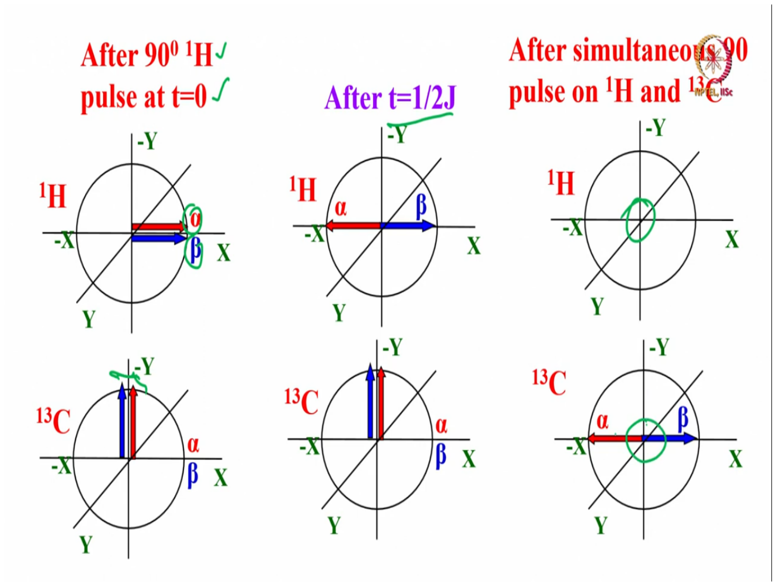

Now, as I told you, you will see how we are going to see the diagrammatically vectorial diagram how this this magnetization vectors start evolving. After 90 degree 1H pulse at time t = 0 both alpha and beta component of the doublet are along the X axis; because the thermal magnetization which was on Z axis, you brought them to the X axis by a 90 degree pulse; and at the same time carbon 13 spins are still aligned along that Z axis.

Carbon 13 we are not touching, we are not applying any pulse on that. So, both doublet components corresponding to coupling with proton which corresponds to the alpha and beta spin states of the proton. So, that doublet will still be along that axis. Now, I wait for time 1 over 2J. And this is going to be antiphase character, and this still continues to be along Z axis. That we have not touched at all. So now what I am going to do is after simultaneous 90 degree pulses on proton and carbon 13; we do not have any signal here. You got a complete signal transferred to carbon 13.

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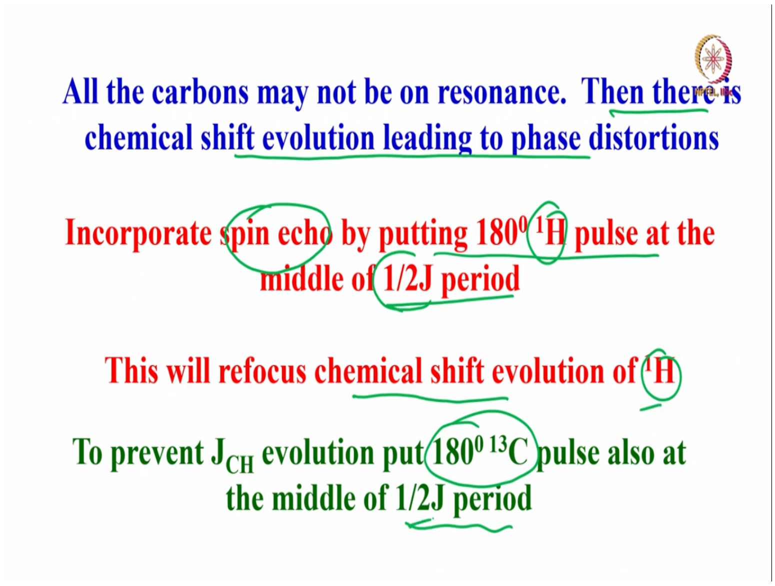

All the carbons may not be on resonance. Then there is chemical shift evolution leading to phase distortions

Incorporate spin echo by putting 180^0 ^1H pulse at the middle of 1/2J period

This will refocus chemical shift evolution of ^1H

To prevent J_{CH} evolution put 180^0 ^{13}C pulse also at the middle of 1/2J period

Now, all the carbons may not be on resonance. This is under the assumption all the carbons are on resonance and that is nu naught - nu R, the difference in the frequency from the centre of the spectrum with the resonance frequency of the peak, nu naught - nu R, is 0. That is our assumption, then only it is on resonance. One carbon we will like that, but there are so many chemical inequivalent carbons; all carbons may not be on resonance.

Then what will happen. Remember we discussed chemical shift evolution and J coupling evolution in the last 2 classes. The chemical shifts do not evolve when the spins are on resonance, whereas, the J coupling evolve during on resonance condition. But during off resonance I have explained to you, both chemical shifts and J couplings evolve. So now then, because of that, there is a chemical shift evolution of the spins, if some carbons or some spins are not on resonance.

So how do you avoid this one? how do you overcome this? For this, we adopt a trick called a spin echo sequence; by putting 180 degree pulse 1H pulse at the middle of I over 2J period. Please remember spin echo cause, we discussed a lot in the previous course itself, a lot of discussion took place on the spin echo. So I do not I have not want to touch up on that, those who are interested can go back to previous course.

Now this will refocus the chemical shifts, spin echo will refocus the chemical shifts of protons. Of course, in product operators I will also show you again how it happens. Now to prevent the JCH evolution, what we have to do is to put a 180 pulse on the carbon 13 also, at the middle of the 2J period. Now 2 things we are doing in the spin echo sequence, which is

90 tau 180 sequence, the 180 pulse we are applying simultaneously on the proton channel and also on the carbon 13 channel. On the proton channel we are going to apply to refocus the chemical shifts, but on the carbon 13 channel we are going to apply to prevent the evolution of JCH.

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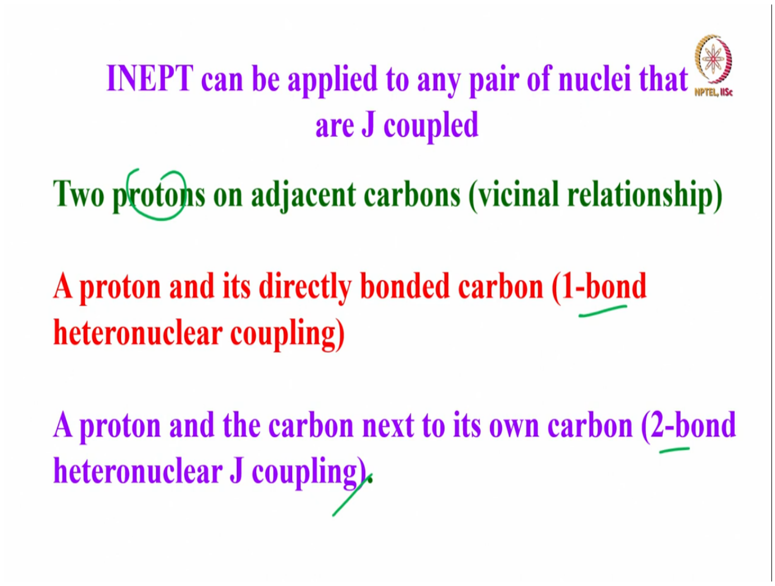

INEPT can be applied to any pair of nuclei that are J coupled

Two protons on adjacent carbons (vicinal relationship)

A proton and its directly bonded carbon (1-bond heteronuclear coupling)

A proton and the carbon next to its own carbon (2-bond heteronuclear J coupling).

That is the thing we should remember. Now INEPT can also be applied to any pair of nuclei that or J coupled. It need not be heteronuclear also; it can between any two nuclei, it can be between two protons, which are on adjacent carbons, that is the vicinal coupling or 1 bond heteronuclear coupling, or 2 bond heteronuclear coupling, anything you consider, you can try.

So, INEPT is only a polarisation transfer between the two J coupled spins. We did not say it should be heteronuclear; it can be homonuclear or it can be heteronuclear. So long as there is a coupling the antiphase doublets of the coupled partner, if there is a J coupling, the polarisation transfer takes place from one spin to another spin. And that spin will develop antiphase character of the doublet.

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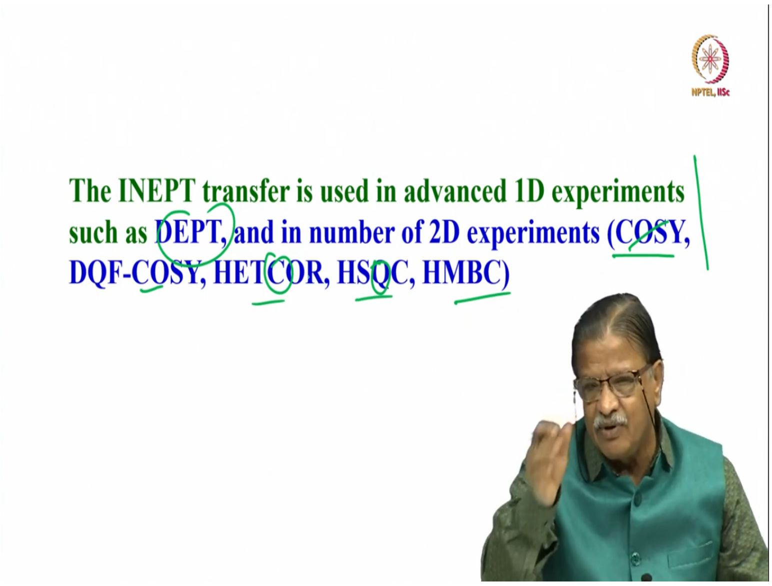

The INEPT transfer is used in advanced 1D experiments such as DEPT, and in number of 2D experiments (COSY, DQF-COSY, HETCOR, HSQC, HMBC)

So, INEPT transfer is used most of the time in many advanced 1D experiments, like DEPT and for example, HETCOR, HSQC, HMBC. All these experiments, and in many of the 2D experiments INEPT sequence is always utilised, because when we are doing the heteronuclear experiment like heteronuclear correlation or HSQC we are detecting in 1 dimension carbon 13. I have not interested in 2D. I will come to that later. 2D we discussed a lot in the previous course, I will just touch upon in this course also. In the 2D what we do is if in one dimension we have carbon 13 and in other dimension we have proton; there can be a correlation. But then carbon 13 if you detect in indirect dimension the sensitivity will be very, very low. We need to enhance the signal intensity; then your experiment can be speeded up. For that we do the polarisation transfer, and transfer the signal from proton to carbon 13 in all these experiments we can do.

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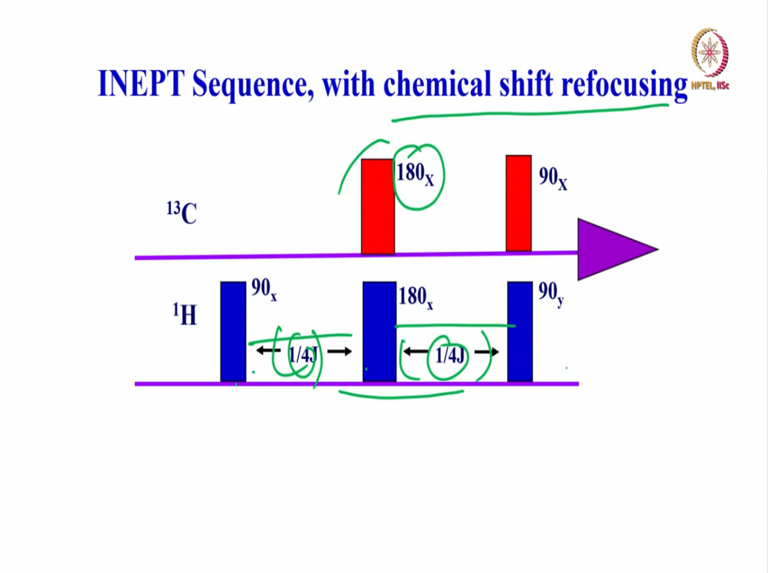

INEPT Sequence, with chemical shift refocusing

So, now, we can do this INEPT sequence. We will see how it is; but with chemical shift refocusing. What did I say for chemical shift refocusing, we need to apply a 180 pulse on the proton channel and also on the carbon 13 channel to prevent the evolution of J coupling there. So, now, this is the sequence; up to this we had already discussed, up to this; But then this is the addition; this 180-tau-90 -tau-is a spin echo sequence.

This is spin echo sequence, and between these 2 delays it is 1 over 4 J. So, simple INEPT sequence has one 180 pulse on the carbon 13 and a 90 pulse followed by the hard 90 pulse; and then on proton channel we have a spin echo 90, 180, 90 and the delay between 90 to 180 and 180 to 90 is 1 over 4J, exactly should be 1 over 4J. Earlier 1 over 2J was there, we divided it into 2 and applied a 180 pulse at the centre.

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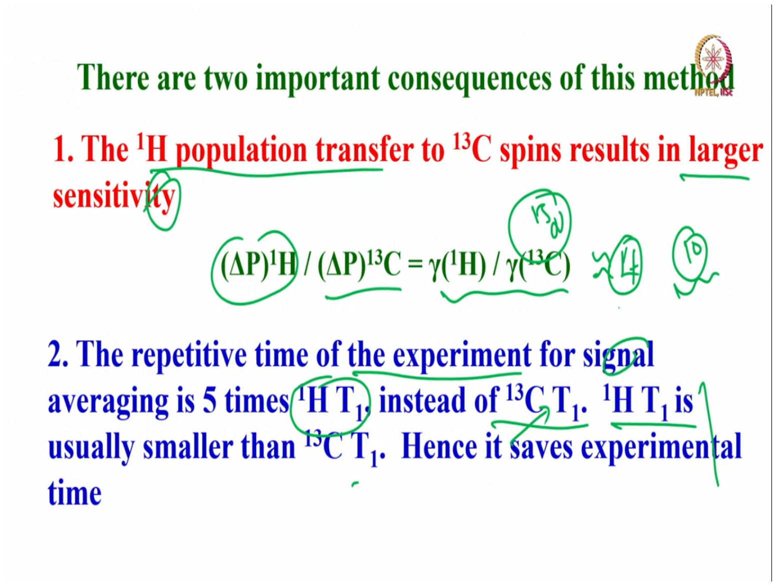

There are two important consequences of this method

1. The ^1H population transfer to ^{13}C spins results in larger sensitivity

$$(\Delta P)^1H / (\Delta P)^{13}C = \gamma(^1H) / \gamma(^{13}C) \approx 4 \quad 10$$

2. The repetitive time of the experiment for signal averaging is 5 times ^1H T_1, instead of ^{13}C T_1. ^1H T_1 is usually smaller than ^{13}C T_1. Hence it saves experimental time

Now there are 2 important consequences of this method. One is the proton population transfer to carbon 13 results in larger sensitivity; there is one thing; very large sensitivity we are going to get, because proton is 4 times larger gamma, the magnetization transfer is more. So, we can look at the population of proton; population difference of proton with respect to that of carbon; it goes by the ratio of the gamma; the ratio of gamma between proton and carbon is approximately equal to 4.

There is 4 times enhancement in the signal intensity, because of population transfer. And another important concept is the repetitive time of the experiment, which we call as in the experimental aspects we discussed in the previous course I told you we need to do 5 times the delay to see that signal intensities are always all uniform, because you have to find out what is the longest which new spin particularly spin is a lot longer T1 5 times that the T 1 you have to give the delay between the successive pulses. In which case you will get all signals of nearly equal intensity. And that means, in the case of this experiment, what we have to do is the repetitive experiment with the signal averaging when you want to do you have to give the delay of 5 times to protons T1 and not 5 times the carbons T1; it is the advantage. I tell you carbon 13, and such nuclei have large T1 compared to that of proton.

So the proton T1 is usually much, much smaller and hence the experiment is speeded up. These are the two important advantages; signal intensity gain of 4 times because of proton carbon gamma ratio, and at the same time the experiment is speeding up. If instead of proton carbon, if I take proton and nitrogen, what will happen? Nitrogen gamma is 10 times lower, that means the sensitivity will get enhanced by 10 times. When the ratio becomes larger and larger, that is the amount of enhancement in the signal intensity; eg. It is 4 times for 13C, and for nitrogen 15 it is 10 times.

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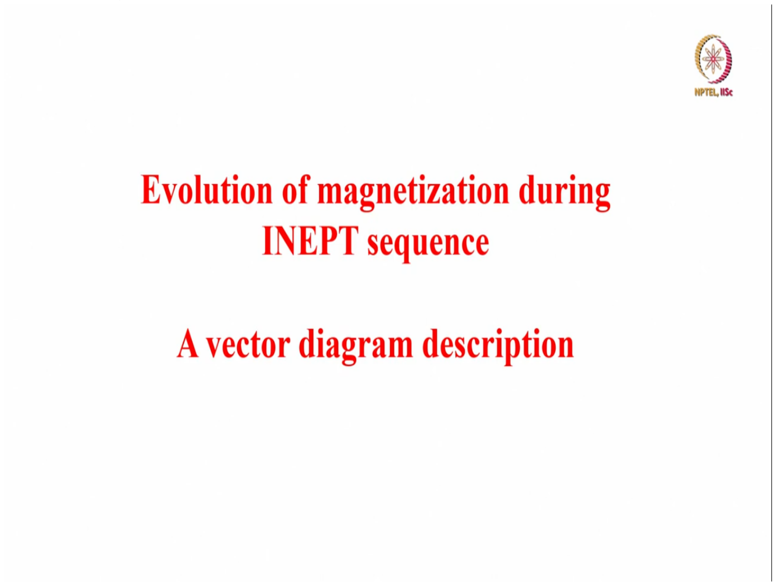

NPTEL, IISc

Evolution of magnetization during
INEPT sequence

A vector diagram description

So now the evolution of the magnetization during INEPT sequence, you can see by the vector diagram description. Now time is getting over. What I will going to do is, this evolution of magnetization during INEPT sequence we will discuss in the next class. But what we discussed today is, as of today, we knew that the polarisation transfer is possible by selectively saturating one of the transitions of the proton.

We consider the heteronuclear case, we found out what is the gain in the signal intensity especially when one of the proton transitions is selectively saturated. That means make the population between these 2 energy states, proton states, which is gives the proton signal equal. Then we calculated the population difference between different peaks, different energy states giving rise to different peaks, we found there is enormous gain in the intensity.

The same thing can be done with the pi pulse where we can do selective inversion of a particular transition. Again, proton transition we saw nearly about 4 times enhancement in signal intensity and of course they were antiphase character minus 3 and plus 5 was the intensity. And then we also found out the problem is there, in sense if we try to do the decouple of these antiphase charactered peaks, all the gain what we got gets nullified, and we are going to get only the same intensity of 2. That is not a good strategy. What we understood instead of that, immediately after collecting the signal by decoupling, why not we give a time delay after the pulse and then start collecting the signal, and that delay should be exactly 1 over 2J, between carbon and proton. We took the example of carbon and proton.

In which case what is going to happen the antiphase character become in phase character, the both of the components of the vectors, minus 3 and plus 5 intensity, come to one direction, they refocus on one axis, and the intensity will add up. Now, if we do the decoupling, instead of getting nullified, you will get gain in the intensity. So, that is what we saw, ratio of proton and carbon 4 gamma is times and that is the signal intensitu, we saw.

And this can be adapted for the sequence called INEPT; we saw that in INEPT, again the intensity if you have antiphase character, it gets nullified. For that I said we can overcome this by using what we call a spin echo sequence, where we are going to apply a 180 pulse at the centre of the proton channel and also at the centre of the carbon 13 channel. In which case what is going to happen is in the carbon 13 channel, if you apply 180 it will prevent the evolution of J coupling, whereas, in the case the proton, you are going to ensure that you will get in phase character for this one. And the chemical shift evolution can be prevented. So, finally, what you are going to do is, you are going to get a spectrum with both the components of the vector aligned along one axis and you are going to get the enhanced signal intensity. This INEPT block can be utilised in number of experiments, especially 2D experiment like HSQC, HMBC variety of heteronuclear experiments, where the polarisation transfer is very, very important. And then how this thing works, I wanted to discuss with the vector diagram. That we will do in the next class. So thank you, we will stop here.