# Advanced NMR Techniques in Solution and Solid - State

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## Module-24
## INEPT and DEPT
## Lecture - 24

Welcome back all of you. In the last class, we discussed about polarization transfer technique, especially hetero-nuclear coupled spin system starting from the basics, we understood what happens if there is a selective saturation of a particular transition; like I take a particular transition and selectively I saturate that and make the population between these 2 energy states equal. Then we saw especially if you do it for the protons transition, we saw enormous change in intensity of the carbon 13.

Same thing we observed when you do the population inversion also. Of course, this is analogous even to the homonuclear case also we observed. And then we found out when this thing happened, the intensity of the carbon 13, J spin doublet, they will be anti-phase character; One will be with minus 3 intensity and the other is plus 5. Now, immediately after the polarization transfer takes place, we design an experiment, take the proton channel, apply a soft pulse for selective excitation. Then afterwards I also applied carbon 13 hard pulse and started collecting the signal. And if you want to do the decoupling, we saw that the anti-phase character of the carbon 13 doublet, that minus 3 and plus 5 intensity peaks will ensure that whatever the gain we got because of polarization transfer gets nullified. So, that is not a happy situation. What we wanted to do is to overcome this situation, we wanted to introduce what is called a spin echo like sequence, where we are going to put two 180 pulses; one on the carbon 13 channel, one on the proton channel simultaneously. So, this will prevent the evolution of the J coupling of the carbon 13. So, this is the advantage of that also and at the same time we also saw that when you do experiment with spin echo like sequence, this antiphase doublet will become in phase doublet, especially, when we apply 180 exactly at the centre, and the delay between these two is adjusted such that it is equal to 1 over 4J. The delay between the first pulse and 180 and the second delay between 180 and another pulse should be exactly equal to 1 over 4 J. Then, we saw that, if that is the situation, in this experiment, the anti-phase character of the doublet became in-phase and it is possible to do the decoupling; it is a very fantastic thing. So, we can incorporate this block in varieties of heteronuclear detected 2D experiments. To understand that pictorially this what I wanted to discuss today.

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Evolution of magnetization during INEPT sequence

A vector diagram description

Let us see the evolution of the magnetization how it is happening during the INEPT sequence, just by vector diagram. We will come to this thing in the product operator later.

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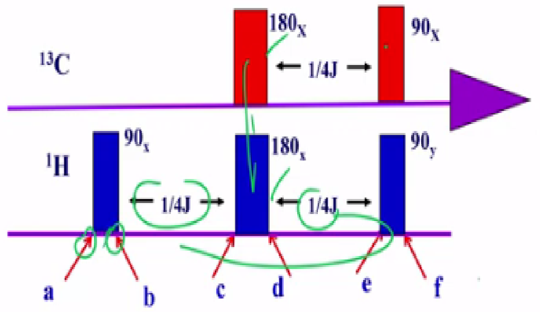
INEPT Sequence, $^{13}$C-$^{1}$H coupled spins



The evolution of magnetization at different can be described by vector diagram

This is the INEPT sequence which we discussed so far.  We give 1 over 4J delay after the proton pulse and two 180 pulses are applied, one on carbon 13, one on proton and then we apply 90 pulse. This is this echo sequence, and then you apply detective pulse here, on the carbon and then start the decoupling. This is an very simple experiment. What is going to happen in this INEPT sequence? Of course, INEPT is also discussed in the previous course. Those want to get more details can refer to that. The evolution of magnetization at the different time of the pulse sequence can be understood. I have identified at different stages of the pulse sequences a, b, c, d, e, f.

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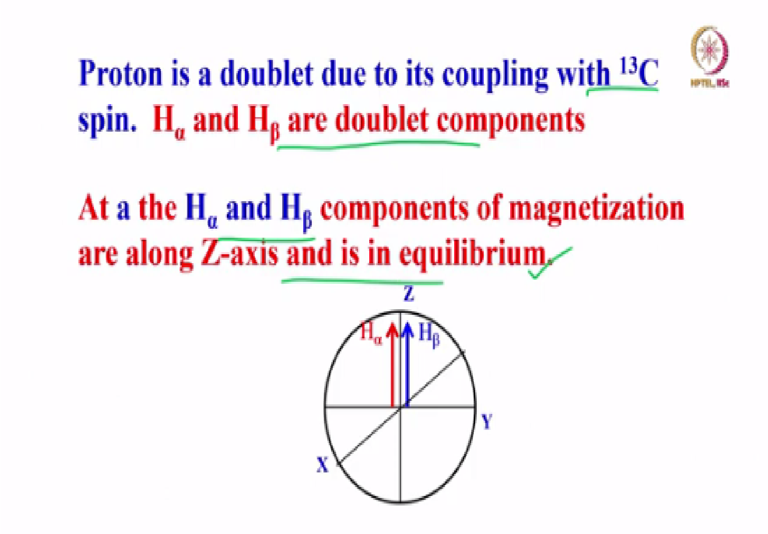

Proton is a doublet due to its coupling with $^{13}C$ spin. $H_\alpha$ and $H_\beta$ are doublet components

At a the $H_\alpha$ and $H_\beta$ components of magnetization are along Z-axis and is in equilibrium.

Let us see vectorially how the magnetization behaves at these different points. The proton is the doublet due to its coupling with carbon 13; and H alpha and H beta are the doublet components. At a, H alpha and H beta components of the magnetization are along Z axis and is in equilibrium, especially at a. It means here, now nothing has been done; we have not even applied the pulse. So, magnetization is in equilibrium; that is correct, this what it is. So, both the components of the doublet are along the Z axis.

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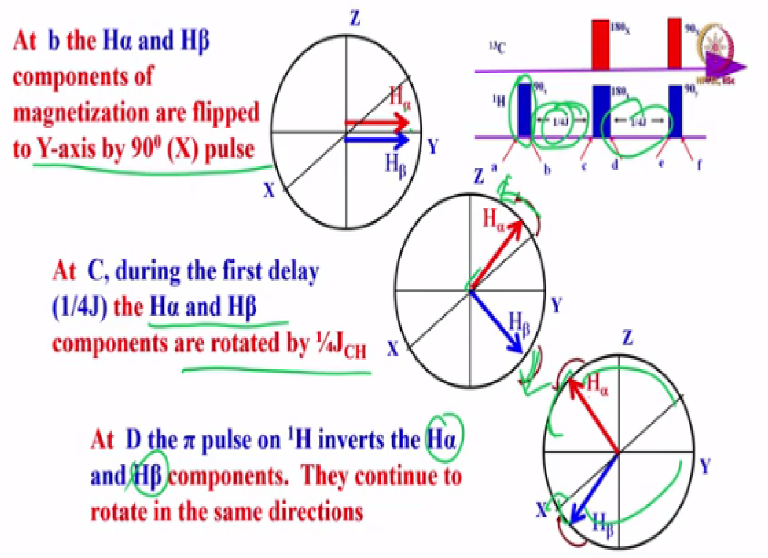

At b the Hα and Hβ components of magnetization are flipped to Y-axis by $90^0$ (X) pulse

At C, during the first delay (1/4J) the Hα and Hβ components are rotated by $\frac{1}{4}J_{CH}$

At D the $\pi$ pulse on $^1H$ inverts the Hα and Hβ components. They continue to rotate in the same directions

So, now, what we are going to do? Let us see at b, what is going to happen, we applied 90 degree hard pulse on the proton channel. And when you applied the 90 degree pulse, what is going to happen? The magnetization flipped to Y axis, because it is a 90 degree X pulse, it flipped to Y axis. We have got alpha and beta component of this, flipped here. So that is immediately after 90 degree pulse, that is what is going to happen.

Now, we have a time t ,during this time 1 over t, 1 over 4J, the spin starts evolving. We discuss about the evolution of J coupling and then the chemical shift. What happens? How does it evolve with the delay, everything we discussed, remember in last 2 classes. Now, at C what will happen? The C delay is exactly 1 over 4J is given; and in the first delay H alpha and H beta components are rotated by 1 over 4J.

Remember just 1 over 4J means H alpha is going to be like this, and the other is like this. We are going to call this one as fast moving, and other as the slow moving components. One starts moving like this, other starts moving like this, at 1 over 4J. Now, what is going to happen? immediately after that, we are going to apply 180 Pulse. 180 pulse on both proton channel and carbon channel, we are applying. First let us see what happens if you apply proton channel 180 Pulse. What does 180 pulse does?

We have been discussing this for quite some time. It inverts the populations, means it will invert alpha and beta components of the doublet; 180 pulse what it does? It inverts the population that means alpha and beta states gets inverted. And now what is going to happen to Alpha and beta components? alpha came here and beta came here; and they continue to move in the same direction. Then what is going to happen? We wait for another 4J after another 4J this has to refocus.

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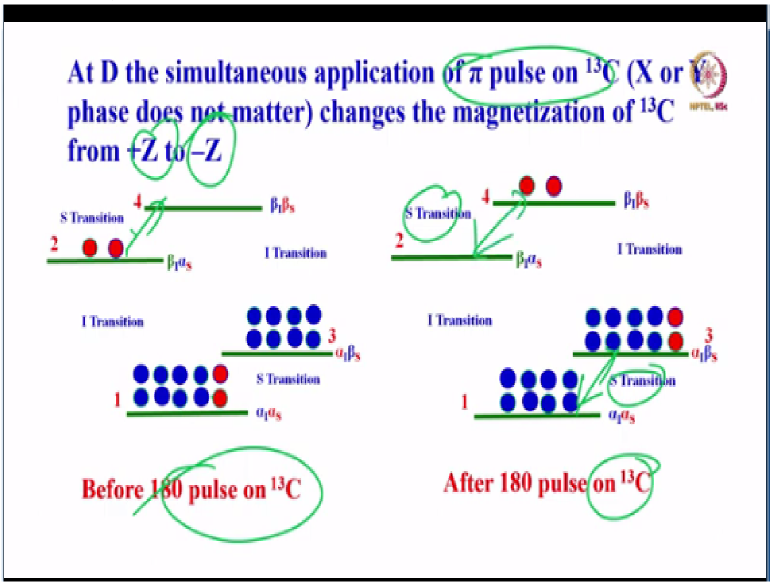

At D the simultaneous application of $\pi$ pulse on $^{13}C$ (X or Y phase does not matter) changes the magnetization of $^{13}C$ from +Z to –Z

We will come to that, before that let us see what is going to happen? We are also applying the pi pulse on carbon 13. It does not matter whether you are going to apply X pulse or Y pulse. And now what is going to happen? A carbon 13 pi pulse, inverts the magnetization of carbon 13, see carbon 13 is still along Z axis. We are not touching that. In the pulse sequence till now we are dealing with only the proton pulse. Please remember, in the pulse sequence here, we are up to this, carbon 13 we have not even touched. We are only discussing this, only the proton pulse, proton 90 delay and this thing. Now, if we apply carbon 13, here still magnetization along Z axis. So, what is going to happen? It will invert the population, the spins which are along Z will come to minus Z this what happens? So now I am inverting the population. This is before applying the carbon 13, 180 pulse; this is the population state, that is what we are understanding, how the population can be distributed between different energy states. This was the thing we started with before doing anything. Now after 180 degree pulse population the carbon 13 gets inverted, which are the carbon 13 transitions; this the S transition, this is the S transition.

See now what is happening 10 and 8 were there; these get interchanged 2 and 0 was here and this get interchanged. Population of the carbon 13 spins from plus Z became minus Z, and it undergoes inversion of the populations between 2 spin states.

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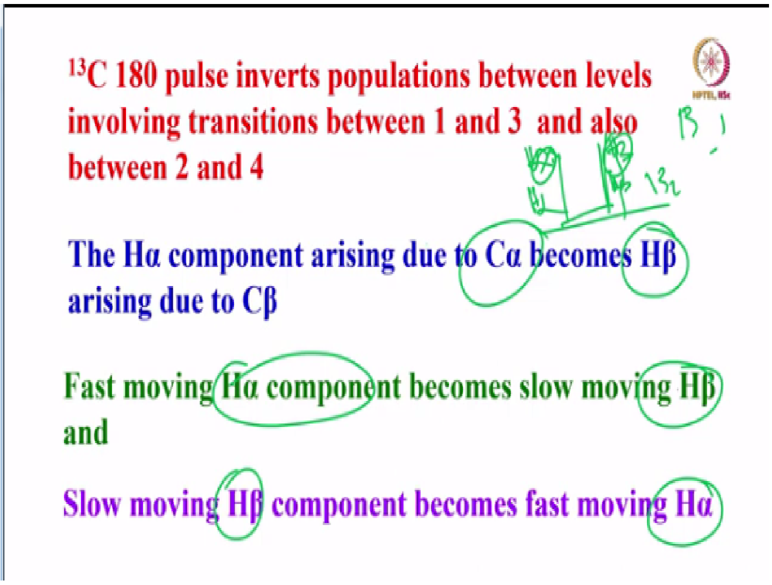

$^{13}C$ 180 pulse inverts populations between levels involving transitions between 1 and 3 and also between 2 and 4

The Hα component arising due to Cα becomes Hβ arising due to Cβ

Fast moving Hα component becomes slow moving Hβ and

Slow moving Hβ component becomes fast moving Hα

That is what the 180 pulse of carbon 13 do. So, when it inverts the levels what is going to happen? H alpha component arise because of C alpha component becomes now H beta. If you consider the doublet of the carbon 13, one component correspond to carbon 13 which sees protons in the H alpha state, this one sees the proton in the beta state; that is what the doublet before applying the 180 pulse.

Why the doublet comes the J coupling we have already discussed a lot. The doublet comes because this carbon 13 sees proton into 2 spin states of alpha and beta. So, now one of the components doublet correspond to proton in the alpha state, this is called the H alpha, this is proton in the beta state this corresponds to H beta they call corresponds to H alpha and H beta state corresponds to I get doublet for carbon 13.

So, now H alpha component arise due to the C alpha becomes H beta and similarly H beta another one, becomes H alpha; that is H alpha becomes H beta, H beta becomes H alpha. So, that means fast moving component becomes slow moving; and slow moving component becomes fast moving when we interchange these two. This is what going to happen. The application of 180 pulse does something like this on carbon 13, because the magnetization changed from plus Z to minus Z; the doublet component which is arising because of C alpha and C beta, now H alpha component becomes C alpha. H alpha arising to C alpha becomes H beta and similarly H beta becomes C alpha. Now, for slow moving and fast moving components it gets completely interchanged.

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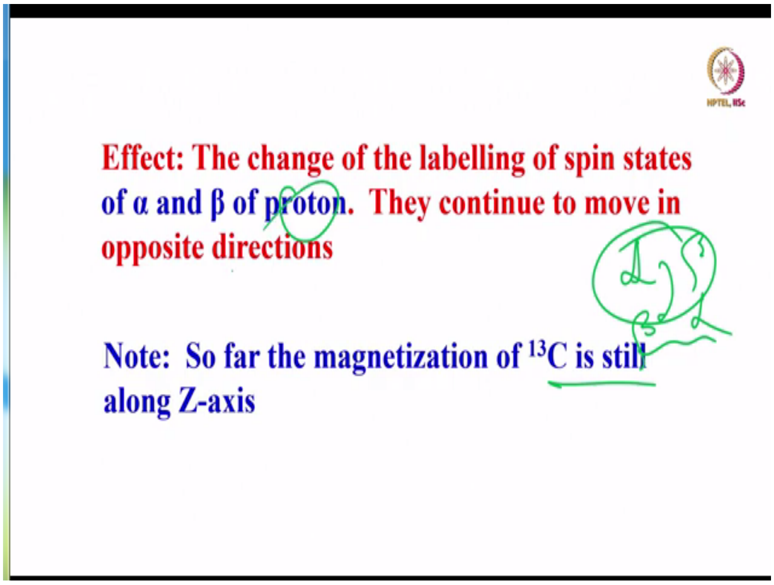

Effect: The change of the labelling of spin states of α and β of proton. They continue to move in opposite directions

Note: So far the magnetization of $^{13}C$ is still along Z-axis

What is the effect of that now? It means the labelling of the spin states of alpha and beta proton change. If I say this is the alpha state of proton, this is the beta state; now they got interchanged. Now this become beta state and this became alpha state. So now what will happen? They continue to move in the opposite direction. Earlier, if we apply proton 180 just it changes from one axis to other axis, just inverted that; but we did not invert the population it will continue to move in the same direction. But now, because of the carbon 13, alpha has become beta, beta has become alpha and they start moving in the opposite direction. That is what going to happen. So, far carbon 13 which was still along the Z axis but we only apply 180 pulse and then brought from plus Z to minus Z. But that effect is seen on the proton; very interesting thing, how alpha and beta component of the proton got interchanged because of the application 180 pulse on carbon 13.

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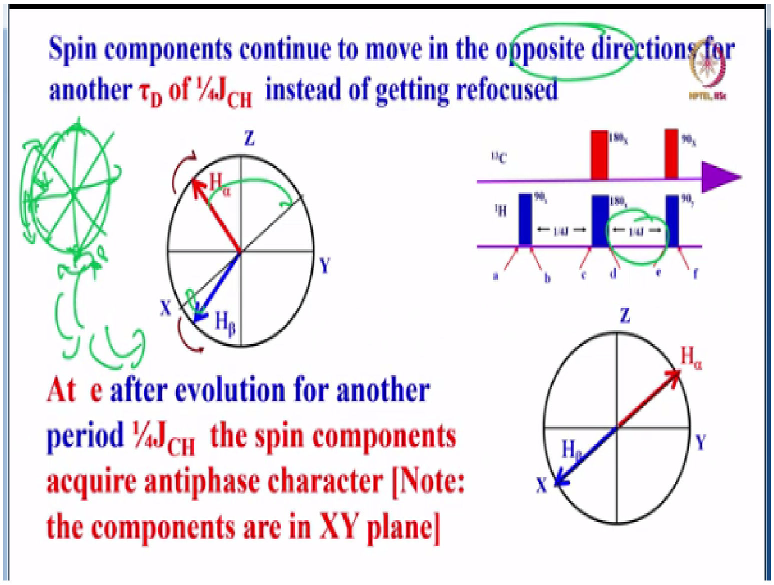

Spin components continue to move in the opposite directions for another $\tau_D$ of ¼$J_{CH}$ instead of getting refocused

At e after evolution for another period ¼$J_{CH}$ the spin components acquire antiphase character [Note: the components are in XY plane]

This is what it is now, spins continue to move in the opposite direction, because of interchange of alpha and beta components. Now, wait for the time another time which is equal to 1 over 4J. Now, what is going to happen? They start moving opposite like this, see very interesting, you must understand, we started like this, after 1 over 4J it was like this, and then we applied the proton 180 this came like this and this came here. So, they were like this and started moving in the same direction.

But now, carbon 13 180 pulse did something for this, this came here, this came here. This was H alpha let us say this was H beta now they get interchanged. So, because they interchanged they started moving in the opposite direction. So, now, what has happened instead of both moving this direction, this starts moving this way, this starts moving this way.

Very interesting thing is going to happen, at e after evolution 1 over 4J. The spin components again acquire antiphase character at H alpha H beta, again this will come here and this will come here. So, they get antiphase character. But remember, the spin components of the H alpha and H beta are still in the XY plane. Now exactly after this delay, we have ensured the spins become antiphase in character; the doublet component become antiphase in character.

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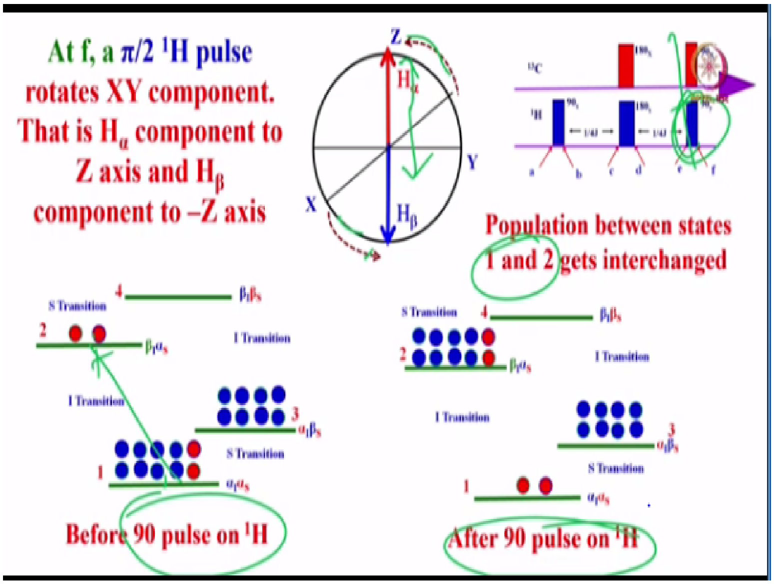

At f, a $\pi/2$ $^1H$ pulse rotates XY component. That is $H_\alpha$ component to Z axis and $H_\beta$ component to –Z axis

Population between states 1 and 2 gets interchanged

Before 90 pulse on $^1H$

After 90 pulse on $^1H$

Now, what is going to happen? We apply a proton 90 pulse, what it is going to do? proton 90 pulse. The magnetization is still in the XY plane, then it rotates in the XY plane. The H1 pulse rotates the XY component; now H alpha components, what it will do? It will take back the Z axis; what it will do for the H beta component it will take back to minus Z axis. Remember, I told you about the pulse phase and the receiver phase.

When I was talking about the pulse phase I showed you what happens if you apply a pulse along plus X, what happens if you apply pulse along minus X, how the magnetization can be made to do according to the phase of the pulses we apply. So, now exactly what we are trying to do by applying a proton 90 degree pulse; the H alpha component is taken to plus Z axis here and H beta component is taken to minus Z axis; that is what happens.

So, they moved backwards like this, in the opposite direction and the population between states 1 and 2 get interchanged. A very interesting thing happened. We started with this thing, because alpha and beta spin state get interchanged, the population difference between spin states of proton 1 and 2, between states 1 and 2 get interchanged. So, now population states between 1 and 2 is the proton transition, now interchanging that.

So, this 2 will come here this one will come here. This is how diagrammatically you can see what happening; this is before 90 degree pulse, this is after 90 degree pulse on proton, where interesting thing happened. What did we do? this is nothing but selective population which I was talking, we did this experimentally by applying rf pulses. Now, up to this we ensured that a carbon 13 spin, C alpha C beta component, because of that, the H alpha H beta component correspond to the protons; they get interchanged; they started moving in the opposite direction; and the 90 degree pulse ensured that H alpha component went to Z axes and H beta component went to minus Z axis; very interesting thing happened now. So this is what it is. Now let us calculate the population difference obviously there is the gain what we saw.

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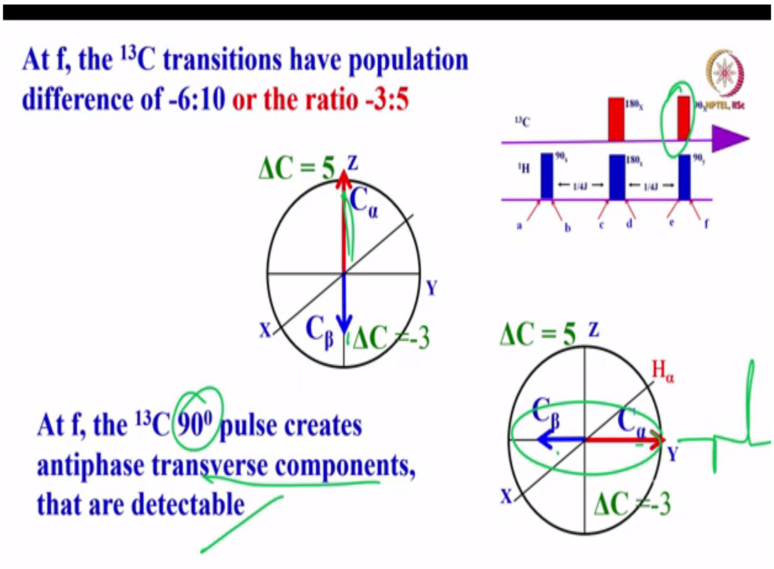

At f, the $^{13}$C transitions have population difference of -6:10 or the ratio -3:5

At f, the $^{13}$C 90$^0$ pulse creates antiphase transverse components, that are detectable

So at f, the transitions or population difference of minus 6 and 10, you can calculate here population difference, this is minus 6 and this is 10; that is what we observed; minus 3 and plus 5, is exactly the same ratio we got now. So population of carbon 13 at this stage is going to be minus 3 and plus 5 at f. Till now we have been talking only about the proton; carbon 13, 180 is only for interchanging the spin states, that is we did. Now, we have to see one more thing. At f we are also applying 90 degree pulse on the carbon 13, what does that do?

The carbon 13 90 pulse creates antiphase transverse components that are detectable. Now so far the components were along Z axes, you cannot detect that. But you have to bring it to the XY plane to detect, because Z component is not we are not detecting. So, what you have to do is apply a 90 degree carbon 13 pulse for detecting the signal; and now, we are going to get both these component minus 10, plus 5 again antiphase character is retained and we are going to get the signal in the XY plane; very interesting thing happened now.

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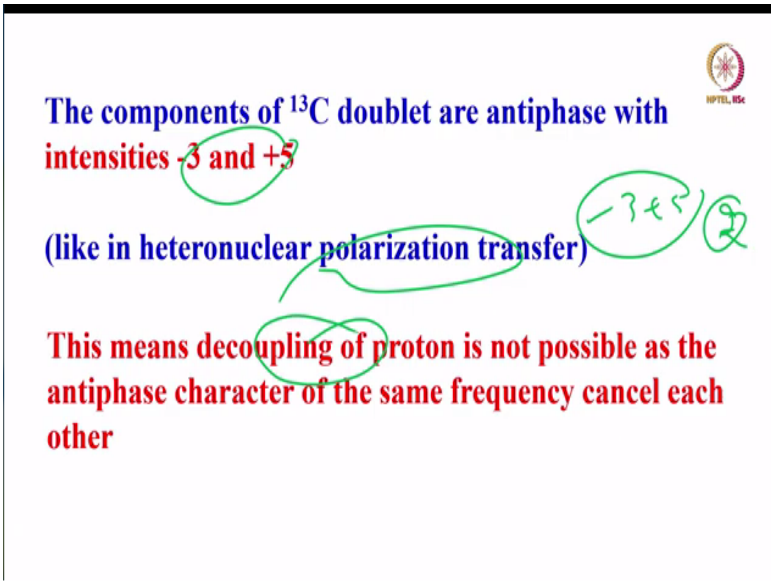

The components of $^{13}C$ doublet are antiphase with intensities -3 and +5

(like in heteronuclear polarization transfer)

$-3+5$

2

This means decoupling of proton is not possible as the antiphase character of the same frequency cancel each other

But again the same problem, components doublet are antiphase in intensities minus 3 and plus 5, like what we saw in the hetronuclear polarization transfer, especially for population inversion and especially selective population transfer we observe this thing. This means, you cannot do the decoupling because minus 3 and plus 5 if you do the decoupling, you will get only plus 2; whatever the gain you got is last, and is of no use.

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What about natural abundance $^{13}C$ signal ? It need to be suppressed

There are ways to address these iss

So, instead of cancelling the intensity, what we have to do is we have to somehow make sure that we need to get this signal. we will do that that; how we have to do? we will have to give a certain delay. Before that there is one more question we have to answer, what about natural abundance carbon 13? we can look for the carbon 13 signal, which also is present; that also we cannot ignore, we have to suppress that, that is also present. The natural abundance carbon is also present in addition to polarization transfer. The carbon 13 signal from natural abundance carbon is also present and we have to suppress that.

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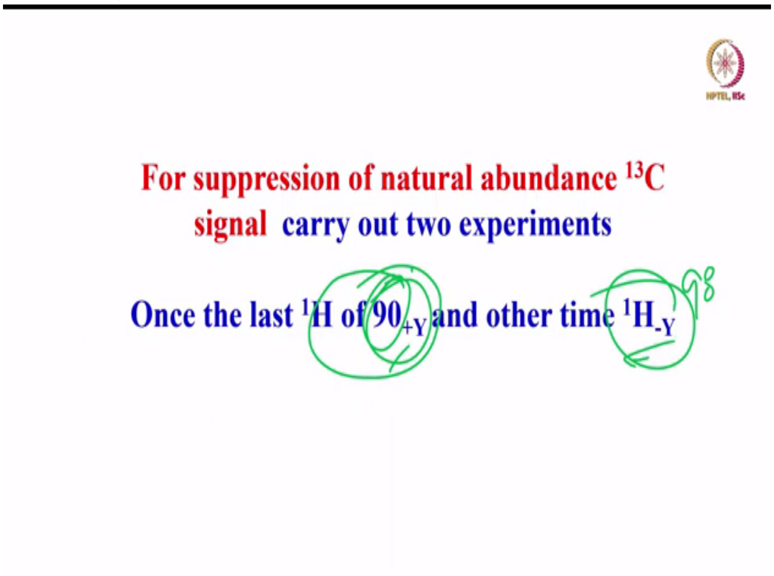

For suppression of natural abundance $^{13}$C signal carry out two experiments

Once the last $^{1}$H of $90_{+Y}$ and other time $^{1}$H$_{-Y}$

How do you do that? There are ways of doing it, what to do is we have to do 2 experiments, the last proton pulse once you apply along Y axis plus 90Y and other time apply along minus Y, plus Y and minus Y axis, you have to apply, both 90 degree pulses. 90 degree plus Y pulse and 90 degree pulse minus Y, you apply then co-add the data.

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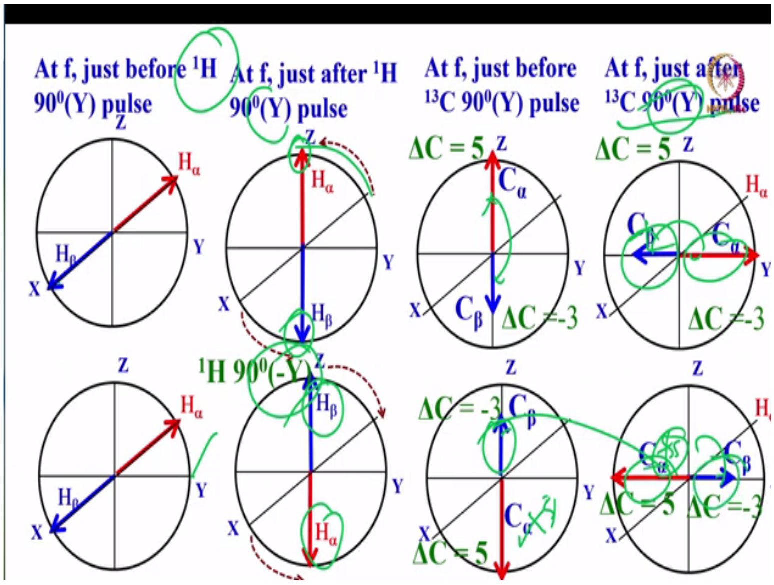

At f, just before $^{1}$H $90^{0}$(Y) pulse

At f, just after $^{1}$H $90^{0}$(Y) pulse

At f, just before $^{13}$C $90^{0}$(Y) pulse

At f, just after $^{13}$C $90^{0}$(Y) pulse

Let us see what is going to happen? At f just before proton pulse this is what we observed alpha and beta components are antiphase in character. Now, we applied proton 90 pulse and we took the magnetization alpha component along Z; beta component to minus Z; that is what we observed. At f, now with carbon 13 90 pulse this what we got for the plus Y. Now after carbon 13 90 pulse also this is the thing we observed. This is exactly what we were discussing till now, this what plus Y pulse; remember proton pulse and carbon 13 pulse we saw it was plus Y.

Now, let this be same, we are not touching that, and here instead of proton plus 90 Y we apply minus 90 Y. Now, what is going to happen? now alpha component came here, beta component came here because here minus 90 is along Y axis; we are applying a phase of the pulse as 90 minus Y now. Now, what is going to happen, it will start continuing like this, this will become minus 3 and this will become plus 5. And when you apply the carbon 90 pulse, now minus 3 comes here and plus 5 comes here.

What happened? In one experiment the minus 3 component was here, plus component was along plus Y axis in other experiment, apply minus Y pulse plus 5 came along this axis and minus 3 came along this axis; both are opposite to each other.

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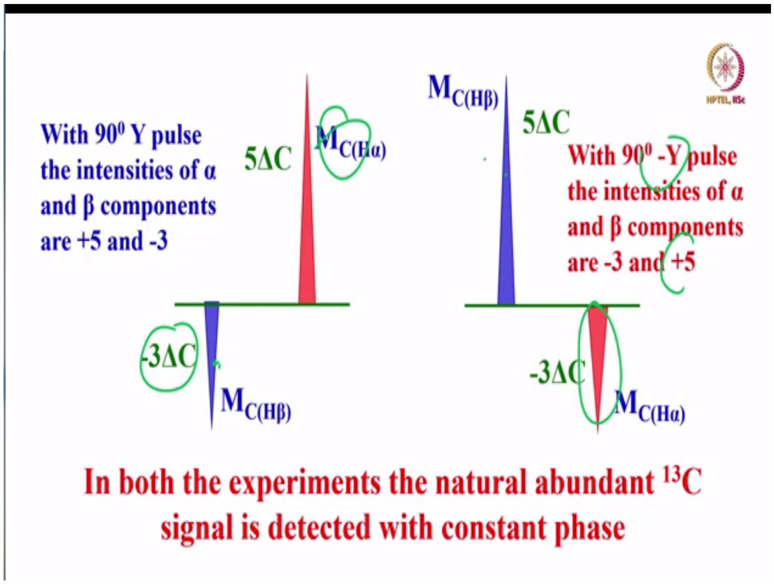

With $90^0$ Y pulse the intensities of α and β components are +5 and -3

With $90^0$ -Y pulse the intensities of α and β components are -3 and +5

In both the experiments the natural abundant $^{13}C$ signal is detected with constant phase

Now what we will do is; now this is the components intensity minus 3 and plus 5, for 90 degree plus Y pulse. Now, what happens if I take for minus Y; exactly we saw this gets interchanged this became plus 5 and this became minus 5; that is what I showed in the vector diagram when we apply plus Y pulse; if we apply minus Y pulse these 2 components interchange; that is all, nothing happened this became plus 5 this became minus 3.

Now, both the experiment you do, in both the experiment natural abundant carbon 13 is signal is always in a constant phase; that is not getting changed. That remains same with always a constant phase.

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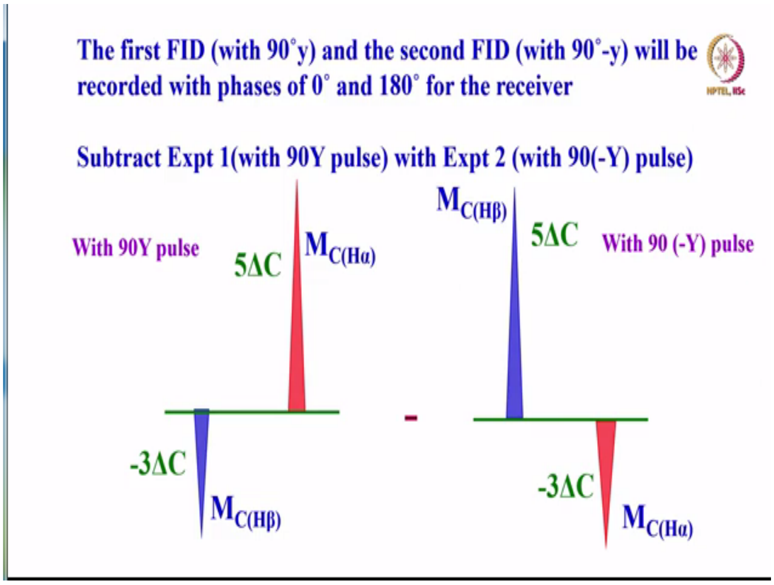

The first FID (with 90°y) and the second FID (with 90°-y) will be recorded with phases of 0° and 180° for the receiver

Subtract Expt 1(with 90Y pulse) with Expt 2 (with 90(-Y) pulse)

Now what we do is we can add or subtract; let us subtract experiment 1 with experiment 2. What is going to happen now? If you take this difference of experiment 1 with experiment 2 here nothing is going to happen.

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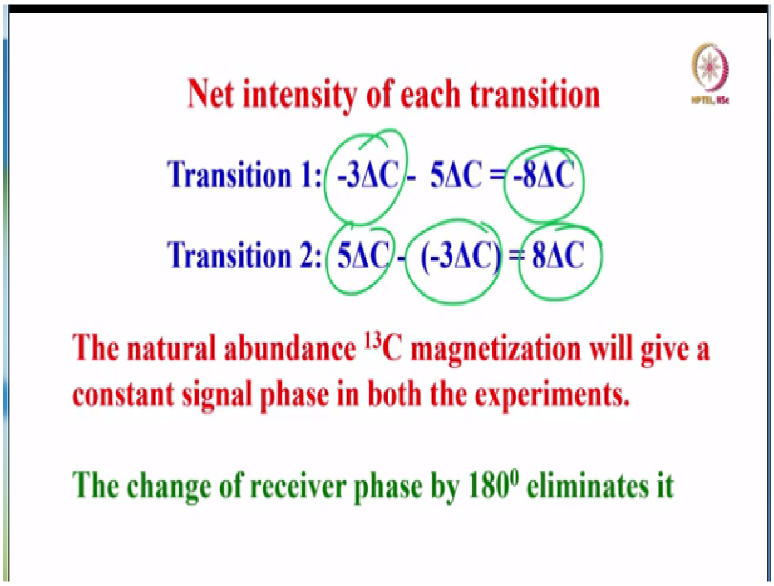

Net intensity of each transition

Transition 1: $-3\Delta C - 5\Delta C = -8\Delta C$

Transition 2: $5\Delta C - (-3\Delta C) = 8\Delta C$

The natural abundance $^{13}C$ magnetization will give a constant signal phase in both the experiments.

The change of receiver phase by $180^0$ eliminates it

Come back here, let us calculate the net intensity of each transition mathematically, when you do the subtraction; remember here never add it up, if you add it up, you are going to have a difference in intensity because this is minus 3, this is plus 5; you will get 2. But I am subtracting, minus 3 and then it will become minus 5, it will become minus 8; I am subtracting. So when I do the subtraction this minus 3 and minus 5 become minus 8. Similarly, other components plus 5 minus of minus 3 = + 8.

So, what did we do? We got the gain in the intensity of the signal, and antiphase character everything whatever is there, we can take care of this one. But natural abundance carbon 13 will give a constant signal in both the experiments. If you do the 2 experiments and subtract this one, then this intensity of the signal will add up; it is the antiphase character because of that nullification of the signal or reduced intensity will not be there.

But still for natural abundance carbon what will happen? They will come with the same phase, that is not getting changed. Only here the phase is changing from minus 3 to plus 5 and plus 5 to minus 3; but for natural abundance carbon the phase will not get it changed; the phase remains same.

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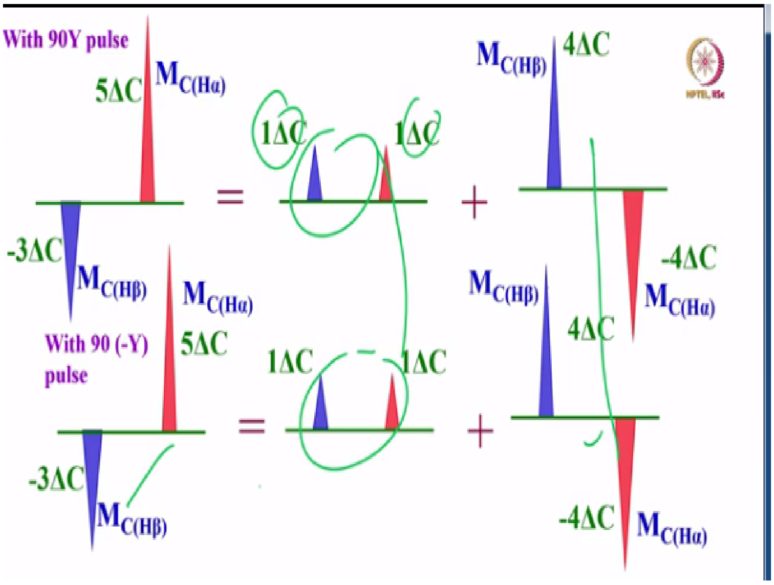

So, now what we can do is with 90 degree Y pulse this is what we get; and this is a natural abundance carbon, plus this; both will come. The natural abundance of the carbon is just 1%; not enhanced in intensity. Now, in the other case if you apply 90 - Y pulse again, natural abundance carbon signal remains same, and this becomes this. When you subtract what will happen? here we got the gain in intensity. Whereas this one if you subtract this with this, it gets nullified. So, you can subtract the natural abundance signal coming from natural abundance carbon. Whereas this will add up; that is the beauty of it.

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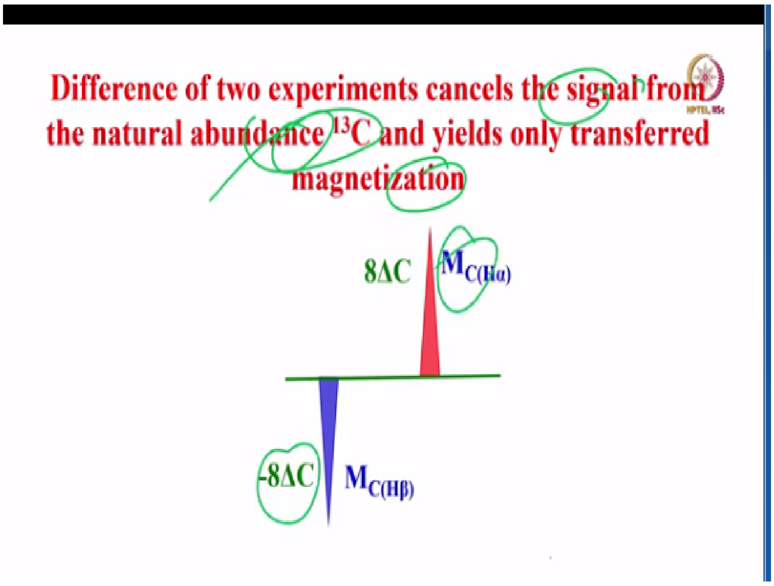

Difference of two experiments cancels the signal from the natural abundance $^{13}C$ and yields only transferred magnetization

So, difference of 2 experiments cancels the signal from the natural abundance carbon and yields only the signal that is transferred from the proton. The magnetization transfer from the proton to carbon, only that signal is detected, and natural abundance carbon signal is completely eliminated. And this is finally we are going to get. An minus 8 and plus 8 is the signal intensity, that gain you are going to get.

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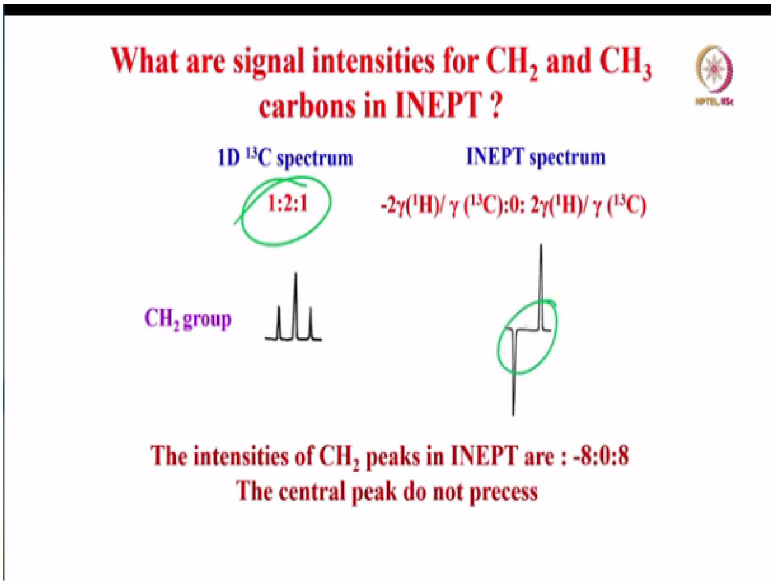

What are signal intensities for $CH_2$ and $CH_3$ carbons in INEPT ?

The intensities of $CH_2$ peaks in INEPT are : -8:0:8

The central peak do not precess

So and finally, what I am going to show you is the signal intensities, how much we are going to gain for different carbons? especially when you are looking at the CH3, CH2, CH and CO carbons. How the INEPT helps, of course CO carbon, there is no proton, we will not worry about it. At the moment, we will see only CH3 and CH2 carbon, CH carbons. How the intensities will change. For CH3 it is a quartet, CH2 is a triplet and in the case of other one CH is a doublet with the antiphase character.

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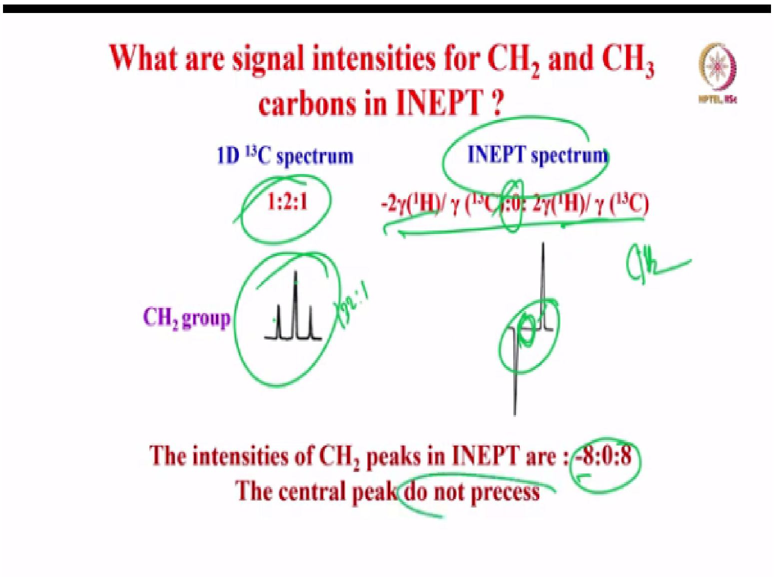

What are signal intensities for CH$_2$ and CH$_3$ carbons in INEPT ?

1D $^{13}$C spectrum

INEPT spectrum

1:2:1

$-2\gamma(^{1}H)/\gamma(^{13}C):0:2\gamma(^{1}H)/\gamma(^{13}C)$

CH$_2$ group

The intensities of CH$_2$ peaks in INEPT are : -8:0:8

The central peak do not precess

Now, here what is happening is, the central peak will disappear. In the case of the INEPT for CH 2 when you do this, This is carbon 13 natural abundance and this is INEPT, normal carbon 13 1D, 13C is spectrum. Now 1 is to 2 is to 1 intensity is there, whereas, see here with INEPT this is the ratio which you will see, because I told you the central peak will not precess, you do not have to worry about it; that is 0 intensity.

Only outer component has a change in the intensity which goes by the ratio of the gammas of proton and carbon. And this is the enhance intensity are going to get. So minus 8, 0, plus 8 are the enhanced intensity; and central peak do not precess and do not give any signal at all.

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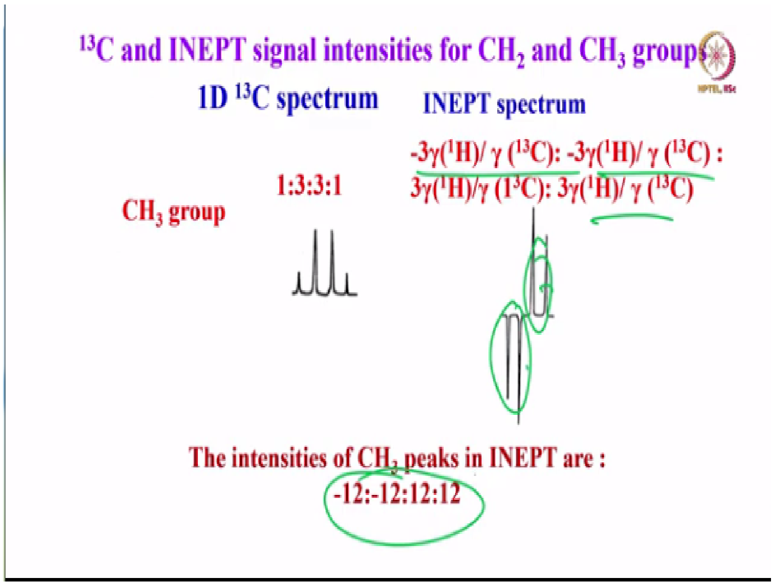

$^{13}$C and INEPT signal intensities for CH$_2$ and CH$_3$ groups

1D $^{13}$C spectrum

INEPT spectrum

$-3\gamma(^{1}H)/\gamma(^{13}C):-3\gamma(^{1}H)/\gamma(^{13}C):$

$3\gamma(^{1}H)/\gamma(^{13}C):3\gamma(^{1}H)/\gamma(^{13}C)$

CH$_3$ group

1:3:3:1

The intensities of CH$_3$ peaks in INEPT are :

-12:-12:12:12

Same thing you can look for CH3 group; CH3 group again there is no central component, as I told you both the components, viz., the high frequency low frequency components move in the opposite direction and the this is the intensity ratio. And if we look at this intensity ratio, the intensity turns out to be minus 12 is to minus 12 is to 12 is to 12 so this is the intensity gain for carbon 13 of the CH3 group.

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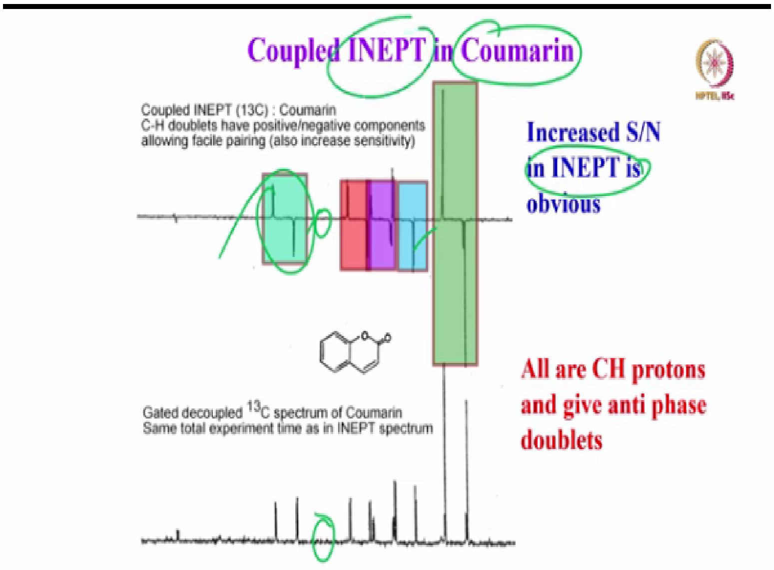

## Coupled INEPT in Coumarin

Coupled INEPT (13C) : Coumarin
C-H doublets have positive/negative components
allowing facile pairing (also increase sensitivity)

Increased S/N in INEPT is obvious

Gated decoupled $^{13}C$ spectrum of Coumarin
Same total experiment time as in INEPT spectrum

All are CH protons and give anti phase doublets

This is just to show you an example. How INEPT is going to help you.  This is an experiment on a molecule called coumarin, taken from the literature. Here is the carbon-13 spectrum of coumarin without INEPT, this is with INEPT; that is the difference. You see the enhancement in the intensity because of the INEPT technique; Ofcourse the antiphase character you see, this is the not a decoupled one; one positive or negative intensity signal is clearly visible.

And you can see there is an enormous gain. Look at the noise, here noise here, there is enormous gain in the signal to noise ratio because of INEPT. So, this is an advantage of INEPT, especially when you want to observe the dilute spins. This an experiment which is coupled INEPT; not decoupled, it is a coupled INEPT.

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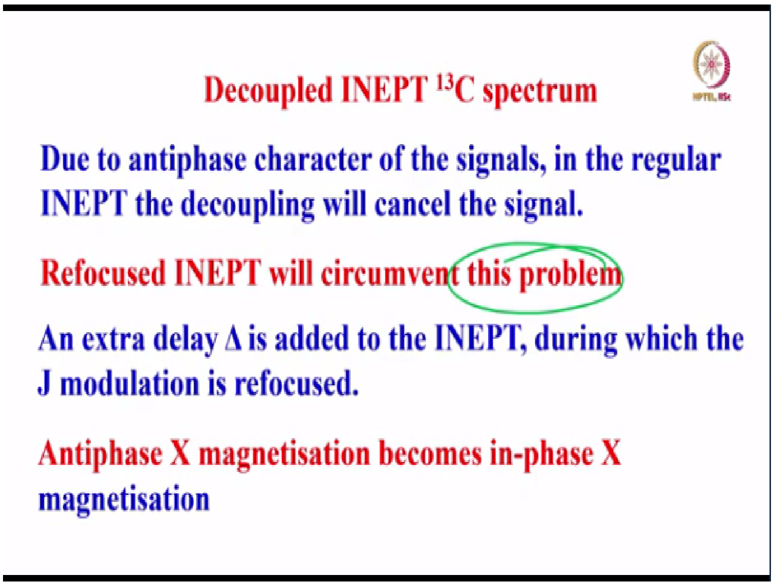

## Decoupled INEPT $^{13}$C spectrum

Due to antiphase character of the signals, in the regular INEPT the decoupling will cancel the signal.

Refocused INEPT will circumvent this problem

An extra delay Δ is added to the INEPT, during which the J modulation is refocused.

Antiphase X magnetisation becomes in-phase X magnetisation

Now, you can also do the decoupling, due to antiphase character I told you if I tried to do the decoupling immediately there is a problem. So, what we can do is we can try to do what is called a refocused INEPT. That is an experiment which will circumvent the problem of antiphase character of carbon 13 signal; and instead of reduced intensity, then we can make sure that antiphase character become in-phase character, and we are going to get the enhanced intensity.

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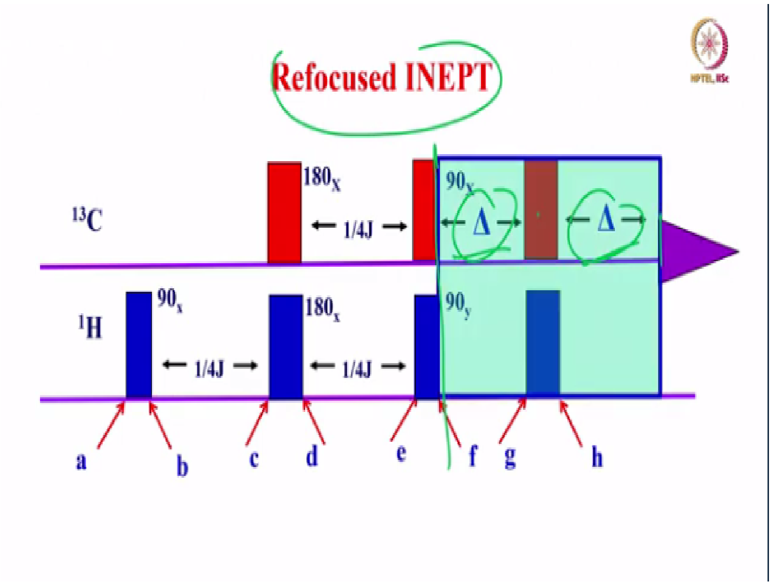

## Refocused INEPT

This is the refocused INEPT sequence. Up to this is clearly known; up to this we understood. Now, what we do is, extra delay we are going to give; again like a spin echo sequence and the carbon 13 give a time delay here and then apply 180 pulses on both proton and carbon after a certain delay start collecting the signal. Now, what is going to happen? this is the extra, additional thing.

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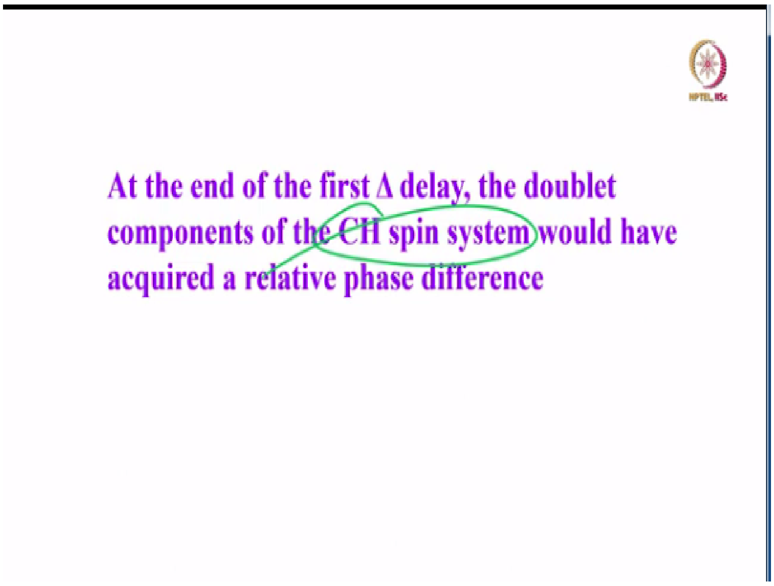

At the end of the first Δ delay, the doublet components of the CH spin system would have acquired a relative phase difference

At the end of the first delay, the doublet component of the CH spin system would have acquired the relative phase difference; there is a phase difference between these things.

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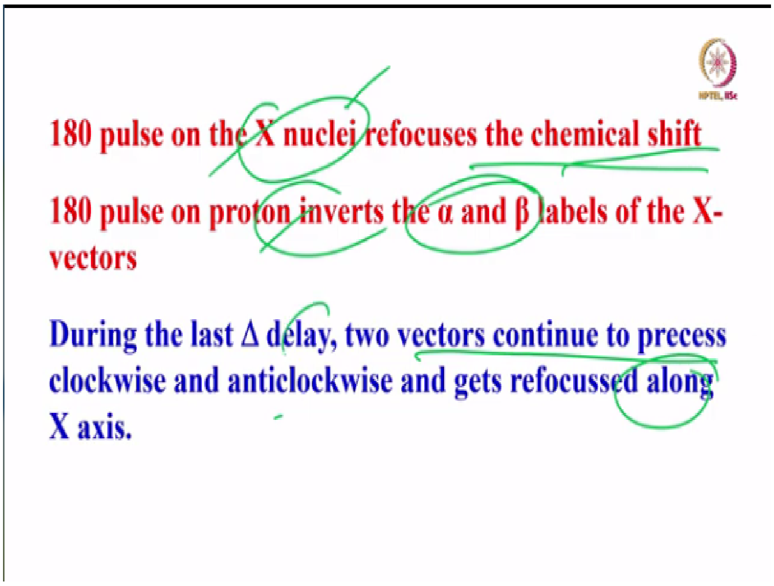

180 pulse on the X nuclei refocuses the chemical shift

180 pulse on proton inverts the α and β labels of the X-vectors

During the last Δ delay, two vectors continue to precess clockwise and anticlockwise and gets refocussed along X axis.

But 180 pulse on the X nuclei refocuses the chemical shift; 180 pulse on the proton inverts the alpha and beta spin labels of the X vectors. And during the last delay, the 2 vectors continue to precess clockwise or anti clockwise and gets refocused along the X axis. Remember, we discussed this about precession, the evolution of the J coupling, evolution the magnetization how the in phase character become antiphase character and how in phase dispersive can become antiphase dispersive, etc. Everything we discussed in the last 2 classes evolution. That is why the evolution of chemical shift and evolution of J couplings, I discussed a lot. It is without going to the details I am telling you, the 180 pulse on the X nucleus refocuses the chemical shift, 180 pulse on proton inverts the alpha beta states. And as a consequence, during the last delay, these vector components continue to process the way it is, and then get refocused along the X axis. Then that is what we wanted; the antiphase character when they get refocused both of them are on the X axis, there is a gain in intensity, there is no question on nullification at all.

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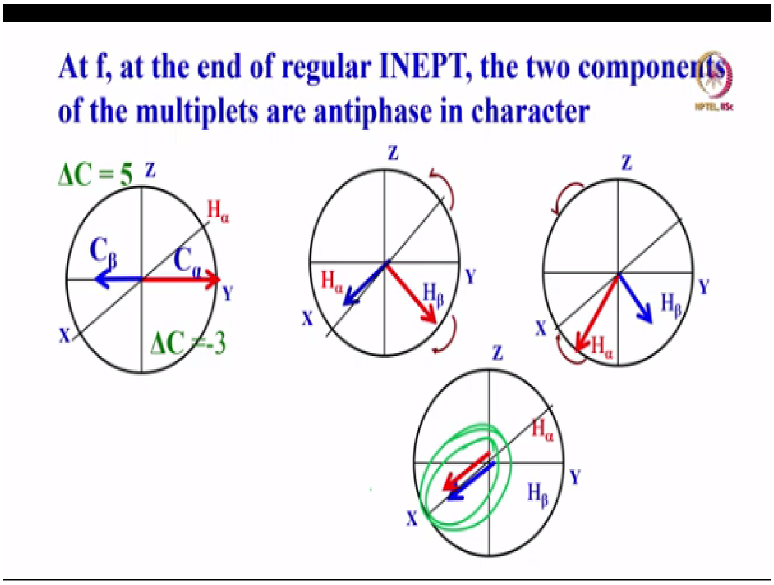

At f, at the end of regular INEPT, the two components of the multiplets are antiphase in character

That is what happens after a delay. If you do that, then finally it will come back here; that is what the last 2, 180 pulses, how it is behaves, I have shown you with a vector diagram without going into the details of it. Remember, what is going to happen after that during the time delay, alpha and beta components start moving like this. And then again they get interchanged, and after some time, they can refocus along X axis.

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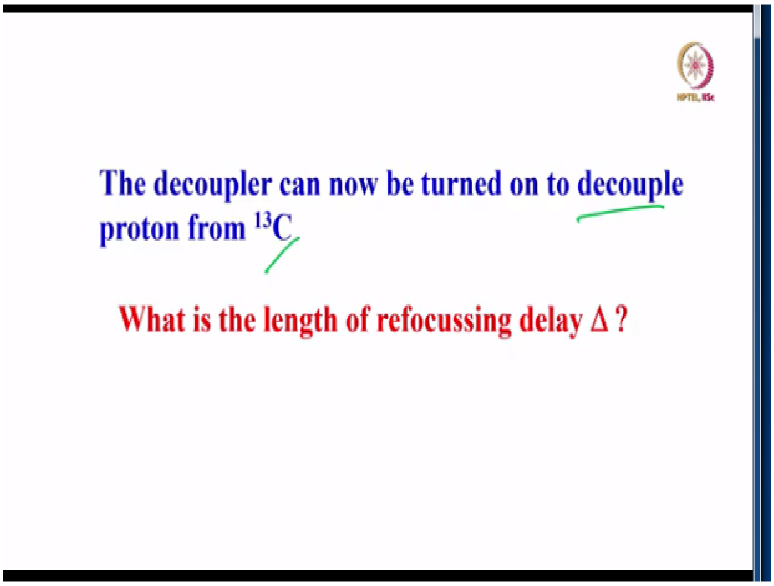

The decoupler can now be turned on to decouple proton from $^{13}C$

What is the length of refocussing delay Δ ?

So the decoupler now we can switch it on, and start detecting the carbon 13; you can break the coupling between proton and carbon after this delay. Now, the question you might ask me what is the length of this delay?

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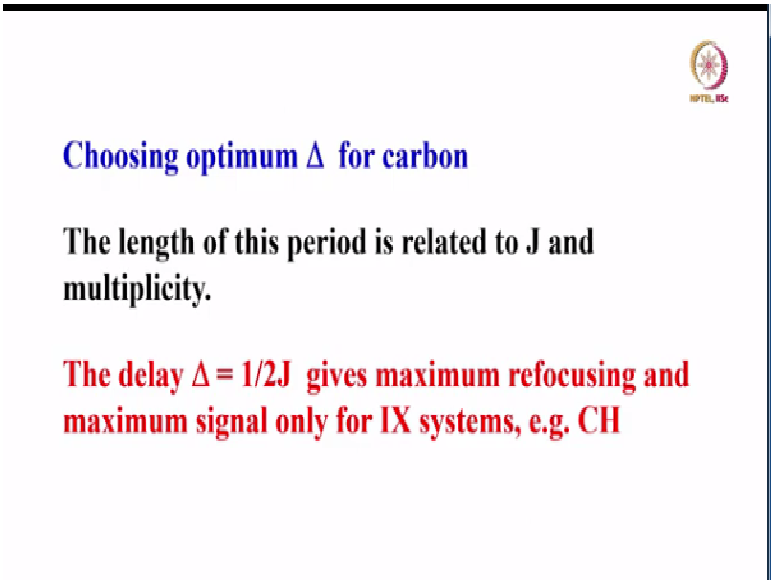

Choosing optimum Δ for carbon

The length of this period is related to J and multiplicity.

The delay Δ = 1/2J gives maximum refocusing and maximum signal only for IX systems, e.g. CH

Because you do not know what is the optimum delay, because J coupling we should know; it depends upon J coupling JCH; but JCH for CH3, CH2 and CH are different. How do you optimize this? What is the right J then? So, we have to use delta = 1 over 2J which is maximum refocusing for IX, for CH.

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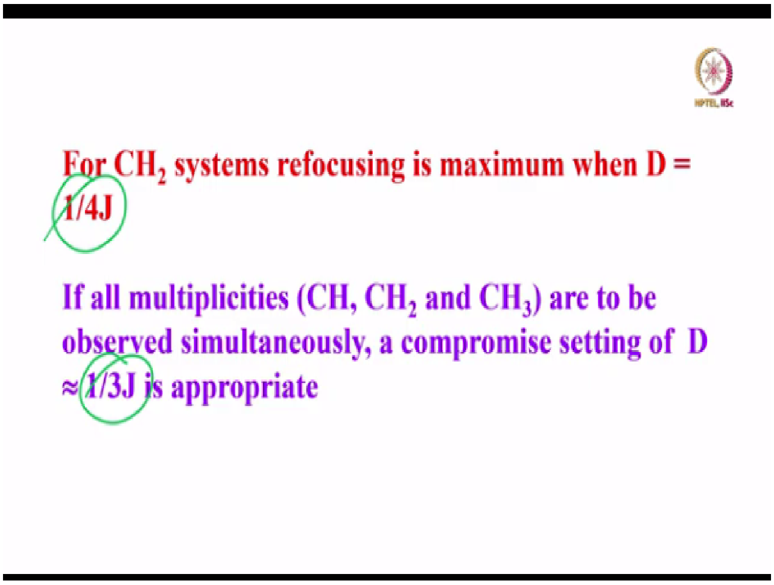

For $CH_2$ systems refocusing is maximum when D = 1/4J

If all multiplicities (CH, $CH_2$ and $CH_3$) are to be observed simultaneously, a compromise setting of D ≈ 1/3J is appropriate

And for CH2 is 1 over 4J and for CH3 1 over 3J. Then you might ask me a question. Do I have to do the 3 different experiments to do this?

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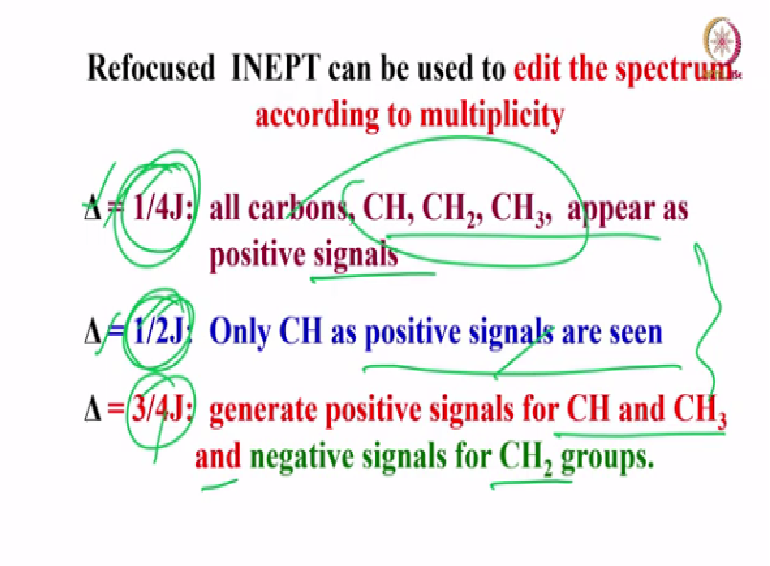

Refocused INEPT can be used to edit the spectrum according to multiplicity

$\Delta = 1/4J$: all carbons, CH, $CH_2$, $CH_3$, appear as positive signals

$\Delta = 1/2J$: Only CH as positive signals are seen

$\Delta = 3/4J$: generate positive signals for CH and $CH_3$ and negative signals for $CH_2$ groups.

Now what we can do is, a sort of in principle you can do the experiment, for identifying the carbons. For example, if delta = 1 over 4J; all carbons appear as positive signals. If I put delta = 1 over 2J only CH as positive signals are seen, others are not seen. If we use only 3 over 4J then generate a positive signal for CH and CH3 and negative for CH2. So, in fact, if you want to worry about the allpositive signal being seen, without worrying about which 1/ 4J if you use it, you get all the 3 peaks with positive in intensity.

But you can also use this delay and do the experiment for what is called spectral editing. You can identify which is CH carbon which is CH2 carbon which is CH3 carbon. Using an appropriate delay, use the exact 1 over 2J for last delta, then you get only CH peaks; all others are not there at all. Then identify which are the CH peaks in the complex carbon 13 spectrum, this is an advantage of this thing.

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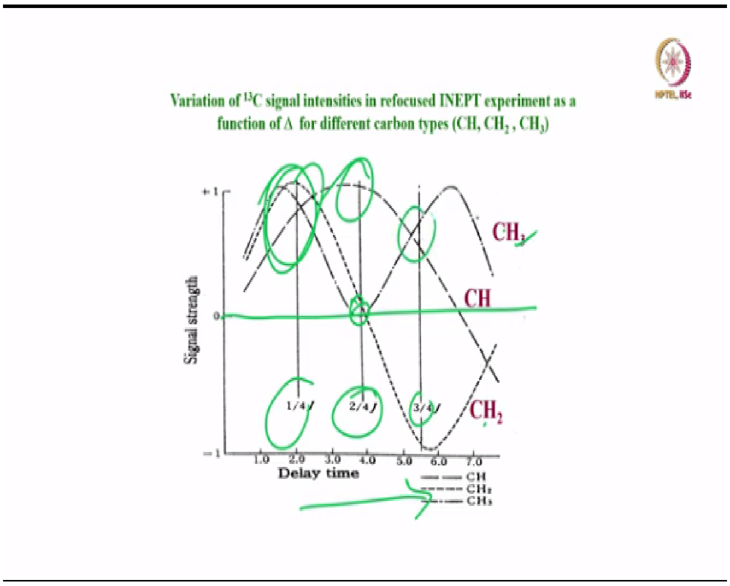

Variation of $^{13}C$ signal intensities in refocused INEPT experiment as a function of Δ for different carbon types (CH, $CH_2$, $CH_3$)

So, this has been taken from the literature. You see how the intensity of the carbon 13 varies in the refocused INEPT, and for different carbon as a function of delay. This is the delay keeps varying here, and intensity of the carbon for different CH3, CH2, CH are varied; and look at exactly 1 over 4J; this is 0 all are positive. At exactly 2 / 4J that is 1 over J, CH2 and CH3 are 0, CH only is seen positive; and at exactly 3 / 4J, then these 2 are positive and CH2 is negative. So you can use this for spectral editing.

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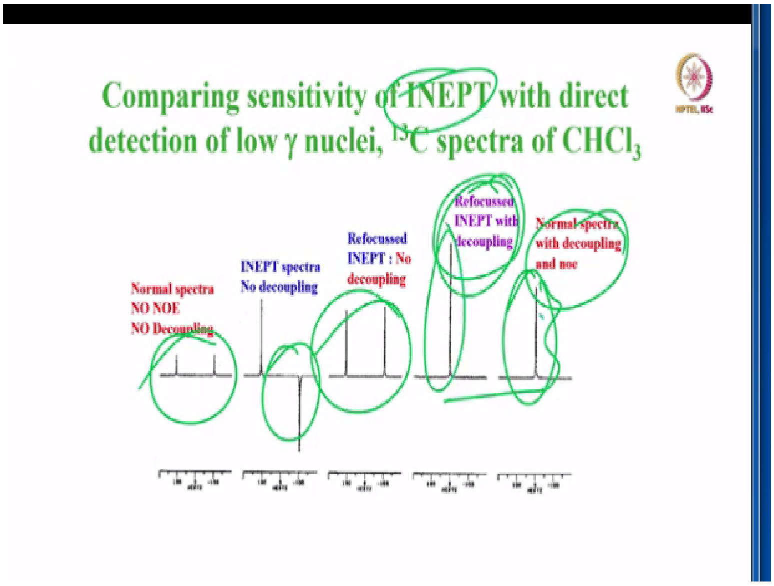

Comparing sensitivity of INEPT with direct detection of low γ nuclei, $^{13}C$ spectra of $CHCl_3$

And this is comparing the sensitivity of the INEPT with the direct detection of a low gamma nuclei. See this is the carbon 13 spectrum, no NOE, no decoupling, whereas, this is a carbon 13 INEPT spectrum, no decoupling; this is a refocus INEPT again. Of course, still you can do decoupling or not it is choice is yours, and it eis a refocusd INEPT with decoupling; and this is a normal carbon 13 spectrum with decoupling and NOE. See the difference. This is normally what people observe, when you do the carbon 13 NMR what you simply do is the decoupling experiment, and the decoupling power is kept on all the time. As a consequence, there is an enhancement in the signal intensity, due to NOE. And this is in spite of that you get the signal. But now, if you do refocused INEPT with GATED decoupling and then see enhancement in the intensity.

This gives rise to enormous signal gain and reduce your experimental time drastically. That is why in all the heteronuclear experiments of 2D like HSQC, HMBC, etcetera, we always use polarization transfer technique. INEPT is one of the modules which is there in the pulse sequence, and we transfer the magnetization from abundant spin to rear spin or dilute spin, and then start collecting the signal. This is what normally we are going to do.

And then of course and this we can discuss a lot, all these things again and again, quite a bit but there is no point in going much more into the detail. But I will just tell you one important expt called DEPT.

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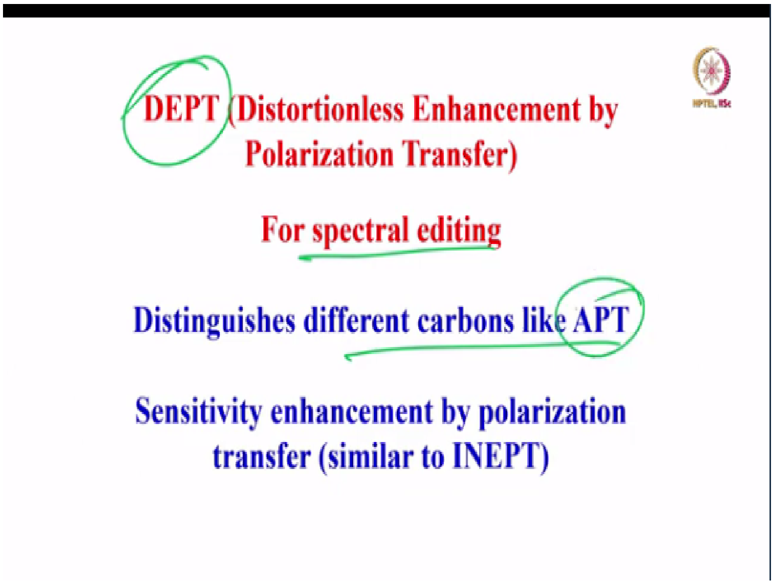

DEPT (Distortionless Enhancement by Polarization Transfer)

For spectral editing

Distinguishes different carbons like APT

Sensitivity enhancement by polarization transfer (similar to INEPT)

DEPT is an experiment called distortionless enhancement by polarization transfer. This is used for spectral editing, This distinguishes different carbons like attach proton test which I did not discuss in this course, I have discussed in the previous course, this is similar to INEPT called DEPT.

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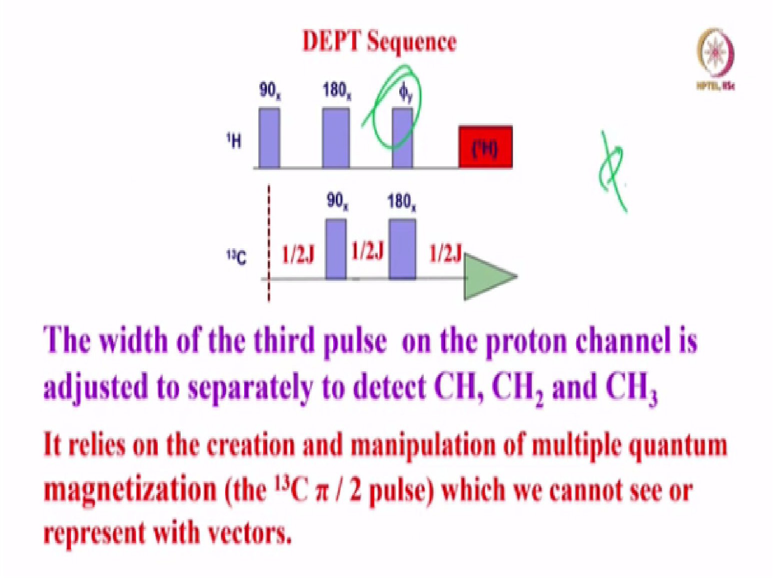

DEPT Sequence

The width of the third pulse on the proton channel is adjusted to separately to detect CH, $CH_2$ and $CH_3$

It relies on the creation and manipulation of multiple quantum magnetization (the $^{13}C$ $\pi / 2$ pulse) which we cannot see or represent with vectors.

Now, DEPT sequence is like this. You can find out from looking at the sequence itself, you can understand there is a polarization transfer that takes place, it is similar to INEPT sequence you can see. Depending upon the width of the third pulse here, this we call it as a phi. Now, in this sequence, depending upon what is the angle of the last pulse

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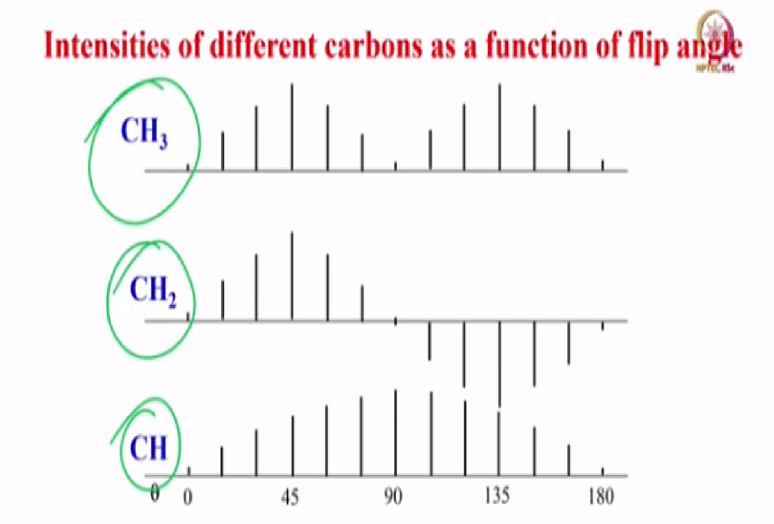

Intensities of different carbons as a function of flip angle

We can have different types of DEPT experiment where you can identify only CH carbons, you can identify only CH2 carbons; you can identify only CH3 carbon; analogues to what we saw in the INEPT for different carbons. So, this is the experiment called DEPT; distortionless enhancement in polarization transfer.

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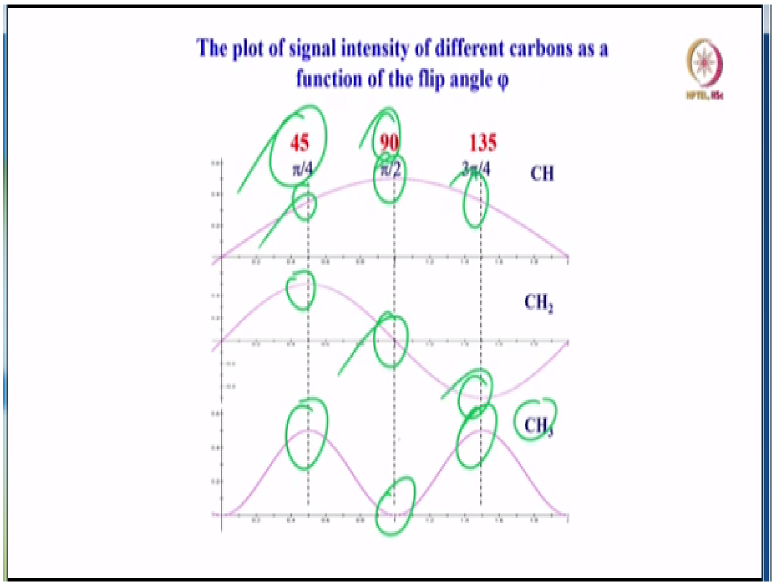

The plot of signal intensity of different carbons as a function of the flip angle φ

Again you can see here, when you keep the last pulse 45 degree, it is called DEPT 45; you can see that what we are going to observe; this is positive this is positive, this is positive. Whenever you are going to a DEPT 45, this especially the CH2 is 0, CH3 is negative and CH is positive. When you are going to do DEPT 135, then this is positive, this is positive and this is negative. So, now, you can use different types of DEPT experiments; DEPT is simple similar to INEPT like sequence, we saw.

But there is a difference in understanding the behaviour of magnetization, especially if you consider the last pulse, the flip angle of the pulse, the angle by which you tilt the magnetization by the last pulse, we can vary. The last last pulse can be 45 degree, 90 degree or 135 degrees, accordingly, different carbons have different intensities, some are positive some are negative, some get nullified; some carbon like this.

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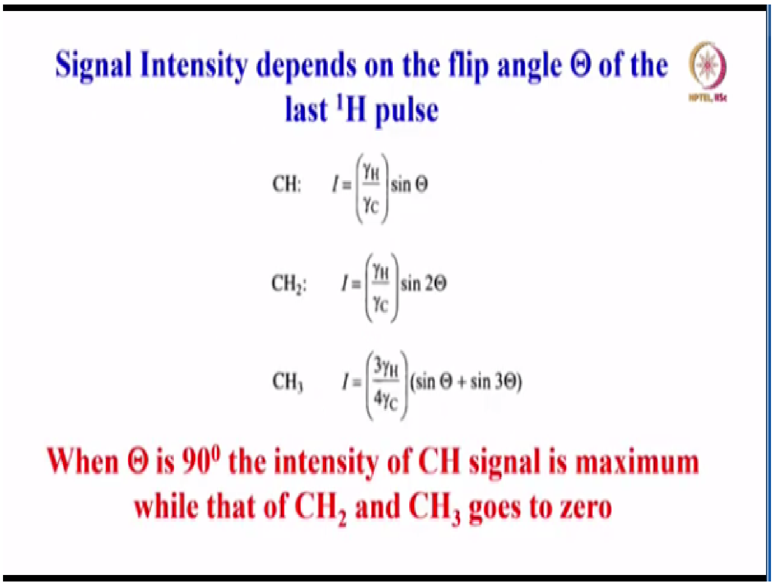

Signal Intensity depends on the flip angle Θ of the last $^1$H pulse

CH: $I = \left(\frac{\gamma_H}{\gamma_C}\right) \sin \Theta$

CH$_2$: $I = \left(\frac{\gamma_H}{\gamma_C}\right) \sin 2\Theta$

CH$_3$ $I = \left(\frac{3\gamma_H}{4\gamma_C}\right) (\sin \Theta + \sin 3\Theta)$

When Θ is 90$^0$ the intensity of CH signal is maximum while that of CH$_2$ and CH$_3$ goes to zero

You can use this idea to do what is called the spectral editing in carbon 13. Generally, this is what is used for identifying different carbons. INEPT is used generally for polarisation transfer experiments in most of the complex pulse sequences; but in a one dimensional experiment if you want to try, and if you want to see, that you want to see the simple carbon spectrum and want to know which carbon is which, then you do not need to do go too complicated 2D sequences etcetera. Simply do the DEPT experiment. The different pulse sequences, different pulse angles of the last pulse 45 degree, 90 degree, 135 degree you can make the identification of all the carbons.

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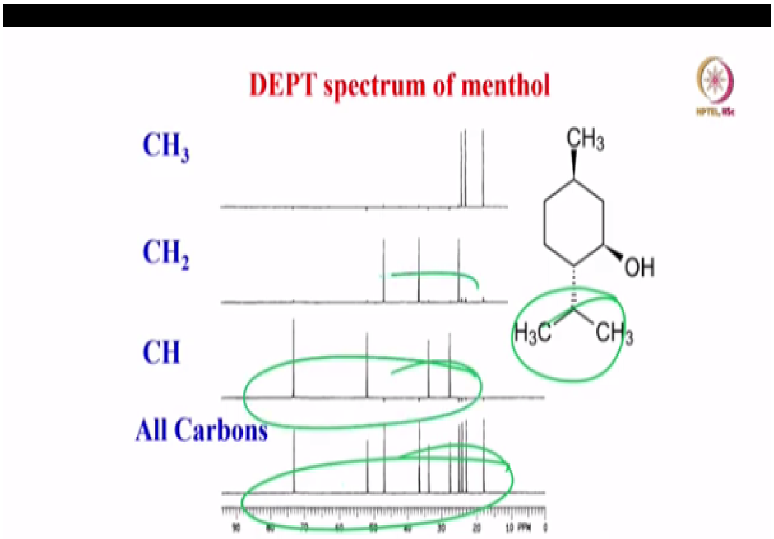

DEPT spectrum of menthol

And this is an example for a molecule called menthol, where you can see all the carbons are present here; and these are only CH carbons and this DEPT sequence is only for CH2 and this is for only CH3.

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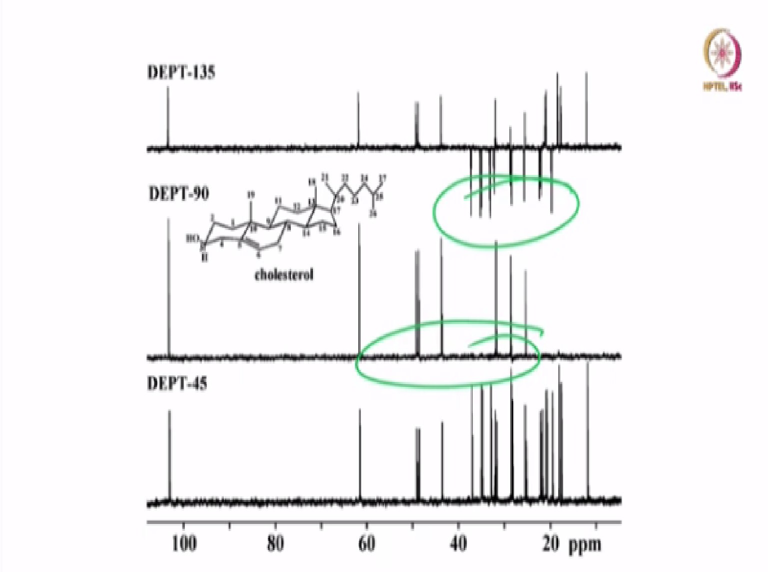

How we got it? You know that; the DEPT 45, DEPT 90, DEPT 135 if you do, in DEPT 90 you observe only CH carbons, DEPT 135 you see CH carbons are all negative intensity and CH3 are positive and you can identify which is which like this.

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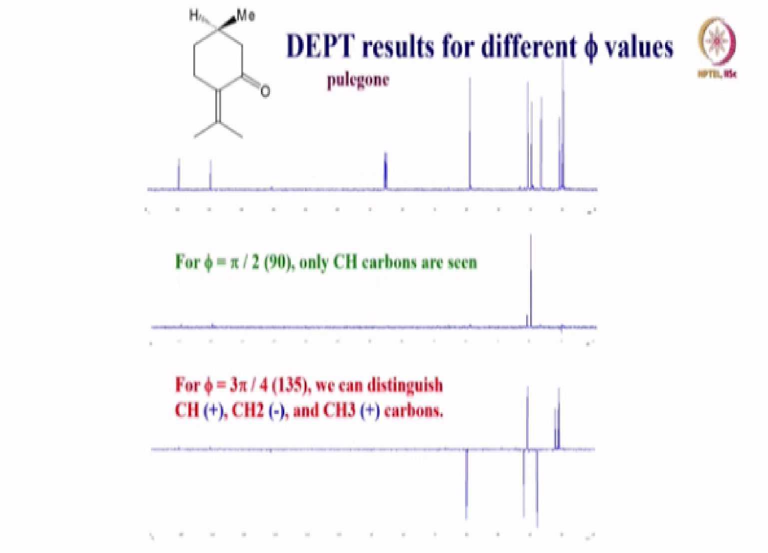
DEPT results for different ϕ values

pulegone

For $\phi = \pi / 2$ (90), only CH carbons are seen

For $\phi = 3\pi / 4$ (135), we can distinguish CH (+), CH2 (-), and CH3 (+) carbons.

So this is what is called pulse editing sequences; and of course, now you can multiply and subtract different spectra you obtained for dept 45, 90, 135 and then co-add data accordingly, Then you can get only carbon 13, CH3 only peaks, only CH 2 peaks; it is possible to obtain.

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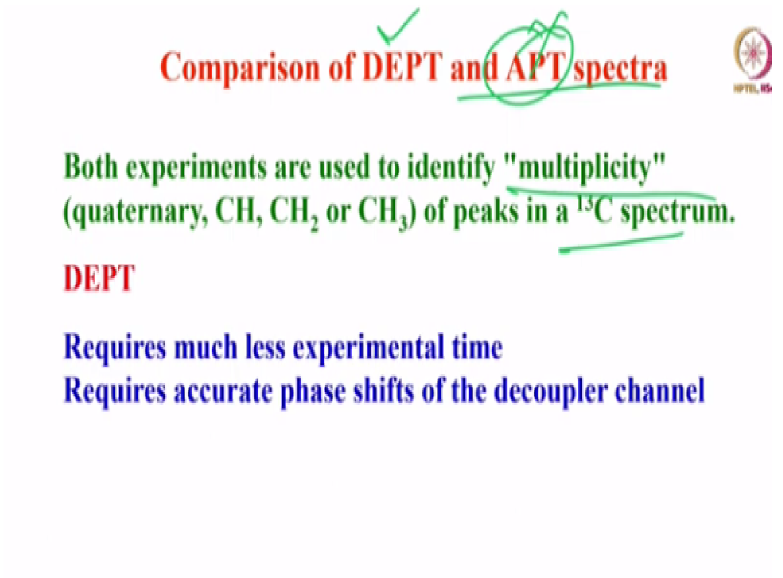

Comparison of DEPT and APT spectra

Both experiments are used to identify "multiplicity" (quaternary, CH, $CH_2$ or $CH_3$) of peaks in a $^{13}C$ spectrum.

DEPT

Requires much less experimental time
Requires accurate phase shifts of the decoupler channel

So that is what you are going to do; and then APT I have not discussed; there is no need to worry about this. But just I want to tell you, both the experiment DEPT and APT and all these things, are used to identify the multiplicity. Nowadays, nobody uses APT, we have to use only a DEPT experiment to identify different carbons; always do the 3 experiments, depending upon the type of carbon, 3 or 2 experimentx; DEPT 45, DEPT 90 and DEPT 135 for identification. But this contains polarization and enhancement already; that is why it is called distortionless enhancement polarization transfer.

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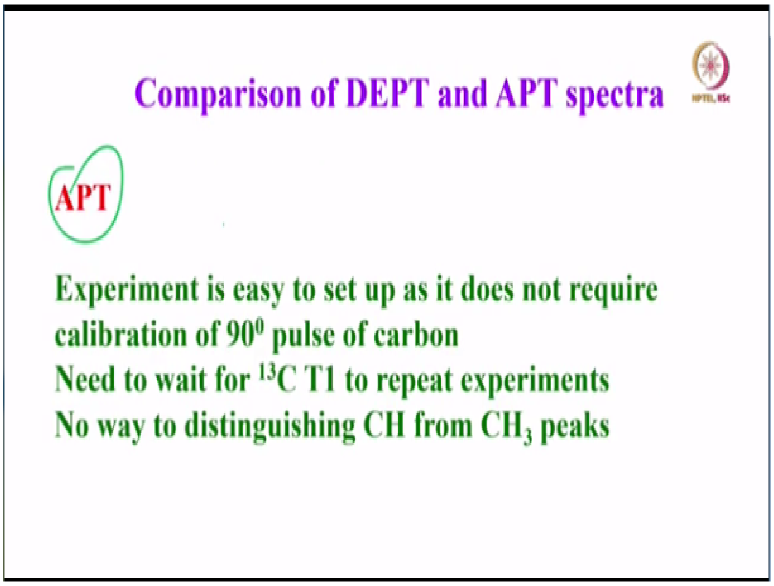

Comparison of DEPT and APT spectra

APT

Experiment is easy to set up as it does not require calibration of $90^0$ pulse of carbon
Need to wait for $^{13}C$ T1 to repeat experiments
No way to distinguishing CH from $CH_3$ peaks

So, this is what I wanted to do. I will stop here, with this polarization technique I have completely covered and I explained to you how polarization transfer is very much useful, right from starting from the pulse sequence of polarization transfer for a hetero-nuclear case, selective population inversion, and selective population transfer etcetera. Then we got a INEPT sequence; like we got first a simple sequence. And then brought in the spin-echo sequence and then we got into an INEPT sequence,e and how we refocusd the antiphase character doublet into the in-phase character for doing the decoupling. All those different methodologies, how we can adapt this we observed. And we saw that INEPT, depending upon the last delay delta, you can have different intensity for the CH carbon, CH3 carbon, CH2 carbon, that is one way of using INEPT for editing the spectrum.

And of course, there is the polarization transfer, as a consequence there is significant enhancement in the signal density, we saw for carbon 13 doublet, what is the enhancement in signal intensity; for a triplet what was the enhancement for CH3 what is the enhancement. Tor doublet we got 4 times for CH 2 we got 8, and for CH 3 we got 12. Of course you do not need to use any big mathematics you see, depending upon the number of protons attached that was the enhancement in signal intensity.

Of course, this is normally not used for spectral editing, it is used for enhancement in the signal intensity, in the 2D experiments. DEPT, I showed you is a simple polarization transfer experiment; depending upon the flip angle of the last pulse, 45 degree 90 degree or 135 degree DEPT experiments you can identify different carbons based on the intensity, whether it is positive or negative based on the signal phases, positive phase and negative phase are in one case completely one of them get nullified.

So, this is what I wanted to discuss in the polarization transfer techniques. Next, we will go to a different topic. I hope I gave you the gist of something which we use in 2D experiments. Thank you.