One and Two Dimensional NMR Spectroscopy for Chemists

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Lecture – 46
INEPT and DEPT

Welcome back, in the last class we discussed about spin echo and attached proton test to identify different carbons based on the number of protons attached to it. That was called attached proton test. Afterwards I wanted to introduce polarisation transfer; we saw what happens to the polarisation transfer when two homo nuclear weakly coupled spins were considered, we saturated one of the transitions, then we saw the change in intensity of the other coupled spin. We also inverted one of the transitions of the coupled spin, we saw the change in intensity of the other coupled spin.

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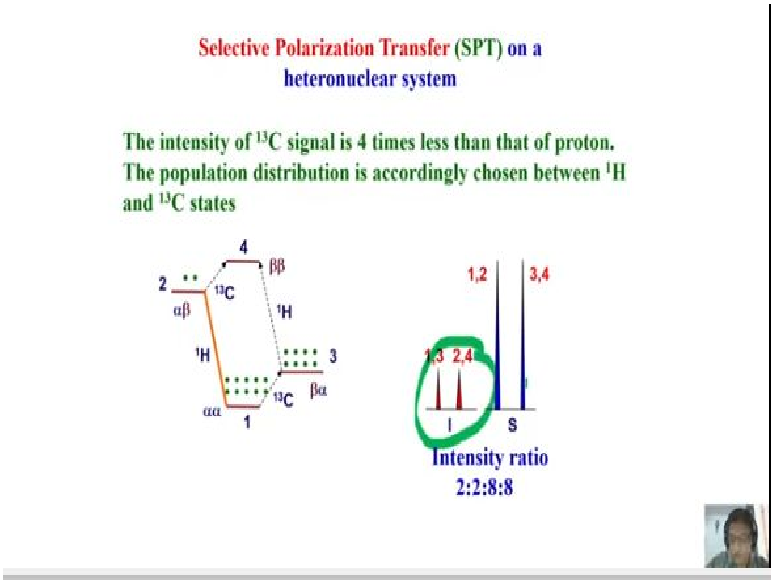

Selective Polarization Transfer (SPT) on a heteronuclear system

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

Intensity ratio 2:2:8:8

Now, we extended the logic to what happens to heteronuclear case, because in the case of heteronuclear, gyromagnetic ratio; I am now taking the example of proton and carbon, carbon gyromagnetic ratio is 4 times smaller than that of proton; there again we selectively saturated one of the signals, selective polarisation transfer also we did. When we did the selective polarisation transfer by saturating one of them, we observed this. We had taken for the purpose of understanding 10 spins here, 2, 8, and 0, so that intensity ratio for 2 protons signals and 2 carbon signals in the doublet pattern remains 2 :2 : 8 : 8, because as you know this one is always much smaller compared to proton, 4 times smaller, that is why, that population ratio chosen.

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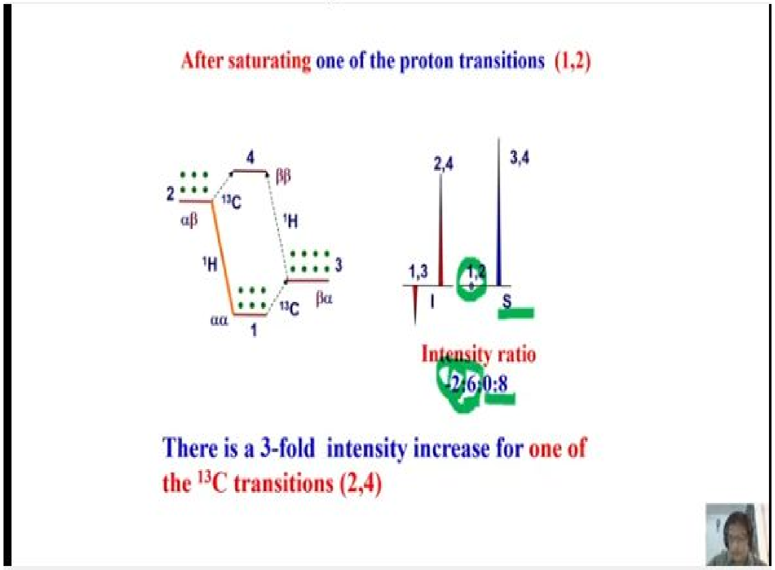

After saturating one of the proton transitions (1,2)

Intensity ratio

-2:6:0:8

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

We also observed what happens if I saturate one of them; we saturated this peak, this proton peak, because we are transferring the signal, or the magnetisation from proton to carbon; so we will saturate proton and see what happens on carbon. We saturated this signal, especially this peak, 1 to 2. See when we saturate, the populations which were more here about 10; and 2 here, now we made it equal, 6, 6; that is all, we saturate only one proton peak, see the effect now, calculate the intensity ratio.

This one in this case, I saturated, this is equal to 0, whereas in this case, the signal 8 minus 0 remain same as 2; not affected at all. What about the carbon signal? look at it, this is 6 and 10, the difference is minus 2 we have seen, 2 here. What about here; 6 minus 0, intensity is 6, so we saw there is 3 fold intensity increase for one of the carbon 13 signals, by saturating a proton signal. We saturated a proton transition and enhancement in both the carbon doublets we saw. Of course one is negative in sign, I did not see the enhancement, up till now we have not seen, we will see later.

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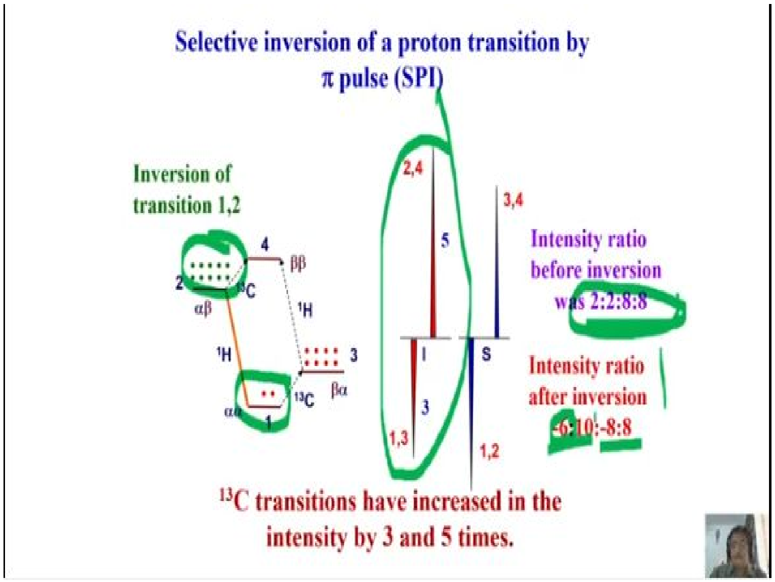

Selective inversion of a proton transition by π pulse (SPI)

Inversion of transition 1,2

Intensity ratio before inversion was 2:2:8:8

Intensity ratio after inversion -6:10:-8:8

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

And we saw for one of the signal, one of the component of the doublet, enhancement in signal was seen.

Now selective inversion of a proton transition; let us see what happens, in the heterionuclear case. There we saturated, now we will invert it, the 10 spins which I assumed to be here, I took it there, but 2 spins which were there, I brought it here, that is all I did. How do you do; by applying a pi pulse, we know that, when you apply a pi pulse, a magnetization which was here will come back; from z axis to minus z axis, just gets inverted, as a consequence the spin population gets inverted that is all.

Now, recalculate the intensities, very interesting thing happens. Now the intensity ratio turns out that, before inversion it was 2, 2, 8, 8; 2, 2 for carbon and 8, 8 for proton, now after selective inversion of one of the proton peaks, it became -6, +10, -8, -8; what a change !!. See, carbon signal which were 2, 2 became one is minus 6 and other is plus 10; there is an enormous change in the intensity, of course one is negative phase, do not worry, we will worry about this negative signal later, but there is an enhancement in the signal intensity, that is what is important.

Look at it, all we did is, saturated; I am sorry, inverted one of the proton signals, one of the protons peaks of the coupled doublet, then we see the carbon 13, there is a drastic change in the signal intensity. Now, this is really 3 to 5 times enhancement is there, 2 becomes 6, and 2 become 10 here, with the opposite phase, okay.

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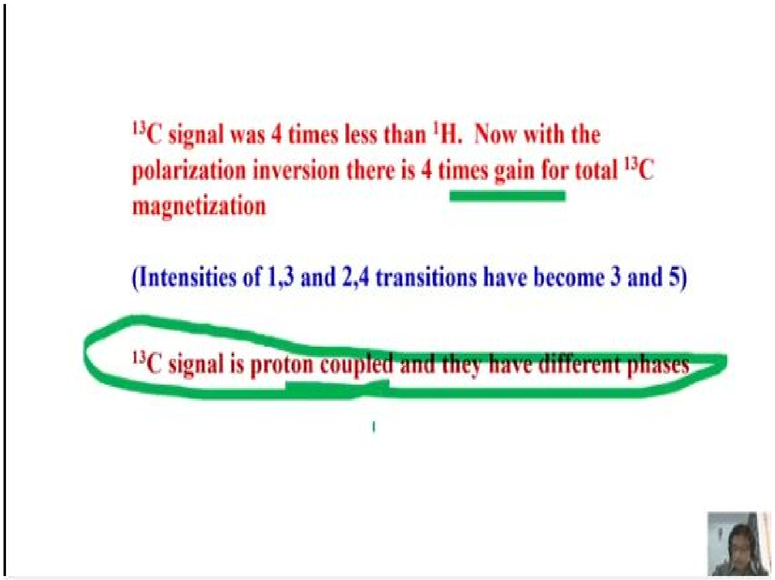

^{13}C signal was 4 times less than ^{1}H. Now with the polarization inversion there is 4 times gain for total ^{13}C magnetization

(Intensities of 1,3 and 2,4 transitions have become 3 and 5)

^{13}C signal is proton coupled and they have different phases

So, intensities have become 3 and 5, gain is much, much more, it is approximately 4 times gain is there. It depends upon the gamma of the proton, gain is depending upon the gamma of the proton. Carbon 13 signal is proton coupled, here, you see, as a consequence the doublet component we saw as opposite phase. But this is not what we want.

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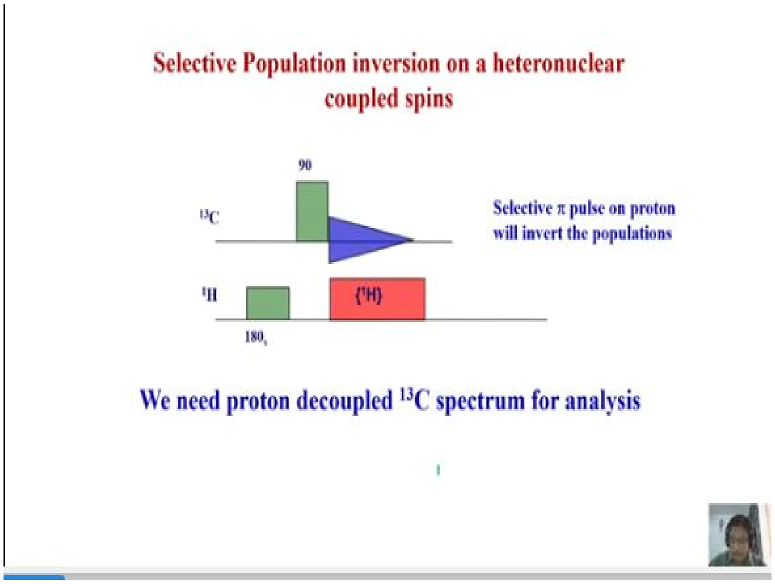

Selective Population inversion on a heteronuclear coupled spins

Selective π pulse on proton will invert the populations

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

Remember, if we, in the previous example, when we did the APT and other things, you are understanding, what happens. If I take this spin echos sequence, you understood from the vector diagram, when you have anti phase components of the multiplets, immediately we start decoupling, the vector addition of the opposite signals is such that the gain is nullified, that is what we observed, we do not get the gain, then whatever we have gained is lost but that is not our aim.

Our aim is to maintain the gain, at the same time, we wanted to do decoupling also, otherwise you know spectrum becomes complex. Our aim was to get the decoupled carbon 13 spectrum at the same time, see that gain is retained. So what we are going to do further, see this is what it is, if we are not doing the decoupling, we are going to get doublet.

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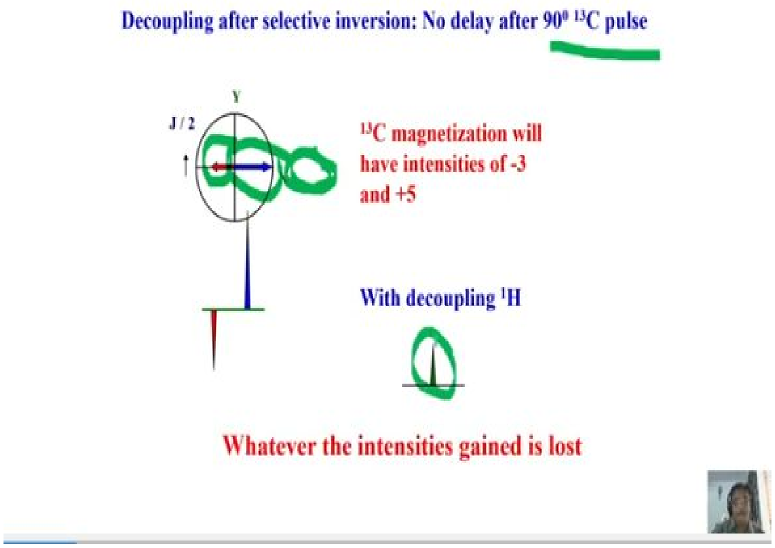

Decoupling after selective inversion: No delay after 90⁰ ^{13}C pulse

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

With decoupling ^{1}H

Whatever the intensities gained is lost

But what happens if I start doing the decoupling here? Look these are the signals, just after 90 degree carbon-13 pulse, there is no delay, this is what you observed, -3 and +5 as the 2 components of the carbon 13 doublet which are opposite in phase, anti-phase character, -3, +5. Now, I do the decoupling, my receiver is here, detector is here, I am sitting here and watching, they add up; the vectors in the opposite direction add up, in such a way the difference is only retained, gain is totally lost, that is not what you want.

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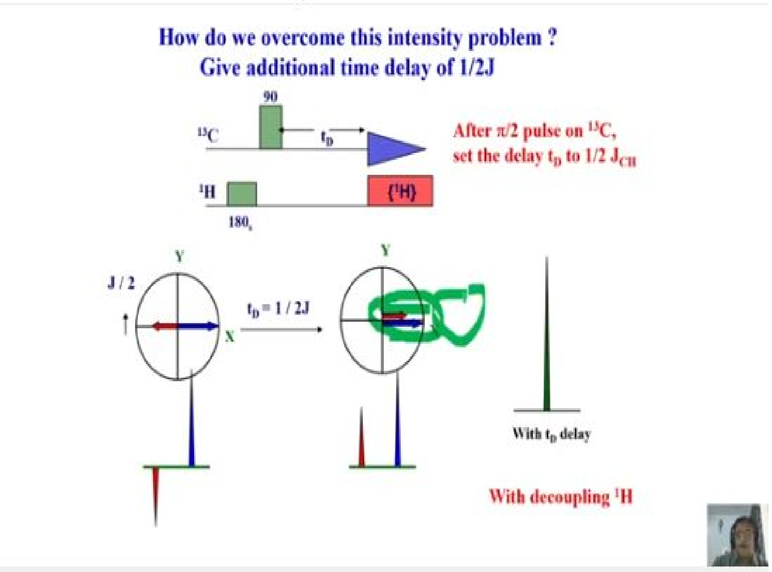

How do we overcome this intensity problem ?

Give additional time delay of 1/2J

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

Of course, how do you overcome this? I said we can give a delay, when we give a delay like this, what is going to happen is; we understood by the energy level diagram previously, the,minus and plus components, if we give some delay, they will start moving in the other directions, after some time they will come and overlap here, in the sense they come in the same phase on the x axis, this is where my detector is there.

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Insensitive Nuclei Enhancement by Polarization Transfer (INEPT)

And now if I do the decoupling, they are not going to get nullified, they get added up, okay. So this is what we observed, we are going to get the gain in the intensity just by putting a delay there and antiphase components we converted into inphase components, you understood now.

There are 2 ways for doing a polarisation transfer; selective population transfer, selective saturation by saturating one of the signal, we transferred the population; that is SPT, selective population transfer.

Other is selective population inversion, we can invert one of the signals and see how the signal is transferred and SPI is very good because we got maximum signal intensity; and these 5 plus 3 added up, it is 8, so earlier intensity was 4, I am sorry, 2; 2, 2, 8, 8, so 4 times gain in the intensity we have got. Why 4 times gain; gamma of proton to gamma of carbon is 4 times, so we get almost 4 times gain in the signal intensity here. So, now we use this for understanding INEPT.

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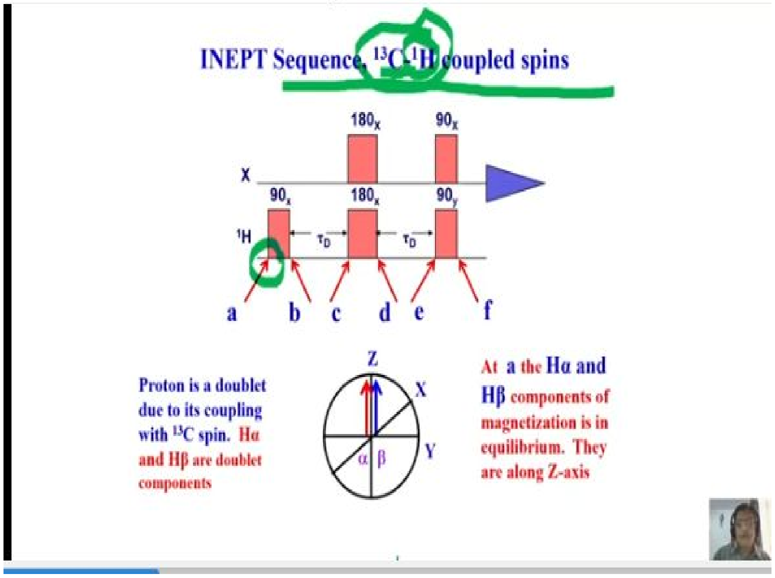

INEPT Sequence, ^{13}C-^{1}H coupled spins

Proton is a doublet due to its coupling with ^{13}C spin. Hα and Hβ are doublet components

At a the Hα and Hβ components of magnetization is in equilibrium. They are along Z-axis

What is an INEPT? INEPT sequence is like this, it is the sequence for doing the polarisation transfer; and here I am taking the example of the carbon proton coupled spin system. How the polarisation transfer is going to help us, not only in gaining the signal intensity but in the spectral editing. We can incorporate that sequence in most of the 2 dimensional, 3 dimensional experiments, this INEPT sequence is always utilised. Let us understand how the magnetisation behaves in different parts of the pulse sequence.

Let us start with a, at the point a, what is happening in the proton magnetisation; remember proton is coupled to carbon, so it is a doublet, there are 2 alpha and beta components here, okay.

So both alpha and beta components are aligned along z axis, they are in thermal equilibrium, Now there is no difference at all, just thermal equilibrium, magnetisation is split into doublet because of carbon 13; 2 components, alpha, beta.

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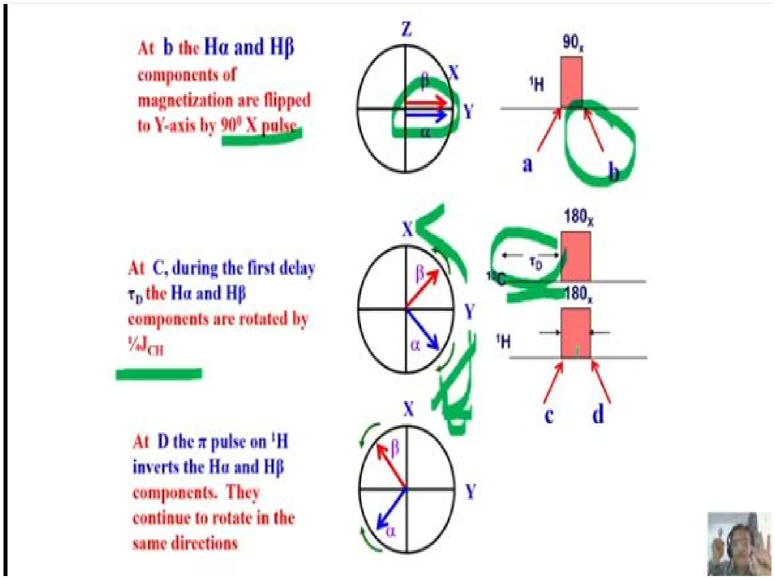

At b the Hα and Hβ components of magnetization are flipped to Y-axis by 90^0 X pulse

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

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

Apply a 90 degree pulse, this is the situation at a, here this one, okay. Now apply a 90 degree pulse, being the magnetisation vector to y axis, I have done that, now flip the magnetisation to Y axis by applying a 90 degree X pulse, that is position b at the point; this is what happen at the point b; immediately after the 90 degree pulse. Now, what I will do is I will start waiting, I do not do anything, I will simply keep quiet, then what happens?

This is the point C, there is a delay between B to C, these alpha and beta components of proton start dephasing, one moves like this, other moves like this, depending upon their Larmor frequencies; because when it is the doublet, you know one is at the higher frequency, other is at the lower frequency from the center of the chemical shift position, we know that. Okay; so that is what happens, as a consequence they start rotating.

Let us say after a time delay τD, they have rotated by 1/ 4JCH. Now at time D; that is just after time C, what we have done is we have applied two 180 pulses simultaneously on proton and also carbon. Let us see what happens to the proton components; we are going to apply a 180 pulse along X, see this is Y, this is X, now magnetisation is moving here and here, in the Y axis. Now, in the X axis I rotate by 180, what happens?

This component is here, this component is here; one which was here came here, the one which was here came here; but they continue to precess in the same direction. You understand what is happening to the proton; proton spin vectors which was brought here, they start dephasing like this and like this, they start dephasing like this; and the rotation on the X axis like this is like turning the pan cake. The vector start moving, it was here and here, now they are here and here, simply gets inverted. So, now beta component is here, alpha is here, they continue to move in the same direction. That is what is happening as far as the proton vectors are concerned.

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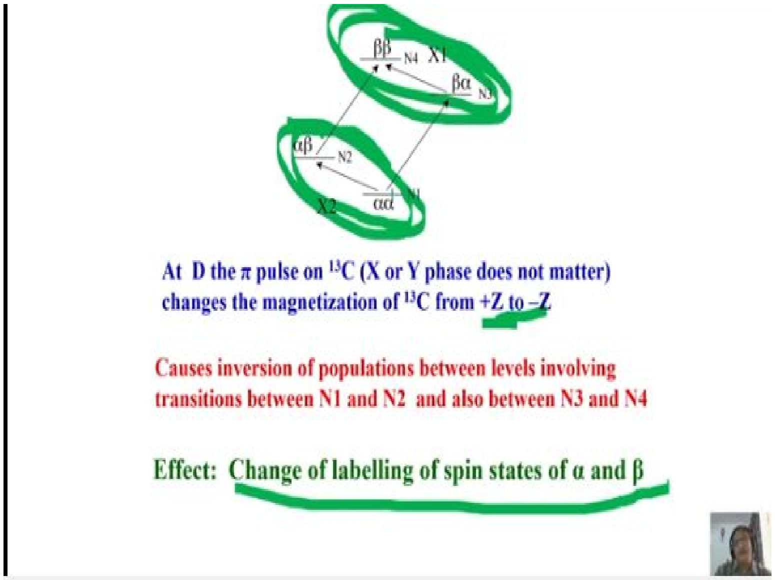

At D the π pulse on ^{13}C (X or Y phase does not matter) changes the magnetization of ^{13}C from +Z to -Z

Causes inversion of populations between levels involving transitions between N1 and N2 and also between N3 and N4

Effect: Change of labelling of spin states of α and β

So, now at the same time, we apply pi pulse on carbon 13, what is happening with pi pulse and carbon 13? Now we have not chosen any particular resonance, right, one particular peak, so we are applying a pi pulse. I do not know which phase we are going to apply, does not matter, what will happen; you are inverting the magnetisation. So that means these 2 and these 2 spin populations should get inverted, inverted that means, carbon 13 which was still along the Z axis, we are not touched it till now, remember, you are only talking about proton doublet component, carbon doublets are still here. Now because of 180 pulse, from +Z direction, they came to -Z direction. It is as good as telling the population of the spin states are reversed, or okay you can say they got inverted. Now, N1, N2 populations and N3, N4 populations gets reversed.

Essentially it means, we are labelling this spin states of alpha and beta, whatever was there, it is changed now, alpha and beta, spin states labelling gets changed.

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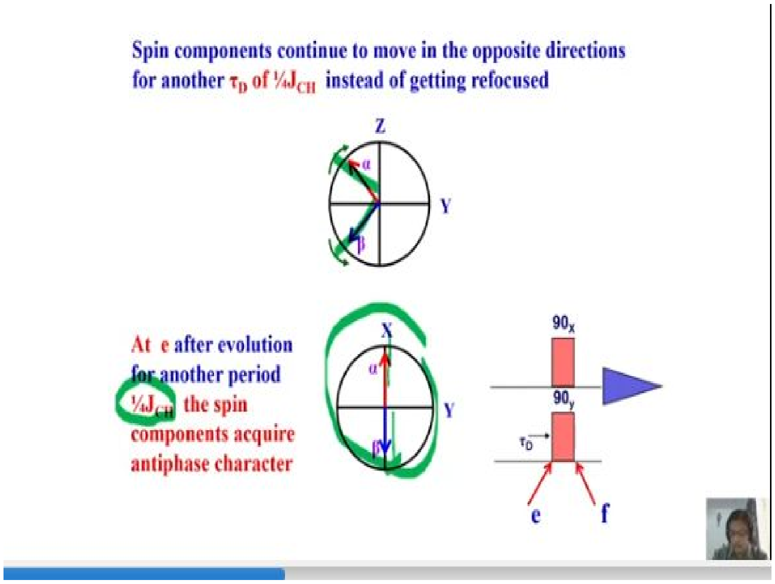

Spin components continue to move in the opposite directions for another τ_D of $\frac{1}{4}J_{CH}$ instead of getting refocused

At e after evolution for another period $\frac{1}{4}J_{CH}$ the spin components acquire antiphase character

So, the spin component continue to move, what did we do; by interchanging carbon 13, now we interchange the labellings of spin states of proton. so that started moving in the opposite direction, remember before that they were coming here, because you are applying a 180 pulse, we interchanged the spin population. They are coupled spins, you know they are coupled to protons. This doublet components of carbon is because of proton; and proton doublet is because of carbon. Now we are inverting this means, we have changed the labels of spin states of alpha and beta of proton; that is what happens. Now, the spin component continues to move in the opposite direction, what does it mean? alpha and beta spin component varied means, fast and slow moving components will interchange, so the ones which are going in the direction like this, they start move in the opposite direction, the fast component becomes slow, slow component becomes faster now, okay, now let them continue to evolve like that for some time.

After sometime, you see that again another 4/ J if you allow it to evolve, one which was here and here; they come exactly opposite, in the sense the alpha component is along this axis and beta component is along this axis; exactly opposite, antiphase character, you understand. The vectors are in opposite direction, this is the antiphase character, this is at a point e, after time delay, when 180 pulses were applied on both, proton and carbon.

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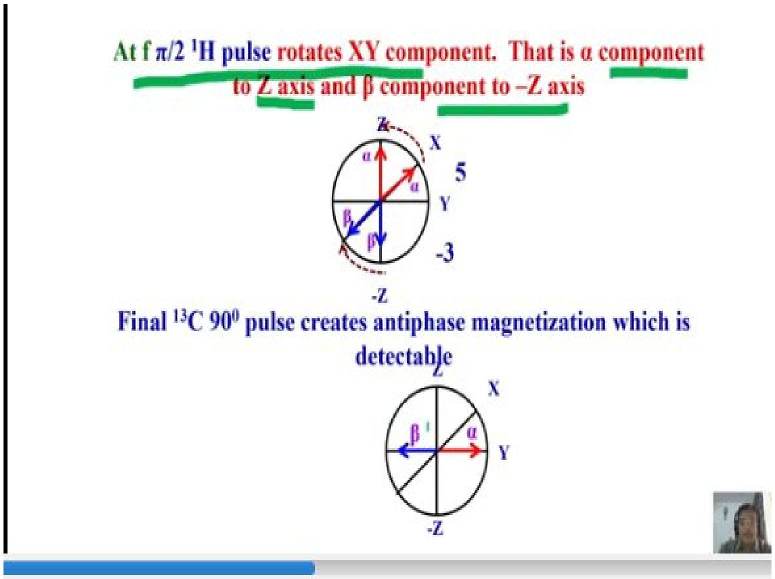

At f $\pi/2$ ^1H pulse rotates XY component. That is α component to Z axis and β component to –Z axis

Final ^{13}C 90^0 pulse creates antiphase magnetization which is detectable

Now, at this point, we do another trick, immediately when they are at this position, antiphase position, we do two things, we apply a pi/2 pulse on proton here, pi by 2 pulse proton, what is going to happen now? they start rotating, the magnetisation which was on XY rotates alpha component to Z axis and beta component to minus Z axis. Remember, what is going to happen, here alpha component which was here along this axis, as soon we apply a 90 pulse on X axis, you can work out by right hand thumb rule, it goes to the plus Z axis.

This component goes to minus Z axis, they start rotating like that. Now finally carbon 13 pulse you are going to apply, which creates antiphase magnetisation which is detectable, carbon 13 pulse is applied, 90 degree pulse, so it creates antiphase magnetisation, you are detecting the signal.

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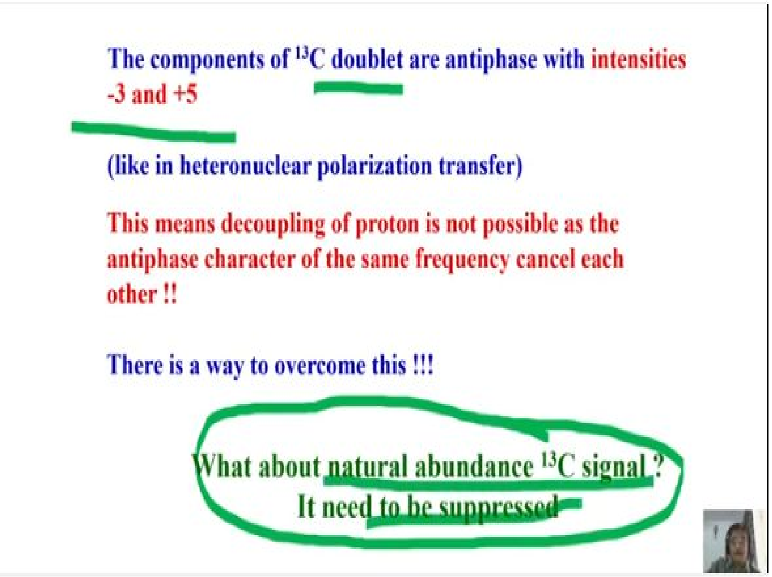

The components of ^{13}C doublet are antiphase with intensities -3 and +5

(like in heteronuclear polarization transfer)

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

There is a way to overcome this !!!

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

Now, what is going to happen; the components of carbon 13 doublets are antiphase, intensities are minus 3 and +5, remember; okay, this is moved like this, carbon 13 finally brings it to this axis. carbon 13 90 pulse, now as a consequence, the carbon 13 signal which has acquired antiphase character, this is minus 3 and this is plus 5, on this axis is my detector, it is detectable, it is the detectable magnetisation, okay.

So, what does it mean, we cannot do the decoupling, remember I said earlier, if you have antiphase components and you start doing the decoupling, because of antiphase component vector addition is there, in the opposite direction, whatever the gain you got is lost. We will overcome this later but we should also remember there is one important point there, in the sense, in addition to this type of polarisation transfer, there is also natural abundance carbon 13 signal which is there. When you are detecting carbon, not only you are detecting the gain here because of polarisation transfer, there is also natural abundance carbon signal, we have to suppress that.

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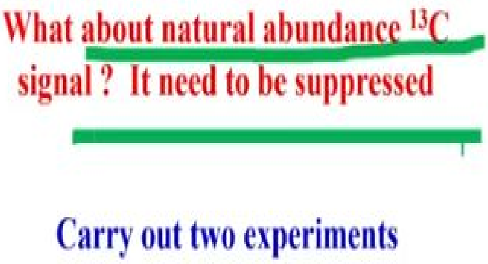

What about natural abundance ^{13}C signal ? It need to be suppressed

Carry out two experiments

So, how do you supress that; we can carry out 2 experiments for suppression.

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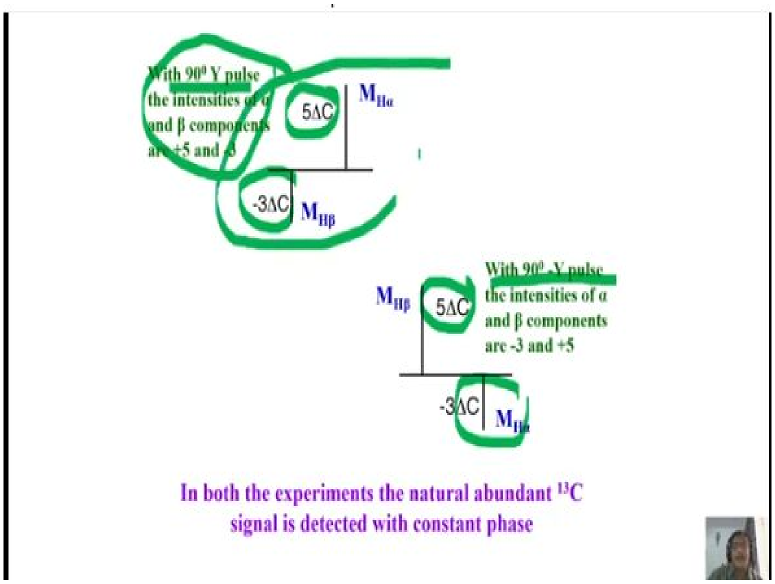

With 90^0 Y pulse the intensities of α and β components are +5 and -3

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

One; we do an experiment with 90 degree Y pulse, what happens; then alpha and beta components are +5 and -3 in the intensity ratio, understand. the carbon doublet components of the carbon, one is +5, another is -3. I have assumed that I have applied pulse on Y axis, 90degree Y pulse. On the other hand I can also do another experiment with 90 degree -Y pulse here. In the last pulse what I am applying, I can apply once 90 +Y, another 90, -Y; minus. In the 90 +Y experiment, this is positive and this is negative, in the 90 -Y, the components gets interchanged. Now +5 is here and this is negative, alpha beta components get interchanged, intensity gets reversed. Now, what you are going to do? In both the cases, in addition to these what are we getting?

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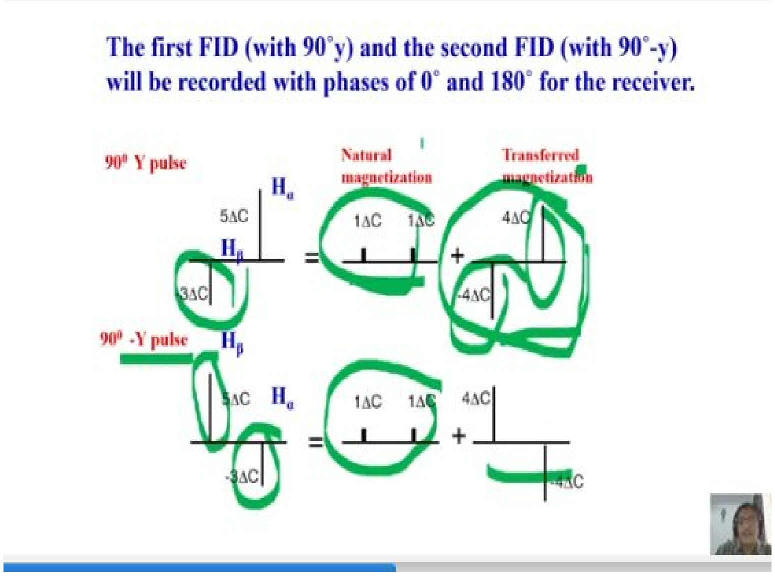

The first FID (with 90°y) and the second FID (with 90°-y) will be recorded with phases of 0° and 180° for the receiver.

In this experiment we get this signal, okay, natural component is also there, this is one intensity, this is +5, this adds up, because this is all in phase, natural signal of carbon is in phase; what is going to happen; this will add, now this and this if it is present, finally what is going to happen the transferred mangetisation, turns out to be 4 and this gets subtracted, it will become -4, because natural magnetisation is adding for that and it is creating some trouble for us okay.

So, what we will do is; in fact, this is even a worst situation, at this place if you do decoupling, you will get completely 0 signal. okay now, apply 90 degree in minus Y pulse, now alpha, beta components are interchanged, now this is +5, this is -3 in intensity. Of course natural component with inphase is still present, now if you add you will get this thing, again very interesting thing. These are 2 experiments we did, in both the cases, we are going to get these things.

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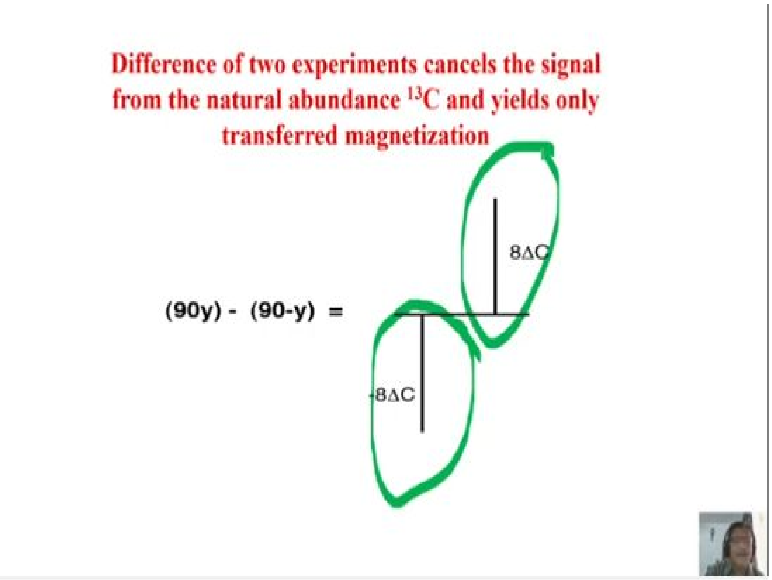

Difference of two experiments cancels the signal from the natural abundance ^{13}C and yields only transferred magnetization

(90y) - (90-y) =

But what you will do is; we will take the difference of the 2 experiments, this and this, now if I take the difference, what will happen, this will get nullified, because it is in the same phase, same intensity and this will get added up and this is what we get. If I take the difference of the 2 experiments, we get this signal, -8 intensity and this signal +8 intensity. Of course antiphase character is still maintained. But now, you will see the gain, 8 intensity, what was intensity when we started before, it was 2, compared to proton, one fourth t was less; but now it is 4 times gain.

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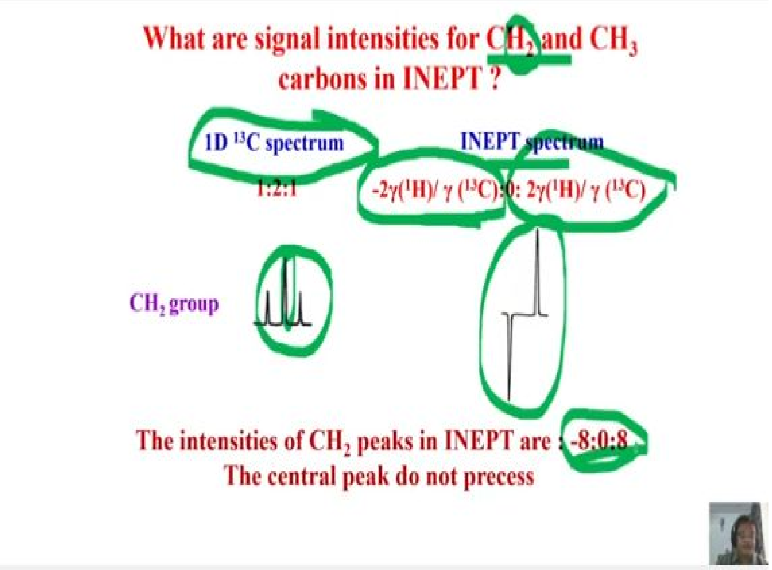

What are signal intensities for CH_2 and CH_3 carbons in INEPT ?

1D ^{13}C spectrum

1:2:1

INEPT spectrum

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

CH_2 group

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

The central peak do not precess

Why 4 times gain; it has a relation with gamma, gyromagnetic ratio. We can understand how it is related to gyromagnetic ratio as a consequence, it became more. Now you look at the intensity pattern for different signals. In the conventional carbon 13 NMR, 1D NMR. what is a pattern for CH2, carbon coupled to 2 equivalent protons, is a triplet, no doubt about it, we know that. Whereas in the INEPT case, we get a character signal like this, why? Reason is the central component of this triplet do not precess at all. It is always along this axis, only fast moving slow moving components start dephasing, moving in the opposite directions.

But the one which is at centre will not precess at all, it is along the same axis always, so as a consequence that is not affected. Whereas, here this one, this is the gain of intensity by this ratio, this is the gain of intensity by this ratio, I worked out, if you work out, it turns out to be -8, 0, +8, this is exactly what we saw here, so this is the gain we get for CH2 peak.

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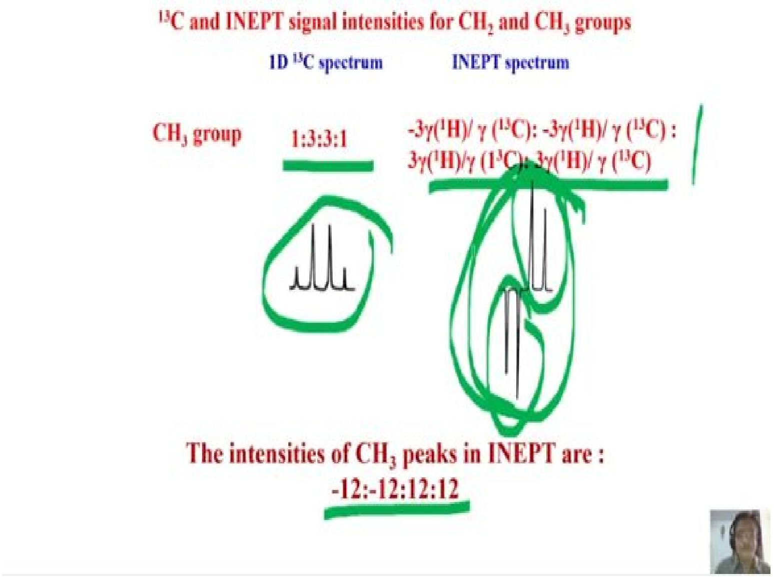

^{13}C and INEPT signal intensities for CH_2 and CH_3 groups

1D ^{13}C spectrum

INEPT spectrum

CH_3 group

1:3:3:1

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

The intensities of CH_3 peaks in INEPT are : -12:-12:12:12

And what about the CH3 peak, CH3 peak it is 1, 3, 3, 1 intensity in the conventional carbon 13 spectrum without INEPT. With INEPT exactly it behaves like this, now there is no central component, 2 components moving in the higher Larmor frequency, 2 with lower Larmor frequency, they start moving in the opposite directions. As a consequence it behaves like this, there are 2 negative peaks and 2 positive peaks. Again, intensity ratio can be given like this, 3 times gamma over this one, you work out and it turns out that intensity in the case of CH3 is -12, -12, 12, 12, a fantastic thing, beautiful, right.

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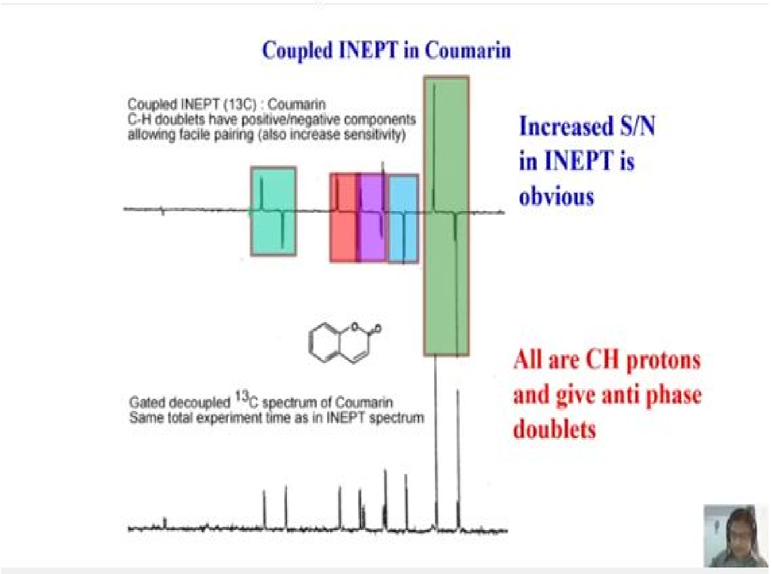

Coupled INEPT in Coumarin

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

Increased S/N in INEPT is obvious

All are CH protons and give anti phase doublets

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

Now, in the case of INEPT, we can have a coupled INEPT and decoupled INEPT, because this is so far what we have observed is coupled INEPT, till now we have not decoupled, moment we will do the decoupling, we know the signal get lost and this is what happens, you see, we get antiphsae components always, this is antiphase, this is antiphase, this is antiphase, this is antiphase, they are always, of course it is a coupled INEPT.

Only thing is you have to take the centre of this for the carbon chemical shift and of course you will get 2 peaks like this. But that is not a great achievement because the signal to noise ratio only when we add up there is a gain, so coupled INEPT is not most advantageous thing as far as we are concerned.

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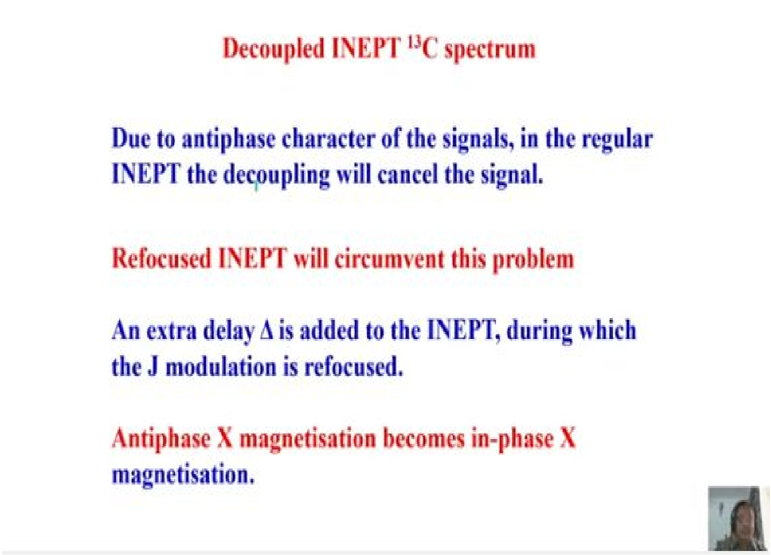

Decoupled INEPT ^{13}C spectrum

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.

So, what we will do; we have to do the decoupling, how do you do the decoupling? We have to make sure the antiphase components which were in the opposite directions should come in phase, so they would not get cancel out. How to do that; this is done by what is called refocused INEPT.

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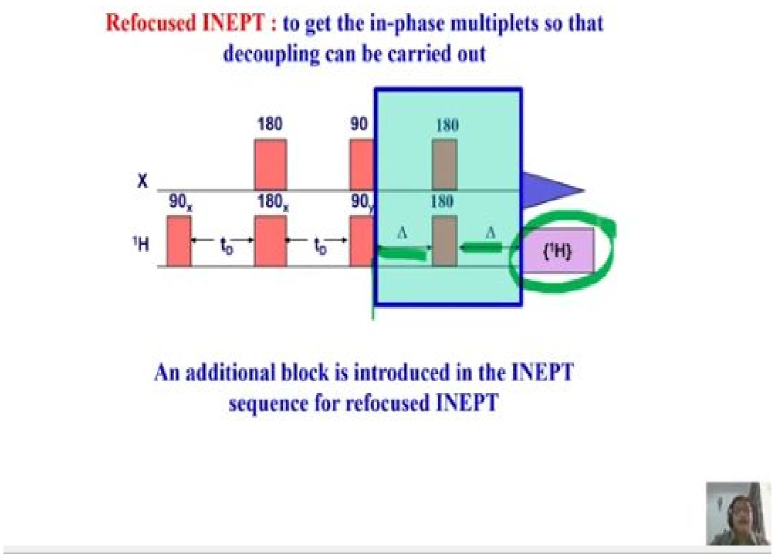

Refocused INEPT : to get the in-phase multiplets so that decoupling can be carried out

An additional block is introduced in the INEPT sequence for refocused INEPT

What we do in this case, up to this is known, now we add an extra block like this. What is this extra block doing? this extra block with this delay, 180 pulse on both proton and carbon with another delay , then collect the signal, at the same time switch on the decoupler. This will take care of cancellation of the signal due to antiphase components.

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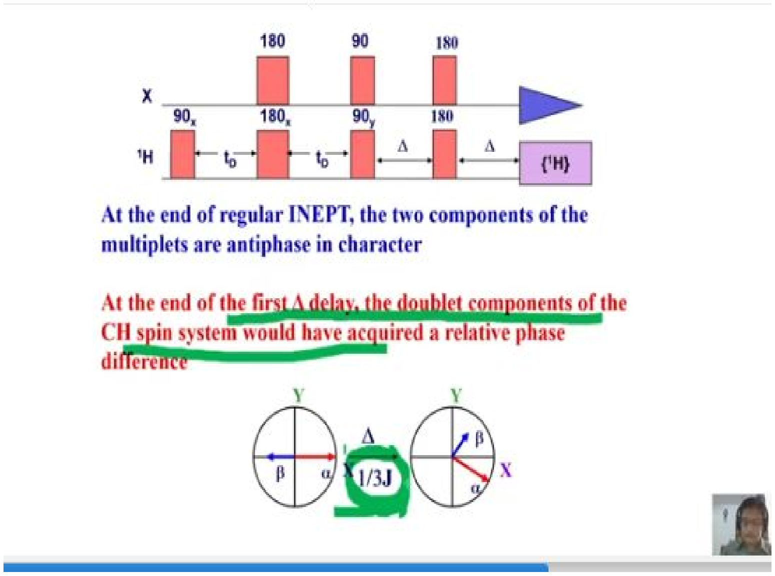

At the end of regular INEPT, the two components of the multiplets are antiphase in character

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

How it works? Very simple, of course we know we have already worked out in the previous case in the many examples of APT and others we worked out; what happens when we give a delay. Just to tell you at the end of the first delta delay, doublet components of the CH spin system would have acquired a phase like this, okay. Now, we give a delay, delay how much we give is in my hands, we know how to give, this 1/3J is normally accepted value. Here J's are different.

For example, 1 bond J coupling between CH group is different, carbon CH2 group is different, CH carbon coupling is different, if I take CH3 group, CH carbon coupling will be different. It is because they are sp, sp2, sp3 carbon couplings. I pointed out in carbon 13 NMR, the coupling strengths are different, so what to do, how do you adjust the delta; by and large you have to assume that they are nearly equal and take average of it; one third of J is by and large which is known to be good. This serves your purpose, put that delay.

When you put one third of J, what happens? if it is one fourth it comes here, half it comes here, one third of delay is somewhere here, magnetisation vectors have moved away. They have acquired a phase difference, 2 components; beta and alpha they have moved from here. It started from here, it move like this, this started from here it move like this; and then after sometime, you will see they would have acquired some phase difference, okay.

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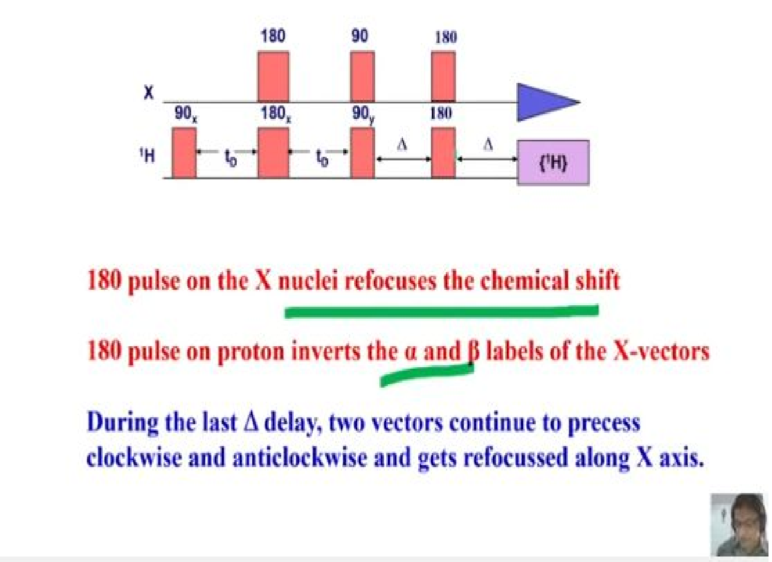

180 pulse on the X nuclei refocuses the chemical shift

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.

And 180 pulse on the X, channel, X nuclei, refocuses the chemical shift. The 180 pulse on the proton inverts the alpha, beta spin labels of the X vectors, see they started acquiring the phase and after sometime, the 180 pulse and X nuclei refocuses the chemical shift and again the labels get interchanged. When the label get interchanged, in the last delay what happens?

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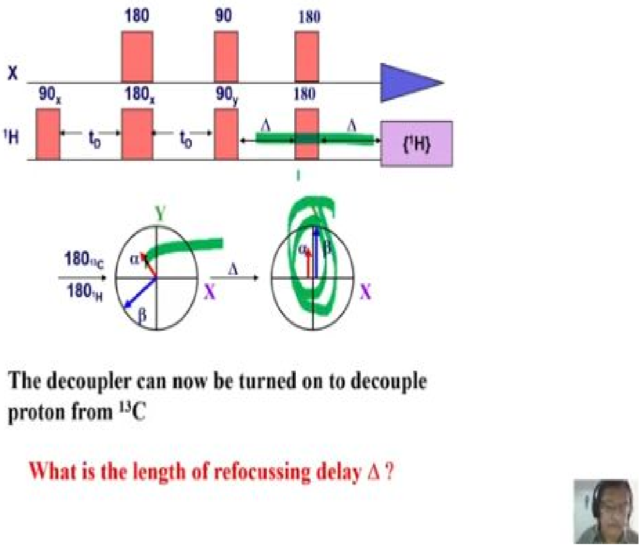

The decoupler can now be turned on to decouple proton from ^{13}C

What is the length of refocussing delay Δ ?

The spin vectors continue to move in the same direction. After sometime both of them will get aligned along this axis. Now, what happens? they are in phase. you understand what is happening now? they are in phase. The antiphase component by applying 2 pulse and giving 2 delays, we converted them into inphase magnetisation.

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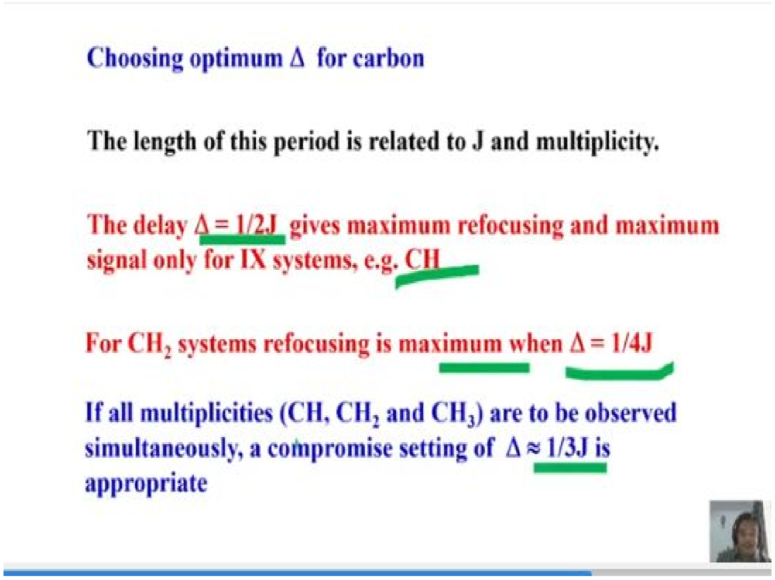

Choosing optimum Δ for carbon

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

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

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

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

Now, you can choose your optimum delay which is equal to some value, now both are in phase, vector addition enhances the signal, it will not nullify now. As a consequence we can get a signal with proton decoupling, we can get the carbon with proton decoupling. So, what is the length of this J delta, of course as I said average value is one third; but you can also do the editing with this, delta 1/ 2J gives you only maximum signal for CH. For CH2, delta 1 /4J gives maximum signal and for 1/ 3J all are positive I would say, all are seen there.

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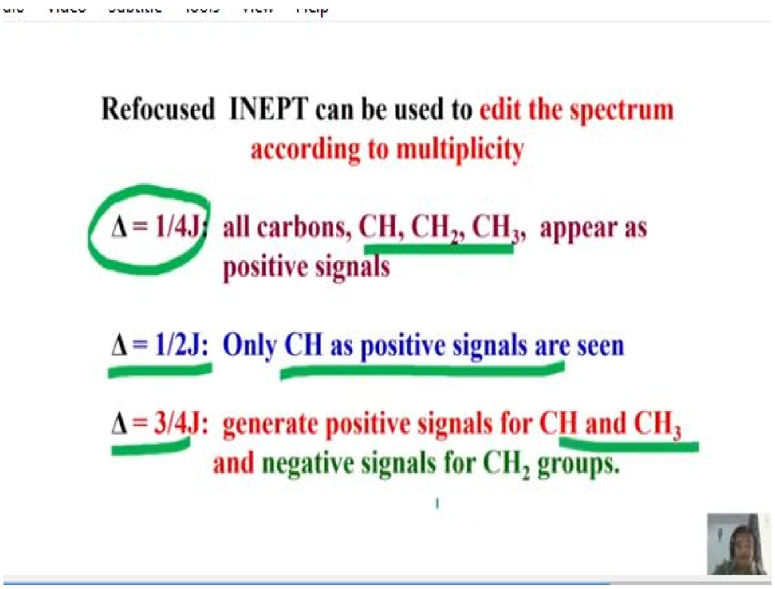

Refocused INEPT can be used to edit the spectrum according to multiplicity

$\Delta = 1/4J$: 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.

So, we can use this idea for doing editing also, this is called refocused INEPT. In the refocused INEPT what you can do is; take delta which is equal to 1/ 4J, all carbons appear as positive, no problems, put delta is equal to 1/ J, only CH is positive, all others are not seen, only CH is seen as positive signal, all other becomes 0, put delta is equal to 3/4th of J, CH and CH3 are positive and CH2 is negative.

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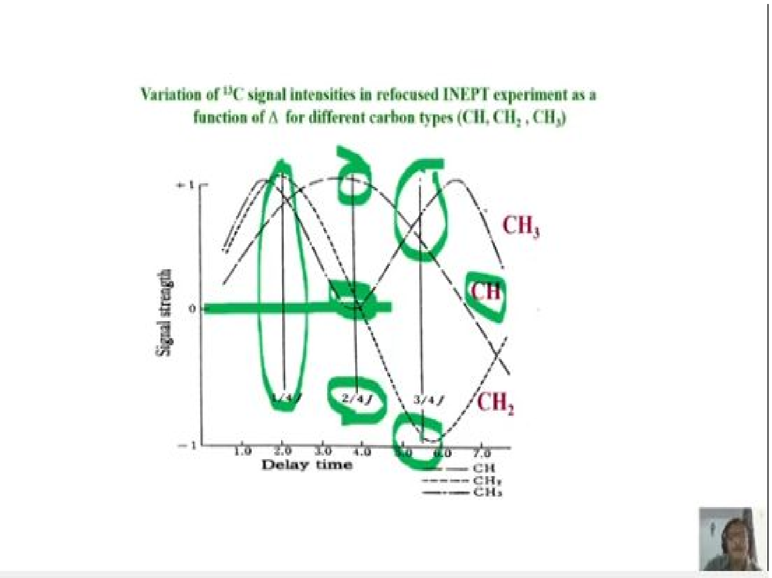

Variation of ^{13}C signal intensities in refocused INEPT experiment as a function of Δ for different carbon types (CH, CH_2, CH_3)

You can see that by the intensity plot as a function of delay time, And you see that, look at it, when it is 1/4th of J, all are positive, exactly at 1/ 2J, see CH2 and CH3 both are 0, both are 0, it is here, whereas CH is positive signal, maximum positive. Now, come to 3/4th of J, what is happening; CH2 is negative, CH3 and CH are positive, so you can do this for identification of carbons based on the proton multiplicities, how; by choosing the delay delta.

We can choose appropriate delta and get all positive or you can get only CH, nullify this or you get 2 positive, 1 negative. Similar to what you get in other refocused, in other experiment like APT.

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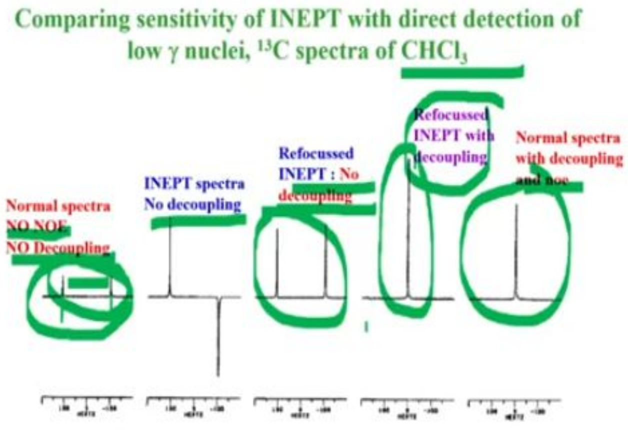

Comparing sensitivity of INEPT with direct detection of low γ nuclei, ^{13}C spectra of $CHCl_3$

So, what is advantage here, look at it, this is normal NOE spectrum, normal spectrum without NOE, without decoupling, It is carbon 13 spectrum of CHCl3, it is a doublet, because of coupling to single proton, no NOE, no decoupling, simply acquire the spectrum with the one pulse, we are going to get doublet, this is JCH; and this is the intensity pattern; signal to noise ratio. Same thing INEPT spectra, no decoupling, +4, -4 intensity is there.

Refocused INEPT; no decoupling, only thing we made antiphase components as inphase components, there is not much difference to be discussed about here between this and this. Whereas look at the refocused INEPT with decoupling, now these 2 will collapse, add into a single peak, see enhanced intensity. You get the point, the refocused INEPT has enhanced intensity. Now really we have to compare this with the normal carbon 13 spectrum with decoupling and with NOE.

I said in the decoupling when I was explaining carbon 13, generally practiced is we acquire carbon 13 spectrum with decoupling and NOE; that is the decoupling experiment of carbon where we are going to see NOE also.

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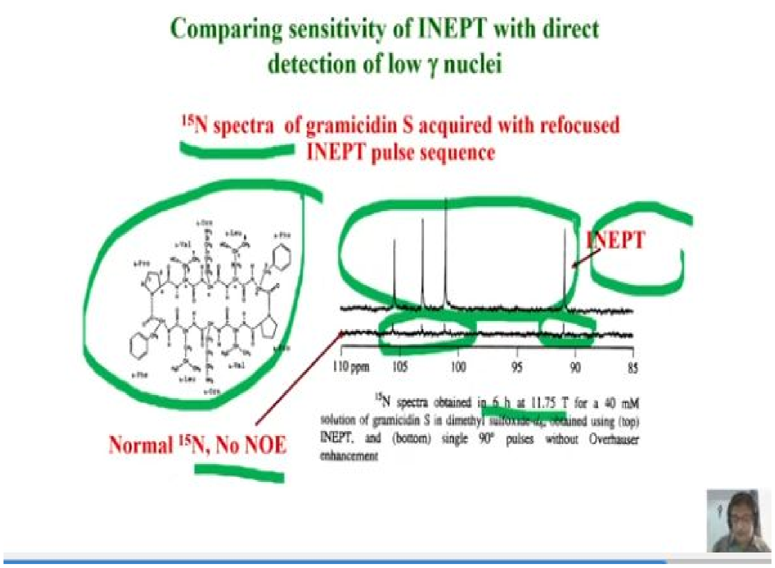

Comparing sensitivity of INEPT with direct detection of low γ nuclei

^{15}N spectra of gramicidin S acquired with refocused INEPT pulse sequence

^{15}N spectra obtained in 6 h at 11.75 T for a 40 mM solution of gramicidin S in dimethyl sulfoxide-d_6, obtained using (top) INEPT, and (bottom) single 90° pulses without Overhauser enhancement

Normal ^{15}N, No NOE

That is actual signal which we get. Now compare that signal with INEPT, dont you see the enhancement of the intensity, this is the advantage of INEPT. So this is the polarisation transfer technique just to compare a spectrum like this for a molecule like this, big molecule. Of course I told you this polarisation transfer experiment, I took example of proton and carbon, does not mean it is applicable only to this, this is a general method I discussed, it can be between any abundant spin to any low abundance spin, polarisation transfer is possible.

It can be proton to nitrogen or proton to phosphorous, phosphorus to something else, anything we can do, you understand. So any low abundant spin you can take, aor low gamma nuclei also and you can do this thing. Okay and look at it, this is the conventional 15N spectrum, no NOE, this is what you see, it takes ages to acquire, it takes nearly 6 hours to do this thing, whereas INEPT experiment gives enhancement like this, see the advantage of polarisation transfer.

NMR is basically insensitive technique, compared to other spectroscopic techniques but still we could do something and get the enhancement just by playing with the magnetisation and spin dynamics and we got. And this is a significant advantage and if the intensity goes up by 3 to 4 times, your experimental times comes down by square of that. Instead of doing the experiment for 1 day, we may finish it in less than 1 hour, it is the biggest advantage, time saving factor.

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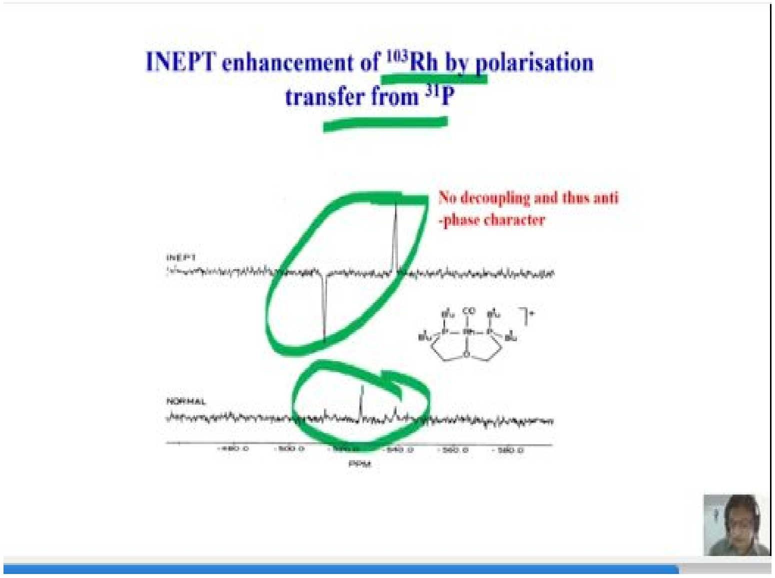

INEPT enhancement of ^{103}Rh by polarisation transfer from ^{31}P

No decoupling and thus anti -phase character

So, you see in an example like Rhodium polarisation transfer from phosphorous, this is of course not decoupling, but with anti phase character, this is the conversional spectrum, see the difference in the intensity, lot of enhancement.

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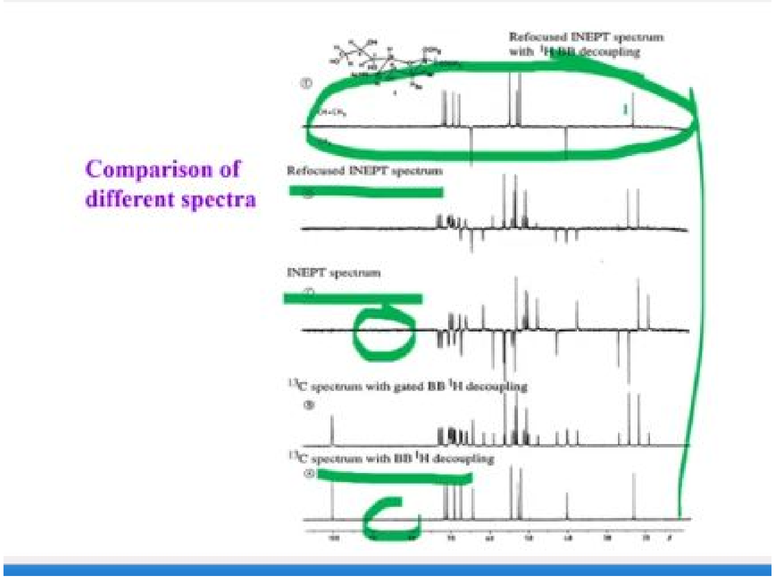

Comparison of different spectra

Refocused INEPT spectrum with ^{1}H BB decoupling

Refocused INEPT spectrum

INEPT spectrum

^{13}C spectrum with gated BB ^{1}H decoupling

^{13}C spectrum with BB ^{1}H decoupling

And this where you can compare varieties of spectra, different types carbon 13 decoupling, no decoupling, INEPT, refocused INEPT and all these. You do not compare just the intensity, you have to compare the signal to noise ratio, this signal to noise ratio we need to compare; and no doubt if you can get the refocused INEPT spectrum and with the broad band decoupling is definitely a better spectrum.

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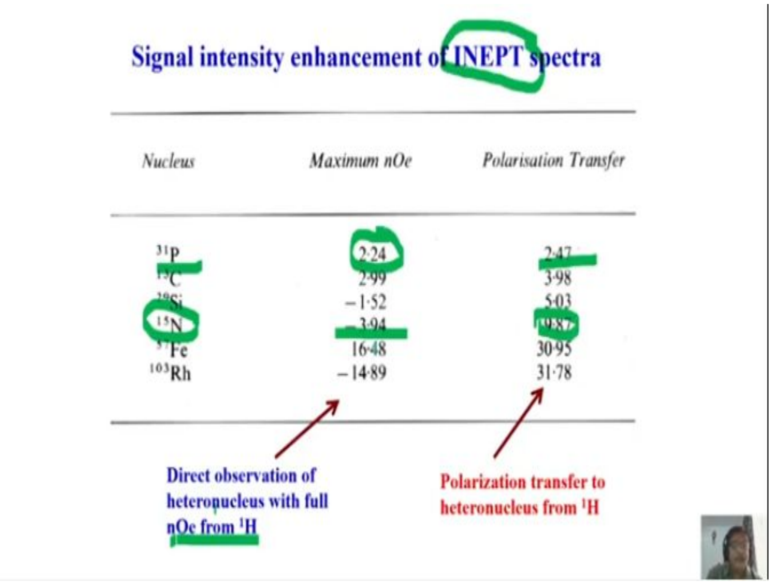

Signal intensity enhancement of INEPT spectra

Nucleus	Maximum nOe	Polarisation Transfer
^{31}P	2·24	2·47
^{13}C	2·99	3·98
^{29}Si	−1·52	5·03
^{15}N	−3·94	9·87
^{57}Fe	16·48	30·95
^{103}Rh	−14·89	31·78

Direct observation of heteronucleus with full nOe from ^1H

Polarization transfer to heteronucleus from ^1H

So, signal intensity maximum enhancement you can see different for different nuclei; phosphorous 31 if you directly observe with NOE and decoupling of proton, we get 2.24 times enhancement, if we do polarisation transfer, we get 2.47. Most important is look at nitrogen 15, see -3.94 noe, if you do the normal detection with full NOE from NOE from proton and decoupling, whereas with polarisation transfer, it is nearly 10 times gain, see the advantage, 10 times gain in the intensity, your experimental time enormously comes down, same thing is for the carbon, so this is advantage of INEPT.

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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)

So, as an extension of this, I discussed about DEPT, you know what is DEPT, distortion enhancement polarisation transfer for spectral editing. The advantage of DEPT is it distinguishes carbon like APT which I discussed, at the same time, this has the sensitivity enhancement. Sensitivity enhancement comes because of polarisation transfer similar to INEPT, this combines the benefits of both the type of experiments.

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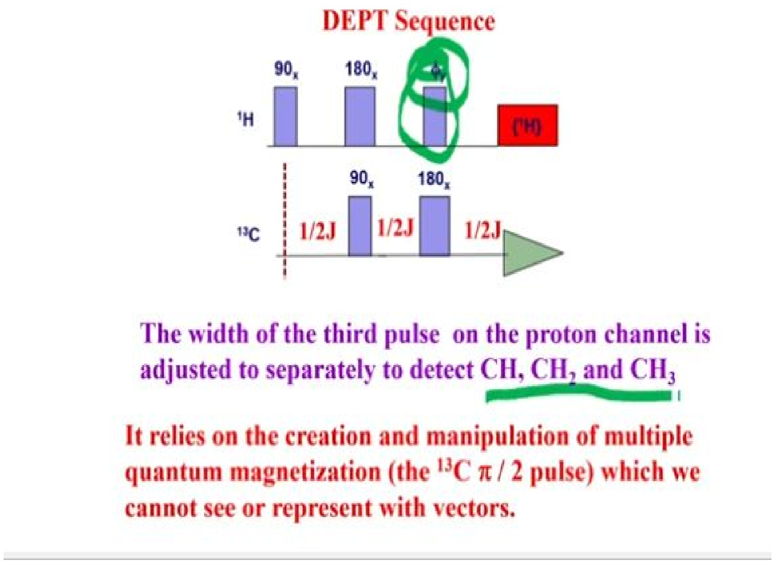

DEPT Sequence

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 π / 2 pulse) which we cannot see or represent with vectors.

So, this is most advantageous; that is why in carbon 13 NMR at the end of the class, I said about DEPT, I do not want to discuss again. But remember most important is the pulse sequence of that is the last flip angle pulse on the proton channel is with different flip angles. What we can do is; normally, 3 experiments are done in the DEPT. This phi is the one which defines the pulse angle, you do 3 experiments; phi, 45, 90 and 135. This is the commonly done DEPT experiments. It is to detect CH, CH2 and CH3 magnetisation.

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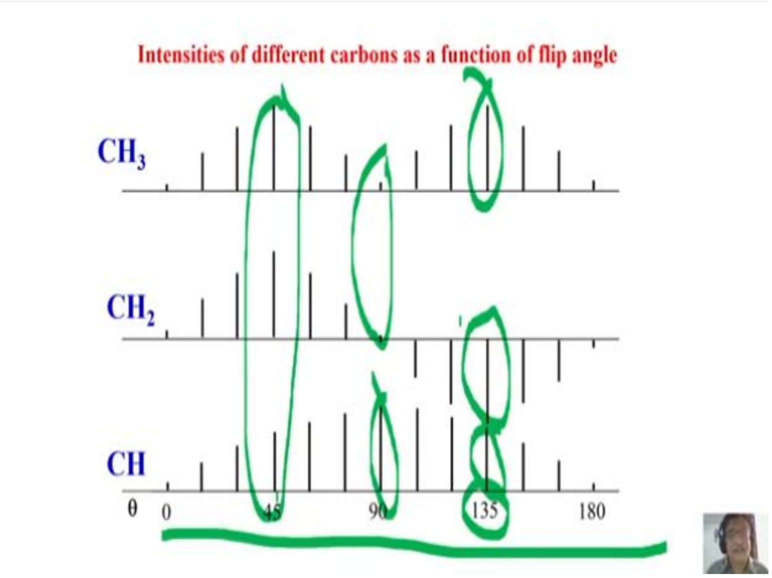

Intensities of different carbons as a function of flip angle

So, what people have done, you see these are the comparison of the intensity of different carbons as a function of flip angle theta, flip angle of this last pulse. Okay when it is done, you see intensity keeps going like this; and this is the place where theta is going to be 45 degree, all the 3 signals are positive. When theta is equal to 90 degree flip angle, these 2 are practically 0, we get only CH peak.

When theta is equal to 135 degree, this is positive, CH is positive, CH3 is positive, CH2 is negative, so based on the number of protons attached to carbon, whether odd number or even number, similar to what you saw in APT, you can distinguish the carbon by the sign of the signal whether it is positive or negative.

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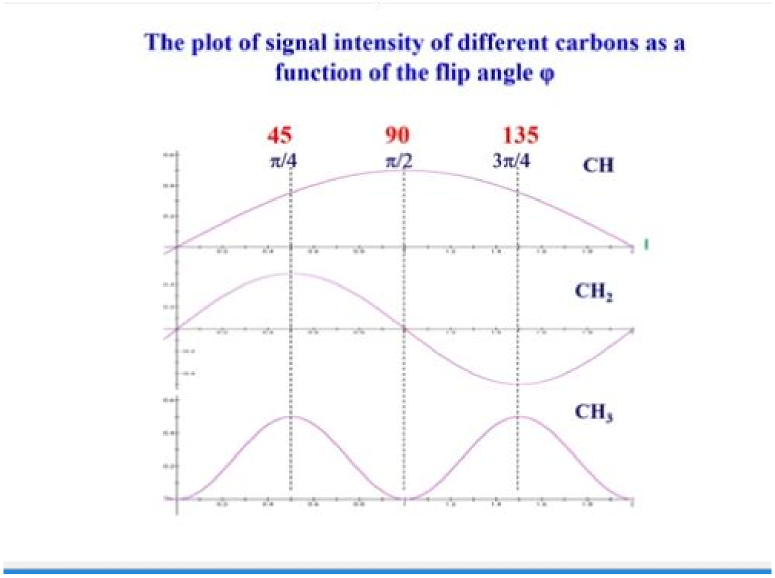

The plot of signal intensity of different carbons as a function of the flip angle φ

This is what I showed in the graph how this signal intensity is varying with different flip angle.

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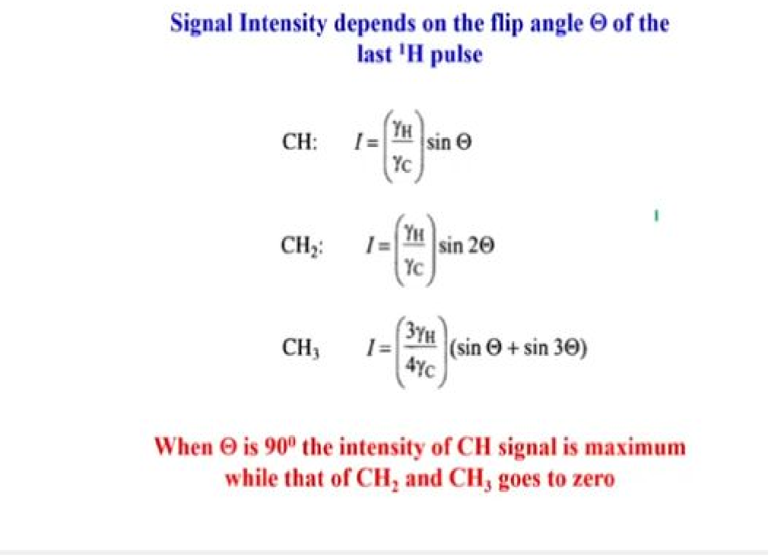

Signal Intensity depends on the flip angle Θ of the last ^1H pulse

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

Of course, it depends upon sin theta, whereas other earlier I said depends upon cosine of the theta.

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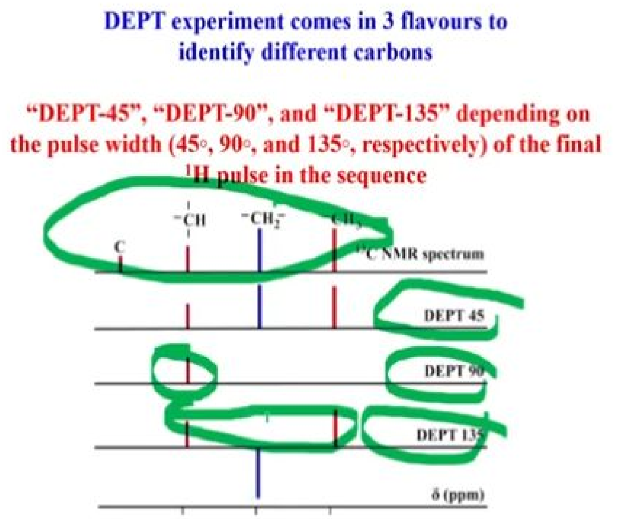

DEPT experiment comes in 3 flavours to identify different carbons

"DEPT-45", "DEPT-90", and "DEPT-135" depending on the pulse width (45°, 90°, and 135°, respectively) of the final ^{1}H pulse in the sequence

So, this is simple comparison, it is a normal carbon 13 NMR spectrum, it is a DEPT 45, where everything is observed, DEPT 90 only CH peak is observed, DEPT 135, these 2, carbons attached to odd number of protons are positive, CH2 is negative, okay.

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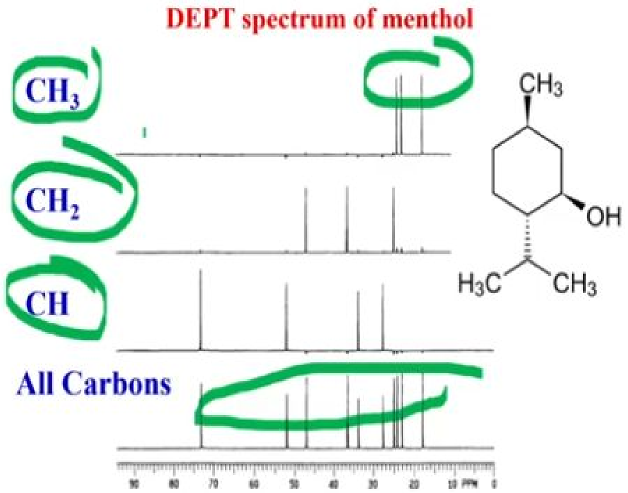

DEPT spectrum of menthol

And real comparison of this spectrum is like this in a menthol molecule. This is CH3 only carbon, CH2 only carbons, CH only carbons, and all carbons. And how did I get only these, this is what I said, 3 experiments we do, as I said once we get only CH, once we get all are positive, once we get 2 positive and 1 negative in 135 experiment. Now, there is a multiplication factor for this, so that you can scale the intensities equally.

Then making addition and subtraction of corresponding 1 or 2 spectra, we can do editing in such a way from this normal spectrum, we can subtract; CH only spectrum, then you get other carbons. Then you add up CH and CH3 and then scale it, subtract from this, you get only CH only spectrum. Like this you can play with the scaling factor, do addition and subtraction of these DEPT spectra of different flip angles, you can managed to get only CH carbons, only CH3 carbons, and only CH2 carbons spectra individually.

This is called spectral editing. Most important, very, very advantageous for you for making assignment of the carbons. Now you understood the polarisation transfer technique, the biggest advantage is you got the enhancement in the intensity, and able to identify the carbons based on the protons attached to it, at the same time, most important we are able to get the decoupled spectra. So biggest advantage; that is why always use DEPT experiment for your analysis.

Okay, so I have told you all about polarisation transfer, not all I would say, it is only a tip of a iceberg, lot more things are there for you to understand, please go back and read some more literatures, some more books, this is only the concept, and fundamentals I have told you. You have to understand. But this idea what I have given you should be sufficient for you to start analysing DEPT and other spectra. So we will continue in the next class with a different topic.