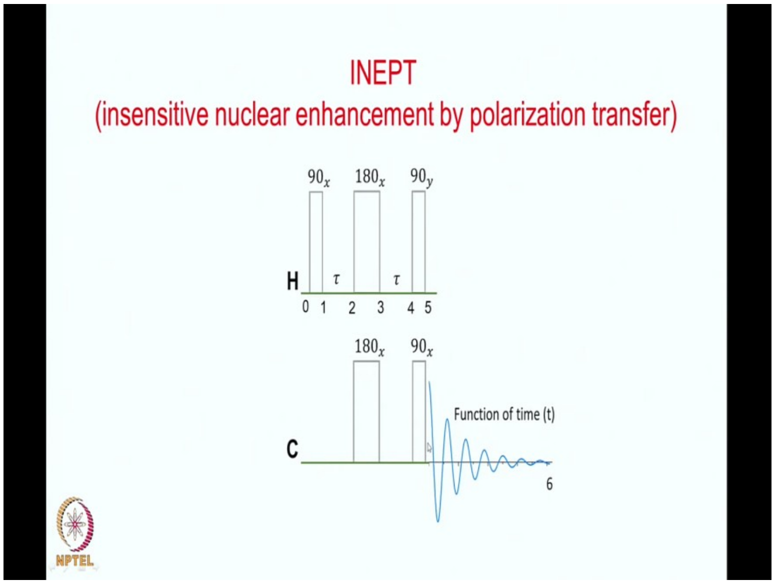
NMR Spectroscopy for Chemists and Biologists
Dr. Ashutosh Kumar
Professor Ramkrishna Hosur
Department of Biosciences and Bioengineering
Indian Institute of Technology Bombay
Lecture No 39
INEPT

So in the previous classes, we have discussed in detail that the product operator formalism for describing density operator evolution through an NMR experiment and we have seen how it becomes easier to use this formalism for analysing the NMR experiments. Explicitly we considered the spin echo in the previous classes and I am going to extend this further describe to you one more experiment as an example and that is the INEPT.

(Refer Slide Time: 1:02)



INEPT
(insensitive nuclear enhancement by polarization transfer)

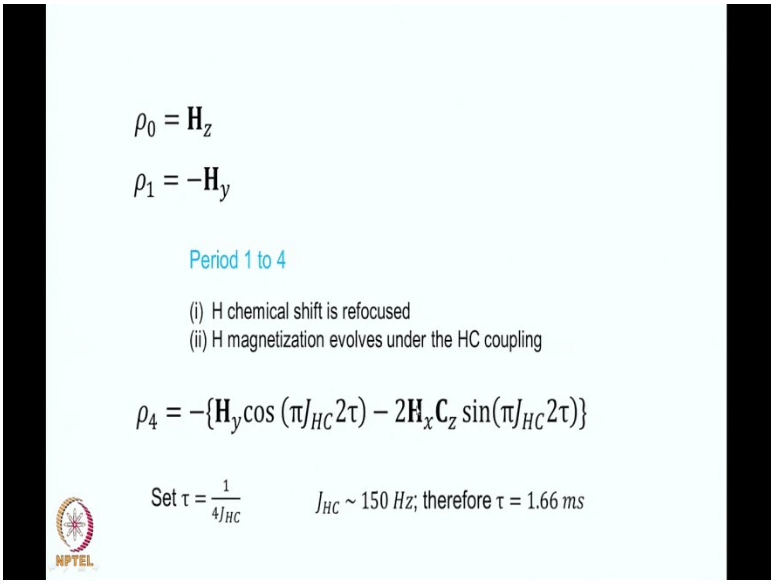
INEPT stands for Insensitive Nuclear Enhancement by Polarization Transfer. This experiment has actually been described to you in the previous classes by Professor Ashutosh Kumar and this is the polarization transfer experiment where the magnetization is transferred from proton to an insensitive nucleus like Carbon 13 or Nitrogen 15.

So here I will consider proton and carbon 13 here to see how the polarization or magnetization gets transferred from the proton to the carbon and we detect the carbon. So the pulse sequence for this is shown here, so on the proton channel you apply these pulses, you have a $90x$ followed by τ and then you have a 180 degree pulse which is applied simultaneously on both proton and the carbon channels, then you have another time period τ and which is followed by a 90 degree pulse on proton channel and a 90 degree pulse on the carbon channel and this is the FID.

Notice, here you will have to be careful with regard to the phases of the pulses, here it is a 90*x* pulse, this is the 180, this can be *x* or *y* does not matter, but this has to be *y*. If this is *x*, this has to be a *y* pulse. This can be *x* or *y* does not matter, this carbon pulse 90*x* pulse can be 90*x* or 90*y* does not matter, but it is very crucial here that if this is 90*x*, this has to be a 90*y*. There has to be a 90 degrees phase shift between these two 90 degree pulses.

And the various time points here are represented like this 0, 1, 2, 3, 4, 5 and this is the time point 6 which is in your detection period. You also recall that this is very similar to the spin echo sequence, the spin echo also had this 90, 180, 90 and separated by the time τ and except that we are now using it as a hetero experiment for transferring polarization from proton to carbon. How does it happen? Let us see this, we will analyse that this using the product operator formalism.

(Refer Slide Time: 3:36)



$$\rho_0 = \mathbf{H}_z$$

$$\rho_1 = -\mathbf{H}_y$$

Period 1 to 4

(i) H chemical shift is refocused
(ii) H magnetization evolves under the HC coupling

$$\rho_4 = -\{\mathbf{H}_y \cos(\pi J_{HC} 2\tau) - 2\mathbf{H}_x\mathbf{C}_z \sin(\pi J_{HC} 2\tau)\}$$

Set $\tau = \frac{1}{4J_{HC}}$ $J_{HC} \sim 150\ Hz$; therefore $\tau = 1.66\ ms$

Okay, so at time point 0 we consider the density operator which consists of the proton magnetization along the *Z* axis and so this we call right here now as H_z, so do not confuse this with the Hamiltonian. This is the operator for the proton z magnetization and therefore I write it as H_z. When I apply 90*x* pulse on the proton channel notice there is no pulse applied on the carbon channel.

So therefore, the carbon magnetization which is along the *Z* axis is unaffected. So therefore, we do not even write the carbon magnetization here we only write the proton magnetization here H_z and the pulse is applied only on the proton channel and therefore we get a $\rho_1$which is -H_y.

And during the period 1 to 4 what happens? This is a spin echo, we have seen that this is the spin echo, the spin echo sequence is exactly in that manner. So two things happen here, first the proton chemical shift is refocused completely so the 90*x*-τ-180*x*-τ at the end of that there is a spin echo. So at the point 4, time point 4 this is the spin echo and the proton chemical shift is therefore refocussed.

However, the proton magnetization evolves under the proton carbon coupling, okay. So this Hamiltonian is operating, this is not refocused so the coupling is not refocused. In the spin echo experiment when the 180 pulse is applied on both of the channels both the nuclei, therefore, we will directly write ρ_4 because we do not consider the chemical shift evolution at all because it is refocussed therefore, we from ρ_1 to ρ_4 we will only consider only the evolution under the coupling Hamiltonian between proton and carbon.

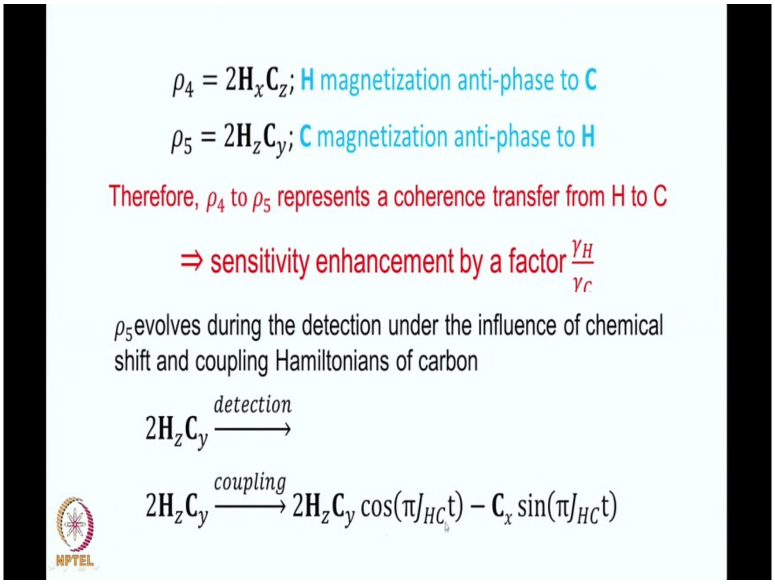
So how does this H_y evolve, this is through the product operator formalism the ρ_4 turns out to be like this, H_y evolves as

$$\rho_4 = -\{\mathbf{H}_y \cos(\pi J_{HC} 2\tau) - 2\mathbf{H}_x\mathbf{C}_z \sin(\pi J_{HC} 2\tau)\}$$

Notice now these are the operators okay this represents the anti phase magnetization of proton with respect to the carbon and this is the in-phase magnetization of proton of along the Y axis. So these are the operators Hy and 2 H_xC_z are the operators and τ is the time of course and J_{HC} is the coupling constant between proton and carbon.

Now, what we do is, we set $\tau = \frac{1}{4J_{HC}}$, so here, if I put a $\tau = \frac{1}{4J_{HC}}$ what happens? So this HC HC will cancel so this will become $\cos\frac{\pi}{2}$. And similarly here, this will become $\sin\frac{\pi}{2}$ therefore, this goes to 0 and only this term remains okay.

(Refer Slide Time: 7:04)



$\rho_4 = 2\mathrm{H}_x\mathrm{C}_z$; H magnetization anti-phase to C

$\rho_5 = 2\mathrm{H}_z\mathrm{C}_y$; C magnetization anti-phase to H

Therefore, ρ_4 to ρ_5 represents a coherence transfer from H to C

⇒ sensitivity enhancement by a factor $\frac{\gamma_H}{\gamma_C}$

ρ_5 evolves during the detection under the influence of chemical shift and coupling Hamiltonians of carbon

$$2\mathrm{H}_z\mathrm{C}_y \xrightarrow{detection}$$

$$2\mathrm{H}_z\mathrm{C}_y \xrightarrow{coupling} 2\mathrm{H}_z\mathrm{C}_y\cos(\pi J_{HC}t) - \mathrm{C}_x\sin(\pi J_{HC}t)$$

Therefore, ρ_4 becomes 2 H_xC_z okay and notice this minus sign and this minus sign becomes plus and therefore here I have 2 H_xC_z and this is now the proton magnetization anti phase to carbon okay this is we learnt it from the product operator descriptions. Now, when I apply a 90 pulse on proton and carbon we get ρ_5 and ρ_5, so applying a 90y pulse on proton right so therefore this H_x goes to H_z and C_z goes to Cy because I am applying this on the carbon channel I am applying along the X axis so therefore this goes to C_y.

Now, this is carbon magnetization anti phase to proton and that is the interesting thing. Therefore, ρ_4 to $\rho_5$represents the coherence transfer from proton to carbon which all the polarization which is present on the proton has now become carbon magnetization. What is the meaning of this? This means there is a significant sensitivity enhancement of the carbon from magnetization right sensitivity enhancement by factor $\frac{\gamma_H}{\gamma_C}$because proton magnetization is determined by the gyro-magnetic ratio of the proton and the carbon magnetization is normally determined by the gyro-magnetic ratio of the carbon.

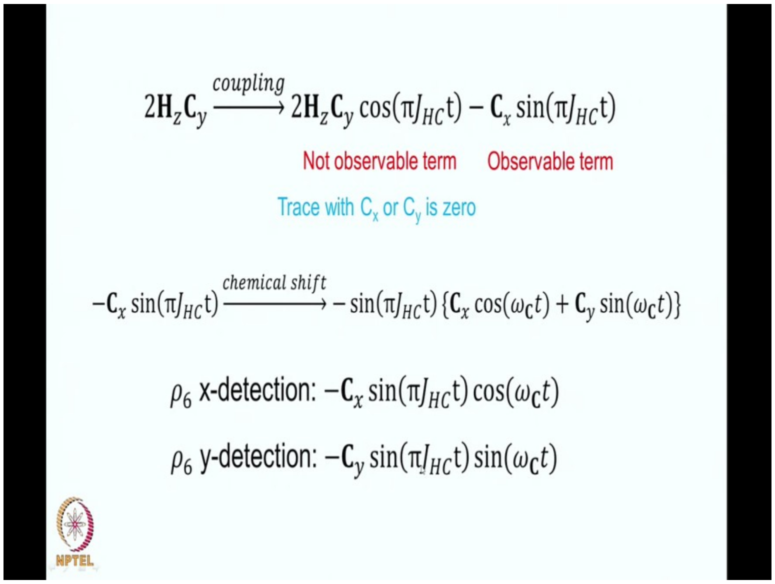
But here the proton magnetization is appearing as carbon magnetization therefore, there is a sensitivity enhancement of the carbon signal by a factor $\frac{\gamma_H}{\gamma_C}$ and this is the factor of 4 right so this is a factor of 4. If this were a nitrogen for example, then this will be factor of 10 and that implies the substantial saving in time and the great improvement in the signal-to-noise ratio in your experimental time because experimental time goes as a square of this factor, okay. Now, after this rho 5 evolves during the detection period under the influence of chemical shift and coupling Hamiltonians of carbon okay. So these 2 H_zC_y during the detection period evolves under the chemical shift as well as the coupling.

Let us first consider the coupling evolution because you remember I had told you that it does not matter which evolution you will consider first and which evolution you will consider later, okay. So, we will consider the coupling evolution first, there is a purpose in it so I will illustrate this to you very quickly. So $2H_zC_y$ now this is the product operator and you are considering its evolution under the influence of the coupling Hamiltonian between proton and carbon. So this gives you

$$2\mathbf{H}_z\mathbf{C}_y \xrightarrow{coupling} 2\mathbf{H}_z\mathbf{C}_y \cos(\pi J_{HC}\mathrm{t}) - \mathbf{C}_x \sin(\pi J_{HC}\mathrm{t})$$

now it is a function of time during the FID, during the FID this is what we are getting.

(Refer Slide Time: 10:11)



$$2\mathbf{H}_z\mathbf{C}_y \xrightarrow{coupling} 2\mathbf{H}_z\mathbf{C}_y \cos(\pi J_{HC}\mathrm{t}) - \mathbf{C}_x \sin(\pi J_{HC}\mathrm{t})$$

Not observable term Observable term

Trace with C_x or C_y is zero

$$-\mathbf{C}_x \sin(\pi J_{HC}\mathrm{t}) \xrightarrow{chemical\ shift} -\sin(\pi J_{HC}\mathrm{t})\{\mathbf{C}_x \cos(\omega_C t) + \mathbf{C}_y \sin(\omega_C t)\}$$

ρ_6 x-detection: $-\mathbf{C}_x \sin(\pi J_{HC}\mathrm{t})\cos(\omega_C t)$

ρ_6 y-detection: $-\mathbf{C}_y \sin(\pi J_{HC}\mathrm{t})\sin(\omega_C t)$

So the same thing is written once more here and now you notice that this particular term is not observable, this is anti phase magnetization is not an observable term, right. So therefore, if we did not have this coupling evolution we would not have any magnetization which is observable, this is not observable term because you recall our discussion that to be observable it is a trace with C_x and C_y has to be non-zero.

Now, if you take the trace of this with either C_x or C_y, this is 0 and we had actually described this earlier also that the anti phase magnetization is not an observable operator, whereas this one is an observable term. Therefore, the coupling evolution leads to an observable magnetization here, observable term, so this is the observable term and this is the not observable.

Now therefore, for after this we need not consider the evolution of this at all for the chemical shift because this is anyway not observable so this will go away. Now, we will consider only this term therefore we will now consider

$$-\mathbf{C}_x \sin(\pi J_{HC}\mathrm{t}) \xrightarrow{chemical\ shift} -\sin(\pi J_{HC}\mathrm{t})\,\{\mathbf{C}_x \cos(\omega_{\mathbf{C}} t) + \mathbf{C}_y \sin(\omega_{\mathbf{C}} t)\}$$

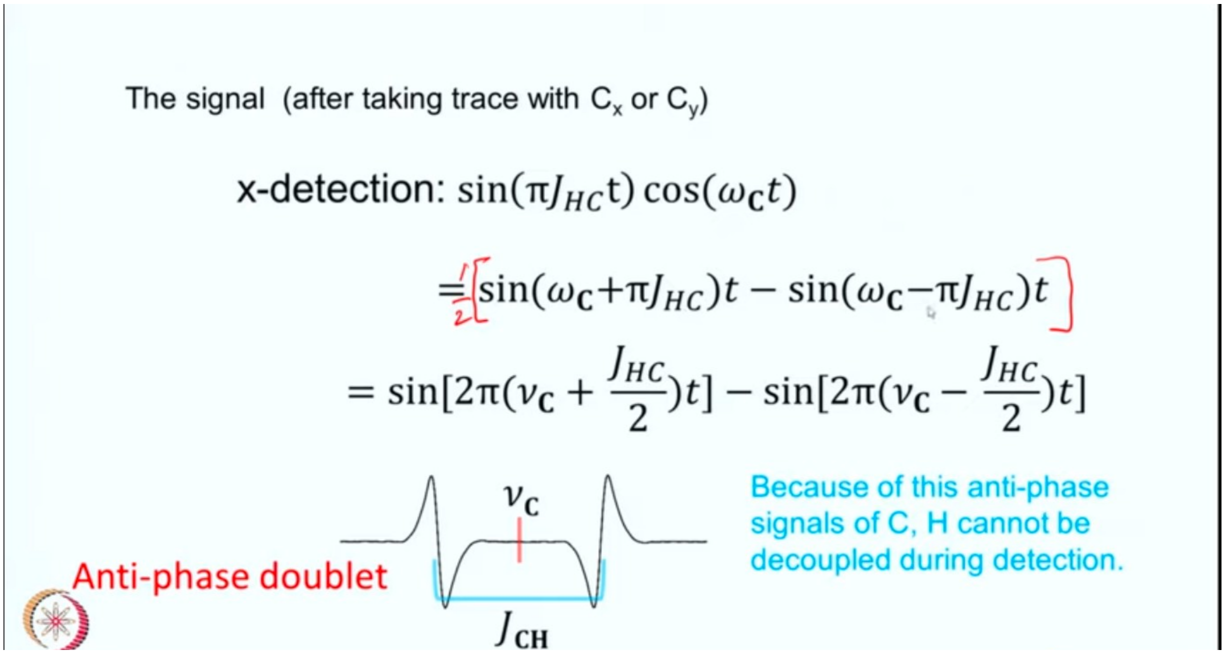
this is the normal chemical shift evolution under the influence of the carbon chemical shift Zeeman Hamiltonian.

Okay now, so I have a C_x term and a C_y term right so if I observe the *x* magnetization I get this term, if I observe the *y* magnetization then I get this term. So therefore, $\rho_6$the density operator if I were to do *x* detection then I get this

$$\rho_6 \text{ x-detection: } -\mathbf{C}_x \sin(\pi J_{HC}\mathrm{t}) \cos(\omega_{\mathbf{C}} t)$$

Okay, what is our signal? To see what is the signal, I have to take the trace of this with C_x or C_y okay. So when I take the trace of this C_x C_y operators this C_x terms will vanish I will only be left with coefficients which actually are functions of time and that is my observed signal.

(Refer Slide Time: 12:43)



The signal (after taking trace with C_x or C_y)

x-detection: $\sin(\pi J_{HC} t)\cos(\omega_C t)$

$$= \frac{1}{2}\left[\sin(\omega_C + \pi J_{HC})t - \sin(\omega_C - \pi J_{HC})t\right]$$

$$= \sin[2\pi(\nu_C + \frac{J_{HC}}{2})t] - \sin[2\pi(\nu_C - \frac{J_{HC}}{2})t]$$

Anti-phase doublet

Because of this anti-phase signals of C, H cannot be decoupled during detection.

So therefore, the signal what we get after taking the trace with the C_x or C_y so this will be for *x*-detection

$$\text{x-detection: } \sin(\pi J_{HC} t)\cos(\omega_C t)$$

Now let us try to expand this and the

$$= \frac{1}{2}\sin(\omega_C + \pi J_{HC})t - \sin(\omega_C - \pi J_{HC})t$$

will be expanded as the sum of two sine terms okay. So when we expand this as sum of two we get this here I have to put this as there is a factor of ½ here, have to put it in. So I will get two frequencies here right there are two sine terms here so there are two frequencies.

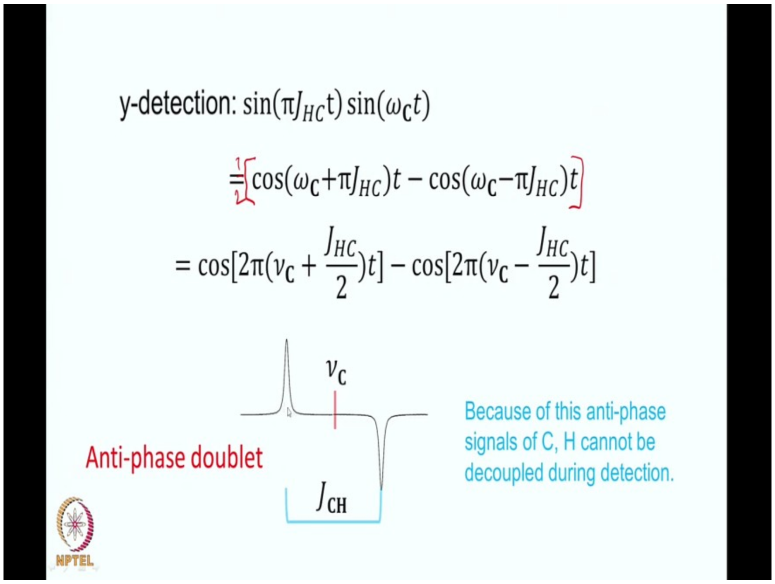
Ofcourse these are in radians so if I take out the if I want to express them in hertz, in terms of numbers then I take out this 2π here. I write this as

$$= \sin[2\pi(\nu_C + \frac{J_{HC}}{2})t] - \sin[2\pi(\nu_C - \frac{J_{HC}}{2})t]$$

So, if I would do a Fourier transformation of this signal, then I will get two frequencies right the frequency spectrum, but this frequency spectrum will have dispersive line shape because these ones are sine terms. This is what we have seen in the very early discussions in the course. So this sine term is giving me two dispersive signals and now because of this minus sign these two also have opposite phases. So this goes like this positive and negative and then this will be opposite in sign compared to this and therefore this goes in this manner and we have two dispersive signals which are anti phase in nature, it is called the anti-phase doublet with dispersive line shapes.

Okay, now during the detection, during the detection period these two signals will be present as they are if you do not decouple, we cannot decouple this. If you want to decouple this then of course these two will collapse fall on top of each other and then this will be 0 signal. So therefore, in this situation we cannot decouple the during the detection period, okay. And this is the center of this is the chemical shift and the separation between these two is the coupling constant J_{CH} that is from here, notice the center is here okay the center is not here or here this center is here, this center is here. So from here to here it is J_{CH}.

(Refer Slide Time: 15:46)



y-detection: $\sin(\pi J_{HC}t)\sin(\omega_C t)$

$$=\frac{1}{2}[\cos(\omega_C+\pi J_{HC})t-\cos(\omega_C-\pi J_{HC})t]$$

$$=\cos[2\pi(\nu_C+\frac{J_{HC}}{2})t]-\cos[2\pi(\nu_C-\frac{J_{HC}}{2})t]$$

Anti-phase doublet

Because of this anti-phase signals of C, H cannot be decoupled during detection.

Now, if I were to do a y-detection then my term what I have is

$$\text{y-detection: } \sin(\pi J_{HC}t)\sin(\omega_C t)$$

Once again the operator part has gone off because after they take the trace with the C_y that the $Tr\{C_y^2\}=1$. So therefore that will not appear and I will have only have the time dependent function here which actually is the part of the FID.

So once again here I will have a factor of

$$=\frac{1}{2}\cos(\omega_C+\pi J_{HC})t-\cos(\omega_C-\pi J_{HC})t$$

These are the two frequencies were coupled, but now the difference is these ones are cosine functions so I representing them in this in terms of hertz, so again I write it here as

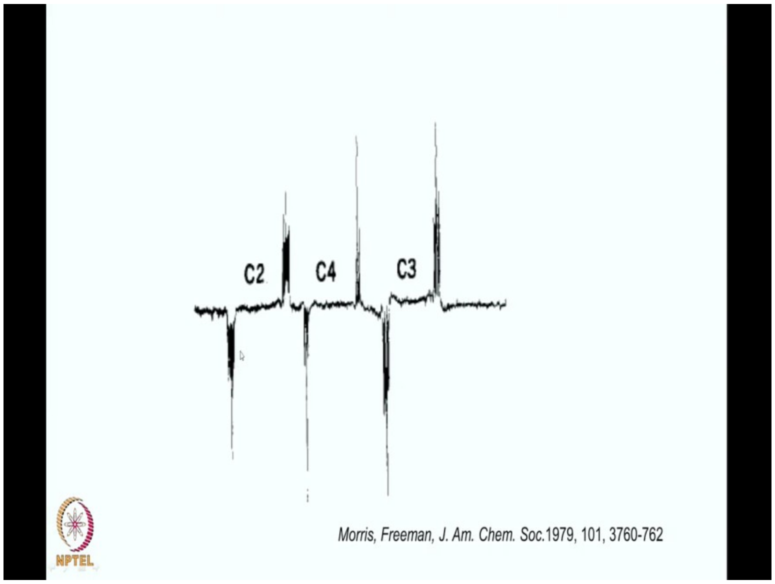
$$=\cos[2\pi(\nu_C+\frac{J_{HC}}{2})t]-\cos[2\pi(\nu_C-\frac{J_{HC}}{2})t]$$

Reason of course both are cosine terms and once again these two have opposite signs.

So this one is If I were to take it as positive and this will be because if the minus sign this will be negative. Now it will represent absorptive signals because these are cosine terms. So the cosine terms will produce me an absorptive signal here at this particular frequency and this will produce a negative signal at this particular frequency which is this.

So once again because of this anti phase signals CH cannot be decoupled during detection. What happens if you decouple? Because this they will overlap, they come on top of each other. This will come here and this will come here, therefore, they will cancel. So therefore, you cannot decouple if the signals are anti-phase in nature. The anti-phase doublet will appear like this.

(Refer Slide Time: 17:37)

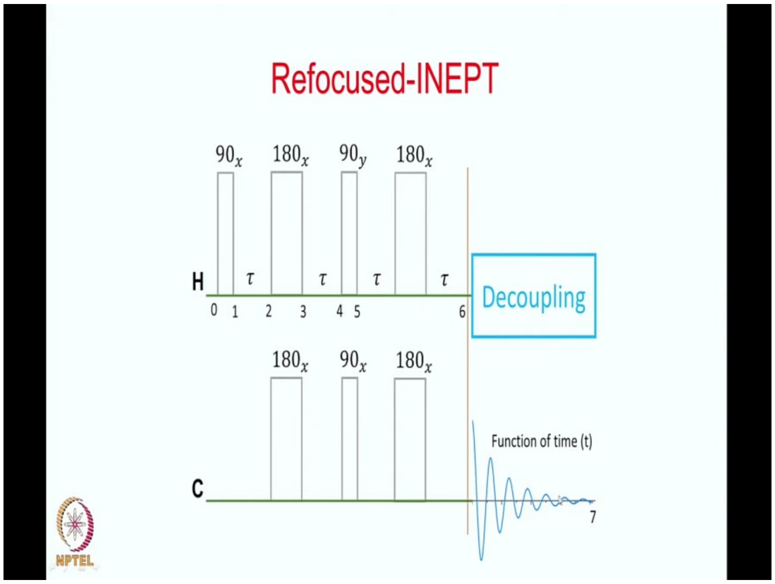


Morris, Freeman, J. Am. Chem. Soc.1979, 101, 3760-762

So here is an experimental example this was the very first data which was recorded, this came from Morris and Freeman and this is published in 1979 in Journal of American Chemical Society. And you can see here there are three anti-phase doublets, so one here this is negative and positive and negative and positive, negative and positive. And of course you are seeing some fine structure here and of some molecule which is atom because this is the carbon-carbon couplings which also evolved during the detection period.

So we are not done nothing about the carbon-carbon couplings, so wherever there is a carbon-carbon coupling it will lead to a splitting and therefore the whole set here will contain this carbon-carbon couplings here. So there are three carbons C2, C4, C3 and they are coupled to each other and that results in this kind of a fine structure here and we have anti-phased doublets for each of the carbons.

(Refer Slide Time: 18:34)

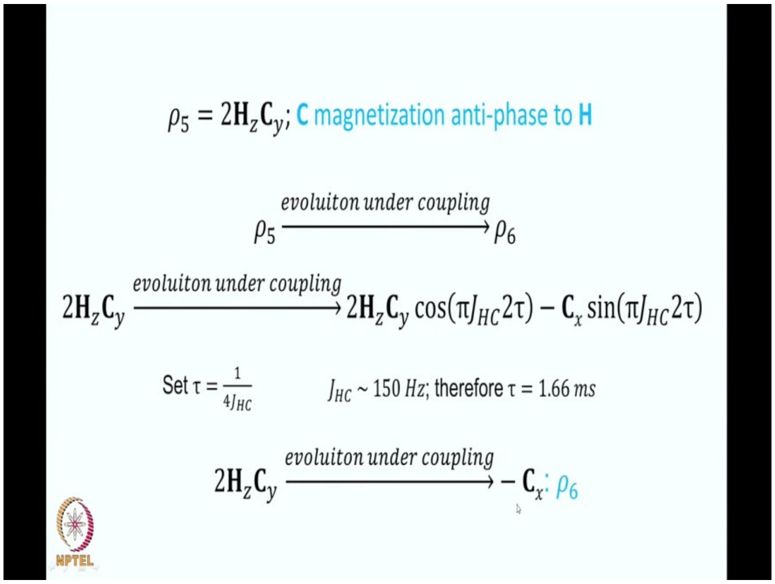


Refocused-INEPT

Now, but this anti phase nature is often a disturbance and they can if there are two anti-phase which are close by then they may lead to cancellations and therefore we do not want that thing to happen. So therefore, we need to do something further to improve upon this. So therefore, what we do? We do what we called as the refocused INEPT. So here the pulse sequence is extended, see the INEPT was ending here and you are having the FID collected from here.

Now for the refocused INEPT what you do is, you extend this pulse sequence by introducing another spin echo sequence here, τ-180-τ and the 180 pulse is applied on both the sequences and both of the channels both the proton and carbon. So we have tau 180 tau applied and the data is collected as a function of time on the carbon channel and you can also decouple here on the proton channel. How it happens we will, I will show you.

(Refer Slide Time: 19:35)



$\rho_5 = 2H_zC_y$; C magnetization anti-phase to H

$$\rho_5 \xrightarrow{evoluiton\ under\ coupling} \rho_6$$

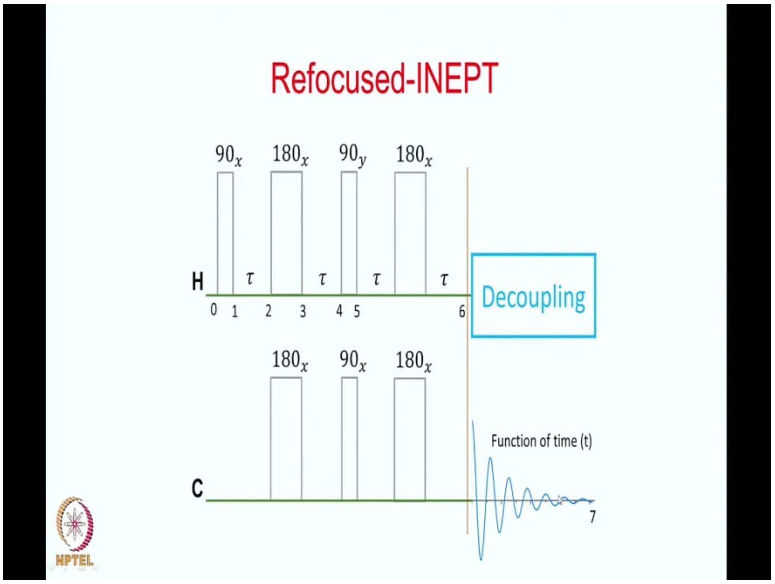
$$2H_zC_y \xrightarrow{evoluiton\ under\ coupling} 2H_zC_y\cos(\pi J_{HC}2\tau) - C_x\sin(\pi J_{HC}2\tau)$$

Set $\tau = \frac{1}{4J_{HC}}$ $J_{HC} \sim 150\ Hz$; therefore $\tau = 1.66\ ms$

$$2H_zC_y \xrightarrow{evoluiton\ under\ coupling} -C_x;\ \rho_6$$

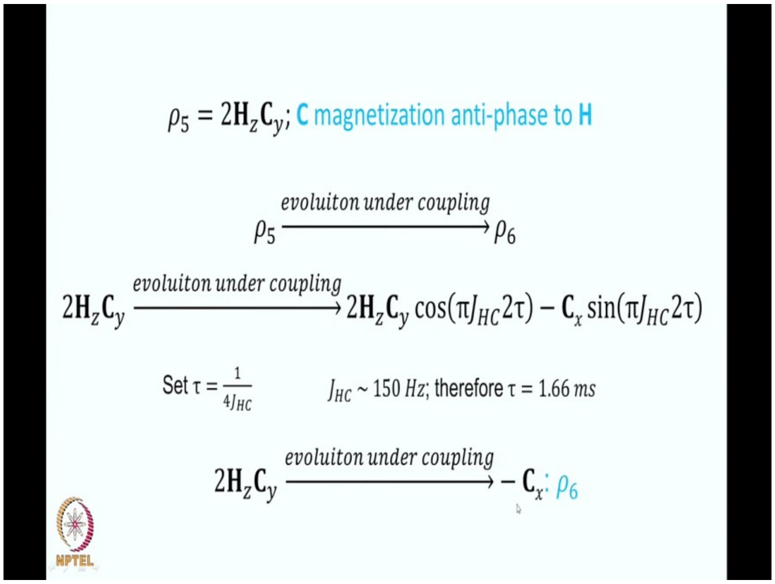
So until ρ_5 the product operator description is the same, so we do not want to do that once more here. Now $\rho_5 = 2H_zC_y$ this is the carbon magnetization anti phase to proton right so this is what we had.

(Refer Slide Time: 19:57)



Now, evolution under coupling, where is the ρ_6 here? See the ρ_6 is at this point, this is the extended point. We want to calculate was the density operator at this point. So ρ_5 to $\rho_6$6 what happens? There is no chemical shift evolution because of the 180 pulse the magnetization is on carbon, magnetization is here and this gets refocused chemical shift is refocused because of the 180 pulse, but and then because of the 180 pulse is on both carbon and proton channels, this evolution under the coupling continues and that is not refocused. Therefore, at this point ρ_6 we have to consider evolution under the coupling only, okay.

(Refer Slide Time: 20:35)



$\rho_5 = 2\mathbf{H}_z\mathbf{C}_y$; C magnetization anti-phase to H

$$\rho_5 \xrightarrow{evoluiton\ under\ coupling} \rho_6$$

$$2\mathbf{H}_z\mathbf{C}_y \xrightarrow{evoluiton\ under\ coupling} 2\mathbf{H}_z\mathbf{C}_y\cos(\pi J_{HC}2\tau) - \mathbf{C}_x\sin(\pi J_{HC}2\tau)$$

Set $\tau = \frac{1}{4J_{HC}}$ $J_{HC} \sim 150\ Hz$; therefore $\tau = 1.66\ ms$

$$2\mathbf{H}_z\mathbf{C}_y \xrightarrow{evoluiton\ under\ coupling} -\mathbf{C}_x;\ \rho_6$$

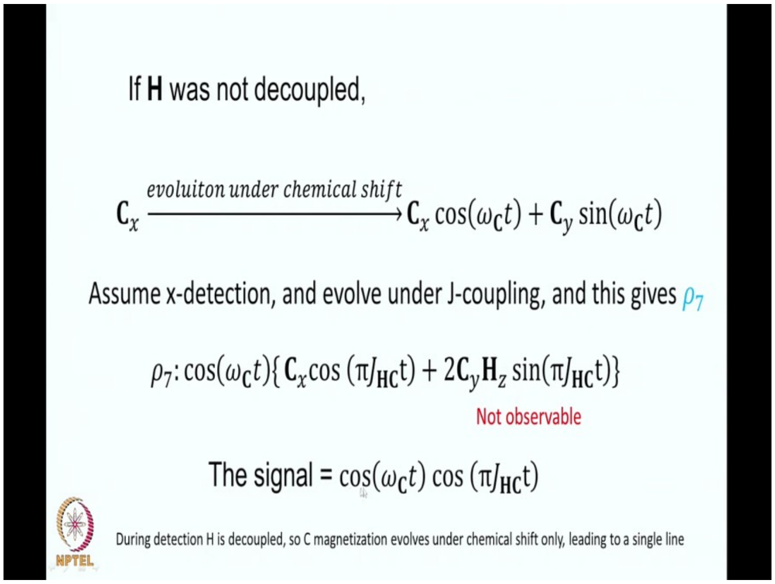
So therefore, if I do evolution under coupling that gives me the ρ_6 and what is that? $2H_zC_y$ evolution under a coupling Hamiltonian leads me to

$$2\mathbf{H}_z\mathbf{C}_y \xrightarrow{evoluiton\ under\ coupling} 2\mathbf{H}_z\mathbf{C}_y\cos(\pi J_{HC}2\tau) - \mathbf{C}_x\sin(\pi J_{HC}2\tau)$$

Now once again I adjust my τ because this is under my control I adjust my τ to be equal to $\frac{1}{4}J_{HC}$ for assuming J_{HC} is of 150 hertz and this will be only, τ will be approximately 1.66 milliseconds or 1.7 milliseconds.

So this is the approximate, therefore if it is 140 hertz or so 150 hertz or things like that so approximately one can adjust this to 1.67 milliseconds. Now, when you put that this term goes to 0 as before because this is $\cos\frac{\pi}{2}$ and this will become 1, okay. Therefore, $2H_zC_y$ will become just $-C_x$ all the time dependent these terms are gone, I only have ρ_6 is therefore simply equals to -Cx.

(Refer Slide Time: 21:52)



If **H** was not decoupled,

$$C_x \xrightarrow{evoluiton\ under\ chemical\ shift} C_x\cos(\omega_C t) + C_y\sin(\omega_C t)$$

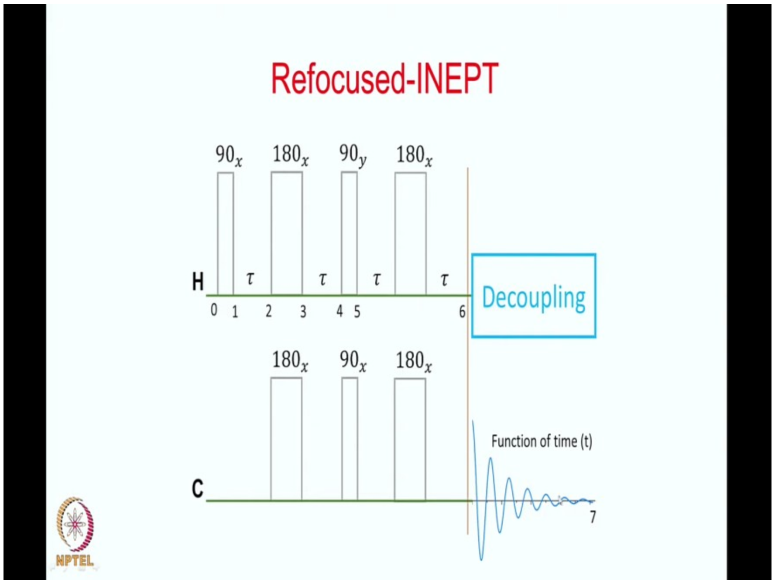
Assume x-detection, and evolve under J-coupling, and this gives ρ_7

$$\rho_7: \cos(\omega_C t)\{C_x\cos(\pi J_{HC}t) + 2C_yH_z\sin(\pi J_{HC}t)\}$$

Not observable

The signal = $\cos(\omega_C t)\cos(\pi J_{HC}t)$

During detection H is decoupled, so C magnetization evolves under chemical shift only, leading to a single line



Refocused-INEPT

Now, suppose the proton was not decoupled okay suppose the proton was not decoupled, now C_x will evolve under a chemical shift as well as coupling during the detection period right, so now we are considering what is happens during the detection period ρ_6 was at the end of the spin echo right. Now this will now evolve under the coupling right, this is ρ_6. The ρ_6 was here there is no pulse applied here, so this will evolve during the FID it will evolve under the influence of the chemical shift in the coupling and depending upon what you do. So if I wait considering without proton decoupling, then I will consider the evolution under a chemical shift as well as the coupling.

Okay, let us consider first the evolution under chemical shift so this will give me just I ignored the sine here so it does not matter.

$$\mathbf{C}_x \xrightarrow{evoluiton\, under\, chemical\, shift} \mathbf{C}_x \cos(\omega_{\mathbf{C}} t) + \mathbf{C}_y \sin(\omega_{\mathbf{C}} t)$$

Assuming *X* detection, we will ignore this term, this because we are only demonstrating, in principle of course when you are collecting the data and evolving so you will have to consider all the terms which are present but this is to demonstrate how things develop depending upon what term you select. So, if I were to *x* data that is *x* detection and evolve under the J coupling now so shift evolution has produced C_x and C_y.

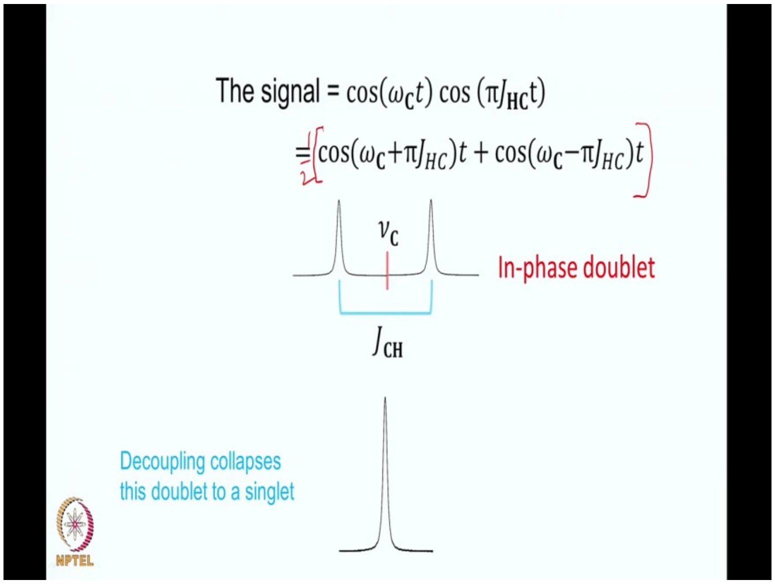
Now, consider the evolution of the *x* part under *J* coupling and this gives me ρ_7, so the ρ_7 gives me

$$\rho_7 \colon \cos(\omega_{\mathbf{C}} t)\{\, \mathbf{C}_x \cos\,(\pi J_{\mathbf{HC}} \mathrm{t}) + 2\mathbf{C}_y \mathbf{H}_z \sin(\pi J_{\mathbf{HC}} \mathrm{t})\}$$

Once again this term is not observable, this is anti phase carbon magnetization with respect to the proton and this is not observable because this trace will be 0 with respect to C_x or C_y, therefore my signal will be

$$\text{The signal} = \cos(\omega_{\mathbf{C}} t)\,\cos\,(\pi J_{\mathbf{HC}} \mathrm{t})$$

(Refer Slide Time: 24:10)



The signal = $\cos(\omega_C t)\cos(\pi J_{HC} t)$

$$=\frac{1}{2}\left[\cos(\omega_C+\pi J_{HC})t+\cos(\omega_C-\pi J_{HC})t\right]$$

Decoupling collapses this doublet to a singlet

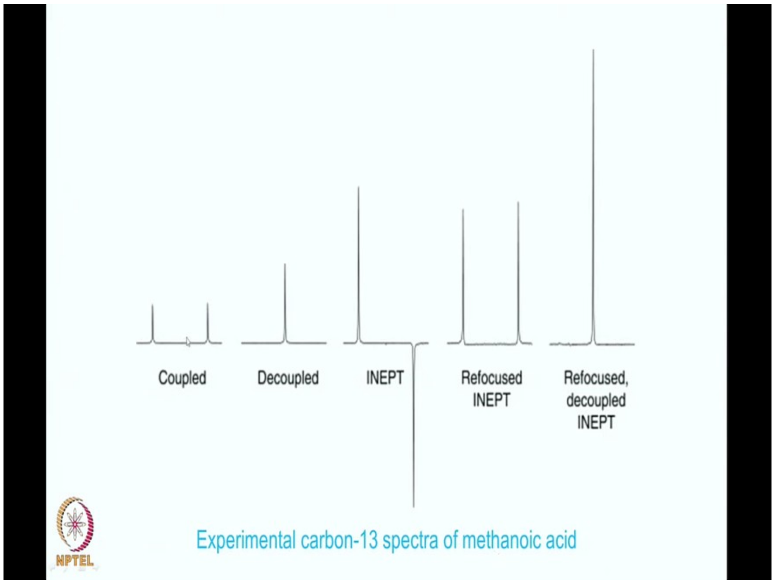
So once more I write the signal as this and I will decompose this into two terms into the two frequency terms put here once more a factor of half. So, what we get here now? So this is the same as before we get two frequencies

$$=\frac{1}{2}\cos(\omega_C+\pi J_{HC})t+\cos(\omega_C-\pi J_{HC})t$$

but now notice I have a plus sign here, earlier this was a minus sign, now I have a plus sign which means both these signals have the same phase right. So both are positive therefore this is an in-phase doublet of carbon.

So we are starting from the proton magnetization which is the refocused INEPT during the detection period so I have here in-phase doublet as a measurement. Now this separation is J_{HC}or J_{CH}, now I can decouple this. If I decouple these two will collapse into a singlet, decoupling collapses this doublet into a singlet and this will be at the center here and with the twice the intensity because both these will contribute to the intensity and intensity of this will be twice. So this will be a big advantage for the carbon signal.

(Refer Slide Time: 25:40)



Experimental carbon-13 spectra of methanoic acid

So now, here it is a comparison of an experimental spectra under the various conditions. This is the carbon 13 spectra of methanoic acid there is only one carbon and one proton so you have a coupled spectrum which is a doublet normal coupled spectrum, carbon detection, direct carbon detection okay and if you decouple this you have this appearing in the center here the carbon, this is a decoupled spectrum.

Now, if we did an INEPT experiment now it is the magnetization is now coming from the proton remember, it is not the, you are not taking the carbon magentization as it is done here. The magnetization is coming from the proton and therefore this has a $\frac{\gamma_H}{\gamma_C}$ signal enhancement therefore, in the normal INEPT you have this anti phase signals which are one positive here and one negative here.

Now, if I did a refocused INEPT this will turn out to be positive it will go up like this, so this was positive here and this is positive here and now refocused and decoupled. Now you decouple this so this will do collapse again once more here and you have a huge signal coming here. So this is the significant advantage in the signal to noise ratio through this refocused decoupled INEPT experiment and that is how this becomes an extremely important tool, extremely important technique for heteronuclear experiment for all organic chemistry people this has become an extremely useful technique for determining how many carbons there are in their molecules, how to get signal to noise ratio and we can do with the natural abundance.

We can do carbon-13 spectra at natural abundance, you use proton magnetization to enhance the signal to noise ratio and you can identify the number of carbons in your molecule and if you couple the record proton coupled spectra also, then of course we can see the mutiplet structures in all of those things. So, I think we will stop here.