NMR Spectroscopy for Chemist and Biologist
Professor. Ramkrishna Hosur
Department of Biosciences & Bioengineering
Indian Institute of Technology, Bombay
Lecture 19
Spin Decoupling in FT NMR and Relaxation Measurement

Good morning, in the previous classes especially those by Professor Ashutosh Kumar when he discussed about the analysis of NMR spectra, you have learned about the spin-spin coupling constant, how do they effect the appearance of the NMR spectra? How is it useful to identify the groups in your molecules and how it is useful to determine the structure the of the molecules, often it also becomes a necessary to record spectra without spin-spin coupling, how to do that?

So we need to do something special to remove the spin coupling and this is called as a spin decoupling, so we are going to look at that aspect now. How we can decouple the spins and get simplified spectra, essentially those spectra will have only chemical shifts and you can count the number of peaks to identify the number of spins and so on. This is particularly useful in the case of carbon NMR where you can simply count the number of carbons in your molecule if you have recorded the spin decoupled NMR spectrum.

In organic chemistry this is an extremely useful technique, people record spin coupled spectra for identification of the functional groups, what type of carbons they are, and you record protons decouple carbon spectra to identify the number of carbons in your molecule, it is also useful in homonuclear NMR to identify the pairs of proton which are couple to each other, if there is one proton which is distinct it coupled to some other one if you remove the coupling then there will be changes in the spectra and you can identify the pairs of proton which are coupled to each other, this is again a very useful thing to find out the structures of the molecules.

How to do spin decoupling?? This is an important topic, it is lot of discussion can be made on this one but we will look at the basic concepts how is routinely used how it can be applied to different kinds of situations.

Spin decoupling we may recall the coupling between two nuclei, let us say A and M, two spins A and M is written as $J\ I_A.I_M$, this is the interaction between the two spin A and M, this is the coupling interaction and J is the coupling constant, this two may be separated by one bond, two bond, three bond and so on and so forth which was discussed by Ashutosh Kumar in his lectures and J is the coupling constant between the two spins A and M.

Notice this is the dot product here, if one can make this dot product go to 0 then essentially this interaction will not appear in your spectra, you cannot change J when you say spin decoupling, you are not changing J, you are only seeing to it that this does not appear in your spectrum, so to do that we can manipulate this part of the interaction, this is $I_A.I_M$.

So how should we do it? Let us look at the situation at the equilibrium, at equilibrium we know that the magnetization is along the Z axis that is the direction of the magnetic field, so your magnetic field is here and your protons or the spins are oriented in the direction of the magnetic field at equilibrium, both the spins A and M are oriented with the respect to the magnetic field along the positive Z axis.

We say that the two spins are quantized along the Z axis along the direction of the field, this two spins have their independent chemical shift and the coupling constants, so typically such a kind of things will give you two line for A and two lines for the M, and this will be your A chemical shift and this will be the M chemical shift, this is A and this is M this is A.

So, if one can somehow orient this M spins along a different direction, for example if you do a kind of spin lock on the M spin so that this magnetization comes along the Y axis and the A magnetization continues to remain on the Z axis, in other words A and the M spins are now quantized along orthogonal axis, how does one do this? This is done by what is known as the spin lock.
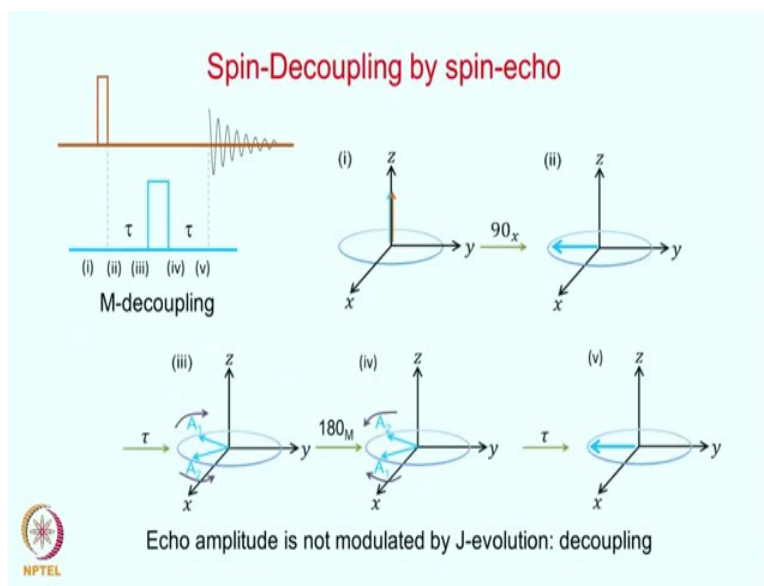
(Refer Slide Time: 06:52)
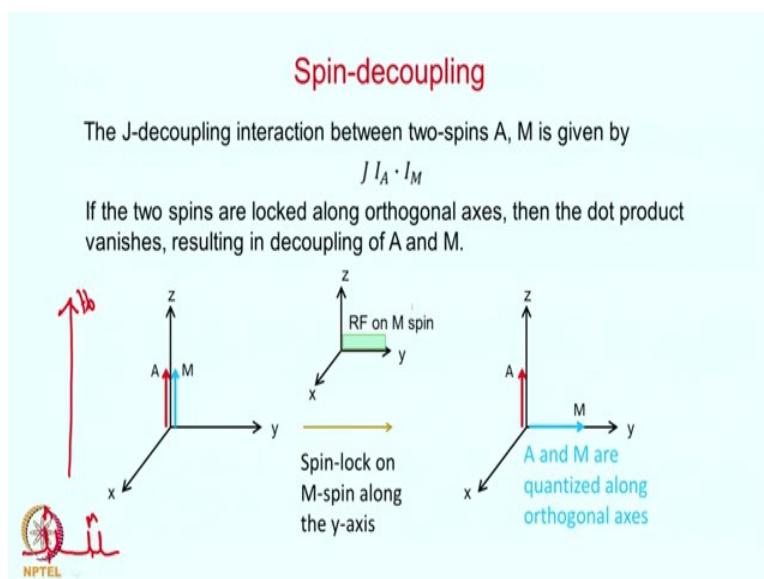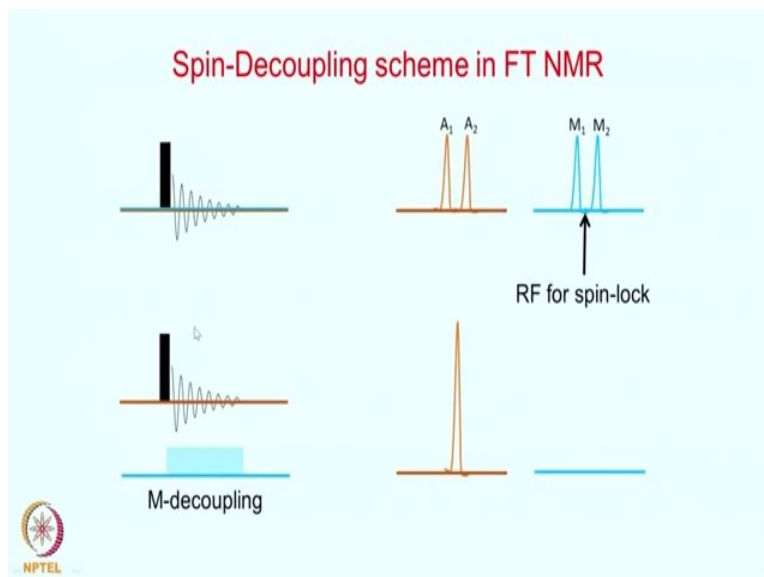
You apply an RF on the M spin, so here you have spectrum recorded by after applying normal $90°$ pulse when we apply collect the FID Fourier transformation will give you two lines for the A spins and two lines for the M spin, now you apply an RF here, very selective RF which means it

is a low power *RF* and it saturates the *M* spins completely, it normally saturated actually it is little higher power than what is require for the saturation and that is indicated by this *RF* here, this is applied at the middle of the *M* doublet, so at the chemical shift of *M* you apply this low power *RF* so perturb only the *M* spin and does not disturb the *A* spin at all.

So, therefore what happens to the magnetization which was along the *Z* axis, the *M* magnetization if we applied *RF* along the *Y* axis here *M* spin along the *Y* axis this magnetization will rotate like this, it will rotate like this and eventually come down and orient itself along the *Y* axis as indicated her. So therefore that is the way you try to achieve the orthogonal orientation of the two spins and this is what we say we want to quantize the two spins *A* and *M* spins along orthogonal axis.

So, now you see as a result of this if you record the spectrum here if you apply the *M* spin lock decoupling, it has to be applied for a long time so long your FID is there here do we apply all along when you are collecting the data this should be on, so after the 90˚ pulse therefore the coupling is not seen in this FID therefore the *A* doublet will collapse into a singlet and which will have twice the intensity because both this intensity is now merged into this, on the other hand the *M* spin will not have any intensity for the is saturated and this will be 0. So this is how you achieve decoupling, you apply an *RF* while on the *M* spin while you are acquiring the data.

(Refer Slide time: 09:21)

Spin-Decoupling by spin-echo

M-decoupling

Echo amplitude is not modulated by J-evolution: decoupling

We can also achieve decoupling by using what is called as the spin echo which we have discuss earlier, how does allow us to get the spin decoupling? How do we achieve the spin decoupling by the spin echo? You recall your vector picture of the spin echo, how the spin echo works, and remember in spin echo we had an 90˚ pulse here and 180˚ pulse on both this planes A and X we consider A and X spins and the 180˚ pulse was applies here on both the a and the X spins, in this particular case we are talking about A and M spins, in the normal spin echo you apply the 180˚ pulse on both the A and M, however if you want to go decoupling what you do is you apply this 180˚ pulse only on the M spin, you do not apply to the A spin at all.

So, what is the consequence of that, let us look at this stages here, this is the stage of the magnetization here is 1, 2, 3, 4 and 5 and this vector diagram still you what is the orientation of the magnetization at this individual time points. At time point 1 both the spins are oriented along the Z axis as was indicate before, now when you apply the 90˚x pulse both the spins will get rotated on to the negative Y axis say both are here. Now let us look at the A transitions as we did in the case of the spin echo we looked at the A transitions A1 and A2, there were two transitions A1 and A2, you were sitting at the rotating frame of the A spin which means you are sitting at the chemical shift of the A spin and look at the two transitions how they are moving.

So, we said earlier also the A2 which is a higher frequency goes in the anticlockwise direction, so this goes in this rotates in this direction and A1 which is the lower frequency compare to the chemical shift, so that is the slower one that goes in this direction. After time tau they have separated by this much amount, the two transitions have dephase by this much amount. Now when you apply the 180 degree pulse on the M spin as here indicated here what happens? Once again you recall our discussion on the spin echo when you apply to one of this spins M spins the two transitions change their levels, $A1$ become $A2$ and $A2$ becomes $A1$.

So, therefore this becomes $A2$ and this becomes $A1$ and the $A2$ continues to go in this manner, $A1$ continues to go in this manner that original sense because these are slow and fast moving transitions, so after the next time τ that is at this point at this point this one's would again moved by the same amount this and this will come here, this will come here and therefore they come back here at along the -Y axis as we started of here. Which means the coupling has had no effect on the moment of the two transitions, therefore at this point it is as though there has been no

coupling, there is only simply chemical shift, chemical shift of the *A* spin which has been rotated by 90˚ from the *Z* axis to the -*Y* axis.

In other words during this entire period the coupling has been removed the spin has been decoupled the same thing happens if you apply 180˚pulse on the *A* spin only and not on the *M* spin, if you did that then what happens here is this two transitions will move here this *A1* will move here and *A2* will move here and they continue to go in this direction and here eventually they will orient themselves along the positive *Y* axis.

So, in either case they are refocused the two transitions have refocused and you will not have any effect of the coupling on the observed magnetization at the time of the echo, this spin *J* coupling modulation is remold in another words if you apply a 180 pulse on any one of the spins not on both the spins the coupling effect is removed and you achieved what is known as the spin decoupling.

This is required in many multiple experiments; notice here we are not actually collecting the data, in the previous case we are collecting the data and here the *RF* was applied while you are collecting the data here *RF* was applied on the *M* spin and you can collect the data there, so that the A transitions are seen as decoupled from them transition but that would mean you should be able to apply the *RF* power selectively on the *M* transition.

This becomes a particularly useful thing when you are doing heteronuclear experiments because if this is the proton channel and this is the carbon channel then there is no difficulty in applying a 180 pulse on the carbon channel here or if you are doing carbon channel here and the proton channel here there is no difficulty in applying 180 pulse on one of the channels so that the from here to here there is no coupling evolution here at all.

You may collect the data here with coupling or without the coupling it does not matter, so in many of this multidimensional experiments and other pulse sequences which we will see such kind of the combination is of evolutions with and without spins coupling will be coming up, and that is the important part of the multiples design.

So, so much for the spin decoupling and we will see many examples of this in various multiples experiments which will be discussing at the later time. Now we switch to a different topic now

which is measurement of relaxation times we have discussed this relaxation time earlier in the first few lectures and we showed the importance of the T_1 and T_2 relaxations in determining the evolution of the magnetization, recovery of the magnetization along the Z axis and decay of the magnetization in the transfers plane in the previous lectures and these are the important parameters of the individuals spins systems and we must know how to measure the T_1 and the T_2 of the given spins systems.

(Refer Slide Time: 16:22)
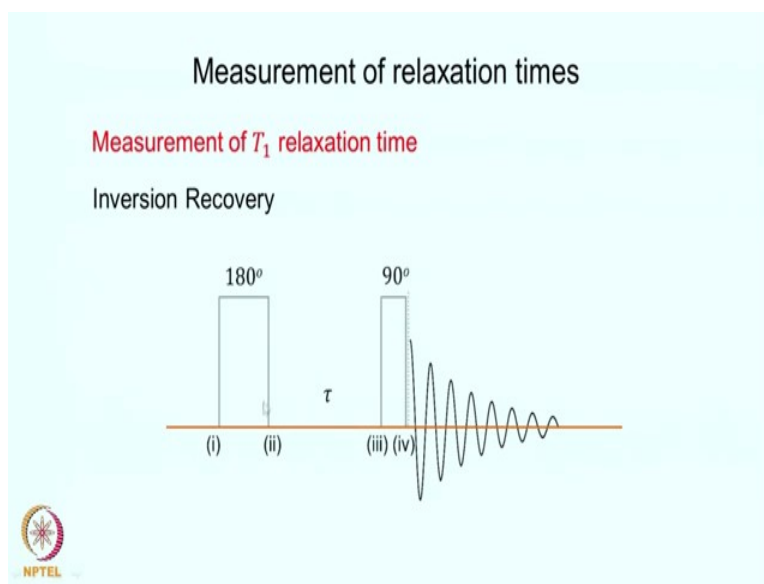
Most common technique for the measurement of T_1 is the so called inversion recovery experiment, we have already looked at some application of this sort of the strategy in water suppression but we will look at that same thing again and how one can use it to measure the T_1 relaxation times.

The pulse sequence is pretty simple, so we have here an 180° pulse in the beginning which is applied to all the spins, this are hard pulses, we have applied to all the spins then you wait for a time tau and then at the end of it you apply the 90° pulse and after that you collect the data as in the FID, so this are the time points 1, 2, 3, 4, and the data collected is actually 5, this τ is the period which is adjustable and depending upon how we adjust it you can have different kinds of signals in your NMR spectra.
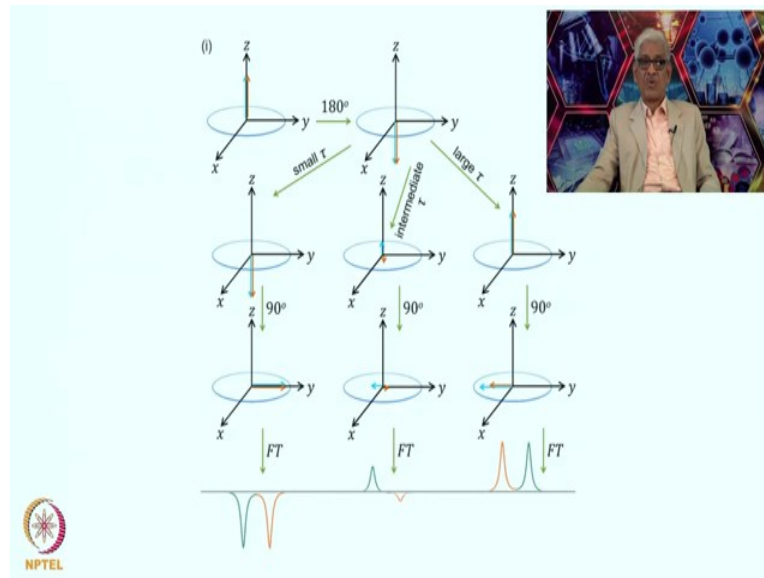
Let us look at that in little bit more detail, so here is the initial situation, we again consider two transitions here, we are not considering coupling at this point but it does not matter, so we are considering two transitions which are belong to two different spins and when we apply 180˚ pulse to this transitions what happens the magnetization is rotated from the Z axis to the -Z axis.

So both of them come here, now these ones will start recovering and as it starts recovering notice these are on the Z axis now, so there is no precession since it is along the Z axis the transitions will start going up along the negative Z axis and eventually they will go to the positive Z axis but they are not in the transfers plane therefore there is no precession.

Now depending upon the time tau they would have recovered to different extents along the Z axis, for a small tau example then both this are still on the negative Z axis not quite as intensity as this to be generate, small tau they were slightly different and if you apply 90˚pulse and measure this then what we will get? We will get the two signals but with the negative intensity because this are on the negative Z axis here, when you have applied the 90˚pulse they are along the negative Z axis, therefore they will have a different sign in your spectrum.

Positive Z axis magnetization leads to positive signals and negative magnetization leads to negative signals, that is the initial point, similarly if you have a very large τ, this is the situation if all of them have come back to the Z axis are recovered completely along the Z axis they both will be here as they started of here they will be here and if you apply 90˚x pulse to this or the y pulse

whatever then magnetization gets rotated along to the -Y axis and this is what you observed in your spectrum both positive signals you are going to observed this is in the contrast to this.

So this is complete recovery and this is the initial very small value or the intermediate values there are the intermediate situations. For example for this the two spins here have two different relaxation times the cyan one has already gone partly along the positive Z axis, the orange one is still lying along the negative Z axis.

Therefore, when you apply the 90x pulse the cyan one moves to the negative Y axis here and whereas the orange one here push to the positive X axis here, therefore when you Fourier transform this you will get this as positive and this one as negative, so this will vary from different spin to spin depending upon their relaxation times. Now what is recovering along the Z axis is dictated by the spin lattice relaxation time T_1. Therefore, if you can monitor this as a function of a τ you get the intensities of each one of this as a function of τ, it naturally allows you to estimate the relaxation time of the individual spins.

(Refer Slide Time: 20:47)

$$\frac{dM_z(t)}{dt} = -\frac{(M_z(t) - M_o)}{T_1}$$

where M_z is the z-magnetization at time t, and M_o is the equilibrium magnetization. Integrating, assuming $M_z = -M_o$, at $t = 0$,

$$M_z(t) = M_o\left(1 - 2e^{-\frac{t}{T_1}}\right)$$

So mathematically of course you have seen this when you have solved the block equations then you have or even when you are considering the recovery of the populations during the initial phases and it is the same equation which is put here, so the rate of change of magnetization is proportional to the deviation from the equilibrium and it is dictated by the time constant T_1.

Now in the inversion recovery experiment at time is equal to 0,

$$M_z(t) = M_0 \left(1 - 2e^{\frac{-1}{T_1}}\right)$$

So at time is equal to infinity you can say here that this term actually goes to 0, $e^{-\infty}$e and therefore it is 0 then $M_z = M_0$ and that is what is here.

Now what is infinity? What is time $T = \infty$? this depend upon how much you want magnetization to recover and come closer to the M_0 value, typically if you see if this is factor of φ if φ time T_1 for example then it will happen with almost 99.9 percent of the magnetization has recovered and which will be almost this will be equal to 99.9 percent of M_0.

So therefore you see after that it does not make any difference so it reaches the plateau here at this point already most of the magnetization has recovered, after that if you take more time it does not matter at all because it will going to reaches the maximum value which is already reached. Therefore, you will get a curve if you plot this and then it will slowly recover to the equilibrium value at large values of τ.
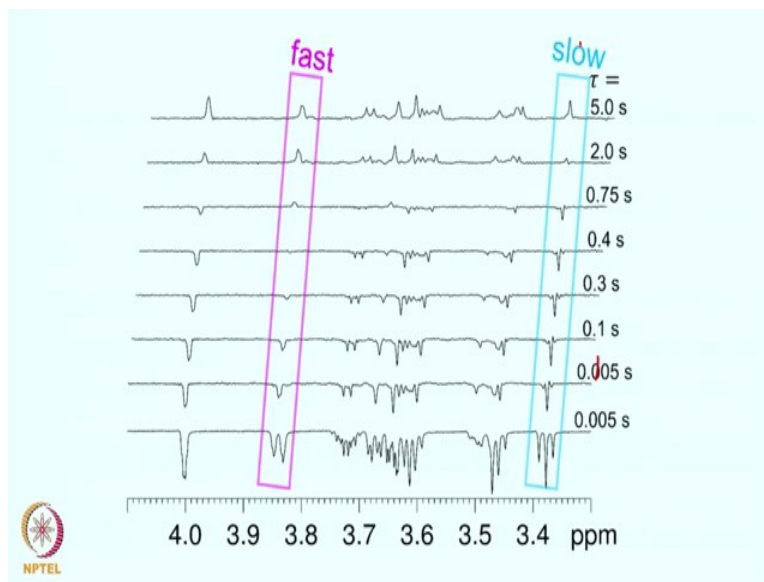
So this is are the experimental spectrum to demonstrate this, so, you have a some particular molecule and you can you have done the experiment at various values of τ, so here it is 0.005 seconds this is the coupled spectra and this is 0.001 this thing is extra 0 here. So there and then it is 0.1 second, 0.3 second, 0.4 second, I think I should remove this extra 0, this 0 is extra, then you have 0.05, 0.1, 0.3, 0.4, 0.75, 2 and 5 seconds you can see by 5 seconds almost entire magnetization has recovered but completely though.

So it is still long way to go, but never the less we can see differences in the recovering rates of the individual spins, the one which is in the magenta color, so this one here this one is recovering faster than this one, this is the slow recovery, this is the faster recovery but even though this is not reach the complete recovery state, it has to still go longer for it to complete recovery.

(Refer Slide Time: 24:37)
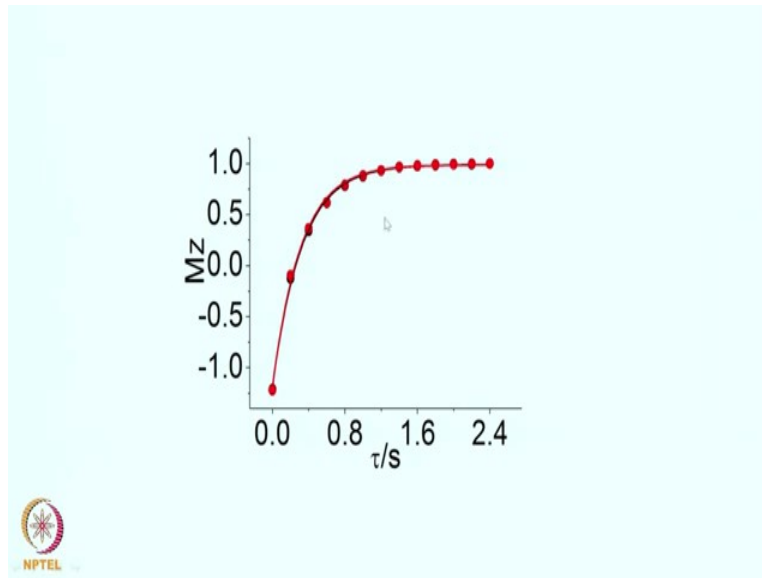
And if you plot this as a function of τ as we discussed earlier you get a curve which is like this, now you fit this to that equation which we show earlier you get the value of T_1.

(Refer Slide Time: 24:51)

At a particular value of $t = \tau_0, M_z = 0$,

$$e^{-\frac{\tau_0}{T_1}} = \frac{1}{2} \text{ or } T_1 = \frac{\tau_0}{\ln 2}$$

This equation allows a quick estimation of the T_1 relaxation times. Alternatively, the above equation can be recast as

$$M_o - M_z = 2M_o e^{-\frac{t}{T_1}}$$

$$\ln(M_o - M_z) = \ln 2M_o - \frac{t}{T_1}$$

This equation shows a linear curve whose slope yields a value of T_1

Now at particular value of $t = \tau_0, M_z = 0$ but it passes through the minimum when it is recovering from the negative Z axis to the positive Z axis it goes through 0 at some point in time, so at that time point $M_z = 0$ and if you put that $M_z = 0$ then you get this equation here

$$e^{\frac{-1}{T_1}} = \frac{1}{2}$$

That means at that point the magnetization is 0 and from here also we can get $T = \dfrac{\tau_0}{\ln 2}$

So this is the quick way of finding out what is approximate value of T_1 for a given proton or for a any given spin.

So for accurate measurement you have to do it for a various values of tau, although priori you do not know what value of tau you should use to get tau not but quickly one does sees the variations and see where the rough tau null will be and you can estimate the value of T_1 and accordingly then you choose the various τ values, you must choose the tau values so that you have the right kind of points for you to fit your data to the equation given.

Now so this equation allows quick estimation of the T_1 relaxation time, alternatively we can also put the above equation in a different form,

$$M_0 - M_z = 2 M_0 e^{\frac{-1}{T_1}}$$

$$\ln(M_0 - M_z) = \ln 2 M_0 - \frac{t}{T_1}$$

if you plot $\ln(M_0 - M_z)$ verses T you will get a linear equation linear curve that is straight line, so the slope of that straight line gives you the relaxation time T_1 and the intersect will give you $\ln 2 M_0$, so we will have to collect this data for various tau values and you can plot this $\ln(M_0 - M_z)$ against the τ values.

(Refer Slide Time: 27:01)

Measurement of T_2 relaxation time

$$M_{xy} = e^{(-t/T_{2^*})} \qquad \frac{1}{T_2^*} = \frac{1}{T_2} + \frac{1}{T_{2(\Delta H)}}$$
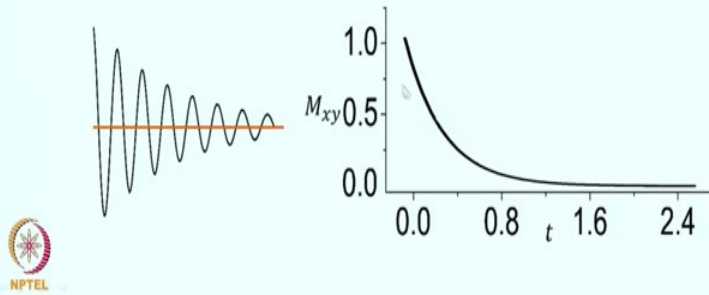
Now measurement of the T_2 relaxation time, T_2 relaxation time is now the transverse relaxation time, this is the FID, the FID is going like this so this is the decaying and this decay is due to two factors as we discussed earlier, one is the spin relaxation time T_2 and the other one is the contribution from the field inhomogeneities, field inhomogeneities in your sample cause different lines to precesses a slightly different frequencies and that in-homogeneity appears as the line width factor or we can treat it as a separate contribution to the T_2.

Therefore the measure relaxation rate here, and the time constant what we get here is generally

represent as a T_2^i, $\frac{1}{T_2^i} = \frac{1}{T_2}$ which is the true relaxation time plus $\frac{1}{T_2}$ which is the contribution from the field inhomogeneities the field inhomogeneities caused de-phasing and that leads to the decay, de-phasing means its leads to the cancellation of the magnetizations and therefore it appears as an intensity has gone down. So therefore this has to be removed to measure the T_2 precisely. So, how do we do this? So this is the observed decay what we will get from here.

(Refer Slide Time: 28:18)

Measurement of T_2 by spin-echo
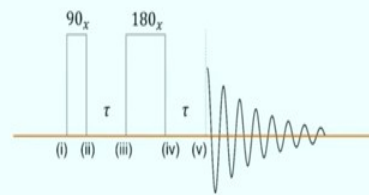
Echo amplitude (M_{echo}) is collected as a function of τ, the peak intensity in the NMR spectrum represents the echo-amplitude for the individual spins.

Since the field inhomogeneity effects are refocused,

$$M_{echo} = M_0 e^{(-t/T_2)}$$

This allows precise measurement of T_2

Here we make the use of the spin echo, you remember the spin echo refocuses the field inhomogeneities, so this is the pulse sequence foe the spin echo, once again you have seen the echo amplitude is collected is unaffected by the field inhomogeneities and therefore whatever decay what you will measure here in the echo amplitude as a function of this tau will be truly due to the spins and relaxation time T_2.

So what you do is you echo amplitude is collected as a function of a tau, there is a peak intensity in the NMR spectrum represents the echo amplitude for the individual spins, when you take this FID and Fourier transform it you will get different line and the different line the intensity if each line represents the echo amplitude for that particular frequency, therefore we measure the peak intensity for all the spins and plot it as a function of τ then you will fit it to a equation

$$M_{echo} = M_0 e^{\left(\frac{-t}{T_2}\right)}$$

Notice the field inhomogeneities effects are gone and therefore here you have exclusively the T_2 coming out as a result of spin echo amplitude changes as a function of tau, this allows precise measurement of T_2, however there is one difficulty and that is during this period tau suppose the molecule shifts from one place to the another place and if there is the field inhomogeneity there.

So it is it has to completely refocus, whatever it has decayed here has to completely refocused here by the precessional frequency of the particular spin has to remain the same and this tau as well as in this tau, but if during this tau the sample is in the first τ if the molecule is here and in

the second τ if it is here and if the fields are different in the two places then the refocusing will not happen, refocusing from this precession of this during this tau will not be completely refocused here. Therefore, the field inhomogeneity effects will not be removed, so some of that contribution will be present. Therefore, this diffusion has to be avoided, so therefore how do you achieve this and this is dependent obviously on the magnitude of your tau value, how much time you give.

(Refer Slide Time: 30:56)

Carr-Purcell modification:

Diffusion during the τ period causes improper refocusing resulting in reduction in echo-amplitude. This can give wrong T_2 values.

Carr- Purcell modified the pulse sequence to counter this effect.

$$90 - \tau - 180 - \tau - echo - \tau - 180 - \tau - echo - data\ collection$$

The τ value is kept at the minimum to avoid diffusion effects.

The number of echoes is varied so as to get different time points for the exponential fitting procedures for T_2 estimation. This yields a more reliable value of T_2.

NPTEL

The Carr-Purcell made a modification top the pulse sequence, let us not do the spin echo in a simple way like that as indicated but let us do in the following way, you give a 90°pulse-τ-180°-τ-echo-τ-180-τ-echo and you go on doing this and at the end after certain number of echoes you do the data collection and now from here to here it is echo but what you do is you keep this τ period extremely small, very very small, something about like 1 millisecond or sometimes even less than a millisecond.

So if you keep this small then there is not enough time for this molecule to defuse from one portion of the sample to another portion of the sample, therefore complete refocusing will happen here and after the echo it will again decay the τ is kept the same 180 pulse, τ and again an echo, so once there is no time for the molecule to defuse therefore the echo amplitude at each point is not modulated by the field inhomogeneity at all.

So therefore then you say how do I get different time points if I want to measure the as a function of tau as in the previous case what we have tom do is, so we have to repeat this, keeping small value of τ as required here repeat the number of echoes, suppose for 10 milliseconds I have to root 10 echoes and after the 10 echoes you collect the data one data point, for 20 milliseconds I have to root 20 echoes, so after 20 milliseconds whatever echo comes I will collect that data point, so like that 30 milliseconds, 40 milliseconds , 50 milliseconds if you have to do you simply increase the number of echoes here keeping this τ the same as the result the diffusion portion will not alter your data but you still gets different time points for the different measurement of your T_2.

So the τ value is kept at the minimum to avoid the diffusion effects and the number of echoes is variate so as to get a different time points for the exponential fitting procedure for T_2 estimation, this is the more reliable value of T_2.

(Refer Slide Time: 33:08)
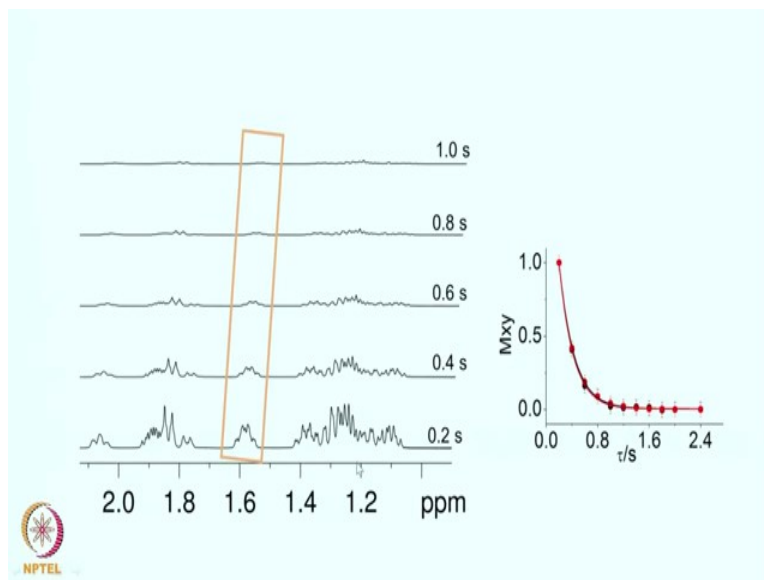
This is the experimental demonstration of a particular sample, so you have here a normal one day spectrum; this is done with a point 0.2 seconds, 0.4 seconds, 0.6 seconds, 0.8 seconds and 1.0 seconds. Notice here this are all the total τ values, it does not tell you how many echoes have been collected here, that is not relevant for you we keep the τ value so small so the diffusion effects are removed and the total number of echoes is adjusted so that I get a time point which is equal to 0.2 seconds, here it is 0.4, 0.6, 0.8 so on and so forth.

And now you are able to fit this intensities of all of this transitions for the individuals once you can fit this and you get a relaxation fitting like this and you get a T_2 value, obviously for the different transitions here we get the different values of T_2 as you can say they are all decaying at different rates, so this is because of the different T_2 values. So, I think we will stop here, so we have completed here the discussion on the relaxations time and he measurement of the relaxation time, how one can achieve good quality spectra and how one can remove the coupling and that was the main theme of the today's talk, we will stop here.