NMR Spectroscopy for Chemists and Biologists
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Lecture No. 26
Rotating Frame Experiments

So welcome to today's class. We were discussing polarization transfer and we will take some some advance topic in polarization transfer. So, in last class we had looked at like how we can enhance the sensitivity of its nuclei by selective polarization inversion. So, that means *A* and *A* spins were coupled we were selectively inverting the population of one of the transition in *A* spin and because of that population inversion, the intensity of *X* spin got enhanced. Then we looked at another method called INEPT where we were transferring the polarization from *A* spin to *X* spin with help of series of these pulse sequences.

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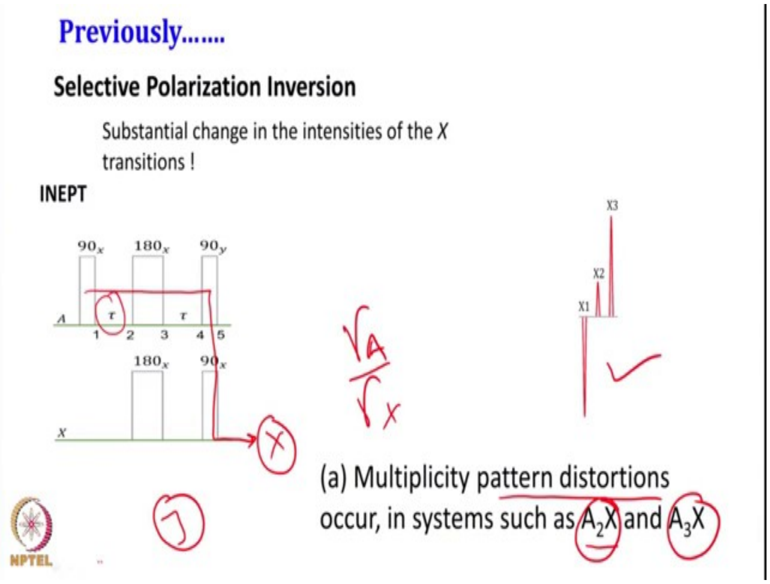


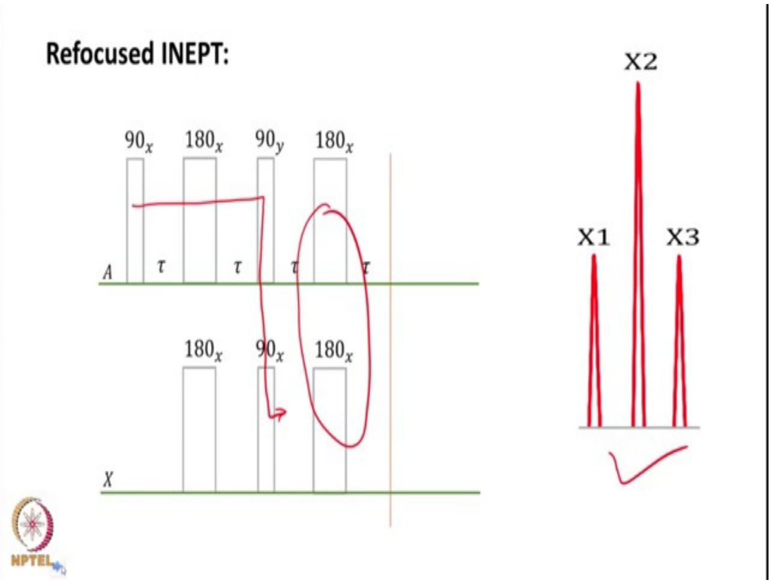
Previously……

Selective Polarization Inversion

Substantial change in the intensities of the *X* transitions !

INEPT

(a) Multiplicity pattern distortions occur, in systems such as A_2X and A_3X



We were starting with 90°pulse, then we were evolving the magnetization for τ period and τ is $1/4J$ and then we were applying simultaneously 180° pulse on *A* spin and *X* spin, then again evolving it for τ period which is again $1/4J$, so total τ is $1/2J$. Then we were applying a 90°*y* pulse on A spin and 90°*x* pulse on *X* spin. Because of that magnetization was coming from here to here and we were detecting on *X* spin and because of that actually sensitivity of *X* spin got enhanced and that can be in order of the γ_A/γ_X, so typically 4 times enhancement we can get it. We saw that there are some problem associated with this kind of INEPT because it depends up on *J* value, so *J* dependency is there.

And then we can get it distortion in the peak pattern because of coupling how many protons or how many *X* are coupled, so we can have a different kind of distortion like if you have A_2X and A_3X different kind of peak pattern will arise and some of them can be positive, some of them can be negative. So looked at this, to get rid of this we just saw that we had additional spin echo system, as spin echo sequence to the INEPT which is called refocused INEPT, we can get rid of this different sign of the multiplicity peak and all can be positive. So here what we were doing? So same like INEPT we are starting with a 90°pulse, then applying 180°simultaneous pulse.

Now magnetization was here at INEPT and then we are doing this is spin echo that means τ-180-τ and because of that all the lines which some of them were negative, some of them were positive, now all are positive. So this refocused INEPT removes the problem that were associated with INEPT and all the lines here with a positive signal, so we had finished up to this point.

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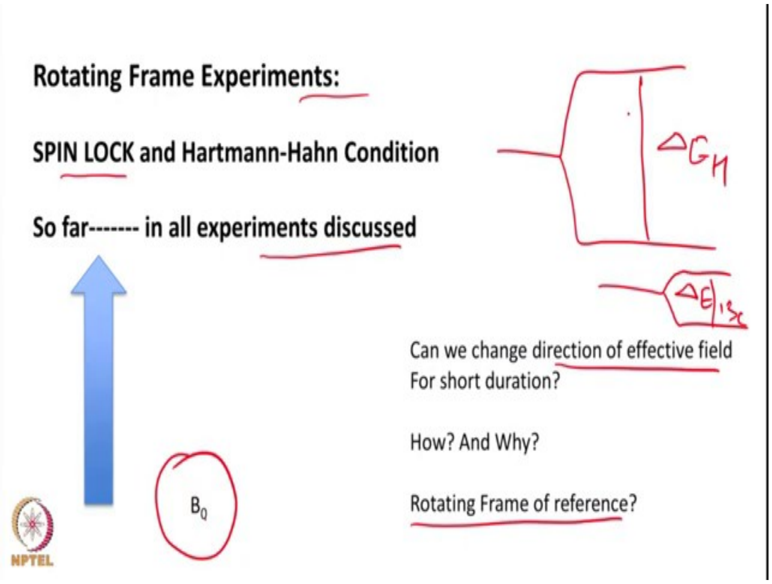


Rotating Frame Experiments:

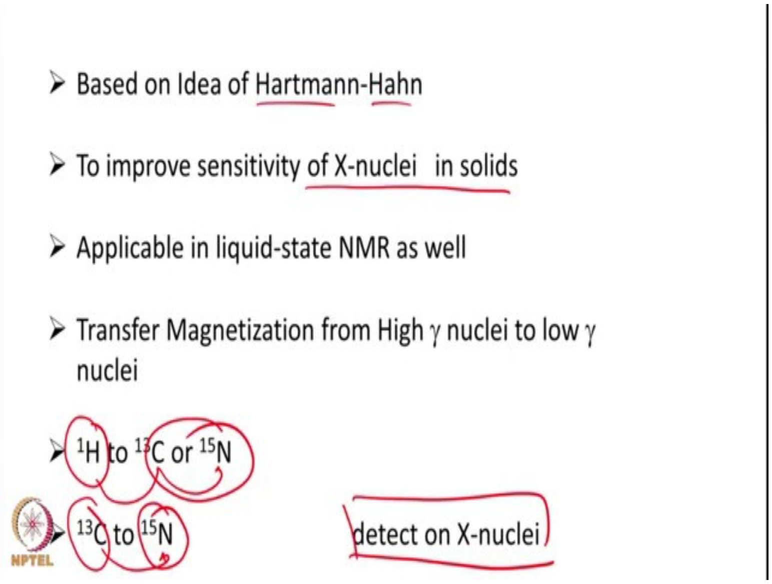
SPIN LOCK and Hartmann-Hahn Condition

So far------- in all experiments discussed

Can we change direction of effective field
For short duration?

How? And Why?

Rotating Frame of reference?



- Based on Idea of Hartmann-Hahn
- To improve sensitivity of X-nuclei in solids
- Applicable in liquid-state NMR as well
- Transfer Magnetization from High γ nuclei to low γ nuclei
- ^{1}H to ^{13}C or ^{15}N
- ^{13}C to ^{15}N

detect on X-nuclei

And then we looked at how we can do transfer of the magnetization from *A* nuclei to *X* nuclei or proton to carbon using a concept called cross polarization. So we will build up those concepts today and we will take it forward for some of those topics that we want to discuss today. So as we said that we have to do these in rotating frame of reference and two experiments that we are going to discuss today is spin lock and Hartmann-Hahn matching condition. I briefly mentioned what is actually Hartmann-Hahn matching condition.

So today I am going to explain this in little more detail. So what we have seen, that till now we have we have actually discussed, how the spins interacts with main magnetic field which is B_0 and this interaction or this interaction Hamiltonian is called Zeeman Hamiltonian. So here B_0 is in Z direction and our spins are interacting because of this phenomenon we have different chemical shift for different spins.

Now and so here we have looked, so but can we change this effective field direction for short duration? So what is the benefit of changing the, changing the direction of the effective field, how we can change it and why we can change it? So you might have heard of concept of rotating frame reference or we discussed earlier, so what we are trying to do from the lab frame we are transforming it to rotating frame by changing the direction of the effective field for a short duration.

So what we achieve by doing this, so this idea is based on something called Hartmann-Hahn. So these are two scientists who actually conceptualize this and proposed the idea. This is actually used for improving the sensitivity of X nuclei in solid. So as I said like in Z direction, when the magnetization is in z direction it is difficult to match the energy gap between proton and carbon. So here say if it is for proton this is for delta E for proton, for carbon is it going to be 4 times less.

So this is our ΔE for ^{13}C, if they are interacting with the B_0. But suppose we change the direction, effective field direction, by doing that we can match the energy gap between proton and carbon and we somehow match it then we, in last class we conceptualize that we can transfer the polarization from proton to carbon and that will enhance the sensitivity. So how we are going to do that for improving the sensitivity of X nuclei? So this was conceptualize in solid, but this is also applicable in liquid-state. So the crux of the matter is we want to transfer the magnetization from high γ nuclei to low γ nuclei.

That means high γ nuclei such as proton to carbon 13 or nitrogen 15 or carbon 13 to nitrogen 15 and we can transfer this and this transfer will enhance the sensitivity or signal of the the low gamma nuclei such as these nuclei and we need to detect on X nuclei in solid that is why we need to transfer it so to enhance the sensitivity. Now why to detect, because the dipolar coupling something called dipolar coupling that we have seen the 2 spins are connected through a space by a coupling called dipolar coupling. So in solid this dipolar coupling is used and for proton dipolar coupling is very very large, so that leads to the line broadening. Therefore, in solid generally X nuclei are detected.

So since X nuclei are detected, therefore it is imperative to enhance the signal of X nuclei and this is the way to enhance it transferring the polarization from more sensitive nuclei such as proton to less sensitive nuclei such as carbon or nitrogen.

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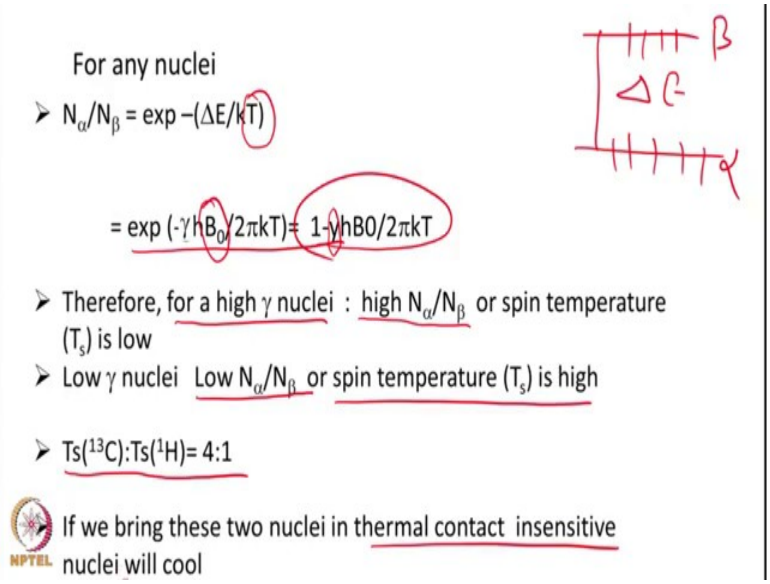

For any nuclei

- $N_\alpha/N_\beta = \exp -(\Delta E/kT)$

  $= \exp(-\gamma h B_0/2\pi kT) = 1-\gamma hB0/2\pi kT$

- Therefore, for a high γ nuclei : high N_α/N_β or spin temperature (T_s) is low
- Low γ nuclei Low N_α/N_β or spin temperature (T_s) is high
- $Ts(^{13}C):Ts(^{1}H)= 4:1$

If we bring these two nuclei in thermal contact insensitive nuclei will cool

Now so essentially if you look at the conceptual that we have learned in previous classes, so sensitivity is given by the difference in the population between the ground state and the excited state or or the first excited, or we can say α state and β state. So α state we have more number of particles, β state we have less number of particles. So, a nuclei will be called sensitive if the difference is more, so there are more in α state and less in β state. And that depends up on what is the Δ E between these 2 states and also on T.

$$\frac{N_\alpha}{N_\beta} = \exp - \left(\frac{\Delta E}{kT} \right)$$

So T we cannot change too much. So actually if you expand this

$$¿\exp\left(\frac{-\gamma h B_0}{2\pi kT}\right)=\frac{1-\gamma h B_0}{2\pi kT}$$

T is generally ambient temperature that we see. Therefore, in NMR the difference between $\frac{N_\alpha}{N_\beta}$ comparatively quite less. And if you look, if you expand this we get this formula where there is a B_0 dependence. So that is why the separation between these 2 states depend on B. Now, so that means if the separation at the same *B*, ΔE is more for proton, so proton is more sensitive. If it is less, then it is less sensitive, so that is why proton is more sensitive and carbon 13 and nitrogen 15 is less sensitive because here is a γ and $\gamma_{A/H}$ for proton is is high compare to carbon 13 and nitrogen 15.

So for a high γ nuclei such as proton it has high $\frac{N_\alpha}{N_\beta}$. That means ratio for proton is high and therefore, their spin temperature is low. So that means protons are sitting at low temperature spin bath, whereas carbon 13 which is low gamma nuclei, they have low ratio of n alpha and n beta and they are sitting at high temperature spin bath. So spin bath ratio is typically 4 to 1 because gamma of proton is 4 times more than carbon 13. So T_S that is spin temperature of carbon 13 is actually 4 times more than the spin temperature of proton.

Now by a mechanism suppose we want to bring these 2 spin bath, proton spin bath at low temperature, carbon spin bath at high temperature or proton is more sensitive, carbon is less sensitive, somehow we bring these 2 spin bath together, then what will happen, the temperature will flow or spin temperature will flow from carbon to proton or we say sensitivity will flow or polarization will flow from proton to carbon and carbon sensitivity or polarization can be enhanced. So if you bring these 2 nuclei in a thermal contact, insensitive nuclei will cool and therefore, if they cool their sensitivity can be enhanced.

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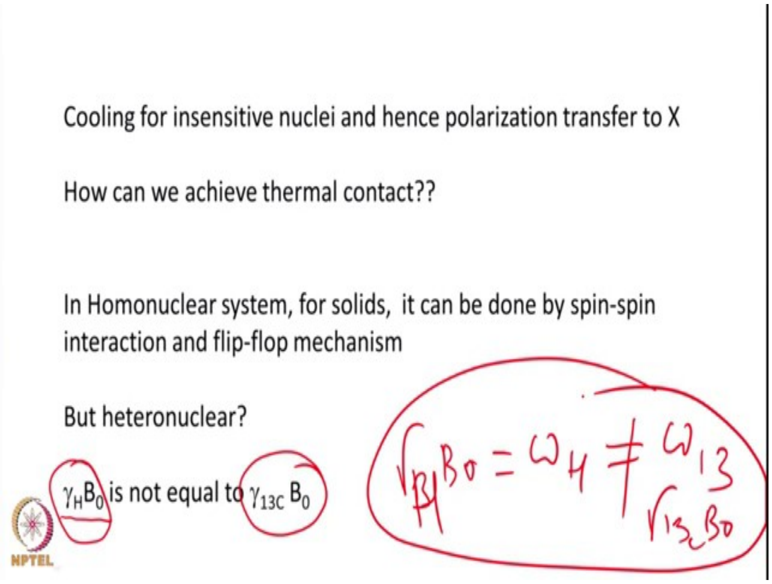

Cooling for insensitive nuclei and hence polarization transfer to X

How can we achieve thermal contact??

In Homonuclear system, for solids, it can be done by spin-spin interaction and flip-flop mechanism

But heteronuclear?

$\gamma_H B_0$ is not equal to $\gamma_{13C} B_0$

$\gamma_H B_0 = \omega_H \neq \omega_{13}$

$\gamma_{13C} B_0$

So this this is what we want to achieve. We want to cool the spin temperature of insensitive nuclei by putting them in thermal contact with low spin temperature nuclei such as proton. So how we can achieve that? So that actually in homonuclear system, for solid it can be done by spin-spin interaction. So what happens since in solid spins are closer and they are not tumbling too much, then actually dipolar coupling is active and spins are interacting.

So by something called flip-flop mechanism, actually they can transfer this magnetization but say heteronuclear system such as like such as proton and carbon coupling,

$$\gamma B_0 = \omega_H \neq \omega_{13C} = \gamma_{13C} B_0$$

Now, so that means if we keep in same field these two nuclei X and ^{13}C, even the thermal contact cannot be achieved and polarization transfer cannot happen.

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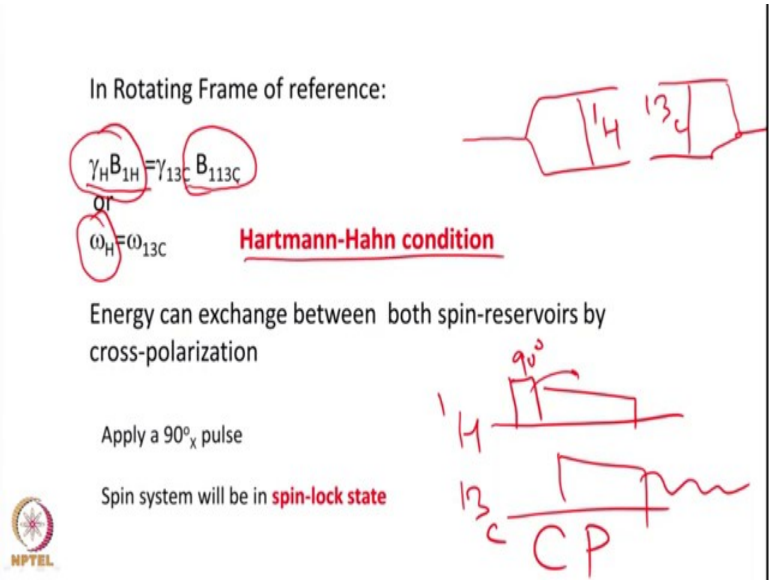

In Rotating Frame of reference:

$\gamma_H B_{1H} = \gamma_{13C} B_{113C}$

or

$\omega_H = \omega_{13C}$

Hartmann-Hahn condition

Energy can exchange between both spin-reservoirs by cross-polarization

Apply a 90°_x pulse

Spin system will be in **spin-lock state**

So then we have to do a trick. We have to shift from lab frame to rotating frame of reference. And now that is what we are saying, we want to change the direction of effective field by applying a B_1 field and B_1 field is applied on proton as well as on carbon. So suppose we achieve this condition where we applied two B_1 field, one for proton and one for carbon, then in that case we can achieve that $\gamma_H B_1 = \gamma_{13C} B_{113C}$ and effective field applied along like a transverse plane or rotating frame of reference on ^{13}C.

So if you do that, we can achieve $\omega_H = \omega_{13C}$ and this condition is called Hartmann-Hahn matching condition. That means to visualize you simply now by applying this we want to match this for proton with of carbon 13. So this is for ^{13}C and this is for proton. By applying 2 RF pulse or effective field in rotating frame of reference one on proton and one on carbon 13. So if you do that, this frequency of proton and carbon 13 will match. This matching condition is known as Hartmann-Hahn matching condition and this will lead to polarization of transfer between proton and carbon.

So energy exchange will happen with both spin-reservoir by something called cross-polarization. So polarization is coming from proton to carbon 13, therefore it is called cross-polarization. How we have to do that? So essentially we have to apply a 90°pulse, so let us see we write two channel, one channel is for proton and another channel is for ^{13}C. So, if we apply a 90°pulse on proton, and then what we are doing 90° here and then we are locking it. So we are locking proton and carbon, so because of that now here we brought it to *x-y* plane and then we locked it and then we can detect *X*. So this is called cross-polarization experiment CP, cross-polarization and this actually enhances the sensitivity of carbon. That happens in solid.

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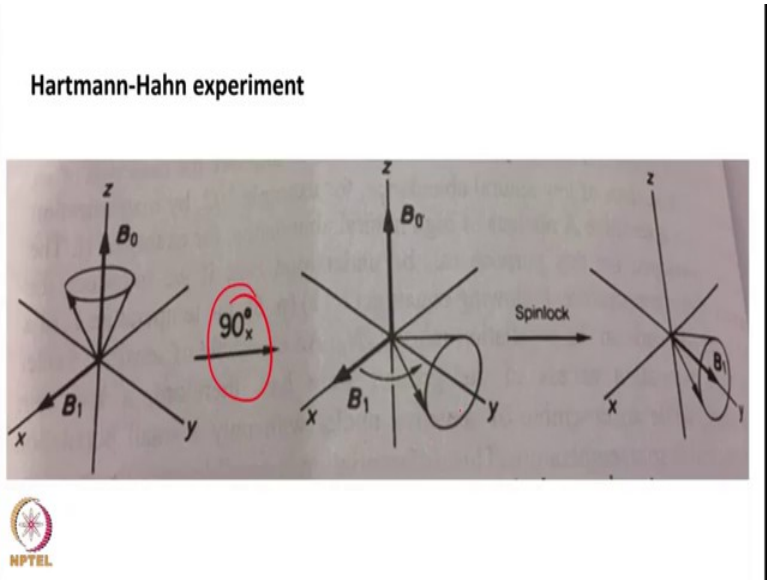

Hartmann-Hahn experiment

So essentially vectorially if you see it, what we are doing, here is our B_0 field to start with we had magnetization in B_0 field in Z direction and here is our B_1 field which is like transverse reaction, we applied a 90˚pulse which is say on *x* pulse, now our spin is in this direction and rotating in *x-y* plane like here. Now we are locking it, we are applying a pulse so that it is locked in the *x-y* plane and by locking proton and carbon both in this same direction that means we are maintaining thermal contact between *X* and ^{13}C and that actually is the cause of sensitivity enhance. So that we can do easily in solids.

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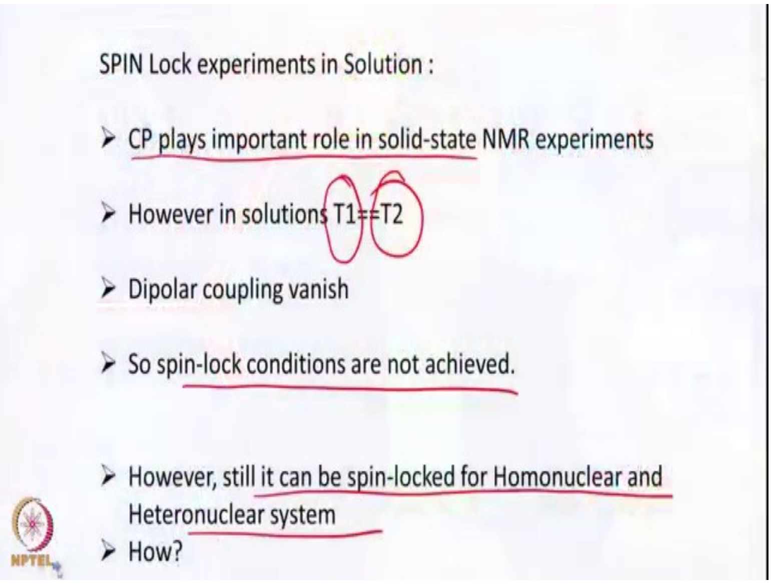

SPIN Lock experiments in Solution :

- CP plays important role in solid-state NMR experiments
- However in solutions T1==T2
- Dipolar coupling vanish
- So spin-lock conditions are not achieved.
- However, still it can be spin-locked for Homonuclear and Heteronuclear system
- How?

CP which means cross polarization plays an important role and in generally in solid state NMR experiment. All experiment essentially where we are detecting on carbon starts with cross polarization, we will transfer the magnetization from proton to carbon, that is the first step in solid-state experiment. But in liquid state, actually CP may not work because here what happens typically for liquid state the *T1* relaxation time and *T2* relaxation times are more or less equal. And therefore, actually the like spins does not have dipolar coupling or simply you can visualize at the spins are orienting normally at very rapid scale and therefore the dipolar coupling which is distance dependent will vanish because now direction is changing all the time and distance is changing, so dipolar coupling vanish completely.

Therefore, if you do cross polarization the spin-lock condition cannot be achieved. So that cannot be achieved, so that means whatever we were saying, transferring the polarization from proton to carbon in liquid if you do like this we cannot enhance it. However, we can do another trick and then we can achieve the spin-lock for homonuclear and heteronuclear system but how we can do it, let see it.

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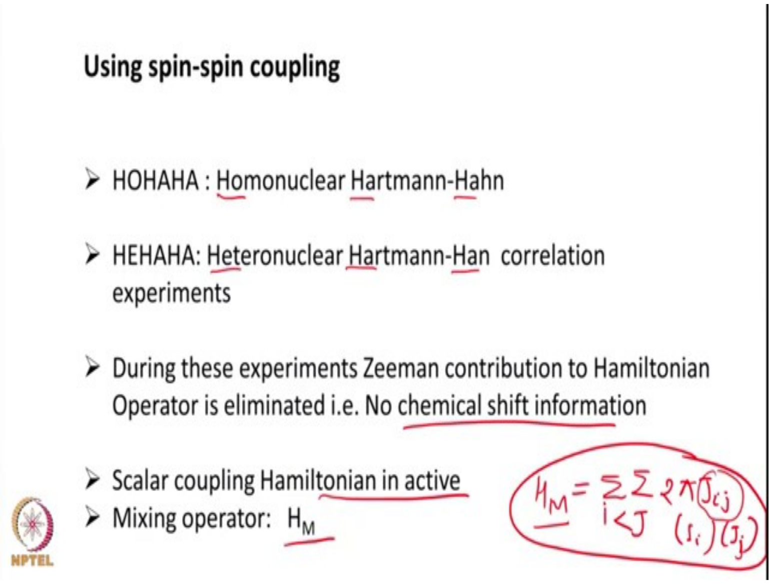

Using spin-spin coupling

- HOHAHA : Homonuclear Hartmann-Hahn
- HEHAHA: Heteronuclear Hartmann-Han correlation experiments
- During these experiments Zeeman contribution to Hamiltonian Operator is eliminated i.e. No chemical shift information
- Scalar coupling Hamiltonian in active
- Mixing operator: H_M

$$H_M = \sum_{i<J}\sum 2\pi (J_{ij}) (I_i)(J_j)$$

So here as you know dipolar coupling is not active, but we have 1 coupling which is active and that is *J* coupling, the scalar coupling, so scalar coupling is active. Using this scalar coupling we can still achieve the transfer of polarization for a homonuclear system and heteronuclear system. So for homonuclear system the experiment is called HOHAHA. Homonuclear Hartmann-Hahn experiment and for and heteronuclear it is called HEHAHA that means Heteronuclear Hartmann-Hahn. HOHAHA is Homonuclear Hartmann-Hahn. So these two experiments can be done to achieve the polarization transfer. So what we actually do? During these experiments actually we have to remove the Zeeman contribution, Zeeman contribution as we have seen B_0 contribution. So there is no chemical shift revolution.

What we have to do? We have to remove that Zeeman contribution but we have to keep active the scalar coupling, scalar coupling Hamiltonian but still our coupling, scalar couplings are active in the Hamiltonian. So scalar coupling can act as a mixing operator, so if you look at so this essentially sum of the

$$H = \sum_{i<j} \sum 2\pi J_{ij} (I_i)(J_j)$$

so that means 2 spins say *A* spin and *X* spin, there is a coupling, scalar coupling is active even if we remove the Zeeman contribution. Now that actually helps in mixing of the of the or polarization transfer between 2 nuclei.

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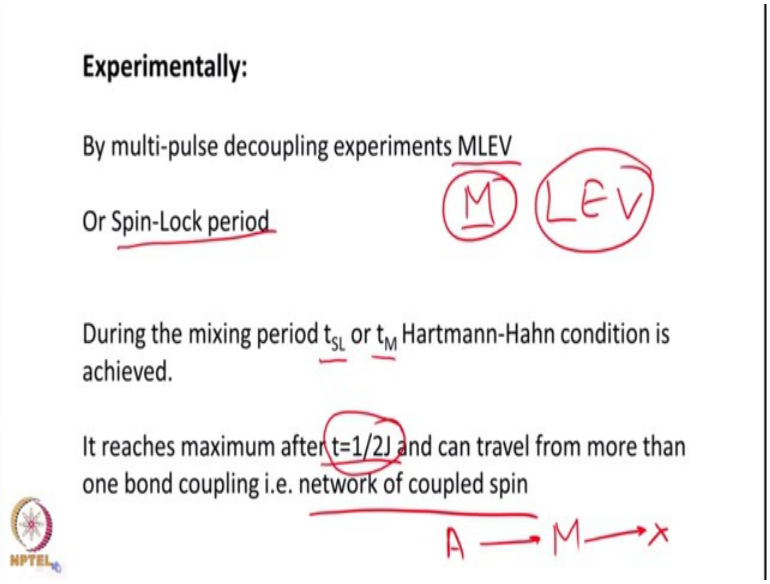

Experimentally:

By multi-pulse decoupling experiments MLEV

Or Spin-Lock period

During the mixing period t_{SL} or t_M Hartmann-Hahn condition is achieved.

It reaches maximum after $t=1/2J$ and can travel from more than one bond coupling i.e. network of coupled spin

So how experimentally it is done I will just explain you. So we are saying we are removing the Zeeman contribution, but keeping active the scalar coupling contribution. So experimentally it is done by something called multiple pulse decoupling experiment which is called MLEV. So MLEV is is actually has come from name of scientist whose name is Malcolm and Levit. So Malcolm Levit actually has developed this sequence and that is how it is MLEV or we can lock it the spins for a period and that is called a spin lock period.

So what actually it is done, so during this period either it is a spin lock period or the like a mixing period T_{SL} or this T_{SL} means spin lock period and it is a mixing period. Essentially we want to achieve Hartmann-Hahn matching condition and this Hartmann-Hahn matching condition if we achieve the transfer will happen. Transfer will happen from one nuclei to another nuclei, more sensitive nuclei to less sensitive nuclei or even at in the homonuclear fashion. And this transfer, so essentially what we are doing, we are starting with the z magnetization, we are bringing into x-y plane and we are locking it there.

So this locking actually has a time dependence or so it actually reaches to maximum when the locking period or the spin lock period is more or less ¿$1/2J$. And if we achieve this, that magnetization can transfer or travel from more than one spin, so like if you take a AMX system it can transfer from A to M and then to X. So that means multiple spins can be coupled or network of spin can be transfer in network of spin, coupled spin can be achieved.

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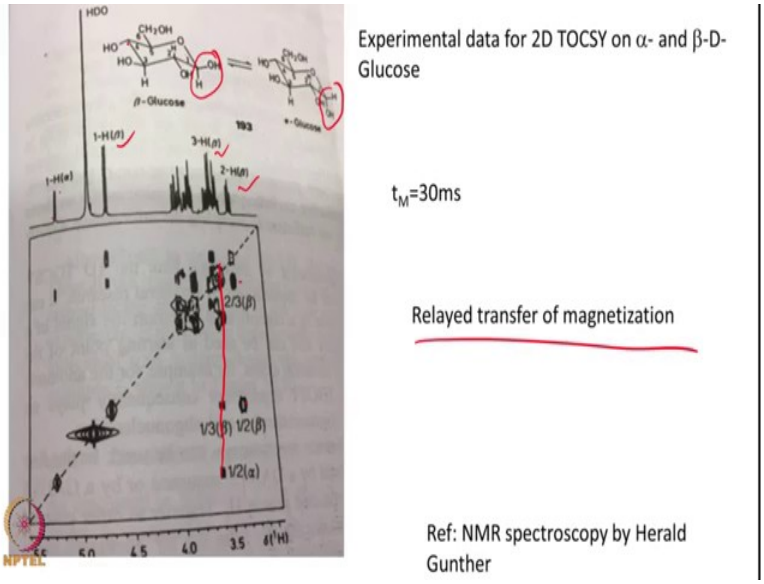

Experimental data for 2D TOCSY on α- and β-D-Glucose

t_M=30ms

Relayed transfer of magnetization

Ref: NMR spectroscopy by Herald Gunther

So how actually it is done, the experiment is simple, do not worry at the moment about this. Here it is a pulse sequence for 2D but essentially if you remove this t_1 period here, it is a sequence for transferring from magnetization transferring from one spin to another spin. So what essentially we are doing applying a 90˚pulse. Applying a 90˚pulse means bringing them to *x-y* plane and then we are spin locking it, and locking in the *x-y* plane.

So because of locking that means we are applying a weak RF pulse. Weak RF pulse bringing them to to like a rotating frame of reference and here the Hartmann-Hahn matching condition happening and then we detect it and that is FID. So similar in case of TOCSY total correlation spectroscopy that we are going to learn after few class, so this is also essentially the achieving this Hartmann-Hahn matching condition. So start with a 90˚pulse and then a series of decoupling pulses is applied which is called MLEV and then you detect it. So this is mixing time and this is spin lock time, so essentially the concept is similar.

Now we, same experiment can to extended to 2 dimension just we have to add a t_1 evolution here, that again we are going to discuss after few class. So that means from 1 dimension we can add 2 dimension and as we can actually achieve the magnetization transfer relay from one spin to another spin to third spin to forth spin something like that. So that is what essentially is HOHAHA or TOCSY experiment. So for an example I will just give you where actually it is used. So say we have a glucose molecule D-glucose which actually which is in equilibrium $\beta-$¿

glucose and αglucose the difference you can notice is at this position. So now there are so many protons here, if you record 1D, we get all these peaks.

So here is a water peak and these are other proton that are these are coming from βconfirmation, β glucose, these are β glucose, this is α glucose because here is the difference. Now, if you record 2D on glucose molecule, say with a mixing time of 30 milliseconds, so this 30 millisecond when we spin lock in x-y plane. Now because of this mixing there is relay transfer of magnetization from one spin to another spin. You can see it, here this is in between 2, 3, 1, 2 like if you go in this line there are transfer happening 1 to 2, 1 to 3. So that means relay transfer happens. So we can transfer from magnetization say from here to here to here and so and so forth.

And that helps in getting the structural information for any of such molecules, details we are going to discuss when we start actually looking at the 2D experiments on such as COSY TOCSY and all those. But at the moment this is this concept is important because we can now achieve the correlation between 3 bond, 4 bond coupling.

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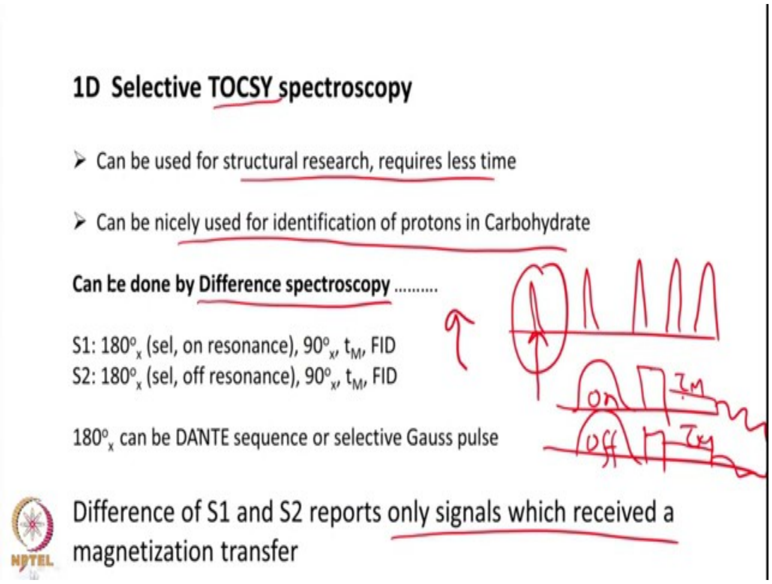

1D Selective TOCSY spectroscopy

- Can be used for structural research, requires less time
- Can be nicely used for identification of protons in Carbohydrate

Can be done by Difference spectroscopy ....

S1: 180°_x (sel, on resonance), 90°_x, t_M, FID
S2: 180°_x (sel, off resonance), 90°_x, t_M, FID

180°_x can be DANTE sequence or selective Gauss pulse

Difference of S1 and S2 reports only signals which received a magnetization transfer

Simplified version of this can be also done in 1D, 1D fashion so now I am going to discuss with you 1D selective TOCSY experiment. So TOCSY is total correlation spectroscopy, do not worry too much at the moment about name. So it is a same spin lock period, 90˚ spin lock period and detection. Now this can be used for structural information, why actually, generally say 2D takes 3 hours but the 1D version of same experiment, you can achieve and you can save lots of time. So this polarization concept, actually polarization concept can be used for transferring the magnetization in 1D fashion and you save lots of time.

And the like whatever example we showed in the last slide, it can be nicely used for identification of protons say in carbohydrates. So how we are going to do experiment? Let us see, we do 2 experiment and we take difference between them, so that is called difference experiment. So essentially we are trying to do something like this 2 experiment, experiment number 1 we want to apply a 180˚selective pulse on resonance, so say I have 5 peaks here, 1, 2, 3, 4 and 5 peaks.

So in first experiment I apply 180˚pulse selectively on this spin then we apply a 90˚pulse, so 180-degree selective then we apply a 90-degree pulse on all spins, then we have a mixing period here spin lock period or mixing period here tau mixing and then we detect FID, so this is our first experiment.

In the next experiment we are, so this on resonance, in next experiment we are doing same thing, we are applying 180-degree pulse but not this time on resonance but off resonance somewhere here. And then we apply a 90-degree pulse and then we have a mixing time and then we detect FID and then we take difference between these 2, whatever spectra comes take a difference between S1 and S2, so this will, this will deport only where magnetization transfer has happened because here we have inverted and then we mixed it so difference between these two actually gives us information where the magnetization transfer happens and that will tell you the the connected proton.

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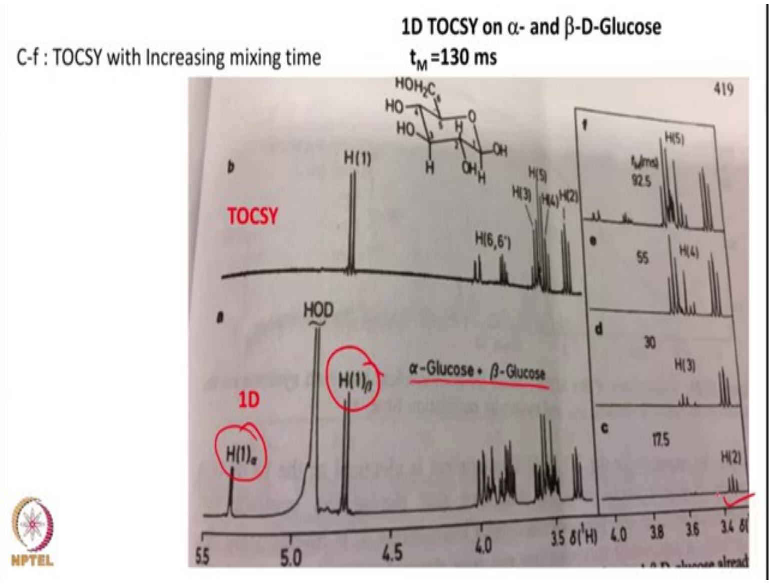

C-f : TOCSY with Increasing mixing time

So here is a one of the example. So let us see, with same molecule β glucose if you take. Now just we got say β glucose is always in equilibrium with α glucose, so here we getting the spectrum coming from a mixture of alpha and beta glucose. Here are those peaks that appears, we have seen in the last slide. So these are, this is the $H_1\alpha$ and this is $H_1\beta$ so that tells that we have both confirmation in same solution. This is water peak.

Now we did a TOCSY, so TOCSY means total correlation spectroscopy, so all those are directly coupled will come. Therefore, if you notice here this peak is not coming because this is from different conformers, so all those has come 1, 2 like here 6, 6 prime, 3, 5, 4, 2, so here 1, 2, 3, 4, 5, 6 all protons have come.

Now we have a complete spectrum, so now what experiment we are doing same TOCSY with increasing mixing time we are doing. So if we say start with 1, we selectively invert 1 and then we want to apply a 90°pulse and then we are increasing the mixing time. So first one which is near to that will be appearing and that is how we get a 2, signal coming from 2. 180° applied on 1, then we started mixing we got 2 then we are getting 2 plus 3. So now we know that 2 and 3 are connected. Similarly, we are getting 2, 3, 4 we are getting our 2, 3, 4, 5 and if we keep increasing we can get everything.

So now by increasing time, so here we are, we can assign it which is closer to one proton. Now the nearest one appears at a shorten mixing time, the furthest one will appear at a longer mixing time. So we can get a like we can see the relay in the magnetization transfer from 1 to 2 to 3 to 4 to 5 and 6, and that is the beauty of this experiment. Now we can do spectral editing by doing the multiple 1D TOCSY experiment.

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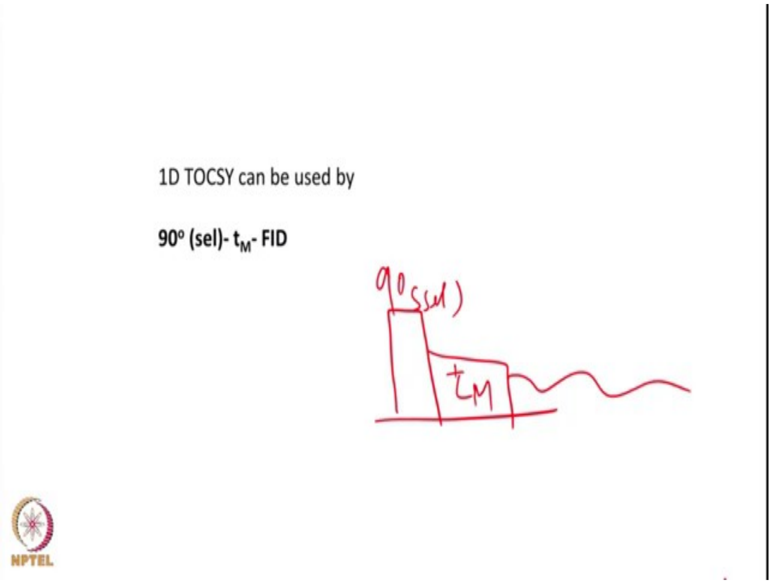

1D TOCSY can be used by

90^o (sel)- t_M- FID

So essentially, alternatively TOCSY that I discussed we can also do by a simple experiment so 90-degree is selective pulse, say 90˚is selective we do want spin number 1, then we are doing a mixing time here, τ_M or t_M and then we do FID. So essentially same experiment you can do by just selectively applying a 90˚pulse on spin number 1, then mix it and then we detect it. So if keep mixing for, see the proton number 2, then we see proton number 3, then we see proton number 4 and so and forth. So that is the beauty of 1D TOCSY experiment can be done easily and in a quicker time.

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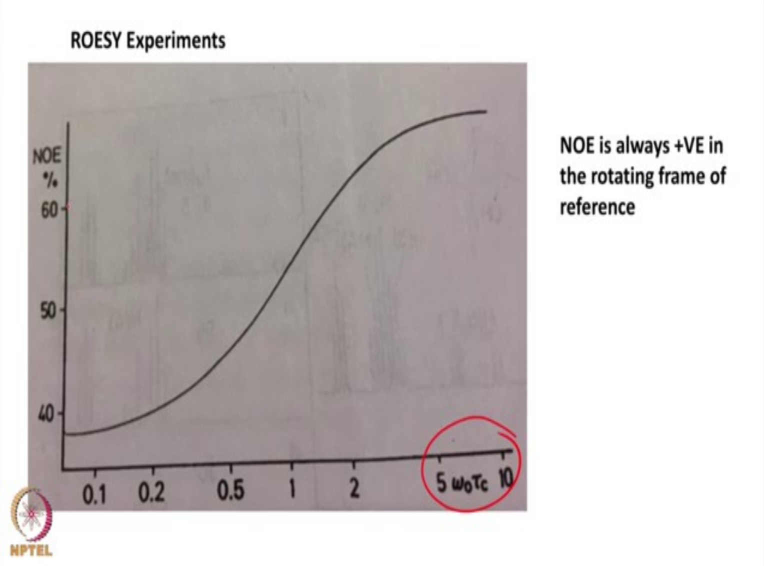

ROESY Experiments

NOE is always +VE in the rotating frame of reference

We had discussed this earlier that generally for large molecule we have a negative NOE, but there is a one good part of rotating frame of reference, here NOE is always positive and that can be used to do something called like a again something called a NOE based experiment. So in rotating frame these experiments are called ROESY Rotational Frame Overhauser Effect Spectroscopy that is a ROESY. So if you look at here, we can get still positive NOE enhancement even for the bigger molecule which was difficult in the NOESY NOE enhancement that we have seen in the previous lectures but if you do in rotting frame we still get positive enhancement and that positive enhancement is almost 67 percent.

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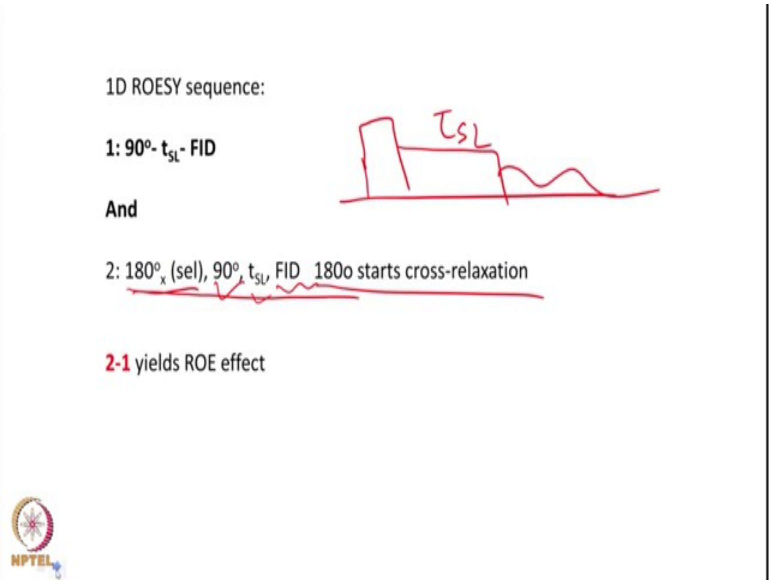


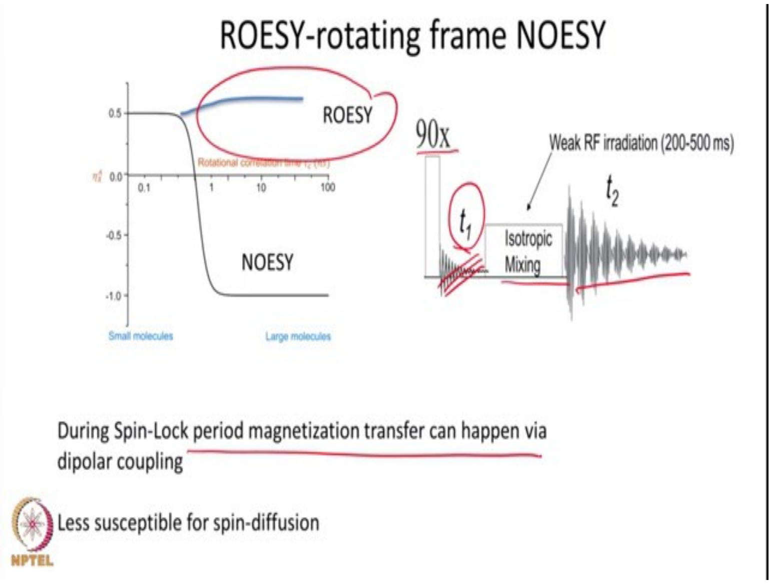
1D ROESY sequence:

1: 90°- t_{SL}- FID

And

2: $180°_x$ (sel), 90°, t_{SL}, FID 180o starts cross-relaxation

2-1 yields ROE effect



ROESY-rotating frame NOESY

During Spin-Lock period magnetization transfer can happen via dipolar coupling

Less susceptible for spin-diffusion

So using this concept whatever we just discussed, this is 1D ROESY experiment we apply a 90° pulse, then we have a like a time period, so we apply a 90° pulse, then we have a time period where we spin-lock it, 90° pulse then we spin-lock it τ_{SL} and then FID. So now this actually spin locking is slightly different then TOCSY, TOCSY uses J coupling for transferring the magnetization, here we want to make sure that the transfer happens by a cross relaxation rate, so still it can use dipolar coupling. So one can have this period where or the alternate experiment where we selectively invert one of the spins, so by applying a 180° pulse, then a 90° and then spin lock and FID.

So now if you apply 180-degree pulse it starts cross-relaxation, so if you take the difference of these experiment number 2 minus experiment number 1, one can get the ROE effect and that is exclusively coming from the dipolar coupling contribution and that is called ROESY, so still we get NOE enhancement by putting them in rotating frame and spin locking it, the signal enhancement is always positive up to 67 percent and one can achieve a nice dipolar coupling based correlation that we had seen earlier.

So here in this regime that is what we are talking ROESY works better and pulse sequence for 2D even one can have a 90° pulse, a t1 evolution again I said just after few lectures we will get into 2D but at the moment if you delete this you have a 90° pulse isotropic mixing which is generally achieved by applying a weak RF pulse and then we detect. So this is ROESY experiment and during this spin lock period magnetization transfer happens by a dipolar coupling.

And good part of this ROESY experiment that it is it is less susceptible to spin diffusion, therefore, we can achieve a longer transfer through dipolar coupling and this is quite a bit used for identification of of the distance proton that are connected through dipolar coupling in many of the spectrum.

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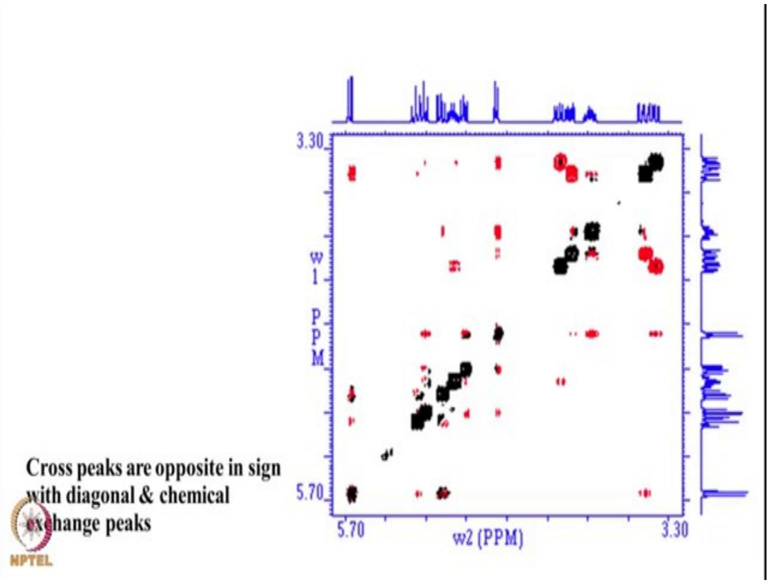

Cross peaks are opposite in sign with diagonal & chemical exchange peaks

And here one of the spectrum that we saw, here the cross peaks comes of opposite sign and then the diagonal peak, so this is ROESY spectrum for any of the molecule and now for peptides or for oligosaccharides there is no need of NOESY experiment people do ROESY experiment where they mix it for longer time and that is less susceptible to spin diffusion which was problem of NOESY spectrum.

So I think we covered more or less the polarization transfer, and now we will move to next topic from next lecture, so if you have any question on these topics, do not hesitate to write us, we will be happy to happy to answer all of your questions. Thank you very much.