Advanced NMR Techniques in Solution and Solid-State
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Module-37
DQ and ZQ Relaxation Pathways
Lecture – 37

Welcome all of you, in the last class, we started discussing about nuclear Overhauser effect. I said what is NOE, in the short form which is called NOE. First predicted by Overhauser. He said saturate electrons, ie. conducting electrons, saturate electron spin population, then the polarization of the nuclear spins get enhanced by 1000 fold; a marvelous phenomenon.

That means, the Boltzmann population of electron spins get transferred to that of the nuclear spins. As a consequence, nucleus spins sensitivity of detection goes up by 1000 fold, by transferring the polarization. This was called dynamic polarization; dynamically saturate the electron spins, then transfer the polarization to nuclear spins. Later it was shown by various other stalwarts of NMR, that it is not only restricted to electrons polarization transferring to nuclear spin by saturating electrons. It can be between two nuclear spins. Saturate one of the nuclear spins and you can see the change the intensity of the other. It is also possible between two nuclear spins. So that was experimentally demonstrated. Then what are the salient points of this NOE? it does not require a covalent bond. Basic requirement is that two nuclear spins have to be close in space, the spatial proximity is an important condition. And that the change the intensity of one of the signals when other spin is irradiated which is in close proximity can be positive or negative. It is called positive NOE or negative NOE. If this phenomenon has to happen, the spatial proximity is important condition, because it depends upon 1 over r power 6, where r is the distance between two nuclear spins. It falls of drastically as r increases. So, spatial proximity plays a dominant role is very very important; that we are to discuss.

Another thing is this spins need not be J coupled; it is only through space interaction and dominant interaction is the dipolar interaction. That is what I said is the dominant interaction. And the NOE is the enhancement factor, what I mean is the change the intensity of the signal either increase or decrease, depends upon several factors. One is the relaxation pathway that is the pathways that are available for the spins to relax; that is what he said here the pathways available for relaxation.

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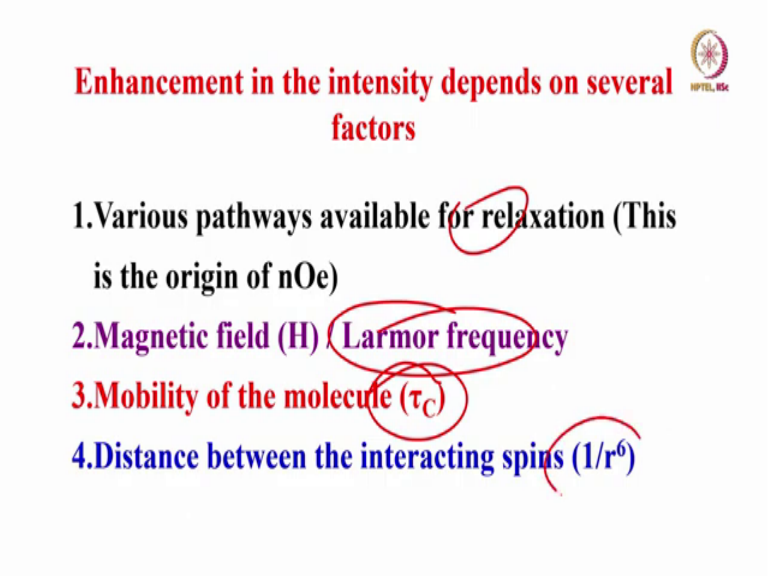

Enhancement in the intensity depends on several factors

1. Various pathways available for relaxation (This is the origin of nOe)
2. Magnetic field (H) / Larmor frequency
3. Mobility of the molecule (τ_C)
4. Distance between the interacting spins ($1/r^6$)

And then it depends upon the Larmor frequency. That is the frequency at which you are doing the experiment; or in other words the magnetic field strength. It depends upon the mobility of the molecule tau C. How big or how small the molecule is. And most important depends upon 1 by r power 6; that is distance between two spins which are close in space.

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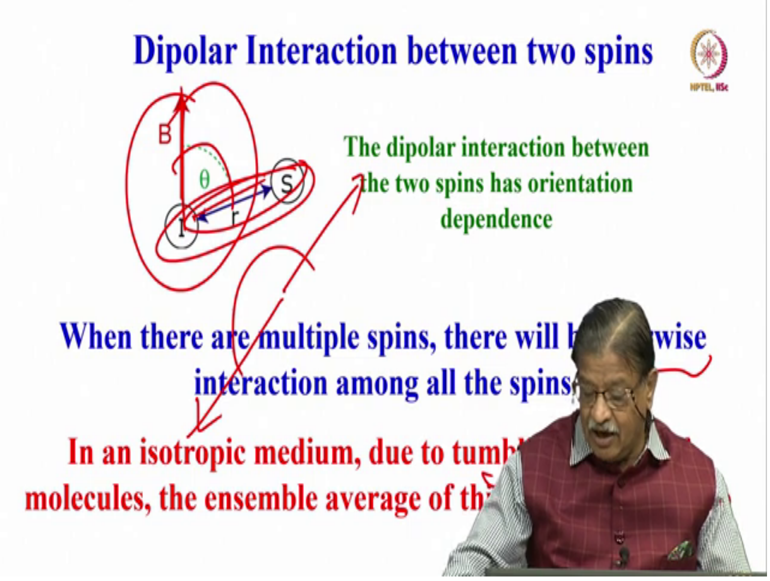

Dipolar Interaction between two spins

The dipolar interaction between the two spins has orientation dependence

When there are multiple spins, there will b wise interaction among all the spins

In an isotropic medium, due to tumb molecules, the ensemble average of th

This also we understood, now, the most dominant interaction is dipolar, what is the dipolar interaction? I think when we introduced internal interaction parameters, I mentioned it. For the benefit of everybody slightly I will tell this here. Look at this one, this is the internuclear vector I and S, it is this the magnetic field direction. Now, we have two spins and the dipolar interaction has an orientation dependence. Orientation, that is angle that the internuclear vector makes with respect to the magnetic field. It is very important, it depends on that. So dipolar coupling depends upon orientation, it is an orientation dependent parameter. Then you may ask me a question, why right now, we took the example of two spins? I took internuclear vector, only one of them, fine. What happens if there are n number of spins? How do we understand then? When there are multiple spins, there will be pairwise interaction among these spins. What I mean is that, if I have 1, 2, 3, 4, 5 several spins are there, this can interact with this, this can interact with this, it can interact with this, this can interact with this. All possible interactions, you can think of. This is called pairwise interaction. And there are multiple spins, there will be pairwise interaction among all the spins depending upon their spatial proximity and when we are far away interaction strength is less and you may not be able to see also.

And generally in the isotropic solution, you will not see that. Only the anisotropic medium you will see it. So, in isotropic medium what happens, there is a rapid tumbling motion of the molecules due to thermal agitation, even at room temperature. Molecules are undergoing motion due to rapid tumbling motion; Shat happens this parameter is completely averaged out. Why? I told you it orientation dependent phenomenon.

If I have a internuclear vector in this direction, let us say. At any given instant of time you have another vector in the opposite direction; take the vector addition of these two it becomes 0. Like that when there is isotropic motion going on, every possible orientations are there; you take the ensemble average of these things. When you take the ensemble average, this interaction totally becomes 0.

So, in the solution state is dipolar coupling is not going to be seen at all. There is a important point. Only in the oriented state, that is partially aligned state or in the solid state you see this type of interaction; this dipolar interaction is not going to be seen in the isolation state.

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Allowed and Forbidden Transitions

Now, before going further, of course, we discussed this in the very first class, let me tell you what is allowed and forbidden transitions in NMR? Allowed transitions we already discussed, you know that; the selection rule says the change in the magnetic quantum number between two spin of states, when I was looking at two spin states, should be either +1 or –1.

I took two spin half states, in which case, I said that as there are two states beta and alpha, – 1 by 2 and + 1 by 2; Here the transitions from here to here is allowed or here to here is also allowed. Because, the change between – 1 by 2 to + 1 by 2 is – 1; and between + 1 by 2 to – 1 by 2 is + 1, – 1 and + 1 both are allowed, these are allowed transitions.

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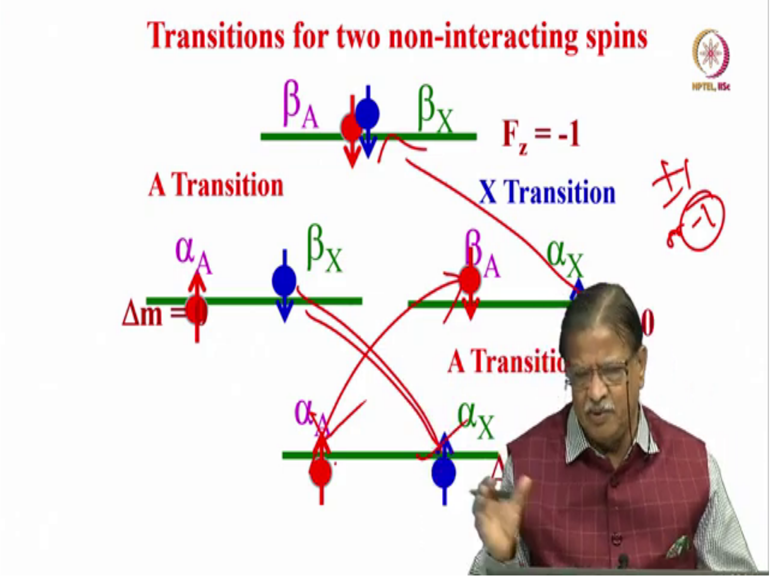

Transitions for two non-interacting spins

So, how many allowed transitions are there if I take two non-interacting spins. We discussed this when we analyzed the AX spin system, the two possible orientations alpha beta beta, alpha alpha here, beta beta, alpha alpha. The magnetic quantum number of this state is – 1, beta beta, this is – 1 by 2 + 1 by 2 alpha beta this is 0, this is a + 1 by 2 – 1 by 2 this is 0 and these + 1 by 2 + 1 by 2 this is 1.

Now, which are the allowed transition? we know by selection rule, allowed transitions are change in the magnetic quantum number should be the + 1 or – 1. This what is. Now which are the allowed transition? you can see that this is allowed, this is A spin transition. We knew that when we analyze AX spin system and this is A transition, this also followed this A transition.

Again A spin is undergoing transition from alpha to beta and beta to alpha; and this is allowed because X spin is going from alpha to beta and also coming from beta to alpha. And this is alpha going to beta, beta coming to alpha, again X spin this also allowed. What is that you are observing here. In all these transitions, the change in the magnetic quantum number is either + 1 or – 1. They are all allowed transitions in NMR. We took the example of two spins.

We also analysed for three spins in the AMX case, when you analysed the Pople notation and quantum mechanical analysis treatment of three spins systems. We understood this allowed transitions and worked out for weakly coupled system; n number of transitions are allowed. But but normally, of course, these are the things which you do not know; more details are there.

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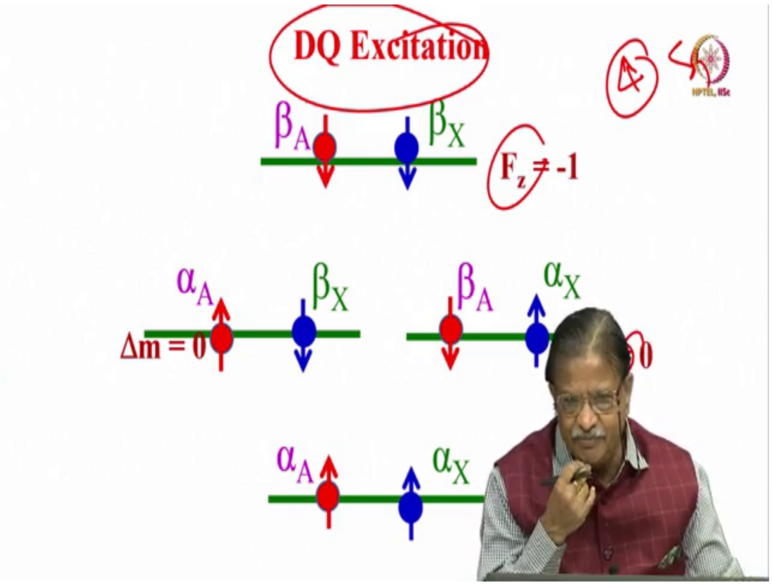

We do not have to worry we already discussed, these are allowed transitions normally in any NMR spectrum. You see the four transitions for two coupled spin system. These are called four single quantum transitions. We will discuss more about it when I come to multiple quantum NMR later. This is called single quantum excitation, this is a single quantum transition. Like single quantum excitation and detection can we think of DQ excitation and DQ detection? Is it possible to see double quantum? What do we mean by double quantum? We have four energy states with Fz – 1, + 1, 0 and 0. Now, Can I expect the transition from beta beta to alpha alpha; two spins simultaneously undergoing flipping. i.e these 2 beta state spins should go up 2 alpha state spins should come down simultaneously, can it happen? Normally it is not allow, it is the double quantum transition, because it requires two times the energy, double quantum of energy, two quantum of energy, it is forbidden transition in NMR. We can detect that also, when you come to MQT, I will tell you. It is not difficult there is an indirect way of detection.

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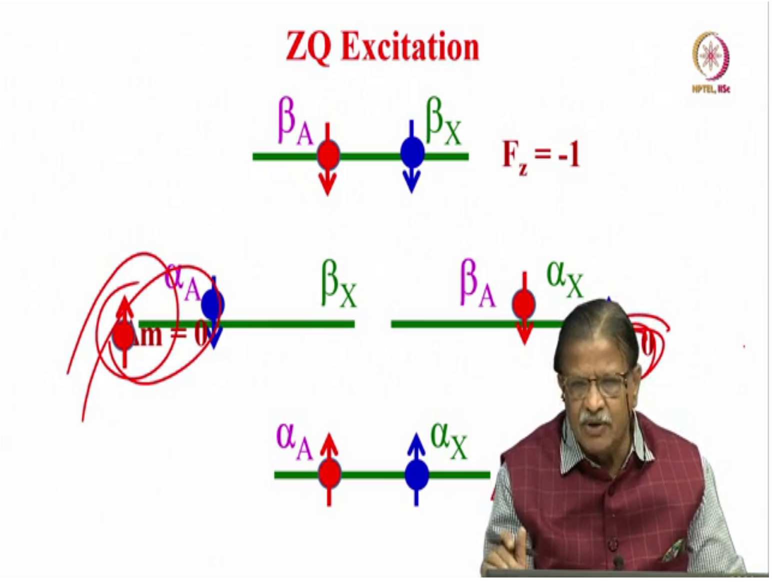

ZQ Excitation

But this is forbidden. What about this transition, transition where two spins 00 can go to 00 alpha beta can become beta alpha. Then what will happen? deltam equal to 0 here, delta m is 0 here. So, is this transition allowed? No, because change in the spin quantum number is 0 here, between two spin states, I am sorry, changing the magnetic quantum number between two spin states is 0, not allowed. This is also forbidden transition. So, there are two forbidden transitions in the two coupled spin system. One is double quantum other is 0 quantum, both are forbidden.

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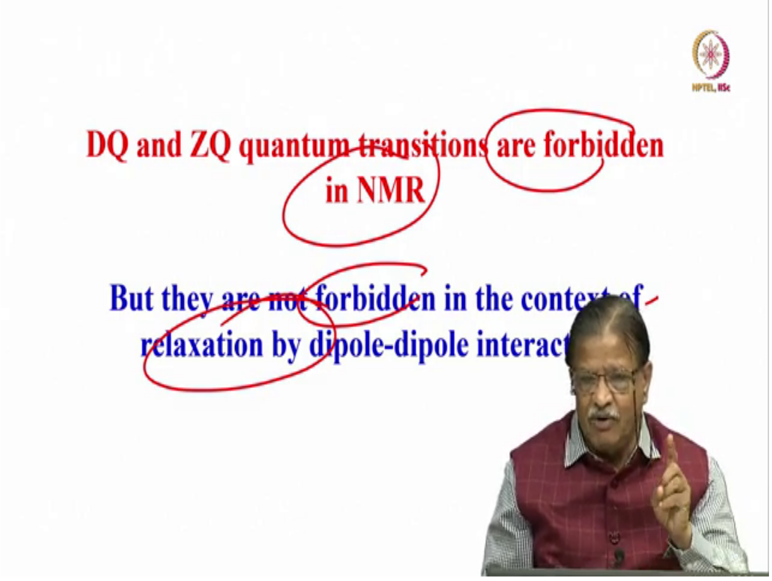

DQ and ZQ quantum ~~transitions~~ are forbidden in NMR

But they ~~are not~~ forbidden in the context of relaxation by dipole-dipole interact

But one important thing, these are forbidden as for the detection of the signal is concerned. But they are not forbidden in the context of relaxation by dipole-dipole interaction. If the spins are undergoing relaxation, because of dipole-dipole interaction these type of interactions are allowed; only for relaxation, not for detection; double quantum, 0 quantum detection in the normal way in conventional NMR is not possible. But the interaction helps the spins to relax in that pathway. What I am trying to say the spins will relax from this to this easily. If this is a transition, in fact it has to emit radiation for you to see the transition. So, you should be able to see that, but you do not see it because it is not allowed. Whereas, this transition aids for the spins to relax through dipole-dipole interaction, aids spins to relax. Thus it is allowed in the context of relaxation. This rule of forbidden transition is only for detection, not for aiding relaxation.

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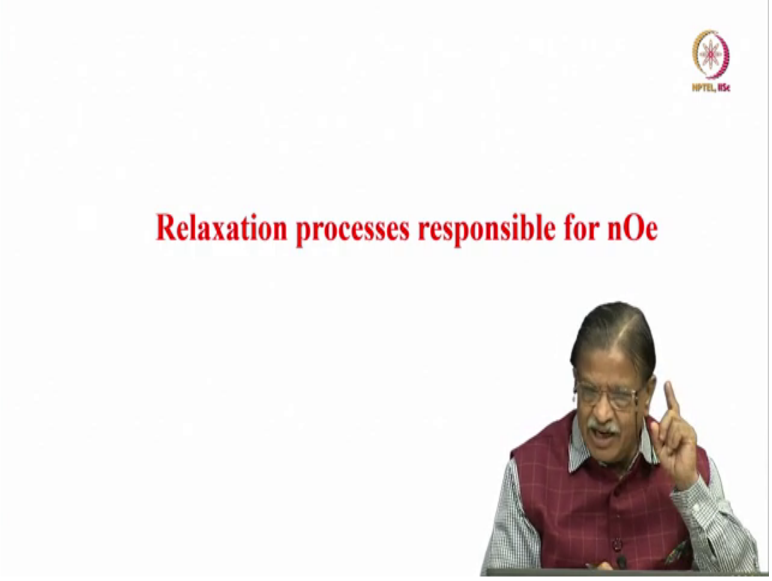

Relaxation processes responsible for nOe

We this we will try to understand some of the relaxation processes that are responsible for NOE. Please understand very important point. How these relaxation pathways aids in in NOE phenomena.

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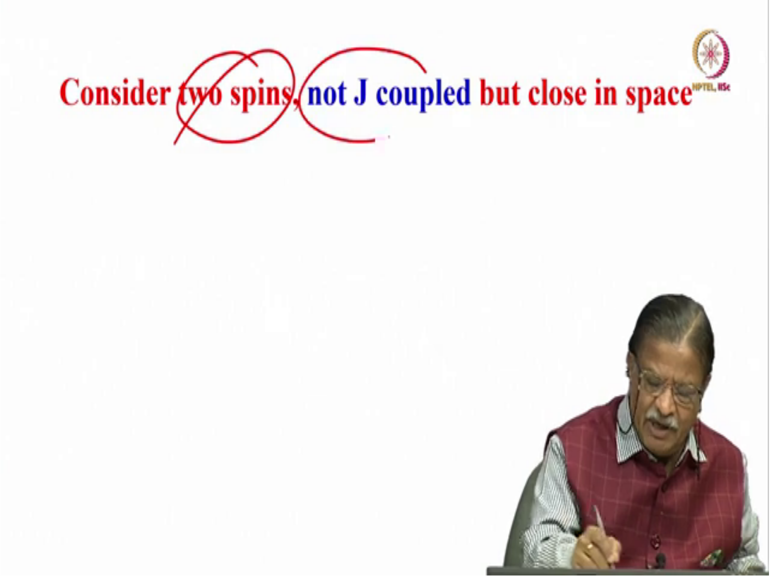

Consider two spins, not J coupled but close in space

Now, consider two spins, a special case two there are two interacting spins. They are not J coupled, that is not a condition for NOE. That is what I said, they need not be J coupled. All I need is, close spatial proximity, that is important. I will see that that two spins are close in space no J coupled, does not matter.

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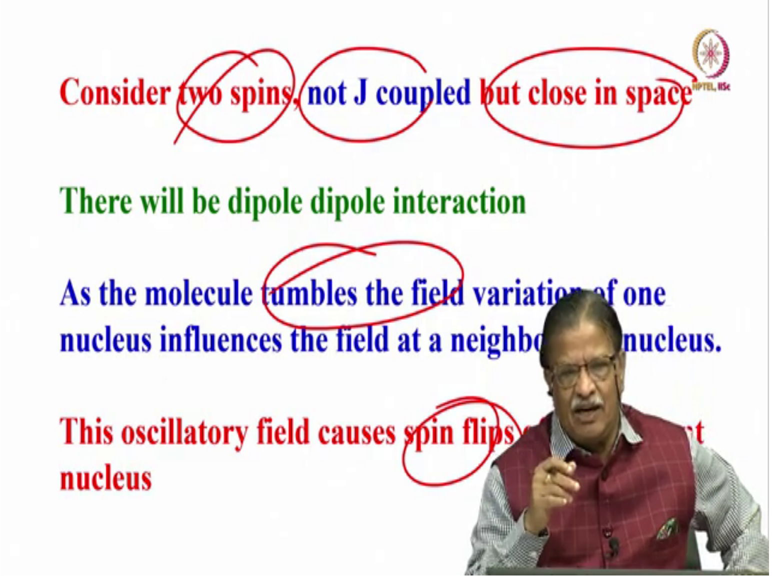

Consider two spins, not J coupled but close in space

There will be dipole dipole interaction

As the molecule tumbles the field variati f one nucleus influences the field at a neighbo nucleus.

This oscillatory field causes spin flips t nucleus

Now, there will be dipole-dipole interaction between these two; as I said, when the two spins comes close to each other that is what we discussed, even for relaxation also. The molecule is undergoing tumbling motion. When one spin undergoing motion, we discussed a long stretch about dipole-dipole relaxation phenomenon; the spin orientation with respect to the magnetic field do not change. But when the spins undergo tumbling motion, the magnetic field induced at the state of the other spin changes; it creates local field at the site of the neighboring spin. This is an oscillatory field, this oscillatory field causes spin flips of the adjacent spin. As a consequence it aids relaxation, this we discussed a lot. So, imagine the dipole-dipole interaction is a dominant relaxation phenomenon. It is because of the tumbling motion of the molecule, one of the nuclear spin creates a fluctuating magnetic field, oscillatory field at the site of the neighboring spin which is close in space, aids in relaxation.

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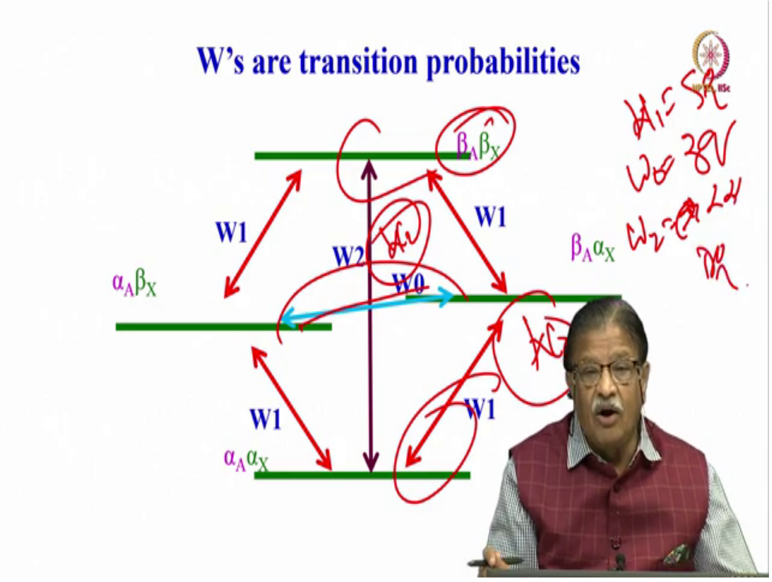
W's are transition probabilities

So, this is relaxation dominant pathway, with this will go back to what are the transition probabilities. We discussed the transition probability in the context of relaxation, where we said transition probability W alpha beta for this spins going from alpha to beta state W beta alpha for this spins coming from beta to alpha to work out the phenomenological equation for the relaxation. That is what we did. Now, we will define the transition probabilities for two spin system.

Again we have energy states; beta beta, beta alpha, alpha beta and alpha alpha which correspond to –1, 0, 0 and +1. Now, this is z\a allowed transition, single quantum transition, his is allowed single quantum, this is allow single quantum, this is allowed single quantum. We know that this should correspond to A transition, this corresponds to X transition; these two are X and these two are A transitions. That we have been discussing since a long time.

And there is a probability for this nuclear spins to undergo transition between these two states. For all the single quantum states, we call it as w1, w1 is the probability of spins to undergo single quantum transition, either this to this or this. Since this one, this one, this one, all four are w1's, it is the transition probability for single quantum transition, there is a transition probability. Now, what about this one, this is a transition probability for double quantum. You see this one, this is the transition probability we see now for double quantum now. This also allowed, there is probability; there is a definite probability for this also. So, this is w2 because it is a double quantum. Now, we can think of the transition probability for the spins to undergo transition from beta alpha to alpha beta, this is 0 quantum. So, I call it as w0. So, there are three transition probabilities.

I am going to define here, w1 is single quantum transition probability between two states, w0 is zero quantum transition probability between alpha beta to beta alpha states; and w2 is the double quantum transition probability from beta beta to alpha alpha. This is double quantum. All these transition probabilities are respectively called as w1, w0 and w2.

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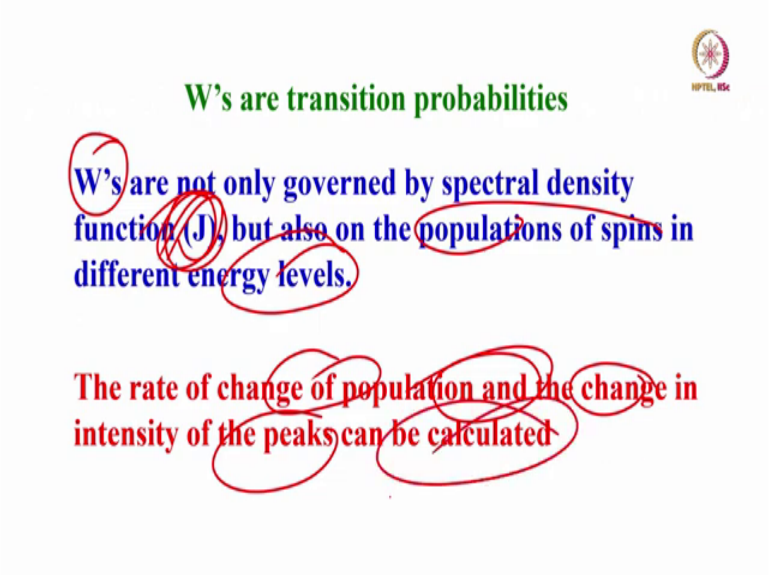

W's are transition probabilities

W's are not only governed by spectral density function (J) but also on the populations of spins in different energy levels.

The rate of change of population and the change in intensity of the peaks can be calculated

Now, what are these w transition probabilities?, they are governed by what is called a spectral density function. Transition probability, we discussed, we did not discuss transition probability at that time, but we said spectral density function, we discussed earlier with respect to the relaxation. These w's are related to J, spectral density function, which defines the transition probability; that is what we discussed indirectly the relaxation.

Depending upon Jw we wrote three possibilities for omega tauc > 1, = 1, < 1; we discuss all those things. So that is the Jw spectral density function. So, w's are governed by spectral density function and also the population of the spins in different energy levels, both are important, w depends upon spin populations in different energy levels. At the same time, it also is governed by J, the spectral density function.

The rate of change of population and the change in the intensity of the peaks, we can also calculate. Simple for two spin case or to energy levels. We calculated that; w alpha beta, w beta alpha and we showed when we were trying to understand relaxation, when the basic expression basic equation worked out the transition probabilities and everything. The rate of change of population also we understood.

Then we introduced rate of deviation from the population from thermal equilibrium, we introduced the term. So, we understood all those things in the last couple of classes when we discussed relaxation. So, if I know Jw, w is everything, the rate of change of population and the change in intensity peaks we can work it out, you can calculate, it is not difficult.

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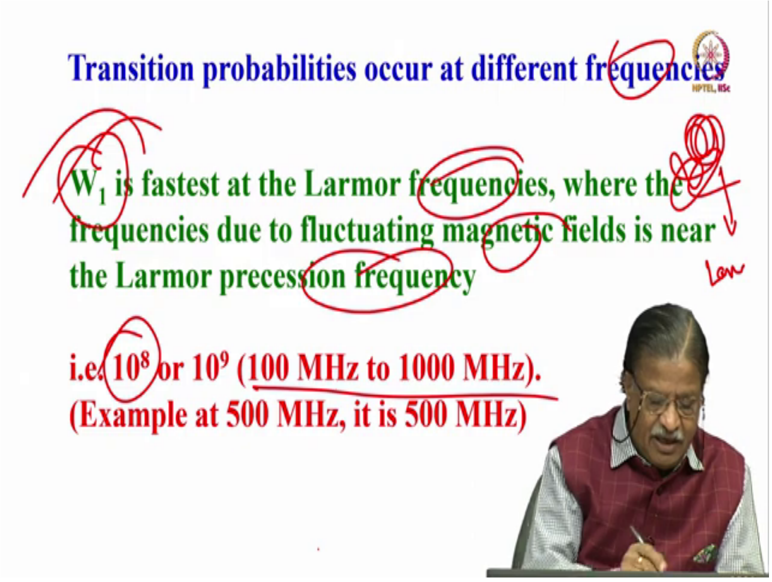

Transition probabilities occur at different frequencies

W_1 is fastest at the Larmor frequencies, where the frequencies due to fluctuating magnetic fields is near the Larmor precession frequency

i.e. 10^8 or 10^9 (100 MHz to 1000 MHz).
(Example at 500 MHz, it is 500 MHz)

Now, the transition probabilities occur at different frequencies. That is the thing, this is given by this one, w1 is the fastest transition property; it is at the Larmor frequency. What is the Larmor frequency is it a resonating frequency; the Larmor frequency is the resonating frequency of the spins in a given magnetic field. And the fluctuating magnetic field will be more at the precssional frequency; as a consequence w1 is fastest at the Larmor frequency, when the frequency of the fluctuating fields are near the Larmor preccession frequency. You understand, the spins are undergoing fluctuation, random motion, chaotic motion whatever it is. When its frequency is equal to Larmor frequency then w1 is dominate; that is the thing which is existing. So, when I say 100 MHz; 1000 MHz if we take, the resonant frequency is, Larmor frequency 10 to power of 8 or 10 to power of 9 at 500 or 1000 MHz then transmission probability can be larger for that.

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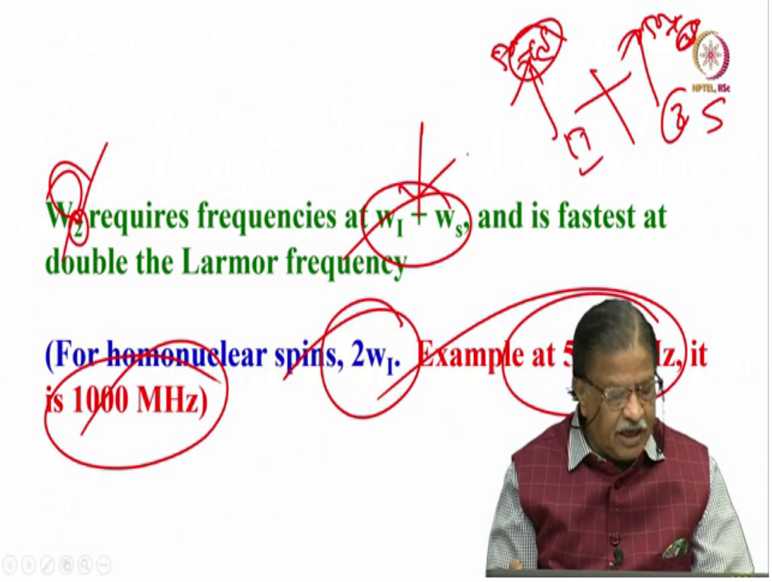

W_2 requires frequencies at $w_I + w_S$, and is fastest at double the Larmor frequency

(For homonuclear spins, $2w_I$. Example at 5 z, it is 1000 MHz)

For w2 it requires double the resonating frequency; that means resonating frequency of the two spins. What is sum of the resonating frequency of the two spins. I am taking sum, sum of the resonant frequency of the two spins; that is spin 1 and spin 2 or I and S. What is sum of the resonating frequency of these two?

If this is a 500 MHz; this also is 500 MHz + delta which is a chemical shift. So, for 2 homonuclear Spins it is two times the Larmor frequency. A small change in the chemical shift is there; but two times Larmor frequency. I have a 500 MHz spectrometer. So, w2 correspond to 1000 MHz. I am taking the sum, not the difference. So that is why it is w2.

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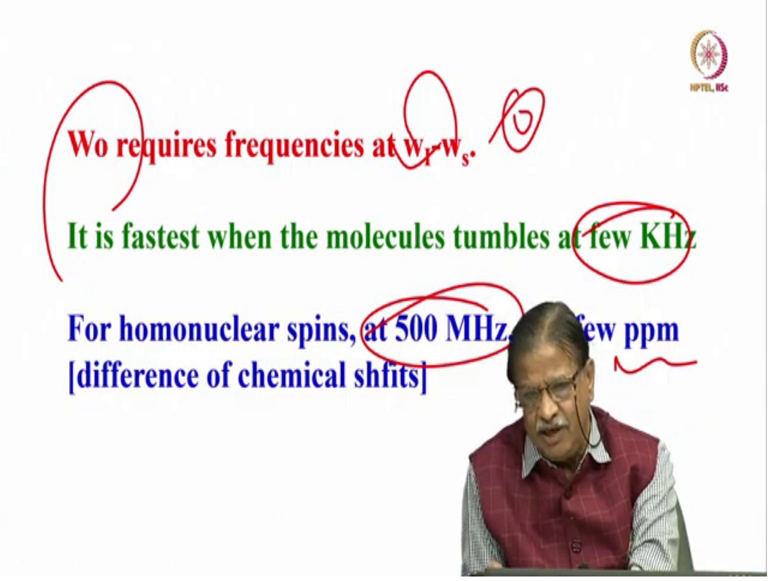

Wo requires frequencies at w_I-w_S. 0

It is fastest when the molecules tumbles at few KHz

For homonuclear spins, at 500 MHz, few ppm
[difference of chemical shfits]

If I go to w0, it is a difference in the Larmor frequency. What is the difference in the Larmor frequency between two homonuclear spins? it is close to 0, because only small changes chemical shift which is of KHz to few KHz only, that is w0. For homonuclear spins at 500 MHz it is only few PPM, two spin separated with 2, 3 or 4 PPM let us say, that 500 MHz 4 PPM is 2000 Hz. If that is the Larmor frequency present, then this is dominant. At 500 MHz, few 1000 is the fluctuating frequency then w 2 is dominant. At 500 MHz if fluctuating frequency at 500 MHz Larmor frequency that w1 is the relaxation phenomena. So, three different transition probabilities. The dominant Transition probability for w1 when spectral density function or the fluctuating field is at the Larmor frequency. If the fluctuating field is twice Larmor frequency w2 is the transition probability that is dominant, w0 dominant when the difference in the Larmor frequency which is the order of difference in chemical shifts is of the order of few KHz.

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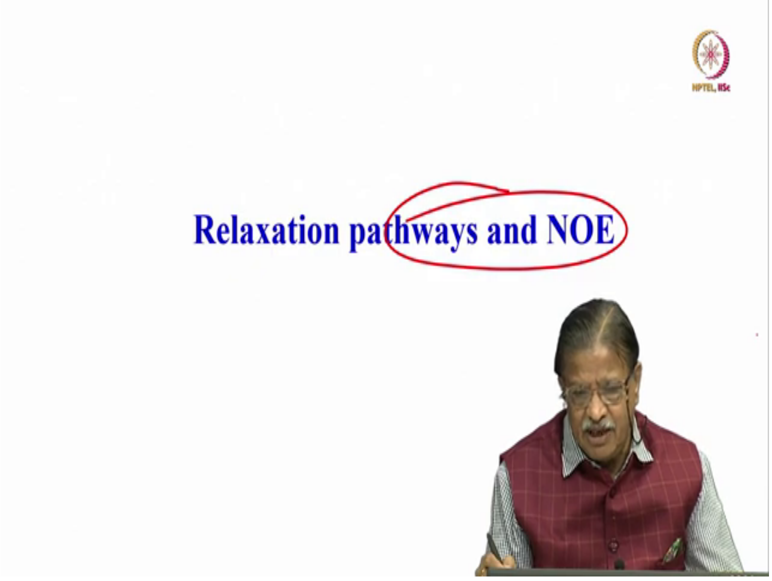

Relaxation pathways and NOE

With this idea, let us now try to understand relaxation pathways. How NOE builds up? Because I said it depends upon the relaxation pathway, NOE depend strongly on the relaxation pathway. Let us see what is the relaxation pathway which is responsible for enhancing the signal intensity.

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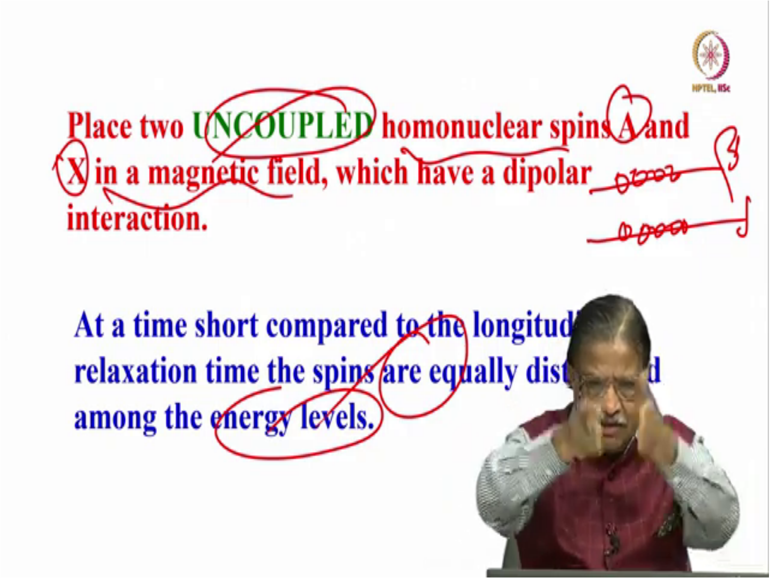

Place two UNCOUPLED homonuclear spins A and X in a magnetic field, which have a dipolar interaction.

At a time short compared to the longitud relaxation time the spins are equally dist d among the energy levels.

Now, let us take two uncoupled nuclear spins, A and X, uncoupled; no coupling at all, They need not be coupled. but close in space. Why close in space, because they should have a dipolar interaction and should be dominant. If it is very far away then there is no dipolar interaction and there is no point in discussing NOE. So, we have considered a situation two uncoupled spins are there, homonuclear which close in space, kept it in a magnetic field.

There is a dipolar interaction. Now for a short time compared to the longitudinal relaxation time; the spins are equally populated; equally distributed among the energy levels. This is what you understood when we tried to understand the nuclear spins. This is the first slide a second slide I showed you; take a sample which is outside somewhere, the spins are randomly distributed, there is no preferential orientation. That is all possible orientations are there. Take the sample, put it in a magnetic field. That is what I told you; instantaneously, immediately after putting the sample in the magnetic field. If it takes spin half nucleus, both beta and alpha states are equally populated. That is what I said, it is saturated situation. Spins are equally populated or no population difference at all; exactly that is what I have been telling.

Take two uncoupled spin system, put it in strong magnetic field, these two spins should have a dipolar interaction with close spatial proximity. And immediately after putting in a magnetic field, you will see there is a saturation state; there is no population difference that is the condition.

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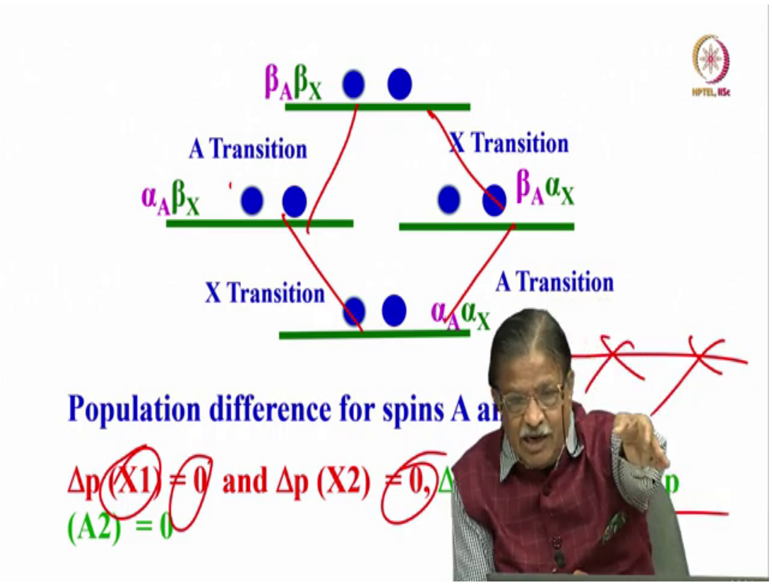

Population difference for spins A a

Δp (X1) = 0 and Δp (X2) = 0, Δ p (A2) = 0

So this is the situation. Now, immediately after putting the sample in a magnetic field, there are two spins here, two spins here, two spins here, two spins here, it is only a number we have taken for calculation. Do not ever be under the impression that only two spins, millions and millions of nuclear spins are there in the sample. It is not just two; for my easy calculation, I have taken a number 2, 2, 2, 2 like this.

Now, what are the X transitions and what are the A transitions? This is an A transition, which undergoes transition. This is a transition alpha going to be beta; here alpha A going is to beta A; here alpha A going to beta A or beta A going to alpha A. Now, the X transition, alpha X going to beta X; and beta X coming to alpha X; this also X transition, alpha X going to beta X and beta X is going to alpha X. So, all the four transitions are allowed here.

All are possible. Now, when I take the population difference for A spin and X spin, what is the intensity? This A spin intensity is 0. And X spin, this X spin intensity is 0, A spin intensity is 0, A spin intensity is 0, the Saturation condition. That is what I said. And there is no population difference, you would see deltaP of A = 0, deltaP of X = 0. So finally, you do not see any signal at all, you will see no signal. Absolutely 0 signal.

That is why as soon as they put the sample in a magnetic field; for the spins which have longer relaxation time, you try to do the experiment you do not get the signal. You have to wait for sometime.

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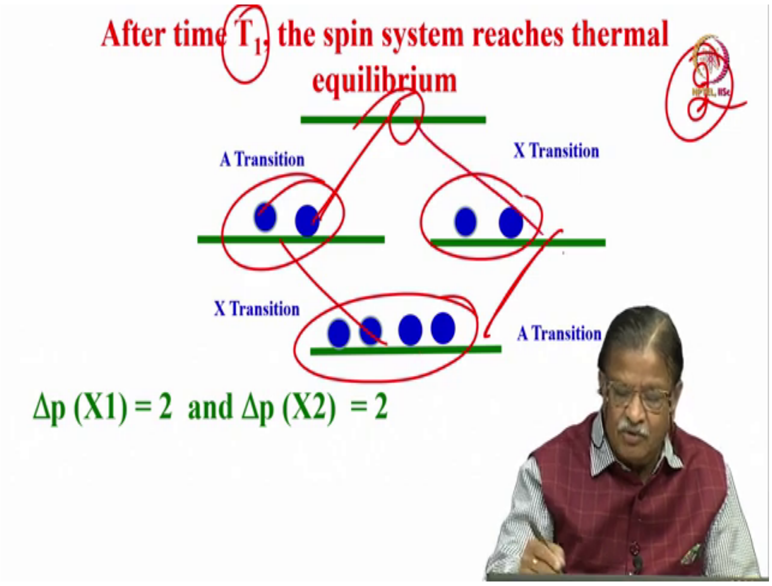

After time T_1, the spin system reaches thermal equilibrium

Δp (X1) = 2 and Δp (X2) = 2

That is what we are going to do. Now, interestingly, what we do is we will wait for some time, what is this sometime? What is this time? This is what is called T1. We discussed this. Wait for some time for the spins to attain thermal equilibrium; as soon as you put the sample in a magnetic field wait for some time the spins will come to thermal equilibrium; that is what we call a such a situation, where Boltzmann population is attained.

The Boltzmann population distribution is attained among all these spin states. Now, I have taken the example of two spins four energy states; I waited for sufficiently long time after putting the sample. Let us see what is the spin population attained after time T1, where the spins are now in thermal equilibrium. I have put four spins here, two here and two here and no spins here.

Never be under the impression there are 0 spins; millions of spins are there. For my calculation again the 2, 2, 2, 2 which was there; they have redistributed themselves after attaining thermal equilibrium, Now, let us calculate the population difference. What is the population difference for A transition? A transition is this one and this one, 2 – 0 =2; 4 – 2 = 2; so the intensities are 2 each.

Similarly taking X, X is 4 – 2 and 2 – 0, X intensities are also 2. So, 2 X transitions have 2 intensity and 2 A transitions also will have same intensity. So, X1 and X2 is has the same intensity. Similarly, A1 intensity I did not write also has the same intensity. So, both the transitions are of equal intensity 2 2, 2, 2 intensity or 1 is to 1 equal intensity. This is the situation we have got after thermal equilibrium.

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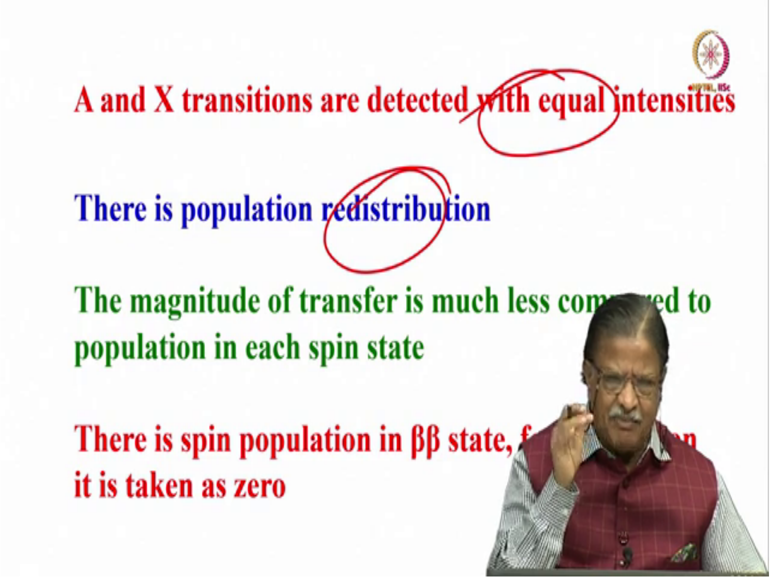

A and X transitions are detected with equal intensities

There is population redistribution

The magnitude of transfer is much less com ed to population in each spin state

There is spin population in ββ state, f n it is taken as zero

Now, what we do what happened here? A and X are detected with equal intensity because there is a population redistribution. And the magnitude of transfer is much less compared to population of each spin state; does not matter. But there is a spin population in the beta beta state for calculation is taken as zero; I have been stressing again and again do not ever be under the impression.

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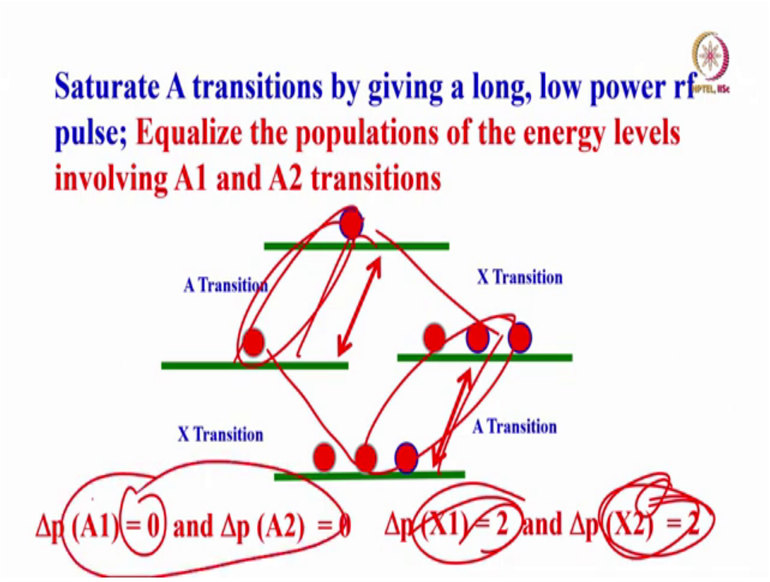

Saturate A transitions by giving a long, low power rf pulse; Equalize the populations of the energy levels involving A1 and A2 transitions

$\Delta p\,(A1) = 0$ and $\Delta p\,(A2) = 0$ $\Delta p\,(X1) = 2$ and $\Delta p\,(X2) = 2$

We will do an interesting thing now, saturate A transitions by giving a long low rf pulse; very weak rf pulse. This is possible, called selective saturation, selective pulse you can apply. And only A spin we can saturate; that means this transition and this transition are saturated. What do you will be saturation? I make population between these two equal; when the population between these two will become equal 4 and 2 will become equal.

You have to make it this as 3, this is as 3; that is saturation; what about this one? this is 2 0 this is 1, this is 1 you make it. Then you have saturated both the A transitions; let us see what happens now. This what you carefully see now, what is happening, I am applying a small weak rf power, so that I am irradiating or saturating A transitions. When I do that now, it became 1, 1 and 3, 3. The saturation has equalized the population between these two states and these two states.

Calculate the intensities now, what is the transition? It is a saturated state. So, A transition has 0 intensity; 3 – 3, 1 – 1, 0 intensity. What about X transitions? X transition, 3 – 1, this is 2. What about this transition? 3 – 1; 2. So X transition intensity, still 2, 2, what did we do? We saturated one of these spins. One of the spins both the transitions of A spins and the calculated; what happens to the change in intensity. But we see intensity of the X transition remains unaltered whereas A transition becomes 0 because we saturated that.

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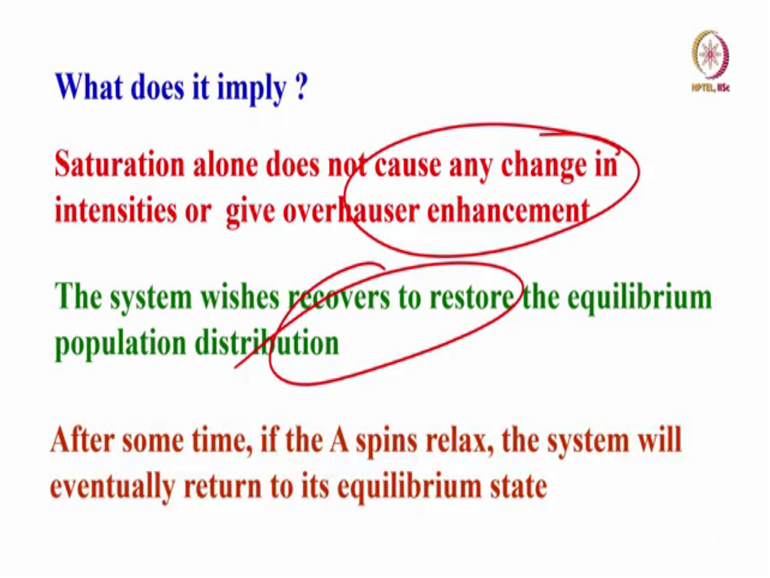

What does it imply ?

Saturation alone does not cause any change in intensities or give overhauser enhancement

The system wishes recovers to restore the equilibrium population distribution

After some time, if the A spins relax, the system will eventually return to its equilibrium state

So, what do you understand from this, saturation does not cause any change in the intensity and it does not give overhauer effect. ust because I saturate one of the peaks, you do not get enhancement in the signal intensity, there must be something more is happening. If that thing happened, system will recover to an equilibrium in a different way, where the population distribution takes place in a different way.

We discussed w1 probabilities and we may saturate the energy states and calculate the population difference; and we saw there is no change in intensity. So, a system now wants to recover, restore to equilibrium in a different way to get the population redistribution. If after sometime the spins relax, the system will eventually return to thermal equilibrium without any change in intensity.

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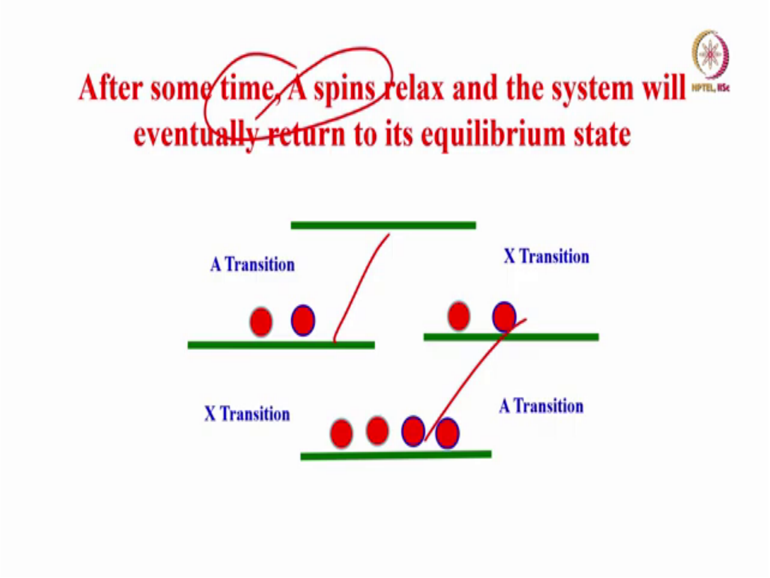

After some time, A spins relax and the system will eventually return to its equilibrium state

Now, this is what happens; you distributed the populations 3 3, 3 3, now, you waited for sometime it comes back that is all; you saturated A, made them equal waited for some time then spins relax back and comes here. So, now we started from this state and we came back to the same state. That means there is no change in intensity of the peak after saturation, if you irradiate a particular transition and saturate.

Spins get redistributed, calculate the intensity there is no change in intensity, wait for some time spins come to thermal equilibrium. So, you have not gained NOE but what we said if I irradiate a particular frequency, there must be change in intensity for NOE; either increase or decrease, but nothing is happening now. What do we understand? So, saturation alone does not help and single quantum transitions what we saw here; w1 probabilities, that alone will not help in NOE.

So, there is something more is going to happen; The relaxation pathways are important now; that has to be different we understand that, what is that relaxation pathway. Now the since the time is up, we will take up the relaxation pathway, how the spins redistribute themselves and give rise to change in the intensity of the signal. So that we will take up in the next class.

But right now today we understood what is NOE, and then concept of NOE. What are the important salient features of NOE? And for NOE spins need not be J-coupled. For NOE what is required is spatial proximity of the two nuclear spins, it is one of r power six dependence. And what we did is in the example, we took two spins which are close in space and not J-coupled, put in the magnetic field. And for the purpose of calculation thermal equilibrium population have different spins, the different energy states we took and calculated population difference and the intensity of the peaks. We found both are equal intensities for both A transitions and both X transitions. That is fine. But what we do is we irradiate one of A transitions selectively and saturated that; and again redistributed the spin populations and calculate what is the population difference.

Now, we saw intensity of the X spin or the spin which is close in space which is not irradiate did not change, only irradiate spin, which is saturated becomes 0 intensity. So, saturation alone did not help in gaining the intensity or losing the intensity. Wait for some time, we know that spins will attain thermal equilibrium, they come back to where they were; the populations were 4 2 2 0; the redistribution took place, like where we started with.

So, then what is that, which is responsible for NOE enhancement. If w1 probabilities are not the dominant thing, there must be some other mechanism of relaxation of the nuclear spins, the population redistribution takes place in a different way while the nuclear spins, undergoing relaxation, not through single quantum, but some other way. That we will try to understand and see whether that can give rise to enhancement or decrease in intensity. The relaxation pathway selection, the dominant relaxation pathway for NOE, we will discuss in the next class, I will stop here. Thank you.