One and Two Dimensional NMR Spectroscopy for Chemists

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Lecture – 47
Decoupling and NOE

Welcome back, in the last class we discussed about polarization transfer technique, how we can utilize the polarization transfer technique like INEPT to transfer the magnetization from abundant spin to rare spin, so that sensitivity of our detection gets enhanced. As a consequence, our experimental time also gets reduced. So I showed with a few examples how the signal intensity was enhanced, also many examples in different nuclei.

And also we can adapt this technique for spectral editing like Attached Proton Test and DEPT experiments, which generally people follow in carbon 13 NMR, for identification of carbons based on the number of protons attached to the carbon. So this is a another important thing which we discussed. Today, I will go to discuss about NOE, another important thing where we can get information about the spatial proximity of the nuclear spins. So, if there are 2 nuclear spins which are in spatial proximity, NOE helps us in getting the information about the spatial proximity.

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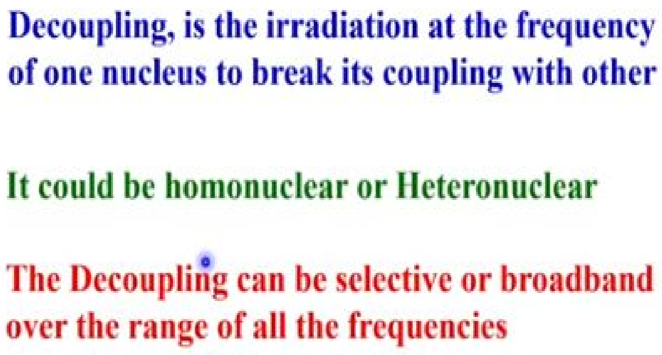

Decoupling, is the irradiation at the frequency of one nucleus to break its coupling with other

It could be homonuclear or Heteronuclear

The Decoupling can be selective or broadband over the range of all the frequencies

Now starting with the NOE, what is NOE? If we remember, I discussed about decoupling. What do we do with decoupling; I can irradiate a particular frequency of one nuclei, so that the coupling is broken with the other nuclei. Remember I told you in the homo nuclear case, if we take 2 protons coupled like this, it is an AX spin system, let us consider a weakly coupled. I have to sit exactly at the center of this doublet and keeps irradiating with the second radio frequency signal for breaking the coupling of this nucleus with other nucleus.

And the power should be such that it should be larger than this coupling strenght, then only this we can saturate this spin. As a consequence the population difference between the energy states of this spin becomes equal. So this coupled spin will not see, this in either of the spin states, you see the average spin state, as a consequence it appears as if this proton is not coupled with this proton at all, this was a general homo nuclear decoupling which I discussed.

So, we have to apply decoupling power, sufficient enough at the right frequency of the proton which we have to decouple and then see the effect of it on the other part of the spectrum. This could be both homo nuclear and hetero nuclear, we discussed. Hetero nuclear case we saw it is a broadband decoupling, the entire carbon will be decoupled at a time with protons, as a consequence I said we get single peak for each carbon in the molecule, that is fine.

Now, this decoupling we can make it is either selective, or broadband, the broadband, over the broad range of all the frequencies, that is hetero nuclear, okay this is what happens. So this decoupling can be selective or broadband, in the case of proton, it can be selective also.

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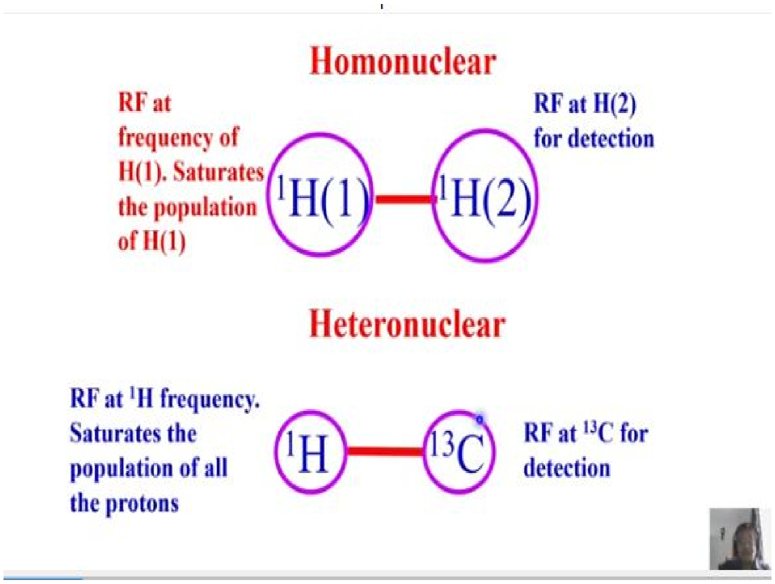

Homonuclear

RF at frequency of H(1). Saturates the population of H(1)

$^1H(1)$

$^1H(2)$

RF at H(2) for detection

Heteronuclear

RF at ^1H frequency. Saturates the population of all the protons

^1H

^{13}C

RF at ^{13}C for detection

So, what we do, let us see diagrammatically. In the homo nuclear case, let us say 2 protons are coupled, I am going to saturate this proton and I will apply RF pulse in such a way I am detecting the signal here. Okay, I am saturating one of the protons which is coupled to this proton, I will see what is the change here. It could be 1 spin, 2 spins; many spins can be coupled here.

When I hit this proton then what happens is; whatever other protons which are coupled to it, you see the change in the spectrum there, okay. The complexity would have reduced, let us say this proton is coupled to this, it will make it a doublet. If this also coupled to another proton, this makes it a doublet of doublet. Let us say, it makes doublet of doublet. Now, when I decouple this proton with this, you know what is going to happen in the spectrum; this will appear like only a doublet, because you are breaking the coupling between this and this, coupling between the proton 2 and proton 3 are retained, that is what happens. This is a selective decoupling you can do. You are simplifying the complexity; this helps us to analyze and interpret the spectrum and also get this structure of the molecule. We know which is coupled to which, which is the proton which is neighbor to this proton; like that we can keep on getting the structural information.

Same thing we can do for the case of the heteronuclei. As I said for the entire proton range we apply a broadband decoupling power, saturate all the protons in such a way, the carbons will not couple to any of these protons. As a consequence all the carbons appear as singlets, this is basically decoupling experiment.

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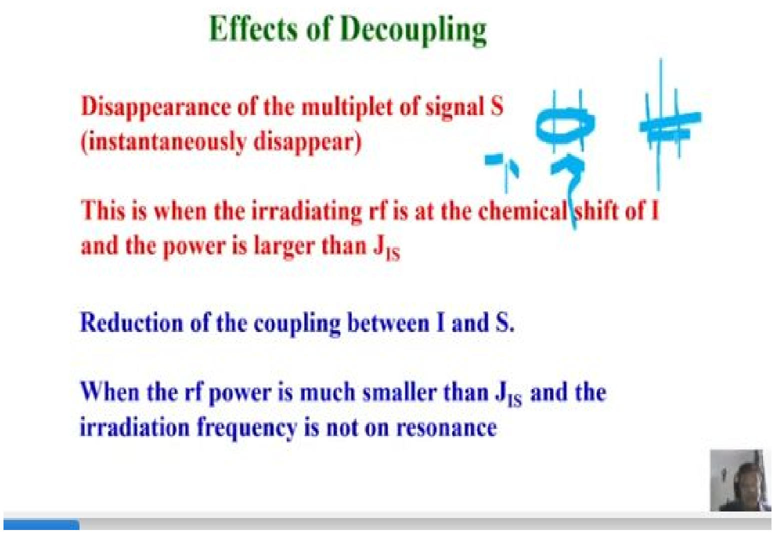

Effects of Decoupling

Disappearance of the multiplet of signal S
(instantaneously disappear)

This is when the irradiating rf is at the chemical shift of I
and the power is larger than J_{IS}

Reduction of the coupling between I and S.

When the rf power is much smaller than J_{IS} and the
irradiation frequency is not on resonance

Now, there are certain effects of decoupling which we cannot forget. One effect is disappearance of the multiplet, I told you let us say 3 protons are coupled, I hit here, this proton which was coupled to 2 of the other proton also, this spectrum gets simplify. If there are let us say, only 2 protons are coupled, I hit this proton here, then this proton what happens, you see it is going to be a singlet; see there are 2 protons here, it is an AX spin system.

I am going to irradiate here, then what happens; this will collapse into a singlet, that is one way, disappearance of multiplicity; and this can happen only when decoupling power is larger than this coupling strength and at the right position. And sometimes if I go far away, if I start hitting here or not with the enough power, it can so happen, there is a reduced coupling here, the splitting will not disappear, but coupling can get reduced, that is another side effect of the decoupling.

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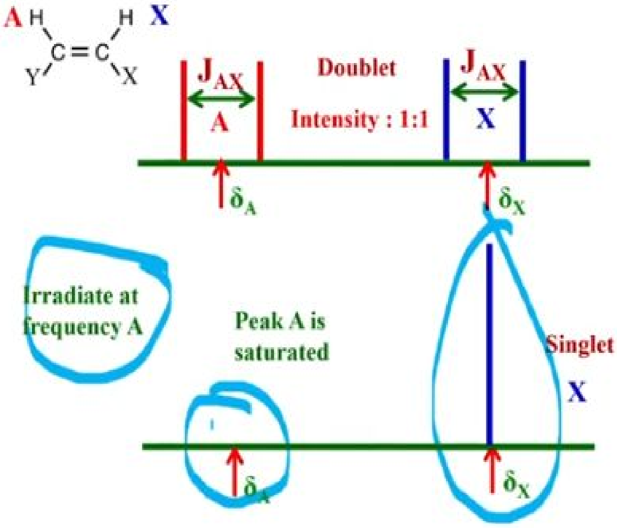

This is exactly what happens in the case of decoupling, as I showed this, we, saturated this peak exactly at the center and you are going to see a singlet; and these 2 doublets collapse and gives a single peak of double this intensity, both the intensity will add up. This I am irradiating at frequency A, I can also do one thing, I can irradiate here and see this one, this will become singlet, so that is what happens and irradiated peak we will not see because we have saturated, population difference is zero.

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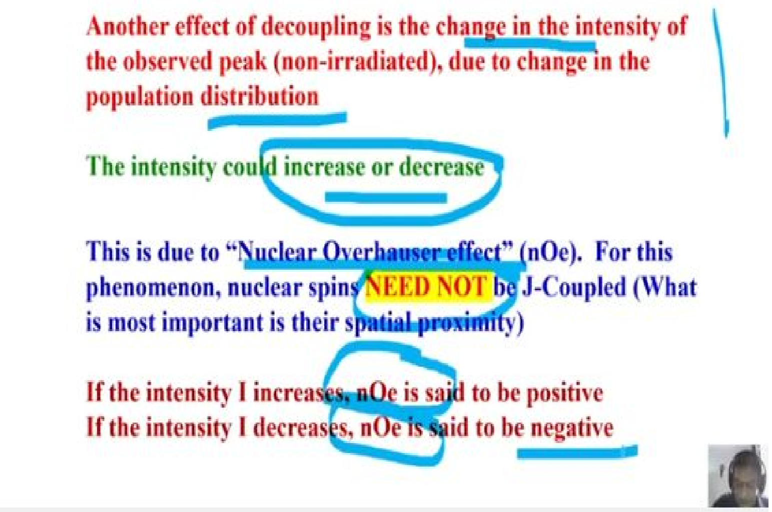

Another effect of decoupling is the change in the intensity of the observed peak (non-irradiated), due to change in the population distribution

The intensity could increase or decrease

This is due to "Nuclear Overhauser effect" (nOe). For this phenomenon, nuclear spins NEED NOT be J-Coupled (What is most important is their spatial proximity)

If the intensity I increases, nOe is said to be positive
If the intensity I decreases, nOe is said to be negative

Okay, now another side effect of decoupling is there is change in the intensity of the observed peak due to population redistribution, population can get redistributed and there is a change in the intensity. Intensity can either decrease or increase, very interesting thing. Normally, in the case of decoupling, you will reduce the multiplicity to make the assignment of the peaks easier.

But in some examples of homonuclear decoupling, instead of reducing the multiplicity, the peak intensity will go up, I am sorry, instead of reducing multiplicity, the multiplicity will go up, you will get more peaks than what is expected, something strange, right; that is called spin tickling, Okay that is called spin tickling ; we will not worry about it, we can also see such type of effects in the spin decoupling. There are several problems in spin decoupling depending upon the power you apply, the place you are sitting or irradiating with the second RF and everything, so lot of effects can be there.

Now, one of this side effects of that is change in the intensity of the observed peak; when you are hitting the other peak. The intensity can increase or decrease and this is because NOE. This is the effect called nuclear Overhauser effect and for this phenomena to occur, remember, nuclear spins need not be J coupled, very interesting thing, this is similar to a decoupling. I can send a second radio frequency weak RF power at the site of the one of the nucleus; and see the effect on the other nucleus that can by change in the intensity of this, it can go up or come down.

Incidentally, these 2 spins need not be J coupled, they can be just close in space that is enough. And this is one of the things, which is called a nuclear ovehauser effect; and this NOE which is positive, if there is enhancement in intensity, this NOE is negative, if it is a reduced intensity. This can happen, remember depending upon where you are going to hit, the amount of power you apply, for the duration the power you are going to apply, the 2 spins which are not J coupled also, you can see that, when you irradiate one of the peaks with a RF power, weak RF power; then, what happens is the other spin which is in close proximity with this can have a change in the intensity, remember this point very clearly.

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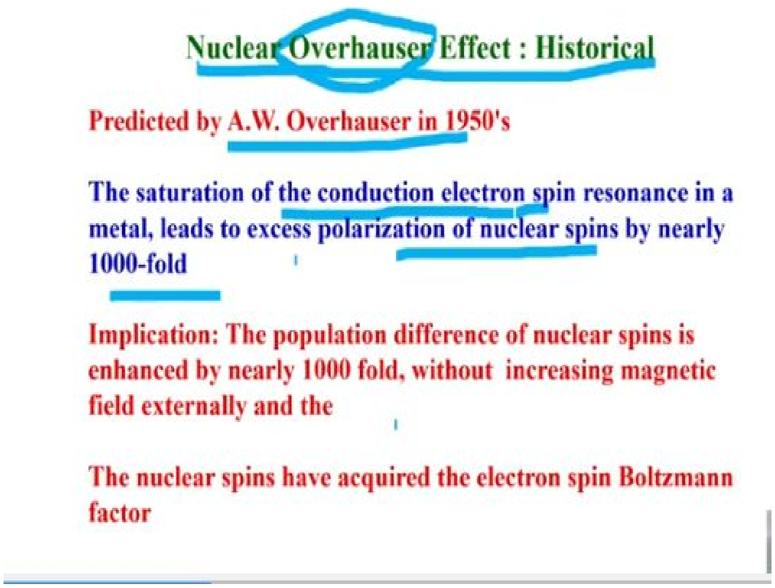

Nuclear Overhauser Effect : Historical

Predicted by A.W. Overhauser in 1950's

The saturation of the conduction electron spin resonance in a metal, leads to excess polarization of nuclear spins by nearly 1000-fold

Implication: The population difference of nuclear spins is enhanced by nearly 1000 fold, without increasing magnetic field externally and the

The nuclear spins have acquired the electron spin Boltzmann factor

This effect was first observed by a person called Overhauser, in 50's. Some historical developments, first he said if we can saturate the conducting electrons, then what happens, electrons have larger polarization, 1000 times more polarization than nuclear spins, then what he said is because of that, the nuclear spins gets polarized, 1000 times. This means, your signal intensity goes up 1000 times if we saturate an electron and observe nuclear signal, very interesting phenomena, right.

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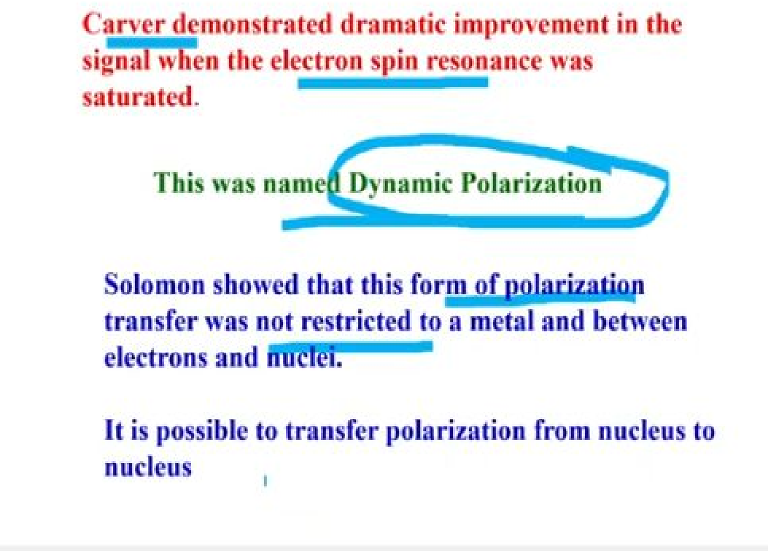

Carver demonstrated dramatic improvement in the signal when the electron spin resonance was saturated.

This was named Dynamic Polarization

Solomon showed that this form of polarization transfer was not restricted to a metal and between electrons and nuclei.

It is possible to transfer polarization from nucleus to nucleus

And this was even demonstrated experimentally by a person called Carver and he called this as dynamic polarization; where he hit electrons spins, when that was saturated, he observed change in the intensity of the nuclear spins. But later on there was another person Solomon who is responsible for giving the NOE equations which I will tell you later, he showed that this form of polarization transfer can occur not only from electron to nucleus, between 2 nuclear spins also, you understand.

First, it was done on metal, where hit an electron, you have lot of unpaired electrons there and then see the change in the intensity of the nuclear spin; because the polarization is 1000 times more for electron, it gets transferred to the nuclear spins. But now it is possible to transfer polarization from one nuclear spin to another nuclear spin by hitting one nuclear spin; so not necessarily from electron to nucleus, nucleus to nucleus transfer of polarization is also possible and this type is called Nuclear Overhauser effect which was demonstrated by Overhauser. This can happen between 2 nuclear spins also.

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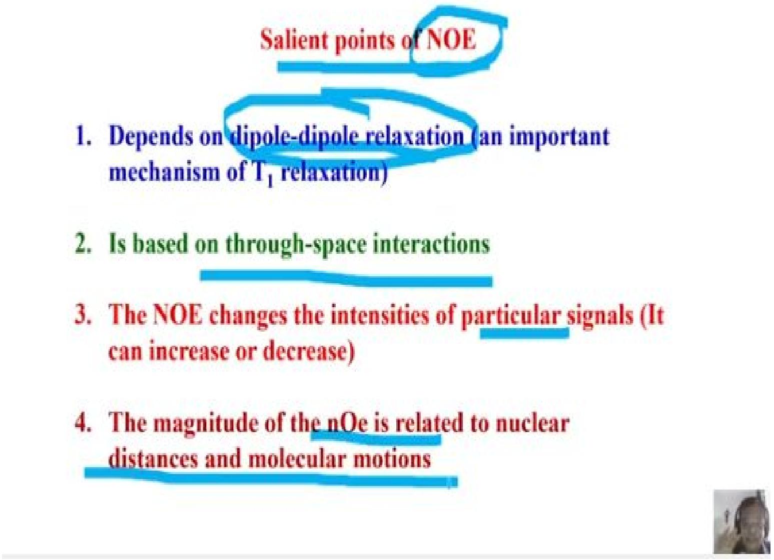

Salient points of NOE

1. Depends on dipole-dipole relaxation (an important mechanism of T_1 relaxation)
2. Is based on through-space interactions
3. The NOE changes the intensities of particular signals (It can increase or decrease)
4. The magnitude of the nOe is related to nuclear distances and molecular motions

Now the salient points of NOE; one of the important things is, it depends upon dipole, dipole relaxation mechanism, very, very important mechanism for relaxation, remember very important mechanism for relaxation. There are many ways the spins can relax, you apply a 90 degree pulse, bring the magnetization to x axis, as I told you it has to go back to thermal equilibrium, when it goes back, it exchange energy with the surroundings. And finally, slowly the energy it has gained it given to the lattice and go back and attain thermal equilibrium. There are several mechanisms to achieve at thermal equilibrium, okay; and dipole-dipole relaxation mechanism is very important for NOE; as far as NOE is concerned. It is a through space interaction, as I told you spins need not be J coupled, okay; and NOE can change the intensity, either decrease or increase; and NOE is related to internuclear distances, and molecular motion, very important.

The NOE depends upon distance between 2 nuclei, with the nuclear spins are far away, even if you disturb this nuclear spin, this cannot transfer the polarisation directly to it, this is not possible, okay. Also the transfer of polarisation depends upon molecular motions, how fast the molecules undergo motion and what is that motional frequency; all those things come into picture.

So, there are several points to discuss about NOE, this is a huge topic, even in NOE, number of NOE's experiments can be done; steady state NOE, transient, driven NOE and NOE in the rotating frame called ROESY, it is rotating frame, overhauser effect; lots and lots of things are there. The NOE itself is a very big topic to discuss, so in next 1 or 2 classes, this and may be in the next class, whatever is possible I will give you the salient points of NOE. Possibly we will stick to steady state NOE. If there is time, may be at the end of the course, I will select the special topic to tell you about ROE etc., some interesting topic; but at the moment we will stick to steady state NOE.

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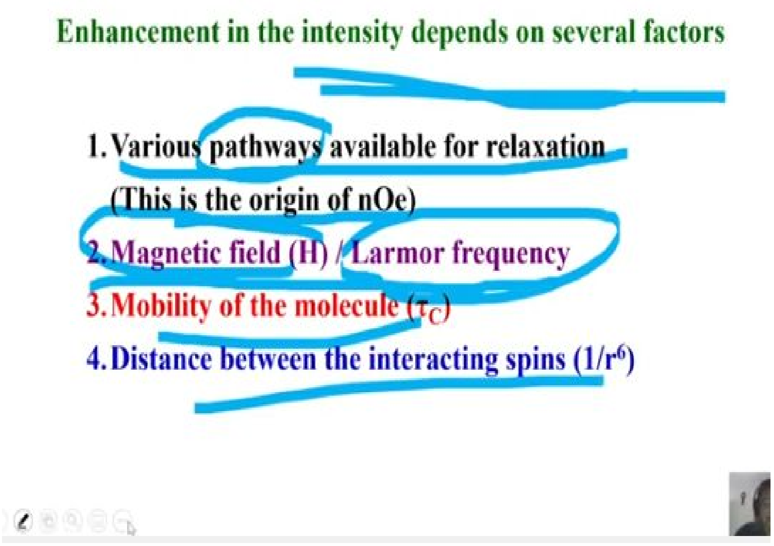

Enhancement in the intensity depends on several factors

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 NOE enhancement of intensity, I said, there is a change in the intensity of the peak; that depends on various pathways available for relaxation, I said dipole-dipole relaxation is the dominant mechanism responsible for this NOE. But in that there are several pathways, how do they relax, what is the pathway it adapts for relaxation is an important concept; and this is the basis and origin of NOE, remember NOE depends upon how this spins are relaxing, what is the pathway they are adapting for relaxation, which we are going to discuss now once we go ahead.

And it also depends upon the magnetic field at which we are recording the spectrum. The NOE, depends upon molecular motions I said, whether it is a very big molecule, or a very small molecule, if it is at low frequency or if you have very, very high magnetic field, etc. the NOE depends upon various factors. It is not that I imply do an experiment like decoupling, send a radio frequency signal and see the change, it does not happen routinely like that.

There are several factors governing this, it depends upon the molecule, size of the molecule and which magnetic field we are studying; that is on the the Larmor frequency and there on the molecular mobility, etc., and also the distance between the interacting spins, so many factors govern this NOE.

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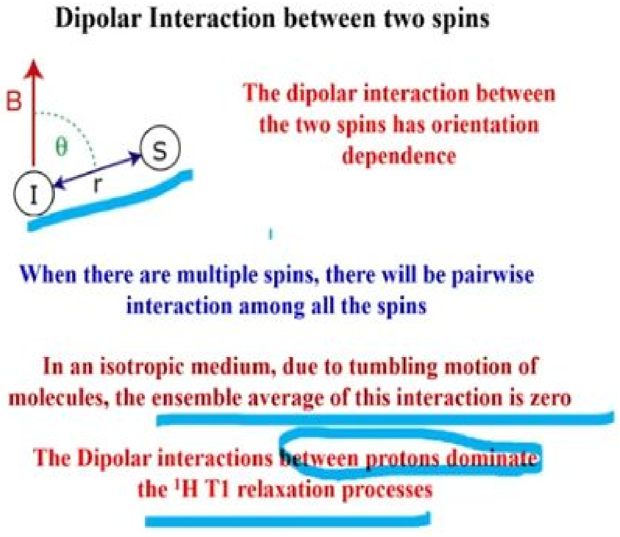

Dipolar Interaction between two spins

The dipolar interaction between the two spins has orientation dependence

When there are multiple spins, there will be pairwise interaction among all the spins

In an isotropic medium, due to tumbling motion of molecules, the ensemble average of this interaction is zero

The Dipolar interactions between protons dominate the ^{1}H T1 relaxation processes

Now, one of the important aspects of NOE is the dipolar interaction. Remember I told you what is a dipolar interaction; the dipolar interaction between 2 spins have an orientation dependence like this. If I and S spins are interacting and this is the r, the distance separating them, let us say it is making an angle theta with respect to the magnetic field. Now, we can get the dipolar interaction between these; we can work out and find out, taking the magnetic momentum of this nuclear spin, the magnetic moment of this nuclear spin, and get the dipolar coupling; thus the dipolar interaction can easily be studied.

But in a given molecule, when there are multiple spins, N number of spins, I have taken only 2 spins here, if I take 30 or 40 spins, any number, N number of spins let us say, each spin can have a dipolar interaction, dipolar coupling with other spins, because the dipolar interaction does not need chemical bond. Dipolar coupling is through space interaction, it does not demand a J coupling, I mean covalent bond between them, it is only spatial proximity what we need.

The same proton is here, one proton, other proton is far away like this, then if it is more than let us say 5 Å, greater than 5 Å, let us say this separation is 10 Å, NOE may not be seen. So it depends upon spatial proximity because it does not go through covalent bond. So, in an isotropic medium what happen?, this dipolar coupling goes to 0, very interesting thing.

The dipolar coupling goes to 0 in isotropic solution because the dipolar coupling is the orientation dependent phenomena. And as a consequence what happens is, in the ensemble average, there is a rapid tumbling motion, this interaction goes to 0, dipole, dipole coupling in solution goes to 0 that is what I said. Remember, even in the beginning in one of the class I said the dipolar interaction, dipolar coupling do not exist in solution state, because of motional averaging; that is what I said, exactly I am repeating the same thing. The dipolar interaction is non-existent in solution state. But the dipolar interactions are present that will aid the relaxation process, dipolar coupling may not be visible in the solution state, it is going to help you or help the nuclear spins for relaxing. So it is a dominating mechanism for relaxation, dipolar interaction is a dominating mechanism for relaxation of the nuclear spins. So, it aids in making the nuclear spins to attain thermal equilibrium, okay.

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Relaxation processes responsible for nOe

Now, with these salient points, remember I said few things, dipolar coupling is an orientation dependent interaction; and depends upon the internuclear distance, and in the solution state, motion of average removes this; and although it is in solution state, dipolar coupling is absent but the dipolar interaction is a dominant mechanism for relaxation, aids the nuclear spin to relax, when you perturb the nuclear spins by sending radio frequency pulse, then after sometime the spins have to attain thermal equilibrium, and this relaxation process helps in making it to achieve thermal equilibrium, so this are the salient points. Now let us understand what are the relaxation mechanisms that are responsible for NOE, okay.

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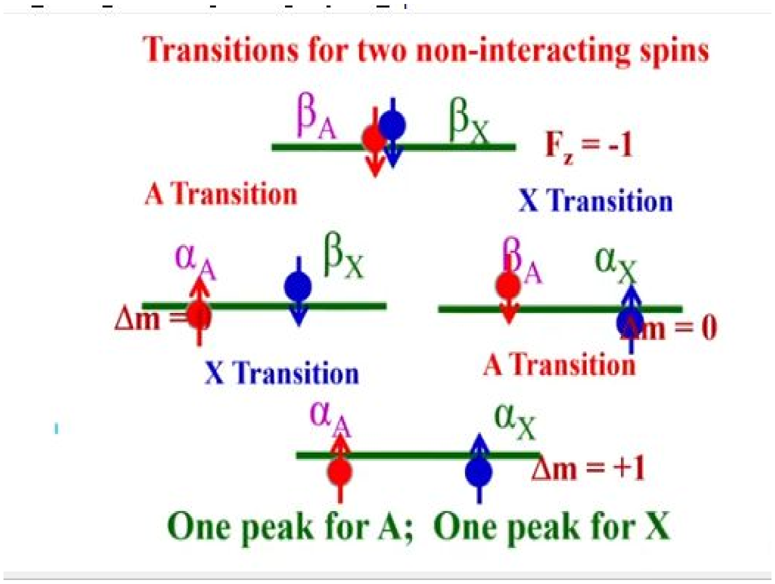

Transitions for two non-interacting spins

One peak for A; One peak for X

First consider 2 non-interacting spins that I have taken. This we discussed already couple of times. We have only 2 spins; considering non-interacting; and there are only 4 possible energy states, right; and 4 possible transitions; 2 for A and 2 for X, this is what we observed. The 2 transitions for A and 2 transitions for X are overlapping for non-interacting spins, remember we discussed this long ago.

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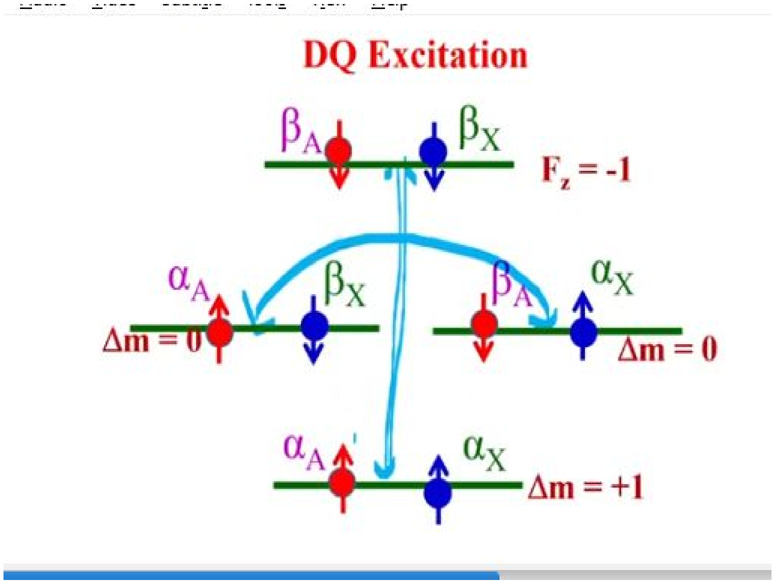

DQ Excitation

For 2 spin systems which are non-interacting, we get like this. But sometimes we can see, I am taking the example of a coupled spin because for double quantum, excitation and detection, spins will be coupled, dipolar or J coupled, does not matter, there must be a coupling. Now, in these 2 energy states, I observed the transitions are allowed between this and this; because I said what is going to happen only change in the magnetic quantum number is +1 or -1 is allowed.

I said this is not allowed, remember this is -1 to +1, change in the magnetic quantum number of these 2 energy states is +2 or -2, it is a forbidden transition. But it can happen, 2 spins can simultaneously come from beta beta state to alpha alpha state. This is called double quantum transition. If I some more make these spins to go up here to double quantum; and detect the signal, it is called double quantum excitation, there is a way to detect that. It is a forbidden transition. But there is a way to detect it, we can detect in some way.

Let us not bother about it at the moment. what about this one? 0 to 0, magnetic moment of this energy state is 0, this is also 0. This energy state to this energy state if you consider, is the transition allowed? this to this is the transition allowed? no, because it is 0 to 0, it is a forbidden transition, here this transition, +1 to -1, it is double quantum, this is called zero quantum transition, this is called double quantum transition; but they are not allowed, okay this is that double quantum. This is zero quantum, they are not allowed transitions.

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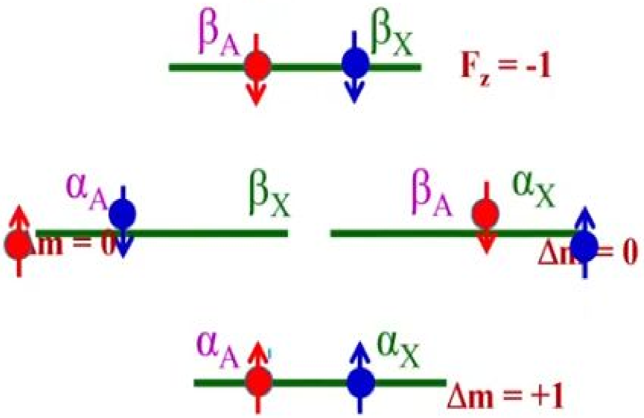
ZQ Excitation



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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 interaction

But they are forbidden NMR as far as the detection of the transition is concerned, remember my point, explicitly I am telling you, for the detections of the transitions they are forbidden; but they are not forbidden in the context of relaxation. The dipole, dipole interaction is there that can aid in the relaxation of the nuclear spins, they are not forbidden in the relaxation context, but they are forbidden from the point of detection of the magnetization.

You can excite double quantum and zero quantum, but you cannot excite and detect, there is indirect way of doing it; but directly you cannot send a RF pulse and then start detecting the double quantum. They are all forbidden transitions but not for the relaxation that is the point I want to tell you.

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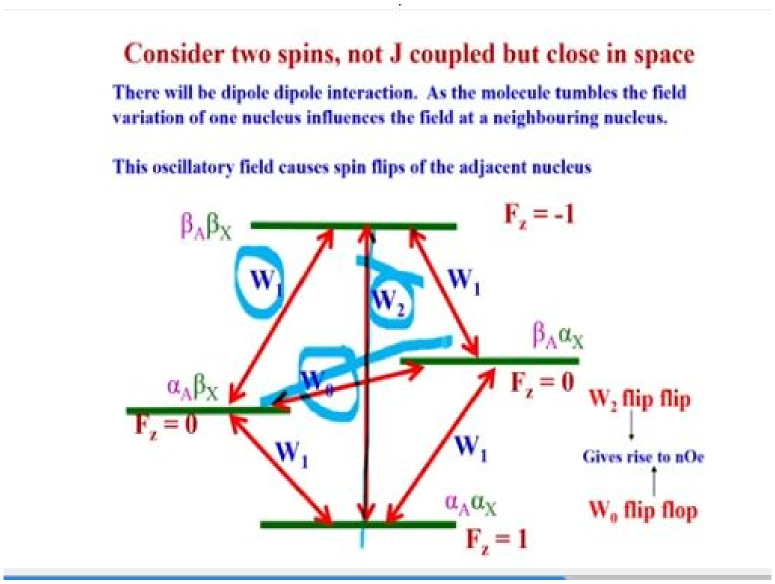

Consider two spins, not J coupled but close in space

There will be dipole dipole interaction. As the molecule tumbles the field variation of one nucleus influences the field at a neighbouring nucleus.

This oscillatory field causes spin flips of the adjacent nucleus

Now, consider 2 spins, not J coupled but close in space. This is the condition for NOE, it need not be J coupled, I told you know. So there is a dipole-dipole interaction as the molecule is undergoing tumbling, the magnetic field that this site have one varies because of the tumbling of the other spin. This causes oscillatory field.

The oscillation frequency can be any of them, it can be W0 or W1 at the Larmor frequency or twice the Larmor frequency or the difference of the chemical shift. So it can happen, that the oscillatory frequency can be any of them, okay. This oscillatory field can cause the spins to flip, one spin can flip the other nuclear spin, okay. Now, let us see what is going to happen here, this W2 to W1 flip is also allowed.

This is what is happening here; Before going further I wanted to tell you one thing, I have written 3 terms here; W1, W2 and W0. What are these things? This is the transition probability for single quantum transition, this is called single quantum transition you know, this is the transition probability for double quantum, this is transition probability W0 for zero quantum, so these are all W transition probabilities, I have taken it as W's.

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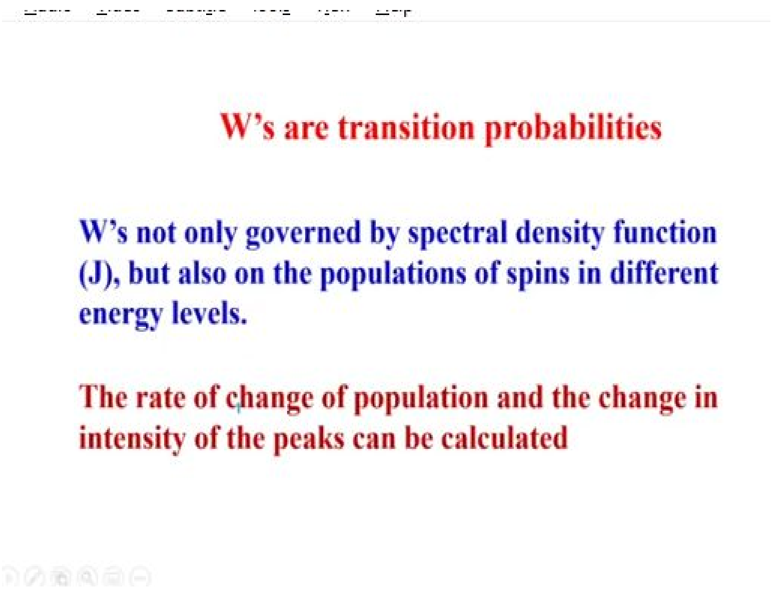

W's are transition probabilities

W's 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, these W's; transition probabilities are governed by what is called spectral density function, and also on the population of nuclear spin in different energy levels, very interesting thing. W's are transition probability governed by spectral density function and also population. What is a spectral density function? we will discuss later, we are coming to this, In this class or next class itself, we will discuss. And also on the population of spins in different energy levels and the rate of change of population and the change in intensity of the peaks can be easily calculated, very easy, okay.

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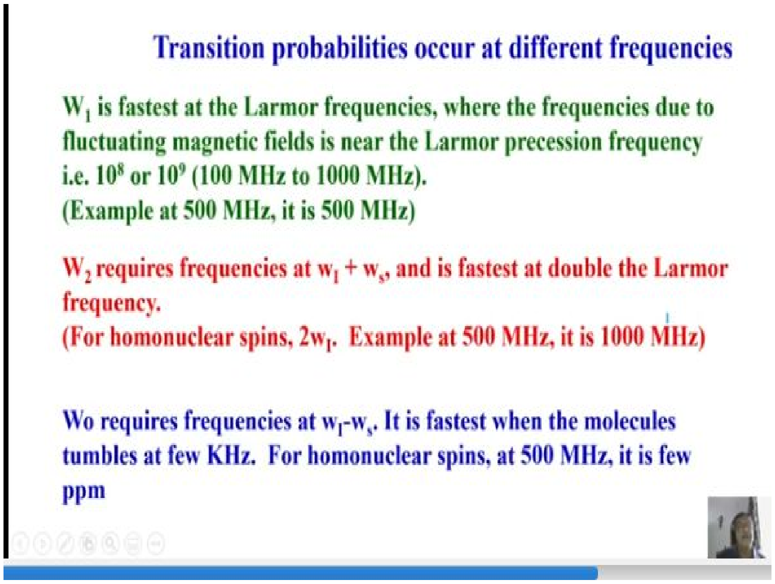

Transition probabilities occur at different frequencies

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)

W_2 requires frequencies at $w_I + w_s$, and is fastest at double the Larmor frequency.
(For homonuclear spins, $2w_I$. Example at 500 MHz, it is 1000 MHz)

Wo requires frequencies at w_I-w_s. It is fastest when the molecules tumbles at few KHz. For homonuclear spins, at 500 MHz, it is few ppm

See, transition probabilities occur at different frequencies, W1 occurs at Larmor frequency let us say 500 megahertz my resonating frequency, the transition probability of single quantum transition is around 500 megahertz; The molecular motions and the vibrations, fluctuating magnetic field that arises is at the Larmor frequency. I can take the fluctuating magnetic field, all the vibrations and whatever the motions we get, all these motion will have the frequency, you can do the Fourier transformation and get the frequencies. And W1 will be at the Larmor frequency and it is the fastest, W1 is always fastest at the Larmor frequency.

W2 is interestingly, is fastest at the sum of the Larmor frequencies of 2 spins; that is double the quantum, double the Larmor frequency. This is at Larmor frequency, this is at Larmor frequency, we have to add up. So, W2 requires frequencies of double the Larmor frequency; so the transition probability for a single quantum is at the Larmor frequency, transition probability for the double quantum is at the double the Larmor frequency.

For example, consider the 2 nuclear spins, a small chemical shift separation is there and we are recording at 500 megahertz; and the nuclear spin to have a dominant relaxation or W2 transition probability, then it may reach 1000 megahertz, the molecular motions and fluctuating magnetic fields should generate the frequencies of double the Larmor frequency, remember, it should generate the frequency at double the Larmor frequency that is 1000 megahertz.

You may ask, what is W0; W0 is only the difference in frequencies, what is difference in frequency; there are 2 protons, one is at chemical shift of 5 ppm, other is a 10 ppm, only difference is few hertz, okay it is not much, it is only a very small value, it is of the order of few hertz or few kilo hertz, so W0 is at few kilo hertz; W1 comes at Larmor frequency, W2 the probability, W2 requires double the Larmor frequency. For nuclear spin to undergo relaxation through W2, we require frequencies around 1000 megahertz. Okay, let us see, 1000 megahertz I said because I have taken 500 megahertz as the example.

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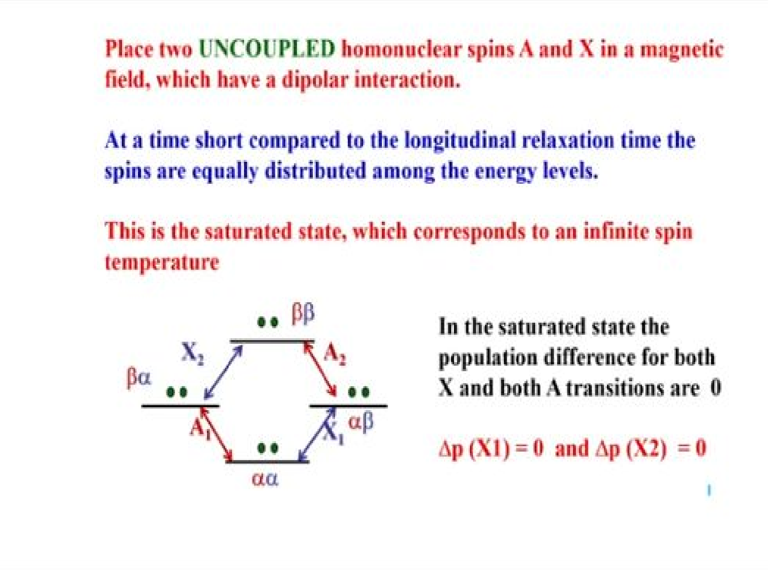

Place two UNCOUPLED homonuclear spins A and X in a magnetic field, which have a dipolar interaction.

At a time short compared to the longitudinal relaxation time the spins are equally distributed among the energy levels.

This is the saturated state, which corresponds to an infinite spin temperature

In the saturated state the population difference for both X and both A transitions are 0

$\Delta p\ (X1) = 0$ and $\Delta p\ (X2) = 0$

Now, I will do one thing, I will take 2 nuclear spins uncoupled, homonuclear. of course, I will put them in a magnetic field, they are so close that there is a dipolar interaction, I cannot put 2 nuclear spins which are far away, too far away more than 20 or 30 angstroms; then there is no interaction. Let us say we have put 2 uncoupled nuclear spins which have a dipolar interaction that is close in space. Now, for a short time, immediately after you put the sample in a magnetic field, what is the state of our nuclear spins in different energy levels?

See, there is no magnetization, magnetization comes only after you put the sample in a magnetic field and wait for some time. See outside the magnetic field, they are all randomly distributed, okay, the energy states are completely degenerate. Now, take the sample put it in a magnetic field, there is a removal of degeneracy, the degeneracy is completely removed, whatever the degeneracy was there is removed.

Now, the spins have to redistribute themselves within different energy states according to Boltzmann population; right. It cannot happen immediately, as soon as you put the sample, not that energy separation is there, there is a removal of degeneracy, spins start occupying different energy states and get populated. It take some time, okay. The time which requires is called a spin relaxation time.

It cannot happen immediately, it requires some time. Let us say I do not wait, I am very impatient, I do not wait and put the sample in the magnetic field and immediately see what are the populations; what you will get; all spins are equally populated, it has not attained Boltzmann population distribution at all. This is called a saturated state, okay, this is called saturated state. Now, if I take the population difference, between for any transition of A or X spin, it is 0. That is why there is no signal, now the spins are saturated, we do not get the signal.

So, you cannot get the NMR signal as soon as you put the sample immediately and within fraction of a nanosecond, you try to see the signal, you may not see the signal. You have to wait for certain time till the spins attain thermal equilibrium.

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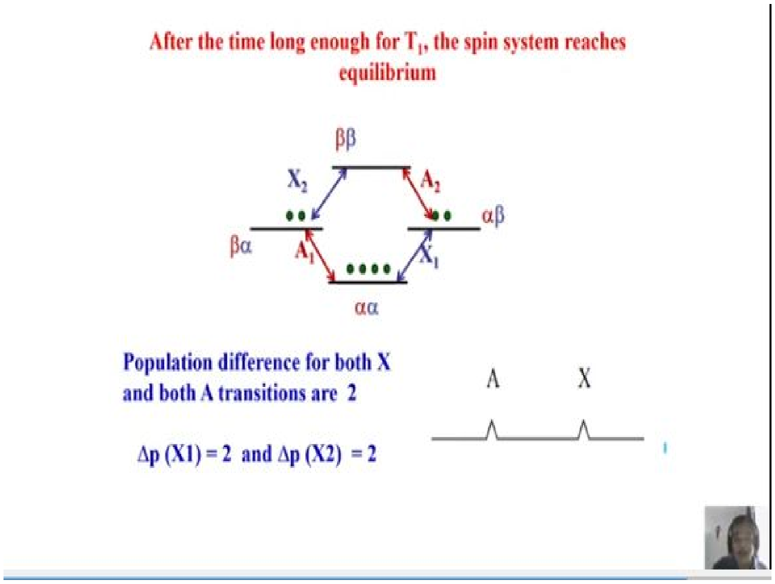
After the time long enough for T_1, the spin system reaches equilibrium

Population difference for both X and both A transitions are 2

$\Delta p(X1) = 2$ and $\Delta p(X2) = 2$

So, wait for a long time, long means few seconds. for this spins to attain equilibrium, it reaches thermal equilibrium. Then what happens? population gets redistributed, I took this example earlier. Same we will take the example; 4 spins here, 2, 2, and 0, so that in the AX weakly coupled spin system all peaks should have equal intensity; that is why I have taken this example. Of course, there are spins here, only for calculation I have taken like this, it does meant there are no spins in the beta, beta state.

Now, take the population difference for both X and A transitions; X transition has population difference 2 here, A transition has population difference 2 here, so you get 2 transitions for A, 2 for X, both are perfectly overlapped and you are going to get only 2 peaks, that is all, of equal intensity, 2, 2, remember this, okay.

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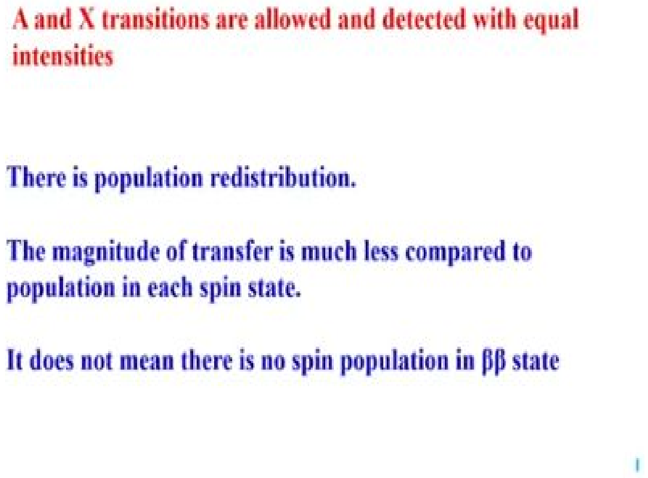

A and X transitions are allowed and detected with equal intensities

There is population redistribution.

The magnitude of transfer is much less compared to population in each spin state.

It does not mean there is no spin population in ββ state

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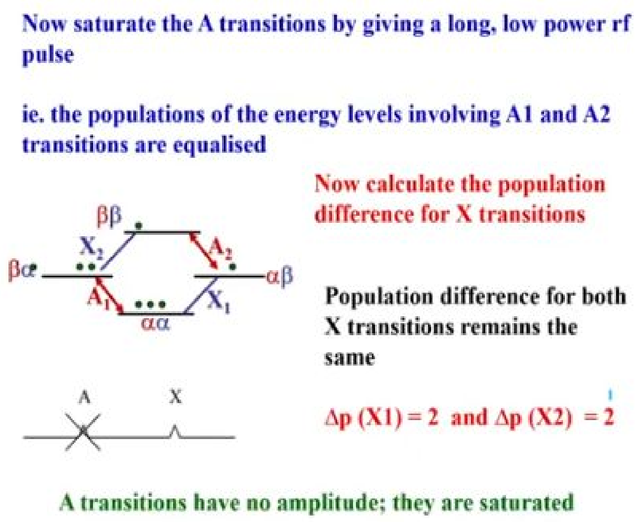

Now saturate the A transitions by giving a long, low power rf pulse

ie. the populations of the energy levels involving A1 and A2 transitions are equalised

Now calculate the population difference for X transitions

Population difference for both X transitions remains the same

Δp (X1) = 2 and Δp (X2) = 2

A transitions have no amplitude; they are saturated

There is a population redistribution and now what I will do is; I am going to saturate one of the transitions here, what I am going to do; I will saturate one of the transitions. Meaning; I will make spins between these two energy states exactly equal, let us say I will take this 4 spins, put 2 spins here, saturate it, this will become 3, this will become 3. I will do that, I take this thing and take it there, okay, saturate, okay, it should have moved here, does not matter. Of course, I am not saturating one transition, sorry, I am saturating both the transitions, all A transitions I am saturating completely. Both the transitions of A are saturated. This 4, 2 became 3, 3. And 2, 0 became 1, 1 okay. Understand, I am saturating both the transitions of A. Now, calculate the population difference of X transition, what happens; 3 -1, 2, 3 -1, 2, X transition intensity has not been affected at all, it remains same.

But what about A transitions; both A, I have irradiated, okay; because both spins are uncoupled you know, there is no coupling at all, just I have written the energy level diagram like this. Now I am saturating both of them, so populations are equal. We will not see any transition at all. So, A transition is now nonexistent; only X transition is seen, of intensity 2. What was the intensity before irradiation; after it attains thermal equilibrium? it was 2, okay, both A was 2, X was 2, now again it is 2, X1 and X2 both are same, intensity of both the X transitions are 2.

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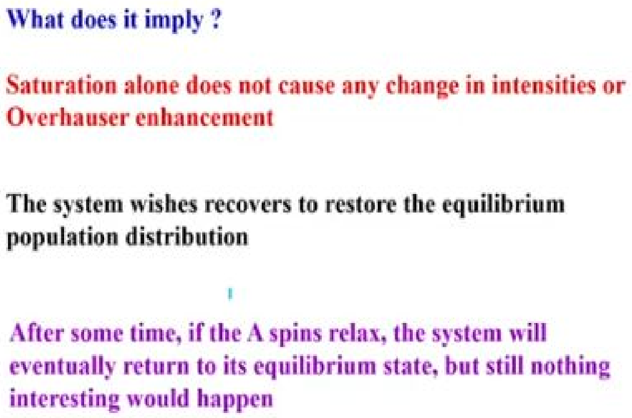

What does it imply ?

Saturation alone does not cause any change in intensities or 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, but still nothing interesting would happen

So, what did we achieve; we did not achieve anything, just because we saturate one of the transitions, we did not get any change in the intensity at all, the intensity remained same. So saturation does not mean there is a change in the intensity or Overhauser effect. So we did not get enhancement and decrease in the intensity, none of them. So saturation does not alone work for you, there must be something more happening to see the enhancement.

Now, wait for some time, what will happen? the system will come back to equilibrium and then after some time, the same thermal equilibrium 4, 2, 2, 0, will be attained. It will come to same state, nothing has happened. No interesting thing; you took the sample, put it in a magnetic field and it attained thermal equilibrium and you saw 2 transitions for A and 2 for X, both are overlapping, same intensity.

We saturated one of the A transitions, not one, both the A transitions, okay, then what is happening; A transition will not be observed because population of the 2 energy states become equal; that means you do not see peaks for A at all. We recalculated the intensity for X, X did not change the intensity, okay the irradiation is done, afterwards what will happen? the spins will go back to thermal equilibrium.

So, nothing interesting happens even if you do the irradiation of one of the spins. You did not find any change in the intensity on the other spin, even though they are in close spatial proximity with the dipolar interaction. Then something more should happen, what is happening for the NOE to occur; that is what we have to understand, right.

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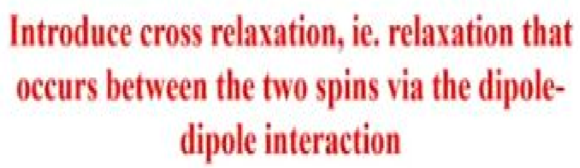

Introduce cross relaxation, ie. relaxation that occurs between the two spins via the dipole-dipole interaction

So, for this you have to introduce the concept of cross relaxation that is a relaxation that occurs between 2 spins via dipole-dipole interaction, you understand. We have to introduce cross relaxation that is a relaxation which is occurring between the 2 spins via dipole-dipole interaction.