Advanced NMR Techniques in Solution and Solid-State
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Module-38
Positive and Negative NOE and Spectral Density Functions
Lecture – 38

Welcome all of you. In the last class we started discussing about nuclear Overhauser effect. I told you, in the case of NOE first, it was predicted by overhauser. He showed that by irradiating electron spins we can enhance the polarization of the nuclear spin by nearly one 1000 times; 1000 fold enhancement in the signal intensity was seen without changing the magnetic field. So, that was the first thing, it was called dynamic nuclear polarization.

Subsequently, it was also shown this phenomenon is possible, even between 2 nuclear spins also. So, what is nuclear Overhauser effect? We understood certain conditions, certain restrictions for that. What are the requirements for that? These are, when you decouple a particular spin, irradiate a particular spin with certain rf power, 2 or 3 things can happen; when you decouple that particular peak, the peak which is coupled to it can collapse; the multiplicitycan collapse into a singlet; or a simplified pattern. Or it can so, happen, it can change the intensity. The change in intensity could be either positive or negative and also, I said, for such a phenomenon the 2 nuclear spins need not be J coupled. All what is required is the spatial proximity between 2 nuclear spins.

The spins have to be close in space. And the NOE phenomena depend upon various factors. I discussed about spectral density function, correlation time and everything. And we started discussing about an example of 2 spins which are uncoupled, kept in a magnetic field. Immediately after putting in a magnetic field, there is a saturation state. All the spins are saturated in such a way there is no population difference at all, no population difference at all.

Finally, what happens; wait for some time that system at attain thermal equilibrium and there is redistribution of spin populations; and for the 2 spins which were kept in the magnetic field which are close in space, we found out that the intensities are equal for 2 transitions, for each of these spins intensities were identical. Just for the calculation purpose we took it as 2 is to 2 is to 2 is to 2.

And then what we did is we irradiated one of the transitions or one of the spins, with a low rf for a long time and then saturate that particular spin. Then measure the intensity of the other spin which is close to it. We found again, there is no change in intensity; and after certain time, spins will come back, revert back to thermal equilibrium. It attains thermal equilibrium, this is what we observed.

So, then we understood just by radiation alone the intensity of the other spin which is close in space, will not change. So, NOE phenomena cannot happen just by irradiation of one of the spins. Then what is that which is responsible to bring in NOE phenomenon? What I said was we have to consider some of the relaxation pathways. We also worked out what are the transition probability for single quantum. double quantum and zero quantum also, we discussed. But then I said, these transitions are disallowed, 0 quantum and double quantum, other than single quantum, other transitions are not allowed as per the selection rules of NMR. But, however, in the context of relaxation they are allowed. So, with this idea now we will bring in another term, called cross relaxation. Instead of spins attaining thermal equilibrium by undergoing transition using the single quantum pathways, what happens if we allow the spins to adopt a different relaxation pathways? For example, the spins can relax through double quantum pathway or spins can relax through zero quantum pathway either of them, we can do. Let us see, if that pathway is adapted for the spins will there be any change in the intensity.

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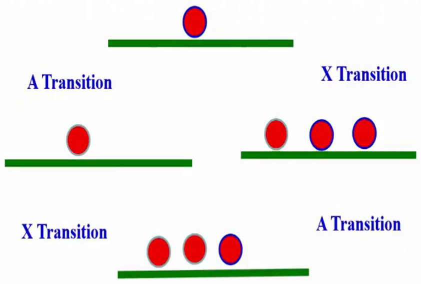
After some time, A spins relax and the system will eventually return to its equilibrium state



So, we will start with that now and see what is going to happen? So, this is what it is.

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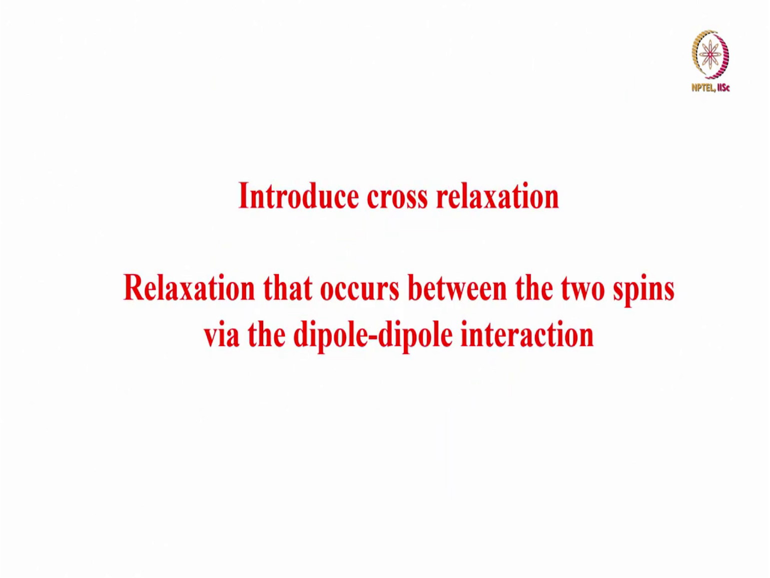

Introduce cross relaxation

Relaxation that occurs between the two spins
via the dipole-dipole interaction

Now, we will introduce cross relaxation; it is the relaxation that occurs between the 2 spins via dipole-dipole interaction. Of course, this is a dominant relaxation pathway. We have been discussing even from the relaxation phenomena which we introduced.

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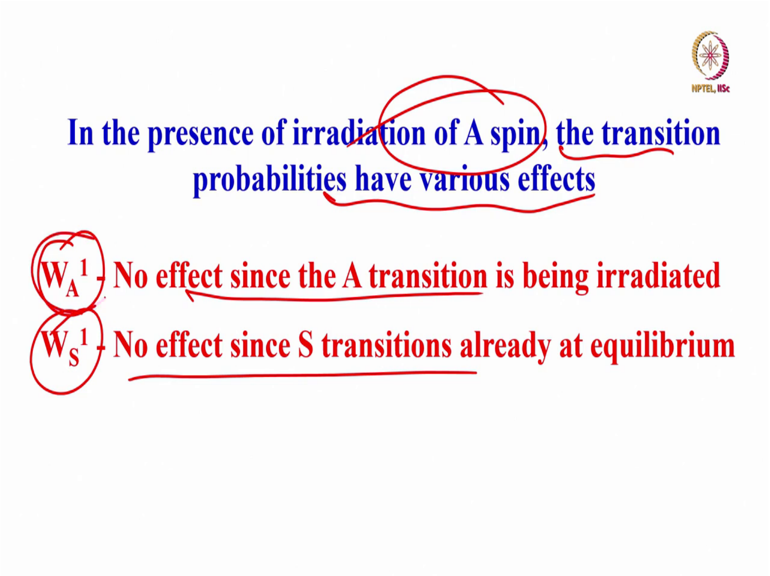

In the presence of irradiation of A spin, the transition probabilities have various effects

W_A^1 - No effect since the A transition is being irradiated

W_S^1 - No effect since S transitions already at equilibrium

Now we will consider the situation. In the presence of irradiation of one spin, for example, A spin. The transition probabilities can have various effects; we will consider like this if WA1 single quantum, if you consider, if you irradiate A spin, there is no effect, because A spins are being irradiated. If A spins are irradiated, you will not see any effect. On the other hand, we observed that no effect is seen because S transition is already at equilibrium.

So, this there are 2 possibilities we can think. You have different effects, when I irradiate one of these spins. Remember when I irradiate A spin it has no effect because it is in a saturated condition, saturated state and W1S is already at equilibrium, no effect.

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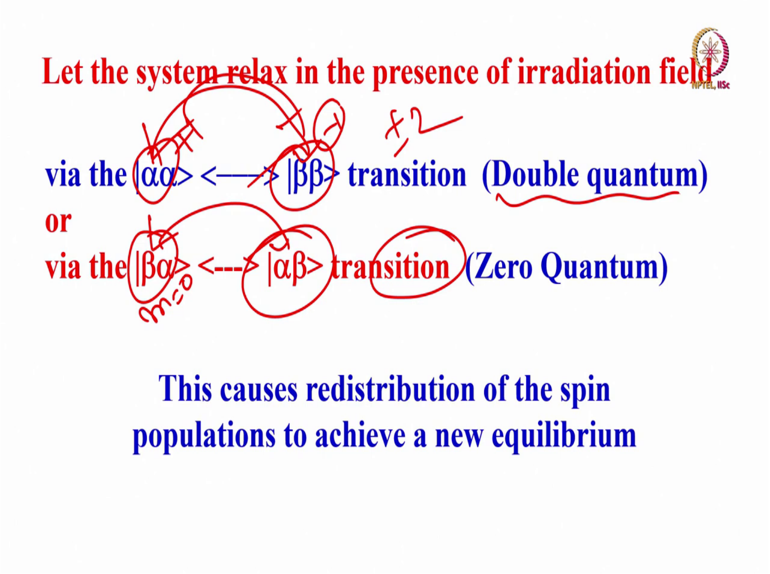

Let the system relax in the presence of irradiation field

via the |αα> <——> |ββ> transition (Double quantum)

or

via the |βα> <---> |αβ> transition (Zero Quantum)

This causes redistribution of the spin populations to achieve a new equilibrium

But now let the system relax in the presence of irradiation field in a different pathway. You can think of 2 possibilities. I told you alpha alpha to the beta beta relaxation you can think of or beta alpha to alpha beta relaxation you can think of, either of them. Here 2 spins are changing their states simultaneously; it is called double quantum. Here again 2 spins are changing state simultaneously, but it is a beta alpha to alpha beta or alpha beta to beta alpha. It is a 0 quantum transition, because the change in magnetic quantum number here is 0, so, 0 to 0 transition, it is 0 quantum. On the other end, it is +1 to −1 change is +2 or −2. It is called double quantum transition, either of them we can think of. Let us see what happens if I do this? If this can happen, there could be redistribution of spin populations to achieve a new equilibrium. Earlier we saw one type of equilibrium when the spins relaxed through single quantum pathway.

Now let us see with this whether there is any redistribution of spins, or the spin population which is different from what we observed.

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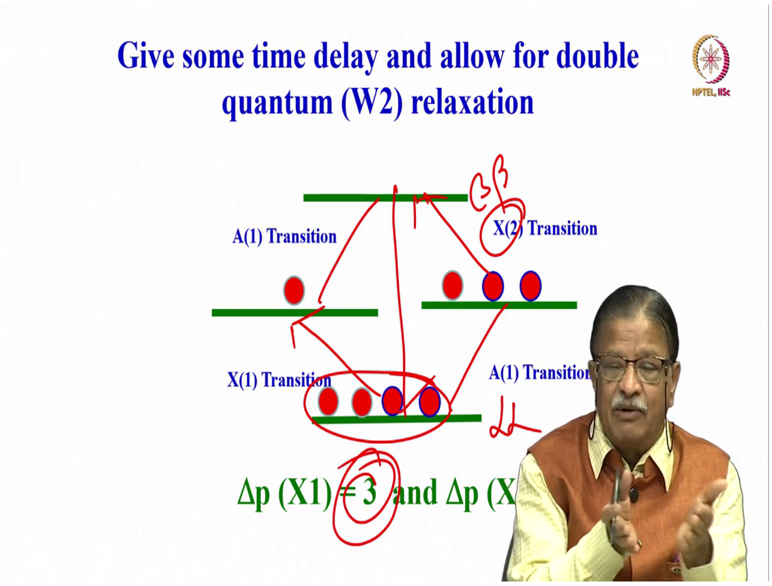

Give some time delay and allow for double quantum (W2) relaxation

Δp (X1) = 3 and Δp (X

Let us see that. Now we have already irradiated this spin. This is the spin population. Now we have saturated A transitions, you see, A transitions have equal intensity. A spin has been saturated. Now, let us see X transitions, by allowing this spin instead of attaining thermal equilibrium by these pathways, why not we allow it to come down directly through double quantum pathway; and see what is the change we are going to get?

Let us see that now. Now the spins will jump from beta beta state to alpha alpha state. It is a double quantum transition. It is relaxing through double quantum pathway. Now let us recalculate the population difference for X, transitions. Of course, A transition If you consider, now it is 1 − 0, it is 1. Similarly, here A transition, if you consider 4 − 3, it is 1. So, A transition has reduced intensity by half, after the spins relax through double quantum pathway. That is not interesting, because we are irradiating that; let us see the effect of this on the neighbouring spin X. What is happening to that? We calculate the populations of spin X, the population difference. This X1 transition is now 4 − 1. It is 3. Similarly, X2 transition is 3 − 0. That is also 3. What was our population difference when we started with equilibrium and after irradiating we saw population difference continue to be 2, 2, 2, 2 only. There was no change.

But now you see there is 50% enhancement in the intensity. We did not do anything. We only assumed that spins are relaxing through double quantum pathway. So, by irradiation of a spin, we saw enormous change in the intensity of the other spin which is close in space with it.

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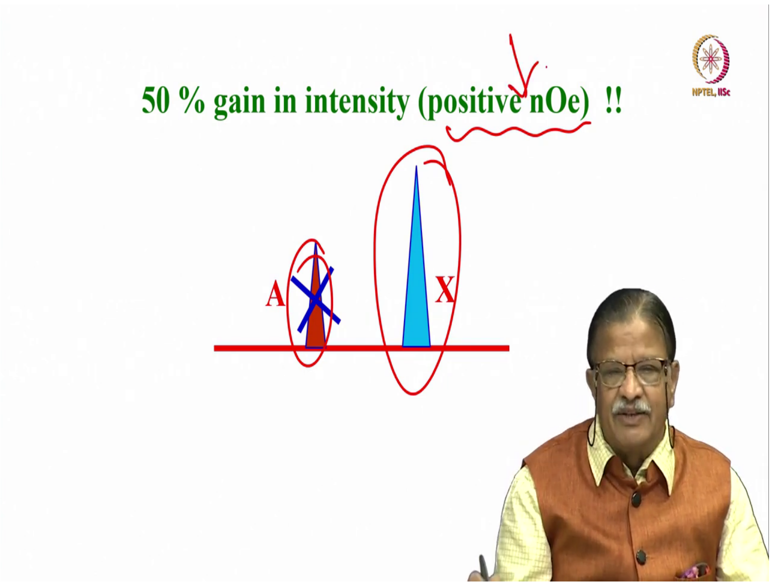

50 % gain in intensity (positive nOe) !!

So, the 50% rise. This is what we are going to do. This came down by 50% we are not interested in that, whereas X has gained in intensity by 50%. This is called a situation of positive NOE; remember, positive NOE. So, what do we understand from that? If the spins adopts or follows the double quantum relaxation pathway, then we can see positive NOE. There is a change in the intensity of the signal. Alright this is a positive NOE case.

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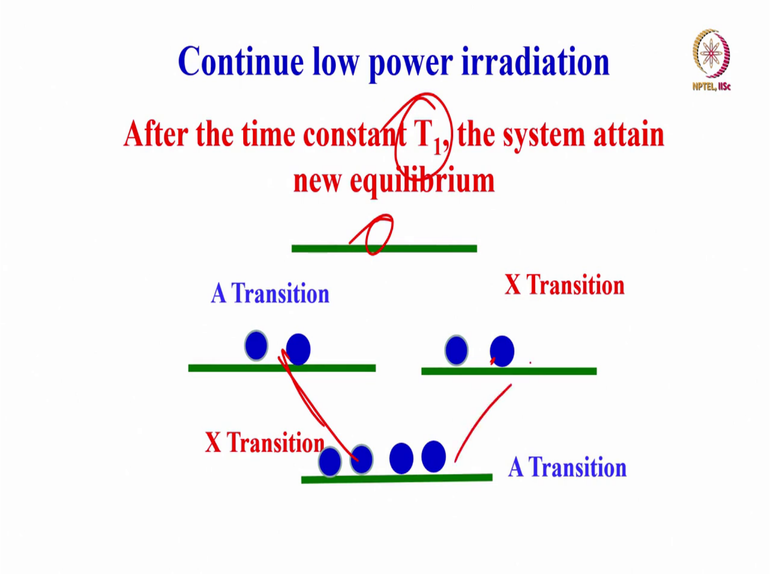

Continue low power irradiation

After the time constant T_1, the system attain new equilibrium

Now let us continue to do the low power irradiation for some time. All these phenomenon are happening while we are radiating one of these spins continuously with low power rf. After sometime, what will happen? Wait for some time, T1 which is the relaxation time required for the spins to come back to Z axis, attaining thermal equilibrium. Now it has come to this state.

This is where we started with after attaining thermal equilibrium; this was this spin population. We took for calculation of intensity as some numbers, so, again, 4 2 2 0 it came. Now we recalculate the population difference, if we recalculate now it is again X is 2, A is 2 2, 2. So, intensity has come to the original state. That is one way. So, this is possible.

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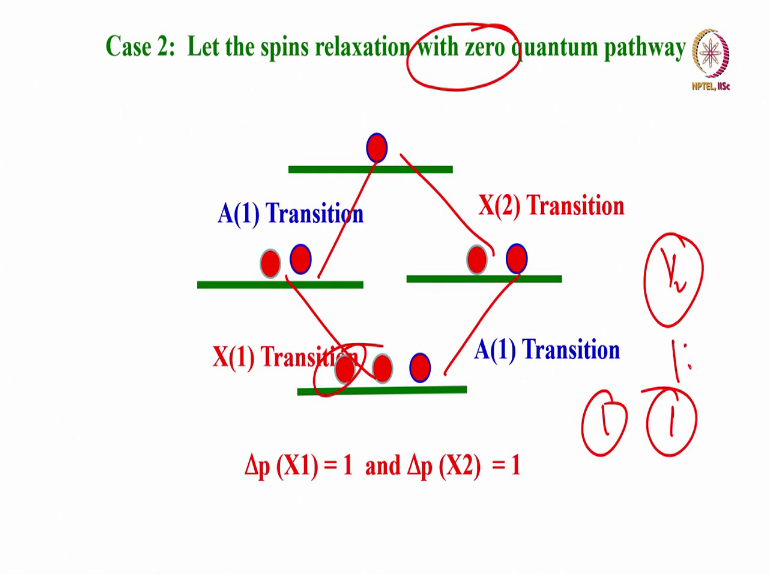

Case 2: Let the spins relaxation with zero quantum pathway

Δp (X1) = 1 and Δp (X2) = 1

Now we can think of another thing. Let the spins relax through 0 quantum pathway. Why I should go through double quantum always? There is no need for it. It can adopt zero quantum pathway also. Let us see if the spins relax like this, one of the spins relax like this after irradiation for some time. Now, recalculate the population difference, look at it A transition again, 2 − 1 is 1. 3 – 2 it is 1. A transitions always continue to be half that has not changed.

In the double quantum relaxation pathway also it was half, reduced by half now also it is same. What about the X transitions? look at; the X transition here this is 3 − 2 it become 1 here, 2 − 1 it became 1. We started with 2 is to 2 intensity. Now, with the 0 quantum pathway we came into, 1 is to 1 intensity.

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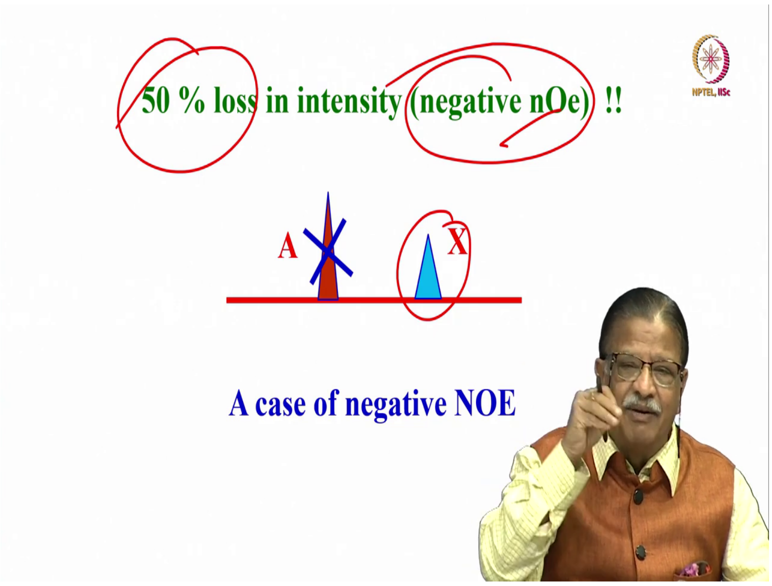
50 % loss in intensity (negative nOe) !!

A case of negative NOE

What is the change you observed? The reduction in the intensity of the signal. In fact the X transition, came down by 50%, It is a loss. 50% reduced intensity. This is called a case of negative NOE. So, now we understood this, you can just by radiating the spin; allowing them to relax through single quantum pathway will not give rise to change in the intensity of the spin; which is close in space with that of the irradiated spin.

On the other hand, if we allow the spins or let us say, the spins follow cross relaxation, it can follow double quantum relaxation pathway or 0 quantum relaxation path, either of them or both of them it can follow. It is a competitive process we do not know; it need not follow only one pathway, it can have both the paths. Which is dominant is important. If it adopts a double quantum pathway, we say positive NOE; if it adapts, 0 quantum pathway we have a case of negative NOE.

So, now we understood even by radiating with a rf power. We are not doing anything only thing we brought in cross relaxation phenomena where spins are allowed to relax through double quantum and 0 quantum pathways.

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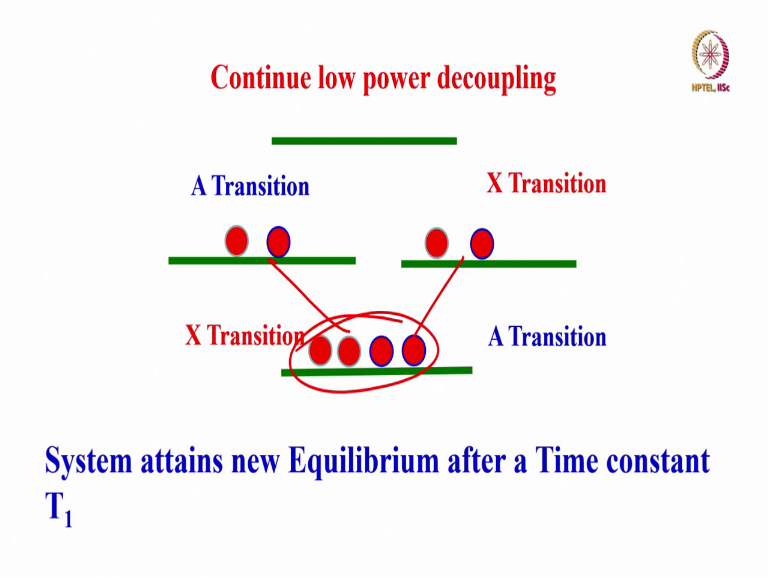

Continue low power decoupling

System attains new Equilibrium after a Time constant T_1

Now continue to radiate with low power rf. What is going to happen, system will come back to original equilibrium. New equilibrium is attained. The new equilibrium is what we started with after putting the sample in a magnetic field. So, that means the same 4 2 2 2 ; the equal intensity, so, in between, when you are irradiating, this phenomena can happen, change in intensity case of positive and negative NOE can happen.

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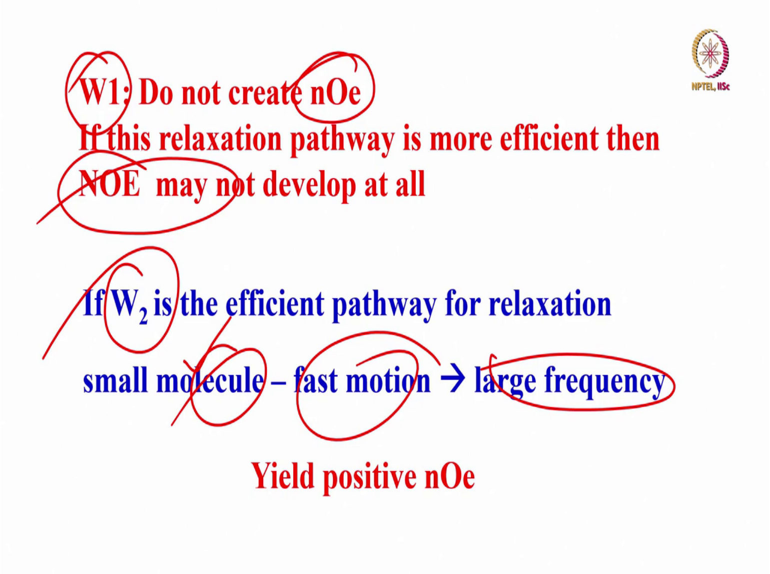

W1: Do not create nOe

If this relaxation pathway is more efficient then NOE may not develop at all

If W_2 is the efficient pathway for relaxation

small molecule – fast motion → large frequency

Yield positive nOe

Now let us understand one thing W1 transition probability that is a single quantum transition probability do not create NOE. If this is the dominant relaxation pathway or this is the most efficient relaxation pathway, then NOE may not develop at all. You will not see NOE. If W1 is a relaxation pathway, dominant relaxation pathway. On the other hand, if W2 is a efficient pathway for relaxation, then you can observe this phenomena. This can be experienced in small molecules where there is fast motion.

And the motional frequencies are very large then W2 is the efficient pathway for relaxation and you get positive NOE. So, you understand a positive NOE case. There should be double quantum relaxation pathway, W2 should be more efficient and this can happen for small molecules undergoing fast motion.

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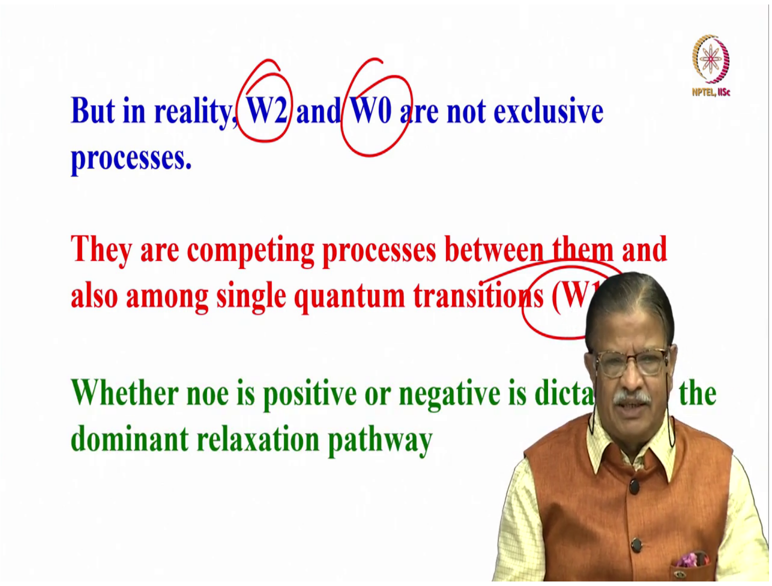

But in reality, W2 and W0 are not exclusive processes.

They are competing processes between them and also among single quantum transitions (W1

Whether noe is positive or negative is dicta the dominant relaxation pathway

So, W0, if you come, if that is the efficient pathway for relaxation that is for 0 quantum, this can obviously happen only for large molecules, undergoing slow motion and frequency distribution for small frequencies, not large frequencies. W0 is very small, in which case it is negative NOE phenomenon. So, positive NOE negative and NOE you understood, it is depending upon the relaxation pathway and also on the size of the molecule and depends upon molecular motions. For small molecules you have positive NOE, W2, relaxation pathway, large molecule, negative NOE and W0 is the relaxation pathway. But remember these are not exclusive processes. I told you both W2 and W0 are competitive processes, but it can be happening simultaneously. You cannot dictate terms for the nucleus to follow particular pathway. So, it is a natural phenomena both can happen simultaneously.

And also W1 can also be happening. All the three relaxations pathways can be adopted by the nucleus spins simultaneously and which is dominant relaxation pathway defines whether you have positive NOE, negative NOE or 0 NOE or no NOE at all. So, these are the things we have to understand. So, this is what we should know, NOE positive, negative or 0 is dictated by the relaxation pathway.

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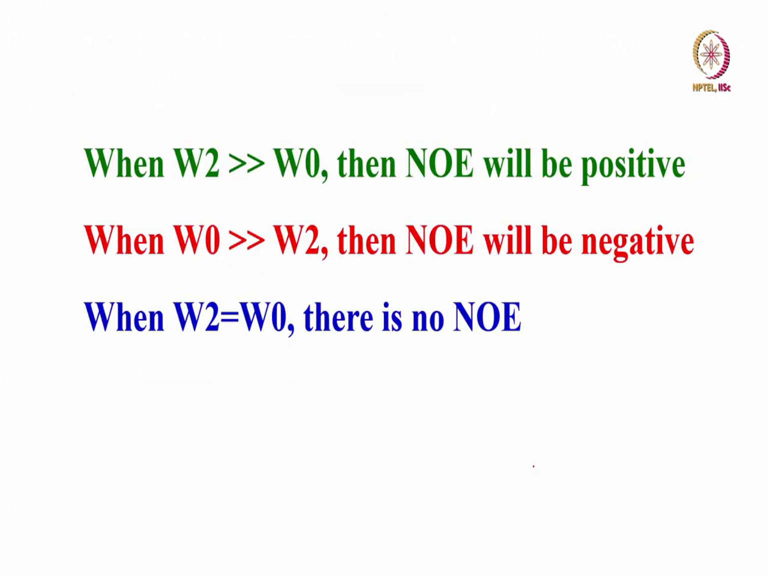

When W2 >> W0, then NOE will be positive

When W0 >> W2, then NOE will be negative

When W2=W0, there is no NOE

So, in summary, when W2 is very much greater than W0, then NOE is positive. If W0 is very much greater than W2; NOE will be negative. When W2 is approximately equal to W0; not exactly approximately, you can say, there is no NOE. This is the basic rule you must remember. What will happen when W2 is very much larger than 0, that is dominant, NOE is positive. If W0 is dominant then NOE is negative, when W2 isapproximately equal to W0 there is no NOE at all.

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Saturating the transitions for a period of time that is long relative to the relaxation times allows a new steady-state of populations to arise as a result of this competition

Eventually this is what is measured

This is steady state noe

Now saturating the transitions for a period of time. That is relatec to the relaxation times where the spin system attain new thermal equilibrium. New steady state that is attained because of steady state population. It is because of these competitive processes, eventually the change in the intensity when you measure this is what is happening. We are irradiating for a short period of time, relatively long time compared to relaxation times and the spins will attain new steady state.

And change in the population whatever it is, we are going to observe; because of competitive processes; and this is what eventually we are going to measure. So, within this the spins attain steady state because of this low power irradiation, saturating the transition for a short period of time. This is called steady state NOE. This is called steady state nuclear Overhauser effect.

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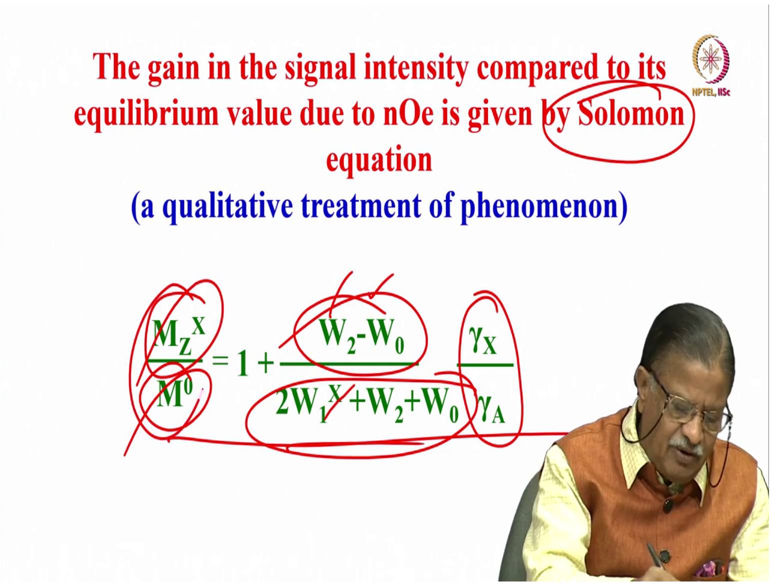

The gain in the signal intensity compared to its equilibrium value due to nOe is given by Solomon equation

(a qualitative treatment of phenomenon)

$$\frac{M_Z^X}{M^0} = 1 + \frac{W_2 - W_0}{2W_1^X + W_2 + W_0} \frac{\gamma_X}{\gamma_A}$$

The gain in the intensity you may ask me, there was a change in intensity, positive or negative. That is what we observed because of relaxation pathways. How much is the gain? How much is the enhancement in the intensity or reduction in intensity? Can we work it out? Of course, you can work it out also, detailed discussion can be given, mathematically. You can work it out, but without going into that, I will give you there is a qualitative treatment for this phenomena.

This is an equation given by Solomon called Solomon equation. What is the Solomon equation? It is this one. This is Solomon equation which says the change of the intensity with respect to the thermal magnetization. If you find out, this is given by the simple expression, W2 − W0 over 2 times W1X + W2 + W0; and it is also multiplied by gyromagnetic ratio of the spins which you are irradiating and the spins in which you are observing the change in intensity.

These two are important, gyromagnetic ratio is important; and W2, W0 and W1, all the three are important. And then, if you know this, you will know what is the change in the intensity. This is the qualitative treatment for Solomon equation. Without deriving I am giving you this and this is what is important parameter to be observed.

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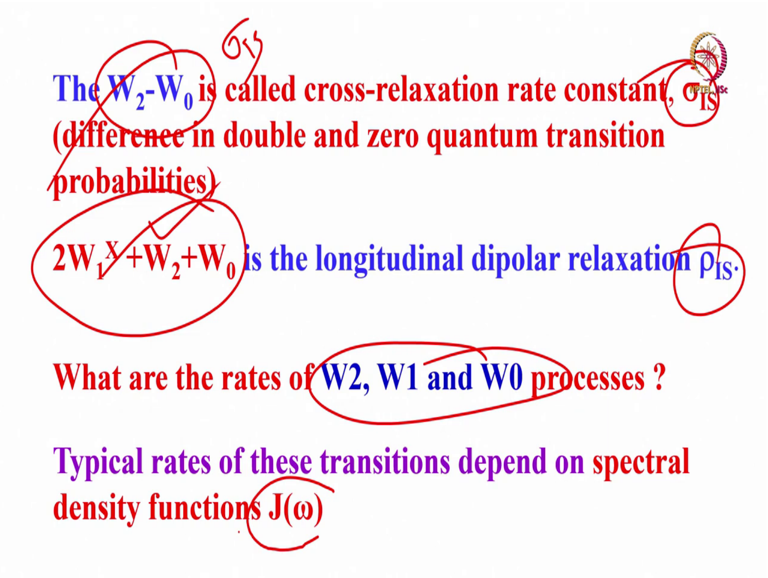

σ_{IS}

The W_2-W_0 is called cross-relaxation rate constant, σ_{IS} (difference in double and zero quantum transition probabilities)

$2W_1^X+W_2+W_0$ is the longitudinal dipolar relaxation ρ_{IS}.

What are the rates of W2, W1 and W0 processes ?

Typical rates of these transitions depend on spectral density functions J(ω)

Now in this thing which is highlighted in the numerator, you see W2 − W1. This term is called cross relaxation. It is called sigma IS, called cross relaxation. It is a difference between double quantum and zero quantum transition probability. So, W2 − W0 is called sigma IS cross relaxation between spin I and S, whereas the denominator you saw W1 X + W2 + W0. This is a longitudinal dipolar relaxation. Ehat is this one? This is not cross relaxation.

This is longitudinal dipolar relaxation rho IS, this comes in the denominator. Now, what are the rates of W2, W1 and W0 processes? We discussed this not with respect to this, in the T1 measurement and relaxation processes, we discussed the concept. We knew for dipolar interaction, dipolar relaxation there are processes which gives rise to certain frequencies which induce, which aids the spin system to give its energy to the lattice through dipole interaction.

And we found out and we discussed that they are called spectral density function, J omega. You remember that we discussed that. It is spectral density function J omega.

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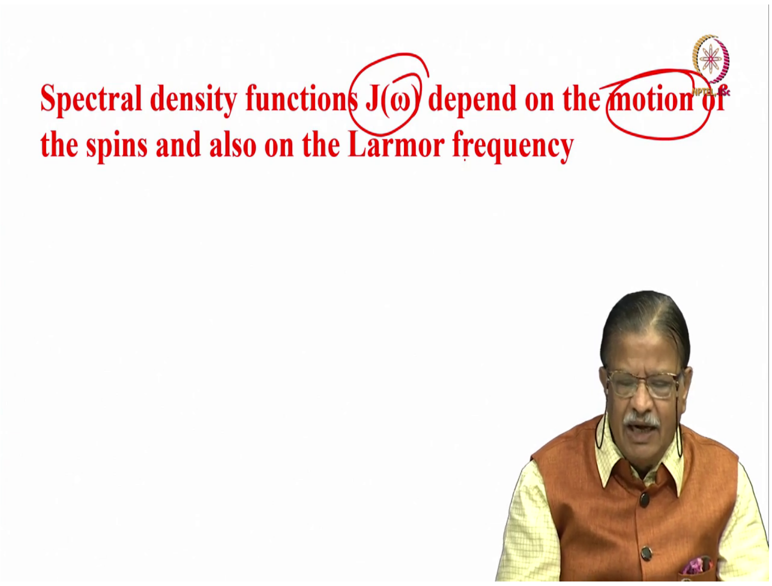

Spectral density functions J(ω) depend on the motion of the spins and also on the Larmor frequency

So, J omega; the spectral density function depends on the motion of the spins and also on the Larmor frequency. We knew this. We discussed thi;s J omega and we also found out it depends upon both molecular motions and Larmor frequency. That is in turn, we discussed about tau c and also omega 0. It depends upon both of them.

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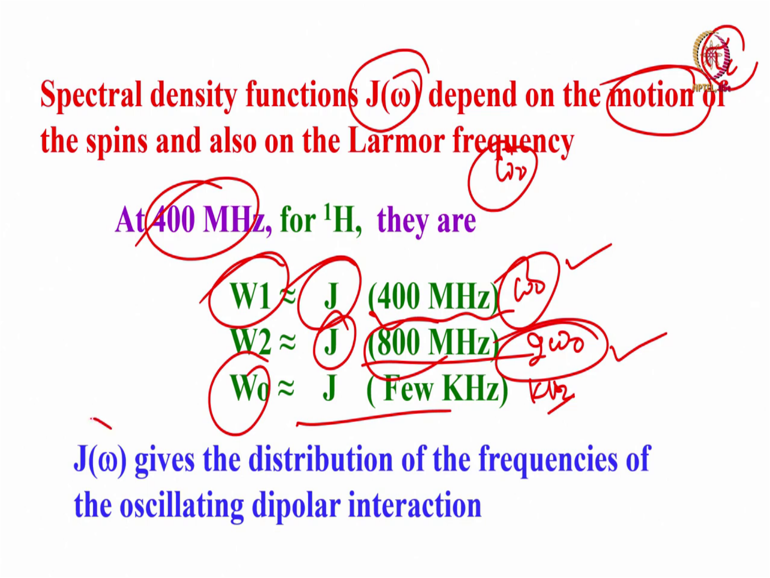

Spectral density functions J(ω) depend on the motion of the spins and also on the Larmor frequency ω_0

At 400 MHz, for ^{1}H, they are

W1 ≈ J (400 MHz) ω_0

W2 ≈ J (800 MHz) $2\omega_0$

W0 ≈ J (Few KHz) KHz

J(ω) gives the distribution of the frequencies of the oscillating dipolar interaction

So, let us consider we are recording the spectrum at 400 megahertz which is the resonating frequency for protons, then what are W1; W2 and W0s? W1 is the spectral density function is approximately equal to 400 megahertz that is what it is. It is a resonating frequency or it is nothing but the Larmor frequency, omega 0. W2; J (w) is double the normal frequency. It is 2 omega 0, whereas W0 is the difference in the chemical shift, very small difference.

So, this is of the order of few kilohertz. This is megahertz, since, it is the 2 omega, twice the Larmor frequency. If you are work working at 400 megahertz, this is 800 and this is 400. You understood, how the spectral density function depends upon the Larmor frequency. Of course further we also know it depends upon tau c. Later we will, of course discussdc, maybe if there is possibility, we can touch up on it.

So, J(w) gives distribution of the frequency of oscillations; oscillating dipolar interactions over a wide range.

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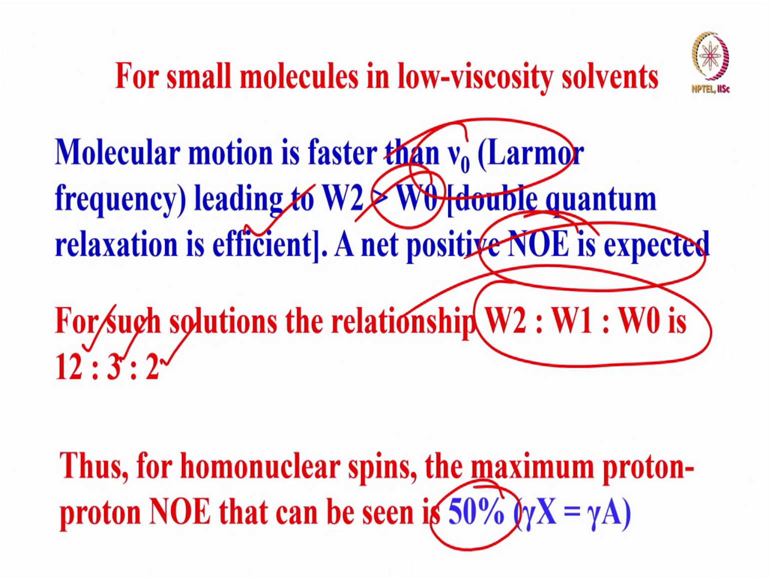

For small molecules in low-viscosity solvents

Molecular motion is faster than ν_0 (Larmor frequency) leading to W2 > W0 [double quantum relaxation is efficient]. A net positive NOE is expected

For such solutions the relationship W2 : W1 : W0 is 12 : 3 : 2

Thus, for homonuclear spins, the maximum proton-proton NOE that can be seen is 50% ($\gamma X = \gamma A$)

And for small molecules in low viscosity solvents molecular motion is faster than Larmor frequency that is, W2 is dominant than W0; double quantum relaxation pathway is the efficient relaxation pathway, so, we are going to get NOE which is positive. Remember the molecular motions are faster than rho 0. You will talk about W2 which is twice the Larmor frequency. Then you will have the case of positive NOE. Positive enhancement you are going to see.

For such solution, the relationship for W2, W1 and W0 has already been worked out. We do not need to discuss this in depth. It is nothing but 12 is to 3 is to 2; that is the relationship for small molecules undergoing molecular motions, faster than Larmor frequency. So, for homonuclear case we will consider, plug in these numbers. What is gamma A, gamma X, they are same for homonuclear case and then put into that equation find out.

You will see that, there is a 50% enhancement; maximum gain you can see for homonuclear case, if you take proton, for example, proton-proton NOE maximum gain you are going to see because of NOE is only 50%; that is what you are seeing.

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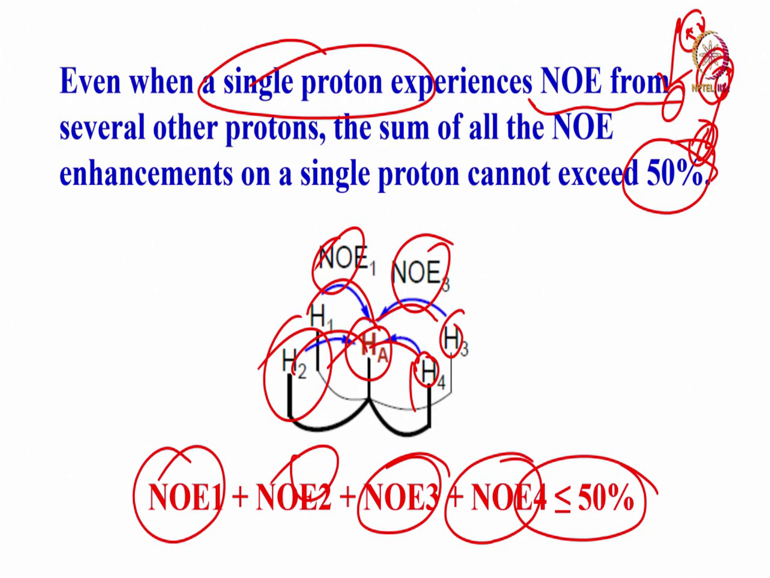

Even when a single proton experiences NOE from several other protons, the sum of all the NOE enhancements on a single proton cannot exceed 50%.

$NOE1 + NOE2 + NOE3 + NOE4 \leq 50\%$

Of course, now you may ask me a question. We are dealing with 2 spins. What happens if there are more number of spins in the space which are close by? If all of them can also give rise to NOE. For example, I have 3 spins. I am irradiating this spin. I looked at the intensity of this. This proton change in intensity I said in the homonuclear case maximum is 50%. Then what about this spin, if it also can get affected, can it not give intensity for that together? Will it not be enhanced?

Enhancement will not be much more than 50%. No, please remember even when a single proton experiences NOE from several other protons, the sum of all the NOE enhancement together for a single proton, cannot exceed more than 50%. That is the maximum enhancement you can get. Whether the proton experiences or get the NOE from one proton or 2 spin effect, 3 spin effect, no problem. The total NOE, sum from all other protons, the total NOE If you calculate it can never exceed 50%.

Take, for example, a situation like this. I am looking at the proton A; this proton 4 can give NOE to it. Proton 3 can give, a proton 1 can give, proton 2 can give, simultaneously. In which case I will call it as let us say NOE 1, 2, 3 and 4. 4 things we get. This is proton 1 gives NOE1. This is 3. This is NOE4. This is NOE2; all are possible let us say.

In this case the NOE what you are going to see at the side of the proton A is sum of NOE1, NOE2, NOE3 and NOE4. All these things together cannot exceed 50%. Please remember if a single proton experiences NOE from several other protons, the sum of the NOE enhancement all put together, cannot exceed more than 50%; that is the important point.

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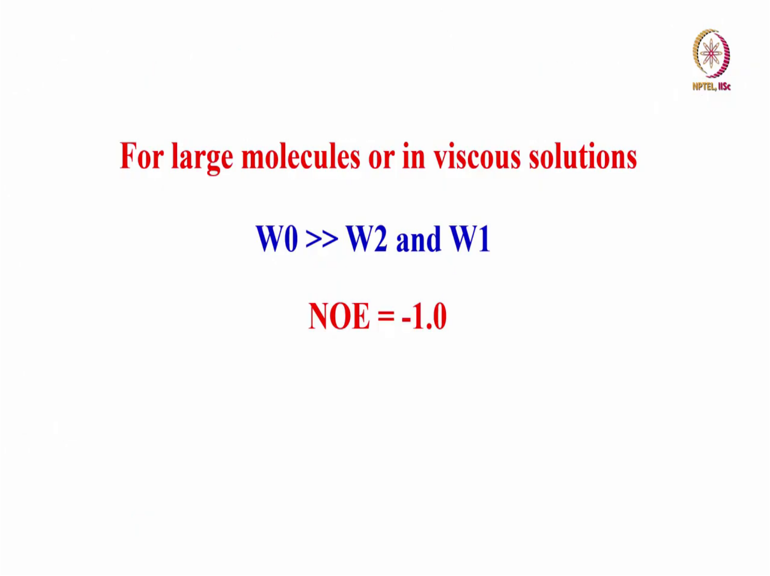

For large molecules or in viscous solutions

W0 >> W2 and W1

NOE = -1.0

Now will it be same for all the molecules, whether NOE enhancement is 50%, is for all the molecules. Again depends upon various other conditions. For large molecules or the molecules in the viscous solutions where the mobility is reduced or restricted, W0 is larger than W2 and W1. In which case NOE is – 1. When W0 is much larger than W2 and W1 NOE is −1.

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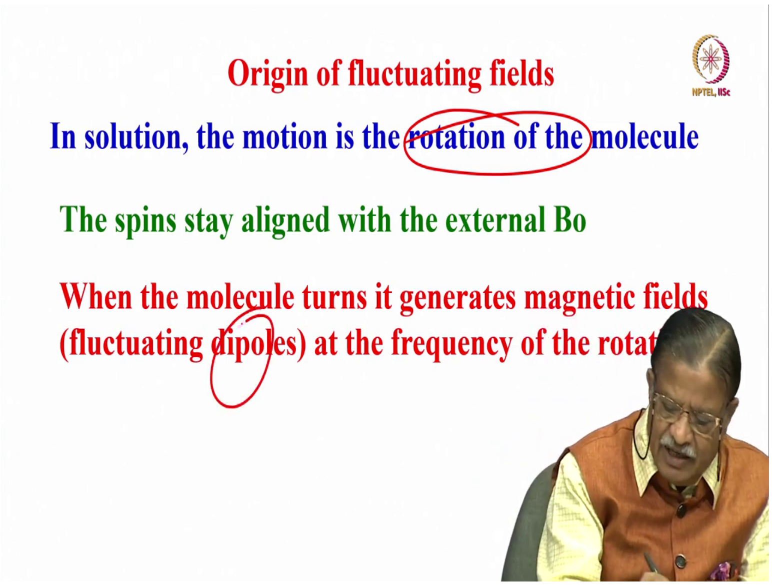

Origin of fluctuating fields

In solution, the motion is the rotation of the molecule

The spins stay aligned with the external Bo

When the molecule turns it generates magnetic fields (fluctuating dipoles) at the frequency of the rotat

So, of course, we discussed about the origin of the fluctuating fields in the context of relaxation and, of course, in the solution, the motion is the rotation of the molecule that is a dominant fluctuating field for dipolar interactions. We observe that when the 2 spins which are treated as a dipole, their orientation with respect to magnetic field do not change when they undergo motion. When they undergo motion tumbling motion, one spin keeps on rotating. Let us say it changes the dipole or field at the site of the other spin. As a concept when the fluctuating field produces frequencies at the Larmor frequency, the frequencies, at the Larmor frequency giving raise to or enabling the spins to give energy to the lattice. So, we know that the fluctuating fields generates magnetic field. This is because of the rotation or some disturbance of the molecule like this.

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These fluctuating fields are the pathways for the system to release energy

Now these fluctuating fields are the pathways for the system to release energy. We discuss this in the relaxation phenomena. So, this is needed, if there is no fluctuating fields, the spins cannot relax, it will take enormous amount of time to come to thermal equilibrium. So, there will be always natural fluctuating fields at some places. So, the spins eventually will come to thermal equilibrium by releasing energy, it could be seconds, millisecond, microsecond or hours or days.

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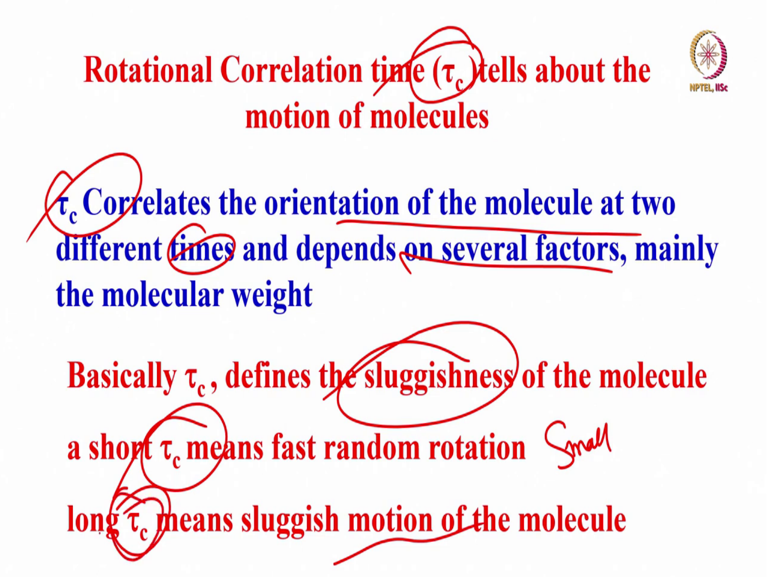

Rotational Correlation time (τ_c) tells about the motion of molecules

τ_c Correlates the orientation of the molecule at two different times and depends on several factors, mainly the molecular weight

Basically τ_c, defines the sluggishness of the molecule

a short τ_c means fast random rotation Small

long τ_c means sluggish motion of the molecule

But finally, it will achieve thermal equilibrium by releasing energy, so, thermal equilibrium has to be attained by releasing energy. So, all these things depend upon rotation correlation time we also discussed, and that tells you about the motion of the molecule. We knew tau c depends upon the size of the molecule, that is on the molecular weight, weight of the molecule.

Tau c correlates the orientation of the molecule at 2 different times and depends upon several factors. We also defined tau c. It is the motion of the molecule by one radian. You know how much the time is taken for the molecule to undergo rotation by one radian, we discussed that. So, there are several factors made responsible for this. For the tau c major contribution is from the molecular weight that is responsible, for that major responsible factor is molecular weight.

Basically, what is tau c is going to tell you. It tells you about the sluggishness of the molecule. How fast or sluggish the molecule is? For example, a short tau c, the random rotation, the small molecules undergo very fast rotation. Long tau c means molecules like this. It is a very big molecule. For example, this could be small organic molecules, small molecules which can undergo rotation fast; undergo motion faster.

This could be a very big bio molecule that is a big protein or a big nucleotide and a big polysaccharide; they undergo sluggish motions.

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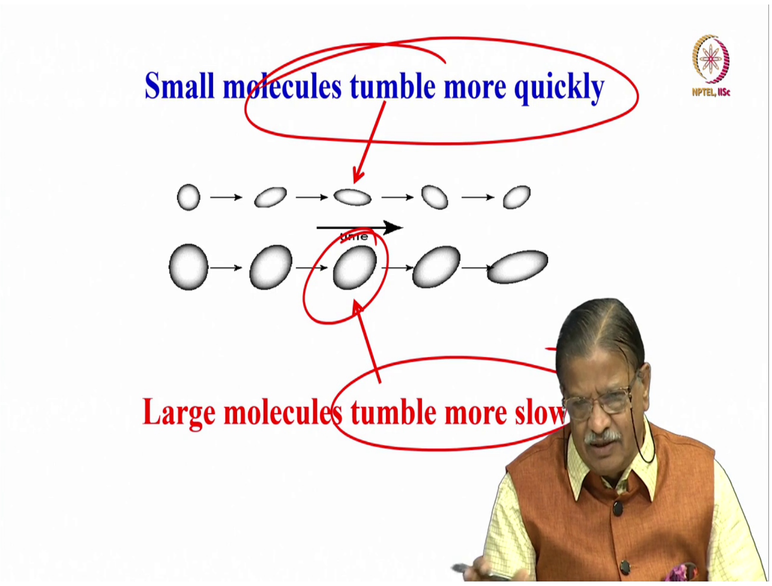

Small molecules tumble more quickly

Large molecules tumble more slow

Pictorially you can see small molecules tumble more quickly; and these are the big molecules that tumbles more slowly. So, the tau C defines the sluggishness of the molecule and how fast tau c whether it is long or short is decided by the size of the molecule.

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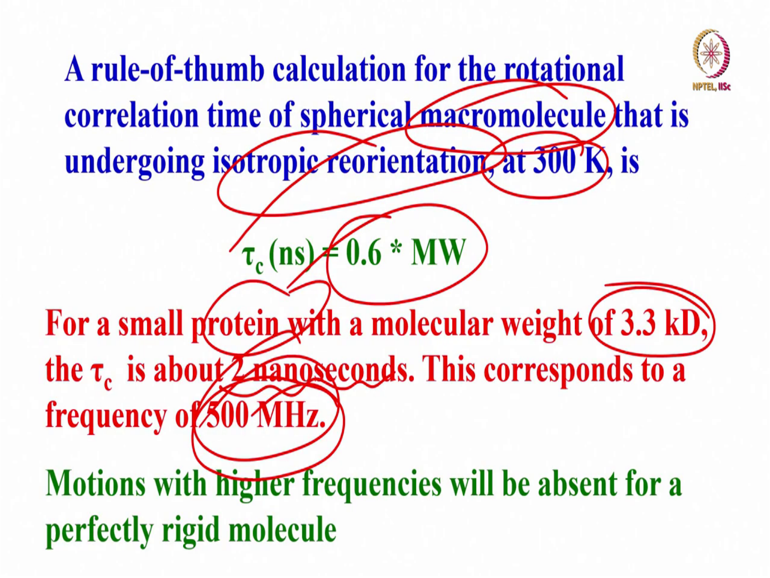

A rule-of-thumb calculation for the rotational correlation time of spherical macromolecule that is undergoing isotropic reorientation, at 300 K, is

τ_c (ns) = 0.6 * MW

For a small protein with a molecular weight of 3.3 kD, the τ_c is about 2 nanoseconds. This corresponds to a frequency of 500 MHz.

Motions with higher frequencies will be absent for a perfectly rigid molecule

So, one rule of thumb for calculation, the rotational correlation time. Approximately I mean it is not that it should always be true. For a spherical macro molecule, if I consider more or less spherical globular molecule which is undergoing isotropic, reorientation isotropic means uniformly it is changing in all directions, at say at 300 K, I am looking at the temperature also, then tau c is approximately equal to 0.60 times, 0.6 times molecular weight.

This is a rule of thumb to calculate what is the tau c. If I know the molecular weight, I know approximately what is the tau c? So, for a small molecule protein, with a molecular weight let us say 3.3 kilodalton tau c is approximately 2 nanosecond. What is 2 nanoseconds? It corresponds to 500 megahertz. You understand now how the molecular motion and tau C decides. See now with this you know what is Jw. You know what is relaxation pathway, and you know whether positive NOE or negative NOE. So, all these parameters are related to each other. So, motion with higher frequency will be absent for such a perfectly rigid molecule. I think we will discuss more about the spectral density function and few other things related to NOE. See the time is up we will come back and discuss again in the next class.

But right now in this class, we understood a lot. We discussed about how the relaxation pathways will enable spins to gain or have positive NOE or negative NOE. We saw that for a double quantum pathway, if it is dominant NOE is positive and if the 0 quantum pathway is dominant, NOE is negative

And positive NOE, we also found out this 50% enhancement will be there for homonuclear spin systems and for the large biomolecules, the negative NOE is −1; 100% reduction in the intensity. And all these W2, W0 the transition probability of the molecules, and there are relaxation pathways; they are all competitive processes. None of them are exclusive, all these things can happen simultaneously.

So, in a molecule W1 will be there, transition probability, single quantum, W0 transition probability 0 quantum and W2 transition probability double quantum. All competitive things can be going on, if W1 is dominant no NOE is seen at all. If W2 is the dominant, positive NOE. If W0 is dominant negative NOE. All these things do happen and we understood what is the rule of thumb. What is the spectral density function which is dominant? What are the fluctuating fields? How they are related to tau c? The tau c depends again on the molecule. Tau c basically defines the sluggishness of the molecule, all those things we discussed. So, we will discuss further; continuing with this NOE the next class a little bit and then we will go to a different topic. So, I am going to stop here. We will meet again in the next class. Thank you.