One and Two Dimensional NMR Spectroscopy for Chemists

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Lecture – 48
NOE - 2

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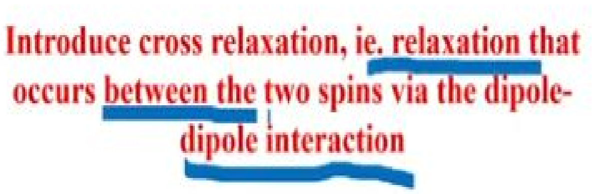

Introduce cross relaxation, ie. relaxation that occurs between the two spins via the dipole-dipole interaction

Welcome back, in the last class, I tried to introduce NOE for which I have brought in the concept of decoupling both homo and hetero nuclear decoupling, how it is done. I said we can saturate one of the spins and see the effect of it on the other spin which is coupled to. In the homonuclear case, we can reduce the multiplicity; collapse the multiplicity; or we can see the change in the intensity or reduce the coupling strength; Or sometimes you can get extra peaks, which is called as spin tickling. So variety of such things we understood; and then I wanted to take 2 spins which are uncoupled but close in space with the dipolar interaction, we worked out the energy level diagram; I briefed you what is W1, W2, W0, which are the transition probabilities for the spins. We wanted to understand because of irradiation by the second radio frequency. I said NOE occurs and then there will be change in the intensity of the observed peak, somewhere we would see the change in the peak, change in the intensity of the peak. So, to test that what we did is; we took 2 uncoupled spins, took the population difference in such a way, which obeys Boltzmann distribution; and then calculate the intensity of both the spins, and the transitions were of the same intensity for both.

Then we saturated the transitions corresponding to one spin; and again, recalculated the population difference, and we did not see any change in the intensity. And then wait for some time after we remove the RF, the system will go back to thermal equilibrium. So nothing much interesting has happened in spite of irradiating by the second radio frequency pulse. So, I said there must be some other mechanism, otherwise how do we get the NOE?

For that we require what is called dipole-dipole interactions, so we are going to discuss today about dipole-dipole interaction and teh relaxation mechanism through double quantum and zero quantum transition pathways.

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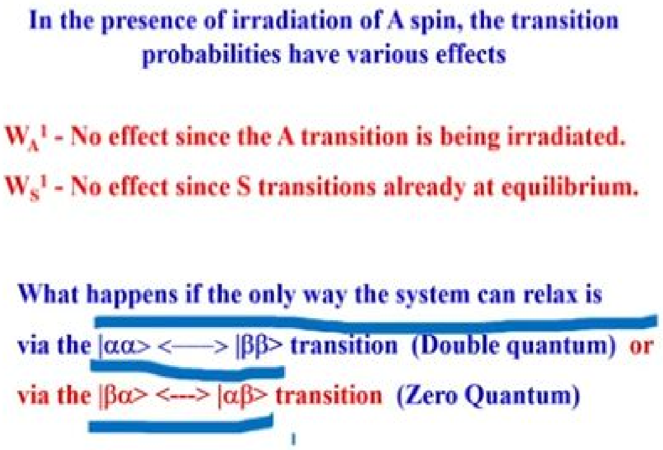

In the presence of irradiation of A spin, the transition probabilities have various effects

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

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

What happens if the only way the system can relax is via the |αα> <——> |ββ> transition (Double quantum) or via the |βα> <---> |αβ> transition (Zero Quantum)

In the presence of irradiation of A spin, the transition probabilities have various effects; one W_A^1 and W_S^1. There is no effect, since the A is irradiated and there was no effect, and S is already in equilibrium, in the presence of irradiation. Now, let us do one thing; since this did not work out for us, why not we think of the way a spin system can relax in a different pathway.

See, after the spin system attain thermal equilibrium, the relaxation pathway chosen was single quantum. See we remember we had the energy levels; we had a different energy levels, we saturated one of them and then when we saturated this, we saw this one and this one, whatever the things which were allowed, there was no change in the intensity; because these are all single quantum transitions you are looking at.

But on the other hand now, let us see what happens, if the only possible way for this spins to relax is either this or this. Of course, this also possible, they are competitive processes; I am not excluding any of them. But let us assume for the time being, the only possible way for this spins to come back to thermal equilibrium is this way, double quantum or this way, it is single quantum, you understand.

Double quantum is alpha, alpha to beta, beta, zero quantum is alpha beta to beta alpha, these were forbidden transition as far as the detection is concerned; but I said in the context of relaxation they are allowed. Now, let us see if we follow that path how does it work.

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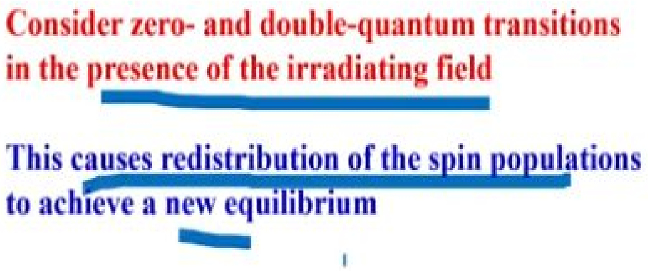

Consider zero- and double-quantum transitions in the presence of the irradiating field

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

Now, consider the zero and double quantum transitions in the presence of irradiating field, see how the spins get redistributed into the new equilibrium.

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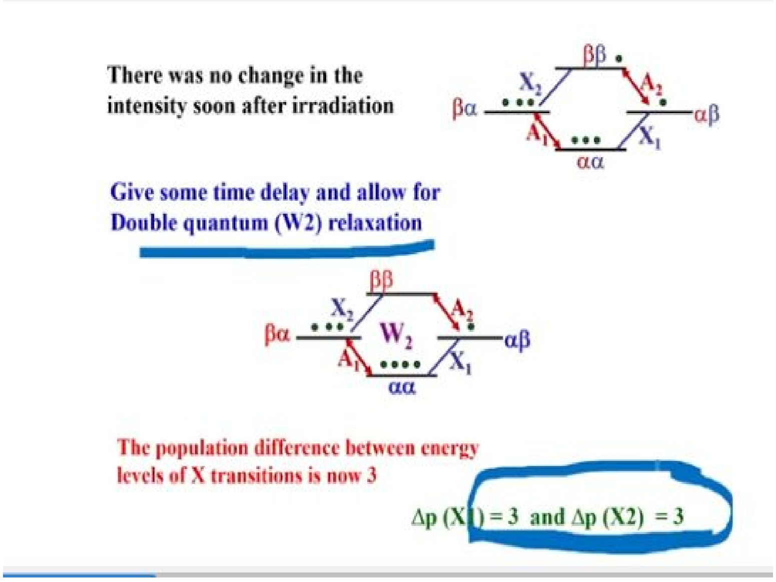

There was no change in the intensity soon after irradiation

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

The population difference between energy levels of X transitions is now 3

Δp (X1) = 3 and Δp (X2) = 3

Now, we have saturated spins, peaks correspond to A1 and A2; the A spin is saturated, Let us start from that point; and after some time when we calculated the intensity A0, this A0 and this was 2, this was 2; there was no effect; that is what is our conclusion. Now, let us do one thing, we will wait for some time after irradiation, allow the spins to relax through double quantum pathway, where W2 is probable. We were thinking it will go back like this and then attain thermal equilibrium. We were always thinking that W1 was the possibility. Let us think of W2 possibility, let us say the nuclear spins comes down like this, it will relax like this and this is the situation. Now what is going to happen let us see, calculate the population difference between energy levels of X, X transition here is 4 – 1; it is 3 and this transition 3 - 0 is 3, what did we did do? We did not do anything; we have saturated the spins. And then after that one spin was here, after saturation of both A transition; but we allowed this not go through the single quantum pathway, we allowed it to directly jump here, to come through double quantum pathway; that is a relaxation mechanism I am allowing it. Now, when I recalculated the population difference and intensity, earlier it was 2, 2, now it became 3, 3. I did not do anything, no magic just in the earlier calculation the population difference was calculated using single quantum pathway, like this. Now when I allow it to come through double quantum pathway, if you recalculate intensities became 3, 3. So, what is happening now; just because we irradiated A spins, X spin has gone up by intensity by 50% it was 2, 2, now it became 3, 50% gain in the intensity of the peaks, you agree.

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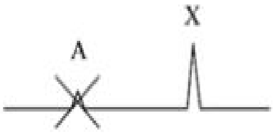
50 % gain in intensity (positive nOe) !!



A case of positive NOE

Now, the 50% gain is called positive NOE okay.

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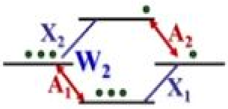
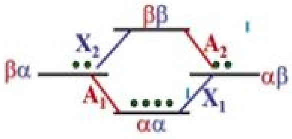
continue low power irradiation →



After the time constant T_1, the system attain new equilibrium



Now, we will do one more thing, keep on continuing for low power irradiation and leave it for some time. After sometime what will happen? it will come back to thermal equilibrium, this is what the situation before applying RF power, so it has to come to thermal equilibrium. You cannot keep on applying RF for infinite time, after sometime you remove RF power, slowly system will come back to equilibrium and this is the population distribution; Boltzmann distribution at equilibrium.

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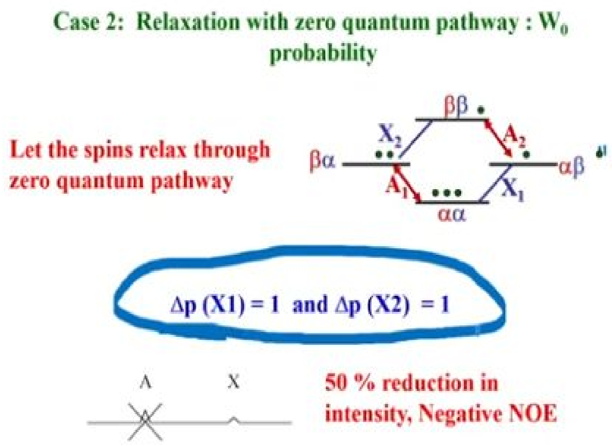

Case 2: Relaxation with zero quantum pathway : W_0 probability

Let the spins relax through zero quantum pathway

Δp (X1) = 1 and Δp (X2) = 1

50 % reduction in intensity, Negative NOE

Now, we will do another possibility; again we consider a situation, spins A; both the possible transitions of A are again saturated, you irradiated and saturated these with low power RF. Now, instead of allowing the spin to come from here to here, that is double quantum relaxation path way, why not allow this to go to zero quantum pathway. That is also allowed as far as the relaxation is concerned; let us see what happens; this spin goes like this.

Now, find out the intensity pattern for X. Now when this is saturated, when these became equal, the zero quantum relaxation pathway occurred and the spin which were saturated here, X spin came through zero quantum pathway and came here, what is happening now? Calculate the intensity for X; here it is 3 - 2 it is 1, 2 – 1 it is 1. The transitions for X, earlier it was 3, 3. If we allow the spins to go through zero quantum, it became 1, 1 now. Let me summarize for you, when the spins were undergoing relaxation through normal pathway, there was no change in the intensity, all were equal, 2, 2, right. Now, when after saturation if I allow the spins to relax to the ground state, alpha, alpha, through 2 double quantum pathway, then the intensities of X transition went up by 50%, 3; from 2 it went to 3. And, on the other hand, if I consider this relaxation pathway, what is happening? the intensity came down to 50%, this is called negative NOE, understand, this is called negative NOE.

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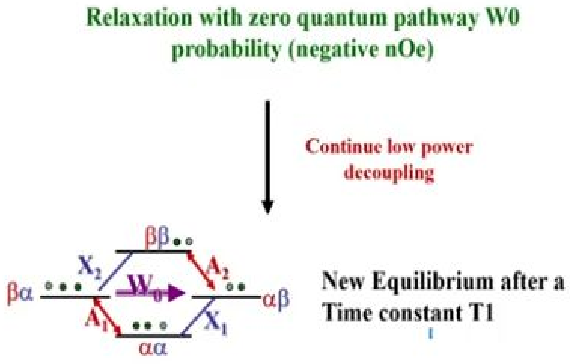

Relaxation with zero quantum pathway W0 probability (negative nOe)

Continue low power decoupling

New Equilibrium after a Time constant T1

So, the relaxation with zero quantum pathway possibility gives a negative NOE, relaxation with double quantum pathway gives positive NOE. Of course after sometime, continue low power decoupling and then after sometime nothing will happen, the system will go to thermal equilibrium.

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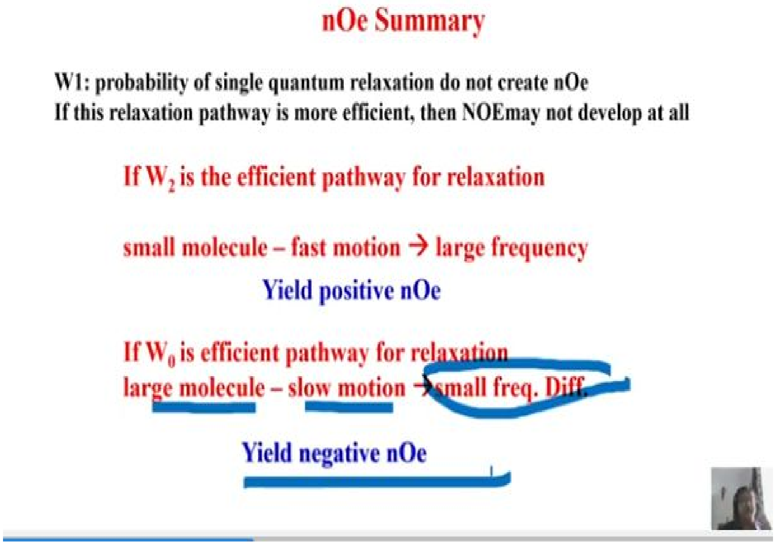

nOe Summary

W1: probability of single quantum relaxation do not create nOe
If this relaxation pathway is more efficient, then NOEmay not develop at all

If W_2 is the efficient pathway for relaxation

small molecule – fast motion → large frequency
Yield positive nOe

If W_0 is efficient pathway for relaxation
large molecule – slow motion → small freq. Diff.

Yield negative nOe

So, what is the final summary of NOE, if there is a W1 probability, single quantum that will not create NOE at all. Suppose in a molecule, now this is the most efficient relaxation pathway, this one, your experiment whatever you do, you will not get NOE; because this relaxation pathway is dominant is W1; and that is in your hands to control, you cannot control. And naturally, if it is that one, you may not get NOE at all.

On the other hand, if W2 is efficient pathway of the spins, then we will get positive NOE. This can happen only for small molecules which is undergoing fast motion and motional frequency is quite large; okay. It should be at the double the Larmor frequency, so motion frequency is quite large, large frequency. If W0 is efficient; that is the relaxation pathway generally, it can happen for large molecules, which undergoes slow motions, small frequency difference; that gives negative NOE.

Remember, 3 cases; with the relaxation is through W1, no NOE; the efficient relaxation pathway is double quantum W2 then positive NOE; the relaxation is efficient through zero quantum pathway negative NOE, this is what happens.

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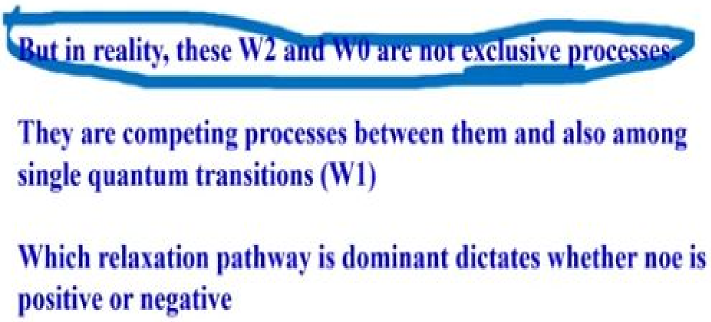

But in reality, these W2 and W0 are not exclusive processes.

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

Which relaxation pathway is dominant dictates whether noe is positive or negative

But in reality what happens; none of them are exclusive processes, they are not exclusive, They are all competing processes, you cannot dictate the nuclear spins to do what you want. In reality everything is going on, it can be W1, W2, W0; all relaxation pathways are allowed. Now, observation of the NOE, detection of NOE in your sample depends upon which is the dominant relaxation pathway, okay.

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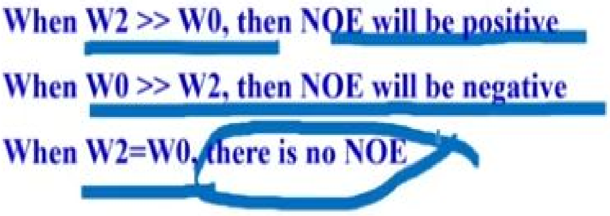

When W2 >> W0, then NOE will be positive

When W0 >> W2, then NOE will be negative

When W2=W0, there is no NOE

If W2 is the dominant relaxation pathway; much, much greater than W0, then NOE will be positive. If W0 is greater than W2, then NOE will be negative, when W2 is equal to W0, no NOE at all. Very difficult situations you come across; right. So this is the important concept, you should know basics of NOE, relaxation pathway of this irradiated spins, how they relax, defines whether it is a positive NOE or negative NOE, okay.

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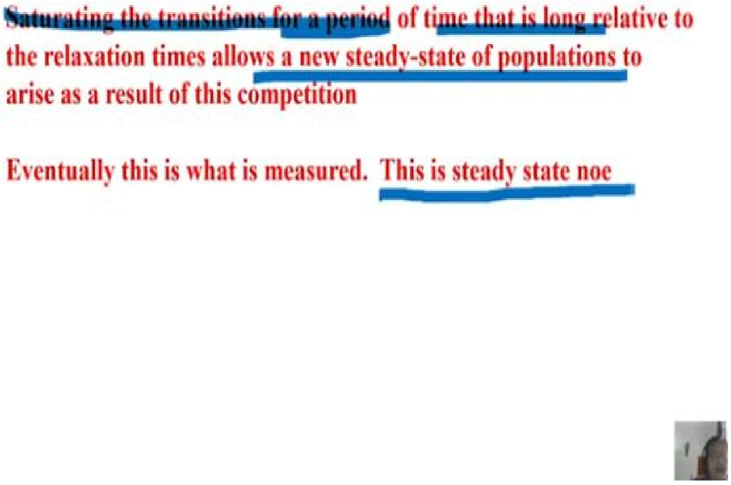

Saturating the transitions for a period of time that is long relative to the relaxation times allows a new steady-state of populations to arise as a result of this competition

Eventually this is what is measured. This is steady state noe

And this is generally, what happens is we are saturating the transition for a period of a time and allow the system to attain steady state; because of various competition mechanisms; that is why this experiment is called steady state NOE. Saturate the transition for a period of time; it should be longer than its T1 time, it cannot be a faster then allow the spins to go through the competitive processes, and then eventually after sometime you will measure the NOE. This is called steady state NOE.

As I said we have transient NOE, driven NOE, various things, rotating frame NOE called ROESY. We do not have time to discuss all those things; but I just tell you this is enough to understand steady state NOE for you, okay.

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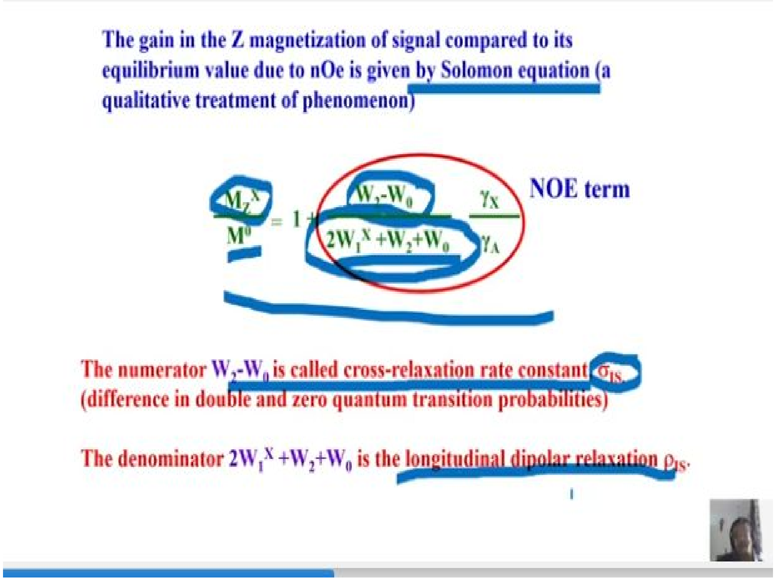

The gain in the Z magnetization of signal compared to its equilibrium value due to nOe is given by Solomon equation (a qualitative treatment of phenomenon)

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

NOE term

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

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

This Z magnetization, the gain in the signal intensity is given by Solomon equation. It is a qualitative treatment of equation, Solomon has done that and he has given an equation like this. So if we look at the spin, MzX, is the gain in the X nuclei with respect to the equilibrium; can be given by a simple equation; and this is NOE term; and depends upon W2 – W0 and all the 3 relaxation pathways.

It is an equation which has been worked out by Solomon. The numerator W2 – W0 is called cross relaxation time; or cross relaxation constant and this is called σIS, denoted as σIS. You do not worry, this is the difference between double quantum and zero quantum pathway. The denominator is there you know, here; this is sum of all the 3; which is called longitudinal dipolar relaxation, okay; this is cross relaxation, this is dipolar relaxation, okay.

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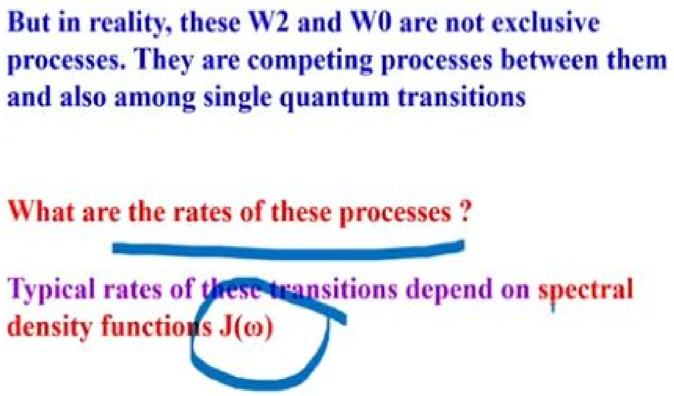

But in reality, these W2 and W0 are not exclusive processes. They are competing processes between them and also among single quantum transitions

What are the rates of these processes ?

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

Now, when I say all the things are going on competitively, how do you know what are the rate of these process? How do you find out what are the possible processes that causes these types of transitions, what is the transition probability, how do you know which is going on fast; W2, W1, W0; which is dominant? what difference makes these processes, this is defined by what is called spectral density function.

What is the spectral density function? it is the function which is given by Jw. Let us say motional fluctuations are there, lot of fluctuating magnetic field will develop because of the various possible motions in the molecule at the state of the nuclear spins. This is the time domain perturbation, it perturbs with time, it is undergoing changes with time. It is a signal you can collect in time and do the Fourier transformation, what you will get? You will get frequencies.

So, this is a type of fluctuating magnetic fields, it has certain definite frequencies, you can do the Fourier transformation of time domain fluctuating signal and you will get the frequency and that is called spectral density function.

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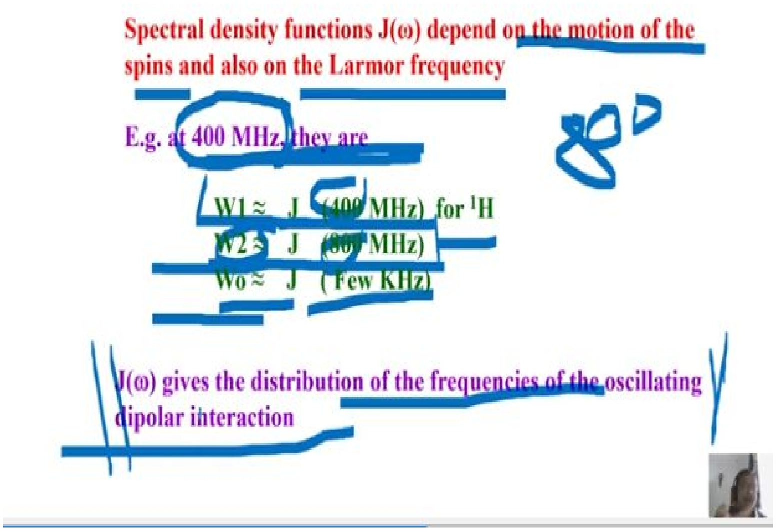

Spectral density functions J(ω) depend on the motion of the spins and also on the Larmor frequency

E.g. at 400 MHz, they are

W1 ≈ J (400 MHz) for ^{1}H
W2 ≈ J (800 MHz)
Wo ≈ J (Few KHz)

800

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

So, without going more into the details, I will tell you what is the spectral density function depends upon. It depends upon the motion of the spins and on the Larmor frequency. It depends upon 2 things; first it depends upon the motion. Of course, I was telling previously also; which is a dominant relaxation mechanism; and it depends upon whether it is small molecule or big molecule, I told you know.

In the case of small molecule, I showed which is dominant; for big molecule I have told you W0 is dominant; fine. Now let us take the example of a 400 megahertz spectrum, record the spectrum in a 400 megahertz spectrometer and calculate the molecular fluctuating signal generated and do the Fourier transformation. Take the time domain function of that and calculate the spectral density function, it is 400 megahertz it is W1. W1 will be 400 megahertz.

And W2 is always double the Larmor frequency I told you, it requires 2 quantum of energy, At 400 MHz, the single quantum of energy is 400 megahertz; the double quantum energy is 800 megahertz. What is W0; it is difference in the chemical shifts, and is of the order of few kilohertz, so at dominant mechanism, at the particular spectrometer frequency it depends upon various things. First, Larmor frequency and motion of the spins. Suppose the same molecule you take it to 800 megahertz, W1 is 800 MHz and W2 is 1600 megahertz, your fluctuating field may not have the large frequency. Then this is not a dominant mechanism, you understand. So on the other hand, you go to, let us say, from 800 go to 100 megahertz; W1 is 100 and W2 is 200, it is quite lightly, the fluctuating mechanisms of the molecular motion is such that you may have 200 megahertz, which may be easily achievable. So it depends upon the Larmor frequency, which depends upon the magnetic field, okay.

So, W2, W1 and W0 are proportional to the Larmor frequency, whatever the spectrometer frequency you are considering, W1 corresponds to that Larmor frequency, W2 is twice a Larmor frequency, W0 equal to difference of the chemical shifts you have to take, okay. And this is the distribution of frequencies of the oscillating dipolar interaction. This is all oscillating dipolar interactions because of its motions generate fluctuating fields, and that have all these frequencies. Is it clear, what is spectral density function? It is nothing but frequency distribution obtained because of the oscillating dipolar interactions. The dipolar interactions are always undergoing oscillations, know, they wont be static.

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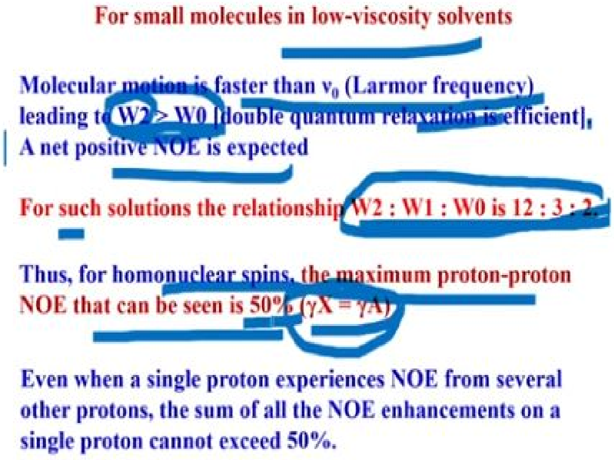

For small molecules in low-viscosity solvents

Molecular motion is faster than v_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% ($\gamma X = \gamma A$)

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%.

And then the time domain function , we do the Fourier transformation that is what you get for frequency. Wo for small molecule in low viscosity solvents, molecular motion is faster than Larmor frequency; leading or dominating is W2. For small molecules, the motional frequency is such that it is always faster than Larmor frequency. In 200 megahertz if you take molecular motional frequency will be 400 megahertz frequency.

So, what is dominant mechanism; W2. So double quantum relaxation is efficient, you get positive NOE. Now if I take bigger molecules; very, very big molecules, then motion may not be very fast; then other one is dominating, so depending upon the field and the oscillating frequency. you know which is dominant whether you get positive NOE or negative NOE you can decide.

Now, for all these things, there is simple relation like this; W2 : W1 : W0 is given by 12 : 3: 2. So depending upon which is dominating, which is this thing, you can put these values in the Solomon equations, you will come to know what is the gain in the signal intensity because of NOE, okay. So, for homonuclear spins, maximum gain is 50% that is the equation I have to show you that; where is that equation? I did not give you that equation, okay, does not matter, I will come to it later okay, so there is a Solomon equation; I think I showed Solomon equation know, the ratio of γX2 to γI, is there; that will define; Along with these factors, there was W2 – W0 which was at the numerator, I said that it is a cross relaxation term, I told you know. So, based on that which term is dominating, because the ratio is given like this for the small molecules, you can find out what is the maximum NOE we are going to get. When both are homo nuclear spins, when gamma's are both are equal, then you get maximum NOE, that is 50 percent.

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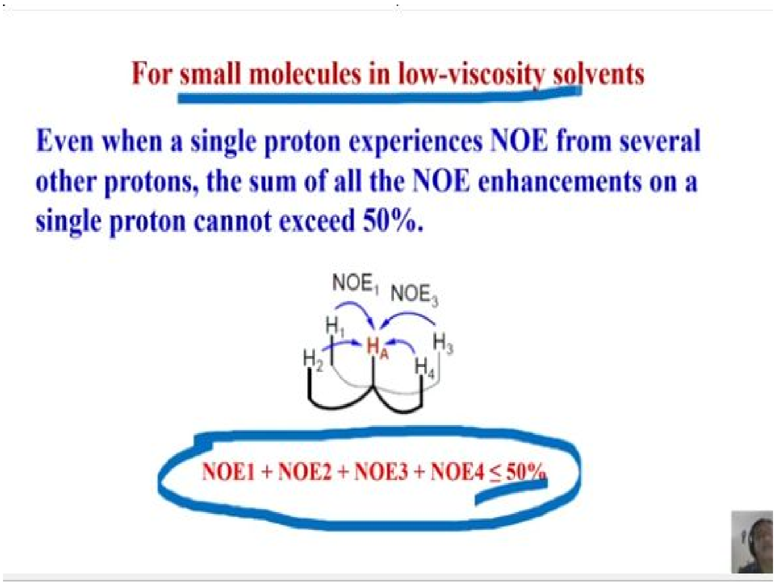

For small molecules in low-viscosity solvents

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 ≤ 50%

This is not just for one spin; In a given molecule for example, one spin can experience NOE with varieties of other protons which are close in space, does not matter, this can have NOE with this, NOE with this, NOE with this. Now each of them cannot give 0.5, 0.5, 0.5 and 05; add up to 2. No, not that we will get 200% enhancement in the intensity. Together each individual NOE if we calculate, maximum NOE cannot be more than 50%. It should be less than or equal to 50%; it cannot exceed 50%.

This is for small molecules in low viscosity solvents, why I say low viscosity; if the viscosity is more the motional frequency is less, it cannot rotate freely you know, tumbling motion frequency is much less.

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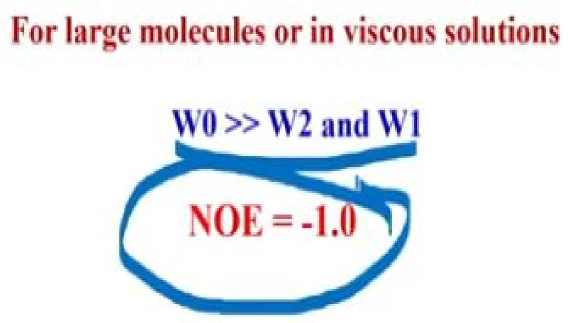

For large molecules or in viscous solutions

W0 >> W2 and W1

NOE = -1.0

So, for large molecules or in a viscos solvent, this is dominant, and NOE is -1. Remember for large molecule NOE is -1, for small molecule NOE is + 0.5, so one case small molecule positive NOE, large molecule negative NOE, maximum enhancement for small molecule positive NOE is 50% and for the large molecules is -1, -100%, okay.

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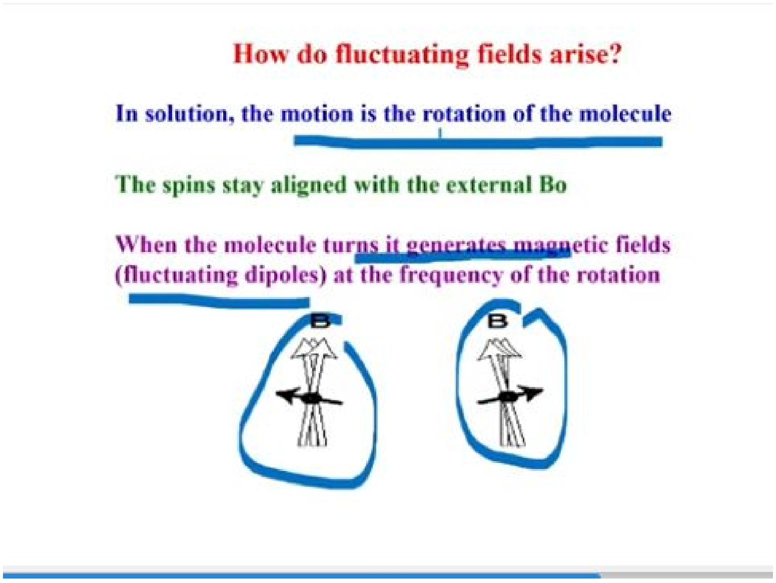

How do fluctuating fields arise?

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 rotation

Now, this is I wanted to show you. How do the fluctuating fields arise? The spins, let us say in solution, motion is rotation of the molecule, the spins are always aligned with respect to the magnetic field. But the spins I would say the molecules undergoes turns, it will not just staying idle, you know, it is undergoing motions. It rotates and then turns and then as a consequence what happen; this small microscopic dipoles which are present gives fluctuating magnetic fields; the fluctuating dipoles gives magnetic fields.

And that has a frequency; okay; that rotational frequency defines this spectral density.

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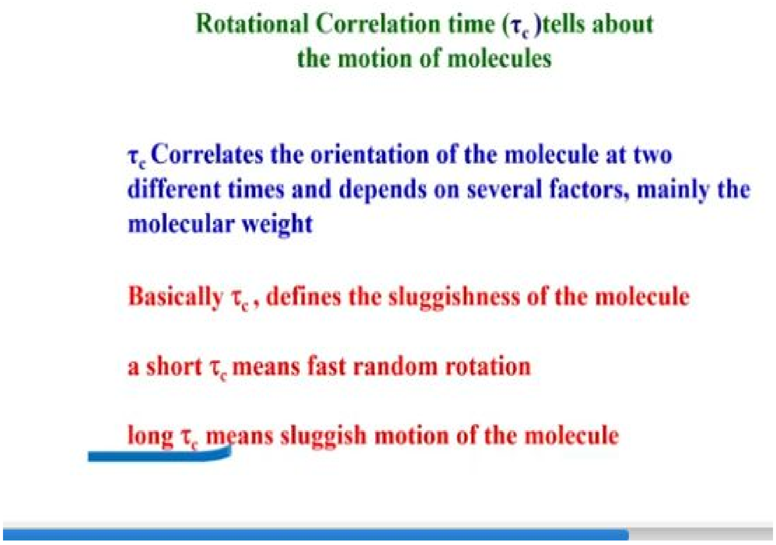

Rotational Correlation time (τ_c)tells about the motion of molecules

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

long τ_c means sluggish motion of the molecule

Now, we have a rotational correlation time, another thing, which tells about the motion of the molecules. Whether the molecules are undergoing fast motion or slow motion, how do you find out? The rotational correlational time is another term which tells you about the molecular rotation. The τc is the time required for the molecule to undergo rotation by 1 radian; okay.

If a molecule undergo rotation by 1 radian, how much time it requires is defined as called correlation time. The definition of correlation time is the time required for the molecules to undergo rotation by 1 radian; and that what it is. so the τc correlates the orientation of the molecule at 2 different times; and depends upon several factors, the τc depends upon several factors.

One is molecular weight, mainly molecular weight, sluggish molecule cannot rotate faster, you see you take a small ball you can rotate very fast, you cannot rotate an elephant faster, right! When the molecule become bigger and bigger, it will become more and more sluggish, it cannot rotate faster. So, size of the molecule also matters; okay, so the rotation correlation times which depends upon the motion of the molecule, depends upon molecular size, molecular weight, okay.

Basically, τc tells you about the sluggishness of the molecule, shorter the τc means fast random motion; τc is longer means, molecule is very big and sluggish, very lazy. Very sluggish molecule, in which case τc is very long, it takes more time to undergo one rotation.

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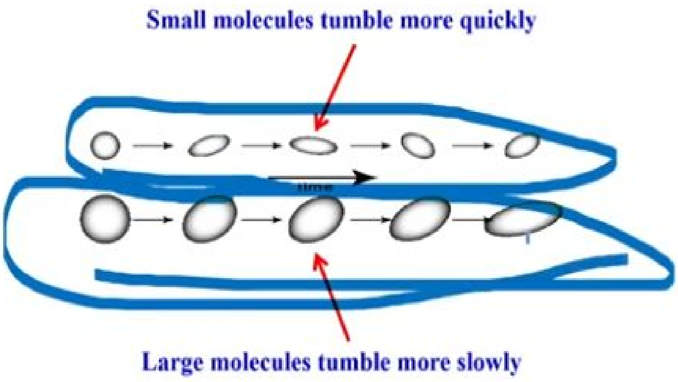

Small molecules tumble more quickly

Large molecules tumble more slowly

And this is the simple example to show, these are the small molecules which can undergo rapid tumbling, these are the large molecules, sluggish molecules; tumble very slowly.

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So, from the rule of thumb we can calculate the tau, and see what is the correlational time. It is always expressed in nanoseconds, it is approximately for a spherical macro molecule like a big protein, it is of the order of up to 25 kilo Dalton, then this is a general thumb rule. The general thumb rule is τc is 0.6 times the molecular weight. So, for a small protein, let us say of 3 to 4 kilo Dalton,τc is about 2 nanoseconds.

So, for this 2 nanoseconds τc, calculate the frequency, it turns out to be 500 megahertz; that is the Larmor frequency of 500 megahertz spectrometer; okay, so motions with higher frequency will be absent for a perfectly rigid molecule; for a perfectly rigid molecule that is absent.

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So, the spectral density at the twice the Larmor frequency is small for proteins; that is what I said. So, that is the reason why we understood what is W2, W0 which is dominant, etc. okay. So the zero frequency component of the spectral density function increases with increase in the molecular weight, since the rotational frequency become lower and lower, this I will show you by drawing a figure.

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Look at this one, the total amount of oscillating field is a constant, whether the area of this, area of this, area of this; whatever you take, is a constant but upper limit of the frequency vary with the correlation time. The frequency varies with the correlation time. So, one can draw a graph of this thing as a function of ωτc. Omega is the Larmor frequency, τc is the correlation time, multiply, this by is the factor.

If it is very much larger than 1, this spectral density function follows this path; and in this region it relaxes very fast. If ωτc is approximately equal to 1, this is the Larmor frequency, then you will not able to give get NOE at all. If ωτc is very much smaller than 1, the spread in the frequency is quite large here, okay, this is very, very large here.

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So, the simple logic is if ωτc is smaller than 1, molecule tumbles fast and you get positive NOE, this is called extreme narrowing condition. If ωτc is larger than 1, molecule tumbles very slowly; and give negative enhancement, this is called a diffusion limit. Please understand this point very clearly. When ωτc is approximately equal to 1, correct value is 1.106, I think. In this limit, NOE goes to 0. so ωτc; the Larmor frequency x correlation time of the molecules, defines or it tells you whether your NOE is positive, negative, or 0. Remember when I started this class, I told you that there are several factors on which the NOE is dependent. It depends upon the correlation time, that is size of the molecule, motion of the molecule, magnetic field which defines the Larmor frequency; and the relaxation pathways. The NOE depends upon various mechanisms; I explained to you.

This is what I wanted to tell you. In NOE we discussed about the relaxation pathway, we know W2 and W0, which is dominating, and gives positive or negative, we understood. And now we understood about the correlation time; and based on the correlation time and Larmor frequency, we know what is the spectral density function. The spectral density function is

Jw, what is that; based on that W0, W1 and W2, we know which is dominating; that depends upon again molecular motions, τc. So, now taking τc and W, both into account, we know whether molecule give positive NOE, negative NOE, or 0.

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And this is dependence of NOE on the factor, $\omega \tau c$. See in this case this is the measured NOE, this is taken form this journal, read this paper. See for small molecules, see this is becoming like this, going like this and here it becomes larger. This side is for a larger molecule, this side is for small molecule; exactly at this place, the cross over point, NOE is 0 at this place, where $\omega \tau c$ is close to 1.106. at this point, NOE is 0. $\omega \tau c$ is 1.106 here and when that is the situation, no NOE and in this case, small molecule it is 0.5 intensity, positive intensity, negative intensity of 100% here. To overcome this zero NOE problem, different type of NOE experiment is there, called rotating frame overhauser effect, ROE. I do not think, I have time to discuss that maybe at the end of class, if there is time, at the end of the course, if the time is left time for me, I will take some special topics like this, which I have not covered.

So, it may not be sequential, but I pick up specific points and then try to give you the idea but at the moment, we will not worry; but please remember one thing, ROE overcomes the problem of zero intensity case, okay.

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And the NOE depends upon the distance also I told you, the strength of dipolar interaction depends on rIS and we need 2 dipoles to have in dipolar coupling. As a consequence NOE depends upon inverse of $(rij)^3$ x $(rij)^3$, it is rij to the power of 6; so the intensity of NOE depends upon inverse 6th power of the distance; remember inverse 6th power of the distance.

So, for small variation in the distance, if you slightly move away from 1 angstrom, 2 angstrom, NOE drastically comes down. NOE is depends upon 2 spins; and W's will depend upon this; as a consequence NOE is distant dependent.

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So, in the case of homo and hetero nuclear spins we can see what the gain is. In the homo nuclear case, gamma A is equal to gamma X like this, so maximum NOE is 50 percent. But in the heteronuclear case, gamma A is different from gamma X, take for example carbon, this is proton, this is carbon, then you can see the intensity is different. NOE is important especially for low gamma nuclei, where gamma matters a lot, there can be maximum enhancement of signal.

This is precisely what we saw in carbon 13 NMR, where we did the decoupling with NOE, we got more signal signal to noise ratio. When we did the broadband decoupling, you remember, in carbon 13 I told you, it had a NOE factor built in; that is why in the hetero nuclear case especially the one which has low gamma, this NOE factor is very important to get a better signal to noise ratio.

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So, we can calculate let us say, this is the saturated spin and this is the observed spin; for intensity change now put it into this is the equation, please note down this equation, it is very important. I0 is original intensity. What is the gain in the intensity as a factor of η, which depends upon gamma, you should put it into that, then for the case of proton, when proton is decoupled, carbon 13 is observed; gamma A divided by 2 times the gamma X, calculate it is 4 divided by 2; the 200% enhancement, in the case of carbon if I decouple proton and observed carbon. That is why we got better signal to noise ratio when we decoupled proton and observed carbon

On the other hand, I can decouple carbon and observe proton that is also possible. In which case gamma will change, in numerator and denominator gamma is interchanged. So now you put those gamma values, it turns out to be 12.5, enhancement is not much, it is only 12.5. Because you are decoupling carbon 13, low gamma nuclei and observing proton, high gamma nuclei. So the advantage is you have to decouple high gamma spin and look at the low gamma nuclei, that is a better enhancement of the signal to noise; that is what happens.

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And this is how the steady state NOE is done. First what you do for a steady state NOE is; I think, now if I start explaining this, it will take more time; what I am going to do this, I think I have covered lot about NOE in the 2 class, in the next class I will just tell you what is steady state NOE, how we measure it experimentally and then I will change over to a different topic.