Advanced NMR Techniques in Solution and Solid-State

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Module-39
NOE and correlation time
Lecture – 39

Welcome back all of you, we have been discussing NOE, since last 2 classes. Of course, we discussed very fundamental concepts of what is an NOE? We knew NOE is the change in the intensity of the one nuclear spin, when other nuclear spin is irradiated. The basic requirement for this is the two nuclear spins should be close in space; and it depends upon the distance. It goes by inversely as r power 6.

As the distance increases slightly, there is a drastic change in the intensity. So, the NOE phenomena, you can think of happens only when one spin is irradiated. That is like, for example, while doing the decoupling, the change in the intensity could be positive or negative; this phenomena called positive NOE or negative NOE.

The dominant mechanism for this NOE is the dipolar relaxation pathway. And then we also saw that dipole interaction is the dominant thing. And then we understood basically, how cross relaxation pathway; cross relaxation phenomena aids in getting positive NOE and negative NOE. We consider two spins which are close in space, we irradiated one of these spins with low power rf for a short time and then the spins attain new thermal equilibrium. Immediately after that we measured the population difference. By irradiating we found, there is no change in intensity when the spins follow single quantum relaxation pathway; w1 is the probabilities of transitions, w1 is a transition probability for single quantum. So, there was no change in intensity. What we did is we brought in cross relaxation pathways like, for example, instead of single quantum pathway. What happens if the spins attain different equilibrium state by going through a different pathway for relaxation like double quantum or zero quantum. Then we worked out, what is the change in the intensity, when it follows w2 probability that is the double quantum probability. When that is higher, when we measured the change in the populations between energy states of the other spin which is in close proximity with this irradiated spin, we saw 50% enhancement in signal intensity. Whereas we also found out what happens if the dominant relaxation pathways is w0; that is alpha beta to beta alpha transition; that is w0 probability, 0 quantum, we found there is a reduction in the intensity by 50%. So, what basically we tried to understand is all the 3 processes are competitive process. There are not exclusive, none of them are exclusive, not that only W2 is present, w0 is present or w1 is present in the molecule; everything could be possible and they are competitive processes. But now, the thing is which is the dominant mechanism that defines whether it is a positive NOE or the negative NOE. We observed that and which is the dominant and w1 is dominant let us say, single quantum relaxation pathway. Then we saw that there is no way you can see NOE, detection of NOE is less probable. You will not see it also.

So w2 and w0 are the requirements we have found out and then we correlated this with the spectral density function and also tau C, the size of the molecule. And we discussed that tau C also tells you about the sluggishness of the molecule. How fast the molecule is undergoing motion, whether it is fast motion or slow motion defines the frequencies and which define whether w2 is dominant or w0 is dominant.

So, all these things we understood. And we came to a situation to understand if we want to know the tau C, when we correlate with this molecular weight, the general rule of thumb was tau C in nanoseconds is approximately equal to 0.6 times the molecular weight, approximately 50%, slightly more than that 0.6 times the molecular weight. And we tried to see what is going to happen.

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For a small molecule I consider, if the molecular weight of 3.3 Kilodaltons and we found out tau C is about not 2 nanoseconds; simply calculate this. This corresponds to 500 Megahertz. On a 500 Megahertz spectrometer then this is dominant. w1 is dominant. So that is what I am going to tell you the tau C depends upon the molecular weight which in turn depends upon the frequency that is going to be produced; that is tau C nanoseconds. And that depends upon which frequency, omega 0 you are doing the experiment and then that tells you whether NOE is positive, negative or 0.

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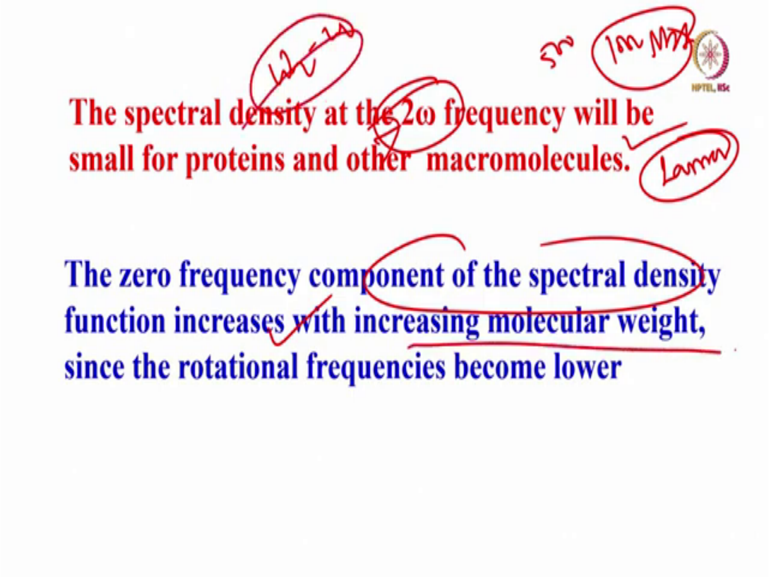

The spectral density at the 2ω frequency will be small for proteins and other macromolecules.

The zero frequency component of the spectral density function increases with increasing molecular weight, since the rotational frequencies become lower

This is what we discussed. Now, let us consider the spectral density twice the omega frequency; twice the Larmor frequency for small proteins and other molecules. Then what happens especially for small molecules, at twice the Larmor frequency, let us say, 500 Megahertz, if you are considering then 2w; that is a 1000 Megahertz is generally absent. So that means what is the relaxation pathway ? we can find out what is the relaxation pathway for that. It is so, when that is absent only the Larmor frequency, whatever you see is dominant. The 0 frequency component of the spectral density function then increases because of this. Finally, we found out w 2 – w 0. So, when this increases it increases the molecular weight so, the rotational frequency becomes lower, this is the important concept.

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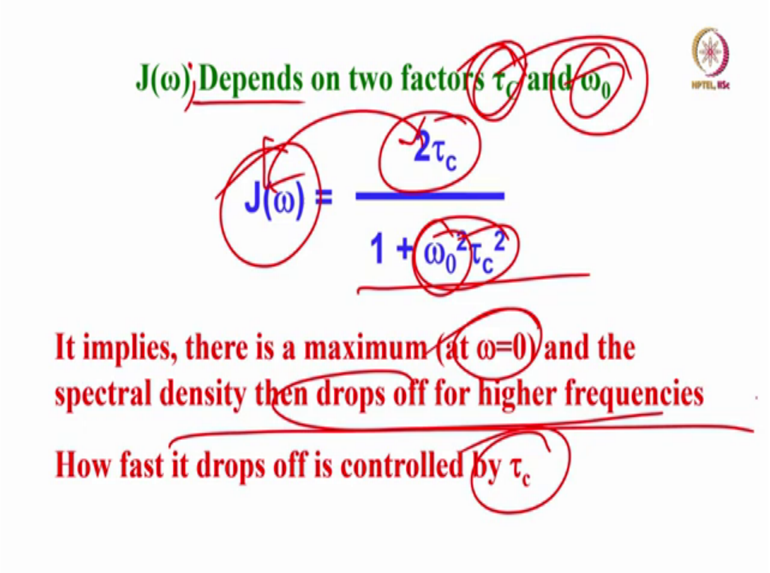

$J(\omega)$ Depends on two factors τ_c and ω_0

$$J(\omega) = \frac{2\tau_c}{1 + \omega_0^2\tau_c^2}$$

It implies, there is a maximum (at $\omega=0$) and the spectral density then drops off for higher frequencies

How fast it drops off is controlled by τ_c

So, we also discussed Jw. What is Jw? Spectral density function. It depends upon tau C and omega 0 and simple equation without working out I am giving you. Jw depends upon twice tau C or on over omega square into tau C square. So, omega tau C is what is important. Let us see if omega tau C is 0; then Jw is equal to twice of the correlation time. So, like that you can work it out. Now what it implies, this equation, is, you can calculate; it is a maximum at omega equal to 0; and the spectral density drops off very drastically at higher frequency, falls off very easy. And so, the spectral density, how fast it is dropping off; how fast it is reducing at higher frequencies, is controlled by tau C. You understand Jw depends upon 2 factors, tau C and omega 0, which is given by this; and at omega 0, omega the spectral density drops off, it is actually maximum and afterwards it drops off to higher frequency. So, how fast it drops off everything is controlled by tau C. that molecular correlation time.

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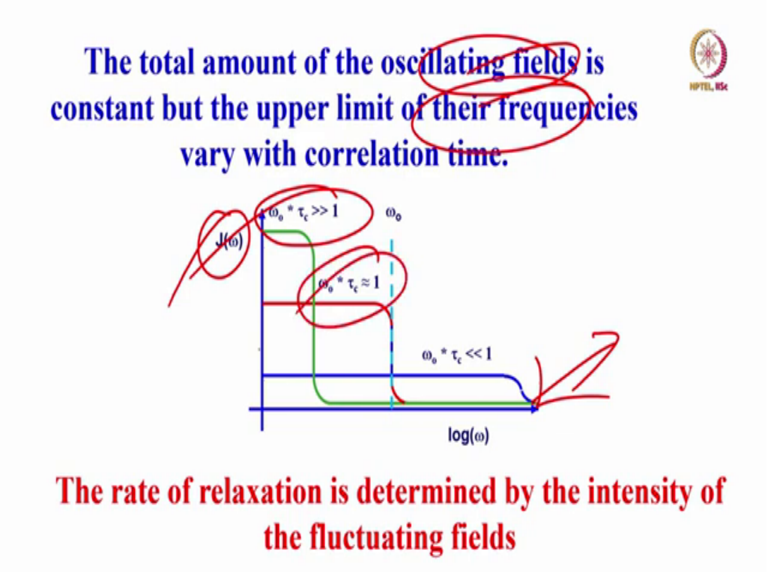

The total amount of the oscillating fields is constant but the upper limit of their frequencies vary with correlation time.

The rate of relaxation is determined by the intensity of the fluctuating fields

But, what is the total amount of the oscillating field? This what we discussed earlier also; that remains constant. The upper limit only is the one which changes in the correlation time, but the total correlation; amount of the oscillating field that is produced at the site of the nucleus remains same. That cannot change and that define the frequency. But what is happening is the upper limit of the frequency is the one which vary with the correlation time.

That is why this equation, this graph I showed this when we were discussing the relaxation phenomenon also. This is Jw, spectral density function and this is what we saw, omega tauc is very much greater than 1. This is an intermediate condition. When omega tau C is approximately equal to 1, where you see no NOE.

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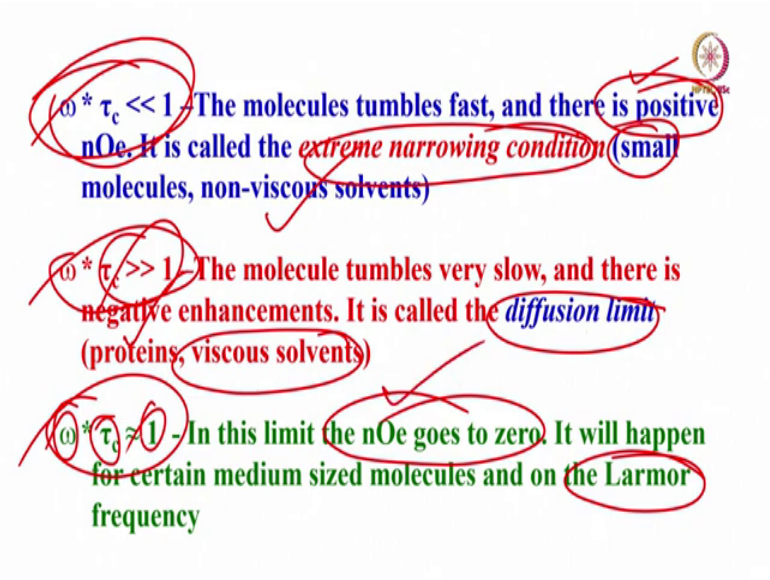

$\omega * \tau_c << 1$ - The molecules tumbles fast, and there is positive nOe. It is called the *extreme narrowing condition* (small molecules, non-viscous solvents)

$\omega * \tau_c >> 1$ - The molecule tumbles very slow, and there is negative enhancements. It is called the *diffusion limit* (proteins, viscous solvents)

$\omega * \tau_c \approx 1$ - In this limit the nOe goes to zero. It will happen for certain medium sized molecules and on the Larmor frequency

And this is omega tau C which very much smaller than one. Now, you will see we have certain conditions under which we can see NOE or not. Let us see omega tau C is less than one when omega tau c is less than one. The molecules tumble very very fast and this is the situation, we also discussed the molecular motion is fast. We discussed for small molecules it happens, positive NOE.

Remember in the previous class we discussed; for small molecules which tumbles very fast, omega tau C, if you calculate, is much much smaller than 1. As a consequence NOE is positive. And this condition is called extreme narrowing condition. The extreme narrowing condition is experienced, you are going to see this extreme narrowing condition only for small molecules and especially in non viscous solvents.

When the molecules you put in non viscous solvents, if it is viscous solvent, of course, mobility is less, only non viscous solvent if you put, the molecules undergo tumbling motion, very fast thermal agitation is going on, because of this fast tumbling motion. Then NOE is then positive and this is the situation we encounter. Now, when omega tau C is larger than 1 greater than 1 what does it mean? Molecule is tumbling very slowly. That means there is a negative enhancement, negative NOE we are going to see. This is called diffusion limit. When this can happen, the diffusion limit can happen only for big molecules like proteins in the sense large molecules. So, it cannot rotate faster like small molecule, they cannot tumble faster; and then especially such molecules you put it some molecule put in viscous solvents, restrict the mobility. In which case you can experience omega tau C larger than 1. It is called diffusion limit. On the other hand, consider the situation omega tau C is approximately equal to 1. If this is the limit in which NOE goes to 0, when omega tau c is approximately equal to 1, you will not see NOE at all. And this can happen for neither too big molecules, not too small molecule, some medium size molecules; and this is at the Larmor frequency.

This can happen, when tau C and omega is approximately equal to one. Then you are going to see this in medium size molecules. You can experience this and you do not see NOE at all. So,when omega tau C = 1 is the situation for which NOE = 0. You do not see NOE at all

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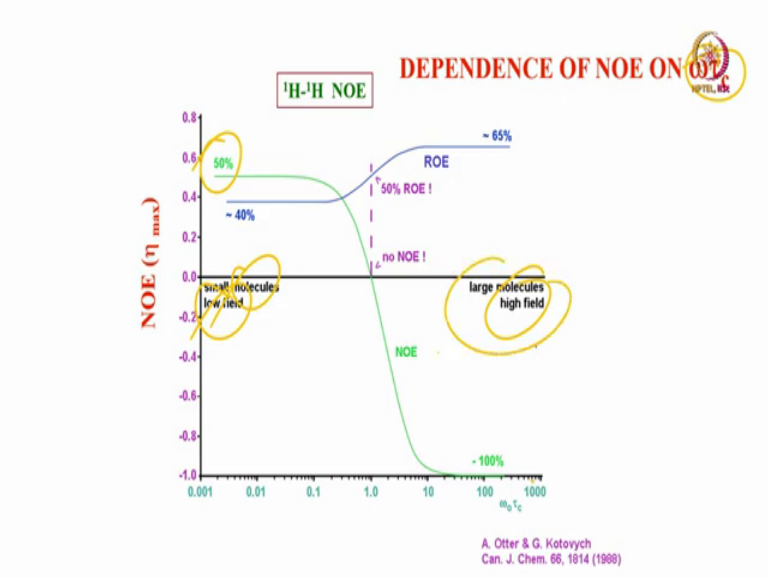

DEPENDENCE OF NOE ON $\omega\tau_c$

A. Otter & G. Kotovych
Can. J. Chem. 66, 1814 (1988)

And the dependence of NOE on omega tau C is given here. Of course, this is taken from this paper. You can see that this is an example of a proton proton NOE. How NOE depends upon omega tau C? And this is the NOE in this axis. It is the intensity of the signal, that is NOE that is measured. It is given by eta, eta the maximum NOE we saw, is plotted as a function of correlation time omega tau C.

Now, see the molecule like this. For example, large molecules are at this side. Small molecules are this side. For small molecules you know if you are in this range, you get 50% NOE. That is another thing very, very small; and then, as you keep crossing this omega tau C limit, then it is a negative NOE – 100. This is for the large molecule. So, once you cross this omega tau C which is equal to 1, which is a condition where there is no NOE. Then you are going to the higher side it is a large molecule. This can happen for large molecules at higher magnetic field. And this is – 100%. That is – 1% enhancement – 1 we call 100% enhancement of the signal intensity. So, this 100% or it is 50%. It is for negative NOE, it is for positive NOE, this can happen for small molecules in low magnetic field. This region comes for the big molecules in high magnetic field.

This is the situation we always come across when you are dealing with NOE. So, please remember it depends strongly on omega tau C, that is the important concept, we should remember. So, omega tau C, and of course omega depends upon the magnetic field and also size of the molecule. So, small molecules at low magnetic field gives raise to positive NOE. Big molecules at high magnetic field give raise to negative NOE.

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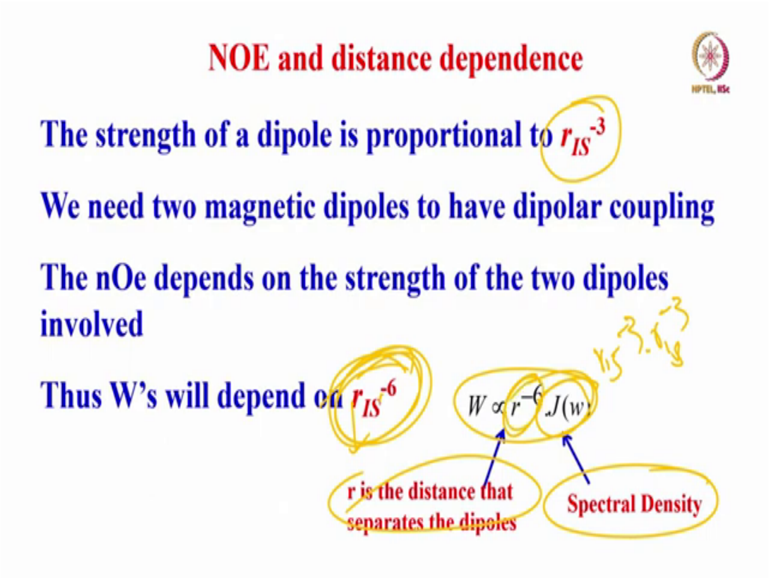

NOE and distance dependence

The strength of a dipole is proportional to r_{IS}^{-3}

We need two magnetic dipoles to have dipolar coupling

The nOe depends on the strength of the two dipoles involved

Thus W's will depend on r_{IS}^{-6}

$W \propto r^{-6} . J(w)$

$r_{IS}^{-3} . r_{IS}^{-3}$

r is the distance that separates the dipoles

Spectral Density

This is the important graph, you please remember. And NOE also depends upon the distance I said, 1 over r power of 6. Of course, we can calculate NOE also. We can do the experiment of and find out what is r IS etcetera. We will discuss those things later if there is time. But remember, the strength of the NOE depends upon r IS to the power of -6 for a dipole. Dipole interaction depends upon the inverse cube of the distance between 2 interacting spins.

That is a dipole dipole coupling, depends upon 1 over r IJ cube, where r Ij, is the distant between two nuclear spins i and j. But now we are dealing with 2 nuclear magnetic dipoles for NOE. So, NOE depends upon the strength of the 2 dipoles involved. As a consequence, w depend upon r ij to the power of– 3 into r ij to the power of – 3. So, it become r to the power of – 6.

So, the strength of the NOE depends upon inverse 6th power of distance between these 2 spins I and S; an important point. That is the reason why slight deviation from the distance, if you change r little bit, because you have r power 6 in the denominator, this will drastically decrease the intensity. So, it is a very, very sensitive parameter, r power 6.

So, this is the equation which is given with the enhancement of signal intensity. W is given by r to the power of – 6, r is the distance between the two nuclear dipoles; that 2 nuclear spin that separates, and also Jw the spectral density. So, this is also dependd on spectral density. So, enhancement in the signal intensity depends upon r and spectral density together.

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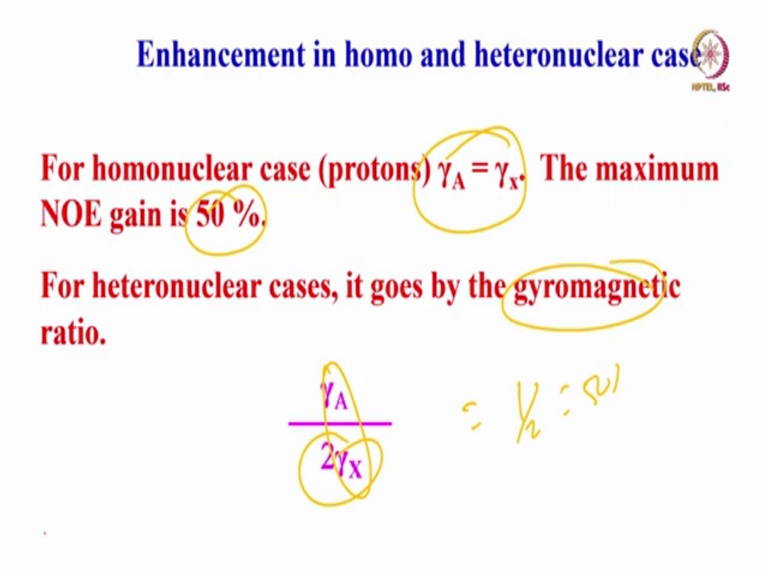

Enhancement in homo and heteronuclear case

For homonuclear case (protons) $\gamma_A = \gamma_X$. The maximum NOE gain is 50 %.

For heteronuclear cases, it goes by the gyromagnetic ratio.

$$\frac{\gamma_A}{2\gamma_X} = \frac{1}{2} = 50$$

So, remember at one stage we said it depends upon the ratio of the gammas. What happens if you consider homonuclear cases? identical, gammas or heteronuclear cases with different gammas? How does the enhancement affect? What will be the enhancement? Let us see. For homonuclear case, of course, we know gammas are identical. The maximum NOE gain you get is 50%. Remember maximum NOE in the homo nuclear case is 50%. On the other hand, for heteronuclear case, it goes by the gyromagnetic ratios. The gyromagnetc, gamma of two heteronuclear spins dictates the enhancement in the signal intensity. This is the formula which you have to use gamma A by 2 times gamma X.

So, both are identical, that is a homonuclear case. It is half; 50% intensity. On the other hand, if it is, let us say some other nuclei with different gamma, quite small let us say gamma four times smaller. Let us consider heteronuclei. Then you see depending upon which you are irradiating and which you are observing it changes. So, we will work it out as you go further. When you look at the intensities you can find out.

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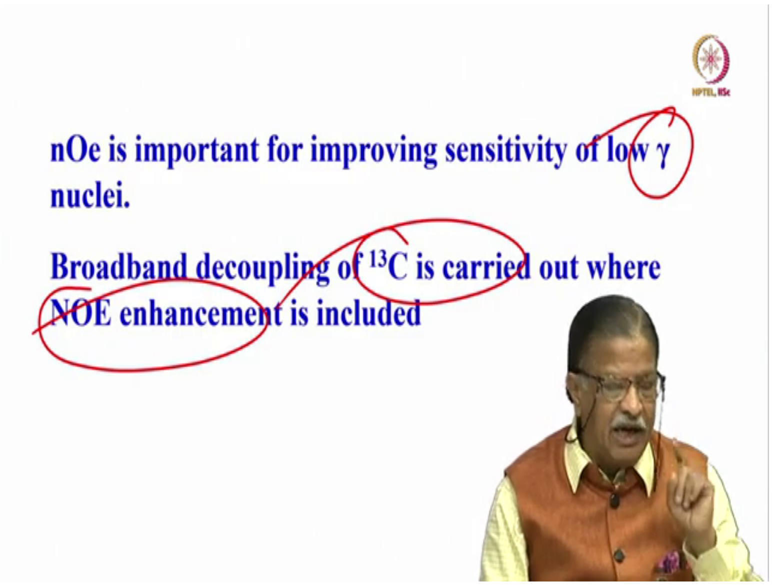

nOe is important for improving sensitivity of low γ nuclei.

Broadband decoupling of ^{13}C is carried out where NOE enhancement is included

I mean, one of the examples of a proton and carbon will take and find out. So, NOE is very important, especially when you are dealing with heteronuclear case; that to, when one of the nuclei has a low gamma. That means carbon 13, let us say 4 times low gamma or nitrogen 15, for that matter. Of course, it is special case it is a negative magnetic moment also. We will come to that later. Such low gamma nuclei, if gamma A divided by gamma X is the ratio.

You know when you consider, the sensitivity enhancement depends upon gamma; and it is very important especially for low gamma nuclei; to enhance the sensitivity. Basically, when we discuss the carbon 13 NMR also I told you, always carbon 13 NMR is experiment the spectrum is obtained; C13 experiment is carried out with broadband heteronuclear decoupling.

That is what I said, I am observing carbon 13 spins. I want to get the carbon 13 spectra, but I break the coupling of all carbons coupled to all the remaining protons. While doing that we wanted to simplify the carbon 13 spectra to get only single peaks for each chemically inequavalent carbon. But another important factor was there. which I did not tell at that time.

There is also what is called NOE enhancement when you do the decoupling; heteronuclear decoupling, carbon 13. You are going to have NOE enhancement and change in the intensity. That benefits actually.

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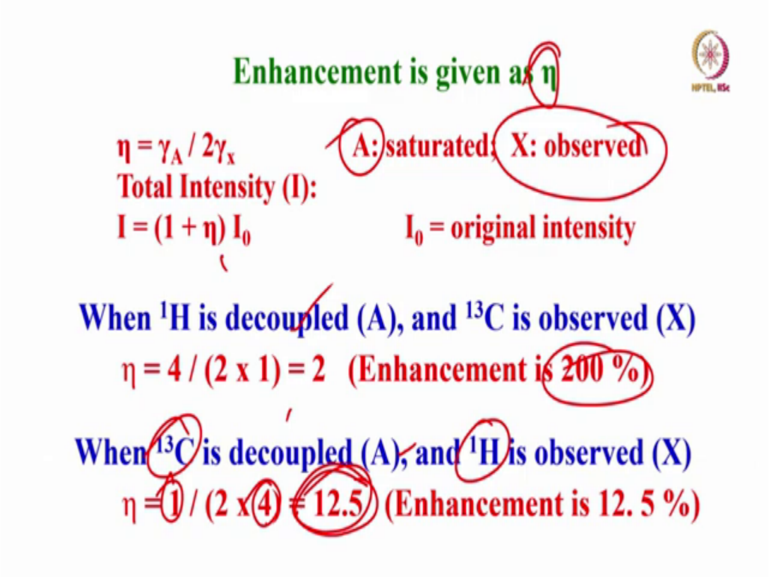

Enhancement is given as η

$\eta = \gamma_A / 2\gamma_X$ A: saturated; X: observed

Total Intensity (I):

$I = (1 + \eta) I_0$ I_0 = original intensity

When ^1H is decoupled (A), and ^{13}C is observed (X)

$\eta = 4 / (2 \times 1) = 2$ (Enhancement is 200 %)

When ^{13}C is decoupled (A), and ^1H is observed (X)

$\eta = 1 / (2 \times 4) = 12.5$ (Enhancement is 12. 5 %)

So, enhancement of NOE, of course, as I said, is defined by the term eta, which is a gain factor. As I told you, eta is given by gamma A over 2 times gamma X; A is a saturated spin. I mean irradiating A and X is the observed spin. Please remember which nuclei are observing which nuclei you are disturbing? irradiated with rf, that defines the enhancement in the intensity; because these 2 parameters are different here.

The total intensity I is given by this thing 1 + eta into I0, I0 is the original intensity. So, if eta becomes more then, it because it multiplied by that you, let us say eta = 2. Then it is 3 times I0. eta = 4, 5 times I0. Enhancement with respect to original intensity depends upon this eta enhancement factor. Now, I will consider the situation. I am decoupling, proton A and observing carbon 13. What does it mean? I am decoupling. Consider gamma A over 2 times gamma X.

Now, my observed Nucleus is in the denominator, and this is what I am decoupling. So, what is eta or you know the gamma; say gamma of carbon 13; 4 times less than that of the proton. So, now I am irradiating proton, the gamma A is in the numerator, it is divided by 4; this is, let us say, 1 by 4th of that. This is 4, this side take only 1 because 1 by 4th of that. this 2 is there already in the denominator. It turns out to be 2. You understand, gamma of proton is 4 times more than that of carbon. If a carbon gamma I consider one, this I consider 4. And because it is 2 is in the denominator it turns out to be 2. That means, if I decouple proton and observe carbon 13, there is a 200% enhancement of the signal. There is huge gain in the signal intensity; that aids you know in the speeding up of our experiment.

Please understand now I am decoupling proton and observing carbon. There is a enhancement in the intensity by 200%. On the other hand, let us consider a situation where I am decoupling carbon 13 and observing proton. That is something you know, it is reverse of that. We do not need to decouple proton and observe carbon always. You can also do another experiment. I do carbon 13 decoupling and observed proton. Now, proton is the observed nucleus that is in the denominator. This 4 is denominator now, 1 is in the numerator; calculate this thing. This turns out to be 12.5%. See when I decouple carbon thirteen and observe proton only 12.5% enhancement in intensity; unlike in the decoupling proton observing carbon, there is 200% enhancement. This is the difference; depending upon which nucleus you are decoupling and which is the nucleus you are observing. Based on that enhancement factor eta depends, especially in the case of heteronuclear because gammas are different.

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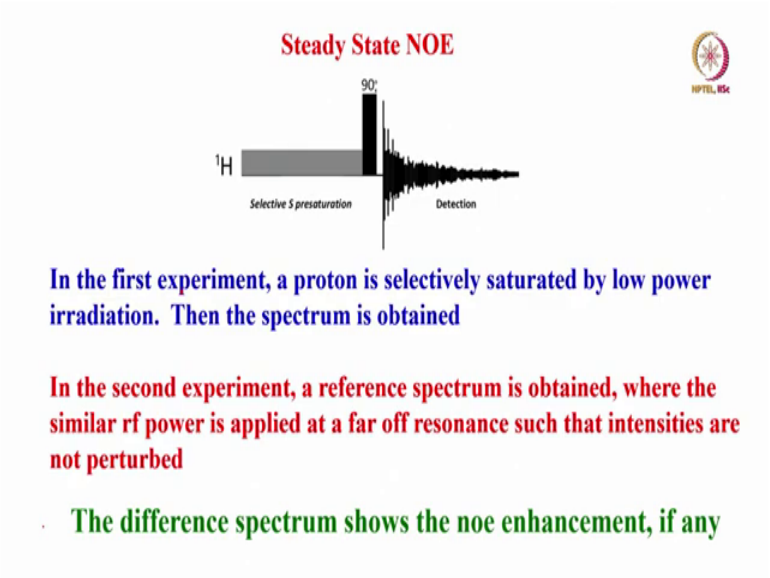

Steady State NOE

In the first experiment, a proton is selectively saturated by low power irradiation. Then the spectrum is obtained

In the second experiment, a reference spectrum is obtained, where the similar rf power is applied at a far off resonance such that intensities are not perturbed

- The difference spectrum shows the noe enhancement, if any

So, now, with this, having said all those things, we want to see how we can measure NOE. All along I have been discussing steady state NOE. I told you about steady state NOE because with the low power rf, we are irradiate saturating the spins and the spins attain a new, steady state after some time and the change in intensity is what we are going to observe. That is what we said and we know it is a steady state. So, we have to call it as a steady state NOE. How do you measure the steady state NOE, is a simple experiment like this, very simple experiment. What we have to do, let us say I am looking at proton, irradiating one of the protons and see the effect on the other neighboring protons. So, we have to selectively pre-saturate that proton selectively with low power rf; keep on sending the low power rf continuously for that proton and disturb the spin state of that one, saturate it. And then that is called selective saturation, a pre-saturation I am doing before continuously, before applying the second detective pulse, 90 degree pulse. I am saturating the selectively; then apply a radio frequency pulse and collect the signal; this is the simple experiment. Simple experiment what we have to do here is simply we have to apply a low power rf. And then apply a 90 degree pulse and start collecting the signal. The low power rf has to be applied for a long time. You should not apply high power rf, then you know you will disturb the spin system and you will be completely doing a different experiment like decoupling and you will not see NOE. So, low power rf. In the first experiment what you have to do? The proton is selectively excited and saturated, you obtain a spectrum. That is one experiment. It is something which we have to do.

I let us say I want to see the NOE between 2 protons. I am irradiating this, that is what I do and then get the spectrum and measure the intensity of the other one; that is what we have to do. In the second experiment, what we do is we take a reference spectrum, because finally, I have to do a difference NOE. What we do is, I apply similar rf but far away sitting somewhere here, very very far away, at off resonance, so that spins are not disturbed because rf power. If there is if there is some disturbance because of rf power that should remain same in both the experiments. So, we do off resonance irradiation which is far away and observe the change in the intensities. Now, when I do this experiment off resonance, these intensities are not perturbed, they are not changed at all. So, this is called a control experiment.

You first do control experiment and then selectively irradiate the peak of your choice. Take the difference between these 2 experiments now, because in this case there must be a change in intensity; you will not notice the change in intensity because you are simply getting the NMR spectrum. But if you want to notice the change in intensity there must be some difference, that you have to see. Only then it will tell you whether there is intensity change or not. So, do the control experiment and then do this normal experiment take the difference you will find out how much is the change in the intensity; This is a simple experiment routinely done It is called 1D difference NOE experiment, one dimensional difference NOE experiment.

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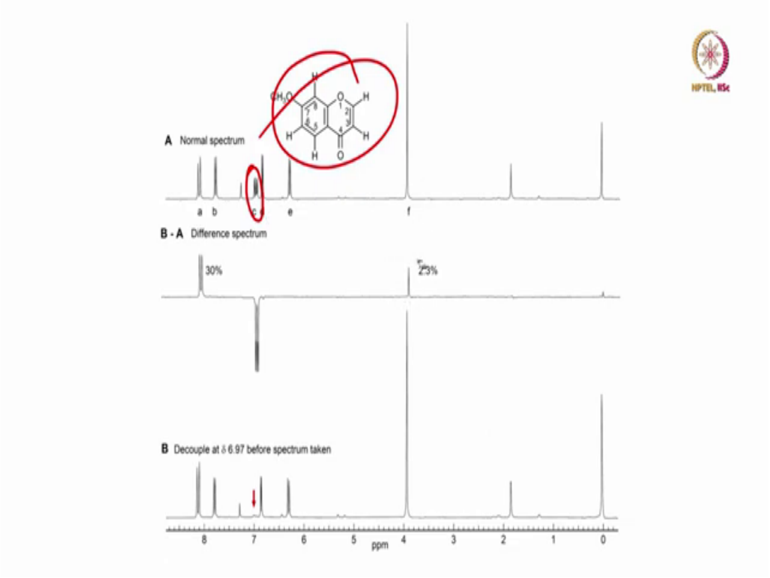

We simply irradiate one of the protons at some frequency, and then do the control experiment by irradiating far away so that no spins are disturbed. Take the difference between these two. In a simple example of this molecule. Let us say we are irradiating one of these protons here. With the irradiated proton, it will be always negative in intensity. You will see that only so you are not going to observe any signal there. And then this is a normal spectrum which you observing. By radiating this one we took a one dimensional spectrum. And then look at it for all other peaks intensity is not there. They are far away. So, for this proton, only this and this are close by, there is a change in intensity here. But then, if what you want to see, you have to do the decoupling and see the change. But here I have already taken the difference that is why you are seeing.

If I do not take the difference if I give you this spectrum, I am doing the radiation here, signal is 0 and you see there is some change. But you are not seeing anything in it; what is the difference between this and this. If I give a normal spectrum and spectrum after irradiation with low power, you do not find much of a difference. I can always readjust the intensities to matches with this one but that is not right. Now, I took the difference between these two and this is what you are going to see. When I take the difference between this and this. This is 0 intensity because it is irradiated, saturated signal; and here because you subtracting this from this, it will be a negative intensity. And look at it wherever there is enhancement in the intensity. There is change, the difference is not 0; but here difference is 0.

This signal is nullified; all the other signals are nullified when you took the difference. But especially this peak and this peak, there was a gain in the intensity. As a consequent when I took the difference of these two, there is no nullification at all. Still there is some signal retained. This is the gain in the intensity. Of course, gain maximum is 50%. That does not mean you have to be always 50% but that depends upon various parameters. You see, we got 30% enhancement here and only 2.3% enhancement in this one.

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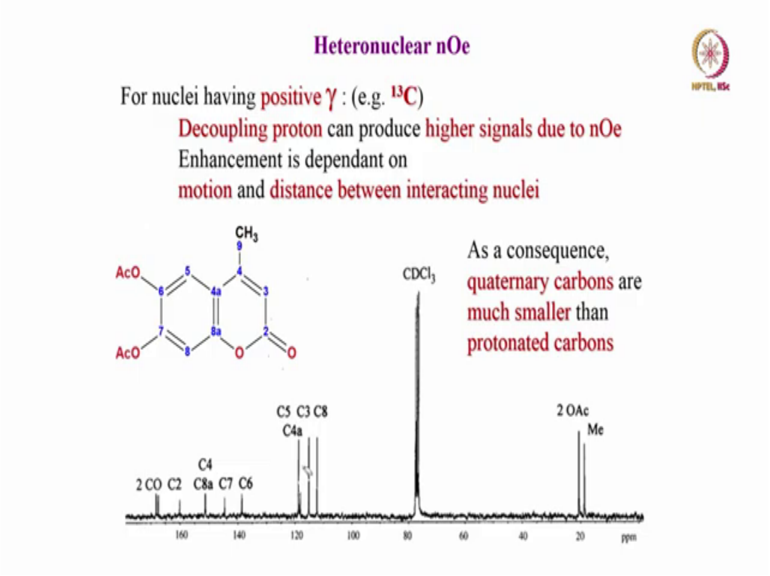

Heteronuclear nOe

For nuclei having positive γ : (e.g. ^{13}C)

Decoupling proton can produce higher signals due to nOe

Enhancement is dependant on

motion and distance between interacting nuclei

As a consequence, quaternary carbons are much smaller than protonated carbons

So, similarly you can do heteronuclear NOE, look at the carbon 13 here. This is what it is. See for example, some of these peaks are very, very weak in intensity. But if you try to do the decoupling of protons then there is enhancement the signal intensity; we saw when we decouple proton observed carbon. We said there is 200% gain.

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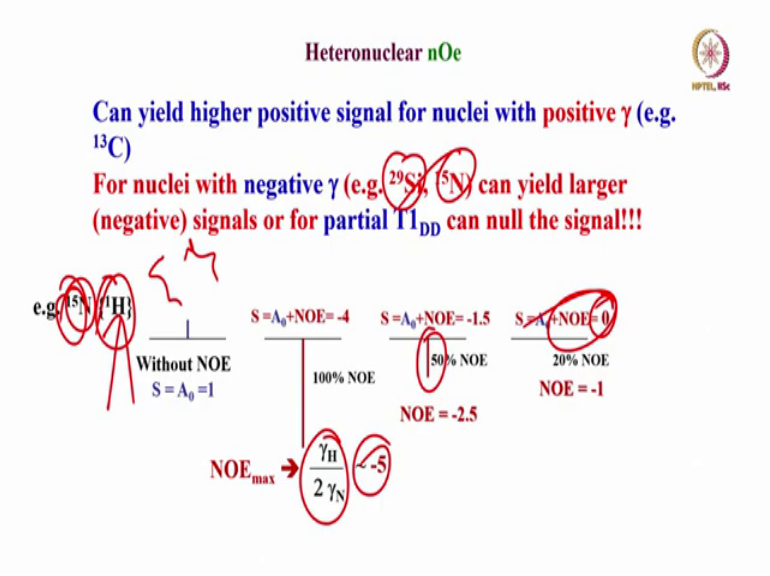

Heteronuclear nOe

Can yield higher positive signal for nuclei with positive γ (e.g. ^{13}C)

For nuclei with negative γ (e.g. ^{29}Si, ^{15}N) can yield larger (negative) signals or for partial $T1_{DD}$ can null the signal!!!

So, this how we can play use this idea of NOE for heteronuclear case to enhance. But there is a peculiar situation we may come across in heteronuclear NOE. Heteronuclear NOE can yield positive signal for the nuclei with positive gamma like carbon and others. There are certain nuclei with a negative gamma, magnetic moment is negative, for example, silicon 29 and nitrogen 15, they have negative gamma.

These two, when you have negative gamma a very interesting thing happens. If you take the ratio, it is negative. That means sometimes you may not even see the NOE, NOE may become 0 also. Look at the situation for this; a hypothetical case. We are observing nitrogen 15, decoupling proton. We are observing 15 N, while decoupling proton, the decoupling nuclei, is always given in flower brackets you know that.

So, without any NOE, the intensity let us say we call it 1. It is a stick plot. Now, with NOE, now the 100% NOE is there, now it is going to be – 4; because if you take, the gamma of nitrogen 15 is 10 times smaller than that of proton. You work out it turns out to be – 5; but 100% enhancement also, if you take – 5 + 1, it will be only – 4. Still, it is negative. There is not much gain in the intensity for you.

If you continue like this in a situation where there is 50% NOE, you will get – 1.5. Whereas, if you consider the situation this can turn out to be 0. Let us say there is a 20% NOE if 20% NOE, if you put here, calculate what is the 20% NOE with this then it turns out to be NOE – 1. Then, in which case, if you take the total intensity of the signal, it turns out to be 0.

So, nuclei with negative magnetic moment or negative gamma, sometimes it so, happens when you do NOE, instead of gain in the signal, it will give less signal or it sometimes even signal intensity will go to 0 also.

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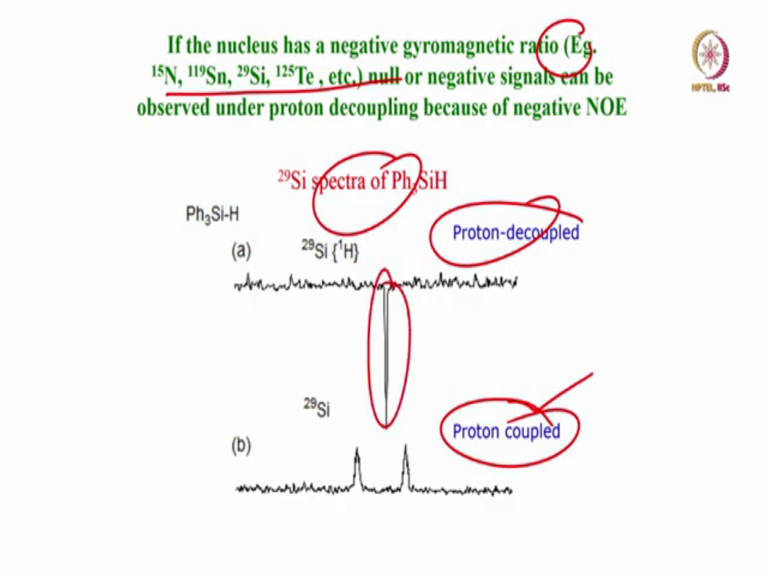

If the nucleus has a negative gyromagnetic ratio (Eg. ^{15}N, ^{119}Sn, ^{29}Si, ^{125}Te , etc.) null or negative signals can be observed under proton decoupling because of negative NOE

So, these are some of the tricky situations which you should know, and in the spectrum which is taken from some literature, you see, the nucleus has a negative gyromagnetic ratio like this, some of this nuclei; a null signal can be observed under proton decoupling because of this. An example of a situation like this, this is a proton decoupled, this is a proton coupled, silicon spectrum. So, here you see when you are decoupling your signal intensity has gone up, when you have coupled signal intensity has come down actually.

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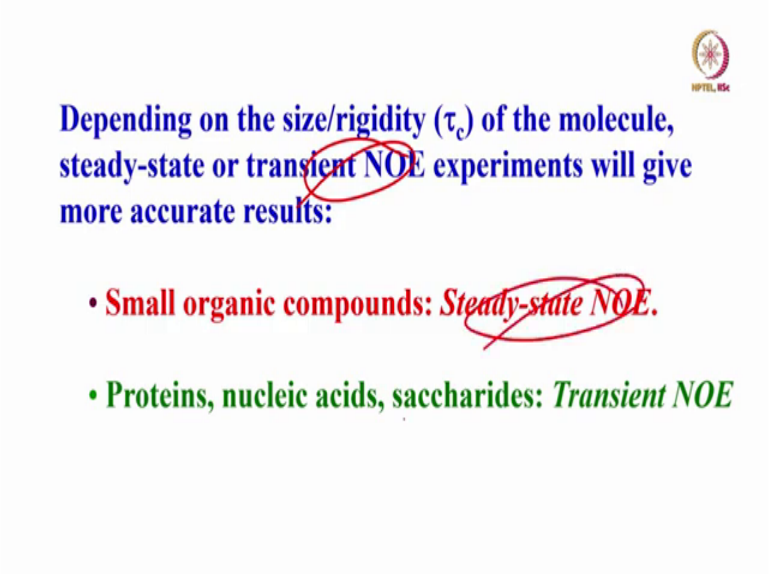

Depending on the size/rigidity (τ_c) of the molecule, steady-state or transient NOE experiments will give more accurate results:

- Small organic compounds: *Steady-state NOE.*
- Proteins, nucleic acids, saccharides: *Transient NOE*

So, you can get negative, sometimes because of negative magnetic moment; so, silicon 29 intensity may even become 0. It may be negative also. So, depending upon the size and the rigidity molecule, tau C of the molecule, steady state or transient NOE experiments are done. I have not introduced the transient NOE. We will discuss when we come to 2D NOE after introducing 2D.

Since I have not discussed 2D, we will not worry about it. For small molecules or organic molecules what we can do is the steady state and what we discussed now. Irradiate one of the protons and irradiate at off resonance. Take the difference. When you take the difference spectrum, you see the change in the intensity of only selected peaks with that you can measure the percentage of NOE.

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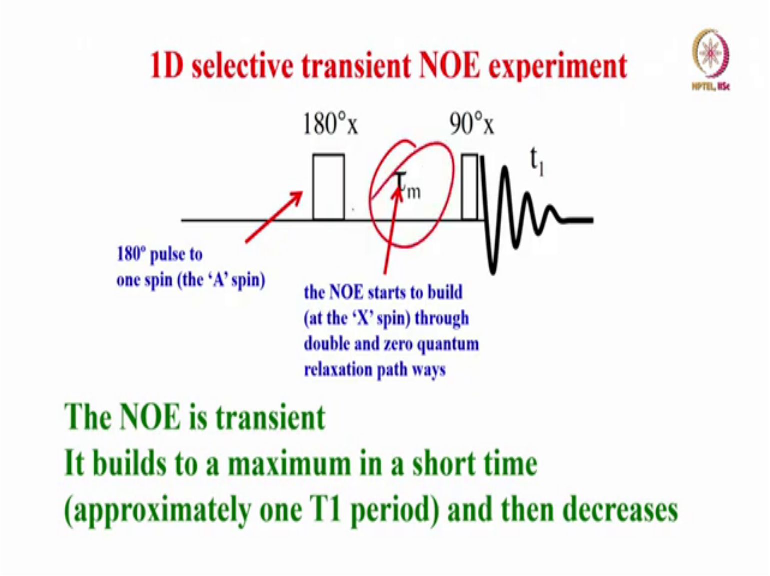

1D selective transient NOE experiment

180° pulse to one spin (the 'A' spin)

the NOE starts to build (at the 'X' spin) through double and zero quantum relaxation path ways

The NOE is transient
It builds to a maximum in a short time
(approximately one T1 period) and then decreases

So, this is the 1D selective transient NOE. In 1D also you can do the transient NOE similar to 2D.

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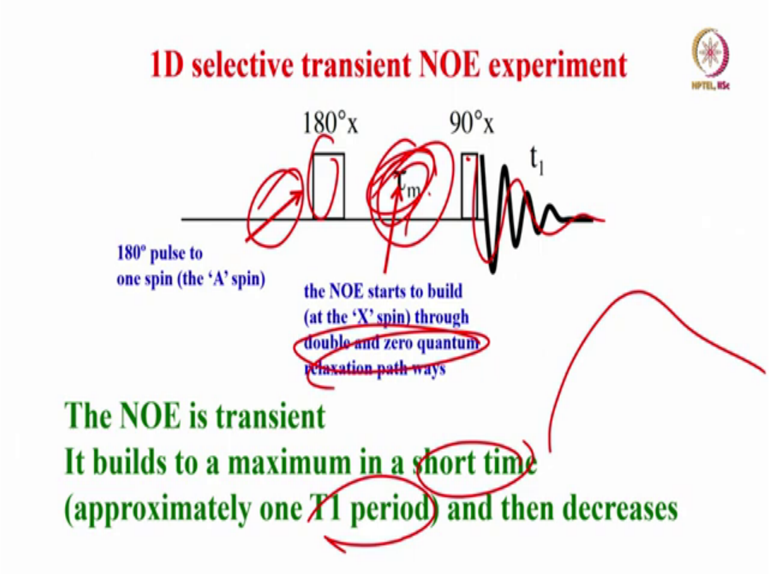

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But anyway I will explain this when I explain NOESY, because now you see I will use the transient NOE, hence I quickly touch upon this. You have to apply 180 pulse on one of the spins and give some mixing tau m and during this time NOE start building up using both 0 quantum and double quantum relaxation pathway, apply detective pulse and collect the signal.

That is all the transient NOE experiment in one dimensional way. And now how the NOE builds up with time has to be obtained depending upon how short the time, how long the time is. And then tau m must be approximately fixed, to be fixed one over T1. So, it will build up like this and then start decreasing. So, these things I need to discuss more about this after I introduce 2D when I talk about 2D NOE, I will introduce transient NOE.

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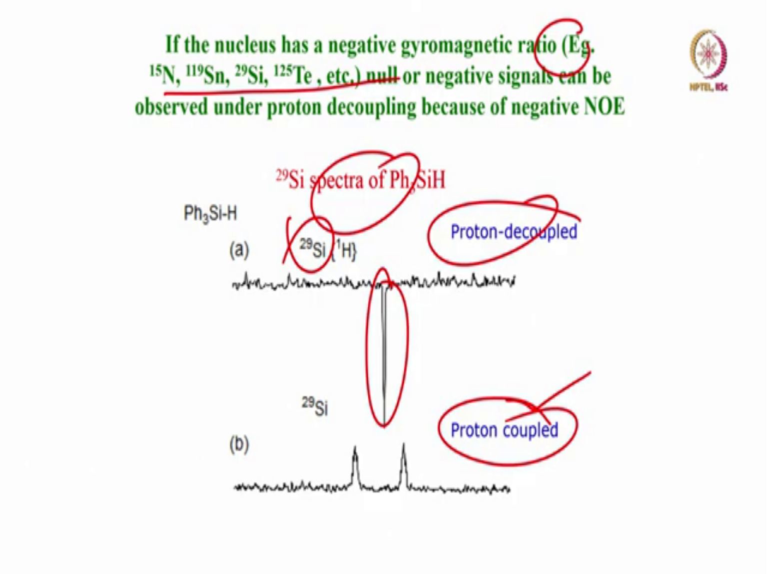

If the nucleus has a negative gyromagnetic ratio (Eg. ^{15}N, ^{119}Sn, ^{29}Si, ^{125}Te , etc.) null or negative signals can be observed under proton decoupling because of negative NOE

^{29}Si spectra of Ph_3SiH

Please remember here what we have been discussing. If you look at some of the spectra, sometimes it so happen, the signal intensity can be nullified when you are decoupling because of negative NOE, negative magnetic moment. As a consequence, while doing heteronuclear case, you have to be extremely careful, homonuclear case there is no issue either you get intensity more or less depending upon the size of the molecule. for the heteronuclear case it also depends upon gamma sign, that is the sign of the gamma.

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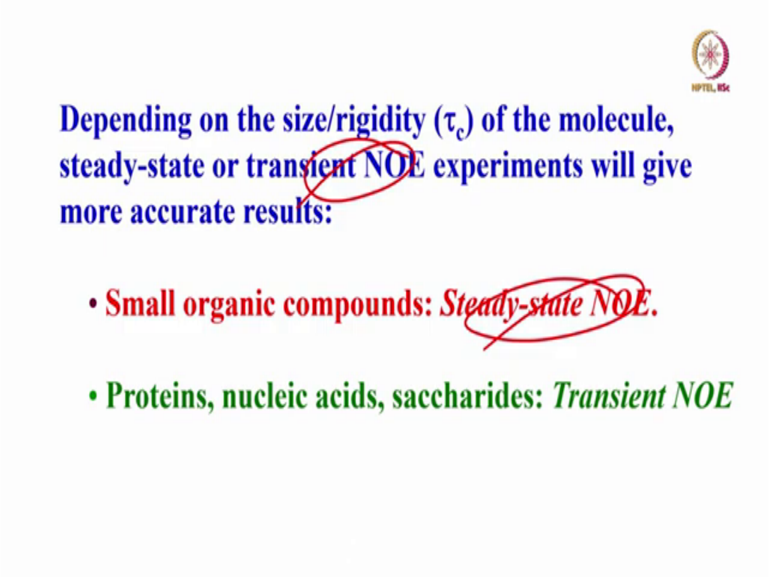

Depending on the size/rigidity (τ_c) of the molecule, steady-state or transient NOE experiments will give more accurate results:

- **Small organic compounds: *Steady-state NOE.***
- **Proteins, nucleic acids, saccharides: *Transient NOE***

So, this is what I just basically wanted to tell you. Since the time is up, I am going to stop and the NOE I do not think we can discuss further. Basically, in this I have given you the idea of what is the concept of NOE? Do not worry this is a huge topic in NMR. There is a book on NOE itself by Noggle. They there are not one, two books on NOE itself, on the topic NOE.

So, such a huge topic so, we cannot cover everything and discuss every aspect of this thing. Basically, I wanted to give the basic concepts and highlights of what one can do. Remember you can use this to get the distance information. Once you get the enhancement. How much is the enhancement intensity? You can correlate it with the distance and get the distance information. How to get the distance information? I did not discuss.

It is all simple arithmetic. You can do that. The most important is the concept and how do you do the experiment I discussed, next is simply you have the well known equations. You have to fit into that and then measure the change in the intensity and get the r. So, this is what it is, But there are certain things here you should understand. NOE we discussed about only two spins. What happens if the third spin present.

So, it is not very simple to understand. 3 spin effect will be there. Third spin also will contribute; whether it adds or subtracts is a different question. So, varieties of things or the problems are there. It is not that we have discussed the example of taking 2 spins means we have understood all about NOE. I just discussed only about the concept, mechanism of NOE, the relaxation pathways that is generally adapted, cross relaxation which is responsible for positive NOE and negative NOE, where we showed double quantum and 0 quantum pathways; one gives positive NOE other gives negative NOE which is dominant. None of them are exclusive processes. All are happening together. The dominant relaxation pathway gives you whether it is positive NOE negative NOE or 0 NOE. And we correlated this with the spectral density function and also tau C which depends on the size of the molecule.

Tau C basically, decides about the sluggishness of the molecule. And afterwards we did a 1D difference NOE experiment, when the 1D difference NOE experiment; pre-saturate the signal for a long time and then apply 90 degree pulse and then collect the signal and do the Fourier transformation. Get the spectrum in the other case under identical condition, the identical rf power irradiate at far off place, which is called off resonance and collect identical spectrum for identical time and scans.

Take the difference between these two. Then, when you take the difference between these two, those where there is no enhancement of the signal intensity. All the peaks go to 0, the irradiated peak will become negative and wherever there is a change in intensity, either positive or negative. That will reflect in the difference spectrum. You can quantify this intensity and then measure, r, the distance information.

So, this is basically the gist of NOE which I wanted to tell you and we discussed a lot about relaxation mechanisms, NOE. All you know one way or other is the information required to understand; then you require relaxation, so, all those things we discussed. I think, next time in the next class we will start with the product operators and then go to 2D NMR. There are lot more to discuss and then go to a different topic. So, I will stop at this stage. Thank you very much.