Advanced NMR Techniques in Solution and Solid-State
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Module-36
Decoupling and NOE Concepts
Lecture – 36

Welcome back all of you, in the last couple of classes, we discussed a lot about relaxation that is one of the important concepts, it is fairly a tough concept to understand, it is not pretty simple. I tried to explain in the most simplest possible way about that two important relaxation phenomenon in NMR called spin lattice relaxation and spin-spin relaxation; both of them we discussed a lot. What are those phenomena? What is that which is responsible for spins to relax. For example, in the case of the T1 we knew, it is the energy spins gives energy to the lattice. How it gives energy? What is the mode of giving energy? What is the form it gives energy? It is in the form of heat. What are the processes which are responsible for the spins to give to the energy to the lattice, we discussed. It required a lot of other phenomenon like fluctuating magnetic fields which are at the Larmor frequency. Then it so, happens the spins will give its energy to the lattice.

And of course, what is the energy conserving phenomenon while giving the energy to the lattice and then what are the several phenomena which are responsible for the spin lattice relaxation to happen; we discussed. For example, dipole-dipole interaction, chemical shift anisotropy interaction, that is CSA; spin rotation relaxation, quadrupolar relaxation, various phenomenon are responsible for the spins to give energy to the lattice and undergo relaxation.

Similarly, we also discussed a lot about spin spin relaxation, spin spin relaxation also. Of course, there is no energy given to the lattice, but spins but between themselves there is a mutual interaction ;it is nothing but the decoherence of the nuclear spins. I showed, the 90 pulse bring the magnetization to the x-axis instantaneously there is a statistical phase coherence. But what is going to happen is, all the magnetization vectors start dephasing with time; they will not be at the same place, because of some local fluctuating fields, some local phenomenon. As a consequence, the spins start moving out, fanning outside in the XY plane. And then intensity of the magnetization that you are going to get along the particular axis where you have the receiver, if you take the vector addition of all the magnetic moment vectors, this keeps coming down.

After some time there is a complete dephasing; you will not see any signal. This phenomenon is called T2, spin spin relaxation. It is the decay of magnetization in the XY plane, the spin lattice relaxation is the growth along z axis. Both are exponential functions. That is what we said; both are exponential functions, one is the decay function and the other is the growth function. And we know that, we said relaxation is a radiationless transition.

How do you make the relaxation measurements? There are several ways of making the measurement of T1 and also for T2; we adopt spin echo sequence, a method which is the Car-Purceel or the Meiboom Gill modified sequence. Or in the case of spin lattice relaxation measurement, we use what is called inversion recovery method. We apply 180 pulse bring the magnetization –z axis, allow it to go back to z axis as a function of time.

The magnetization vectors starts shrinking along –z axis slowly becomes 0 and goes to +z axis. The shrinking phenomenon how much it shrinks we can measure it, as a function of time and then fit it into the equation which is given for relaxation phenomenon; which you worked out you. Then will get the T1 values for all the peaks present in the sample.

Same way we can do for the T2 measurement; instead of 180 -90-sequence, here it is 90-tau-180-tau; first apply 90 pulse bring the magnetization to x axis or to the xy plane, they start dephasing, apply 180 pulse and vary this delay. This is echo time and after an equal amount of time start collecting the signal, it forms an echo. Now, you apply number of such echo sequences; and during the echo process what happens, there is decay of the signal intensity due to natural process. You have to subtract the component from the magnetic field inhomogeneity and pulse imperfections. Then we again fit into the e to the power of a m x or m y equal to mz to power of –t by T2. Then you can measure T2. So, we understood quite a bit about how to make the measurement of relaxation, etc.vIt is a very important concept. With this, we can jump to another topic called nuclear Overhauser effect; that is something which let us try to understand.

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Nuclear Overhauser Effect noe

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What is nuclear overhauser effect? In short it is called NOE. It is also a fairly difficult topic to understand. Let us see how we can understand.

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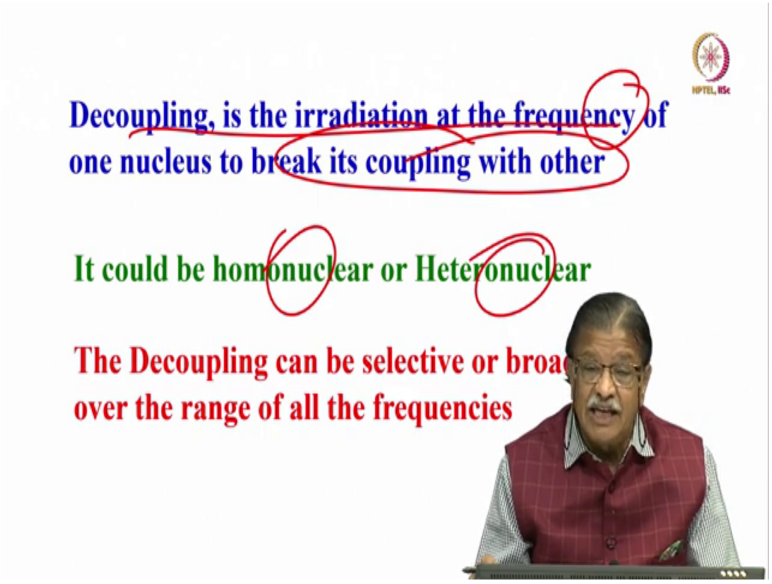

Decoupling, is the irradiation at the frequency of one nucleus to break its coupling with other

It could be homonuclear or Heteronuclear

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

Before that we will understand what is the decoupling, which we have discuss this in the previous class. There is no need to discuss in-depth in this class. But let me tell you in the previous course I discussed. What is the decoupling? It is the irradiation at a particular frequency of one nucleus to break its coupling with other. In fact, I mentioned this when we understood or discussing carbon 13 NMR.

Remember, I said that broadband decoupling; when you detect carbon 13, you apply radiofrequency power rf pulse at the proton frequency such that simultaneously you will break the coupling of all carbons with all the protons. Then you are going to get a single peak for each carbon. Of course, excluding any other spins other than protons, which are coupled it. If there are no other spins coupled, if you break the coupling of carbons with all the protons, you will get a single peak for each carbon. That is the decoupling phenomenon; called the broadband decoupling. So, this is what exactly happens in decoupling when you do by applying the frequency at the site of the other nucleus, you can break the coupling between these two nuclei. It could be homonuclear or heteronuclear; both are possible. You can do homonuclear decoupling and heteronuclear decoupling also.

And of course, this decoupling can be selective or broadband. In the case of carbon 13, it is broadband decoupling. All carbons are decoupled simultaneously with all the protons, that is a broadband decoupling; and it covers a whole range of frequencies. Whereas, in the case of homonuclear that type of broadband decoupling is not possible, because you have to apply the decoupling power for the magnetization in the transverse plane, it is not possible to do without distributing the spins.

So, there is a way to do that is called pure shift experiment. I come to that if there is time at a later stage. We will discuss pure shift also; touch upon it at least. But, in the case of homonuclear even from the early days of NMR, there is one thing we was practiced, it is called selective decoupling.

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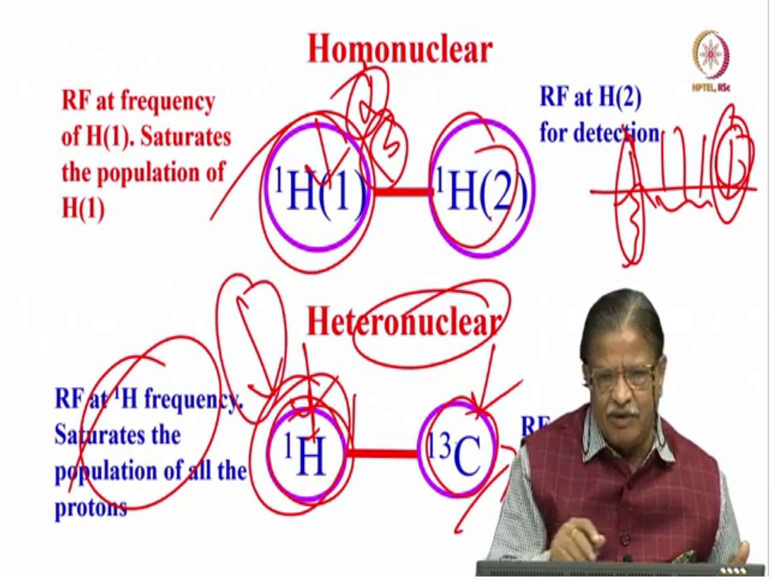

Homonuclear

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

RF at H(2) for detection

Heteronuclear

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

In the sense if there is a complexity of the spectrum, what we do is to selectively irradiate at a particular proton with the little rf sufficiently larger than the coupling, larger to break the coupling between proton which is coupled to other proton. Then you see the effect of this on the multiplicity pattern, if there is any change, at this at the other nucleus. Let us say I have one proton here other proton here, you apply a small radio frequency pulse here like this, on the proton H1 and you see the effect of it and the proton H2. So, what is the meaning of decoupling is, I am saturating the spin states of one particular proton, which I am radiating. This is exactly what we did in the broadband decoupling. We saturate the spin states, two spin states of spin half nuclei**.** And it so happens the spins will be going up and down, between alpha to beta, beta to alpha simultaneously, and so rapidly, the spin which is coupled to it will not see the spin either in the alpha state or in the beta state; in either of them, it sees the average field. As a consequence, what is going to happen? This will not be coupled to this. Or in other words, we say this proton is decoupled from this, because it is not seeing two different fields corresponding two spin orientations, alpha and beta of the coupled spin. This is what is called homonuclear decoupling, selective decoupling.

I can do select decoupling. If there are 10 spins, or 10 different protons that are chemically inequivalent, and I can irradiate and see the effect somewhere here, and these I do not touch upon at all. But if there is a coupling with this, the effect is seen; but I will not touch that proton. Since I am not touching at all, this is called selective decoupling, it is possible to do that selective decoupling.

In the heteronuclear case, of course, I said look at the carbon 13, do decoupling of proton or fluorine or any other nuclei need not be carbon and proton. Anything you can break; you saturate one of the nuclei in which you want to break the coupling. Carbon 13 usually in organic systems, we have plenty of carbons, plenty of protons couple among themselves. What we do is we apply a broadband radio frequency over the wide range at a particular rf power, for this proton and saturate the spin system of these protons, the population of these two energy states and then see the effect of this at the carbon 13. And this is what you are going to detect. You apply rf power here; strong rf power, which is larger than the coupling strength; again make the spin states spins to undergo transition rapidly between two spin states. So that the carbon that will not see in any of the protons in either alpha or beta states. And apply detective positive at carbon 13 and detect the signal. This is heteronuclear decoupling.

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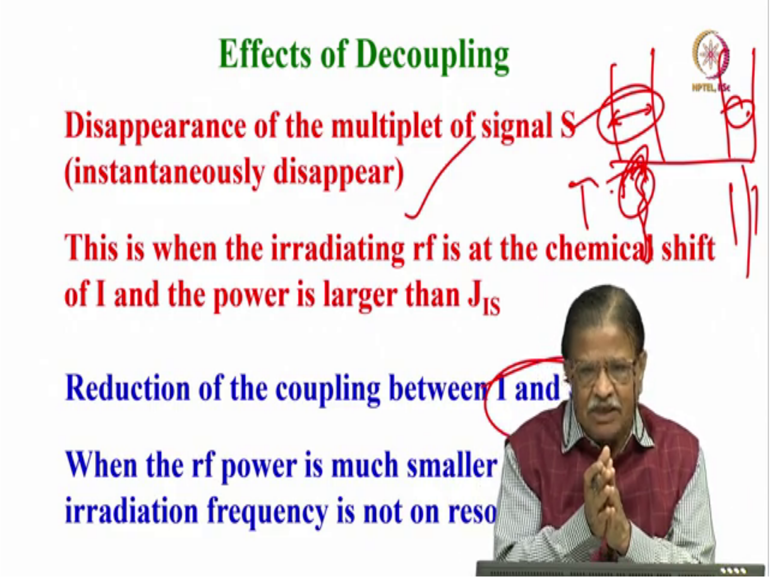

Effects of Decoupling

Disappearance of the multiplet of signal S (instantaneously disappear)

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

Reduction of the coupling between I and

When the rf power is much smaller

irradiation frequency is not on reso

Now, when you are doing decoupling some funny things do happen. What are these things which can happen. Effects of decoupling; first thing the disappearance of the multiplet of signal of S, it will instantaneously disappear because we are saturating that; decoupling means saturation; and I am saturating these two spins when I am decoupling, I will not see the signal, when they are say saturated there is no population difference.

So, decoupling of the spin will not give any signal. This is the first thing. So, it will disappear, you do not see signal at all. So, when I am irradiating rf at some chemical shift of I spin, let us say, which is coupled the S spin; and the coupling constant is J_{IS}. My decoupling power should be larger than this coupling strength, only then this phenomenon happens. There is a disappearance of the signal that can happen only when the irradiation with rf power is larger than coupling strength.

Sometimes it so happens if you are decoupling power is sufficient to not larger, there is a reduction in the coupling strength; there is a reduced coupling you will see, instead of this coupling. Instead of becoming 0 and giving a single peak at the center you will get to reduce coupling like this. This larger doublet will become a smaller doublet like this. This is reduction of the coupling between I and S spin.

When can it happen? It can happen when the rf power is smaller than JIS, much smaller. If not, you should be very much larger than JIS to break the coupling. If it is smaller, some funny thing will happen. Then the radiation frequency is not exactly on resonance also; or when it is smaller than the coupling strength. In other words, what I am telling you is;

generally what when you do the decoupling, you have to see that you apply the rf power exactly at the center of the doublet; that is what is the condition. So, what I am going to show now here; see I have two doublets which are two protons which are coupled. I irradiate at the center and the power should be larger than this; then this will become a singlet and no signal for this. That is this situation. Now, I am irradiate not at the center somewhere here, far away, slightly far away. That is called off-resonance.

Off- resonance irradiated; then it will not become a single peak, there is reduced coupling here; it will not become totally 0. Doublet will not disappear. That is one thing. Or you irradiate at the same place; but the power is less not sufficient larger than JIS; then also can happen. So these are all the side effects of decoupling; it can happen. The peak will completely disappear, the coupled peak will become a singlet, or doublet strength comes down. That is one thing when the power is less or when you are at off resonance. Not exactly on resonance.

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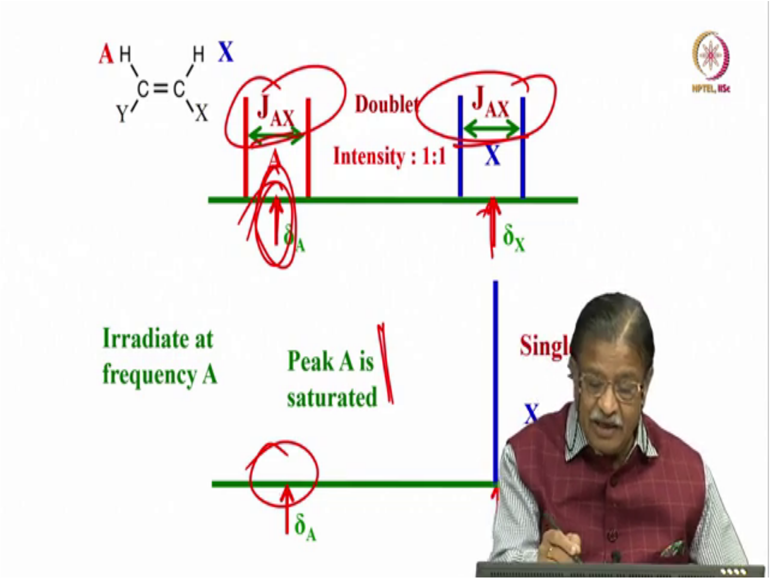

This is a situation like this. See when I am irradiating exactly the center A. See this is the chemical of X, intensity of peaks are equal. This is the chemical shift of A, where this JAX. This we discussed, we understood under Pople notation. Now, I am irradiating the proton A. Obviously, I will also not get signal, because peak A is saturated that we know. As a consequence, I do not see any signal at all. But nevertheless, there is a collapse of the doublet into a singlet, this what at the chemical shift of X, exactly the center; that can happen.

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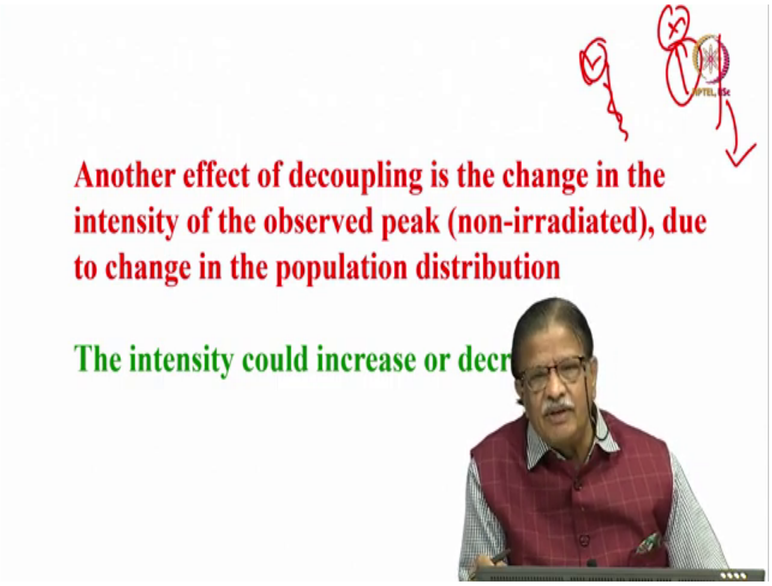

Another effect of decoupling is the change in the intensity of the observed peak (non-irradiated), due to change in the population distribution

The intensity could increase or decr

See another side effect of decoupling is the change in the intensity of the observed peak which is not irradiated due to change in the population of the other spin. See, I am irradiating this spin, spin A and spin X I am not irradiating. I irradiate this spin and I see the change in the intensity in the signal; the change could be either increase or decrease, it can go up or it can come down.

There is another interesting thing that can happen, it is the effect of decoupling that can also be seen. The change in the intensity of the observed peak, non-irradiated due to change the population of the irradiated spin; it can happen. And of course, there is another phenomenon which I did not discuss. It so, happens neither the peak will disappear nor there is a reduction in the coupling strength. It can happen you get additional peaks, more peaks in NMR; funny things can happen. Here, irradiating one peak of the couple proton, if you see other proton, instead of reducing the intensity or complete nullification, you get additional peaks. This phenomenon is called spin tickling.

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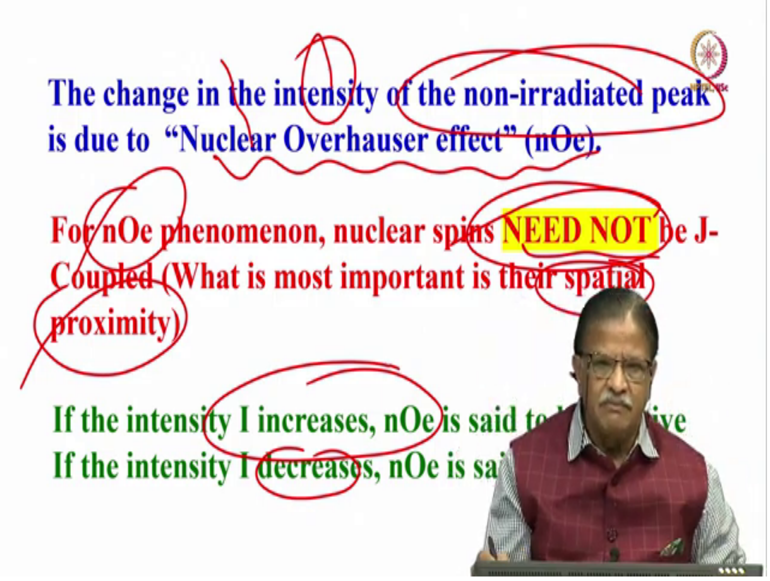

The change in the intensity of the non-irradiated peak is due to "Nuclear Overhauser effect" (nOe).

For nOe phenomenon, nuclear spins NEED NOT be J-Coupled (What is most important is their spatial proximity)

If the intensity I increases, nOe is said t ive
If the intensity I decreases, nOe is sai

We will not worry about those things, but what I wanted or what we are concerned is to see the change in the intensity of the non irradiated peak; this is the important point. We are irradiating one peak seeing the change the intensity of other proton. This effect, the change could be positive or negative; whatever it is, this effect is called nuclear Overhauser effect, it is in effect called NOE, nuclear overhauser effect.

And interesting point you should know for this nuclear Overhauser effect phenomenon, spins need not be J-Coupled. When we are decoupling, I said the spins were coupled. And we are breaking the coupling between them by using the second rf. Irradiating rf at the strength larger than the coupling strength, we broke the coupling; and made it completely decoupled.

That is a decoupling; broadband and selective. But for a NOE phenomenon, I irradiate a peak and see the change in the intensity of the other; but the spins need not be J-Coupled. Very interesting, so, if they are not J-Coupled you may ask me a question then how does the intensity change occurs? But remember, what is important is not J coupling but the spatial proximity. The two spins should be very close, preferably less than 5 Armstrong, then no he can be seen. The spatial proximity is important. No matter how many bonds they are separated. See in a three dimensional conformation it will happen one proton will be here undergoes was conformation, etc. And other proton is well separated with 10, 20 chemical bonds, but still may come close to each other in space; in a three dimensional space; does not matter as long as there is close in space this phenomenon can happen, this NOE phenomenon can happen. And there is a change in intensity you observe that is important point. Now, when the increase of intensity I takes place, we call it as a positive NOE case. The intensity I increases, you say there is a positive NOE. If the intensity of I decreases, we say NOE is negative; both can happen; you understand the concept now.

Important point: for NOE spins need not be J-coupled. What is important is spatial proximity. When you irradiate one spin there is a change in the intensity of the other spin. When the intensity increase, it the positive NOE, or when intensity of the other spin decreases it is negative NOE. These are the concepts, you must remember.

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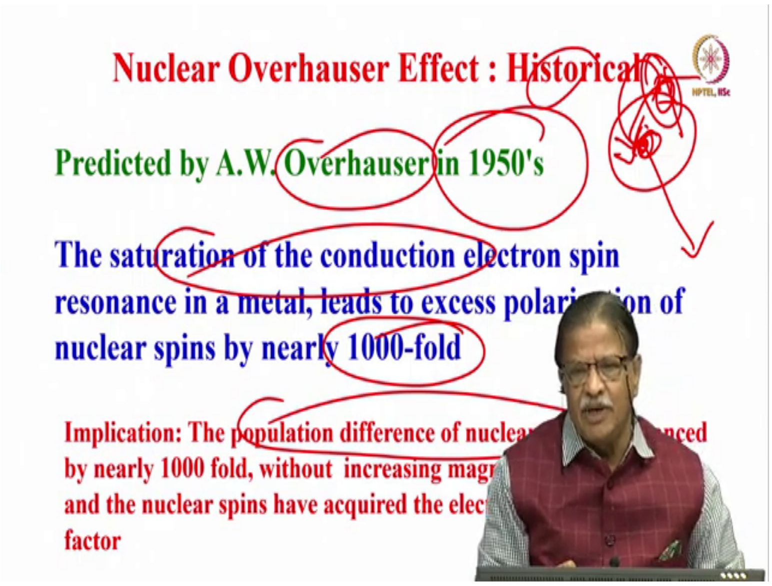

Nuclear Overhauser Effect : Historical

Predicted by A.W. Overhauser in 1950's

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

Implication: The population difference of nucle nced by nearly 1000 fold, without increasing magr and the nuclear spins have acquired the elec factor

Then, how this NOE develop? How did we come to know this phenomenon? A little bit of some historical facts about NOE. Why do we need it? We should know how it was discovered by Stalwatts, great scientists, they discovered this thing and made our life simple to understand. So, this is NOE; little bit about this thing.

It was predicted by overhauser; that is why it is called nuclear Overhauser effect. Remember, he predicted this in 1950; nearly 70 years back itself he said this; what a genius idea. See those days itself he was able to predict this can happen. What he did was not with nucleus and nuclear spins. What he did was he said was, the saturation of a conducting electron if is there in the metal, if a conducting electron spin is there in a metal you saturate that, Then he said nuclear spins get polarized 1000 times more, marvelous phenomenon. Remember NMR detection is very very less sensitive. The sensitivity is a big issue, less sensitive, we have been discussing, inspite of many sensitivity techniques; compared to all other molecular spectroscopy techniques. But look at this idea, we have nuclear spins, we have electrons; he says saturate the electrons. Electron spin of a particular metal; conducting electron; if you are going to saturated spin of that electron in a metal, he says the nuclear spins will gain polarization. The polarization gained by nuclear spins is 1000 times more; a marvelous idea. The implification of this means; the population of the nuclear spins is enhanced 1000 times because the electron spin polarization is transferred to nuclear spin. There is no coupling between these two, but nevertheless electron spin directly transfersthe polarization to nuclear spin. So, nuclear spins acquire electron spin polarization; as a consequence, the intensity goes up by 1000 times. If we calculate by Boltzmann population distribution, electron population Boltzmann population is much larger, compared to nuclear spins. So, nuclear spins now attain electrons spins Boltzmann population; and that is why the detection sensitivity enhanced 1000 times. And this was a brilliant idea given by Overhauser.

He did not say irradiate one spin and observe the change in the other nuclear spin; but what he said is saturate the electron spins; the conduction electron of a metal. Then you see the nuclear spin attain electron spin Boltzmann population; and enhances the intensity by nearly 1000 times, this is the idea given by overhauser in 1950s.

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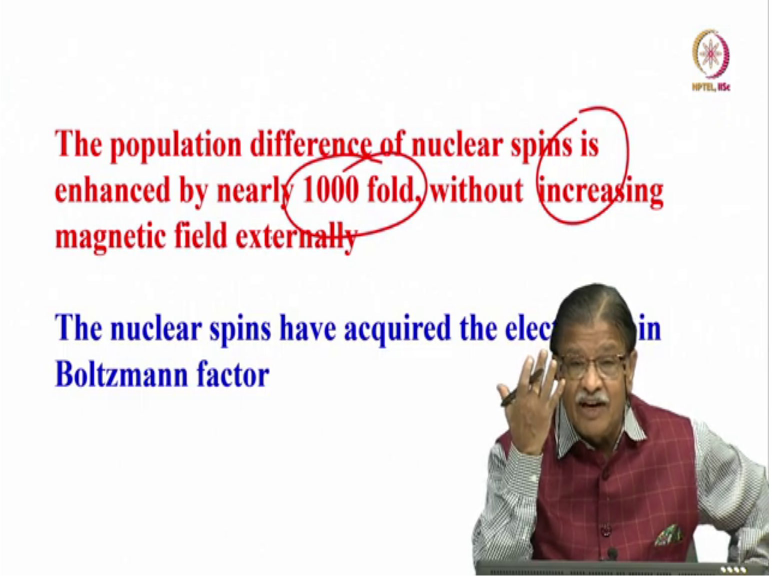

The population difference of nuclear spins is enhanced by nearly 1000 fold, without increasing magnetic field externally

The nuclear spins have acquired the elec in Boltzmann factor

And the population difference is enhanced by 1000 fold. Now, the interesting this is you did not increase the magnetic field externally. Remember, I told you we started increasing the magnetic field and in one slide I give an example, as you keep on increasing the magnetic field, the energy separation keeps increasing; and the population difference also change linearly with the magnetic field.

We even calculate it for different magnetic fields what is the population difference between different energy states. So now, 1000 fold change without increasing the magnetic fields; A phenomenal achievement; that was done. So, the nucleus spin acquired electron spin population without even in enhancing the magnetic field.

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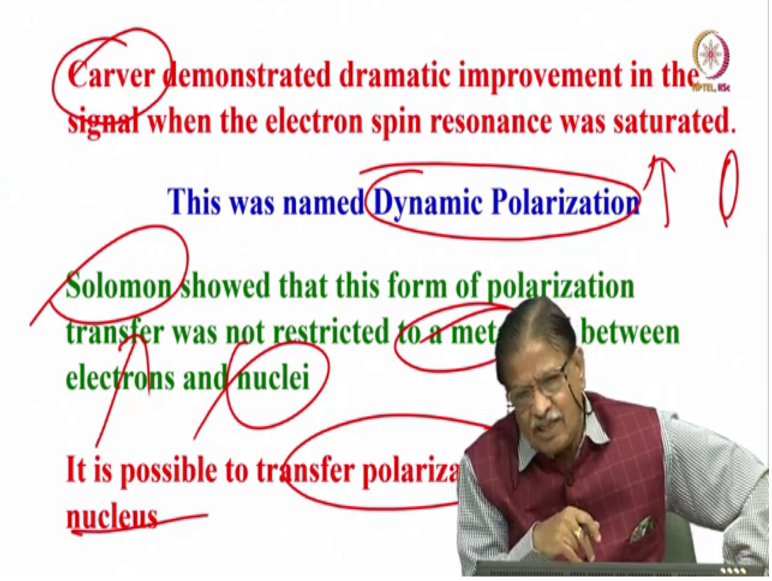

Carver demonstrated dramatic improvement in the signal when the electron spin resonance was saturated.

This was named Dynamic Polarization

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

It is possible to transfer polariza
nucleus

And this was demonstrated experimentally by another person called Carver and he really saw that he saturated the electron spin and observed the change in intensity of the nuclear spin; experimentally; he really observed that was the phenomenon; not just predicted. Overhauser predicted that and Carver observed that; and this was named dynamic polarization. Dynamic polarization happens. Why dynamic? because as soon as you saturate nuclear spins get the Boltzmann population of electrons, dynamically it happens. So, this was called dynamic polarization. And there was another scientist Solomon, Solomon what he did was, he showed this form of polarization transfer need not be for metals, between electrons and nuclei. It is not restricted only to the metals; it need not between electron spin and nuclear spin.

You do not have to saturate the electrons; and enhance the nuclear spin polarization. He said it is possible to transfer polarization from nucleus to nucleus also. Irradiate one nucleus and see the change in the intensity of the other, exactly identical phenomenon. But only thing is here, the Boltzmann population is not the electron Boltzmann population, it is a different phenomenon. It is for the nuclear spin, you have a small change in intensity, not 1000 fold but small change. We will see how much is the change in intensity, we can work out as we go ahead. May be in this class or in the next class we will see what is the change in intensity. So, it is possible not only for transfer of the polarization from electron to nucleus, he showed it is possible to transfer from nucleus spin to nuclear spin. Irradiate one nuclear spin see the polarization transferred to other nuclear spin, change in the enhancement of intensity of that particular spin.

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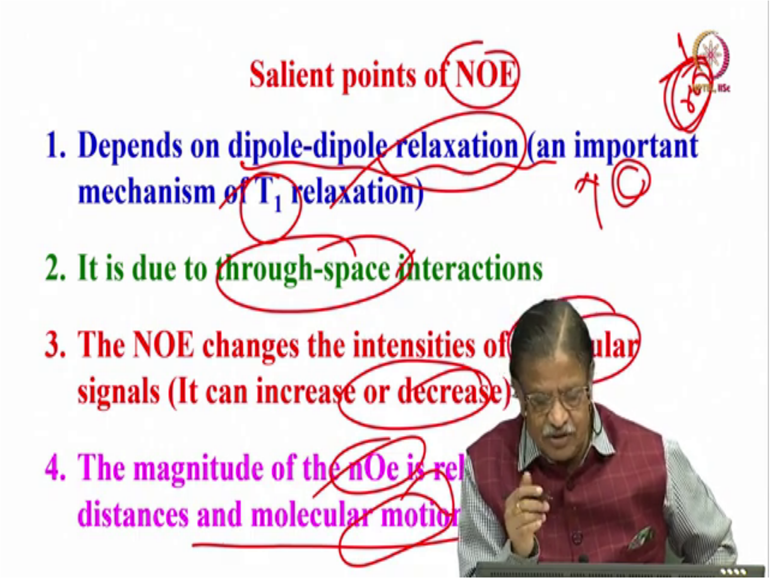

Salient points of NOE

1. Depends on dipole-dipole relaxation (an important mechanism of T_1 relaxation)
2. It is due to through-space interactions
3. The NOE changes the intensities of ular signals (It can increase or decrease)
4. The magnitude of the nOe is rel distances and molecular motion

So, with this we will try to see some salient points of NOE. Each of these points we will discuss as much as possible. Well, I will take all of you through this slowly and steadily as much as possible we will discuss each point. The salient points of NOE. One is, it depends upon dipole-dipole relaxation. Of course, we discussed this in relaxation; that is why I introduced relaxation before and moving.

It is an important mechanism; is the dominant mechanism for relaxation, especially spin lattice relaxation we discussed, the dipole-dipole relaxation is a dominant relaxation mechanism, especially for spin half nuclei for T1, we saw that. We know that nuclear spin undergoes molecular motion; its orientation with respect to the magnetic do not change between the two nuclear spins, they orientation with respective magnetic field remain the same. But because molecules are undergoing tumbling, there is a change in the local field generated at the site of the other spin, which has a frequency analogues to Larmor frequency. If you can calculate the frequency of that motion that induces transfer of energy from spin to lattice, that we observed. The dominant dipole-dipole interaction phenomenon; that is one of the relaxation phenomenons dominant in NOE.

NOE is dipole-dipole relaxation phenomenon. And it is due to through-space interaction; there is no need of chemical bond. I told you already what is needed is the spatial proximity of the two nuclear spins. They should be close in space that is enough. If they are far away there is no transfer of polarization. So, for this to happen, the spin should come close in space then the energy transfer takes place.

When this polarization transfer takes place you see the gain in signal intensity. And so, it is not through covalent bond, it is the direct through space interaction; it is through space that is one point. And NOE changes intensity of the particular signal only particular signal; if you are irradiating the particularly signal it changes intensities of the other spin which is in close proximity; something else which is far away which is not connected to this will not affect at all.

It will affect the intensity of sspin which is close in space, it will change, increase or decrease in intensity, only particular spin, it will change the intensity. And the magnitude of this NOE is related to inter-nuclear distance and molecule motions; that is correct. I told you his spatial proximity is important condition, farther the distance, as we go ahead and tell you NOE depends upon one over r to the power of 6.

If the distance slightly increases, the power of six is in the denominator. So drastically the intensity of the signal comes down. The polarization transfer will not take place. So, NOE phenomenon depends upon the distance between two spins which is the inverse of r power 6 that is the important thing. And of course, it also depends upon the molecular motions we know , because dipole relaxation everything we discussed, the spectral density function everything we discussed.

We will talk about it this time also if there is a time, we have to talk about those things. So, these are all some of the important points; the magnitude of the NOE is dependent on the nuclear spin distances.

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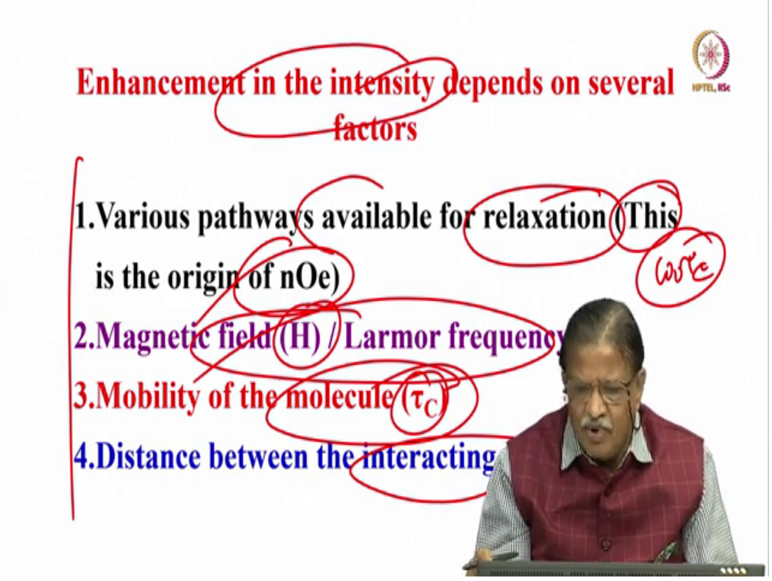

Enhancement in the intensity depends on several factors

1.Various pathways available for relaxation (This is the origin of nOe)

2.Magnetic field (H) / Larmor frequency

3.Mobility of the molecule (τ_C)

4.Distance between the interacting

$\omega\tau_c$

And enhancement to the signal intensity: there is a change in intensity of the signal. It depends upon several factors. One thing, of course, distance between the interacting spins which we discussed, I already said it is one over r power six. That is one thing. And it depends upon the mobility of the molecule tau C. Of course, we discussed about this tau C, J W everything earlier. Tau c depends upon the size of the molecule, depends on molecule mobility.

Most important thing it also depends upon magnetic field, a Larmor frequency that is what we discussed; omega tau C. We discuss omega tau C condition which is greater than 1 equal to 1 less than 1, what will happen? For small molecule, big molecules, medium sized molecules, we discussed relaxation. So, it depends upon the magnetic field. In other words, it depends upon omega 0 Larmor frequency.

And also most important thing, it depends upon various pathways available for relaxation. The relaxation phenomenon we discussed. Mechanism involved in relaxation we understood; there are several path the spins can relax. What are those pathways? How it relaxes in a particular pathway? How the intensity changes? This are the most important things we have to understand.

Actually, this is the origin of NOE. The origin of NOE is how the nuclear spin relax? which is a pathway it adapts for relaxation; that defines. So, NOE is the enhancement in the intensity of NOE depends upon several factors, many things are there; which we will discuss. But most important points is; it depends upon the relaxation pathway which is available; and which is the dominant relaxation pathway; and also it depends upon the Larmor frequency.

For example, if I do the experiment at 600 megahertz spectrometer the same NOE you may not get at 200 megahertz. Larmor frequencies are different, enormously different, 200 megahertz her, here it is 600 megahertz, quite a bit, nearly three times larger. Similarly, mobility, a big molecule like protein give NOE in a different way compared to a small molecule, also depends upon distance.

So, these are the important factors, parameters which dictate the change in the intensity because of NOE.

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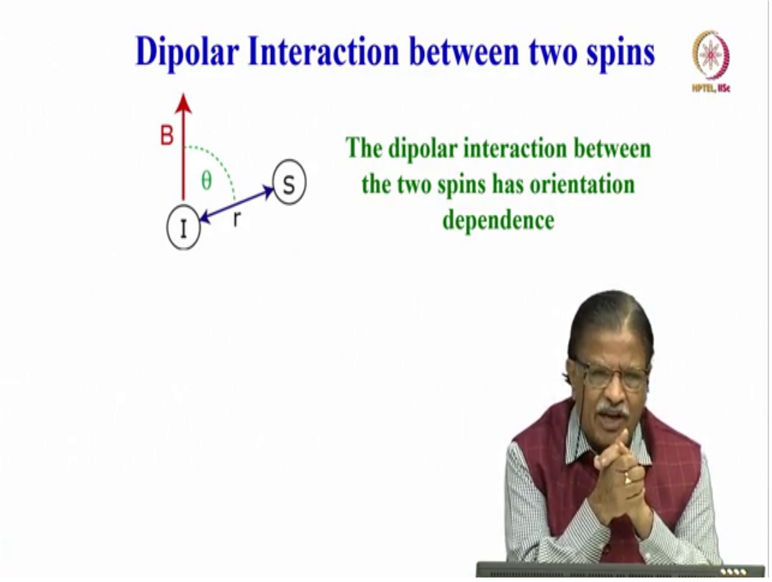

Dipolar Interaction between two spins

The dipolar interaction between the two spins has orientation dependence

So, what we will do is, we will discuss about the dipolar interaction and various things, what will happen everything later? But now since the time is getting up, what I am going to do is? I will stop today, what we discussed today is after discussing all about relaxation and everything since the last class, we started jumping into understanding what is the NOE. NOE as I said is the nuclear overhauser effect. It was predicted by overhauser in 1950's itself.

He said saturate an electron, conducting electron of a metal, you will see the polarization of the nuclear spin gets enhanced by 1000 times; marvelous and a remarkable phenomenon. The nuclear spins now attain electron spin Boltzmann population, what is what we understood. So electron Boltzmann population is attained by nuclear spins. So, after some time, a few other stalwarts said it need not be restricted to electrons or nucleus, it can be between two nuclear spins also.

That is also possible; NOE, like overhauser predicted the enhancement in the signal intensity is also possible between nuclear spins, not only restricted to electrons and nucleus. It is also a demonstrated but only thing is Boltzmann factor is not electron Boltzmann factor this time; that is a different question. But, still there is an enhancement and enhancement depends upon various factors. The factors depends upon pathways available for relaxation. And it depends upon the Larmor frequency, depends upon the mobility of the molecule, tau C and distance between interacting spins. And various factors are responsible for understanding the NOE. They contribute for the change in NOE. Each of these things we will discuss in the next class. So, I will stop today, in the next class we will continue further. What are the relaxation pathways, how it affects everything we will discuss in detail. Thank you.