Application of Spectroscopic Methods in Molecular Structure Determination

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Lecture - 09
Nuclear Overhauser Effect (NOE) and its effects in NMR spectroscopy

Hello, welcome to module 9 of the course on application of spectroscopic methods in molecular structure determination. In this module, we will have a look at the nuclear overhauser effect. We have already briefly mentioned about nuclear overhauser effect during the carbon that is spectroscopic lesson. And in this module, we will look at the origin of the nuclear overhauser effect and it is effects on the NMR spectra.

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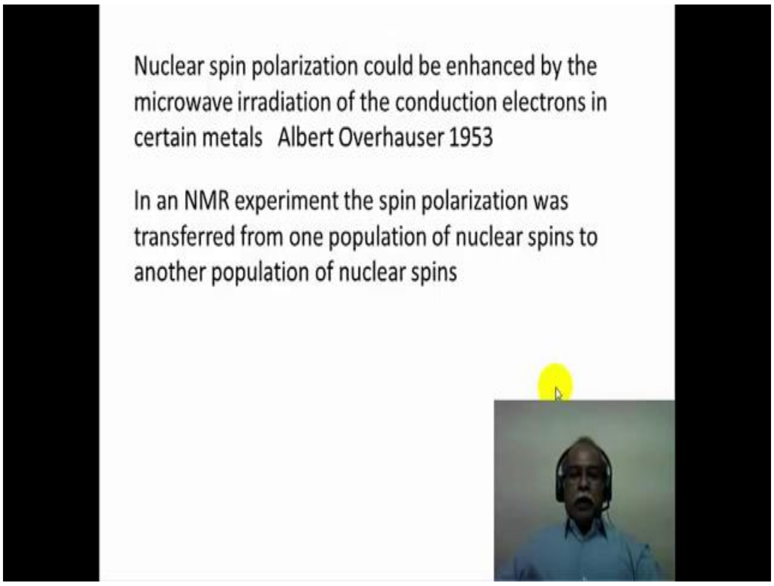

Nuclear spin polarization could be enhanced by the microwave irradiation of the conduction electrons in certain metals   Albert Overhauser 1953

In an NMR experiment the spin polarization was transferred from one population of nuclear spins to another population of nuclear spins

Now, the phenomenon of nuclear overhauser effect was discovered in 1953 by Albert Overhauser, hence the name overhauser effect. He was studying metals and what he observed was that the nuclear spin population could be enhanced by the microwave irradiation of the conduction electrons in certain metals. In other words, he was doing a double irradiation experiment of irradiating the electrons spin using a microwave irradiation and simultaneously observing the nuclear spin polarization. Now in the NMR experiment, we do not deal with electron spin polarization. We talk about spin polarization transfer from one population of the nuclear spins to another population of nuclear spin. In other words, it could be a spin polarization between carbon 13 and proton or proton and proton

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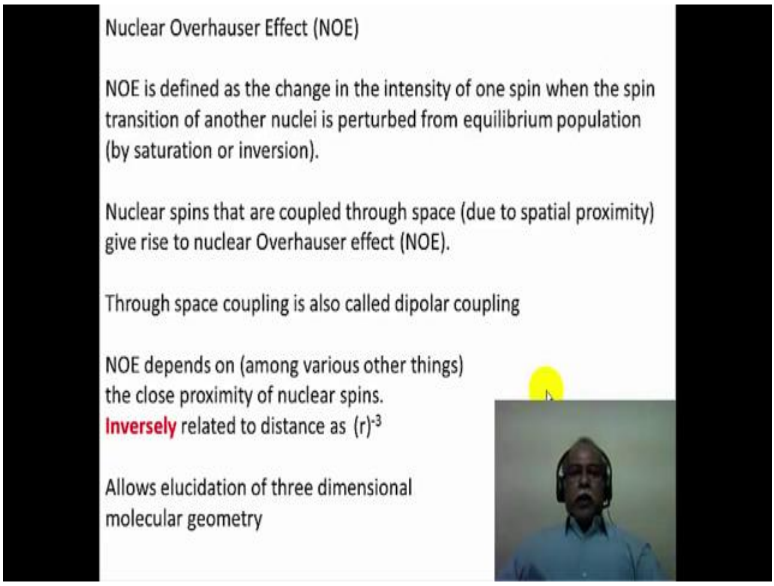

Nuclear Overhauser Effect (NOE)

NOE is defined as the change in the intensity of one spin when the spin transition of another nuclei is perturbed from equilibrium population (by saturation or inversion).

Nuclear spins that are coupled through space (due to spatial proximity) give rise to nuclear Overhauser effect (NOE).

Through space coupling is also called dipolar coupling

NOE depends on (among various other things) the close proximity of nuclear spins.
Inversely related to distance as $(r)^{-3}$

Allows elucidation of three dimensional molecular geometry

So, NOE can be defined as the change in the intensity of one nuclear spin. When the spin transitions of another nucleus is perturbed from equilibrium population. In other words, at room temperature, normally you have the equilibrium population when two spins are present and this equilibrium population of one of the spins can be disturbed if the other nucleus is simultaneously irradiated with the second radio of frequency.

Now, nuclear spins that are coupled through space in other words due to spatial proximity give rise to the nuclear overhauser effect. They need not be bounded directly; there can be a through space interaction and this interaction is known as dipole-dipole interaction. Through space coupling is also therefore known as the dipolar coupling. The nuclear overhauser effect among many other things depends on the close proximity of the nuclear spin.

In other words, the enhancement that one sees is inversely proportional to the distance; if r is the distance between two non-bounded hydrogen then, the NOE is inversely proportional to r to the power 3 or proportional to r to the power minus 3. This kind of a spatial relationship allows one to elucidate three-dimensional molecular geometries and structures and hence the power of nuclear overhauser effect.

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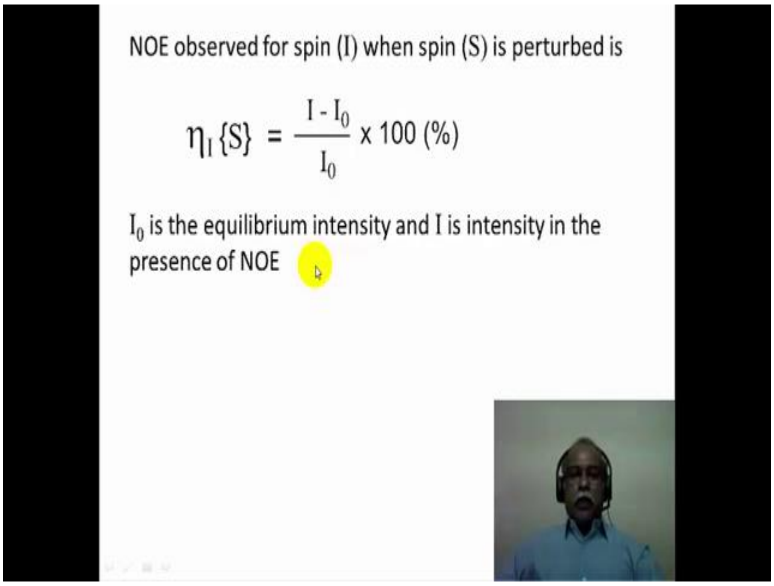

NOE observed for spin (I) when spin (S) is perturbed is

$$\eta_I\{S\} = \frac{I - I_0}{I_0} \times 100\ (\%)$$

I_0 is the equilibrium intensity and I is intensity in the presence of NOE

Now, the nuclear overhauser enhancement that one can observe for a spin I when, another spin S is perturbed is given by this expression. This is the expression that is used for enhancement of nuclear overhauser effect on the spin I when S is saturated or S is perturbed by a second double irradiation using a second radio frequency wave. Now, this corresponds to I minus I 0, where I is the intensity of the signal in the presence of a NOE effect; I 0 is the intensity of the signal without the NOE effect divided by I 0 times 100 gives the percentage of the nuclear overhauser enhancement. Now I 0 is the equilibrium intensity, which is normally measured without the double irradiation and I is the intensity in the presence of NOE which is measured in the presence of a double irradiation experiment.

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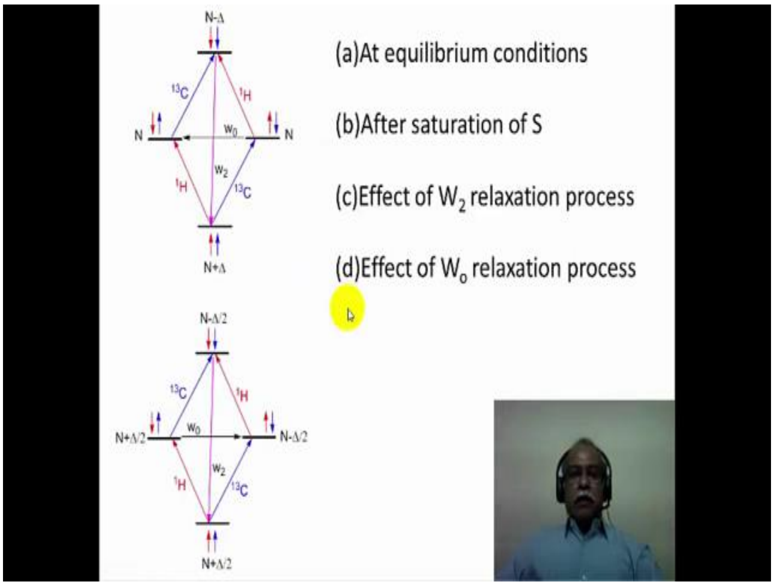

(a)At equilibrium conditions

(b)After saturation of S

(c)Effect of W_2 relaxation process

(d)Effect of W_0 relaxation process

Now, let us look at the origin of the nuclear overhauser effect. Let us consider a spin of proton and another spin, which is carbon 13. In other words, we are considering two spins namely proton and carbon 13 and they are color coded as red for proton and carbon 13 is color coded as blue. Now for the two spins, there are four possible orientations that are possible or four possible energy levels are possible. Both the spins are aligning with the external magnetic field; there should be the ground state; one spin is aligning with the external magnetic field and another spin is opposing the external magnetic field. In this particular case, the blue spin is aligning with the external magnetic field and the red spin is opposing the external magnetic field, and it is other way around, in this particular scenario. Finally, the higher energy level contains both the spins opposing the external magnetic field, when the magnetic field applied in this particular direction.

Now, the blue transition corresponds to the carbon 13 spin inversion and the red transition corresponds to the protons spin inversion and these are singled quantum transition. In other words, there is only one spin that is changing it is orientation with respect to the external magnetic field. Therefore, they are called the single quantum transitions.

Now we have to consider four different types of scenario. We have to consider at the equilibrium condition what is the population of the two spins; and after saturation of one of the spin let us say the proton is irradiated and saturated what happens to the population, and what happens when you have a affect of this particular deactivation, in other words relaxation process. This is a double conduct relaxation. If you look at it both the spins which are opposed to the external magnetic field get inverted to the opposite direction in terms of aligning with the external magnetic field. So, the delta m is actually two in this particular case, and this is allowed transition. We can also have transition from; this is the W 0 transition, the arrow should be actually from this direction to this direction, if the proton is getting saturated. And it is drawn correctly in the bottom figure for example, here.

Now, let us look at the equilibrium condition. This would be equilibrium condition, where you have a slight excess population in the ground state. Let us assume there are four n spins are available totally, total number of spins is equal to four n. So, you distribute equally n number of spins in all the levels, except in the ground level you have slight excess of population which is indicated by delta. And there will be a small population deficiency in the excited state which is n minus delta, whereas other two energy levels have the population of n each. So, you can see that the population difference between these two states is delta, these two states is also delta, the population difference between these two states is also delta and these two states also delta.

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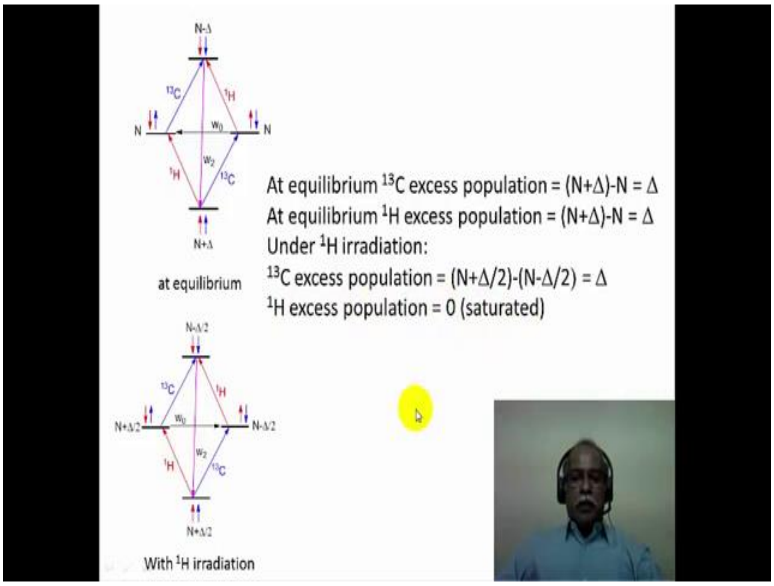

at equilibrium

At equilibrium ^{13}C excess population = (N+Δ)-N = Δ

At equilibrium ^{1}H excess population = (N+Δ)-N = Δ

Under ^{1}H irradiation:

^{13}C excess population = (N+Δ/2)-(N-Δ/2) = Δ

^{1}H excess population = 0 (saturated)

With ^{1}H irradiation

So, at equilibrium, if you look at the excess population of the carbon 13 which would be this difference or the this difference that would correspond to the difference between the population of this state minus this particular state which would correspond to n plus delta minus n which is corresponding to delta. Delta is the excess population in the carbon 13 at equilibrium; let us say at room temperature. Now the equilibrium proton population difference is between these two states or these two states, you can take these two states for example; the population difference would be n plus delta minus n which is equal to delta. So, there is a population difference of excess population of delta in the carbon 13 equilibrium at the carbon 13 population; at equilibrium, the proton population also there is a difference of delta.

Now what happens when the proton is irradiated; in other words, this is this scenario when the proton gets irradiated. We are talking about irradiation so that the population becomes nearly equal. In other words, saturation is what we are talking about in terms of second radio frequency corresponding to the frequency of the proton is being applied, and the proton resonance of the proton spins get excited to the higher excited states such that the population now is equal between the two proton levels. Similarly, between these two levels also the population difference is essentially same. Now in the presence of the proton irradiation, now if you look at the carbon 13 excess population, this would be the difference between this level and this level, which again corresponds to delta. If we look at the difference between these two levels, because it is saturated the population excess populations of the proton will be 0 because of the saturation that we talk about.

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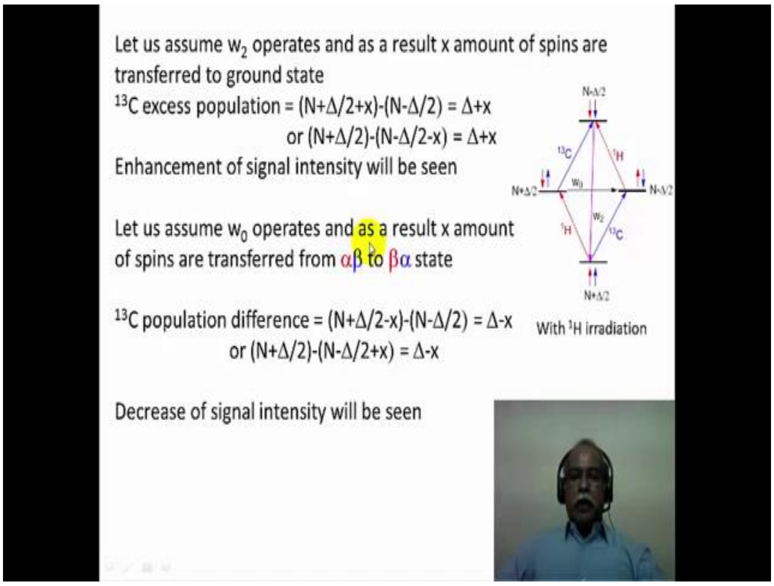
Let us assume w_2 operates and as a result x amount of spins are transferred to ground state

^{13}C excess population = $(N+\Delta/2+x)-(N-\Delta/2) = \Delta+x$

or $(N+\Delta/2)-(N-\Delta/2-x) = \Delta+x$

Enhancement of signal intensity will be seen

Let us assume w_0 operates and as a result x amount of spins are transferred from $\alpha\beta$ to $\beta\alpha$ state

^{13}C population difference = $(N+\Delta/2-x)-(N-\Delta/2) = \Delta-x$

or $(N+\Delta/2)-(N-\Delta/2+x) = \Delta-x$

Decrease of signal intensity will be seen

With ^{1}H irradiation

Now, let us assume that the double quantum relaxation is operational. If the quantum relaxation is operational and let us assume there is a x number of spins that are getting transferred to the ground state from the excited state; in other words, from this state we subtract x number of spins and bring it to the ground state. So, the population in the level ground state will be n plus delta by 2 plus x, the population here would be n minus delta divided by 2 minus x, so that is the population now when the W 2 is in operation.

So, let us now calculate the excess population of the carbon 13 spins between these two levels if you take for example, this would be n plus delta divided by 2 plus x, this is a population of this particular state. Population of this state is n minus delta divided by 2. So, if you work out these numbers, this would be delta plus x in other words there will be slight excess population in compare to the equilibrium population in the carbon 13 levels. Therefore, the signal intensity will be enhanced for the carbon 13 because of the double quantum relaxation being operational.

Now, let us assume that the W 0, which is also a double quantum relaxation that is operating. As result of that let us assume that x amount of the spins are transferred from the alpha beta state to the beta alpha state. Now the population difference, if you calculate, you remove minus x from here and add 2 plus x this sate because x number of spins are being transferred from this state to this state. Now the population difference if you look at n plus delta 2 minus x which would be this particular spin state minus n minus delta by 2, which is equal to delta minus x.

In other words, there is a depletion of population upon the W 0 being operational, and this would essentially decrease the signal intensity, this would correspond to nuclear overhauser enhancement, this would be nuclear overhauser in the reverse direction, in other words a negative nuclear overhauser effect is what is being operational when W 0 is operating. When you talk about carbon 13 accumulation using proton double irradiation; it is W 2, which is predominantly operative. So, one always looks at the nuclear overhauser enhancement with the proton decoupling experiment of the carbon 13 spectroscopy

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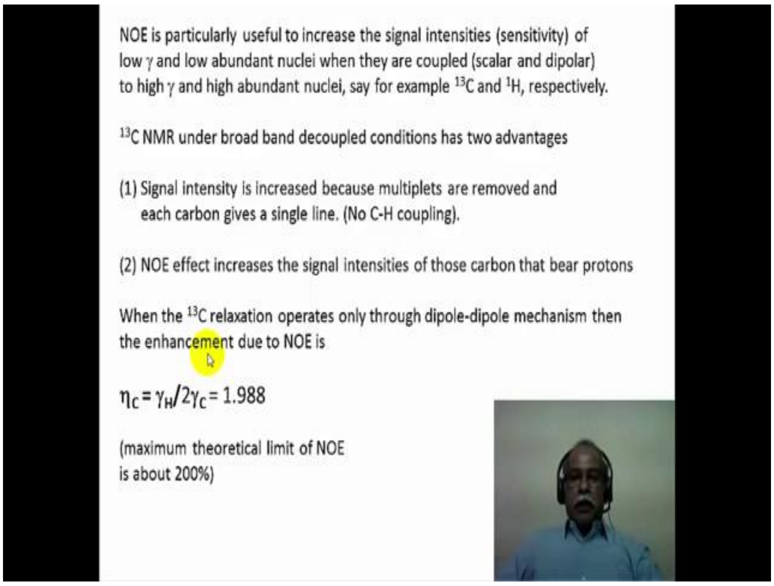

NOE is particularly useful to increase the signal intensities (sensitivity) of low γ and low abundant nuclei when they are coupled (scalar and dipolar) to high γ and high abundant nuclei, say for example ^{13}C and ^{1}H, respectively.

^{13}C NMR under broad band decoupled conditions has two advantages

(1) Signal intensity is increased because multiplets are removed and each carbon gives a single line. (No C-H coupling).

(2) NOE effect increases the signal intensities of those carbon that bear protons

When the ^{13}C relaxation operates only through dipole-dipole mechanism then the enhancement due to NOE is

$\eta_C = \gamma_H / 2\gamma_C = 1.988$

(maximum theoretical limit of NOE is about 200%)

Now, NOE effect is particularly useful to increase the signal intensity or increase the sensitivity of the technique of low abundant as well as low gamma nuclei when they are coupled to either through scalar coupling that is j coupling or through space coupling namely the dipolar coupling to a high gamma and high abundant nuclei. The example that we are talking about is the carbon 13 which is a low gamma as well as low abundant nuclei and it is coupled to proton which is high abundant and a high gamma nucleus, under these conditions NOE is particularly useful. Therefore, carbon 13 under broad band decoupled condition has two advantages. First of all, because the multiplicity is lost the signal intensity will automatically increase because all the multiplets will collapse to a singlet, but there is no carbon hydrogen coupling under those conditions. In other words, you have decoupled the carbon-hydrogen coupling.

Secondly, the NOE effect operates at increases the signal intensity, because of the reason we discuss in the earlier slides as how the signal intensity increases because of the population difference being higher under the double irradiation condition compare to the population difference under thermal equilibrium. So, the NOE effect essentially increases the signal intensities of the carbons that where the protons, this is because of the scalar coupling, the effect would be felt in here. It need not be coupled directly through space coupling also it is possible to have the NOE enhancement.

When the carbon 13 relaxation operates only through dipole-dipole mechanism then the enhancement is given by this expression. In other words, the enhancement of the carbon intensities signal intensity is corresponding to or equal to gamma of hydrogen divided by 2 times gamma of carbon which works out to be 1.988, because gamma of carbon is one-fourth gamma of the hydrogen atom. Therefore, the max maximum theoretical limit of enhancement that one can observe in a carbon 13 spectrum under the conditions of proton double irradiation would be roughly 200 percent or so. In other words, intensity of the signal will be three times because original intensity let us say x if it increases by 200 times then the total intensity that you would observe upon the irradiation of the proton would be three times the intensity under equilibrium conditions.

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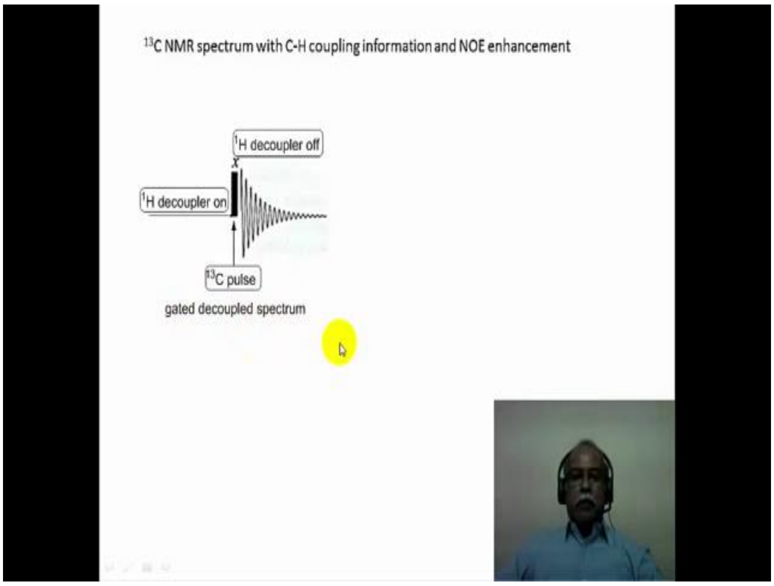

^{13}C NMR spectrum with C-H coupling information and NOE enhancement

gated decoupled spectrum

Now, let us have a look at the diagram that, explains the enhance nuclear overhauser effect being observed in a carbon-13 spectrum. Now let us say for example, this is where the carbon 13 pulse is applied. So, this will be the pre-pulse duration and this will be the free induction decay and then there will be a delays, before the second pulse that takes place. This is the diagram of the pulse sequence in a gated decoupled spectrum. In other words, the proton de coupler is turned on. In other words, the proton spins are now getting saturated at this stage, and it is turned off at this point where the carbon 13 pulse or the radio frequency corresponding carbon 13 is applied as a pulse. And after the pulse is over, the free induction decay starts at the time the pulse of the carbon thirteen excitation stops the proton de coupler is also turned off. In other words, both simultaneously the proton de coupler as well as, the carbon 13 pulse are turned off at this stage and then the free induction decay is observed.

Now, what will happen initially you have saturated the proton by turning on the decoupler up to this point. So, the populations are now almost equal here. So, the NOE is going to setup here. Now the proton now gets decoupled the decoupler is turned off here therefore, the proton carbon coupling information will be retained here. Now the difference is the spin inversion takes place instantaneously, whereas the nuclear overhauser effect or the nuclear overhauser effect takes sometimes to develop in terms of the time duration that is necessary for a NOE to be observed is much higher compare to the spin in version which are instantaneous. So, during the free induction decay the NOE gets developed. So, what you get is a C-H coupling information along with the NOE enhancement and this is spectrum with the C-H coupling information because the coupler is turned off here along with the NOE enhancement is what is seen in the gated decoupled spectrum.

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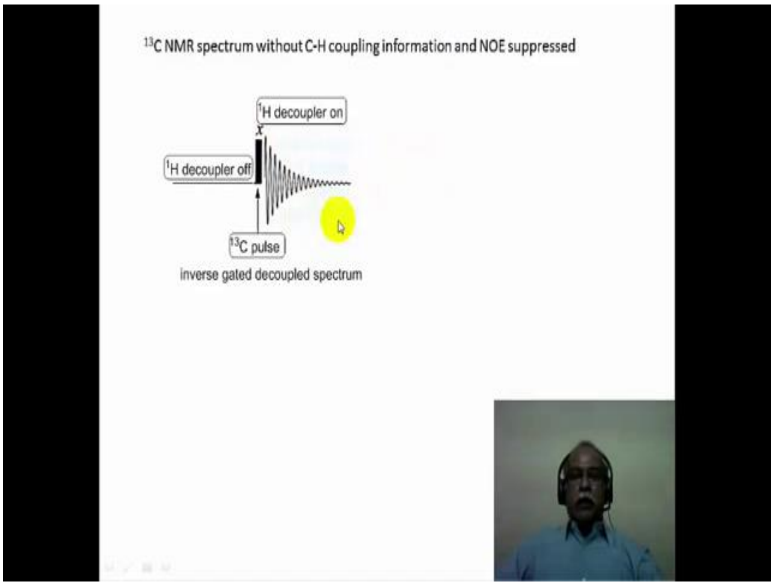

^{13}C NMR spectrum without C-H coupling information and NOE suppressed

^{1}H decoupler on

^{1}H decoupler off

^{13}C pulse

inverse gated decoupled spectrum

Now, in the case of carbon 13 without the C-H coupling information, NOE is suppressed this is a kind of pulse sequence that one has; this is just an exactly the opposite of the earlier pulse sequence. In the case of the pre-carbon 13 pulse, the decoupler is turned off the decoupler is turned on along with the carbon 13 pulse here and the decoupler is on throughout the free induction dyne. In other words, when the decoupler is on the C-H coupling information will be completely lost, here the decoupler is off for example, so NOE also will not be present. So, this is known as the inverse gated decoupling in spectrum; in other words, both this C-H coupling information as well as NOE are suppressed in this particular accumulation of the scans.

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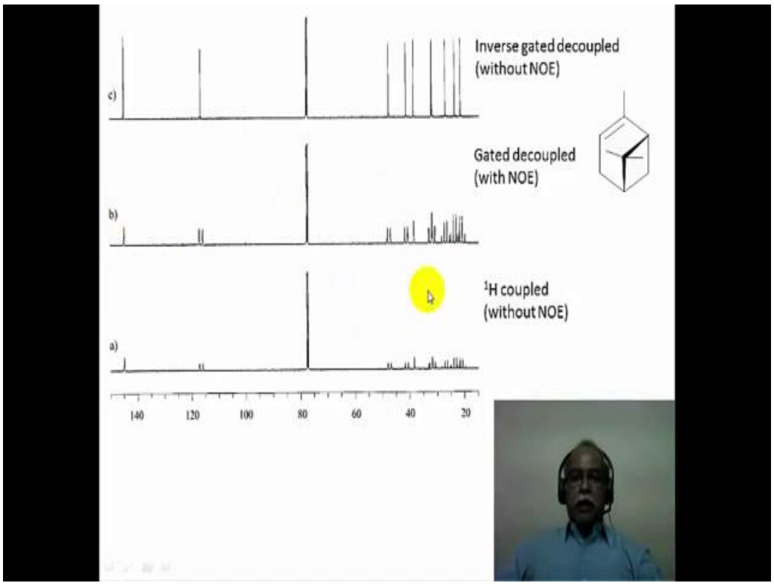

This is illustrated in the traces here, which are the carbon 13 spectra of this particular compound known as alpha pynen. Now if you look at the bottom trace, you have the proton coupled without the NOE; in other words, the proton is coupled and there is no double irradiation of the proton signal prior to the turning on the signal of the carbon 13 pulse. So, there is no NOE build up and this is a kind of signal intensity that one can observes.

Under identical condition, if the gate decoupled spectrum is scanned, in other words if the decoupler is turned here, and then turned off this sequence here, this is what happens, you can see the enhancement of the signal under identical condition in terms of the concentration of the substrate as well as the number of scans here. So, you can see the enhancement of each one of the signal because of the NOE, but the coupling information is retained in everyone of the carbon signal where the coupling is directly between the carbon and the hydrogen.

The last trace in other words the top trace corresponds to the inverse gated decoupled spectrum that is without the NOE, you are observing the signal intensities without the coupling as well as without the NOE because, it is without coupling the signal intensities are much higher than the other two spectrums. And because it is without the NOE, it does not matter whether you have a quaternary carbon or C-H 2 carbon or C-H 3 carbon they all have equal intensity. In fact, this kind of spectroscopic methods that one can use to quantify the carbon 13 spectrum; otherwise, quantifying the carbon 13 spectrum is not easy because of the differential spin relaxation times as well as the differential NOE is of the various types of carbon in an organic compound. So, the inverse gated decoupled spectrum is useful for quantification of the carbon 13 intensities, whereas the gated NOE spectrum is useful to observe the NOE effect simultaneously with the coupling information. And of course, the broad band decoupling essentially gives you the NOE effect and the completely decoupled spectrum of the carbon 13. So, these are the various modes in which the carbon spectra can be accumulated.

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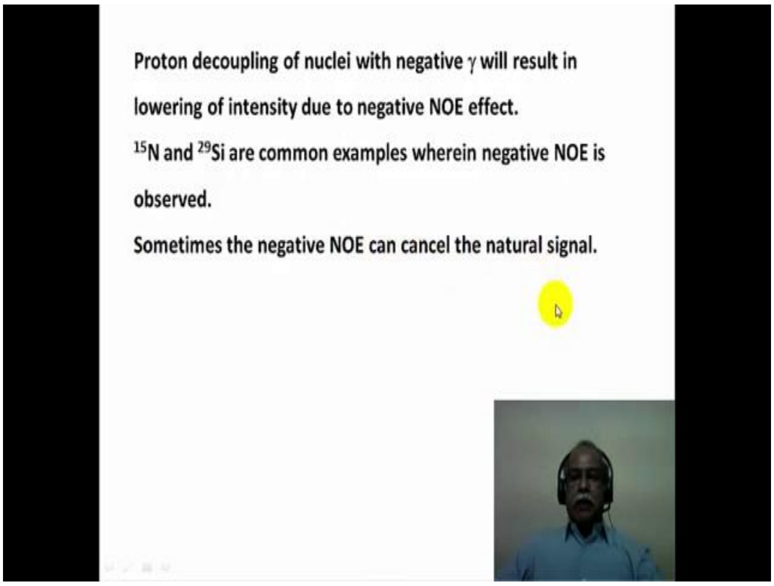

Proton decoupling of nuclei with negative γ will result in lowering of intensity due to negative NOE effect.

^{15}N and ^{29}Si are common examples wherein negative NOE is observed.

Sometimes the negative NOE can cancel the natural signal.

Now, proton decoupling of any nuclei, which has the negative gamma will result in the lowering of the intensity due to negative NOE effect. Remember the NOE effect here, if the gamma of the observing nuclei is happens to be negative then overall the intensities is going to be negative intensity; in other words, there is going to be depletion of intensity. For example, when there is a proton coupling takes place nitrogen 15 or silicon 29 are the common examples where negative NOE is observed, because the gamma value for the nitrogen 15 and silicon 29 are negative in nature. Sometime negative NOE can actually cancel the natural signal of the sample. If the natural signal intensity and the negative NOE corresponds to the same amount then the signal will be nullified that is what meant by saying that the NOE can cancel the neutral signals natural signal.

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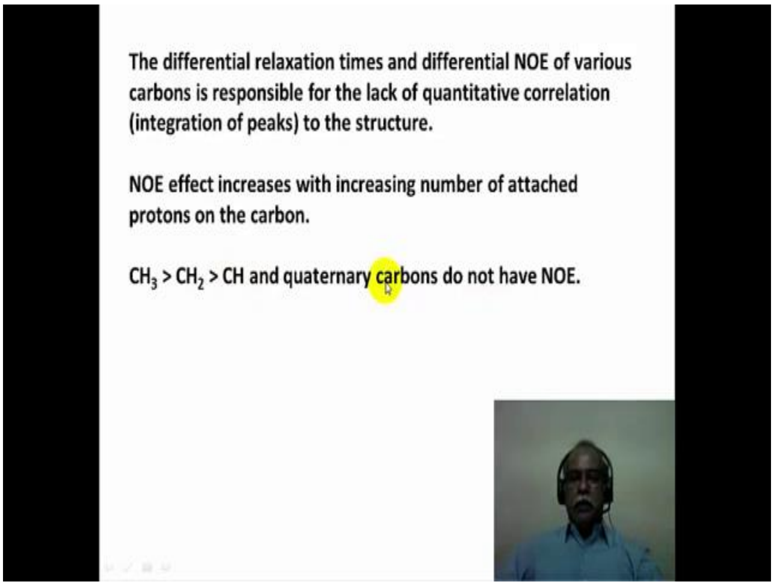

The differential relaxation times and differential NOE of various carbons is responsible for the lack of quantitative correlation (integration of peaks) to the structure.

NOE effect increases with increasing number of attached protons on the carbon.

$CH_3 > CH_2 > CH$ and quaternary carbons do not have NOE.

Now, this is already mentioned the differential relaxation times and the differential NOE of the various carbon is responsible for lack of quantitative correlation; in other words, peak integration is not possible in the case of carbon 13 spectroscopy. However, in the inverse gated spectrum, one can do the quantification in terms of the signal intensity comparison. NOE effect increases with increasing number of protons attached, because NOE will is proportional to the number of nuclear spins that are attached; this is a hetero nuclear cross polarization is what we are talking about in terms of NOE effect. Now the C-H 3 therefore will have a higher intensity compare to the C-H 2, which will have a higher intensity compare to C-H carbons and the quaternary carbon off course will not have any kind of NOE effect.

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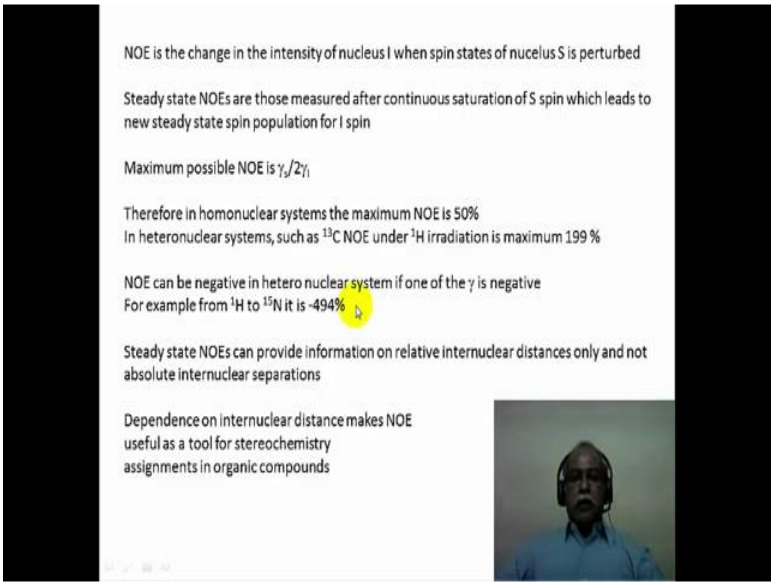

NOE is the change in the intensity of nucleus I when spin states of nucelus S is perturbed

Steady state NOEs are those measured after continuous saturation of S spin which leads to new steady state spin population for I spin

Maximum possible NOE is $\gamma_s/2\gamma_I$

Therefore in homonuclear systems the maximum NOE is 50%
In heteronuclear systems, such as ^{13}C NOE under ^{1}H irradiation is maximum 199 %

NOE can be negative in hetero nuclear system if one of the γ is negative
For example from ^{1}H to ^{15}N it is -494%

Steady state NOEs can provide information on relative internuclear distances only and not absolute internuclear separations

Dependence on internuclear distance makes NOE useful as a tool for stereochemistry assignments in organic compounds

So, let us summarize what we have learned in this particular module. NOE is definition is that it is the change of the intensity of nuclei nucleus I when the spin states of nucleus S is perturbed. Steady state NOEs are those which are measured after the continuous saturation of spin S, which leads to new steady state spin population of the spin I. In other words, you are continuously irradiating the spin S and saturating the levels of spin S just like we discussed the proton spins being saturated during double irradiation that leads to a another steady state population of the observable spin which is the I spin. The maximum possible NOE is given by the expression gamma s which is the nucleus, which is undergoing double irradiation or saturation two times the gamma of the I which is the observable nucleus in this particular case. Therefore, a homonuclear system the maximum NOE observable is fifty percent because the gammas will be the same. One half of this what is the maximum observable NOE effect.

In the heteronuclear system of course, it depends upon the gamma values; if it is a positive gamma of the two nucleus then the carbon just like in the case of carbon 13 and proton, the NOE will be maximum 199 percent, it can be much higher also if the gamma is higher. Now, the NOE can be negative in heteronuclear system, in which at least one of them has a negative gamma. For example, proton to nitrogen 15 has a negative NOE effect to a maximum of about minus 494 percent or so. Steady state NOEs are extremely useful because they depend on the relative internuclear distances only and not at the absolute internuclear separations. They provide information regarding the relative internuclear distances and not the absolute internuclear separations. This is extremely useful because the dependence on the internuclear distance makes the NOE a powerful tool to study stereochemical assignments in organic compounds of which we will see some information at a later stage.

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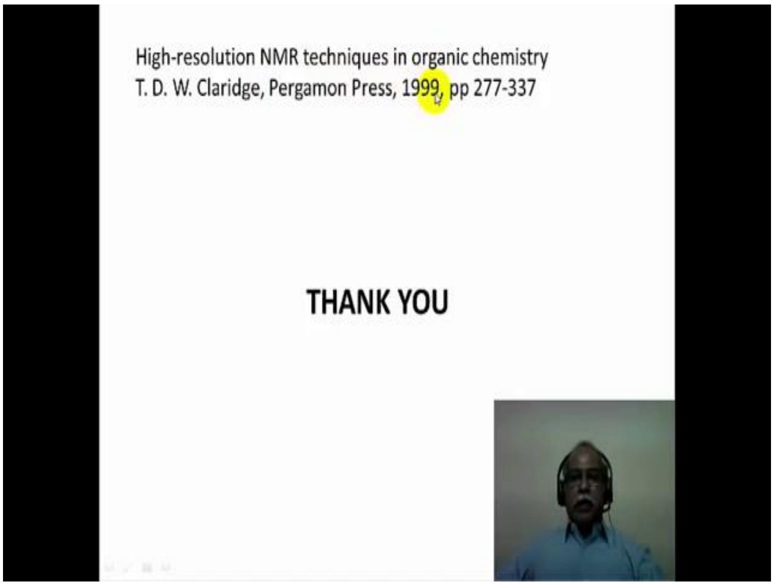

High-resolution NMR techniques in organic chemistry
T. D. W. Claridge, Pergamon Press, 1999, pp 277-337

THANK YOU

Now, if you want to read about the NOE, the origin of NOE, the effect of NOE and carbon 13 spectrum, and the application of NOE, a good source would be high resolution NMR techniques in organic chemistry by Claridge, this is published in 1999.

Thank you very much for your attention.