Principles and Applications of NMR spectroscopy

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Module 6

Lecture No 28

So, we will we will start today with 2D NMR spectroscopy in for heteronucleai. So, we have looked in the last class on how homonuclear spectra, what are the different types such as 2 D TOCSY, 2 D COSY and 2 D NOESY. So, that was in both the axis it was hydrogens, protons and protons. Now will move on to if one of the axis is carbon that means where going to look at correlation between a carbon and a proton. So, that is why it is called as 2 D heteronuclear NMR heteronucleus means two different nuclei.

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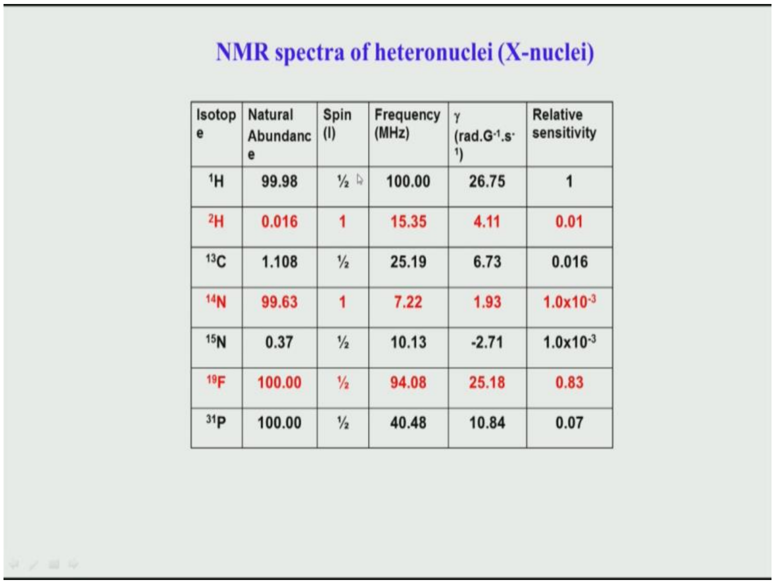

NMR spectra of heteronuclei (X-nuclei)

Isotope	Natural Abundance	Spin (I)	Frequency (MHz)	γ (rad.G^{-1}.s^{-1})	Relative sensitivity
^{1}H	99.98	½	100.00	26.75	1
^{2}H	0.016	1	15.35	4.11	0.01
^{13}C	1.108	½	25.19	6.73	0.016
^{14}N	99.63	1	7.22	1.93	1.0×10^{-3}
^{15}N	0.37	½	10.13	-2.71	1.0×10^{-3}
^{19}F	100.00	½	94.08	25.18	0.83
^{31}P	100.00	½	40.48	10.84	0.07

So, let us see how heteronuclear experiments are required, so this is a table here we shows the different isotopes of different elements and their natural abundance okay. And they shown here is a spin value and frequency. So, based on the magnetic field depending on the magnetic field there is a particular frequency and shown here is the gyromagnetic ratio and the relative sensitivity.

So, let us analyze this table a bit before we go to why we need 2 D NMR or 2 D heteronuclear NMR you can see here for proton which is the maximum having the 100% abundance has a maximum gyromagnetic ratio and therefore it is the most sensitive nucleus in the whole of NMR spectroscopy. So, therefore we called we normalize or we relatively we grade all the other nuclei with respect to the hydrogen.

So, hydrogen will give it as one relative sensitivity as one and you can see here is for a given magnetic field let us say it is 100 megahertz is a proton frequency. Now if you start looking at the other nuclei with respect to the hydrogen. Now you can see that deuterium has a very low sensitivity. We can see the natural abundance is very less, is only about 1.6% and this is actually in the percentage. So, it is only 0.016% and it is 6.5 times less in gyromagnetic ratio.

So, therefore if you calculate your magnetic field and the frequency will be 15 megahertz. So, therefore relative sensitivity based on these two factors, so what has been done here is you multiply this this factor there is natural abundance into the Gamma and then respects with to protons. The scale with with respect to proton and then you can get the value of sensitivity. So, for example for deuterium if you multiply these two it turns out to be 0.01 times one that means it is 0.01 and 100 times less than hydrogen approximately.

Now, let us look at carbon which is very popular nuclei nucleus in NMR. So, the natural abundance of carbon 13 we have seen this earlier is only about 1%. That means 99% of the carbon in the any molecule if you take a collection of a molecules is C12 and if you look at the gyromagnetic ratio is about 4 times less compared to hydrogen. So, therefore if you multiply these two you basically see that is about 16 what 1.6 or 0.16 times of that of proton.

So, that is a very low sensitivity because we have 100 times 4. So, it is about 400 times less and remember we are going to detect both proton and carbon, sorry we detect an excite nucleus. So, if you want to excite carbon and detect carbon you have to have multiply Gamma into Gamma also 16 times it is less sensitive plus there is a factor of 100 further because of the low abundance. So, that comes to about 0.016 times less compares to proton.

So, this is a very low factor infect you will need about a 60 times more it is proton insensitive compare to carbon. So, this is the why carbon sensitivity is low and we need to do something more to increase the sensitivity. So, direct carbon experiment therefore we saw in the previous classes that direct carbon 1D is not a good idea if sample sensitivity is low. So, there are varieties of methods which have been implemented which are known to increase the sensitivity of carbon and other heteronuclei based on these factors.

So, like this we can go on for different nuclei and see what is the different problems and why they are less sensitive and so on. So, you can see this is for a fluorine is actually very sensitive nucleus has almost as much as hydrogen. So, the sensitivity is good because it is almost 100% abundance and the gyromagnetic ratio you see is almost closed to proton. So, therefore the sensitivity wise it is very close to carp proton.

So, next two protons in the whole of periodic table fluorine is the most sensitive nucleus but our focus now will be only on carbon 13 because carbon 13 is the most popular nucleus and it helps a lot very much in getting the structural information of molecules. So, let us look at how proton carbon 2 D experiments can be done and how we can improve the sensitivity of carbon. So, that it the sensitivity can be this spectrum can be recorded in a reasonable amount of time.

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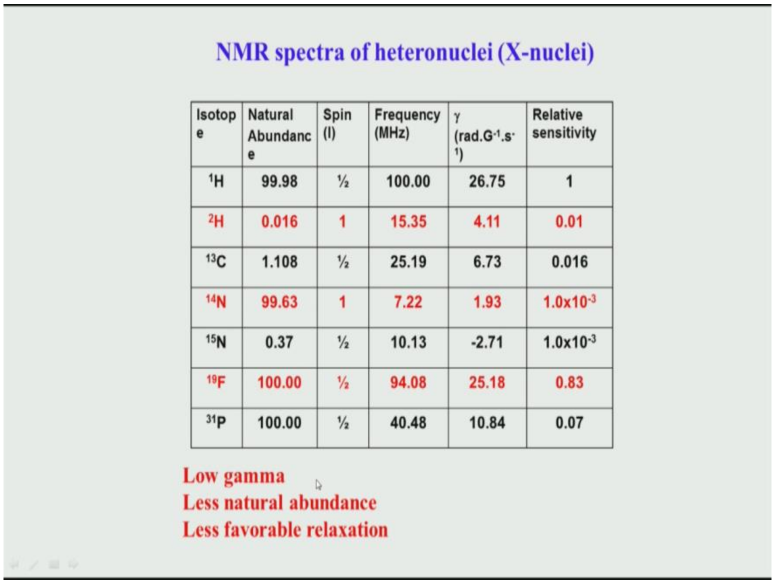

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Low gamma
Less natural abundance
Less favorable relaxation

So, these are the basically three problems which do not favor many nuclei. So, one of the most important two factors as we saw is Gamma value and natural abundance, natural abundance if it is low, then sensitivity is less and Gamma if it is low the sensitivity is also low. So, compared to proton these are the factors which make it less sensitive for X nuclei. So, in NMR typically we use this notation we will say X nuclei or X nucleus to denote the nucleus which is other than hydrogen.

So, this is convention use and also sometimes many of these nuclei have less favorable relaxation properties that means the relaxation values are very short, for example if you look at nitrogen 14 the spin value is one. Similarly if you look at deuterium the spin value is one, so these are called quarter polar nuclei and for quarter polar nuclei you have additional source of relaxation and that reduces the T 2 and T 1 of these systems and makes it less favorable to study by NMR.

So, for as far as I spin half are concerned the additional source of relaxation can be what is called chemical shift an isotropy. So, chemical shift an isotropy is very high for fluorine and phosphorus. So, therefore these types of nuclei are insensitive not only because of this problem natural abundance or Gamma but also because of this favorable relaxation less favorable relaxation properties.

In fact if you see for fluorine and phosphorus abundance is 100% but still we do not use them very often because of this additional problem of CSA induced relaxation even in the liquid state and that scales we say with magnetic field. So, as you go to higher magnetic field it becomes less favorable even more compared to low magnetic field, for example for phosphorus you ever does not go beyond 500 megahertz spectrometer because this CSA induced relaxation is very high and similar is a case with fluorine and nitrogen 15.

So, we will not take into account every about relaxation today or now in this heteronuclear we are going to focus only on the carbon because by for carbon is the most next favorable favorite nucleus after proton in for obtaining structural information. So, will will stick to the in this whole course on primarily on proton and carbon. So, let us see how we can improve this, so that is the reason as I said because of these in unfavorable properties in the nucleus is the detection is very insensitive.

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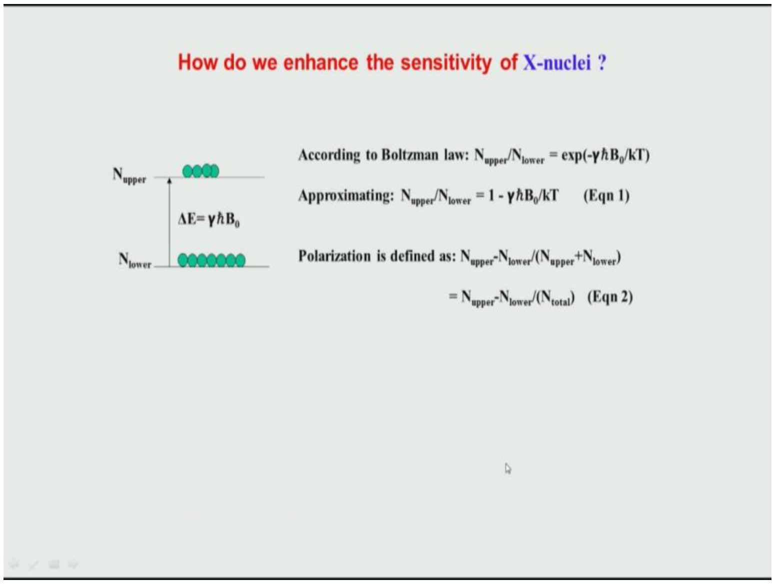

How do we enhance the sensitivity of X-nuclei ?

According to Boltzman law: $N_{upper}/N_{lower} = \exp(-\gamma \hbar B_0/kT)$

Approximating: $N_{upper}/N_{lower} = 1 - \gamma \hbar B_0/kT$ (Eqn 1)

Polarization is defined as: $N_{upper}-N_{lower}/(N_{upper}+N_{lower})$

$= N_{upper}-N_{lower}/(N_{total})$ (Eqn 2)

Now, let us quantified that how much is the less sensitivity of these nuclei how how less is the sensitivity okay. So, how much a how do we get these information from, how do we know that the sensitivity is less why it is less? And that basically comes from this Boltzmann principle. So, this is what we saw in the very in the very first part of the course we looked at this energy levels and we saw that the population is distributed between these two energy levels this is called the lower state or alpha state this spin half positive half and this is for spin value minus half we call it as upper state.

So, the energy gap between these two energy levels is given by this equation, the delta E is Gamma into H cross into B0. So, this is what we have seen earlier, so this is more like a refreshing refresher for that part. So, let us look at a little bit of some algebra here. So, if you go to the Boltzmann law, according to the Boltzmann law the ratio of number of molecules which are sitting or which are locate or which are here in the upper state it is will spin minus half value compared to what is in the lower state if you look at these ratio we will this is related to this expression given by this expression.

So, here if you see here what are the values one is Gamma this is a constant H is H Planck’s constant this is B0 and this is Boltzmann constant and T. So, now typically what happens is the temperature that we work at in our laboratory is typically the room temperature or you may go down to probably up to 0 degrees, but very rarely you go to very low temperatures of the order of make 100 kelvin or even 5 kelvin or 10 kelvin.

So, we are working basically at an ambient temperature conditions. So, under those conditions one can actually approximate this equation and write it in this form okay. So, this is called as high temperature approximation, the idea is the temperature we are working is at a high temperature remember it does not mean high means very high in the normal sense it is even the room temperature is high enough.

So, that if you take this quantity Gamma H cross B 0 divided by K T this quantity becomes very small and according to mathematical rules if E raise to minus X is if X is small, where X is this number, then we can write that there is 1 minus X, so 1 minus X where X is this number which is what we saw. So, this is an approximation and we usually use the word high temperature approximation to denote this expression.

Now if you see here further let us say I just do sum little bit of algebra which will not go in detail but if you do a little bit of rearrangement of this equation you will see that this term can be now count define. So, what we will do is we will define means we will say that there is term called polarization. Polarization will define this term polarization now, polarization is given by this expression N upper minus N lower divided by the total number of nuclei.

So, this is the total number if you add what is of the top and what is added on the bottom that is a total number. So, total number basically means the total number of atoms in your sample in case, so that is given a sample I have a total number of atoms. So, when I put it in a magnetic field some atoms will go to the lower state some nuclei and some nucleus of the atom go to the upper state in the energy levels.

So, this is what we saw this is a distribution, so this if you add of the total value that is the total number of nucleus that is what is written as N total and that difference in this population between the upper lower in the upper state is difference is detect here. So, this term the difference divided by the total number is given is denoted as polarization. Now the polarization this value can be obtained from this equation by a simple rearrangement. So, that is what is shown next that if I combined equation one and two and do a little bit of rearrangement I will get I will get like this okay.

So, this is something which I would encourage you to do it yourself and then figure this out because this is simple rearrangement of this equation and what basically it says now is that the polarization means the difference in the population which is what we saw here is proportional to this value this term okay. So, this is what it means, so now we can see here this polarization is directly proportional to the Gamma okay, Proportional directly to the magnetic field and inversely proportional to temperature okay.

So, these are the things which if we know that proportional that magnet polarization is proportional to these numb factors then we can do something to enhance our goal as is said here is how do we enhance the sensitivity of X nuclei.

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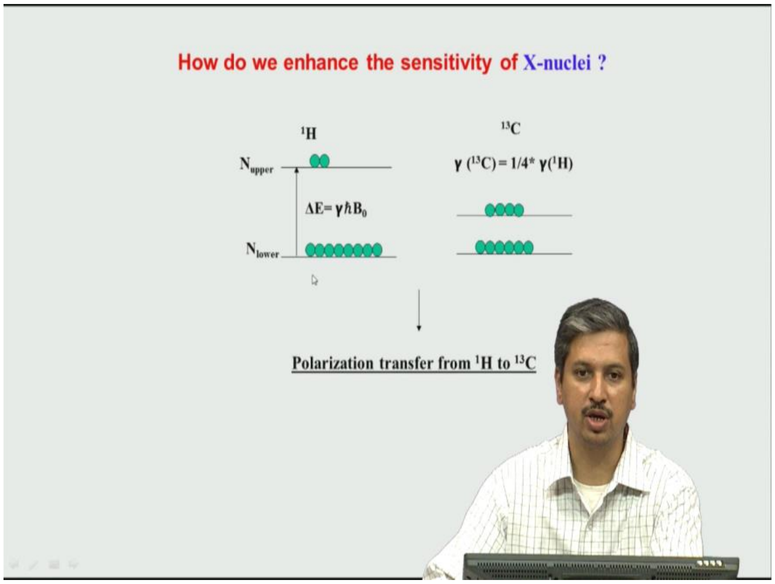

How do we enhance the sensitivity of X-nuclei ?

Polarization transfer from ^{1}H to ^{13}C

So, from here you can see the (sensitivity) why is a X nuclei less sensitive, the X nuclei is less sensitive because of the following what is happening is, in the case of carbon okay. So, if you look at this term now, so we have a hydrogen spin level energy levels. Now hydrogen energy levels is given like this and this is delta E is Gamma H B 0 which is we saw in the last slide. Now if you come if you translate or if you move this picture to carbon you can see in carbon case what will happen in the case of carbon all other factors are same accept Gamma. So, in carbon the Gamma is 4 times less compare to hydrogen.

Therefore the population difference will now be as we saw in the previous slide the population difference is proportional directly to Gamma. So, if the population if the Gamma is 4 times less in carbon compare to hydrogen, then the population also will be 4 times difference population difference will also be 4 times less here compare to what you see here. This is schematically shown here you can see that difference in number of spins is 8 and here it is two okay.

So 8, 2 is 4 times less compare to 8 that means the different if you subtract the number of atoms in the ground state minus number of atoms in the upper state this difference is proportional to Gamma. So, therefore in the case of carbon it will be 4 times less the difference will be 4 times less compare to difference here. So, please keep in mind this is just a notation, this is just in schematic in the sense it is not that in reality there is a difference of 8 atoms but in the proportionality is same.

So, what ever be the difference in protons carbon will be 4 times less. So, what is the importance of this polarization this difference and the reason is that the sensitivity that means how much signal you get in NMR is directly proportional to polarization. So, this is very important, so if you have polarization is 4 times less for carbon compared to proton. So, the signal also will be 4 times less and carbon compared to proton for the same concentration for the same magnetic field at the same temperature.

Keeping all other factors same the polarization difference a polarization is population difference is directly related to the signal intensity. So, from these you can see that the carbon is 4 times less compare to proton because of this reason. So, therefore how can we improve the sensitivity of the X nuclei. So, one potion is we can transfer this polarization from here to carbon to proton sorry proton to carbon. If we can do that then the polarization of carbon is enhanced and if polarization of carbon is enhanced our sensitivity also enhanced.

So, this is basically like saying that suppose I have 1000 rupees with me and I have a person who is having 250 rupees 4 times less I can make the other person have more money by simply transferring the money from my from my side to his side. So, I can give 1000 rupees to him, so he will have 1000 plus 250. So, you see if the by doing that I can transfer the polarization if I do that then he will be richer.

So, in similar way if I transfer the polarization from a higher Gamma nucleus to a lower Gamma nucleus, then I will get the sensitivity enhancement of the lower Gamma nucleus by a factor of 4 in this case but higher factor in case of nitrogen and depending on the Gamma value. So, this is the basic philosophy or idea in all heteronuclear NMR experiments that will do in NMR spectroscopy is that we basically enhanced the sensitivity of X nuclei by exit or implementing this concept of polarization transfer.

So, polarization transfers can be done in many ways but the basic idea is the population difference between one nucleus is communicated or transferred the population difference in second nucleus and the second nucleus now be as sensitive as the first nucleus and then we can proceed further.

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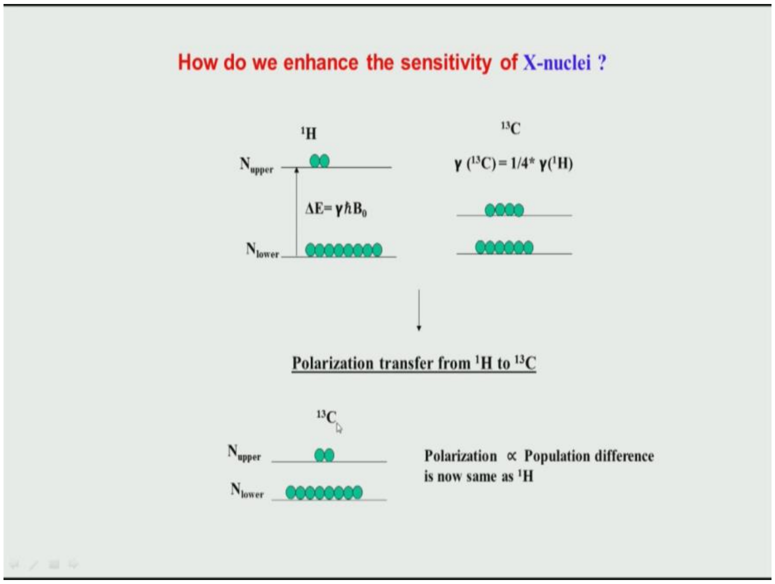

How do we enhance the sensitivity of X-nuclei ?

Polarization transfer from ^{1}H to ^{13}C

Polarization ∝ Population difference is now same as ^{1}H

So, this is basically how it is done, so you can see now that the polarization in after I transfer the polarization from proton to carbon it will look something like this. What has happened I have more nucleus now in the ground state compare to excited state and the difference in the population is now as much as the difference which was here. But look carefully that energy levels are not change the gap in the energy level Delta E that is not going to changed why because we have not changed the Gamma value we have not changed the B0 value, we have only transferred the population like this.

So, the energy gap remains the same, that means carbon does not start behaving like hydrogen we just because we transfer the polarization the carbon remains as a carbon only thing has happened is population level in the difference has change and therefore as to start with if the sensitivity has gone up okay. So, this is the basic idea when we start a heteronuclear NMR experiment, we first try to bring the polarization from the proton to carbon we will see how we do that in different ways and once we do that then the population of carbon is high difference is high in such case now the sensitivity will be also higher.

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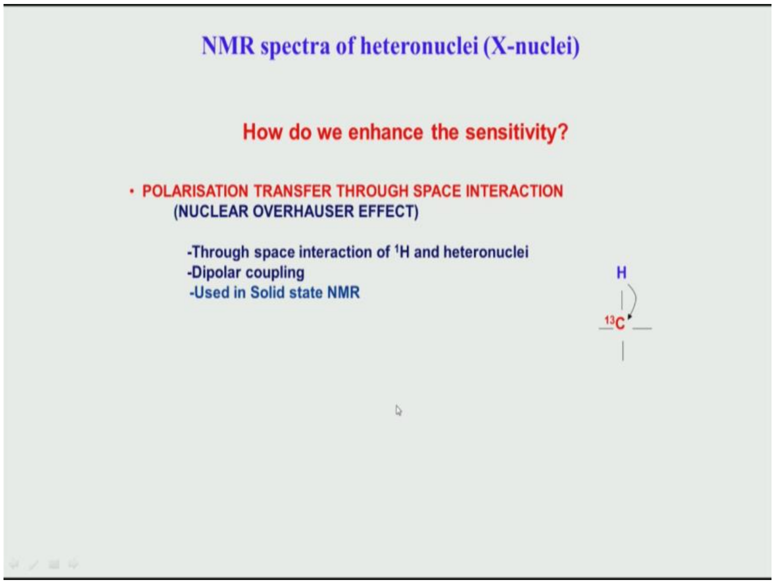

NMR spectra of heteronuclei (X-nuclei)

How do we enhance the sensitivity?

- POLARISATION TRANSFER THROUGH SPACE INTERACTION
  (NUCLEAR OVERHAUSER EFFECT)
  - -Through space interaction of ^{1}H and heteronuclei
  - -Dipolar coupling
  - -Used in Solid state NMR

So, what are the different ways, we can do transfer this polarization. So, this is shown here as a scheme again in a words. So, this is what is says here a polarization transfers can happened through space interaction which means, so you can look at this picture here we are trying to achieve a transfer form hydrogen to carbon okay. So, let us say we have a molecule like this where H is hydrogen is directly bounded hydrogen is directly bounded to carbon.

So, these two are very close in space typically it is a one bond length. So, one bond distance in molecules is how much is about 1 to 1$5 Armstrongs. So, in that distance I can used that short distance two and these two remember both of them are spin half nuclei means they are magnetically active nuclei what it means it means they have a dipole movement, this has a dipole movement this fellow also has a dipole movement.

Therefore I can do a dipole I can exploits the dipole-dipole interaction, so there is a dipole-dipole to dipole interaction between these two nuclei and that gives us a mechanism to transfer polarization from here to here this is similar to what we saw in the NOSEY experiment. So, similar idea we can use to transfer the polarization from here to here. So this is typically what is implemented or used in solid state NMR okay.

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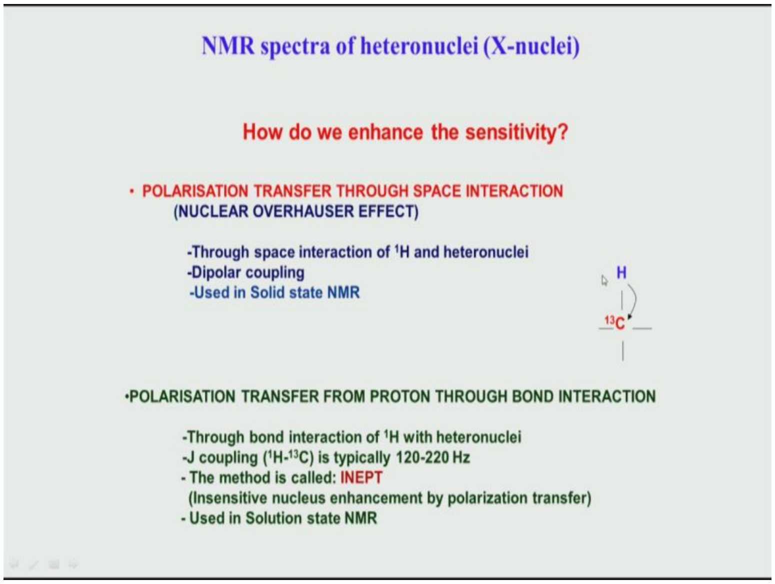

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  (NUCLEAR OVERHAUSER EFFECT)

-Through space interaction of ^{1}H and heteronuclei
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-Used in Solid state NMR

•POLARISATION TRANSFER FROM PROTON THROUGH BOND INTERACTION

-Through bond interaction of ^{1}H with heteronuclei
-J coupling (^{1}H-^{13}C) is typically 120-220 Hz
- The method is called: INEPT
(Insensitive nucleus enhancement by polarization transfer)
- Used in Solution state NMR

But if you come to solution state NMR these dipolar coupling which we saw is not very strong mechanism to transfer energy therefore we rely on direct interaction through bond. So, you see this is a bond is about one bond here and that is there is a J coupling between this hydrogen and this carbon and that J coupling will be used, can be used for transferring polarization from hydrogen to carbon. So, typical value of J coupling is about 120 to 220 hertz.

And remember it depends on the the hybridization state of the carbon, so in aromatics it is higher, Alkynes it is higher, Alkene it is higher but in a spin 3 it is about 120 to 140 hertz J coupling. So, this transfer of polarization using the J coupling through bond, we used the INEPT this stands for insensitive nucleus enhance nuclear enhancements sorry by polarization transfer okay.

So, this is the typical word we use INEPT, so we will not go into detail of how INEPT achieves this polarization transfer. So, there is a mechanism by which you can use J coupling but we will call this whole process as a single word INEPT and we say will will assume that by INEPT we can go this achieve this transfer which is possible or how does not INEPT exactly work at a microscopic level that push part we cannot go into detail in this course.

So, this is a typical step or method used in a solution state NMR to transfer polarization from one nucleus to other nucleus. So, let us see schematically again how this whole thing is now INEPT is used for any 2 D NMR, heteronuclear NMR experiments.

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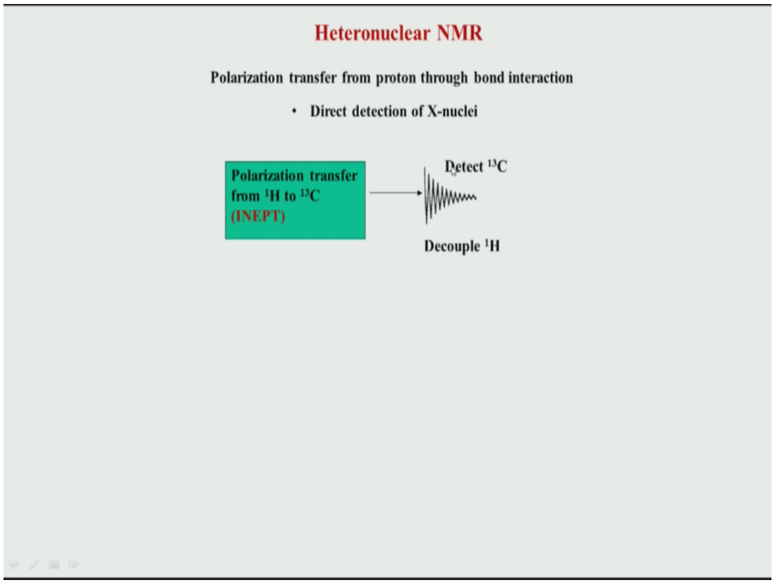

Heteronuclear NMR

Polarization transfer from proton through bond interaction

- **Direct detection of X-nuclei**

Polarization transfer from ^1H to ^{13}C (INEPT)

Detect ^{13}C

Decouple ^1H

So, this is how schematically we can depict a heteronuclear experiments like this, so what you can do the block which I called as INEPT let us I should data is a block as a box that is used first to transfer the polarization from proton to carbon. So, will as I said this is not just a block like this it is going to come we consist of a series a sequence of pulses.

A sequence of pulses together constitute a block which we called as INEPT. So, will see the picture of INEPT little bit latter but as I said we will not go into detail of how this sequence of pulses achieve the transfer. So, let us assume there is does it somehow, so this block of pulses put it the first.

So, what will happened up to the end of the block the polarization whom hydrogen would have been transferred to carbon, then after that I excite the carbon by a 90 degree pulse which is not shown but let us say we have in this scheme 90 degree pulse and that excitation will bring the now the difference of population from hydrogen of two protons. So, the population difference in carbon is much higher compare to what it was earlier.

So, because of that when I excite the carbon it is signal will be now 4 times stronger compare to not having this transfer. So, this is the advantage your sensitivity as gone by 4 times which is a tremendous improvement compares to what if you did not have this transfer block or element. So, how does this 4 times sensitivity improve help us we will see that latter?

So, this is one scheme of transferring the polarization, now if you see here we are also writing here that you have to decouple proton, when the carbon is running and when the carbon is acquired. Why is that so that is because remember whenever we acquire a carbon spectrum we saw that earlier that it is J couple to directly attached hydrogen. So, when I detect carbon I should apply decoupling on protons to remove the J coupling.

Therefore the J coupling instead of coming as doublet the peaks will come as singlets, so that is why we decouple protons. Now I can do this for a 2 D, so remember this will only give me 1 D NMR information. This is not a 2 D experiment drawn, right now it is still the 1 D experiment but the 1 D this experiment is different from the 1 D we saw in the earlier part of the annum this course where we looked at 1 D proton 1 D carbon NMR there it was direct detection means direct you starts form carbon like a 1 D proton you like start from (one) for carbon and excite the carbon and detect carbon but here we have added some extra step that we first transfer the polarization from proton to carbon and then we detect the carbon.

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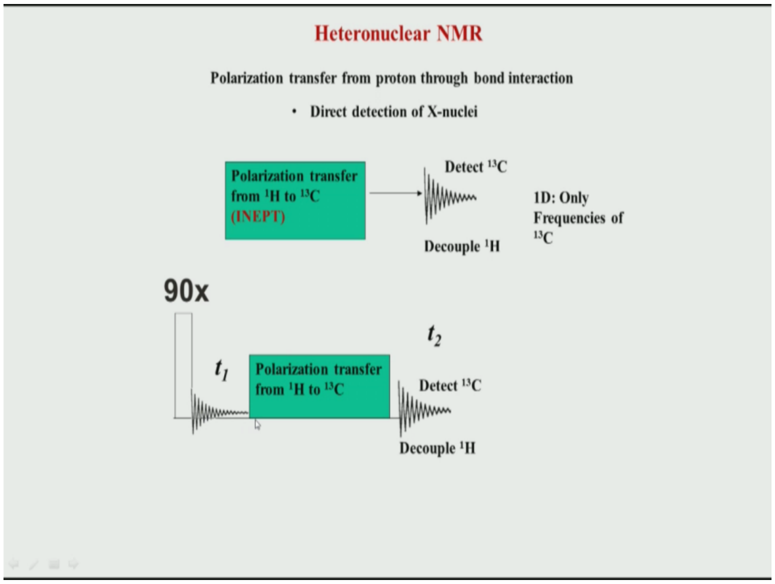

Heteronuclear NMR

Polarization transfer from proton through bond interaction

- Direct detection of X-nuclei

Polarization transfer from ^{1}H to ^{13}C (INEPT)

Detect ^{13}C

Decouple ^{1}H

1D: Only Frequencies of ^{13}C

90x

t_1

Polarization transfer from ^{1}H to ^{13}C

t_2

Detect ^{13}C

Decouple ^{1}H

So, now let us see how it can be done in a 2 D fashion, how can we convert this experiment in the 2 D into a 2 D. So, this is how we can do as shown here. So, first apply a 90 degree pulse on hydrogen. So, this will be a proton okay. We will see this in a short while, this will be a proton pulse not carbon okay. So, you apply this pulse on hydrogen proton then the protons comes to the X Y plane and it is start evolving because of the chemical shift that means it is start oscillating based on its chemical shift value and that period during which it oscillates we called it as T 1.

Then you transfer the polarization to carbon, so after evolution has happened for some duration we will transfer that polarization to this and after doing that polarization transfer. Now after this step my magnetization has come here, it has come to carbon and now I will excite the carbon with a pulse here which is not shown but it is part of this block and when I apply a pulse for the carbon comes into the X Y plane and that is oscillating or moving or evolving with the chemical shift of carbon.

So, this is the carbon detection experiment, so what has happened we have started from hydrogen we evolved the hydrogen with it is chemical shift than transfer by polarization to carbon then detected the carbon.

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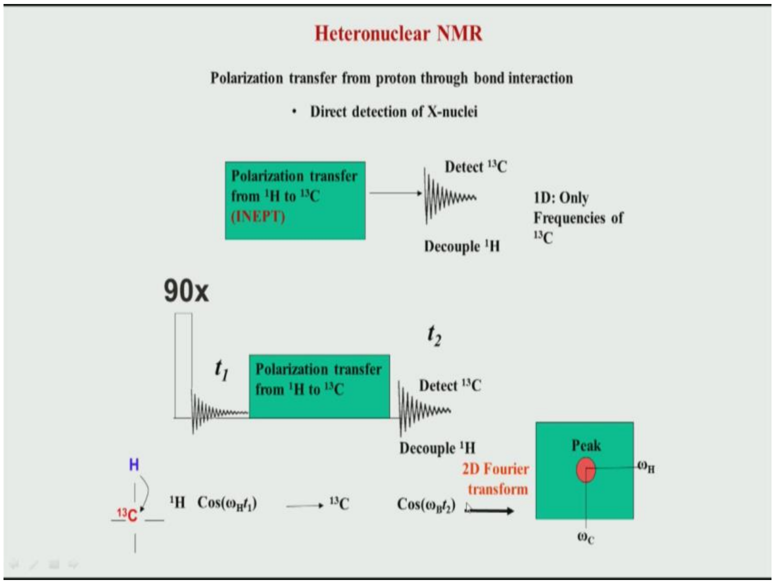

Heteronuclear NMR

Polarization transfer from proton through bond interaction

- Direct detection of X-nuclei

Polarization transfer from ^{1}H to ^{13}C (INEPT)

Detect ^{13}C

Decouple ^{1}H

1D: Only Frequencies of ^{13}C

90x

t_1

Polarization transfer from ^{1}H to ^{13}C

t_2

Detect ^{13}C

Decouple ^{1}H

H

^{13}C

^{1}H $\cos(\omega_H t_1) \longrightarrow {}^{13}$C $\cos(\omega_B t_2) \longrightarrow$

2D Fourier transform

Peak

ω_H

ω_C

So, now if you look at it mathematically what has happened is the following you start from proton you transferred the magnetization. So, let us start from the proton, so we apply a protons pulse first which is this pulse and that will give me a frequency of proton during evolution and that is denoted by cosine Omega H T 1 where Omega H is a chemical shift of the hydrogen, then we transferred the polarization to C 13 and which detected the chemical shift of carbon which I will call it as Omega C or B, B is a sense second nucleus okay.

So, this is how we get the transfer and then now what you can see here my FID finally will be a combination of this FID and this FID. So, when I combine I am getting frequencies of proton which is now getting connected to or correlated to the frequency of the carbon. So, if I do a 2 D Fourier transform like we saw in the standard in a homonuclear case. I will get now this spectrum which is basically gives me a cross peak and in one axis is proton and second axis is carbon. So, if you see here what has happened this is the (carbon) proton is in the, this axis Y axis which we called it a T 1 dimension or F 1 okay whereas carbon has come has come to this axis which is T 2 or F 2.

So, basically that comes from here, so you have T 1 proton along T 1 and carbon along T 2. So, this type of an experiment is called as 2 D HETCOR. 2 D HETCOR means heteronuclear correlation. So, 2 D heteronuclear correlation was one of the beginnings in the first time first experiments in heteronuclear NMR to be proposed because there the idea was simply you transfer polarization and start detecting.

But the problem with this experiment is that it is very less and the sensitivity is low and why is it low it is because you are detecting carbon okay. We will see that how detection of carbon is not a very preferable approach, the first approach would be to detect proton okay. So, you start from proton, you transfer to carbon and again to come back to proton for detection. So, that instead of doing a detection of carbon if I do a detection of proton it is a preferable thing.

But thing that want be possible using this approach in this approach I have to do the way it is shown here that is I start from labeling or evolving proton and detect carbon. So this approach leads to HETCOR but we will see in the next class that this is not the best approach and therefore for the best sensitivity it is better to start from proton and also end in proton but at the same time get the 2 D heteronuclear information. So, that is called inverse detection and that will be we will see in the next class because that would be seen as a much higher sensitivity then this approach of 2 D HETCOR.