Principles and Applications of NMR spectroscopy
Professor Hanudatta S. Atreya
NMR Research Centre
Indian Institute of Science Bangalore
Module 4
Lecture No 18

We will start now with carbon 13 NMR. In the last lecture we looked at how to analyze the 1 D NMR spectrum of protons. Now the next important nucleus in NMR for chemist is basically carbon 13 NMR, carbon 13. So let us look at how carbon 13 spectrum can be analyze what are the details of carbon 13 chemical shift and so on.

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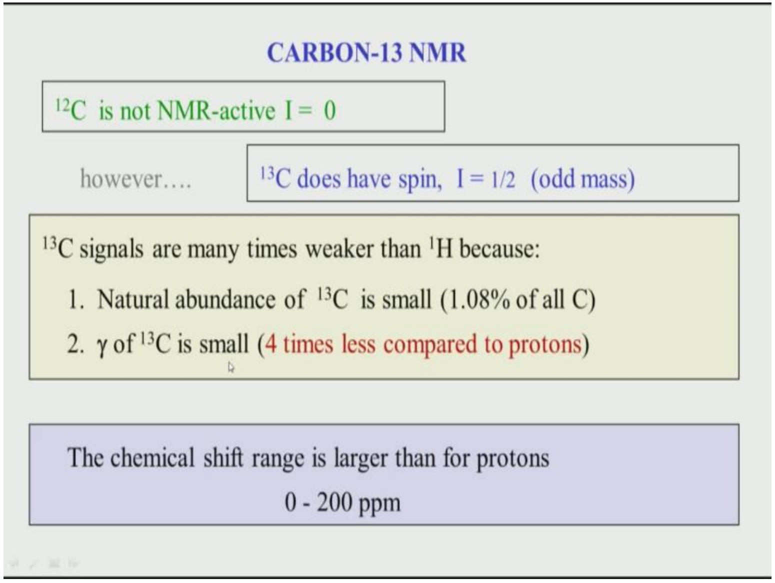

CARBON-13 NMR

^{12}C is not NMR-active I = 0

however….

^{13}C does have spin, I = 1/2 (odd mass)

^{13}C signals are many times weaker than ^{1}H because:

1. Natural abundance of ^{13}C is small (1.08% of all C)
2. γ of ^{13}C is small (4 times less compared to protons)

The chemical shift range is larger than for protons

0 - 200 ppm

So let us start with this thing that we know the carbon 12 is not NMR active. And that is a very clear because it has even atomic number and even atomic mass. So when you combine the spins in the nucleus they basically pair up and the next spin of carbon 12 is zero. So therefore you cannot do any NMR with carbon 12. But fortunately for us there is an isotope of carbon, carbon 13 which has a spin half. And this is because the mass now becomes a odd number.

And according to the rules we saw in the very beginning carbon 13 has a spin half. And C 13 the only problem but is that C 13 is pretty weak, very weak compared to hydrogens. And the reason is for that is given here the two reasons mainly. The number one is the natural abundance of C13 is very small. So typically 1% of all carbons in the molecules will be carbon 13. That means if I take a collection of molecule every after out of every 100 carbons one carbon will be carbon 13.

So basically it means 100 times less concentration of carbon 13 compare to hydrogen's. That is one reason why the sensitivity of carbon as well as. And the second reason is this gamma. So if you remember gamma is basically related or helps us to figure out the population difference between energy levels and the strength of the interaction of the magnetic field with the dipole. therefore we know that the before the gamma is small the sensitivity will be low. Because of this smaller population difference.

In other words the polarization of carbon is much less compare to protons. And that is basically four times less to the carbon gamma value is four times less compare to hydrogen. So therefore if you combine this two factors you can see this already a factor of 400. Four coming from here and 100 means 1 out of 100 is only carbon. So it is not 100% carbon 13, only 1 % so compare to proton, proton is 100% one H nearly.

Therefore it is 400 times less but there is one more factor which makes carbon 13 even more insensitive is when we detect carbon. So normally what happens in 1 D NMR, we apply a the pulse to proton and we detect the proton signal that is the standard 1D NMR proton NMR which we have seen up to now. But if you do the same thing with carbon we are going to excite or apply a pulse on carbon and also detect carbon.

So for during detection again the sensitivity of the gamma comes into the picture and therefore there are twice it has a problem. First when you excite the sensitivity is already low because the population difference is low. But when you detect carbon then also again it is low because of this low gamma. So basically it is like a square means two times gamma comes into a picture. So we can say it is 16 times less sensitive when it comes to detection of carbon.

And multiply again with 100 with the 100 so it is about 2000 times less. In fact actually if you see practically, it is about four orders of magnitude carbon is less. Means almost 10,000 times less sensitive compare to hydrogen. So carbon is therefore very very weak much many times weaker compare to protons. So therefore there have been varieties of techniques to improve the sensitivity which will see a later on as we we look at 2D hetro nuclear NMR.

So we will now what is advantage of this having even if this is weak. What is advantage then of studying carbon? The advantage is the chemical shift range. You see the protons typically is about 10 ppm, 0 to 10 is what we saw earlier, in fact it is even 0 to 5 ppm if you ignore the amide regions. If you look at only the aromatic its about 0 to 8 ppm.

But look at carbon, carbon has a huge range which will see of 200 ppm. In fact go even beyond this it can go even to 300 ppm for certain type of molecule. So this wide range of Chemical shift possibility with carbon makes it very attractive and we will see why it so because it is different functional groups are very nice spread in the chemical shift and therefore carbon 13 NMR is preferred even though the sensitivity is much less because of this factor.

So what is the range of this chemical shift? Let us have a look at that about. Before we look at that, we should look at the frequency of carbons. So remember carbon gamma is now small. So we have to look at how that frequency of gamma changes with respect to proton.

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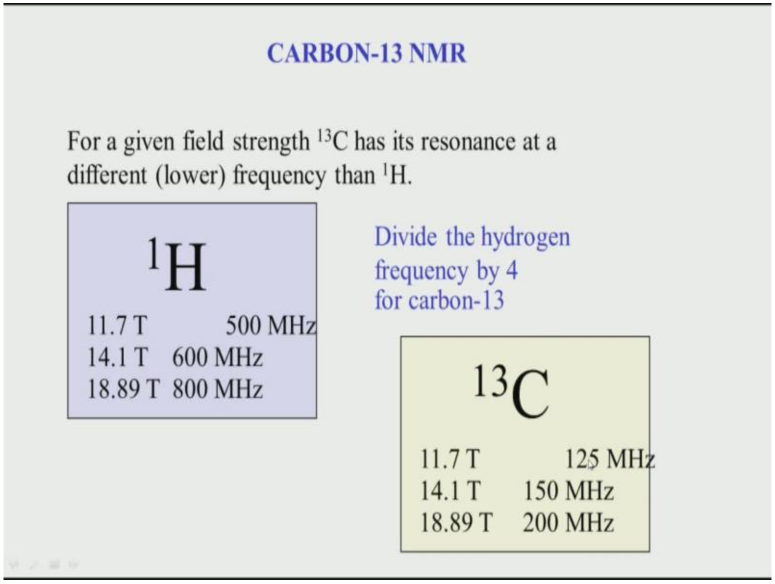

CARBON-13 NMR

For a given field strength ^{13}C has its resonance at a different (lower) frequency than ^{1}H.

^{1}H

11.7 T	500 MHz
14.1 T	600 MHz
18.89 T	800 MHz

Divide the hydrogen frequency by 4 for carbon-13

^{13}C

11.7 T	125 MHz
14.1 T	150 MHz
18.89 T	200 MHz

So this is shown here when you look at protons we have fine let us say spectrometer, which is operating at 500 megahertz then the magnetic field for that is corresponding to this value. How do we get this relation? Remember again the equation omega equal to gamma into B0. So you can see in the B0 value which is based B0 and gamma you get the value of omega.

Omega actually this is omega this is 2 pi into omega 2 pi into frequency. So this is how we get the omega value. Similarly if I change the magnetic field to a different value correspondingly this will change. So for a hydrogen atom this directly the magnetic field directly related to the frequency and the proportionality is omega is equal to gamma into B0.

So therefore when it comes to carbon your gamma is going to change and for a carbon we saw in the last slide that the gamma of carbon is four times less compared to protons. So therefore when you multiply, when you use the equation omega equal to gamma B0 your gamma is now four times less compare to hydrogen.

So if hydrogen is 500 megahertz you have to divide it by four, so you have tp divide the hydrogen's frequency by four then it will become 125 megahertz for the same magnetic field. So this is constant, because we are looking at a given magnetic field. But the frequency you see there is a big difference the frequency, the operating frequency of hydrogen is this much and for carbon is this much.

If you look at now 600 megahertz proportionality it goes down by factor of four. If you look at 800 megahertz it goes down again factor of four. So everywhere remember if you are working at a X frequency for hydrogen X by four is what will be the frequency for the carbon 13 at the same magnetic field ok.

So this is very important because when you convert ppm scale to frequency we have to keep this in accord in mind. For example let us say that there is two peaks in protons which are separated by 1 ppm ok. So that will be the 500 hertz for proton. Because remember that equation we in the last part where we just multiply this frequency 500 into the ppm difference and that is the difference in hertz.

But if you come to carbon then the difference in hertz will be the different. Because there the same 1 ppm difference in carbon peaks will now correspond to 1 into 125 which is 125 hertz. So you see the difference is reduced compare to proton. That means protons is more resolved, more well separated in terms of hertz value compares to carbon.

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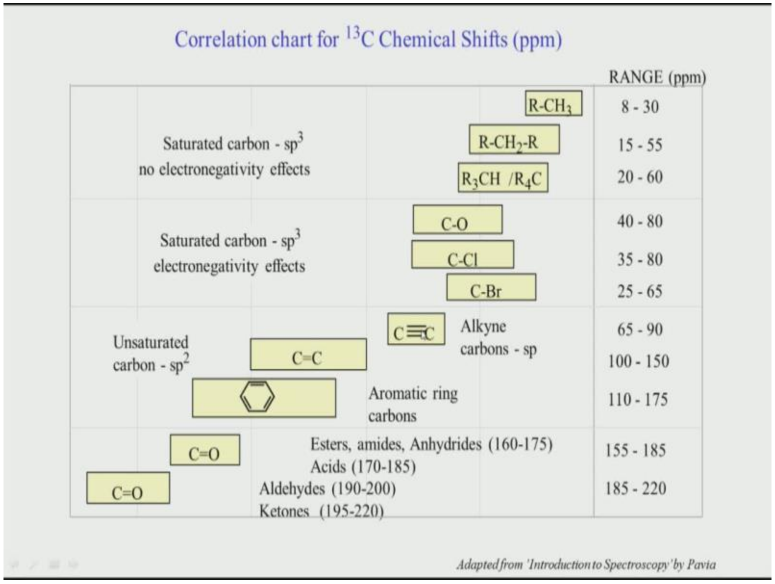

Correlation chart for ^{13}C Chemical Shifts (ppm)

		RANGE (ppm)
Saturated carbon - sp^3 no electronegativity effects	R-CH$_3$	8 - 30
	R-CH$_2$-R	15 - 55
	R$_3$CH /R$_4$C	20 - 60
Saturated carbon - sp^3 electronegativity effects	C-O	40 - 80
	C-Cl	35 - 80
	C-Br	25 - 65
Alkyne carbons - sp	C≡C	65 - 90
Unsaturated carbon - sp^2	C=C	100 - 150
Aromatic ring carbons		110 - 175
Esters, amides, Anhydrides (160-175) Acids (170-185)	C=O	155 - 185
Aldehydes (190-200) Ketones (195-220)	C=O	185 - 220

Adapted from 'Introduction to Spectroscopy' by Pavia

So now ((0:08:20.9)) look at the ranges of chemical shift which is observed. So as I said carbon has a very nice, very a favorable frequency range. It goes all the way from almost 0 ppm, all the way to 220 ppm. And you can see the big range is what is very attractive when carbon 13 spectroscopy is concerned. So you can see in the begin, let us look at this ranges as we go from top to bottom.

So in the top part of the spectrum that is when it comes close to 0 to 30 ppm we can include from 0 to 30 here. So 0 to 30 ppm you have what is called methyl groups CH3. And very strong peaks comes between 0 to 30 and that is same as similar what we see in the protons, protons also remember the methyl comes always between 0 to 2 ppm.

Now you can see this part up from here to here is all corresponding to this SP3 Hybridize, hybridize carbons. Now so second the methylene groups if you look at CH2 the carbon of a CH2 that comes somewhere between 20 or 15 to 50 ppm. So this is a typically the range of the methylene groups CH2 groups in molecules.

Now if you go to methyne. Here is methyne, methyne groups basically comes at 20 to 60 ppm. So this is typically the methyl's carbons are observed in bio molecules such as peptides and proteins. In amino acids if you in the amino acids remember the alpha carbon amino acid is a methyne carbon. And therefore that carbon very typically come somewhere between 50 to 60 ppm.

So that is a for amino acids but general range is about 30 to 60 ppm or methyne carbon. Similarly for quaternary aliphatic carbons ok so aliphatic. Because this is a aliphatic group for that the carbon can somewhere between this ppm. The next if you go to the next zone so you can see we can divide this whole NMR I mean carbon range into four different zones which is shown broadly here.

So first zone was basically the aliphatic to carbons which are SP3 hybridized. Now if you look at what is called as the because of the electro negative carbons which are attached to electro negative atom such as oxygen, halogen they come somewhere in this zone. So you can see here for carbon bond to oxygen for example in in case of alcohols or esters so on esters where there is carbon attached to oxygen, then that particular come somewhere between 40 to 80 ppm.

And then you look at halogens also very much down field shifted, somewhere around 40 to 80. And same is for bromine. Of course as the electro negativity decreases it goes up field. So that is why when we say chlorine, this is more electro negative the range of chlorine is chlorinated. I means carbon attached to chlorine is higher than that of bromine.

Now if you come to next level, next zone the alkynes carbons typically come here. There is another set of peaks which typically you know carbohydrates. When you go to carbohydrates like glucose, maltose they will also come in this zone ok. So that is another zone and after 90 ppm there is a gap. So there is typically region where you will not get any peak in NMR and that is 90 to 100, a little bit 105.

So there is about 20 ppm range. Where typically you don’t see any peak. Then what happens is after this 100 and 105 starts the C double bond C carbons. They start coming at this range and the very popular very one core chemistry. We come across aromatic rings, and aromatic rings are very prominently they come between 110 to 150 typically.

175 is little is little on the extreme, typically 150 or 120 to 150 this range is what typically comes in aromatics. So if you get peaks in this portion of the spectrum in carbon you can as well very

be confident that there is an aromatic ring present in the molecule. So we will use this ranges as we go along as we analyze some more NMR spectra of carbon.

Then comes very important the amide groups now not amides sorry carbonyl groups. The carbonyl groups remember are can be part of any of this can be an ester. It can be an amide in the carbonyl, carbonyl amide. You can have uh anhydrides, carbonyl groups, acids carboxylate. So you see there is very clear this whole zone you can see very clear distinction that this esters, amides and anhydrides they come somewhere in this range.

And if you go little bit down field, you have the acids that carboxylate acids which come in this range. Now if you look at ketones and aldehydes they come even little bit more down field. So you see the ketones typically come very much down field. There is a last, it comes around 200 to 220 ppm, typically some more than 200. And aldehydes are a little bit up filed shifted and they come between 190 to 200.

So any peaks which you get see from 190 onwards has to be either aldehydes or ketones. Typically it is much very high down field beyond to 200 then it is most slightly a ketone. So you can see that very nice distribution of chemical shift across a huge range of carbon uh which can be exploited or which can be used when interpreting or analyzing a carbon 13 spectrum.

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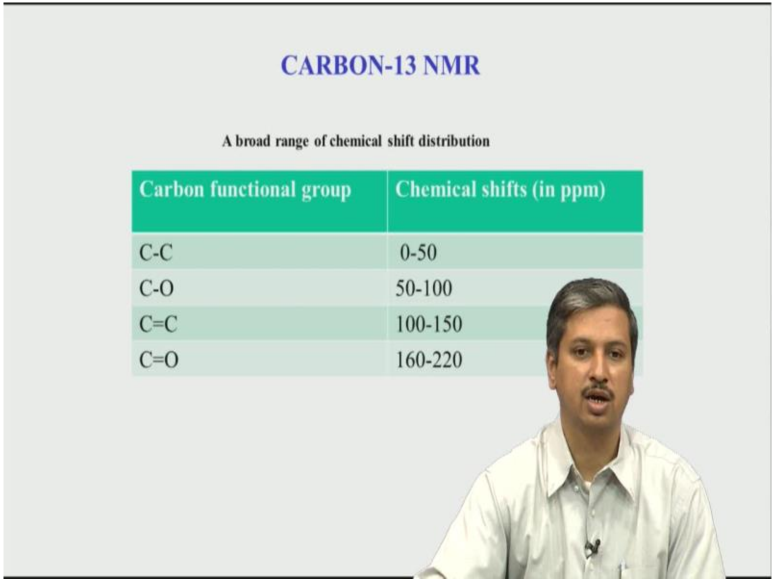

CARBON-13 NMR

A broad range of chemical shift distribution

Carbon functional group	Chemical shifts (in ppm)
C-C	0-50
C-O	50-100
C=C	100-150
C=O	160-220

So now let us look at this broad ranges. So you see this is what typically you can think of instead of thinking of full range from 0 to 220 you can divide as I said into four categories. The first category C double bond C we can see that that is come 0 to 50. Then you have C single bond O. So single C single bond C is 0 to 50 that this aliphatic carbons. C double or C single bond O is 50 to 100.

Then C double bond C which includes ethylene as well as aromatic groups all always in this range and then finally all the carbonyls are in this range. So you can see there is a broad classification of the chemical shifts and this is very useful typically you know when we do this solid state NMR of many materials when you look at materials science, material chemistry typically many times you just want to have a broad quick picture of what are the functional groups present in the sample in the molecule.

At that time we just record a 1D carbon spectrum and then see the based on the peaks present in the different zones. We come out with a hypothesis of what are the functional groups present in this molecule.

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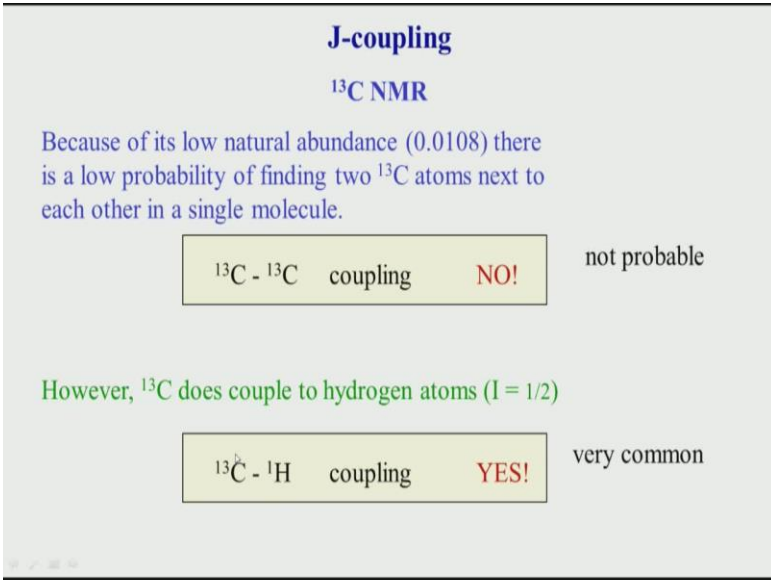

J-coupling

^{13}C NMR

Because of its low natural abundance (0.0108) there is a low probability of finding two ^{13}C atoms next to each other in a single molecule.

^{13}C - ^{13}C coupling NO!

not probable

However, ^{13}C does couple to hydrogen atoms (I = 1/2)

^{13}C - ^{1}H coupling YES!

very common

This is a broad range. Now let us look at J coupling. So we have look at the chemical shift values in case of carbon. So now let us come across look at the J coupling. Now in J coupling you see that one thing you have to keep in mind is that the very low natural abundance of carbon. The carbon as I said is about 1% mean 01, 0 . 01.

So that means if I have 1% carbon the probability that I will find two neighboring C 13 carbons will be 1% of 1%. So which is 0 . 01% very very low it is 0 . 0001. So that measn 1 in 10,000 case only I will find the two neighboring carbon atoms both of them are carbon 13. That means you can never have practically two neighboring carbons such carbon 13 in a natural abundance.

Remember we can always generate a carbon 13 labeled molecule. In fact this carbon 13 labeled molecules are available commercially. And in biology as we see as will see along as we go along you will see in biology you need actually enriched carbon 13 samples. So not in that cases, we are talking about a case where there is no enrichment.

You are taking a standard sample let us say from a nature of natural sources. In nature you won't find a carbon 13 enriched sample. We will find carbon 12 and carbon 13 will be in natural abundance. So in such samples the chance that you will encounter to a two neighboring carbon is carbon 13 is very, very low. So therefore practically we never bother about C 13, C 13 coupling in carbon 13 NMR in natural abundance. But if you look at protons there is definitely a coupling there. Why? because you take any carbon, it is always will be attached to proton. Unless it is a carbonyl, unless is a carbonyl or a quaternary carbon. Without except this two all the carbon are generally attached to two protons.

Now protons is 100% proton is NMR active. So that means if you take any carbon 13 it is always attached to proton. So in such cases if the carbon proton coupling now becomes active because both are NMR active so there is a J coupling in between them. And that J coupling is definitely present in your sample. That is very common means it has, it is always there so that is a big problem or a gain. You can think of it in both ways.

So what typically is done we try get tread of this coupling. Because the coupling can create a little problem. When you look at the data so will see that how it creates problem. So this is a thing we have to now take into account. That is a proton to carbon coupling is very much present in any molecule. So you should always remember that proton 13 whenever you look at 13 carbon is always attached to proton except those two cases which I said that is carbonyl and a quaternary carbon except those two all the carbon are always having a hydrogen attached carbon 13. So therefore you have to worry about this coupling.

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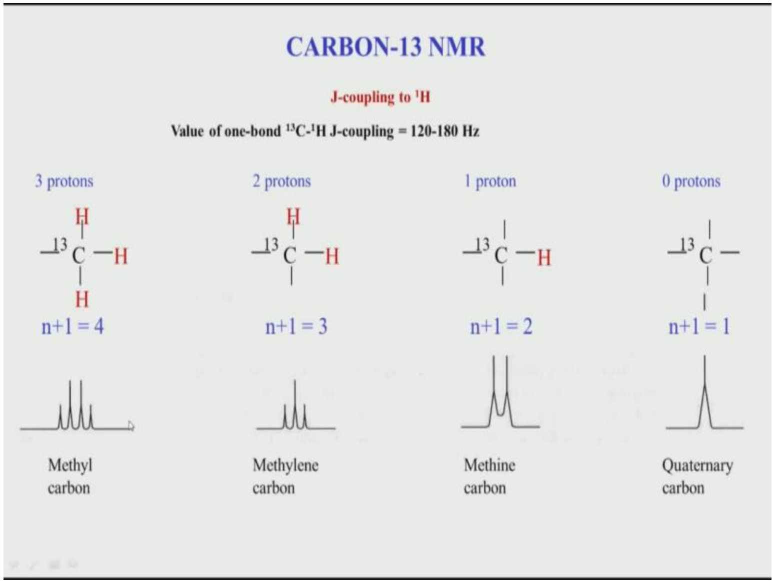

CARBON-13 NMR

J-coupling to ^{1}H

Value of one-bond ^{13}C-^{1}H J-coupling = 120-180 Hz

3 protons	2 protons	1 proton	0 protons
n+1 = 4	n+1 = 3	n+1 = 2	n+1 = 1
Methyl carbon	Methylene carbon	Methine carbon	Quaternary carbon

So now let us look at how this coupling pattern will be. So typically the one bond J, C 13 proton coupling. Means the directly attached proton to carbon coupling will be of this range 120 to 180 hertz. So you can see this is a pretty large value compared to what we saw in the proton proton coupling. So if you recollect the proton, proton coupling values it was in the range of about 5 to 10 hertz. Sometimes may be 20 hertz but not beyond that.

Whereas carbon to proton coupling and we are looking at one bond. Whereas in the case of hydrogen of hydrogen coupling iIt was two bonds for germinal and it was three bond for vicinal. So the germinal and vicinal coupling one of the range of 5 to 15 or 20 hertz. But in case of carbon we say it is directly one bond coupling we are looking at and that do comes in this big range. This is a very big value about.

So what happens is, is typical the N + one rule is applicable here also. So suppose I have a carbon like a methyl group which is attached to three protons and then that carbon signal will be split into four peaks because of the I + one rule, N + one rule. And we saw that N + one rule if you use the Pascual triangle you expect a quartet, a quartet will be 1 is to 3 is to 3 is to 1. Now if you look at methylene group CH2 then there are two hydrogens.

So according to N + one rule you will get a triplet which is 1 is to 2 is to 1. And similarly if you have a methine group then you will get a doublet which is equal intensity 1 is to 1. And in case of quaternary carbon or if it is a carbonyl you get only one peaks because there is no coupling to any hydrogen. Now in a way coupling you can say is very useful because if I get a quartet immediately I know it is a methyl group.

Or if get a triplet I can say immediately methylene. So this coupling as a good information as information about the type of carbon, we use the word multiplicity. So multiplicity is the number of protons attached to a carbon. So here the multiplicity is three for this carbon, multiplicity is two, multiplicity is one and multiplicity is zero. So by looking at the coupling pattern, coupling multiplet in principle it is very useful information you can get the multiplicity.

But if you see the number of peaks have increased. It has four peaks instead of one. And remember the coupling are large here. So when a coupling is large this separation between this multiplet is also large. So that means it occupies a large range of chemical shift unlike proton. In protons what happens is they occupy a small range because the coupling are small.

So they are all together bunched up. But in carbon the suppression is more because the J coupling is more and therefore it can create a problem of overlap. Means two neighboring carbon atom will start having overlap.

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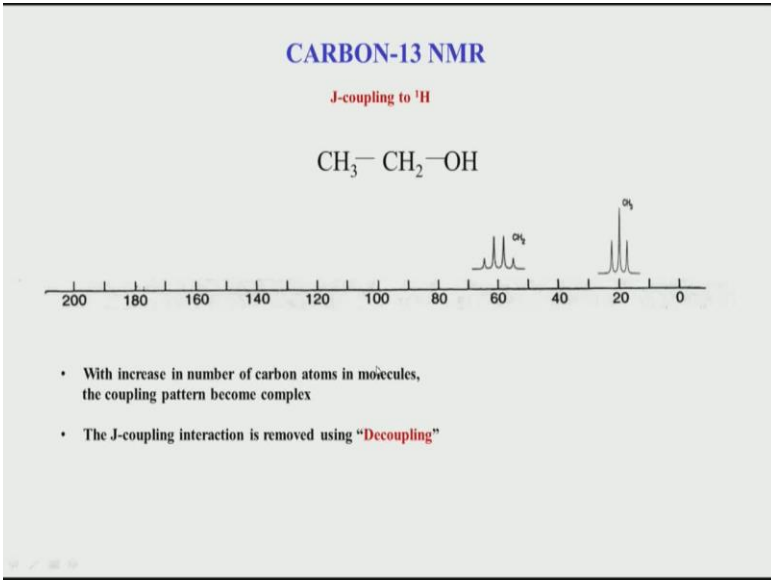

CARBON-13 NMR

J-coupling to ^1H

CH_3-CH_2-OH

- With increase in number of carbon atoms in molecules, the coupling pattern become complex
- The J-coupling interaction is removed using "Decoupling"

So let us that how that can help? So this is case of methanol sorry ethanol. In ethanol molecule we have a CH3 CH2 OH. So the CH3 peaks the carbon of CH3 remember comes somewhere between 0 to 10 which we saw and because it is a CH3. then therefore it should be a quartet so I am sorry there is a labeled here this is CH, this is CH2 and that should actually come here.

This is for the proton spectrum but is by mistake it has to be reversed. So this is for CH3 and that is carbon spectrum, that will come here. And CH2 will come here. Why this CH2 come at a 60 ppm? That comes because of the attachment to oxygen. So with the increase in number of carbon atoms in the molecule in this case there only two carbons.

So therefore they was not a big problem with this coupling. The couplings well separated. But it can happen that in a large complex, in the large molecule where there are many carbon atoms this J coupling can be a problem. So will see the example how, how it becomes complex?

and then how do you remove that interaction? We can remove the interaction. what we can do. We can get tread of this coupling multiplet and only get a single peak instead of multiplet that is possible. And for that technique what we use is called Decoupling. Decoupling is basically a method to remove the J interaction J coupling interaction between carbon and hydrogen.

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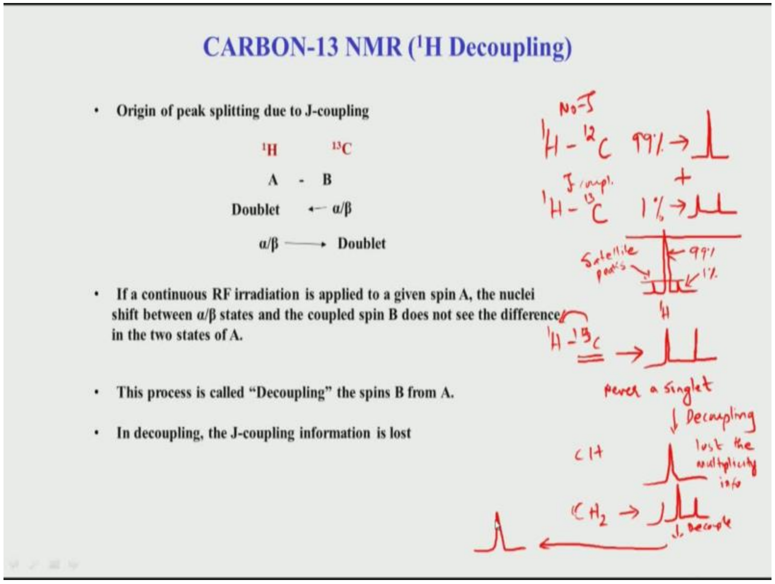

CARBON-13 NMR (^1H Decoupling)

- Origin of peak splitting due to J-coupling

^1H ^{13}C

A - B

Doublet ← α/β

α/β → Doublet

- If a continuous RF irradiation is applied to a given spin A, the nuclei shift between α/β states and the coupled spin B does not see the difference in the two states of A.
- This process is called "Decoupling" the spins B from A.
- In decoupling, the J-coupling information is lost

So this is basically what happens. So you see origin of peaks splitting is remember we discussed is because of only J coupling. So let us say you have a carbon proton A, attached to a carbon B, which is C 13. Then what will happened is this proton will get a doublet. Why does it get a doublet? It has doublet because it is attached to C 13. So remember C 13 is a spin half nucleus. So it has an alpha, beta state.

And because of that alpha, beta state this hydrogen will be split into two peaks. Similarly if I look at the carbon the same thing will happen. In case of carbon 13 it is attached to a hydrogen. And this hydrogen has two possible spin states, alpha and beta. And because of that two spin states this peak will become doublet ok. So that is why that is what I was saying earlier.

That is a mutual reciprocal interaction. Means if a carbon splits a proton into two the proton will also gets split into two because of carbon ok. But what happens most of the times? T his hydrogen is attached to C 12. Because remember according to the natural abundance 99 sole we can look at it like this. So we can look at it like this. So a proton is attached to C 12 99% of the time. Therefore it will give a singlet. Why, there is no coupling, no J here, coupling. But for the 1% of the molecule this proton is attached to C 13 that is for 1%.

So this will be a doublet because of J coupling ok. So that means in any given molecule both of these are presents. So you have to add these two. So if you add these two, what you will get?

You will get a small peak and the tall singlet and a very small peak like this. Because this height, this is now corresponding to 1% whereas this is 99 %.

So this is about 100 times taller compare to this small peak. So we use the word satellites, this is called a satellite peak, satellite peaks ok. So this is what is the case in the case of hydrogen spectrum. Ok this is a hydrogen but this is not the case in case of carbon. If you look at carbon in the case of carbon there is basically a proton attached to C13. But if you see this C 13 is always attached to proton right.

So any way C 12 does not give any peaks. So C 12 does not give any peaks. So whenever you look at carbon 13 all the time this carbon 13 is coupled to a hydrogen. So therefore carbon 13 will always give this spectrum. It will no never be a singlet, never a singlet unless we remove the J coupling ok. So that means you have to keep this in mind is very carefully.

The point is that in the case of hydrogen spectrum we did not see a coupling, we did not see the doublet mainly because the 99 % of the time it is singlet because of this C 12. But 1 % of the time it is doublet. So when you combine these two you get a very small satellite peak, but not in the case of carbon. When you look at carbon spectrum it is always attached to hydrogen right?

Because C 13 and C 12 can never be observed in carbon in NMR. So therefore in the case of C 13 it is always doublet, never a singlet. So that means if have, I have to decouple I means if I want to get a singlet means like this, if I want the peak like this then I have to remove the J interaction. Only if I remove the J interaction then only I will get a nice peak in the center.

But remember if I remove J interaction I lose also information of the multiplicity. But let us say that we don’t care for multiplicity now we only want a single peak, if we want then I have to remove this interaction between the J coupling between the two proton this carbon And hydrogen and then only I will get a ((0:27:15.7)). So how do we actually do decoupling? How do we remove this interaction so that we can get a singlet? And that is done as follows. What you do is you apply a continuous RF irradiation is applied to spin A ok.

So we are looking at here. So we want to decouple this two in a carbon spectrum. So we are not looking at proton now, we are looking at carbon spectrum. So in a carbon spectrum the doublet is coming because the proton has two possibilities, two possible spin state. So how do I remove this doublet to singlet?

One way is remove this alpha beat distinction. So how do I remove the distinction between alpha and beta? Keep the population of alpha and beta level constant same. That is how do I do that? I can apply a radiation. If I apply an RF radiation on spin A which is hydrogen continuously ok. So when I do that the nuclei the population shifts between alpha sates.

Why does it shift? Because remember according to we saw in the, in the very beginning. When we apply an RF radiation which is equal to energy difference the molecules will jump from alpha and go to beta and vice a versa. So this process will keep on happening alpha go into beta back will keep on happening if I keep on applying a continuous radiation.

So basically what I'm doing is I'm equalizing the population. Means earlier it was a boltzmann population but now it will be equal. So when the population becomes equal for the. As far as the carbon is concern it does not have any different on between alpha and beta. Because alpha beta now equal therefore it does not see any difference spin state. So when that happens this peak becomes a singlet. So this process of doing an RF irradiation on one sample so that it boost the J coupling is called as decoupling.

There are varieties of decoupling available methods in literature so therefore decoupling in fact is very ongoing part of research in NMR. The reason being what happens is you want to get a very nice decoupling. You don't want to get half done decoupling. So therefore you need to apply irradiation in a very proper manner. If you apply too much radiation it will cost the heating of sample.

So heating of the sample is not good because their sample will not be stable. So how do we apply a low power radiation so that we get a good decoupling is a process of non going work in research in NMR. So basically what happens in the end, in the when we do decoupling the J coupling information is gone. Because remember if suppose I have, let us look at again this picture. So basically if I look at this, this will give me a singlet. Sorry if I do a decoupling, if I do decoupling this will basically become a singlet.

So I have lost the multiplicity information. So this information is lost. Now suppose I have this is for CH. Suppose I have CH2 where I expect according to the N + one rule a triplet. But again if I decouple I will get basically a singlet. And then that information is also lost ok. So in this manner if I do decoupling for every carbon I keep losing the information of its multiplicity. but I will get definitely a much cleaner spectrum which is a singlet.

So we will see that how the multiplicity aspects can be recovered by some other techniques and will see some examples of how decoupling improves the, the spectral resolutions in the next class.