NMR Spectroscopy for Chemists and Biology
Professor Ashutosh Kumar
Department of Biosciences & Bioengineering
Indian Institute of Technology, Bombay
Analysis of NMR spectra of molecules
Lecture No. 9

Good morning, so welcome to today's lecture. We were discussing the scalar coupling or J coupling or spin-spin coupling, and we will continue discussing today. So in the last class we were discussing, how the splitting happens because of two spins are in neighbor and these are connected through bond and then we went ahead in defining magnetically equivalent and chemically equivalent, we said that magnetically equivalent does not cross the splitting and chemically equivalent which groups have a same chemical shift and all those and then we moved ahead and looking at what actually is the reason for the different coupling strength.

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So we defined that spin-spin coupling over one bond is called ^1J, and then if it is two bond coupling, then it will be called like ^2J, so here is the number of the bond and this is J is J coupling and it is also called geminal coupling, now then three bonds coupling are called vicinal coupling so that is denoted by ^3J ,and long range coupling will be called like more than four bond , they will call long range coupling and that is denoted by like as usual ^nJ is the superscript, now we ended it here and we define the factors that effects the spin-spin coupling. So what are those

factors, so some of those factors that we listed out are the hybridisation of the atom involves in the coupling.

So this hybridisation is one of the important factor and then that also is somehow connected with what kind of bond angle is there between these spins so that also effects the dihedral angle. So, dihedral angle is between two planes and generally this is very important, in case of protein structure. So dihedral angles also dictates the strength of the coupling and then the bond length like single bond it is there or double bond there, that also affects. Today we are going to understand all these and if there is a substituent like if it is a lone pair of there is a π bond or, or some electronegativity group are attached, that also affects the strength of the spin-spin coupling or J coupling.

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So that we will be discussing today giving some examples, so let us start the one bond coupling. So one bond coupling is if like if we consider the coupling between proton and deuterium, so suppose we have a molecule like here we H and D, what is the one bond coupling between them, one connected proton with deuterium. So in this case the one bond coupling between D-H is generally the 42.94 that is 43 Hertz. And as you know that proton, so one bond H-H coupling and D-H coupling, so D-H coupling is 42 so H-H coupling can be quite high and if you look at, this is dictated by gyromagnetic ratio.

So the gyromagnetic ratio of proton is 6.5 times higher than the deuterium so in that case $^1J_{HH}$ is 280 Hz. So now we come to the heteronuclear J coupling, so that as we discussed in the previous slide that depends upon the hybridisation strength, so let us take some of the example of J coupling between ^{13}C and proton. So if it is sp^3 hybridised and you see this bond angle is also 109, so here the ^1J bond coupling in this case is 125 Hertz. Now if this becomes sp^2 hybridised the coupling strength actually increases and it becomes 165 Hertz and when it sp hybridised like then s contribution increasing , so one angle is also increasing and that gives up 250 Hertz.

So, you can look at the methane, like here sp^3 hybridised where 4 protons are attached in this case 125 Hertz and as you increase the s contribution here actually, the coupling constant strength increases. Now other thing that affects the coupling strength is the substituent that can also change the spin-spin coupling and that value change can happen in tune of 20 to 30 Hertz.

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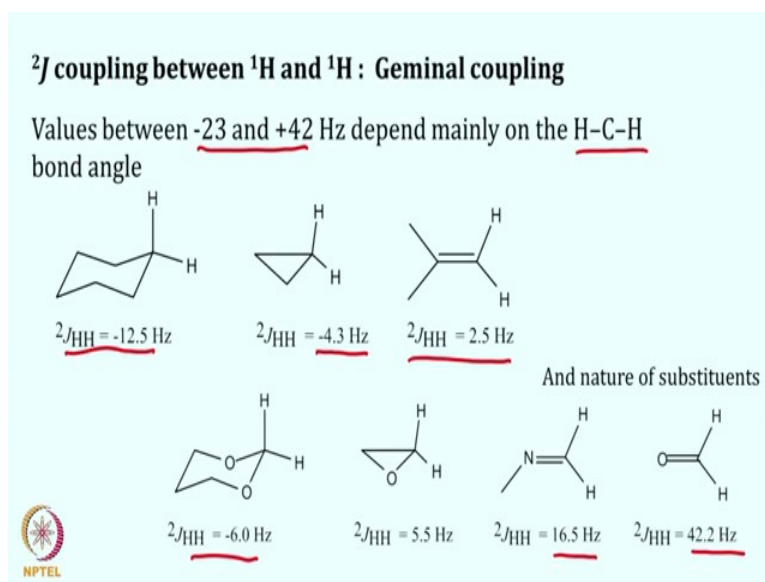
Okay, so just for an example now this was one bond coupling, homonuclear and heteronuclear, now we will come to the two bond coupling , this two bond coupling , proton-proton two bond coupling are called geminal coupling and there is somewhere between now minus 23 to 42 Hertz. As we discuss the splitting strength does not depend whether the coupling is negative or positive with its absolute value, this is dictated by the orientation of the spin, so whether it is negative or

positive. So lets us look at some of the two coupling, geminal coupling that is dictated between the bond angle between H-C-H here.

So if we look at here, in this case two bond coupling is -12.5 Hertz. The same two bond coupling between these two decrease and that becomes -4.3. So if you look at, the bond angle is changing here. Here also two bond coupling because of bond angle change, it is 2.5 Hertz. So as we move from here, the sign of J coupling is changing and bond angle is changing and that is how it is also decreasing in the magnitude. So some more example, so if you look at this molecule here the J bond coupling is minus 6 Hertz and as we change, so this becomes like now you can see it, the substituent is also affecting the J bond coupling. So the difference between this and this are two oxygen are attached and that change is by like 6 Hertz or so.

Here again one oxygen is attached here replaced carbon is replaced by oxygen, and you can look here it is was - 4.3 and now it is become + 5.5, so almost 10 Hertz coupling has changed. Similarly one can look here at here the proton-proton coupling between these two change by almost 14 Hertz and this is because now here this carbon was replaced by nitrogen and the substituent is also affecting a nature of substituent is affecting the coupling strength. So in this case again if N was replaced by oxygen this coupling has increased and now it has become 42.2 Hertz.

So the bond angle, the hybridisation, the substituents are changing the strength of the coupling, that is what we understood here. Now three bond coupling between proton and proton-proton and proton.

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³J-coupling between ¹H and ¹H

The dihedral angle (φ) dependence,
Karplus equation: $^3J_{HH} = A + B\cos\phi + C\cos 2\phi$

φ is the dihedral angle between the two C–H vectors,

A, B and C are constants with empirical values for hydrocarbons found to be A = 7 Hz, B = -1 Hz and C = 5 Hz.

Generally ^3J-coupling constants are greatest when φ = 180° and φ = 0° ($^3J_{180} > {}^3J_0$), and very small when φ = 90°.

So this is generally is gives the dihedral dependence or φ dependence, so this φ is very important and Martin Karplus actually provided an empirical relation which co-relates the three bond H-H coupling with the φ torsion angle in the peptide bond. So this has become very very instrumental tool to know this confirmation of a protein and if one can calculate the three bond coupling between proton and proton, essentially it is like this angle, φ torsion angle between two proton, so here is H-N-H, I will come to that in next slide.

So if one can determine the φ torsion angle between two planes, one from one amino acid and another amino acid you know that dihedral angle and the coupling between this proton and this proton dictates the phi torsion angle by this empirical relation and if you know the three bond coupling, you can determine the phi and that gives the torsion angle in the protein case. So *ABC* are constant and they are having empirical value for the hydrocarbons, so like typically *A* is 7 Hertz, *B* is -1 Hertz and *C* is 5 Hertz and you can calculate putting at the different φ value, how the *J* varies between *180˚* and *0˚* and it is very small when it is *90˚*.
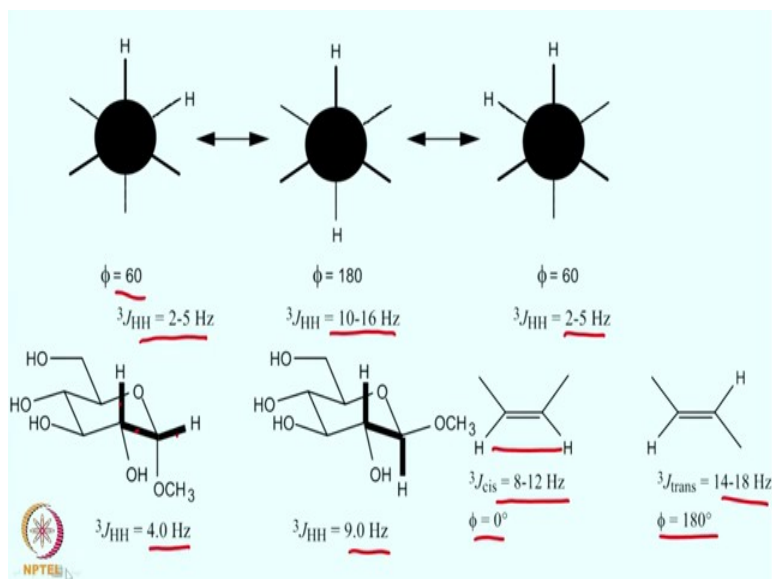
So I will come to the next slide, so the variation of the J couplings on the φ. So like here if the φ torsion angle between these two planes, so this is ethane plane, if the phi torsion angle is around *60˚*, so you have a three bond proton-proton coupling is 2 to 5 Hertz, if it is 180˚ then it is 10 to 16 Hertz and if it is *60˚* it is 2 to 5 Hertz. So depending upon how they rotate, here you can see the J value, the J value three bond coupling is changing, okay and so one if you can experimentally determine this J value, we can calculate the φ torsion angle. like similarly some more three bond coupling in this molecule, the here 1, 2 and 3, these are three bonds, so the coupling between this and this proton is typically of 4 Hertz, if you take the same molecule but orientation is different now this becomes 9 Hertz.

So if you compare this orientation with this orientation, what had change is the angle and that angle change the J coupling value. Similarly like if you look at here, if J bond, between *cis* protons and *trans* protons differs because there is a *cis*, the angle between these φ- φ angle is 0˚ in *trans* it is 180˚, so this is 8 to 12 Hertz and here it is 14 to 18 Hertz. So now it is clear that even the torsion angle changes the three bond coupling in case of proton-proton.
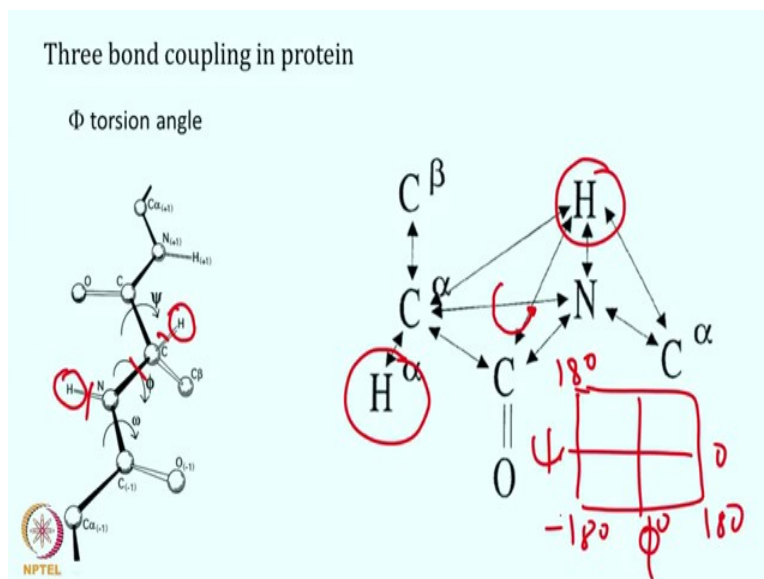
And therefore, this as we were discussing this is very important in case of getting the protein conformation. So in protein suppose if we measure this coupling between this proton, this proton which is NH, proton and this C_α H proton, so here is the torsion angle which is φ, if one can measure experimentally we can determine the φ torsion angle, so this is actually φ torsion angle. So what we are measuring, the coupling constant between these two protons and this three bond one bond, two bond and three bond. So, by measuring this three bond coupling, one can determine the φ torsion angle, and if you remember your basic Ramachandran plot, it is between φ and ψ, so φ plot and ψ plot -180° – (0,0)° - +180°.

So one torsion angle just by experimentally determining from the three bond coupling one can know what is the φ and φ can change between +180° to -180°. So at least from phi you know that what is the φ angle and then one need to determine the ψ angle the you know the conformation of protein or a secondary structure of the protein. So therefore the three bond coupling or the coupling in general is very important to get the molecular structure, for a small molecule a small organic molecule using this coupling one can determine the at least partially conformation of a protein because we can determine the directly φ angle from this three bond coupling.
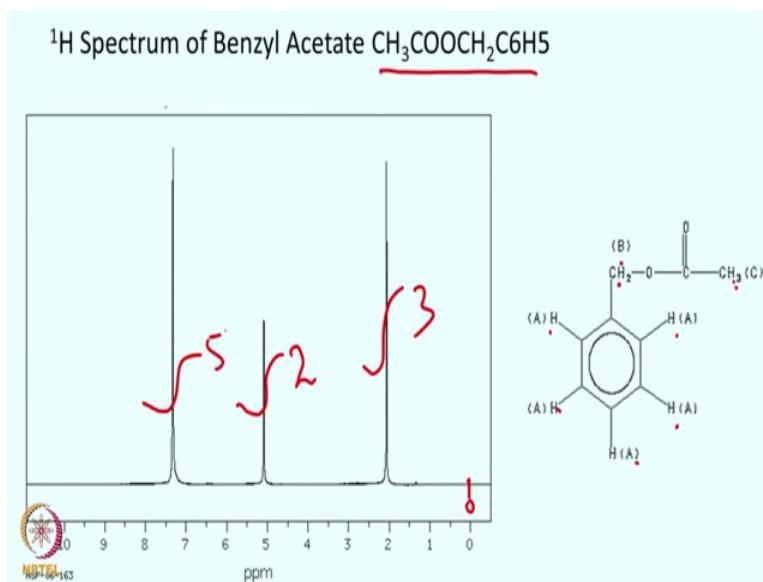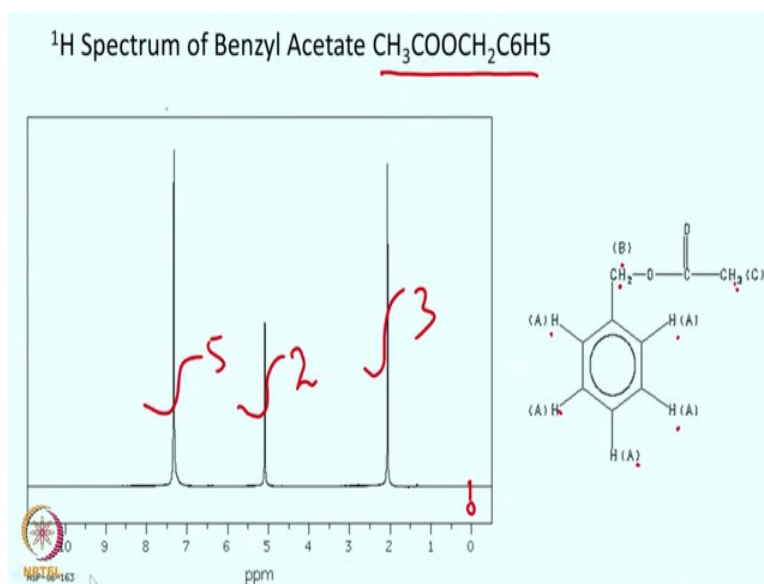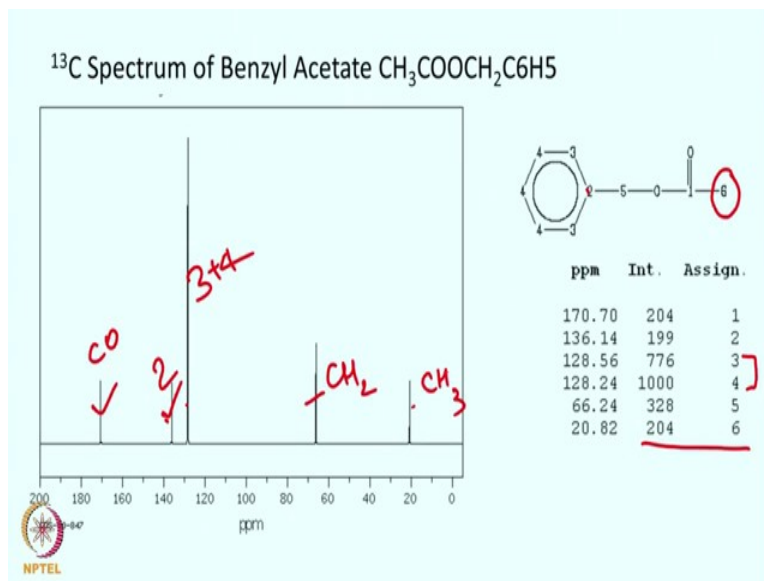
Now what I will do today, I will give some example to make more clear that how the spectrum looks like. So I will give you some example of say, let us take some of the example like benzyl acetate and we and try to understand how the spectrum looks like and how you can interpret those depending upon the structure. So if you look at benzyl acetate is like this, so there is a benzene ring, then CH_2 is attached to this and then COO and then CH_3. So how many types of proton we have, essentially we have a three types of proton.

One type is coming from like, if you are recording on a relatively smaller magnet, we have a like a one proton coming from the benzene ring, here we have five proton, 1, 2, 3, 4, 5. Then second kind of protons, we have here two kind of proton coming from methylene group here and then third kind of from methyl group here. So three kinds of protons we have, so therefore we should have at least three peaks in the spectrum and their intensity will vary depending upon how many protons are contributing. So here we are having contribution from three protons, here we are having contribution from two proton and here we are having contribution from five proton.

So if this is 0 ppm is our TMS then the first one will be like from the methyl proton that will be around 2 ppm and this will corresponds to three proton. Then will be from methylene, this is, this will be a little bit downfield shifted because it is connected to ring as well as the COO group, COO is electronegative group, so that will be downfield shifted or higher ppm value shifted and

that will correspond to two proton. Now here for benzyl ring we have a five proton so that will correspond to five protons, so that will be the spectrum of benzyl acetate.

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^{13}C Spectrum of Benzyl Acetate CH_3COOCH_2C6H5

ppm	Int.	Assign.
170.70	204	1
136.14	199	2
128.56	776	3
128.24	1000	4
66.24	328	5
20.82	204	6

## ^{1}H Spectrum of Benzyl Acetate CH_3COOCH_2C6H5

Now let us look at some of the, some other example and we take the example of suppose like a, say benzyl acetate , the carbon spectrum if we look at, so how many types of carbon now we have, okay. So let us look at the carbon that we have, same molecule here let us go back, let us look at the carbon molecule so we have the carbon. Here we have a six carbons here, one carbon here, seven carbons and eight and nine carbon. Now how many types of carbon we have, so we

go back here and we have like, here if you look at these, these three carbons are chemically equivalent.

These two carbons are chemically equivalent, this is another one, now CH_2 is another one and C=O is another one. So, yeah, and here is the methyl is another one. So we have six type of carbon, therefore we should get six peaks and where are those? So the first one, we will look at the methyl peak, because that will be most off-field shifted or lower ppm value so that comes around 20 ppm so this is for CH_3, okay. The next one here, the next one is from the methylene group and that will be here. Now after that what we have is three aromatic peak, so three aromatic peak like two here, then so three aromatic peaks are coming here, here, so that are quite close here.

If you look at, like these three, three and four are quite close and two is because two connected to CH_2 and all those so this will ortho so that is two and one is carbonyl peak, that will be most downfield shifted. So this is for CO, this is from the aromatic carbon two and these are three and four, they are quite close and then there we have from CH_2 and this is CH_3, so that is benzyl acetate carbon spectrum, okay.

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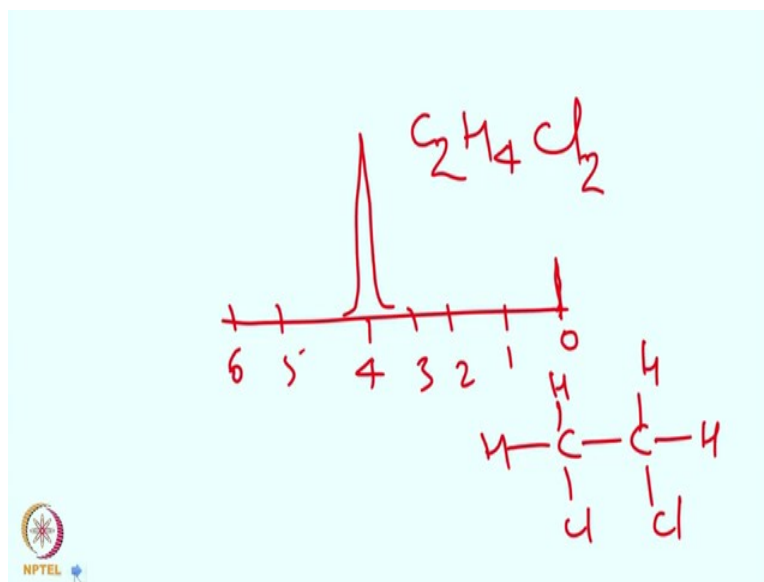
Then we move ahead and take some another example to understand how the spectrum looks like, I will give you and now we have to analyse .Our molecule is suppose, $C_2H_4Cl_2$ and suppose we

have a spectrum which is like this here is our 0 ppm and this is our signal coming from the standard reference and just suppose we get only one peak, okay, so what will be this molecule, let us define. So one peak are coming four protons are there, this is for proton spectrum and this comes suppose around 4 ppm, so here 1, 2, 3, 4, 5, 6, something like this, so what this molecule is.

So now it says that it looks like four, all protons are in the same environment so there are two carbons here are four protons, so we will write four protons here and all four are in the same environment, so it is this molecule, right, one two dichloroethane, let us take some other example.

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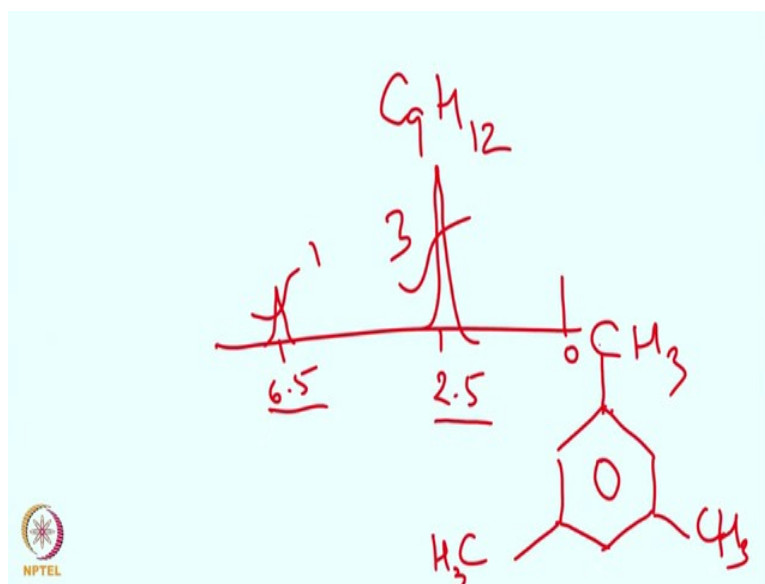
Suppose our molecule is now C_9H_{12} and we got this spectrum which is 0 ppm standard and we got here and here and this ppm value is suppose 2.5 ppm and this one is around say 6.5 ppm. So if you integrate here to get the ratio of 3 to1, now what this molecule can we solve this. So two things is are clear, first thing is if something coming around 6.5 that must be coming from the benzene ring, so let us write a benzene ring. And something coming around 2.5 most likely this is coming from the CH_3 or CH_2 so since now there are 12 protons, so we will look at where we can adjust and proton and all three like here, so six carbons are adjusted here, we have three carbons.

And then six carbons are gone here so we have actually six proton left here. So now that means we can, if we have molecule like this because the methyl's are in the same environment, so therefore it should be something like this. So now we have a three protons left here and these nine so, we have two kinds of proton, one coming from methyl group, so nine methyl's here and three here from the benzene ring , so therefore 9 ratio 3 is 1 ratio 3 and the chemical shift position is 2.5 and 6.5 ppm. So, this is the molecule that spectrum is like this.

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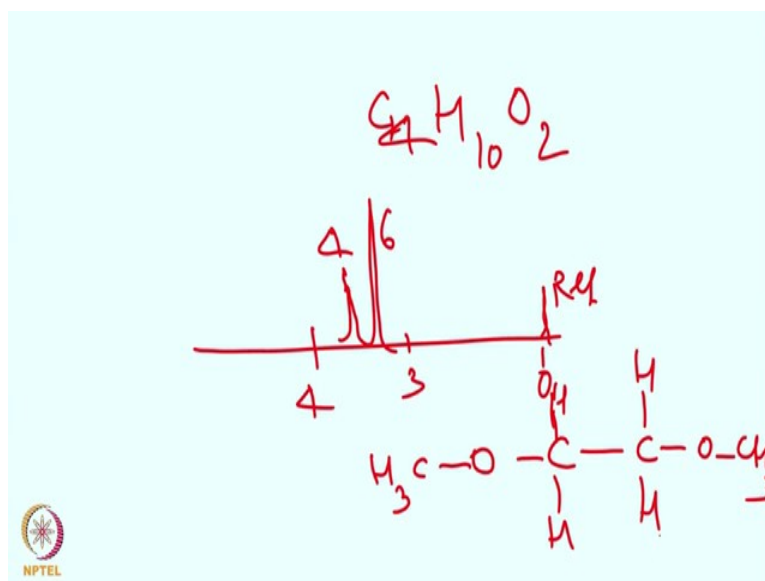
Next, move to next molecule, we have a molecule which is $C_4H_{10}O_2$ and this gives us spectra here say 0 ppm and this gives us spectra too quitely close and that ppm value is between 3 to 4 ppm and the ratio is 6 to 4, what this molecule is so how can we solve it. Now it looks like that only two kinds of protons are there so this is reference and then we have to write it like that so four carbons are there so two carbons here and since they look quite downfield shifted, so probably there is a two oxygen's here so now we have to fill the protons, so six protons are of same chemical environment, so that can be CH_3 and CH_3 and then four protons are of same kind so here will be four protons.

So this is the spectrum of this molecule. So, at the moment we are looked at the chemical shift only and how to interpret the spectrum and generate the spectrum from a given compound so that

we looked at. Now I will give you some examples of this splitting, so we will try to determine the chemical structure of the compound.

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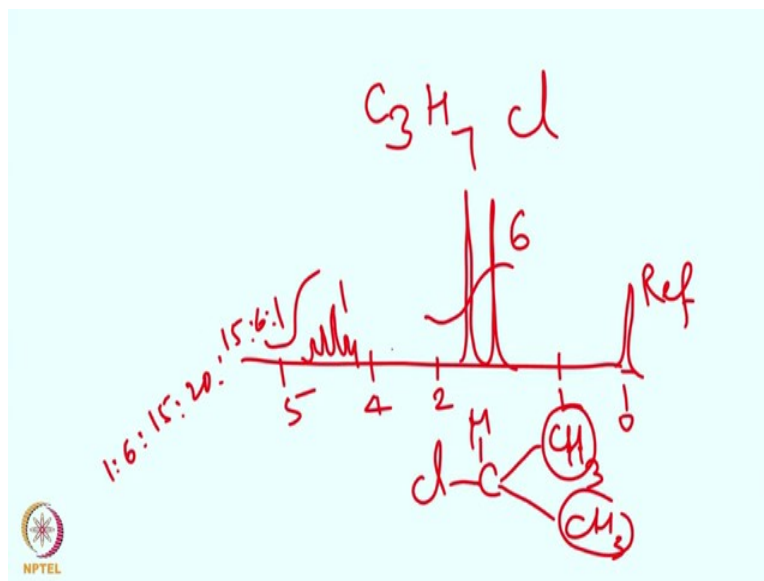
So suppose now consider the spin-spin coupling to determine what molecule is this, so let us take some example like C_3H_7Cl, and we have a spectrum where our reference is at 0 ppm TMS and we get here two peaks, and here we get a multi plate, like this. So here the ratio is 1 and this ratio is 6, so what this molecule could be, so let us try to understand this, okay. So one thing is clear that 6-6 protons are here, one proton is of this type, so only two kind of chemically different protons are there, okay. And then there is a chlorine group attachment to it, so let us attach a chlorine group, Cl group. So then two kinds of protons so it looks like that and since the value is here, this is around like a 1 ppm and this is 2 ppm and this one is 4 ppm and 5 ppm.

So it is very clear that one is methyl proton, so here is CH3 and another CH3, so now 6 protons are gone, one proton which is left is this one. Now this we can explain it, now 6 protons, we will split this into how many? 7 and that ratio, one can know that what will be the ratio of this splitting, so one can identify these molecules very easily. For 6 proton it should be 1: 6: 15: 20:15: 6:1. And this proton will split these chemically equivalent 6 protons into this, so that is why it is doublet.

So these two are equivalent, therefore there should have been one peak, if there was no coupling but because of coupling, we have a splitting into two and the 6 proton split this into 1, 2, 3, 4, 5, 6, 7, so this is shifted.

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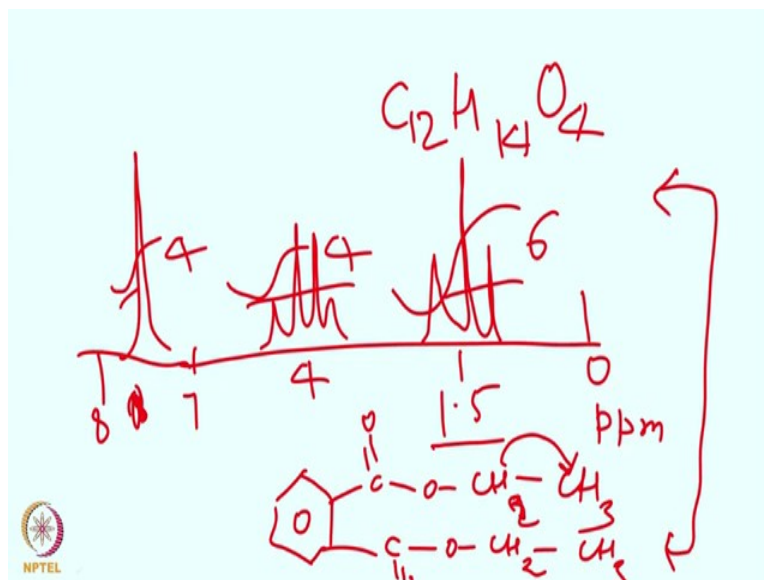
So next I will give one more example before we try to close up, so let us have a molecule which is $C_{12}H_{14}O_4$ and the spectrum for this is coming something like this, here we have a reference compound 0 ppm, then we have something like this and a quartet like this and then we have a singlet like this, and this corresponds to four peaks, this corresponds to also four peaks and this correspond to six peak. And the value here comes around say 7 to 8 ppm and this somewhere around 4 ppm and this comes around say 1.5 ppm, so 1.5 ppm, so what this compound could be?

So, 1.5 is clearly methyl group is there and there are six carbons, so probably two methyl's group are there, here there is the benzene ring, so let us try to write it, a benzene ring first and two methyl's are attached to there, now we see the four peaks are also there, so that means now these methyl's and there is here is also there is a something called methylene group, so probably it looks like, there is a structure which will be something like this, because now methylene groups will come here, that are four. So if you look at two two four this will be six and this corresponds to four proton. And then you can also explain the splitting because each of these equivalent

proton, split this into four and this will split into three, this CH_2 will split CH_3 into three, so six proton will be splitted into triplet.

Now, this four protons will be splitted into quartet and this will be mostly singlet because this is, there is no splitting here. So these are some of the example, rest examples you can do in the tutorials, so we will continue now we have a good exposure of the chemical shift, how to interpret using chemical shift. We all introduced the coupling constant and we looked at how the coupling constant gives the finest structure and we learned looking at the splitting pattern and the chemical shift value how we can identify the chemical structure of a molecule.

Now we are at a stage going little deeper and understand the quantum mechanical formalism of the coupling constant and then we looked at interpretation of some of those. So I hope these things are clear to you and rest the concepts will be cleared in the tutorial session so hoping you to see in the next class where we will start with quantum mechanical description of the coupling. Thank you very much.