

NMR Spectroscopy for Chemists and Biologists

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Lecture No. 08



Interpretation of multiplet structure using first order analysis

Hello, welcome to today's lecture. So we will continue with high resolution NMR spectra of molecules. So previously we discussed about the important parameter such as J-coupling.

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In the last lecture

- Important Parameter: J-coupling : Splitting of Resonances
- How does splitting happens
- Factors Influencing J-coupling
- Weak coupling and strong coupling
- AB, AX, ABX, AMX spin systems
- Magnetically Equivalent, Chemically Equivalent spin systems



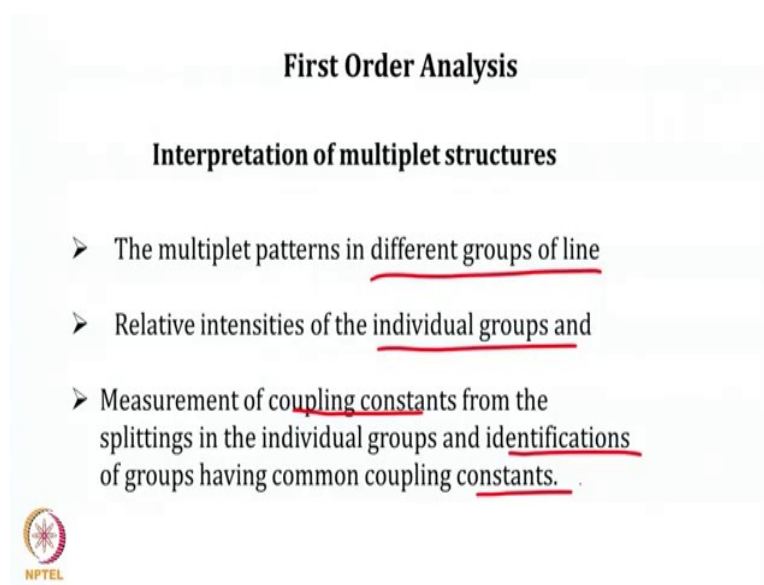
And this J-coupling causes the splitting of resonances. Then we asked this question, how does this splitting happen? So what is the reason behind this splitting? And we looked at; there two spins are coupled via bond that causes the splitting in the resonances. Then we discussed little bit in detail that what are the factors that influences this J-coupling and then we looked at two kinds of coupling, one was weak coupling and the strong coupling. This classification of the weak coupling and strong coupling was not on the basis of the strength of J value but the J value compared to the difference in the chemical shift between two spins. So that was the origin of weak coupling and strong coupling.

Then we went ahead and looked at the various spin systems such as AB spin system, AX spin system, ABX spin system and AMX spin system. And these AB spin system, AX spin system and all those spin system are based on the how far or how closely they are resonating. In that case AB means A and B , the resonance frequency is quite close whereas AX , A resonance

frequency is far from the X , so that is, and in these cases actually these are weakly coupled system because the AX in chemical shift differences, this is quite far.

So after that we defined something called magnetically equivalent and chemically equivalent spin system and we looked at that magnetically equivalent protons does not cause splitting and then we also said that we will look at in details when we will discuss about the quantum mechanical aspects of this, why magnetically equivalent protons does not cause splitting? However chemically equivalent proton spin system can cause the splitting. So today we are going to continue with this


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First Order Analysis

Interpretation of multiplet structures

- The multiplet patterns in different groups of line
- Relative intensities of the individual groups and
- Measurement of coupling constants from the splittings in the individual groups and identifications of groups having common coupling constants.



And then we are going to, in detail we are going to discuss the first order analysis of the spectrum and how we start interpreting the first order spectrum. So interpretation of multiplet structures we will discuss. So multiplet patterns in different group of line that we are going to discuss today, how we are going to interpret and what is the origin of relative intensity of these individual group and how we are going to measure this coupling constant between these lines, so splitting of individual groups and identification of these groups having common coupling constant, that we are also going to also look at how we are going to do. So these all points today we will try to cover up in today's lecture.

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Interpretation of multiplet structures

The centres of the multiplets represent the chemical shifts.

In magnetically equivalent nuclei: A_2, B_2, A_3 the **J-coupling** does not lead to splitting of lines

The integral of any multiplet is proportional to the **total number of equivalent nuclei** in that group.
 CH_2 : 2 Equivalent protons; CH_3 : equivalent protons

The resonance line of a nucleus J-coupled to n equivalent nuclei of another type gets split into **$(2nI+1)$** lines where I is the spin of the individual nucleus in the equivalent group.

So now we see that the centre of the multiplets represents the chemical shift. So what I mean, so this was a peak before the J-coupling consideration. Now if, because of J-coupling this is splitted into two. So centre of this will represent; so now this will be separated by JAB and the centre of this chemical structure will match with the centre of this, the upper peak. So the centre of multiplet structure represents the chemical shift. So the chemical shift for this spectra is essentially this centre point.

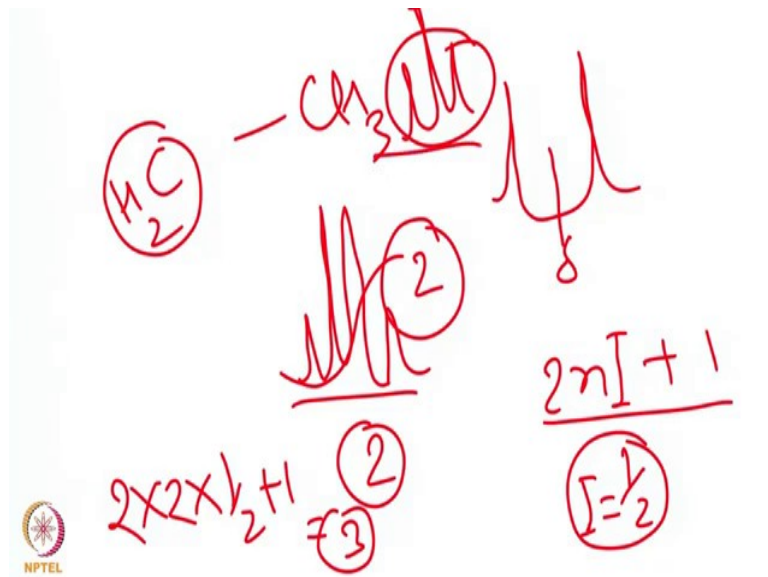
Now the magnetically equivalent spins like as we defined A_2 or B_2 or A_3 , the J-coupling does not lead to splitting. So like, for an example CH_3 in methylene for this actually the peak is going to be only one peak, magnetically equivalent. Or in previously we had given an example of magnetically equivalent. So in those case if the spin system is like A_2 or B_2 or B_3 there will be no splitting of line in these cases.

Now next, integral of any multiplet is proportional to total number of equivalent proton in that group. That means, now what we are saying even if they are splitted, say in methylene it is splitted into four, for ethyl alcohol spectrum if you remember, but this is coming because of the 2 protons. So if you integrate this will, the ratio will correspond to 2 proton.

Similarly, like CH_3 in alcohol, this will correspond, this will be splitted into say, 3 because of CH_2 , now this will corresponds to 3 proton. So integral of multiplet is proportional to the number of, total number of equivalent proton in that group. Next the resonance line of a nucleus, J-coupled to n equivalent of another type gets splitted into $2nI+1$ is the spin of the

individual nucleus in the equivalent group. What I mean by that, I will just explain each of these points one by one. So, so what I mean here is following.

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That first thing we mentioned that this splitting; splitting of these two peaks, the chemical shift will be centre. Second we mentioned that whatever splitting is, but this will match to the number of protons. So that will be contributed by the number of protons that are contributing towards this signal. Then we mentioned that the number of, how many numbers of, it will be splitted, that depends upon what is the I , $2nI + 1$.


So if say, it is because of proton which I , the I of proton is, or hydrogen is half so say there are 2 protons. So 2 protons then it will split into

$$2 \times 2 \times \frac{1}{2} + 1 = 3$$

So CH_2 will split CH_3 in ethyl alcohol into 3. So 1:2:1, that I am going to come how this ratio is coming. So that is the idea that we discussed in the next slide.

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- For a nucleus J-coupled to two groups of equivalent nuclei with n and m nuclei having I_1 and I_2 : total number of lines will be $(2nI_1+1)(2mI_2+1)$ $H-C-m$
- For spin $1/2$ nuclei (say different groups of $^1H, ^{13}C, ^{19}F, ^{31}P$ etc) the multiplet for a particular nucleus would have $(n+1)(m+1)$
- Single group of n equivalent nuclei with spin $1/2$ coupled to a particular nucleus, say A , the different lines in the A multiplet will have the intensities in the proportion of binomial coefficients in the expansion of $(a+b)^n$.



 $3 \times 6 = 18$

 $(2 \times 2 \times \frac{1}{2} + 1)$

 $(2 \times \frac{3}{2} + 2 \times 1) =$

Now, but what happens, for a nucleus which is J-coupled to two groups of the equivalent nuclei with n and m nuclei having two spins like I_1 and I_2 . What I mean by this, suppose we are considering a case of CH where there are 2 groups, like H-C group and here there are n and here there are m . So splitting of these kind of things will be $2nI_1+1$ where, and $2mI_2+1$. So this again we are going to discuss in detail. So how many equivalent that will be, how are we going to interpret this data? So next will be the spin of half nuclei, say different groups like proton, carbon 13, ^{19}F and ^{31}P .

The multiplet for particular nuclei would be $(n+1)(m+1)$. This I am going to again explain you in a moment. And a single group of n equivalent, so n equivalent nuclei with spin half ($I = \frac{1}{2}$) coupled to a particular nuclei say A , the different line in the A multiplet will have the intensity of proportion to binomial coefficient of expansion $a + b$ to n . So let us explain one by one. For a nucleus J-coupled to two groups of equivalent protons n and m nuclei of I_1 and I_2 spin. So like there are two kind of spin. So let us say I_1 is half and I_2 is say, 3 by 2 or something like this.

So then total splitting will be 2 plus, suppose $I_1 = \frac{1}{2}$, $\frac{1}{2} + 1$ and for, because of this, so multiplied with, because of this,

$$\left(2 \times \frac{1}{2} \times 2 + 1\right) \left(2 \times \frac{3}{2} \times 2 + 1\right) = 18$$

so such this will give a such complicated spectrum. Whereas like in heteronuclear coupling so say, proton is half spin, carbon13 is $I = \frac{1}{2}$ so 1 proton coupled to one carbon 13, so for

carbon 13 and proton both are half, so this will $2 \Rightarrow +1$, so that will be total of 3 line so like carbon 13 and proton will cause 2 lines separately. So now let us look at some of the examples here.

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Interpretation of multiplet structures

First-order rules ($\Delta\nu / J \gg 1$)

For nuclei with $I=1/2$:
 The multiplicity of the splitting equals $n+1$, where n is the number of nuclei in the neighbouring group (for $I > 1/2 \Rightarrow 2n+1$)

Pascal's triangle.

$n=0$			1			
$n=1$			1	1		
$n=2$		1	2	1		
$n=3$		1	3	3	1	
$n=4$	1	4	6	4	1	
$n=5$	1	5	10	10	5	1

Handwritten notes: J_{AB} , ν_A , ν_B , $(a+b)^n = \sum_{k=0}^n \binom{n}{k} a^k b^{n-k}$, $2 \times 1 + 1 = 3$

So just to remind you, we are now discussing the interpretation of multiplet structure, and for first order rule. So only we are discussing the first order rule. First order rule means the comparison you have to do with a difference in their chemical shift compared to the J value. So here, suppose resonance A and resonance B there is a difference between them in terms of chemical shift is ν and their coupling constant is J_{AB} so for first order rule to be valid, the difference in ν that is here, divided by the J should be much, much larger than the 1.

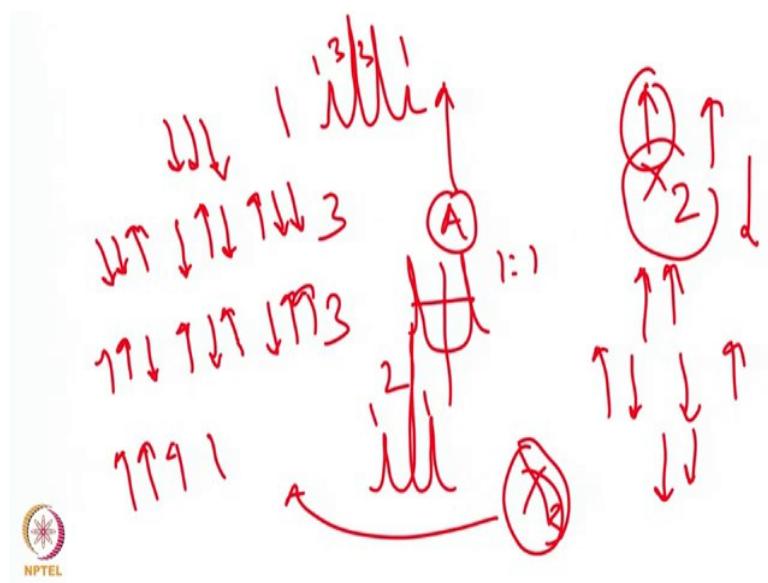
That means change; difference in the chemical shift should be larger than the J . Now for half nuclei, the multiplicity of splitting, that will be $n+1$, where n is the number of nuclei from the neighboring group. And for spin more than half it will be $2n+1$. So suppose for deuterium, which spin is 1, so this will cause the splitting 2 into suppose one deuterium $2 \times 1 + 1 = 3$

So deuterium will split into 3 whereas proton will split into, one proton will split into 2.

So now for $I = 1/2$, as we said that will be splitting according to the coefficient of the binomial expansion of $(a+b)^n$ to the power n . That is given by something called Pascal Triangle. So suppose there is a 0 spin that will be only 1 line. So one line if there is no other spin. But suppose there is 1, 1 proton near to another proton so that n is 1 here, if you look at here. So that will split into 2. And intensity will be dictated by the coefficient of this expansion 1: 1. So that means 1 proton will split the neighboring signal of equal intensity 1: 1.

Now there are, suppose there are 2 protons so they will split their neighboring proton in the intensity ratio of 1:2:1. Now if there are 3 protons that will split into 1: 3: 3: 1 and that is all coming from expansion of this and this is called Pascal Triangle. Similarly, if there are n equal to 4, they will split 1:4:6:4:1 and 5 will be 1:5:10:10:5:1. So I will just give you little bit of understanding of this.

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So what we are saying, suppose there is only one proton near another proton. So 1:1, this is our observation proton or nuclei and the other one is say X here, so AX system, so now X will split A into 2 of equal intensity because X can either be like up or down and that is going to be splitted into 2 of ratio 1:1. Now suppose there are X_2 which are, X_2 here 2 molecule of X , so that means they will be oriented both up, up down, down up and down down.

And that will split A into 3 of ratio 1:2:1, so 1:2:1. That is what we are saying. Suppose X is now 3. So how they are going to split? So their orientation is going to be like up up up, 2 up 1 down, like this, and then 2 down 1 up, and 3 down. So that is 1:3:3:1. So 3 spins, sorry the X_3 will split A into now 1:3:3:1. That is what we are saying.

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Interpretation of multiplet structures

First-order rules ($\Delta\nu / J \gg 1$)

For nuclei with $I=1/2$:
 The multiplicity of the splitting equals $n+1$, where n is the number of nuclei in the neighbouring group (for $I > 1/2 \Rightarrow 2nI + 1$)

Pascal's triangle.

$(a+b)^n = \sum_{k=0}^n \binom{n}{k} a^k b^{n-k}$

$n=0$			1			
$n=1$		1		1		
$n=2$		1	2	1		
$n=3$		1	3	3	1	
$n=4$	1	4	6	4	1	
$n=5$	1	5	10	10	5	1

That the intensity of the line will be according to the Pascal Triangle so 2 spins 1:2:1, 3 spin 1:3:3:1, 4 spin 1:4:6:4:1 and so on and so forth. So that is the intensity of the lines, how they are coming from. Now similarly again I will go little more detail about the energy diagram.

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Analysis of NMR spectra of molecules

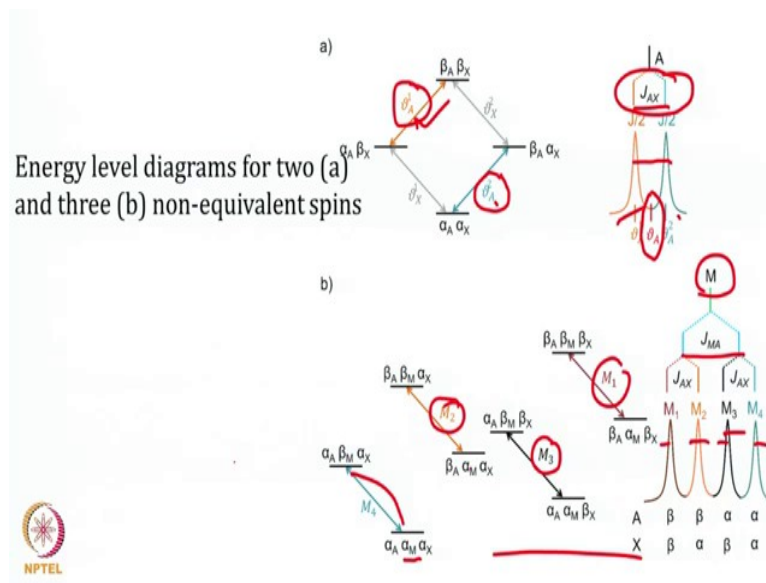
$-\text{CH}_2-\text{CH}_3$, energy level diagram:

CH_2	m_I	CH_3	m_I
$\alpha\alpha$	+1	$\alpha\alpha\alpha$	+3/2
$\alpha\beta \beta\alpha$	0	$\alpha\alpha\beta$ $\alpha\beta\alpha$ $\beta\alpha\alpha$	+1/2
$\beta\beta$	-1	$\alpha\beta\beta$ $\beta\alpha\beta$ $\beta\beta\alpha$	-1/2
		$\beta\beta\beta$	-3/2

↓ 1:2:1 (CH_3) ↓ 1:3:3:1 (CH_2)

That is what we have said, so let us talk about our famous molecule ethyl alcohol and at the moment I will just consider these two, CH_3 and CH_2 . So CH_2 will be in up up state, that is $\alpha\alpha$ state, $\alpha\beta$, $\beta\alpha$ state and $\beta\beta$ state and that will cause the splitting of CH_3 in 1:2:1 ratio. So this is the multiplicity of this. Now CH_3 will be in $\alpha\alpha\alpha$ state, $\alpha\alpha\beta$ state, so that is what I mean by $\alpha\alpha$ state and $\alpha\alpha\beta$ state something like this. So that will be again 3 type, 3 type, 1 type and that is why this CH_3 will split CH_2 in ratio of 1:3:3:1.

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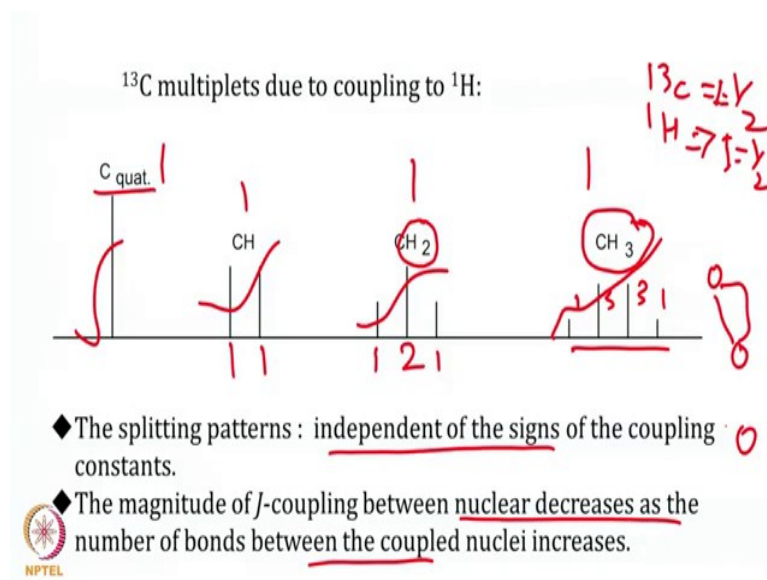
So now that let us look at little bit of energy level diagram what is happening for 2 and 3 non-equivalent spin. So 2 spins here is the case. Energy level diagram suppose for α state of A and α state of X here; so now in this case alpha is flipping and that is giving origin to this νA_2 and here also α is flipping, so this is giving rise to νA_1 . So therefore because of this coupling with X , now 2 lines are coming, one is from here this one, and another is from here to here. And the splitting between them is actually that dictated by this what is the coupling constant between them J_{AX} .

So and the, without splitting this would have been the original frequency here but now because it is coupled to X we have 2 lines and they are separated by that J_{AX} . So now A because of coupling with X , splits into 2 and of equal intensity. Suppose A is coupled to non-equivalent spin so here will be the case for that. So now 2 non-equivalent spins, so that may, each of them will split into 2. So M is 1 which is coupled with A , so now M will be split because of coupling with M , A will be splitted into 2 and then further it would be splits into 2 because of coupling with X .

So now it will give us 4 lines with equivalent intensity which is here, M_1 , M_2 , M_3 and M_4 . So here it is shown here. So in this case M is flipping and that is why $M_\alpha \rightarrow M_\beta$ that is giving line to M_4 here. Then here actually now, if you look at this also, M is going to beta but that has different energy and that will be M_2 so these two are coming from here. Now it is coupled with X as well. Therefore, this giving line to M_3 and M_1 . So 1 line now splits into 4 because

they are coupled to M and X . And that is why we are saying 2 non-equivalent spins will cause splitting into 4 lines.

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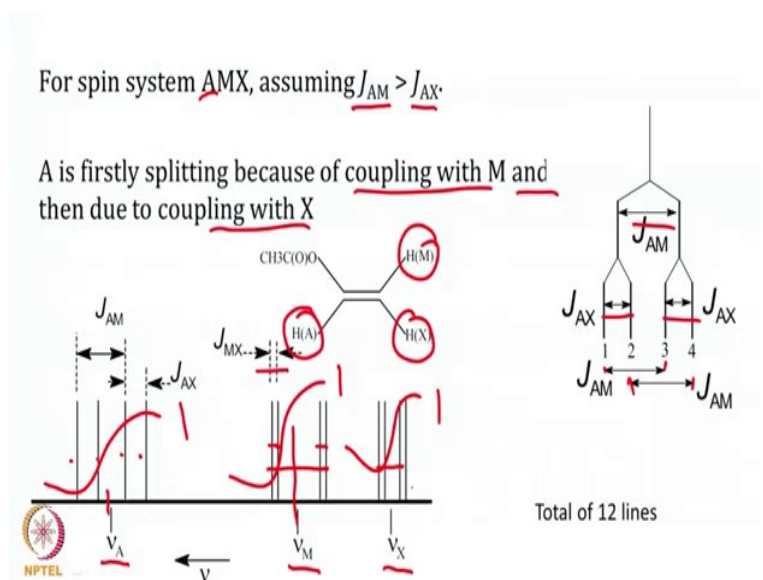
Now let us look at some heteronuclear splitting pattern. So here, suppose we take a molecule where we are detecting carbon 13 and it is coupled with proton. So carbon 13 has its spin half and proton also has carbon 13. Its spin is half and for proton, so $I = \frac{1}{2}$ for this. For proton also spin is half. So now how this coupling is happening? For a CH_3 group that C is now coupled with 3 protons of half spin. And these 3 are equivalent. Therefore, this carbon spectrum will be splits into 4 with a ratio 1:3:3:1.

So this methyl carbon spectrum will be like this. Now coming back to methylene here we have 2 equivalent proton that will split carbon spectrum into 3 and ratio of this will be 1:2:1. Now coming back to CH, CH here there is only one proton which is splitting this carbon as resonances and that will 1:2:1. And quaternary carbon, now this quaternary carbon is not attached to any of the proton. So there this will not be split and this will give single line. So now, but now if you integrate all of them, since all of coming from one single carbon so their integral of this will be 1, 1, 1, 1.

So that means each of this carbon contributing to equal intensity however splitting pattern depends upon how many equivalent protons are attached to different carbon. So there we can conclude that splitting pattern is independent of sign of the coupling. So does not matter the coupling is negative or positive it just how many protons are attached to it. And magnitude of J -coupling between nuclear spin decreases as the number of bond increases. So that we have

seen that 2 bond coupling is quite, quite strong compared to the 3 bond, 4 bond coupling. So as we move far the effect of coupling constant decreases.

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So let us look at the some of the more interesting examples, so like we have defined AMX system. So for AMX system the separation in the resonance frequency between A , M and X is quite far and we assume that the coupling between J_{AM} and J_{AX} is different. So J_{AM} is more than J_{AX} . So how they will split? So now A will be firstly splitted because of the coupling with M because this coupling constant is higher then eventually it will split because of the coupling with X .

So here for an example, now this splitting is happening, so there are say, 3 resonances separate for A , separate for M and separate for X . Here what we are saying that A is coupled with M and X as well. So first splitting will happen into 2, so here are that splitting, that νA will be splitted into 2, one is this line, another is this line. Now each of them will be splitted into 2, so that that is why we have 4 line. But the centre of that will be the original frequency of A . So now A will split and give 4 lines.

Here if we now, M is also coupled with A therefore now the splitting first happens because of A here into 2 and MX is smaller so that means again it will be splitted into 2, and here it will be splitted into 2. While similarly X will be, is coupled with M so it will also be splitted into 2 and further it is coupled with A so that will be splitted into 2. So a system like this here, A , X and M we have spectrum like this.

And that would be, each of them if you integrate here, this would be intensity 1, intensity 1 and intensity 1 because each of them, each contributed by only one proton but the difference in the line coming because they have different J coupling value. So here that that is why we have 12 line, first it is splitting because of J_{AM} , now further it is splitting because of J_{AX} so each of them get 4 line and that is the separation between these lines. So now that is what, here for A 4 line, 4 line for M, 4 line for X total of 12 line.

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Factors affecting spin-spin coupling

Spin-spin coupling over one bond	1J
Geminal coupling	2J
Vicinal coupling	3J
Long-range coupling	$^nJ, n \geq 4$

Factors affecting spin-spin coupling

- ✓ The hybridization of the atoms involved in the coupling;
- ✓ The bond angles; ✓
- ✓ The dihedral angles;
- ✓ The C - C bond length; ✓
- ✓ Substituent effects such as ✓
- Electronegativity, neighbouring π -bond and lone pair effects);

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Ok so what are the factors that affects this spin-spin coupling? We have looked that single bond coupling is called; one bond coupling is denoted as like 1J . The geminal coupling is two bond coupling, vicinal coupling is three, and long range coupling we can call it nJ . So the factors that affect this spin-spin coupling is basically hybridization of the atom, what is the contribution of each of these like sp , sp^2 , sp^3 . What is the contribution? And that dictates actually the what will be the coupling constant.

Then it is it also depends upon what is the bond angle because that bond angle also plays an important role and dihedral angle and what is the length of like, single bond or double bond or triple bond so bond length changes according to bonding pattern and if there are substituents attached like electronegative group or neighboring group or different kind of bond, pi bond and lone pair and all those affects the coupling constant.

So in next class we are going to in detail discuss how each of these parameters affects the coupling constant and how using these concepts we can start interpreting some of the data. So

we are looking forward for you to attend the next class. And if you have any question, do not hesitate to write or ask us. We will try to respond those questions. Thank you very much.