Multidimensional NMR Spectroscopy for Structural Studies of Biomolecules

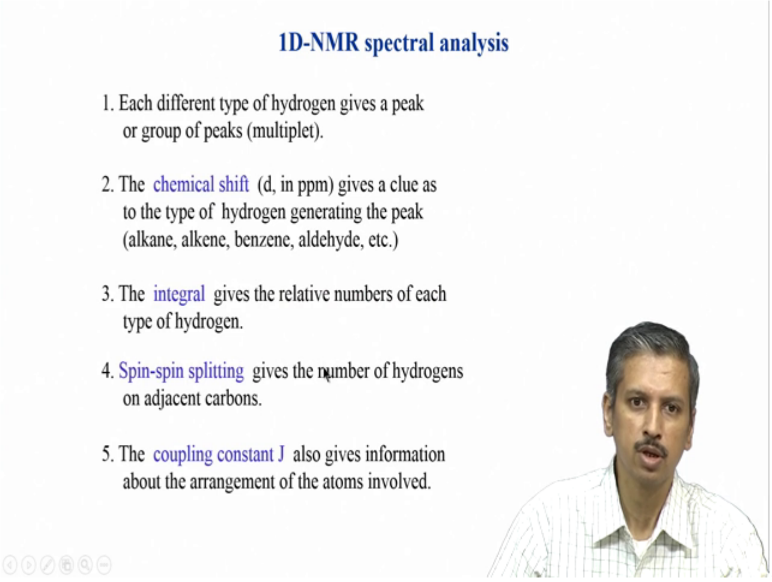
Prof. Hanudatta S. Atreya
Department of Electrical Chemistry
Indian Institute of Science, Bangalore

Lecture - 04

Basic concepts in 1D NMR: Chemical shift and Spin-spin coupling

We will look at now how one dimensional NMR spectrum can be interpreted, what are the different parameters. As, I said this is a course on for bimolecular NMR. So, the idea is to basically introduce very basics of these 2 concepts of chemical shift and coupling. We will not go into details which we have looked at in the in the previous course. So, let us briefly look at how this chemical shifts are calculated and how does spin-spin coupling affect our spectrum.

(Refer Slide Time: 00:55)



1D-NMR spectral analysis

1. Each different type of hydrogen gives a peak or group of peaks (multiplet).
2. The chemical shift (d, in ppm) gives a clue as to the type of hydrogen generating the peak (alkane, alkene, benzene, aldehyde, etc.)
3. The integral gives the relative numbers of each type of hydrogen.
4. Spin-spin splitting gives the number of hydrogens on adjacent carbons.
5. The coupling constant J also gives information about the arrangement of the atoms involved.

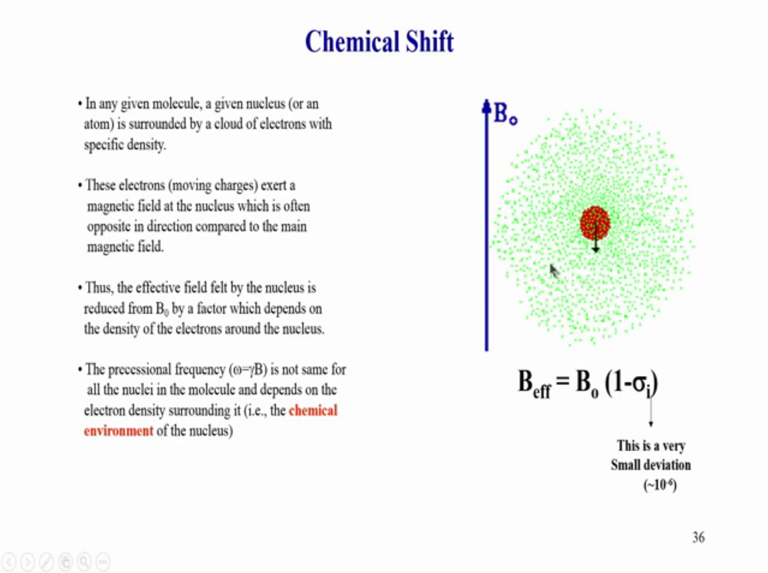
So, chemical shift so, if you look at a 1 D-NMR spectrum, in a general or any NMR spectrum, these are the 4 parameters which you will notice in the spectrum. First see the most important is a chemical shift which is nothing, but the position of the peak and that tells you information on whether it is an alkane, it is an alkene, or it is aldeyhyde and so on it is an amide an aromatic group’s etcetera.

The chemical shift is the most important parameter, it tells immediately about the possible functional groups or structure of the molecule. So, that is what is the first thing to look at. The second is integral that is how strong is the peak. And, that number the integral gives us the relative number of protons or hydrogen’s as we saw in the previous class. Here we would look at the area of the peak and that is why it is call integration.

In proteins and biomolecules this plays a very important role because many of the quantification applications requires this value to be correct. And, the third thing which is the what is called as J-coupling or spin-spin splitting, it is because of the coupling between the hydrogen’s and the carbons or hydrogen’s. And, this is basically the same thing as the fourth parameter here. The spin-spin splitting and coupling constant essentially tells you about, what are the adjacent hydrogen’s and carbon present in a molecule.

And is a very important parameter as far as structure is concerned, because from the coupling constant or from the spin-spin splitting pattern, we immediately get to know what are the number of neighbouring hydrogen’s whether is a cautionary carbon it is a tertiary carbon and so on. So, we will look at this chemical shift little bit in more detail now and see how this is calculated.

(Refer Slide Time: 02:35)



Chemical Shift

- In any given molecule, a given nucleus (or an atom) is surrounded by a cloud of electrons with specific density.
- These electrons (moving charges) exert a magnetic field at the nucleus which is often opposite in direction compared to the main magnetic field.
- Thus, the effective field felt by the nucleus is reduced from B_0 by a factor which depends on the density of the electrons around the nucleus.
- The precessional frequency ($\omega=\gamma B$) is not same for all the nuclei in the molecule and depends on the electron density surrounding it (i.e., the **chemical environment** of the nucleus)

$$B_{eff} = B_0 (1-\sigma_i)$$

This is a very Small deviation ($\sim10^{-6}$)

.

So, what is the first why does the chemical shift arise in a molecule? And, this something which was briefly covered in the previous class. It basically what happens is you have a nucleus sitting in the centre of an atom. And, an atom consists of electron cloud and this electron cloud, which is basically not a single electron. It is it is a cloud of electron that tries to oppose the magnetic field at the centre of the nucleus.

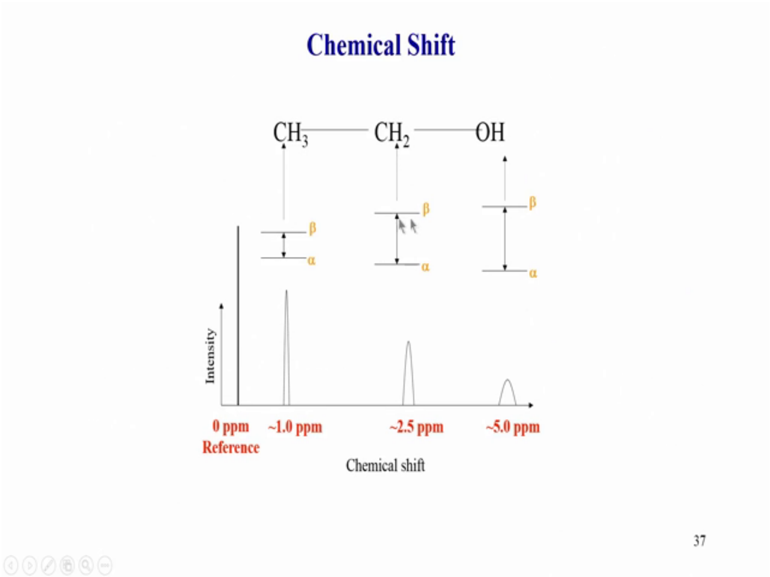
And therefore, the magnetic field experience by the nucleus is not just B 0, it is reduced by some factor and this is why we use the word shielding. So, essentially this electron cloud is trying to shield the nucleus from the external magnetic field. And, that depends now on many parameters; how much is the shielding, what kind of type of shield is it uniform and so and so forth. And, that depends on the structure of the molecule it depends on many other properties.

So, this shielding factor is a very small deviation from the main from 1. So, it is not that the shielding is very high the shielding just is a very small number nevertheless very important number. Without this shielding we would not be able to do NMR at all, even though we have all the possible of we have the nuclear magnets and so on. Even then without having this shielding affect NMR would have been not usable.

So, therefore, it was very important to understand this chemical shift concept this is the heart of the NMR technique as far as structure is concerned. So, we can see this what determines the shielding, how much is the nuclear shielded, that we will see the factors as we go on, but now let us see how it is chemical shift value that is how much is this shielding value calculated based on this parameters.

So, now we can see here again remember this equation. So, we are looking at an effective magnetic field, which is not different from the main magnetic field because of this fact. Now, this can be positive or negative we will for example, assume time being it is a positive, sigma is positive. So, this is smaller than 1.

(Refer Slide Time: 04:37)



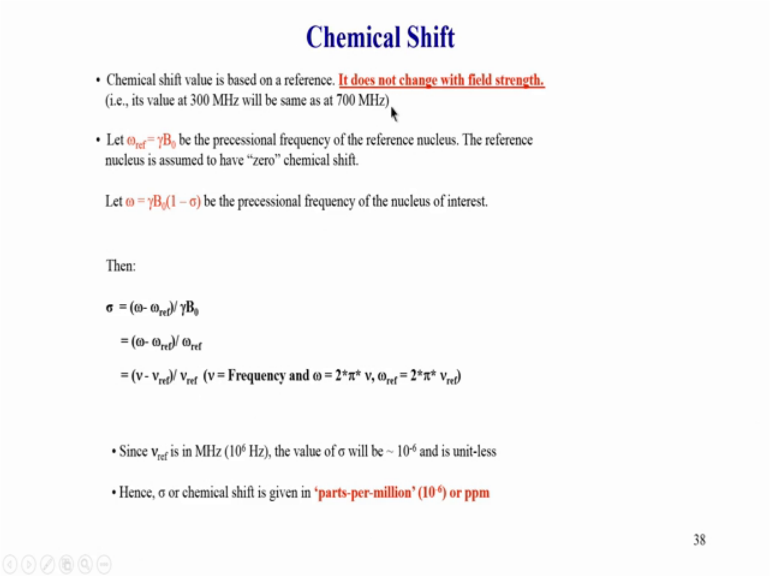
Chemical Shift

So, this is how it depictive the chemical shift effect is depicted here. So, we can see that because of this shielding factor the energy gap for the CH 3 become smaller than the energy gap of CH 2 and smaller higher for the OH. Because, this is the least shielded, this hydrogen is least shielded because of the oxygen here we will see the this concept of inductive effect shortly, but because of less shielding that the energy difference in the alpha and beta states of this hydrogen is proton is high compared to this and compared to that.

So, now this energy difference depends on now remember delta is H into mu. So, the mu that is H into mu the mu value the frequency value changes for this changes for this and changes for this. And, that is why this 3 different atoms or hydrogen comes at different frequencies and that frequency is converted into a scale called as ppm scale.

So, essentially what is going on here is a dth hydrogen atom is experiencing a different frequency of resonance they are not resonating at the same frequency. They are experience different resonating frequencies, but they are converted into a scale which become makes it independent of the frequency scale, we will see that shortly how this comes about.

(Refer Slide Time: 05:55)



Chemical Shift

- Chemical shift value is based on a reference. **It does not change with field strength.** (i.e., its value at 300 MHz will be same as at 700 MHz)
- Let $\omega_{ref} = \gamma B_0$ be the precessional frequency of the reference nucleus. The reference nucleus is assumed to have "zero" chemical shift.

Let $\omega = \gamma B_0(1 - \sigma)$ be the precessional frequency of the nucleus of interest.

Then:

$$\sigma = (\omega - \omega_{ref}) / \gamma B_0$$

$$= (\omega - \omega_{ref}) / \omega_{ref}$$

$$= (\nu - \nu_{ref}) / \nu_{ref} \quad (\nu = \text{Frequency and } \omega = 2 * \pi * \nu, \ \omega_{ref} = 2 * \pi * \nu_{ref})$$

- Since ν_{ref} is in MHz (10^6 Hz), the value of σ will be ~ 10^{-6} and is unit-less
- Hence, σ or chemical shift is given in **'parts-per-million' (10^{-6}) or ppm**

So, this is how the calculation is shown here. So, essentially the main thing is the chemical shift value when we calculate in ppm scale is based on a reference and it does not change with field strength. This is a very important parameters; that means, if I go from 300 megahertz spectrometer to a 700 megahertz spectrometer and take the same molecule ethanol or any other molecule. It is chemical shift value in ppm should not change. And, that is how why that has happened that comes from this formula or calculation shown here.

So, let us say that we have a reference frequency, which is our rotating frame frequency and there is standard B 0 gamma into B 0 and that is the processional frequency of a reference nucleus ok. So, we can take any nucleus as a reference and assume that it has "zero" chemical shift. So, remember this is an assumption many a times people are confused that reference molecule has actually "zero" chemical shift now we are setting in forcing it to be 0 and is an arbitrary.

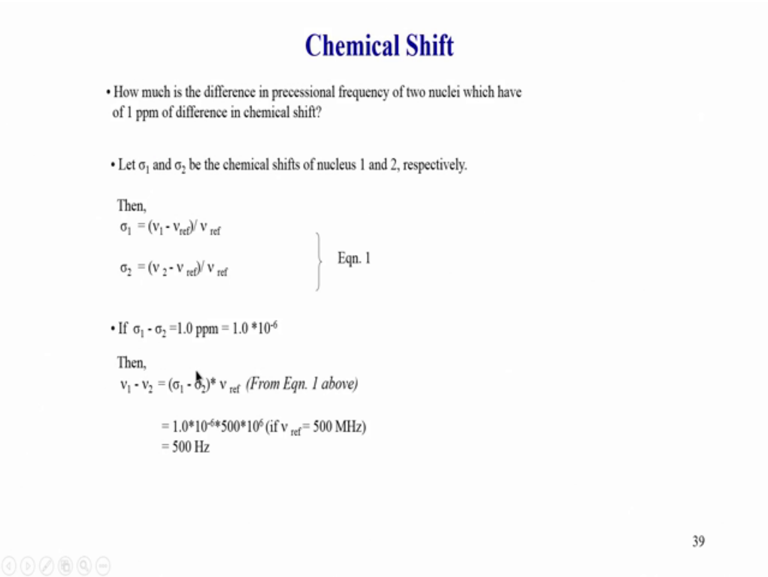
So, let us say that references frequency is has chemical shift 0. So, it has a perfect condition resonance shown like this. Now, our proton of interest is not having 0 frequency it is having a finite chemical shift values that is not 0. So, it is frequency now will be like this. So, what we do next is we can subtract these 2. So, when we subtract these 2 and divide by B gamma B 0, you can simply do yourself this calculation you will get this sigma in this way.

So, this sigma that is a chemical shielding factor chemical shift or shielding factor is now mu minus mu reference divided by mu reference. So, what is mu reference? Mu reference is basically the magnetic field strength, which remember was in 10 to the power 6 in megahertz and this is the difference between the frequencies.

So, what happens is now since mu ref is a megahertz range the value of sigma will become 10 raised to minus 6, because this 10 raised to the power 6 goes up to the numerator as 10 raised to minus 6. And therefore, we use our ppm. So, sigma becomes in the value in the order of 10 raised to minus 6 and that is why we use a word parts per million or ppm.

So, what happens is because you are subtracting reference frequency and again dividing by the reference, the chemical shift value the shielding value in ppm scale becomes independent of the frequency. That is it is becomes independent of this number, whether it is 700 or 300 because you are dividing it by that number.

(Refer Slide Time: 08:33)



Chemical Shift

- How much is the difference in precessional frequency of two nuclei which have of 1 ppm of difference in chemical shift?

- Let σ_1 and σ_2 be the chemical shifts of nucleus 1 and 2, respectively.

Then,

$$\left.\begin{aligned}\sigma_1 &= (\nu_1 - \nu_{ref})/\,\nu_{ref}\\ \sigma_2 &= (\nu_2 - \nu_{ref})/\,\nu_{ref}\end{aligned}\right\}\ \text{Eqn. 1}$$

- If $\sigma_1 - \sigma_2 = 1.0\text{ ppm} = 1.0 * 10^{-6}$

Then,

$\nu_1 - \nu_2 = (\sigma_1 - \sigma_2) * \nu_{ref}$ *(From Eqn. 1 above)*

$= 1.0 * 10^{-6} * 500 * 10^{6}$ (if $\nu_{ref} = 500$ MHz)
$= 500$ Hz

.

So, is kind of scaling. So, that is shown here. For example let us say I have 2 frequencies. So, that question here is how much is the difference? If two nuclei have 1 ppm difference in chemical shift value. So, let us say 1 chemical shift value is sigma 1, which is given by this it is same as the last formula in the last slide. Another proton is having another chemical shift values, which was again is the same formula accept that only thing which is changing here is mu 1 and mu 2. So, you can see that now if I subtract the 2, the difference I am considering as 1 ppm which is what was shown here. So, that is 1 ppm means 1 into 10 to the power minus 6.

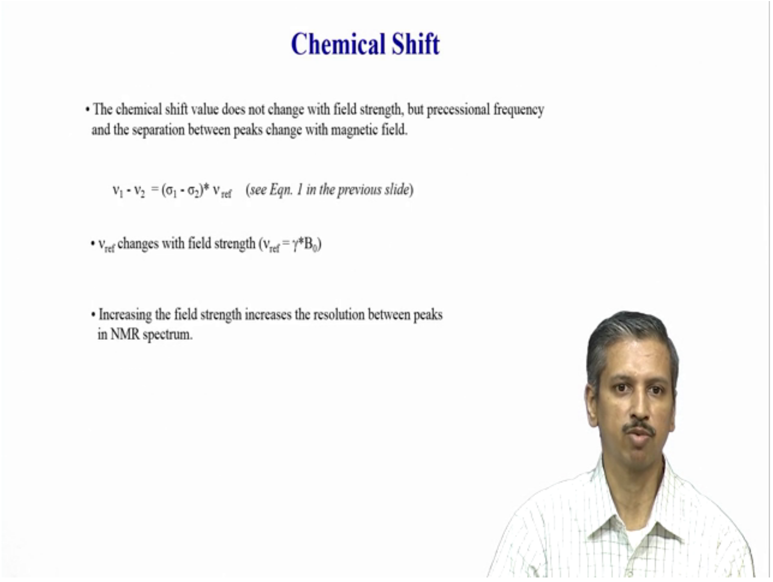
So, therefore, if I subtract these 2 frequencies sigma 1 minus sigma 2, then if you look at this equation if I subtract this what will I get I will get mu 1 minus mu 2 divided by mu ref. So, the mu ref it goes to this side that is how it is written here coming from this equations. Now, that is 10 raised to the power minus 6 is this number? And, that multiply with 500 into 10 to the power 6, because let us say we are at 500 megahertz.

So, if you go to 500 megahertz spectrometer if the 500 mega spectrometer is used, then you are now the difference in the 2 frequencies, which are only 1 ppm apart, now the difference actually is 500 hertz. So, you see the difference now actually depends on the frequency there is a spectrometer frequency, but that is in the hertz scale.

In the ppm scale they are still 1 ppm. So, suppose I go to 900 megahertz the spectrometer or 800 megahertz spectrometer. If I do the calculation there again the difference will remain the same they are still 1 ppm away from each other, but in the hertz scale this number will become 800 megahertz. So, 800 will come here and this will become 800 hertz.

So, you see the difference the in chemical shift value between 2 peaks definitely changes in a hertz scale it become 700 800 depending on the spectrometer, but it is difference in the ppm scale will always be constant. So, that is the beauty of chemical shift scale that it does not affect the chemical shift value in ppm scale, it affects only the chemical shift value in the hertz scale. That is why chemical shift scales are very popularly used.

(Refer Slide Time: 10:47)



Chemical Shift

- The chemical shift value does not change with field strength, but precessional frequency and the separation between peaks change with magnetic field.

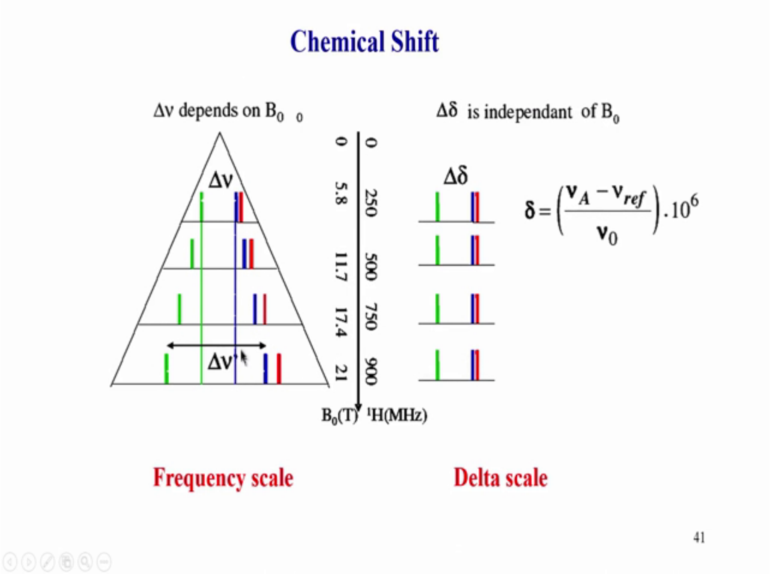
$\nu_1 - \nu_2 = (\sigma_1 - \sigma_2)^* \nu_{ref}$ *(see Eqn. 1 in the previous slide)*

- ν_{ref} changes with field strength ($\nu_{ref} = \gamma^* B_0$)

- Increasing the field strength increases the resolution between peaks in NMR spectrum.

So, this is what is basically shown here is because of this term here mu ref, which came from the previous equation or previous slide mu ref changes with field strength and mu ref is nothing, but this term and this is depending on your B 0. So, if you have 500 megahertz has 1 B 0, 800 megahertz will have a higher B 0. So, the reference frequency definitely changes with spectrometer. So, therefore, when you calculate in hertz scale it changes, but what does not change is a difference in the ppm scale.

(Refer Slide Time: 11:17)



Chemical Shift

$$\delta = \left(\frac{\nu_A - \nu_{ref}}{\nu_0}\right).10^6$$

Frequency scale **Delta scale**

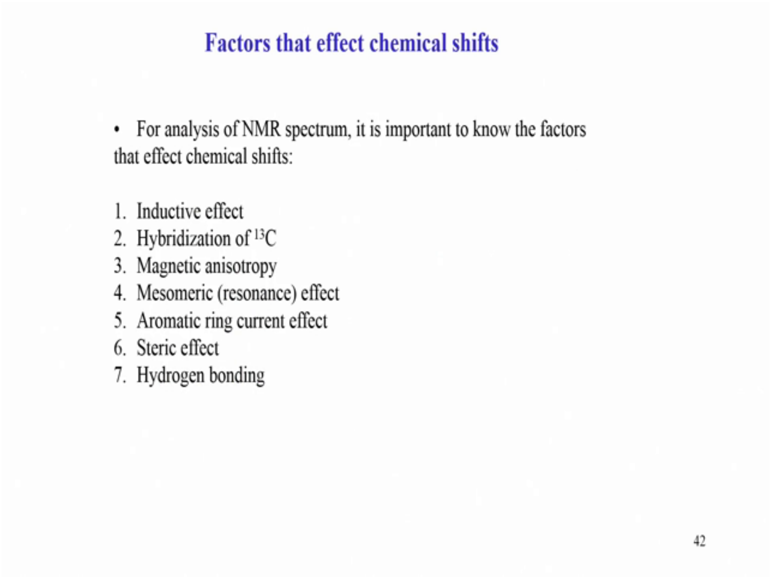
.

So, we can see this is what is depicted here that if you take in a hertz scale. So, this is left side is frequency scale, frequency meaning in hertz scale and right side is the delta scale which is in ppm value. So, you can see 2 peaks or a set of peaks always remain in the same whether you are a 250 megahertz and 500 megahertz, or it 750 or 900 megahertz in different spectrometer the peak pattern, the 3 peaks will not change. But, now if we calculate the difference between the 2 the peak let us a green and this blue peak their difference is not same they are actually changing. And, they are linearly increasing because of this frequency changes because of this magnetic field change.

So, you see the difference now the difference is that although they look same in the delta scale they are actually the frequency scale, the hertz scale they are not the same, the chemical shifts they are factory changing. So, therefore, ppm scale is a very preferred scale, because it bit makes it independent of the spectrometer that you are using and therefore, it is very handy and useful to use whereas, hertz scale tells us that (Refer Time: 12:24) part.

So, the resolution, you see the resolution is going up, because I am separating the peaks more and more. So, as you go higher and higher magnetic field, it is not that the chemical shifts are not changing they are changing in hertz scale and that helps to increase the resolution. And, that is one of the reason why in biomolecules there is a lot of effort to go to higher magnetic field, because the resolution is improving as we go up. And, this resolution linearly goes up and therefore, it is very useful to have a hertz scale or higher frequency in for biomolecules.

(Refer Slide Time: 12:57)



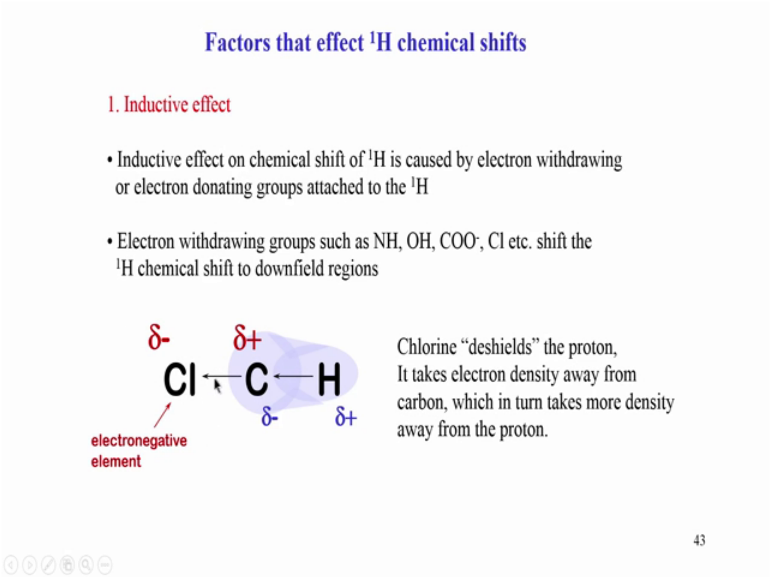
Factors that effect chemical shifts

- For analysis of NMR spectrum, it is important to know the factors that effect chemical shifts:

1. Inductive effect
2. Hybridization of ^{13}C
3. Magnetic anisotropy
4. Mesomeric (resonance) effect
5. Aromatic ring current effect
6. Steric effect
7. Hydrogen bonding

So, what are the different factors which affect the chemical shift? So, this is just now we alluded to one factor which is the effect of inductive effect, but we look at quickly through all this again this is something which has been covered very nicely in various test book in the previous course, but we will have a quick glance because in biomolecules all of these have an effect. And therefore, it will be useful to understand the effect how they affect the spectrum.

(Refer Slide Time: 13:27)



Factors that effect ^{1}H chemical shifts

1. Inductive effect

- Inductive effect on chemical shift of ^{1}H is caused by electron withdrawing or electron donating groups attached to the ^{1}H
- Electron withdrawing groups such as NH, OH, COO^{-}, Cl etc. shift the ^{1}H chemical shift to downfield regions

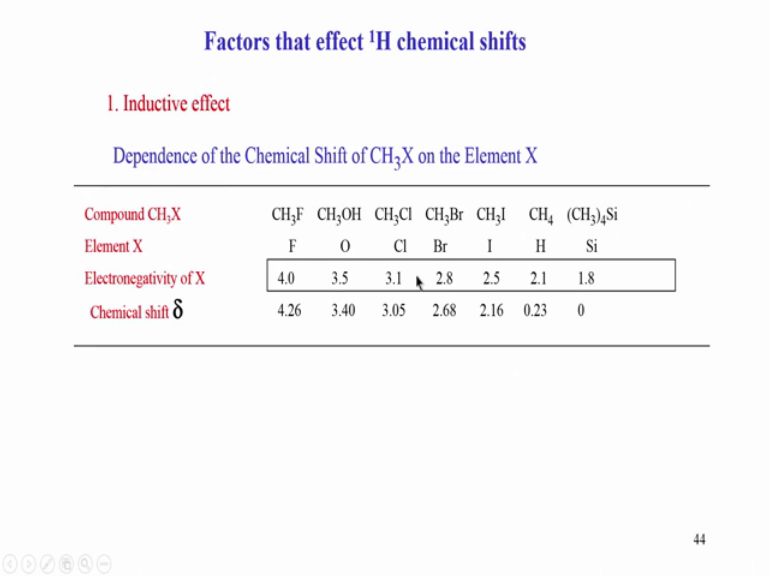
Chlorine "deshields" the proton, It takes electron density away from carbon, which in turn takes more density away from the proton.

The first one is the simplest effect that is the inductive effect and that is nothing, but related to the electronegativity. So, you can see a very simple drawing here, that if you have a carbon molecule in a biomolecules we do not have chlorine. Let us take an example of some molecule which has carbon attached to this chlorine. So, because of this electronegative atom which is highly electronegative, it pulls the electron from the carbon. Therefore, the electron density, the how the electron cloud is distributed changes. And therefore, that intern changes the electron density around this proton.

So, this proton is also affected indirectly because of the electron a negative electronegative effect of this chlorine. So, this can happen with other electronegative atoms like nitrogen, oxygen, carboxylate groups and so on.

So, therefore, what chlorine essentially is doing is it is deshielding it is taking away the electron from the hydrogen. So, therefore, hydrogen becomes partially positive charged. And, by taking away the electron density from hydrogen it is reducing the shielding factor, the sigma value which we saw in the previous slides, it is reducing and that makes it go closer to as much as deshielded as possible.

(Refer Slide Time: 14:41)



Factors that effect ^1H chemical shifts

1. Inductive effect

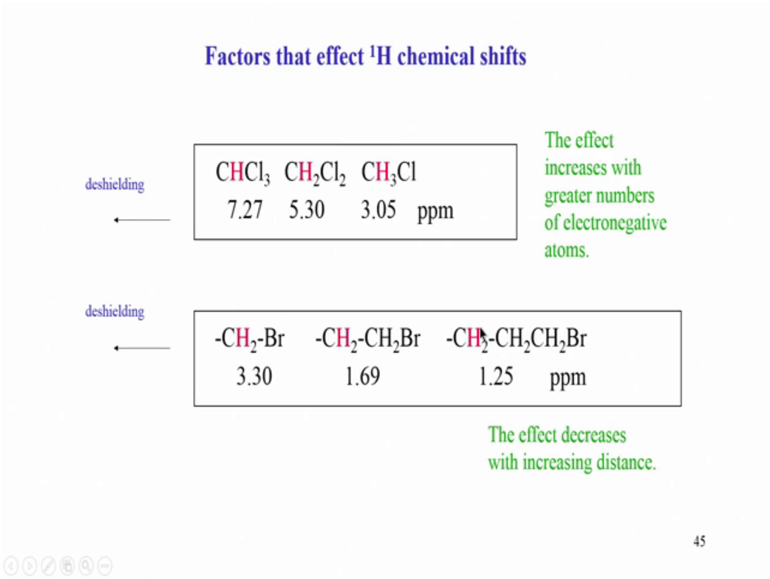
Dependence of the Chemical Shift of CH_3X on the Element X

Compound CH_3X	CH_3F	CH_3OH	CH_3Cl	CH_3Br	CH_3I	CH_4	$(CH_3)_4Si$
Element X	F	O	Cl	Br	I	H	Si
Electronegativity of X	4.0	3.5	3.1	2.8	2.5	2.1	1.8
Chemical shift δ	4.26	3.40	3.05	2.68	2.16	0.23	0

So, that is one of the simplest effects and this is shown further here for some. So, we can see fluorine we know is a most electronegative atom. So, that has the highest deshielding effect on this particular hydrogen. So, we can see that therefore, it becomes very high chemical shift d shielded it goes down field that is to 4 ppm. And, as you go lesser and lesser electronegative atoms the deshielding effect is reduced and you can see that it is going low.

So, this is the trimethyl silane which is the less least deshielded or most shielded is the reference, which we normally take it as it. Again remember this is not that chemical shift value is 0, we have forcing this peak to have a 0 value, with respect to that we are actually calculating the others. There is an arbitrary 0 concept arbitrary reference which is put a 0. So, you can see the shielding effect is very clear very linear with respect to the inductive effect.

(Refer Slide Time: 15:33)



Factors that effect ^{1}H chemical shifts

deshielding ←

$CHCl_3$	CH_2Cl_2	CH_3Cl	
7.27	5.30	3.05	ppm

The effect increases with greater numbers of electronegative atoms.

deshielding ←

$-CH_2-Br$	$-CH_2-CH_2Br$	$-CH_3-CH_2CH_2Br$	
3.30	1.69	1.25	ppm

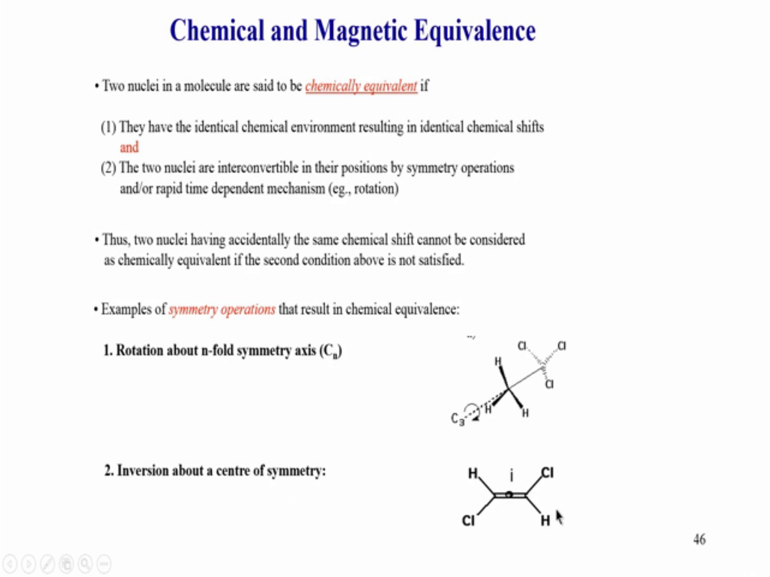
The effect decreases with increasing distance.

Now, this is another example where we have taken a little bit away. So, you see this is a little higher second order. In the sense it is not directly attached to an electronegative, it is away from the electronegative atom 1 by 1 hydrogen here. So, this is the second hydrogen away from here. This is directly attached and whereas, this is 3 2 carbons away this hydrogen.

So, we can see the effective reduce for this which is closest to bromine has a highest shielding effect, de shielding effect. And this is the less deshielding and this is the most shielded, it is almost it is very away from Br that is bromine. So, therefore, it is having the least deshielding effect.

So, this is something which all of us are aware of this is just being in this the second factor which also helps inductive effect is the number of electro negative. So, you can see in chloroform we have 3 carbons, which is 3 chlorines sorry. So, 3 chlorines have the maximum de shielding effect. And therefore, this hydrogen is highly shielded compared to this hydrogen, compared to this which has only 1 chlorine.

(Refer Slide Time: 16:37)



Chemical and Magnetic Equivalence

- Two nuclei in a molecule are said to be *chemically equivalent* if

(1) They have the identical chemical environment resulting in identical chemical shifts
and
(2) The two nuclei are interconvertible in their positions by symmetry operations and/or rapid time dependent mechanism (eg., rotation)

- Thus, two nuclei having accidentally the same chemical shift cannot be considered as chemically equivalent if the second condition above is not satisfied.

- Examples of *symmetry operations* that result in chemical equivalence:

1. Rotation about n-fold symmetry axis (C_n)

2. Inversion about a centre of symmetry:

So, these are the basically different deshielding shielding effects. Now, let us look at this concept of chemical equivalence and this is also very important concept. So, chemical equivalence basically means any 2 hydrogen’s if, they have identical chemical environment, they will end up with the same chemical shift value.

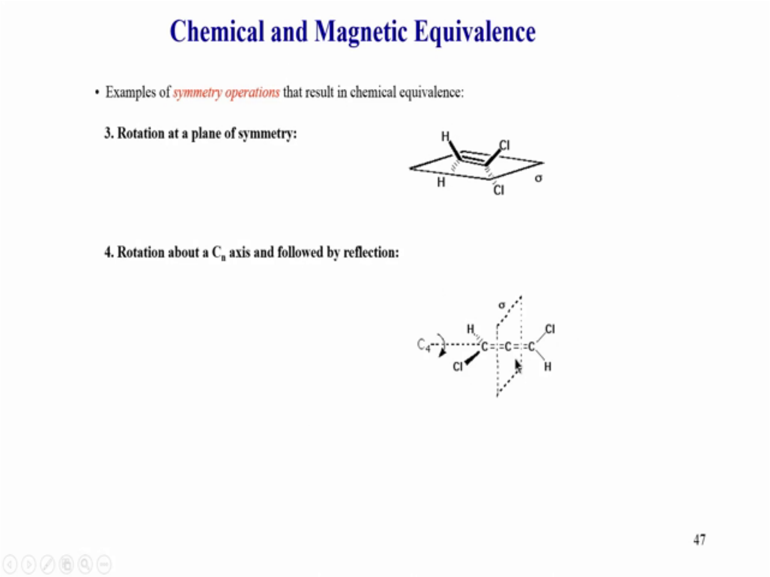
Now, having chemically equivalent is basically mean same chemical shift, but same chemical shift need not mean chemically equivalence. So, the reverse is not true which is written here, that 2 nuclei may have accidentally the same chemical shift value does, they cannot be considered as chemically equivalent because the above condition has to be satisfied.

So, you see here there is a condition very important condition, these 2 hydrogens should be in inter convertible in their positions by symmetry operations, which means I should be able to take those 2 hydrogen is which are equivalent having the same chemical shift. And, if I change their replace 1 with other it should have the same symmetry in the atom in the molecules.

So, let us look at the case here is shown here look at this molecule here it is basically has a rotational symmetry. So, you can look at these 3 hydrogens. If I rotate it and this hydrogen comes here, this hydrogen goes here. This it does not affect anything it remains these 3 hydrogens are kind of chemically equivalence because they are all rotating and therefore, their positions are equivalent. It is equally distance from these 3 whether it is in this position or in this position or in this position. So, because of this rotational symmetry it there because it has a rotation n fold symmetry axis these 3 hydrogens are equivalent.

Similarly, we know the centre of symmetry here if this hydrogen is inverted is has a inversion of symmetry. These 2 hydrogen's have the same chemical environment and, they are related by symmetry operation, they can be converted from here to here here to here without any change in the structure of the molecule. Remember symmetry operation basically means if you rotate or a take a reflection around any axis it should not change the structure. So, that is what is shown here these 2 hydrogens are chemically equivalent.

(Refer Slide Time: 18:41)



Chemical and Magnetic Equivalence

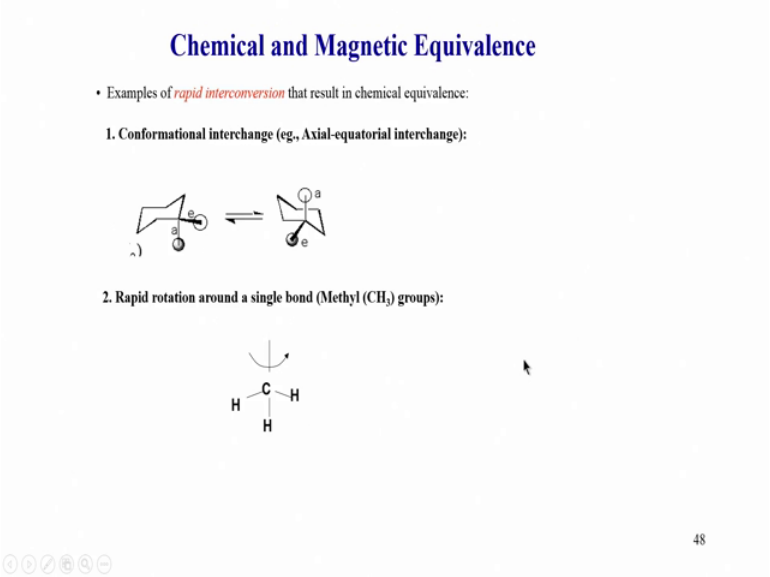
- Examples of *symmetry operations* that result in chemical equivalence:

3. Rotation at a plane of symmetry:

4. Rotation about a C_n axis and followed by reflection:

Now, we can look at more examples. So, this is another case where there is an mirror is an plane of symmetry not an axis of symmetry. So, if you look at this plane of symmetry these 2 hydrogen's are mirror image. Therefore, they are identical they are equivalent. And therefore, that will not change whether I take this hydrogen here or here the structure will not change. So, this is another examples. So, we can keep having different examples.

(Refer Slide Time: 19:07)



Chemical and Magnetic Equivalence

- Examples of *rapid interconversion* that result in chemical equivalence:

1. Conformational interchange (eg., Axial-equatorial interchange):

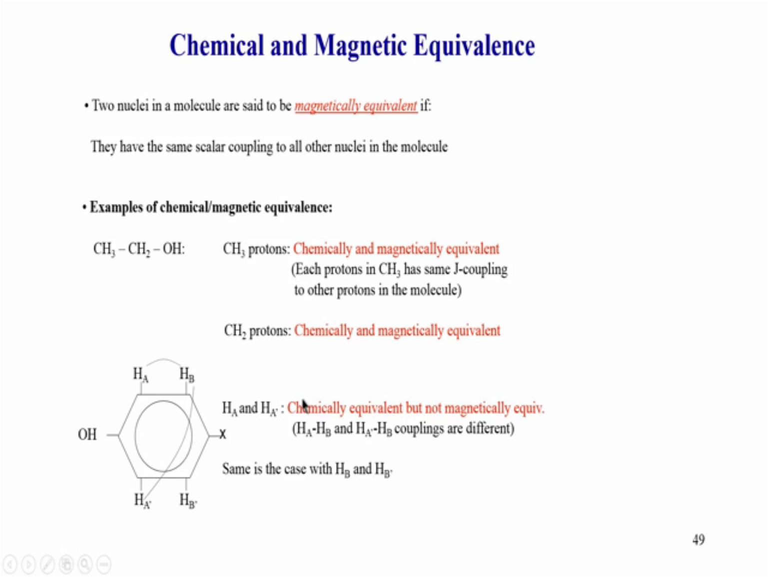
2. Rapid rotation around a single bond (Methyl (CH_3) groups):

48

We will go to what is the most important is the methyl? So, methyl groups are known to be always strong peaks in NMR spectrum. In fact, in biomolecules Methyl's play a very very important role and we will look at the methyl groups as we go along.

Now, here this 3 hydrogen's why are they equivalent? There is no symmetry concept here, they are equivalent basically because they are rotate very fast around this axis. So, you see this is the axis here they rotate very fast around it is axis and because of that rotation they become equivalent, because the average out in their environment, because of this averaging they are essentially equivalent in the chemical shift. So, Methyl groups always you will see in NMR spectrum are essentially a group together. So, they will be 3 times stronger or even more compared to any other protons in the spectrum and they are very easy to identify.

(Refer Slide Time: 19:57)



Chemical and Magnetic Equivalence

- Two nuclei in a molecule are said to be *magnetically equivalent* if:

They have the same scalar coupling to all other nuclei in the molecule

- **Examples of chemical/magnetic equivalence:**

$CH_3 - CH_2 - OH$: CH_3 protons: Chemically and magnetically equivalent
(Each protons in CH_3 has same J-coupling
to other protons in the molecule)

CH_2 protons: Chemically and magnetically equivalent

H_A and $H_{A'}$: Chemically equivalent but not magnetically equiv.
(H_A-H_B and $H_{A'}$-H_B couplings are different)

Same is the case with H_B and $H_{B'}$.

49

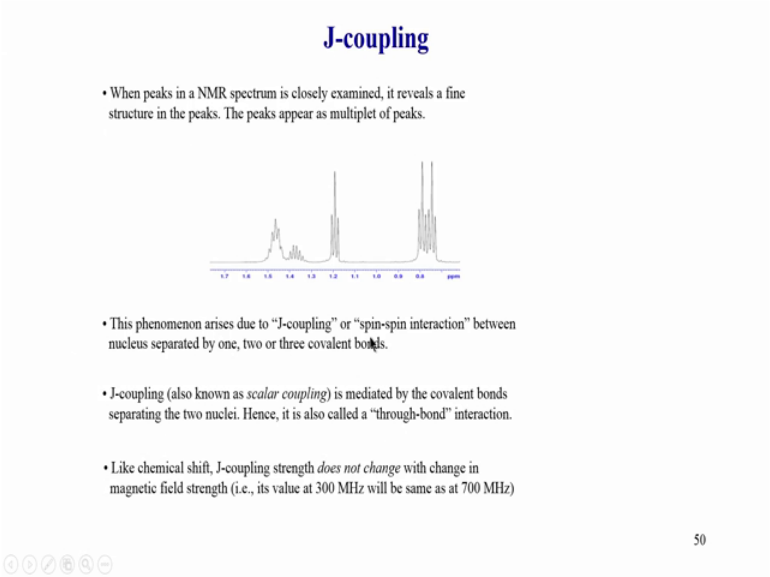
Ah. Chemical Magnet the second concept which is very important in NMR is the magnetically equivalence. So, we saw just now chemical equivalence. Let us look at a example here. So, if you see here this X which is the hydrogen this is some functional group, this can be another functional group, if we look at these 2 hydrogen's there is a symmetry here. If I draw a line here I just shown by this mouse there is a axis of symmetry.

So, you may say that this hydrogen is equivalent to this hydrogen and this hydrogen is equivalent to that hydrogen which is fine. So, they are chemically equivalent, but if you look at this hydrogen it is coupling to this H B here is not the same as it is coupling to this H B here. So, you see this is very far away this is 1 bond, 2 bond, 3 bond, 4 bond away whereas, this is 3 bond away, 5 bond away in fact.

So, you see this 2 hydrogen's are coupled J-coupled we will reach will come too shortly, their coupling is not the same as that J-coupling between this hydrogen is the same. So, therefore, although these 2 hydrogen's are chemically equivalent they are not magnetically equivalent, because they are coupling to the neighbouring hydrogens is not the same.

So, for example, the coupling of H A to H B is different from this H A to this H P. So, although A and A are same they are A A prime. So, this is the nomenclature typically used for denoting spin systems, which will not going to detail, but the idea is they are chemically equivalent. So, chemical shift will be same, but magnetically they are not equivalent.

(Refer Slide Time: 21:25)



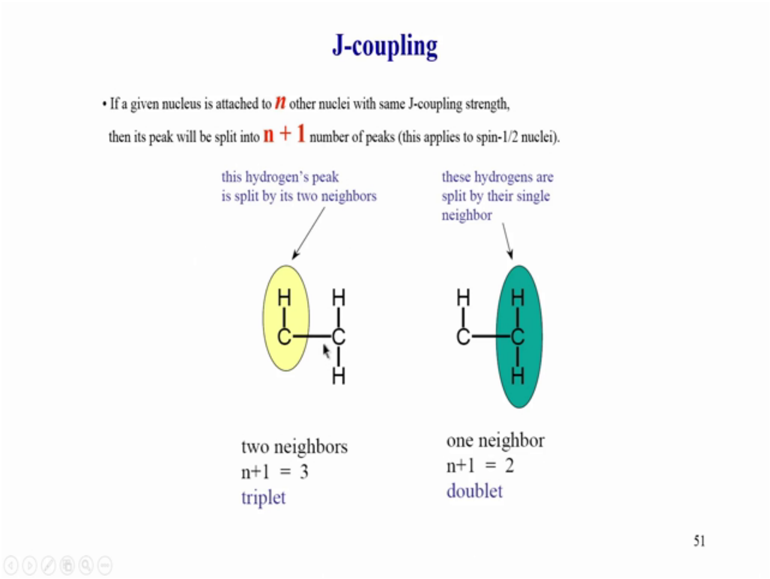
J-coupling

- When peaks in a NMR spectrum is closely examined, it reveals a fine structure in the peaks. The peaks appear as multiplet of peaks.
- This phenomenon arises due to “J-coupling” or “spin-spin interaction” between nucleus separated by one, two or three covalent bonds.
- J-coupling (also known as *scalar coupling*) is mediated by the covalent bonds separating the two nuclei. Hence, it is also called a “through-bond” interaction.
- Like chemical shift, J-coupling strength *does not change* with change in magnetic field strength (i.e., its value at 300 MHz will be same as at 700 MHz)

So, that does not affect much in the spectrum. So, we will not go into detail of magnetic equivalence, but what affects the most is a J-coupling so which is shown here. The J-coupling essentially see if you look at any NMR spectrum, you will never see a single peak you will always a multiple structures like this. And, that is happens because of this phenomenon called J-coupling or scalar coupling.

J-coupling has a very rich history and how it was this code is very interesting. We you can have a look at it in an different textbooks how it was actually discovered and found out. So, we use different words for J-coupling sometimes in test books you will also use we use the spin-spin interaction, you will see the word scalar coupling and so on. So, J-coupling essentially happens only when there is a covalent bond between 2 hydrogen’s. So, that is why we use the word through bond interaction. So, let us go through J-coupling briefly, how does J-coupling affect the spectrum?

(Refer Slide Time: 22:23)



J-coupling

- If a given nucleus is attached to *n* other nuclei with same J-coupling strength, then its peak will be split into n + 1 number of peaks (this applies to spin-1/2 nuclei).

this hydrogen's peak is split by its two neighbors

these hydrogens are split by their single neighbor

two neighbors
n+1 = 3
triplet

one neighbor
n+1 = 2
doublet

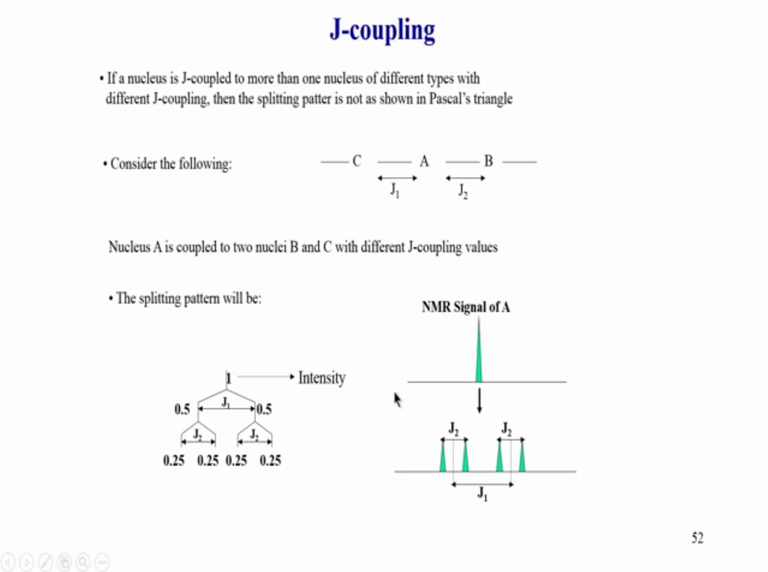
So, this is shown here as an example. So, we let us say you have a spin system or a molecule like this. So, you have a 2 this carbon has 2 equivalent hydrogen. So, let us considered as chemically equivalent and this is not this is one hydrogen we are looking at. So, in the NMR spectrum this hydrogen will be couple to this hydrogen and this hydrogen.

But, because these 2 are equal equivalent this hydrogen we will look at them as 2 equal hydrogen's. So, it is number of peaks for this hydrogen we will follow a rule known as n plus 1 rule. Therefore, n is the number of chemically equivalent hydrogen to which this yellow colour is coupled. So, it will now show as a triplet ok. So, the triplet meaning 3 lines, now the intensity of the 3 lines are not the same, but we will see that soon is using concept called Pascal's triangle.

Now, let us look at these 2 hydrogen's. So, we looked at this yellow colour here, now in the same molecule let us say what happens so, these two. So, these are chemically equivalent as we assume, the remember you not be equal equivalent. In fact, in biomolecules molecules like these sorry moieties like this are never equivalent. So, we will see that when we come to the biomolecule part, but let us assume that right now a some molecule they are equivalent. In that scenario these 2 are together as 1 entity. Now, this one entity is couple to another one hydrogen. So, according to the n plus 1 n is equal to 1, because these 2 together are couple to only 1 more and that is a doublet.

So, these 2 peaks these 2 meaning 1 peak for these 2 hydrogen’s we will show up as a doublet and the peak for this hydrogen we will show up as a triplet in the NMR spectrum. So, this is what is called as the n plus 1 rule, where only applies when there is an equivalent hydrogen’s ok.

(Refer Slide Time: 24:11)



J-coupling

- If a nucleus is J-coupled to more than one nucleus of different types with different J-coupling, then the splitting patter is not as shown in Pascal’s triangle
- Consider the following:

Nucleus A is coupled to two nuclei B and C with different J-coupling values

- The splitting pattern will be:

52

So, we can actually see here more examples. Now, what happens if the coupling between let us say we have 2 protons. So, this is the all protons here. So, this is this is 1 hydrogen A this is another hydrogen B remember this is a just a schematic drawing, do not think of it as a bond here. It is actually a C H let us say and is there is another C H here, another CH here ok.

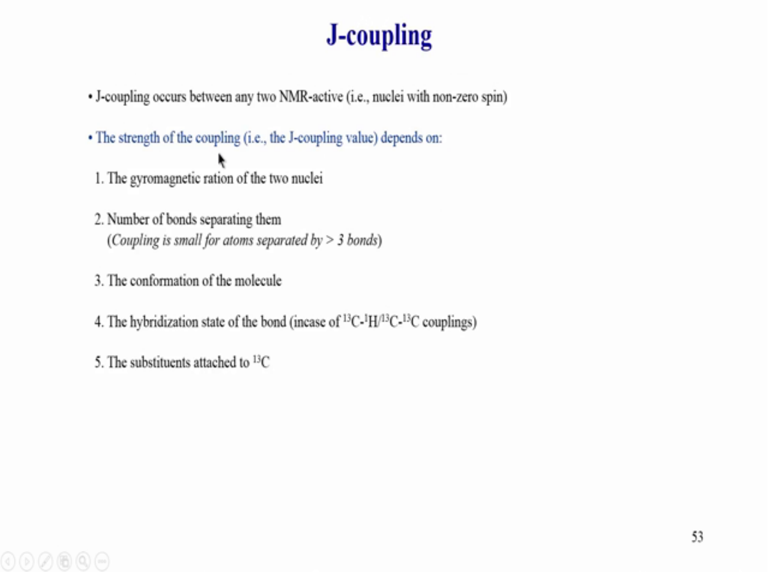
So, this CH hydrogen in centre is coupled to 2 protons so, 1 proton this side and 1 hydrogen this side. Now, these 2 hydrogen’s B and C let us say are having completely different environment. So, they will be having different coupling to this hydrogen here. So, which is written as J 1 and J 2.

So, if J 1 is equal to J 2 then it will be a chemical equivalent case and it will become a triplet, but if it is not same then what does this how does this spectrum look? So, that is how it is analysed here. We can take a particular peak here that is without any coupling. So, let us say that C and B did not exist, if C and B did not exist A would have no coupling and it will only have a single peak. But, now it is coupled to this 2 hydrogen 1 on this side 1 on that side and with different coupling values.

So, in such a scenario this peak first we will get split into 2 peaks and it will shown as J 1 here, because of the J coupling that is shown here, it gets shown split into 2 and each 1 of them is having a half the intensity as previous. So, remember J-coupling simply splits the peak into 50 50. So, 1 peak is half another peak is half of this original peak. So, it is 1 is to 1.

But, then there is another coupling J 2 and that further splits each of this peaks into further 2. So, we can see here now it has become 2 separate peaks. So, first it is split into this dotted line here, and that each peak which was here further split it into 2 more. So, you will get what is called as a doublet of a doublet. This is not simply a triplet, a triplet would have come if they were all equal values J 1 was equal to J 2, but because now the J 1 and J 2 are not the same they become a doublet of a doublet. So, this is the scenario if the couplings are not equal this is what happens.

(Refer Slide Time: 26:25)



J-coupling

- J-coupling occurs between any two NMR-active (i.e., nuclei with non-zero spin)
- The strength of the coupling (i.e., the J-coupling value) depends on:

1. The gyromagnetic ration of the two nuclei
2. Number of bonds separating them
   (*Coupling is small for atoms separated by > 3 bonds*)
3. The conformation of the molecule
4. The hybridization state of the bond (incase of ^{13}C-^{1}H/^{13}C-^{13}C couplings)
5. The substituents attached to ^{13}C

53

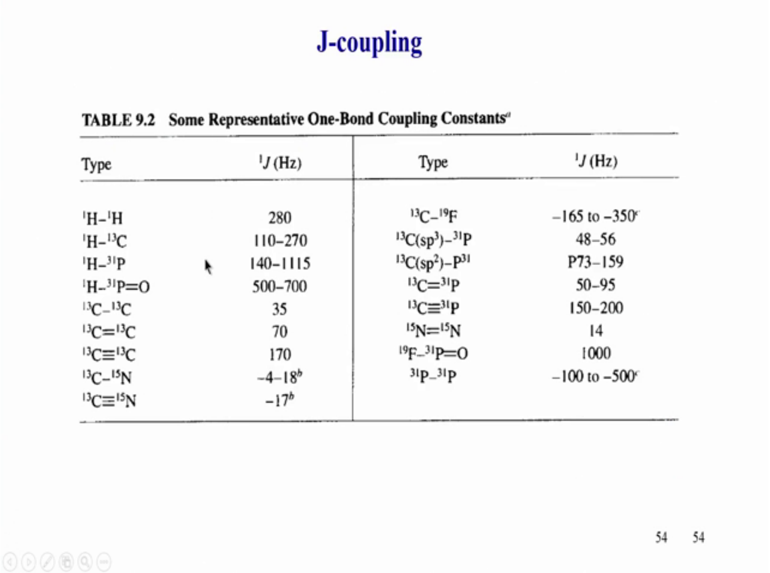
So, now the J-coupling value between 2 hydrogen’s depends on varieties of factors, which is listed here this is similar to chemical shift values. So, we can see that it depends on the gyromagnetic ratio not ration is ratio of the 2 nuclei. So, if there is a hydrogen and carbon the coupling will be different compared to hydrogen and hydrogen and hydrogen nitrogen will be different and nitrogen carbon will be different and so on.

Similarly, it depends on how many bonds are separate remember this is a through bond interaction, which means it depends on number of bonds separating them, if they are separated by more than 3 bonds to hydrogen then the coupling is very small. If, they are separated by 1 bond of course, hydrogen hydrogen 1 bond cannot come it will becomes hydrogen molecule, but you can have hydrogen carbon 1 bond, then it is a very strong coupling. And, if you have carbon nitrogen 1 bond it is also good, but if you have carbon carbon 2 bonds away then it is very weak. It depends on the structure of the molecule which is also we use a word confirmation and very useful this.

So, these are these parameters are very very important in biomolecules. So, one should remember these things, because this is how it will affect our sensitivity or the pattern what we will see in heteronuclear NMR then in bio molecules and depends on the hybridization state. For example, in aromatic the hybridisation SP to whereas, in aliphatic in Methyl's it is SP 3.

And therefore, it depends on what is hybridization state the chemical shift coupling sorry coupling spin-spin coupling will also change. If you look at the substituents again it matters. So, if there is a functional group oh compared to attach to see versus some other functional group, then the chemical shift the coupling of carbon 2 protons will change.

(Refer Slide Time: 28:01)



J-coupling

TABLE 9.2 Some Representative One-Bond Coupling Constants[a]

Type	^1J (Hz)	Type	^1J (Hz)
$^1H-^1H$	280	$^{13}C-^{19}F$	−165 to −350[c]
$^1H-^{13}C$	110–270	$^{13}C(sp^3)-^{31}P$	48–56
$^1H-^{31}P$	140–1115	$^{13}C(sp^2)-P^{31}$	P73–159
$^1H-^{31}P{=}O$	500–700	$^{13}C{=}^{31}P$	50–95
$^{13}C-^{13}C$	35	$^{13}C{\equiv}^{31}P$	150–200
$^{13}C{=}^{13}C$	70	$^{15}N{=}^{15}N$	14
$^{13}C{\equiv}^{13}C$	170	$^{19}F-^{31}P{=}O$	1000
$^{13}C-^{15}N$	−4–18[b]	$^{31}P-^{31}P$	−100 to −500[c]
$^{13}C{\equiv}^{15}N$	−17[b]		

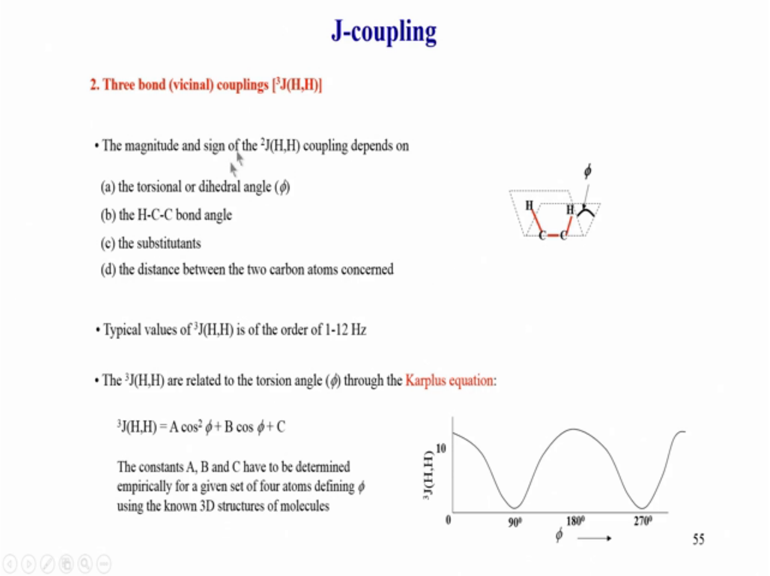
54 54

So, you see there are varieties of parameters which affect the chemical shift and coupling values say coupling here is shown for different types of combinations. As far as our biomolecular course is concerned or this course is concerned, we are mainly interested in hydrogen to hydrogen. Of course, this is a hydrogen molecule not in 3 bond hydrogen, you can see the hydrogen, hydrogen direct coupling is very strong we will not bother about this, this is just a highest value possible.

But, if you can hydrogen to carbon this is exact this is the range which we will be using in our in our bimolecular experiments and we exploit this high coupling. If you look at phosphorus which is a nuclic acids, that is also very strong if you look at carbon to carbon double bond, it is also strong carbon to carbon single bond, is also quite good this is useful in biomolecules. And, bimolecular NMR all these couplings are very useful and exploited in designing various experiments.

We would not look at fluorine in this course fluorine is not present in biomolecules. Fluorine is present in different chemical molecules which is not part of this course.

(Refer Slide Time: 29:09)



J-coupling

2. Three bond (vicinal) couplings [$^3J(H,H)$]

- The magnitude and sign of the $^2J(H,H)$ coupling depends on

  (a) the torsional or dihedral angle (ϕ)

  (b) the H-C-C bond angle

  (c) the substitutants

  (d) the distance between the two carbon atoms concerned

- Typical values of $^3J(H,H)$ is of the order of 1-12 Hz

- The $^3J(H,H)$ are related to the torsion angle (ϕ) through the Karplus equation:

  $^3J(H,H) = A\cos^2\phi + B\cos\phi + C$

  The constants A, B and C have to be determined empirically for a given set of four atoms defining ϕ using the known 3D structures of molecules

And finally, in the one thing is the how does that J-coupling depends on the confirmation. So, this is the very famous equation known as the Karplus equation, this says that a coupling between 2 hydrogen which are 3 bonds away. So, look at this picture here they are actually 3 bonds away. So, if you draw this molecule you can show like a book like a 2 planes here.

So, 1 plane representing 1 hydrogen 1 carbon this carbon and the second plane representing this hydrogen, this carbon, and this carbon. So, you see now the angle between the 2 plane is known as this torsion angle, a very very important parameter when it comes to biomolecular structure. So, the torsion angle or also used the word dihedral angle. Now is basically this angle and this coupling between these 2 hydrogen’s depends on this angle by this equation.

The very famous Karplus equation way back in 60s was proposed and it has this form, we can see this has a form like this is a cosine function cosine square plus cosine. So, you can see if I know the J value I can guess the phi value from this. Of course, it has multiple possibilities, but still I can narrow down what are the possibilities.

Similarly, if I know the dihedral angle value from there this plot suppose I know it somewhere like 140 degrees, if I go up here I can get the actually the chemical shift sorry the J-coupling of those 2 hydrogen. So, you say interrelation or a connection between J-coupling and the dihedral angle, which is very very very useful in biomolecules it is worth repeating that as we go along we will see the alpha helical structure the beta sheet all depend on this J value which is useful.

So, we will in the next class we will look at the final aspects of 1 D NMR, which is C 13 NMR and look at a brief very few practical aspects and then we will go on to 2 D NMR.