NMR spectroscopy for Structural Biology NS

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Lecture: 06

Chemical Shifts and Coupling Constant

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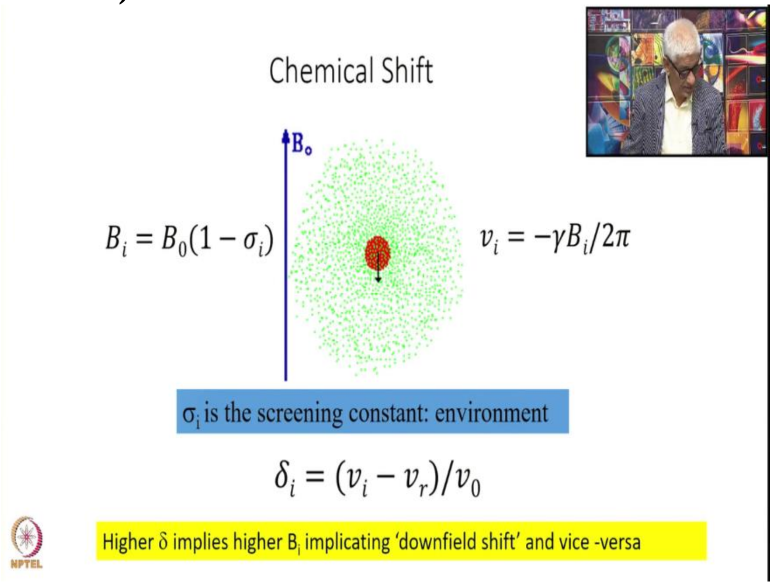

Chemical Shift

$B_i = B_0(1 - \sigma_i)$

$\nu_i = -\gamma B_i / 2\pi$

σ_i is the screening constant: environment

$\delta_i = (\nu_i - \nu_r)/\nu_0$

Higher δ implies higher B_i implicating 'downfield shift' and vice -versa

We were discussing about the chemical shifts and this is just a quick recap on what we did last time. So, we have a nucleus which is here and you have an electron cloud around it which is indicated by the green dots here and we have an applied magnetic field which is B_0 indicated here as B_0 in the previous class I might have set it as H_0 but it does not matter it is B_0 here and this B_0 field induces a current in this electronic cloud.

And which produces a magnetic field which opposes the externally applied magnetic field and therefore the field seen by the nucleus is $B_i = B_0 (1- \sigma_i)$. So, that is the σ_i is the so-called screening constant and this can vary depending upon the environment of your molecule. And the precessional frequency of a particular nucleus will then be ν_i or this is if you put it in terms of frequency in hertz that is $\nu_i = -\gamma B_i / 2\pi$ and ν_i is the field which is which is given here.

Now to this will then depend upon the magnetic field strength therefore since you want to characterize only the screening or the electronic environment around the nucleus we define a an entity which is given here $\delta_i = (\nu_i - \nu_r)/ \nu_0$, ν_r is a kind of a reference compound. And you measure the absorption frequencies with respect to a particular line of a reference compound and then this will be a very small number compared to nu naught, nu naught is your spectrometer frequency this corresponds to B_0.

So, you divide it by this nu naught then of course this will be a number which will be very small and you multiply therefore by 10^6 although that is I wrote it in the previous one and that is called as the ppm δ_i is expressed in terms of ppm. Higher δ_i means higher B_i implicating downfield shift and therefore it is less screening and you will have a spectrum which is at a lower higher frequency of absorption.

And the lower δ implies that greater shielding that will means interrupt field shift and vice versa. So, this is of course we already did this last time and let me explain that a little bit more here.

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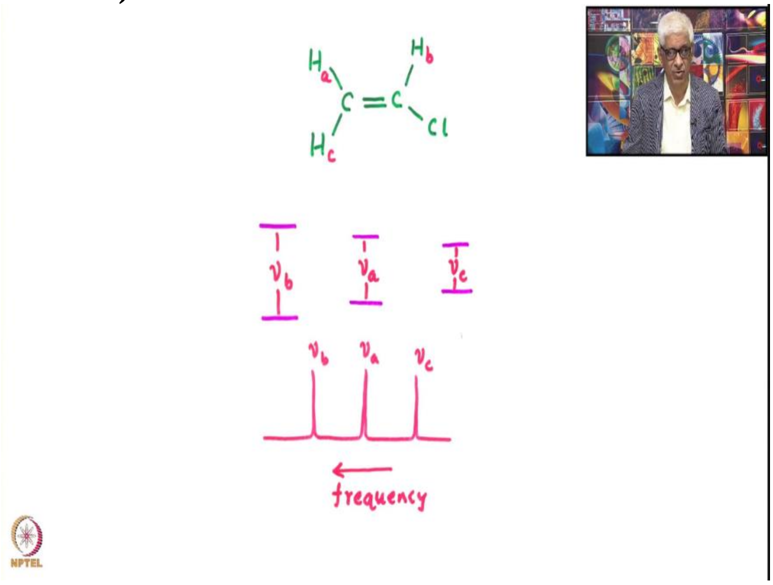

We take this example this is vinyl chloride. So, vinyl chloride has 3 protons this has 3 protons here this is electric label them as H_a H_b and H_c. There is a double bond here and chlorine is an electron withdrawing group therefore it changes the electron density in these proton around the protons. So, we said whatever is the electron density that determines how much will be the screening.

So, therefore if the electron density is lower, then the screening will be less and therefore that will be at a higher frequency of absorption or the higher δ value. So, the frequency absorption is going like this. So, the chemical shift here the H_b which is the closest to the chloride which is the with the electron withdrawing group therefore this will have the lowest electron density here.

Therefore the energy separation the B_i will be highest for this particular nucleus and this ν_b therefore will be the highest because the energy of absorption will be high the energy separation will be higher. This will be followed by nu a which is indicated here this is the trans and therefore the effect of the chloride will be after this it will be more at this point which is trans to the chloride group.

And this will have the next frequency which is nu a and the one which is resist to it that will be the van der Waals interactions and things like that the screening will be more here therefore the lowest delta will be for the ν_c. Therefore the frequency increases in this order and the chemical shifts numbers delta values will increase in this order. So, this is how the chemical shift occurs because of the screening by the electron cloud around it.

And clearly this is an important parameter which will describe the electronic environment and therefore the structures of the molecules.

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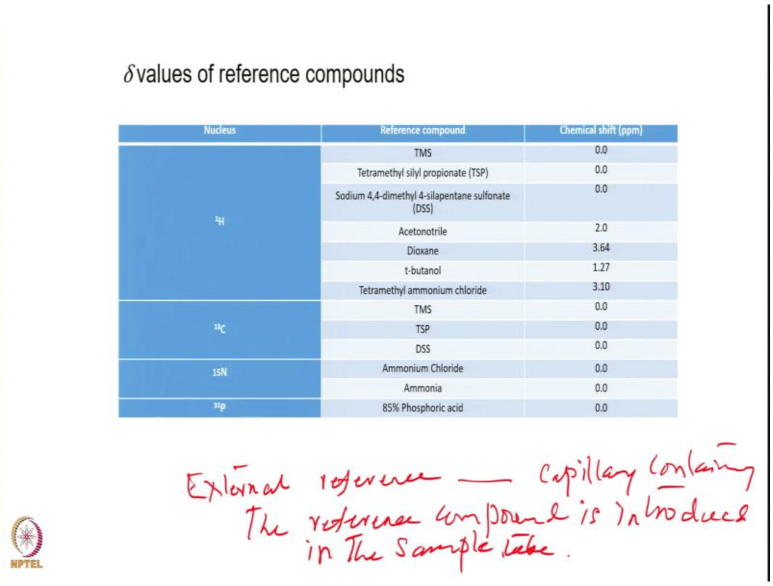

δ values of reference compounds

Nucleus	Reference compound	Chemical shift (ppm)
^1H	TMS	0.0
	Tetramethyl silyl propionate (TSP)	0.0
	Sodium 4,4-dimethyl 4-silapentane sulfonate (DSS)	0.0
	Acetonotrile	2.0
	Dioxane	3.64
	t-butanol	1.27
	Tetramethyl ammonium chloride	3.10
^{13}C	TMS	0.0
	TSP	0.0
	DSS	0.0
15N	Ammonium Chloride	0.0
	Ammonia	0.0
^{31}p	85% Phosphoric acid	0.0

External reference — capillary containing
The reference compound is introduced
in The sample tube.

And there and a chemist used a larger number of molecules to characterize these chemical shifts in the functions and what are the reference compounds? I said there is with respect to a reference compound we define the chemical shift. So, for the proton there are several reference compounds which are used TMS is tetramethylsilane and this is it has one line. So, that is taken as chemical shift that line is taken as 0.0 ppm and TMS is one which is used whenever your solvent is chloroform or DMSO and things like that and it is a single line and which is the most up field because the screening is the highest for that there are 3 methyl groups and therefore silane $SiCH_3$.

So, therefore this is the zero ppm $(CH_3)_4$. Now tetramethylsilyl propionate this is the molecule which is used for soluble systems. If you are working in water then typically you use this tetramethylsilyl propionate and that is that also is one line and is other things are deuterated and you will have one line and that is you have 0.0. Another common compound which is used is DSS sodium 4, 4- dimethyl 4-silapentane sulfonate.

This has many lines but if you deuterate all the protons accepting 1 then you will have only one line otherwise you have the most up field line which is taken as 0.0. Other compounds are also used acetonitrile is used sometimes dioxane is used tertiary butanol is used and various different kinds of compounds are used for referencing. And what numbers which are given here are with respect to the TSP or the TMS.

And you have this one as the reference chemical shift and others will be a reference according to that. For carbon 13 (^{13}C) similarly you use TMS, TSP or DSS and these are all at zero frequency. For nitrogen 15 (^{15}N) you use ammonium chloride or liquid ammonia and both are their chemical shifts are taken as 0.0 and for phosphorus you use 85 phosphoric acid and that also the line is taken as 0.0.

So, all your you either you add these things internally inside the sample itself or you put this in a capillary and insert that in your sample tube then it is called as an external reference. If you put that inside the molecule itself then it is called as internal reference. So, I can write that here. So, you can have an external reference in that case the capillary containing the reference compound is introduced in the sample tube.

If you directly put this molecule inside your sample then it is an internal reference. Sometimes you use if you are working with water you look at the water signal itself you look at the water signal itself and make us make that as a reference compound. Often you do not like to put additional molecules inside your sample especially you are working with biological systems with proteins or nucleic acids or any other biological system.

You do not want to introduce an external molecule external perturbation because it can change your conditions it may change the pH or things like that. So, when you do not want to do that then what you do is reference your water molecule water signal with respect to that and use the water signal itself as a reference compound that is that often is done. Now so, having worked with large number of organic molecules the chemist come out with certain kinds of understanding as to what sort of groups will have what kind of ppm in their values.

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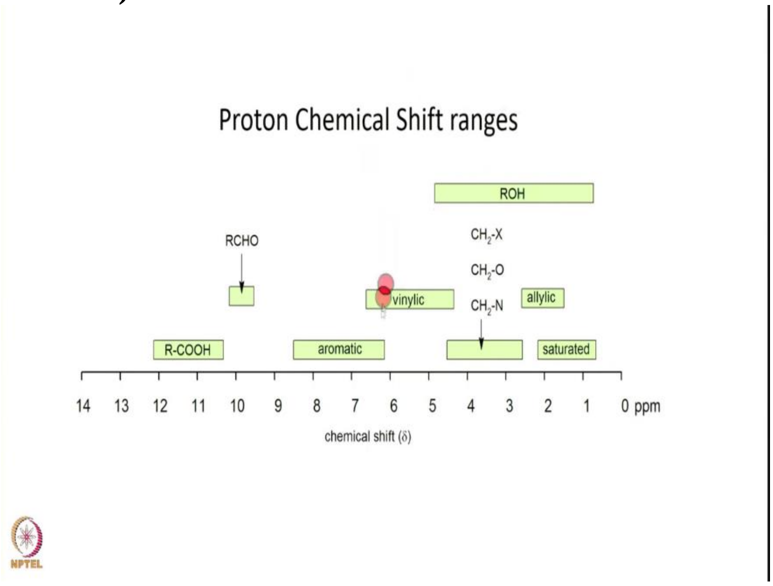

Proton Chemical Shift ranges

And this actually figure shows that sort of information here. So, zero ppm is what we said earlier this is the TMS or TSP or whatever and if you have saturated compounds and those signals will appear between 1 and 2 ppm you have the allylic. Ones they appear here in this region and you have the CH_2 –X which are the electron withdrawing groups here CH2 –O CH_2 –N or this R –OH alcohol.

Now what we are talking about is the signals from R not to the OH okay and those ones will appear in this area then you have the vinylic compounds and which I showed you the vinyl chloride earlier and those ones will appear in this region 5 to 6 ppm. And if we have the aromatic compounds the aromatic compounds will appear between 6 to 8 ppm or 8.5 ppm all these benzene rings various kinds of rings which are there the protons in those ones will appear in this area.

And if you have the aldehyde group the R –CHO then we are talking about this particular proton here this proton will appear at this place here and R –COOH this is the carboxylic acid group this proton will appear here. And here of course the OH also will appear in this area it can vary from here this is wide range here depending upon what the R is you will have a wide range of things and depending on what the conditions are?

What is the pH of the solution you can have this OH proton appearing in a wide range of frequencies? So, therefore it is very sensitive to the conditions of your sample and that proton chemical shifts can vary quite a lot depending upon the environment in your solution. Similar exercise now let me explain to you I explained last time about the aromatic ring effects.

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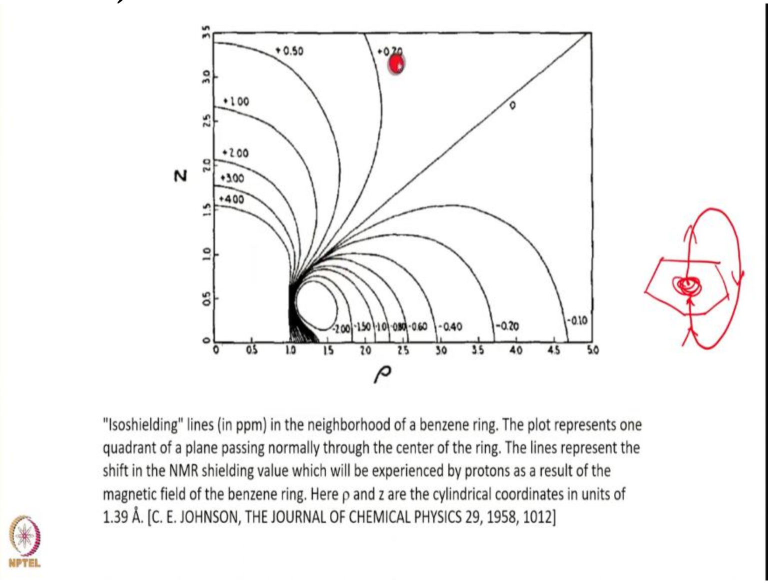

"Isoshielding" lines (in ppm) in the neighborhood of a benzene ring. The plot represents one quadrant of a plane passing normally through the center of the ring. The lines represent the shift in the NMR shielding value which will be experienced by protons as a result of the magnetic field of the benzene ring. Here ρ and z are the cylindrical coordinates in units of 1.39 Å. [C. E. JOHNSON, THE JOURNAL OF CHEMICAL PHYSICS 29, 1958, 1012]

The aromatic ring effects what is shown here is if I have a ring I showed here a ring and it has the electron cloud above and below and it produces an external induced magnetic field. Because of the currents inside this the field goes like this and like this and you can have a proton anywhere present. So, depending upon the proton you will have different kinds of chemical shifts what are shown here are the iso-shielding lines.

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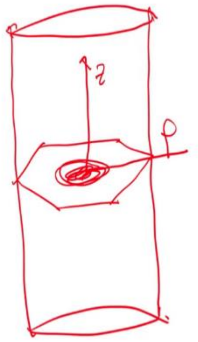

ρ, z are the
Cylindrical
co-ordinates

So, let us say I have a ring here and then I draw a cylinder around this if I draw a cylinder the electronic current is here. Now let us say I consider a position I mean this I define the coordinate system here and this coordinate system here this is called the z coordinate and this is called as these are the cylindrical coordinates. So, ρ and z are the cylindrical coordinates. So, depending upon where your proton is suppose I say I have a proton here ok. So, it will have a certain coordinate z coordinate and the ρ coordinate.

So, this is the coordinate of these are the cylindrical coordinates for this particular proton what will be the chemical shift there. So, what is what has been done is the previous slide which I showed here. So, what is plotted is the z versus ρ. One particular one particular quarter of this.

So, this is this entire circle instead of the entire circle you particularly take one quarter of this. So, you have the cylinder lying on top here like this. So, I take this particular quarter here.

So, if I plot this how the Z axis is on this going up the Z axis on this axis on the right orthogonal is the row axis and what are the chemical shifts in those ones? So, now you see here as you go up as you go up along the z axis. So, that is if you are on the top here if you are there then you have positive shifts here + 4.00 3.0 2.0 1.0 in all these positions at all this position it will have the same 1.00.

If you are here along this contour then you will have 0.00 if you are here 4.00 if you are here then it is 4.0. So, this is all positive numbers here and this is the zero axis this is the no change here and if you are on this side as the ρ is increasing here you start getting negative numbers which means you are on the horizontal plane of the plane of the ring. If you are on the plane of the ring then you get negative shifts and that is to be expected.

Because the field is opposing the field is opposing in the horizontal plane in the vertical axis the field is adding to your main H_0 field and therefore there it causes the positive shift the iso-shielding curves and this is on the negative side. So, this is the calculation which has been done by theoretical procedures using the theory of chemical shifts in great detail and therefore this is extremely valuable to predict depending upon where you are with respect to the ring.

Respect to during where you are you will have different kinds of chemical shifts this is important to know how far it can go and these are in certain units of 1.39 angstroms you see each one of these the z and the rho are given in units of 1.3 and angstroms for the some particular reason they have used this number one point is the chemical length of a carbon-carbon bond. So, a particular bond which they have chosen.

And even this in this is taken from this reference the journal of chemical physics you see in 1958 quite old and of course thousands are valid all along. So, so this is an important effect which is the ring current effect. This is also called the anisotropy because of the ring there is an anisotropic in the chemical shifts depending upon the orientation of the ring depending upon the location of your proton with respect to the ring you will have different chemical shifts and that is drawn in the form of a contour.

So, because there are 2 coordinates which are responsible one is the on the ρ and one is z depending upon the combination of these you will have different kinds of shifts. So, this is what explained to you.

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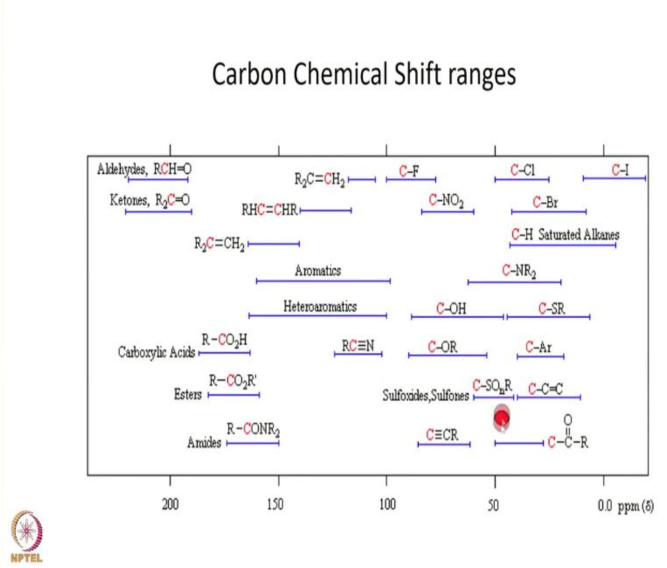

Now similarly for the approach that is for the protons. Similarly you also have the carbon chemical shifts. Now the carbon chemical shifts also you see this carbon has a much wider range of chemical shifts. While proton went from 0 to 10 or 12 ppm carbon goes from 0 to 200 in 230 ppm this is a quite a wide range of chemical shifts you have here. And once again depending upon the nature of the carbon your different kinds of chemical shifts the highlighted carbon is indicated in red colour here.

So, you have R –CO –C that appears in this area C = C-C that appears here in this range and this range and C aromatic you have this one here C –SR, $–NR_2$ CH saturated alkanes CBr and CI. So, C-C. So, all of these; these are the halides here iodine chlorine fluorine and those ones will appear in this area R_2C CH_2 is if you look at this carbon it will appear here and if you have the aromatics the aromatic carbons will appear from 100 to 150 ppm.

Heteroaromatics means if you have this heteroatoms substituents over there they will appear here and RC triple bond n that will appear here sulfoxide, sulfones see this is the result of enormous amount of data collection there is a huge database such thing databases are available in the literature and there are also many many volumes of database of the chemical shifts reported and from this you draw this out of a picture.

So, amides appear here R –CO and R_2 RCO_2 –R’. So, these are the acids esters here and these are the carboxylic acids and they appear in this region 150 to 200 ppm typically the carbonyl carbons. So, you see the typically the carbonyl carbons appear in this area aromatic carbons appear in this area aliphatic carbons appear in this area. And mostly we will be dealing with these kinds of chemical shifts when we do with proteins or nucleic acids.

Because there you have the aliphatic carbons they will all appear in this area and the aromatic carbons the carbonyl groups in peptide bonds and things like that they will all appear in this area aromatic carbons in this area and so on so forth. Phenylalanines and all of these aromatic ring rings in amino acids they will appear in this area. So, this sort of a classification is extremely useful for understanding the structures of molecules.

First of all you have to assign the individual resonances to particular nuclei both proton and carbon and once you have done that then you can go forward to see what sort of structural indicators they are. And now there is another important parameter which is called as the spin-spin coupling.

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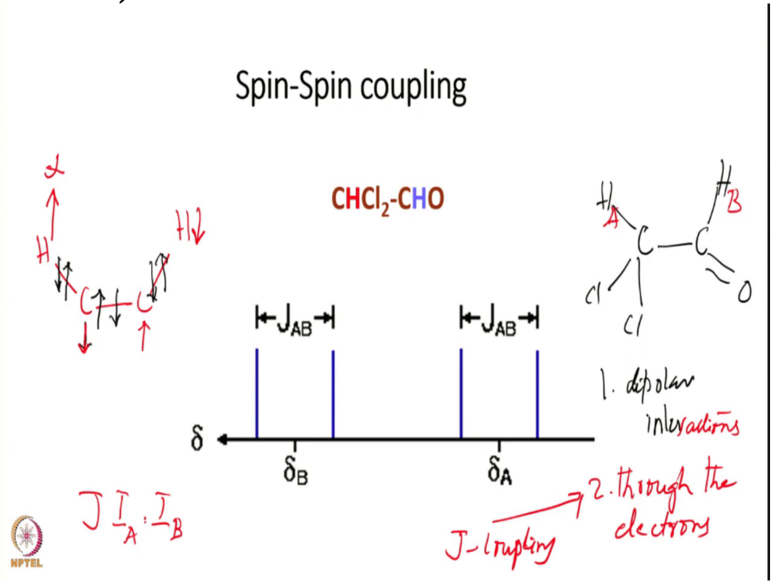

Spin-Spin coupling

$CHCl_2$-CHO

Now the protons while they are surrounded by the electrons and that is of course important to screen the magnetic field but the protons themselves they also interact with each other. Suppose I take this $CHCl_2$ and CHO this proton and this proton they are separated by 3 bonds. So, let me write here C let us say CH ok this is this molecule right. So, now you have this proton and this proton and here you have Cl, Cl these 2 protons interact with each other.

Because they are all magnet they are magnetic moments they are magnetic dipoles therefore they interact with each other either through directly, directly through space interaction. So, there are 2 kinds of interactions possible dipolar interactions ok. Dipolar interaction or there is other what is called as through the electrons in the intervening bonds. Now dipole interaction is a separate story this is actually most applicable in case of solid state NMR and relaxation phenomena.

And we will also talk about it a little bit later and mostly for what we see in organic molecules these the interaction through the electrons and this is also called as J coupling spin-spin coupling is also called as J coupling. What is the result of this I am just showing you the spectrum here you have the $δ_B$ this is the chemical shift you have the chemical shift of the particular nucleus let us say we call this one of them as A other one as B.

So, let me say this is A and this is B and you have the chemical shift of B here and the chemical shift of A here but this line is. Now not appearing at delta B it is actually getting split into 2 lines this is split into 2 lines too and the separation between them is the so called spin-spin coupling constant and that is J_{AB}. Why does this happen how does this happen. So, we can represent this as in this manner.

Let us say I have a proton here I write a proton here and this is my H C C H. So, this is the orientation of the spin here if I call this as let us say the alpha state is the alpha oriented parallel to the field. So, there are also electrons here right in the bonds there are electrons. So, these electrons what happens is the proton nuclear magnetic moment polarizes the electron spin electron magnetic moment and makes it orient in a direction opposite to the α state of the nuclear orientation.

Now since these 2 are in the same bond. So, this bond has 2 electrons the second one will be oriented in this manner because they have to be anti-parallel that contributes to the lowest energy state and therefore it will be anti-parallel. Once this one is there then suppose this were carbon 13 (^{13}C) suppose is the ^{13}C this also has a nucleus and. Now it will orient this nuclear I should use a different colour here again.

So, this is the nuclear magnetic nuclear spin it orients it like this and this in turn orients the electron here it will put in this direction and the pairing electron it puts in this direction and now once again let us put the carbon will again be up. So, the carbon here will be up if it has to be open. Now then I put the proton once more and the electron I put it here like this and then like this and what will happen to this.

So, therefore this will become like that. So, these 2 protons are anti-parallel to each other. So, therefore they are that oriented in opposite direction. Now in terms of the interaction between these 2 how do we describe this we describe this interaction as J I_A . I_B. Now if they are opposite to each other if they are opposite to each other this interaction will be it will produce a negative number right I_A . I_B is I_A I_B cosine 180 I_z^A I_z^B cosine π.

So, if they are exactly one d opposite to each other this will be negative. Therefore if they are opposite to each other they will have a different energy level okay so because of this. Now if J is positive this interact this state will be the lower end energy compared to the other state where this can be positive also it is not that the thing is not possible. It is also possible that this fellow will become negative down here this goes up the other configuration is also possible.

The other configuration will be you have this here the other one is let us say it is also like this is another configuration okay this is configuration one this is the configuration 2 this is also possible this will have a different energy compared to this and this energy will be lower compared to this okay. So, in other words the proton α state will be split into 2 levels.

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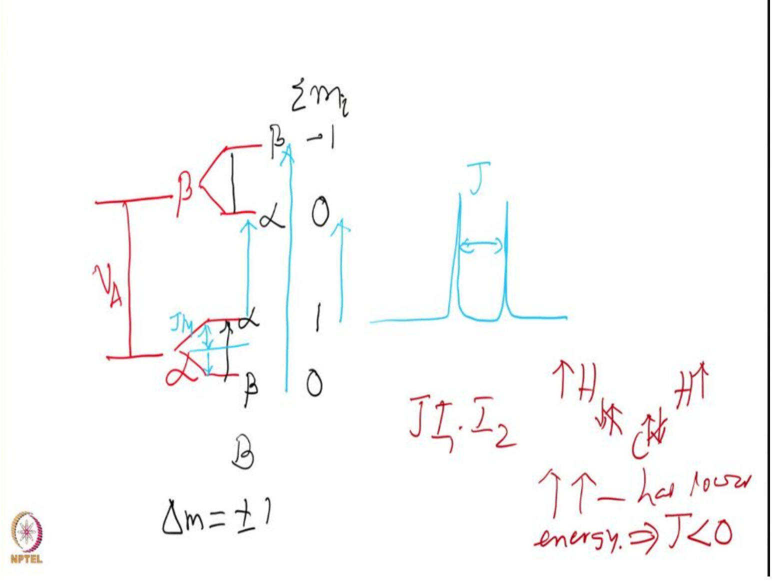

So, now I draw here the α state of the proton this will have 2 energy levels because it will split into 2 because of the orientation of the other spin. Now this is let us say I call it as ν_A and this is α state of ν_A and here the I will write here the spin state of the x nucleus let me write the x nucleus and if this is β this will be down the energy because these are opposite orientations and this is alpha here this is the orientation of the other spin this is the beta spin the B spin orientation.

So, therefore these 2 have different energies obviously instead of one line coming here there will be 2 lines coming there. If I call this as the beta state. So, here again it will split into 2 okay it is split into 2 why and the beta with the alpha goes down. So, this one this is the alpha and this is the beta because I_1 . I_2 if the 2 are in the same direction. If the 2 are in the same direction it will be positive energy if they are in the opposite direction then it will be negative energy.

So, therefore the βα goes down ββ goes up αα goes up αβ goes down. So, therefore they we will have this orientations like this. And what is this energy separation here this will be of the order of few hertz and that is J it which is indicated similarly here. Now what are the transitions possible here but the transitions will be dependent on $\Delta m= -1$.

So, what instead of this transition I will have 2 transitions. Now which are the 2 transitions possible I can have a transition m = 0 to 1. So, what is the m value for the ββ the m value here let me write the m = -1, the β value m value is 0 here because of the βα 1 is down other one is up the sum of the 2m = 0 here.

Similarly this one is 1 and this one is 0 the total m value the summation of the m values I am writing here of the individual spins this is. Now I should have a transition where delta m is equal to plus minus 1 okay this is $\sum m_i$ of the 2. Now which transitions are possible I will have a transition possible from here to here 1 to 0 and 0 to -1. I will have another transition possible here there are 2 transitions possible.

So, therefore this leads to a separation ok how much is this separation here this is J by you can you can calculate what is the value of that this will be $\frac{J}{4}$ this will be $\frac{J}{4}$ the separation will be $\frac{J}{4}$ this will also be J by and then energy which is going down will be $\frac{J}{4}$ o that is J by 2 total. So, now if you look at this therefore you will have 2 lines for each one of those.

So, we will have 2 green 2 sine lines. So, one is between these 2 states this is from here to here which I indicated and the other one is from here to here zero to minus one up and down both are possible okay therefore $\Delta m= \pm 1$, will have these 2 state. And the separation between them will be J . This is how the splitting occurs between the 2 energy levels.

So, we can do the same thing for the other spin as well and that is beta spin I wrote it for the ν_A notice this is in megahertz this ν_A is in megahertz and this is in hertz therefore this is a very small number this is a very small number. So, similarly one can write for the ν_B as well.
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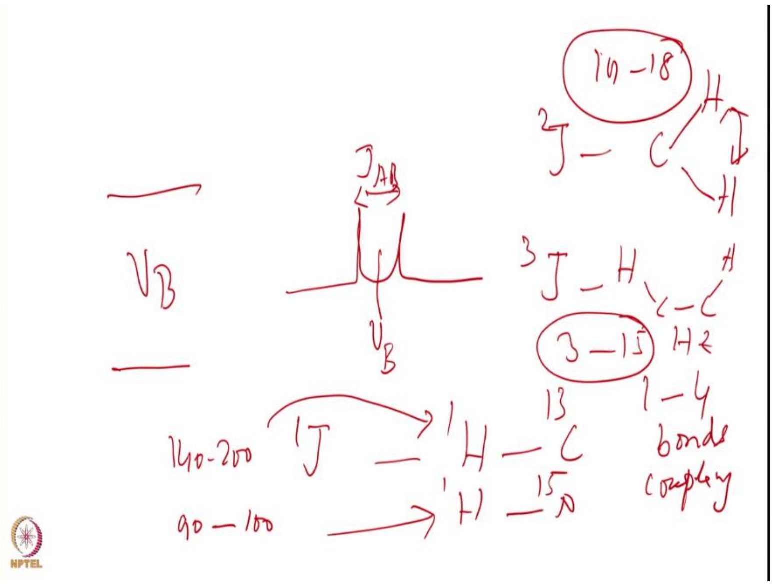

So, I can write for nu B as well I will also have 2 lines corresponding here and the separation between them will be this is nu B and this separation will be J_{AB}I showed you for the 3 bonds you can have coupling from one bond you can have one bond coupling that is let us say proton to ^{13}C or you can have proton to nitrogen 15 you can have 2 bond couplings this is one you can also have 2 bond couplings that is like CH CH here.

So, that is a coupling between these 2 this is a 2 bond coupling I showed you the 3 bond couplings that is H C C H. These are 3 bond couplings the same mechanism applies in all of those cases. And notice here this how far does it go how far this relay goes it does not go beyond 4 bonds okay because if you go beyond 4 bonds then the effect will be relatively small then you do not see the coupling as much.

So, typically so, you will see from 1 to 4 bonds coupling and they have different ranges. So, if I want to write the these are typically of the order of one bond couplings of the order of carbon couplings are of the order of 140 to 200 hertz that is a and then you have 90 to 100 hertz for the proton this one and this is for this one. And the 2 bond couplings are of the order of 14 to 18 hertz and 3 bond couplings will also be of the order of 3 to 15 hert.

And incidentally one more thing one has to notice is the 2 bond couplings can have a negative J value. Now go back to this previous slide here. Now I showed you the value of J interaction is J $I_1 . I_2$ right. So, if J is positive $I_1 . I_2$ if it is negative then there is a decrease in energy if J is negative then it will be $I_1 . I_2$ a negative will be positive right. So, the total will become positive. In the case of 2 just if you take 2 bond let us say H C H and I draw the spins here like this and let us say this one is like this, this one is like this electron spin and I have here this one let us say like this, this one like this and this is the most stable state let us assume this is the most stable state.

Now you see the 2 protons spins are parallel to each other and this if the stable state this actually has to be energy has to be negative. So, this parallel state has lower energy. So, this implies that J is less than zero because it decreases the energy value and therefore the geminal couplings the. So, called geminal couplings are negative whereas the vicinal couplings on the one bond couplings they are all positive. So, I think I will stop here again we go to the next one in the next class.