NMR Spectroscopy for Chemists and Biologists

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Factors affecting Isotropic Chemical Shifts

Lecture No. 6

So, welcome to today's lecture. Today we are going to discuss more details of high resolution NMR spectra of molecules which we were doing in the last class.

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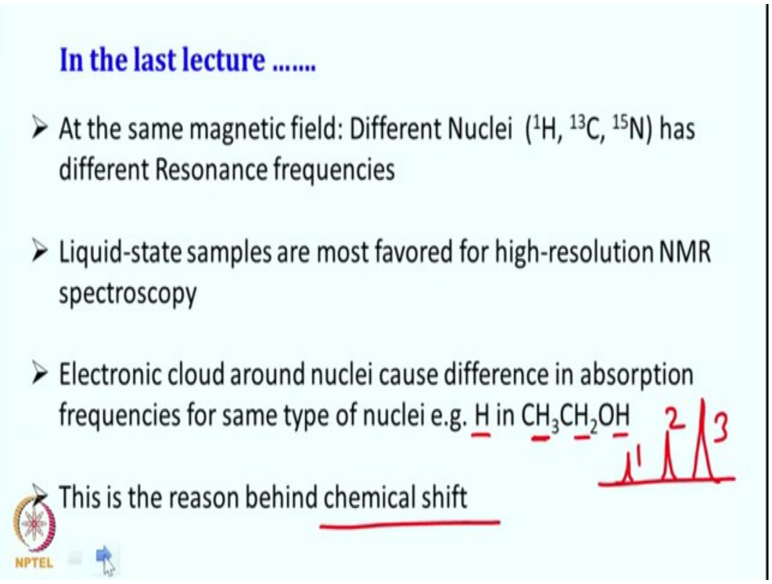

In the last lecture …….

- At the same magnetic field: Different Nuclei (^1H, ^{13}C, ^{15}N) has different Resonance frequencies
- Liquid-state samples are most favored for high-resolution NMR spectroscopy
- Electronic cloud around nuclei cause difference in absorption frequencies for same type of nuclei e.g. H in CH_3CH_2OH
- This is the reason behind chemical shift

So, in the last lecture we looked at, that at the same magnetic field, different nuclei such as proton, Carbon13, Nitrogen15 has different resonance frequency and that gives us possibility to record; in the same sample different spectrum for different nuclei. We also looked at liquid state samples are most favored for high resolution spectrum because liquid sample can tumble in the solution and because of this tumbling, lots of motional averaging happens and many of the anisotropic interaction are averaged out.

And therefore we have a sharp line whereas solid generally gives broad line and gaseous sample has less number of spin to contribute towards the signal. Therefore, the intensity from the gaseous sample is less. So, for high resolution NMR spectrum liquid state samples are most favored one. Then we looked at electronic cloud around the nuclei cause difference in absorption frequency for same type of nuclei we give a example for like proton. Say proton in CH_3 has a different electronic cloud around than CH_2 and than OH.

And therefore, for alcohol we have 3 spectrums, 1 corresponds to CH_3 another corresponds to CH_2 and one correspond to OH and their intensity ratio was also 3 to 2 to 1. So, this also we looked at and this differential electronic cloud around the nuclei is the reason behind the chemical shift.

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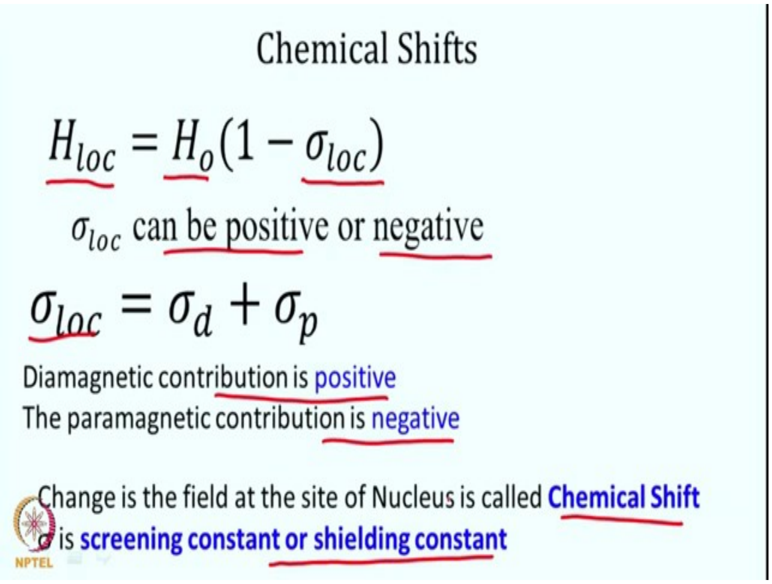

Chemical Shifts

$$H_{loc} = H_0(1 - \sigma_{loc})$$

σ_{loc} can be positive or negative

$$\sigma_{loc} = \sigma_d + \sigma_p$$

Diamagnetic contribution is **positive**

The paramagnetic contribution is **negative**

Change is the field at the site of Nucleus is called **Chemical Shift**

σ is **screening constant or shielding constant**

After that we were discussing about like how because of this differential electronic cloud around a particular nuclei give rise to different effective absorption frequency, so or the magnetic field.

$$H_{loc} = H_o(1 - \sigma_{loc})$$

So, H_{loc} is the magnetic field experienced by a particular nuclei is different than the H_0 which is main magnetic field and that depends upon what is the screening constant or shielding constant around that nuclei. So,σ_{loc}.

$$\sigma_{loc} = \sigma_d + \sigma_p$$

Now, we also looked at that σ_{loc} can be positive or negative and this sigma local depends upon 2 contribution, 1 coming from the diamagnetic contribution another coming from the paramagnetic contribution. So, generally diamagnetic contribution is positive and paramagnetic contributions are negative.

So, now we looked at the change in the field at the site of nucleus is called chemical shift and this this sigma which is called a screening constant or shielding constant. So, this is the reason why actually same kind of nuclei experience different local magnetic field and that gives you different absorption line.

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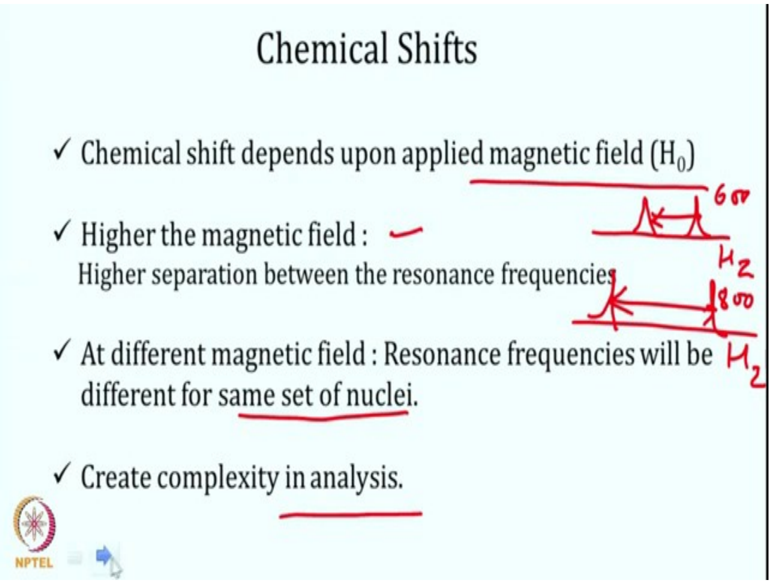

Chemical Shifts

- ✓ Chemical shift depends upon applied magnetic field (H_0)
- ✓ Higher the magnetic field : Higher separation between the resonance frequencies
- ✓ At different magnetic field : Resonance frequencies will be different for same set of nuclei.
- ✓ Create complexity in analysis.

Next, we were discussing that the chemical shift depends upon applied magnetic field which is H_0. So, if higher the magnetic field, higher will be absorption, separation between the resonance frequency. So, like if we record a spectrum on 14 tesla the separation is going to be like that. If we express this resonance frequency in hertz and if we apply this is on say 600 megahertz and if we record that on 800 megahertz the separation between line will be far.

So, that is the case and therefore the higher magnetic field are better to resolve this spectrum, but this creates a problem if we express the resonance frequency in terms of hertz because at different magnetic field resonance frequency are different for same set of nuclei. So, this creates little complexity in the analysis.

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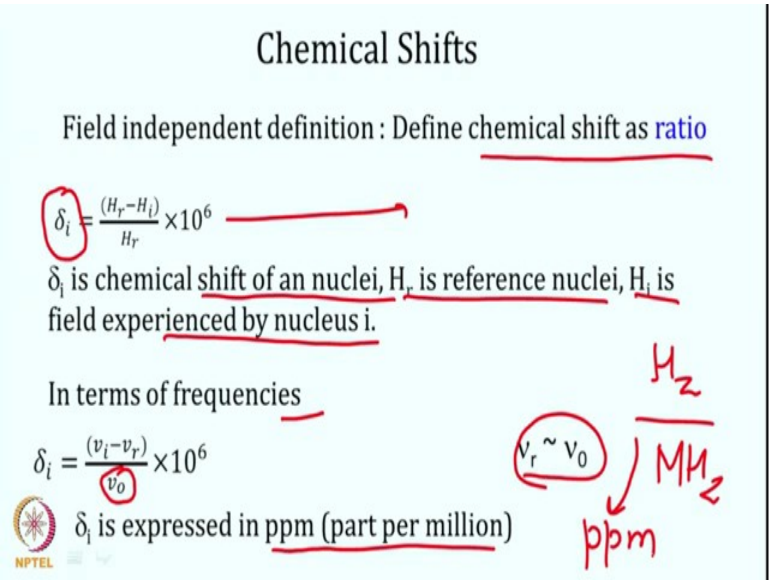

Chemical Shifts

Field independent definition : Define chemical shift as ratio

$\delta_i = \frac{(H_r - H_i)}{H_r} \times 10^6$

δ_i is chemical shift of an nuclei, H_r is reference nuclei, H_i is field experienced by nucleus i.

In terms of frequencies

$\delta_i = \frac{(\nu_i - \nu_r)}{\nu_0} \times 10^6$

$\nu_r \sim \nu_0$

$\frac{Hz}{MHz}$

ppm

δ_i is expressed in ppm (part per million)

For getting rid of such complexity, one has to express or explain the chemical shift in a field independent manner. So, how one define this in a field independent manner? By defining chemical shift as a ratio. So, this ratio which is, this is chemical shift say I am interested in getting the chemical shift of a particular nuclei what has to be done. Then you express that as a reference magnetic field and difference with that with a particular interest of nuclei and divide that the magnetic field of the reference compound and that is in parts that multiplied with a 10 to power 6 that is million.

$$\delta_i = \frac{(H_r - H_i)}{H_r} \times 10^6$$

So, now here sigma i is the chemical shift of a particular nuclei, H_r is the reference nuclei, H_i is the field experienced by the i^{th} nuclei which we are interested. So, this is actually chemical shift for particular nuclei. Here, if you look at, now we are taking the ratio of 2 magnetic field but if we want to explain this in terms of frequency what one has to do that subtract the i^{th} nuclei frequency from the reference nuclei and then divide that with a with a main magnetic field which is ν_0. This is because the difference between the reference nuclei and main magnetic field is not going to be too large.

$$\delta_i = \frac{(v_i - v_r)}{v_0} \times 10^6$$

And therefore, we can take this as v_0. So, if we explain this then sigma that delta which is chemical shift can be expressed in part per million because the difference in the chemical shift between the reference nuclei and the choice of nuclei is in hertz and main magnetic field is in megahertz. So, hertz divided by megahertz is essentially part per million.

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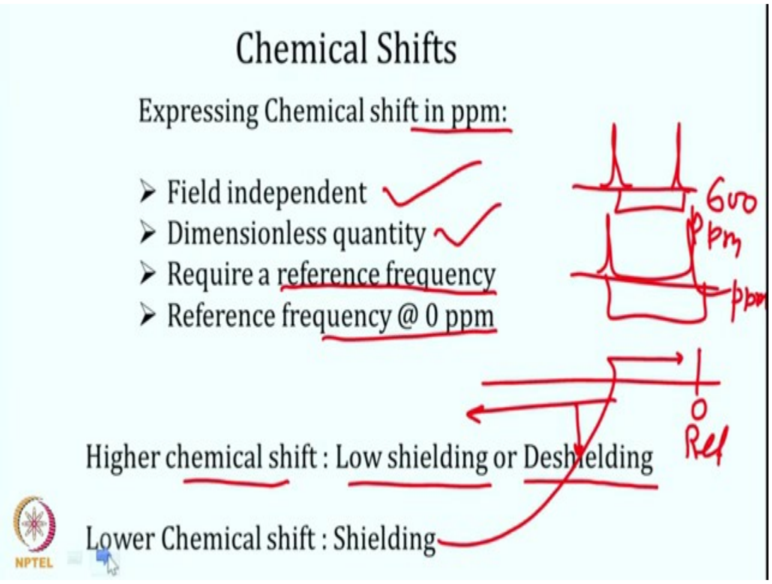

Chemical Shifts

Expressing Chemical shift in ppm:

- Field independent ✓
- Dimensionless quantity ✓
- Require a reference frequency
- Reference frequency @ 0 ppm

Higher chemical shift : Low shielding or Deshielding

Lower Chemical shift : Shielding

If we express this chemical shifts in terms of ppm we have some merit in that. So, now defining this in ppm actually it removes the field dependence. So, now chemical shift has become really, really field independent whether you record an spectrum on 600 megahertz or record an spectrum on 800 megahertz there is no field dependence. So, at all field you can express your resonance frequency in part per million and it is going to be same. So, here like if we are recording on 600 megahertz, the ppm value of these 2 peaks is going to be same whether you record on 800 megahertz.

If ppm value is going to be same which is not the case when we were representing the chemical shift in terms of hertz. The another important point since you are dividing 2 units, so chemical shift in ppm is an dimensionless quantity but for expressing this chemical shift in terms of ppm one require an reference frequency and generally we put that frequency at 0. So what we mean we take an compound which we say that this is are my reference compound and now all other chemical shift will be actually defined how far they are from that reference frequency. Therefore, we put our reference frequency at 0 ppm.

So, now in broader term we can conceptualize that if there is a higher chemical shift that means low shielding or deshielding or if there is a lower chemical shift that is shielding. So, what I mean to say if our reference frequency here is 0 ppm reference. So, if we are away from this 0 that means the compound is deshielded or there is a lower shielding and so this is deshielding and if it comes near 0 so that is shielding means there is a more electronic cloud near this compound and therefore it is shielded and it comes near 0. Okay.

So, now so we need an reference compound. An reference compound should have ideally the maximum shielding so that we can have that is a reference and we can express that chemical shift as a 0 ppm.

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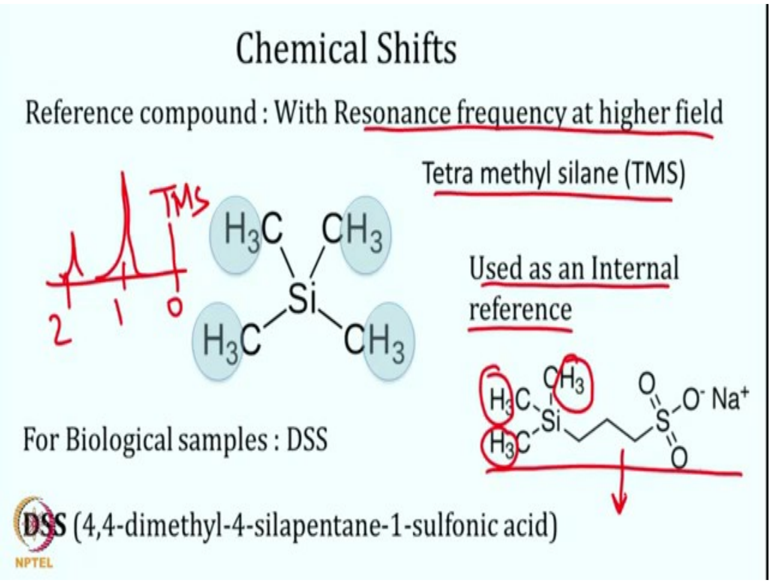

Chemical Shifts

Reference compound : With Resonance frequency at higher field

Tetra methyl silane (TMS)

Used as an Internal reference

For Biological samples : DSS

DSS (4,4-dimethyl-4-silapentane-1-sulfonic acid)

So, generally reference compound should have the resonance frequency at higher field and for most of the case this tetra methyl silane, TMS is used as a reference compound. So, as a TMS a silicon, there are 4 methyl group attached to it. So, if you look at these 4 methyl H, H, H; there are like a electron, high electron density around these protons and that is that is why it is more shielded and its resonance frequency is a is defined as a 0 ppm.

So, this is used as a internal reference. So, what internal reference means, this compound is added to the to the experimental sample and now we look for the peak which is coming from TMS, we put that as a reference frequency and with respect to that all other frequency are explained. So, that means your 0 ppm signal comes from TMS and then we have another absorption line. So, it can be 1 ppm or 2 ppm or whatever it is. So, here, TMS gives signal gives at 0 ppm. Now, TMS is can be e dissolve in many organic solvent.

However, it is not very soluble in the water therefore for protein experiment TMS is not preferred internal reference. We use another compound called DSS. It is a sodium salt of similar moiety. So, if you look at here, here also we have CH_3 CH_3 CH_3 and it is a sodium salt. So, actual DSS is 4, 4-dimethyl-4-silapentane-1-sulfonic acid. So, this is water soluble and it is used in protein sample as an internal reference. Now, these are internal reference. So, that means they should not interact with my compound of interest and they should not cause any harm or any perturbation to that.

So, it has to be inert compound. However, in many case if they are interacting with the experimental compound then we do something called external referencing, means we record an experiment where only reference is recorded and then in another experiment in the same solvent, same buffer at same pH we record our compound. So, that signal from TMS is or DSS is kept at 0 and rest explained in terms of difference from that. So, that is the difference between internal reference and external reference.

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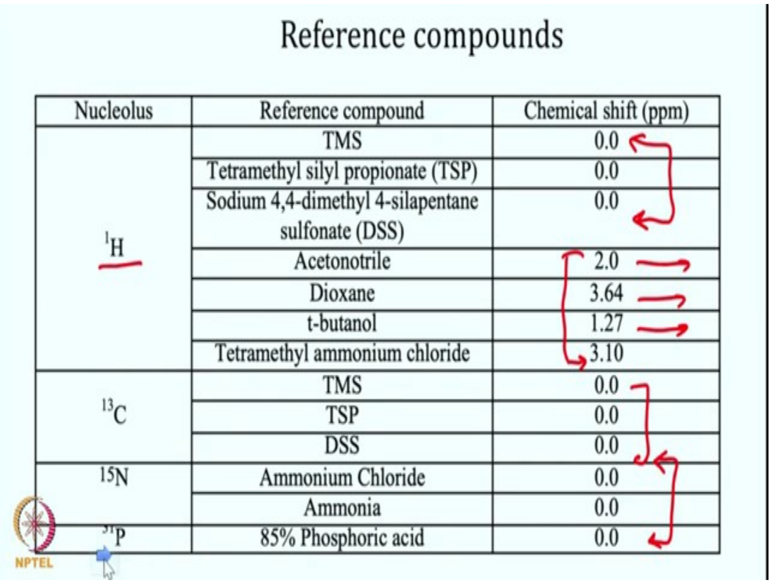

Reference compounds

Nucleolus	Reference compound	Chemical shift (ppm)
^{1}H	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
^{15}N	Ammonium Chloride	0.0
	Ammonia	0.0
^{31}P	85% Phosphoric acid	0.0

So, there are many compounds which are used as a referential reference compound. So, one of favorite is TMS. Now, then there are another like tetramethyl silyl propionate, TSP or DSS that we just looked at. So, all these compounds their reference frequency is kept at 0 ppm and these are basically reference compound for proton. So, if you look at their reference frequency is at 0 ppm. With respect to that, these can also be used as a reference like that they do not change their chemical shift too much, acetonitrile or dioxane or like tertiary butanol, so or tetra methyl ammonium chloride.

They have different resonance frequency than TMS and that can also be used as a reference compound. Now, for ^{13}C again TMS or TSP or DSS is used, you put that their chemical shift at 0 ppm. Other than that for ^{15}N ammonium chloride or ammonia can be put and you put that frequency as a 0 ppm or even for ^{31}P phosphoric acid, 85% phosphoric acid is used. So, all these is calibrated to are put as 0 ppm and then other frequency are measured with respect to these compounds.

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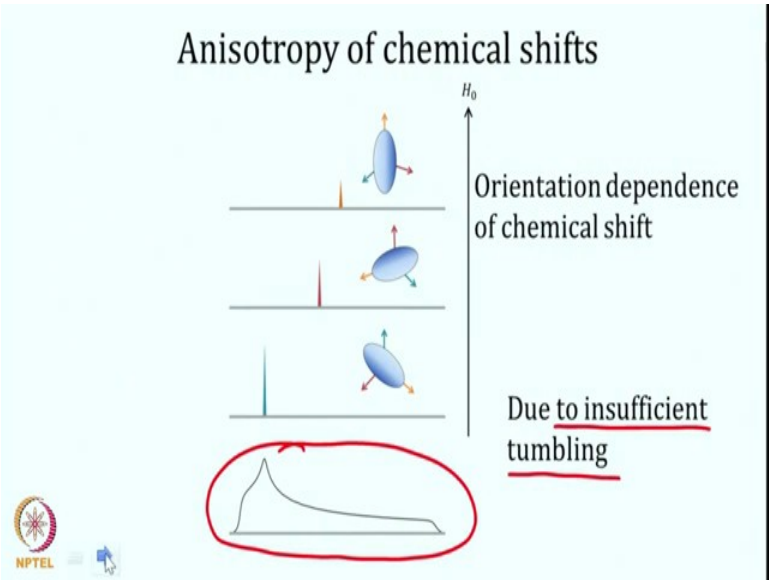

Anisotropy of chemical shifts

Orientation dependence of chemical shift

Due to insufficient tumbling

Now, after getting the concept of the chemical shift let us move to another concept called anisotropic of the chemical shift. So, we understood that in solid there is a there is no tumbling. Therefore, spins were aligned in many other direction and many spins were closely packed. So, because of no tumbling all these spins had some orientation effect like here. If you look at their spin here it has orientation for this this orange spin along the magnetic field. However, in the other spin here, red spin is aligned along the magnetic field.

And in third case, it is this cyan color spin is aligned around the magnetic field. So, all this essentially contributes to the chemical shift and therefore when we record an spectrum for non-spinning static sample all these spins oriented in the different magnetic field contributes to the chemical shift. Therefore, the average chemical shift or resultant chemical shift that appears gives a very broad line and this is called peak pattern. So, now this broad line is because of non-tumbling or insufficient tumbling of the molecule.

So, even in oriented liquid sample if where there is a incomplete tumbling, you get some kind of anisotropy, anisotropy means orientation dependent chemical shift. And that is very much used for many of the experiment that we will discuss later. So, insufficient tumbling causes the line broadening.

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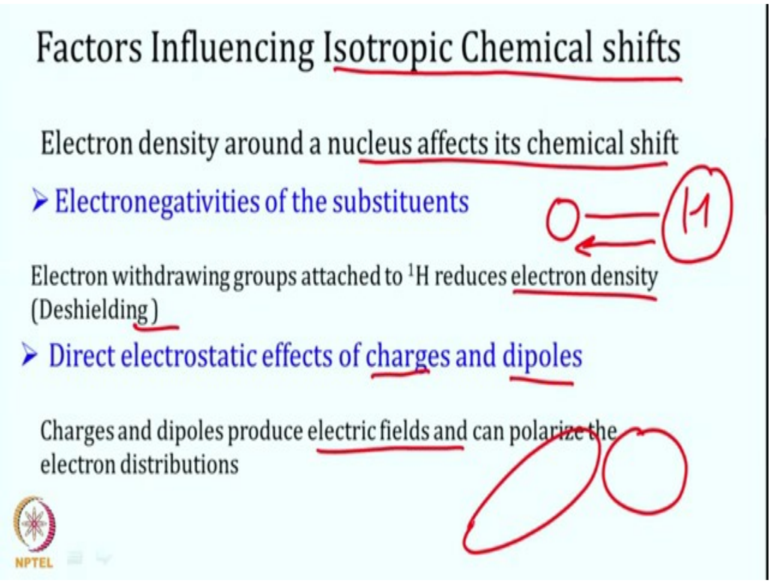

Factors Influencing Isotropic Chemical shifts

Electron density around a nucleus affects its chemical shift

- Electronegativities of the substituents

Electron withdrawing groups attached to ^{1}H reduces electron density (Deshielding)

- Direct electrostatic effects of charges and dipoles

Charges and dipoles produce electric fields and can polarize the electron distributions

The other chemicals, type of chemical shift that we looked at is isotropic chemical shift where there is a complete tumbling and it gives a sharp line that is called isotropic. Isotropic means no orientation dependence in the chemical shift.

So, what are the factors that influence this isotropic chemical shift? So, anything that changes the electron density around a nucleus can affect the chemical shift. So, some of them can be electronegativity of the of this substituent. Electronegativity means how it can pull the electron towards itself, like say here is our OH. So, if you are interested in knowing the chemical shift of this proton now, O is an electronegative atom. So, it will try to pull the electron towards itself. Therefore, it will change the electronic cloud near this proton and thus it will be kind of a deshielded means there will as electron cloud around this, okay.

So, that is the for proton. Now, the, that will create the electron density deshielding. The another one can be direct electrostatic effect of charge and dipoles. Suppose in sample there is some charge or some dipole. Now, the charge or dipole can produce the electric field and that can polarize the electron distribution around this compounds. So, that polarization of electrons like if there was a spherical electron density but if you put a dipole it made it something like this.

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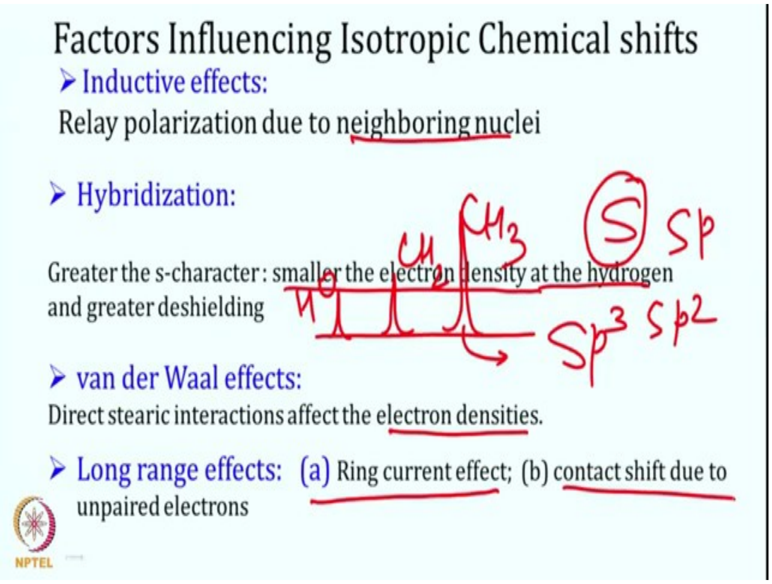

Factors Influencing Isotropic Chemical shifts

- Inductive effects:

Relay polarization due to neighboring nuclei

- Hybridization:

Greater the s-character: smaller the electron density at the hydrogen and greater deshielding

- van der Waal effects:

Direct stearic interactions affect the electron densities.

- Long range effects: (a) Ring current effect; (b) contact shift due to unpaired electrons

And that will also change the resultant chemical shift around a particular nuclei and that will change the chemical shift. The third one can be inductive effect. What is inductive effect? It is a relay polarization to the neighboring nuclei. So, suppose it is not directly connected but it is coming from like little far neighbor that can also induce the polarization in the magnetic field and that can be little far away and that also causes the change in the chemical shift. The third one is hybridization.

You know there is a mixing of orbital happens and that is called hybridization. They are like *s*, *sp*, sp^2, sp^3 or something like that. So, if you look at this hybridization the contribution of different orbitals changes like in s there is completely spherical shape, when it mixes to *p* and becomes *sp* or sp^2 or sp^3. Contribution of *s* orbital changes in different hybridization. So, for *s* characteristic there is a smaller electron density at the hydrogen and that is why it is deshielded but when it makes sp^3 the contribution of the s changes.

And therefore, the electron density increases around the magnetic field around that particular nuclei and therefore different magnetic field will be experienced by this nuclei and that also changes the electron density and that is a classical case that we looked for CH_3 which is sp^3 hybridized, CH_2 and OH. OH is a classical case of electronegativity, therefore it is deshielded, here there is a more contribution coming because of sp^3 hybridization and that is why it is shielded and therefore it is coming near 0 ppm.

Next effect is van der Waal effect. So, this a direct steric interaction that affects the electron density. van der Waal like if 2 atom are approaching closer to each other then electron density will also be influence by the, like increasing density of the contributing nuclei. So, if something supposing a protein some moiety is buried inside and same moiety is exposed there will be different electron density around that particular nuclei and therefore, that also changes the chemical shift. The next one is ring current effect or contact shift due to unpaired electron.

So, I will just go in detail what is ring current and what is contact shift due to unpaired electron.

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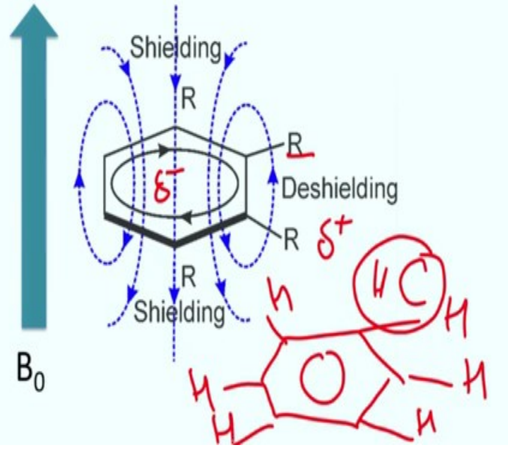
π electron clouds in aromatic rings : Produce secondary magnetic field.



So what happens. Let us take a benzene ring. So, in benzene ring there is a π electron cloud around the magnetic field. Now, this π electron is circulating. Now, because of this circulation, if you look at this circulating, this produce additional magnetic field and because of that the electron density around this all protons are all over the ring is not same. Here, you have a less electron density so you have essentially, a partial positive charge at the edge of the ring and a partial negative charge at the centre of the ring.

And that causes also different chemical shifts for different protons or different moiety attached in a ring. So, here you have a deshielding and at the centre, you have a shielding. This is called ring current effect and therefore even the even the aromatic protons which are essentially like if you look at they are C-H. So, they have a different chemical shift than usual C-H because of this something called ring current effect, okay.

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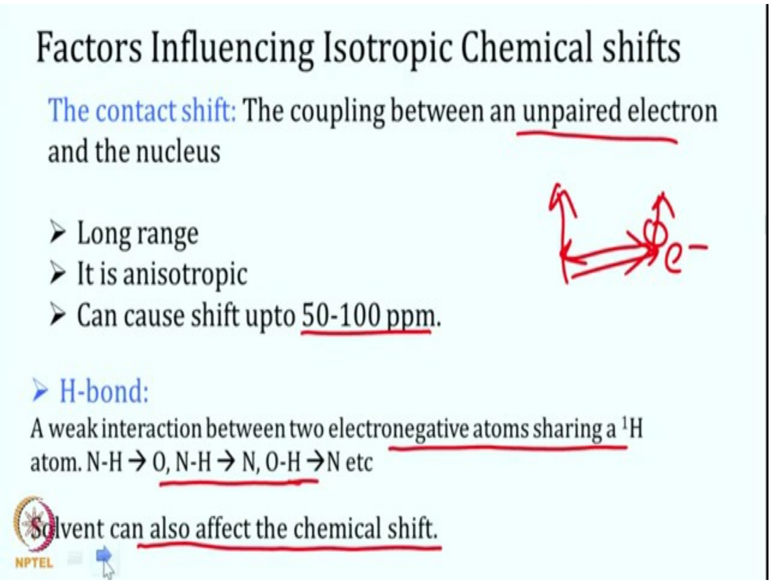

Factors Influencing Isotropic Chemical shifts

The contact shift: The coupling between an unpaired electron and the nucleus

- Long range
- It is anisotropic
- Can cause shift upto 50-100 ppm.

- H-bond:

A weak interaction between two electronegative atoms sharing a ^{1}H atom. N-H → O, N-H → N, O-H → N etc

Solvent can also affect the chemical shift.

So, next one is the contact shift. Contact shift actually arises because of the coupling between unpaired electron and nucleus. So, what happens? Suppose in a solution there is some unpaired electron right here unpaired electron and we have a nuclei. Now, there is a long range coupling between these two. It is a, it is called Fermi contacts and all those and it is also anisotropic depends upon because this a vectorial quantity. It depends upon what is the angle between it, where this electron is.

This unpaired electron affects the chemical shift and actually this is quite long range. So, chemical shift can shift all the way up to 50 to 100 ppm. So, this is one of the major factors that influence the chemical shift of a particular nuclei. Next one is hydrogen bonding. Hydrogen bonding also affects the electron cloud around a particular proton or particular nuclei. So, if it is hydrogen bonded it shifts it shifts differently in alpha helical N-H and in β-sheet and that actually changes the chemical shift for a particular protein. Now, other than these factors that we discuss the solvent can also affect the chemical shift.

Therefore, precise selection of a solvent is also important for interpreting the chemical shift of different nuclei.

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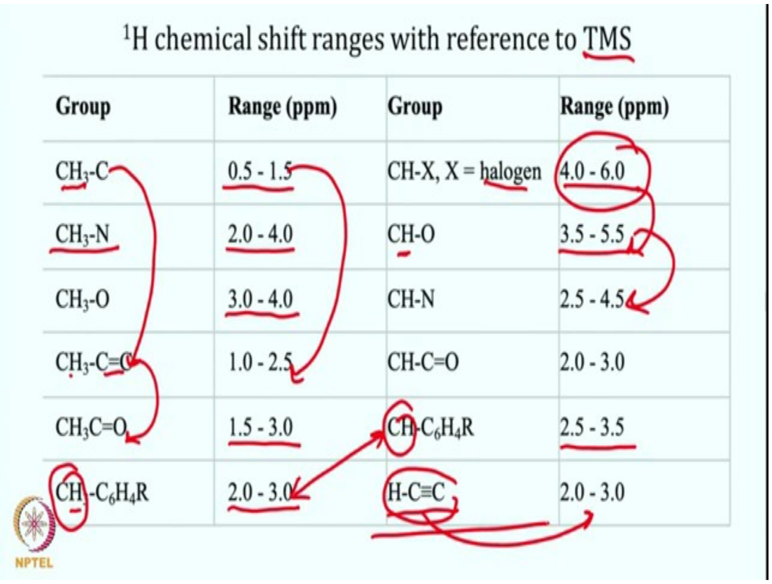

^{1}H chemical shift ranges with reference to TMS

Group	Range (ppm)	Group	Range (ppm)
CH_3-C	0.5 - 1.5	CH-X, X = halogen	4.0 - 6.0
CH_3-N	2.0 - 4.0	CH-O	3.5 - 5.5
CH_3-O	3.0 - 4.0	CH-N	2.5 - 4.5
CH_3-C=C	1.0 - 2.5	CH-C=O	2.0 - 3.0
CH_3C=O	1.5 - 3.0	CH-C_6H_4R	2.5 - 3.5
CH-C_6H_4R	2.0 - 3.0	H-C≡C	2.0 - 3.0

So, if you go little bit into details and we measure the chemical shift with respect to or as reference compound which is TMS. So, there are groups of this chemical shift and details can be found in any textbook of NMR spectroscopy but just for giving you an idea if we just looking at this CH_3 proton, so you look at this CH_3 proton which is essentially kind of a shielded and therefore, its chemical shift comes quite close to 0 which is 0.5 to 1.5 ppm. But if we attach here an N.

Now, here it was C and here N. N is electronegative, therefore electron are pulled away from the CH_3 and therefore it gets deshielded and chemical shift comes at 2 to 4 ppm. Now, we attach O to this so what O is doing now, it is pulling more electrons to itself because it is electronegative and therefore the chemical shift changes from 2 to 4 to 3 to 4. Now, if you attach a like C here if you attach a double bond, like here if you look at here there was only 1 carbon. Here, there are 2 carbon but attached with a double bond.

Double bond has a π electron. So, if you look at the chemical shift changes from 0.5 to 1.5 to 1 to 2.5 because of this π electron present and it can also influence the chemical shift of this CH_3. Instead of C if you attach O, O is electronegative. It can pull more electron towards itself and therefore it chemical shift will be 1.5 to 3. Now, if you add aromatic compound that aromatic compound also as we said that has a π electron and it can also pull to 2 to 3. So, therefore if you look at what group is attached and how the electron cloud is disturbed around a particular proton.

It influences the chemical shift. Similarly, if you look at CH and if you attach a halogen, halogen are known to be quite electronegative so they can pull more electrons towards their self and therefore, it will be more deshielded. And therefore, chemical shift comes around 4 to 6 ppm. In CH here, CH is a kind of a only 1 proton is here. If you attach oxygen here that will also change but halogens are more therefore it is slightly less than this. If you attach N it is less electronegative, therefore it comes more towards shielded region and so and so forth.

So, here if you compare this with this now, CH_3 and CH_2 rest remains same. Now, CH_3 there are 3 protons, so therefore in-built here is more shielding. In CH there is a less shielding. Therefore, this goes towards higher ppm value or deshielded. Similarly, if we compare this with a triple bond like triple bond is has more pi electron and therefore, it shifts more towards higher ppm value or deshielded value. So, these are broad concept how you know whether your proton of choice will be shielded or deshielded depending upon what is attached to it whether these compounds are giving electron or taking electron or withdrawing electron or pushing electron that dictates how their resultant chemical shift is going to change.

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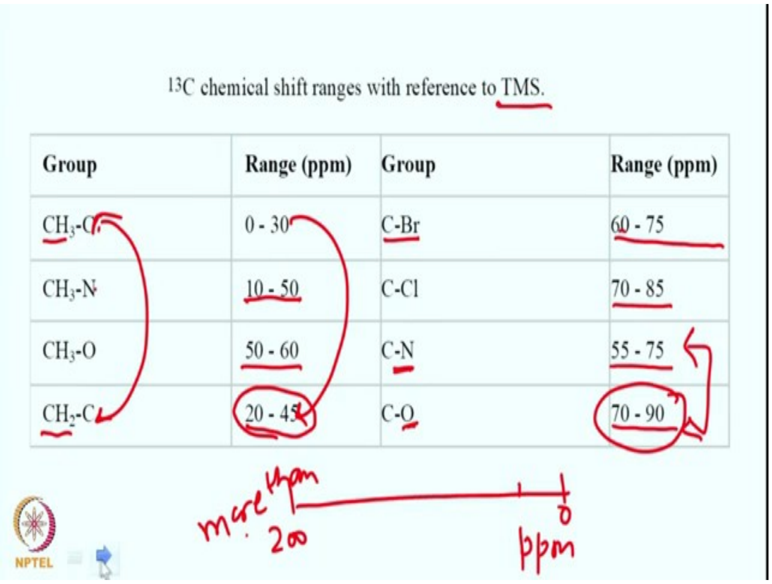

^{13}C chemical shift ranges with reference to TMS.

Group	Range (ppm)	Group	Range (ppm)
CH_3-C	0 - 30	C-Br	60 - 75
CH_3-N	10 - 50	C-Cl	70 - 85
CH_3-O	50 - 60	C-N	55 - 75
CH_2-C	20 - 45	C-O	70 - 90

more than 200

0

ppm

Similarly, for carbon 13 there is a range of chemical shift and those range are expressed in terms of like how they are far from TMS. TMS is also an reference compound. So, again if you look at again CH3 it is a sp^3 hybridized, CH3 is shielded and therefore for carbon. So, generally carbon ppm range is not like proton. It comes from 0 to all the way up to 200 or even more than 200, 200 ppm it comes. So, therefore, this has a wide range and in that case CH_3 should be shielded and therefore it comes around, 0 to 30 ppm. But if you attach instead of carbon, if you attach nitrogen it will pull electron towards itself it will be more deshielded and therefore chemical shift comes around 10 to 50 ppm.

Attach oxygen to same moiety more deshielding, it comes 50 to 60 ppm. And now instead of CH_3 it becomes CH_2 so if you compare this CH_3 as a 3 proton attach here it is a 2 proton attach, the chemical shift changes towards higher ppm value that is, it is less shielded compared to CH3. Now, similarly if you attach more electronegative like a Br then it goes to more deshielding and chemical shift comes at 60 to 75 ppm. Now, if you attach chlorine right, so it is even more, it goes to 70 to 85 ppm but if you attach N or O. so, N is somewhere 55 to 75. O is more electronegative so if you compare these 2, this has more deshielding effect or with electron withdrawing effect and therefore it chemical shift comes around 70 to 90 ppm.

So, that is what we had discussed. Now, more or less I gave you a concept of chemical shift, how a group attached around a particular nuclei causes shielding or deshielding and all those are measured with respect to a reference compound. Many cases it is TMS, in protein cases since TMS is not water-soluble so DSS is used.

And we looked at what is the range of different chemical shift, details of this chemical shift range can be extracted from any NMR textbook. In the next class what we are going to do, is looking at another important concept which is actually spin-spin coupling.

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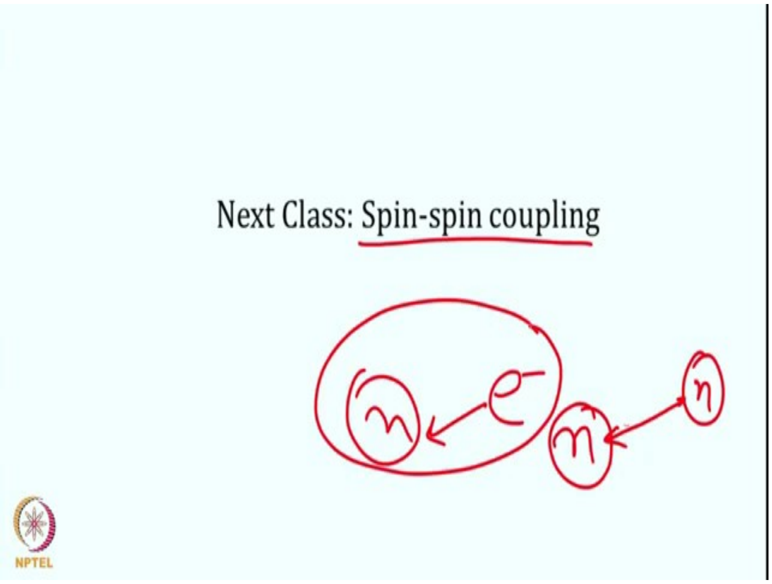

Next Class: Spin-spin coupling

So, here what we had looked at, how the electron around a particular nuclei is influencing its resonance frequency. Now, in the spin-spin coupling we are going to say how one nuclei is going to affect its neighbor nuclei. So, this is like nuclear like a nuclei-nuclei talking rather than nuclei electron talking.

So, our next class we are going to discuss about spin-spin coupling. I look forward for your active participation. If you have a questions please write to us we will try to respond each and every question in coming lectures. Thank you very much and looking for the next lecture.