NMR spectroscopy for Structural Biology NS
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Lecture: 05
NMR Spectra of Molecules

So, now we are going to talk about practical aspects or things that which are common use for the chemist and the biologist and that is what we say NMR Spectra of molecules.

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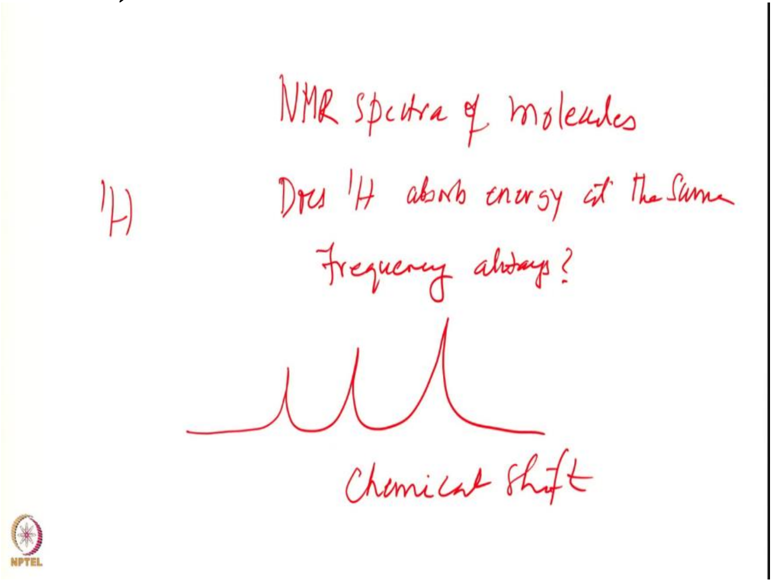

NMR Spectra of molecules

^1H

Does ^1H absorb energy at the Same Frequency always?

Chemical Shift

So, suppose we take the proton the question here is the proton the same in every molecule so far as NMR is concerned. Does it absorb energy at the same because it has the same magnetogyric ratio. So, therefore it has the same magnetic moment. So, does every proton absorb energy at the same frequency. So, does proton absorb energy at the same frequency always? Actually this was in the initial days this was what one had thought.

So, they turned out that actually this is not quite true there is a story which goes here this is the discoverer of this particular parameter called as the chemical shift and that was Professor S. S. Dharmati who actually started this NMR activity in TIFR, TATA Institute of Fundamental Research. He was actually a postdoc with Felix Bloch. When he was working with Felix Bloch at that at that time the intention was to measure the magnetic moment of the proton.

So, he asked Dharmati to put water in the magnet and record the NMR Spectrum but Dharmati they put alcohol. So, you put alcohol in the magnet and he observed three signals. He observed three signals here. So, the Felix Bloch could not believe this, what is this with the same proton which is how it can be absorbing at three different frequencies. And this is this was actually a puzzle and in fact this was the start of what is called as the concept of chemical shift.

Why does this happen? Now this is because every nucleus has an electron around it. If I take a molecule there are various electron clouds there are various bonds and various structures.

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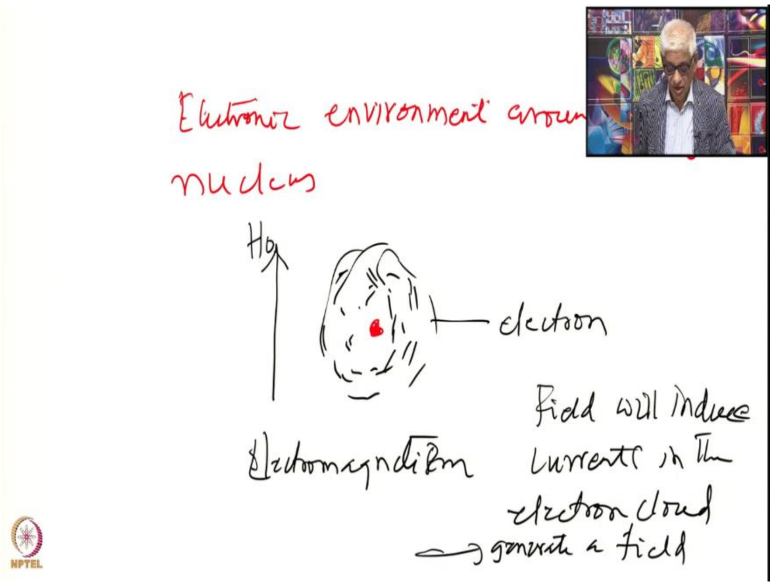

Electronic environment aroun

nucleus

H_0

electron

Electromagnetism

Field will induce currents in the electron cloud

→ generate a field

So, there is electronic environment around every nucleus. Suppose I put the electron in the nucleus here and now I. So, I put lot of dots here and then what is this? This is electron. Electron cloud there is an electron cloud around the nucleus and now to and now I am putting this system in the magnetic field this is the perturbation. There is electron cloud around the nucleus and I am putting the magnetic field.

Now what is the response of the electron cloud with respect to this? We know from electromagnetism there is a law called Lenz law that if you apply this field it will induce currents in the electron cloud. So, the field will induce currents in the electron cloud. And when there is a current going it will produce a magnetic field. So, this will generate a magnetic field will generate a magnetic field. And this field will tend to oppose induced field will oppose the externally applied magnetic field.

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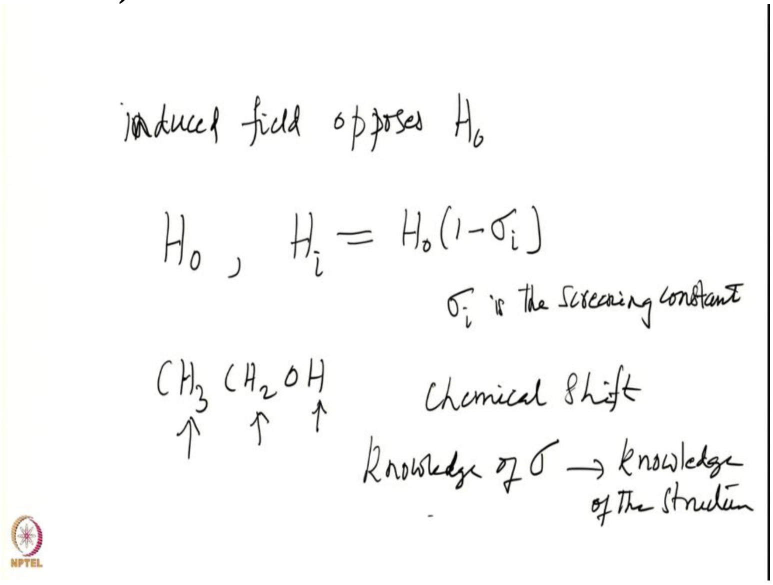

induced field opposes H_0

H_0 , $H_i = H_0(1-\sigma_i)$

σ_i is the screening constant

CH_3CH_2OH

Chemical Shift

Knowledge of σ → knowledge of the structure

The induced field proposes H_0 by and large there may be special situations where it may go in the same direction but those are very special cases we will not go into that. For most of the cases in chemistry and biology which we will be dealing with we have a field which opposes H naught. So, if it opposes H_0. So, therefore the field is seen by a particular nucleus the field seen if the field is H_0 then the field seen by a particular nucleus is not the same as H_0.

This is equal to $H_i = H_0\,(1-\sigma_i)$ it is reduced by a certain amount and σ_i is called as the screening constant. So, therefore there is a shift. So, if there is an electron density variation electron environment variation around the nucleus which can happen depending upon the structure of the molecule. Then you will have different fields for different nuclei although it is all proton the different protons will experience a different field and therefore they will absorb energy at different fields.

And this is why you get three different lines you got three different lines in alcohol CH_3CH_2OH the electron cloud around this proton this proton and this proton they are all different and that is why you see they all have different absorption of energy and that is called as the chemical shift. Now you will see that this actually depends upon not only the screening constant but also the H_0 itself.

So, however what we want to do the chemist or the biologist what he is interested is not in the H_0 dependence but on the chemicals on the environment dependence. You want to characterize the σ's. The sigma's are very characteristics the electronic environment therefore the knowledge about the sigma will give you the knowledge about the structure. So, knowledge about σ of σ gives you knowledge about the structure.

So, this is crucial. So, in order to achieve this; what we do is we eliminate this H_0 dependence and this we can do it by a kind of eliminating this frequency.

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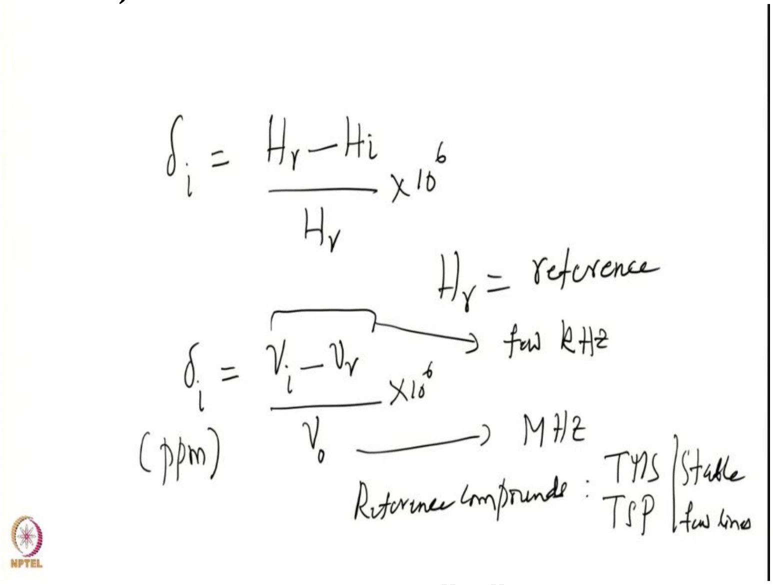

$$\delta_i = \frac{H_r - H_i}{H_r} \times 10^6$$

H_r = reference

$$\delta_i = \frac{\overbrace{\nu_i - \nu_r}^{\text{few kHz}}}{\underbrace{\nu_0}_{\text{MHz}}} \times 10^6$$

(ppm)

Reference Compounds : TMS | Stable
TSP | few lines

Now I can write this as I can write this as $\delta_i = \frac{H_r - H_i}{H_r} * 10^6$. Now what is H_r? H_r is for a reference you choose a particular reference. Some reference viewport and H_i is your nuclei of interest with respect to this reference how much is the deviation between these two and divided by this by H_r which is the reference field and this ∂_i is called as the chemical shift.

Now you see the field dependence is gone in this when we do this the field dependency is gone and it will depend upon what is the reference chemical shift screening constant of the reference chemical shift that we can use it as a constant is a kind of a referencing system therefore it does not matter. Now also what is the magnitude of this you can see you can also write this in terms of frequencies.

I can also write this in terms of frequencies $\delta_i = \frac{\vartheta_i - \vartheta_r}{\vartheta_0} * 10^6$, ν is the frequency divided by now what I will do I will represent replace with this H_r by ϑ_0 why because ϑ_r and ϑ_0 are very, very close see this difference is of the order of few kilohertz and this is megahertz. So, individually in ϑ_i and ϑ_r are in the range of megahertz. Therefore I can easily replace this H_r by I could have a ϑ_r here but I replace this when ϑ_0 simply to make sure that is a spectrometer frequency.

I calibrate with respect to the spectrometer frequency itself but to measure the chemical shift I take a reference compound with respect to the reference I take the difference and then I quantitative everything with respect to a reference. Then of course I will also multiply here by 10^6 because this is a very small number ϑ_0 is in megahertz and this is in kilohertz you can imagine what is you know suppose this is 100 Hertz this is 100 Hertz and this is 100 megahertz and this is extremely small number.

Therefore we must do a reasonable representation we multiply this by 10^6 and therefore we will say this is in what is called as parts per million δ_i is represented as parts per million because how many parts per million of this is the deviation is the screening that is the chemical shift. So, therefore now the field dependence is gone. Now whatever we therefore get in this chemical shift is purely dependent on the screening constant that is structurally very, very relevant.

So, what are the kind of reference compounds we use various kinds of reference compounds reference compounds are reference compounds are used this is the like one often write one as TMS is tetramethysilane for solvents which like CDCl3 and DMSO and things like that I use the TMS tetramethysilane or you can also use other things like TSP which is Trimethylsilylpropanate. So, so various kinds of reference compounds are there.

So, this will not list all of those. So, depending upon whatever is required for the considering the solubility you use an appropriate reference compound. What are the other requirements of the reference compound it has to be stable preferably it has only one line or it has the fewer lines and in such situations you will have a good reference compound and that is what is used in all of these cases. Now we will see a briefly about what all parameters determine the chemical shift.

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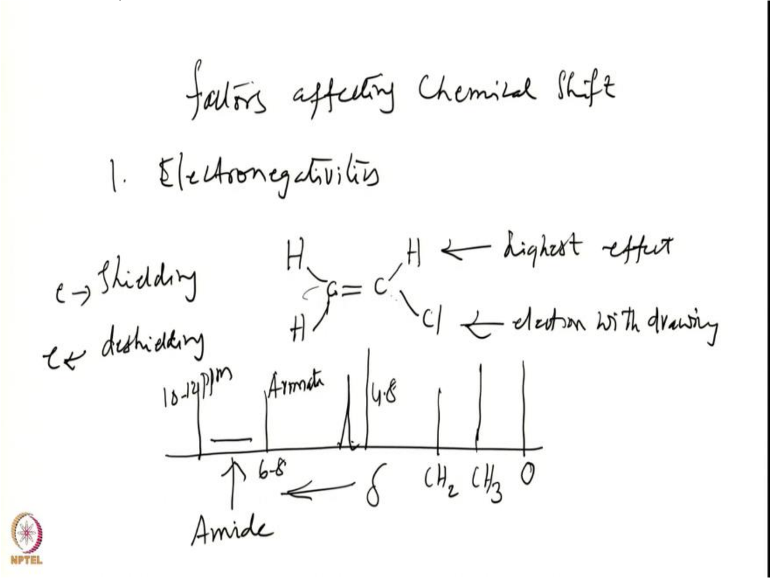

Factors affecting chemical shift, first electronegative groups now suppose I take a molecule which is like this. So, we have here chloride here the chloride is an electron withdrawing group. So, this is electron withdrawing therefore it will tend to withdraw the electrons from this group from this cloud. Therefore the electron density in all of these proton positions is going to be different.

The one which is maximally affected is this. So, this is this has the highest effect that means electron density will be lowest here and for the other two of course there will also be differences depending upon how the electron withdrawing effect goes through the double bond and things like that. So, therefore we write this chemical shift as in the kind of a plot here. So, this is the frequency if a particular place the electron density is low then it will be called as a de-shielding.

So, electron density is low means shielding. So, you have the electron cloud it is shielding effect the shielding if it is withdrawn then is de-shielding. So, this is electrons are withdrawn here and electrons are present here holding. So, the electron density is higher then it contributes to the shielding effect electron density is lower then it is because the shield de-shielding effect.

Typically for protons with respect to a particular reference let us say this is the TMS or TSP or whatever you have the range of about 10 to 12 ppm up to this you have the spread of the chemical shift. The various functional groups appear in this area. So, if you have an oxygen then it will have a highest electron withdrawing effect. So, then you will see Peaks appearing somewhere here.

So, the water appears here water peaks will appear at 4.8 ppm this is 4.8 ppm alcohol CH_3CH_2OH the OH proton appears somewhere here. Then you have the methyl will appear here. So, these are the methyl groups then you will have the methylene groups here CH_2 and you have the aromatics it will appear here. So, these are around 6-8 ppm then you will have here the amide groups things which are attached to nitrogen amides these will appear in this area up to 10 ppm.

And various kinds of other protons will appear in this area. So, therefore depending upon the structure of your molecule you will have different electronegativities effects coming and this is it will get relayed into the chemical shift into different position the de-shielding or the shielding effects. I am only listing here some of the very important parameters. I will now also show this next one is the hybridization.
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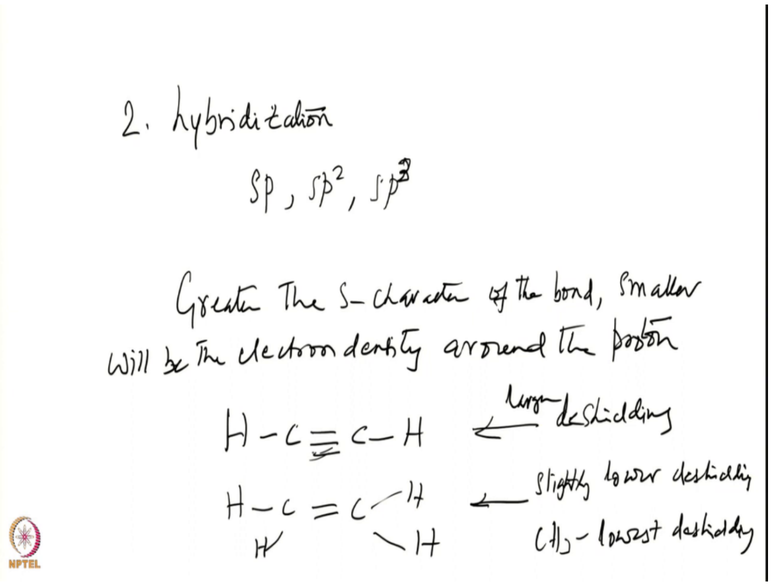

2. hybridization

sp, sp^2, sp^3

Greater the s-character of the bond, smaller will be the electron density around the proton

Hybridization means what kind of hybridizations do we have in your molecules? You have three kinds of hybridizations sp, sp^2, sp^3 of course in higher element you will may also have the dsp^2 dsp^3 and so on so forth but we are not concerned about those ones those ones happen in transition metal elements and so on so forth. But most organic chemistry we will have only these three sp, sp^2, sp^3.

Now if I have a proton which has this sp kind of hybridization the electron density will be highest around the proton it will pull out more electrons into the this one. So, therefore there is a rule which says here depending upon the hybridization of the greater the s character greater the s character of the bond smaller will be the electron density around the proton say for example if I take this acetylene molecule.

So, most of the electron density is concentrated here near the carbons. So, the carbon pulls out the electron density. Therefore the amount of electron density at the proton will be is smaller and similarly if there is a double bond here withdrawing effect by the carbon is relatively less and therefore there will be more electron density and the so the other thing will be like this is a double bond situation.

So, therefore this has the highest sp character in this in the in the bonds here therefore lowest electron density at the proton relatively higher here and relatively higher for the CH_3. So, if I take just the CH_3 in the alcohol that is what was CH_3 therefore if the electron density is lower. So, that means more de-shielding here larger de-shielding and here slightly lower de- shielding and if I have CH_3 lowest de-shielding. So, therefore what is the consequence let us look at that in the case of CH_3CH_2OH.

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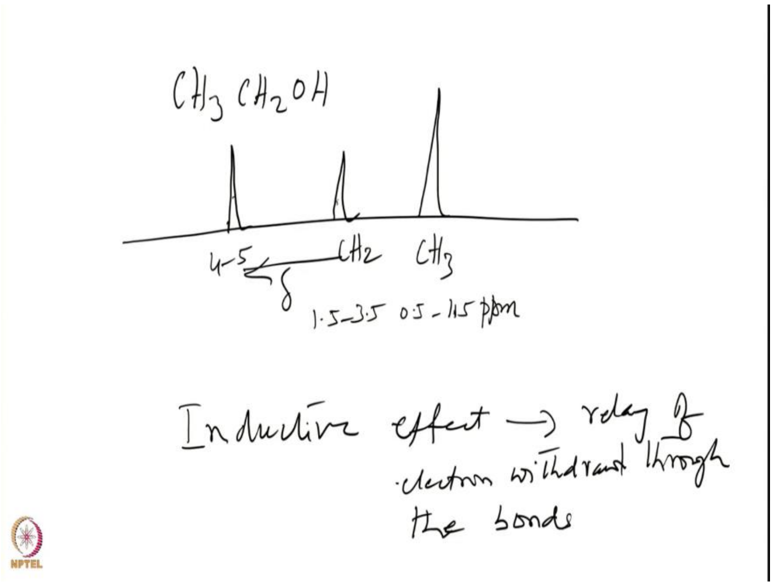

So, if I put CH_3CH_2OH if I wish to draw this spectrum which is the one which has the lowest electron density the OH proton. So, that will appear here at the lowest field this is the δ highest frequency therefore the lowest field and if this will be followed by CH_2 and followed by CH_3. Highest shielding effect here less shielding effect here even less shielding in other words more de-shielding here less de-shielding compared to this and even less de-shielding compared to this. So, it is the same language.

So, therefore typically you will find that the CH_2 protons appear between 1.5 to 3.5 ppm CH_3 protons appear around 0.5 to 1.5 ppm and these ones will appear at 4 to 5 ppm this is about 1.5 to 3.5 and this is around 0.5 to 1.5 ppm this is what will happen. Similarly if we have the LDI's or oxygen I mean containing groups the amide containing groups you will have different kinds of chemical shifts.

So, these are the two most important parameters which will influence your a chemical shifts and this electronegativity or the so called inductive effect. The inductive effect is basically is the relay. Relay through the; is basically relay of electron withdrawal through the bonds. So, therefore as you go further and further the inductive effect will die down and then the effect will not be as large.

So, this will be in the immediate vicinity you will see large amount of effects. And then of course we will also have what is called as ring effects. This is typically in aromatic rings and things like that you will have the ring effects.

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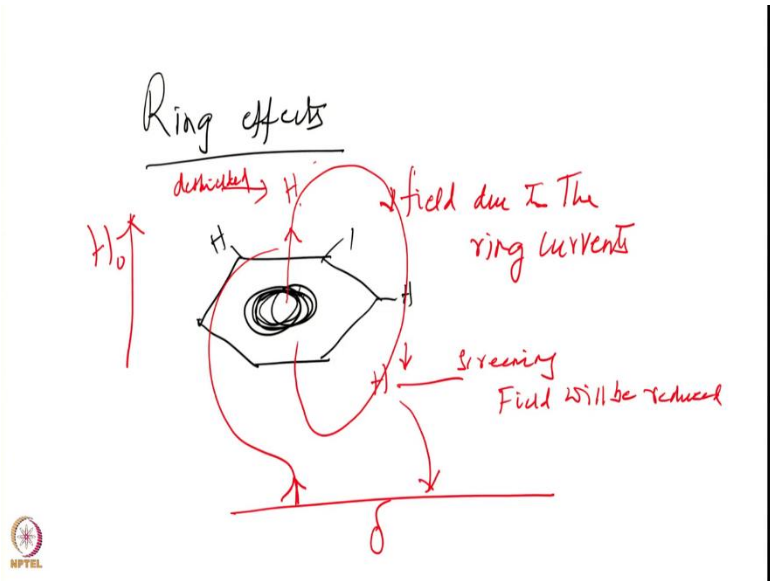

What are the ring effects here. So, I will let us say an aromatic ring like this and is an electron density down here electron cloud in the center. So, I will have protons here protons here and so on so forth but there is electron cloud here there is an electron cloud above and below the ring. So, then when the currents are induced in this electron cloud this will produce a magnetic field which will go like this.

This is the induced field this is the field due to the ring currents. So, now and your main field is here main field is let us say is H_0 here and this field will tend to either go with this or oppose this. Suppose you have a proton which is coming here. So, therefore this fellow will see a field which is in line with the H_0. So, this it will see a higher field therefore that is equivalent to de-shielding.

If I have a proton so this will be de-shielded. If I have a proton here which is in this direction. So, the field is going in this direction induced field S field is going like this. So, this will be screening goes in the opposite direction. So, the field will be reduced this is shielding. So, this proton will appear this proton may appear here and this one may appear here. So, the ring currents can cause this kind of shifts in your magnetic field in your absorption frequency this is your δ.

So, depending upon the screening you will have ring current effects on your molecule. So, this is a proton. Suppose there is effects I mean in a molecular structure you will have various kinds of protons coming in there and around the aromatic rings. So, what is the effect of the aromatic ring on the neighbouring protons? How does it change the spectrum.

So, that is the effect which is reflected in this parameter. So, there is various places one can see these various ranges of chemical shifts they are their tables I will possibly show these tables of the chemical shifts for the different molecules in the next class. So, I will have a slide to show you where these; what are the ranges of the chemical sheets for different kinds of protons.

So, that is the one of the most important parameter in the chemical of the NMR Spectra of molecules and this is what made NMR a very important technique for structural chemistry. Molecular structure you know organic chemistry or structural biology or whatever the chemical suit becomes an extremely important parameter. Because looking at that chemical shift itself you can figure out what sort of a functional groups are present what sort of a structure the molecule might have.

So, this provides an initial input with regard to the structure of the molecule. So, the actual of course this is I talked to you about the proton chemical shifts the same thing will apply to the other nuclei as well ^{13}C shifts or nitrogen 15 (^{15}N) shifts it will appear the same the same principles hold good whether it is a proton shift or carbon shift or nitrogen shifts or phosphorus whatever nucleus you are doing the general principles are the same.

And we will take up the next parameter in the next class. So, we will deal with the spin-spin coupling in the next class. So, we will stop here.