Principles and Applications of NMR spectroscopy
Professor Hanudatta S. Atreya
NMR Research Centre
Indian Institute of Science Bangalore
Module 1
Lecture No 19

In the last class, we saw uhh how uhh the proton carbon spectrum is decoupled by applying weak RF radiation on the hydrogens, and that helps in simplifying the spectrum and because now instead of seeing many multiplexed structures, many peaks they collapse into a single peak. But the cost of this, you lose the information of the multiplicity. So let me show you an example of how the uhh the carbon spectrum looks cleaner or simpler when we do decoupling, so this is shown in this picture.

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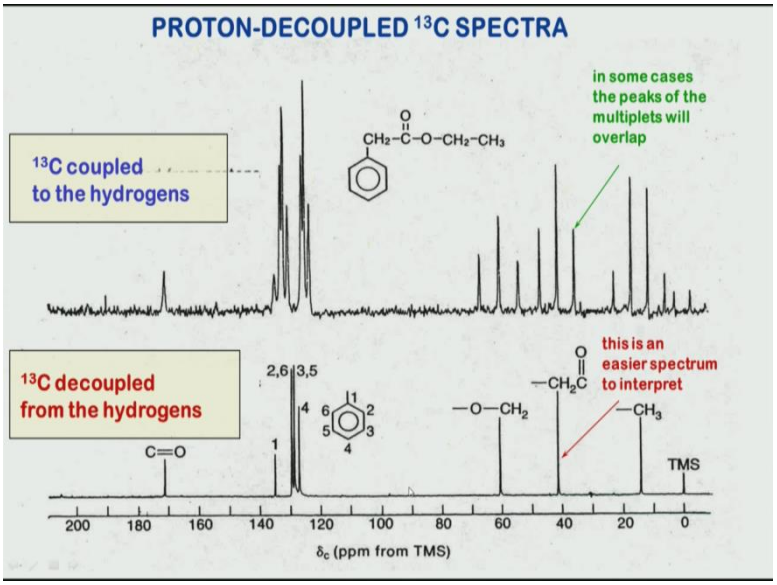

This is taken from the book Pavia where this example is shown. So you can see here that if I do a decoupling uhh so if I do not do a decoupling when I record a normal spectrum of carbon, uhh this is what the spectrum you will get for this molecule, which is shown here.

So you see different carbons are there, this is aromatic region which is coming from this and this is a carbonyl. Uhh so but you see there are many peaks now because of this J-coupling. In some cases the peaks are overlapping, so you know this triplet can start overlapping, but this triplet and so on. So that looks very, uhh little complicated but now if I do a decoupling from hydrogen that means I remove all the J-interaction, J-coupling between carbon and hydrogen everywhere, then you will get something like this, which looks much cleaner. You can see here, I am getting a single peak for every carbon.

So by simply counting the number of peaks, I can figure out how many carbons are there in the molecule. So as far as interpretation is concerned, it is easier to interpret and and I do not have to bother about uhh the overlapping peak structure. So you can see how the cleaner spectrum comes. So most of the time, when we look at analysing the molecules we will be looking at this type of a spectrum which is decoupled spectrum. Ok? Now looking at the coupled.

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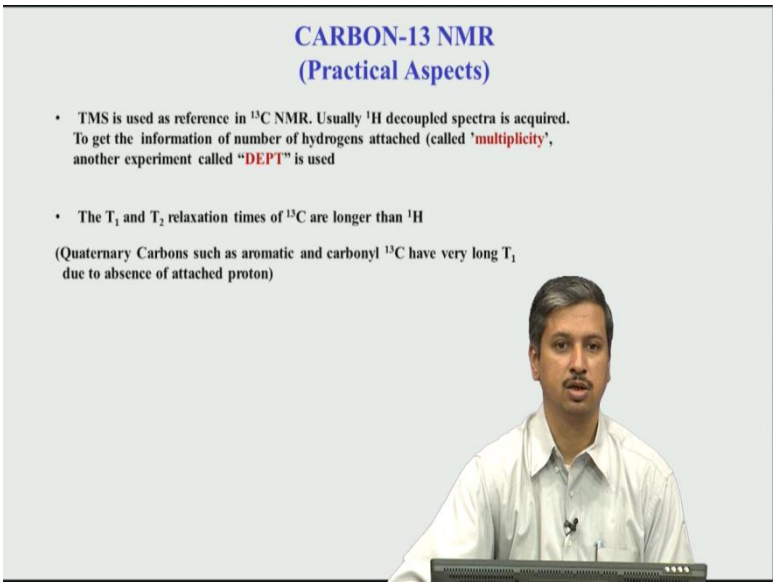

So let us see what are the practical aspect what are the practical aspects when you uhh record a carbon 13 NMR spectrum. So similar to what we saw for the proton, uhh it is important to understand what parameters are uhh will affect the carbon spectrum. So first thing is as we do in proton, uhh we use TMS also in carbon as a reference. We saw that in the last slide also; and if you have a peak corresponding TMS carbon, you set it to 0 and with respect to that, all other carbon peaks are monitored or calibrated. So that what is called calibration.

And then usually, as I said one proton decoupled spectra is acquired. Now the one thing we we saw that, we lost the information of how many Hydrogens are attached to the carbon that is useful because remember looking at a peak, I can directly say whether it is uhh uhh is a methyl peak or is coming from methylene or it is from methine and so on. That information is lost if I do decoupling but there is a technique uhh which called DEPT, D-E-P-T. It is an acronym for a bigger name which we will see later and that experiment helps you to get this information.

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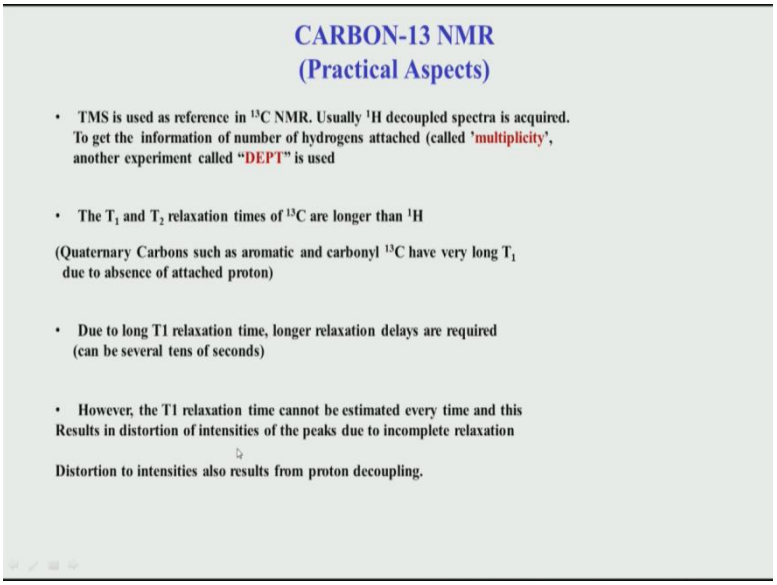

So this is a separate experiment. 1D experiment which can be recorded to get the information for every carbon, that how many Hydrogens are attached to that carbon uhh but a normally if you directly if you are not interested in multiplicity and only in the chemical shift and the number of carbons you can do decoupling and get a cleaner spectrum. So one thing one has to know uhh practically is that the T1 and T2 relaxation of Carbon is much longer than Hydrogen. This is theoretically because of the low gamma of the carbon.

So whenever the gamma is low and there is what is called dipole (interact), dipolar interactions are weaker and therefore whenever dipolar interactions are weaker the T1 and T2 are longer. So T1 T2 relaxation both of uhh for both the carbons it is longer than hydrogens. In fact if you go to carbon such as where there is no hydrogens attached such as quaternary carbons and aromatics or if you look at carbonyl they have pretty-pretty long , very long T1 relaxation uhh for this in absence of proton. So basically what is the consequence of this is that in the same molecule, I am going to have a range of T1 and T2 values.

So that is a big problem in a molecule because typically we expect that if a molecule is uhh for a given molecule expect all the atoms to have a similar relaxation times, but it turns out, for carbon it is not so and what is the effect of this. The effect of this is that because of the long relaxation time, you have to use what is called a longer relaxation delay. Now uhh if you remember, what is relaxation delay? A relaxation delay is the delay that we use between two consecutive scans.

So if you recorded 1D NMR spectrum, and remember we apply a pulse, then we record a uhh FID, then we have to wait for some time, before the molecule is relaxes back to full equilibrium situation, then you apply the next pulse. So this cycle of applying the pulse, recording the data and the delay to when it comes back to equilibrium is repeated N number of times. That is called scans.

So in that picture, the time delay the between FID is collected and before the next pulse, we use what relaxation delay and that relaxation delay, if you remember, we said, we told, that it depends on the T1 value. So typically the relaxation delay is about 4 to 5 times the T1 value of the sample. Uhh so protons is typically uhh second, that means if I want to have a long relaxation delay, 1 to 2 seconds is a typical T1 of protons for small molecules. So for hydrogen proton NMR we typically use a relaxation delay of 5 to 10 seconds. Typically it is not more than 5 practically.

But in the case of carbon, that is not that is cannot be done; because in a carbon you have a much longer time relaxation time as written here, it runs into several tens of seconds. It could be 10 seconds, 20 seconds, so in that case if you want to use a relaxation delay, you have to use about 100 seconds. Let us say minute of relaxation delay, 60 seconds. So that increases a time, a lot because if your one scan is going to take a minute. So if you want to do a 100 scan experiment, it will be 100 minutes. So you see the problem is because of a longer delay, our time is also increasing a lot.

So practically what happens is we do not worry about this five time relaxation delay because this long relaxation is only for the case of those carbons which do not have any attached proton that is in absence of attached proton. For those protons, we have a very long relaxation delay but those high carbons which have sorry those carbons, so those carbons which have protons attached , they will have a short relaxation. So uhh thus the whole problem , the problem is there is a range of uhh T1 T2 values but for those carbons with are which are attached to a hydrogen, it will have a short relaxation (shor) relatively shorter and therefore all the NMR experiments typically when we do for carbon, we worry about only those which are attached to hydrogens.

So that will be of the order of let us say a few seconds, T1 relaxation. So your relaxation delay can be about 10 seconds so instead of using minutes. Now what is the consequence of that? The consequence of that is that for those uhh carbons, for those carbons where the T1 relaxation is very long, that time for those carbons we are not using the correct relaxation

delay and because of that the intensity of those peaks will now get distorted. Distorted means they will no longer be correct intensity.

And why is it so? Remember, it depends on the relaxation delay and the recovery of the magnetisation. If I do not recover the magnetisation completely, my signal intensity is lost. So let me uhh again uhh show you this with a with a (pict-) which we workout this.

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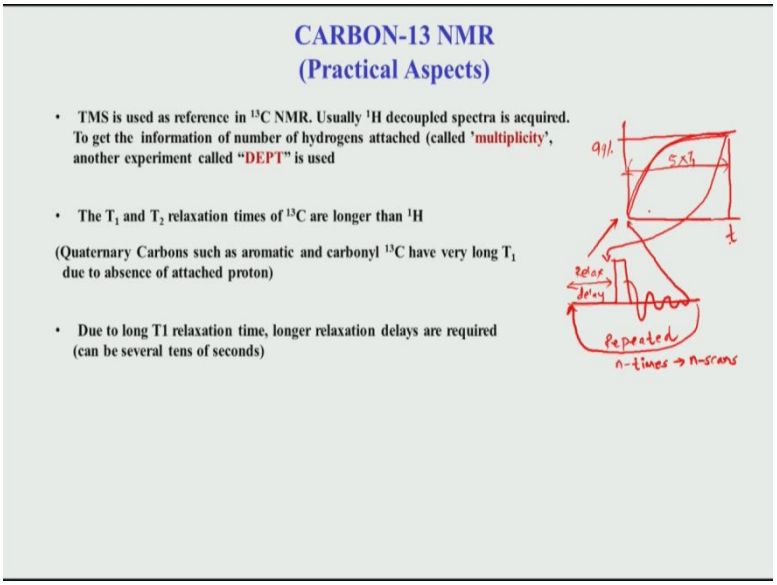

So, if you look at this relaxation, so we are looking basically what we are talking about is a pulse followed by an FID. Ok? And this portion is called relaxation delay and this is relaxation delay and what happens is after the end of this cycle you go back to this position and this is repeated. N times; And that is called N scans.

So, now if this relaxation delay, if I, this is basically a recovery of the signal which is, signal starts when it is here. The signal is basically 0 and then it slowly recovers and goes to full at this position, this is almost 99 percent, when it comes to this time, after a long time. And this is the situation at this position here. So (befo-) that means after the FID is over, it is 0 then when it comes back to this position and then when it uhh this portion, this portion it relaxes like this. So therefore this time, this time is typically is five times T1. Ok?

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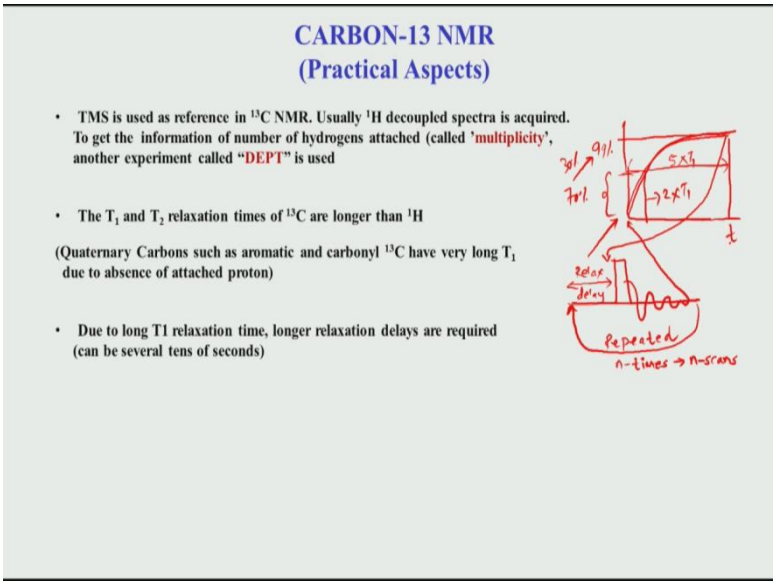

But suppose I do not give five times T1, I give only three times T1 or two times T1 so this will be probably around two times T1. So when I do this two times T1, my signal is only this much. This could be probably around 70 percent. So you see from here to here I have lost around thirty percent of the signal. So therefore if I do not use a correct relaxation delay, my intensity is no longer now correct value because intensity has decreased by some thirty percent or depending upon how much is T1 and that is a big problem and therefore the intensity is not fully come back to the equilibrium and when the intensities are not the correct value, you cannot use it for quantifying.

What does it mean? That means use, they are no longer proportional to the number of carbon. So remember this is a big difference from proton NMR. In proton NMR, we saw that the number of protons is related to the area of the peak, so from the area of the peak you are able to recover the the number of protons but you can not do that in the case of carbon NMR. In the case of carbon 13 NMR, you will have to now depend uhh you can not use the intensities any more because of this incomplete relaxation. The (proto) carbons are no longer the area of the carbon peak is no longer proportional to the number of hydrogen because we have not correctly captured the intensity. The intensity has got somewhere abruptly cut because of this incomplete relaxation.

So this is a practical problem and this one other reason why intensity goes down is because of an effect called Nuclear Overhauser Effect. We will see that later, in 2D NMR. So there because of this Nuclear Overhauser effect, which comes because of the decoupling of

protons. So when you do a proton decoupling that means when you apply a weak RF radiation on protons, it affects the Carbon intensities. So these factors of decoupling, incomplete relaxation, etcetera. results in the problem that the carbon intensity you can never be it never be considered as a quantitative and normally we do not use carbon for NMR, for quantifying a number of protons.

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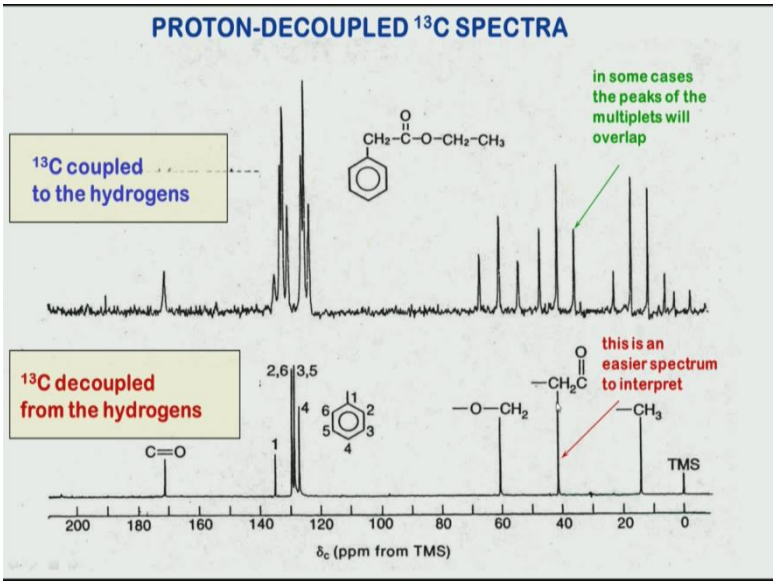

We use always hydrogen proton 1D NMR for quantification. So this is what you can see in the previous slide, you see here uhh here you saw that here also you can see that this is a CH 2, this is Carbon, this is also 1 Carbon but look at this intensity, they are no longer same. If they both are single carbon you would have expected both of them to have the same intensity. Similarly look at methyl, it is having a smaller. So the intensities are no longer related to the number of carbons and all this is coming because of those two factors, this is incomplete relaxation delay and NOE effect; and therefore, Carbon 13 NMR cannot be quantitatively analysed like a Proton spectrum.

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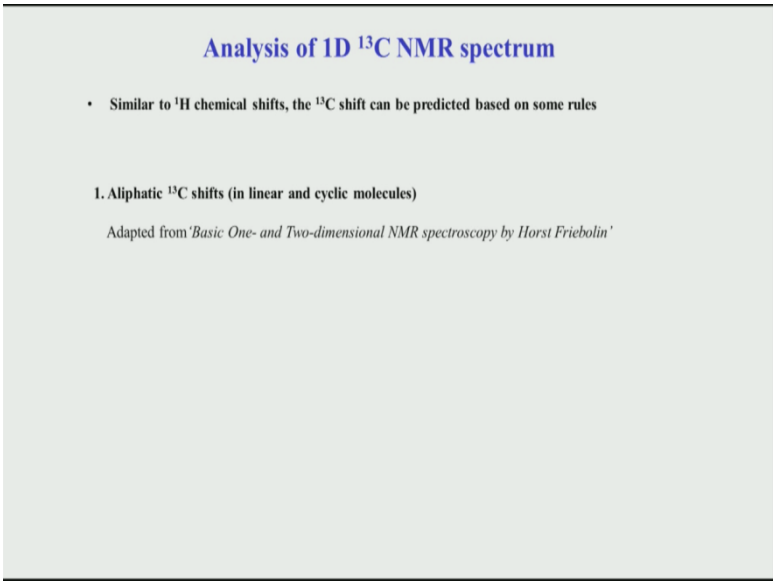

So these are the practical aspects of carbon NMR so now let us move to how do we interpret for analyse carbon 13 1D NMR spectrum. Uhh So the next Few slides what I am going to show is basically a set of (val) Table taken adapted from this book and this has much more extensive tables which you can refer to what we are trying to do here is that given a structure of a molecule we are going to see how we can predict the chemical shift value. So this is again similar to what we did for hydrogen proton NMR there also we were given the structure we had some rules, empirical rules, Shuleray rules, Pascal Seimen rules and so on. Based on that that given a structure we tried to predict the chemical shifts.

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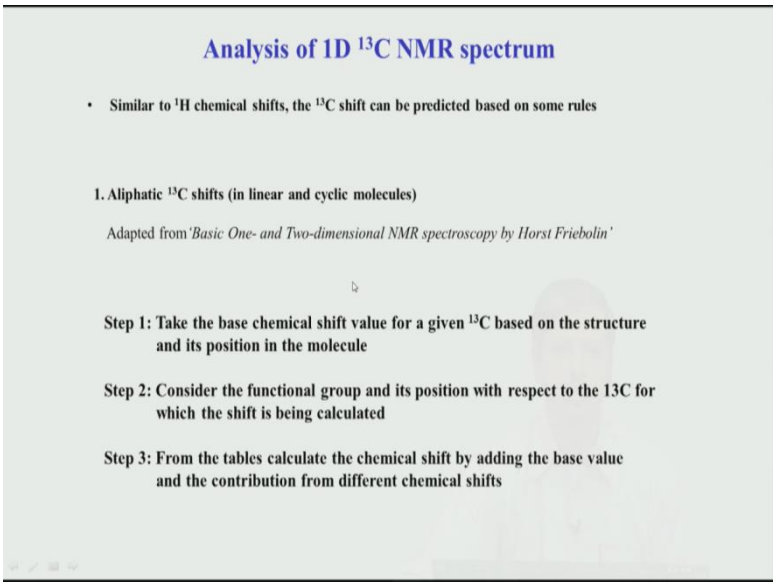

So let us look for carbon what uhh how do we do that? So this is what you have to do, these are the basic three steps. Uhh very similar to what you do for hydrogen proton so basically you start from a base chemical shift value that means for a given structure let us say it is aliphatic or it is ethylene, given functional group or aromatic. For a given functional group of molecule functional group in a molecule you start from a base value which will be given in a table then to that base value you start adding the values based on what are the different substituents present in that molecule.

So for example, if it is a halogen substituent or it is a nitro group or it is a amine group; based on the functional groups you start adding the the the chemical shift to that. So again this is taken from a table and then finally you get the total value of the chemical shift which is based on the base value plus a contribution from different functional groups.

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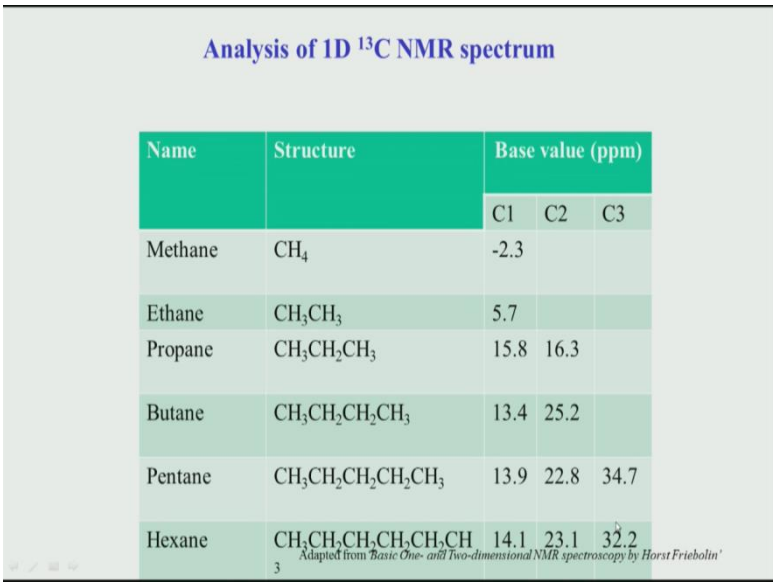

So let us look at a few examples, we are not going to exhaustively look at all the examples as I said this is more exhaustive uhh tables are given in this book so we just an illustration of how this can be done. So this is a base value which we will consider for a aliphatic system cyclic and acyclic. So for a cyclic case you can see that if you have a methane, methane carbon, the carbon methane the base value will be assigned as -2.3 ppm.

So suppose you have an ethane molecule then what you do is for ethane that for the C1 this carbon or this carbon, the base value is considered as 5.7. Now look at propane in this case of propane this and this are one and the middle guy, middle carbon is C2 so if you look at C1 carbon if you are analysing the chemical shift of C1 carbon then you consider a value of 15.8

as base value. But if you're considering the middle carbon which is C2 then for that carbon you should start from a base value of 16.3 and then you keep adding based on the substitution wherever is present that we can that we will see.

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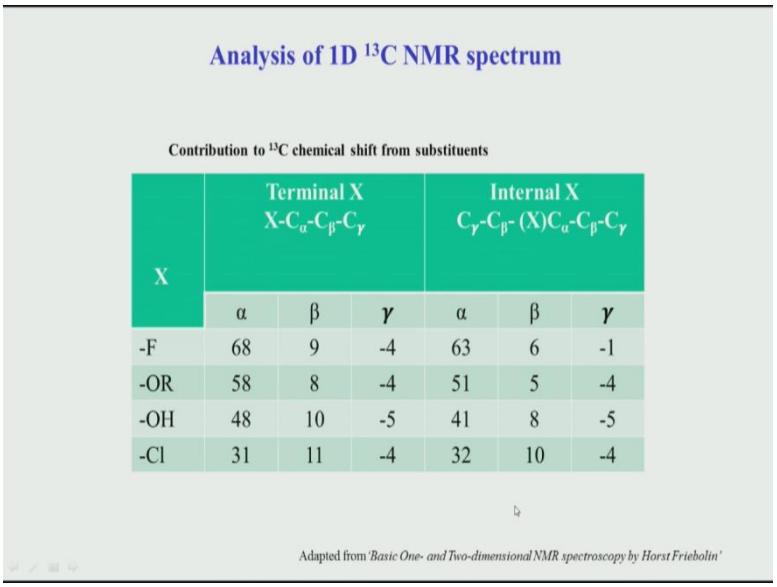

So like this you can do it for all the acyclic molecules and you can see that if there are based on different acyclic group. You will have different positions and different base values. okay? So let us see what happens. Now look at the substituent, so in the previous this one only the carbon and the the acyclic system. But now I should start adding the substituent let us say I add a substituent at this position, X. So that X position, what is the influence of this on this this and this carbon? For that you have look at this table that uhh if you want to look at the effect of X, contribution of this X on this C alpha and then you have to look at this column here. So if you have a substitution is a Fluorine then you have to take 68 as a contribution. This you have to add to the base value. So base value shown in the previous slide and this is now the slide containing the contribution values which both of them now have to combine.

Similarly if you have an (alcohol) OH group substituent at this position, you have to add 48 ppm to the base value. If it, for C beta, for a OH present here, you to add 10 ppm to the base value. So like this you can do it. Similarly you may have a functional group in the middle of the molecule not in the terminal terminus. So in case it is in the centre somewhere in the middle now based on the position, you have to then add these values.

So example, let us say you have uhh H, you have a fluorine present in this. Here is a branched here there is a fluorine here so what is the effect of the chlorine on C beta? So for that you

have to look at this side, the effect of fluorine is six ppm whereas effect of fluorine on directly attached carbon is very high, 63 ppm. You see this is the inductive effect, the moment the fluorine, next carbon in (do) dies down rapidly and if you go to further away, fluorine has even lesser effect.

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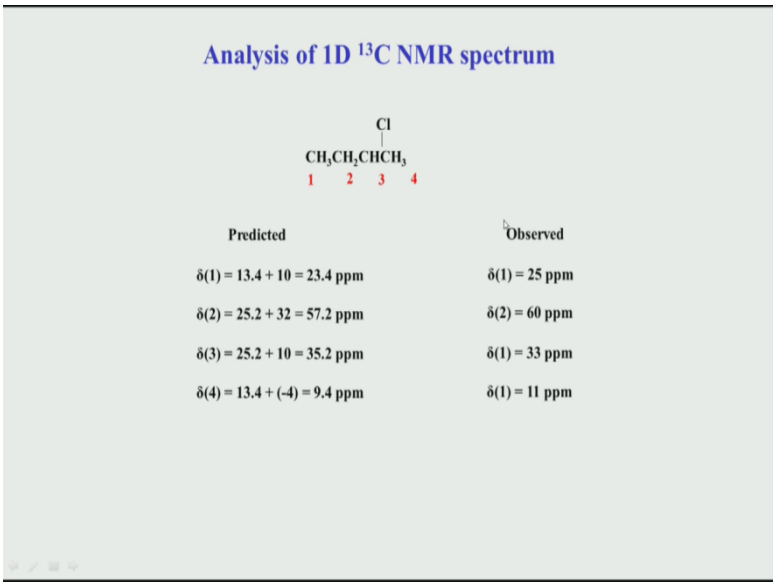

So these are the values which values which we have to add to the base value. So we will look at a example, let us say we have this a molecule where this chlorine is attached to this second here in the CH position. So now this we have to use previous table, so this is a branch kind of a system because it is in the middle, not in the terminus. So we have to basically look at this table here, these values.

So if you do that, let us predict the values. So this all taken from the previous table, so I will take base value as 13.4 and look on calculating for this carbon. For this carbon I am looking at the base value 13.4 because remember in a butane, in the butane system, we saw in the first slide, that the base value should be taken as 13.4. Now you add the contribution of chlorine, if it is at this position, so this is now with respect to chlorine. This is alpha, this is beta, this is gamma. So if you look at the previous table, for a chlorine it is at a alpha-beta position, you have to look at this value of 10 ppm. Okay?

So if you look at that, then you have to add the 10 ppm, you will get 23.4. Similarly if you look at delta 2, which is this carbon, you will have to add 25 that is the base value for this carbon because again coming from the butane and then do you add this contribution, you get

32. And for delta 3, it is directly attached to this chlorine and if you take this value, you get 35.2.

And similarly for delta 4, it is -4. So you have to basically look at that uhh actually uhh there is a slight error here so what we are looking at is this here. So here the numbers are reversed, so it should be, one this is for one, this is for two, this is for three and this is for four. Yeah!

So uhh there is a typo here, so you have to just reverse this order. Now this is, if you look at the absorbed value for those same carbons, they match very well. You can see that they match very well with absorbed values again, remember, they are not exact, this is all empirical rules. When we talk about empirical rules, it means it can be approximately correct, so this basically helps you to predict this. So given a structure of a molecule, the idea here is that you should be able to predict this chemical shift and you will be having the experimental value so it helps you to assign, which peak will correspond to which carbon.

So this is basically the gold behind this exercise, is that given a spectrum and given the structure, suppose I want to do a mapping or a matching of which peak in my carbon spectrum corresponds to which peak in my molecule in the sorry, in which peak in my carbon spectrum correspond to which atom in the molecule. For that I need to, I can use this empirical rules and try to predict the chemical shift values of different carbons because I know the structure and I know the rules and I will be seeing some peaks in my spectrum.

So I can do a matching and then figure out which peak is corresponding to which carbon and that uhh sorry there is a typo here. So which basically means, this is called assignment. This whole process of trying to match a spectrum uhh peaks in the spectrum with uhh absorbed (sa) with a predicted chemical shift, we use the word assignment.

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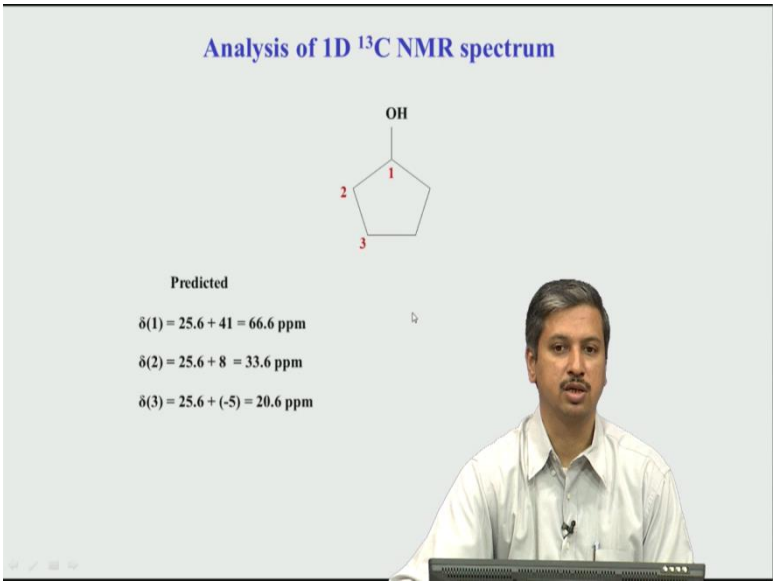

So let us look at some another cyclic system. So for cyclic system also there are set of rules uhh given in this uhh book Alfred Rowling and similarly the idea here is just a it is an illustration uhh so you can see suppose I have uhh system like this. In this case this one is directly attached to oxygen so therefore this is the base value uhh which is uhh (given) given in this in that book and based, based on this base value, I add the contribution because of this OH.`

So you see when the OH is directly attached to a carbon, that carbon of course, will expect expect to get the most deshielded because of the electronegative effect and therefore this contribution is very high. So the chemical shift of this carbon therefore is expected to come around this ppm. Then (again), if you recollect uhh in the last class, we saw the chemical shift ranges, there also we saw that if you have a C single bond O, the carbon of chemical shift comes somewhere, somewhere in this range 60 to 80 ppm.

Now if you look at the second carbon here, which is away from here so the inductive effect is reduced, I mean is is now less compared to influence here and again according to the table, it is 8 ppm. So if you add this you get this value. And now if you look at the third carbon which is even further away and that has a contribution of -5 as a base value. So if you add this you get this. So this is basically the three peaks which you expect.

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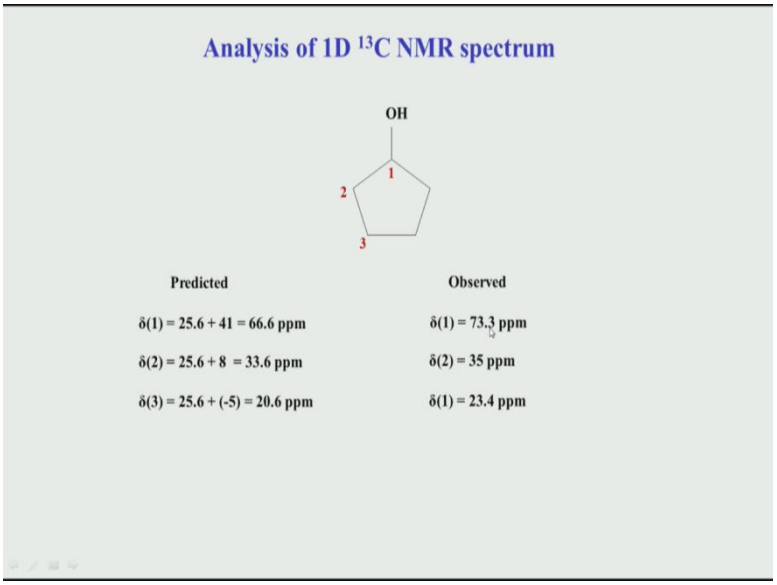

What about this peak? This is same as this, because there is a symmetry here. Similarly, what about this peak? This is same as this carbon. So we are only going to consider one set which is here is the same as this. So let us see how it compares with the absorbed values. It turns out the absorbed values is actually here shown on this side and you can see it matches very nicely with this carbon not very well with this and this but definitely let us say, in your molecule, in your spectrum, if you are seeing only three peaks, and you get this value and the predicted is what you expect and in the absorbed is you see three peaks only.

Obviously, then very easy to do the mapping. This is called mapping or assignment, very easy to assign because this 66.6 has to be corresponding to this which means that whatever peak you get at this ppm in the experiment, that corresponds to this carbon. Similarly, whatever you peak you are seeing in this ppm will be matching with this and that corresponds to this carbon and whatever spectrum you see in this, correspond to this.

So you see this is a very useful tool uhh for predicting chemical shifts based on the structure uhh and what you match it with your absorbed value so that you can assign uhh which peak corresponds to which carbon atom.

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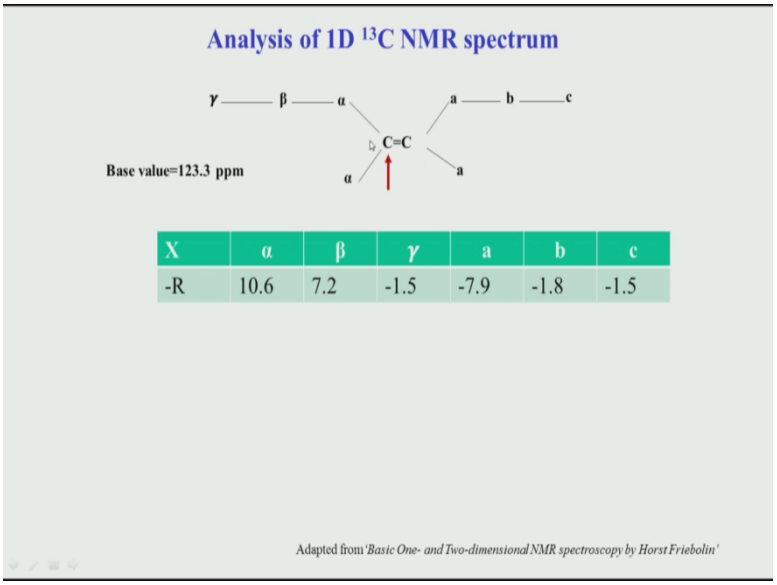

X	α	β	γ	a	b	c
-R	10.6	7.2	-1.5	-7.9	-1.8	-1.5

We can do this also for this kind of a system, again taken from this book. I get more exhaustive tables, I am only limiting it to a few functional groups because this is only for illustration and again it can see for this kind of a molecule, if you have this where there are substitutions and this is now a C double bond C. Again, if you (look) remember, in the last class, we saw that C double bond C comes somewhere between 100 to 150 ppm. So the base value is taken for this calculation as this value 123 and based on the substituents at different positions, you have to add the values.

For example, if you have an aliphatic substitution, aliphatic meaning only hydrogen uhh or CH two three or CH three. Based on that you have to basically add this value to wherever they are present to the base value. We are now trying to calculate the chemical shift of this carbon. Okay? So this (carb) where the arrow mark is shown, we are going to calculate for this carbon based on substituents and dispositions.

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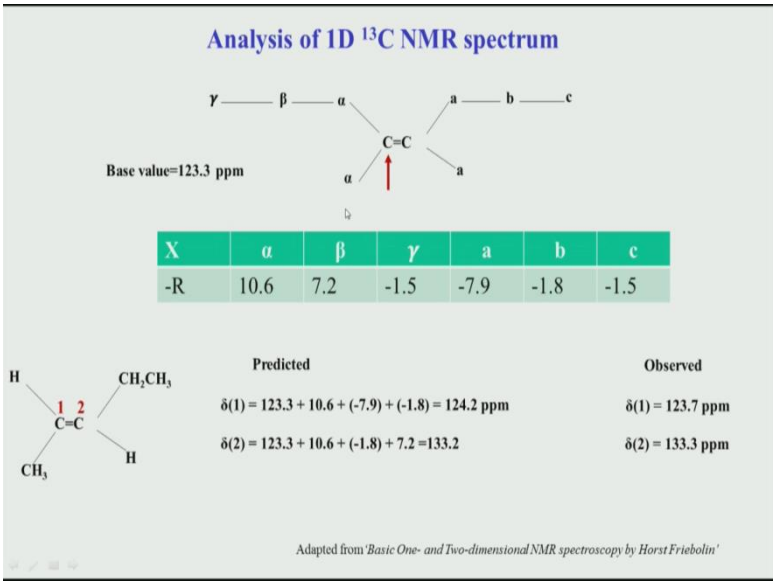

So let us look at this uhh molecule here, there is a hydrogen here, there is CH three here. There is CH2CH3H. So you can see that now, look at this corresponding picture here. So A is CH2; B is CH3 and this alpha is CH3. So if you use all these values here, you can do the prediction, so for this carbon, we are going to look at all the carbons. For this carbon it will be the base value, plus the different contributions coming from this table.

So if you do this math you will get, this value for this chemical shift which is 1. Similarly, if you look at Two again you have to look at this picture and with respect to two that this are different values. So again, you can use this table, with respect to two you will get 133.2 and you can see this again matches very nicely with the observed value. So that means in your spectrum, when you record, if you get a C double bond C in the C double bond C region comes somewhere around 100 to 150.

So in that region, if you see two peaks, corresponding to these two positions, this is observed value, then your want to assign it, which carbon is which, are very easily assigned at uhh this ppm peak at this ppm corresponds to C1 in this molecule and this peak which will be observed is C2 because it is based on this prediction. So this is all based on this table here and this a more exhaustive function group substituents are given in this book.

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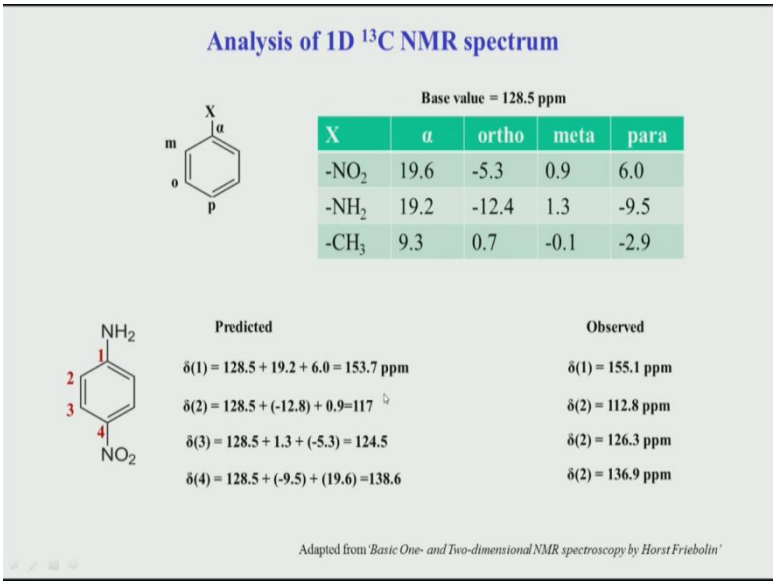

So let us look at some another example, in aromatic system. So in the case of an aromatic system, uhh the same uhh principle. You start from a base value which is one twenty eight, again remember, aromatic peaks come always between 100 to 150. So the base value is somewhere in that region and that one twenty eight point five. Now based on the substituents at ortho position or at this position or ortho, meta and para there is this uhh sorry there is a typo here. This is Meta and this is Ortho.

So this is ortho position, this is meta position. You can see that there are different values and then you can also calculate again based on these rules. So if you look at ala alanine sorry you amine group, then what is the value of one that is coming from here, that is nineteen point two that is added to this base value and you basically get this peak and there is at para position, there is a nitro group so that will also contribute to this carbon here and you will get this value which is what you get. Similarly if you look at this carbon, this carbon, same philosophy same idea. You can use this table and different substituent positions and you can calculate these values.

Now let us see how it matches with the absorbed values again look, very nicely it matches. Almost within 2 to 3 ppm range and it matching with the absorbed values. So you can see that if I get a spectrum with four peaks in this region and if I have to assign, which peak correspond to which. I can use simple rules of prediction and then match it with this molecule.

So we will look in the next class at how uhh there are different prediction softwares which are available in literature, basically the idea is, looking at these empirical rules, uhh we are going to predict which carbon has which chemical shifts and there are many softwares available. It will give you the websites uhh URLs of these softwares which you can go yourself and try it out and it is a very very useful learning experience to given, and you generate the structures as an exercise and will generate the chemical value and you can look in the literature for the published values and you can compare them and see how it nicely it works.

So we will see that in the next class.