Spectroscopic Techniques for Pharmaceutical & Biopharmaceutical Industries
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Lecture 33
Structure Calculation & 2D-NMR Spectroscopy

Hello students welcome back to the lecture of this course. The last 3 lectures I was discussing about the principles of NMR spectroscopy and I started discussing about how to get the structure of a molecule using NMR spectroscopy. I will continue with that and then I will again go to 2-D NMR spectroscopy and I will let you know what is the potential of 2-D NMR spectroscopy and how it can be used for elucidation of structure.

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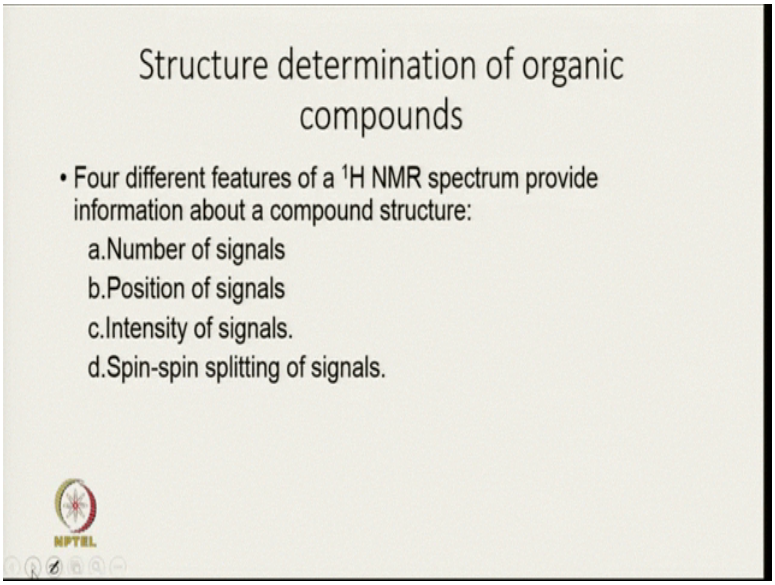

In the last lecture I discuss how 4 different features of NMR can be used to get information about a compound structure. Those 4 features are number of signals, position of signals, intensity of signals and spin-spin splitting of signals.

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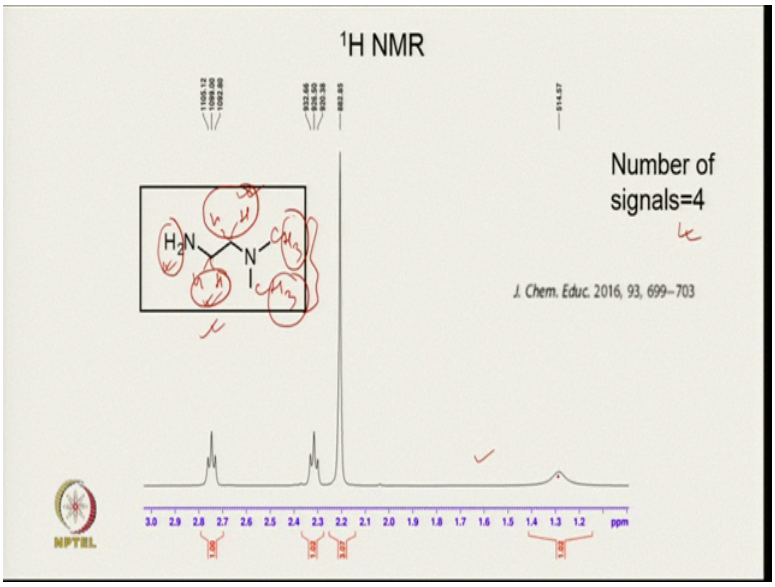

I will give you one example. So let us look at 1D NMR of this compound NH2, CH2, CH2 and CH3 twice. As you can see there are 2 proton attach to nitrogen, 2 proton attach to carbon, 2 proton is attach to this carbon and your 6 protons are attached to these 2 carbons. These 6 protons are equivalent because they have same environment. These 2 protons are equivalent, similarly these 2 protons are equivalent and these 2 protons are equivalent. And so there is 4 different groups, one consisting of these 6 proton, another consisting of these 2 protons the third one consist of these 2 protons and forth one consist of these 2 protons. So there are 4 different kind of protons and so number of signals is going to be 4. That is what we expect in 1D NMR and this is your 1D NMR and you can see there are 4 different peaks, 4 different peaks.

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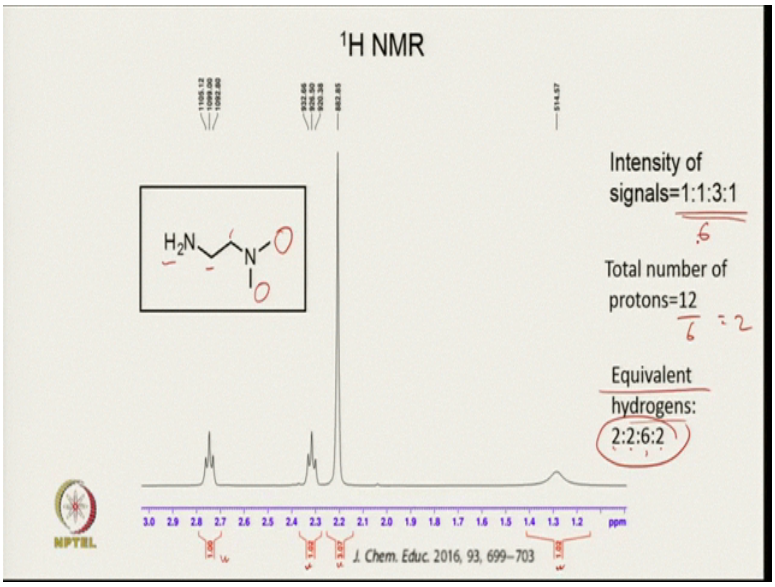

So let us go and understand how to assign this. The intensity of signals, if you look at this is the ratio of intensity of signals and you can see the ratio is 1 is to 1 is to 3, 3 is to 1, 1 is to 1 is to 3 is to 1 and if you remember here, there is 2 protons, 2 protons here. So total is 4, 2 protons here, 6 and then 3 here, 3 here. So 6 plus 6, 12 protons, 12 protons and intensity if you sum this, this is equal to 2 plus 3 plus 1 is 6. So let us divide this by 2. So 12 divided by 6 is equal to 2. So one basically few look at the equivalent hydrogens. So we need to multiply this by 2. If I multiply this by 2, then I get the first group consist of 2 protons. The second group consist of 2 protons. The third group consist of 6 protons and forth group consist of 2 protons. The way we got is simply we multiply this by 2.

So first thing is that when you get 1D NMR, you look at the intensity and then the look at your compound and see how many protons are there. Now you sum all the intensity ratio and if suppose this comes to be 6 and there are 12 protons, it means that, you know each the intensity ratio corresponds to 2 protons, corresponds to 2 protons. So if intensity ratio is 1 that corresponds to 2 proton. If intensity ratio is 3, then that corresponds to 6 protons. So that is how we can get information from intensity observe increase of proton NMR. And when you do that is quite easy to get the assignments. This has your intensity 3, means there should be 6 protons which are equivalent and if you look at the structure only these protons are, these protons are equivalent and their number are 6. So from the intensity itself, you can assign this peak.

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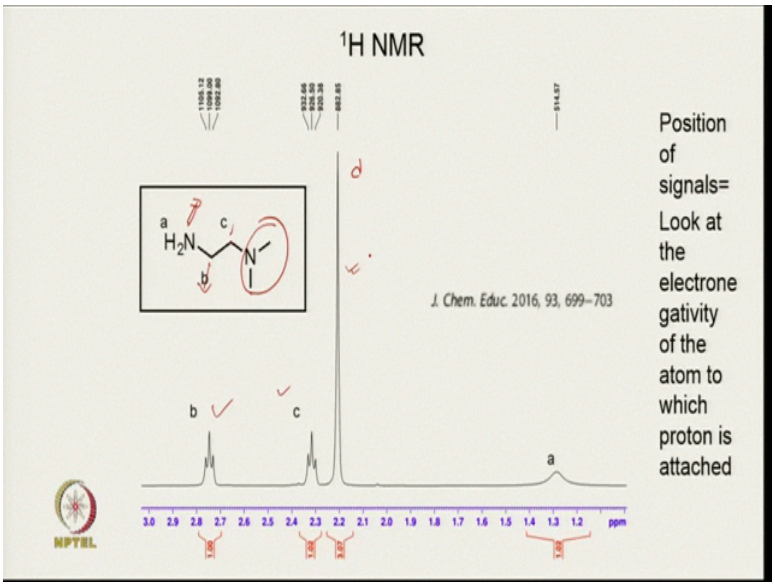

You can also look at the position of signal. So for that, you need to look at the electronegativity and if you look at the electronegativity. Then the, this should come at smaller chemical shift, this should come at your highest chemical shift because this is attached to electronegative atom. This protons are attached to NCH3 twice group, which is less electronegativity than NH2 group and so these protons will come at a higher position, higher chemical shift position. Whereas these protons will come at lower chemical shift position.

So now you can also assign bc, bc here and d we have already this and this will be a based on the, based on your position or based on the chemical shift value. There are another thing which you can look at that is splitting. In this b and c, you see these are 2 protons here, which will couple with the 2 protons here. So they will be split into 3, so both b and c protons are expected to split into 3. And here again, this is CH3, CH3 and they are not coupled to each other, they are not coupled to any nearby proton and so you expect a single peaks. So you expect a single peak. So this is the way you can assign the peaks of the 1D NMR, peaks of the 1D NMR.

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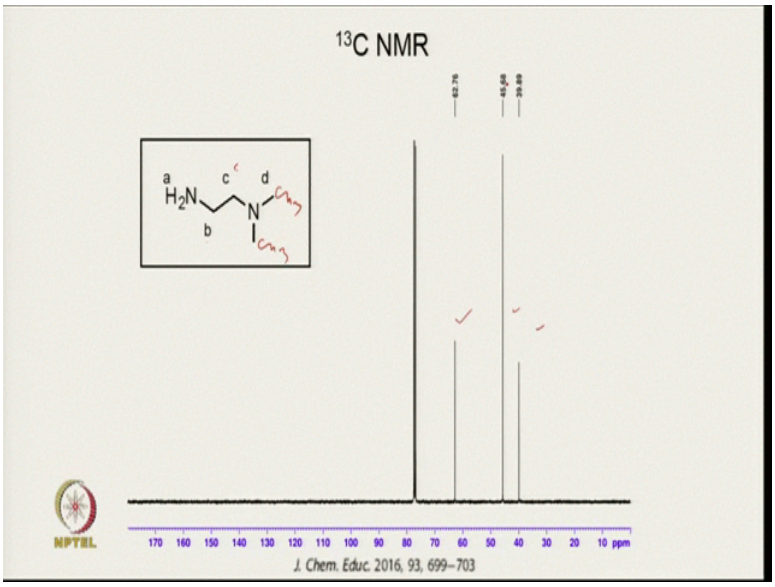

13C carbon nuclei is also NMR active and so we can also look at 13C NMR. And here you can see that they are 3 different kind of carbon, one is d, c and d is as CH3, CH3. And so they are equivalent to each other, since both has same environment. So you expect 3 peaks and that is what you see here, there are 3 different peaks 62.76, 45.66 and 39.68.

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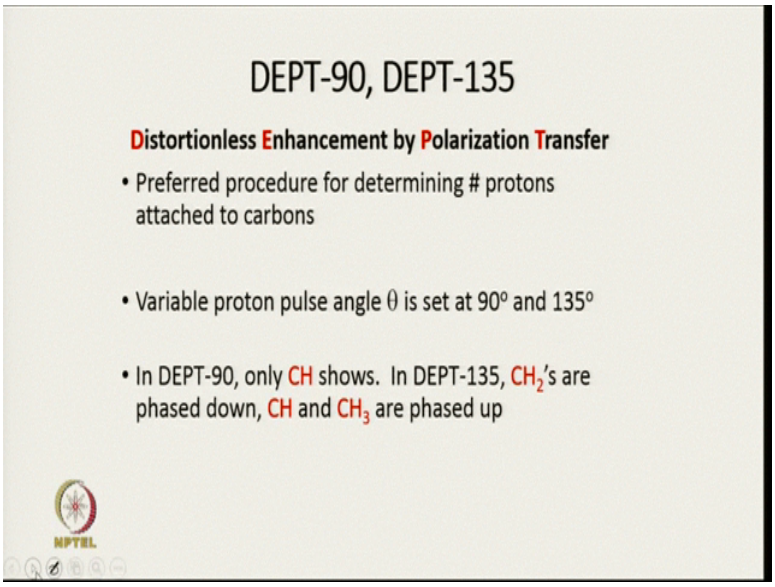

Now if we want to assign these protons, if we want to know which of this carbon gives this peak, we need to do another experiment called DEPT experiment, DEPT experiment can be of two type, DEPT 90 and DEPT 135. DEPT stands for Distortionless Enhancement by Polarization Transfer and it is a preferred method for determining number of protons attached

to carbons. There are 2 different kind of DEPT, DEPT 90, DEPT 135. This 90, 135 is basically a proton pulse angle.

A proton pulse angle is set at 90 degree, it is known as DEPT 90. Whereas if proton pulse angle is set to be 135 degree, then it is known as DEPT 135. In DEPT 90 only CH shows, so you will not see any peak due to CH2 or CH3. Whereas in DEPT 135 (CH3), CH2's are phased down, whereas CH and CH3 are phased up. So you will get a positive signal and negative signal, depending on kind of carbon, kind of carbon. Here, when I say kind of carbon, I means carbon is attached to how many protons.

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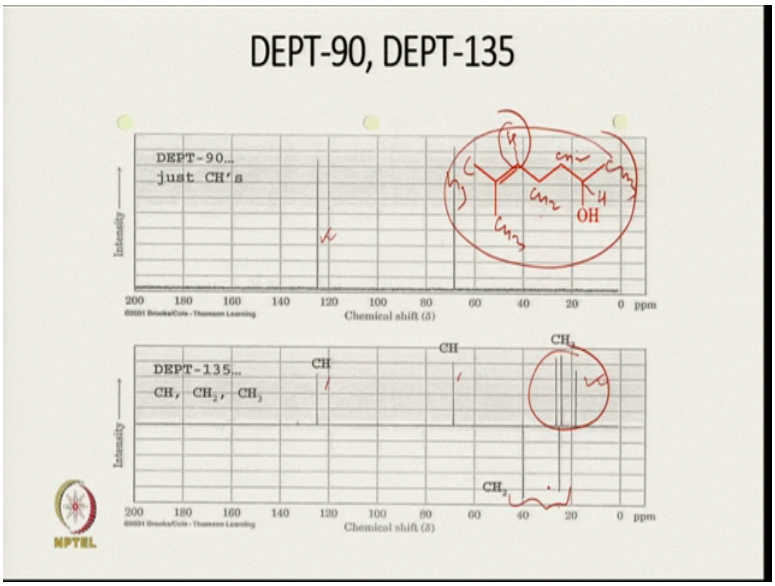

So this is an example of DEPT 90 and DEPT 135 of this molecule. And now you can see that if we look at the CH I have only 1 CH group, only 1 CH group. So I will get only 1 peak in DEPT 90, only 1 peak in DEPT 90. But if I go to DEPT 135, then you look here, this is CH3, this is CH3 and this is CH3 and this is CH2 and this is CH2. So you will get peak due to your all 3 CH, CH2, CH3. CH 2 peak will be down.

So this 2 peaks are due to CH2 and if you look at the CH2 there are 2 different kind of CH2, this is CH 2 and this is CH2. So you got 2 different peak for CH2. CH will come at higher chemical shift in comparison to CH3 and this is your CH peak, this is your CH peak and this is your CH3 peak, this is your CH3 peak and since there are 3 different kind of CH3 and so you got 3 different CH3 peak. CH is, there is 1 H here also. So there is 2 CH group and so you are getting 2 peaks corresponding to CH. And you have 3 CH3 group and so you goes 3 peaks for CH3 and 2 peaks for CH2. Based on that you can assign different carbons

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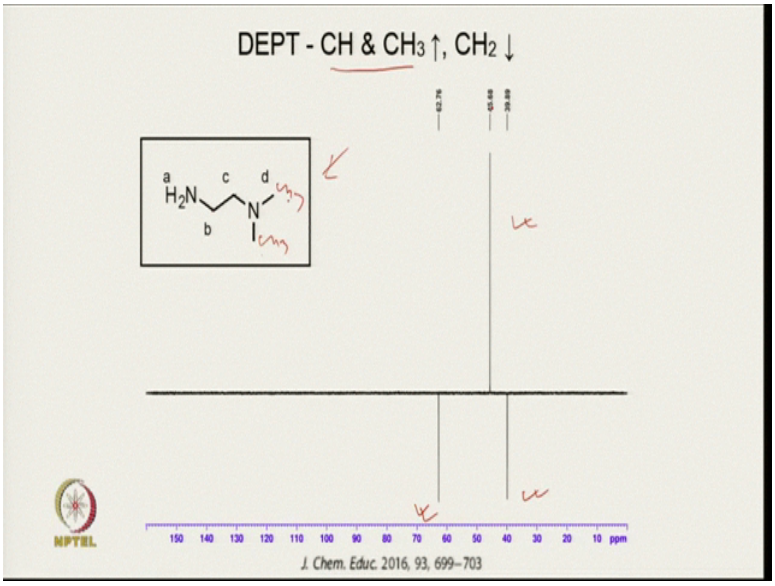

Now come back to the same compound which we are dealing with. And if I do DEPT 135, I will get peak of CH and CH3 up and CH2 down. So you see there are 2 CH2 here, belonging to these 2 CH2, where is CH3 is this and this has only 1 peak. So 45.66 is now assign to CH3 group, now assign to CH3 group. So carbon, this carbon has chemical shift 45.66. This is the way you can assign proton, you can assign carbon.

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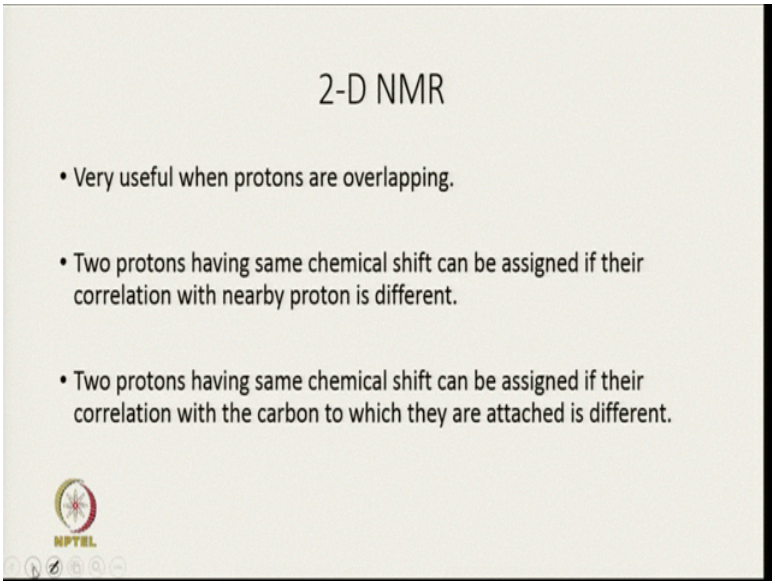

So, but still that may not be enough and for assignment and structure calculation, you may need to use 2-D NMR and they are very useful when protons are overlapping and protons are overlapping. So 2 protons having same chemical shift can be assigned if their correlation with

nearby proton is different or nearby proton is different and then you can look at the proton proton correlation, to proton proton correlation to distinguish between 2 different protons.

2 protons with same chemical shift can be assigned if their correlation with carbon to which they are attached is different and in that you can look at your heteroatom correlation, means carbon and proton correlation. So the two different kind of 2-D NMR experiment you can think of. One which correlates proton with proton, and another which correlates proton with carbon or proton with nitrogen.

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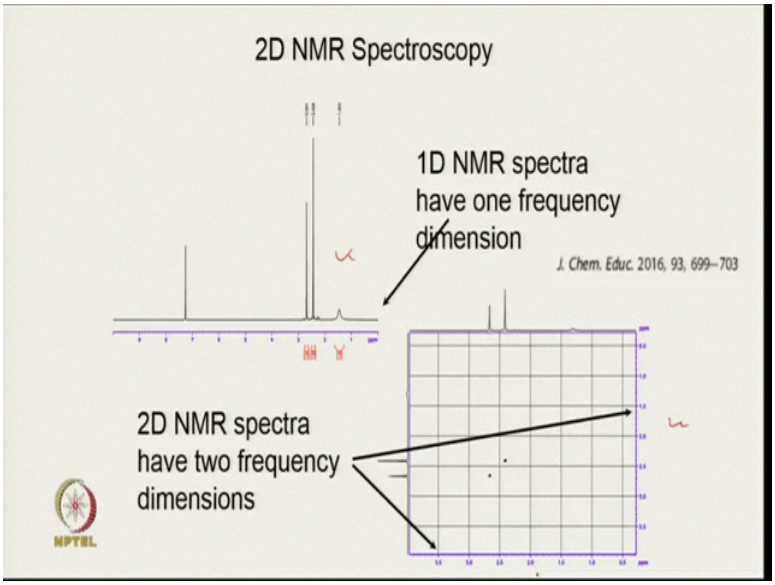

So 1D NMR we have already seen. In 2-D NMR there will be two dimension, one dimension can be proton, the other dimension can be proton or carbon if you are particularly doing with organic compound. Then people try to look at a correlation between proton and carbon, there are sometime if you go to more complex molecules. For example, your proteins, in that case, people also look at 3-D NMR and 4-D NMR. So basically you are trying to differentiate between different proton based on their correlation with heteroatoms and other protons in the system.

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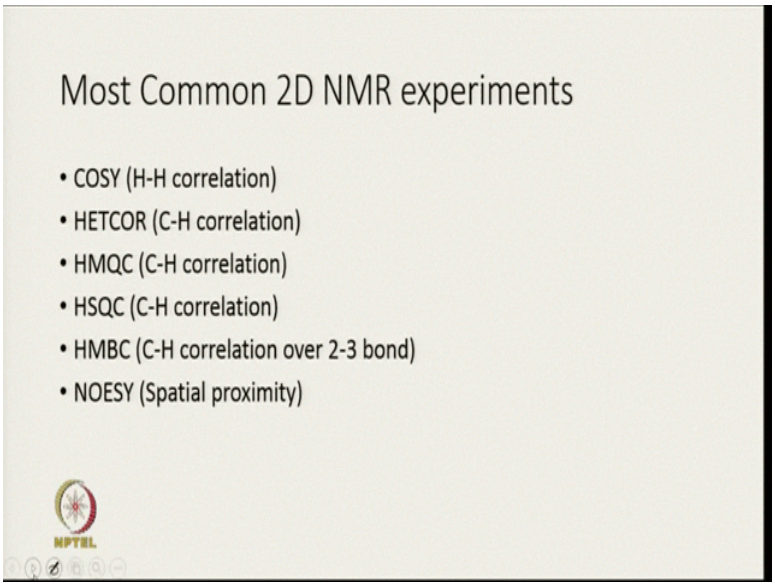

So most common 2-D NMR experiments are COSY, where proton and proton are correlated and then there is experiment called HETCOR, in which carbon and proton is correlated. HMQC is better version of HETCOR and again, this is related to CH correlation. HSQC CH correlation. HMBC, here the carbon and hydrogen are correlated which are separate by 2 to 3 bonds. Then the text 2-D experiment is NOESY, NOESY looks at the nearby proximity of two protons, nearby proximity of two protons.

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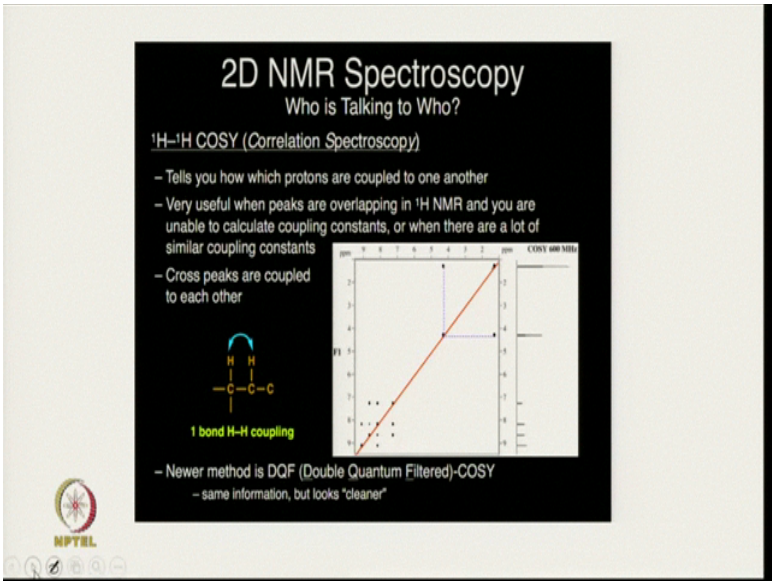

So 2-D NMR spectroscopy is important because it gives you an idea about who is talking to whom. The first simplest one is proton, proton COSY. It tells you how and which protons are

coupled to one another. It is very useful when peaks are overlapping in proton NMR and you are unable to calculate coupling constants, or when there are a lot of similar coupling constants. There will be two different kind of peaks in 2-D NMR, one is your diagonal peak and another is cross peaks, what you are looking at is cross peaks. Cross peaks are coupled to each other when there is coupling, then will see a cross peak. And one of the important variation of this, this spectroscopy method is DQF COSY, which is double quantum filtered COSY, same information, but it is more cleaner.

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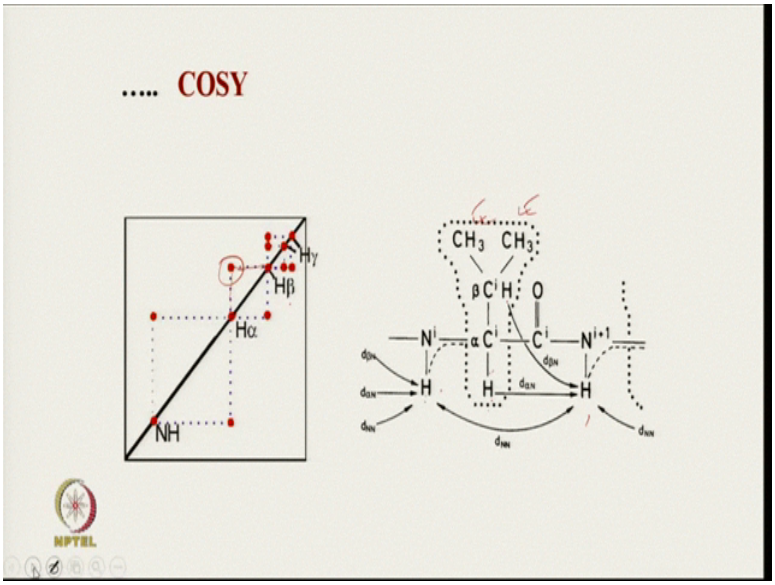

Let us look at this molecule. Now you can see there is a correlation between you are this proton and this proton and that is what you can see. Now as I told in COSY there is a diagonal peak and there are cross peaks. Diagonal peaks are not that much useful, what is useful is cross peaks because that tells you about that this proton is talking to, which proton, which next proton. So here is the 4-5 cross peaks. Because there are 5 different atoms, so this is one, this is the second and this is the third and then your gamma, forth one, now you look at their correlation.

So now you see NH is basically if you look at this NH is correlated to H alpha and that is how you can assign it. H alpha is correlated to H beta, so you can see there is a cross peak here, there is cross peak here and they are correlated to two different resonances and the way we can get it, draw a vertical line, draw a horizontal line. And it coincides with this peak and this peak, it means this two are correlated. So H alpha and H beta protons are correlated. And

then H beta is correlated to 2 gamma, two different gamma and that is your CH3, CH3 groups. So correlation between different protons can be seen through COSY.

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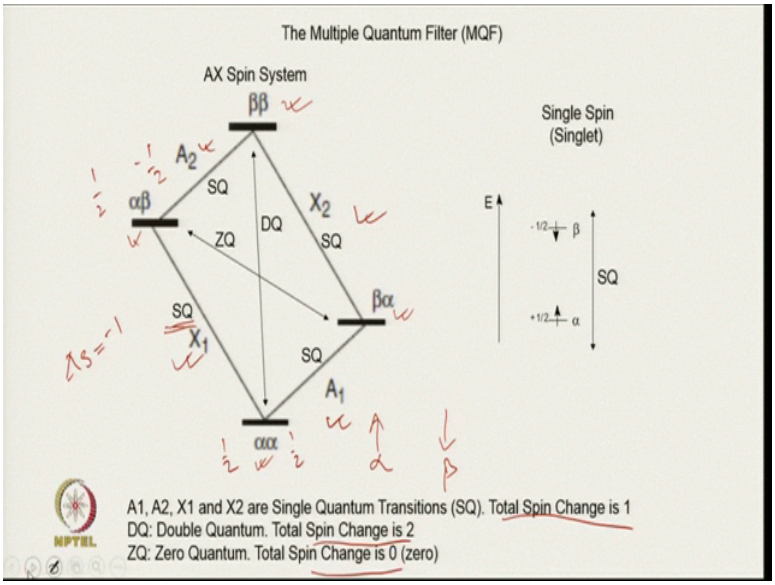

Then comes the DQF its double quantum, double quantum filtered COSY. Now let us understand about what we mean by double quantum or multiple quantum filters. So if there are two spins, they can be in alpha alpha state, beta alpha state, alpha, beta state and beta beta state. Alpha means you are talking about up spin, beta means you are talking about down spin. So if both are up, then you have a alpha alpha and if both are down, then this is beta beta. If I spin is down and S spin is up, then you have beta alpha. When I spin is up and S spin is down, then you have a alpha beta.

And now if you go from alpha, alpha to alpha beta. This your less transition corresponds to change in spin to delta S is equal to 1. So it is basically you are going from, so this is half and here is half, your half and this is minus half. So you are going from half to minus half. So delta S is minus half, minus half. So delta S is minus 1. And if delta S is minus 1 or plus 1, then basically you have a single quantum, single quantum transition. So if total spin change is 1, then you have single quantum transition. And that you can see with this one, this one, this transition and this transition.

So A1, A2, X1, X2, the total spin change is 1. If you look at the DQ, DQ is basically this one, and here you see your going from alpha to beta, beta. And here the total spin change is 2 and that transition is called double quantum transition. If you go from beta alpha to alpha beta or

alpha, beta to beta alpha. The spin change is 0 and that kind of transition is known as 0 quantum transition, 0 quantum transition.

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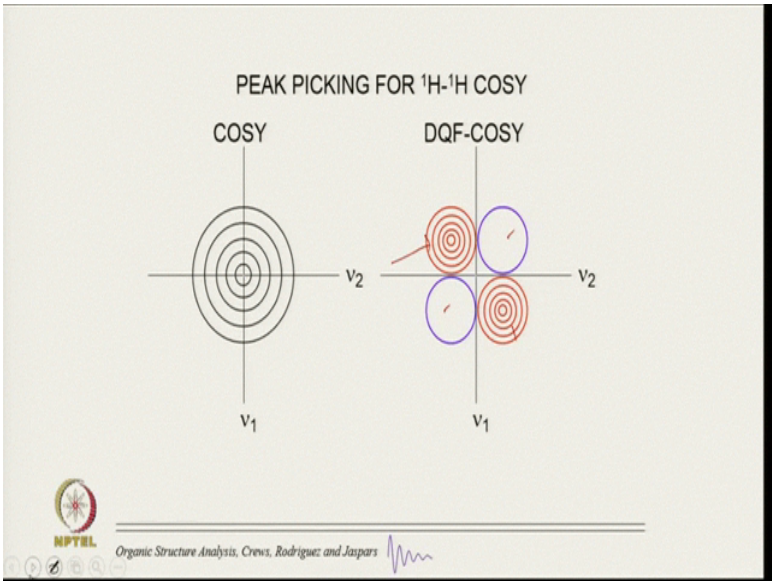

So if we look at normal COSY it will look like this. But if you go to double quantum filtered COSY it will look like this. So this two are positive peaks and this two are your negative peaks, this two are negative peaks. And they give much cleaner spectra and so now where it is your DQF COSY is carried out.

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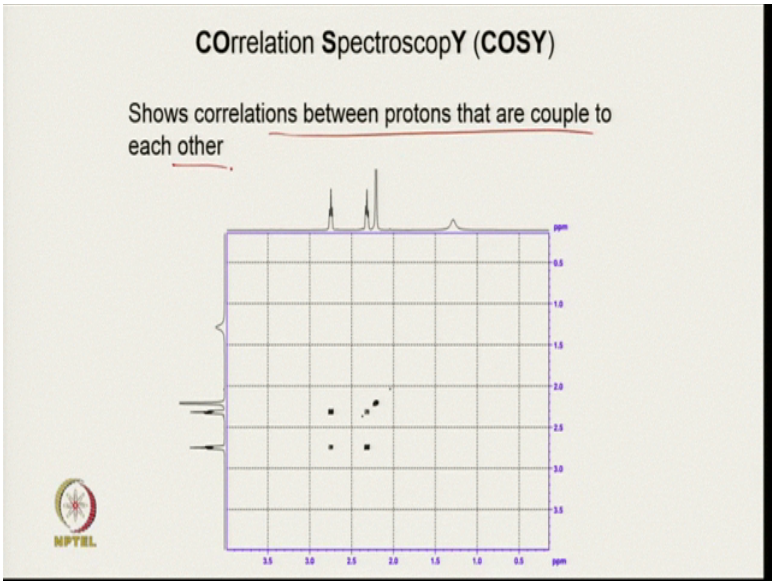

So this is COSY, which shows the co-relation between protons that are coupled to each other.

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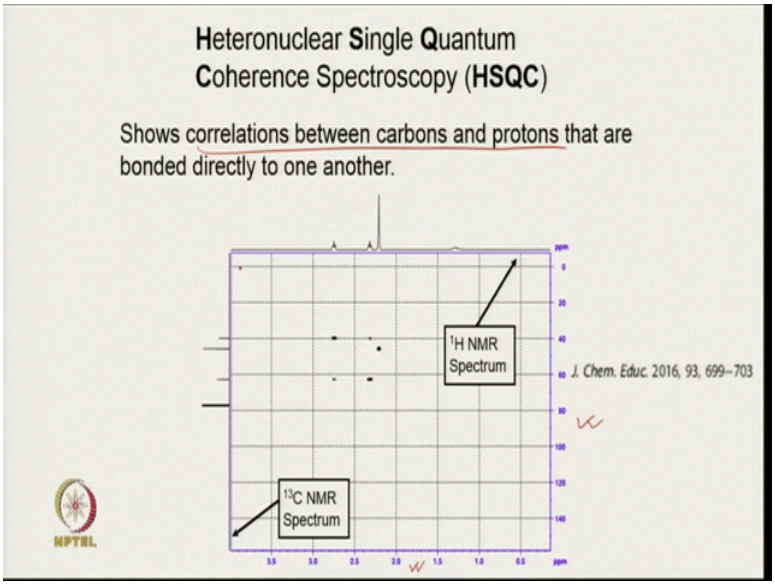

Then HSQC, as we discuss before that its shows correlation between carbon and protons that are bonded directly to one another. So this side is your carbon chemical shift, whereas the side is proton chemical shift and you are looking at the correlation. So this side is you see, this is proton NMR spectrum and this side is your carbon NMR spectrum.

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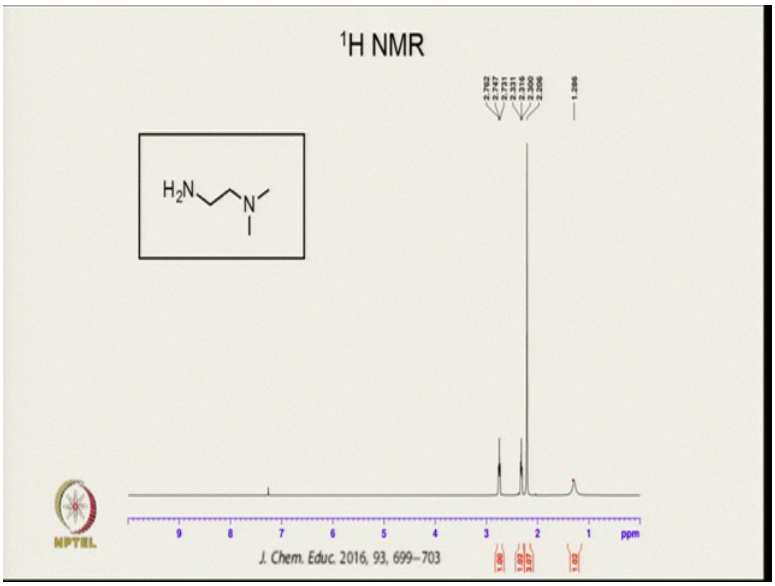

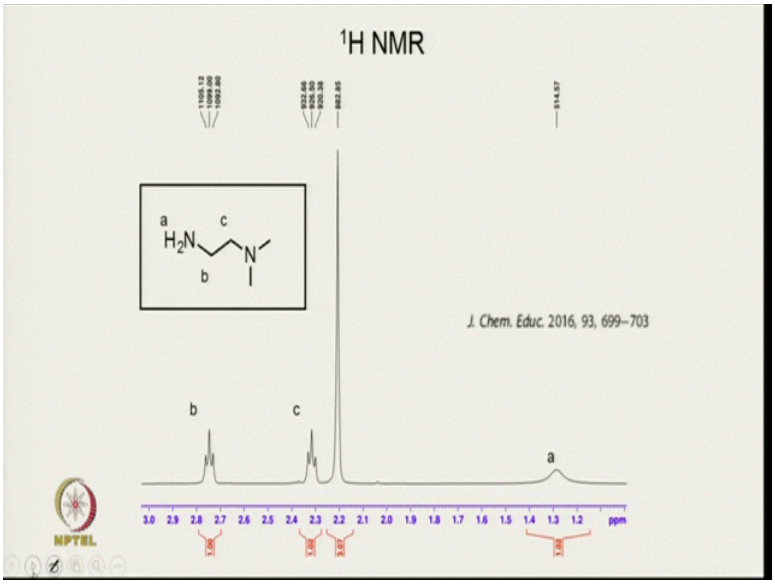

Now again come back to this compound which we were discussing. This has already been assigned and that is what we did previously. So this is your proton NMR and this has been assigned to, if you take ABCD different kind of protons and this is your assigned spectrum, 1D spectrum.

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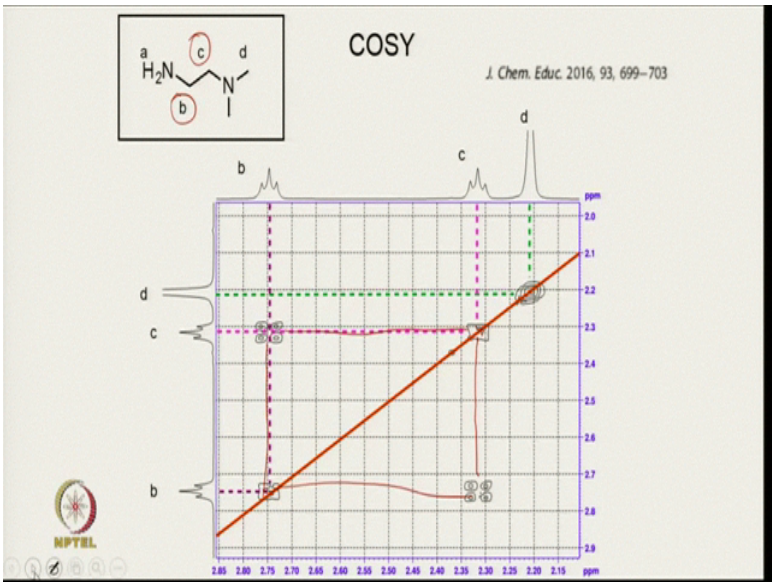

Then if you go and take COSY you will get this kind of COSY this is DQF COSY. And now you can see the correlation. So if you look at this cross peak and put a horizontal lines, and vertical lines. So now you know that this one is correlated to, this two peaks are correlated, this two peaks are correlated, this belongs to c, this belongs to b. So b and c are correlated and that is expected because these b and c are nearby to each other and so their protons are coupled and so you can get the corresponding peaks in COSY.

So COSY gives you an idea about the protons which are near to each other, protons which are near to each other. And here you see d is not, d is only your correlated to d. There is no cross peaks and so there is no protons which are coupled to the protons labelled by d.

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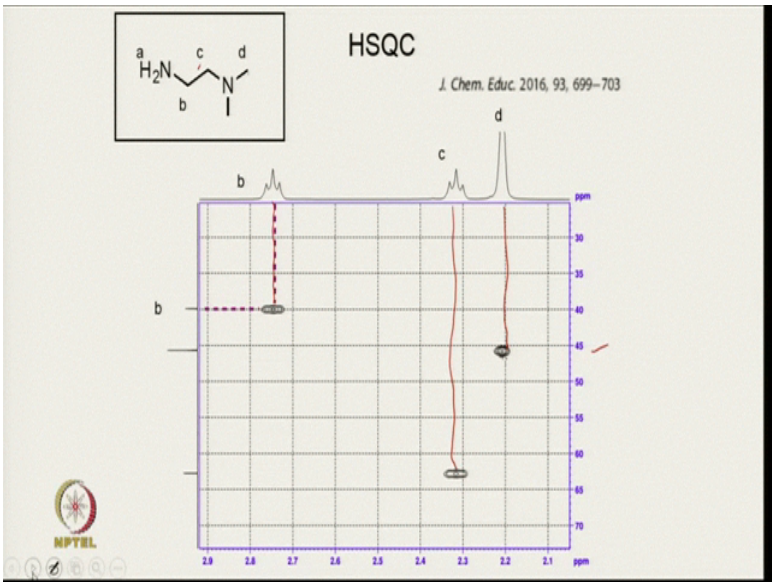

Now let us look at its correlation, its correlation. So you can see that there are 3 different kind of carbons, so you got 3 different peak. And now you can correlate this carbon is basically correlated with this carbon is, basically attached to this proton, this carbon is attached to this proton and this carbon is attached to this proton. So now you can, this is for d. So carbon chemicals shift for this proton d carbon, carbon chemical shift of d carbon is 45 ppm. And carbon chemical shift for c carbon is your around 62.5. Whereas carbon chemical shift of b carbon is 40 ppm. So this is the way you can assign all the peaks, this is the way you can assign all the peaks.

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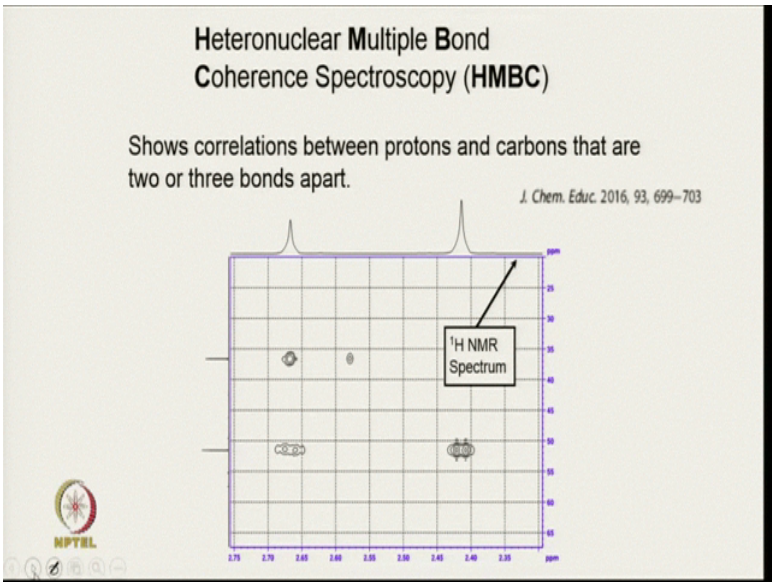

Now you can also carry out HMBC and this is particularly useful if your compound is quite big. It shows correlation between protons and carbons that are 2 to 3 bonds apart, 2 to 3 bonds apart. So this side again, there is a proton NMR spectrum and the Y axis is your carbon NMR spectrum.

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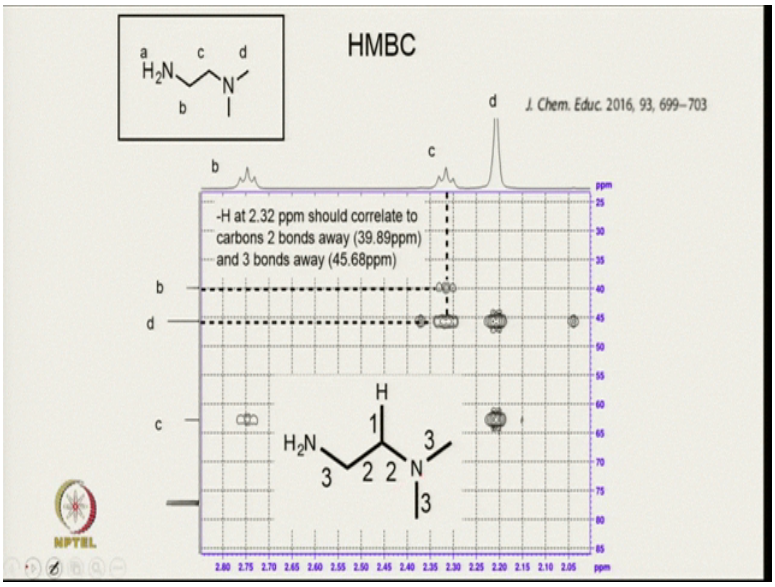

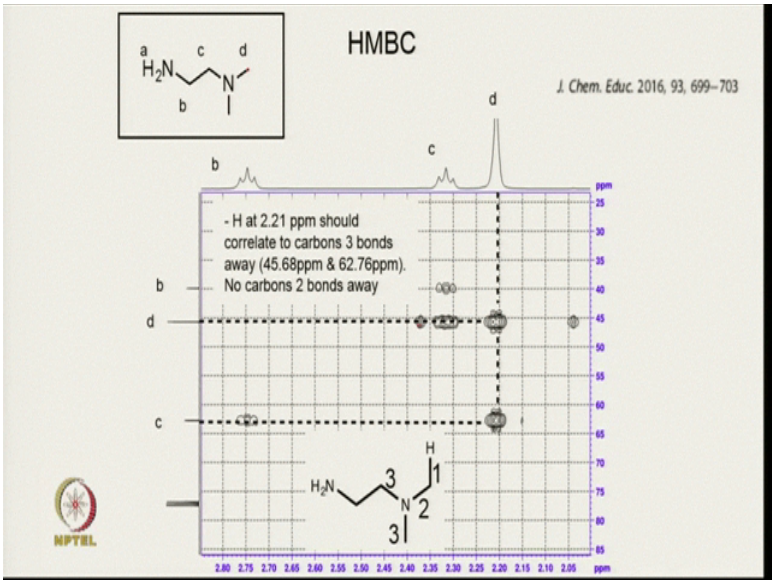

Now look at here correlation, now you see this is your HMBC for this compound, your b c d. Now you can see that b is correlated to, if you look at 1, 2. So here you see this c is correlated to b. Since it is 1 bond apart and then you can see that, look at this, this b is correlated to c and c is correlated to d, c is correlated to d. And now you see here, this d is correlated to not only c, it is also correlated to your d. So this d is correlated to okay, this is d d is correlated to c and d is correlated to this one also. And now b is correlated to d, b is also correlated to d and you see this is 1 bond, 2 bond, 3 bond apart, 3 bond apart.

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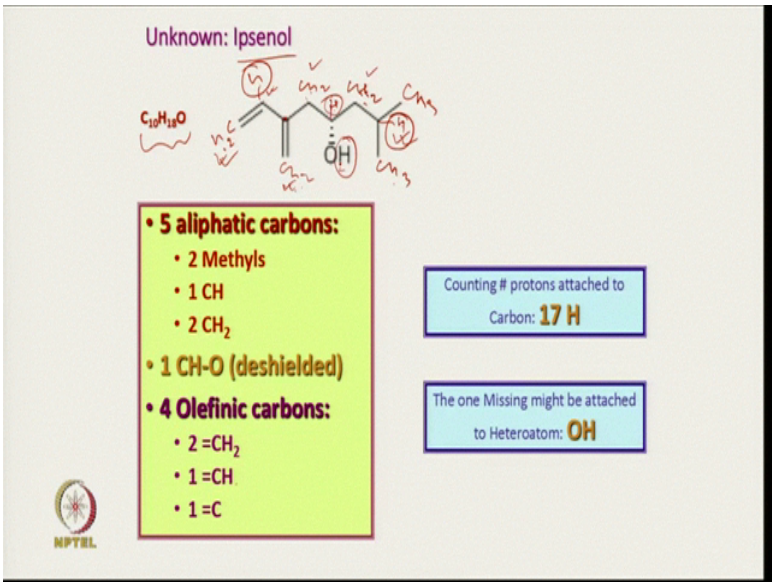

Now what I will do that, I will give you one structure and we will discuss about how to get, how to get all the resonance and structure from the NMR spectrum. So this is a compound which has molecule formulas C10H18O. And this is the, your structure of this compound

which is Ipsenol, Ipsenol. Now let see how we can assign it. So first thing we need to know how many aliphatic carbons, so it has 2 methyl group here and this is CH2 group and this is CH2 group, there is a CH group and this is CH2 group and there is H here.

So it has 2 methyl group, 1 CH, SCCH, this one is CH, 2 CH2. This CH2 this CH2 group, this is aliphatic carbon. So this is 1 CH group, sorry, this is 1 CH group and this is not aliphatic. Then 1 CHO group. So this is CHO, so OH group. So this is 1 group. 2 double bond CH2 group, so CH2 CH2 and 1 double bond CH 1 double bond CH and 1 double bond C, so 1 double bond C. So these are all the, this is 1 double bond C, which is not attached to any proton. So this is the way the structure is. So if you count hydrogen atoms you have 3 here. 3 6 oh there is 1 proton here also, 1 proton here also. So this is your 3, 4, 3 7, 2 9, 10, 2 12, 14, 15 and 2 17 and 1 18.

So there is 18 hydrogen, 18 hydrogen, but proton such as 2 carbon is 17 if you neglect this, then proton attached to carbon is 17. In all 17 hydrogen 2 is as a methyl group, 1 is CH. So this is not CH, this one is CH, 2 CH2, this 2, this 2 are CH2 group. 1 CHO. So this is 1 CHO and 4 Olefinic carbons 2 CH2 and 1 double bond CH 1 double bond CH and 1 double bond C. So this is thing. So here we have looked at how many protons which are attached to carbon. The one missing might be attached to heteroatom and so this is your heteroatom OH and that is how you can count about 18 H.

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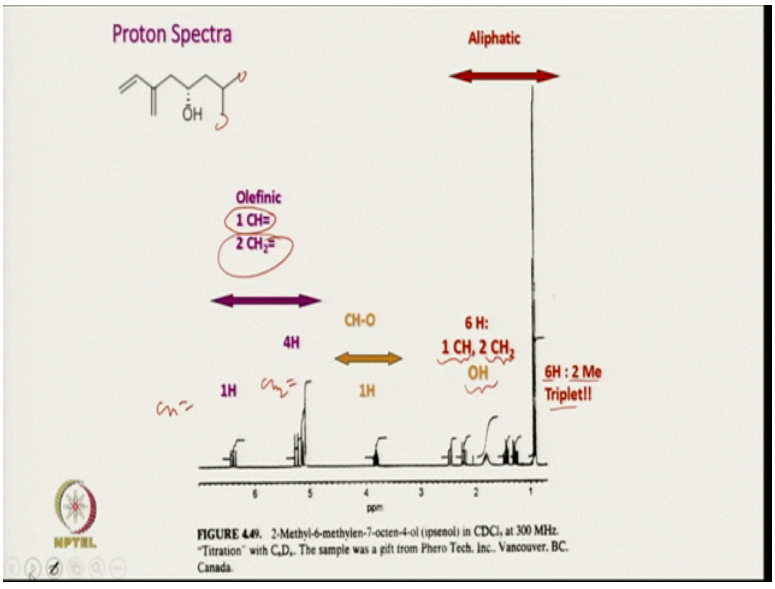

FIGURE 4.49. 2-Methyl-6-methylen-7-octen-4-ol (ipsenol) in $CDCl_3$ at 300 MHz. "Titration" with C_6D_6. The sample was a gift from Phero Tech. Inc., Vancouver, BC. Canada.

Now look at its proton spectrum, this is your proton spectrum of this compound. Now if you go by intensity ratio. This will corresponds to 1H, this will corresponds to 4H and since they

are in at a higher chemical shift. So they can be assign as Olefinic 1 CH double bond, 2 CH2 double bond. So this can be assign and from this, you can again see that these 4 has same environment and this 1 has different environment and so these can be assign to CH2 double bond and this will be assign to CH double bond.

So again by intensity ratio, you can assign this to 1H and this chemical shift region is your H attach to CO group, H attach to CO group. And then you have the intensity ratio is here quite high, it is like 6H. So it means you have 2 methyl triplet here. So this can be assigned off. And there are 6H, which will come from 1 from CH, 2 into 2, 4 from CH2 and 1, from OH. Now it is a bit difficult to assign here, so what will do, that we will do go for 2-D experiments. These are aliphatic region.

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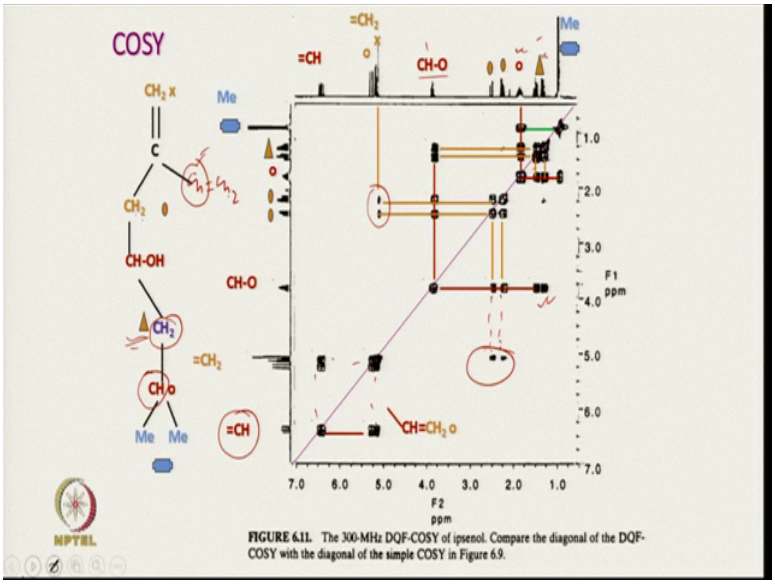

FIGURE 6.11. The 300-MHz DQF-COSY of ipsenol. Compare the diagonal of the DQF-COSY with the diagonal of the simple COSY in Figure 6.9.

So let us go and look at the COSY, COSY of this structure. Now these are fixed which are on diagonal and now I know at this is for CH3, CH3. So let us see the things we each we have already assigned. This is for methyl, this is CHO double bond, CH2 double bond CH. Now let see if I relate this, this is related to this peak, this is related to this peak, which is due to methyl group is related to this one. And so this O, this red circle corresponds to this CH group which is attached to 2 methyl group. So now I am able to assign this peak.

Now let us go and look at. So this, now this one, this red circle is now correlated to this, these peaks that corresponds to this triangle, this triangle and if you go and look at the structure, basically this CH is attached to this CH2 and now we know that this CH2 basically corresponds to triangle. So now this CH2 is also assigned, this CH2 is also assigned. Now

you see this is correlated to these 2 peaks is also correlated to CHO. So that is what is correlated and so it is now quite easy to assign peaks, assign peaks which was difficult to assign by 1D NMR.

So till now we have already assign, now look at this and see that this H is coupled to which proton, H coupled to which proton. So you see this is coupled to 2 different kind of proton, this proton we have already seen that there is a coupling between these 2 protons. But there is also coupling with these 2 peaks and that corresponds to these 2 peaks. So if you look at this, so this is basically coupled to this CH2 group, CH2 group. So now the chemical shift of this is also assigned or these protons are also assigned, these protons are also assigned.

Now let us go where these 2 protons are correlated, these 2 protons are correlated to this and this is a very weak peak. So if you go and see the structure, this is also related to double bond CH2 and so you can see this CH2 is correlated to double bond CH2. This CHO is correlated to your this double bond CH2. And this is weak peak because it is 2 bond apart, this CH2 and CH2 are 2 bond apart and so a very weak peak will be seen. And if you remember this structure also has this thing.

So this CH2 is also correlated to this CH and the way you can say is this you remember, this CH2. So they are also attached to this weaker peak, the weaker peak and that corresponds to your CH, this corresponds to this CH2. So this is also correlated to this by this one. And if you go here the CH2, which is here and this CH2 is correlated to, this CH2 is also correlated to this CH. So this CH is correlated to CH2 and so these CH protons are shown here, these CH protons are shown here. So there is a correlation between CH2, which is this CH2 and this CH and that can be seen here, can be seen from here.

And this is how we correlate, correlate different protons and we assign all the peaks, we assign all the peaks. So 2-D NMR is used for more complex organic molecules. Since lot of chemical shifts are overlapping, particularly in the aliphatic region, it is difficult to assign a protons which comes from, which comes from, which are attached to similar kind of carbons and in that case based on proton, it cannot be assigned and so it is better to go for COSY kind of spectrum.

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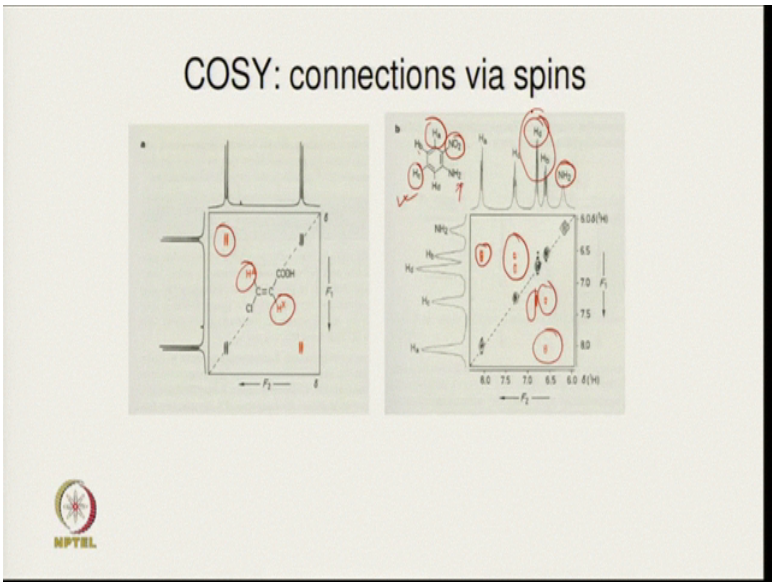

So here I show you some more COSY spectrum of some more molecules. So here is your molecule C double bond C COOH and this is H and here is CL and the second carbon you attached with H and CL. So in COSY you will look at the correlation between HA and HX and you can see here that, here is the correlation between HA and HX. So you will see 2 peaks which are cross peaks, 2 peaks which are cross peaks, one both showing the correlation between HA and HX.

Now if you look at this compound which is Ortho, Ortho Nitro aniline. In this case there are 4 different kind of proton which is attached to carbon and there is one more which is attached to your NH2 group, NH2 group. And if you look at here, then your HA, HC, HB, HB, HD, HB and NH2. So the lowest one is NH2 and since others are attached to carbon, double bond carbon. So they have higher chemical shift value, higher chemical shift value. HA has highest chemical shift value because it is nearby 2 an electro, electron withdrawing group, electron withdrawing group.

And that affects HA and HC more because they are in Ortho and para-position and so you get as a HC. Now you can look at the correlation HA is correlated to HB, HA is correlated to HB. Now HC is correlated to, HC if you look at HC it is correlated to these 2 peaks and that is your HB and HD. So HC is correlated to HB and HD. Now you see this HD it is correlated to HC, HD is correlated to HC and this a HD is correlated to HC and HA, HC and, HC, HB correlated to HA. So based on that if you have a confusion. There is a bit confusion between HD and HB and so you can assign the HB and HD peaks looking at the COSY spectrum.

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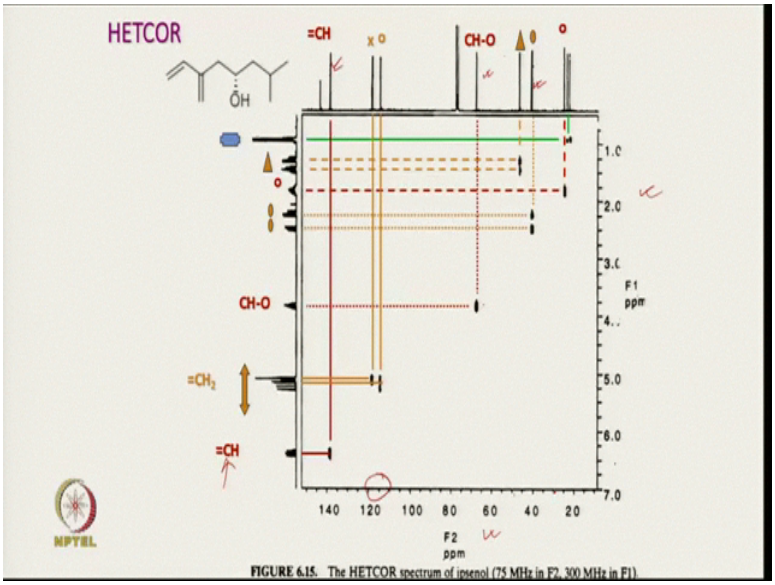

FIGURE 6.15. The HETCOR spectrum of ipsenol (75 MHz in F2, 300 MHz in F1).

Now we will look at the HETCOR, this side is your proton spectrum. So your Y axis is proton and X axis is your carbon, carbon spectrum. Now we can also assign the carbon, we can also assign the carbon. So we know that this corresponds to CH. So carbon of this proton can be assigned and that corresponds to around 138 ppm. Now this CH2, here you see the this side is your, so now you can assign these protons also and they have your carbon chemical shift around this values. Now CHO, you see this will around this carbon is going to have chemical shift, carbon chemical shift around your 75 ppm.

These 2 protons correlated to this carbon, which comes at 40 ppm and again, this proton comes, is correlated to this carbons which comes around 30 ppm. And then these 2 protons are correlated to this carbon, which comes around 48 or 50 ppm and this methyl protons is correlated to carbon which is at 20 ppm. So we have seen how COSY is used to assign the different protons and now we have seen how HETCOR can be used to assign your carbon attach to those particular protons. Similarly in place of HETCOR you can use your HNQC or HSQC to look at the correlation between carbon and proton and this is the way you can assign the different NMR signals.

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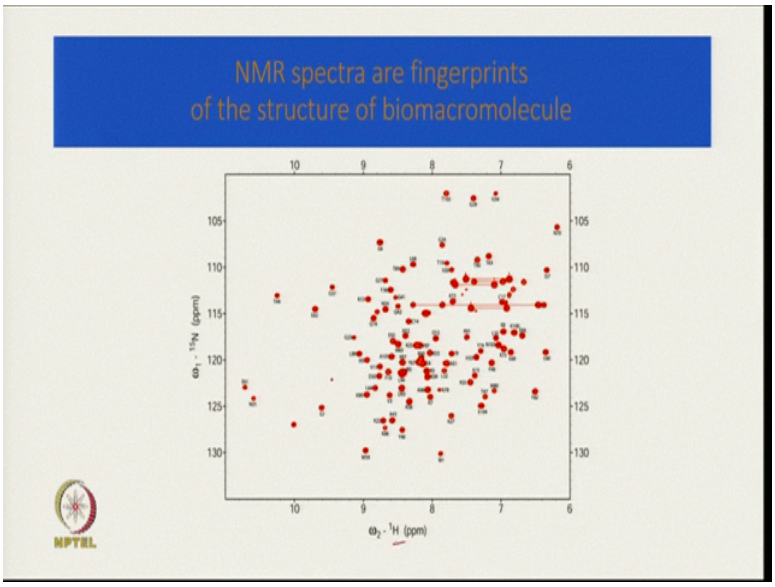

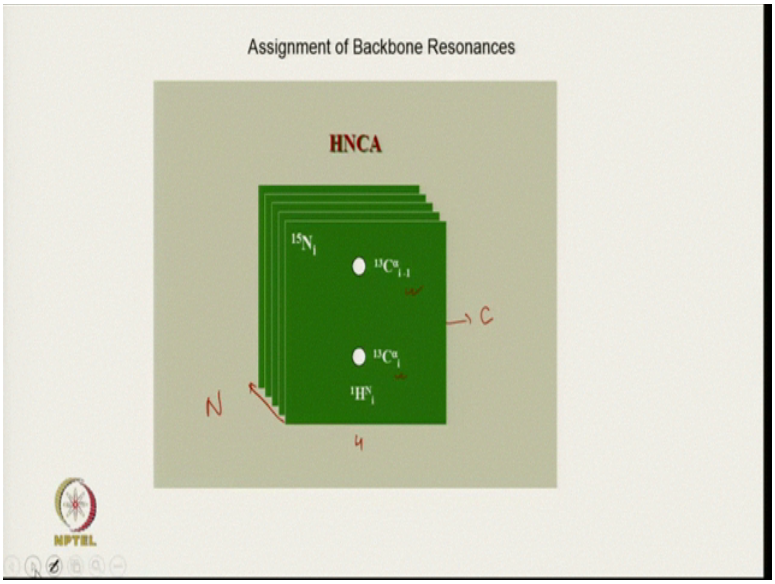

NMR can also be used as to see a structure of bio molecule, for example proteins they are basically fingerprints of the structure of the molecule. Here we show you the HSQC, where 15 N has been correlated with proton for a protein, for a proton. And you can see that there are several peaks and that basically corresponds to your amide nitrogen and proton correlation. So first thing is how to assign it? The assignment is done using your 3-D experiments. For example HNCA, where correlation between protons attached to nitrogen and C alpha is same. Basically here you are looking at the correlation between proton attached to nitrogen and C alpha.

So here what has been done is your there is on X axis, there is proton and Y axis, there is carbon and on Z axis you are taking nitrogen and that is why it is known as 3-D NMR method. And for one value of N, basically one value of nitrogen chemical shift, you can look at how proton and carbon is correlated. So proton will be correlated with 13 C alpha of Ith minus N and 13 C alpha of I minus 1 A minus N. So proton is correlated to C alpha of Ith minus N and or C alpha of I minus 1 A minus N. Now what you do is you try to correlated each of this 15 N plane with other planes and try to find out where there is your correlation and so you can find out, you can assign a backbone resonances.

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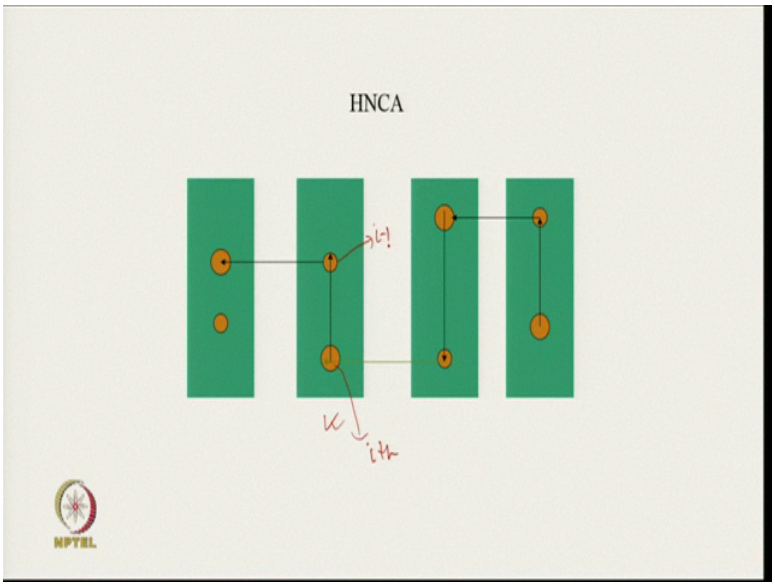

Now the way you do is, as now you take all the planes, all the different planes, which differ in 15 N chemical shift and now try to look at that which plane coincides with the plane, coincides with the plane. Where C alpha of preceding residues is correlated with C alpha of the residue of Ith residue. Now look at here, this big circle is given for Ith a correlation between proton and C alpha of the semi-spaces.

Whereas this smaller circle is given for correlation between proton, amide proton of Ith residue and C alpha of preceding residue. So if you look at here, this is 2 peaks and what does this tells you, this is your Ith residue and this is for I minus 1 residue. The smaller circle is matched, smaller circle of Ith residue is matched with bigger circle of I minus 1 residue and that is how you see this one is matched to bigger, smaller is matched to bigger and this is the way, you know how you can assign the backbone.

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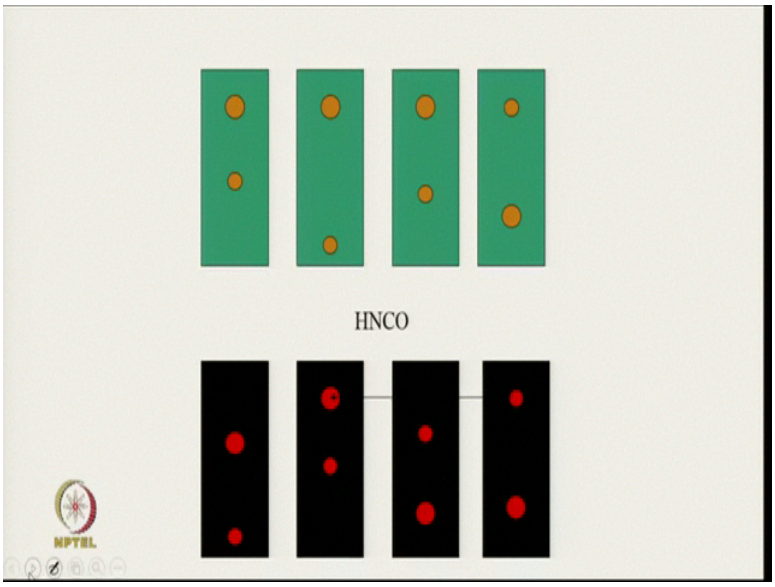

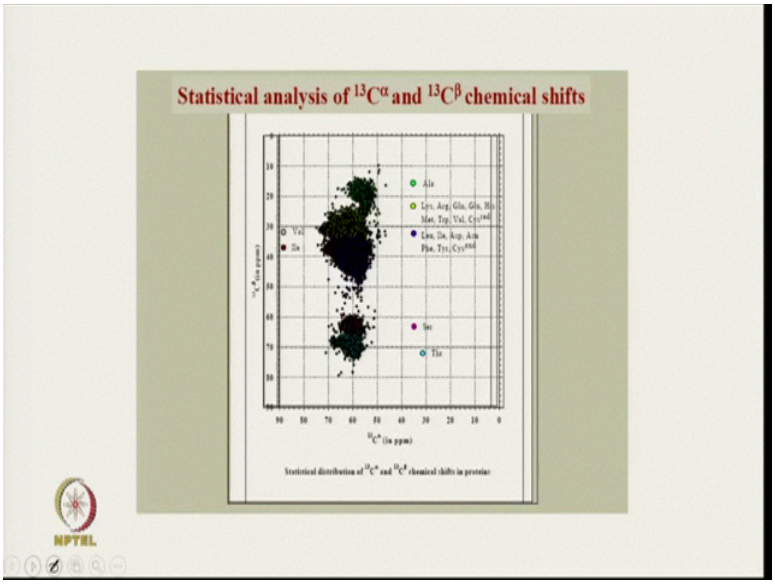

Similarly, you can do for HNCO experiment and once you assign all the peaks in HSQC. Now you can go and determine just based on chemical shift what will be the secondary structure that is done by comparing 13 C alpha chemical shift and 13 C beta chemical shift of the given protein and their deviation from the chemical shift of the amino acid and they are in random coil.

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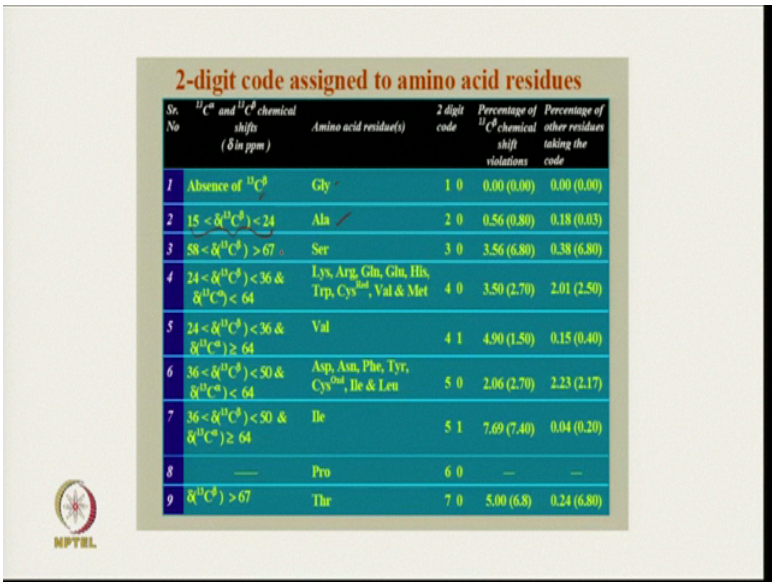

And your 13 C alpha, 13 C beta chemical shift based on chemical shift also you can assign which amino acid it may belong to and that is going to help you when you are assigning a peaks based on HNCO or HNCO experiment and these are the few facts which has been seen that chemical shift of 13 C beta varies between 15 to 24 for alanine. This C beta peak will be absent in the glycine, serine it varies from 58 to 67. And these are the few things which you can use during the assignment.

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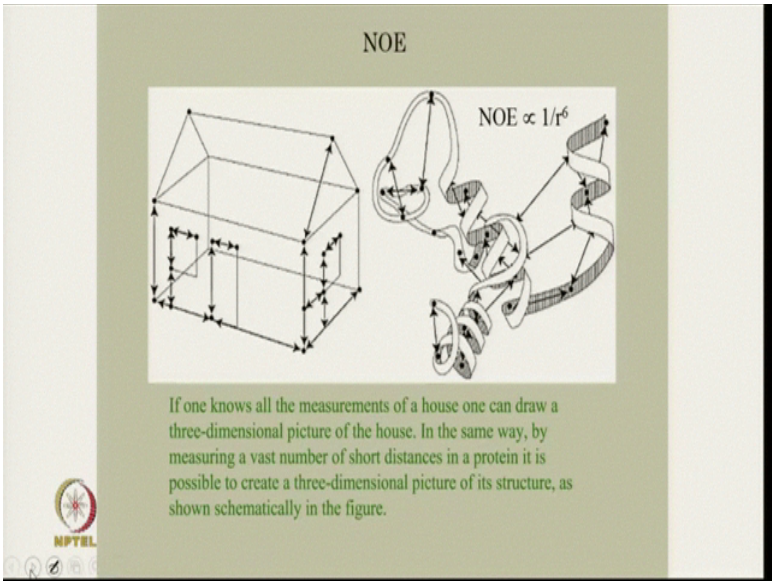

Now, once you have assigned all the peaks corresponding to your protons in a protein, you go for NOE and NOE gives you distance between 2 protons and as you, as distance are used to

for construction of a house. Similarly here distance constraint is used to fold the protein using your computational software. So in the first you generate a simple random coil consisting of all the amino acid and then you put constraint, distance constraint and dihedral constraint, and that is used to fold the protein. And that is how NMR structure of a protein is generated.

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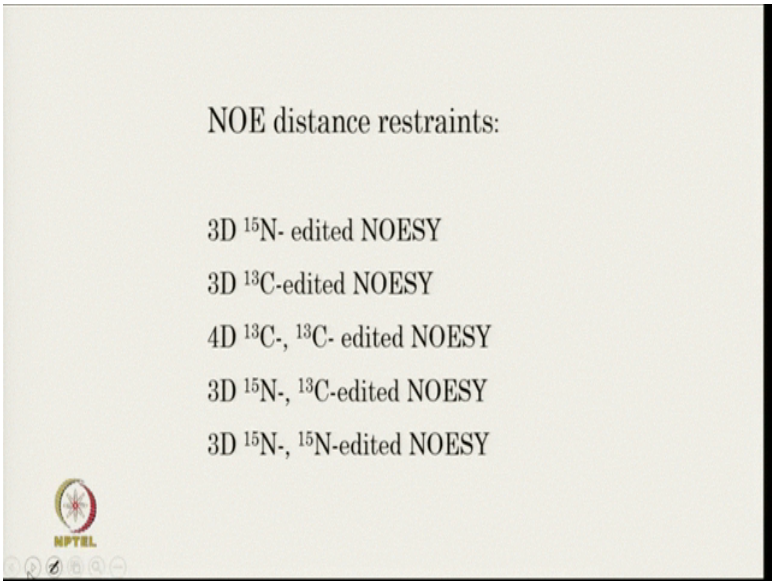

The different NOE distance restraints can be 3-D 15 N edited NOESY, 3-D 13 C edited NOESY, 4D 13 C, 13 C edited NOESY, 3-D 15 N, 13 C edited NOESY and 3-D 15 N, 15 N edited NOESY.

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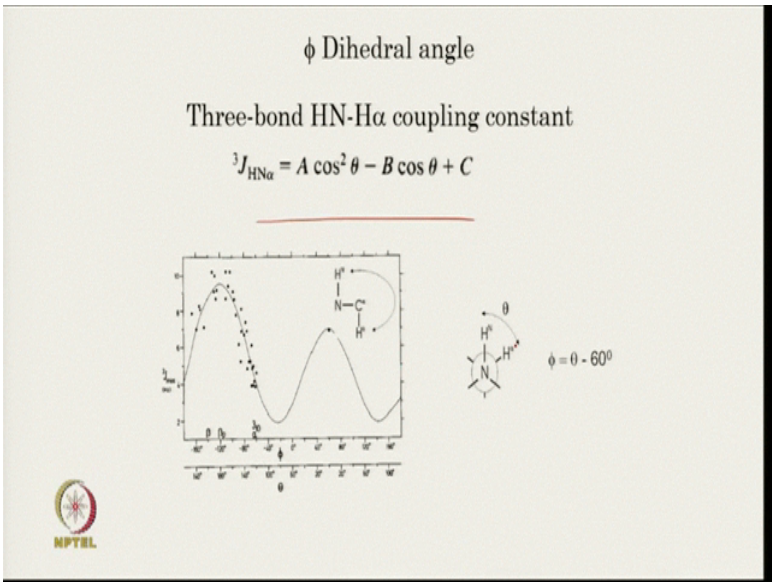

We can also use the dihedral information obtained from 3 bond, HN H alpha coupling constant. I have already told you about the Karplus equation which is this and that can be used to get this dihedral angle theta. And that can also be used as a constraint, when you are solving NMR structure.

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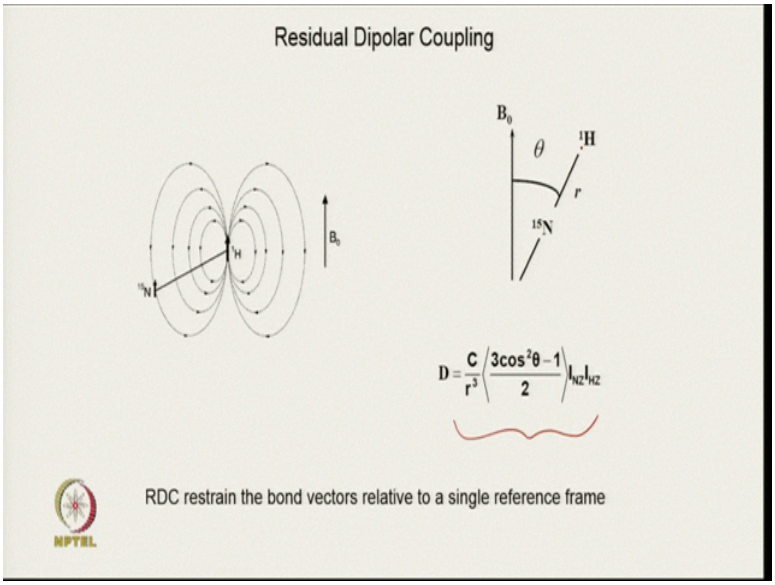

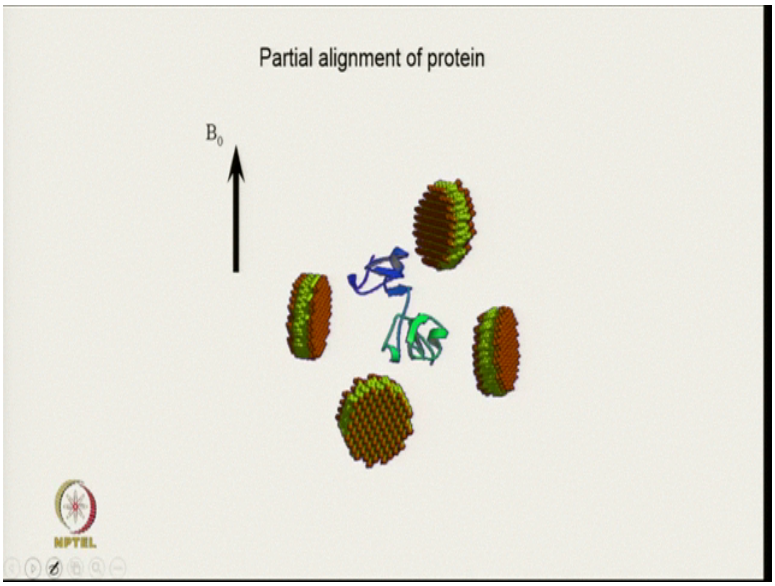

The third constraint comes from residual dipolar coupling measurement, where dipolar coupling is basically dependent on theta and theta. Where theta is the angle of NH vector with the external magnetic field. And these angles can be used for the, your structure calculation. And the way we measure dipolar coupling is to put it inside a by cells, and, where now the protein will be partially. In that case protein will be partially aligned with the magnetic field. And that is how you can get the information about theta.

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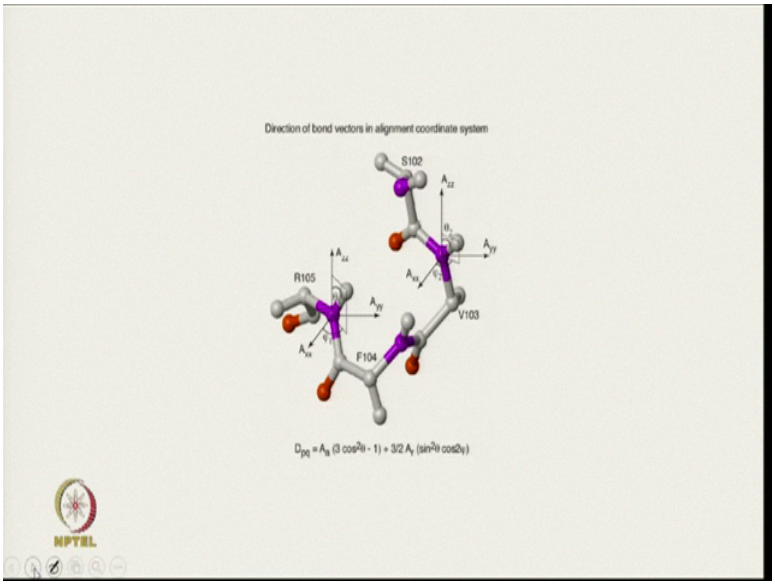

This is the seen, how dipolar coupling can be related to the different angles and using those angle information, we can basically get more accurate NMR structure.

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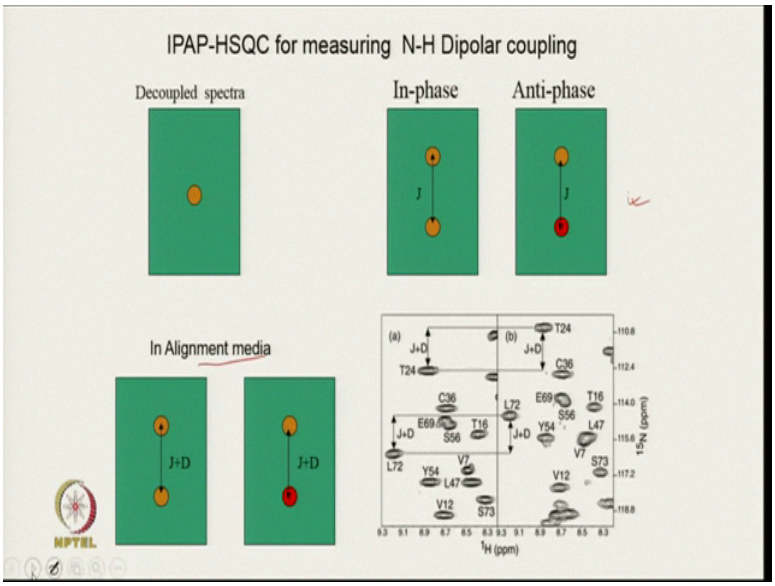

So this is the way in which dipolar coupling measurement is done. This is your decoupled spectra, if we take coupled, so you can get in-phase and anti-phase. This is your J value and if the, you know, you take coupled spectra in alignment medium, your difference will be equal to J plus D. So once you take the spectra in absence of an alignment medium, you get the value of J. In the alignment media, you get the value of J plus D. So you can know what is the

dipolar coupling value and that is how you can get another constraint which can be used to get a much better quality structure.

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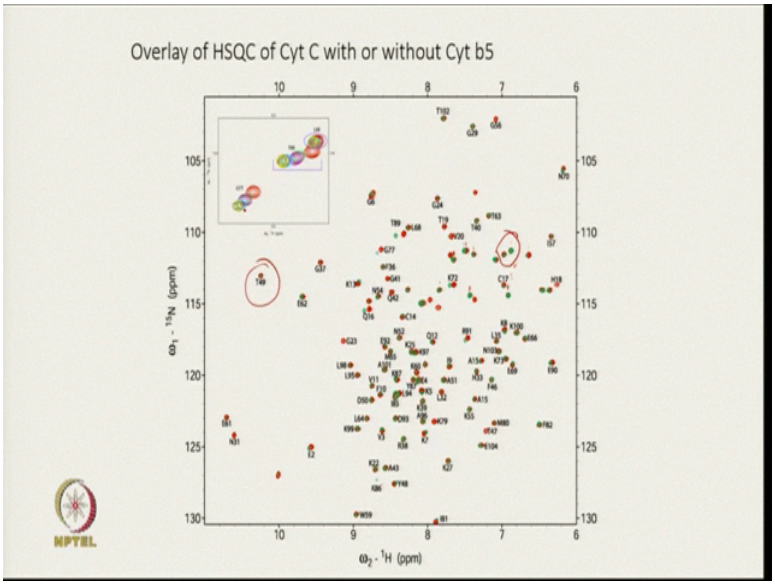

The second thing which you can do using HSQC is that you can also get the information about interaction. So here there is overlay of HSQC of cytochrome C with or without cytochrome B5. So what we are trying to look at is how cytochrome C interacts with cytochrome B5. And there is overlap of HSQC peak and you can see that lot of peaks are quite overlapping with each other. But, there are certain peaks which is moving, for example T89, L68, G77 and this information where peaks are moving can give you an idea that which part of cytochrome C is interacting with cytochrome B5.

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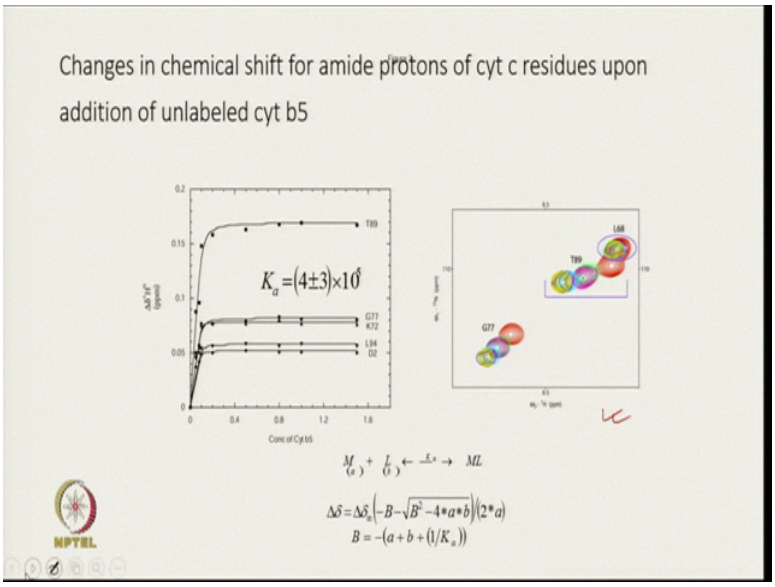

So using that chemical shift a deviation you can measure the binding constant and this is your one of the typical peak which we used to get the binding constant of cytochrome C, with cytochrome B5.

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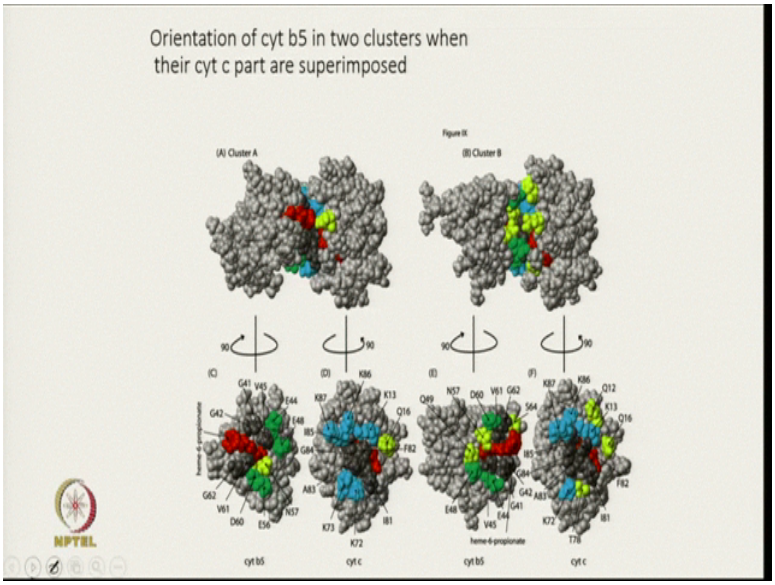

So once you know that this molecules, this interface is involved in binding with the another protein. Then you can just put that into software, here haddock has been used, haddock is a software which utilizes that information about interface to do the docking. And if you incorporate those information you will get a much better complex structure. So NMR can also be used to look at the binding side or getting a complex structure. So thank you very

much and I hope that I have given a flavor of how to use NMR in a structural calculation of protein and organic molecule. The scope of NMR is quite vast at a difficult to talk about every aspect in just 4 lectures. But I hope that this is good enough background for you to go and look at NMR quite closely. So thank you very much.