Advanced NMR Techniques in Solution and Solid-State

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Module-50
Coupled and Decoupled HSQC, HMBC, INADEQUIATE, 2D Jresolved
Lecture – 50

Welcome back. In the last class, we discussed two dimensional experiments especially correlated experiments, direct correlation, like COSY, TOCSY, heteronuclear correlation, HETCOR experiment, like that. And I said, COSY establishes the correlation among immediately coupled neighbours. TOCSY gives rise to the correlation among all the coupled spins in a single experiment, heteronuclear correlation establishes connectivity among the heteronuclei and HETCOR experiment establishes the one bond connectivity.

COLOC is the longest correlation spectroscopy, establishes connectivity among the carbons and protons which are separated by more than 2 bonds or 3 bonds, etcetera. And we discussed about various advantages and disadvantages of all these experiments. The way they are represented also. I said the COSY is symmetrical with respect to diagonal, TOCSY is symmetric with respect to diagonal. Whereas, the heteronuclear correlation experiments there is no symmetry, one has a large chemical shift along carbon 13 dimension let us say, and other has a different chemical shift range, compared to proton. Hence, it can never be symmetric and there is no diagonal. Important please remember, in heteronuclear correlation experiments, there is no diagonal and actually there is no symmetry, they have different spectral widths on both F1, F2 dimensions.

And I said the detection takes enormous amount of time. As a consequence, inverse detection can be done, the inverse detection is done in an intelligent way; take the proton magnetization give to carbon or nitrogen the dilute spins or low abundance spins, then take it back and detect proton. In the process there is coherence transfer between these two nuclei, and you can establish connectivity, especially the chemical shift connectivity among directly bonded or even remotely bonded.

And they are accordingly called HSQC, HMQC, HMBC experiments. HSQC establish a direct connectivity between two heteronuclei which are separated by one bond. For more than 2 or 3 bonds we use HMBC experiment. So, right now today what we will do is, we will start discussing about HSQC spectrum, how we get, everything quickly go through, one simple interpretation, etcetera, because the time is running short, what we will do is we will see HSQC, HMBC. HMBC is identical there is a small subtle difference, which I do not want to touch upon.

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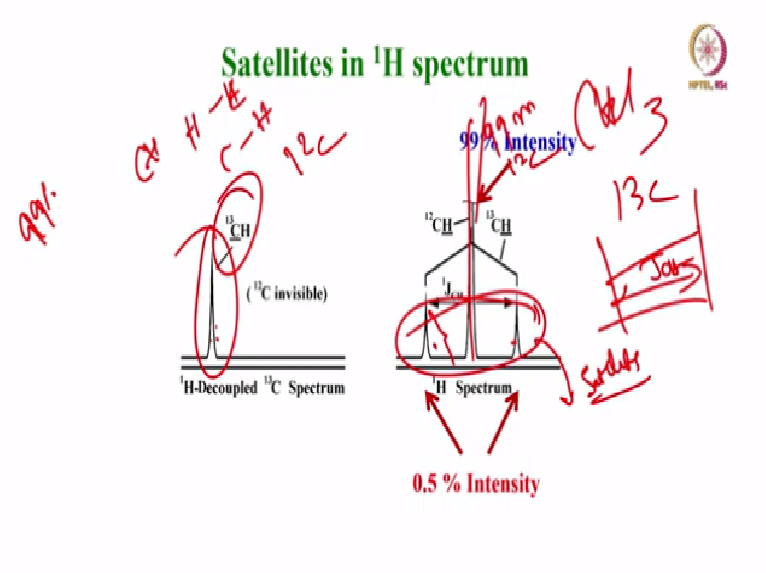

Satellites in ^1H spectrum

Before going into HSQC I want to tell you something about satellites in proton spectrum. Why I am bringing satellites here, it is very important to understand coupled and decoupled HSQC. Let us say I have CHCl3. If you look at the proton, you get only a single peak. If you look at the carbon 13 what you will get? You will get 2 peaks, a doublet, how is it possible? Interactions in NMR always mutual, that is what I said, when a proton is coupled to carbon, carbon is also coupled to proton, both are true.

Why in proton you get a singlet, whereas you get doublet in carbon. Here it is proton which is coupled to carbon, JCH, but you are directly detecting carbon 13. In this case, you are detecting proton which is attached to carbon 12, which has 99% abundance. So, you will not see any coupling, carbon 12 has 0 magnetic moment, spin is 0. What about 1% of carbons, 1% of the molecule has carbon 13, carbon in 13 carbon state, that will give rise to coupling.

So, in the proton spectrum, you will see the large peak which is coming because of 99 molecules which have carbons in carbon 12 state, and only one molecule is in carbon-13 state. And this gives rise to doublet which is exactly equal to doublet in the carbon 13 spectrum. And this intensity is 1% compared to the big peak, that is why they are called satellites. So, the satellite is in the proton spectrum is very important to understand, because we will see when you go to HSQC how they are useful?

So, if you take the CHCl3 spectrum, 99% of the signal intensity is coming because of carbon 12 attached to proton and only 1% is for carbon 13 attached, and that is further split into a doublet because of coupling with carbon13, thus intensity is 0.5%.

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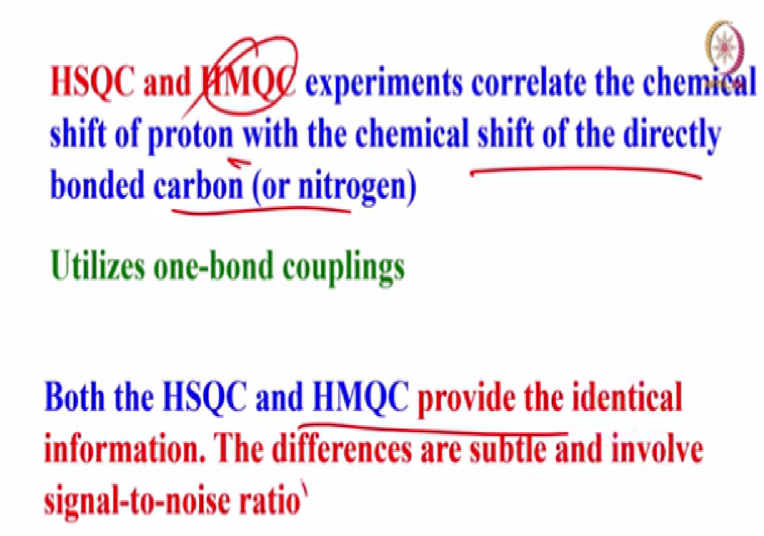

HSQC and HMQC experiments correlate the chemical shift of proton with the chemical shift of the directly bonded carbon (or nitrogen)

Utilizes one-bond couplings

Both the HSQC and HMQC provide the identical information. The differences are subtle and involve signal-to-noise ratio

Now HSQC and HMQC; with this we will go further. They correlate chemical shift of proton and chemical shift of the directly bonded carbon, HSQC and HMQC, both utilizes 1 bond coupling, there is a small, subtle difference, both HSQC and HMQC provide the identical information, a small difference, especially signal to noise ratio, and H-H coupling is coming in one of the experiments, we will not discuss that.

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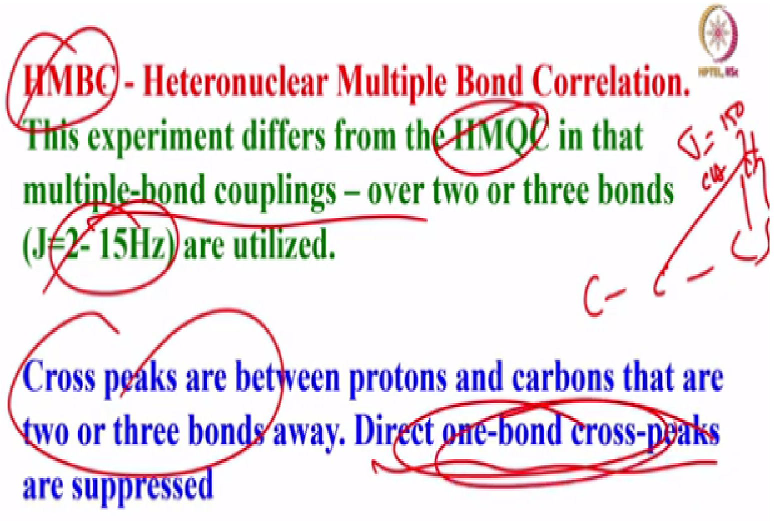

HMBC - Heteronuclear Multiple Bond Correlation. This experiment differs from the HMQC in that multiple-bond couplings – over two or three bonds (J=2- 15Hz) are utilized.

Cross peaks are between protons and carbons that are two or three bonds away. Direct one-bond cross-peaks are suppressed

Whereas, other hand HMBC, Heteronuclear Multiple Band Correlation experiment differs from HMQC or HSQC, in that it establishes connectivity among heteronuclei which are separated by 2 or 3 bonds. In the one bond J coupling is very large, let us say, a carbon and protons one bond J coupling is more than 150 Hertz 150 to 250. Whereas 2 bond, 3 bond is only 2 to 15 Hertz. So, HMBC can be utilized to establish the correlation between 2 heteronuclei that are separated by 2 bonds or 3 bonds; that is called HMBC experiment.

So, cross peaks are between protons and carbon that are 2 or 3 bonds away in HMBC, cross peaks are between directly abundant proton and carbon in HSQC. Now, what you may ask me a question, in HMBC, what will happen to these 1 bond cross peaks. If I look for the molecule like this, there is a proton here, there is a direct one bond correlation that also gives cross peak, and this also gives cross peak, then what will happen to this direct which anyway we are getting from HSQC. But what we do is in HMBC we suppress this one bond correlation. We detect only long range correlations. Correlations are 2 or 3 bonds, direct 1 bond correlation peaks are suppressed.

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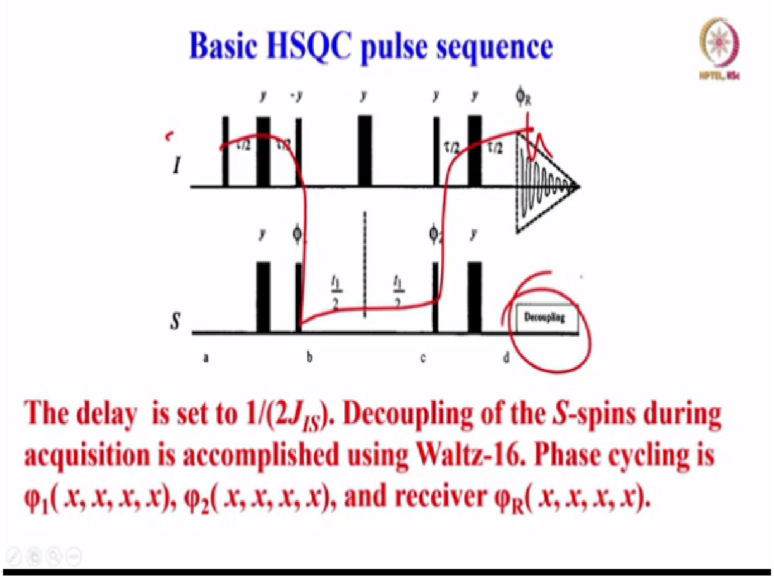

Basic HSQC pulse sequence

The delay is set to $1/(2J_{IS})$. Decoupling of the *S*-spins during acquisition is accomplished using Waltz-16. Phase cycling is $\varphi_1(x, x, x, x)$, $\varphi_2(x, x, x, x)$, and receiver $\varphi_R(x, x, x, x)$.

These is a basic HSQC pulse sequence. Here it is like an INEPT sequence, if you remember, the INEPT what we discussed. In the INEPT sequence where the polarization transfer takes place from proton to carbon here; and they are allowed to evolve here. Of course, we have a spin-echo like sequence here, and then reverse transfer is done here. Magnetization which is taken from proton given to carbon and from carbon given back to proton.

The magnetization transfer goes like this, from proton here it comes to carbon; then it goes to proton again. And we detect proton by decoupling carbon 13. This is the way magnetization gets transferred in HSQC type experiment. Rest of the things are subtle details for experimentalists, is you do not worry.

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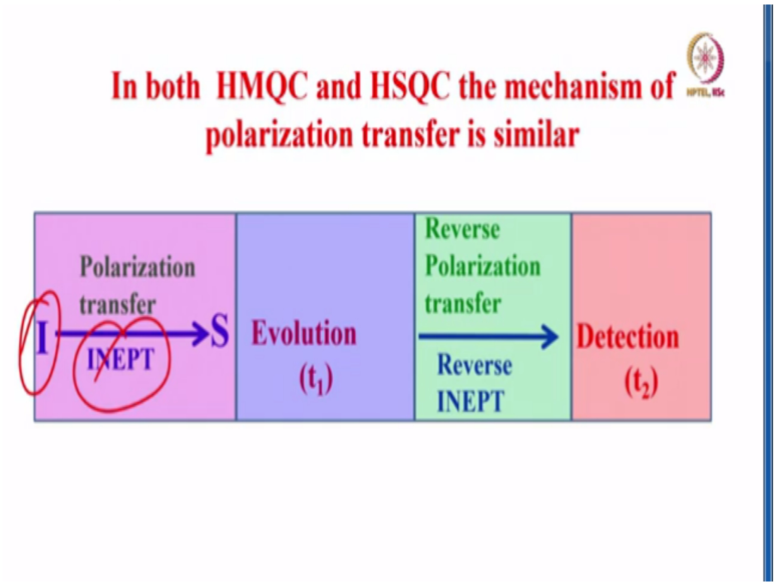

In both HMQC and HSQC the mechanism of polarization transfer is similar

Polarization transfer

I → S

INEPT

Evolution (t_1)

Reverse Polarization transfer

Reverse INEPT

Detection (t_2)

In both HMQC and HSQC the mechanism of polarization transfer is identical; HMQC and HSQC all are identical. What we do is? We take I spin do the polarization transfer using INEPT to S spin, allow it to evolve during time t1, then do the reverse polarization, using reverse INEPT, give the magnetization from carbon to proton back; and start detecting proton during the detection period. This is the basic pathway that is adapted for HMQC and HSQC experiments.

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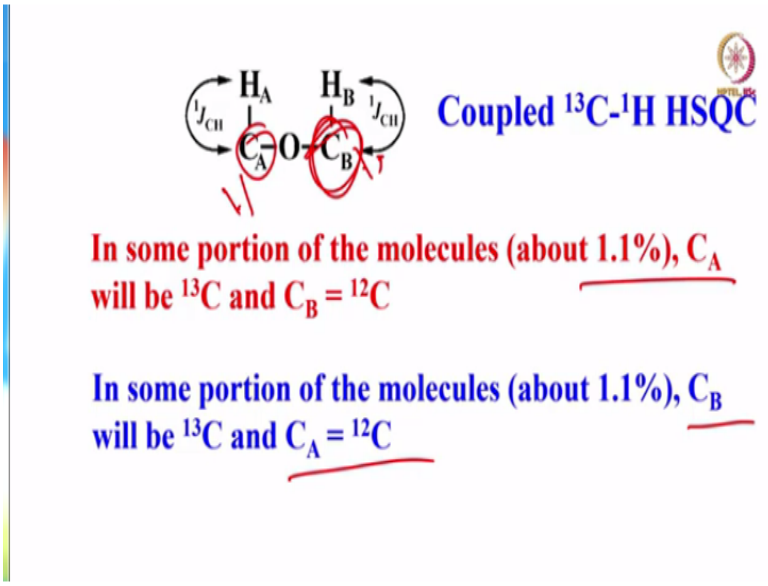

Coupled ^{13}C-^{1}H HSQC

In some portion of the molecules (about 1.1%), C_A will be ^{13}C and C_B = ^{12}C

In some portion of the molecules (about 1.1%), C_B will be ^{13}C and C_A = ^{12}C

These experiments can be both coupled HSQC, coupled HMBC, decoupled HSQC or decoupled HMBC. You can see that carbon proton are coupled in the 2D experiment; and they are also decoupled. When they are decoupled it very easy you can directly get 1 peak, 1 peak will be there go vertically or horizontally both you can identify chemical shift of proton and carbon. But in the coupled version there is multiplicity also, you can do that you can get information like that also.

Take a molecule like this. Now, you please understand, that is why I introduced satellites, carbon 13 has 1% abundance. Now in a molecule like this, you always see there is 1% of the molecule here, which is carbon A is in carbon 13 state, carbon B is in carbon 12 in some portion of the molecules. And in some portion of the molecules, carbon B is in carbon 13 state and carbon A is in carbon 12. So this is 1%, this is 1%. So, the probability and when I explained the carbon 13 remember I said these are 2 different isotopomers, and you get the superposition of the spectrum of two isotopomers in carbon 13 NMR; that is what I said. So, this is what it is.

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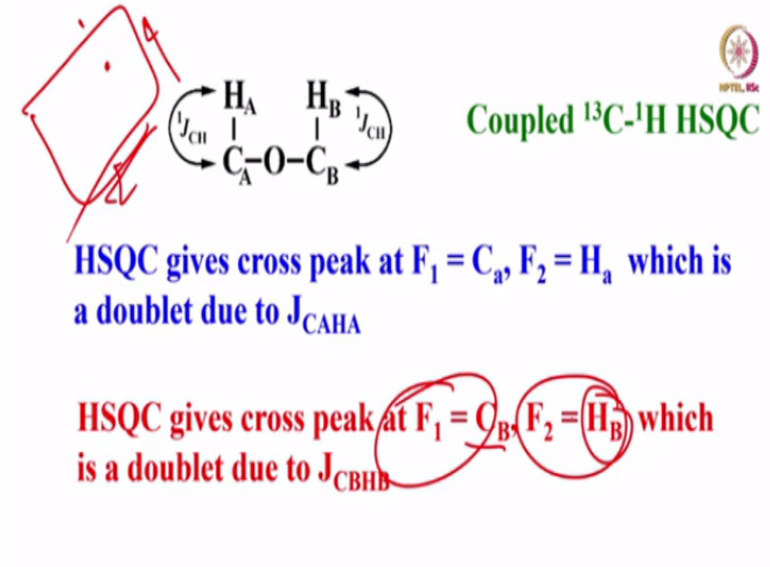

Coupled ^{13}C-^{1}H HSQC

HSQC gives cross peak at $F_1 = C_a$, $F_2 = H_a$ which is a doublet due to J_{CAHA}

HSQC gives cross peak at $F_1 = C_B$, $F_2 = H_B$ which is a doublet due to J_{CBHB}

Now we see a coupled HSQC spectrum. In the HSQC spectrum what will happen is, there is a cross peak in the F1 dimension; this is the HSQC spectrum, there is a cross peak in this F1 dimension, this is F2. In F1 dimension let us say this is carbon A which is directly bonded to carbon B. You may see this spectrum, instead of drawing, you will see in the next slide. HSQC gives a peak at F1 for carbon B.

And it correlates for proton in the F2 dimension, which is a doublet because of carbon HB coupling. See look at this molecule in the F1 dimension, you are going to have a peak here, let us say there is a peak here. But in the F2 dimension actually it is not a single peak, in this axis is only one peak is there, but in this axis, there are 2 peaks. That doublet comes because of the coupling of carbon with protons. In the natural abundant carbon 13, it is OK, it is directly observed. But in the proton dimension how it will come? It will come as satellites, that is why I introduced satellites before.

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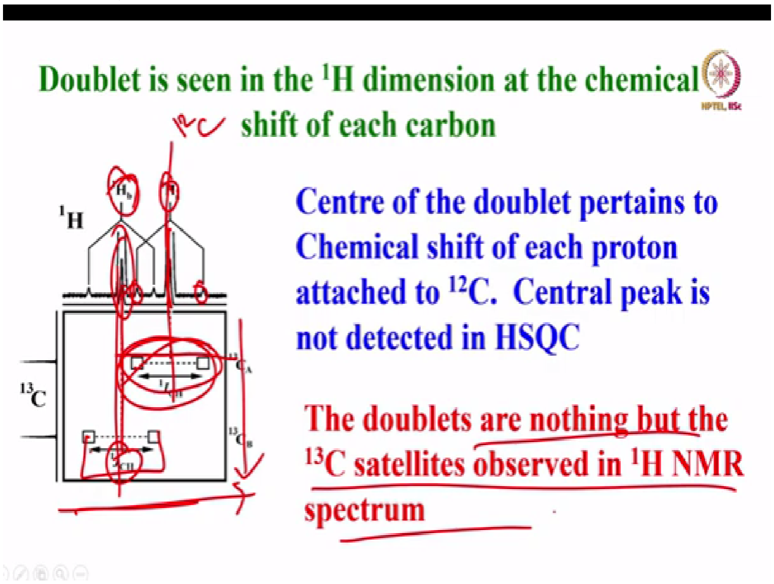

Doublet is seen in the ^{1}H dimension at the chemical shift of each carbon

Centre of the doublet pertains to Chemical shift of each proton attached to ^{12}C. Central peak is not detected in HSQC

The doublets are nothing but the ^{13}C satellites observed in ^{1}H NMR spectrum

So, look at the spectrum of this molecule, doublet is seen in the proton resonance, this is the carbon dimension, this is the proton dimension. In the proton dimension you get a single peak that corresponds to carbon attached to 12 carbon, a strong peak, but there are two chota peaks here, these are satellites, these satellites are coming because of coupling of carbon to proton; carbon 13 to proton which splits into a doublet.

Similarly, for the Hb proton, there is a peak here, exactly the centre correspond to carbon 12 attach the proton. Whereas, in the F2 dimension here, in this dimension you get a doublet this is corresponding to coupling between carbon and proton Hb; proton attach to the carbon B is going to splits this into a doublet. Here proton attached to carbon A splits this into a doublet. Through coupled HSQC you will see the coupling pattern in the F2 dimension and a single peak for each carbon in the F1 dimension.

Of course, you can interchange the dimension also. There are several experiments like that. I am taking this simple experiment which is commonly employed. So, centre of the doublet here correspond to the chemical shift here, this centre corresponds to chemical shift of carbon B, so doublets are nothing but carbon 13 satellites observed in the proton spectrum. Remember that is what I explained to you, the satellites in the proton spectrum.

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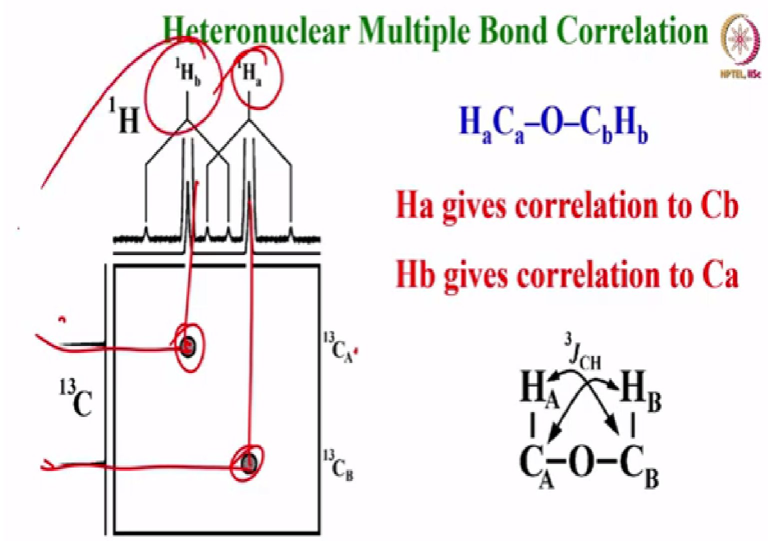

Heteronuclear Multiple Bond Correlation

H_aC_a–O–C_bH_b

Ha gives correlation to Cb

Hb gives correlation to Ca

Now, in the case of HMBC something more interesting will happen, here it is a long 2 bond correlation, 3 bond correlation. Now this carbon correlates to proton here, Ca, so carbon b here, there is a peak here in this dimension correspond to carbon chemical shift, but it is correlation with this carbon B. So, the correlation for the carbon B is with proton a; not with proton b. Remember in the previous example, the correlation was with directly bonded proton. But here in this case, it is for remotely bonded; this is carbon A you can see this is a chemical shift of carbon A, whereas if you go vertically up it is correlated to proton b, whereas proton b? it is here. So, this is a 2 bond correlation, this is a HMBC. HMBC gives correlation with the next proton attached to next carbon, not for directly bonded proton. Similarly look at this carbon B, you go go horizontally like this, you get carbon 13 chemical shift, of carbon B. But if you go vertically up, you get you do not get carbon B but you get carbon A. See here carbon B gives correlation to proton A; carbon A gives correlation to proton B understand. The very interesting thing you should observe, when you are looking at the heteronuclei here. This is a heteronuclear correlation. Let me just quickly say because it is very important experiment, which every time people will adapt. The chemical shift of carbon Carbon B, in this direction carbon 13 chemical shift, in this dimension we will get proton chemical shift. But it is not proton b, but proton a. Similarly, here carbon 13 which is carbon A, this gives carbon A chemical shift. If you go vertical here you do not get proton a, you get proton b chemical shift. That is the advantage here.

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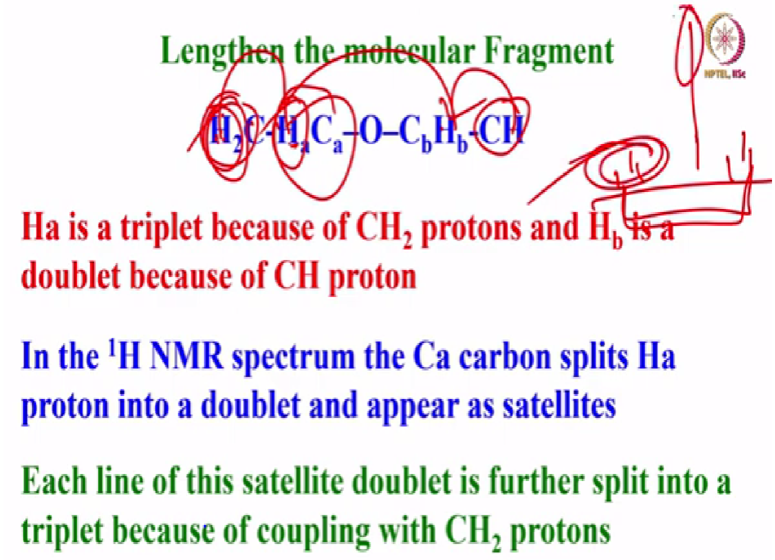

Lengthen the molecular Fragment

$H_2C\text{-}H_aC_a\text{-}O\text{-}C_bH_b\text{-}CH$

Ha is a triplet because of CH_2 protons and H_b is a doublet because of CH proton

In the ^1H NMR spectrum the Ca carbon splits Ha proton into a doublet and appear as satellites

Each line of this satellite doublet is further split into a triplet because of coupling with CH_2 protons

So, it gives you multiple bond correlation. You might ask me what will happen if I keep on increasing the length of the molecule. Now I bring CH proton; another interesting thing happens here, this proton to this proton coupling is not there, it is practically very small. Here these two protons can couple to this, this CH2 proton is coupled with this, it will become a triplet; and this will coupled to this it will become a doublet. Similarly, this will couple to this it will be a doublet, this will couple with this it will be a doublet. All those things are known; That is what we discussed. So, now Ha is a triplet because of CH2 protons and in the 1H NMR spectrum what you are going to get in the satellites, carbon directly coupled with this proton. ok let us consider this carbon, this carbon directly coupled to this proton in the HSQC it will be a doublet but now, this proton is coupled to this, it will be triplet, satellite will give rise to the proton couplings.

Remember the previous course, we discussed about the satellite analysis, how to extract the coupling among equivalent protons, everything we discussed. So, here the multiplicity pattern is seen in the satellites, it is because of the coupling of proton with proton, this doublet separation is due to coupling between carbon and proton. But the multiplicity in these satellites is because of coupling between protons. Here coupling between proton and carbon, It is coupling between protons and this C12 attach, this is C13 attach satellite.

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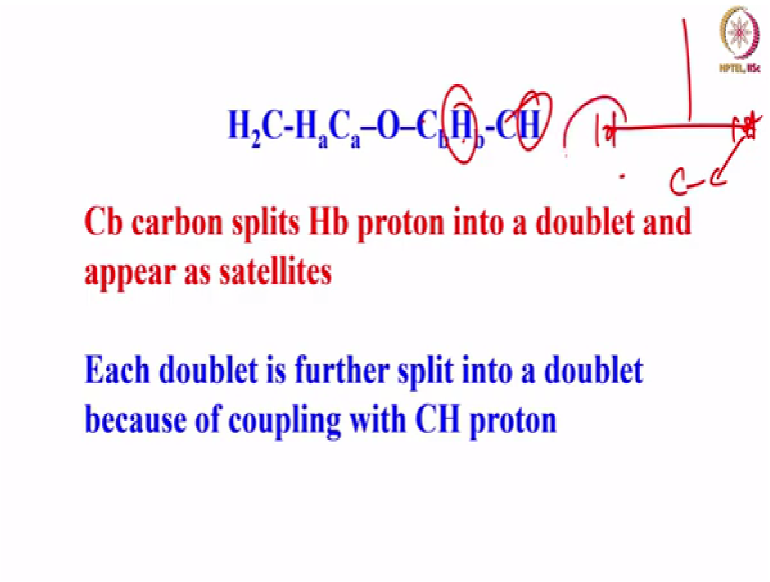

$H_2C\text{-}H_aC_a\text{-}O\text{-}C_bH_b\text{-}CH$

Cb carbon splits Hb proton into a doublet and appear as satellites

Each doublet is further split into a doublet because of coupling with CH proton

So, this is what it is. Similarly, same thing if you look at Cb, Cb will be a doublet because of this satellite. and each layer of satellite will be a doublet because of coupling with this proton. Very interesting thing will happen. Now, if you want to correlate for the long range HMBC what will happen? We will come to that later. But please remember, in the satellite spectrum satellite you can see the coupling with the next proton.

Carbon which is attached to the next directly bonded carbon is there, the proton which is attached to the next carbon, that will couple with this proton. So, 2 bonds coupling that 3 bond couplings can also be seen. Those proton-proton couplings are seen in this satellite spectrum.

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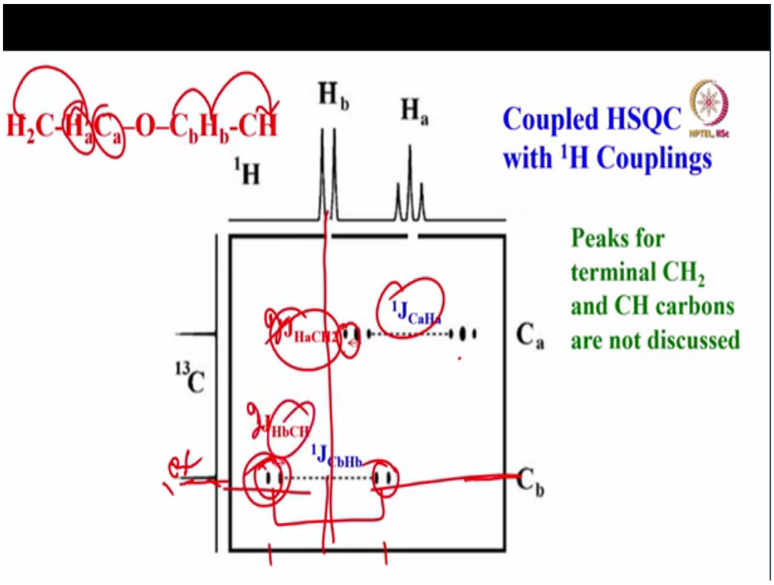

$H_2C\text{-}H_aC_a\text{-}O\text{-}C_bH_b\text{-}CH$

Coupled HSQC with ^1H Couplings

Peaks for terminal CH_2 and CH carbons are not discussed

Now a coupled HSQC with proton coupling. Let us see how the spectrum comes, look at it this, what it is? This is now carbon 13 go along this axis this is carbon 13 chemical shift of C

a. But this is F1 axis, in the = F2 axis what is happening is? In this dimension you have only one chemical shift along this, but in this dimension you will see 2 here, this is a triplet, this is a triplet. Exactly the centre of this corresponds to chemical shift of proton a.

But each of the satellites you see as triplet, why they are triplets? This carbon is a doublet here because of this large coupling, and each line of the doublet is a triplet because of coupling with two protons. As a consequence, the satellite spectrum is a triplet, each satellite is a triplet, you understand. Now, we go back to this carbon b, carbon b is very easy to understand again, it is very simple, look at this carbon b.

If you look at the carbon b now this is the F1 axis only one chemical shift for this, go along this axis you will get carbon 13 chemical shift F 1 axis. But now, in the F2 axis, we have 4 peaks doublet here; and doublet here. The centre of this exactly corresponds to chemical shift of proton, why these 2 are doublets, these 2 are satellites one bond coupling with carbon gives rise to this separation, and the each line of satellite is a doublet because of its coupling with neighbouring proton, this is how coupled HSQC works.

This is because 1 bond JCaHa and this separation correspond to 2 bond HaH2. Similarly, here as 1 bond, J CbHb here, 2 bond HbCH here, this is 2 bond coupling.

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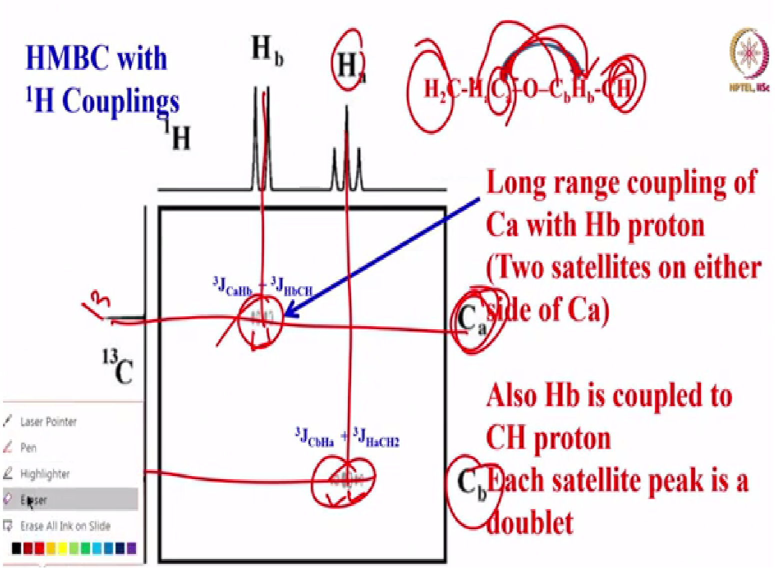

HMBC with ^1H Couplings

Long range coupling of Ca with Hb proton (Two satellites on either side of Ca)

Also Hb is coupled to CH proton Each satellite peak is a doublet

So, now what will happen to HMBC? There is something interesting. HMBC what it does is; this is the chemical shift of carbon a along this axis, but it is given correlation to Hb. The carbon a is given correlation to Hb here. This Hb is coupled to this proton and is a doublet. So, again what you saw the doublet at the site of carbon a; you see this one because it is correlated to Hb; carbon a is correlating with the proton b and it is going to be doublet because it is adjacent proton.

Similarly, carbon b correlation with proton a. See this is carbon b chemical shift ,this carbon a. And each line of this carbon a, will be a triplet because of coupling with 2 protons. So, you will see 2 triplets here, overlapped. See the long range couplings are smaller it is not clearly visible; this what is going to happen. So, this is the thing you should remember, what is going to happen is in the case of this on,e see this is a long range correlation here for this case.

The long range correlation here, is the coupling of carbon with proton, two satellites on either side, also Hb is coupled to CH proton. Each satellite peak is a doublet, that is for this one.

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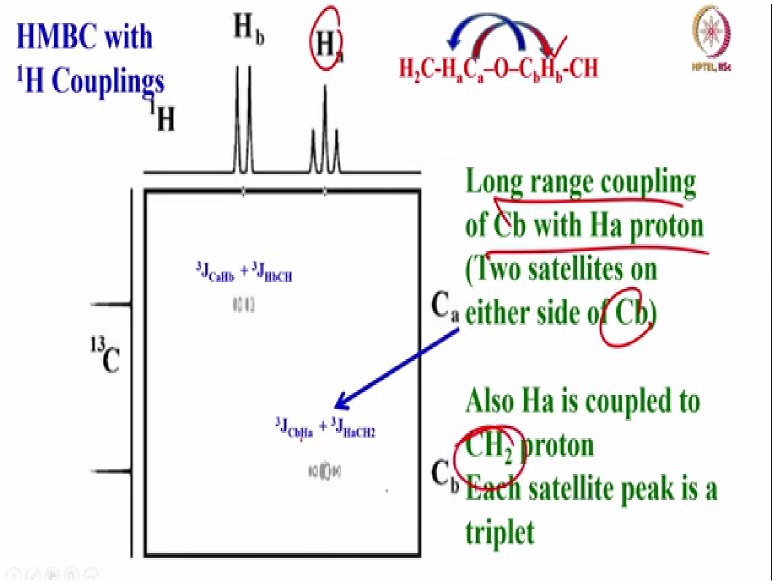
HMBC with ^1H Couplings



Long range coupling of Cb with Ha proton (Two satellites on either side of Cb)

Also Ha is coupled to CH_2 proton Each satellite peak is a triplet

Now, for this one long range correlation to see Cb proton Ha, 2 satellites on either side because of Cb and then each of them is a triplet because of coupling with CH2 protonz; this the way we have to understand.

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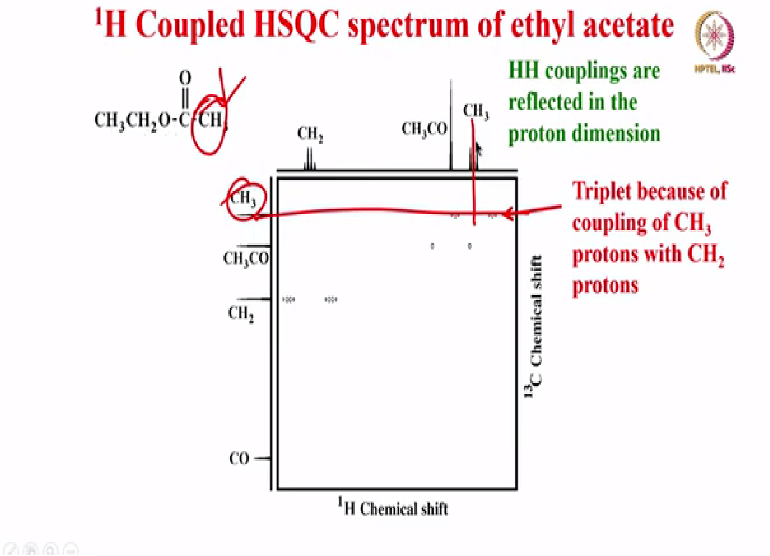

^1H Coupled HSQC spectrum of ethyl acetate

HH couplings are reflected in the proton dimension

Triplet because of coupling of CH_3 protons with CH_2 protons

Now simple analysis or interpretation of a spectrum like this; This is the proton coupled HSQC spectrum of ethyl acetate, here we have CH3 CH2 CH3CO. Look at this one, CH3 here. This is, not correlated to anything only for this carbon. So, this is CH3 carbon; and along the centre of this if you go you get this proton chemical shift; and on either side it is a triplet because this is actually this carbon, because this is coupled with these 2 protons, these I am sorry, this is not this carbon CH3; this is this CH3, because of this CH3, What I was telling, this is not this CH3.

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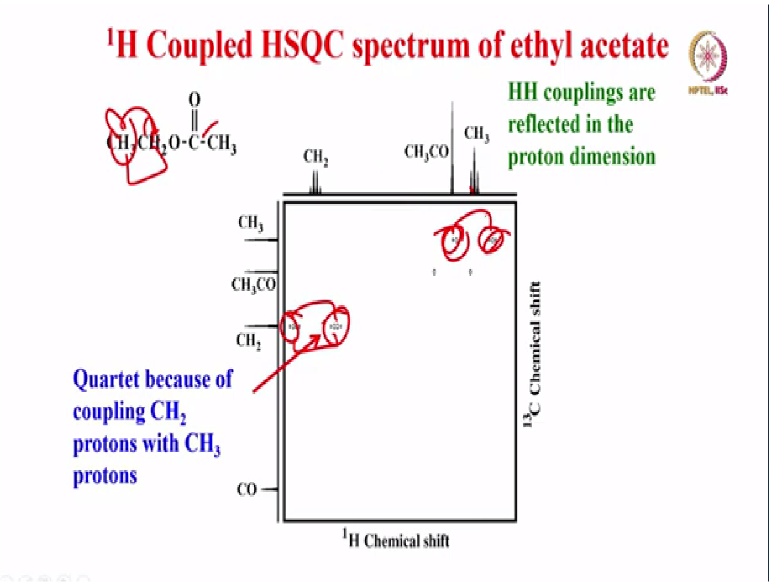

^1H Coupled HSQC spectrum of ethyl acetate

HH couplings are reflected in the proton dimension

Quartet because of coupling CH_2 protons with CH_3 protons

Now it is coupled to this proton. So, it is a triplet on either side. One bond coupling is there between this carbon and the CH3 and 2 bonds proton coupling makes it a triplet. Now coming to the CH2, CH2 one bond coupling is here; this is CH coupling. And each of them is a quartet because it is coupled to CH3 and CH2 is coupled CH3 is a quartet; and CH3 is coupled CH2 is a triplet. That is what you are seeing proton-proton couplings. whereas, if you come to CO carbon. This is CH3 protons directly coupled to this; this is not CO, I think this is CH3CO

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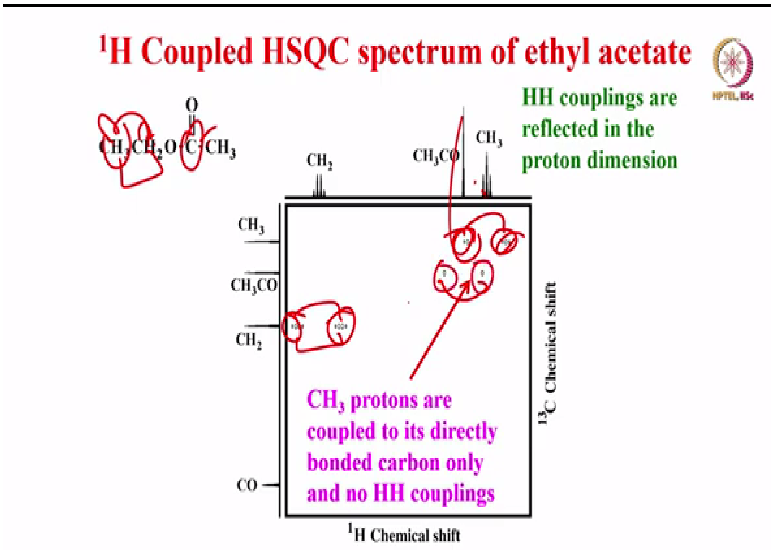

^{1}H Coupled HSQC spectrum of ethyl acetate

HH couplings are reflected in the proton dimension

CH_3 protons are coupled to its directly bonded carbon only and no HH couplings

CO cannot give, because there is no direct attachment. You will see that only in HMBC. This is CH3. This is coupled to this proton; and there is no proton-proton coupling; satellites are only two singlets.

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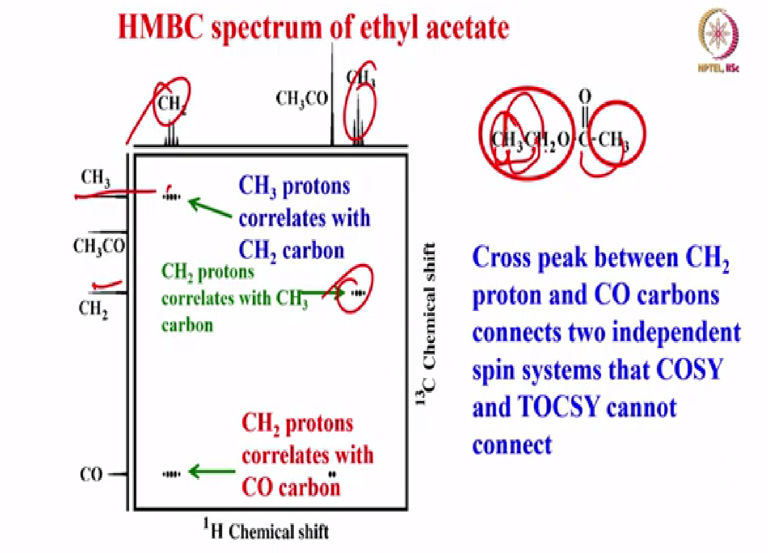

HMBC spectrum of ethyl acetate

CH_3 protons correlates with CH_2 carbon

CH_2 protons correlates with CH_3 carbon

CH_2 protons correlates with CO carbon

Cross peak between CH_2 proton and CO carbons connects two independent spin systems that COSY and TOCSY cannot connect

This is what it is. The same thing if you wanted to see the HMBC; this is the CH3, it is correlated to this proton, this CH3 is correlation to CH2 proton, this is CH3 carbon. Similarly, CH2 carbon correlated to CH3 proton, and each time we are going to get long range coupling; carbon correlation to this proton, and this carbon correlation to this proton; but these proton-proton couplings are seen.

Similarly, if you come to this one; this is CO carbon, this can have long range correlation with this CH3 protons or CH2 proton, either of them. So, this how HMBC has to be interpreted; the interpretation of HMBC is correlation to the next neighbour.

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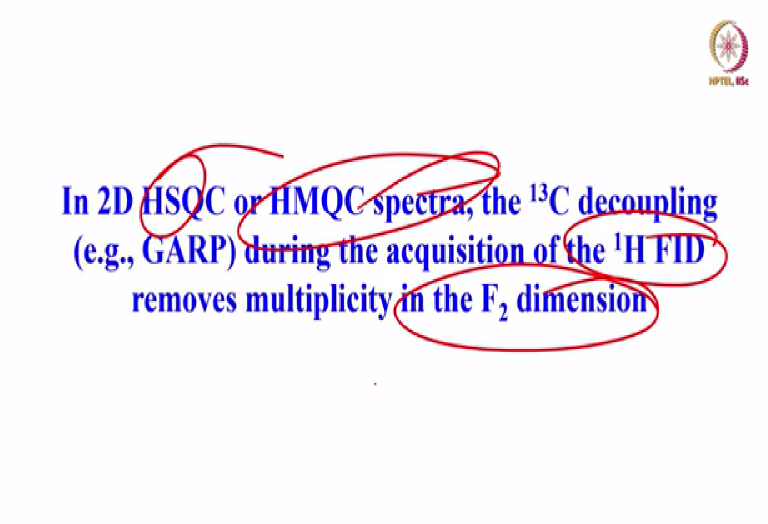

In 2D HSQC or HMQC spectra, the ^{13}C decoupling (e.g., GARP) during the acquisition of the ^{1}H FID removes multiplicity in the F_2 dimension

So, in 2D HSQC or HMBC spectra the carbon 13 decoupling during the acquisition of the FID removes multiplicity in the F1; in the F2 dimension you will get singlet.

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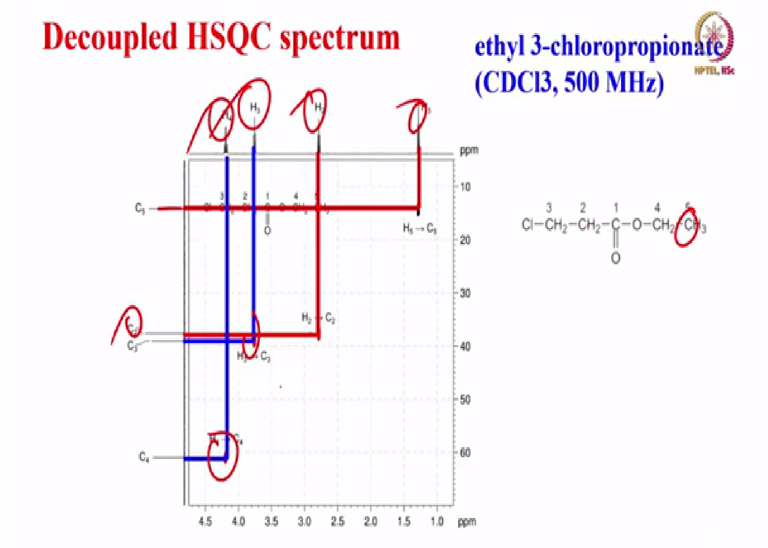

Decoupled HSQC spectrum

ethyl 3-chloropropionate (CDCl3, 500 MHz)

In fact in both the dimensions one can do the decoupling and you get a fully decoupled spectrum, where direct correlation is possible. Look at this one, here proton 5 is correlated to carbon 5. Here, carbon 2 is correlated to proton 2, here carbon 3 is correlated to proton 3, carbon 4 is correlated to proton 4. It is a decoupled HSQC. So, direct correlation, no couplings no messy things are there.

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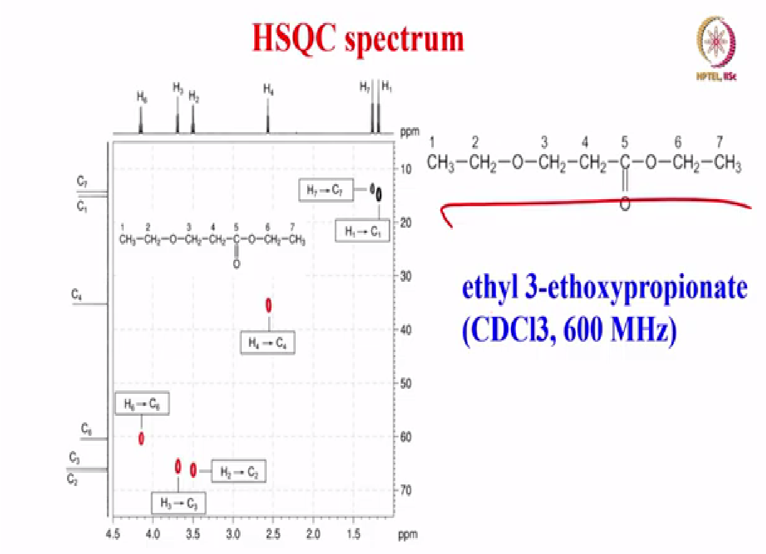

HSQC spectrum

$\overset{1}{CH_3}-\overset{2}{CH_2}-O-\overset{3}{CH_2}-\overset{4}{CH_2}-\underset{\|\atop O}{\overset{5}{C}}-O-\overset{6}{CH_2}-\overset{7}{CH_3}$

ethyl 3-ethoxypropionate (CDCl3, 600 MHz)

Similarly go to the HSQC spectrum the same molecule which you saw earlier for COSY and TOCSY; look at this, here is HSQC spectrum.

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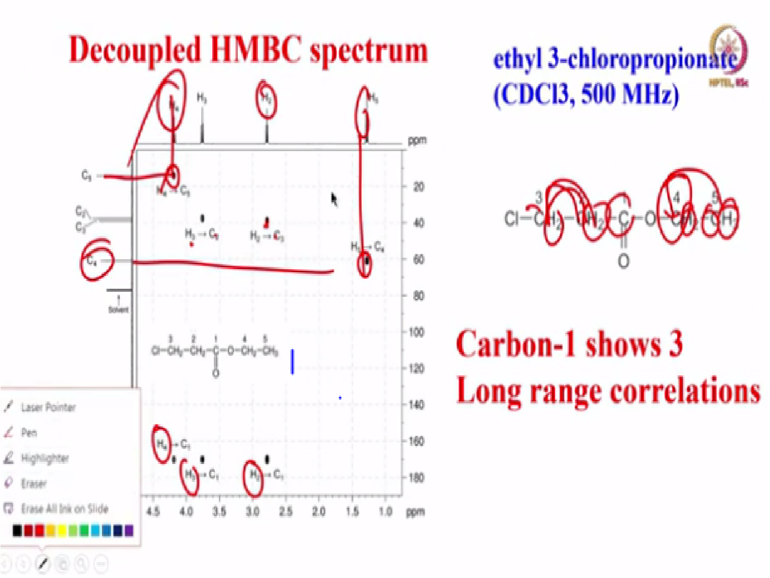

Decoupled HMBC spectrum

ethyl 3-chloropropionate (CDCl3, 500 MHz)

$Cl-\overset{3}{CH_2}-\overset{2}{CH_2}-\underset{\|\atop O}{\overset{1}{C}}-O-\overset{4}{CH_2}-\overset{5}{CH_3}$

Carbon-1 shows 3 Long range correlations

Of course, this the expanded version we will see. This is the decoupled HMBC; this was HSQC now, we will see HMBC. HSQC you already saw that. We should see the HMBC. Come to this proton 4 isolated, this proton 4 is coming here, it is correlated to carbon 5. So, otherwise in this dimension carbon 5 is correlated to proton 4. Now, this proton 5 should correlate to carbon 4; look at this is proton 5, this correlated proton 4; very easy.

Similarly, you can look at all other things. Very interesting that this carbon 1 has a correlation to 3, 4 and 2. It has correlations to proton 2, proton 3 and proton 4, because there is a long range coupling for this. Like this, you can start analyzing the HMBC spectrum. Look at this one proton 2, is correlating the carbon 3; see this proton 2 is correlating to carbon 3, similarly carbon 2 is correlated to this and carbon 3 is correlated to this; long range correlations can be very easily seen here.

Look at this one especially 2 and 3 I want to show you again, because very important to see the long range correlation, sometimes it is very confusing to understand. So, let me repeat it. Carbon 3 is correlated to proton 2 long range here, see proton 2; and this is carbon 3. Proton 3 here now correlated to carbon 2 here, it is a proton 2 is correlated to carbon 2 here like that, you can understand how easily you can start interpreting the HMBC spectrum.

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2D-INADEQUATE

Incredibe Natural Abundance DoublE QUAntum Transfer Experiment

So, with this we have completed heteronuclear correlation experiments. But another very interesting experiment which I want to tell you is a very complex experiment, it is very time consuming experiment, it is called 2 dimensional INADEQUATE. INADEQUATE stands for Incredible Natural Abundance DoubLE QUAntum Transfer Experiment. It is called 2D INADEQUATE experiment. Why do we use this? It is the correlation experiment; homonuclear correlation. Then you ask a question homonuclear correlation already we have seen two experiments COSY and TOCSY. Why are we bringing in other experiment?

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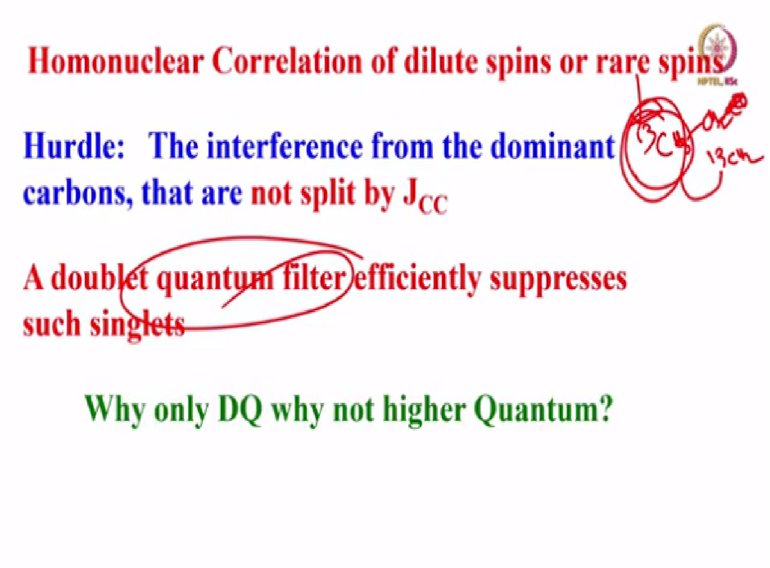

Homonuclear Correlation of dilute spins or rare spins

Hurdle: The interference from the dominant carbons, that are not split by J_{CC}

A doublet quantum filter efficiently suppresses such singlets

Why only DQ why not higher Quantum?

This correlates dilute spins or rare spins. What do you mean by that? take for example, CH3 CH2 OH. If I want to correlate this carbon to this carbon chemical shift, it is not easy, it is very difficult. only 1% of this will be in carbon 13 state and this is 1% in carbon 13 state. When we discuss carbon 13 we discussed these things. The probability of seeing both in carbon 13 is 1 into 10000; very difficult. And we have to correlate that. When you are trying to correlate, there is a very big hurdle. What is the big hurdle you are getting? You are getting the interference of the dominant carbons that are not split by JCC. For example, there are independent isotopomers, remember, carbon 13 spectrum as I said earlier is a superposition of the spectrum from individual isotopomers, and carbon 13 -carbon 13 coupling you are going to see from one of the isotopomers, where both the carbons are in carbon 13 state, they will be split by J coupling. Whereas individual carbons CH3 CH2 OH if you take, this is only one isotopomer, this is another isotopomer and only this carbon 13 is dominant here, there is no coupling; and these peaks are much stronger compared to carbon 13 carbon 13 doublet; J split doublet, the biggest interference coming because of the dominant carbons from carbon 13 state, the single carbons, which are not split by the neighbouring carbon.

So, how do you eliminate that? We have to subtract that; only then we are going to get this long range correlation. I mean correlation among dilute spins is possible. For this, we have to use a double quantum filter, we can filter out all these things. When we use a double quantum filter, what will happen? all singlets will be removed. Remember what we discussed in the DQF-COSY, there was a double quantum filter, all singlets, references peaks, all get eliminated.

So, use double quantum, then all these carbon 13 peaks which are unsplit, which are not split by J coupling get eliminated. Then you get peaks only from two carbons which are coupled between themselves. Now the question you may ask me is, why only double quantum? Why cannot we use triple quantum or higher quantum?

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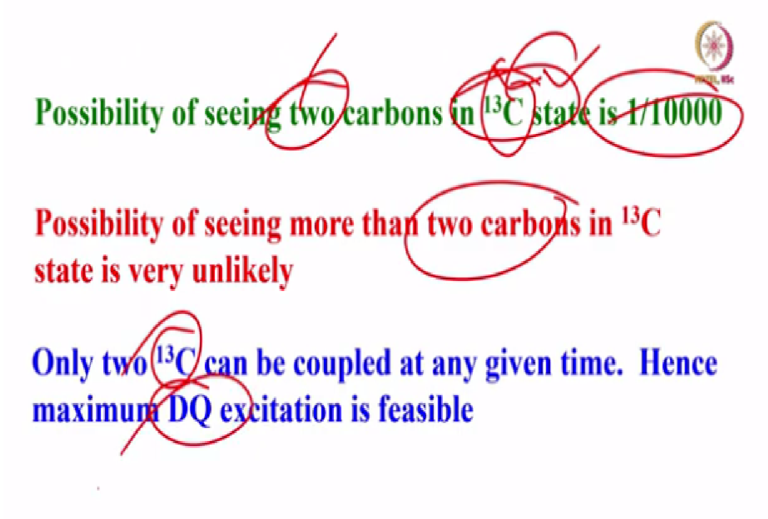

Possibility of seeing two carbons in ^{13}C state is 1/10000

Possibility of seeing more than two carbons in ^{13}C state is very unlikely

Only two ^{13}C can be coupled at any given time. Hence maximum DQ excitation is feasible

For that you should know just now, I said possibility of seeing 2 carbons in carbon 13 state is 1 in 10000. If you have 10000 molecules only 1 molecule can have a possibility of both the carbons in carbon 13 state; and very remote probability when you have more carbons to see many of them simultaneously in the carbon 13 state. So, probability is only these 2 carbons in carbon 13 state. So, if I use double quantum, then I can filter out all singlets and I can extract only coupling among these carbons, very easy.

So that is why DQ filter is used and anything about it is not needed. Only DQ carbon 13 to carbon 13 coupled at any given time is useful. So, DQ, maximum is enough to subtract this one.

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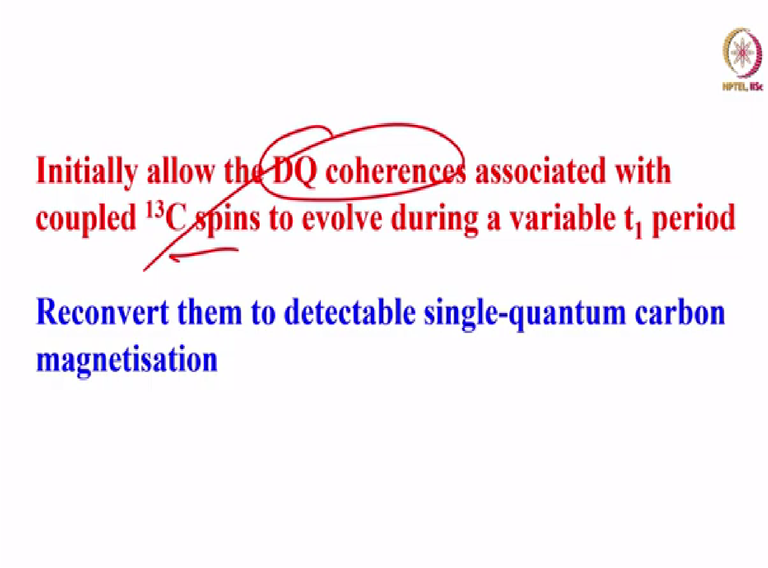

Initially allow the DQ coherences associated with coupled ^{13}C spins to evolve during a variable t_1 period

Reconvert them to detectable single-quantum carbon magnetisation

So, what we have to do in this experiment is, initially we allow the DQ coherences associated with the coupled spins to evolve during a variable time similar to COSY and other DQF-COSY, you have we seen that. And then after some time, we reconvert the DQ coherence into detectable single quantum; that is what we did in DQF-COSY. Identical you can do here also, but it is for natural abundant dilute spins.

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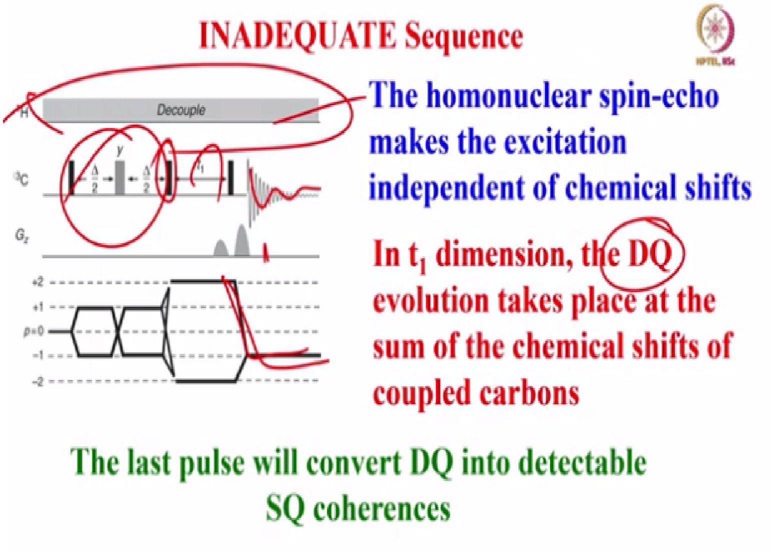

INADEQUATE Sequence

The homonuclear spin-echo makes the excitation independent of chemical shifts

In t_1 dimension, the DQ evolution takes place at the sum of the chemical shifts of coupled carbons

The last pulse will convert DQ into detectable SQ coherences

So, this is INADEQUATE sequenc. This like a sequence where homonuclear spin-echo is there. It makes excitation independent of the chemical shifts. So, you do not have to worry about chemical shift evolution here; they get refocused. You know homonuclear spin-echo chemical shift get refocussed, but not J couplings, that is what we have been discussing. Whereas in the t1 dimension, the DQ evolution takes place. After the next pulse the multiple quantum evolution takes place, Use a DQ filter from +2 to -1; using the phase cycling or gradients; and then choose this pathway to convert. That means you are converting DQ coherence to single quantum coherence, and then start detecting this while doing the decoupling. The advantage is you can continuously do the decoupling right from the beginning till the end. The advantage of that decoupling power is it can not only decouple, but also gives rise to the NOE enhancement. Please remember NOE as heteronuclear case I discussed. I said there is a possibility if you are decoupling proton and observe carbon, we saw that about 200% gain in the intensity. We saw, there is the enhancement of the signal intensity, because of NOE.

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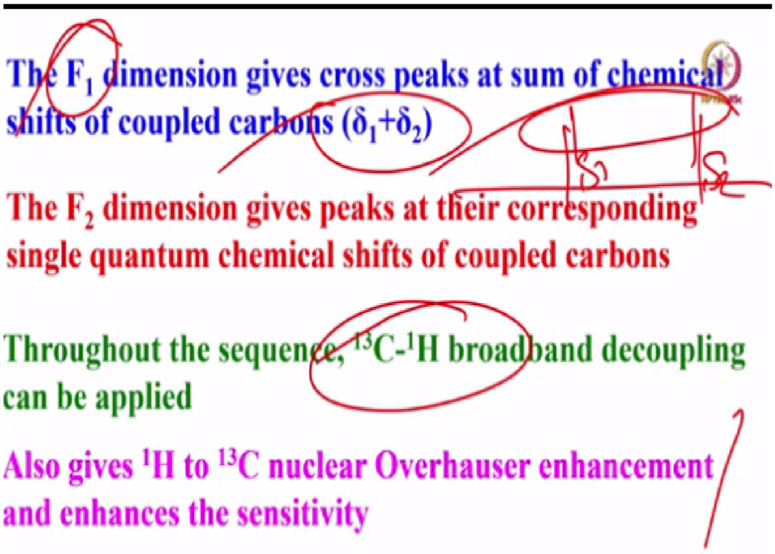

The F_1 dimension gives cross peaks at sum of chemical shifts of coupled carbons $(\delta_1+\delta_2)$

The F_2 dimension gives peaks at their corresponding single quantum chemical shifts of coupled carbons

Throughout the sequence, ^{13}C-^{1}H broadband decoupling can be applied

Also gives ^{1}H to ^{13}C nuclear Overhauser enhancement and enhances the sensitivity

So, in the F1 dimension what happens is, you get cross peaks at this sum of two chemical shifts. We are looking for double quantum filter. There are 2 carbons and the chemical shift is 1 and other is 2. In the direct dimension you get two peaks individually, in the double quantum dimension, they come up with sum of the chemical shifts. F2 dimension gives two peaks at their corresponding chemicals shifts; and in F1 dimension, we get peaks at the sum of the chemical shifts.

So, throughout the sequence, carbon 13 proton decoupling is applied to get the NOE enhancement and also broadband decoupling of all carbons attached to it all the protons.

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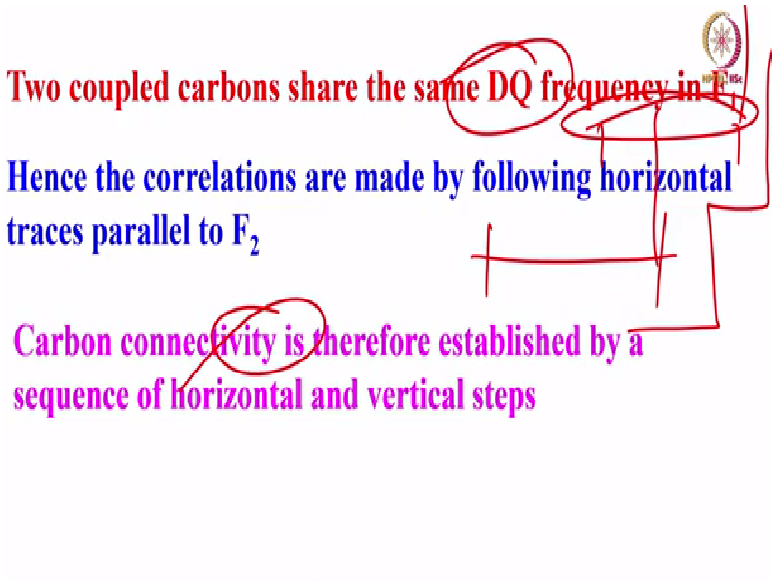

Two coupled carbons share the same DQ frequency in F_1

Hence the correlations are made by following horizontal traces parallel to F_2

Carbon connectivity is therefore established by a sequence of horizontal and vertical steps

So, now biggest thing you should understand is the two carbons coupled with the same double quantum frequency, the two carbons are there, they have two carbon chemical shift positions, but in the F1 dimension, they share the same DQ frequency in the F1. Hence, you can make the correlation by horizontal traces go horizontally find out which are the 2 which are coupled that give rise to double quantum and then go vertically up you will see the correlation. The carbon connectivity you can establish by sequence of horizontal vertical, horizontal vertical, like a step ladder keep on going horizontally and vertically you will identify all the carbons which are coupled among themselves. The connectivity you can establish. Remember two coupled carbons, I am taking the example in the F2 dimension two individual chemical shifts in the F1 dimension they come up with sum of the chemical shifts. And go horizontally because two carbons share the common double quantum frequency, identify two carbons along the F2 dimension; go vertically up, you will find out the coupled carbon, go vertically up from horizontal again you will find another carbon which is coupled to that.

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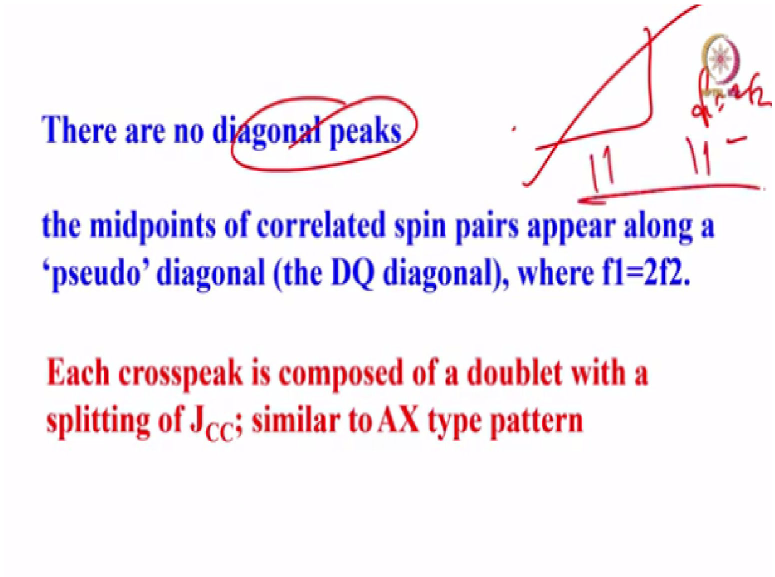

There are no diagonal peaks

the midpoints of correlated spin pairs appear along a 'pseudo' diagonal (the DQ diagonal), where f1=2f2.

Each crosspeak is composed of a doublet with a splitting of J_{CC}; similar to AX type pattern

Like that you can keep on going through this ladder, step by step ladder horizontally vertically, you can identify all the connectivity. You can establish all the connectivities. Again here, though it is homonuclear, remember, there are no diagonals here, diagonal peaks are totally absent here, unlike COSY, TOCSY. But one thing when we are talking about double quantum, very interesting thing happens. I will show when you see the F2 dimension and F1dimension.

If you draw a line exactly at the centre of the doublets along the F2 dimension, you will get a diagonal; and that is called a pseudo diagonal, where if you take the slope of it, for example, if you do like this, if you take a slope here, it is F1 = 2F2. Slope is 2, that is why so you are going to get a double quantum diagonal in this case. I will show you the spectrum of it how it is and each peak doublet correspond to AX type pattern.

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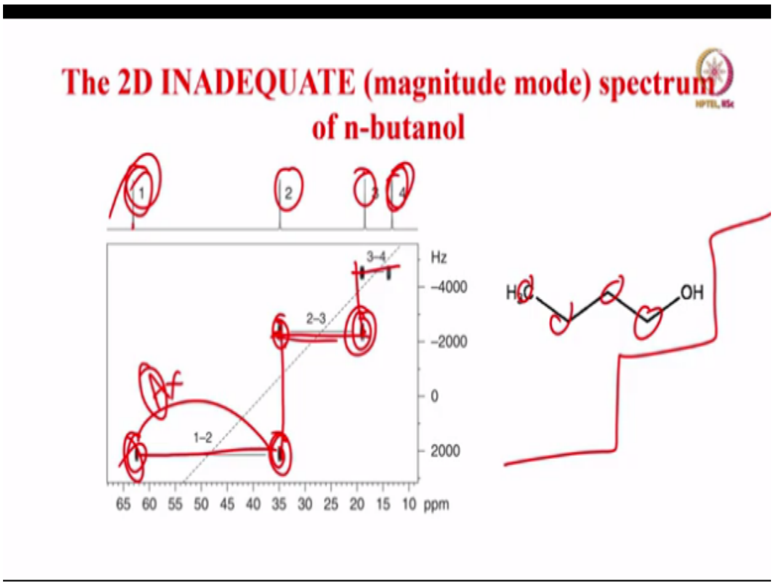

The 2D INADEQUATE (magnitude mode) spectrum of n-butanol

We will see the spectrum now, it is the simple molecule. Now, how many carbons are there? 1, 2, 3 and 4, these are already identified for you. The carbons 1, 2, 3 and 4. Normally, your idea is to identify the direct connectivity between carbons 1 to 2, 2 to 3, 3 to 4, you have to identify carbon connectivity like proton connectivity, we did. So, what we do is, I know this has been already identified taken from the book of Timothy Claridge.

Now, look at this proton 1 which is identified, there is doublet here, its shares the double quantum frequency with another another carbon, go horizontally you identify a doublet. These two forms the AX spin system. So, AX spin system means, this is a doublet, this is a doublet. So, you got 2 doublets. Two peaks for two corresponding carbons which are coupled gives rise to AX pattern.

Now in the double quantum dimension, there is a peak at sum of the chemical shifts. Go vertically up you see one peak; OK this is 1 and 2, and 2 is here. Now horizontally you go, again this and this becomes a doublet, this is a doublet, this is a doublet; again another AX spin system 2 and 3 forms an AX spin system. So, now if you go horizontally you can identify this coupled partner, go vertically up you find another carbon; go vertically up horizontally again, you find another carbon. Like this sequentially keep going like this, you can identify each of the carbons from their connectivities. So, the 2D connectivity is always given in the magnitude mode for various reasons. And what I wanted to show you is 2D there is no diagonal, but there is a pseudo quantum double quantum diagonal.

And in the F2 dimension you get doublet corresponding to AX pattern. AX spin doublets go vertically up, identify its coupled neighbour, again go horizontally, again two coupled spin system, the AX pattern, go vertically up and again, like this keep on going ladder wise; step by step horizontal vertical, horizontal vertical you can establish the connectivity of all the carbons connected among themselves, you can establish the carbon connectivity that is the dilute spin connectivity. That is a very important experiment called 2D INADEQUATE.

There are variations of this again, so all the 2D experiments which we discussed, a number of variations are there. But what in this course I am giving is, basic introduction, basic concepts, basic ideas, how they work. Basically, how we utilize these things. For the improved version you can go back to the literature and start reading all the books. So, with this, my time is up, I am going to stop here. But I do not know whether I will be able to continue 2D again in the next class for the reason the time is up and I have to cover multiple quantum, solid state NMR and various other things. Let me see how much time is going to be left. If possible I will come back to 2D again and cover J-resolved. And then if possible NOESY and ROESY I will cover if there is a time if I will come back. Of course, J-rseloved we discussed in the last course also in depth. It is very simple. It is only the separation experiment, where you get chemical shift in one dimension, J coupling in other dimension. So, NOE another experiment, the transient experiment. Of course steady state we saw by selective irradiation of one of the peaks, and seeing the changes in the intensity, by subtraction; that is a different experiment. But the transient NOE you can do in the 2D way. I do not think I have time to cover so much now. But what we will do is, we will wait till the end of next class, I will go to multiple quantum NMR I will go to solid state NMR. Hopefully I will be left with some half an hour, 1 hour slot and 1 half an hour slot if it is available, again we will come back. Anyway you already have all the information about 2D, in just one class I will discuss about other 2D experiments. So, 2D I will not continue further, I will stop it. We will go to MQT in next class. Thank you very much.