One and Two Dimensional NMR Spectroscopy for Chemists
Prof. N. Suryaprakash
Department of NMR Research Center
Indian Institute of Technology – Bangalore

Lecture – 55
Heteronuclear Correlation and Inverse Detection

Welcome back; In the last couple of weeks we have been discussing about various 2 dimensional experiments. I have already introduced and discussed about COSY, TOCSY and other sequences, and took several examples of that.

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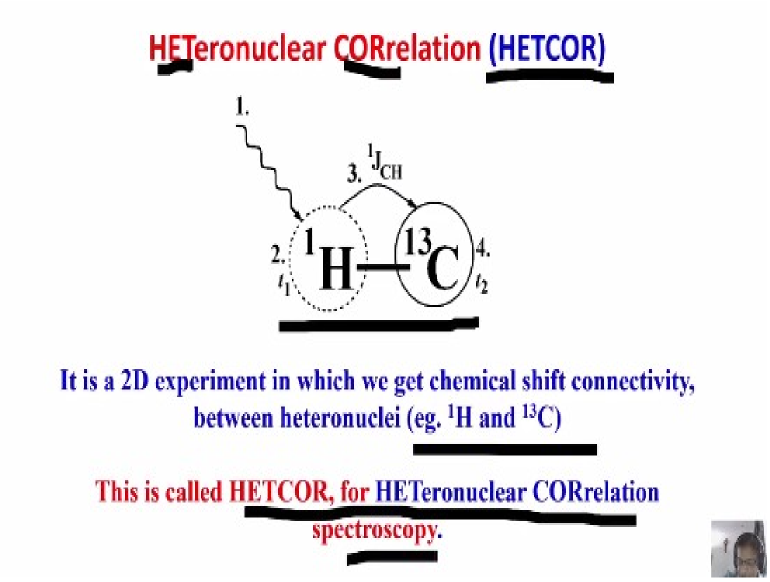

HETeronuclear CORrelation (HETCOR)

It is a 2D experiment in which we get chemical shift connectivity, between heteronuclei (eg. ${}^{1}H$ and ${}^{13}C$)

This is called HETCOR, for HETeronuclear CORrelation spectroscopy.

Today, we will go to heteronuclear correlation experiment. This is called HETCOR. It is written as HETCOR, but this I am just giving you an idea. It is a correlation experiment what we can get out of this. But remember this HETCOR nobody is going to use nowdays. The improved version of that is called HSQC; that is what people are going to use. So, we will use that and we know how to do, what to do with the HSQC later.

But just to give you the information about what HETCOR does; is this. It is another correlation experiment similar to COSY; homonuclear COSY we saw. Only thing is there you got the chemical shift connectivity among the coupled spins in the homonuclear case. where one proton is coupled to another proton through chemical bond, where there was a correlation; you got cross peaks. But here in this experiment it is a heteronuclear correlation.

For example, you consider CH3 group, the carbon is coupled to three protons. From the heteronuclear correlation I can see which carbon is connected to which proton. I can correlate the chemical shift of a particular carbon to that of a proton which it is attached to. It is a beautiful information. For example, if I make the proton assignment, if I know what are the proton chemical shifts, I can use this experiment to make the assignment of the carbons. Which carbon belongs to which proton. Only drawback is it gives correlation to protons attached to carbons. If there are quaternary carbons or non-protonated carbons, you will not be able to see that in the experiment at all. You will see only carbon peaks which are attached to protons. This is called heteronuclear correlation, or called HETCOR.

Of course, I took the examples of proton and carbon. This is not the thing which we have to do all the time. This correlation can be between any two heteronuclei; that is between abundant spin and rare spin. That is an important concept I am telling you, because here we adapt polarization transfer. We take the magnetization from one, of course not in HETCOR, but in HSQC, what we do is we take the magnetization from abundant spin give it to low abundant spin and then take it back to the highly abundant spin and detect abundant spin. So, that is why this is very important thing, you can do between abundant spin and the less abundant spin.

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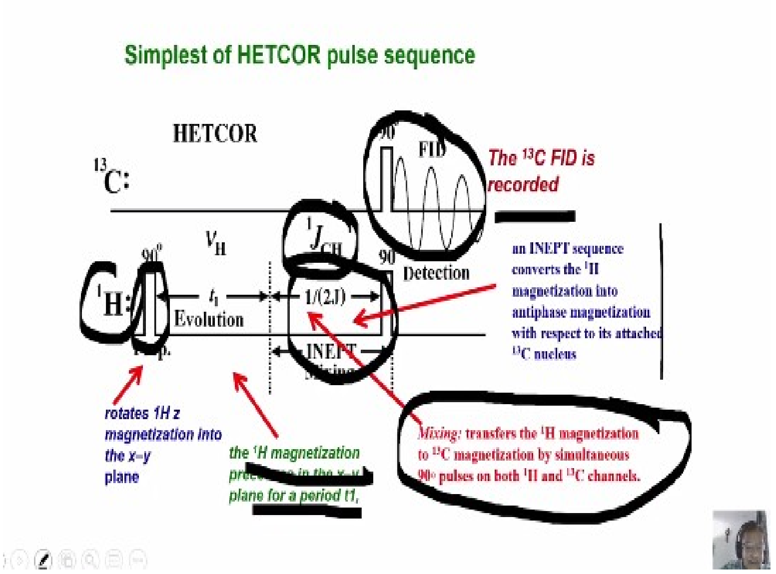

Simplest of HETCOR pulse sequence

The ^{13}C FID is recorded

an INEPT sequence converts the ^{1}H magnetization into antiphase magnetization with respect to its attached ^{13}C nucleus

rotates 1H z magnetization into the x–y plane

the ^{1}H magnetization precesses in the x–y plane for a period t1,

Mixing: transfers the ^{1}H magnetization to ^{13}C magnetization by simultaneous 90° pulses on both ^{1}H and ^{13}C channels.

But in the HETCOR, it is a simple correlation experiment, where you can detect the carbon directly. This is HETCOR sequence; very simple pulse sequence. You apply 90 degree pulse on proton; this is the preparation period; you allow it to evolve for a time t1 and in between, of course, you can also put a mixing where you are going to put INEPT polarization mixing so that polarization can be enhanced here also.

And then here 90 degree pulse bring the magnetization to xy plane. In the t1 period proton magnetization precesses, in the xy plane for a time t1. Polarization transfer takes place now you are applying a 90 degree pulse for detection; and at the same time you are applying 90 degree pulse for carbon and you start seeing the carbon by proton decoupling. This is a direct detection of carbon, remember.

Unlike, HSQC inverse experiment where abundant spin is detected. This is a direct detection of low abundance spin. This is what we are going to see. So here simply you take a 90 degree pulse; allow it to evolve for a time t1, after that for a small time delay which is = 1 / 2 times the JCH; do the polarization transfer, enhance signal intensity and then detect the carbon 13 signal with proton decoupling. This is simple pulse sequence of HETCOR.

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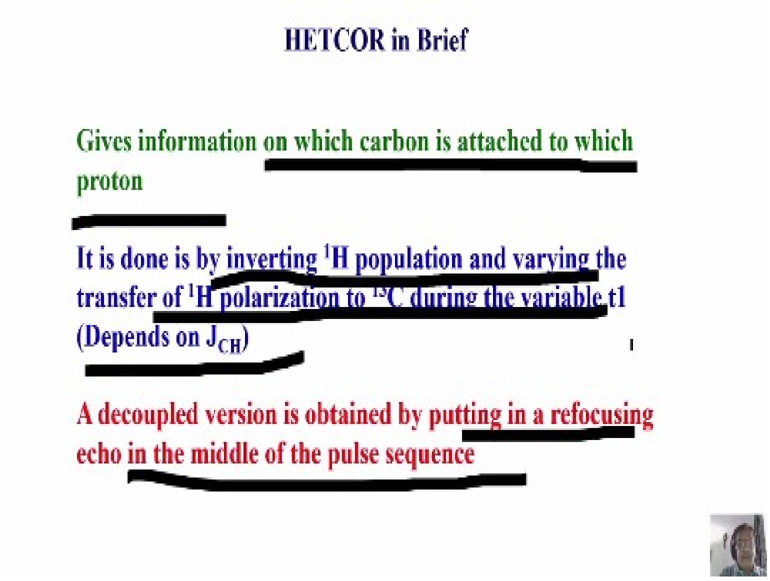

HETCOR in Brief

Gives information on which carbon is attached to which proton

It is done is by inverting ^{1}H population and varying the transfer of ^{1}H polarization to ^{13}C during the variable t1 (Depends on J_{CH})

A decoupled version is obtained by putting in a refocusing echo in the middle of the pulse sequence

So one important thing is it gives information of which carbon is coupled to which proton. It is done by inverting proton population and varying the transfer of polarization to carbon. Remember, the selective polarization transfer we discussed; how we can enhance the signal intercity. Exactly same idea here, but it depends upon JCH. You can have a coupled version and decoupled version also.

So, decoupled version can be done by putting in a refocusing echo in the middle of pulse sequence; that is what it is. So we discuss what happens in the case of sequences like this immediately after polarization transfer if you start start doing the decoupling, the anti-phase components will be there. It will nullify the gain which we have achieved. As a consequence, we can do some peaks to see that we get in-phase components, which adds up and gives rise to enhanced intensity; that is what is normally done.

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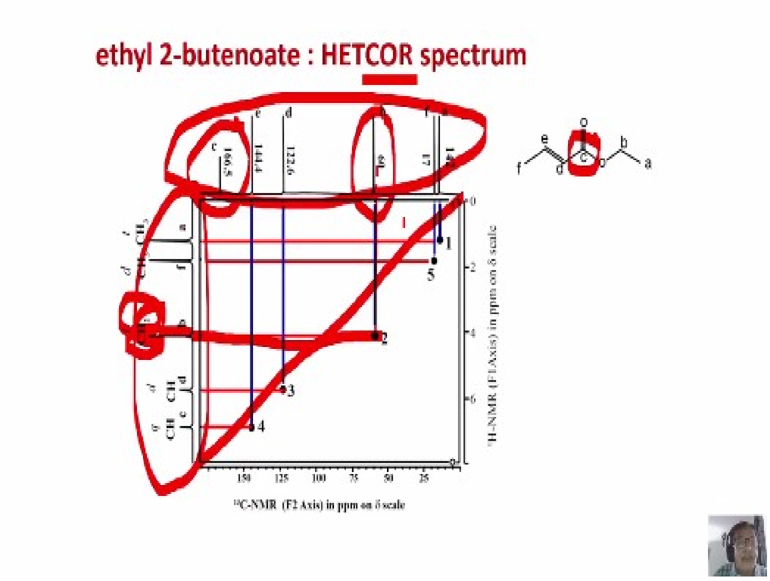
ethyl 2-butenoate : HETCOR spectrum

And without going into the details, this is the molecule that is taken; look at it this is 2 ethyl butanoate; we analyzed its proton spectrum. This is the carbon spectrum; this is a proton spectrum; the projections re plotted on the top of it; and this is really the 2D HETCOR spectrum. Now look at this, what you are observing here. We analyzed, you remember, the proton spectrum of this molecule.

This is CH3 peak we know; this is 1 and this is a proton axis go horizontally along this axis. You are going to hit a peak here; and then go vertically up; you are going to hit that peak. See go vertically up here, you are going to hit this peak; go horizontally along this axis you will hit this proton. So that means the CH3, these three protons attached to carbon has the proton chemical shift here. Its carbon chemical shift you can get from here. The simultaneous detection of both the nuclei is possible. You can correlate the chemical shift information of the protons attach to that particular carbon. Now look at this one, this carbon which is again CH3 group, which corresponds to F. Look at this one, you see a cross peak here; that means this CH3 go horizontally along this axis. This is a proton chemical shift and here you hit up on a cross peak, go vertically you will see this peak, this is a carbon peak. So this carbon chemical of this CH3 carbon is correlated to this CH3. You get the chemical shift of this proton and chemical shift of carbon if you know this peak. It is very easy. There is also CH2, CH2 is here; it is B, it is written. Look at this one, go horizontally along here and then you go vertically up; see go horizontally here you see this peak; go vertically up you see this peak.

That means this proton of this CH2 is correlated to this carbon. This is if I take the CH2 group, its chemical shift in the carbon dimension is this, the carbon chemical shift is this and the proton chemical shift is this. So directly we get both the chemical shift information. So, all the carbons which are directly attached to protons can easily be identified. So you can see this and this is for CH carbon. This is a proton chemical shift, this is a carbon chemical shift. But if you see there is one peak in the carbon spectrum; if you see the projections here or plot the carbon spectrum on the top of it, which has no correlation at all, there is no correlation to any proton. You look at this carbon, it has to be this carbon, because this carbon has no directly attached proton. As a consequence in the HETCOR experiment this carbon will not give rise to any cross spectrum. This is the important point if you remember. You get the peaks only for the carbons that are attached to protons in HETCOR. But it is a fairly a difficult experiment, long time consuming experiment for the reason you are directly detecting the carbon. You know direct detection of carbon which is a very low sensitive nuclei takes the enormous amount of time. That is why there is an improved version, called inverse detection which I am going to tell you later as we go ahead. So, this is an important thing we should remember, but you notice one thing, this is also correlation experiment similar to the homonuclear correlation experiments like COSY and TOCSY. There the spectrum was symmetric with respect to diagonal remember; this is the diagonal.

In the spectrum, the correlation peaks were symmetric with respect to diagonal. On either side of the diagonal you will get peaks. But here first of all there is no diagonal. Secondly, there is no symmetry in the cross peaks, because in the homonuclear case both the dimensions have chemicals shifts of identical spread. So very easily you would get the cross peaks on either side and diagonal will be there. Here, it is not like that this is a carbon chemical shift much larger, about 300 PPM. This is a proton chemical shif,t about 10 PPM there is no question of symmetry, no question of diagonal or anything. So, in the heteronuclear correlation experiment you will not have a diagonal peak. First point there is no symmetry with respect to diagonal like COSY the homonuclear experiment; and you will not get peaks for non-protonated carbons. These are the salient point of HETCOR experiment.

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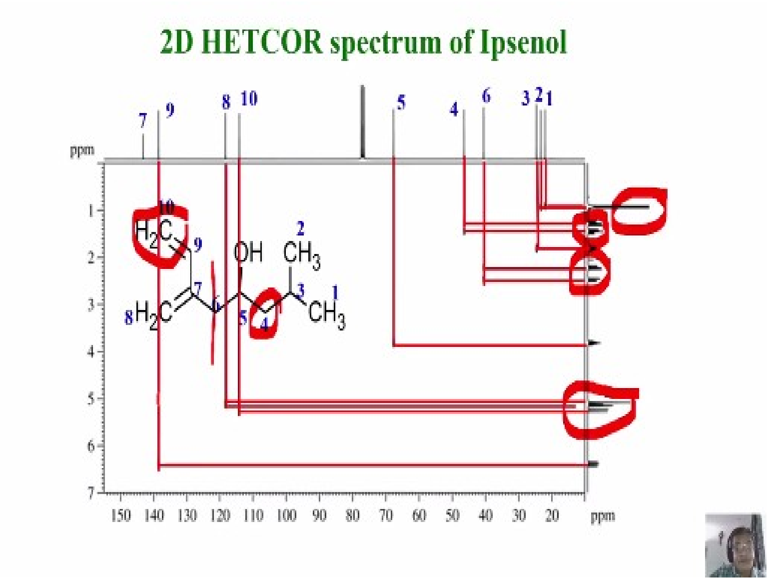

2D HETCOR spectrum of Ipsenol

So now look at the HETCOR spectrum of molecule this is simple molecule called ipsenol. Look at this there are three CH3 here, there are two CH3 and I do not know what is third one, third one is here. It is CH I think so it is here, but it does not matter look at this, you can go along the vertical axis here, you get carbon chemical shift. From the same contour draw a horizontal axis you will hit up on this, you will get proton chemical shift.

See very interesting things that you should appreciate here is, in this region believe me there are two proton peaks hidden. It may be difficult for you to distinguish here because they are closely resonating peaks. But on the other hand, if you go back you see these two peaks are well resolved in the carbon dimension. That is the beauty, you can measure carbon chemical shift very precisely, from the center of this peak. And you will precisely get the proton chemical shift. That is one of the biggest advantages of that. Now if you come to carbon 6, carbon 6 is attached to two protons, they are inequivalent. You can see that one carbon peak, and it is correlated to two protons. This information you can very easily get from the heteronuclear correlation experiment. Same way, proton is correlated to two peaks here. One chemical shift as far as the carbon dimension is concerned, but in the proton dimension it has two protons. These two protons are attached to the same carbon. So it gives the same chemical shift for carbon, and there are two different peaks in proton dimension. Like that you can start doing analysis. And you see the proton 10 again interestingly, it has got two peaks here correlated to two peaks; maybe they are non equivalent here. So you can identify all the protons attached to all the carbons very easily.

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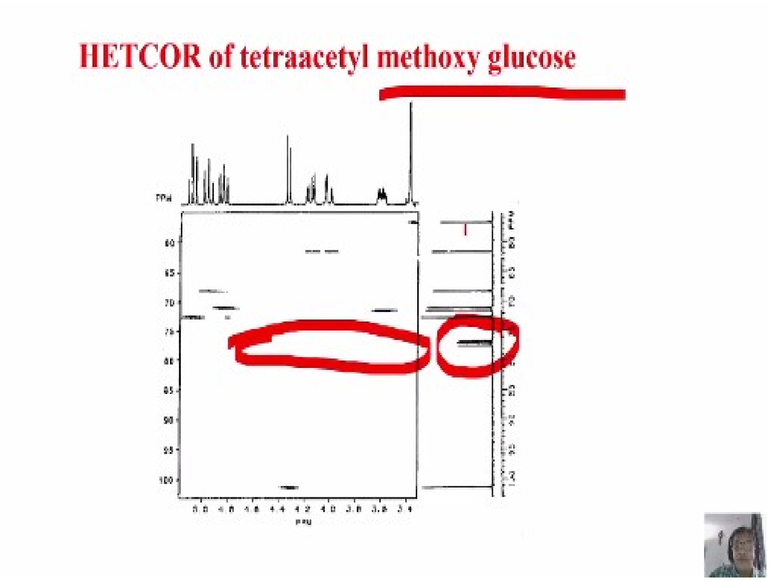
HETCOR of tetraacetyl methoxy glucose

Of course you can keep extending analysis of the HETCOR spectrum. It is very simple take the example of tetraacetyl methoxy glucose, there are 1, 2, 3, 4, 5, 6 carbons. This is CDCl3 you can forget it. You cannot get cross peak because it is a deuterated carbon, not protonated so there is no peak for CDCl3. And you can count 1, 2, 3, 4, 5, 6 carbons are there. Each carbon you go along this axis, and this axis, you can get proton and carbon chemical shifts very easily.

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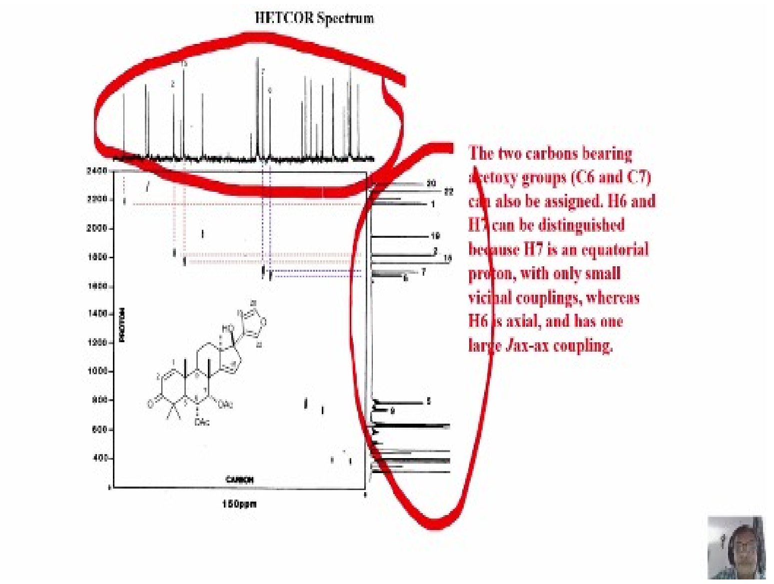
HETCOR Spectrum

The two carbons bearing acetoxy groups (C6 and C7) can also be assigned. H6 and H7 can be distinguished because H7 is an equatorial proton, with only small vicinal couplings, whereas H6 is axial, and has one large Jax-ax coupling.

And I wanted to show you even for a very complex molecule like this. See there are so many carbons is present here; in the proton dimension; no this is carbon dimension; and this is proton dimension. And in the carbon dimension, you see so many peaks are present, but you are able to assign each and every one of them, that is the beauty.

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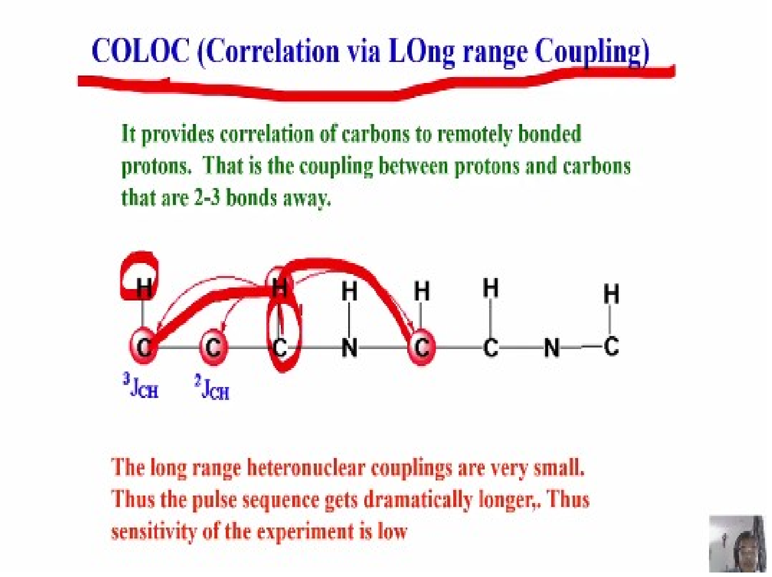
COLOC (Correlation via LOng range Coupling)

It provides correlation of carbons to remotely bonded protons. That is the coupling between protons and carbons that are 2-3 bonds away.

The long range heteronuclear couplings are very small. Thus the pulse sequence gets dramatically longer,. Thus sensitivity of the experiment is low

There is another experiment called COLOC experiment similar to heteronuclear correlation where it is a correlation of long range coupling. It is a correlation between proton and carbon, heteronuclear correlation via long range coupling. For example, in the previous experiment what did you observe? there was a correlation of carbon to directly attach proton. That is HETCOR, but now this carbon can correlate to this proton and this can correlate to this carbon. And this correlation we can subtract, we can eliminate that, and do an experiment where we can get correlation peak only for the carbons which are coupled to remote protons. That is also possible. That type of experiment is called a COLOC experiment, called correlation via long range coupling. Just for example two bond coupling between proton and carbon is there that can be correlated. Three bond coupling between this carbon and this proton can be correlated. But mind you this is the most difficult and time consuming experiment. Thus, you are trying to correlate the long range correlation. Of course you may ask my question what happens to one bond coupling? we can efficiently suppress that while doing this experiment.

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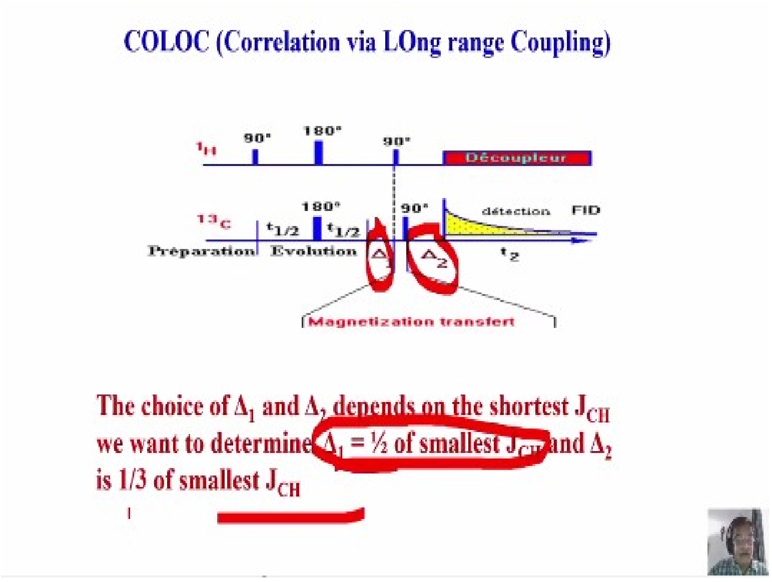

COLOC (Correlation via LOng range Coupling)

The choice of Δ_1 and Δ_2 depends on the shortest J_{CH} we want to determine Δ_1 = ½ of smallest J_{CH} and Δ_2 is 1/3 of smallest J_{CH}

It is a very low sensitivity experiment. So it is not easy to do this. For COLOC, this is a pulse sequence; and you can understand this is a very nice experiment you can go back and see it is similar to HETCOR experiment; only thing you are going to put a delay here, the delay just before and after this pulse. So that delay you can choose, optimize this for the smallest coupling between proton and carbon.

And once you adjust this delays delta 1 and delta 2 corresponding to smallest coupling, of course different carbons may have a different coupling, but smallest two bond and three bond coupling approximate value you should know and optimize the delay equal to half of J coupling for the delta 1 and one third for J for this one, then you can establish long range correlations. I am not showing that spectrum.

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“Inverse” Spectroscopy

Now I will go into what is called inverse spectroscopy. What is inverse spectroscopy that is something very, very important to know?

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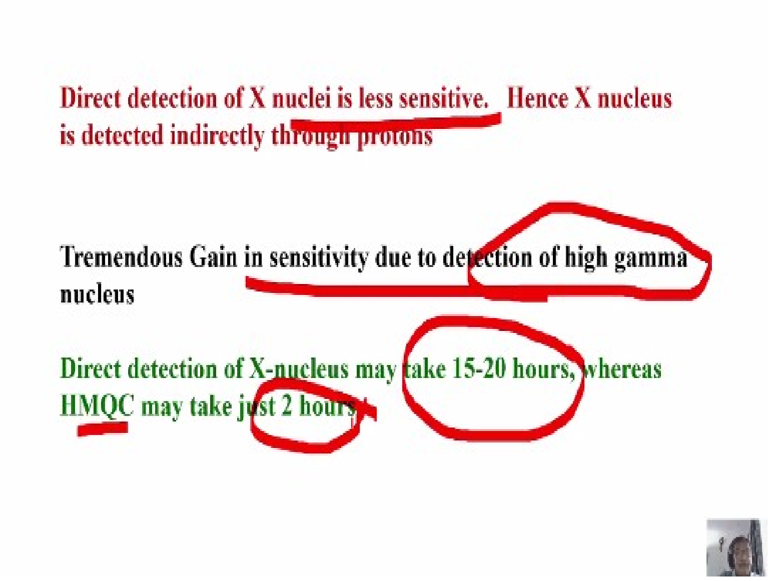

Direct detection of X nuclei is less sensitive. Hence X nucleus is detected indirectly through protons

Tremendous Gain in sensitivity due to detection of high gamma nucleus

Direct detection of X-nucleus may take 15-20 hours, whereas HMQC may take just 2 hours !

For example, if you want to detect the X nuclei which is less sensitive you know that. I want a detected carbon I have already told you, it is so less sensitive it takes the enormous amount of time to get the carbon with the same amount of signal to noise ratio as that of abundant spin. On the other hand, if we indirectly detect it through protons, how is it, what will happen? It is a trick we adopt. Take the proton, give this magnetization to carbon and again take it back. In this process you can get the information about the carbon.

Now we are detecting proton not carbon, and detection of proton is much more better you can get the spectrum in less time; that is the advantage. This is called inverse detection of carbon through proton. These experiments are called inverse experiments. Whatever the experiments I said HETCOR, COLOC etcetera for long range correlation, the same experiments you can do in a much faster time, with better sensitivity by using inverse detection.

This is a very important experiment and what is important is the tremendous gain in sensitivity due to high gamma of the proton. Proton has a very high gamma, transfer the magnetization of proton to carbon and take it back. The beauty is direct detection of carbon may take almost half a day to one day. Whereas if I do this inverse detection by HMQC, HSQC, I will tell you what it is soon, then you can do the same experiment in less than two hours.

Nowadays even less; even you can get in 30 minutes, with specially designed probes and spectrometer with special pulse sequence. And everything you can get it even faster, you can get in less than 30 minutes now. See the advantage we have taken the benefit of large magnetization of proton and give it to carbon and do the inverse detection, this is the beauty.

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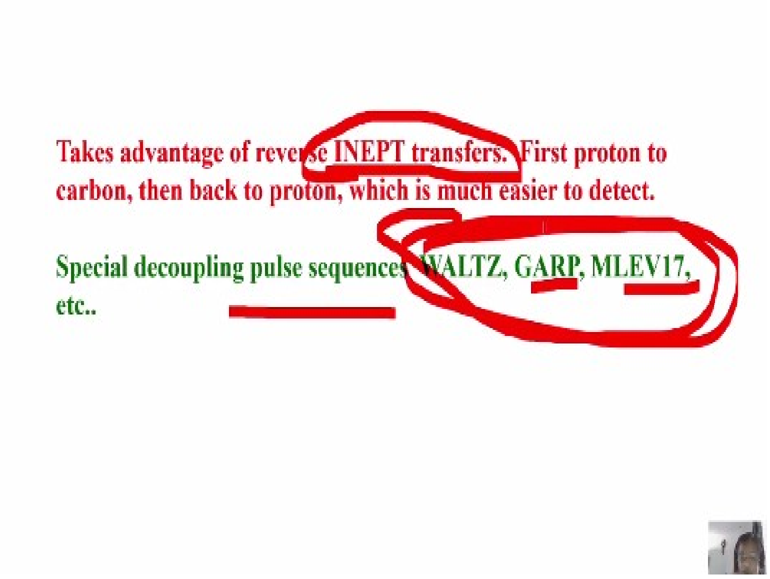

Takes advantage of reverse INEPT transfers. First proton to carbon, then back to proton, which is much easier to detect.

Special decoupling pulse sequences WALTZ, GARP, MLEV17, etc..

It takes the advantages of the reverse INEPT transfer. What you will do is, you will first do the polarization transfer from proton to carbon; then do the reverse INEPT transfer. Take the magnetization back to proton and now we can directly detect the proton. So we have combined this with varieties of decoupling sequences like, WALTZ, GARP, MLEV these are all pulse sequences used for decoupling.

I told you about heteronuclear decoupling by CW. I also mentioned I think there is GARP, WALTZ pulse sequences are there, which you can apply for decoupling. So, finally you do the first INEPT transfer, and back reverse INEPT transfer, and while detecting the proton you can also do decoupling.

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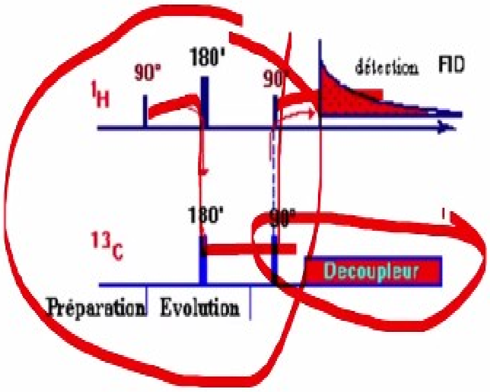
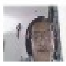
Pulse Block for Inverse Detection





This is a pulse sequence very, very easy to understand. This is a preparation period. Apply proton 90 pulse, and up to this what happens you know. It is evolution and you are going to take the magnetization from proton to carbon, from carbon to proton back, and then start detecting the proton, while doing decoupling of carbon; very interesting. When you are detecting carbon you are decoupling proton. Now we can do the reverse. This is inverse pulse sequence. For most of the inverse experiments, this block remains same. Take the magnetization from proton to carbon and how it works we already discussed in the heteronuclear INEPT sequences, how INEPT works everything by vector diagram, we understood. So I do not want to go into the details. I hope you are all with me. So 90 degree to 180, and then transfer the magnetization here to carbon. From carbon to bring it back to proton again, and detect the proton while doing decoupling. This is simplest pulse sequence used in inverse detection.

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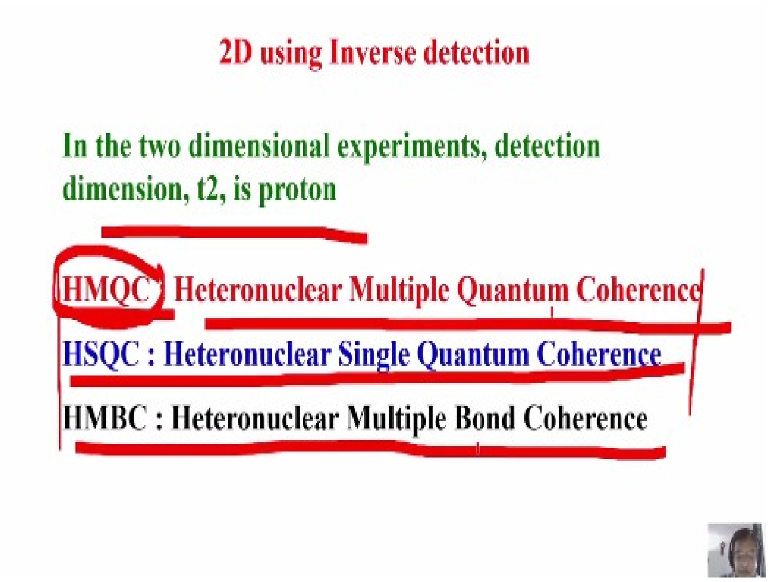

2D using Inverse detection

In the two dimensional experiments, detection dimension, t2, is proton

HMQC : Heteronuclear Multiple Quantum Coherence

HSQC : Heteronuclear Single Quantum Coherence

HMBC : Heteronuclear Multiple Bond Coherence

In this 2 dimensional experiment the detection dimension, t2 is proton, I have been telling you. Now we have three classes of experiments which we can discuss, here. One is HMQC heteronuclear multiple quantum coherence, HSQC heteronuclear single quantum coherence, HMBC heteronuclear multiple bond coherence. And the HMQC and HSQC are more or less similar. And they give similar information, except that there are some technical advantages of this over this. That is the advantage. Apart from that, the information contained in both the experiments is same. That is the important point you should remember; whereas HMBC gives you long range correlations.

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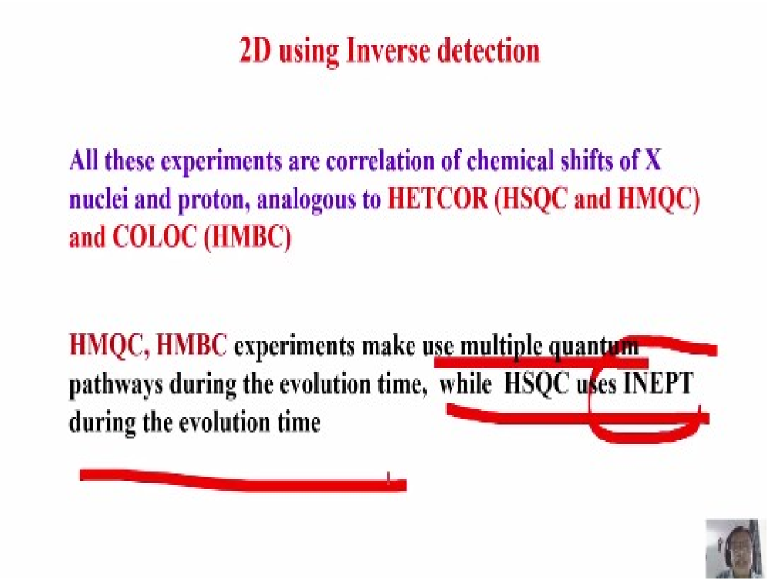

2D using Inverse detection

All these experiments are correlation of chemical shifts of X nuclei and proton, analogous to HETCOR (HSQC and HMQC) and COLOC (HMBC)

HMQC, HMBC experiments make use multiple quantum pathways during the evolution time, while HSQC uses INEPT during the evolution time

We will see how we can do inverse detection. All these inverse detection experiments can be correlated to direct detection experiments. There is a similar experiment for direct detection.

HETCOR was there you know I told you HETCOR, pulse sequence where proton, carbon was correlated, that is analogous to inverse detection sequence called HSQC and HMQC.

There is experiment called COLOC; correlation of long range coupling, that is an experiment where two bond, three bond proton carbon couplings can be correlated. Protons and carbons can be correlated; which are two to three bonds away. Instead of directly bonded in HETCOR, this experiment is analogous to what is called HMBC.

Please understand, we have HETCOR experiment where direct correlation between proton and carbon of directly bonded nuclei, carbon and proton can be detected. You can understand CH is coupled carbon chemical shift and proton chemical shift of that attached proton and carbon you can get easily. That correlation is obtained by the HETCOR experiment. From COLOC this carbon can be having coupling with proton that is two bonds away, another proton that is three bonds away. This proton correlation we can obtain. That is the long range two to three bonds away correlation obtained, again between carbon and proton. But the similar experiment is possible by inverse detection called HMBC. As I said HSQC gives us INEPT during the evolution time and HMQC experiment make use of multiple quantum pathway. This is where, it is very difficult for you to understand, when you have a multiple quantum pathway by energy level diagram. It is bit difficult to do, but is not extremely impossible.

So this is what I wanted to tell you. HSQC is routinely followed. It uses INEPT polarization transfer during evolution time. Please remember, inverse detection is what you have to do. If you are doing the proton carbon correlation, if you have a molecule, if you want to analyze carbon spectrum and want to know which carbon is coupled to which proton; do not do HETCOR experiment or COLOC experiment. please do HSQC. Directly bonded carbon proton can be correlated.

If you want to get correlation to identify carbonyl carbon, which carbonyl is two bond or three bonds away from the proton, do long range experiment that is HMBC. Then you can even get information about carbons which are not directly attached to protons, but remotely coupled. Then you do HMBC. Except this inverse experiment please do not try to do any direct experiments, that is no more advantages and now nobody practices that.

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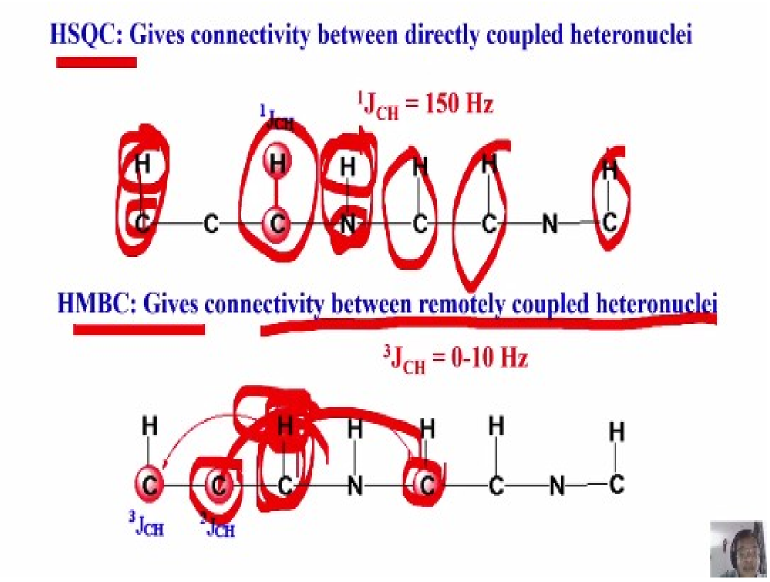

HSQC: Gives connectivity between directly coupled heteronuclei

$^1J_{CH}$ = 150 Hz

HMBC: Gives connectivity between remotely coupled heteronuclei

$^3J_{CH}$ = 0-10 Hz

Pictorially this is what it is. Carbon proton, one bond coupling is obtained by HSQC one bond correlation. And I have let us say 10 carbons or 5 carbons here, 5 carbons are attached to protons. I am going to get 5 peaks in HSQC experiment. And in the carbon dimension you get this chemical shift, in a proton dimension for this cross peak you will get this chemical shift.

So for each cross peak, if you go on F1 dimension and F2 dimension, correspondingly we get chemical shift information of the carbon and corresponding proton. But if you have a nitrogen, proton like this, remember that is also possible. You can do nitrogen 15-1H HSQC experiment, especially you find it very useful in biology, like in proteins and peptides, lot of NHs are there, can do HSQC, nitrogen 15-proton HSQC, where you can correlate nitrogen 15 chemical shift with the proton which is attached to it.

Now you come to HMBC, it gives connectivity between remotely coupled hetero nuclei. For example, this carbon correlation to this proton can be seen; and this proton correlation to this carbon can be seen. This is of course two bonds, this is three bonds; so three bond correlation.

We can do an experiment to suppress this type of one bond correlation, in the long range correlation experiment, it is HMBC. You can suppress directly bonded carbon proton correlation peaks. You can detect only this, this is HMBC experiment where you get long range correlation, remotely bonded carbon proton correlation can be obtained.

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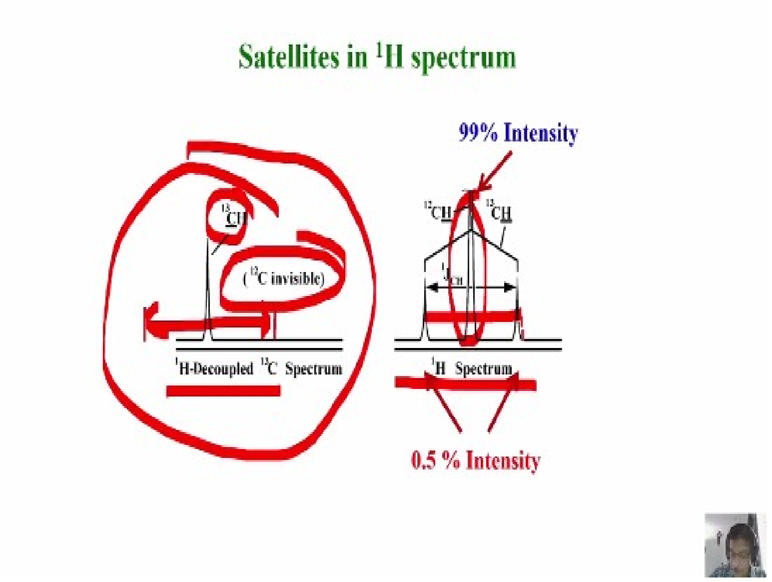

Satellites in ^1H spectrum

Now I will try to bring in to you the information about the analysis of what is called proton decoupled and proton coupled HSQC and HMBC experiments in which case you need to get some idea about proton carbon couplings in the proton spectrum, which we already discussed. You know what are satellites I discussed. If I take a simple example for CHCl3 simple molecule, I must get a single peak for proton. It should be a doublet, but why it is a single peak; because 99% of the molecules are in carbon 12 state. They exist as carbon 12 isotopomer. So it gives a single peak because 12C has no magnetic moment, so no coupling to proton. But remember 1% of the molecule has carbon 13. This is the carbon 13 observation. If I come to proton observation you will see this is a carbon 12 peak and two satellite peaks.

So when I observe carbon 13 here, carbon 12 is invisible I decouple proton I get a single peak. If I do not do decoupling I will get two peaks. Measure the separation, this exactly matches with this separation. This is CH coupling. You can detect in the carbon coupled spectrum; because we do the decoupling generally, we do not see the coupling. Hence we got single peak. But in the proton you see carbon coupling with proton, and you see H satellites. 1% intensity peak. 1% further divided into 2; it is 0.5% intensity peaks are seen. This is the proton spectrum, you see carbon satellites. Carbon proton couplings are the satellites and this is 99% intensity peak. These peaks are 0.5 intensity peaks. This is what I wanted to tell you to get an idea. This you knew, but I repeated it make you clear. So we are going to do it.

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Coupled and Decoupled ^{13}C-^{1}H
HSQC, HMQC and HMBC

Let us look at the coupled and decoupled spectrum of HSQC, HMQC and HMBC. If you understand HSQC of course HMQC is similar, let us not worry about it. At least you should understand how HSQC of coupled carbon 13- proton and decoupled carbon 13- proton spectrum looks like. Similarly for HMBC. This is very important for you to interpret the heteronuclear correlation spectra like HSQC and HMBC. You must have an idea as how to analyze this.

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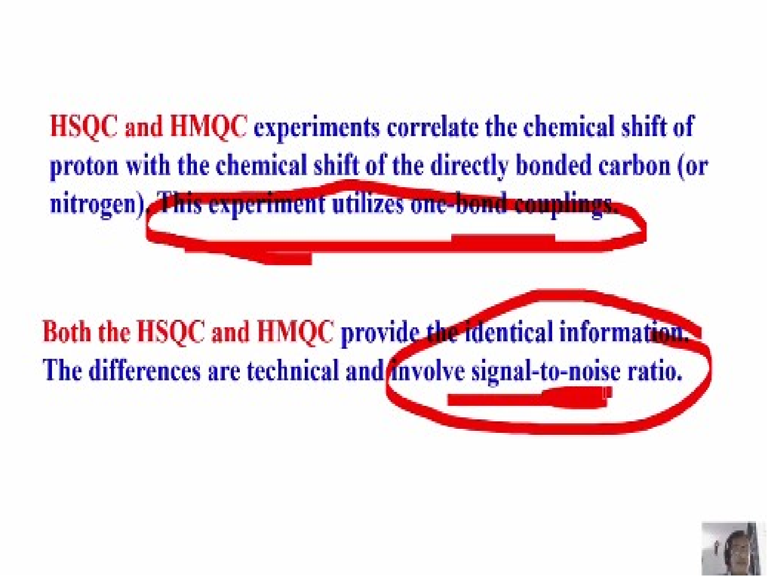

HSQC and HMQC experiments correlate the chemical shift of proton with the chemical shift of the directly bonded carbon (or nitrogen). This experiment utilizes one-bond couplings.

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

Now HSQC and HMQC as I said always utilizes one bond coupling, no need to repeat that and HSQC, HMQC gives a similar information, but for technical reason this has a signal to noise ratio difference that I told you, no problem.

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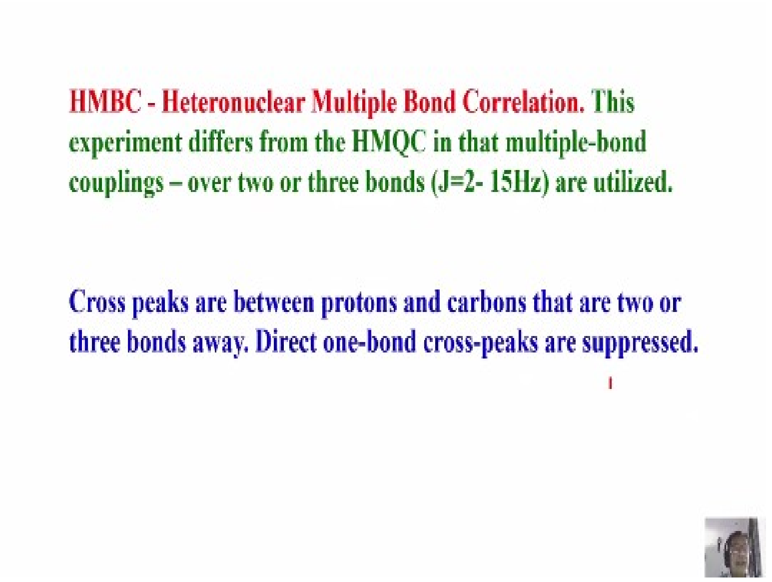

HMBC - Heteronuclear Multiple Bond Correlation. This experiment differs from the HMQC in that multiple-bond couplings – over two or three bonds (J=2- 15Hz) are utilized.

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

Now coming to HMBC what does HMBC do? I told you again it correlates over two to three bonds away.

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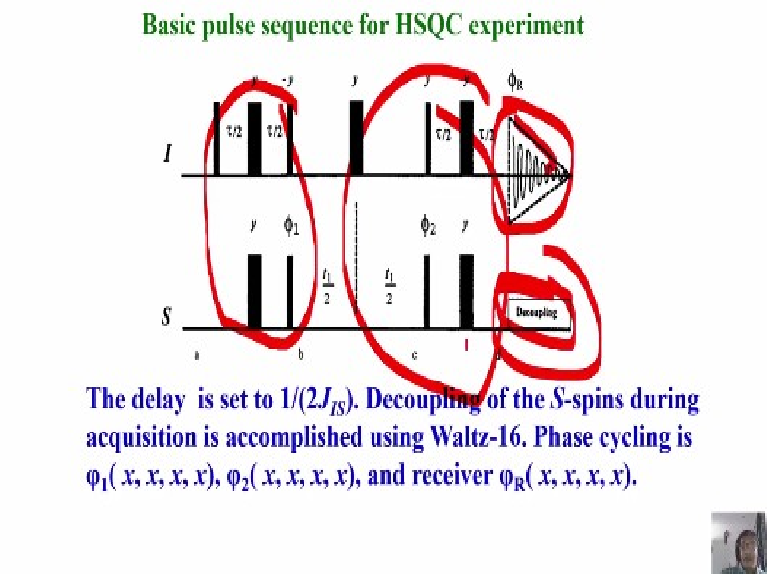

Basic pulse sequence for HSQC experiment

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 $\varphi_R(x, x, x, x)$.

This is a basic pulse sequence for HSQC. Can you notice some similarities what we discussed long back? couple of classes back? We discussed this one. If you understand we carefully see what is this? does it not look like you INEPT block. Exactly, then this looks like a reverse INEPT. We can do this. What it does is? this pulse sequence is very simple. Here we take the polarization from protons give it to carbon. And then after certain time t1/2 in this evolution period you will ensure the magnetization is taken back to proton by reverse INEPT. One is a forward INEPT other is a reverse INEPT. In the process what you will do is you will take the magnetization from proton give it to X nuclei; from X nuclei take it back, and give it to proton. Now we are decoupling carbon and detecting the proton.

Why I have to do decouple carbon? when I am seeing proton. Carbon is only 1% abundant, But these satellite peaks are the ones which will be seen in the correlation experiments; we have to avoid that. That is why I showed carbon 13 satellites in CHCl3 spectrum; you understand. The basic pulse sequence you have already discussed INEPT sequence by vector diagram, you can continue to do that, there is no problem.

And if you do not worry about the way magnetization behaves and all those things, how the spin behaves, do not bother. Please remember, in this block we take the magnetization of proton give it to carbon. In this block we take it back from carbon to proton, and then start seeing the proton signal while doing decoupling of carbon. We observe proton and decouple carbon. That is the interesting part of this experiment. Inverse experiment means, we detect protons and indirectly get the information about the coupled carbon.

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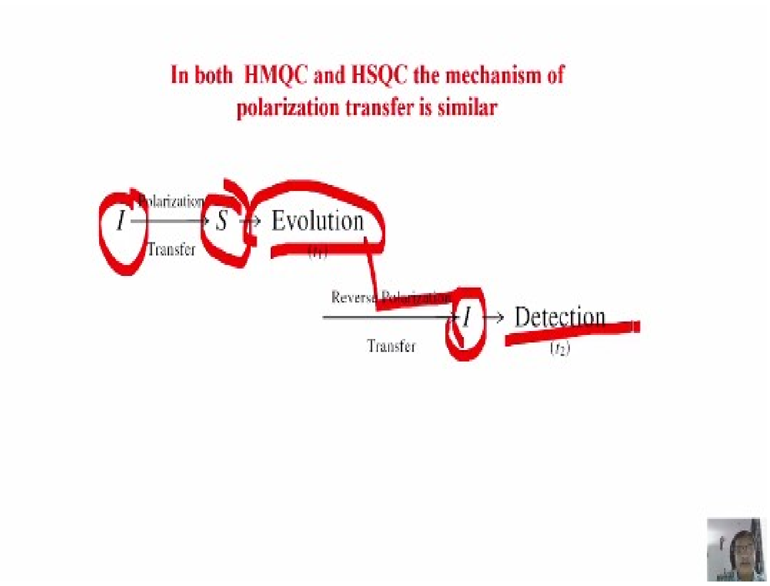

In both HMQC and HSQC the mechanism of polarization transfer is similar

Basically look at this one this is a flow chart in both HMQC, HSQC or HMBC whatever the mechanism we tried to do, of course here is a polarization transfer mechanism. What we do here is you take polarization from I spin, transfer to S spin, allow it to evolve during t1, then you do the reverse polarization transfer to I, and detect. Very simple, two steps that is all nothing more; Very fantastic.

But this experiment is so good and so powerful now everyone will do only this. HETCOR experiment is no more practiced; they are all outdated now. Only for academic interest to understand the concept we do that, otherwise experimentally it is not done anymore.

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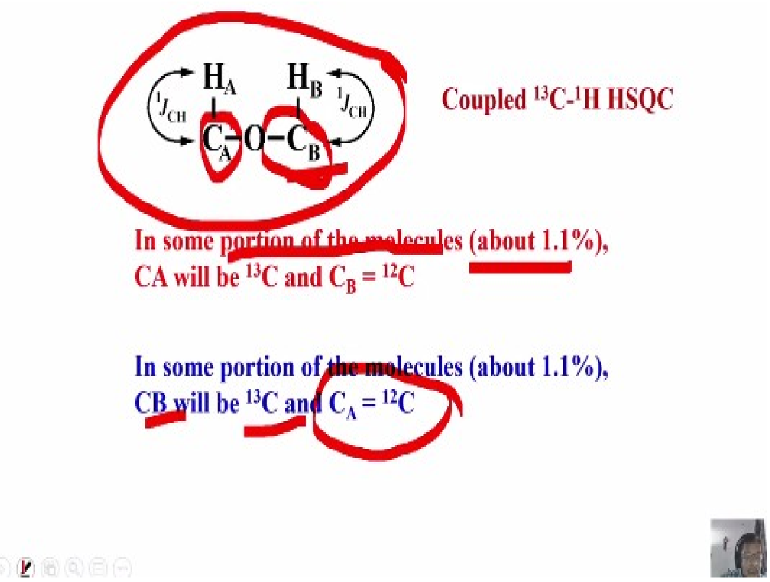

Coupled ^{13}C-^{1}H HSQC

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

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

Now let us look at the carbon 13-proton HSQC spectrum. I consider simple molecule like this. Now I will consider situation, because I have to look at the carbon 13. In this molecule there are several isotopomers. In some portion of the molecule, let us say carbon 13, CA will be in carbon 13 state. CA exists as carbon 13 and CB will be carbon 12. Obviously, we know joint probability of both of them existing as carbon 13 is very low, that we discussed already in some of the molecules. That is one type of molecules we get. It is one type of isotopomers we call. In some portion of the molecules or samples CB carbon B; will exist in the carbon 13 state and carbon A exist in carbon 12 state. That means we have two different types of isotopomers present, that is all we discussed. Remember in carbon 13 NMR with individual carbons isotopomers spectrum only you will get and final spectra is a superposition of all of them that is what I said. Exactly here also now we have taken this molecule as an example, but remember when CA is 13, CB is C12; and CB is 13, CA is C12, both joint probability is very less.

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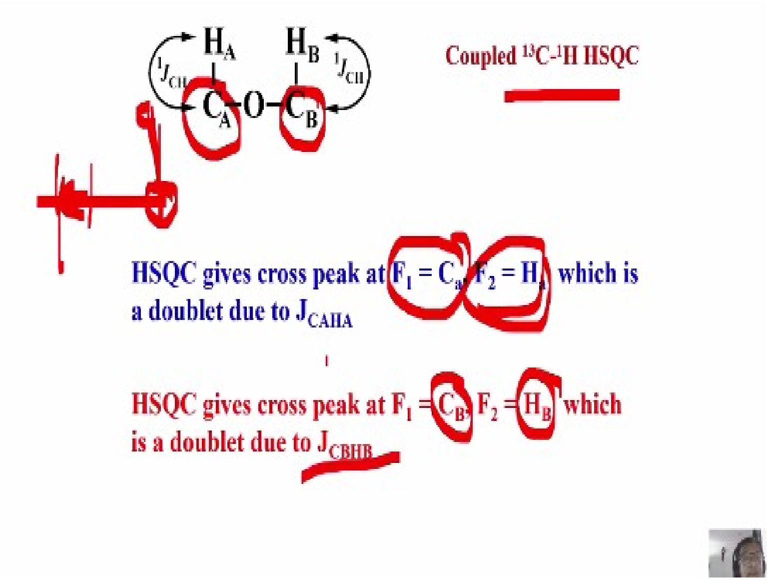

Coupled ^{13}C-^{1}H HSQC

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 in the HSQC spectrum what happens? In the F1 dimension CA gives a peak or carbon F2 dimension you get proton peak; that is what it is. The is correlation is like this. If you get carbon here in the F1 dimension or let us say in this F1 dimension this is carbon, the F2 dimension this is proton that is all. I said in the HETCOR experiment we got a cross peak like this you know.

You go vertically up you get carbon chemical shift, go horizontally you get proton chemical shift that is what we get. But now if it is a coupled HSQC not decoupled. There is a coupling between carbon and proton. But in the carbon 13 dimension its detection is easy in the proton spectrum is what? they are satellites. Two peaks; that is, a J coupling is there and they come small peaks, as satellites; that is what it is.

In the carbon 13 dimension when this carbon is coupled to this proton it will be a satellite doublet. Similarly, this one also will be a doublet in the proton dimension, appear as satellites. See in the carbon dimension you get chemical shift of carbon; in the proton dimension you get chemical shift of proton, but in that dimension we have a doublet which gives you proton carbon coupling. Let us see the spectrum now; very easy.

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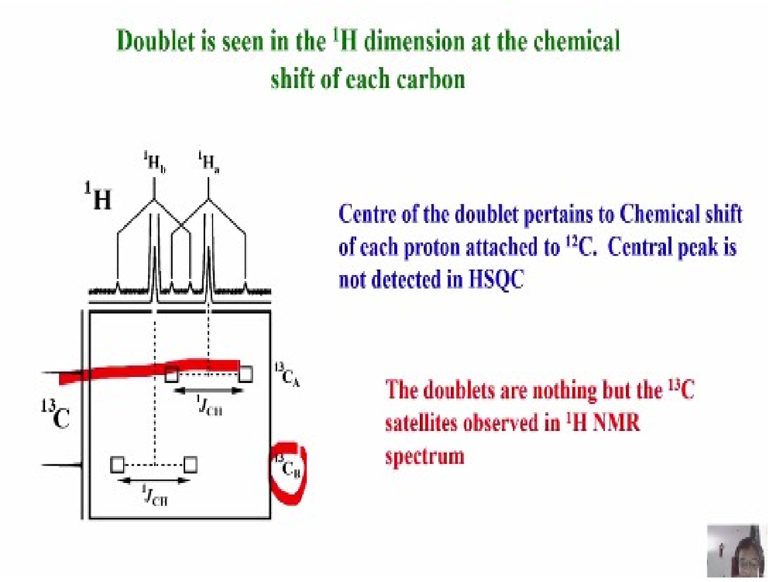

Doublet is seen in the ^{1}H dimension at the chemical shift of each carbon

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

Look at this one. Now this is the carbon 13 chemical shift, if you go along this axis. This is CA carbon A. Now look at this carbon B; if you go along this axis you get only one peak. So this corresponds to carbon chemical shift. But let us go to proton dimension this is a proton dimension this is a proton peak corresponding to C12 carbon attached, and look at it on either side you are getting a peak.

What are they? These two are satellite peaks of this proton coupled to carbon. They appear as satellites, because we are doing coupled HSQC. Similarly, for this proton B you have two peaks on either side they are satellites. These two peaks are satellites for this carbon, these two peaks are satellites for this carbon. So center of this gives you this proton chemical shift, center of this gives you this proton chemical shift and this difference is J coupling.

This difference is J coupling between carbon and proton; carbon A and proton attached to it. This is a J coupling between carbon B and proton attached to it. This is a coupled carbon 13 spectrum. So in the F1 dimension we are going to get satellites. Carbon coupling is there in the proton dimension, which appear as satellites. Whereas in the carbon dimension you get a single peak, nothing else.

So we get these things and these doublets are nothing but carbon 13 satellites. Now we will talk about HMBC. What will happen to the HMBC? we will see, quickly we will do that although time is up.

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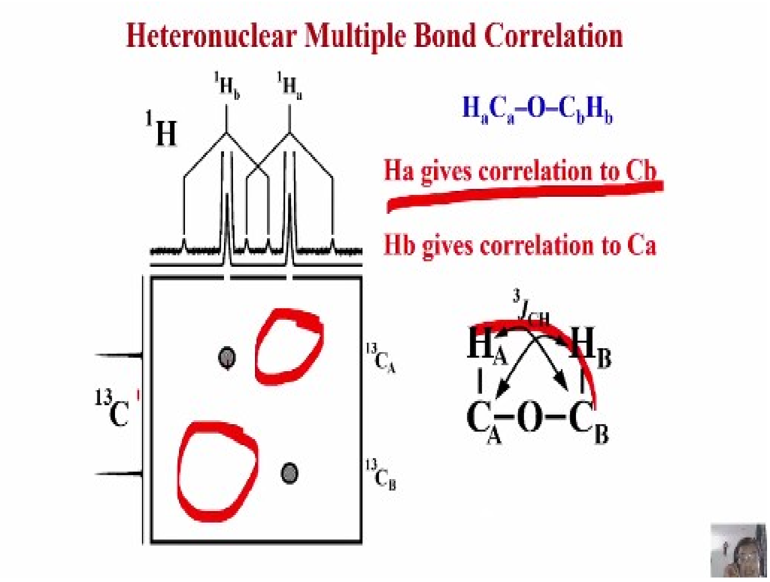

Heteronuclear Multiple Bond Correlation

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

Ha gives correlation to Cb

Hb gives correlation to Ca

Now in heteronuclear multiple bond correlation what we have to see? This carbon should correlate to this proton; and this carbon should correlate to this proton. That is called long range correlation. Now exactly if you look at this, carbon 13 chemical shift here. earlier you got a doublet here corresponding to one bond carbon proton correlation, but now that is suppressed.

What I am seeing is long range correlations so this carbon A gives a peak at proton B position. You see this carbon A, come horizontally go vertically up you see proton B. This is carbon A, it is attached to this proton, but it is giving correlation to this proton, that is called long range correlation. This is multiple bond three bonds away. This carbon is giving correlation to proton which is three bonds away.

Similarly, CB is giving correlation to 1, 2, 3 bonds away this proton. So CB this is a chemical shift of carbon B, but you go up vertically like this, this is correlating to proton A. This is a longrange correlation carbon B giving correlation to proton A. Here carbon A giving correlation to proton B. This is a beautiful experiment; you understand what is happening here.

You are getting long range correlations very easily; Ha gives correlation to Cb this one; Ha gives correlation to Cb, Hb give correlation to Ca. And direct correlation peaks are suppressed; no peaks here. This is what is called heteronuclear multiple bond correlation. We can come back again, I will continue further in the next class. Alright, another one slide I will explain in another one or two minutes.

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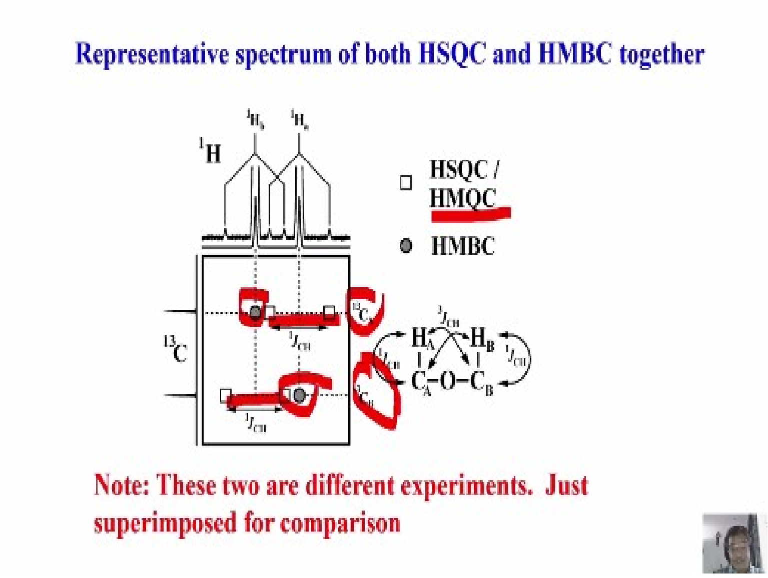

Representative spectrum of both HSQC and HMBC together

Note: These two are different experiments. Just superimposed for comparison

Now in reality of course they are two different experiments, plotted together. How it comes, this will make your doubts clear. Now in HSQC what you are going to see in the carbon dimension is very clear for you, this is carbon A, carbon B and just go across this, go across this, you get carbon chemical shifts, done. This are this, does not matter. Now in the HSQC experiment you get two peaks, the coupled HSQC exactly at the center.

Similarly, for carbon B you get two peaks exactly at the center, what are these? These are proton carbon coupling which are directly bonded. This CB coupled to this HB gives you this doublet. This CA coupled to this HA gave you doublet. This is coupled HSQC and gives satellite peaks. On the other hand, if I look at HMBC this CA gives correlation to HB; this CB gives correlation to HA. Both are different experiments. Just for comparison just to make you understand this is shown here. I hope it is clear for all of you. These are two different experiments just superimposed for comparison. Remember, HSQC gave you direct correlation to directly attached carbon proton, but in this coupled case in the F2 dimension it is a doublet because of carbon proton J coupling; this is what you observed in both the carbons, but in the HMBC case carbon A correlates to proton B; and carbon B correlates to proton A. You may ask me this is also coupled HMBC why there are no peaks, why there are multiplicity or satellite peaks. In principle we should see the coupling is measurable. If it is very small within the linewidths you will not see most of times. So it should be in principle possible to see because it is not a decoupled HMBC.

Okay with this I will stop today. We will come back and then continue with HSQC and then we go to J resolved experiment both homonuclear and heteronuclear, and I do not know whether I have time to talk about NOESY. I will come back to that NOESY later, as there are lot more things we can talk. We can talk about inadequate, adequate experiment, like that. In inadequate is to get carbon-carbon correlation in the natural abundance, but most of the times you do not require that.

So I do not think I have time to talk about it. Next, I will continue with this with the decoupled HSQC and HMBC; how to get the cross peaks and then I will talk about some one or two examples of J resolved experiment. Let us see how we proceed further.