One and Two dimensional NMR Spectroscopy: Concepts and Spectral Analysis
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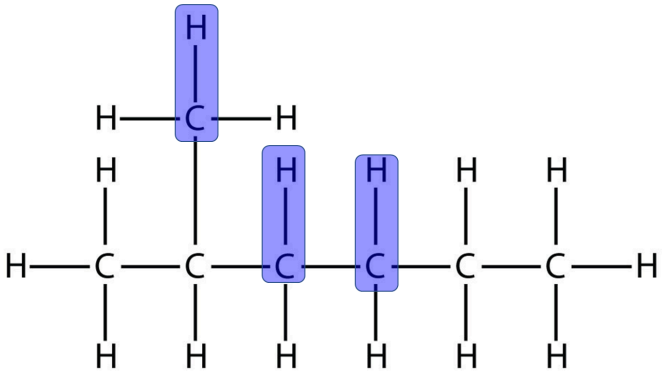
Lecture 44: HSQC-II

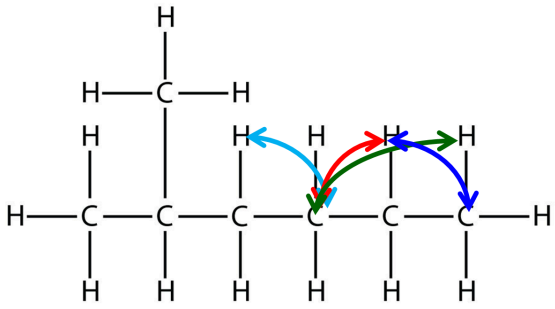
Since the last class, we started discussing about heteronuclear correlation. I discussed about what is the heteronuclear correlation, a very basic pulse sequence where we can correlate any two different heteronuclei, proton carbon, proton nitrogen, proton fluorine, etcetera. But it could be two abundant spins, abundant or dilute spin, whatever it is. We can do heteronuclear correlation. And I explained to you how heteronuclear correlation works in a simple pulse sequence. We apply a 90 degreee proton pulse, bring the magnetization to x-axis and then allow it to evolve for some time. After t1 period, do an INEPT so that you create an anti-phase magnetization for the proton, anti-phase coherence. And then, simultaneously applied to 90 degree pulses on both proton and carbon, the coherence jumps from proton to carbon and detect carbon. A very simple experiment to understand. But it also is very easy to interpret. I said heteronuclear experiment, heteronuclear correlation spectrum, do not have diagonals and or not symmetric because the chemical shift ranges are different. For different nuclei, they have different chemical shift ranges. So, the two dimensions are entirely different and there is no question of any symmetry or diagonal. That is one. There are the few advantages. And interpretation is very simple. You sit on a peak in a heteronuclear experiment, go up vertically up and horizontally across. If this is vertically when you go up, if that is a proton axis, you get a chemical shift of proton for that peak. And go horizontally, if it is a carbon axis, then you are going to get a carbon chemical shift. For example, if it is a CH3 group, you go vertically up, you get CH3 proton chemical shift, go horizontally get CH3 carbon chemical shift. And I showed one or two examples how we can interpret it, very easily. But there is one drawback with this. HETCOR is a direct detection of the X-nuclei. That could be very time consuming. Then new experiment has been invented and discovered. Over the years people start doing different types of experiments to speed up the experiment and get better signal to noise ratio. And they are called inverse experiments. So, from today we start discussing about inverse experiments. The main problem I told you, the direct detection X nuclei is less sensitive. Remember, in the very first class or second class when I was discussing about sensitivity of detection of NMR signal, I said proton is highly abundant and compared to any other nuclei has highest gamma and is more sensitive. And carbon-13 compared to that, I said is 6000 times nearly less sensitive. And if I want to detect the carbon-13 directly, it is even more difficult, because it is less sensitive, abundance is small. So, as a consequence, that will

take an enormous amount of time. On the other hand, why cannot we detect X-nuclei indirectly through protosn? It is something funny. Instead of detecting carbon, why should I not detect through proton indirectly? There is a way we can do that. The advantage of that is we are not detecting carbon, we are detecting proton. That means, gamma is high for proton, sensitivity is 4 times larger than that of carbon, the experiment is faster. But how we do is a challenge, we will do that. So, direct detection of X-nuclei may take, let us say, few hours, 10 hours, 15 hours experimental time. Whereas inverse detection experiment will take half an hour to one hour. See the saving in the instrument time, saving in your experimental time, there is enormous saving in the experimental time, because we are not directly detecting the X-nuclei. How do we do that? It is a very simple way. We do the polarization transfer through INEPT. This is always done from abundant spins to rare spins, dilute spin. Abundant spin, if let us say, take proton and carbon is less abundant spin, I give magnetization to carbon. Then I detect carbon. So, then again, detecting carbon, that is a problem. What we will do is, you will take the magnetization of proton, give it a carbon and take it back from proton again. Take it back from carbon to proton again. You understand the problem, give the magnetization of proton to carbon and take it back. In this process, I am detecting proton, but in this process, I get the information about carbon 13. This is the advantage. Directly, I am detecting proton, high-sensitive nuclei, saving experimental time, but I also get the information about dilute spins. This is an advantage. And of course, I can do the decoupling experiment, and do the coupled experiment, and all such inverse experiments, can be done. That is all well known. So, in the inverse experiment, in the two-dimensional experiment, usually detection dimension is proton because I am directly detecting proton, that is sensitive nuclei. If I detect carbon 13, then it is not, no, it is not an inverse experiment. It is a direct detection, it is like HETCOR, that will take more time. So, in the inverse experiment, detection dimension is always proton, that saves time. And number of experiments are there, which have been designed. For example, HMQC, Heteronuclear multiple quantum coherence, HSQC, Hetero nuclear single quantum coherence, HMBC, Hetero-nuclear multiple bond coherence. These are some of the common hetero-nuclear correlation experiments generally employed for analyzing the spectrum. What HSQC does?



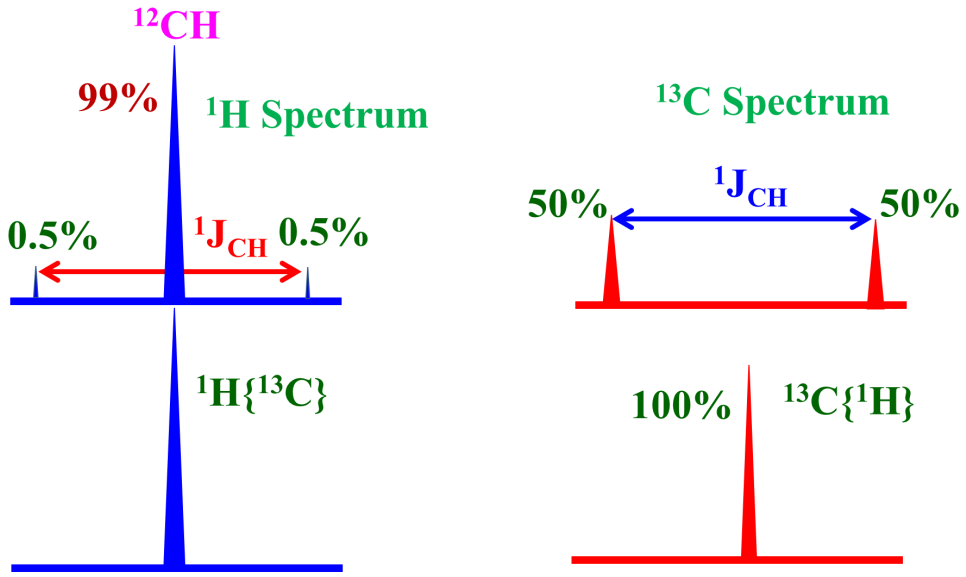
HSQC gives connectivity between directly coupled heteronuclei and it uses one bond heteronuclear coupling. For example, in a molecule like this, assume one bond coupling is 150 Hz, that is only for experimental purpose to set up. In a molecule like this, I can get a correlation between this carbon and this proton or this carbon and this proton, does not matter, this carbon, this proton, this carbon, this proton. I can get the correlation information, one bond directly bonded, that is HSQC. And it uses the concept that there is a one bond coupling between carbon and proton. You know, a heteronuclear J coupling, indirect coupling. And this can be any nuclei. I took the example of carbon, does not make it mean it is only for carbon and proton. You can have a carbon proton HSQC, Nitrogen 15-1H HSQC, any other dilute spin to any other abundant spin. One of them should be abundant spin because you have to do the polarization transfer and detecting that nuclei, the sensitive nuclei. So, you can have fluorine nitrogen, fluorine carbon, all sorts of HSQC experiments are possible. If I do nitrogen 15N-1H HSQC, I can get the correlation between one bond proton and nitrogen and proton, one bond carbon proton, varieties of such experiments are possible. Of course, I also said one more thing, HSQC, HMQC and HMBC, three experiments are there. What is the difference between HSQC and HMQC? One is multiple quantum through the multiple quantum pathway we adapt, other is HSQC single quantum. Both of them provide identical information. Small difference is there in HMQC, we will also get HH coupling. There will be some homonuclear couplings among protons may also evolve. So, small difference is there in the observation. As far as the appearance and spectrum is concerned, except some couplings like HH coupling, others will also come through. As far as the heteronuclear correlation information is concerned, both of them give identical information. And if there are any differences between them, it is only small subtle differences.

Let us see what about the long range couplings in carbon 13. You can also take the carbon 13 NMR spectrum. I can get three bond coupling like this, I can get three bond, two bond, one bond like this. Varieties of long range couplings are possible. How do you correlate them? Is there any way I can correlate? In the HSQC, I said directly one bond coupling between carbon and proton if it is there, I will correlate only directly connected heteronuclei. What about long range coupling? If this proton correlates with this, in a molecule like this, is it possible? this long range. Yes, it is possible. That is called heteronuclear multiple bond correlation. That means several bonds are they are remote coupling between two nuclei, proton and less abundant spin, any other spin, remotely coupled can be correlated. Then what do you use here? In the HSQC, use one bond carbon-proton coupling. One bond coupling is needed for getting the correlation information. In the HMBC, we use long range correlation, two bond or three bond, which is from 0 to 10 Hz. Similarly, if I take CH coupling, two bond is also about 10 to 15 Hz. So, this is also 10 to 15, 0 to 10 Hz like that. So, we use long range coupling constant to correlate protons and carbons, which are remotely coupled, long range correlation.

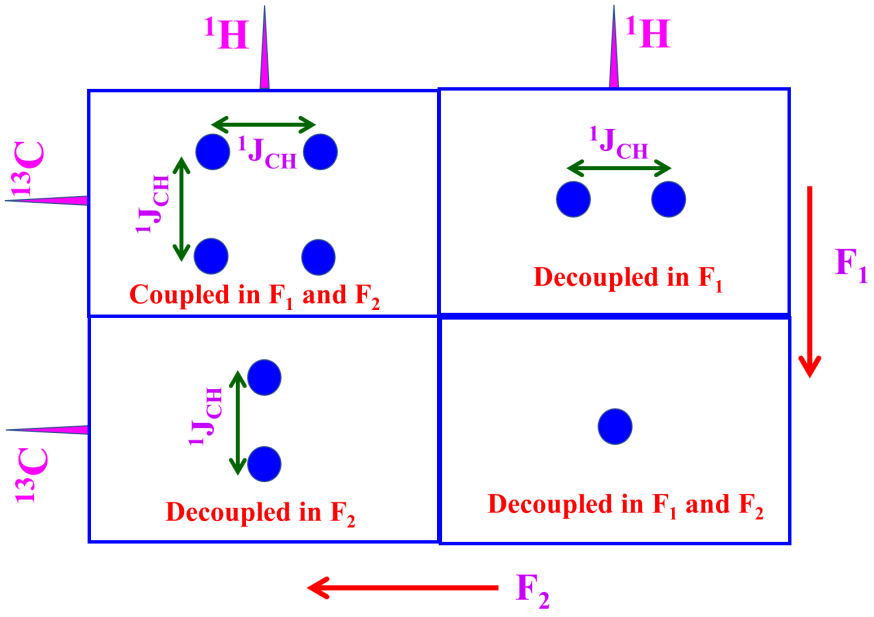


For example, in a molecule like this, HSQC, I told you will give direct correlation like this, but HMBC gives correlation of this carbon to this proton, this carbon to this proton, and this carbon to even this proton, one to three bonds away. And each carbon can give correlation to this. Like that, varieties of correlations you can think of. HMBC can give long range correlations. One carbon to many other protons, two bond, three bond separated also. HMQC makes use of multiple quantum pathway during evolution, that is the only difference. HMQC is slightly different from HSQC and HMBC. The only thing is, in the case of HSQC and HMBC, we use INEPT for polarization transfer. During the evolution time, we use INEPT. So, that is the advantage, we will gain here, polarization transfer is there. So, normally, HMQC very few people use, very rarely, HSQC and HMBC are commonly employed experiments. So, we will start discussing HSQC, HMBC in this class for some time, try to take lot of examples. So, HMBC gives cross-peaks between protons and carbon that are two or three bonds away. And then you may ask me a question, if I have a molecule like this, and I have proton here and this proton here, this can give correlation to this, this can give correlate, whatever is directly bonded, that is also there. Will it not give correlation, will it not confuse us? Then we do an experiment, we design the experiment in such a way, the direct one bond correlation peaks are suppressed. We will remove this one, retain only long range correlations. One bond direct correlations will be suppressed and long range correlations are retained, that is what is done in HMBC, that is why it is called heteronuclear multiple bond correlation. Multiple bond means, separated by multiple bonds, remotely bonded, that correlation we can do, one bond directly bonded or suppressed, they are removed. So, HMQC experiment few subtle points to understand you. Sublte point is HMQC experiment is more robust as far as experiment in imperfections and miscalibration is concerned. For example, may be slight deviation from 90 degree pulses etcetera, there is imperfection in the pulses. HMQC is tolerable. HSQC is more favorable when you want high resolution work, that is another information. You see HSQC if you want very high resolution, because HMQC generally gives broad signal because of HH coupling which are coming into the picture, that will broaden the signal. Unless of course, there are ways to remove the HH coupling by using several type of experiment. If there is a time I will discuss that

also. But remember that usually gives broad signal because of homonuclear couplings among protons are also coming into the picture. So, HSQC gives high resolution, more favorable for that. And suppression of the resonances, another important thing is we have to do. When I am correlating the carbon 13 with proton, I have to correlate this one with proton. But remember carbon 12 is highly abundant 99 percent, that is what I said. If I do an N15 proton correlation, Nitrogen 15, Nitrogen 14 is also there, that is 99 percent abundant. They give huge peaks. Somehow, we have to reduce this. There could be signal coming from protons attached to carbon 12 and N14 abundant spins. We need to suppress them. Otherwise, it is very difficult to get correlation of less abundant carbon 13 or 14N, because these huge peaks will interfere. How do you suppress that? There are ways of suppressing this. One way is by phase cycling, otherway is by gradients. There are two ways. These two things we will discuss today. So, we have to suppress protons signal bound to carbon 12 or nitrogen 14 by using either phase cycling or gradients. When we are talking about the heteronuclear experiments like HSQC, HMBC, HMQC. You may ask me a question, are there any analogous data detection experiments? Of course, there are. HSQC is HMQC is nothing but HETCOR experiment. I think very first experiment we discussed yesterday or in the previous class is about HETCOR experiment. That is a direct detection experiment analogous to HSQC. HSQC is faster because of polarization transfer, saves time, otherwise identical. What about remotely bonded HMQC experiment? I did not explain, but is there any identical experiment for direct detection? Of course, it is there and that is called COLOC, correlation of long range carbons. This is analogous to HMBC. There are also direct experiments, analogous to HSQC experiment, inverse experiments, but direct experiment nobody does nowdays. So, that is I am not discussing, but I will give you some information about coupled and decoupled carbon 13 HSQC. HSQC experiment when we do, we can do different types of experiments. This has been modified over years by various workers in the field. We can have a different type of experiment. We can get coupled HSQC, we can do decoupled HSQC and decoupling in the F1 dimension, coupling in F2 dimension, decoupling in F2 dimension, coupling in F1 dimension or decoupling in both the dimensions, varieties of experiments are possible. We will discuss what is coupled and decoupled carbon 13 proton HSQC. I can do 1H decoupling in the t1, which removes multiplicity in the F1 dimension. I can do carbon 13 decoupling in the t2, again it removes multiplicity. Either way I can do individually or I can do both or I can do nothing and get the coupling information in both dimensions, everything is possible. We will understand that what is that experiment by taking a single CH bond, a hypothetical case, we have carbon 13 proton. Take for us for example, CHCl3. In CHCl3, you have carbon and proton directly bonded, forget about Cl3. This is a CH bond I have written. What are the types of experiment we can do HSQC? I will show here.

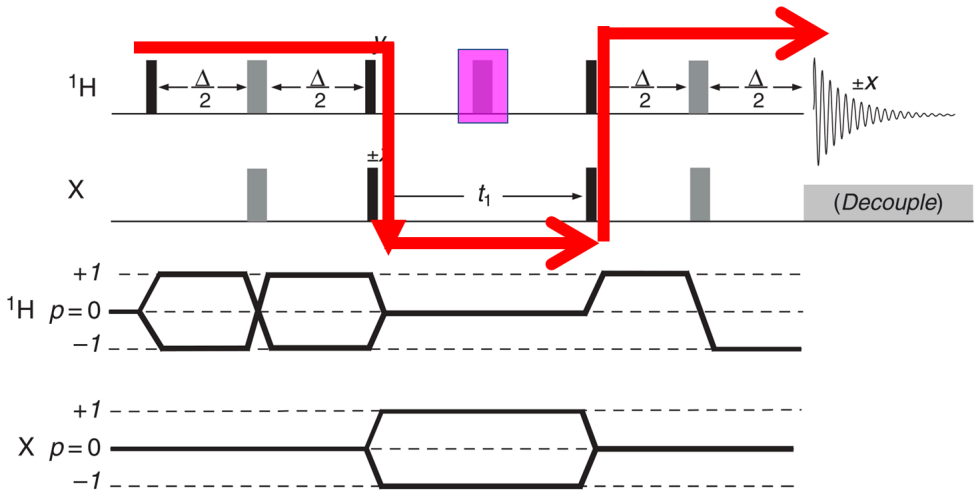


I will take the 1H spectrum of that first. What do you get? We all know if I take CHCl3, 1H spectrum gives a single peak, this one. This is from 99% of the molecule coming because of carbon 12 attached to proton. That is carbon 12 is NMR inactive, but only proton will be detected no coupling. What about 1% of the molecule which has got carbon 13? That is here carbon 13 coupled to proton, one molecule is there 1% , that will become a doublet because of coupling with proton and that is what the peaks we are getting here. I told you already they are called satellites. So, if I take the proton spectrum of the CHCl3 molecule, we get one peak for 12CH attached proton and two satellite peaks for carbon 13 attached protons. So, that is how it is. We go to the next one. If I detect carbon 13, of course, this directly gives you one bond, proton carbon coupling. What about carbon detection? It will give only a doublet. As I said, when I am detecting the dilute spin, the question of satellites does not arise. I am directly detecting carbon 13. So, it will be coupled to proton, it will be just a doublet of equal intensity. So, if I detect the carbon 13 spectrum of this hypothetical molecule, we get a doublet of intensity 50-50 because it is split into doublet. Now, what is the separation giving you? This gives you J coupling between carbon and proton. Alright. Now, I will do one thing. I will do the detection of proton with carbon 13 decoupling. What will happen? I break this carbon-proton coupling that will not be there. I remove it. I told you about decoupling long back. So, when I do the decoupling, carbon-proton coupling is broken and you get only a single peak. So, this peak is already 99% intensity. With this, it will become 100% intensity. It adds to that. You get a single peak by breaking carbon 13 coupling. What about this molecule? Here, I am detecting carbon 13 and I do proton decoupling. So, these two doublets will collapse into a singlet. 50-50% intensity will go. This will become 100% intensity for one peak. This is what we can do. We can detect proton, do carbon 13 decoupling. I can detect carbon 13 and do proton decoupling. Both the experiments are possible.



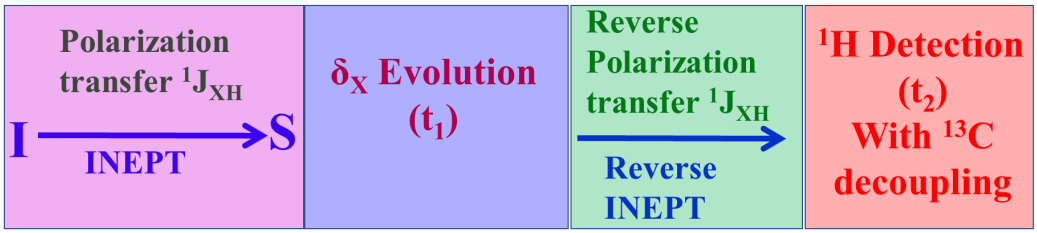
Now, let us use this and see how many types of HSQC experiments we can do. Look at this. In this dimension, we have carbon 13. In this dimension, we have protons. I will consider a situation like this. Coupled experiment, I am not decoupling. In either dimension, what does it mean? I will get like previous example. If I look for proton NMR, I get carbon coupling. If I look carbon NMR, I get proton carbon coupling. So, both are possible. In this dimension, for proton, carbon 13 satellites are there. 1JCH. In this dimension, for carbon, simply splits into a doublet, I get carbon 13 proton coupling. So, this is the situation. In both the dimensions, they are coupled, carbon and proton are coupled. This is called a coupled HSQC. When I do an experiment, this is called coupled HSQC experiment. I can do one thing. I can decouple in the F1 dimension. Which is F1 dimension? This one written here. I can decouple in this dimension and then get the coupling in the F2 dimension. So, when I decouple in the F1 dimension, what will happen? These doublets will collapse into singlet. So, you get a singlet at the center. Whereas, this coupling is maintained. So, in this dimension, it is still a doublet. Only in this dimension, the doublet is removed and you are going to get a singlet exactly at the center , because coupling is removed. This is called an experiment where decoupling is done in the F1 dimension, coupling is retained in the F2 dimension. What is the next possibility? You can think of a situation decoupling in the F2, coupling in F1, decoupling only in the F2. What is F2? This one. If I decouple in this dimension, this coupling is broken and you get a peak exactly at the center. This is called decoupling in the F2 dimension. What is the next possibility? decouple in both the dimensions, then what will happen? In this dimension doublets will collapse into a singlet and this dimension will also collapse into a singlet. You get only a peak exactly at the center, one peak. This is what we saw in the HETCOR experiment. When we try to interpret, we always saw one

contour and we went vertically up and horizontally analyzed and identified proton and carbon chemical shifts. That is what normally is done. This is a decoupled experiment, decoupled in both F1 and F2 dimensions. Then how do you do this? You have got the idea now how we can do the decoupling experiment. We can have varieties of possible experiments. Depending upon the information that you want to derive, we can have different experiments.

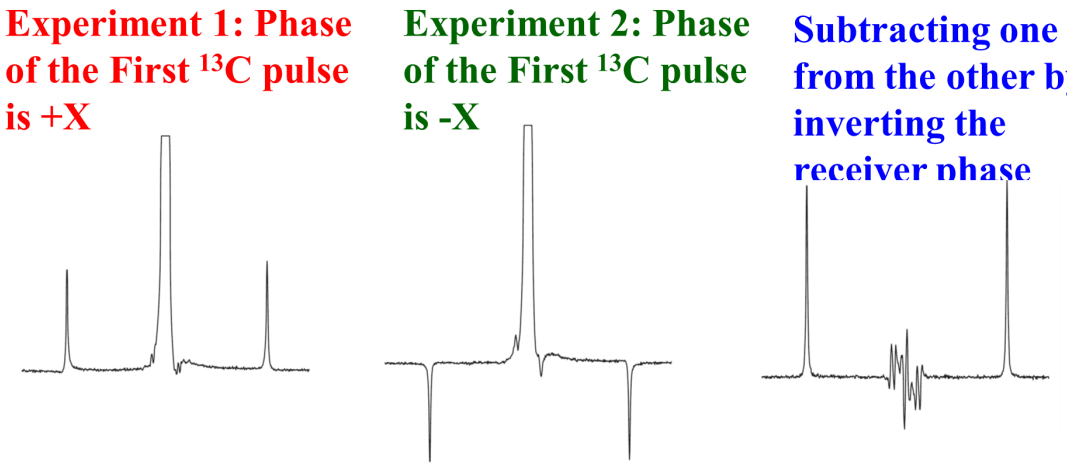


Let us understand what is the basic HSQC pulse sequence. What is the basic HSQC pulse sequence? This much you know already. What is this? It is an INEPT sequence. We have seen that already. We understood 90 on the proton with two delays which we have to manipulate with respect to JCH and simultaneous 180 on this and then simultaneous 90 on this to transfer the coherence from proton to carbon and detect. That is what we saw that. That is an INEPT experiment where the polarization transfer takes place. That is called INEPT. Then in the HETCOR nuclear spin echo, I told you, if you apply 90 degree pulse only one of them, what will happen? You can break the coupling, because that J coupling will refocus. That is what I said. Applying a 180 pulse in one of the channel in the HETCOR experiment means you are breaking the coupling. You are doing decoupling, pulse decoupling you are doing. Then afterwards what happened? This is exactly reversed here. See same thing it came like this. Now we are going backwards. What do you call this one? This is an INEPT sequence and this is reverse inept. That means you brought the magnetization from proton to carbon, and then take it back like this. So, it is called a reverse INEPT. So, HSQC is a combination of INEPT with a 180 pulse at the center of the t1 period to evolve which results in decoupling and then reverse INEPT. This is what it is. We take the magnetization from proton and transfer to carbon by INEPT and then allow it to evolve in the t1 period with a 180 pulse on the center which causes decoupling. It results in decoupling of carbon and proton and then take it

back to proton and then start collecting the signal here while collecting that signal you are doing decoupling.



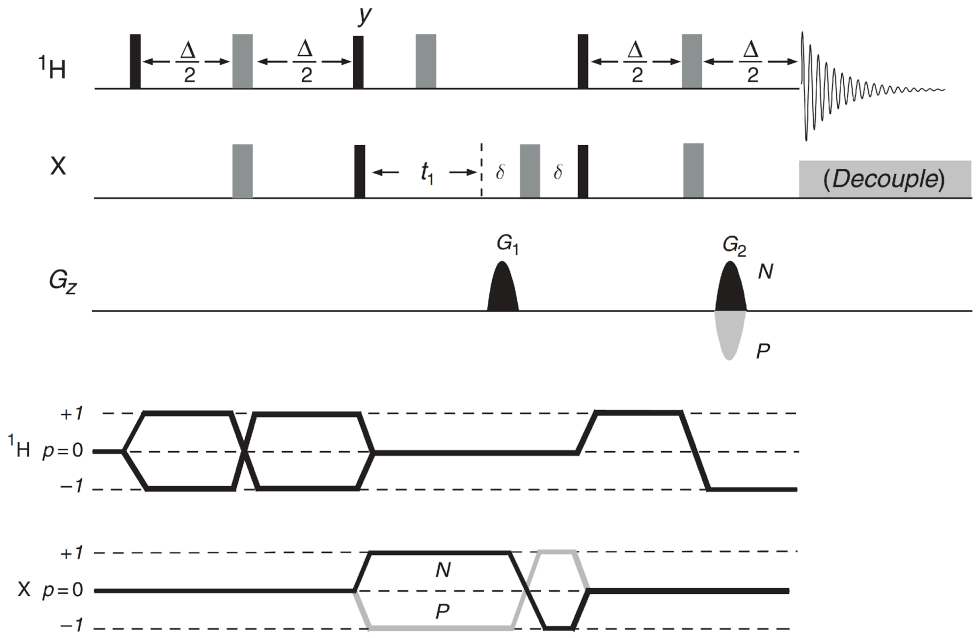
Look at this. I am collecting the carbon signal and decoupling proton. What are you going to get? You get the decoupled carbon 13 spectrum. All protons coupled to all the carbons are removed for each chemically inequivalent carbon, and gives a single peak. But in the process what has happened? You have taken the magnetization from proton to carbon, decoupled here in the t1 dimension and then taken it back. Here you are decoupling. So, in t1 dimension also you are decoupling, t2 dimension also you are



decoupling. That means like what I showed you in the previous slide, four possibilities. You are getting the last one. You get only one peak at the center. In both the dimensions the coupling is broken and this is what the basic HSQC pulse sequence does. Of course, do not worry about this thing. This has been discussed in the previous class. And then basically we use a two pulse sequence. Why? I told you we have to suppress the carbon-12 peak, proton peak coming because of attached to carbon 12. That is important thing. We have to suppress that, so, what we do is we use what is called a phase cycling. Phase cycling I did not discuss in this course extensively. But remember in the one of the previous classes of my advanced course, I discussed about phase cycling. I discussed various things about pulse field gradient etcetera to select a certain coherent pathway. That is what phase cycling does. But anyway without going into the details, remember

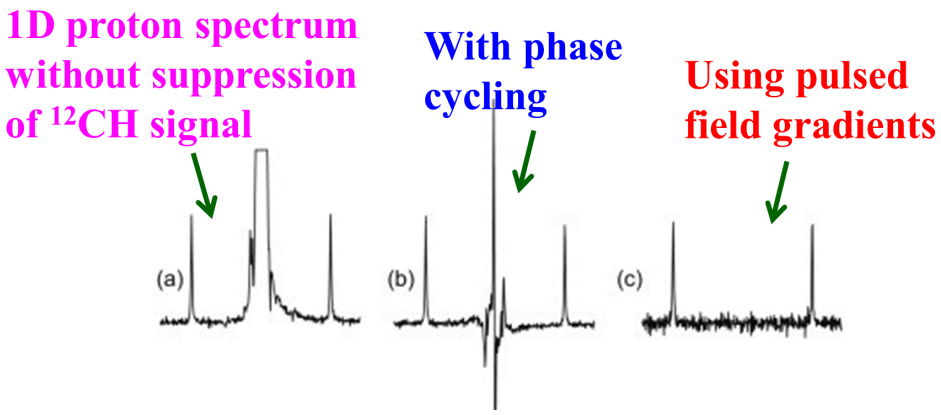
how we do it. We do a first 90 X pulse experiment and then we invert the receiver accordingly, and in the second experiment we do 90 minus x and then the take the difference between the two. This is what we do. In both HSQC and HMBC experiment what happens? The mechanism of polarization transfer is identical. Does not matter. So, this is what it happens. Transfer of magnetization from proton to carbon. This is identical for HMBC also. Proton to carbon, allow the spins to evolve. Then do the decoupling whatever you want in the t1 period and then reverse INEPT take the polarization from carbon to back to proton, start detecting the X nuclei while doing proton decoupling. See we are detecting proton, that is an advantage. Look at it, the sensitivity of detection here. You are detecting proton and decoupling carbon 13. That is important thing. So, this is what it is. So, another important point I wanted to tell you is when the HSQC is recorded, we have all the signal whether it is carbon 12 or carbon 13 attached, system does not distinguish, and the instrument does not distinguish, you are going to get the signal from both these things. All protons will give a signal. The unwanted resonance is 99 percent. That if you suppress, you can efficiently detect the correlation of carbon 13. That is what I said. So, interference of the C12 and N14 peaks have to be suppressed. This is what I said in the basic 2 pulse sequence, 90 pulse experiment associated with inversion and then co-addition of the data will suppress the signal corresponding to protons attached to carbon 12.

How do you do this? First experiment you do with 90 plus x, second experiment you do with 90 minus x, and then each time when you do, you change the receiver phase also accordingly. Afterwards, do the co-addition of the data. This is what happens. I will show you example how it works. Take an experiment 1 where I am doing an experiment, with first pulse on carbon 13 is plus x. I get a signal like this. This is major thing coming carbon 12 attached proton and I am interested in these satellite peaks. Huge peak is coming and then that will be suppressed, because the major component is coming from the abundant spin C12 attached to proton. That is one thing. That is one experiment I will do with first pulse plus x. What I will do, another experiment, the first carbon 13 pulse is minus x. What happens in that case? Carbon 13 satellites get inverted like this. How it works everything is a different question. See in another experiment, I do the phase cycling with plus x pulse and minus x pulse, the C12 signal remains same, C13 signal is inverted because we are looking at C13 coupling. What I will do? I will take the difference between these two. When I take the difference between these two see this component I am trying to suppress and I retain only this signal, that intensity will be better now. That is what we have to do and that is how we suppress this. This is one way of suppression by using what is called phase cycling. We suppress this by using phase cycling. But remember this phase cycling does not suppress efficiently. Look at it, not very efficient. It gives some artifacts. There is some problem. To overcome that, another experiment is designed. Instead of phase cycling, we use gradients here.



That is an interesting thing. See everything is remains same, but only thing is during this time after the t1 period after 180 pulse, we use what is called a sort of an echo like sequence and during this echo time, one pulse is applied and here another pulse is applied during this time a gradient pulse is applied. That was very important. The G1 gradient is applied within the spin echo to refocus that carbon chemical shift and G2 is applied during INEPT refocusing period. What happens, the first gradient acts when carbon has a coherence p is equal to plus 1. This is what is important here. You see here n and p peaks are there, coherence plus 1 and minus -1, they are called. That we discussed extensively in one of the previous courses. Here simply remember G1 acts when the coherence is positive, p is plus 1 single quantum coherence. Then it will become G1 into gamma 13C. The second gradient acts when the single quantum magnetization is p is equal to minus 1, the coherence transfer pathway is minus 1. Then multiplying with this, it will become G2 into minus gamma. And if I have to maintain a particular coherence pathway for HSQC, the sum of these gradients phase induced because of the gradient must become 0. That means G1 into gamma 13C minus G2 into gamma H should be equal to 0. The gamma ratio I know between proton and carbon is 4, I put this value for gamma. Now if I have to maintain ratio of G1 and G2 for carbon 13, it is 1:4. If I make 1:4, this becomes 0. G1 should be 4 times, then this is 1, then it will become 0. So, proton should be 1 times, gamma, this gradient pulse for carbon should be 4 times. Same way for nitrogen 15 like this. When you do this experiment, this is called anti-echo experiment. And when we do this, we select when we have once n peaks, other time p peaks. And then when we do this experiment, apply the gradient once 4:1, once 4:-1. That means we select n peak and p peaks. It is called echo anti-echo method. We collect the signal by doing this. This is

what happens. Look at this thing. This is a first experiment without suppression with phase cycling and this is with the gradients.



See the advantage. In the phase cycling, there is some disturbance here, not completely suppressed the artifacts. But in the gradient, C12 attached proton signal is completely suppressed. This is an advantage. So, parent signal coming from carbon 12 peaks are suppressed efficiently by pulse field gradients. So, this is an experiment which is done and then HSQC is recorded. So, the time is up. I am going to stop here. So, what we discussed today, we discussed extensively about HSQC experiment. I told you what is an HSQC experiment, what is HSQC pulse sequence, how it works, we discussed HSQC. As I said, we take the magnetization from proton, give it a carbon and then take it back to proton and detect proton. In between, during the t1 period, I can apply a 90 pulse to do the decoupling and I will be detecting the proton, I do the carbon decoupling, so that I will get the carbon decoupled proton spectrum. So, I can do the decoupling in both the dimensions. And I showed you various ways of doing the decoupling. You can have decoupling the F1 dimension or F2 dimension or one of them or both, or none in both the dimensions or both the dimension, varieties of possibilities are there. But major problem comes in the HSQC experiment is the parent signal coming from the carbon 12 attached protons or N14 attached protons. This you have to suppress by phase cycling with a two pulse sequence. Once we have to apply plus x pulse, once we have to minus x pulse, correspondingly, you invert the receiver phase and co-add the data, you will suppress the 12C peaks. That is not very efficient, then we use the gradient method and the gradient we know how to calculate which should be 1 is to 4 for carbon and 1 is to 10 for nitrogen and proton. And then once we apply a positive gradients for once, and the other time we apply negative gradient. And then we collect n peaks and p peaks, it is echo and the decoupling method we adopt. Again, co-add the data then you will suppress efficiently and I showed that gradient method is better and suppresses the carbon-12 peak parent signal. So, with this I am going to stop today and in the next class we continue with this HSQC and afterwards we will go to a different 2D experiment. Thank you very much.