One and Two dimensional NMR Spectroscopy: Concepts and Spectral Analysis

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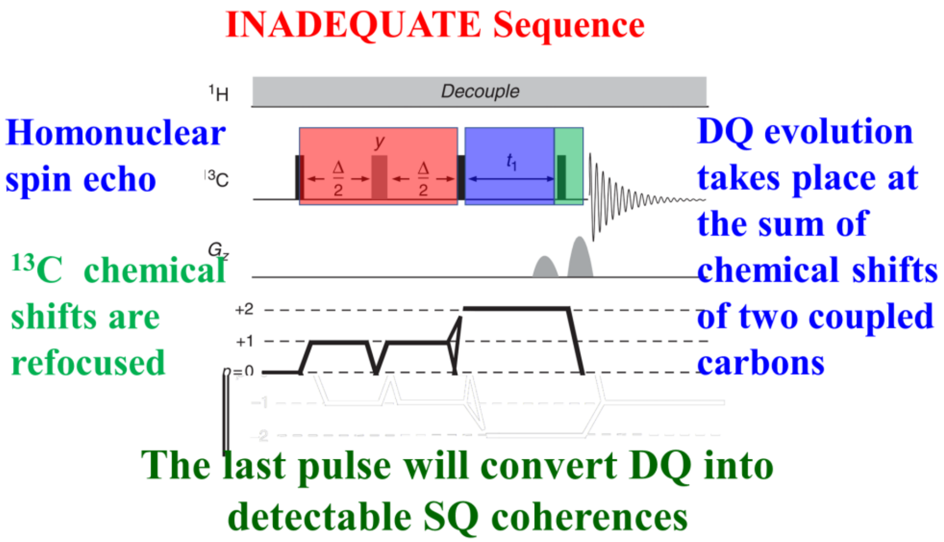
Lecture 48: 2D INADEQUATE

Welcome all of you. In the last couple of classes, we started discussing about heteronuclear 2D experiments, wherein we discussed a lot about HSQC, which stands for Heteronuclear Single Quantum Coherence or correlation whatever you call, and HMBC, both we discussed. In the HSQC, you can correlate between two spins, heteronuclear spins. One is an abundant spin and the other is a dilute spin. That is what we saw. And of course, it can be done for any two heteronuclei. In HSQC, what is done is, the polarization transfer from abundant spins to rare spins and then during the t1 period, we apply a 180 pulse so that we break the heteronuclear couplings and then transfer the magnetization back to protons and detect proton, while decoupling carbon 13 or ^{15}N, or any other heteronuclei. And that is a very simple one. And we also understood there is a strong interference from the C2 attached proton signal in carbon 13 or ^{14}N attached in nitrogen 15. These parent signals need to be suppressed, for that we adapted what is called a phase cycling method or pulse field gradients, both are possible. And then we observed that varieties of experiments we can do in the HSQC. You can do the decoupling in one dimension F1 or F2 or decoupling in both the dimensions or do not do decoupling in any dimension. So, there are varieties of experiments possible. We took the example of a simple molecule and saw how the spectrum comes, how we can interpret the HSQC spectrum based on a coupling patterns, especially and when we get a one bond coupling coupled spectrum. And in the decoupled experiment, we broke the coupling between carbon and proton and we knew how to interpret. In one dimension you are going to get the carbon-13 or nitrogen 15 chemical shifts and other dimension abundant spin like proton or fluorine, and that chemical shifts we are going to get. And in case there are homonuclear couplings present in the molecule, I also gave one or two examples to see how we can get the proton-proton couplings also from the HSQC spectrum. Analogous to what you are going to get in the analysis of the satellites of a dilute spin in the 1H spectrum, if HH couplings are present. That is what we observed.

Similarly, we extended our interpretation for HMBC with a multiple bond correlation, where there is a correlation between the abundant spins and the dilute spin which are separated by 2 to 3 bonds away. In which case again we can have two types of spectra coupled and decoupled. I told you in the HMBC most of the time we do not do the decoupling. We get the coupled spectrum. That is what we are going to observe. And then of course, in HMBC there is one thing, there are two filters. One is Jaccept filter other the Jreject filter. Jaccept filter is the one where the long-range correlations are allowed through and the Jreject filter is the one where one bond HSQC type correlations are rejected. That will not be allowed through. And of course, this can be done by several methods. And of course, we can have suppress the proton-proton couplings by using BIRD sequence. We can also have the constant time experiments and we you can do phase cycling, and varieties of experiments we can think of. All of them we discussed with many examples. We also analyzed varieties of spectra of both HSQC and HMBC. They are all only heteronuclear correlation, where one of the spin is an abundant spin. Now, I will go today I will go into a different experiment where we are going to see the correlation between two dilute spins. This is what we are going to start today. For example, this experiment is called two-dimensional INADEQUATE experiment. As I always say for understanding INADEQUATE, one should have adequate knowledge of NMR. Of course, it stands for Incredible Natural Abundance Double Quantum Transfer Experiment. It is called INADEQUATE experiment. This is what we are going to discuss today. Of course, as I told you in the 2D correlation experiments, COSY/TOCSY, we always correlate homonuclear abundant spins. Of course, we also have heteronuclear correlation like HETCOR. We also have inverse experiments like HSQC and HMBC where the experiment is done faster and correlates heteronuclear spins, where we saw in the examples at least one of them is an abundant spin and the other is a dilute spin. Now, we come across a situation, let us think of a molecule where you have several carbons present in that, I want to see which carbon is coupled to which carbon. Or in other words in a given molecule, the carbon structure or carbon skeleton of a molecule if I want to identify, that is which carbon is sitting next to which carbon or which carbon is bonded to the next carbon, how do you do that? This is a correlation experiment which we have to do. Remember, both carbon-13 is in natural abundance and 2D correlation of natural abundance is extremely difficult. Whereas, in the two dimensional INADEQUATE experiment, we can do something. And we can understand what we can do, how we can do, how we can correlate two carbon 13 spins in their natural abundance, very interesting thing. And why we have to do this type of experiment? Of course, why we cannot do direct determination of carbon-carbon connectivity? As we already know it is not easy. Carbon is already 1% in abundance, simultaneously seeing two carbons present as carbon 13 is very difficult and to see the correlation between these two carbons, and to establish that in a two dimensional experiment is a difficult task. It is a very very challenging task. So, as a consequence what is done is, it is determined usually through protons indirectly like in HSQC experiment. Transfer the magnetization from protons to carbon and then take it back and see what happens during the process, like that. Of course, problem with this type of 2D INADEQUATE experiment is, it requires longer instrument time because we are detecting, remember carbon 13, natural abundance 13C- 13C correlation. And in addition to that you require enormous quantity of the sample, large quantity of the samples are required so that you can acquire the data faster. So, this is a very difficult task. Of course, finally if I have to do it, only option left over for me is INADEQUATE. It is last option. When we do that experiment, we can establish the correlation between to dilute spins. Of course, I already told you whenever we were analyzing the carbon 13 NMR spectrum, the possibility of seeing two carbons in the carbon 13 state in a given molecule is 1 in 10,000 because of 1% abundance. Each carbon is 1 in 100 abundance. So, the joint probability of seeing both the carbons as carbon-13 is 1 in 10,000, very very small. So, the possibility of seeing three carbons in 13C, I told you already when we discussed carbon 13, is 1 over 10,000. to again 1 over 100, 1 in a million, very difficult. So, it is like telling this type of possibility of seeing more than 2 carbons simultaneously is difficult. So, we will at least try to find out the possibility of seeing 2 carbons in the carbon 13 state. It is like detecting carbon 13 correlation in a given molecule is analogous to detecting carbon 13 satellites in the carbon 13 spectrum. Remember, carbon 13 is 1% abundant and the direct detection itself is very weak, very less sensitive and if you want to see carbon 13 satellites in carbon 13 itself, imagine a hypothetical situation, how difficult it is? It is exactly analogous to that. Of course, major hurdle when I try to do the 13C-13C correlation is the parent signals from the main carbon, main signal, because that is always present. And if you want to see the correlation between two carbon 13, each individual isotopomer has of carbon in carbon 13 state. That gives a parent signal from 12C. That has to be suppressed, that is a very important thing. Then it is possible for us to establish correlation between two carbons. And the two coupled carbons you can consider as an AX spin system, when the 2 carbons are coupled.

Generally, of course, chemical shift is quite large. Let us say if you say even 10 ppm or 20 ppm, if it is a 20 ppm in carbon 13, even if it is 400 MHz spectrometer, 20 x 100, it is going to be 2000 Hz. Couplings are very small, 30 Hz, 40 Hz, 50 Hz, 100 Hz. You can consider them as fairly weakly coupled or sometimes AB, but most of the time we have seen it can be treated like a 2 weakly coupled AX pin system. Then what we can do, if I have 2 weakly coupled spin system, there are 4 possible spin states that we have been discussing alpha-alpha, alpha-beta, beta-alpha, and beta-beta and the transition from alpha-alpha to beta-beta is not allowed because it is a double quantum, twice the energy is required. But somehow we can select the pathway, we can take the spins to, instead of single quantum to a double quantum state, filter it, bring it back after applying a double quantum filter, bring it back to minus 1 state, and then start detecting the signal. This is exactly what we did in double quantum filtered COSY, DQFCOSY. Similar to that, if I can use double quantum filter, I can efficiently suppress singlets coming from individual carbons, that parent signal can be suppressed very easily, that is one way. So, that is why it is called Double Quantum Transfer Experiment. And this is an inadequate sequence. Of course, you may ask me question, why only double quantum, why not higher quantum? Of course, there is no need for it because beyond double quantum the possibility of three carbons existing is one in million, I told you. So, there is no need to worry about it. So, only double quantum field is just sufficient. Even triple quantum also can filter single like parent signals, but double quantum is just sufficient, we do not need to worry more. This is an inadequate sequence. Just by looking at the sequence, you can always understand.



INADEQUATE Sequence

Homonuclear spin echo

^{13}C chemical shifts are refocused

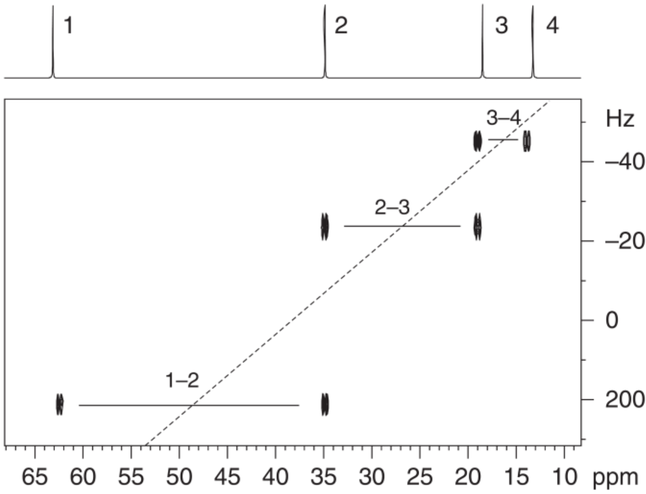
DQ evolution takes place at the sum of chemical shifts of two coupled carbons

The last pulse will convert DQ into detectable SQ coherences

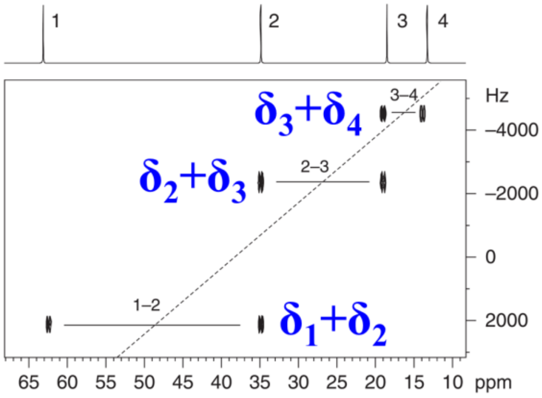
Of course, this is a very simpler sequence. By looking at the sequence, you can imagine what is that. It is like a spin-echo sequence. In any pulse sequence, if you have 180 pulse with a delay on either side, you can think it like a spin-echo like sequence. And then of course, you have evolution period t1, apply RF pulse for detection and then start collecting the signal. Of course, this is more about details about selection of what is called the coherence transfer pathway. Always, you know, coherence when magnetization is along z axis is considered as pathway zero. And then you can take it to single quantum by applying a 90 degree pulse. A 90 degree pulse will take the magnetization to either plus 1 or minus 1 pathway. And then when it comes like this, we can also use this by applying a 180 pulse at the middle, we can take this plus 1 or minus 1 to either plus 2 or minus 2. And then of course, you always detect the signal at minus 1, that is what I always tell. So, we bring the magnetization from plus 2 state to minus 1 state by using the gradients of appropriate strengths. We know how to select it or we can use phase cycling, select a particular coherence transfer pathway. This is what is called coherence transfer pathway selection. I have not discussed this in this course, but in one of the previous courses, it was extensively discussed. Those who are interested can see that. This is basically the pulse sequence. And if you want to understand conceptually, what is happening is like this.

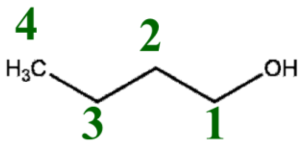
After applying a 90 pulse, you have a homonuclear spin echo. In this case, we are applying on carbon 13. We are worried about homonuclear spin echo on carbon 13. In this case, what will happen? Carbon 13 chemical shifts are refocused. Please remember, I discussed about homonuclear spin echo and heteronuclear spin echo. In the case of homonuclear spin echo, always chemical shifts are refocused, whereas J couplings continue to evolve. That is what you always remember. And this is what is happening. And afterwards, we are going to apply 90 degree pulse. Then after applying 90 degree pulse during this time, what is going to happen? The double quantum evolution takes place. What is the double quantum? The double quantum always evolves at the sum of the chemical shifts. Now, I consider two carbons, let us say carbon 1 and carbon 2, if I consider, this is one chemical shift, and this is one chemical shift. Always double quantum evolves at some of the chemical shifts and the separation between them gives rise to some of the couplings between the active and passive spins. But anyway, don't worry about all those things. In the multiple quantum techniques which I discussed in one of the previous courses, extensively I discussed multiple quantum methodologies. How do you do, excite and detect multiple quantum of homonuclear and heteronuclear spins etc. But you remember one thing, after the spin echo sequence, we apply a 90 degree pulse and during this t1 period, double quantum evolution takes place where the carbons evolve at the sum of their individual chemical shifts. Afterwards, we apply another pulse, it is a detection pulse, 90 degree pulse. This converts the double quantum into single quantum, because you always detect single quantum, we cannot directly detect double quantum. So, it is converted into single quantum, we collect the free induction decay. And of course, this is all about gradients, everything for selection of coherent pathway, you do not worry about it. Basically, you worry about only this part of the pulse sequence. But you can always see at the top what is happening is we are applying a radio frequency pulse on proton, it is a decoupling pulse. We are continuously decoupling proton while doing this experiment. There is a lot of advantage for it. So, this is the basic thing, decoupler pulse also gives rise to what is called NOE. So, we are going to do this type of experiment. INADEQUATE is a very simple experiment, a spin echo sequence followed by 90 degree pulse where double quantum evolution takes place and apply another 90 degree pulse, convert double quantum into single quantum, detect the signal while continuously decoupling proton all through the pulse sequence, right from excitation to detection. And this is the concept of 2D INADEQUATE. In summary, what a 2D INADEQUATE does is initially allow the double quantum coherences associated with two carbon-13 spins which are coupled, to evolve during a variable t1 period. The t1 period is varied, because a two-dimensional version. And reconvert them back to detectable single quantum magnetization, carbon magnetization, okay. And we are detecting carbon by doing, of course, the decoupling of protons.

So, in this case, what is going to happen? In the F1 dimension, there is evolution of carbons at the double quantum frequency. So, where the peaks come, when there is evolution? It is at the sum of the chemical shifts, you always get the peaks at the sum of the chemical shift delta 1 plus delta 2 of the coupled carbons. The two carbons are coupled, having chemical shift 1 and 2 here, C1 and C2, and the each peak in the double quantum dimension pertains to the chemical shift which is the sum of chemical shifts of individual carbons. That is where the cross peak comes in the F1 dimension. Of course, this is how the spectrum looks like.

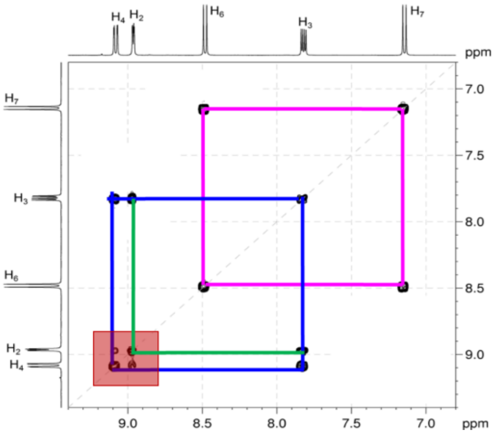


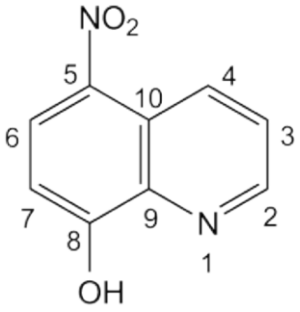
And hypothetical molecule or a realistic spectrum, we have four peaks, four carbons. And in the F2 dimension, what you are going to get? Peaks at the corresponding single quantum chemical shift. See, you have four peaks here, 1, 2, 3, 4, four carbons. Each carbon if you come down here along this axis, it will give you the chemical shifts, carbon chemical shifts for individual carbons. That is fine. But in the F1 dimension, what is going to happen? You are going to go along this axis and this is for 3 and 4 peaks coupled and this axis correspond to double quantum dimension or double quantum chemical shift corresponding to the sum of chemical shift 3 and 4. This one for correlated peaks between 2 and 3 and this axis gives me the chemical shift corresponding to the sum of carbon 2 and 3 chemical shifts. This is chemical shift corresponding to sum of 1 and 2 carbons chemical shifts. That is how the F1 dimension always gives chemical shifts at the sum of the coupled carbons. Whereas in the direct dimension, it is analogous to your single quantum carbon 13 spectrum where you get carbon 13 chemical shifts. If you carefully observe here, each of this peak is a doublet because it is an AX spin system, two carbons when they are coupled, I said AX spin system. I have already discussed in a POPLE nomenclature. The AX spin spins, when two spins are coupled, each of them will be a doublet of equal intensity. Exactly this is a doublet, this is a doublet, these are all doublets. So, that is how it comes. Another interesting thing what is going to happen is, we are applying carbon 13 proton broadband decoupling all through the sequence. This is an advantage, there is a benefit of NOE and the enhancement in the sensitivity. That is an advantage. When I come to NOE, maybe next class or another class after that, I am discussing more about NOE. There we will show what happens if I irradiate a particular proton and the NOE takes place to the adjacent nuclei. Depending upon whether it is a homonuclei or heteronuclei, what is the percentage of enhancement we can calculate. We will discuss that later. But please remember now, like I said in the carbon 13 NMR when I was analyzing, the benefit of applying decoupling power all through the sequence is NOE enhancement in the signal. The sensitivity becomes better, that is all. Now, how do you interpret the INADEQUATE spectrum? Correlations are made by following the horizontal traces, parallel to F2 axis and then you have to go vertically up. This is what we do. The carbon connectivity is established by sequentially going through horizontal and vertical pathway. Similar to your COSY. COSY we know we go to diagonal peak went like this and then go like this, we went like a stepwise manner. Exactly here also, we have to do it. But another thing what you should understand is, you are going to get a diagonal here, which is hypothetical, there is no diagonal. I have written a diagonal written like this. It is called the double quantum diagonal coming at the center of each of these peaks. If you draw a line, you get a diagonal that is called a pseudo diagonal, double quantum diagonal. It does not exist. Just to show you people I have written here. So, you can establish the connectivity like this, horizontally and then vertically. We will really do that analysis and then find out. Of course, I told you there are no diagonal peaks, but still the midpoint of all these spin pairs if you join, take it and draw a line I told you just now, a pseudo diagonal can be created. That is called pseudo double quantum diagonal. It comes at places where F1 is equal to twice F2. If the F1 frequency is equal to twice of F2, you have a pseudo diagonal. In the INADEQUATE actually there exists no diagonal, but you can draw a pseudo diagonal joining a central point of all these coupled pairs that is a analogous to a diagonal called a pseudo diagonal. It is like this. So, each cross peak is a doublet with a splitting of J_{CC} carbon coupling similar to your AX spin case. So, there is a pseudo diagonal, doublet. Now, you understood almost how to interpret. I will tell you here how we can do that. Simply start with one of the peaks here, which you know something which we are confidently you can assign. Assign that peak, let us say this is 4 here, come vertically down, you hit a peak and of course, horizontally if you go you hit another peak. This is a F1 axis where sum of two chemical shifts between this and this will be there. That means it is correlating to this. This carbon is correlating to this one. So, this is the double quantum dimension, the sum of chemical shift and each of them is a doublet, CC coupling is there. With that what we will do is after we establish connection from 3 to 4, come down again, from 3, you hit another peak. Of course, it is same because chemical shift of same carbon is there. Now, horizontal, from photon 4 or carbon 4, you came horizontal, hit a peak that is carbon 3, another carbon because these two together is giving a double quantum here. Come down vertically, you hit a peak that is same chemical shift, same carbon, there is no difference. Now, from this point go horizontally, hit another peak that is another carbon and come down it is the same chemical shift. Go horizontally again, you hit another carbon. What we did here, please remember always the analysis of the diagonal in the INADEQUATE spectrum is very simple. All you have to do is start with a particular peak, identify a chemical shift of that and then go horizontally, you will know it is coupled to which carbon, that carbon chemical shift is known. These two are correlated, coupled to each other. Come down and then again go horizontally, you will hit another carbon. Come down, go horizontally, hit another carbon. So, always when you are coming vertically down, you are in the same chemical shift. When you are going horizontally, you will hit another carbon, which is coupled to this carbon. Like this, you can trace out all the carbon connectivities. The interpretation of the INADEQUATE spectrum is fairly simple. All you need to do is identify one carbon, go horizontally, vertically, horizontally, vertically, keep going like a step function, you will get all the assignments made. That is what it is. Let us see the 2D INADEQUATE spectrum, which is always recorded in the magnitude mode of n-butanol, a molecule like this. This is what I said, we analyzed this just now. Now, I know the structure of the molecule also.



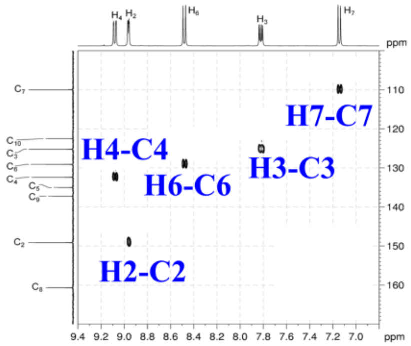


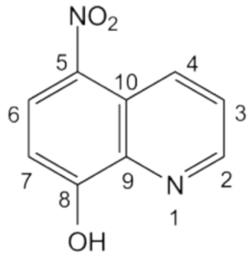
This is CH3. CH3 always comes at high field. So, confidently we can say this is the carbon 4. Identify that and this axis corresponds to sum of chemical shifts, delta 3 plus delta 4. This is corresponding to 3, come down and come here, this is delta 2 plus delta 3, come down here, this is delta 1 plus delta 2 because this is 1. This is how we can do it. We will now go ahead and try to analyze a given molecule, get all the carbon connectivities in a simpler possible way. Of course, before you go to INADEQUATE, you will have some knowledge about the assignments of protons or carbons. How do you do that? Of course, you use other experiments like COSY, TOCSY or HSQC or Multiplicity edited HSQC, HMBC, whatever it is. You can use that, so that you have some idea where to start. So, we will adapt that strategy. We will take for this molecule COCY spectrum, carbon 13, multiplicity edited HSQC, 13C-1H HMBC and then using these, we also analyze INADEQUATE spectra. All the four we can analyze for this molecule, then you will know about the structure of this molecule. Let us start like this. This is a proton-proton COCY. How do you interpret this one?



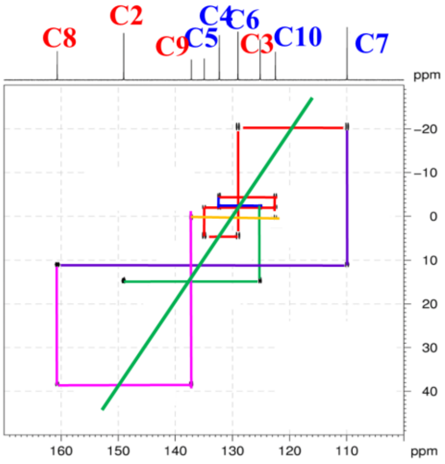


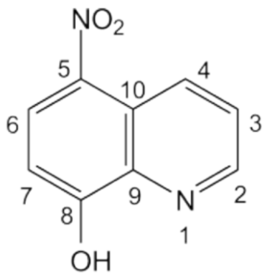
Of course, if you carefully see in this axis, there are only two protons. I have already told you 100 times how we can interpret the phenyl protons chemical shift or spectrum corresponding to the protons from the phenyl group. Here, there are only two ortho protons. Each proton experiences single ortho coupling. So, this will be experiencing ortho coupling to this, this is experiencing ortho coupling to this. So, each of them should be a doublet and the identical separation should be there. So, if we carefully look at it, we have two doublets of identical separations. I would fairly say these are the two carbons which you can see from the COCY, you can identify that very easily, no difficulty at all. One spin system is identified already. This correlation is established between proton 6 and 7. Now, what about the others? You have three more. In the three more, carefully if you see, you can understand this proton experiences two couplings. But remember when the nitrogen is attached here, this ortho coupling will be smaller than this one. We have analyzed in earlier case example. So, it should be a doublet of a doublet. Where can you see doublet of a doublet here? Clearly, you can see this bunch which is a doublet of a doublet. I can confidently assign that to H3. Start with that, come down, you are hitting two peaks, two cross peaks. Go down, complete it. It could be H3, and is coupled to another one. Now, again go by this. This will be ortho coupling larger. This is smaller. From the separation, you can say which is 4 and which is 2 easily. That is why I will say this is H4. That separation is much larger, larger ortho coupling compared to the other one. So, I will complete that square. So, I can assign H3 and H4. Then of course, H3 also correlates to H2. There is a cross peak. Further, this H2 is also correlated to this one. H2 is also correlated to H3. Of course, H2 is also correlated to this one. H4 and H2 both are correlated to H3. Now, we can make the assignment of all the protons for this molecule using COSY. We will go further. Next, it is a multiplicity edited HSQC spectrum.





But of course, you see all the peaks are of similar signs, same sign. There is no peak with negative sign. What does it tell you? I told you, multiplicity edited, if there is a negative sign, they are all corresponding to carbon attached to two protons. They are CH2 carbons. CH and CH3 carbons are always opposite in sign to CH2 carbons. That is what I told you when we interpreted the multiplicity edited HSQC spectrum. Now, all these things tells me they are all odd proton attached carbons. Of course, looking at the molecule, they are all single proton attached carbons. Very easily, you can make the assignment because I know already we have assigned the proton. So, this is H7 correlated into C7. Go along this axis, you get proton chemical shift and along this axis, you get carbon chemical shift. H3C3, H6C6, H2C2, H4C4. All the carbons and their attached proton chemical shifts can be obtained for five of them. There are 3 plus 2, equal to 5. But other carbons, you do not know how do you place it. How do you get the carbon skeleton of the molecule? It is not so simple. So, what we will do is, we will go to INADEQUATE now.





Very easily, we can start making the assignments. Of course, protonated carbons we have assigned already here in the multiplicity edited HSQC. We have protonated carbons assigned here, most of these things. So, out of the carbons here. Out of, forget nitrogen, there are 6 plus 3, 9 carbons. Out of 9 carbons, 5 are protonated, we could assign. The remaining 4 only we have to make the assignment. What we will do is, the one which we are confident, we know definitely, we cannot make this carbon that correspond to CH3 carbon, start with that. And of course, it is a double quantum diagonal, very, you can see that. Start with carbon 7. If we carefully see it is a doublet, it is AX spin system. Start with that, go horizontally, you hit that, that has to be carbon 6. Come down, you go horizontally, then you see carbon 5. Go up, go horizontally, you hit carbon 10. At the same time, carbon 10 has 2 correlations, one for carbon 4, other for carbon 3. See this one, carbon 4, carbon 3. And then you complete this, you go to carbon 2 and C7 also correlates to other one, that is C8. And you can complete it and you go to C9. All the carbon skeleton can be easily assigned. This is a beauty of INADEQUATE experiment.

What you could not get it from HSQC, what you could not get it from other experiment, you can trace each carbon skeleton which carbon is sitting next to which can be assigned by using INADEQUATE experiment. So, finally using this, of course, this is another molecule where we have to get the structure using INADEQUATE other things. Since the time is getting over, of course, this needs bit more explanation, I will come back in the next class. We will again take this and then continue further. So, since the time is getting up, I am going to stop here. Today, what we did is, of course, to summarize, we started discussing about a new experiment called 2D INADEQUATE which is used for correlation of dilute spins like 13C-13C. All other experiments we have done HSQC, TOCY, COCY, HMBC, they are different type of correlation of homonuclear spins or one of them is abundant spin, other is a dilute spin. But in this case, we can correlate only dilute spins. Pulse sequence is very simple, a spinecho sequence followed by another 90 pulse which will create the multiple quantum and then you would create a double quantum filter. Filter out this thing and then use the third pulse which converts into single quantum and then start detecting the signal. And you are going to take pseudo double quantum diagonal in the INADEQUATE, use that and then assignment becomes very simple. Each peak is like AX spin system, doublet, start with one of them known to you, go horizontally, come down vertically, horizontally, vertically like that, keep continuing, you will be able to make assignment of all the carbons connected in the molecule. You require some help of COCY, TOCY, etc, and other experiments. You can combine them and do it. And we tried to analyze one of the molecule.s We will go slowly again and then understand it in the next class. Thank you very much.