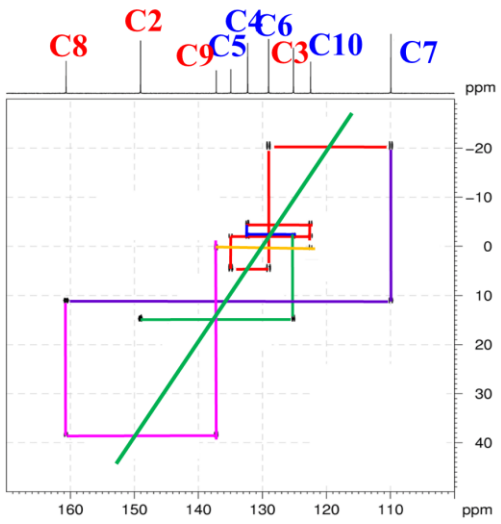
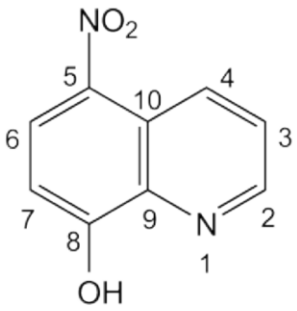
One and Two dimensional NMR Spectroscopy: Concepts and Spectral Analysis
Prof. N. Suryaprakash

CSIR Emeritus Scientist, Solid State and Structural Chemistry Unit
Indian Institute of Science – Bengaluru

Lecture 49: 2D-INADEQUATE and 2D J-Resolved

Welcome all of you. In the last class, we started discussing about a different two-dimensional experiment called 2D INADEQUATE. This is the experiment used to correlate two dilute spins. This is completely different from other type of experiments where you are correlating two homonuclear spins or one homonuclear and other heteronuclear spin, one would be abundant and one dilute. They were other type of TOCSY, COSY, HSQC, HMBC experiments. In this 2D experiment, INADEQUATE experiment, we are correlating two dilute spins and this pulse sequence is a very simple sequence, which I told you. It is a spin-echo sequence followed by the evolution of the magnetization, double quantum evolution and then we apply a 90 degree pulse, convert them to single quantum and then after filtering through double quantum, collect the single quantum and detect the carbon 13 while decoupling proton. And the two carbons present simultaneously as carbon 13 has a probability of one in 10,000. So, the double quantum is just sufficient, maximum we can see two carbons. More than that what happens is very, very difficult, quite unlikely. So, we do the double quantum filtering and the spectrum is very easy to interpret. We saw that. When you get a spectrum, of course, there are various things we discussed. You start with one of the peaks, you are confident that you know there is a cross peak, go vertically down that is same chemical shift or horizontally meet another cross peak that correspond to the chemical shift of the other carbon. Like that you go ahead and then trace the complete carbon skeleton of the molecule and I started analyzing one of the spectra in the last class. It was in a hurry. We will repeat that and then continue from that spectrum again.

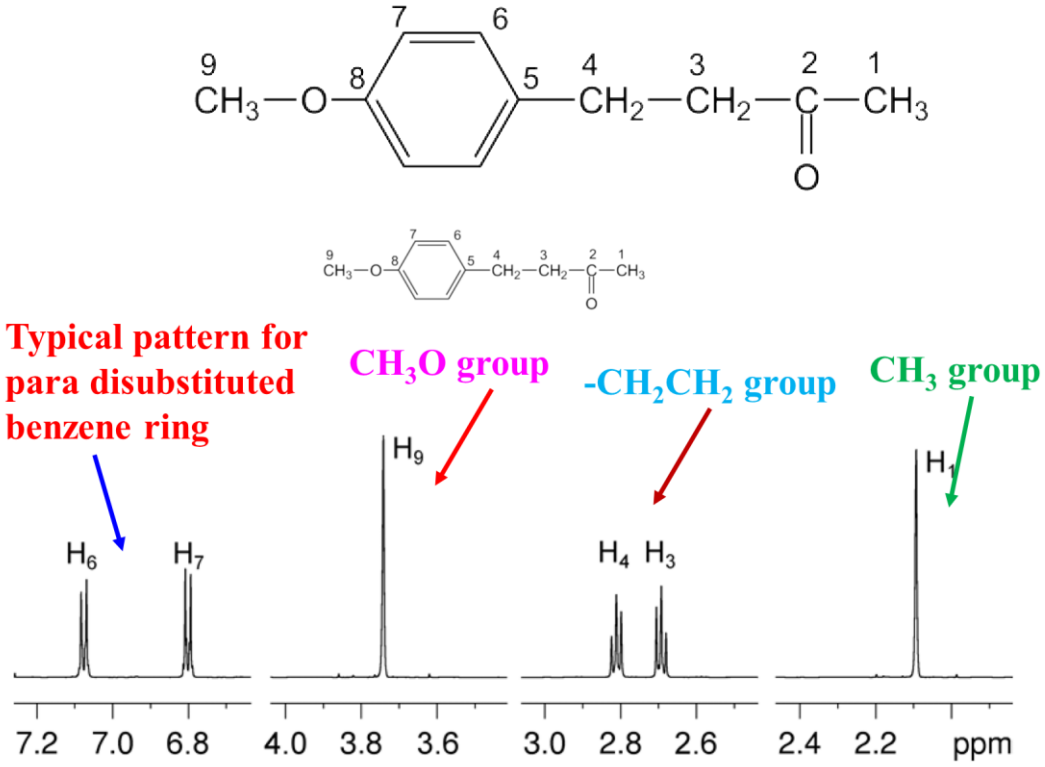




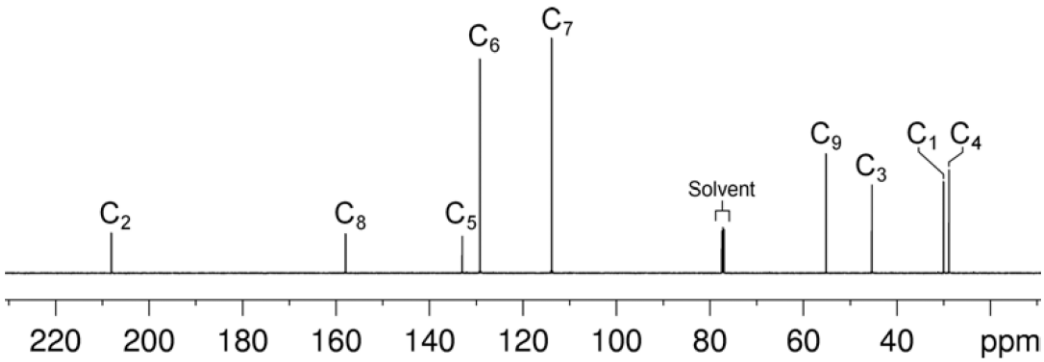
Coming back to this, this is INADEQUATE spectrum. In the previous class, we analyzed this proton spectrum and multiplicity edited HSQC, etc. In the 2D, I have drawn a diagonal here which I said is a pseudo double quantum diagonal and each cross section in the F1 dimension pertains to sum of the chemical shifts of the two coupled carbons. It is a double quantum dimension, evolves at the sum of the chemical shifts. From this one, in the detection dimension come vertically down, you will get only corresponding chemical shifts, single quantum chemical shifts. That is how it is. So, what we will do is this molecule will quickly go through and in the proton, that is H7 and carbon 7 from multiplicity edited HSQC also we know it is carbon 7 and if you carefully see each of them is a doublet, I told you because they are all AX pin systems. Two carbons when they couple, they form AX spin system. So, C7 go horizontally, you are going to hit a peak, and then that is carbon 6. Of course, you can see 7, you can also come down here, I will see that, you can also hit another one. Now, come vertically down, hit a cross peak, of course, same cross chemical shift corresponding to the same carbon. You have to go horizontally again, then you hit a peak that correspond to carbon 5. In the same chemical shift go up, go horizontally, you hit a peak that is C10, because you are going like this, started with C7, C6, C5, C10. And then if you go further from, C10 vertically up, and here come horizontally, you will see another peak that is C4. Then you come down, go horizontally again, you get carbon 3. From carbon 3 come down, go horizontally, you get carbon 2. See, started with 7, 6, 5, 10, 4, 3, 2, everything we assigned. Afterwards, we are ending with a nitrogen, we cannot get a peak. So, there is a dead end here, reaches the end. But what about the other carbons here, we have to assign this also. We can come from 7 again or from 10, from 10 also we can establish the correlation. But if you carefully see here, from 10, from 7 you can come horizontally, come vertically down horizontally, it is coupling to other one, this has to be C8. Come vertically, you will hit another carbon, this has to be C9. So, when there is a dead end here because of the other heteroatom sitting in the molecule, we can start from other point. So, finally, all the carbon skeletons could be traced out. Of course, you can also from C10, you can come down, C10 correlates for not only C5, C9 and C4, it correlates to many things. Now, from C9, you can go back and then you can hit C10. So, either way you can come from either of the direction. So, this is how the structure of the molecule, we can get, the complete carbon skeleton. Just we adopted two-dimensional experiments like COSY, HSQC and HMBC, got the complete carbon skeleton of the molecule, assigned protons, everything. Of course, we did not assign nitrogen and NO2 because you have not done the nitrogen 15 NMR.

Let us start assigning this molecule, how we can utilize inadequate to get the structure of this molecule. This is a 1D spectrum, proton spectrum. Very clearly, you can identify this is proton 1 because this is terminal group attached to C=O and no other coupling and isolated singlet that is proton 1, a CH3, very easily we can do. And of course, these two correspond to ring protons, typical proton expected for benzene ring, that is true. And of

course, one more is there which is uncoupled a singlet attached to OCH3 coming in the methoxy region.

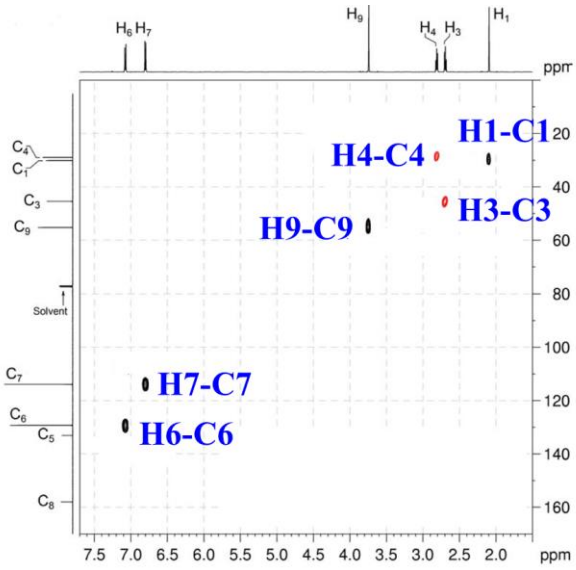


Then we have two CH2s. Of course, each of them is a triplet, making this triplet, this is making this triplet. So, we got two triplets here. So, basically looking at the proton spectrum, 1D spectrum itself just sufficient for you to make the assignment. See all these things have been clearly assigned which group is which. We assigned CH3O, CH2, CH1 everything.



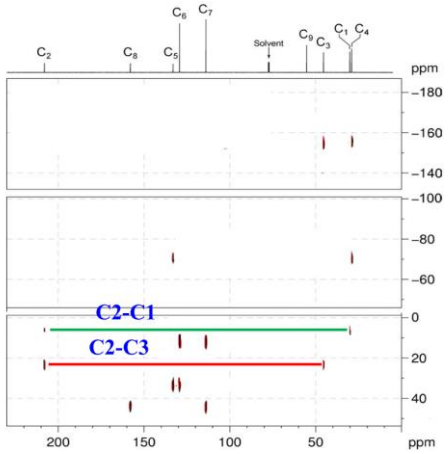
Carbon 13 NMR you can do. We can already assign few protonated peaks and non-protonated peaks. All these low intensity peaks, they all correspond to quaternary

carbons, you can easily make out. And these are all protonated carbons from the benzene ring and these are other protonated carbons, very easily you can make out. To make it very clear, let us start with multiplicity edited HSQC spectrum of this molecule.

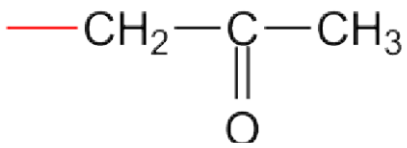


If you do multiplicity edited HSQC, you can see two of them are red in color, negative peaks. Obviously, they correspond to two CH2s. We could see there are two CH2s, each of them is a triplet, they are coupled between themselves and there is no other long range couplings for that. They are different isolated spin systems in this molecule.

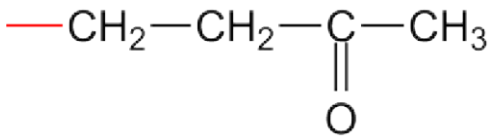
So, if you look at it, confidently we can start with H1, then we know H1C1, this is H3C3, H4C4 and all those things. So, the multiplicity edited HSQC confirms, that 3 and 4 are CH2 carbons here, and rest are all CH and CH3 carbons. And there is a quaternary carbon here, you do not see it in the HSQC, but in the 1D carbon 13, if you look at it here, near 208 ppm, that is a quaternary carbon pertaining to carbon 2. This is one carbon, another carbon, other carbon. There are 3 carbons, non-protonated which are present here. C5 and C8, you can see here, C5 and C8. C8 will not correlate to anything because it is a CO carbon. Similarly, these two are also, they are not, but in the carbon 13 dimension, you are seeing all the peaks. This is the INADEQUATE spectrum.



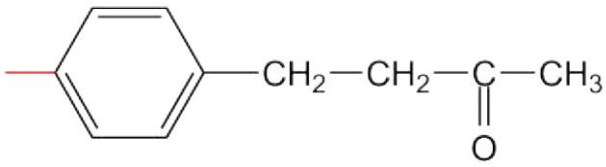
Of course, if you draw a horizontal line here, you can see it is pseudo diagonal, you can get it. Now, what we will do is we shall start the assignment. How do you make the assignment? First of all, we should know confidently one peak, which we are sure of that. C=O is at 208 ppm, correlates to C3 and C1, where is 208? it is here. See, it is correlating to C3 carbon. This one C3 and also it correlates to C2. What does it tell you? It tells me that this correlation puts C=O between these two. This gives us to a group like this. I can start thinking C=O is giving correlation, strong correlation to CH2 and CH3. I guess this must be the group.



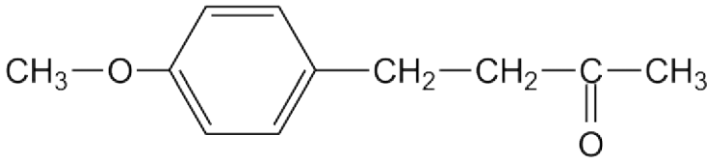
We will continue further. And afterwards, you will see C3 is correlating to CH2 also, correlates to C4. C3 is there, CH2, this correlates to C4. That means, this fragment you identified, C=O was assumed to be between these two. Now, that will extend this fragment like this, because C3 is also correlating to C4 here.Go horizontally, vertically, you will see C4. That tells me not only C=O was here and because this CH3 is correlated to C4, this CH2 proton is correlated to this one. Now, this extends this to be this group. So, this is the fragment now.



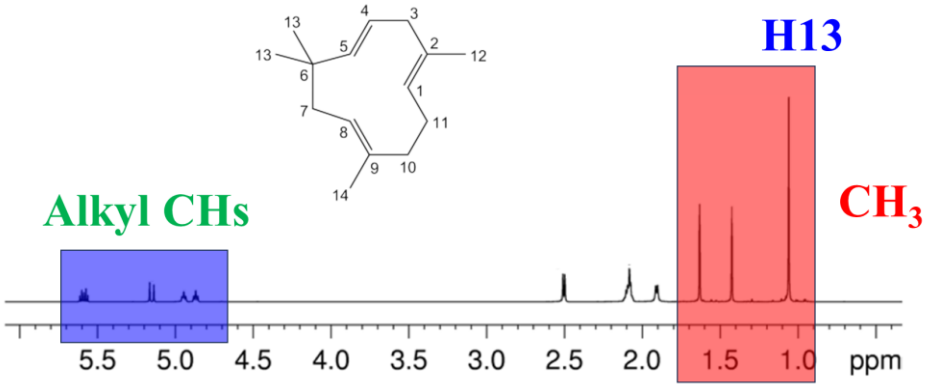
We will go further. The CH3 carbon C4 also has a correlation with the quaternary aromatic carbon, this one. See from here, this is C5, quaternary carbon. Go horizontally, you see it is the quaternary carbon. You can make out from the intensity also. It is a quaternary carbon. It correlates to that. Further, the C5 is correlating to carbon 6, a strong peak, and then it is also correlating to C7, has a correlation with the protonated carbon C8. C7 correlates to C8 here, and C8 is this one. So, most of the carbons started with this one, you could assign all the carbons, trace it out very easily. C6 correlates with C7, C7 is correlated to C8. So, all these things, entire this thing could be assigned. Only thing is the one which is not correlating to anything is C8 carbon, because there is no directly attached carbon next to it. That is not correlating to anything else. Where is that carbon? C9 is here. You see, it is not correlating to anything. There is no carbon attached to it. It is an isolated peak. How easily you can assign that carbon here. Up to this is known and of course OCH3 is coming here. So, this has to be attached to the aromatic proton. We can assign like that. With these assignments, now we can get the fragment like this.



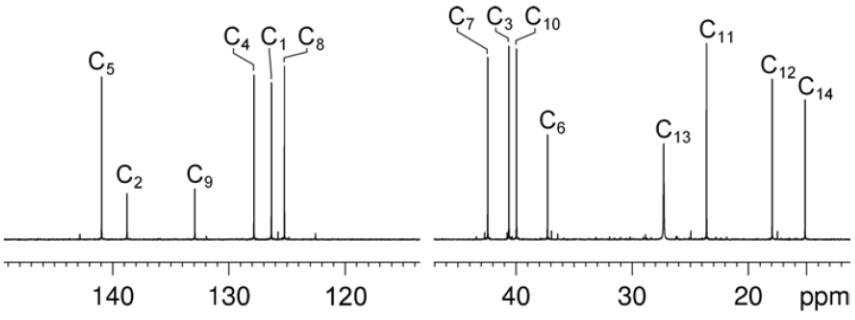
This we got and then we extended this and we know this is correlated to phenyl group. So, from that we got this one. The remaining is a methoxy OCH3 which is not correlating to anything else and it has to be bound to benzene ring at the para position to the other substituents. So, that is why it is. So, as a consequence there is no correlation to OCH3. Also I told you is isolated peak. That means this is attached to the phenyl ring. OCH3 is there first O and then CH3. As a consequence, there is no correlation of the C8 carbon to any of the phenyl carbons also. So, as a consequence, now I can say this is the final structure of the molecule because this O has to be between this and this. Otherwise, this C8 would have correlated to this one. This tells me this must be OCH3 and this is the structure of this molecule. Using the INADEQUATE, tracing the carbon skeleton, you could get the structure of this molecule.



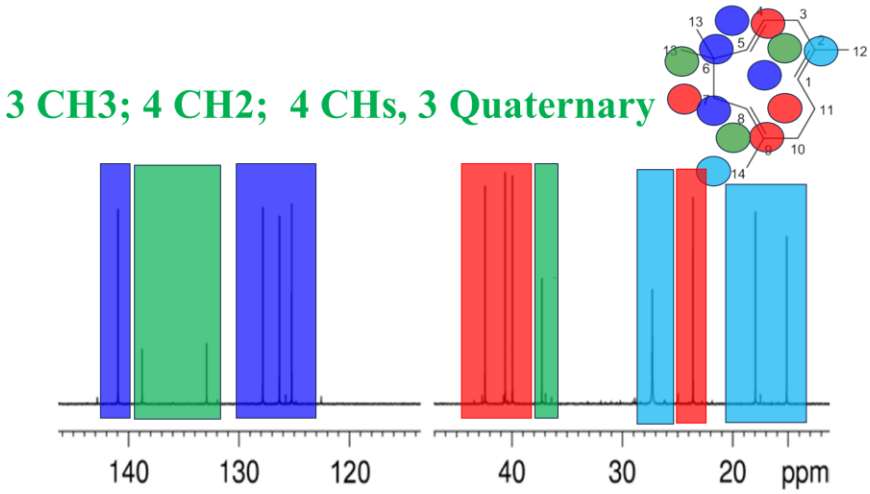
We will go to another molecule. This molecule is called humulin.



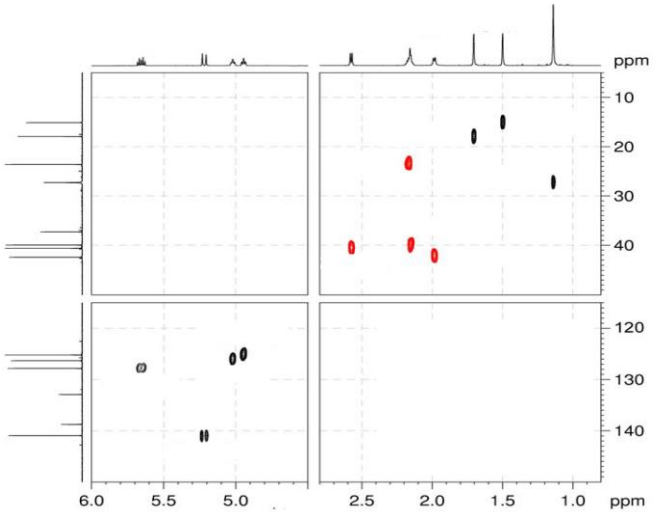
We can quickly go through this. This is the proton spectrum where there are three CH3s here, 1, 2 and 3. These are alkyl CHs, we have there many CHs. And we have, that is H13. Of course, this is what we could make rough estimate and many of these things we do not know. This is carbon 13 NMR spectrum, 1H decoupled.



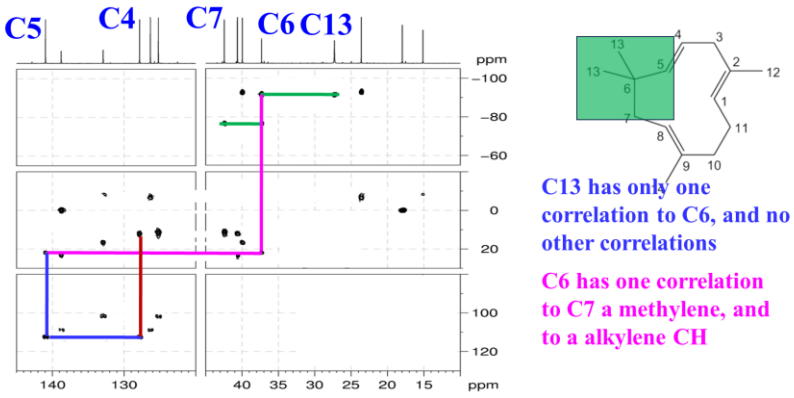
We have to start making the assignment. Carbon 13 NMR if you take proton decoupled, just by looking at it you can say there are many quaternary carbons. Lot of them are protonated carbons. All these high intensity peaks are protonated carbons and C6, C13, C2 and C9, are all quaternary carbons. By their the intensities you can find out. Fine. If I take the proton decoupled spectrum in the another solvent C2D2Cl4, at 338 K, this is what we are going to get. We can start making the assignment. These are CH3s, because there are 2 CH3s here and 2 here and there 4 CH2s are there. 4 CH2s, 4 CH3s are there and now 4 CHs are there, alkyl CHs and the remaining quaternary are there. So, we have 3 CH3, 4 CH2, 4 CHs and 3 quaternary carbons. We can make rough estimate from this structure. From the proton decoupled carbon 13 we can start looking at it and say, but how do you confirm it? We will see that later. These are all one group, these are all CH3, these are all CH2s, these are all CHs, the remaining are quaternary. The colour coding has been done now.



We can identify whether they are CH2s are not. Multiplicity edited HSQC identified 4 CH2s.



The 4 CHs very clearly, and 1 bond CH corelations can also be easily assigned now. So, as a consequence using this one of the carbons which we are sure of that, the CH3 carbon coming at the high field, and if you look at the intensity that two CH3 carbons which are equivalent, I would call it as carbon number 13, number 13 of this molecule. So, I can start assigning that C13 and then remaining things we can start making the assignment. Once I know that, 4 CH2s, in the HSQC spectra we have assigned many already. The assignment is very fairly simple. We do not need to break our head very easy. I assume that all of you know and then we can start making the assignment for each of them because we know the proton spectrum, a sort of we have an idea about the proton spectrum. Of course, this is a challenge, since the molecule is little bigger. But still with an experience we can do it. Let us analyse the INADEQUATE spectrum of this molecule that is more important.

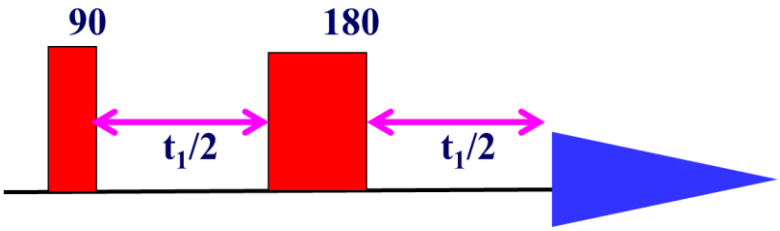


Where do you start? Start with C13, carbon numbered 13, because we know where is C13 and what is the C13 chemical shift here based on the proton spectrum. So, this is the C13 peak which is 4th from the right. Start with that 4th from the right, that is carbon numbered 13 and then go horizontally you are hitting a peak. So, carbon number 13 can correlate only to 6, that is directly bonded. So, that is carbon 6, you can make the assignment. So, after that you have to come down. And of course carbon numbered 13 cannot correlate to anything as apart from that. This is a terminal group only it correlates to carbon 6, that is done. Come down, go horizontally, it can correlate to two things, it can be carbon 7 and it can also correlate to one more thing if you see, if you come down that is carbon 5. Why did I say carbon 5 and why did I say carbon 7? Based on already I know CH2 and CH3 from the multiplicity edited spectrum, which is CH2 and which is CH. So, based on that I know this CH2 has to be there. So, then obviously based on the possible structure I said this is C7 and this is C5. C13 has only one correlation to C6 and now other correlation. The C6 has one correlation to C7, a methylene and to an alkyl CH that is what we got. So, this group is identified very easily. Obviously, because you can clearly see, that this C6 cannot correlate to anything as apart from C5 and C7 and to C13.

So, the whole group is identified because of INADEQUATE already, you can do that. Now, from C5 continue further, go horizontally vertically up, you are going to hit C4. So, we are continuing and that is an alkyl CH carbon that also we know from the multiplicity edited HSQC. Now, we continue further C4 to C3 and from C3 come down, go horizontally, we are hitting one more peak that is C2 and C2 is correlating to C12. Also, C2 correlates to other carbon, you see here. The C2 has two correlations here and here and this one goes vertically like this, this has to be C1 because C2 is correlated to this and this also. Like that go ahead, completely you can make the assignment for all the carbons. This was the interesting thing so I did it, and the rest of the things I did not do that. I was assuming that you can go like this in a stepwise manner. All the other carbons which are here can be assigned. Of course, in the previous slide, they were also assigned. Only these things which you can continue like this and assign. All the carbons can be assigned by this simple method. This is a 2D INADEQUATE experiment. So, now you got the idea. So far, we discussed about the 2D INADEQUATE experiment. This gives rise to correlation among the coupled dilute spins like two carbon 13. So, if you know the correlated peak, simply you have to start from the top, one of the identified correlated peak, come horizontally or vertically down, hit another peak. Horizontally means it is a different carbon. If you come down vertically, the same chemical shift along the axis, F1 axis. And then if you go horizontally, hit another carbon chemical shift that is coupled to that, that is sitting next to that. Come down, come down like that. Keep on tracing all the carbon skeletons of the molecule, it can be done. So, what you will do? You know proton chemical shifts by simply using 1D NMR in simple molecules, little bit complex use COSY, even bit more complex use TOCSY, you can assign all the protons and then you have to assign carbons. You can do what is called HSQC experiment to assign directly bonded carbons and protons. All the carbons attached to proton can be identified. If you want to assign the non-protonated carbons, establish long range correlations, then you go to HMBC. After doing all those things, if you need to assign carbons connected, which carbons sitting next to which carbon, that is still left, for that you do INADEQUATE experiment. So, combining all the three experiments, you can get the complete structure of the molecule. You see, understand the beauty of this. All you have to do is you have to think which is the experiment you have to do. Do the judicious choice of selecting a particular experiment and then using that and then other couple of experiments make the complete assignment of all protons carbons present in the molecule. Of course, if some other heteronuclei are present like nitrogen or phosphorus etc., there are different types of experiment you can think of. But this is how with the 2D homonuclear and heteronuclear experiments you can make the assignments. So far I have told you all correlation experiments, how do we correlate varieties of things. With this, we will switch out a new topic now. So far, we are discussing about correlation experiments. But remember when I introduced the 2D NMR, I classified into two groups. One is the correlation type experiment. These are all the correlation types like what we discussed so far. Other is

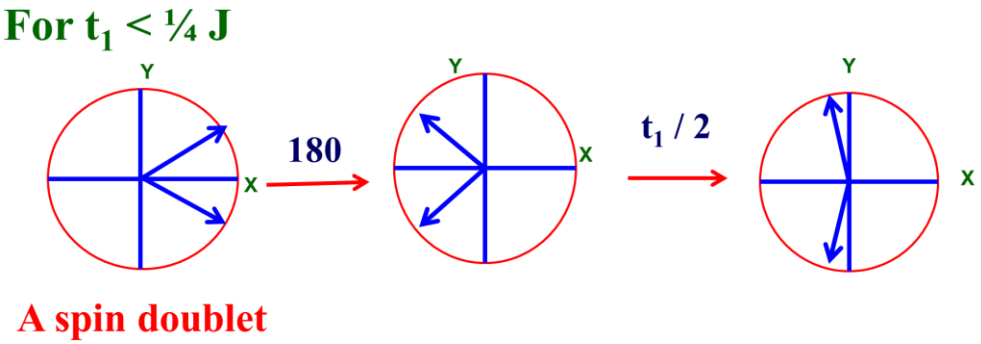
called the resolved experiment. In the correlation experiment, you do not separate the parameters, NMR parameters. You will identify which carbon is coupled to which, which proton is coupled to which carbon, which proton is coupled to which proton, like that. You can correlate the chemical shift information. But if I have to separate out the interaction parameters of NMR like chemical shift and J couplings. If I want to separate out into two different dimensions, I can do that.

That is called resolved type experiment. So, I will jump into another type of experiment called 2D-J resolved experiment. And this is the experiment. What it does, I just now said, COSY, TOCSY gives you homonuclear correlation, HSQC, etc. gives you heteronuclear correlation of coupled spins. But J resolved experiment separates the chemical shift and coupling interactions in two different dimensions. This is the beauty, where you can get the resolution much better. Removing the two parameters which are crowded into two different dimensions will help you to get the better resolution. What is a homonuclear 2D-J resolved experiment? How does it work? What is the pulse sequence for that? This is a pulse sequence.



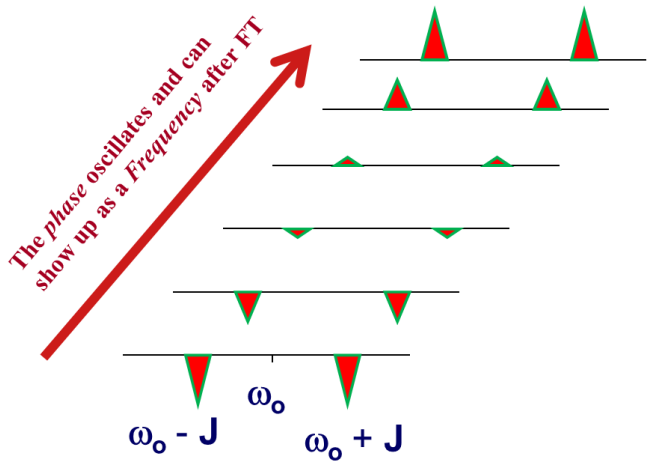
If you look at it immediately, you will know that. We discussed this. This is analogous to a spin-echo sequence, 90 tau, 180 tau. It is similar to spin-echo. So, homonuclear 2DJ resolved is nothing but a spin-echo sequence. So, this is a 90 pulse. You give a delay which you call t1/2, apply another 180 pulse, t1/2, and then start collecting the signal. It is a proton, let us say, homonuclear, or could be fluorine, phosphorus, does not matter. These are all homonuclear 2DJ resolved experiment. Basically, it is nothing but the variation of a spin-echo sequence. In spin-echo, we fix the t1 constant. Here, we can vary it. The chemical shifts are refocused here, like what we discussed in the spin-echo sequence. Always, we said the homonuclear spin-echo refocuses chemical shifts, but couplings evolve. Here also, irrespective of whatever the t1 value you use, delay, the chemical shifts are always refocused. How it works? We will take the example of one or two simple cases, and see how the J resolved spectrum comes. We will take the simple example of a doublet of AX spin system. We will consider one of the doublets, A or X, does not matter. What we will do first? In the pulse sequence, what I showed, first you apply 90 degree pulse. It brings the magnetization to X axis or Y axis, give some time.

What will happen to these two vectors which are coupled, J split vectors, starts moving in the opposite directions.

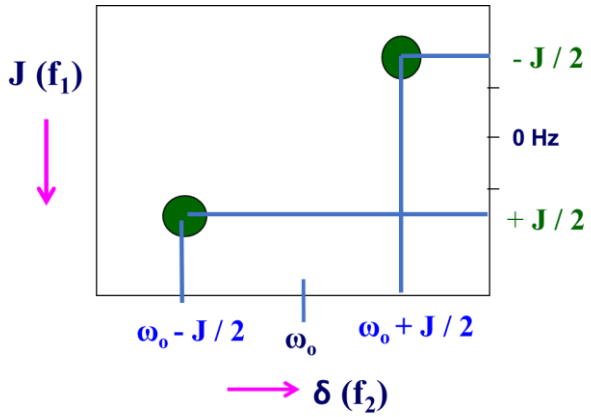


One is fast moving and another is slow moving component. How much it moves? We also calculated, we knew how to calculate, theta is equal to pi into t into J that we discussed also, long back. We know how to calculate. If I know the t, I know how much it has moved. So, I set the parameter, such that after a time t, this has moved by J by 2 *t1, other is moved by minus J by 2 * t1, in the opposite directions. Both the vector components are moving. Fantastic. Let us consider a situation for different values of t1, how much it has moved. For t1 equal to 1/4J. then it moves by 45 degree. If it is less than 1/4J, it is less than 45. A spin doublet start moving like that. This one moving like this, other is moving like this. Fast moving and slow moving components, spin vectors are moving in the opposite direction. What did we do after the delay? We apply a 180 pulse like a spin echo sequence. Then what will happen? Depending upon where you are going to apply, remember I discussed about the spin echo. If you are applying along this axis, you do not rotate like this. If you are applying along Y axis, you rotate it like a pancake. The spin vectors are completely rotated like this, 180 degree flip. So, this component came here, this component came here. Give another time delay, t1/2. They start moving. I told you, chemical shift will refocus, but J coupling will not. J coupled vectors start moving in the opposite directions, again, they start moving. They will not refocus. That is what happens. Now, what I am going to do is, I do not fix the t1 constant. I vary the t1. When I vary the t1, what will happen? Not only the doublet components start moving it, they started moving at different angles, but at the same time, their intensity changes because of the strength of the coupling. The intensity of the two components of doublet, vary as a function of delay and also J coupling strength, both are dominating factors. This is how it happens pictorially. I am considering only a doublet. The one component of the doublet, other component fast and slow moving. Initially, it will be like this. Slowly, they go like this, negative, zero and then positive. So, it forms an oscillating curve like this, goes like this. Both the doublet components identically start varying their

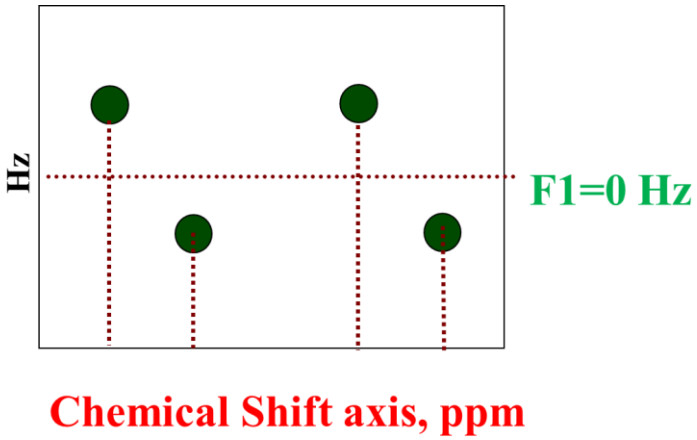
intensity, identical intensity variation from the center of this doublet.That is the chemical shift.



How much is the intensity? How it is changing as a function of t1? You can find out. It is a well known equation that we have been discussing. Even with the spin-echo sequence, I said this. So, it is question of the function. And how much is the delay and the J value. So, all we have to do is you have to take the projection of the intensity along one of the axis, and how much it has moved. So, you know that and we know how the intensity is varying. So, in the t2 dimension, what is happening? This is variation in the t1 dimension, I am telling you. What is happening in the t2 dimension? We still have a chemical shift and J coupling evolution. It is a conventional one-dimensional spectrum that is not getting changed. That continues like that. Chemical shifts and couplings continue to evolve. We will do that. We will vary t1 and then fix at t2, collect a two-dimensional spectrum like any 2D experiment. t1 is varied with 180 pulse in the middle and then we collect this thing and do the Fourier transformation. I am considering only a doublet.



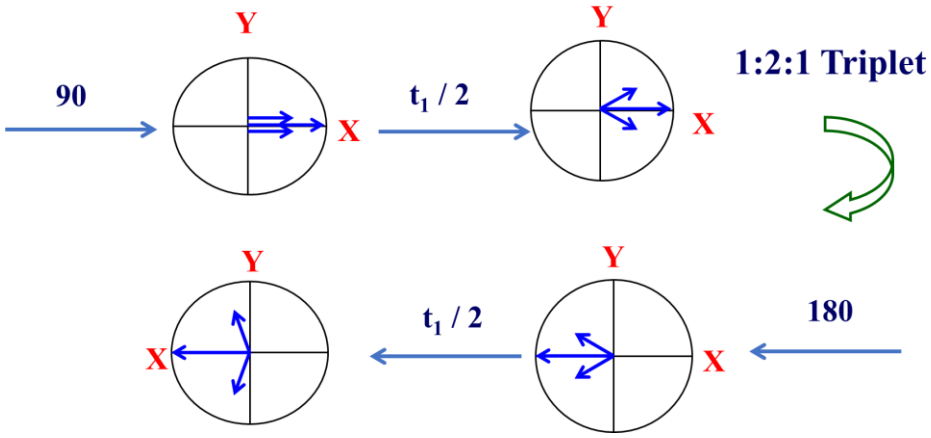
What will happen? In this dimension, if this is the chemical shift, you have one peak coming to the half of J to the right, half of J to the left. This is what we understood for a coupled spin system. When you have a doublet, a weakly coupled, I told you, this is half J, this is half J. One peak is to the half J to the left, one peak is half J to the right, center is the chemical shift. That is fine. So, both J is there and chemical shift is there in this dimension, in this F2 dimension. But in the indirect dimension, chemical shifts are refocused. Only J is evolving and does not matter, from the center you take the frequency. If you do the Fourier transformation, this separation gives you J coupling. Fantastic. So, in the indirect dimension, you can measure the J coupling. In the direct dimension, J and chemical shift are present. For both the spins now, I took only one set of doublets of the two coupled spin. If both are present, what will happen? You see like this. Let us say A spin doublet or X spin doublet, whatever it is, each of them is a doublet and center of them gives a chemical shift for both of them and from the center of the F1 dimension, on either side, you have a J coupling. So, chemical shift and J coupling, both are present here, here only J coupling and that is how it is.



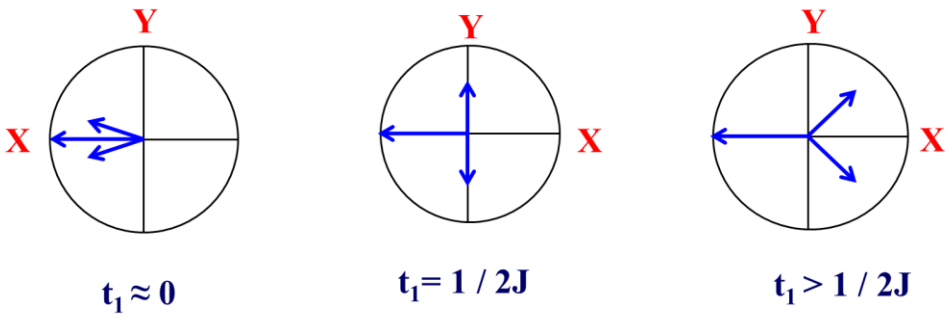
So, in the F1 dimension, you do not get chemical shift as they are refocused. This is how it is. So, as the information in two dimensions are different, spectrum is not symmetric. That is why analogous to your homonuclear COSY, TOCSY etcetera, this is not symmetric. Because the two parameters are completely different. Here J coupling is of the order of 10 to 15 Hz, whereas the chemical shifts in this dimension is of the order of several 1000s of Hz. So, you cannot get symmetric spectrum like COSY or TOCSY, they are not symmetric. So, J result spectrum is not symmetric.

Let us take another simple example of what is called homonuclear 3 spin coupled AX2. What will happen to AX2? Now I am looking at A, A will be a triplet, because of this. That splitting pattern we have already understood. 1:2:1 triplet. We will consider triplet, you apply 90 degree pulse, bring it to x axis, give a delay t1/2, they start moving. Remember I already told you when you are explaining spin echo and J modulation,

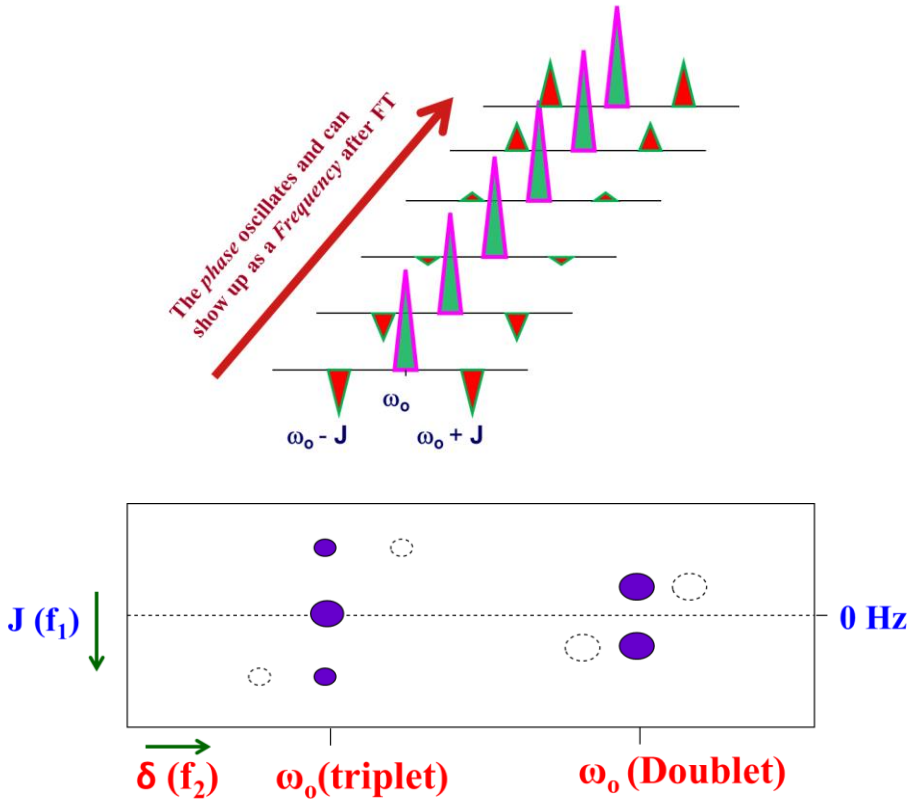
central component of the triplet do not precess, it is always along the same axis. Only the outer components start moving like this, one like this, one like this. Apply a 180 pulse, again they get reversed and after some time central peak remains same, whereas this one start moving another side and this is on this this side, this is what happens. How the evolution of magnetization vector for different values of t1 happens is like this.



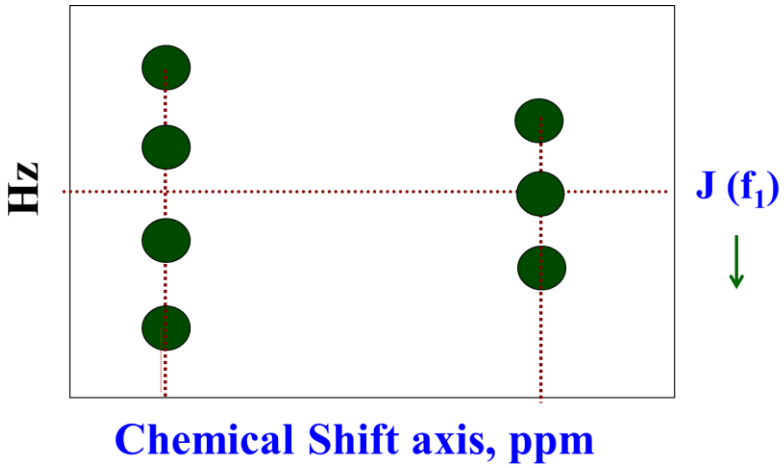
Central peak always remains same, always focused on the same axis. At tis equal to 0, this is what it is. At tequal to half J, you have anti-phase components because they are moved by 90 degree. At 1/2J, they are moved like this. Central component is not precessing, and ius always along the same axis. So, intensity of the outer component is the only thing which varies. Central component do not vary because that is fixed, it is not moving. But it can also change in the intensity, because of relaxation. This is the intensity identical to what we observed in our two doublet case. So, in the t2 dimension, we still have chemical shift and J coupling. The central peak will not precess and the change in intensity is only due to relaxation T2.



The two outer components vary periodically as a function of t1 and strength of coupling like doublet case.



And this is how the example of A3X2 spin system if I consider, this is a triplet because this spin is split by a CH2 group. This is a quartet split by a CH3 group. In the case of CH3 also it evolves similar to CH, there is no difference. We have understood that in the spin echo sequence, this is how it comes.



This is a chemical shift axis, this is the J coupling axis. And further what happens, I can doing the tilting. More about this tilting and everything we will discuss later. But I think the time is getting over, I am going to stop here. So, you understood today what we discussed. We discussed lot of things about INADEQUATE experiment. And afterwards, I introduced a new experiment called 2D J-Resolved wherein we are resolving the two

parameters in two different dimensions. In the indirect dimension, you get the J coupling. In the direct dimension, you have got J coupling plus chemical shifts, both present. Why in the indirect dimension you do not get chemical shift? Because it is a spin echo sequence. Homonuclear spin echo will refocus as chemical shift, but J coupling will continue to evolve. That is what happens. So, instead of fixing the delay, vary the t1, do a 2D experiment, then what will happen? The intensity of the doublet or outer components of the triplet, keep varying as a function of t1 and the strength of the coupling, intensity depends upon both. There is no symmetry in this case because the parameters are different. In the indirect dimension, you just get only peaks corresponding to J coupling from the center of the chemical shift, one peak to the right, one peak to the left and two different cross-sections of the t1 dimension. In the t2 of course, we have both present. Further we can do tilting and everything, I will discuss in the next class. So, basically what we do is the homonuclear spin echo sequence, we get the J coupling along one axis and J coupling for chemical shift in the other axis. We have separated the parameters. In a crowded spectrum, I can extract only J couplings from the indirect dimension. So, this is the biggest advantage which you cannot get it from a crowded spectrum, especially, proton spectrum if you consider homonuclear J couplings, there will be enormous complexity at times, but this J result helps. So, this is simple J result experiment. There are hundreds and hundreds of modifications have been done to get this type of information in a variety of 2D experiments. We do not have time to discuss everything, but only one of these and the heteronuclear experiment and another one are two simple 2D J result experiments I will discuss in the next class. So, I will stop here. Thank you very much.