

One and Two dimensional NMR Spectroscopy: Concepts and Spectral Analysis
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Lecture 04 - Energy levels and allowed transitions

Good morning all of you, welcome back for this course. In the last two or three classes, we discussed extensively about the fundamental concepts of NMR spectroscopy, where I introduced the concept of angular momentum, spin angular momentum and also magnetic moment. And at the same time we discussed about the Zeeman interaction. The energies between two different states will be seen, after the degeneracy is removed when we put spins in a magnetic field. And then we also discussed about Larmor precession frequencies. and what is the Larmor precession frequency, etc. And then we discussed extensively about population difference between two energy states for spin half nuclei. And I said there must be population difference for us to see the NMR signal. And if there is no population difference there is no NMR. And we also understood that spin angular momentum I is a very important factor to see NMR. If for a given nucleus if I is 0, they are NMR inactive nuclei and we cannot see NMR for such nuclei. All those things we discussed extensively.

We also understood how to induce the resonance by applying a radio frequency signal. And I said the radio frequency signal should be applied in a direction perpendicular to the magnetic field. The magnetization or spins will be aligned along the magnetic direction, the direction of the magnetic field. And you have to apply the RF pulse in a direction perpendicular to it. Then the magnetization will be tilted to the another axis. All the three x y and z axis are orthogonal to each other. And the tilting of the magnetization obeys what is called right hand thumb rule. If the thumb points the direction of the axis in which you are applying the RF pulse, then curly fingers tells you the direction in which the magnetization flips to the particular axis. So, we extensively discussed about application of the RF pulse in different axis; everything has been discussed at stretch in one of the previous courses. But I do not want to go to the details of that, but I just want to tell you we need to apply RF pulse. The radio frequency which we are going to apply is in the form of a pulse.

And the pulse duration, I said, is of the order of 5 to 10 microsecond for proton and also it is of the order of 10 to 15 microseconds for carbon 13. This is for 90 degree pulse to tilt the magnetization from z axis to x or y axis. And therefore, 90 degree tilting RF pulse we

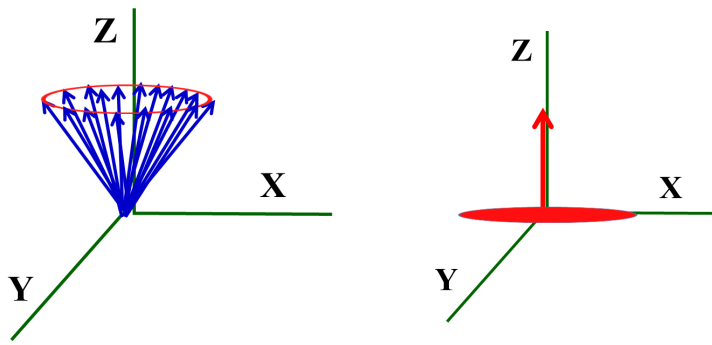
apply. That is why it is called a pulsed NMR spectroscopy. It is completely different from what NMR used to be practiced about 4 to 5 decades back; about 50 years back. That is called CW-NMR. It is a pulsed NMR and after we collect the signal we do the Fourier transformation that is why it is called pulsed Fourier transform NMR.

We discussed little bit of this introduction and everything yesterday. We will go further today and then I wanted to tell you we understood something about how do we induce the resonance by applying a radio frequency signal. And we saw that the spins which are in the ground state go to the excited state and spins in the excited state come to the ground state. And the difference in the spin populations is what we are going to detect. That is a simple way of understanding, but may not be the right way.

As I said the NMR spectroscopy is neither absorption or emission spectroscopy. It is a coherent spectroscopy. We have to see the coherence of the nuclear spins and there is a different way of understanding it by using what is called bulk magnetization concept. So, I am going to introduce today the concept of bulk magnetization. This is the real way of understanding how do you see the signal in NMR spectroscopy. For this we have to understand what is called random phase approximation.

First we look at the individual nuclear magnetic moments. The nuclear magnetic moments which I said μ . They keep oscillating at equilibrium. They are not not in the steady state. As I said they spin start precessing in the magnetic field. And the oscillation is going on; that is called a precession. As a consequence individual magnetic moments are not aligned in the direction of the magnetic field. They are constantly changing its position. They keep on precessing like this. This is what we observed. This is what I called as Larmor precession that is what I explained also. And now we will go into a slightly different way of understanding.

As I said there are two possible orientations for spin of nucleus. One is alpha state (plus half) and the other is beta state (minus half). As I already told you the alpha and beta states are spin up and spin down states; conventionally called alpha and beta. Now, consider nuclear spins which are in the alpha state. That means, all of them are aligned in the direction of the magnetic field. Not exactly aligned but making an angle like this. All these nuclear magnetic moments will be aligning and precessing making an angle; certain angle θ with respect to the magnetic field. So, individually each of them is a vector. And this spin ensemble do not have any phase coherence at all, but they will be precessing. They appear like a cone opening upwards.



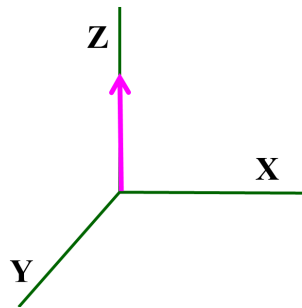
This is true of all the nuclear spins which are in the alpha state. Now, what we will do is we will resolve the vector. The magnetic moment is a vector μ is a vector, I told you. We will resolve them into three components x y and z. Vector can be resolved into three components. Now, the nuclear spins, the magnetic moments are precessing like this along the z axis; in the direction of the magnetic field, which is assumed to be along z axis. The magnetic field is assumed to be along z axis. Now what will happen to the x y components of this? because continuously they are all precessing, the x y components, if you take the ensemble average then what will happen? All these vector components in the x y plane get completely averaged out to 0.

There would not be any x and y components; there would not be any magnetization in the x y plane. That is what is going to happen. So, this is what it is. This is because of random phase approximation. We can make an approximation; because of the random phase all the components in the x y plane are averaged to 0. So, we have only the component of the magnetic moments aligned along the z direction. But now what is the vector addition of all the nuclear spins in the x y plane; here they are averaged to 0, but not along the z axis. They are in the same direction, the vector sum of the nuclear magnetic moments in the z axis will be added up. Only in the x y plane it is 0. So, when all the magnetization is added up in the x y plane, it is 0, no magnetization; here M_x and M_y or μ_x and μ_y is 0. whereas here everything is there.

Now the vector addition of this I can consider. It is a small tiny magnet like this. See a small tiny magnet; this is what is called a bulk magnet. I consider small tiny magnet like this. All these magnets along the z axis, the component of the vectors of magnetic moment vector along z axis will add up and give a small magnet like this, which is aligned along z axis, and x y component is totally 0; fine. We will go to a situation where all the nuclear spins are in the beta state. As I told you in the beta states they will be like a cone opening downwards. Again if you take the ensemble average, I can resolve this beta into 3 components. Magnetic moment oriented in beta state into 3 components, x, y and z. Because of the random phase approximation all the components of the vector along x and y are again averaged out to 0. There would not be any magnetic moment along x and y directions. Again in the minus z direction all of them get added up; that is what going to happen. And then we are going to get a small magnetization, a small tiny

magnet along minus z direction. Remember for all the magnetic moments which are oriented in the direction of the field for alpha state there is a tiny magnet generated, because of the vector addition and with random phase approximation. For beta spins it will be magnetization or tiny magnet in the direction opposite to the magnetic field.

Now, we have 2 small tiny magnets for 2 spin states; one along z axis and the other along minus z axis. This is an important concept. But remember I also said there is a small population difference between alpha and beta states. That means the magnetization along plus z axis or this tiny magnet which is along z axis is more than that along the direction opposite to it. Now, if you take the difference of these two, again it is a vector addition difference of the components between plus z direction and minus z direction, there is a small net magnetization. There is a tiny magnet along z axis; there is a net magnet this is what is called magnetization. It is the concept of bulk magnetization, it comes because of random phase approximation. All the X and Y components of the alpha and beta spins states all the vectors along alpha and beta vectors average out in X Y plane and only we have component in the z axis. There is a co addition because there are excess spins, excess population along z axis. The difference gives us a tiny magnet which is called a bulk magnet. We call it as bulk magnetization and this is usually denoted as M_0 ; alright.



And always it is oriented parallel to the direction of magnetic field, the static magnetic field B_0 . We have a huge magnet and along Z direction. This can be treated like a tiny magnet, unlike individual magnetic moment vectors, which were always continuously changing. You know they were precessing along the direction of the field; but this bulk magnet is always static. it is stationary. it is not moving in equilibrium. M_x and M_y components are 0; whereas, M_z component which is in equilibrium, which is also called M_0 the equilibrium magnetization, is non zero, it is not moving. Again I am telling you, remember individual magnetic moments are moving when I take into account the random phase approximation then I can generate a small magnet, a tiny magnet, because of the difference in population and addition of vectors along plus z minus z directions. Then this tiny magnet is a bulk magnetization which is stationary along z axis and is perfectly aligned along z axis along B_0 field.

Now, if I have to detect a signal in NMR, what we have to do? We have to create what is called a non equilibrium state. Remember in the equilibrium state the magnetization is aligned along the z axis; aligned perfectly along the magnetic field direction. Now, if I have to detect the signal I have to create what is called non equilibrium state. How do you create that non equilibrium state? There is a way of creating. That is by doing what is called the application of an oscillating RF field in the direction perpendicular to the magnetization. The bulk magnetization, let us say, this is z axis and bulk magnetization is here. You have to apply rf in a direction perpendicular to it; either along the x axis or y axis; so that the bulk magnetization can be tilted. Then you created a non equilibrium state. You will perturb this spin system. How do you do? that by applying a radio frequency pulse. We apply a radio frequency pulse in a direction perpendicular to it which is the order of 5 to 10 micro second to tilt the magnetization to x or y axis, it is called a 90 degree pulse. And the frequency of oscillation, as I told you already, previously also, should match with the Larmor precession frequency. Then there is going to be a resonance. The tilting of this bulk magnetization we you can do it with different flip angles; that is what I said last time also. In the previous class I said we can tilt the magnetization from z axis to x axis to y axis minus x axis and again take back to z axis. I can make the magnetization to come from z axis slowly like this and take it back to z axis, I can make it keep on rotating by keep on applying the pulses; 90, 180, 270, 360. Like that you can make the magnetization to go round and round; that is what you can do.

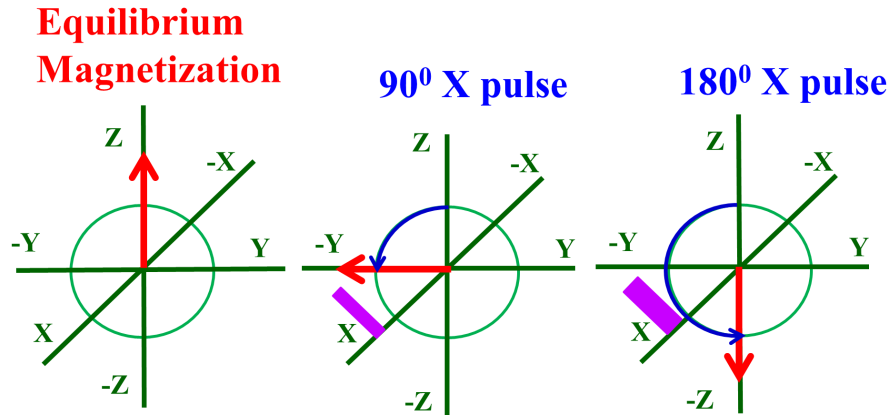
This is an equation which we have to use.

$$(\gamma B_1)\tau_p = \theta$$

This is the angle theta and how much magnetization is tilted by 90 degree or 180 is defined by this equation. This is called gamma b1 into tp. The gamma b1 is called the RF power; tp is called the pulse width. So it means the power and the pulse width defines the angle of tilting of the magnetization. If I have to tilt the magnetization by an angle

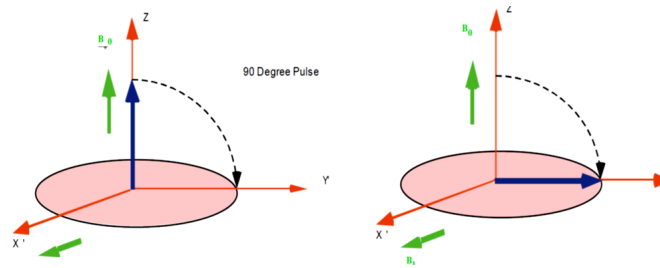
theta, then I have to manipulate. I have to play with the RF power and I have to play with the pulse width. If I can do that I can tilt the magnetization from z axis to any angle that is what we can do now.

Let us consider the equilibrium magnetization is along z axis. I will show you how we can tilt it.

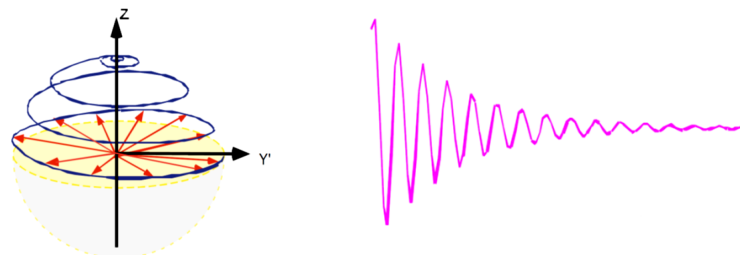


I am going to apply a 90 degree x pulse; that means I am applying a pulse along the x axis, a 90 degree. What do you mean by a 90 degree pulse? that pulse should be sufficient enough to tilt the magnetization along x axis. it will be tilting along minus y axis because of the right hand thumb rule, which I told you already. So it has to tilt towards minus y axis. This is direction in which I am applying the RF pulse and the magnetization which was here tilted to minus y. I increase the width of power of the pulse such that I can make it a 180 x pulse, then the magnetization start tilting to minus z axis. From plus z it came to minus z axis. You can see here from plus z with 180 pulse shifted to minus z. Now I increase the power of the pulse such that I can take the magnetization completely one rotation like this. From z axis I can rotate with 270 degree from plus z it comes to minus y then minus z and then to plus y. Then apply 360 pulse it will completely go one round, one rotation like this. See the beauty of it and this is what happens. This is what we call it as a flip angle of the pulse. I can apply different flip angles of the pulses and tilt the magnetization by any angle theta from the z axis with respect to x or y axis. This is what is the concept we have to use for understanding NMR. So we have the bulk magnetization which is stationary has to be perturbed by applying the RF pulse at any angle x or y axis in a direction perpendicular to it. If you apply by any angle you can tilt the magnetization and this is how we can rotate it in the anticlockwise y-z plane. Of course you can apply in the other direction and rotate in the other direction. Instead of x axis you can apply on y axis. All the possible combinations you can think of.

Okay, now I am going to introduce what is called the free induction decay, and how do you get the NMR spectrum. Look at it, after the 90 degree pulse what happens? the entire magnetization is brought to x-y plane. See magnetization is like a tiny magnet which is the z component of vectors, an addition of magnetic moment vectors, which we already discussed. The magnetization comes to the x or y axis or x-y plane; and as soon as you bring it to the x or y axis there is what is called instantaneous phase coherence. All the spins are aligned along this particular axis. There is complete phase coherence.



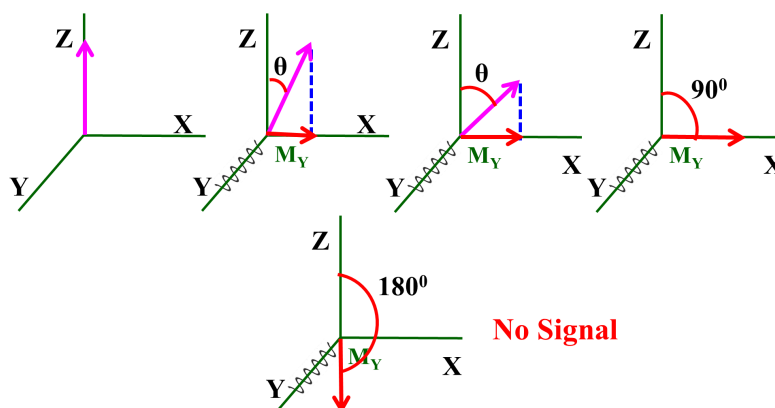
But what happens? this phase coherence is not maintained for a long time. The spins start undergoing dephasing. It was here, brought here, then start moving. The different nuclear vectors start moving like this. They will completely get dephased in the x-y plane. After the dephasing in the x-y plane, because it is a perturbed system from equilibrium, you perturb it, it has to go back to equilibrium. Then while undergoing decay in the x-y plane it starts going back along z axis. It grows along z axis, okay. And this is an exponential function both decay in the x-y plane and growth along the z axis both are exponential functions. And pictorially you have to understand. I bring the magnetization from z axis to x or y axis, I am sitting here. I am observing the magnetization; but you see there was a instantaneous phase coherence, and all the vectors were along this axis. But with time the phase coherence get disturbed. You see these inter nuclear vectors; all the magnetic moment vectors start dephasing. They start spreading in the x-y plane, simultaneously there is a decay and it will start growing along this axis. Both are simultaneously happening; decay and growth. And you see if you look from the top of the magnet, it appears for you as if there is a spiral going on like this.



It is like decaying in the x-y plane, simultaneously start growing along z axis and when it is happening when I am sitting here, using electronics I collect the signal. It is decaying in the x-y plane. I apply RF pulse then I keep quiet. The magnetization which was

disturbed will decay in the x-y plane, start going along z axis and it is an exponential function. I am going to have a receiver here, it induces EMF in the receiver coil and I am going to collect that as a function of time. Okay, this will be freely processing after I apply the radio frequency pulse and you are not doing anything. It is free at the same time it is decaying while inducing EMF in the receiver coil. That is that is why it is called free induction decay. It is called FID and exponentially it decays like this as a function of time. This is what we are going to collect in the NMR spectrometer and the conventional one dimensional NMR spectrum is like this. You apply a radio frequency pulse RF pulse and give a short delay and afterwards you start collecting the signal like this as a function of time. This is what is called acquisition time and you give enough time here for the nucleus to attain equilibrium. That is called a relaxation delay so you have a relaxation delay. Apply a radio frequency pulse and then start collecting the signal this is basically the one-dimensional conventional NMR experiment that is being done.

Okay, now the question you may ask me is do we need a 90 degree pulse always to generate the free induction decay? Remember here in this, I showed you how to apply a 90 degree pulse and then bring the magnetization to x-y plane and start collecting FID. But the question is do we always need 90 degree pulse? the answer is no, because you can see here initially the magnetization is along z axis I can tilt by any angle, I told you $\gamma B_1 \cdot t_p$. I told you it is equal to theta. I can adjust the power and the pulse width in such a way I can tilt the magnetization instead on to x-axis, by a small angle theta. This is a vector. We can resolve into two components and this is what you are detecting. So there is a component here this component, and this component we do not require at all. We do not collect it, whereas the cosine component is the one we are going to collect. There is some signal now. increase theta by a small value to higher value now. What if I did that, let us say new component along this axis become more and go to 90 degree the entire magnetization is brought back to brought to x-axis from z axis. With the 90 degree pulse there is maximum signal here and then go 180 since you are sitting here signal is here you do not see anything at all so 180 pulse will not give any signal. The maximum signal you are going to see is only when you apply a 90 degree pulse. When you apply 180 degree pulse you will not see any signal in NMR. Please understand, 90 degree pulse is the one which gives you maximum magnetization. Maximum perturbation you can do for the spin system is when we are going to collect maximum signal.

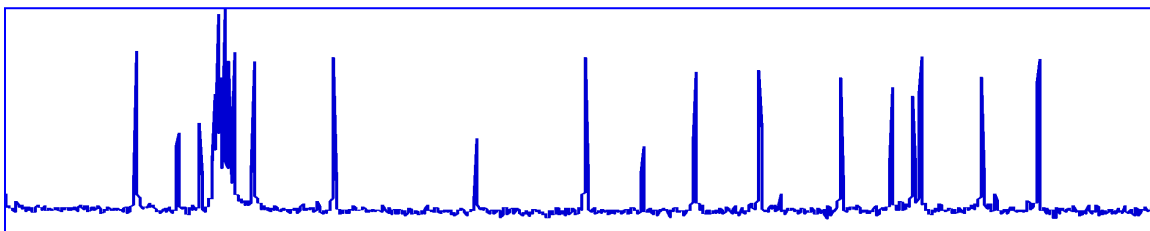


Okay now FID will be created by an RF pulse of any flip angle that is what you should remember, it need not be 90 degree but for better signal more signal to be collected 90 degree is better. It is because entire magnetization will be along x-axis or y-axis where you are keeping a receiver. For any angle, other than 180 degree, there will be always some component in the transverse plane to detect the signal.

Fine, now we have collected the signal what are you going to do with that? See they are all time domain signals. There are lots of frequencies present in that. But how do you know what are the frequencies present? So many transitions could be there; so many energy levels will be there in the given molecule, a lot of different nuclear spins could be there. How do you distinguish the frequencies present in that. See for example our ears cannot hear or I identify all the frequencies at a time. So it is like a particular music for a particular frequency. But all all of a sudden if there is a huge noise you do not know who is talking. How to overcome this? There is a mathematical formula which is given by Jean Baptist Fourier. What we do is called Fourier transformation. We can do transformation. The Fourier transformation of the time-domain signal. The time and frequency are called Fourier pairs. I can do the Fourier transformation of the time domain signal and get the frequency. I can do the inverse Fourier transformation of the frequency domain takes back to time domain. Both are possible. They are called Fourier pairs. So, what I am going to do is, we collect the signal like this in the time domain and do a mathematical operation like this called Fourier transformation from time to frequency.

$$f(t) = \frac{1}{2\pi} \int_{-\infty}^{\infty} \tilde{F}(\omega) e^{j\omega t} d\omega, \quad \tilde{F}(\omega) = \int_{-\infty}^{\infty} f(t) e^{-j\omega t} dt$$

And then when you do that from time to frequency conversion by doing Fourier transformation, you get a spectrum like this.



This is what is really you see when you record NMR spectrum, this is what you are going to see real NMR spectrum. This is called one dimensional NMR spectrum of a particular nuclei. I have taken the example of proton here. Let us say a proton NMR. If you have a some molecule, you give to an NMR operator, what he will do? He will put the sample in

a magnet and apply a radio frequency pulse, collect the signal in time domain like this, and then do the Fourier transformation. He will give you a spectrum like this.

This is finally, what you are going to get for our interpretation. Now, you have got the NMR resonance. NMR spectrum you have obtained. This is what all of us will want. Now, the question is what is the type of spectrum you are going to get? We have to understand there are some intricate details. If you take two energy states, are they well defined states? You have understood the Heisenberg uncertainty principle. According to which there is a spread in the energy level, it is not well defined in a state like this, there is a spread in the energy of the states.



Now, I consider two spin states alpha and beta and I calculate the energy difference delta E and of course, we know delta E into delta t is greater than or equal to h cross. It is the uncertainty condition of Heisenberg. Energy and time uncertainty is there. Now, if we detect a NMR signal, because of this it is not a delta function. If it is a delta function, there is no width. It is sharp. Exactly at the 0 value, length is infinite anything outside this, it is 0. So, that means there is no width at all for delta function.

The NMR signal is not a delta function because it has got natural line width. Why this natural line width is coming? It is due to the lifetime of the spins in the excited state. How much time, how long it stays in the excited state? That is what defines the width of the NMR peak. If let us say delta t is larger, then this may be smaller. This is smaller, this may be larger both are possible. So, now we will see if the shorter is the lifetime of the nuclear spins, if the spins do not stay in the excited state for a longer time, if it decays very fast, then what will happen from this equation? The lifetime and the excited state, this is smaller because of this condition has to obey, there is a spread in the energy state. There is spread in the energy level as a consequence. Then if I have a peak like this, I can get a resonance from here to here, I can get from here to here, I can get from here to here, from here to here. Varieties of possible transitions are, there because of the width of the energy state. And since if it does not stay for a longer time, this spread becomes more, the line becomes broader. That is what is going to happen. The shorter the time the spin stays in the excited state, broader is the signal peak, width become broader. That is why if you want to see NH and OH peaks in your molecule, if I take proton NMR spectrum, the NH proton and OH proton peaks are usually broad, because Nitrogen-14 and Oxygen-17, are quadrupolar spins.

These quadrupolar spins ensure that the spins relax faster. That means the lifetime of the spins in the excited state is very short. As a consequence, you will see that NH and OH peaks in the spectrum are usually very broad; that is the about the lifetime.

Now, we can also discuss about what are called selection rules. Any spectroscopy for that matter will have what is called a selection rule; that is the transitions that are allowed between different energy states. Since now the time is getting up what I will do is, I will stop here. We will come back to the this one later, in the next class.

But today what we discussed in this class is; we started discussing the concept of bulk magnetization. By using random phase approximation, we understood that alpha and beta spin state magnetic moment vectors can be resolved into three components. Because of the random phase, x y components completely get averaged out. There would not be any components of magnetic moment vectors in the x y plane. Whereas, the vectors add up along z axis. Similarly along minus z axis for the beta state the components of the vectors add up. Due to excess population in the alpha state compared to beta state, there is a tiny net magnetization developed along the direction the magnetic field. And I said the bulk magnetization. This is called bulk magnetization, which is always parallel to the magnetic field direction. And it is static. It is stationary. If you have to get the NMR signal, this bulk magnetization which is stationary has to be perturbed. How do you perturb it? By applying a radio frequency signal in a direction perpendicular to the z axis; either along x axis or y axis. Then as a consequence the magnetization gets tilted to x axis or y axis depending upon where you are applying the rf pulse. By using right hand thumb rule we can understand the direction of tilt. And the signal as soon as you bring it to the x axis or y axis there is an instantaneous phase coherence. That means, all the nuclear vectors are on the particular axis. Then with time they start dephasing. While dephasing simultaneously they start going along z axis. Both are exponential processes. When it decays with time, we are going to collect the signal as a function of time. And time and frequency are related by mathematical operation called Fourier transformation. We do Fourier transformation. Then the time domain signal will be converted into frequency domain signal. And the number of peaks correspond to different frequencies present in the molecule of your interest. Each peak corresponds to a particular frequency. And that is what we are going to get; that is the NMR real NMR spectrum. And this, as I said, each peak if you consider they are not delta functions. Not that line width of each of this peak is 0. There is certain line width for each of these peaks, because of the natural line width. This is coming because of the Heisenberg uncertainty principle, $\Delta E \Delta t$ is greater than or equal to h cross. Shorter the time the spin stays in the excited state broader is the signal.

As I said, OH and NH protons are attached to nitrogen and oxygen which are quadrupolar

spins. The quadrupolar relaxation is faster, it ensure that the spins relaxes faster from the excited state. As a consequence if you take the proton NMR spectrum of any of your molecule containing OH groups or NH groups, usually these peaks are very broad. And then afterwards we need to understand the transitions which are allowed and there is a selection rule for this. And that is what we are going to discuss in the next class. Thank you very much.