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 03 - NMR Spin Physics-II

Welcome all of you. In the last two classes, we discussed several concepts related to NMR spectroscopy, fundamental concepts, basics which are extremely important to understand NMR. We started with a spin of nuclei, what is a spin, what is spin angular momentum, what is a spin quantum number, what is a magnetic quantum number, what is a magnetic moment, what is the interaction of the magnetic moment with the external magnetic field, how it works? and what is the energy separation, what is the resonating frequency υ, which is nothing but gamma into B0 over 2 pi; we calculated. We also calculated what is the frequency at which this NMR comes in the electromagnetic spectrum and we did discuss the classical analogy also. We discussed what is called the Zeeman effect, Zeeman splitting which the resonance condition. We call it as Larmor precision frequency which is nothing but the resonance frequency, which is nothing but the energy separation between the two energy states. Quantum mechanically we understand the energy separation which is nothing but the Larmor precession frequency. This is what we understood. We also calculated what is the resonating frequency for different nuclei in a given magnetic field.

We understood what is gamma, we understood what is the sign of gamma, what happens to the sign of gamma is positive or negative, how do the two nuclear spins start precessing depends on whether the gamma. IF they have opposite signs the nuclear spins will precess in the opposite direction. If the nuclear spins have both positive gamma, they rotate in the clockwise direction. The nuclear spins with negative gamma processes in the anti clockwise direction. This is what we discussed extensively. We also understood something about Boltzmann distribution.

We will continue with the Boltzmann distribution and try to understand what is the sensitivity of the detection of NMR signal. It is very very important. I told you NMR comes in the low energy region of the electromagnetic spectrum. I showed you in the last column of this table which I showed energy was very very small, very weak energy very weak interaction compared to all other spectroscopy technique. That means the sensitivity of detection is very small. We will understand what is its sensitivity now.

And again we go with Boltzmann equation. We have two energy states alpha and beta which is given like this and Boltzmann equation is given like this;

$$N_\beta/N_\alpha = e^{-(E\alpha-E\beta)/kT}$$

Nbeta over Nalpha is the spin population ratio is given by E to the power of minus E alpha minus E beta divided by KT. This is the energy separation and Nalpha and Nbeta are the population of the spins in the states alpha and beta. But also we know that the ratio of the populations vary exponentially with the energy difference according to this equation. This is the ratio of spin population. You see how exponential it is varying.

We know we can calculate what is E alpha minus E beta which is nothing but delta E and also I know delta E is equal to gamma into B_0 over 2 pi which is equal to υ. I calculated υ is equal to gamma into B naught over 2 pi that is the resonance condition; Because nu is related to delta E, I know what is delta E, I know I can calculate this thing. What I am going to do is, put the Boltzmann constant, which is very well known. it is a constant easily available and at a particular temperature I am going to do the experiment. Let us say temperature is expressed in Kelvin. These are the parameters I know. I know gamma I know B_0, I know h, I know 2 pi. I can calculate the energy separation. I know at what temperature I am doing the experiment. Boltzmann constant is also known to me. Now what I am going to do is we will use these parameters and calculate what happens to the sensitivity. The population difference is governed by the Boltzmann population distribution. According to this equation there are two parameters which are responsible for it. One is energy which depends upon magnetic field. As the magnetic field linearly increases delta E, the separation increases.

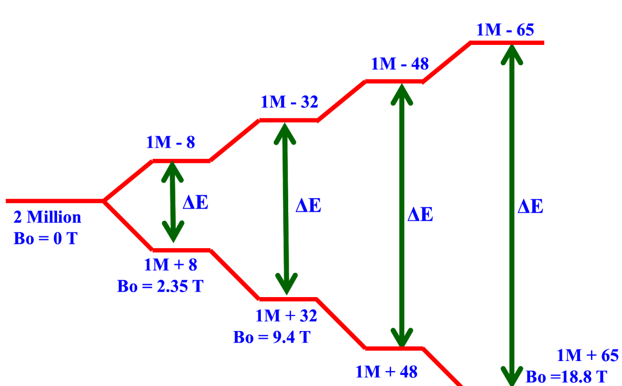
So, now there are two factors that govern the population difference. One is the magnetic field and other is the temperature. For detection of NMR signal there must be spin population difference I told you. When I calculate the population, I wrote the some two energy states what are the population in the alpha state and beta state. I said there is little excess of population in the ground state or in the lower energy state. And there is a difference in population. This is essential to say that in NMR, no population difference means no NMR. With this now we can calculate the population ratio between two energy states. It again depends upon the magnetic field which is in Tesla. I told you what is a magnetic field. So, delta is equal to υ into gamma

$$\Delta E = 3.952 \times 10^{-25}\ J$$

into B_0 over 2 pi. So, it linearly varies with the magnetic field. So, what we will do is we calculate the delta E for a given magnetic field. Let us say magnetic field is 14 Tesla, I know gamma of the proton. I know what is Boltzmann constant, I know what is 2 and pi. I just plug in these valuee for my delta E equation. I gave you delta E equation, delta is gamma into B naught over 2 pi. I said now delta E is turns out to be this value.

Now K_B the Boltzmann constant. I know it is 1.381 into 10 to the power of minus 23 kilojoules. The temperature at which I am doing is 298 K. I plug in these values I know K, I have calculated delta E. I know what is T just plug in these values. Calculate n beta over n alpha. This is exponential value. I calculated delta E I know K_B the Boltzmann constant. I know T at which I am doing the experiment. You just plug in these values. This is what you are going to get. The population ratio between two spin states n beta and n alpha turns out to be 0.999904 very close to 1, almost 1. It means this population ratio between two states is nearly equal. There is not much of difference in spin populations and I can even calculate by plugging the value, for example for 2 million nuclear spins at 14 tesla. Just plug in this value for this equation 14 tesla and I calculated the population difference, which turns out to be this value. In the beta state we have this many spins and in the alpha state we have this many spins. What is the difference? the difference is only 96. that means at temperature where I did the experiment, let us say room temperature, now if I calculate the population difference it turns out to be 96 among 2 million nuclear spins. Remember where is 2 million nuclear spins, where is 96. It is extremely small, almost negligible. That shows the difference in spin population, that is to be detected is very very small. Very small population difference we have to detect. That is why NMR is highly insensitive technique very very insensitive technique. Now what will happen to the sensitivity when I increase the magnetic field, let us see. This as I told you there is an expression in the delta E, linear related to υ0; as υ0 increases the energy separation increases.

When the energy separation increases, the population difference increases. That is what is going to happen. Now I consider two energy states these are the two energy states and there is a large population difference because this energy state is larger than this one. The large energy separation means the large population difference; the small energy separation means the small population difference. So, as the magnetic field increases linearly, the energy separation between two states increases. That means the population difference increases. When



the population difference increases, the sensitivity will increase. So, it means higher the magnetic field, higher the sensitivity, okay. If you want to increase the sensitivity of the detection of the NMR signal, you have to go to higher and higher magnetic field. The higher the magnetic field, higher the sensitivity.

This is what I said in one of the slides earlier in the previous class. Now, let us calculate the population difference at different magnetic fields and see what is the sensitivity gain we are going to get. Consider a situation where magnetic field is zero. I take the example of two nuclei, two million nuclear spins. When magnetic field is 0, that means there is no separation of energy levels; that means there is a degeneracy, there is no population difference.

Now, I will increase the magnetic field, make some value 2.35 Tesla and I calculated the population difference, it turns out to be 16, that is all. See among 2 million spins, at 2.35 Tesla magnetic field, the population difference is only 16. Increase the magnetic field again 3 or 4 times, now it becomes 64. Again increase it to some other value 14 Tesla, you see the population difference now is 96. Then go further, make the magnetic field 18.8 Tesla, calculate the energy separation and the population difference. This population difference is only 130. Remember, you started with 0 Tesla and increased the magnetic field tremendously. From 2.35 Tesla almost we have made it 8 to 10 times larger, but the population difference which was 16 at 2.35 Tesla, became only 130, not a big number. But still it makes significant contribution. The effect is really significant as far as the detection of signal is concerned.

As we go ahead, I will show you how when we record the spectrum at different frequencies, we will get better sensitivity and better resolution. So, this is the magnetic field vs sensitivity. How the sensitivity changes with the magnetic field; the magnetic field versus sensitivity. At 7 Tesla, the resonating frequency is 300. Let us consider this sensitivity as 1 for my reference for calculation.

Magnetic field (Tesla)	**Resonating Frequency**	**Sensitivity**
7.0	**300**	**1.0**
9.4	**400**	**1.54**
11.7	**500**	**2.15**
14	**600**	**2.83**
17.5	**750**	**3.95**

I go to magnetic field 3 times larger, sensitivity is 5.2 times more because the sensitivity goes by B_0 to the power of 3/2. So, if I know the magnetic field, if I change the magnetic field, Bo to the power of 3 by 2 is the enhancement in the sensitivity. So, the sensitivity depends on the magnetic field. That is one thing which we have to understand. As I said, the sensitivity of detection depends upon the strength of the magnetic field.

Now, what are the other factors that is governing the sensitivity? One is magnetic field, I told you because the energy separation is larger, larger population difference, more sensitivity, it is all fine. There are other factors, one is temperature. I said, you remember N beta by N alpha is e to the power delta E over kT, k is the Boltzmann constant, T is the temperature. Remember in this equation, T is in the denominator. Now, what happens? Let us put some value; put T as 0, let us say I go to the T equal to 0 k, I calculate the populations. So, now because T is equal to 0, it is Nbeta over Nalpha, Nbeta over Nalpha into e to the power of minus infinity, which is 0.

So, when Nbeta over Nalpha is equal to 0. Nbeta is equal to 0; that means number of spins in the beta state, higher energy state is 0. What does it mean? It means all the nuclear spins are in the alpha state, the lower energy state. When all the spins are in the lower energy state, what is the population difference? The population difference is very high. That is what is going to happen, higher the population difference and higher the sensitivity. That means if you go to the lowest temperature; at 0 k, all the nuclear spins will be in the lower energy state, occupied in the low energy state, alpha state. As a consequence, sensitivity will be better. You cannot go to 0 k, but understand the concept. As you keep on lowering the temperature, the population difference become more and more. So, the sensitivity becomes more and more; that is one concept.

Now, let us do other trick. What happens if I put temperature infinity? Remember T is in the denominator. You have put T is equal to infinity. Now N beta over N alpha, if you calculate, it turns out to be equal to 1. Now, when it becomes 1, then what is going to happen? N alpha is equal to N beta. That means the number of spins in both alpha and beta states are equal. When N alpha is equal to N beta that means there is no population difference at all, absolutely no difference. As a consequence, you will not see signal and this is called saturation. Saturation is a condition where the populations in both energy states are exactly equal. So, no signal will be seen. That is what you should understand.

There are other factors which is responsible for giving better sensitivity. One is gamma, higher the gamma, higher the sensitivity. As I told you the nucleus with highest gamma is the proton among all the stable isotopes, and proton that is hydrogen has a highest gamma. I told you it depends upon the mass of the nucleus, this is the lowest one. Now, this is not heavy at all compared to all other elements in the periodic table. So, proton has a highest gamma, and the highest sensitivity is because of highest gamma.

The sensitivity depends upon three more factors. One is magnetic moment, population difference and coil magnetic flux. These are the three parameters. Interestingly, all these three factors depend upon gamma. That means the sensitivity depends upon gamma into gamma into gamma. So it depends upon gamma cube. Sensitivity is linearly dependent upon gamma cube because of these three parameters. So higher the gamma, higher the sensitivity. This is what is the reason why I said proton among all the nuclei in the periodic table has the highest sensitivity. Let us compare now the sensitivity of proton with other two nuclei with a different gamma, okay. As I said proton is highest one, we will consider that as a standard, as a reference.

We will compare the sensitivity of the proton with carbon and nitrogen. We will take the ratio. Of course, all other parameter gets cancelled out because B0 is constant. I am not changing 2 pi, everything gets cancelled out. Only gamma is the term which remains. Gamma for the proton is this and gamma for carbon is this which goes by cube. What is the ratio of proton to carbon to gamma? If we calculate, it turns out to be close to 4. Ratio is 4; and 4 cube, is 64. Thus the ratio almost 64. That is why if you look at the proton, it is 64 times more sensitive than carbon. Whereas carbon is 64 times less sensitive. It is very difficult to detect carbon compared to proton because of low gamma.

Now, let us see what happens to nitrogen. Nitrogen has 10 times lower gamma, almost 10 times, compared to that of proton. So, 10 cube, 1000. So, it is almost 1000 times smaller. Of course, it has a negative magnetic moment. That is a different question. That is not a point to be understood now; because that comes only for NOE and others. But right now, sensitivity is 1000 times smaller. Remember, I can do the experiment of proton in just 1 minute, under identical conditions, assume that everything remains same, no change, I need 64 minutes for carbon, I need 1000 minutes to take the nitrogen spectrum under identical conditions. See the difference? Assuming that number of nuclei, number of nitrogen, everything remains same in a given molecule.

So, this is the sensitivity factor coming into the picture. So, carbon 13 is 64 times less sensitive compared to proton and nitrogen is 960 times less sensitive compared to proton.

And sensitivity also depends upon another parameter called natural abundance. The natural abundance of the NMR active nuclei. For example, hydrogen has 3 isotopes, proton, deuterium and tritium, but incidentally this has 99. 99 percent abundant. Fluorine and phosphorus has100 percent abundant, 1 isotope. And these are the 3 nuclei which are relatively more sensitive for detection. It is easy to detect these nuclei than other nuclei. I tell you why. If you look at carbon 13, its sensitivity is 1.1 percent, nitrogen is 0.37. Remember, oxygen is even smaller 0.037. Imagine very, very low abundance.

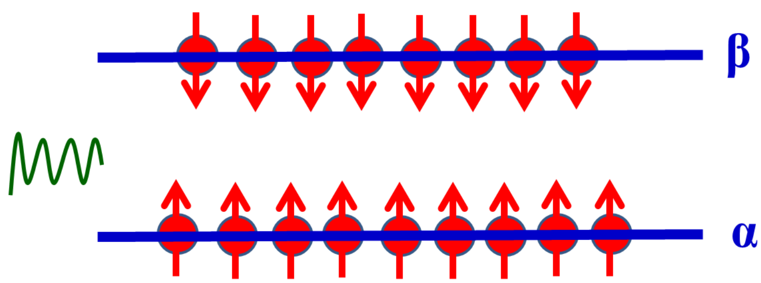
What does it mean? If I take an example of a molecule containing only 1 carbon; For example, take CHCl3, only 1 carbon is there in this molecule. Let us say I have 100 molecules in a sample in a bottle, I have 100 molecules. Then possibility of this CHCl3 having carbon 12 as one of the isotope is 99, because its abundance is more. 99 molecules will have carbon in its carbon 12 state, whereas only 1 molecule will have carbon in carbon 13 state. Understand? 100 molecules are there out of which 99 are in carbon 12, carbon in the carbon 12 state, and only 1 has carbon in the carbon 13 state. So, that is why it is very difficult to detect. So, natural abundance is a very important factor.

Now, what we will do is, we will plug in this factor to calculate the sensitivity of carbon and nitrogen which we did just now. There we considered only gamma, but now we will also factor in the natural abundance. Bring in natural abundance as a factor. Now, this ratio was known, 99.1, 63 is what we calculated and if you do that, it turns out to be 5,672. Remember, now if I bring natural abundance as a factor, the detection of carbon 13 compared to proton is nearly 5,700 times less sensitive. It takes more time. Go to nitrogen, it is 1000 times smaller, you know, this sensitivity is already smaller because of gamma and the natural abundance is even smaller. It turns out to be nearly 2,60,000 less sensitive. Nitrogen 15 is even more difficult to see because of its very low natural abundance.

See the point, what happens? Not only gamma, the natural abundance contributes a lot. So, it is very difficult to detect these things, because of their natural abundance. So, if we go to the table of NMR active nuclei. In this table, you see different color codes. We have spin half, spin 3/2, to like that. Varieties of nuclei are there, but in the periodic table, you can see almost every element except this range, all the elements of the periodic table has at least one element or one isotope of that element which has nuclear spin. That means they are NMR active. That shows every element of the periodic table here can be individually studied by NMR. Now you can understood the ubiquitous nature of NMR.

If you take any material, biological or organic material, it must contain one of these elements, it must contain one of these nuclear spins. So, if you can use this, that is the reason why NMR can be used to study everything thing, right from chemistry, biology, pharmacy to even materials, because all these things should contain at least one element of this or one isotope of this element. With this, now we will compare the relative sensitivity of the different nuclei. Let us say at 250 megahertz, if I consider proton is 100%, fluorine is this one, look at this nuclei. In this range, it is so difficult to see signal at all. Whereas carbon-13 is very small here. So, this is a comparison of relative sensitivity. Assuming one is 100%, what happens if I want to see nitrogen-15, you might not even see the signal compared to that, because I told you 260,000 times it is less sensitive. So, this is what it is.

Now, we will understand how to get the resonance, how to induce a resonance. So far, we understood nuclear magnetic moment, energy separation, everything. But we understood what are N and M, if you understand R, that is the resonance? Then you understand NMR; nuclear magnetic resonance. So far, we discussed a lot about nucleus, lot about magnetic moment, etc. We will understand the resonance now. How do you induce resonance? See, for inducing resonance, you must apply a radio frequency pulse.

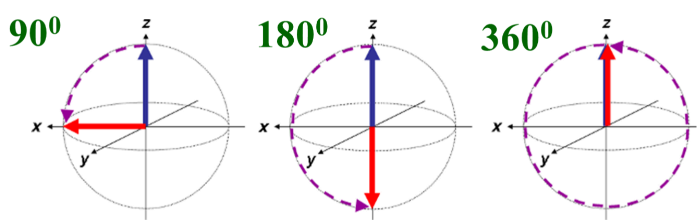


The radio frequency pulse should be applied to the Larmor frequency, ie. at the resonating frequency. So, when I apply radio frequency pulse like this, the nucleus spins, which are in the lower energy state, go to higher energy state and the spins in higher energy state will come to lower energy state. And now, what is happening? We are going to detect the signal, which is the difference between these two populations. This is what is going to happen. That is why I said we are going to detect the signal, which is nothing but the difference in the spin populations.

That is why the population difference is very important in seeing the signal. But what is this RF pulse? How do you apply this RF pulse? Where do you get this RF pulse? Of course, we generate the RF pulse with a transmitter and it is a rectangular pulse and its power is very small, 50 to 300 watt and frequency is the Larmor frequency. The width of the pulse is another important thing. Normally, it is 5 to 10 microseconds. Remember, pulse width which you apply for excitation or to induce resonance. It is only of the order of 5 to 10 microsecond for proton and for carbon that is little bit more. And of course, for different nuclei you can calculate what is the pulse because.

Why I am using is a 90 degree pulse. I will tell you that when we go ahead further. For a 90 degree pulse for proton, approximately, we need 5 to 10 microsecond RF pulse with the power of 50 to 300 watts. The RF pulse is applied in the form of a short burst. Then for the magnetization what is going to happen? it is tilted from Z axis to XY plane. What is the magnetization? I am going to explain to you now. Right now, I have not discussed about magnetization, but when we introduce bulk magnetization and random phase approximation, I will tell you what is bulk magnetization. Till then, you understand the magnetization is going to be tilted from Z axis to the XY plane.

Initially, magnetization will be along Z axis and you apply radio frequency pulse along one axis, let us say X axis here and you tilt the magnetization to Y axis; and here we are going to detect the signal. And this amount of tilting, how much magnetization is tilted is given by this equation gamma b_0 over 2 pi. Gamma b_0 is RF power, Tp is the pulse width and the angle at which you are going to tilt, that is theta, is given by this power and width of the pulse. If I have a pulse, let us say of 5 microseconds and tilt it by 90 degree. Again it is linear. If I go to instead of 5, I will make it 10 microsecond,I will tilt it by 180 degree. If I make it 15 microsecond, I tilt it by 270 degree. So, magnetization which was here, I can tilt it here, take it here, take it here, take it here. I can make it keep rotating like this. I can make the magnetization keep on rotating, it can dance to the tunes. That is what we do and this is what happens.



The magnetization can be tilted by any angle by varying the pulse width and the power. Initially, let us say it is along this axis, apply radio frequency pulse along Y axis, I can bring it to here. Apply 180 pulse, I bring it here. Apply 360 pulse, I take it like this and take it back to Z axis, so it depends upon the pulse you are going to apply. Always all the three are orthogonal to each other. Remember X, Y and Z, all the three are orthogonal to each other. Apply a pulse along X axis, bring the magnetization from Z axis to Y axis and you put the detector on the Y axis, that is what you are going to detect. So, if you apply 90 degree pulse, you bring the magnetization by 90 degree from Z to X axis. Apply 180 pulse, take it to minus Z axis, apply 360, you can bring it back to Z axis. This is what is going to happen. The direction of tilting of the magnetization follows what is called a right hand thumb rule. The direction of thumb is the axis in which you are going to apply the pulse and the curly fingers tells you the direction in which it is going to be tilted, that is important. So, with this how we apply 90 degree pulse, how we bring the magnetization to X axis, Y axis, we have understood. Simply you have to apply the radio frequency pulse at a particular axis, perpendicular to the Z axis where magnetization is there. We have to disturb it, we have to create what is called an equilibrium state and then magnetization comes to other axis, Y axis. All the three are orthogonal to each other and then we start detecting the signal. How it works everything, we have to understand by what is called a random phase approximation, which I am going to explain in the next class. Remember, these are very important concepts to to understand a NMR.

In the course, when we go ahead further, we interpret lot of spectra, analyze spectra, varieties of nuclei, everything, different 1D experiments, 2D experiments, etc. But this is the concept which is very important, you have to understand. So, what we discussed today, we discussed lot about varieties of nuclei, how we get the signal, what we are going to see, all those things we discussed quite a bit. How do you induce the resonance, what is the sensitivity of different nuclei compared to proton. It essentially depends upon varieties of factors, what happens at T is equal to 0, what happens at T is equal to infinity, that is a saturation. At T is equal to 0, all these spins are in the ground state, we have maximum sensitivity. As the magnetic field increases, population energy separation becomes larger and larger. Larger the energy separation, larger the population difference. Larger the population difference, higher the sensitivity. All these things we understood. Sensitivity depend upon three factors like magnetic flux and gamma. It also depends upon gamma cube. The ratio of the gamma between proton and carbon is 4, so gamma cube is 64, so 64 times is much smaller. Sensitivity of carbon is 64 times smaller than that of proton. If you factor into account the natural abundance, it is 64 into 100 approximately, 6400 times smaller. If you go to nitrogen, it has 10 times smaller gamma. So gamma cube is 1000. If you take sensitivity factor into account, we show that sensitivity of nitrogen 15 is nearly 2.6 lakhs times smaller than that of proton. All these things we understood and we understood how do we induce the resonance by applying a radio frequency pulse in a direction perpendicular to the magnetic field. And the population difference is what we are going to see. The spins undergo transition from alpha to beta state and beta to alpha state etcetera. This is what we understood and after that, this is just basic the concepts. If we have to understand NMR resonance, it is just not population undergoing transitions from alpha to beta, beta to alpha states. You have to understand by a term called bulk magnetization. What will happen to bulk magnetization by applying the RF pulse; because NMR spectroscopy is a coherence spectroscopy.

So, we are going to detect the coherence to detect the signal. So, this we will explain in the next class and then we also understand little bit of selection rules and everything. Then we go into parameters which are responsible to give rise to NMR signals etcetera later. So, I am going to stop it here. Thank you very much. We will see in the next class.