One and Two Dimensional NMR Spectroscopy for Chemists
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Lecture No -5
NMR detection and sensitivity

Welcome back all of you, since last 2 or 3 classes we have been trying to understand some of the basic concepts of Spin Physics and in the last class I was telling you about Larmor Precession.

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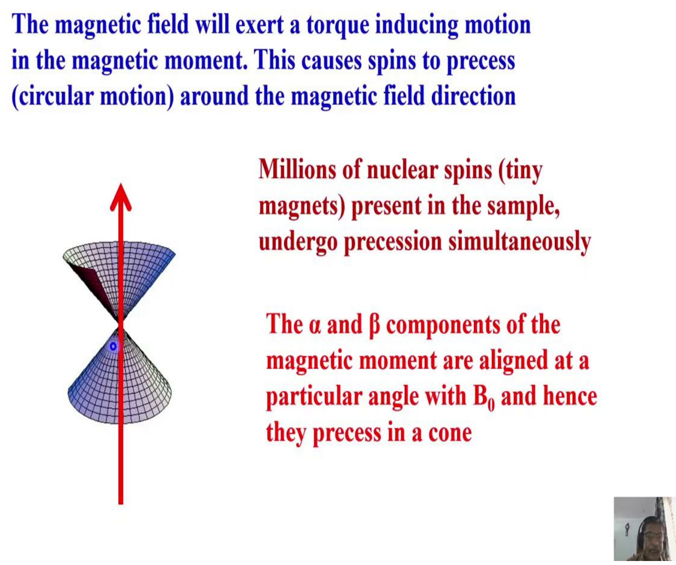

And in that I said one of the important concepts is, the magnetic field will exert a torque induced motion in magnetic moment. The torque induced magnetic moment motion will be there in the magnetic moment. This causes the spins to precess. I said there are two forces acting on it, one is the applied magnetic field which wants the spins to get aligned along the direction of the field, and of course there is a restricted orientation. As a consequence the torque is exerted, the magnetic field will exert torque inducing motion in the magnetic moment. So, millions of such spins will be there, not one or two, they will be rotating, Depending on, because they are spin half nuclei, there are 2 possible orientations, like this like this, which we also understood. We knew the direction of quantization. You could calculate the angle theta in one of the earlier classes.

So millions of such nucleus spin will be there in the sample, they all undergo precession simultaneously at the same frequency. The spins which are oriented in the direction of the field will start rotating like this and those which orient in the direction opposite to the field, all the spins rotate like this in a cone. They make a cone around the magnetic field. And of course, this is for the alpha component and this is for the beta component. And this precession as I said is Larmor Precession. It is a Larmor Precession.

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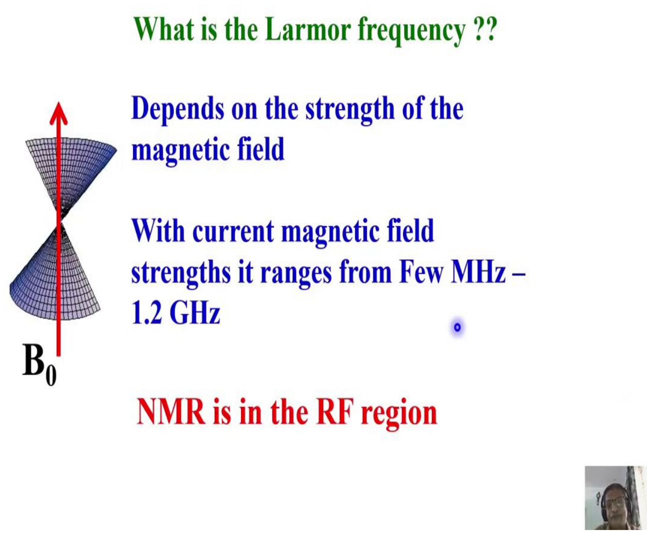

Now the question is what is the precession frequency? They are rotating, they are precessing. I Understand, the nuclear magnetic moments are precessing around the magnetic field. But what is Larmor precession frequency, and of course it depends on the strength of the magnetic field,. Higher the magnetic field, higher the precession frequency. Larmor precession frequency increases linearly with the magnetic field $B_{0,}$ the external magnetic field.

Now as I said in the current day, we have varieties of spectroemeters of various frequencies, upto even 1.2 gigahertz which is going to come to the market soon. At least 1 gigahertz or 1.2 gigahertz I know. So, as a consequence, if I say these spectrometers frequencies 1 gigahertz or 1.2 gigahertz, that is the precession frequency. Imagine magnetic moments are precessing at such a high speed. Hertz means cycles per second. In one second it has to rotate at 1.2 gigahertz that is 1.2 into 10 to the power of 9, imagine that is a precision frequency. The spins will be rotating so fast and it linearly varies with the magnetic field. Of course, again I said, because its precision frequency corresponds to resonating frequency of the nuclear spins, earlier I showed you an example where the spectrometer number on the magnet what you see corresponds to resonating the frequency of proton.

300megahertz, 400megahertz 500megahertz magnets I showed. So if I say I have a spectrometer at 1.2 gigahertz that means my precession frequency is 1.2 gigahertz. This again tells me the NMR is in the radio frequency region of the electromagnetic spectrum.

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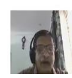
What is the energy involved ?

Radiation	Wavelength, λ (nm)	Frequency, ν (Hz)	Energy (kJ mol^{-1})
Cosmic rays	$<10^{-3}$	$>3 \times 10^{20}$	$>1.2 \times 10^{8}$
Gamma rays	$10^{-1}–10^{-3}$	$3 \times 10^{18}–3 \times 10^{20}$	$1.2 \times 10^{6}–1.2 \times 10^{8}$
X-rays	$10–10^{-1}$	$3 \times 10^{16}–3 \times 10^{18}$	$1.2 \times 10^{4}–1.2 \times 10^{6}$
Far ultraviolet	200–10	$1.5 \times 10^{15}–3 \times 10^{16}$	$6 \times 10^{2}–1.2 \times 10^{4}$
Ultraviolet	380–200	$8 \times 10^{14}–1.5 \times 10^{15}$	$3.2 \times 10^{2}–6 \times 10^{2}$
Visible	780–380	$4 \times 10^{14}–8 \times 10^{14}$	$1.6 \times 10^{2}–3.2 \times 10^{2}$
Infrared	$3 \times 10^{4}–780$	$10^{13}–4 \times 10^{14}$	$4–1.6 \times 10^{2}$
Far infrared	$3 \times 10^{5}–3 \times 10^{4}$	$10^{12}–10^{13}$	0.4–4
Microwaves	$3 \times 10^{7}–3 \times 10^{5}$	$10^{10}–10^{12}$	4 × 10[illegible]–0.4
Radio frequency	$10^{11}–3 \times 10^{7}$	$10^{6}–10^{10}$	$4 \times 10^{-7}–4 \times 10^{-3}$



Now what is the energy involved? Look at this table where varieties of Spectroscopy techniques are in the electromagnetic spectrum, varities of frequency ranges have been given and of course wavelength and frequencies have been classified which all of you know all these things. And energy is another thing which is important, the energy of interaction. If you look carefully radio frequency comes at the bottom of this table.

See energy which is highest is cosmic rays, gamma rays. For example X-rays, energy is in kilojoules per mole. It is 1.2 into 10 to the power of 4 to 1.2 into 10 power of 6, very large, large amount of energy. If we come to the radio frequency region energy is very small of the order of 10 power of -3 into 10 power -7, very, very low energy region. That means NMR spectroscopy falls in the very low energy region. Very, very low energy means the interaction energy of magnetic moments with the external magnetic field is very weak.

The weak interaction energy is really the strength of NMR, you understand. The reason is you can monitor even a minute perturbation at the site of the nucleus. As you go ahead and when we discuss more about chemical shifts and the couplings you understand. Electronic charge distribution at the site of the nucleus, there is minute change because of the substitution, we will see the effect of it. The weak interaction is really the strength of NMR. We can monitor many interactions at the site of the nucleus.

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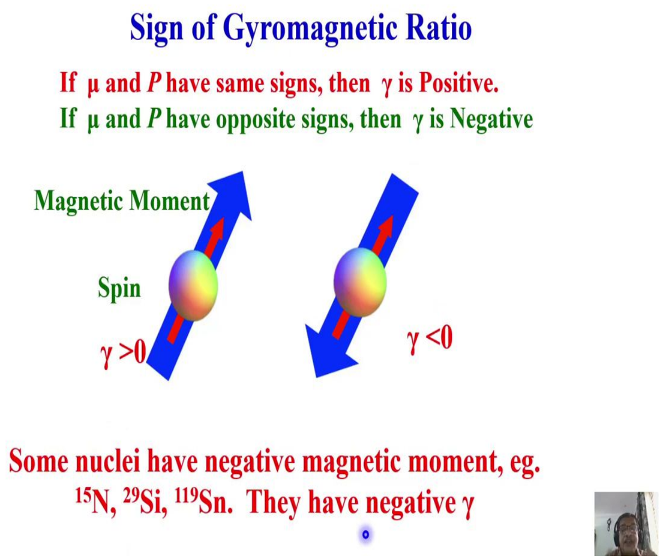

So, this is one of the important concepts. Next, I was telling you about the gyromagnetic ratio. This gyromagnetic ratio has a sign. Let us say we have a magnetic moment μ and angular momentum P, if both μ and P have same signs then gamma is positive. If μ and P have opposite signs then gamma is negative. Now look at this figure, this is a magnetic moment, ok, now both spin and magnetic moment are in the same direction here, they are in the opposite direction here. This is P and μ. This is when both of them have same signs. You have gamma greater than 0, in this case you gamma is less than 0. So what is the importance of this gamma. So, what if it is positive or negative, how does it matter for us. Tremendous implications, especially when you are measuring the couplings you will know, what is a sign of the coupling, It is very important. or when you are measuring what is called the residual dipolar couplings or if you want to detect forbidden transitions involving nuclei with negative magnetic moment, like nitrogen 15 the sign of precession is very, very important.

So, what happens is, there are many examples of nuclei like nitrogen 15, Silicon 29, tin 119, all these nuclei have negative gyromagnetic ratio.

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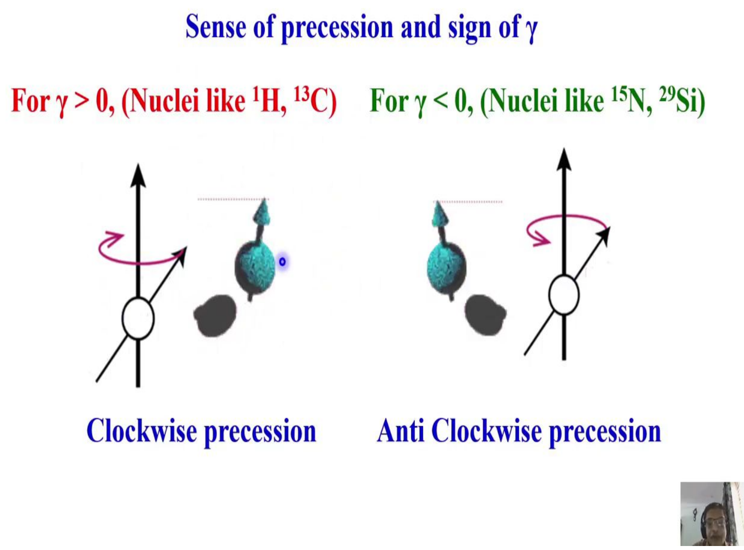

The reason is, you see what is going to happen now, depending upon the sign of gamma, the sense of precession the Larmor precession of the spins in the magnetic field is different. Look at this, for gamma greater than 0 the spins are rotating like this. It is shown as clockwise rotation, whereas for gamma less than 0, see it is rotating anticlockwise. So, it is rotating in opposite direction. For example for nitrogen 15 spins it will always rotate in the opposite direction compared to Proton and carbon like that, which is positive gamma.

This you have to understand, this can be very useful in the design of some, for example double quantum, triple Quantum, multiple quantum forbidden transitions, which you want to detect. The multiple Quantum transitions involving a heteronuclei, like Proton and nitrogen, carbon and nitrogen, like that. then sense of precession will decide, Ok. that is very important.

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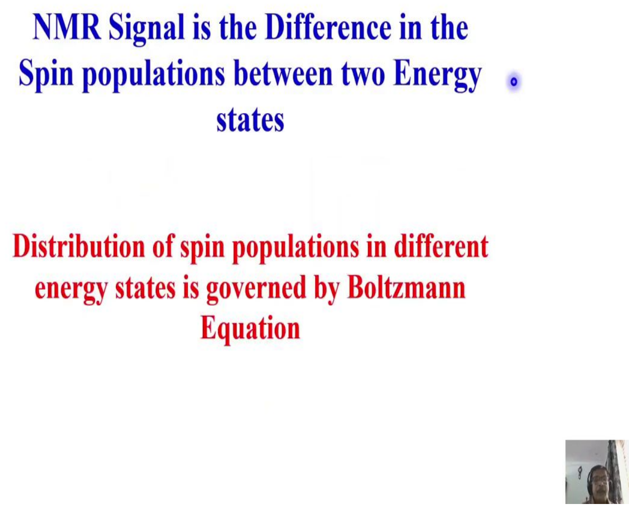

Next, OK, we have understood something in quantum mechanics, spin physics, classically we also have the picture. We know it is Larmor precession, Larmor precession increases linearly with the magnetic field. You also understood the sense of the precession, depending upon the sign of gyromagnetic ratio, this is fine. Now all these things are fine, there is a difference in energy everything, but we need to detect the signal.

How do you detect the signal? The detection of signal is nothing but the detection of population difference between two energy levels. Remember, in the earlier class I said there is possibility of more spins aligning in the direction magnet, that is parallel to the magnetic field than opposing it, that is the lowest energy option I said. So, now we have to find out how many spins are in this direction. How many spins are in this direction? The difference is what you are going to see. You see the problem now. Your NMR signal is nothing but difference in the spin population between 2 energy states.

Now the question is how these populations are distributed. I know, ok. I detect the population difference between 2 energy states and the next question is, how do I know what is the distribution spin of populations. I have to understand. That is governed by what is called Boltzmann equation. See population difference between 2 energy states is governed by this Boltzmann equation.

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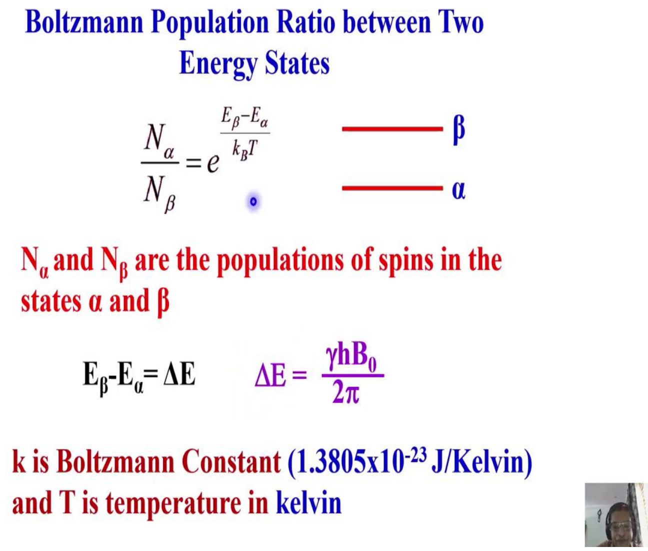

So what is this Boltzmann ratio, which define the population ratio between two energy states. This is simply given by this expression, N alpha by N beta is equal to e to the power of energy difference between beta and Alpha states over K B into T. K is the Boltzmann constant, T is the temperature. But do not get confused in some books it is written as e to the power of minus E beta minus E Alpha, depending upon which one is your upper state and which is your lower state.

Normally it is N of upper state over N of lower state, N beta over N alpha. So, I take the ratio in the opposite way, N beta over N alpha, then it is e to the power minus E beta minus E alpha over K B into T. In other words, in simple form the ratio of the population is given by e to the power of delta E by KT or minus delta E by KT. In this example the energy level the way I have drawn, for me my lower state is alpha and upper state is beta, so in the conventional way it will be opposite I have written like this. No problem, that is right. but no sign does not matter. Interpretation is same, the population difference would not change whether you take this way or that way. How do you calculate that depends upon this term. And N alpha and N beta are the population of states alpha and beta I said that delta is energy, fine. What is delta E? How do you know delta E? Delta E is nothing but gamma h into B 0 over 2 PI, we found out already what it is; while we were trying to get resonating frequency we understood what is delta E. The Delta E is given by gamma into h over B 0.

It is gamma h cross, h cross is written as h over 2 PI, h is being a Planck's constant. Alright, So I know delta E, I can calculate Delta E because I know gamma, I know h, I know 2 PI, and I know B0. B0 is my magnetic field, it should be expressed in Tesla. I know what is KB, Boltzmann constant. This one is the Boltzmann constant, this value is also well known. Temperature must be expressed in Kelvin. I know what is T. All the parameters I know, so the only thing is B0 is in my control. The magnetic field strength is in my control. I can vary the magnetic field to any value I want and calculate what is the energy separation, what is delta E, difference in the energy.

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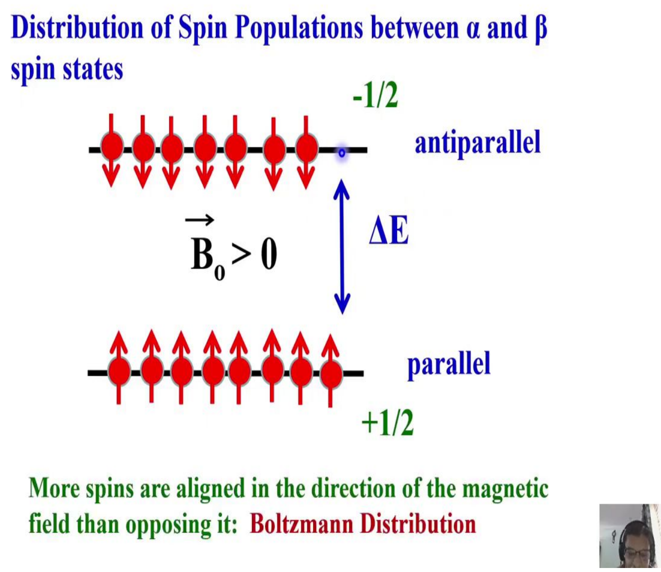

Ok, let us try to do that. Now if I try to do that and find out this spin population. It will be like this, there will be more spins in this parallel orientation than anti parallel orientation. You see that, there is small difference I am showing you, this is the delta E difference. And this is plus alpha state I am sorry alpha state, plus half, beta state minus half. Ok parallel or antiparallel orientations and I calculate the delta E

Now Boltzmann population tells me there are more spins aligned in the direction of the field than opposing it. That is the definition of Boltzmann. Boltzmann population distribution tells me that.

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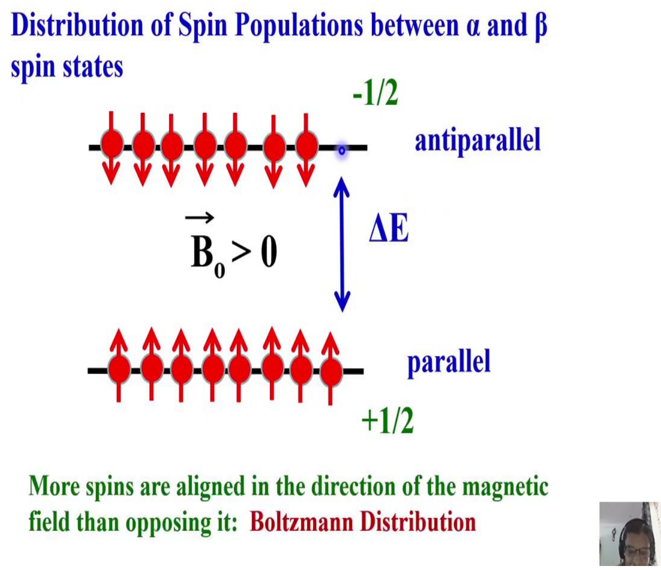

Let us find out what are the factors that governs then. If you look at this equation, what are the factors which governs population ratio. One is the energy separation, because it is in the numerator. Larger the energy separation, this population ratio becomes larger, you understand. When does the energy separation becomes larger? it is linearly proportional to the magnetic field. So, when the magnetic fields keeps on increasing, the energy separation becomes larger and larger, that is what we saw.

Larger the energy separation then according to this equation greater is the population ratio. The population ratio becomes more and more, that is one point. What is the second possibility to increase the population? It is also governed by T, temperature. Temperature is in the denominator I can vary the T and I can make it to Infinity or make it 0 does not matter, I vary the T, the way I want. Let us see what happens? This reduces, this keeps on increasing, so I can control this and I can control this, so both are in my hands.

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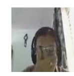
Factors that Govern the Population Difference

Magnetic Field
Temperature

For detection of NMR signal there must be spin population difference between two energy states

No population difference !!   No Signal !!!



Population difference or the population ratio is governed by 2 important factors. One is the magnetic field, other is the temperature. These two factors govern the population difference. Now for detecting NMR signals there must be population difference. If there is no population difference, no NMR, no signal. Look at this, you can come across this kind of situation like that, sometimes it so happens you may come across the situation, where there is no population difference at all, but not in the natural situation. You can create artificial situation by equating the spin populations of both the states by someway. Experimentally we can do, I tell you how later, that is called saturation state. You go to saturation state, where there is no population difference at all, that means you will not see signal. So important factor is you must have greater the population ratio or the population difference between alpha and beta states, then larger the NMR signal, the sensitivity is higher. You will detect better signal. So we can play with these two parameters. Now how we can play with parameters? How we can increase intensity, let us see.

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Population ratio between energy states

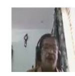
Mag. Field in Tesla

$$\Delta E = \frac{(267.512 \times 10^{6}\ \text{rad/Tesla/s}) \times (6.63 \times 10^{-34}\ \text{J s}) \times 14.00\ \text{T}}{2\pi}$$

$$\approx 3.952 \times 10^{-25}\ \text{J}$$

Using this value of ΔE, $k_B = 1.381 \times 10^{-23}\ \text{J K}^{-1}$ and at 298 K

The Population ratio = 0.999904



First let us see what the real population difference between two energy states is, if you take the population ratio, not difference, I am talking the population ratio. So, now delta E I said gamma into this h Planck's constant here and B0 over 2 Pi, ok, B0 is magnetic field which I have taken in Tesla, 14 Tesla, and 2 PI. Simply it is a number do not have to worry. all units are properly written here. Simply plug in these values into the calculator and find out what is delta E.

You find out what is Delta E? It turns out to be of this order 10 the power of minus 25 joules. That is the energy difference. Ok. Now, we know delta E, I know KB, put it into Boltzmann population ratio equation. N alpha by N beta is equal to e to the power of delta E by KT, put it there. Now I know KB, I know temperature, if I put this into that equation, the population ratio turns out to be 0.999904, understand. Population ratio is 0.999904. What does it mean?

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At Room Temperature

Eg: For 2 million nuclear proton spins in a 14 T Field at thermal equilibrium, at room temperature

The population ratio will be 0.999904.

Upper Energy state will have 9,99,904 spins

Lower Energy state will have 1,000,096 spins

That means the number of spin population in both the energy states, is more or less same, almost equal, is it not, it is close to one. Almost I would say it is one. Very small difference in the sixth decimal place, so it is very small. So the population difference is negligible small, ok, you can even workout the number. At room temperature, let me take this example, 2 million populations I will take. Actually it is much more, you have go into Avagadro number, very high number.

But I will take for understanding purpose, let us say there are 2 million nuclear spins in the molecule and the magnetic field is 14 Tesla, and my temperature is thermal equilibrium, that means room temperature, understand. These are the conditions. I know magnetic field, I know temperature and take the example of 2 million populations and I want to calculate the ratio, ok, I will do that.

If I do that, it turns out that population ratio is this much, that already we worked out. So how many spins are there in upper energy state? How many are there in the lower energy state you can find out. Of course I made a rough calculation. I did not go to get the ratio and taking million numbers everything approximately I made a calculation and you find out what is the number of spins in the upper energy state, what is the number of spins in the lower energy state and find out the difference. it approximately matches, upto 3rd or 4th decimal places I managed it.

And then if I take of course large or bigger calculator I can workout. I roughly mental calculation, I did and this is what the number I got. But don't worry, this is not very much different, right. if you want you can find out N alpha by N beta is this ratio and if the total number is 1 million and it is simple Primary School mathematics you can workout.

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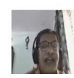
At thermal equilibrium, the spin populations of both the energy states are nearly same, with very little difference in populations

This small difference in population is detected

NMR is highly insensitive !!!



Now at thermal equilibrium the spin populations of both energy states and nearly same, that what it says and very little difference in population. What does it mean? 2 minions the difference in the population is less than 100, 95 or 96, that means, imagine, if I have to take large number of spins taking into account calculate all the spins available in a given sample, find out the population ratio, you hardly get only few 100's. The difference is so small and that is what you want to detect.

So that means NMR is highly insensitive technique. The population difference is so small if you want to detect the NMR signal it is very difficult for you, because it is highly insensitive technique. Then you may ask some question, what do I do then? How do I use NMR? Do not worry, I will show you there are many, many ways of increasing the sensitivity. Mind you that small population difference that you say, is more than sufficient.

We can get the signal, detect the signal and then to do all our experiments, whatever you want. Though the population difference is negligibly small, it is still detectable. Of course there are ways to increase the sensitivity we can do that.

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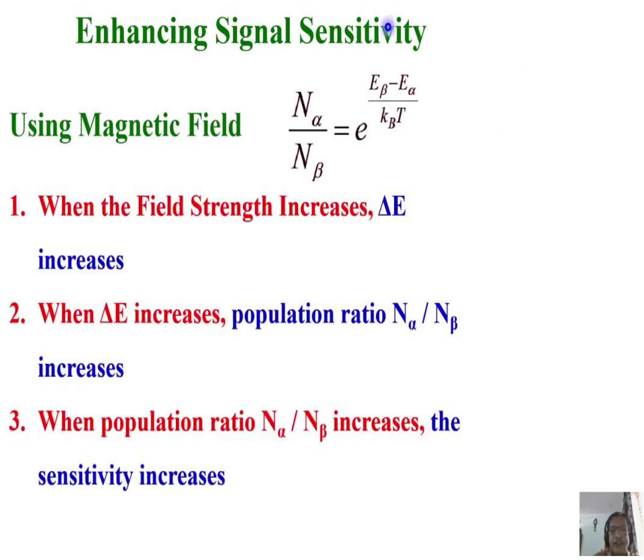

Numbers of ways are there to enhance the sensitivity. I can make signal intensity more and more by various things, look at this Boltzmann equation. Now this is in my hands, this energy separation become larger and larger with magnetic field. So, larger the magnetic field, larger the energy separation. I will play with magnetic field now. What happens if I play with the magnetic field? When the field strength increases delta E increases.

But remember when I take this ratio, delta E is larger, population ratio of N alpha divided by N beta also increases, because this is in the numerator. When the population ratio increases, the sensitivity increases because we detect difference in population, that is all it is simple. What we have do is to keep on increasing the magnetic field, maximum possible then you can achieve, then your sensitivity keeps increasing.

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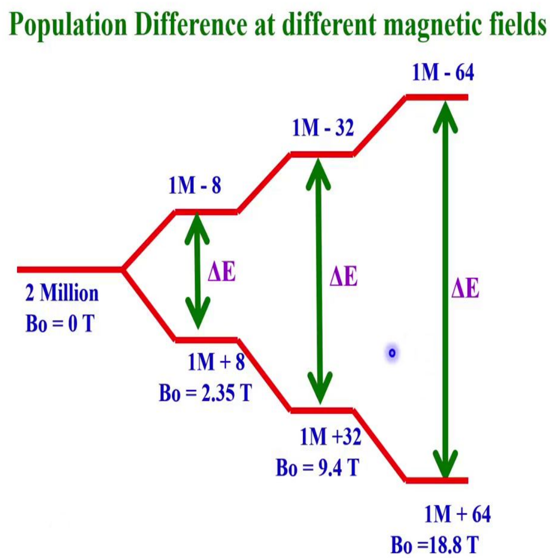

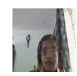

You may ask me a question. Ok, let us say from 5 Tesla, 2 Tesla, etc., I take the magnetic field to 20 Tesla. I will increase 10 times the magnetic field. We will solve the problem of sensitivity forever. Next question is how much magnetic field we have to increase? If I have to increase magnetic field by some number, some ratio, at 2 times, 3 times or 4 times. How much change will be there in the population difference, we can calculate. In the equation which was given to you, just change the magnetic field and calculate. I took the example of 2 millions populations, 2 million spins, when the magnetic field is 0, there are degenerate states. No population difference at all, it is 0. Now I will put this in small magnetic field, not small reasonably big, 2.35 Tesla, it is not a small number. In the magnetic field of 2.35 Tesla I calculated the population difference, it is only 16, population ratio is 16, not the difference, but I made it as a small number. I took as a difference, so that when I subtract these 2, you will get 16 is the difference.

So population ratio 16 but I am showing you as the difference not the ratio, please remember this. This is a small number you can work out. I had no time work out, so I am giving you this value. Now I increase the magnetic field from 2.35 to 9.4, 4 times I made the difference. Population difference becomes 4 times more. Differences was 16 here, it became 64. Ok, further increase, another double it. So, from here, from this place to here, now it is 18 times.

From here to here 8 times, so I have increased the magnetic field 8 times. 2.35 Tesla became 18.8 Tesla, but what is the difference of population you are seeing, only 128. My God, look at the difference you are seeing. From 16 spins difference in 2 million by increasing the magnetic field 8 times you made it only 128, what great thing you have achieved. Just from 16 spin population difference you took it to 128.

But you may say it is a small number, but it is significant, as far as NMR detection is concerned. yhis difference is still significant, remember I took ensemble for this thing, but you are taken ensemble of molecules and so many nucleus spins will be there. If you calculate, but still it will be a small number, but significant enough to see the difference in the sensitivity that is one thing.

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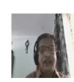
Magnetic Field vs Sensitivity

Sensitivity $\propto B_0^{3/2}$

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
18.7	800	4.35
21	900	5.2



So we will look at what happens, I keep on changing the magnetic field 7 to 14, sensitivity goes by 2.3 times. By increasing the magnetic field from 14, I go to 21, so it became 5.2 times. Remember, let us say if the sensitivity goes by 2 times, your experimental time, instrument requirement comes down by a factor of 4. It means let us say I have a sample which takes 4 hours for me to get a reasonably good signal. If I double the sensitivity then I require only one hour. Imagine the saving of the time. But you have to play with the magnetic field, which is not an easy thing to achieve. When you increase the magnetic field, that is the higher and higher the field it is technically highly demanding, not easy. Nevertheless, nowadays the technology is so advanced we can go upto 21, or even 24 Tesla magnetic fields that are available, so that the sensitivity can be significantly increased. From here to here 5 times sensitivity enhancement, ok take 5 times. So you are experimental times comes down drastically.

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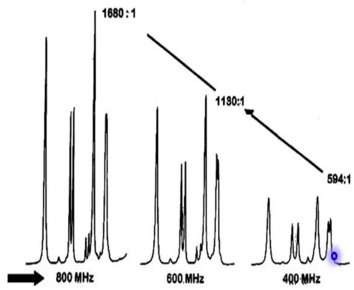
Magnetic Field and Sensitivity

$$\Delta E = \gamma \hbar B_0$$

Doubling B_0 increases S/N threefold

S/N ~ $B_0^{3/2}$



So this spectrum shows you how the sensitivity goes up by change of magnetic field. See at 400 megahertz signal to noise ratio is about 600, but here it is about 1700, nearly threefold enhanced in the sensitivity, just by doubling the magnetic field. See the type of spectrum what you get here, and what is the type of spectrum you got just by doubling the magnetic field, a significant achievement.

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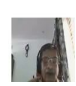
Signal Sensitivity and Temperature

$$\frac{N_\alpha}{N_\beta} = e^{\frac{E_\beta - E_\alpha}{k_B T}}$$

When the temperature is lowered, the power of exponential increases drastically

At low temperatures, the population ratio is enhanced, and hence sensitivity



So, now this is when you play with magnetic field. What happens with the temperature, remember temperature term is in the denominator. Let us play with the temperature, when temperature is lowered the power of the exponential increases, remember in this expression I have taken the opposite way, so the power of the exponential increases drastically, because this comes down. When it increases drastically means what happens? at very, very low temperature the population ratio will get enhanced. Ratio of these two becomes significantly larger, understand. So you take this for large number and multiply by this, so the population of alpha state is significantly larger, several orders of magnitude, multiplied by population of the beta state. that is what it says. So according to this equation if you go to very, very low temperature there is a possibility that I can take, not possibility; there is definitely, I can increase the population in the lower energy state because this number become significantly larger, and multiplied by this spins in the higher energy, so this will become quite large, the population ratio will definitely be increased. So that makes the sensitivity go up.

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Saturation

$$\frac{N_\alpha}{N_\beta} = e^{\frac{E_\beta - E_\alpha}{k_B T}}$$

When the temperature is increased, the power of exponential decreases drastically

At Infinite temperature, $N_\alpha = N_\beta$. This is called saturation. No signal will be seen

And you may ask me a question, what happens if I raise the temperature infinitely. Instead of lowering, I keep on increasing temperature to very high value. Now keep putting higher and higher value for temperature. Let us assume too large a value, close to infinity. Then because this is in the bottom, denominator, then what will happen, this becomes 0. The exponential power become 0, when this indices becomes 0. Then it is e to the power 0, it is 1, what does it mean? N alpha is equal to N beta. That is, if the temperature keeps on increasing, when it become very high temperature, your spin population of both the states are equal. This situation is called saturation, that means there is no population difference at all. Both energy states have equal population. If you go to very, very high temperature, that is the situation, where no signal will be seen. I hope you are all getting the point. How to enhance the sensitivity I have already told you, play with magnetic field. We calculated the population ratio, doubling the magnetic field will take to 4 times.

We calculated the number. Though the number appears small, but in the realistic spectrum we saw nearly three fold raise in the sensitivity by doubling the magnetic field. At the same time temperature is lowered, we know that, go to very, very low temperature, the population in the lower energy state will become significantly high.

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Sensitivity and γ

$$\Delta E = \gamma \hbar \vec{B}_0 / 2\pi = h\nu$$

Larger the magnetic field, larger the energy, higher the sensitivity.

And as a consequence the sensitivity also increases, of course sensitivity also depends upon gamma, another term. In addition to delta E and temperature, the sensitivity also depends upon gamma. It is given by this equation. Ok, larger the magnetic field, larger the energy and higher the sensitivity. Larger the magnetic field, larger the energy higher the sensitivity.

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Higher the γ, Higher the Sensitivity

^{1}H Nucleus has highest γ among all the stable nuclei

^{1}H has highest sensitivity among all the Stable NMR active nuclei

Now what happens with gamma, higher the gamma higher the sensitivity, how? I tell you now. So, we will come back and discuss more about sensitivity enhancement or dependence of the sensitivity on gamma, may be in the next class we will discuss. But I hope you are all with me in this class. I think I have not lost any of you, I am trying to try my level best to take all of you with me. In this class and discuss starting from the Larmor precession frequency, we calculated lot of things.

We found out the sensitivity issue, it depends upon delta E and also T, and we have a worked out population ratio used in the Boltzmann equation, and we know how to play with the magnetic field, how to play with the temperature to enhance the sensitivity. Another important factor that sensitivity also depends upon is gamma, what will happen, how it depends upon gamma is one thing that you have to understand, so we discuss that in the next class.