NMR Spectroscopy for Chemists and Biologists
Dr. Ashutosh Kumar | Professor Ramkrishna Hosur
Department of Biosciences and Bioengineering
Indian Institute of Technology, Bombay
Resonance Absorption
Lecture 02

Welcome to the second lecture on NMR spectroscopy. In the first lecture, we covered the basic concepts such as the nuclear spin, nuclear magnetic moment and how they interact with the magnetic field in a brief manner. We also talked about the angular momentum, spin angular momentum of the nuclei, which is an intrinsic property of the nuclei. And, the magnetic resonance phenomenon appears as a consequence of the presence of both, angular momentum and the magnetic moment, which are related in a linear fashion. We may be repeating, a little bit of what we said last time, for sake of continuity.

We said, the nuclear angular momentum is quantized, so which means it has specific values, which are determined by the nuclear spin quantum number *I*, which is also called just the nuclear spin. And, the *z* component of the angular momentum is also quantized. This is by choice. It could have been *x* or the *y*. But one is chosen, to take it is a *z* magnetization, which is quantized. If you have a nuclear spin *I*, then the *z* component of the magnetization, which is represented by another quantum number called *m*, that takes a certain number of values, specifically$2I+1$, that start from *-I to +I*, with integral increments.

I can have a value *0*, *½ integrals* or *integrals*. This depends on the nuclear spin. For most of chemistry and biology applications, we will be dealing with spins, which have $i=\frac{1}{2}$. These are like- proton, carbon 13, nitrogen 15, phosphorus 31, and so on. When there is no magnetic field applied, all the orientations of the nuclear spins, have the same energy. Their energy levels are degenerate. So, they can be oriented in any direction in space because of the superposition principle of the wave functions in quantum mechanics.

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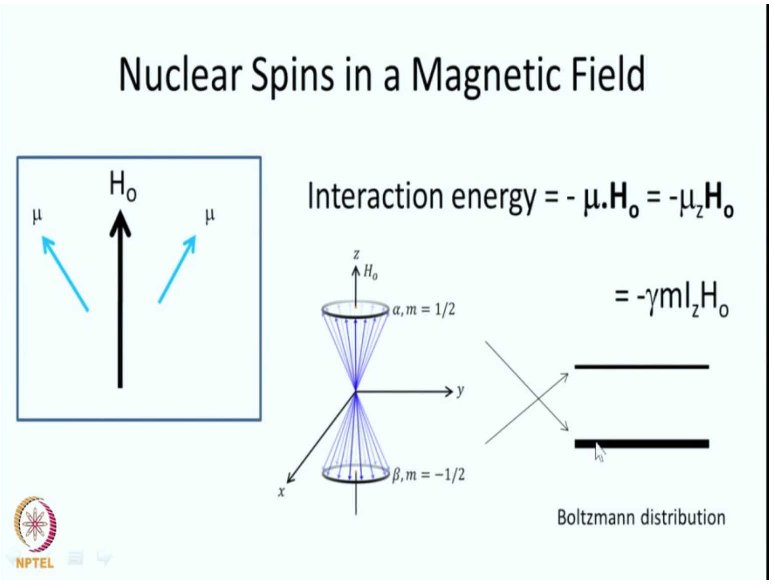

Nuclear Spins in a Magnetic Field

Interaction energy = - $\mu.H_0$ = -$\mu_z H_0$

= -$\gamma m I_z H_0$

Boltzmann distribution

However, when a magnetic field is applied, the degeneracy of these different orientations gets lifted because of the interaction of the nuclei with the magnetic field. This is explicitly given by this equation here. The interaction energy is given

$$Interaction\, energy = \mu . H_0 = -\mu_z H_0 = -\gamma m I_z H_0$$

Where, μ is the magnetic moment and H_0 is the field applied. For ¿$\frac{1}{2}$, there are two orientations as I indicated to you. These states are represented as α and β. α is the state which is, for *z* component is parallel to the magnetic field. And, the β is the state, where the spins are oriented anti-parallel to the magnetic field. This is the β state.

The α state has a lower energy, for positive ϒ. And, in most cases we are dealing with positive ϒ. Except nitrogen 15, which has a negative ϒ value. And, the β state has a high in energy, here. This spins will get distributed between these two energy levels. And, this is determined by the Boltzmann statistics.

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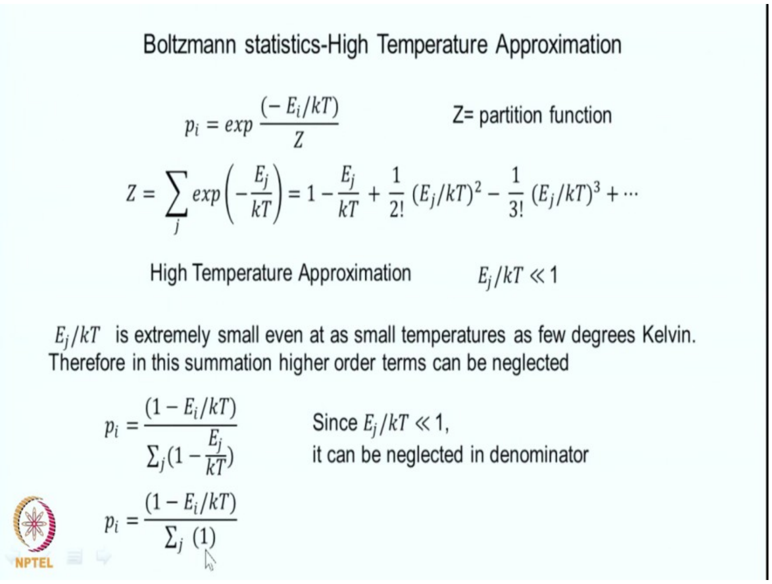

Boltzmann statistics-High Temperature Approximation

$p_i = exp\,\frac{(-E_i/kT)}{Z}$ Z= partition function

$$Z = \sum_j exp\left(-\frac{E_j}{kT}\right) = 1 - \frac{E_j}{kT} + \frac{1}{2!}(E_j/kT)^2 - \frac{1}{3!}(E_j/kT)^3 + \cdots$$

High Temperature Approximation $E_j/kT \ll 1$

E_j/kT is extremely small even at as small temperatures as few degrees Kelvin. Therefore in this summation higher order terms can be neglected

$p_i = \frac{(1-E_i/kT)}{\sum_j(1-\frac{E_j}{kT})}$ Since $E_j/kT \ll 1$, it can be neglected in denominator

$p_i = \frac{(1-E_i/kT)}{\sum_j (1)}$

Now, what is Boltzmann statistics? Typically, if you consider a state *I*, if there are many states, if you consider a state *I*, represented by this index *I*. The probability that the nuclear spin is in that state is given by this expression-

$$p_i = \exp\frac{(-E_i/kT)}{Z}$$

Where, *k* is the Boltzmann constant and *T* is the temperature. And, *Z* is a function called as the partition function. *Z* is defined in this manner,

$$Z = \sum_j \exp\left(\frac{-E_j}{kT}¿¿\right) = 1 - \frac{E_j}{kT} + \frac{1}{2!}(E_j/kT)^2 - \frac{1}{3!}(E_j/kT)^3 + \ldots ¿¿$$

Where, j is an index which runs over all the states, all the $2I+1$ states.

Now, the magnetic moments are generally so small for nuclei, that this energies are extremely small. The interaction energy is quite small compared to the value of *kT*. So, therefore in this expansion, which goes up to infinity, these higher order terms do not contribute much at all. So typically, one can neglect these higher order terms and that is called as the high temperature approximation. $\frac{E_j}{kT}$ is far, far less than 1. And therefore, you can neglect the higher order terms in this expansion for Z.

So, what happens then? So, the p_i, the probability that the spin is in the *I* state, which has energy *Ei*, is given

$$p_i = \frac{(1 - \frac{E_j}{kT})}{\sum_j (1 - \frac{E_j}{kT})}$$

Now, in the denominator, this $\frac{E_j}{kT}$ is still very small compared to 1. Therefore, I can further neglect this term in the denominator and then I will get

$$p_i = \frac{(1 - \frac{E_j}{kT})}{\sum_j (1)}$$

What does that mean? This is simply equal to the number of states in your system.
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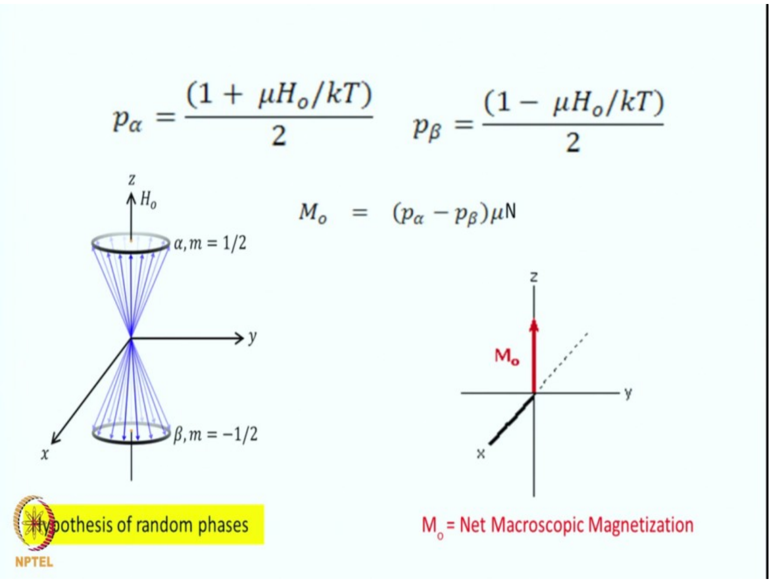

$$p_\alpha = \frac{(1 + \mu H_o/kT)}{2} \qquad p_\beta = \frac{(1 - \mu H_o/kT)}{2}$$

$$M_o = (p_\alpha - p_\beta)\mu N$$

Hypothesis of random phases

M_o = Net Macroscopic Magnetization

For, $I=\frac{1}{2}$, there are only two states as we mention, which we represent as $\alpha \wedge \beta$. So, if I put this energy terms explicitly, the probability for the α state is given by

$$p_\alpha=\frac{(1+\mu H_0/kT)}{2}$$

And, p_β is

$$p_\beta=\frac{(1-\mu H_0/kT)}{2}$$

This is the interaction energy, okay. For the α state and this is for the β state. This is typically true for protons, carbon 13. And, nitrogen 15, it will be wrong, because the γ is negative for nitrogen 15.

Now, so in the ensemble, the spins will be distributed between the two states- $\alpha \wedge \beta$. Now, look at this. We said that, the magnetic moments are oriented with respect to the magnetic field. Its magnitude is not equal to the *z* component of the magnetic moment. And therefore, you also said there is an angle between the magnetic moment orientation and the *z* field. And, therefore, the ensemble spins are distributed in a cone here. All the α spins are oriented in the surface of the cone here. And, likewise the beta spins are oriented at different points in the come here.

There is no specific preference for any specific position on this surface of the cone for any spin. Any orientation, or any position on the surface of the cone is equally allowed and they have the same energy. This is called the hypothesis of random phases, okay. Because if, I want to take a projection of this orientation on the, on the *x-y* plane, the component will have an angle here and that is the phase of the magnetic moment, the magnetization. Therefore, if any phase is allowed, then we say it is hypothesis of random phases.

Now, look at this. For all the spins which are present on the surface of the cone here, we take projections on the *x-y* plane. The *z* component will be the same for all of them. The *x-y* component will be distributed in the plane, *x-y* plane, like this all over, in this plane. And, they will be randomly oriented, which means any orientation in the *x-y* plane has an opposite orientation in the *x-y* plane. Therefore, those magnetization components will cancel out. This happens for both the alpha state as well as for the beta state. Whereas, the *z* component will all co-add. They will add here and they will also add here.

But, since the population in the β state is lower than the population in the αstate, there will be no complete cancellation of these two opposite orientations along the *z*-axis. Therefore, there will be a net magnetic moment, which is given $by\, M_0$ and that stays along the positive *z* axis, which is the direction of the magnetic field. So, I represent this as-

$$M_0 = (p_\alpha - p_\beta)\,\mu N$$

Where, μ which is the magnetic moment. And, is also, you have to multiply number *N*, which is the total number of spins in the ensemble. So, this represents the total magnetization. If you do not have this, then we have what is called as the mean magnetic moment of the nuclear spin system.

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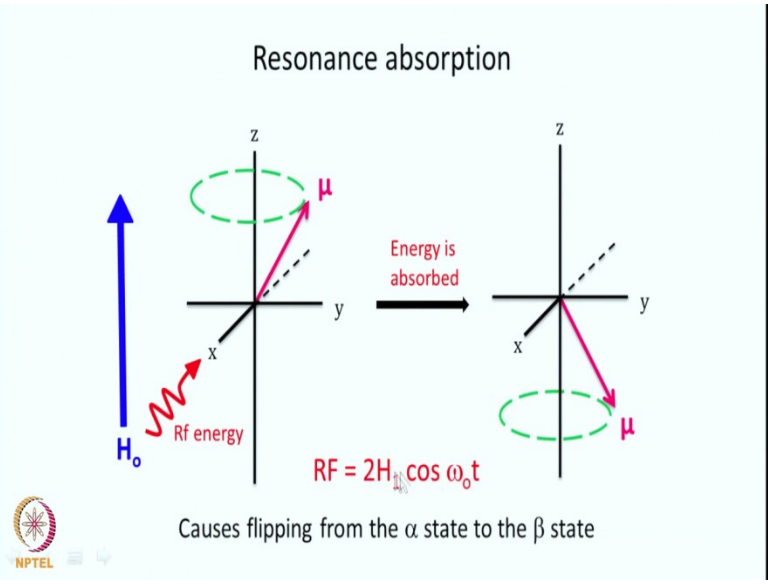

Resonance absorption

RF = $2H_1 \cos \omega_0 t$

Causes flipping from the α state to the β state

Okay. Now, how does the resonance absorption of energy happen? Let us look at it in the classical way. When we apply a radiofrequency now, we apply the radio frequency,

$RF = 2H_1 \cos\omega_0 t$

Where, H_1 is the amplitude of the radio frequency and ω_0 is a frequency of the radio frequency. And, this is represented by $2H_1 \cos\omega_0 t$. Therefore, this is an oscillating wave as indicated by this. And, this is applied in the transverse plane.

And, when there is an interaction between the RF and the magnetic moment, there will be absorption of energy. When the absorption of energy happens, what should happen? There should be redistribution of the spins, between the α and the β states. Which means, there will be some transitions, which will happen from the alpha state to the β state., if we are flipping off the magnetic moment from the α state to the β state. It can also happen with the β state to the α state.

But, the number of spins that will flip from the β, α state to the beta state, will be more when there is absorption of energy, okay. That interaction means that, because it is flipping from the α state to the β state. It can also happen in the reverse direction. But, the total, in the net, there will be more spins which will be flipping from the α state to the β state. And, that amounts to absorption of energy.

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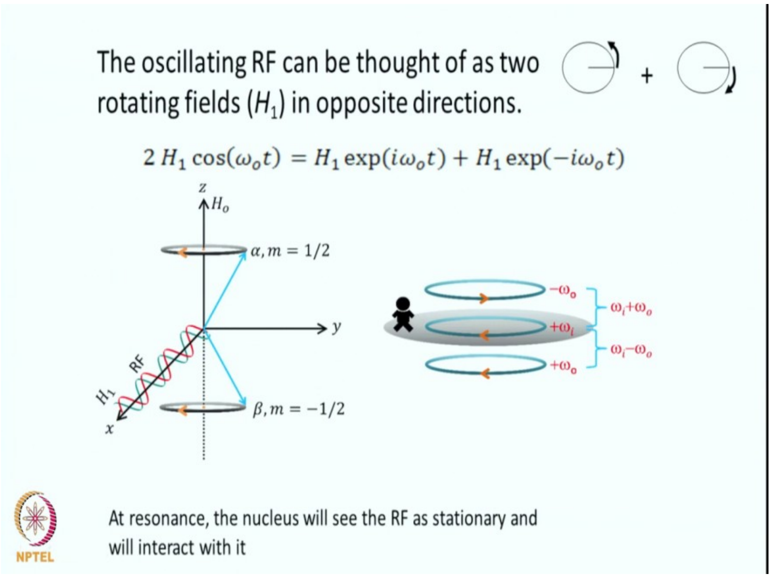

The oscillating RF can be thought of as two rotating fields (H_1) in opposite directions.

$$2\,H_1\cos(\omega_o t) = H_1\exp(i\omega_o t) + H_1\exp(-i\omega_o t)$$

At resonance, the nucleus will see the RF as stationary and will interact with it

Let us understand it in a classical sense, in a slightly different manner. We said we applied an RF, which is represented by the term $H_1\exp(i\omega_0 t)$ in the *x-y* plane. I assumed that it is along *X-axis*. We put it along the *X-axis* here.

$$RF = 2\,H_1\cos(\omega_0 t) = H_1\exp(i\omega_0 t) + H_1\exp(-i\omega_0 t)$$

What do these represent? These two represent rotating fields in the *x-y* direction, in the *x-y* plane. If, this represents a rotation in this direction, this represents a rotation in the opposite direction. Now, the spins themselves are rotating like this in both the states. In both the α and the β states, the spins are rotating like this. These are going like this and these are going like this. Let us look at that here.

This is the magnetic moment, which is spinning in this direction. And, these are the components of the two *RF*. The one *RF*, which is going in this direction. The second *RF* component, which is going in this direction. They are the two rotating fields. Now, suppose I were to sit on this *RF* component. And, look at the spin. What does it see? What do I see? I will see that the spins are precessing with the frequency $\omega_i - \omega_0$, because they are going in the same direction. This direction and this direction are the same. Therefore, the net frequency of the precision will be the difference between these two frequencies.

Whereas, if I am sitting on this one, the net frequency will be the addition because the two are going in the opposite direction. Notice, these are in megahertz. This is also in megahertz. Therefore, this frequency addition will also be in megahertz, which means the spins here will not be able to see this field properly at all. This will be in kilohertz. As you come closer and closer, it will become smaller and smaller. And, when you reach a condition, these two frequencies are the same, then it is called as resonance.

Under those condition, the *RF* will be stationary as seen by their nucleus. Or, if I see sitting on this *RF*, I will see the nucleus is stationary. Then, if it is stationary, which means there is no field along the *Z-axis*. The only field that is present will be the field along the *X-axis*. And, that is in the transverse plane. So, what will happen now? The nuclei will try interact with this *RF* field because that is a magnetic field which is there, which is a small magnetic field. The interaction leads to changes in the energy of the system.

What is the meaning of changing in the energy of the system? It means the redistribution of the populations once more. There will be some transitions occurring from the αstate to the β state, β state to the α state. But, if the energy of the system has to change, there has to be a net absorption of energy, which means there will be more spins going from the alpha state to the beta state, as indicated in the previous slide. So, this is a classical description to show how the nuclear spins interact with the *RF* field.

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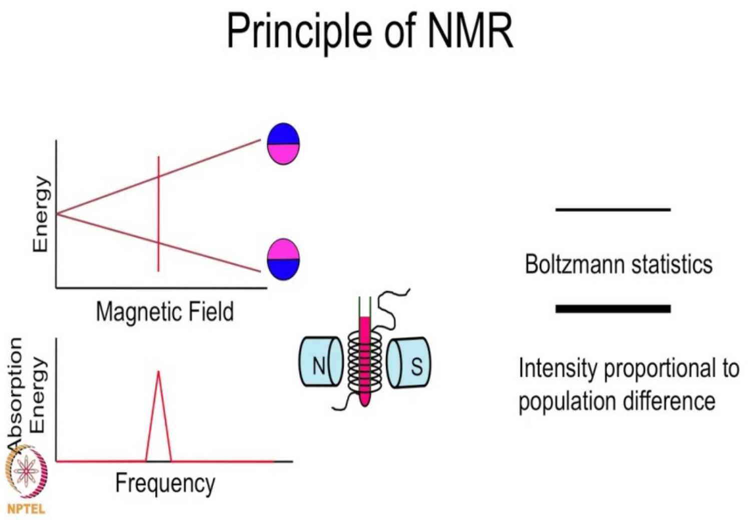

Principle of NMR

Boltzmann statistics

Intensity proportional to population difference

Okay, therefore summarizing this, the principle of NMR can be shown in this particular slide. This maybe a repetition from previous lecture, but nevertheless it actually consolidates what we were trying to say. If we have, in the absence of the magnetic field, the nuclear spins are in a particular state. Here, they have the same energy. The two orientations of the spins, which has $I=\frac{1}{2}$ are the same. And, when you apply the magnetic field, the two energy levels become non-degenerate. And, their separation between the energy levels will depend upon the magnetic field because the interaction depends on the magnetic field.

And, they will be distributed between the two states. And now, the energy absorbed will depend upon how much is the excess population here compared to this. And, this distribution is determined by Boltzmann statistics, as I already mentioned. If, I supply energy to this system, now, we look at in quantum mechanical sense. Earlier we looked at it from the point of view of the classical description. Now, this is the quantum description of the energy, energy level diagram.

So, if I supply energy which corresponds to this energy difference, there will be absorption of energy at this particular frequency. And, this is called as the signal. This is the signal in NMR. The intensity of this signal will be proportional to the energy absorbed. And, therefore it will be proportional to population difference between these two states.

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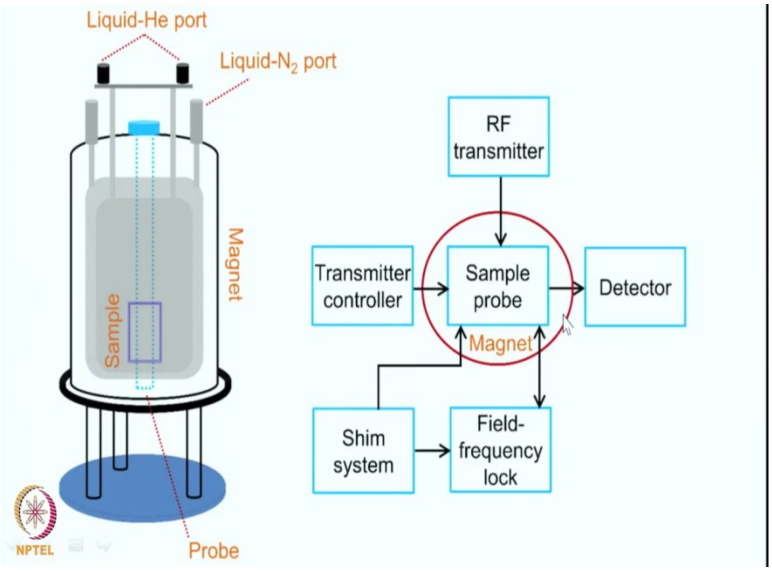

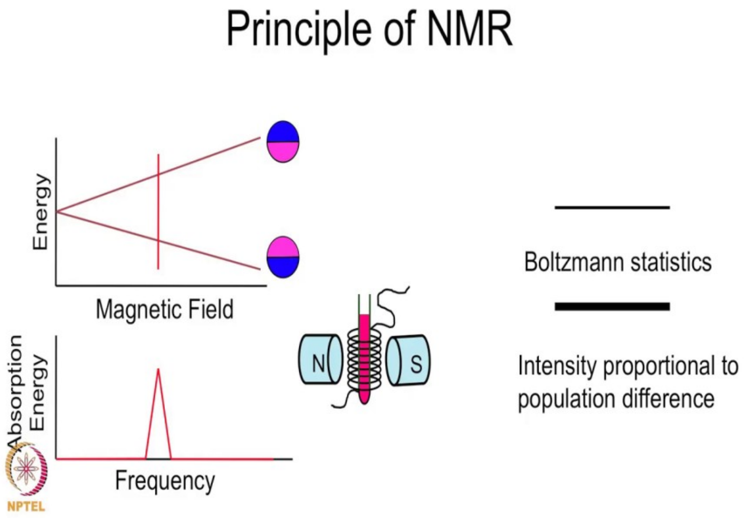
Principle of NMR

Boltzmann statistics

Intensity proportional to population difference

Now, this is a schematic of the NMR spectrometer, modern NMR spectrometers. So, we have a magnet here. These days superconducting magnet. In the previous years what I showed you in the schematic, this was an electromagnet or a permanent magnet. This was the case, in the early days when the superconductor magnets were not available; all the NMR experiments were done with these electromagnets. And, today the magnets are superconducting magnets. This is the revolution in the technology. We can go to very high fields and the homogeneities of the magnetic fields are very good. Therefore, you get good signal with good intensity and very sharp lines.

And then, you have the sample is sitting inside this magnet here at center. And this is called as the sample; and there is a sample probe. And, you have a transmitter controller, which supplies the radio frequency energy. And, then the signal that comes out from there is detected by this detector. This is *RF* transmitter. And, you have a shim system here. The shim system means you have to adjust the homogeneity of the magnetic field. You have to have homogenous magnetic field. The H_0field which we said, has to be the same or the entire sample volume.

So, this has to be adjusted. And therefore, there are various kinds of coils here, which produce currents, producing magnetic fields to correct the variations in the magnetic field distributions in the sample volume. And, this is the detector of that is called a field frequency lock. This detects if there is any instability in the field because of various kinds of disturbances outside the magnet. If there is a small disturbance because of some movement of some object, then the field will get disturbed. And, then, there will be a counter current supplied in this shim system. And, which will bring the field back. And, that is called as the field frequency lock.

So, these are the basic components of the modern NMR spectrometers. Of course there are many, many more complexities here that we do not need to discuss here, okay.

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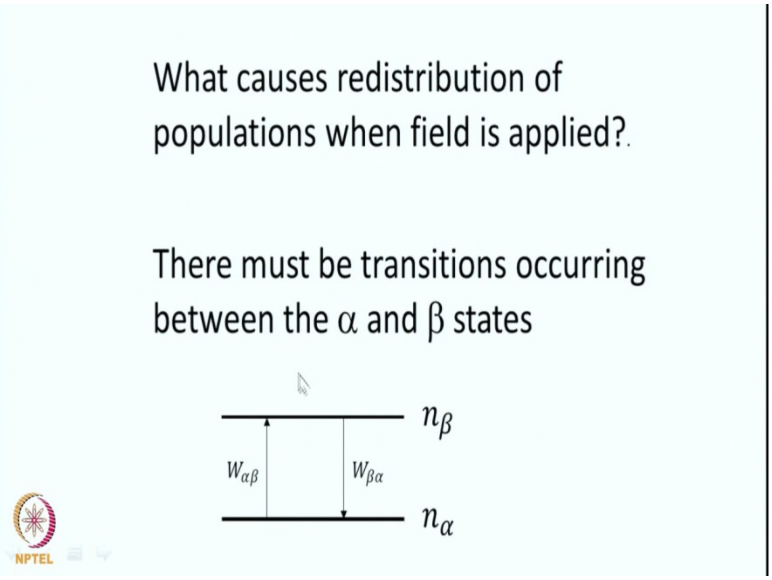

What causes redistribution of populations when field is applied?.

There must be transitions occurring between the α and β states

So, now we return to this question. What causes redistribution of the populations when field is applied? We said, when *RF* is applied, the *RF* interacts with the spin's magnetic moment and causes transitions. Now when we applied main magnetic field, RF is not applied. How does the transitions occur? How does the populations redistribute themselves between two fields, α and the β?

Obviously, there must be transitions happening, which causes the redistribution of the populations. Let us represent this in the following manner. Let us call this state as α state. And, this is the β state. The number of spins here is η_{α}. The number of spins here is η_{β}. And, there is a transition occurring here with, from α state to the β state. And, $W_{\alpha\beta}$ is a transition probability for the transition from α to the β. And likewise, there can be transitions, which are coming from the β state to the α state.

Now, you notice, when we go from the equilibrium to the situation, when there is redistribution of the populations here. In the absence of the field, the two energy levels were degenerate and that was here, okay. And, this was equal. And, when the field is applied, the β states went up here and the α states went down here. If, there is more population here, there are more spins which have gone down than the number of spins which have gone up.

Therefore, the system is actually lost energy, right. Because there are more spins which have lost energy. A fewer, fewer spins have gained energy, which means in the net, there is a loss of energy. Where does this energy go? Who takes this energy? Unless, there is somebody to take this energy, the process will not happen.

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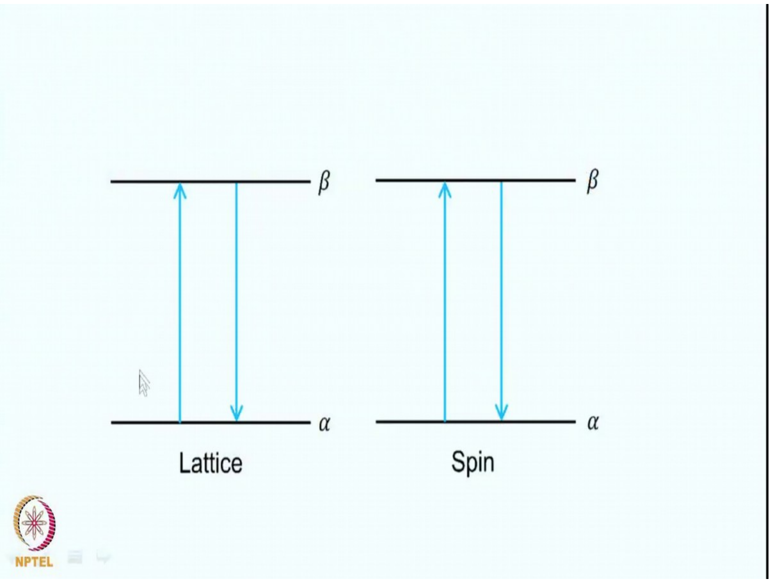

And, that brings us into the concept of the lattice. There is a lattice in your sample. Everything other than your particular spin system is called the lattice. And, lattice has all kinds of energy levels in it. So, every time there is transition, there is a release of energy from here, the lattice takes up that energy and causes the transition, upward transition here.

If there is a downward transition here, of the spin system, there will be an upward transition here, of the spin system. If there is an upward transition of the spin system here, there is a downward transition of the spin system. Therefore, the spins and the lattice are coupled. There is energy exchange between the spin and the lattice. And, which allows the redistribution of the populations in their spin system.

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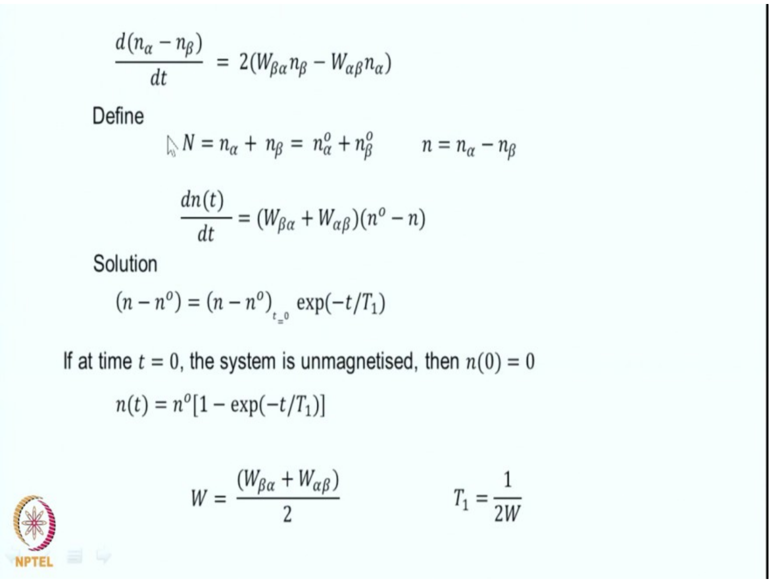

$$\frac{d(n_\alpha - n_\beta)}{dt} = 2(W_{\beta\alpha}n_\beta - W_{\alpha\beta}n_\alpha)$$

Define

$$N = n_\alpha + n_\beta = n_\alpha^o + n_\beta^o \qquad n = n_\alpha - n_\beta$$

$$\frac{dn(t)}{dt} = (W_{\beta\alpha} + W_{\alpha\beta})(n^o - n)$$

Solution

$$(n - n^o) = (n - n^o)_{t=0} \exp(-t/T_1)$$

If at time $t = 0$, the system is unmagnetised, then $n(0) = 0$

$$n(t) = n^o[1 - \exp(-t/T_1)]$$

$$W = \frac{(W_{\beta\alpha} + W_{\alpha\beta})}{2} \qquad T_1 = \frac{1}{2W}$$

Okay, now how does this happen? Let us look at this in a little bit more quantitative manner. *dn* and this written, if there is perturbation of the spin system, which the spin, number of spins in the two states are not at equilibrium and they would like to tend to equilibrium through this transitions. So, therefore, one can write here, the rate equations for these transitions.

$$\frac{d(\eta_\alpha - \eta_\beta)}{dt} = 2(W_{\beta\alpha}\eta_\beta - W_{\alpha\beta}\eta_\alpha)$$

This represents the number of transitions coming down. This represents the number of transitions going up. And therefore this variation goes by this rate equation.

Now, we define here some terms.

$$N = \eta_\alpha + \eta_\beta = \eta_\alpha^0 + \eta_\beta^0$$

And, these are the populations of the α and the β states, any point in time. Therefore, this is obviously a time dependent. That is why you have this time dependent equation here. And, these are the equilibrium populations of the two states.

$$\eta=\eta_\alpha-\eta_\beta$$

The above equation represents the equilibrium populations of the alpha and the beta states. Then, this equation can be recast in this manner

$$\frac{dn(t)}{dt}=(W_{\beta\alpha}+W_{\beta\alpha})(\eta^0-\eta)$$

This is simple algebra from here. If you recast this putting in these equations, you will get this equation. The solution of this equation can be obtained readily. And, that gives you,

$$(\eta-\eta^0)=(\eta-\eta^0)_{t=0}\exp(\frac{-t}{T_1}\text{¿})\text{¿}$$

What is T1? We will soon see.

Now, if at time, $t=0$ the system is unmagnetized, then $n(t)=0$. And, therefore, this equation reduces to n

$$n(t)=n^0\left[1-\exp\left(\frac{-t}{T_1}\right)\right]$$

$$W=\frac{(W_{\beta\alpha}+W_{\beta\alpha})}{2}$$

$$T_1=\frac{1}{2W}$$

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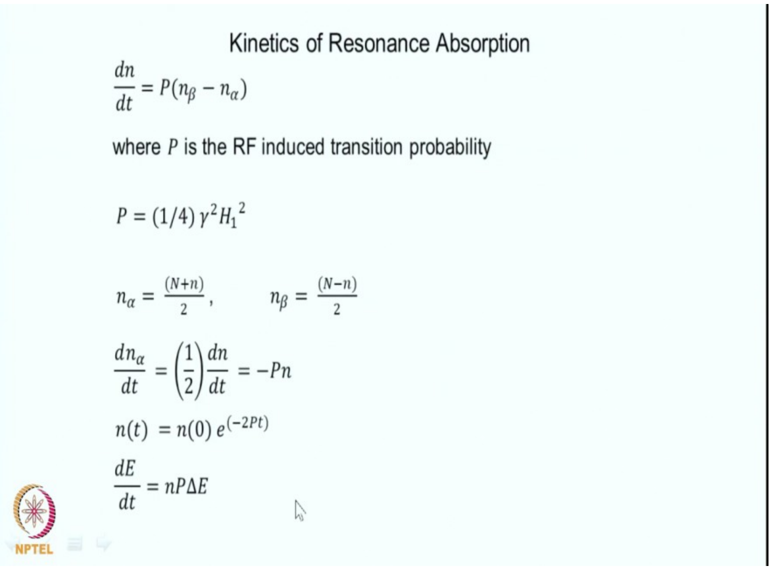

Kinetics of Resonance Absorption

$$\frac{dn}{dt} = P(n_\beta - n_\alpha)$$

where P is the RF induced transition probability

$$P = (1/4)\,\gamma^2 H_1{}^2$$

$$n_\alpha = \frac{(N+n)}{2}, \qquad n_\beta = \frac{(N-n)}{2}$$

$$\frac{dn_\alpha}{dt} = \left(\frac{1}{2}\right)\frac{dn}{dt} = -Pn$$

$$n(t) = n(0)\,e^{(-2Pt)}$$

$$\frac{dE}{dt} = nP\Delta E$$

Okay. So, now we look at the kinetics of resonance also. So, we looked at how the system attains equilibrium because of transitions, which are supported by coupling to the lattice. Now, let us look at the transition induced by the *RF*. The *RF* induces transitions, from both α to the β and β to the α and these two rates will be the same. This comes from the principles of quantum mechanics. When you calculate the transition probability, then that transition probability is represented by P.

$$\frac{dn}{dt} = P(\eta_\beta - \eta_\alpha)$$

Where, *RF* and *P* is the *RF* induced transition probability.

One can also calculate, what is *P* is, *P* is obviously proportional to the amplitude of the *RF* field. So, it is proportional to the power. Therefore, it is given by

$$P = (1/4)\gamma^2 H_1^2$$

This actually follows from the rule of the transition probability; a formula of the transition probability, *RF* induced the transition probability. Now, we all, we have, we already know,

$$\eta_\alpha = \frac{(N+n)}{2} \wedge \eta_\beta = \frac{(N+n)}{2}$$

So, you recast this, you get

$$\frac{dn_{\alpha}}{dt}=\left(\frac{1}{2}\right)\frac{dn}{dt}=-P_{n}$$

And,

$$n(t)=n(0)e^{(-2Pt)}$$

So, this shows you how the population distribution changes with the *RF* that you apply. At long time, you continuously apply the *RF*, this obviously goes down to *0*. And therefore, your population difference also goes down *0*, in which case you will not have any energy absorbed.

Now, we can write a similar equation for the energy absorbed. Energy; if E is the energy of the system. Then, $\frac{dE}{dt}$ represents that the change in the energy of the system that will amount to energy absorbed. And, that is given by the change in difference in the energy multiplied by the population difference multiplied with the transition probability. So,

$$\frac{dE}{dt}=nP\,\Delta E$$

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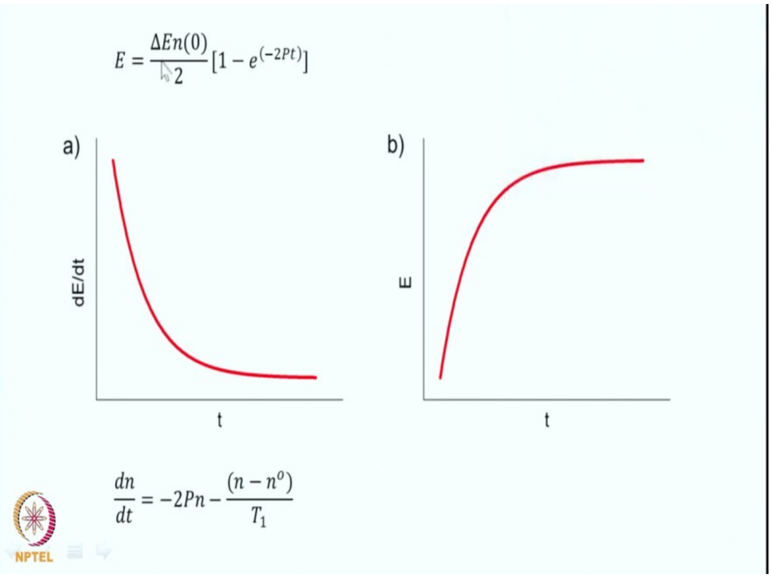

$$E = \frac{\Delta E n(0)}{2}[1 - e^{(-2Pt)}]$$

$$\frac{dn}{dt} = -2Pn - \frac{(n - n^0)}{T_1}$$

And, if you obtain the solution of this, then we get the energy of the system is given by

$$E = \frac{\Delta E n(0)}{2}[1 - e^{(-2Pt)}]$$

ΔE is a fixed number because that is the energy difference between the two states. n_0 is a fixed number, because this is the time t is equal to 0. Whatever was the population difference between the two states. And, this is the term which is dependent on time. So, this describes how the energy will be absorbed. How the energy of the system will change as the *RF* is applied.

So, if you plot this equation, then you will find that $\frac{dE}{dt}$ goes in this manner, exponential decay. And, the E in the system goes in this manner and reaches the saturation at some stage, okay. After this, there is no change in the energy of the system, which means there will be no energy absorbed.

Now, at this point the system, no energy is absorbed means there will be no signal, okay. But, in an NMR experiment, we always find that there is a signal is present. When you are apply the *RF*, energy is continuously absorbed. So, something else also must be happening. So, so far we included, the RF induce the transition in these equations. So, there is something which is compensating, which brings backs the population difference and allows the system to continuously absorb the energy. And, that is the relaxation.

We had ignored the relaxation till now. We had, we simply had

$$\frac{dn}{dt} = -2Pn - \frac{(n-n^0)}{T_1}$$

This defines the transitions occurring because of relaxation. The system tries to recover from the perturbation tries to approach the equilibrium population distribution. And, this happens with the rate constant, $\frac{1}{T_1}$ as we described earlier.

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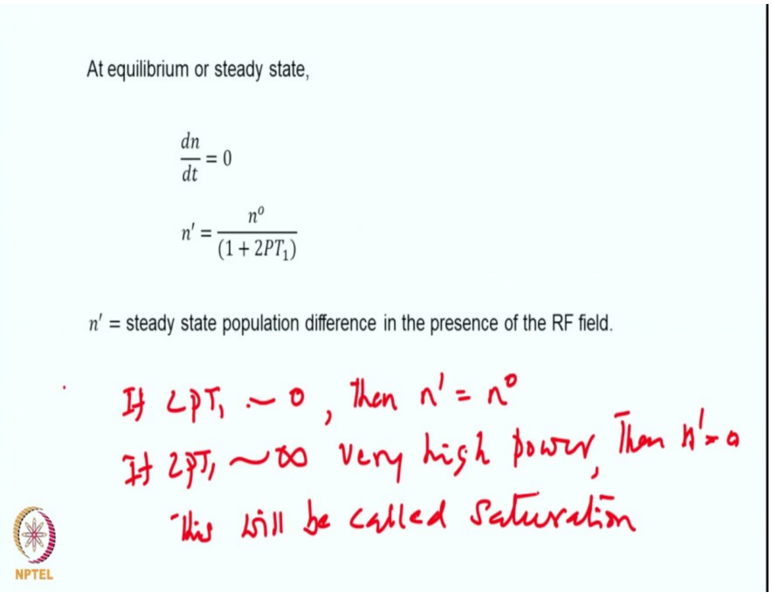

At equilibrium or steady state,

$$\frac{dn}{dt} = 0$$

$$n' = \frac{n^0}{(1+2PT_1)}$$

n' = steady state population difference in the presence of the RF field.

If $2PT_1 \sim 0$, then $n' = n^0$

If $2PT_1 \sim \infty$ very high power, Then $n' \approx 0$

This will be called Saturation

Now, at equilibrium or steady state,

$$\frac{dn}{dt} = 0$$

Which means the population difference remains the same. And, there is continuous energy absorbed, okay. And, if I call that steady state population as *n'*. From the previous equation, I will get

$$n' = \frac{n^0}{(1+2PT_1)}$$

P is the power and T_1 is the relaxation time, which is called as the spin lattice relaxation time.

Now, here I want to say, if $2PT_1 \approx 0$, which means I apply a very low power. P is the power as I mentioned. It is related to the power. Then, $n' = n^0$. In other words, if the power is very low, I reach the equilibrium population very soon. So, it will always remain as though it is at equilibrium. Therefore, there will be continuous absorption of energy.

If $2PT_1 \approx \infty$, which means I apply very high power, which means very high power. Then, $n' = 0$, okay. So, in that case, this will be, this will be called saturation. The signal will get saturated. After that, we will not be able to observe the signal. Therefore, what one needs to do? We have to apply very low power to be able to observe the signal continuously over a long period of time.

In fact, I must tell you a story. Here that, Gorter, who actually did this experiment earlier. He actually lost a Nobel Prize because he missed the signal. Because, he used a system which had very long T_1. The T_1 is very long, which means even at a small power *P*, $2PT_1$ is very large. So therefore immediately, he was reaching a condition of saturation therefore, there was no signal coming. This is unfortunate. He had chosen a sample. Therefore, it turns out that the sample choice is also very important. So, you have to be lucky to be able to observe a signal. And, you have to choose a right kind of samples. So, we will stop here and continue with further topics in the next class. Thank you.