NMR spectroscopy for Structural Biology NS

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Lecture: 03
NMR Basic Concepts - III

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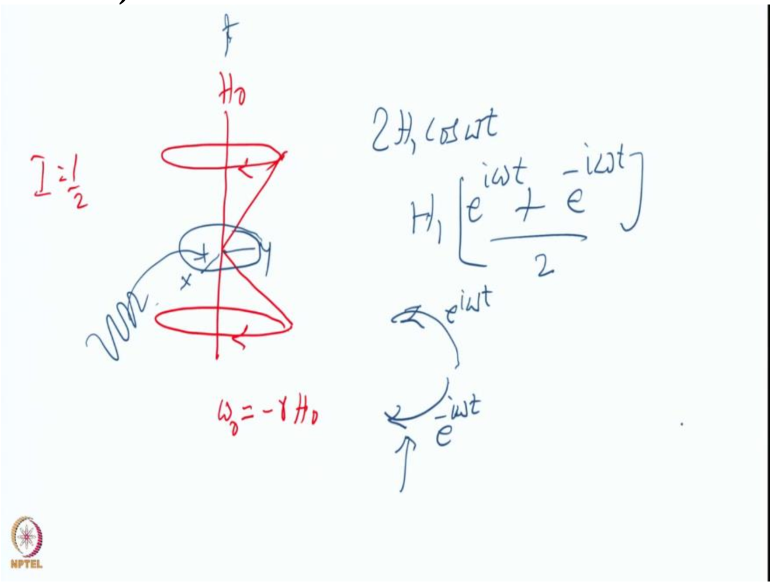

So, we continue from the description of the application of the RF and we said there are two components rotating magnetic fields one going in the direction which is the same as the precessional motion and the other one is going in the direction which is opposed to the precessional motion. And we consider that particular component which is in the same direction.

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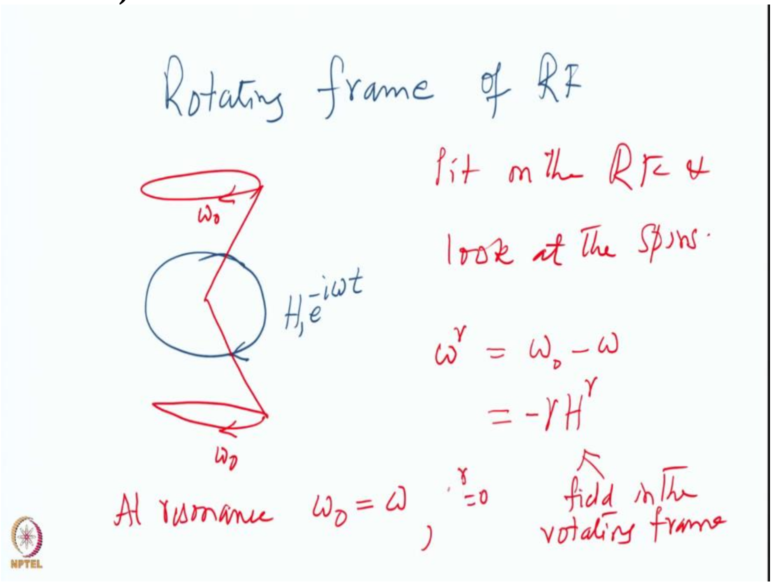

Now we what we do so to understand this interaction we go into the rotating frame which rotating frame we go rotating frame of RF in other words. So, I had this RF which was going in this direction we considered this one this is $H_1 \left[\frac{e^{i\omega t} - e^{-i\omega t}}{2}\right]$ the spins were like this, they are going like this and this is also going in the same direction. So, therefore now if suppose I sit on the RF rotating frame of RF means I sit on the RF and look at the spins.

So, when ω is different from ω_0 this frequency is ω_0 when ω is not the same as ω_0 what will happen what do I see if I sit on ω and look at ω_0 what will the precession frequency. So, omega ω_0 frequency if I call it as $\omega^r = \omega_0 - \omega$ right. So, it is like going in the two different trains one of them is going with the one frequency otherwise going with another speed.

So, it is the difference between the two speeds is what we see that they are going with this particular speed. So, similarly if I sit on the RF and look at the spins it will appear to me that the spins are moving with the frequency which is $\omega_0 - \omega$. And if I want to consider this as a magnetic field just as we said $\omega_0 = -\gamma H_0$. So, $\omega^r = -\gamma H^r$ will also be equal to minus gamma another field which I call it as H r and this will be the field in the rotating frame.

Now as I said we can keep on changing this ω, ω is in our control. So, we can keep changing ω slowly change ω and see you match the resonance condition at the resonance condition what happens at resonance $\omega_0 = \omega$. So, what will happen the spin is stationary right. If the spin is stationary $\omega_0 = \omega$ there is no precessional motion and therefore the field is 0, H^r is 0. So, this will imply H^r is equal to 0. So, then what is the field that it sees.

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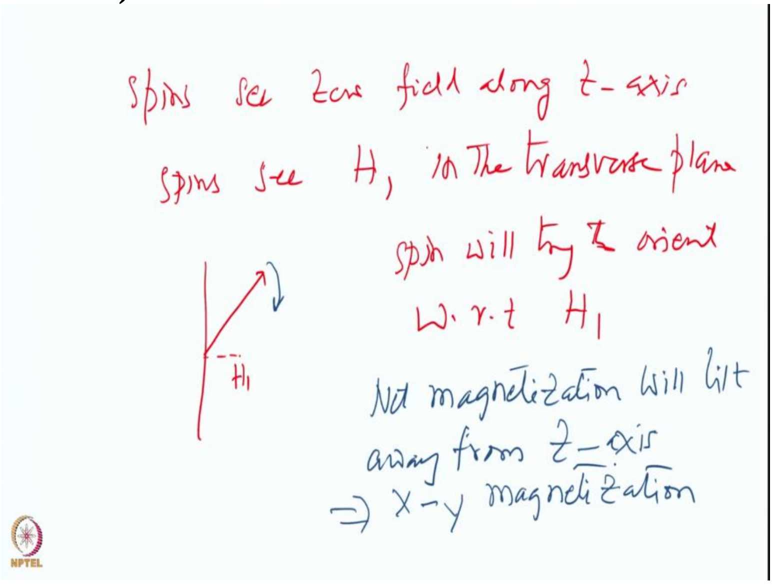

Spins see zero field along z-axis

spins see H_1 in the transverse plane

spin will try to orient w.r.t H_1

Net magnetization will tilt away from z-axis

$\Rightarrow$ x-y magnetization

The spin sees see zero field along the Z axis and they also see H_1 in the transverse plane. So, if I draw it here. So, I have this spin here and I see an H_1 field here. So, this pin will interact with this H_1. So, it has to orient itself with respect to the H_1. So, spins will try to orient with respect to H_1 what does that mean. So, they will have to change the direction I will change the colour here.

They will have to move towards the H_1 because the H_1 is in the transverse plane it moves in the transverse plane. So, therefore the magnetization removes in the transverse plane the net magnetization will tilt away from Z axis. This is the consequence of the interaction between the RF and the fields. So, this implies we have created xy magnetization which was 0 in the beginning at equilibrium once we apply the RF field we have created xy magnetization.

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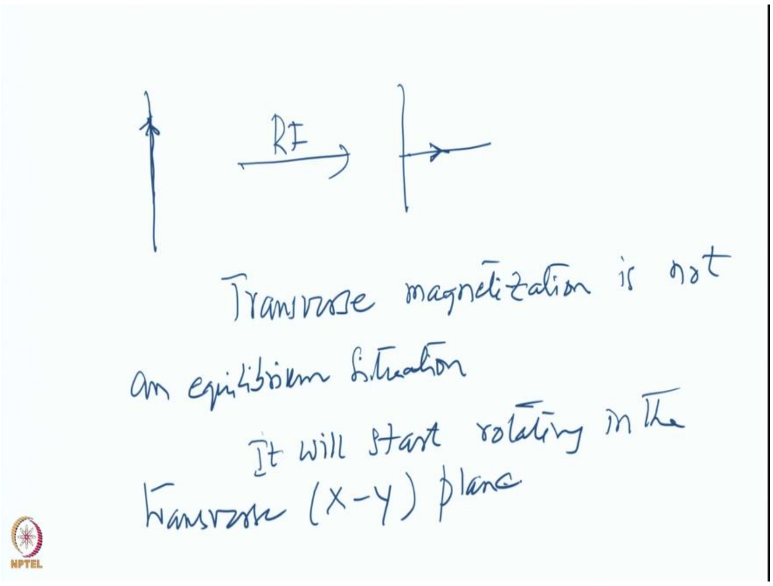

Transverse magnetization is not an equilibrium situation

It will start rotating in the transverse (X-Y) plane

So, let me draw that here. So, initial magnetization was here and when I apply the RF I created some magnetization in the transverse plane. If I continuously apply this RF entire thing will have to orient itself with respect to the RF but this will take a long time and that will take a long time but we do not apply it for that long. So, what happens is therefore the magnetization will start precessing in the transverse plane.

So, this transverse magnetization it is not an equilibrium situation because equilibrium situation was magnetization was only along the Z axis. So, this is how absorption of energy happens and we create transverse magnetization. And if we look at this transverse magnetization the transverse magnetization will start precessing in the transverse plane this will start rotating in the transverse plane transverse meaning xy plane.

And therefore and if you have a detector in this transverse plane this rotating magnetization will induce a signal.

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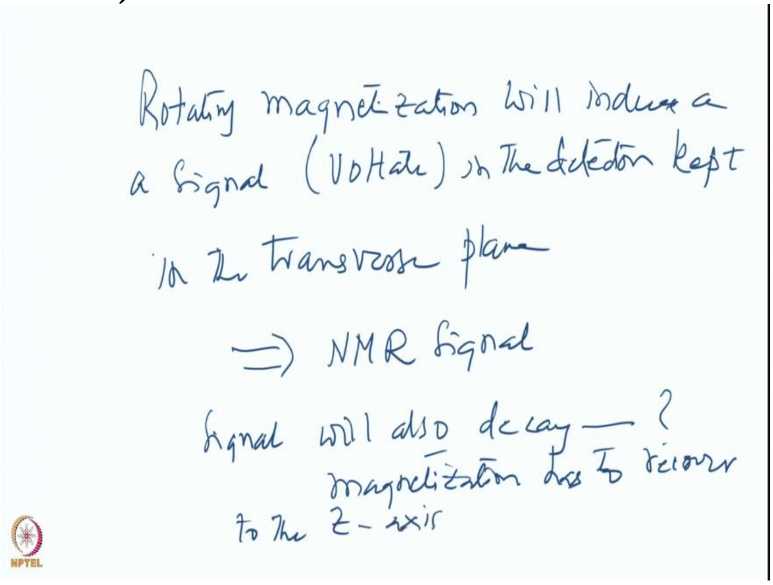

Rotating magnetization will induce a a signal (voltage) in the detector kept in the transverse plane

$\Rightarrow$ NMR signal

signal will also decay — ?
magnetization has to recover to the z-axis

The rotating magnetization will induce a signal meaning what it is an electrical signal it is a voltage. So, this is the voltage in the detector kept in the transverse plane this is my NMR signal. So, this is the NMR signal. So, you see we saw how an NMR signal is observed quantum mechanical picture told you that if you supply energy it will lead to absorption of energy. What does absorption of energy mean?

Absorption of energy means the magnetization which was along the Z axis in the absence of the RF will tilt away from the Z axis and create some transverse magnetization this transverse magnetization will have to recover back to equilibrium. And while doing so it is precessing along the transverse in the transverse plane it rotates in the transverse plane and this induces a voltage into the detector because always the rotating magnetization induces an electrical voltage this is a common law of physics here. So, therefore we get the NMR signal.

So, that is how we get signal but now this signal will also decay because the magnetization has to recover to the Z axis and therefore there is a decay here.

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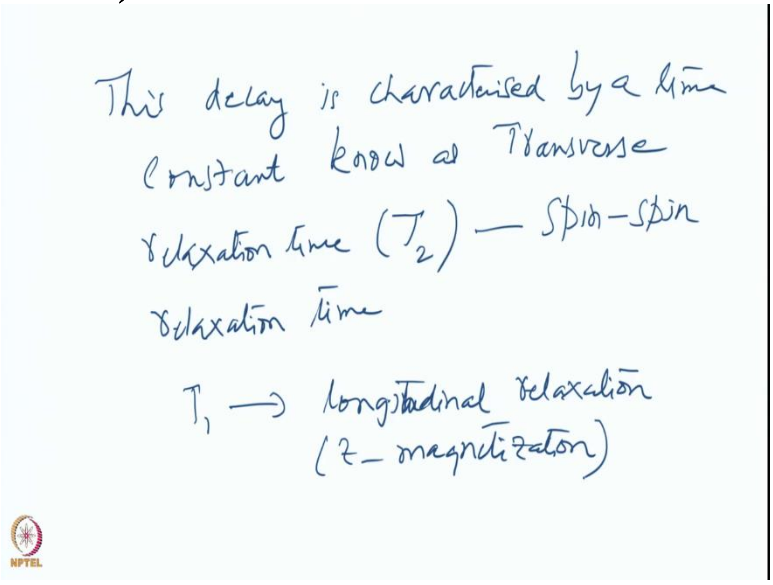

This decay is characterised by a time constant know as Transverse relaxation time (T_2) — spin-spin relaxation time

$T_1 \rightarrow$ longitudinal relaxation (z-magnetization)

This decay is characterized by a time constant known as transverse relaxation time and we often represent this as T_2. So, this is also called as spin-spin relaxation time. So, therefore we have introduced these two concepts here that one is the spin lattice relaxation time and other one is the spin-spin relaxation time. Spin lattice relaxation time is also called as the longitudinal relaxation time.

So, the T_1 is also called as longitudinal relaxation time. Because it has to do with the populations the populations dictate the z magnetization because this is related to z magnetization and therefore it is called as the longitudinal relaxation time and transverse relaxation time has to do with the spins in the transverse plane. Now there is one more concept which we need to understand here that is transverse magnetization represents what?

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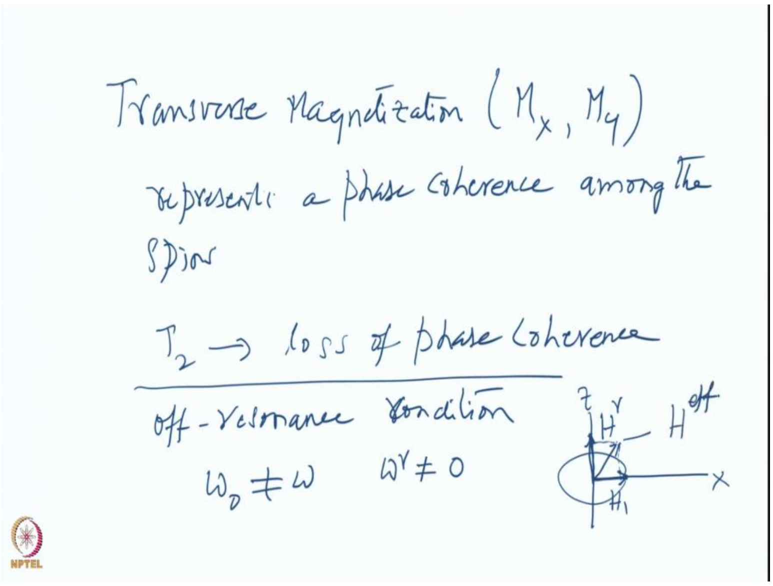

Transverse Magnetization (M_x, M_y)
represents a phase coherence among the spins

$T_2 \rightarrow$ loss of phase coherence

off-resonance condition
$\omega_0 \neq \omega$ $\quad \omega^r \neq 0$

It represents a kind of a phase coherence between the spins. Transverse magnetization which may represent as M_x or M_y represents a phase coherence among the spins you remember we talked about the hypothesis of random phases the phase has to do with the angle a spin magnetization component makes along with the X axis or the Y axis. So, that was a phase. So, in the equilibrium situation this because of the randomness of the orientations of the spins there was no correlation between the phases of the individual spins and therefore there was no transverse magnetization.

The phases were random and therefore there was no transverse magnetization in the case of absorption of energy it creates a transverse magnetization it creates a phase coherence among the spins. Therefore T_2 also represents loss of phase coherence. So, these are important concepts which will be very necessary as we go along at the later stages. Look at one more concept that is which we are suppose we are not at resonance but off resonance situation and that means that $\omega_0 \neq \omega$.

So, therefore $\omega^r \neq \omega$. So, how do we represent this? So, we have let us write here this is the H^r corresponding to the ω^r and this is my Z axis and let us say this is my X axis and I have here the H_1 which is going with the frequency ω of course it is not stationary now it is going with the frequency ω. So, this is precessing here and H^r is the field along the Z axis in the rotating frame because we are sitting on the RF.

And looking at it and now what is the field effective field the effective field is a vector addition suppose I have this vector here like this H^r is represented like this then I will have here an addition vector addition which is this and this is called as $H^{effective}$ the effective magnetic field.
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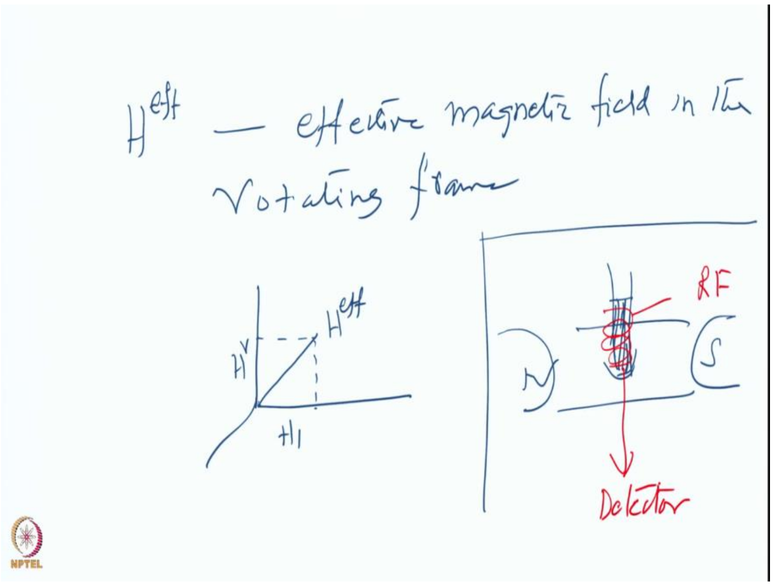

So, H^{eff} is the effective magnetic field in the rotating frame. So, I can represent it in this manner. So, I have here H^{eff} and this is my this is H^r this is H_1 of course this effective magnetic field also keeps rotating because the H_1 is rotating therefore effective magnetic field also will keeps rotating along the in the transverse plane it keeps changing depending upon the magnitude of H_1 it will have a different orientation. So, long as the ω is rotating frame frequency is nonzero it will keep rotating but its orientation can change depending upon the magnitude of H_1 and H^r.

So, this is the off resonance condition and depending upon that we have different creations of the transverse plane the magnetization in the transverse plane. So, now we have this brings us to basics of the NMR spectrometer. So, the NMR spectrometer consists of a magnetic field so you have the magnetic field. So, let us say. So, we have this north south magnetic field and you have the sample here and then you have a coil around which is the RF it means the different colour.

So, I have a coil here which is the RF, RF coil which gives out the radio frequency energy the magnetic field is there. So, then I will have a connected with that is a detector which detects the magnetization. So, this is the basic component of the NMR spectrometer there are so, many other things which are which are important here of course the technicalities we will not go into that.

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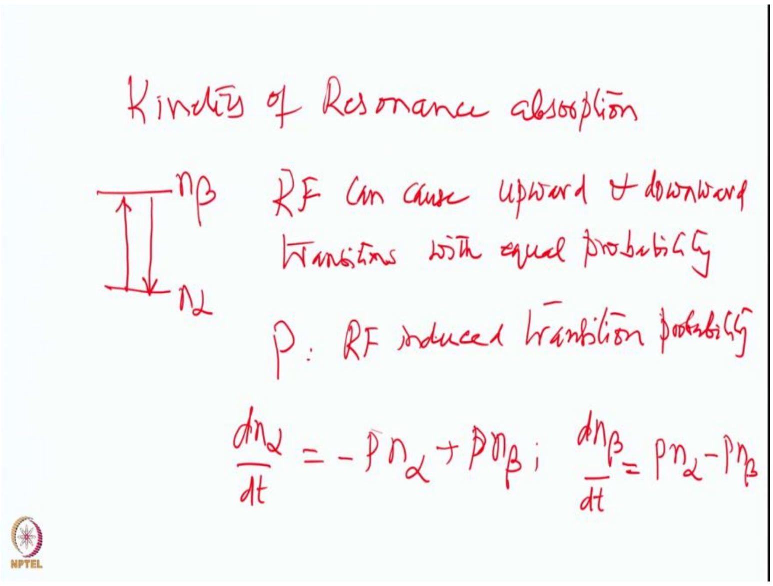

Kinetics of Resonance absorption

n_β

n_α

RF can cause upward & downward transitions with equal probability

p: RF induced transition probability

$$\frac{dn_\alpha}{dt} = -pn_\alpha + pn_\beta ; \quad \frac{dn_\beta}{dt} = pn_\alpha - pn_\beta$$

So, now let us look at kinetics of resonance absorption. So, what does that mean absorption of energy we have put in the RF right RF is causing absorption of energy. So, we consider the two states here the RF induces transition from here to here but notice it can also induce a transition from here to here as well the radio frequency can cause upward and downward transitions with equal probability.

So, we represent that probability as p this is a RF induced transition probability. So, now we can write equations for the transfer of energy in both ways. When it absorbs energy let us say I have this population here n_α and I have the population here n_β. So, if I write here $\frac{dn_\alpha}{dt}$ what is what is it dependent on? It is dependent on the transition probability and the populations.

So, this is the first order kinetics then the chemical kinetics people you already have studied this. So,

$$\frac{dn_\alpha}{dt} = -pn_\alpha + pn_{\alpha\beta}$$

when it causes the transition from β state to α state it increases the population there. Similarly I can write for

$$\frac{dn_\beta}{dt} = pn_\alpha - pn_{\alpha\beta}$$

So, therefore this can be combined into one equation.

So, we will not derive this all of these things there but I will just give you the basics there and we will go into the solution.

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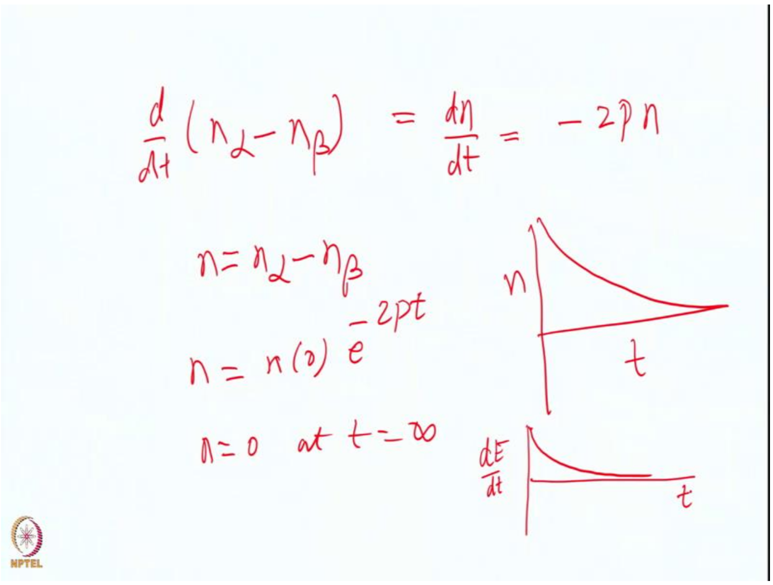

$$\frac{d}{dt}(n_\alpha - n_\beta) = \frac{dn}{dt} = -2pn$$

$$n = n_\alpha - n_\beta$$

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

$$n = 0 \quad at \quad t = \infty$$

So, if I write $\frac{d}{dt}(n_\alpha - n_\beta) = \frac{dt}{dn} = -2pn$
d by dt n alpha minus n beta. So, what will I get d alpha by d beta this is I call it as dn by dt which is the population difference and it turns out that this will be equal to minus 2p n. So, simple algebra here. So, you will generate this sort of an equation $\frac{dt}{dn} = -2pn$ and what is n? $n = n_\alpha - n_\beta$. Now if we solve this differential equation we will I am not going to solve it here but I will give you the result what it will come out to be.

So, the result is the solution of this is n = 0 at time t, $n = n(0)e^{-2pt}$ n (0) is the population difference at time t = 0 and at time t this will be e^{-2pt}. So, what happens at t = ∞. So, n = 0 at t = ∞ but of course you do not continuously pump in energy for so long t = ∞ meaning you are putting the RF continuously for so, long.

You do not put in energy for so long you put it on for a certain time and it is dictated by the transition probability p at this one. So, therefore depending upon the value of p how much is the population difference. So, therefore you will see that if this is very large if this time constant is very large then you will have this energy population difference going to 0. If n = 0 there will be no absorption of energy therefore the signal has to vanish.

So, if I want to plot this here n versus time it will be like this. So, this is the initial population difference at time t = 0 then eventually it will go down to 0 and this depends upon the value of p relative values of p and t at what time you are looking at. However we always see some signal right in NMR experiment we always see some signal. So, what does it mean? So, how do we reconcile this?

We always see NMR signal. Notice here we have ignored one thing. We ignored the fact that there is what is called a spin lattice relaxation. Spin lattice relaxation is trying to bring this population difference back. When you induce transitions by applying the RF it causes the transfer of population from the alpha state to the beta state and when the two populations become equal then $n_\alpha - n_\beta = 0$ when there will be no absorption of energy.

So, if I want to plot this same thing in the form of energy I say I will say here energy here rate of absorption of energy $\frac{d}{dt}$ this will also follow the same pattern here. There will be no absorption of energy as the population start getting equal. So, eventually it will go to 0 then there will be no signal. However in an NMR experiment it does not happen that how long you keep the sample in your magnet you keep on getting the signal and this happens because there is what is called as the spin lattice relaxation.

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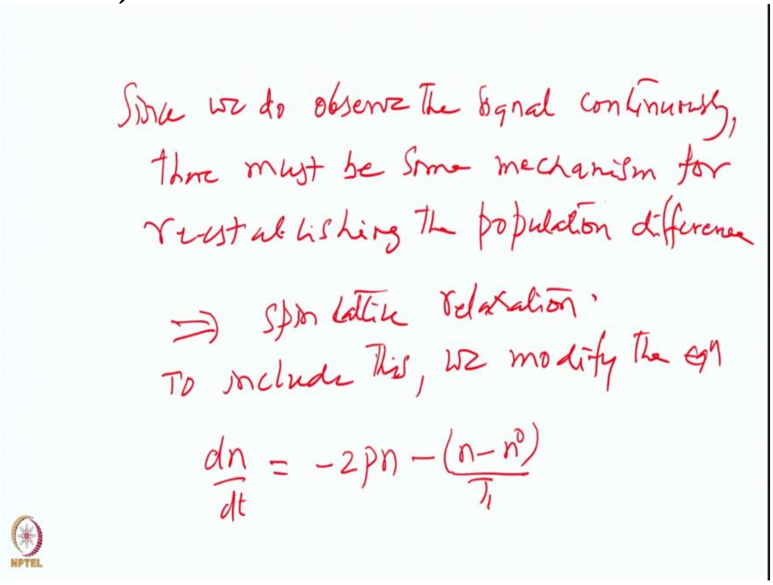

Since we do observe the signal continuously, there must be some mechanism for reestablishing the population difference

⇒ spin lattice relaxation.

To include this, we modify the eqn

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

Since we do observe signal continuously there must be some mechanism for re-establishing the population difference. And this is the spin lattice relaxation which we had ignored. Therefore we will have to modify our equation. So, include this to include this we modify the equation as $\frac{dn}{dt} = -2pn - \frac{(n-n^0)}{T_1}$ dn by dt is equal to minus 2p n this is the original what we had then we have this other term which is n minus n naught divided by T$_1$.

So, if you want to continuously observe the signal without n becoming 0 then what we should say we look at the steady state where n is not 0.

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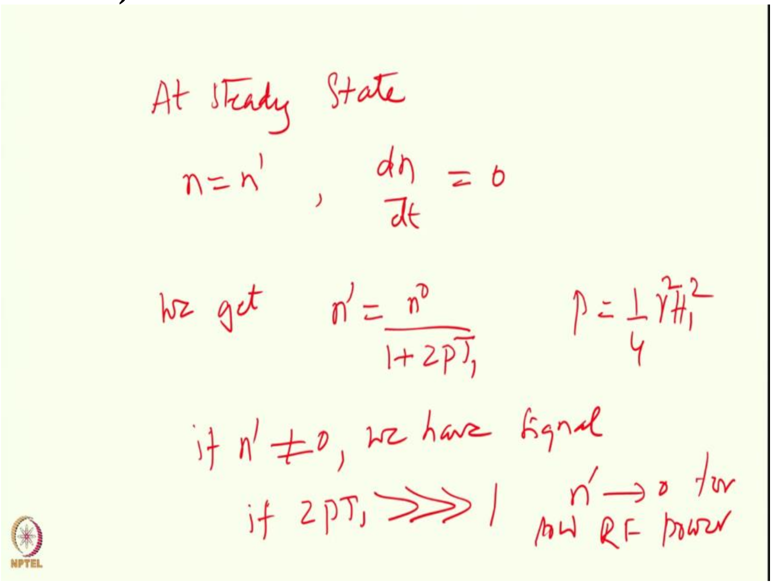

At steady state

$n = n'$, $\frac{dn}{dt} = 0$

we get $n' = \frac{n^0}{1+2pT_1}$ $p = \frac{1}{4}\gamma^2 H_1^2$

if $n' \neq 0$, we have signal

if $2pT_1 \ggg 1$ $n' \to 0$ for high RF power

So, at steady state n is equal to n dash and $\frac{dn}{dt} = 0$. So, when we do this we get we after solving that equation when we said this we get $n' = \frac{n^0}{1+2pT_1}$ n dash is equal to n naught divided by 1 plus 2P T 1 you see now if depending upon what is the value of T$_1$ versus the value of p. So, you get a steady state population difference n dash and. So, long as n' is non-zero if $n' \neq 0$, we have signal.

Therefore $2pT_1$ 2 times P times T_1 becomes an important factor in determining what is the NMR signal. In fact there is a story here Gautier who actually discovered this phenomenon in the very beginning however he had he did the experiment in the beginning but he could not detect the signal for the simple reason that he chose a sample which had a very high T 1 value. When the T 1 value is very large what happens if $2pT_1 \gg 1,$ 2p T 1 is far larger than 1 then n' goes to 0 even with very low RF power n dash goes to 0 for very low RF power.

And why is RF power because RF power dictates the value of p. Depending on how much value of RF power we have. So, let me write here the there is also an equation for p.

$$p = \frac{1}{4}\, \gamma^2\, H_1^2$$

P is equal to 1 by 4 gamma square H_1 square and H_1 is my amplitude right. So, therefore if H_1 is very, very even when it is very small when it is small if true pT_1 is very, very large then n' goes to 0.

And unfortunately Gautier chose a sample which had a very large T_1 very long T_1. So, therefore with very low power he was actually saturating the signal n' was going to 0 therefore he could not see the signal at all. So, unfortunately he missed the Nobel Prize. So, I think we will stop here.