NMR spectroscopy for Structural Biology NS

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Lecture: 01
NMR Basic Concepts - 1

Welcome to this course on NMR in structural biology. NMR has emerged as a technique with applications in all branches of science. It was discovered way back in 1945 in the solution phase by professor Felix Block at Stanford University and his group and professor E.M Purcell at MIT, Boston and that was the beginning of the NMR in the solution phase. Previously, of course some observations were made by Rabi in the gas phase.

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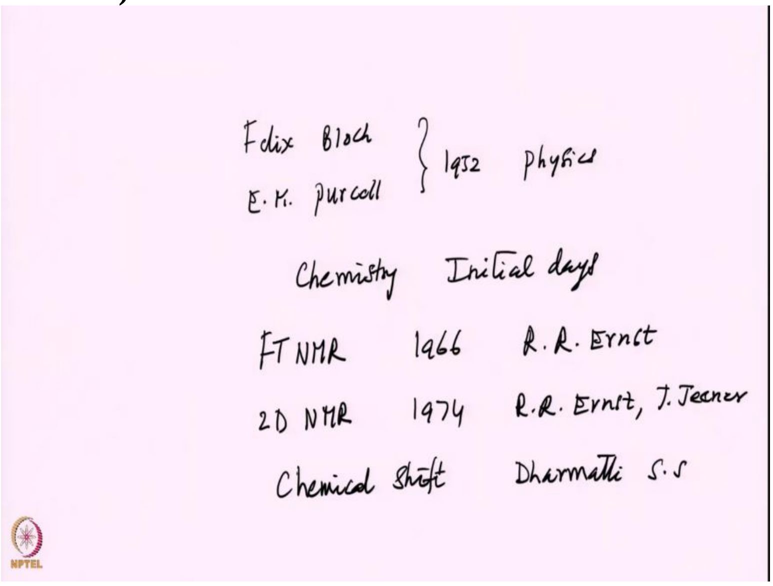

Felix Bloch
E.M. Purcell } 1952 Physics

Chemistry Initial days

FT NMR 1966 R.R. Ernst

2D NMR 1974 R.R. Ernst, J. Jeener

Chemical Shift Dharmatti S.S

And it was such an important discovery is that all of these people got Nobel Prizes Felix Bloch and E.M Purcell they shared the Nobel Prize in 1952 for physics for the basic discovery. Subsequently a whole lot of discoveries have happened and that have led to more Nobel Prizes and structural biology has emerged as one of the most important contributions of NMR. In the initial phases it was mostly applications in chemistry initial days.

And with developments such as Fourier transform NMR in 1966 by Richard Ernst and Anderson 2 dimensional NMR in 1974 by again Richard Ernst and J Jenner a Belgian scientist this revolutionized the applications of NMR in chemistry and structural biology opened up the floodgates in biology. While these were technological advancements there were basic discoveries of various types like chemical shift and this was discovered by an Indian scientist S. S. Dharmati.

Who was initially a Post-doc at Stanford with Felix Bloch and he joined Tata Institute of Fundamental Research in the 1950s that was the time when the chemical shift was discovered.

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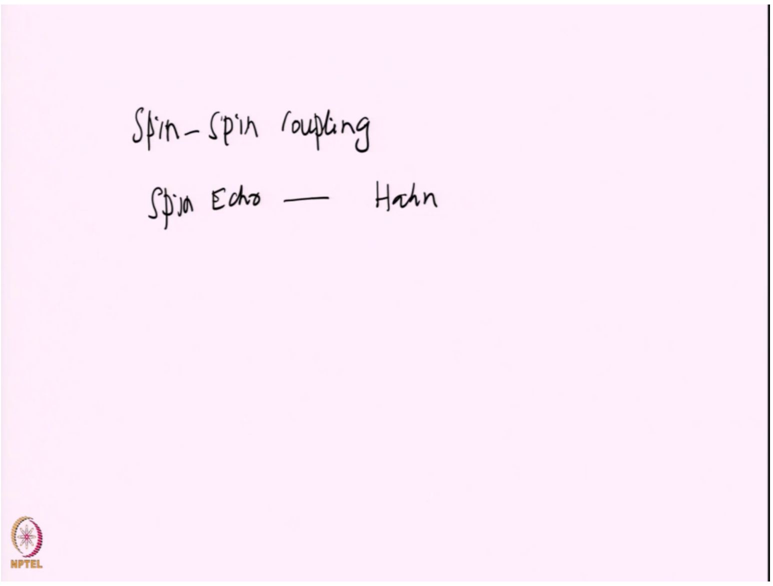

Spin-spin coupling

Spin Echo — Hahn

Then we had discoveries like spin-spin coupling these were important parameters NMR parameters. Then you had things like spin echo this was discovered by Hahn again in the 1950s. The 1950s saw a whole lot of developments discoveries in NMR all of them have led to great contributions in NMR and its applications. We will start this course with the basics of NMR and eventually progress towards the applications at the highest level namely in structural biology.

It is very important to understand the fundamental principles of the technique. So, that you can appreciate the applications you can design new techniques as per the needs as per the problems and that is what has led to unimaginable developments and applications of NMR.

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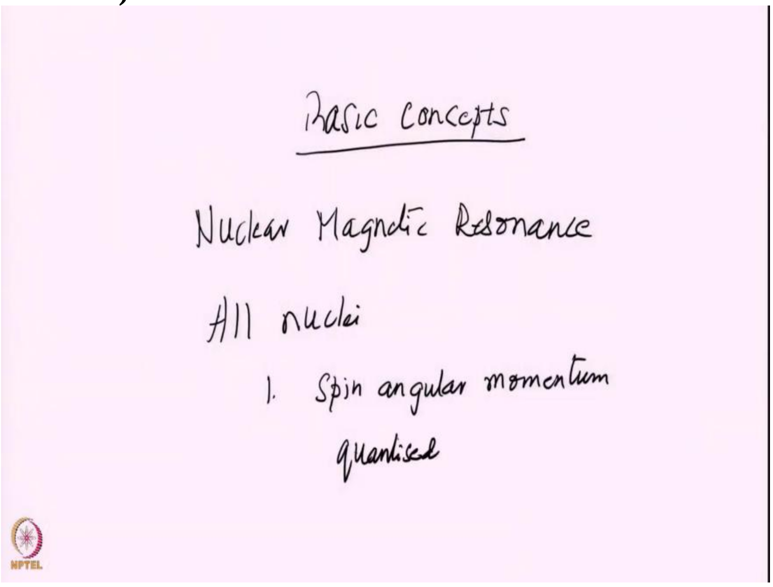

Basic Concepts

Nuclear Magnetic Resonance

All nuclei

1. Spin angular momentum

quantised

So, let us start with the basic concepts. So, we will start here and what does NMR stand for nuclear magnetic resonance. All nuclei have 2 fundamental properties intrinsic properties one spin angular momentum. This is a quantum mechanical concept it did not start from the nuclei because it also was there for the electrons and is slowly even discovered that the spin angular momentum is a very general concept.

This is a quantum mechanical concept the theory of angular momentum as we will only pick up the results from this theory. Spin angular momentum the nuclei have different energy states depending upon the angular momentum it processes. This is quantized what does, that mean it has very discrete values.

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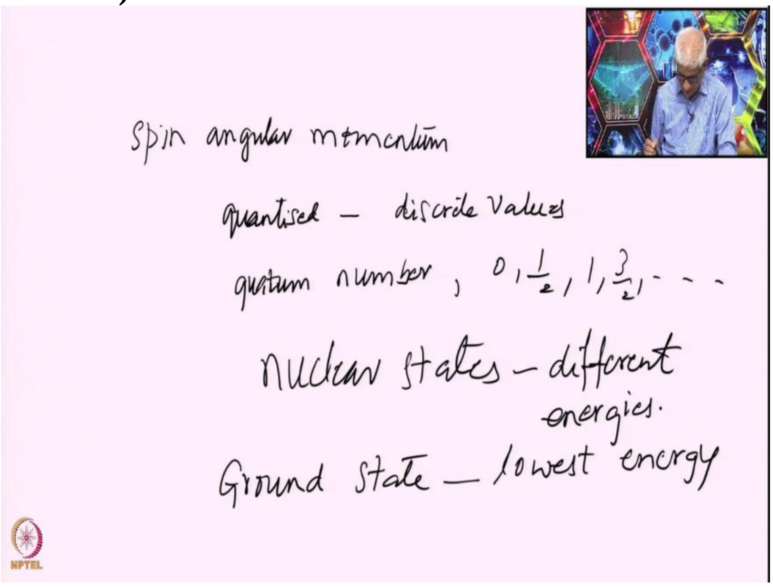

Spin angular momentum

quantised — discrete values

quantum number, $0, \frac{1}{2}, 1, \frac{3}{2}, \ldots$

nuclear states — different energies.

Ground State — lowest energy

Though spin angular momentum we said this is quantized which means it can only take discrete values. And it can have various values it is indicated by particular number known as the quantum number there is a spin angular momentum quantum number. It can take various values 0, ½, 1, 3 by 2 etc various values it can take. Now every nucleus can exist in many different states the nuclear states the so called nuclear states corresponding to different quantum numbers just as it happens in atomic theory.

You have the electrons in the first orbital second orbital third orbital etcetera and they have different energy. So, all of these have different energies most often we deal with the ground state of the nucleus ground state which is the state of lowest energy.

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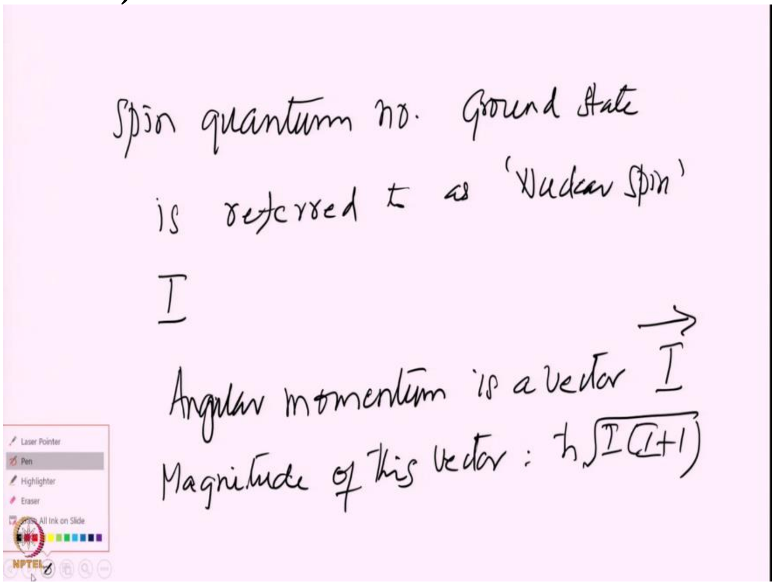

Spin quantum no. Ground State

is referred to as 'Nuclear Spin'

$\underline{I}$

Angular momentum is a vector $\vec{I}$

Magnitude of this vector : $\hbar\sqrt{I(I+1)}$

And the spin quantum number here the spin quantum number corresponding to the ground state of the ground state is referred to as nuclear spin. In the common language it is referred to as nuclear spin and is represented by the symbol I. Angular momentum is obviously a vector and nucleus pin is a value which is a number this is the quantum number which is a number and angular momentum corresponding to this will be a vector.

So, angular momentum itself is a vector is a vector. So, we represent it like that. Now what is the magnitude of this vector the magnitude of this vector is,

$$\hbar\sqrt{I\,(I+1)}$$

I is a number here and what is ħ?

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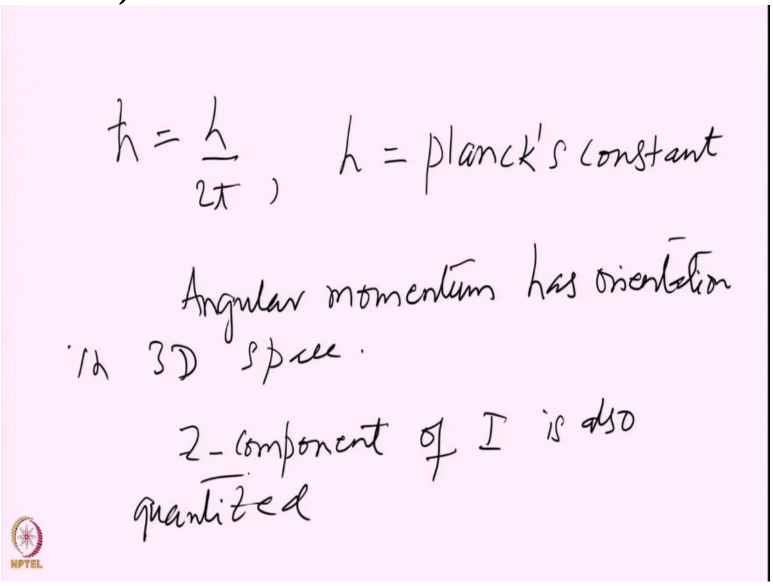

$\hbar = \frac{h}{2\pi}$, h = planck's constant

Angular momentum has orientation in 3D space.

z-component of I is also quantized

$$\hbar = \frac{h}{2\pi}$$

h is the Planck's constant. Now once we said it is a vector it has orientation. So, the angular momentum has an orientation, orientation in 3D space it turns out from the theory of angular momentum that the z component of I is also quantized is also quantized what is the meaning of this.

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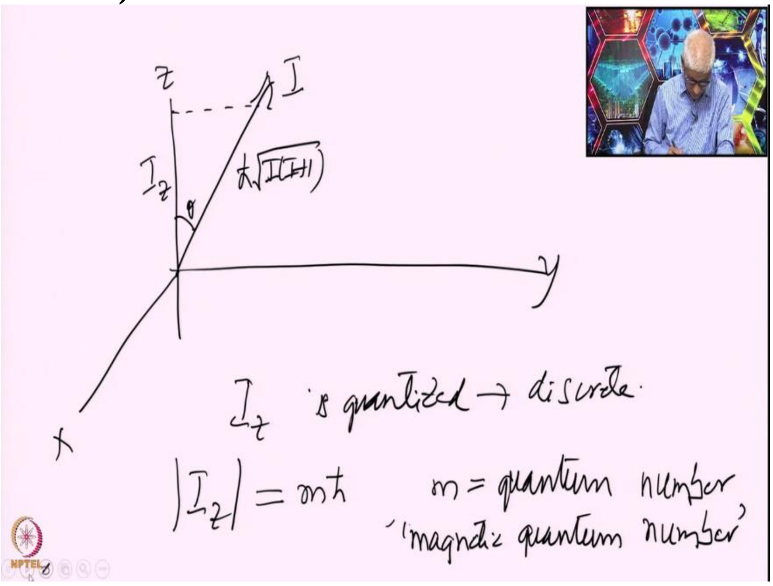

I_z is quantized → discrete.

$|I_z| = m\hbar$ m = quantum number
'magnetic quantum number'

So, if a, way to draw the angular momentum vector like that suppose this is angular momentum vector it has a magnitude which is $\hbar = \sqrt{I+1}$ and it makes an angle let us say call it as θ if this is the Z axis X, Y, Z axis if they are then you have a component here this is the I_z component this is quantized. So, I_z is quantized or I_z is discrete. So, the z component is often represented the magnitude of the z component I represent like this magnitude of I is represented by $|I|$.

Magnitude of z component is represented by like this and this is given by what is m,

$$|I_z| = \mathrm{m}\hbar$$

m is a number is a quantum number another quantum number and this is called as magnetic quantum number and what values it can take it can take a certain number of values.

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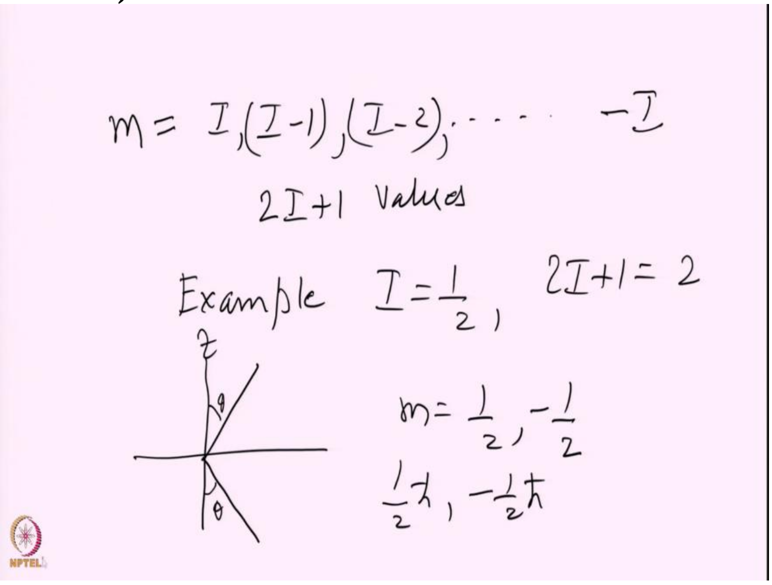

$m = I, (I-1), (I-2), \cdots -I$

$2I+1$ Values

Example $I = \frac{1}{2}$, $2I+1 = 2$

$m = \frac{1}{2}, -\frac{1}{2}$

$\frac{1}{2}\hbar, -\frac{1}{2}\hbar$

It can take values m is equal to I, I – 1, I – 2 so on up to –I, that means it can take 2I + 1 values. So, in other words if we have a spin of I; if we have a nucleus which has a spin of I in the ground state nuclear spin I it can have 2I + 1 orientations in space for example if I is equal to ½ how many will be there 2I + 1 is equal to 2. So, there will be 2 possible orientations. So, I can represent them like this.

So, I can have one orientation like this another orientation like this because what are the values it can take m can take values ½and -½. So, the z component will be

$$|I_z| = \frac{1}{2}\hbar, -\frac{1}{2}\hbar$$

So, if this is the Z axis therefore I will have to write this as on one on the + Z axis other one on the - Z axis. So, therefore the z components the magnitudes of the z components are equal but they are on the Z axis in the opposite direction.

If I take the absolute value then the both these orientations will have the same absolute value but the orientation are different this angle will be different and this angle of course this will be the same and what will be this angle?

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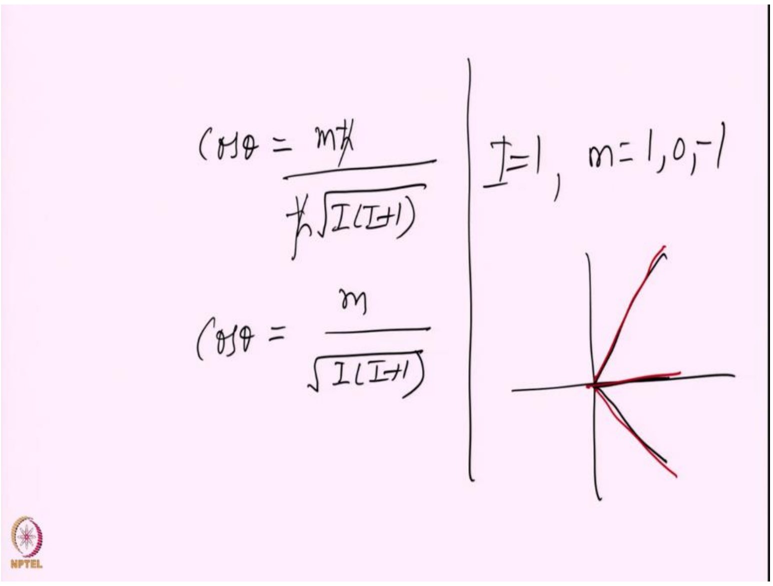

$$\cos\theta = \frac{m\cancel{\hbar}}{\cancel{\hbar}\sqrt{I(I+1)}}$$

$$\cos\theta = \frac{m}{\sqrt{I(I+1)}}$$

$I=1, \quad m = 1, 0, -1$

Because now

$$\cos\theta = \frac{m\hbar}{\hbar\sqrt{I\,(I+1)}}$$

because the length of this vector is ħ I into I + 1 under root and the z component is mħ therefore cos θ is mħ this ħ, ħ will cancel therefore cosine theta is equal to m divided by under root I into I + 1.

$$\cos\theta = \frac{m}{\sqrt{I\,(I+1)}}$$

So, therefore you can see how many angles are possible for the case of I is equal to half I have 2 possibilities.

$$I = \frac{1}{2}$$

So, if I have I =1, then there will be 3 possibilities m will be 1, 0, -1 and then I can draw this thing like that one here one there and one here let me use a different colour here to indicate this. So, I have one here one there and one here you can actually again calculate what is the magnitude of this angle and your 3 possible orientations. If I have I = $\frac{3}{2}$ then there will be four possible orientations.

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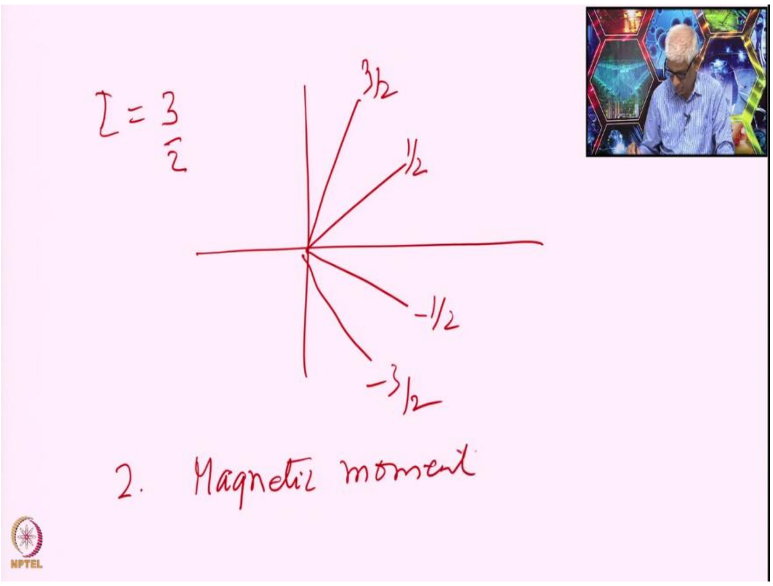

2. Magnetic moment

So, $I = \frac{3}{2}$, I will have four possible orientations here, here, here and here. So, this will be m is $\frac{3}{2}, \frac{1}{2}, -\frac{1}{2}, -\frac{3}{2}$. So, these are the m values and correspondingly the z component will be m ħ. So, this is the first important property of the nuclei which is very fundamental to NMR. What is the next property? Next property is next property fundamental property is magnetic moment.

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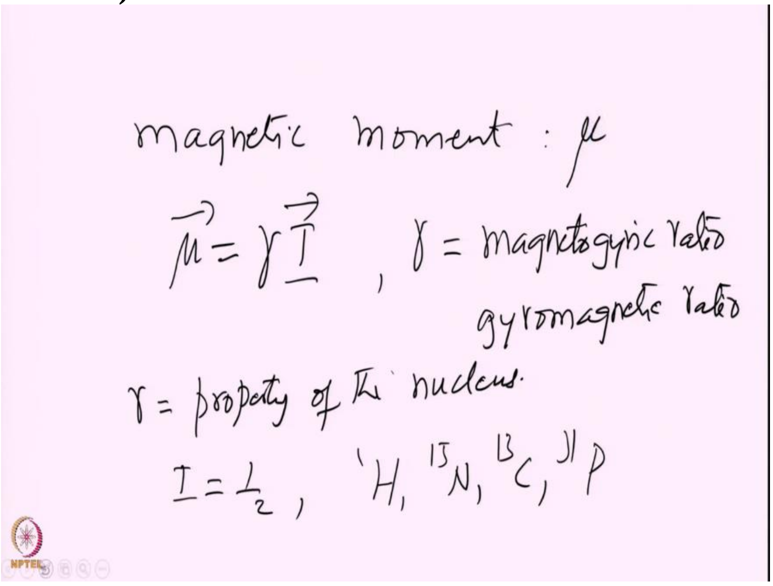

magnetic moment : μ

$\vec{\mu} = \gamma \vec{I}$, γ = magnetogyric ratio
gyromagnetic ratio

γ = property of the nucleus.

$I = \frac{1}{2}$, ^{1}H, ^{15}N, ^{13}C, ^{31}P

Nuclei have a magnetic moment it turns out that the magnetic moment which is represented by the symbol mu is written as mu this is proportional to the angular momentum μ is proportional to γI this is a vector this is also a vector and γ is a constant which is called as the magnetogyric ratio or sometimes it is also written as gyromagnetic ratio both are the same. And this is the property of the nucleus γ is a property of the nucleus. What does that mean, different nuclei which may have the same I value.

May have different magnetic moments which means for example I = ½ how many nuclei are possible here you have large number of possible I will give you the rules for that. For instance you have proton nitrogen 15 (^{15}N), carbon 13 (^{13}C), and phosphorus 31 (^{31}P) all of these are I=½, but they are different magnetic moments and that is because the magnetogyric ratio γ is different.

So, therefore the magnetic moment is an important property with an intrinsic property of the nucleus which determines the application of NMR to various systems. So, that is a very important property.

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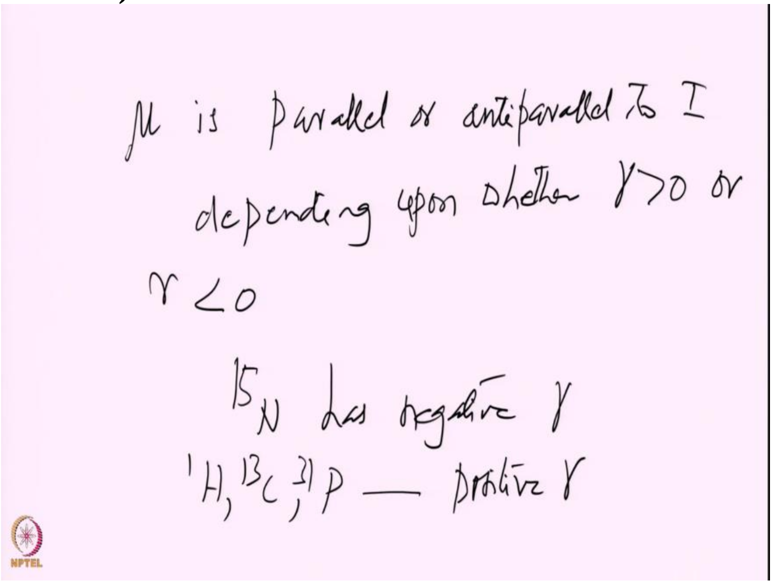

μ is parallel or antiparallel to I
depending upon whether $\gamma > 0$ or
$\gamma < 0$

^{15}N has negative γ
^{1}H, ^{13}C, ^{31}P — positive γ

And also you notice that the magnetic moment is parallel μ is parallel or antiparallel to the parallel to I depending upon whether $\gamma > 0$ or gamma < 0 this can happen γ can be positive or negative. Most nuclei they have it positive but some nuclei have it negative also for example ^{15}N has - γ proton and ^{13}C, ^{31}P is positive proton these are I am labelling only taking only those nuclei which are of common interest in biology structural biology.

So, these ones have +γ. So, this is the fundamental property of the nuclei we have 2 entities one is the spin angular momentum and the ground state of the nucleus is characterized by number which is called as a nuclear spin and that determines its angular momentum. And a nucleus is also an intrinsic property known as the magnetic moment the magnetic moment is proportional to the I value.

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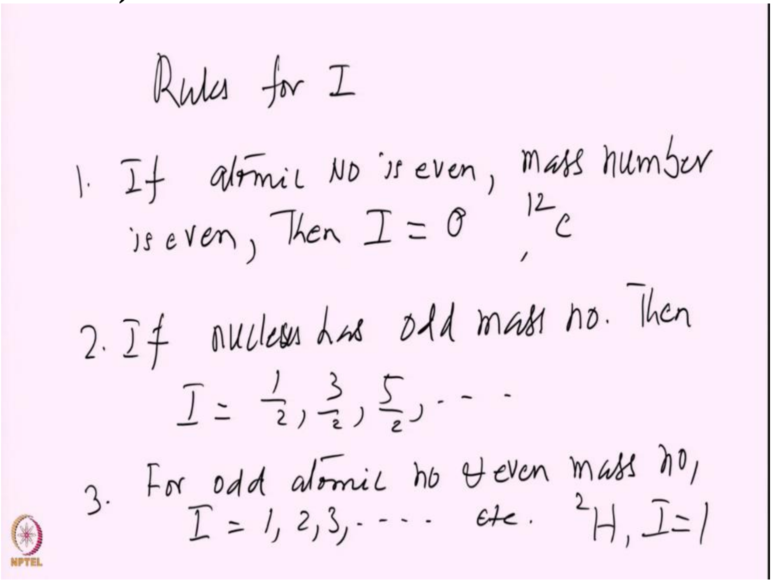

Rules for I

1. If atomic No is even, mass number is even, Then I = 0 ^{12}C,
2. If nucleus has odd mass no. Then $I = \frac{1}{2}, \frac{3}{2}, \frac{5}{2}, \ldots$
3. For odd atomic no & even mass no, $I = 1, 2, 3, \ldots$ etc. ^{2}H, $I=1$

Now is it possible to know what nuclei what kind of are there any rules for the angular momentum nuclear spin can you calculate the angular momentum. There are certain rules empirical rules, rules for I. How do we know what sort of I a particular nucleus will have so if one if atomic number is even and mass number mass number is the same as the isotope number. If the mass number is even then I will have values 0.

For example carbon 12 if the I = 0 then of course it does not have any magnetic moment and therefore this nucleus will not be NMR active. We will say then it is not NMR active. Number 2 if nucleus has odd mass number then I = $\frac{1}{2}$, $\frac{3}{2}$, $\frac{5}{2}$ etc. These are empirical rules which have been established. There are theories to calculate this of course but we cannot go into those ones in this course.

Then number 3 for odd atomic number and even mass number I will take integral values 1, 2, 3 etc. For most of the time we will be dealing with nuclei which are I =½ occasionally we may come across I = 1 deuterium for example has I = 1 sometimes we may deal with deuterium as well in biological NMR. So, this is the nuclear properties. Now what happens if you put these nuclei an ensemble of nuclei in a magnetic field.

I told you earlier that all possible orientations of the nuclei have the same energy in the absence of any external perturbation all orientations have the same energy.

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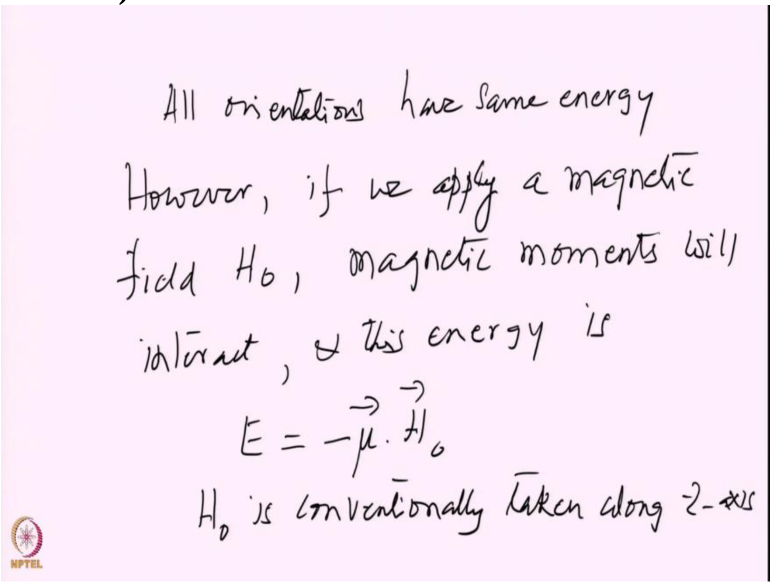

All orientations have same energy

However, if we apply a magnetic field H_0, magnetic moments will interact, & this energy is

$$E = -\vec{\mu} \cdot \vec{H}_0$$

H_0 is conventionally taken along z-axis

Let me write that here all orientations have same energy. However if we apply a magnetic field h naught what happens the magnetic moments will interact with them magnetic moments will interact and this energy is $E = \vec{\mu} . \overrightarrow{H}_0$. These are vector quantities and H_0 is conventionally considered to be applied along the Z axis H_0 is the magnetic field and H_0 is conventionally taken along Z axis. So, as a result what happens the different orientations will now have different energies.

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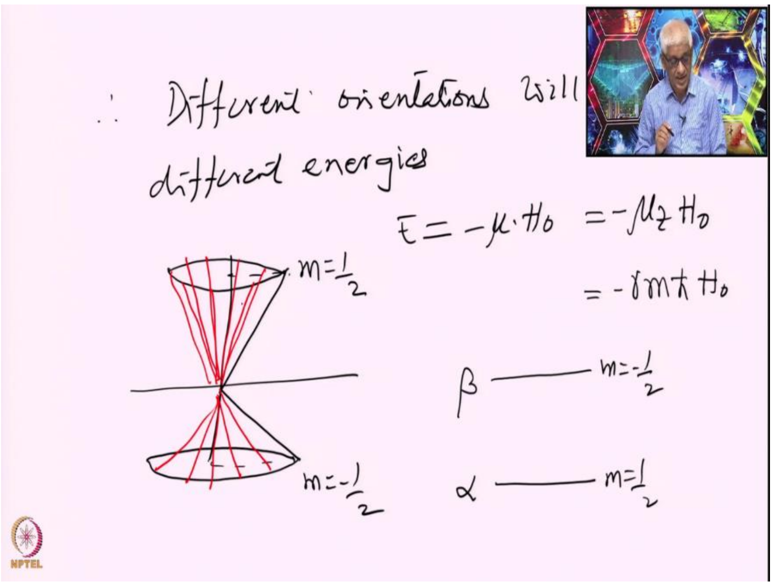

∴ Different orientations will different energies

$E = -\mu \cdot H_0 = -\mu_z H_0$

$= -\gamma m \hbar H_0$

So, therefore different orientations will have different energies. So, let me draw that here. So, I have the nucleus pin oriented like this oriented like this the mu dot mean is $E = -\mu . H_0$ means this interaction this component and this component here they have different energies. So, this if I want to write this is $E = -\mu_z . H_0$, μ_z is the z component of the magnetic moment.

And this is then given by $E = \gamma\, m\, \hbar\, H_0$ the magnetic energy magnitude is given by this. And therefore now we see depending upon the value of m the energy value is different therefore these 2 have different energies. So, here I have m=½ and this is m= -½ and what will be the energies for m = +½ the energy value is negative and for m= -½ the energy value is positive provided H_0 is along the Z axis and this is m = ½ and this is m= -½.

And conventionally we label this as α and β these states discrete states are now labelled as α and β states. Now this is so far as the z component is concerned. However what about the orientation in space the orientation in space when it is not only 2 dimensional it is 3 dimensional. What happened to the x and the y components of the magnetic moments do we know them?

No, we do not know them. Only the z component is properly defined the x and the y components what does it mean suppose I wait to draw a cone here like that I draw a cone here similarly I draw a cone here and now I can draw these vectors angular momentum vectors here, here, here, here, here anywhere on the surface of the cone. So, if I have an ensemble of spins which is there in the solution we have larger number of spins right.

The molecules have been millions and trillions you have 10^{19} - 10^{20} if you take molar sample 10^{23} kind of things that many spins are there and where are they located. You cannot change the orientation with respect to the Z-axis that orientation remains the same. So, which means they will all be distributed on the surface of the cone in a random way.

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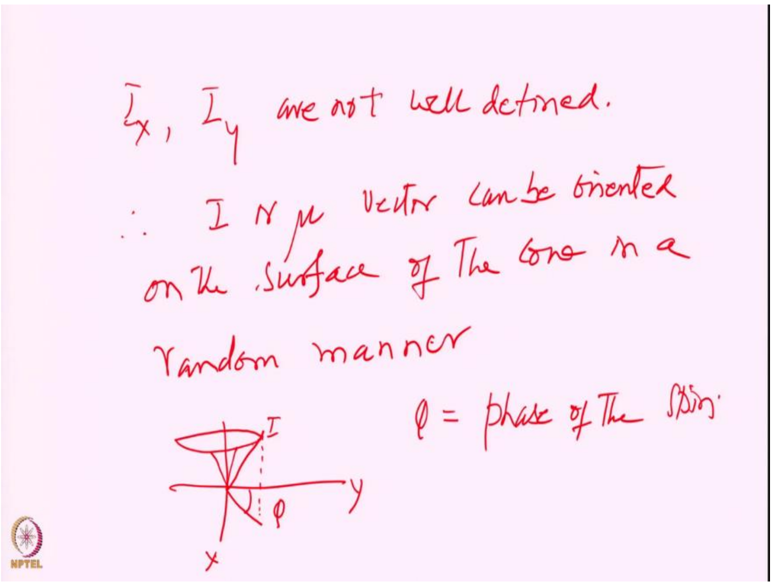

I_x, I_y are not well defined.

∴ I or μ vector can be oriented on the surface of the cone in a random manner

ϕ = phase of the spin.

x and the y components are not well defined I_x, I_y are not well defined therefore the I vector or the $\vec{\mu}$ can be oriented on the surface of the cone in a random manner. If this is the case then what happens suppose I take an orientation like this I take a projection of this, this is my I or μ both are same. Now I take a projection of this onto the xy plane this is x, y let us say and the z is on the top.

So, then I have this projection if I take a component here if this angle is particular value let us say ϕ I am taking the cone like this. Now this angle will be different depending upon where I have my vector on the on the cone surface whether it is here or it is here or it is here it will have a different angle on the in the xy plane. So, therefore this phi is called the phase of the spin.

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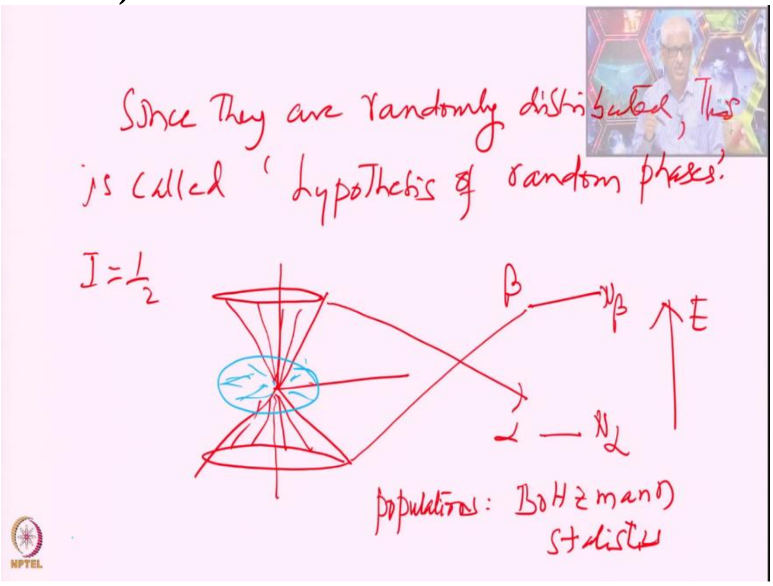

Since they are randomly distributed, this is called 'hypothesis of random phases'.

$I = \frac{1}{2}$

Populations: Boltzmann statistics

Since they are randomly distributed since they are randomly distributed this is called hypothesis of random phases all right. Now let us consider a situation of I = ½ I have this here one orientation like this other orientation like this and I have this cones here. So, I draw the cone here and all the spins are oriented on the surface of the cone here, here, here, here. Now this energy is α is the lower end energy.

And this energy is β this is energy here this is the α state and this is the β state and obviously they have a different population's right. So, if I want to write the populations of this as N_α and I call it as N_β what determines these populations? The populations are governed by Boltzmann statistics. So, there will be more number of spins along there on in the α state compared to the β state.

Now let us look at one of those let us look at the alpha state for example. Now if I wait to take the projections of all of these components on the xyz planes what happens all the xz components will add all the z components will add because they are in the same direction. What about the xy components? xy components are along distributed in the 360° phase anywhere on the phase.

So, if I draw this if I draw this phase here they have xy plane they are anywhere present. So, for every one orientation there is a correspondingly opposite orientation therefore they will all cancel. So, there will be no xy component of the magnetization from either from the alpha state or from the beta state. Now what about the z component? The z component there will be positive z component as well as negative z component.

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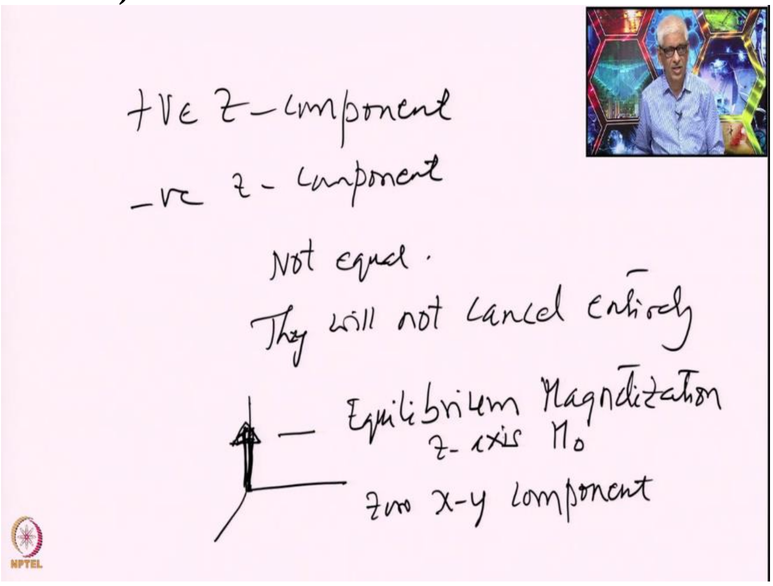

+ve z-component

-ve z-component

Not equal.

They will not cancel entirely

Equilibrium Magnetization

z-axis M_0

Zero x-y component

So, there will be a positive z component and a negative z component these are not equal therefore they will not cancel entirely. As a result there will be a net magnetization net component along the Z axis. So, I will represent this as this thick arrow here this we call as the equilibrium magnetization. No transverse component equilibrium magnetization is along the Z axis 0 x-y components this is often represented as m naught and x-y component is 0.

So, therefore at equilibrium the magnetization of the spin system is along the Z axis and there will be no components along the xy plane. This is an important concept and we will take it forward from here in the next class. We will stop here.