Multidimensional NMR Spectroscopy for Structural Studies of Biomolecules

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Lecture – 01
Introduction to NMR spectroscopy

Hello everyone welcome to the course on Multidimensional NMR Spectroscopy with it is applications to Biomolecules. In this course we will begin first with very basics of an 1 D NMR 2 D NMR principles of NMR spectroscopy. And, then slowly build our knowledge and to move on to 3 D NMR and it is application. So, let us first see why NMR spectroscopy is so, popular what is the unique about it and what are its basic principles.

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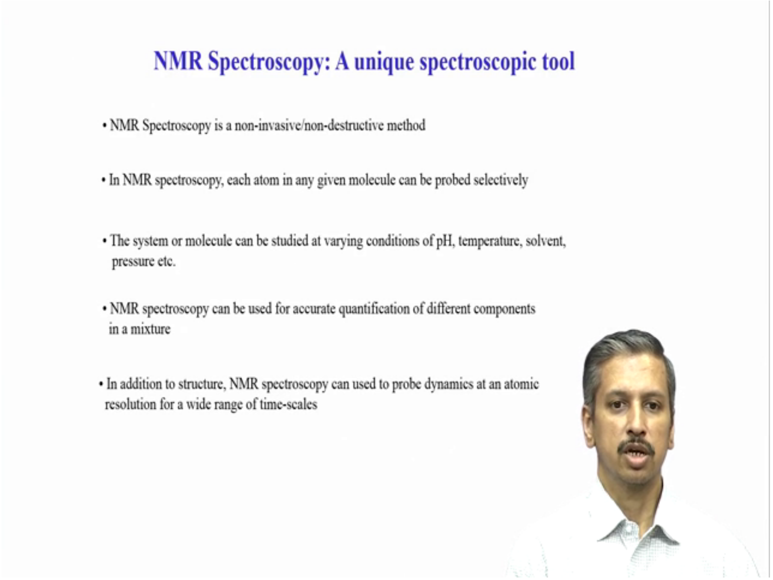

NMR Spectroscopy: A unique spectroscopic tool

- NMR Spectroscopy is a non-invasive/non-destructive method
- In NMR spectroscopy, each atom in any given molecule can be probed selectively
- The system or molecule can be studied at varying conditions of pH, temperature, solvent, pressure etc.
- NMR spectroscopy can be used for accurate quantification of different components in a mixture
- In addition to structure, NMR spectroscopy can used to probe dynamics at an atomic resolution for a wide range of time-scales

So, one of the thing which makes NMR very popular is it is a non-invasive and a non-destructive technique, which means if you have a sample it you and you provide the sample. The sample comes back intact and nothing is destroyed or nothing is broken in the sample. And therefore, the molecules are not affected by recording the data.

So, this is something unique to NMR because some other techniques the sample is evaporated or sample is physically modified for doing the experiment. And, in NMR spectroscopy you can study each and every atom or you can probe each and every atom in a given molecule very selectively. And, that is very important for studying structures of molecules and a very high resolution. So, that is something again unique to NMR and therefore, it is very popular method. The molecules now further can be studied under different conditions of p H temperature and solvent.

One can vary the p H, one can vary the temperature, as long as the sample is still intact the molecule has not degraded one can vary such conditions and again non-invasively, non-destructively study the properties of the molecules at that different conditions. Ah. NMR spectroscopy is also very useful for a accurate quantification of different components.

For example, let us say you have a mixture of molecule and each molecule has different concentration. NMR can be used to quantify the concentration of each molecule in that mixture. And, that is again something unique to NMR, because many other techniques cannot quantify accurately. And therefore, this is a technique which is used very popular; NMR is very popular in a field called metabolomics where quantification is a very key factor.

NMR spectroscopy can also probe dynamics at different timescales. So, as we know that and molecules are not static in nature, they are very dynamic and that dynamics is reflected in many of the NMR parameters. And therefore, indirectly you can actually or directly you can look at those dynamics of the molecules at a very high resolution and the interesting part is you can actually look at wide range of timescales.

So, if the interaction between a molecule and another molecule is happening in seconds or milliseconds one can look at the different timescales of such interaction. So, these are basically the points which make NMR very popular and very useful for chemists and biologists.

So, let us now see what are the principles involved in NMR spectroscopy.

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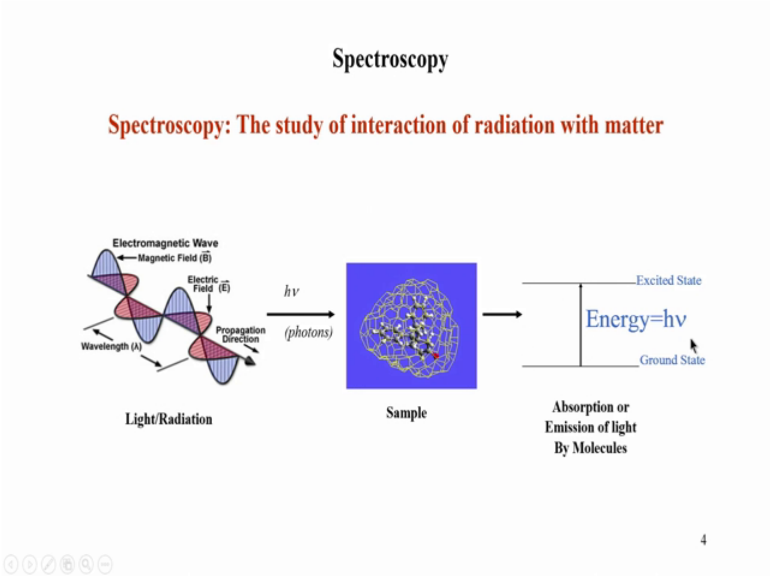

Spectroscopy

Spectroscopy: The study of interaction of radiation with matter

Light/Radiation

Sample

Absorption or
Emission of light
By Molecules

So, this is basically we will start from very basic principles. Now, NMR spectroscopy like any other spectroscopy involves interaction of radiation with matter. So, that is, what is essentially the heart of spectroscopy? So, you have a sample you have light, and as the light interacts with the sample, and the light the sample can either absorb the light, it can either emit the light, or it can scatter the light. So, this is shown here on the left side you can see the schematic drawing of a wave, electromagnetic wave.

So, when I say radiation it basically means electromagnetic radiation. And, you can see it has 2 components as we know this is one is electric field component and a perpendicular to that is the magnetic field. So, the parameters which characterize a wave when we talk about a wave what is the how do we describe it? We describe it by this parameter wavelength or by it is frequency. How many waves are travelling per second? So, frequency is proportional to energy of the wave and wavelength is also another important parameter.

So, when this radiation or light is shown on a sample or molecule. This molecule now will interact with this light may or may not interact. And, if it does not interact the light simply passes through, but if it interacts with the light then it absorbs the energy. And, the molecules now we can say that inside the molecule we can there are discretized energy levels. Now, this is a concept which we know from quantum mechanics, that at a very at an atomic scale the energy levels are quantized and they are discrete they are not continuous. Say, if the light falls on this molecule and if the energy of the light which is given by this h into nu, where nu is the frequency of the light frequency of this wave. And, this wave this energy, if it matches a particular energy gap in the molecule then the molecule will now take the energy and go into an excited state.

So, the molecule now gets excited. And, the energy is taken up or absorbed by the system by the molecule. So, this is called absorption of light. Now, subsequently this energy the molecule which has gone to the excited state will also emit radiation back and come back to the ground state. And, during that process it will emit back the energy which has been taken and that we can call it as emission.

So, essentially when you shine light on a sample you either have absorption of light or emission of light, it can also scatter the light which is not shown here, which we will not be concerned with. We are mainly concerned with absorption and emission as far as NMR is concerned or it can simply pass through means there is no effect of the light on the sample.

In such cases we say that the sample is transparent to the light, it does not absorb the light or does not see the light.

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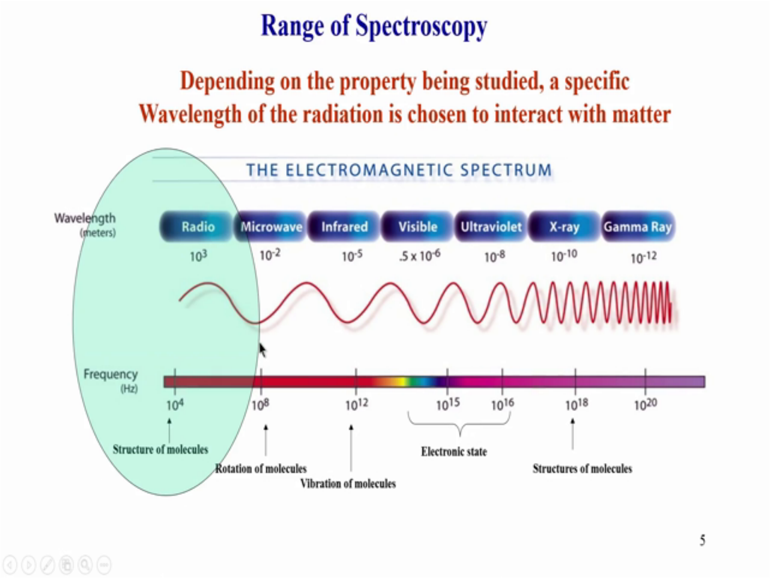

Range of Spectroscopy

Depending on the property being studied, a specific Wavelength of the radiation is chosen to interact with matter

So, when you go look in the electromagnetic region as I said we are looking at electromagnetic radiation, we have a wrong range of wavelengths possible. Meaning we can have a light which has a frequency in this range, a wavelength in this range, 10 raise to minus 12 metres you can see the units written here, or you can have a energy or a wave which is having a frequency or a wavelength of this and so on.

So, we can take different parts of these regions and you can take a wave in this particular region or this particular and then shine it on the molecule. What happens is each, of each type of radiation has a very specific effect on the molecule. So, molecule for example the electronic states of the molecules are affected, if you shine a visible or ultraviolet light on a molecule. Here you can see if I shine an infrared electromagnetic radiation on a molecule, it will affect the vibration. In the vibration of the molecules are affected by this radiation. When you come to very low wavelengths or very high wavelength, very low frequencies we use the word radio waves.

So, this is similar to the radio FM radio which you listen. And in this particular case the structure of molecule is basically affected or not affected it responds to the radio waves. So, our NMR spectroscopy course of a NMR is essentially in this part of the electromagnetic spectrum. So, we call this entire range as electromagnetic spectrum and our focus will be on the radio wave part of the spectrum this is higher NMR comes into picture.

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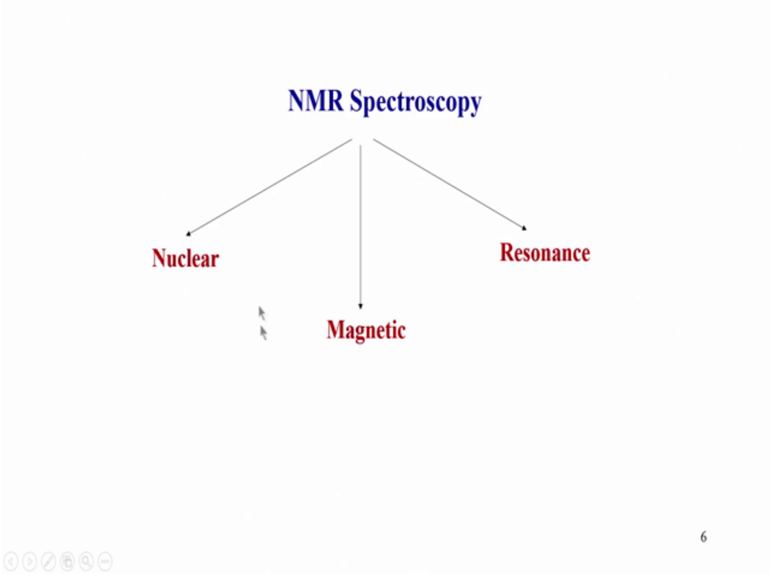

NMR Spectroscopy

Nuclear

Magnetic

Resonance

So, now NMR Spectroscopy when you use this word you can see there are 3 components or 3 parts in this word nuclear, magnetic, resonance.

So, we should now try to understand each of this term and see what they individually mean and so, that which together constitute the technique of NMR. So, let us start from this first one which is nuclear or a nucleus.

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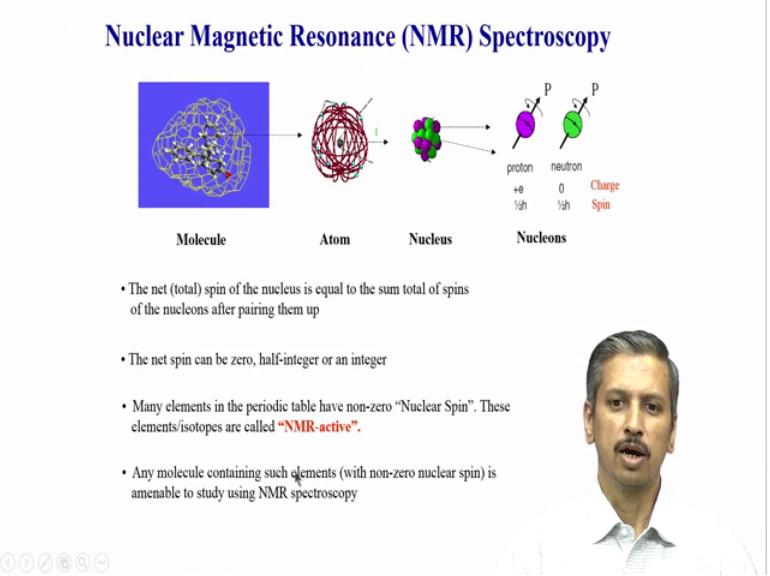

Nuclear Magnetic Resonance (NMR) Spectroscopy

- The net (total) spin of the nucleus is equal to the sum total of spins of the nucleons after pairing them up
- The net spin can be zero, half-integer or an integer
- Many elements in the periodic table have non-zero "Nuclear Spin". These elements/isotopes are called **"NMR-active".**
- Any molecule containing such elements (with non-zero nuclear spin) is amenable to study using NMR spectroscopy

So, if you look at a molecule schematically again here molecules are made up of Atoms, which something which all of us know. And, the Atoms now consist of a nucleus at the centre and surrounded by electron or electron cloud. Now, if you further zoom into this nucleus; nucleus consist of tightly packed protons and neutrons which we use the word nucleons.

Now, further if you look at this protons and neutrons they have 2 properties which is important for us; one is a charge which is project a positive for a proton, because it nullifies the electron negative charge. Neutron is 0 charged is neutral that is as for a charge. Now, the other important property is what is called as a spin. The spin essentially is again not necessarily that it is spinning, but it is a property an abstract quantity associated with a particular proton or a neutron. So, that spin has a value half into h, h is a Planck's constant and this is just a half h is the notation half h cross.

So, this is as far as both protons and neutrons are concerned they both have the same value, but charge wise they are not the same. Now, in the inside a nucleus we have protons and neutrons they start pairing up. So, you can actually see that this proton will pair up or in opposite direction and will cancel the spin.

Say, if that happens the total spin value will become 0, because half and minus half will cancel each other. So, this is what we do for a given nucleus in a given nucleus we start pairing up the spins of protons and neutrons. And, then calculate the total value of the spin of this nucleus. So, for example, let us say a hydrogen atom. In an hydrogen atom there is only one proton, there is no neutron. So, there is nothing for a proton to pair with and therefore, it is spin remains half. So, nucleus of a hydrogen or a proton has a spin half.

But, if you take another nucleus let us say carbon 12, it has even 6 protons and 6 neutrons. And therefore, if they pair up they all match they pair up exactly and the total spin value becomes 0 for the carbon 12 nucleus. So, remember we are trying to look at the total net spin value of the nucleus after pairing up the protons and neutrons. So, the net spin can be 0 as we saw in, as we just now saw in carbon 12, or it can be half, which is what is in the proton or it can be integer. For example, 2 proton 1 proton and 1 neutron they pair up parallel to each other. And, when they are parallel to each other the spin half and half will add up and that will become 1. So, that becomes an integer. So, we can have an integral spin also.

So, many elements in a periodic table, which there are as we know where different periodic table elements group 1 group 2 and so on. All of them can be now grouped into different categories for example, if some particular nucleus has a non-zero value. The total value of the spin is not 0 we use the word that particular element in the periodic table is “NMR-active” which means it can be studied by NMR. And, if a molecule containing elements which has 0 spin for example, carbon 12 is something we just saw we say it is NMR inactive.

But, remember carbon 12 maybe spin 0, but it may have an isotope which is carbon 13 which may not be spin 0. So, therefore, typically in a periodic table either the element main element does is not NMR active. And, if it is not typically has some isotope which is NMR active. So, roughly all the elements in the periodic table actually can be studied by NMR spectroscopy. So, that is beautiful part of NMR there is no element which is difficult to study of course, the difficulty varies because based on the nucleus, but it is possible to study.

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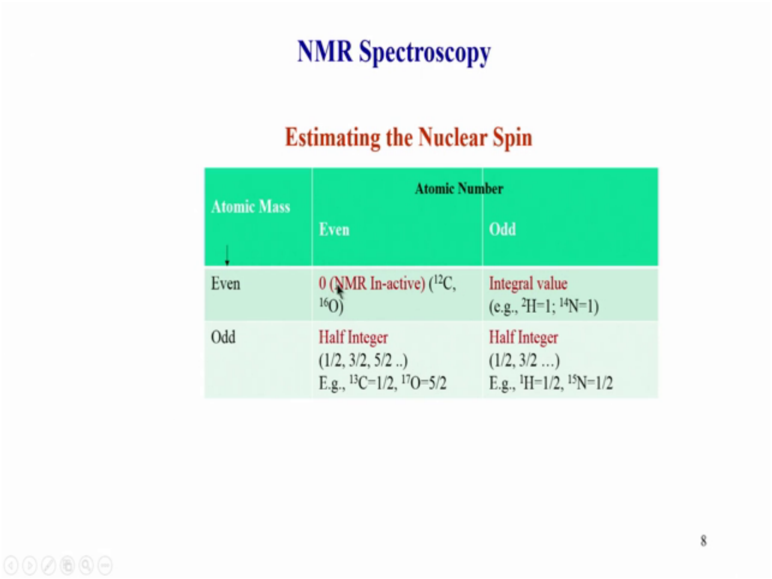

NMR Spectroscopy

Estimating the Nuclear Spin

Atomic Mass ↓	Atomic Number	
	Even	Odd
Even	0 (NMR In-active) (^{12}C, ^{16}O)	Integral value (e.g., $^{2}H=1$; $^{14}N=1$)
Odd	Half Integer (1/2, 3/2, 5/2 ..) E.g., $^{13}C=1/2$, $^{17}O=5/2$	Half Integer (1/2, 3/2 …) E.g., $^{1}H=1/2$, $^{15}N=1/2$

So, this is give the table here gives a rough idea of how to actually estimate the spin value for the nucleus. So, you can see here this atomic mass and Atomic number is put in this row is Atomic number the column is atomic mass. So, you can have an even Atomic mass or you can have an even an even Atomic number. So, if you have this combination; that means, Atomic mass is even and Atomic number is also even in such kind of elements like carbon 12 and 16 oxygen, oxygen 16 the total spin value is 0 for the nucleus of these elements.

So, these elements cannot be studied in their native natural form by NMR, but carbon 13 and this oxygen 17 these isotopes can be studied by NMR. Because they do not have 0 spin, but carbon 12 and 16 is 0 spin and it belongs to this even category. Now, let us look at this odd even combination. So, you have atomic number odd, but the atomic mass is even. And, that is what you can see nucleus such as deuterium this is nitrogen 14. And, these have integral value; that means, the spins do not cancel each other, they actually add up and they become spin half plus half becomes 1.

So, these are integral values. Now, if you look at the third combination where the mass number is even atomic mass is odd. And, this is the standard many molecules atoms fall in this region 13 C, 17 O; we can see the spin value is half integers so, 1 by 2, 5 by 2, 3 by 2 and so on. So, that happens if your atomic number is even for example, carbon 13 the atomic number is 6 and the atomic mass total is 13 because it has an extra neutron that is 7 neutrons.

Now, if you look at the last odd combination and this is where the proton comes in, where it has odd number of atomic number is odd which is one in case of hydrogen and the atomic mass is also one which is odd. So, you can see this spin is half, where total spin of the nucleus it is half and N 15 is also half.

So, in the entire typically in this course or in general in NMR, we normally focus on this Half Integral spin. Because, this integral value spin nuclei are very little complicated and we use the word quarter polar nuclei and that is typically not studied much unless it is in solid state whereas, here the solution state we study spin half the maximum number of times.

So, in like in biology we the course this particular course we will be mainly focusing on these 2 hydrogen nitrogen and carbon this 3.

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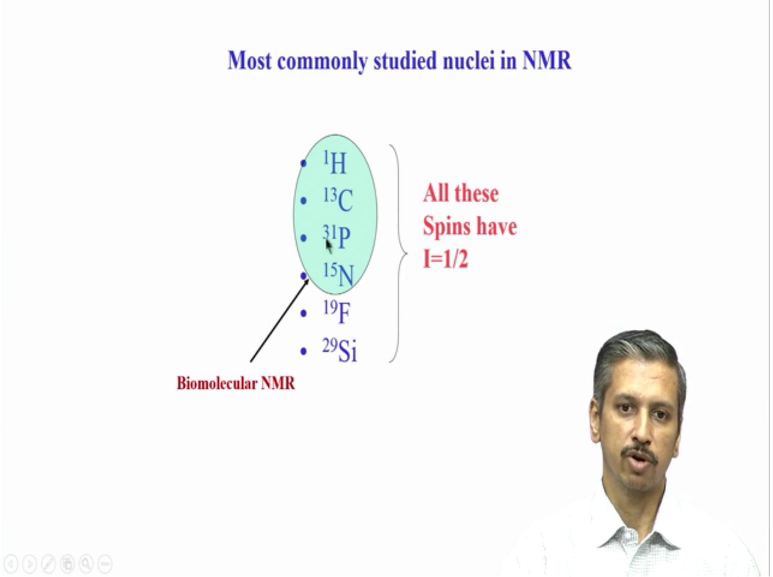

Most commonly studied nuclei in NMR

- ^{1}H
- ^{13}C
- ^{31}P
- ^{15}N
- ^{19}F
- ^{29}Si

All these Spins have I=1/2

Biomolecular NMR

So, if you look at this is what is this state here the most popular nuclei which is studied in NMR are shown here. So, hydrogen which is the most popular this is at natural abundance. Carbon 13 does not exist in natural abundance, it is C 12 which is the 99 percent abundance, but carbon 13 is NMR active carbon 12 we cannot anyway so NMR. So, carbon 13 is used in in molecules and used for structure determination.

In case carbon 13 is low in sensitivity, because is 1 1 1 1 percent, we can enrich the molecule, we can label a molecule and enrich the molecule we C 13 and this is what we use the word isotope labelling, we will see that in the later half of the course. The phosphorus 31 is NMR active in it is natural abundance, meaning this is not an isotope this is what naturally is present in phosphorus. And therefore, it is abundant and it can be studied nitrogen 15 is very important for biomolecules, because remember in biomolecules like in proteins and nucleic acids. Typically hydrogen carbon and nitrogen are the most abundant phosphorus comes in DNA nucleic acids also, but not as popular or abundant as nitrogen and carbon.

Fluorine and silicon these are mainly for materials or drug molecules, which will not go into in this course, but these are also very popular nuclei and they are very well studied and very well studied approachable by NMR spectroscopy. Now, one thing is all of these spins all these nuclei nuclei here have spin half. And that makes them very favourable, because the spin half nuclei is the most NMR friendly nucleus you can think of for studying. So, since as I said in biomolecules these are the 4 nuclei which we will be looking at as we go on.

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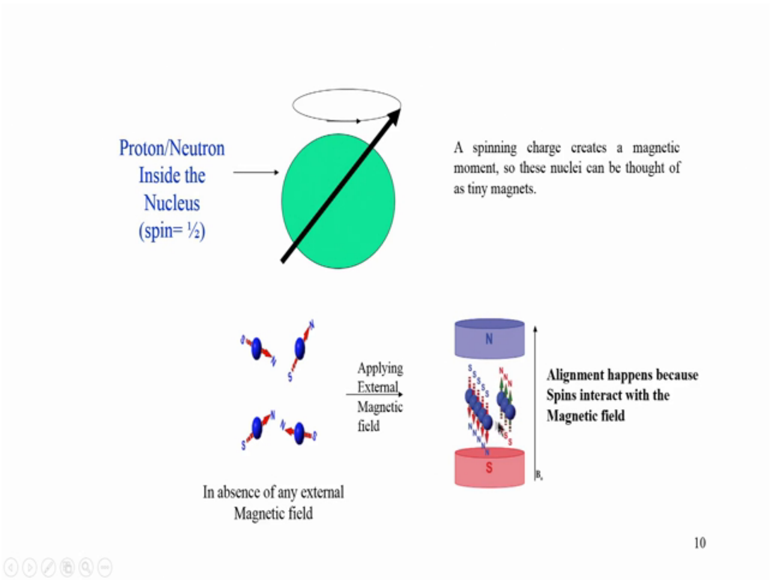

Proton/Neutron Inside the Nucleus (spin= ½)

A spinning charge creates a magnetic moment, so these nuclei can be thought of as tiny magnets.

Applying External Magnetic field

Alignment happens because Spins interact with the Magnetic field

In absence of any external Magnetic field

So, now let us look at the nucleus part is what we finish in the NMR terminology. So, as I said there are 3 terms nuclear magnetic resonance. So, nucleus is something we looked at just now.

Now, let us look at what happens in a magnetic field. So, now, we have to bring in the concept of a magnetic field. Now, if you look at this the very rough or a very approximate description of a nucleus this is a 4 total nucleus. We said that it has a charge because it is positive and also it has a now a total spin value. So, whenever you have a spin a nucleus or any object which has a spin, we say that it is now a spinning charge. And therefore, it becomes a magnet. So, this actually a nucleus is a tiny magnet nucleus is a very small component in an atom, but each nucleus now becomes a magnet because it is having a spin and charge.

So, this small magnets, any magnet which we taken in consider in chemistry and physics we associate what is called as a magnetic moment. So, that is what is written here, we need we every nucleus now has a magnetic moment associated with the nucleus. Now, when this nuclei I mean collection remember we do not deal with one nucleus. So, if I take a glass of water or a sample I will have a large number of molecules in the sample. So, each molecule or each atom will have a nucleus. So, all these nuclei are oriented randomly in absence means in a general in a normal equilibrium situation when there is no external magnetic field.

Now, let us say I take this sample I put it in a strong magnetic field it is a magnetic field here has to be strong, remember the earth magnetic field is already present throughout, but we are looking at something much stronger than earth’s magnetic field. So, when we take this collection of molecules in a magnetic field the molecules now are not randomly oriented, but they have become aligned or either parallel or anti parallel to the main magnetic field. So, why does this alignment suddenly happen when this is random here, it happens because the spins interact with the magnetic field.

So, there is an interaction or there is the magnetic spins are affected by this field which is present in this area this zone. So, that interaction makes them either go parallel or anti parallel and this is very very important for NMR, because this is what we exploit which is what we use for carrying out the NMR experiments. So, now, let us look at this little more deeper in a from a mathematical point of view.

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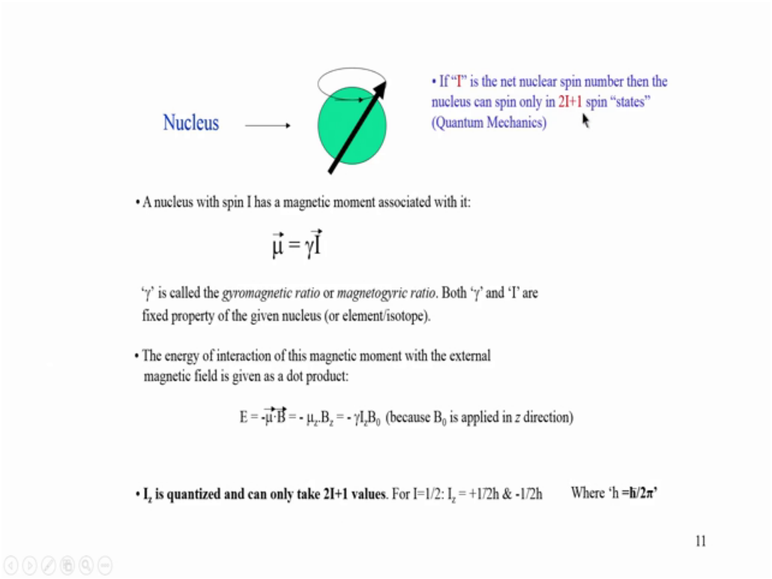

- If "I" is the net nuclear spin number then the nucleus can spin only in 2I+1 spin "states" (Quantum Mechanics)

- A nucleus with spin I has a magnetic moment associated with it:

$$\vec{\mu} = \gamma\vec{I}$$

'γ' is called the *gyromagnetic ratio* or *magnetogyric ratio*. Both 'γ' and 'I' are fixed property of the given nucleus (or element/isotope).

- The energy of interaction of this magnetic moment with the external magnetic field is given as a dot product:

$$E = -\vec{\mu}\cdot\vec{B} = -\mu_z.B_z = -\gamma I_z B_0 \text{ (because } B_0 \text{ is applied in } z \text{ direction)}$$

- **I_z is quantized and can only take 2I+1 values**. For I=1/2: $I_z = +1/2h$ & $-1/2h$ Where '$h = \hbar/2\pi$'

So, this magnetic moment can be written as like this, mu is equal to gamma into I. So, remember throughout this course we were using h as h by 2 pi. So, this is which we should keep in mind that h is not just one single h there is Planck's constant we use h by 2 pi. So, we use the word h bar or h cut.

So, this magnetic moment and now which is associated with this nucleus can be represented by these 2 parameters. The one is called gamma which is gyromagnetic ratio and this is something specific to a particular type of nucleus. So, example hydrogen will have a specific gamma value, if it is carbon 13 it will have another gamma value and so on. So, gamma value is something very unique to a given nucleus and that is something we cannot change. I here as I shown as a vector is a angular momentum. So, again remember look at this picture here it is showing the spin as a spinning nucleus. And, this spinning nucleus essentially has an angular momentum associated with it and that angular momentum is denoted as I.

So, the total the magnetic moment depends on the angular momentum and this gyromagnetic ratio. So, this is our magnetic moment for the interaction with the magnetic field. So, now, when we apply a magnetic field, which is what I showed in the previous slide, the interaction I just mentioned that the spins will interact with the magnetic field and, that interaction is captured in this formula. This is the energy of interaction E is given by minus mu, which is the magnetic moment this one dot in B magnetic field. So, this is a dot product it is not just a simple multiplication it is a dot product. So, this is something which one should be aware of and these are 2 vectors. So, I am taking a dot product.

Now, what happens subsequently is because the B value that is a magnetic field let me just go back to the previous slide, the magnetic field is in these directions. So, we can see this arrow here pointing in this vertical direction. I can call this as z direction z axis. So, if I call this vertical this direction as z axis my magnetic field is essentially along z axis. So, the mu the interaction of the magnetic moment only happens along the z axis. So, the z component of the magnetic moment is what comes into picture we do not worry about the x and the y component and that has no role to play.

So, the same equation is now simplified to this form. Now, mu z if you look at this formula mu z is nothing, but gamma into the I z, I is a vector. So, if I take the z component of mu I should also take the z component of I and that is what is written here. Now, according to quantum mechanics this I z can have 2 I plus 1 value. So, this is what is shown in the top here. So, if I have an I which is for example, half if I put into this 2 into half plus 1 my I z value. Again remember we are trying to calculate the I z value not the I value, I value is given to us as half spin half nucleus. So, the I z value can either take I mean can take 2 values. So, half into 2 plus 1.

So, I z becomes 2 possible value. What are those 2 possible? We will see in the next slide this is basically either half or minus half. So, it can take 2 I. So, example let us say carbon 13 is also spin half. So, carbon 13 spin nucleus also will have 2 parts in the 2 z values 2 I z values plus half and minus half.

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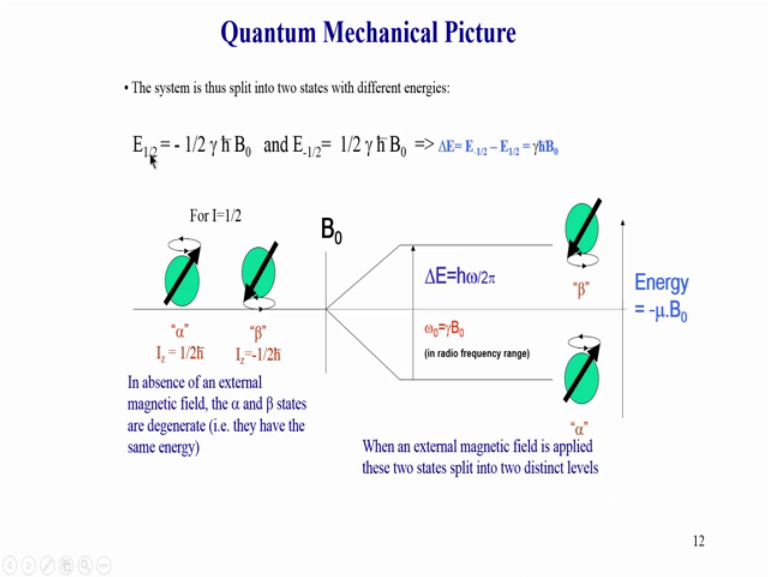

Quantum Mechanical Picture

- The system is thus split into two states with different energies:

$E_{1/2} = -1/2\,\gamma\,\hbar\,B_0$ and $E_{-1/2} = 1/2\,\gamma\,\hbar\,B_0$ => $\Delta E = E_{-1/2} - E_{1/2} = \gamma\hbar B_0$

In absence of an external magnetic field, the α and β states are degenerate (i.e. they have the same energy)

When an external magnetic field is applied these two states split into two distinct levels

So, this is what is shown here; that means if I have the spin is is in plus half state my energy of interaction will be minus half gamma h cross B 0. And, if it is in minus half state then if you look at the previous slide formula the minus will go away, because this is minus half. So, minus half into minus is plus and you end up with this.

So, you see now what has happened is the 2 spin states, that is a hydrogen can exist in 2 spin states. Now, they are no longer same in energy when I apply a magnetic field. Before I apply the magnetic field they were randomly oriented and the energies are all degenerated. In fact, they did not even exist differently, but by adding a magnetic field I created those 2 states or I created a interaction I separated the 2 states into different energy levels ok. Because of this equation here we can see one is positive energy another is negative energy. So, I can say that one is a positive that is this half, that is higher energy and negative is lower energy and soon as.

So, we name these states as alpha and beta this is just an arbitrary nomenclature. So, call the lower one the first half as alpha and the upper energy as beta. So, by doing this applying a magnetic field I have created at 2 states in the system which earlier did not exist.

Now, the molecules have a possibility of either being in the ground state or they can go to the excited state. So, this is the magnetic part of the NMR terminology. So, if you recollect NMR 3 words which I said nuclear magnetic resonance. So, the magnetic field you have plays this role, the role of the magnetic field is to create these 2 energy levels in the system, by simply applying the magnetic field there is nothing else to be done to the sample.

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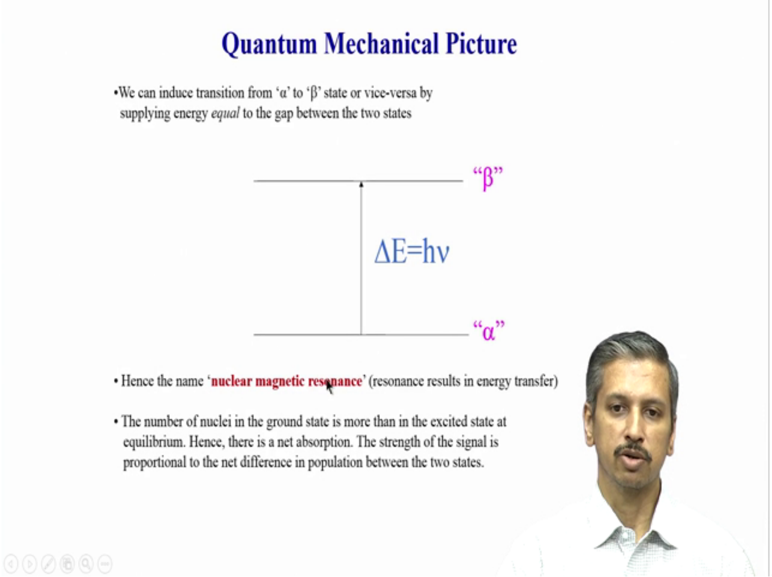

Quantum Mechanical Picture

- We can induce transition from 'α' to 'β' state or vice-versa by supplying energy *equal* to the gap between the two states

- Hence the name '**nuclear magnetic resonance**' (resonance results in energy transfer)
- The number of nuclei in the ground state is more than in the excited state at equilibrium. Hence, there is a net absorption. The strength of the signal is proportional to the net difference in population between the two states.

And, now what is the next thing which we need to do is to now, we can see we have created these 2 energy levels, we now need to excite the molecule from a ground state to the excited state. And, this is something which I mentioned in the beginning in a spectroscopy you shine light, which has energy denoted as h into nu. And, if the molecule has a energy gap which is matches this energy level when the energy of the photon or the radiation. Then, the molecule will absorb the energy and go from alpha to beta; that means; when I create this difference because of the magnetic field. The molecules now which are sitting here will get excited and go to beta if I apply the right amount of energy right energy quantity.

So, that transition we use the word resonance. So, that basically is a third term which I was which I was mentioning in NMR in the resonance condition. So, resonance condition is that you take a molecule sample, which has a non-zero spin value that is hydrogen or carbon 13 put it in a magnetic field, the magnetic field now creates this 2 energy levels. So, molecules get distributed here and here. So, some are sitting here some are sitting here. And, now when I have apply an energy which matches this energy gap, which is external energy which have to supply, it is a radiation have to supply, that radiation now will interact with the sample and the molecules which are sitting here will go up molecules which are sitting there will actually come down.

So, there is a motion going on like this and that word, that transition we use the word resonance. So, this is where the term this is how this word is completed nuclear magnetic resonance. So, nuclear magnetic resonance essentially involves taking a nucleus putting in the in a magnetic field. And, applying a suitable energy which is matches the energy gap and that results in a transition.

Now, one thing which you have to important is to note is that as I said molecules which are here they go up and which are sitting here they actually come down, but are they equally distributed are the number of molecules here equal to that the answer is no.

There are more molecules actually in the ground state then the excited state. We will see that soon and that difference that is more molecules here than here is what is actually very important for NMR. If the molecules numbers were equal, if they were number of molecules here and here were equal then NMR would not be possible, because whatever energy is absorbed from here to here is released from the molecules coming down. So, there is a no net absorption.

So, the whole NMR spectroscopy concept lies on absorption of energy, which happens only if there is more number of molecules in the ground state compared to the upper state. Now, what guarantees or what tells us that there will be more here and more less here, who what kind of law governs that and that is what we will see in the next class.