Structural Biology
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Lecture - 26
Introduction to Spectroscopy and NMR

Hi everyone, welcome again to the course of structural biology as we continue with structural biology techniques and complete X-ray crystallography. Today, in the new module, I am going to teach you NMR spectroscopy. But, as we have gone through the detailing of X-ray crystallography in the case of NMR spectroscopy, I am not going like that; I am just giving you a basic concept that will help you to understand what is NMR spectroscopy and what is its application? And this will help you to take a further interest, knowing more about NMR spectroscopy.

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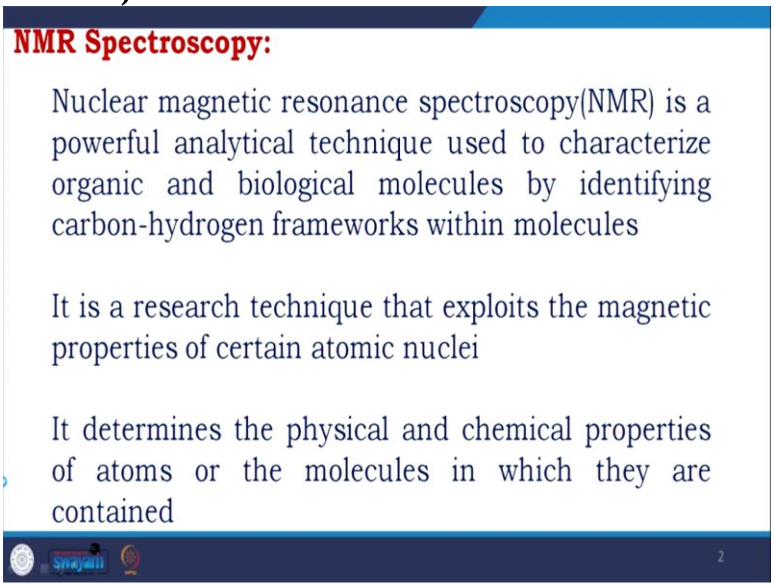

To start with, NMR spectroscopy or nuclear magnetic resonance spectroscopy is a powerful analytical technique used to characterize organic and biological molecules by identifying carbon-hydrogen frameworks within molecules. So, as the definition says, it is an analytical technique. It is a powerful technique, and it has an immense contribution, especially for organic chemists and mainly in the entire branch of chemistry and case of biology understanding biological macromolecules their structure in polymer and various other fields.

Here we are talking about identifying carbon-hydrogen frameworks. I will keep explaining, and you understand that the world is majorly made up of carbon and hydrogen. So, this is where NMR spectroscopy plays an influential role in identification. It is a research technique that exploits the magnetic properties of specific atomic nuclei.

So, as the name suggests, here, we are getting the nucleus correlating to its magnetic property so that we could identify a particular atom or element it determines the physical and chemical properties of atoms or the molecules in which they are content means, it could identify an individual atom or molecules a where a group of atoms or there or even a polymer which is made of repeating units of monomers or combination of different monomers.

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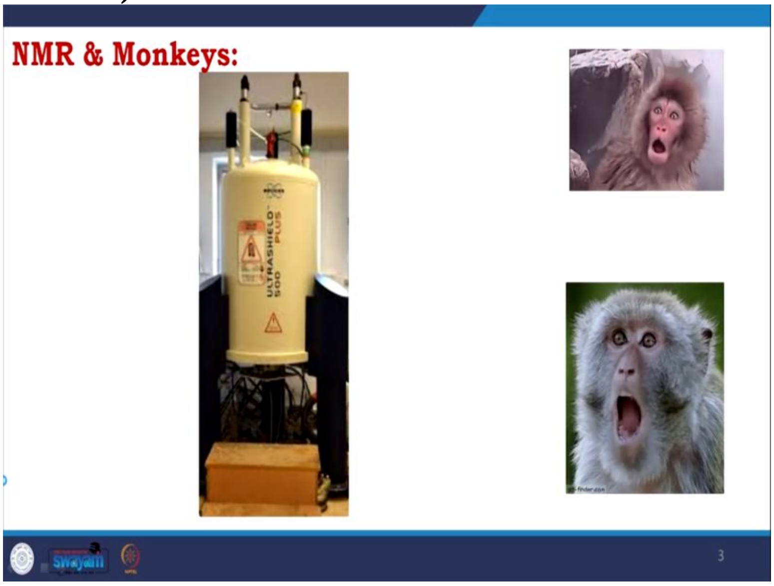

When I talk about NMR, I never forget the story I experienced as a Ph.D. student while doing my Ph.D. at the University of Illinois, Chicago. So what happened, there was a presentation from a company that sells NMR instruments, and the first format picture was there is an NMR machine like that you could see in the screen and a lot of monkeys surrounded the machine. One of our professors just jokingly asked the speaker. Hence, you think we all are listening to you are monkeys, and the speaker representing the company of that NMR instrument, replied, sir, looking at the big area of knowledge you all have. Please pardon me, but when we look at the potential of an NMR instrument, what it could do, and how much it explodes, unfortunately, we all are monkeys. I think I am explaining a little bit you will understand. When we are doing protein crystallography, we are limited to carbon, nitrogen, oxygen detection. Similarly, if you; look at many instrumentations and their relative principles.

You will see that they have their limitations concerning the identification. The instrument or the technique, nuclear magnetic resonance, could easily cover the entire periodic table with only one condition. The element or its isotope have to be NMR active; what I mean by NMR active, let us look at and look at the general application to start with.

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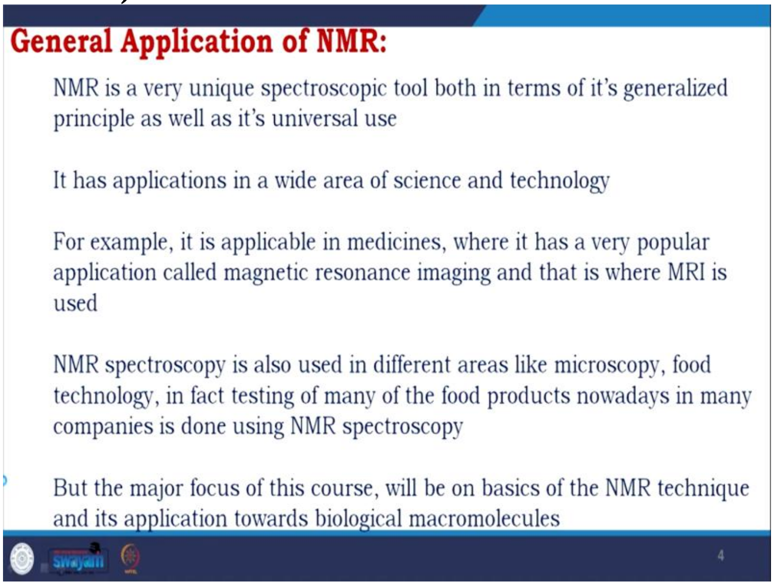

So, I discussed that NMR has diverse applications we will keep talking about, but this is the introduction. NMR is a unique spectroscopic tool, both in terms of its generalized principle and its universal use. So, I introducing you why the principle is generalized? Because, as I told you, it could cover the entire periodic table, it is not restricted to few elements to detect.

And we will discuss that throughout this 5 class module. But on the other side, the application of NMR is also very universal; it has application in a wide area of science and technology. For example, it is applicable in medicine, where it has a very popular application called magnetic resonance imaging or MRI and MRI have a real revolution in medical science. NMR spectroscopy is also used in different areas like microscopy, food technology.

And in fact, testing of many of the food products is now done using NMR spectroscopy in many companies. But the major focus of this course will be the basics of the NMR technique and its application towards biological macromolecules. So, how we proceed with, we will start with the generalized application talking about spectroscopy going focused to NMR spectroscopy looking at the principles and techniques majorly used.

And then how it applies to small molecules because one of the very critical uses of NMR is a small molecule. It has revalorized the place where you know, identifying pure small molecules has performed so that we will talk about them. Then we will go to the biological macromolecule protein.

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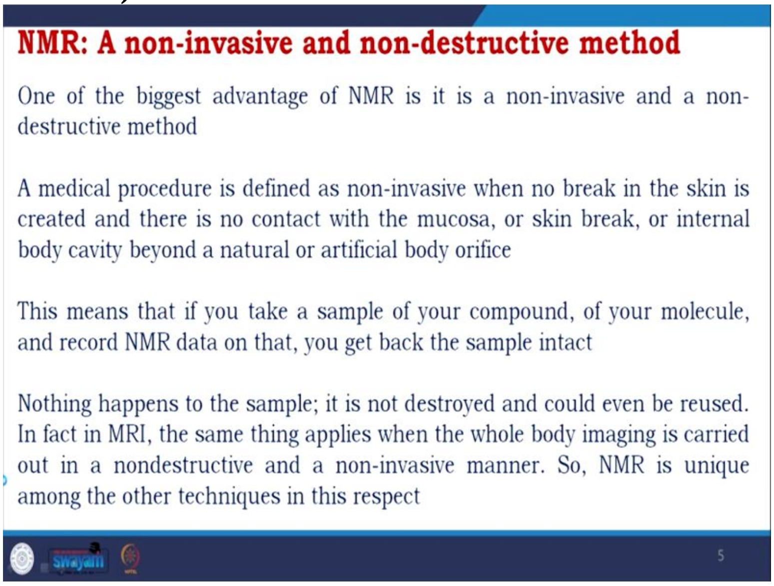

It is a non-invasive and non-destructive method. So, one of the most significant advantages of NMR is it is a non-invasive non-destructive method. So, when I say non-invasive, you could understand that non-destructive does not distract the sample, but what is non-invasive in medical science? Non-invasive is a medical procedure defined when no break in the skin is created, and there is no contact with the mucosa or skin break or internal body cavity beyond a natural or artificial body orifice.

In a word, when we see its technique is applied to a body part and even no minor injury happened. That is called non-invasive. Non-invasive methodologies are getting popular day by day because of their easy to use. Because of their, you know, not putting effect especially on chronic diseases like diabetes and all where you have to check, and every day you have to put medicine.

So, in those sectors, non-invasive techniques are getting popular NMR is a non-invasive technique, and this means that if you take a sample of the compound of your molecule and record NMR data on that, you get back to the sample being intact, nothing happens to the sample, it is not destroyed and could even be reused. That is why you know, if you think it is applied to a living organism, you get the whole body scan.

I hope you get the brain scan and all these things and nothing happened to the person on whom that tests have been done. So, in fact, in MRI, the same thing applies when whole-body imaging is carried out in a non-destructive and non-invasive manner. So, this being a non-invasive and non-destructive method is unique for NMR comparing to other techniques.

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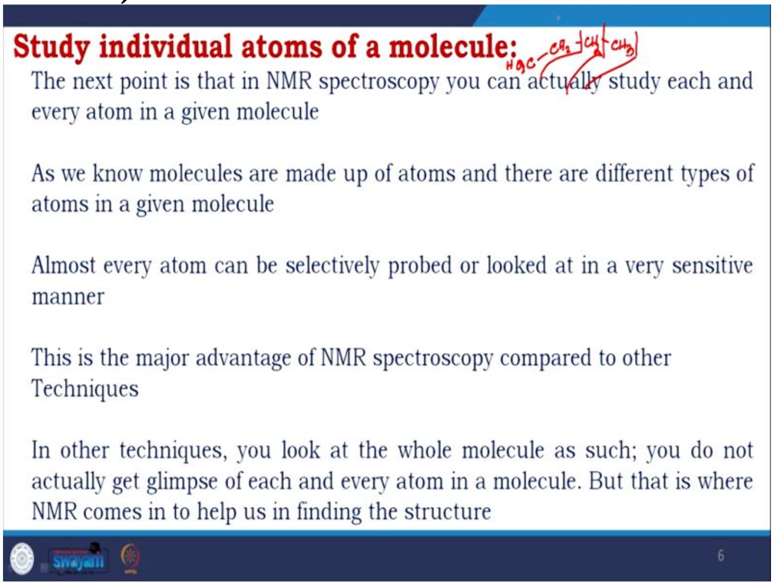

The next point is that in NMR spectroscopy, you can study every atom in a given molecule. As we know, molecules are made up of atoms. There are different types of atoms in a given molecule. Almost every atom can be selectively probed or looked at in a very sensitive manner. This is definitely a major advantage of NMR spectroscopy compared to other techniques.

In other techniques, you look at the whole molecule as such. You do not actually get a glimpse of each and every atom in a molecule, but that is where NMR comes in to help us find the individualistic structure. So, if I have to explain it if you have a compound like propionic acid, four carbon which is propionic, then three-carbon two carbon individualistically, you could differentiate.

Similarly, you could differentiate if there is a hydroxyl group. So, once you do other techniques, it is not that they do not identify. They identify the functional groups and all but this overall identification of every atom and their change in the chemical environment. This is the speciality of NMR, which we are going to discuss in very details.

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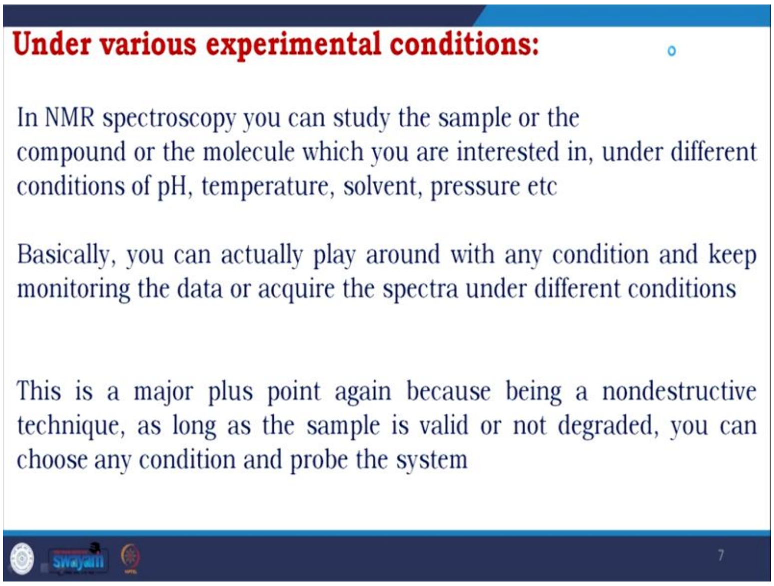

Also, in NMR spectroscopy, you can study the sample or the compound or the molecule, which you are interested in under different conditions of pH, temperature, solvent pressure, so, with different treatment, whatever the effect is coming on the molecule on the polymer, anything with the change of pH change of temperature, with different solvent everything you could get to study using the technique of NMR spectroscopy.

Basically, you can play around with any condition and monitor the data or acquire the spectra under different conditions. So, this is even interesting. So, in many techniques, you treat with something you make the sample after sample preparation, you go for taking data, you cannot change it, but in case of NMR you could have keep the sample let us say you have a protein. You keep the protein at seven pH and then keep increasing the pH and acquire data or keep decreasing the pH and acquire data. You increase the temperature you start from 0 degrees you go to different temperatures and see the effect of the temperature on this protein molecule. So you could take data at various conditions and take it in 1 sample that is even more, you know, helpful.

Again, this is the major plus point because being a non-destructive technique, as we have already discussed, as long as the sample is valid or not degraded, you can choose any condition and probe the system. So, it is easy to do a lot of conditions, a lot of variations, more user-friendly way than even with some instruments where you could do different conditions. Still, you have to prepare samples for individual conditions.

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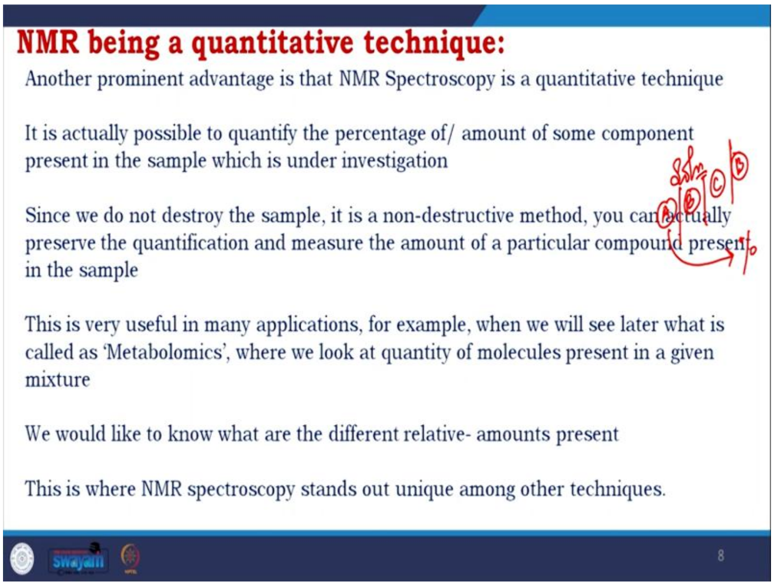

In addition to that, NMR is also a quantitative technique, so, another prominent advantage is that NMR spectroscopy is a quantitative technique. What is mean by quantitative technique? That means it is possible to quantify the percentage of or the amount of some component present in the sample under investigation. What do we mean? Suppose there is a solution. There is compound A, B, C and D as we have previously talked about. You could have identified them individually at the structural level and seen their effect in various conditions. Still, more interestingly, you could also say the percentage of compound A in this sample mixture. So, that is why it is called a quantitative technique. Since we do not destroy the sample, it is a non-destructive method.

You can preserve the quantification and measure the number of particular compounds present in the sample. This is very useful in many applications, for example, when we will see later what is called metabolomics. So, NMR metabolomics is very upcoming and it is a revolution because you could have even identified components from a very complex mixture of cells.

So, we will discuss that in the application part where we look at the quantity of molecules present in a given mixture. So, this quantitation helping the technique NMR metabolomics and nowadays, people are actually combining the technique of mass spectrometry with NMR for metabolomics.

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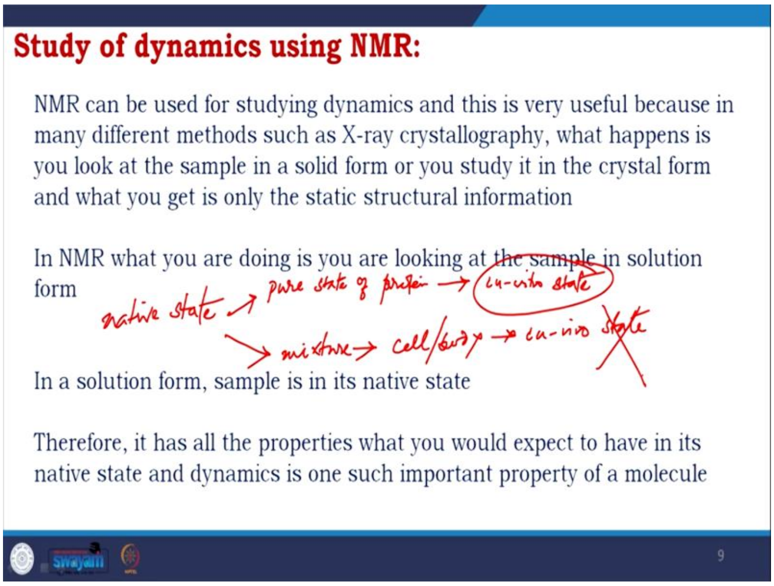

So, NMR can be used for studying dynamics and this is very useful because in many different methods such as X-ray crystallography, what happens is you look at the sample in a solid form, or you study it in that crystal form and what you get is only the static structural information, which means a set of coordinates you are already knowledgeable about x-ray crystallography technique.

I have discussed a lot about what is happening in protein X-Ray crystallography. So, you know that we have the crystal; we diffract the crystal and get the coordinates. We will discuss the details of the coordinates, but you know that we have the coordinates, so you have one coordinate whereas, in NMR, you are looking at the sample in solutions, so you are studying dynamics.

And in addition, in the solution form, the sample is close to the native state where you are doing many other experiments. So, it has all the properties that you would expect to have in its native state and dynamics is one such important property. Now, when I say about, I would also alert you about something that you could probably miss conceptualized in this statement. So, when I say native state, if you remember correctly, I have discussed this before. One of the native states is the pure state of a protein which we call in-vitro state. The other one is in the mixture; mixture means in a cell in a body called in-vivo state. When I say native state here; I mean in-vitro state, not the in-vivo state. So, yes, it is not precisely the function of a protein inside a human body or body like the body of a mouse or at least in a cell; it is about the pure protein.

But still, there are clear advantages over other techniques like crystallography in crystallography. When you are working, you are putting a lot of precipitants which you call crystallization solution. You put them because you want to achieve the phase where it is crystallized, so you push them to precipitate that we call incrystallo state and incrystallo state having a high concentration of precipitants is very different from in-vitro state, and that is where the beauty of NMR technique.

So, I hope I could successfully explain. So, here native state means in-vitro state and as we know that, there are many experimental techniques; where the experiments are performing in-vitro state, it is always at one stage to compare them and understand the different properties, which is not possible using the technique of X-ray crystallography.

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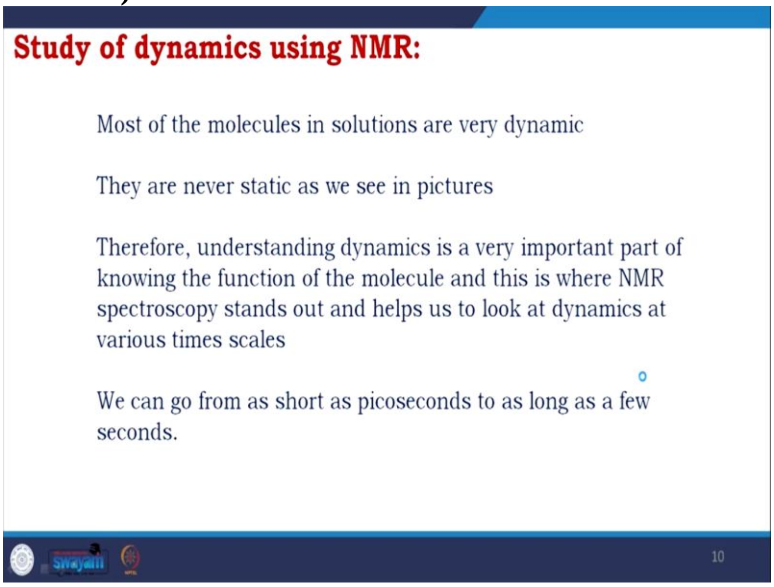

Most of the molecules in the solution are very dynamic. They are never static, as we see in the pictures. Therefore, understanding dynamics is an essential part of knowing the molecule's function. This is where NMR spectroscopy stands out and helps us to look at the dynamics at various timescales. So, we have discussed it before when we have a static structure.

It might be a high-resolution structure. It might be swung us very integrate details of the atomic interactions, but it is not showing us different conditions; it is not showing us what changes are happening when it is in solution. That is where NMR is giving us the opportunity. We can go from as short as picoseconds to as long as few seconds. So, you could catch those biological phenomena by acquiring data from the solution using the technique of NMR spectroscopy.

So, NMR is a spectroscopy technique, so we want to start with the basic spectroscopy principle. So, we talk about the various application of NMR. Now, we are trying to understand the details of the technique. First, we would love to go through the first little bit of spectroscopy because we know that in the next module, we are continuing with the other low resolution spectroscopy techniques: UV viz spectroscopy, circular dichroism, fluorescence spectroscopy, Raman spectroscopy, IR and all. So, this is a continuation so that we will start understanding spectroscopy. Spectroscopy is about the interaction of light with matter.

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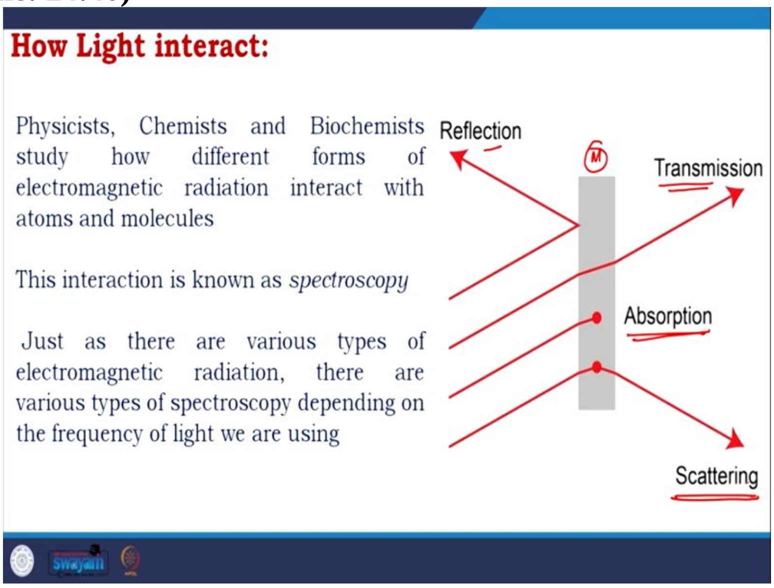

Physicists, chemists and biochemists study how different forms of electromagnetic radiation interact with atoms and molecules. If you see this is the matter and the energy coming to the electromagnetic wave, there are different effects. If it is reflecting, it is called reflection. If it goes through, it is called transmission. If it is absorbed with the matter, it is called absorption. If it is scattered, it is called scattering. So, the interaction of the electromagnetic wave with the matter is not one dimensional, there are many forms of the interaction many effects of the interaction and as I say, physicists, chemists and biochemists study how different forms of electromagnetic radiation interact with atoms and molecules and what are the different effects that would help us to understand or characterize the molecules.

If there are any fingerprints, that is what we are looking for, and this interaction, we call it spectroscopy, just as there are various types of electromagnetic radiation, there are various types of spectroscopy depending on the frequency of light, we are using the effect what a swing there and all many different things is.

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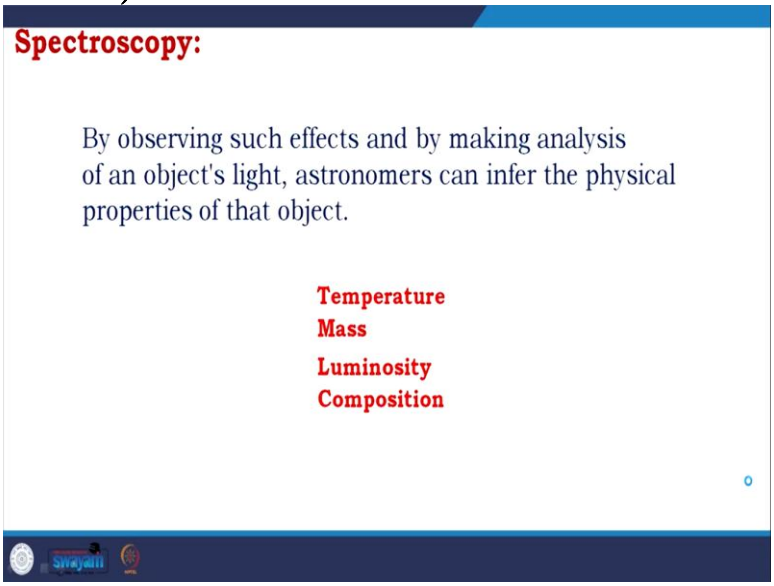

So, spectroscopy by observing such effects and by analyzing an object’s light astronomers at the starting of where spectroscopy developed can infer the physical properties of that object; they used to see that change of temperature, they used to see the change of mass they used to see the change of luminosity. They used to identify the composition and those early days characterization study later in different forms comes to us as different spectroscopy techniques.

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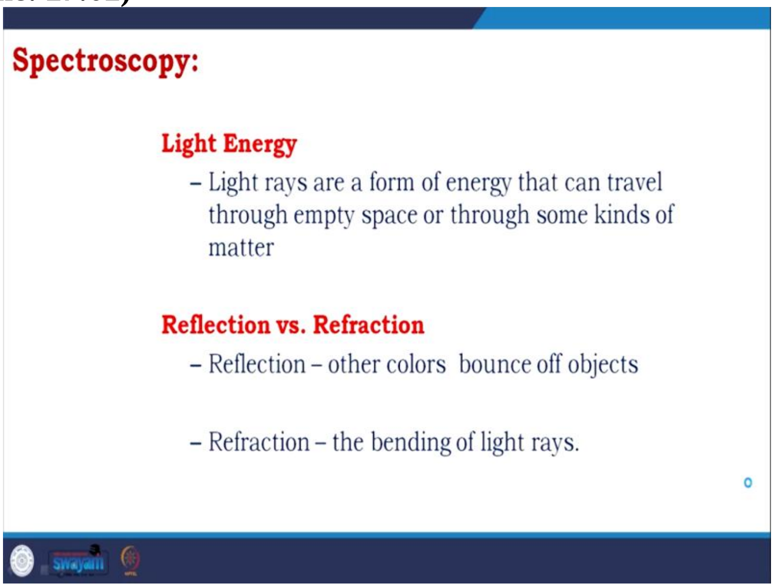

In spectroscopy, light rays are a form of energy that can travel through space or some kind of matter. If we see two terms, reflection versus refraction, reflection is other colors bounce off objects and refraction, the bending of light rays.

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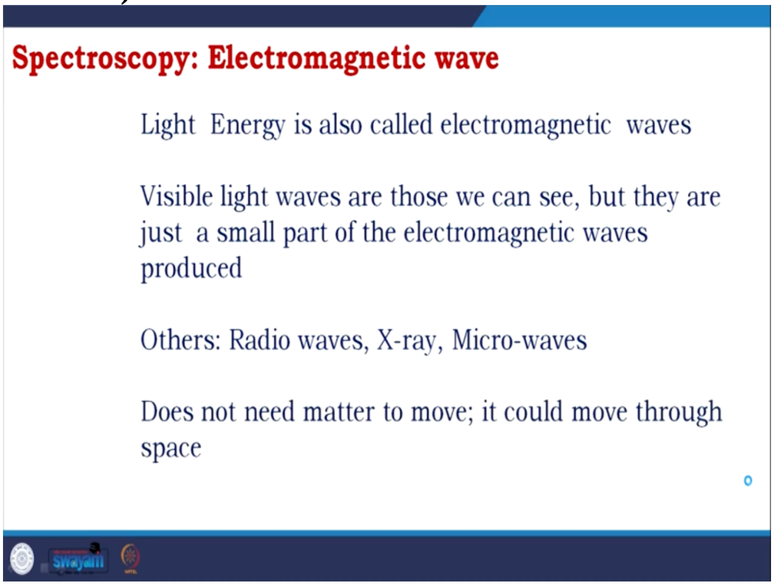

Light energies are also called electromagnetic waves, visible light waves, but they are just a small part of the electromagnetic waves produced. Others are radio waves, X-ray microwaves, and many it does not need matter to move because it could move through space.

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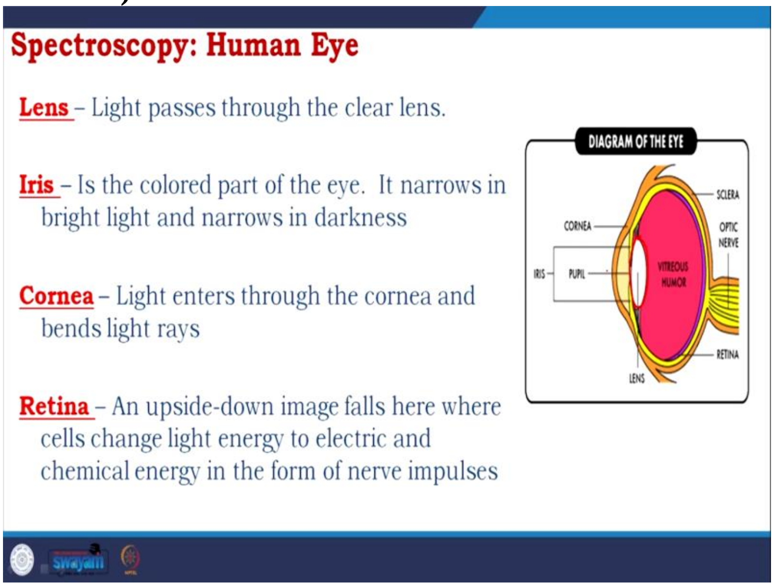

When we talk about spectroscopy, this is a good opportunity to talk about the human eye and you will be amazed to see how this technical, biological formation has developed. It has a lens where the light passes through the clear lens; Iris is the colour part of the eye; it narrows in bright light and it narrows in darkness. Cornea, is the part where light enters and bends light rays.

Retina is an upside-down image of false hair, where cells change light energy to electric and chemical energy in the form of nerve impulses in biology. You see something but it is not only a technological scene, if you see something, your brain has to read that. So the seeing through eyes to be converted to reading of the brain, which is done by retina if you see this retina, it changed the light energy which is coming to electric and chemical energy in the form of nerve impulses the nerve goes then and make this message established in the brain.

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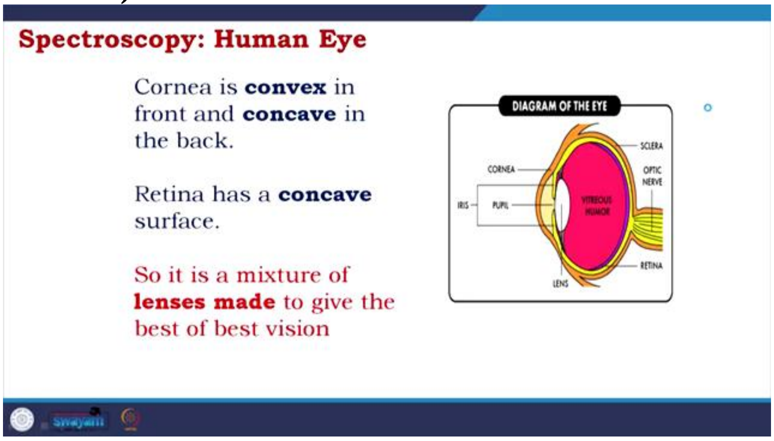

Cornea is convex in the front end concave in the back retina has a concave surface. So it is a mixture of lenses made to keep the best of the best vision.

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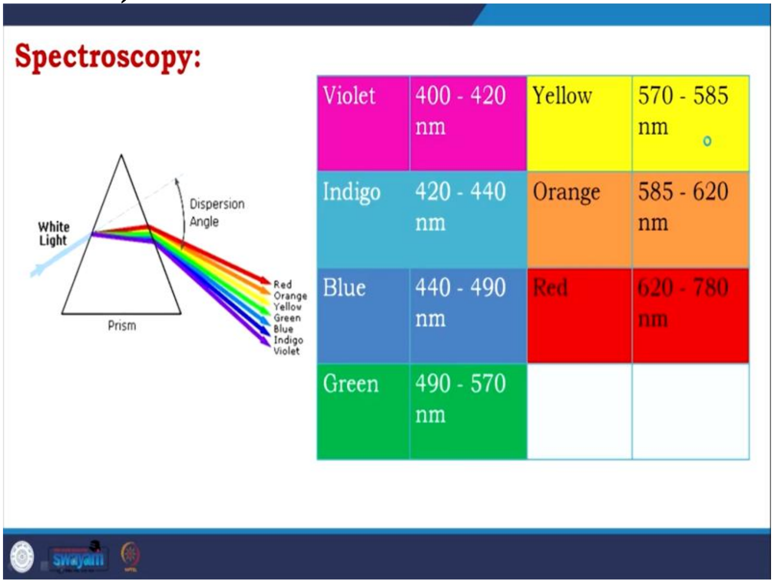

So, if you see the prism, white light comes here. They have making disperse an angle and white light it divided to several light rays, red, orange, yellow, green, blue, indigo, violet, where violet is the lowest nanometre containing rays 400 to 420, indigo 420 to 440, blue 440 to 490, green 490 to 570, yellow is coming in the middle 570 to 585, orange 585 to 620 and red is at the top 620 to 780.

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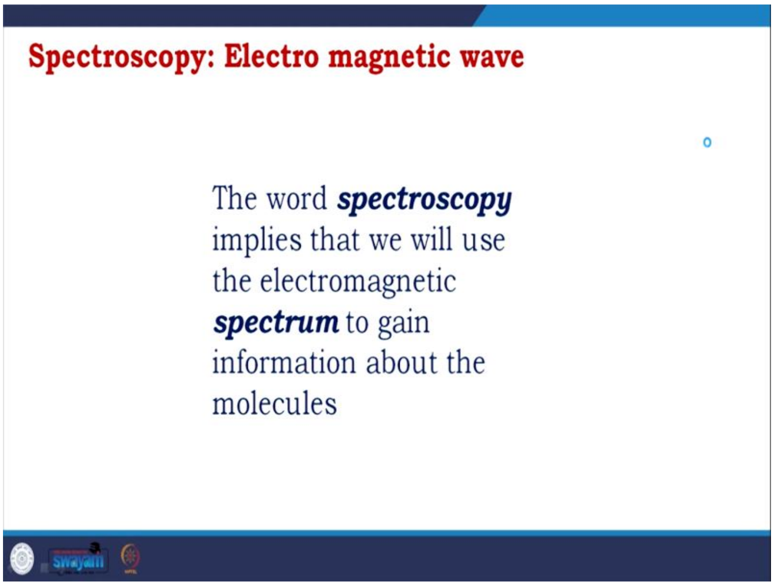

The word spectroscopy implies what we will use the electromagnetic spectrum. So, it implies that we will use the electromagnetic spectrum to gain information about the molecule. So, we put the ray, it interacts with matter and a lot of changes happen. Looking at those changes, if we could characterize the properties of molecules or anything, we want to study our sample that is called spectroscopy. This is basic instrumentation of spectroscopy where you see, you have first a light source, we talked about normal light, but it could be different light also different rays, which we will talk about later.

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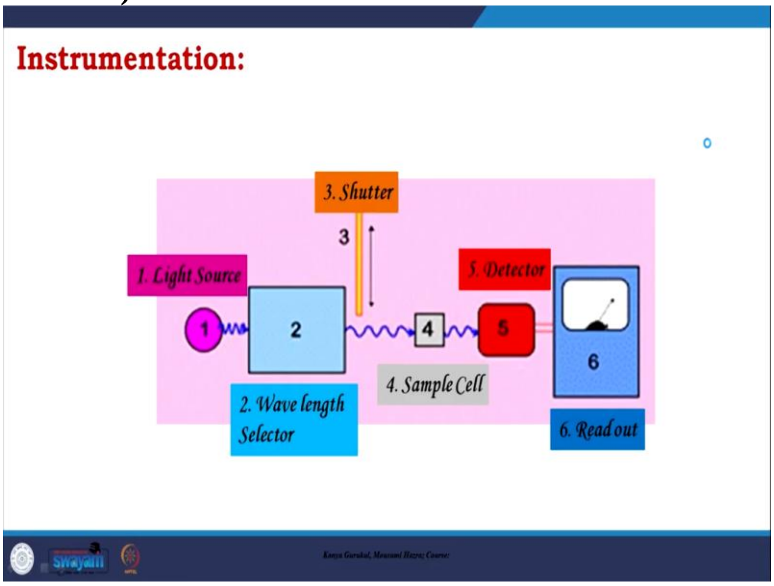

Then when the light comes, it would be coming to the wavelength selector, then there is a shutter, the shutter would control the light ray to go and hit the sample. After that, there is a sample cell where the sample would be kept, then the effect would be kept in the detector, and finally, we will get the readout. So, that is the architecture of the very basic instrumentation of spectroscopy. We will go to different spectroscopy and we will try to discuss the individualistic setup of that instrument.

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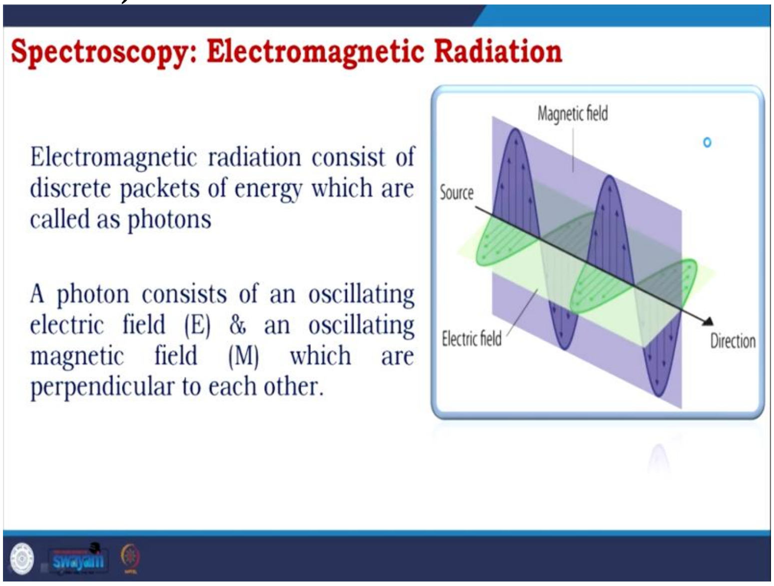

Spectroscopy about electromagnetic radiation we talked about electromagnetic radiation consists of discrete packets of energy, which are called as photons. A photon consists of an oscillating electric field E and an oscillating magnetic field M, which are perpendicular to each other.

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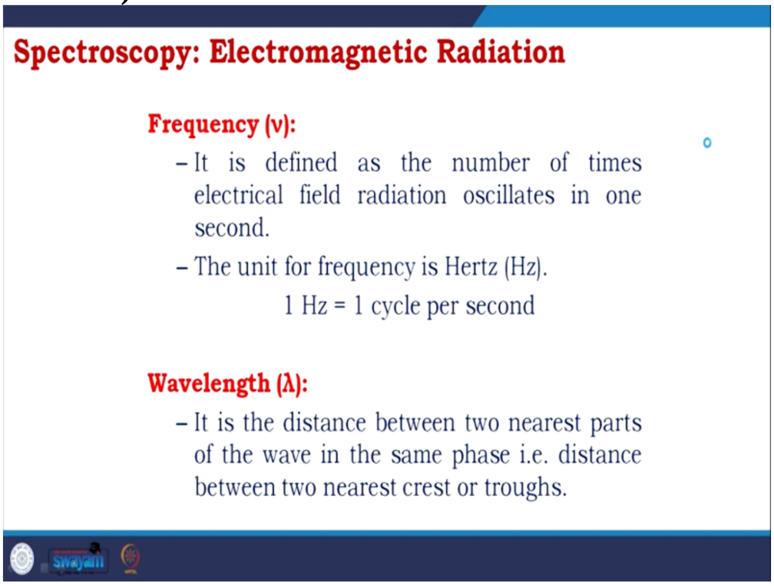

We also have to talk about a few things. Frequency, frequency is defined as the number of times electric field radiation oscillates in one second. The unit of frequencies is Hertz.

1 Hertz = 1 cycle per second.

Wavelength is the distance between two nearest parts of the wave in the same phase, the distance between two nearest crests or troughs.

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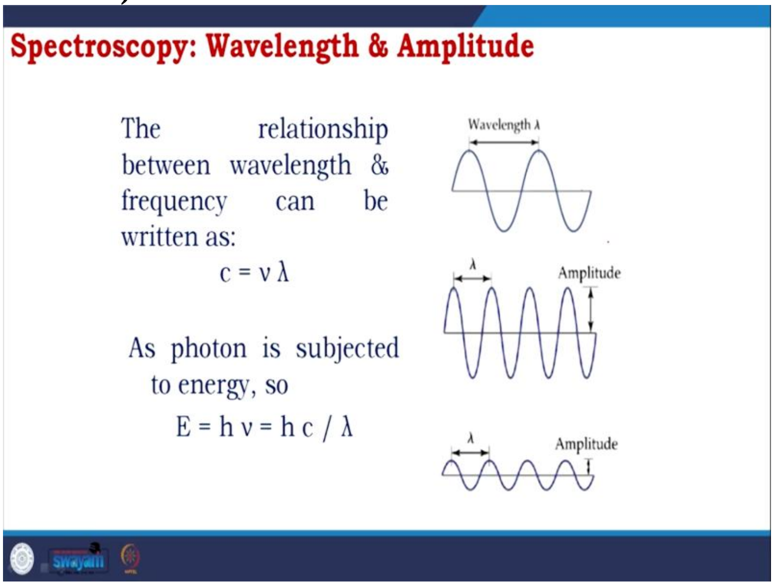

The relationship between wavelength and frequency can be written as

$c = \nu\lambda$

as the photon is subjected to energy, so,

$E = h\nu = hc/\lambda$

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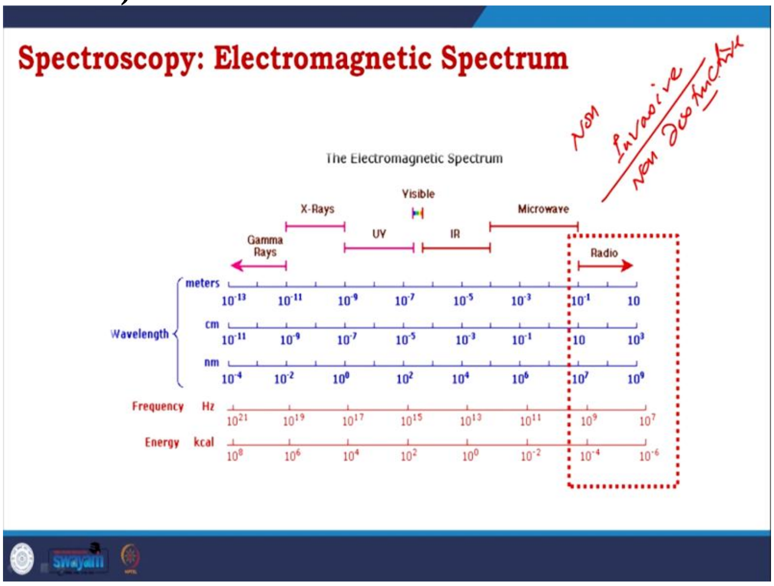

This is called the electromagnetic spectrum, where you see the different rays where they are. So, if you see gamma rays in nanometers, they are 10^{-4} to 10^{-2}, in centimeters 10^{-11} to 10^{-9}, in meters 10^{-13} to 10^{-11}. Between then 10^{-2} nanometer to 10 nanometers, it is X-rays and then 10 to 10^{2} it is UV, 10^{2} to 10^{4} it is visible IR. Then from 10^{6} to 10^{7}, it is microwave. 10^{7} to 10^{9} is radio. So, these are wavelengths and if you see, when we get the wavelengths with increasing wavelength, it is decreasing frequency and decreasing energy. So, gamma rays are around 10^{8} to 10^{6} kcal, whereas radio waves are 10^{-4} to 10^{-6} kcal/mole.

So, we are focusing on radio waves, because, in NMR, we are using radio waves. You will also understand what I mean because this is about larger wavelength when I was talking about the invasive non-destructive technique. So, lower frequency and lower energy, because low energy does not harm the sample. So, you could keep reusing it also; you could apply longer exposure because of the low energy.

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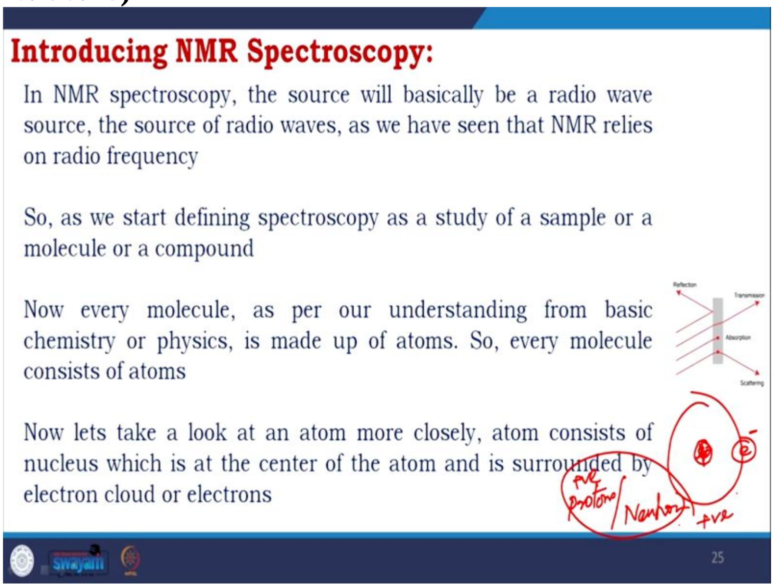

So, in NMR spectroscopy, the source will basically be a radio wave source the source of radio wave as we have seen that NMR relies on radiofrequency. So, as we start defining spectroscopy as a study of sample or a molecule or a compound. Now, every molecule as per our understanding form basic chemistry or physics is made up of atoms. So, every molecule consists of atoms.

Now, let us take a look at an atom more closely. Atom consists of nucleus, which is at the center of the atom and is surrounded by electron cloud. So, if we go for a basic, this is the nucleus and this is the electrons. This is electrons where negative charge and nucleus is positive charge because it has protons and neutrons. Protons are positive neutrons are neutral. So, overall they are positive. So, that is the basic setup.

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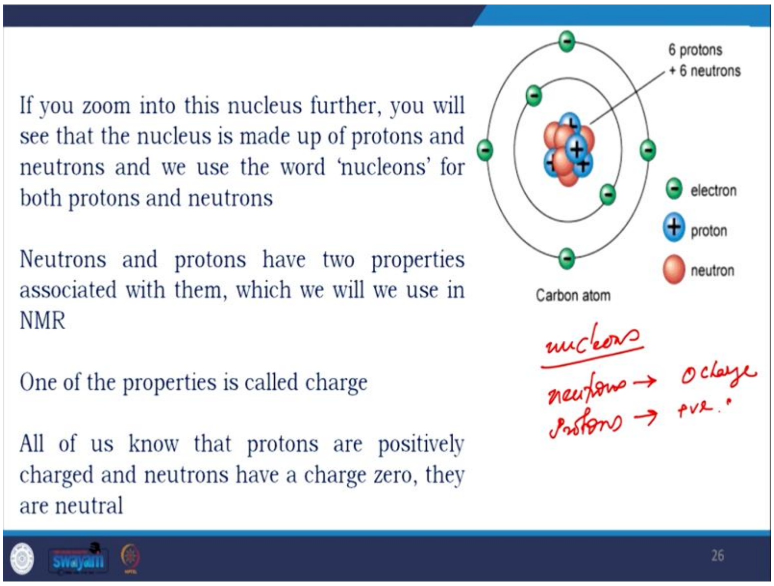

We could easily see that, here you could see, for the carbon atoms, 6 electrons are there, 6 neutrons are there and 6 protons are there. So, if we zoom into the nucleus further, we will see that nucleus is made up of protons and neutrons and we use the word nucleons for both protons and neutrons being together. Neutrons and protons have two properties associated with them, which we will use in NMR.

One of the properties is called charge. So, what do you know about charge so, nucleons it is about neutrons, which are neutral 0 charge, protons they are positive charge, So, overall it is positive.

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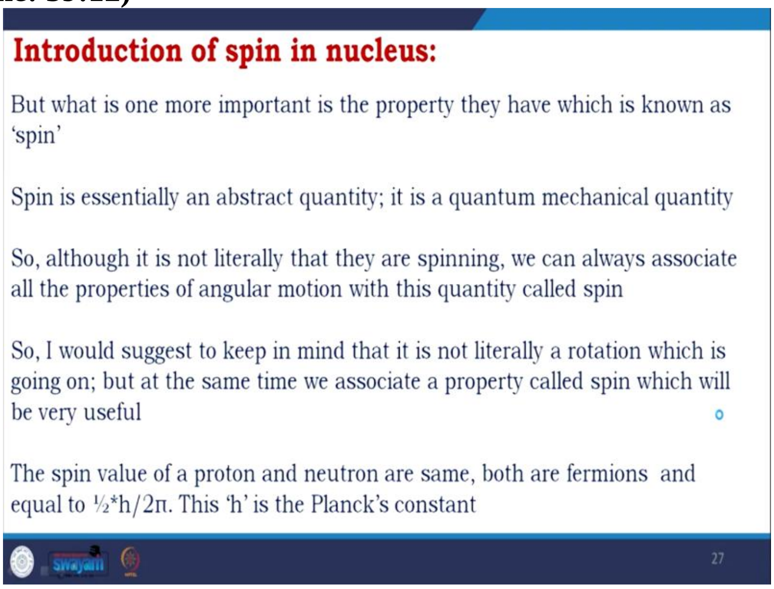

But beside charge, there is one more important property, which I will introduce, and where NMR is mostly based on his spin. Spin is essentially an abstract quantity it is a quantum mechanical quantity. Although it is not literally that they are spinning, we can always associate all the properties of angular motion with this quantity called spin. So it is like the angular motion, which is its property.

The spin values of a proton and neutron are the same; both are fermions in terms of that and equal to 1/2 h/2π, where h is Planck's constant.

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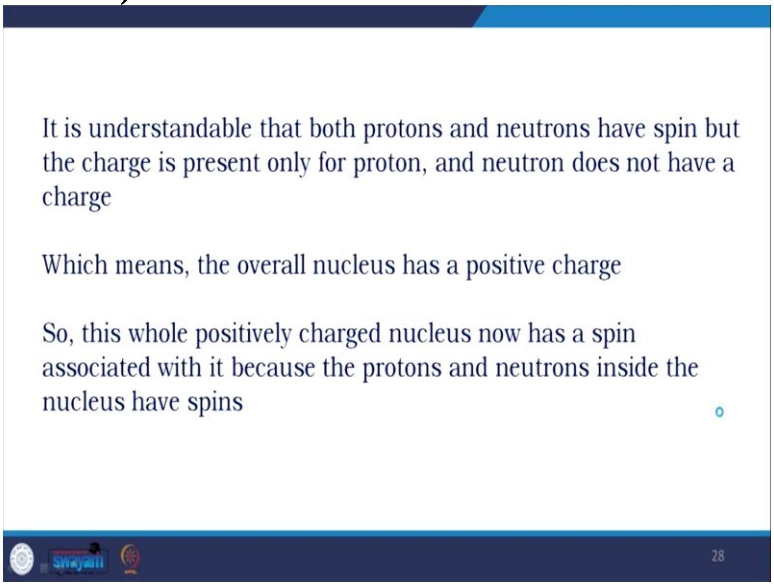

Now, it is understandable that both protons and neutrons have spin, but the charge is present only for proton as I told and neutron does not have a charge, which means the overall nucleus has a positive charge. So, this whole positively charged nucleus now has a spin associated with it, because the protons and neutrons inside the nucleus have spins.

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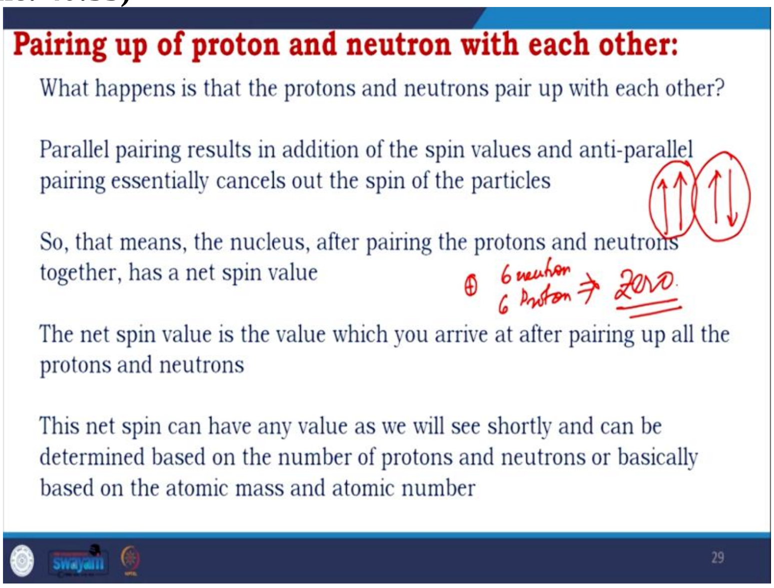

Now, we will look at pairing up of proton and neutron with each other. So, what happens is that the protons and neutrons are paring up with each other. Parallel paring results in addition of the spin values and anti-parallel paring essentially cancels out the spin of the particles. So, it would be parallel pairing or it would be anti-parallel pairing. That means the nucleus after pairing the protons and neutrons together has a net spin value.

The net spin value is the value you arrive at after pairing all the protons and neutrons. So, you have the nucleus, let us say carbon, you have 6 neutron, you have 6 proton. So, if you pair up, it would have cancelled each other. This net spin can have any value as we will see shortly and can be determined based on the number of protons and neutrons or basically based on the atomic mass and atomic number.

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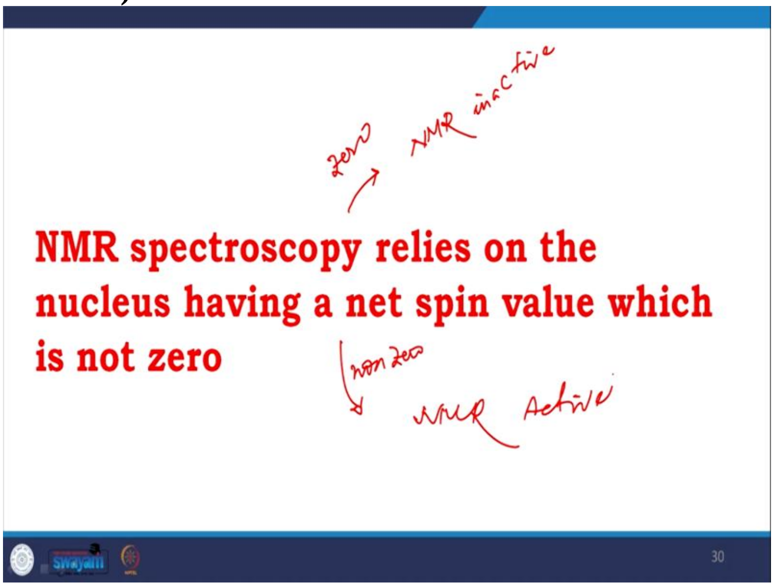

Let us see and remember that very honestly and critically, NMR spectroscopy relies on the nucleus having a net spin value, which is not 0. So if it is 0 that is NMR inactive. If it is nonzero, it is in NMR active easy to remember, but very critical.

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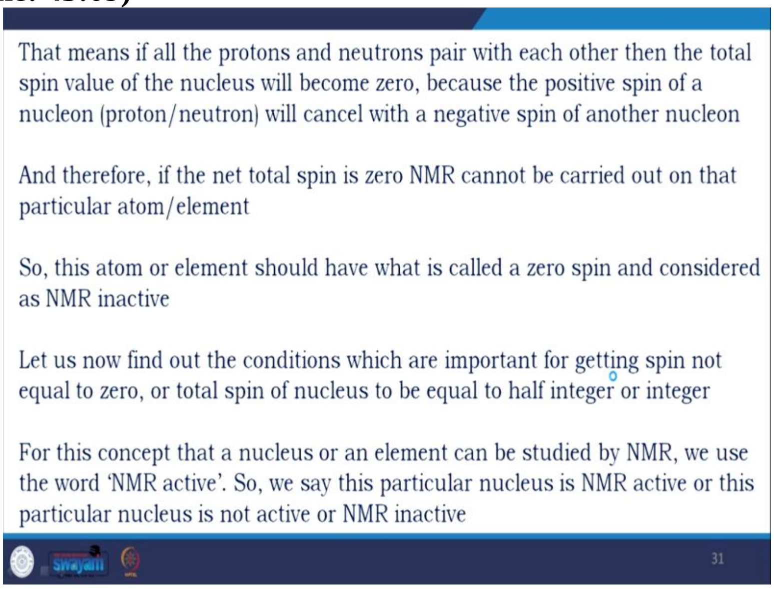

Let us now find out the important conditions for getting spin not equal to 0 or the total spin of the nucleus equal to half integer or integer.

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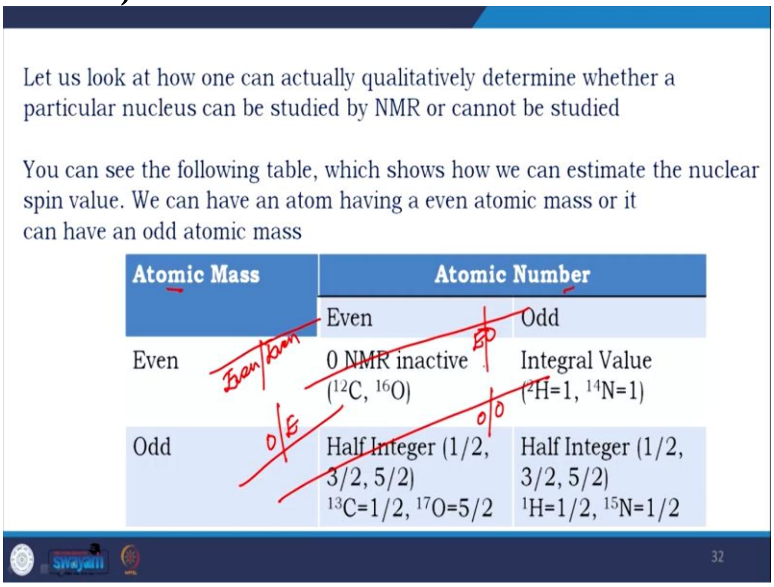

Let us look at how one can qualitatively determine whether a particular nucleus can be studied by NMR or cannot be studied. You can see the table here which shows how we can estimate the nuclear spin value. We can have an atom having a even atomic mass or it can have an odd atomic mass. So, there are 2 things atomic mass and atomic number, atomic mass could be even-odd atomic number could be even-odd.

So, there are 4 conditions one is even-even. So, they are 0 (NMR inactive) like 12C like 16O (oxygen). So, this is even, even or E/E then O/E, half integer 1 / 2, 3 / 2, 5 / 2, 13 C = 1 / 2 17O is 5 / 2, and then we will go for E/O even-odd integral values 2 H = 1 14 N = 1, then we go for O/O half integer 1H equal to half, 15 N equal to half. So, we have four conditions E/E, O/E, E/O, O/O, and this is how we could determine the NMR active or NMR inactive elements.

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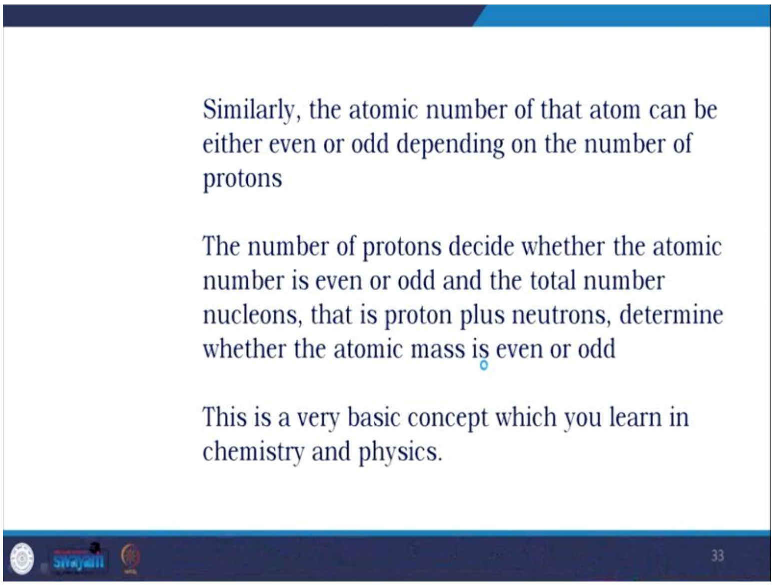

Similarly, the atomic number of that atom can be either even or odd depending on the number of protons. The number of protons decides whether the atomic number is even or odd and the total number of nucleons that is proton plus neutron determines whether the atomic mass is even or odd. This is a very basic concept you learn in chemistry and physics if you have a basic idea about the atom and atomic numbers.

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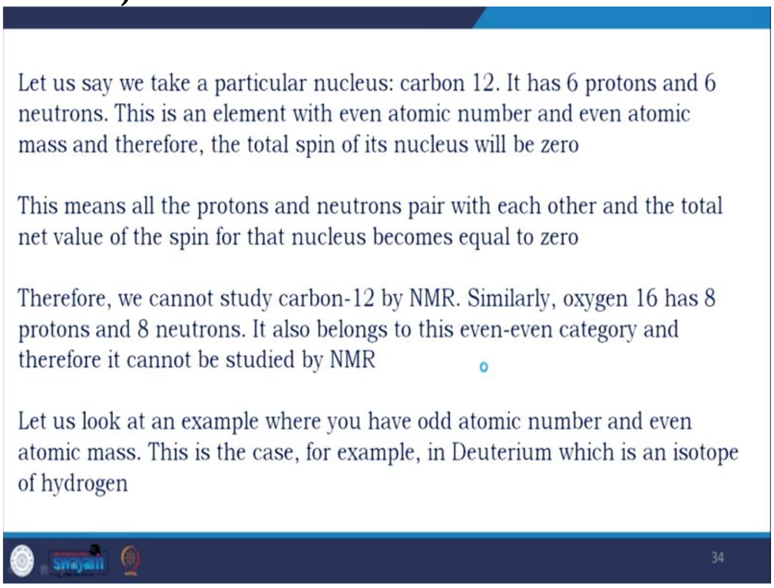

We will go through few examples. Let us say we take a particular nucleus carbon 12 it has 6 protons and 6 neutrons this is an element with even atomic number and even atomic mass and therefore, the total spin of its nucleus have to be 0 very confidently 0. This means all the protons and neutrons pair with each other and the total net value of the spin for that nucleus becomes equal to 0.

Therefore, we cannot study carbon 12 by NMR. Similarly, oxygen 16 has 8 protons and 8 neutrons it also belongs to this even-even category and therefore, it cannot be studied by NMR. Let us look at an example where you have all the atomic number and even atomic mass this is the case for example in Deuterium which is an isotope of hydrogen.

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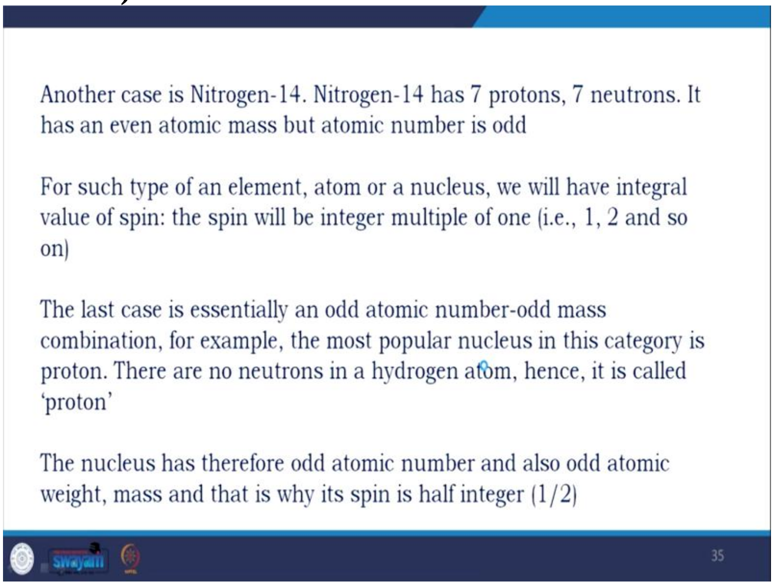

Another case is nitrogen-14 nitrogen 14 has 7 protons 7 neutrons it has an even atomic mass, but atomic number is odd here for such type of an element atom or a nucleus we will have integral value of spin; the spin will be integer multiple of 1, 1, 2 like this. The last case is essentially an odd atomic number-odd mass combination for example, the most popular nucleus in this category is protons there are no neutrons in a hydrogen atom since it is called proton. The nucleus has therefore, odd atomic number and also odd atomic weight mass and that is why it is spin is half integer.

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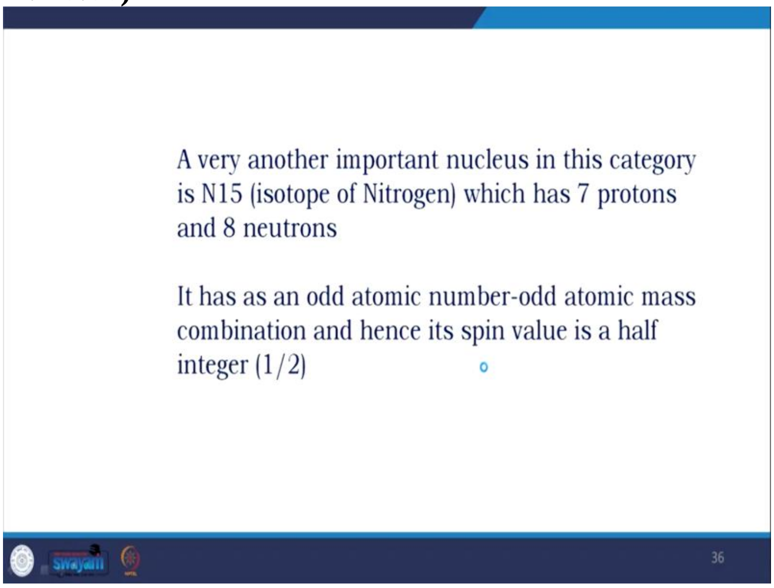

A very another important nucleus in these categories N15 isotope of nitrogen, which has 7 protons and 8 neutrons, it also as an odd atomic number or atomic mass combination and hence its spin value is half integer.

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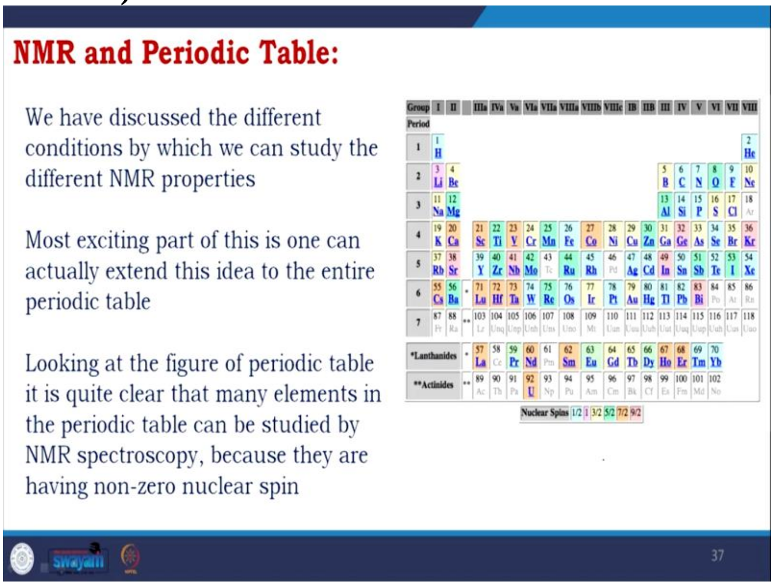

Now, we have discussed the different conditions by which we can study the different NMR properties. Most exciting part of this is one can actually extend this idea to the entire periodic table. Now, if you look at this figure in the periodic table, it is quite clear that many elements in the periodic table can be studied by NMR spectroscopy because they are having non-zero nuclear spin.

You see the nuclear spins half, 1, 3 / 2, 5 / 2, 7 / 2, 9 / 2 all are having different colour codes which you could see here. Now, I could again once talk about the concept of this NMR and monkey you could see this periodic table, look at the huge potential and you could fill for 1 minute that with the potential of NMR technique or the generalized versatile principle of NMR we are in true sense remaining monkey to explore it.

And if only the presence are covering the periodic table is not enough, I will talk about the isotopes, what is about isotopes, as you will remember I told that C 12 is completely NMR in active, but not C 13, C 13 is the isotope of carbon, which is very very less abundant in nature, but it is NMR active.

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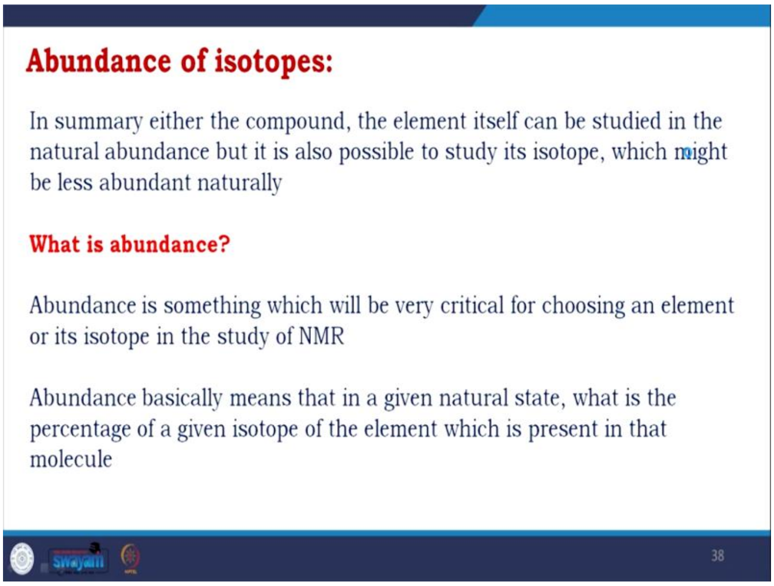

So, in summary, either the compound the element itself can be studied in the natural abundance part, it is also possible to study its isotope which might be less abundant naturally. So, we are talking about abundance what is abundance, abundance is something which will be very critical for choosing an element or its isotope in the study of NMR abundance basically means that in a given natural state, what is the percentage of a given isotope of the element which is present in that molecule.

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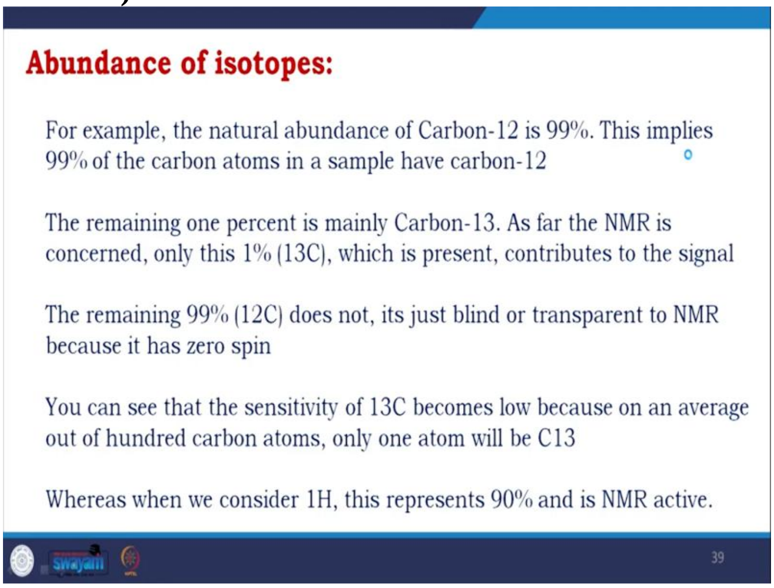

We will talk with you know examples for example, the natural abundance of carbon 12 is 99% this implies 99% of the carbon atom in the sample have carbon 12. The remaining 1% is mainly carbon 13. As far the NMR is considered only this 1% 13C which is present contributes to the signal in the technique of NMR spectroscopy. The remaining 99% does not it is just blind or transparent to NMR because it has 0 spin.

You can see that the sensitivity of 13C becomes low because on average out of 100 carbon atoms only one atom will be C13 because of that, when you were using carbon to study NMR you get very low intensity signal because of the less presence of the C13 and that is called abundance. Whereas, as I told when we consider 1H it represents 90% and is NMR active.

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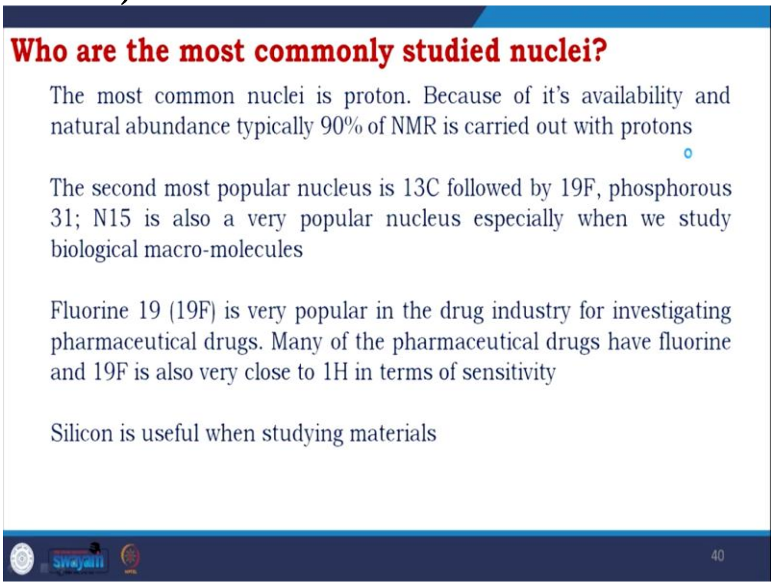

So, the most common nuclei is proton because of its availability and natural abundance typically 90% of NMR is carried out with protons. The second most popular nucleus is 13C followed by 19F fluorine is coming as very important probe phosphorus 31 N15 is also very popular nucleus, especially when we study biological macromolecules protein. Fluorine is a very popular in drug industry for investigating pharmaceutical drug. Many of the pharmaceutical drugs of fluorine and 19F is also very close to 1H in terms of sensitivity silicon is used for studying material.

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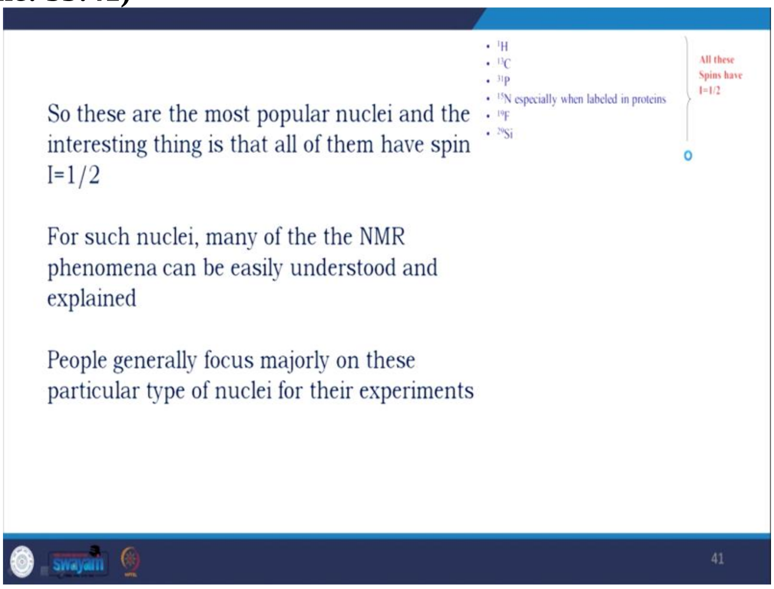

So, if you see 1H, 13C, 31P, 15N, 19F and 29 silicon, they are specially used and all of these have been equal to half. For such nuclei, many of the NMR phenomena can be easily understood and explained. People generally focus majorly on this particular type of nuclei for their experiments. So, I have started introducing NMR spectroscopy. I talked about the very unique applications of NMR.

And it having very versatile principle, which helped this technique to include most of the members of the periodic table if not the main isotope there less abundant isotopes, you could get NMR active signal from nearly every element so, it is extremely helpful in identification. It has very easy to use principle and then we will look at how this is going to develop principles where we will be able to work on some compounds. What are the principles, how the instrumentation works, which are the important part of instrumentation and many thing else in next class. Thank you very much.