Principles and Applications of NMR spectroscopy

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Module 1

Lecture No 01.

So, welcome every one; this is going to be course on a principles and applications of NMR spectroscopy. I am Hanudatta Atreya from NMR Research Center in IIC Bangalore. I work primarily in the area of bimolecular NMR looking at different structures of proteins and nucleic acids. in this course, we are going to basically introduce you to a very basic aspects of what is NMR spectroscopy? What are the principals involved? how NMR spectroscopy can be applied to different areas of a chemistry and biology. So, the I will start from the very basic applications of NMR spectroscopy, as we can see NMR spectroscopy is a very unique spectroscopic tool.

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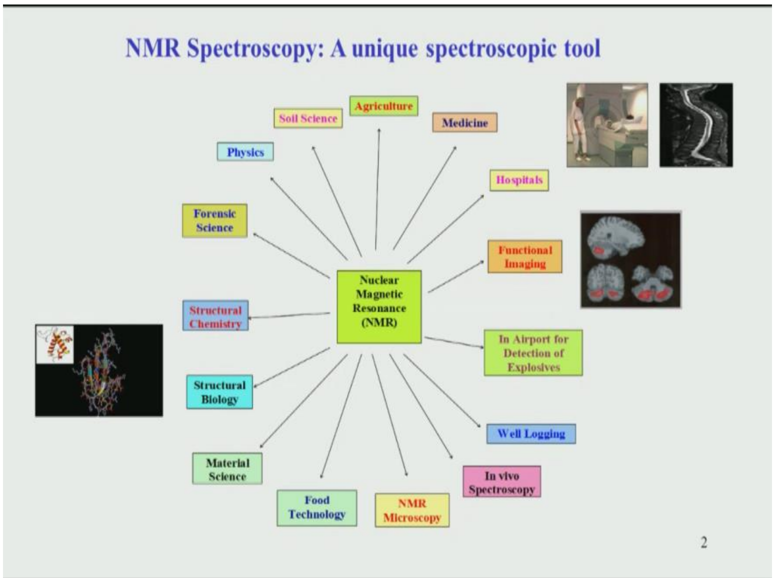

It has applications in a wide area of science and technology, for example it is applicable in agriculture, in medicines, this is a very popular application called magnetic resonance imaging and that is where MRI is used and I will see shortly in this course towards the end (MRI) Magnetic Resonance Imaging is essentially NMR. NMR spectroscopy is also used in different areas like NMR microscopy, food technology; in fact in testing many of the food products nowadays in many companies abroad is done by NMR spectroscopy but the main focus the major focus of this course will be on what is called structural biology and a structural chemistry.

These are two major applications which has now come up in last twenty years and we will see how nicely NMR spectroscopy can be used in this area.

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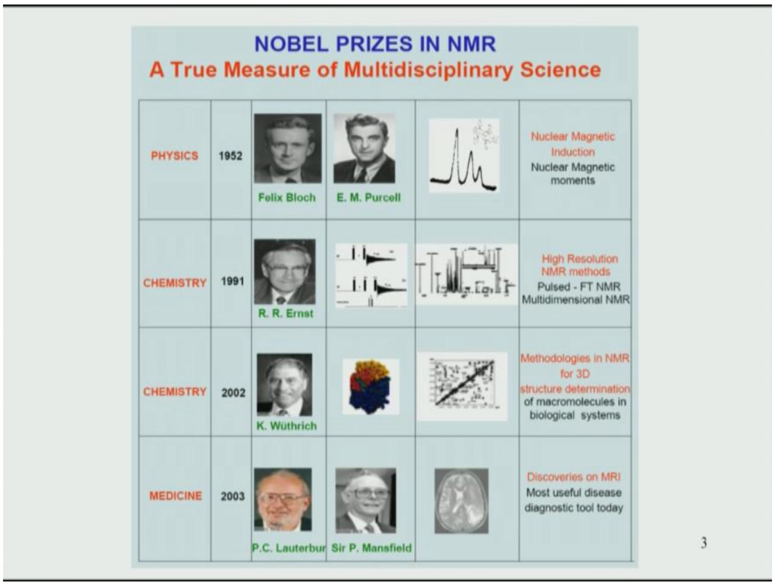

So, having said that the NMR spectroscopy applicable, is applicable to various fields. Therefore, there lot of Nobel prizes which have gone given to different discoveries made in NMR. Starting from in the very the beginning of NMR which is 1952 Nobel Prize was given to Felix Bloch and Purcell who actually independently and simultaneously discovered the phenomenon of NMR spectroscopy.

Richard Ernst got Nobel Prize in 1991 and the Richard Ernst is a very common name in chemistry and physics. Everybody is familiar with what is called Fourier transform NMR (FTNMR), which is, technique is used in routinely in chemistry and Richard Ernst discovered and developed many of the two dimensional NMR methods which we will see in the during this course. Kurt Wuthrich, Kurt Wuthrich from ETH that is Switzerland.

He got Nobel Prize, got Nobel Prize in 2002 and his main contribution was developing new methodologies for structure determination of bio molecules. So, this where basically NMR took off from being a tool of chemist to a being a tool of biologist. Mainly structural biologist and this was a phenomenal growth. it has seen a phenomenal growth after that look we look at how NMR can be used for a structure determination of small biomolecules such as peptides and proteins in this course and a major use of NMR as I mentioned in the previous slide is in the area where we look at imaging. So, this is the Nobel Prize which was awarded in 2003 to Peters Mansfield and Paul Lauterbur.

They, they had done, they had done similar contributions to MRI and MRI basically involves imaging of the whole body of the human or animal and actually capturing the Anatomy of the human system. So, we can see the huge range of applications which NMR has right from physics to looking at chemistry and biology and looking at the major imaging. So, why is NMR such a very unique tool? So, this is what we will see now in this next slide.

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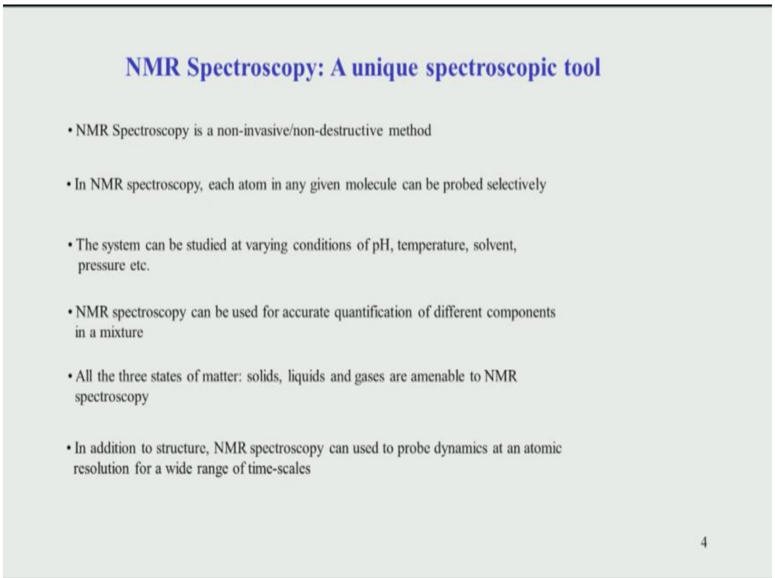

It is a very spectroscopic tool among all the other techniques which you might be aware of, the first and foremost is an NMR spectroscopy is a non-invasive and a non-destructive method. What does is this mean? This means that if you take sample of your compound of your molecule and record NMR data on that you get back the sample in that. Nothing happens to the sample; it is not destroyed and therefore is a non-invasive.

It is very nice non-invasive technique. In fact in MRI, the same thing applies because the whole body imaging is done in a non-destructive and a non-invasive manner. So, NMR is unique among the many other techniques in this respect.

The next point is that NMR spectroscopy you can actually study each and every atom in a given molecule. so, as we know molecules are made up of atoms and there are different types of atoms in a given molecule like in a drug molecule or in a biomolecule. Each and every atom can be selectively you can probe them or look at them in very sensitive manner. This is the major; we know the very good advantage of NMR spectroscopy compared to other techniques.

Where in in other techniques, you look at the whole molecule as such you do not actually get glimpse of each and every atom in a molecule but that is where NMR helps to come in a, to helps in finding the structure. the other important point about NMR spectroscopy is that you can actually study sample or the compound of the molecule which you are studying in under different conditions of pH.

You can change the temperature, you can change the solvent, you can change the pressure. So, you can actually play around with any conditions of your sample and keep monitoring the data or looking at the spectra's at different under different conditions. So, this is major plus point again of NMR because being non-destructive technique as long as sample is valid or not degraded you can use any different conditions and probe the data.

NMR spectroscopy again and the next very important point is it is a very quantitative technique. So, you can actually quantify that the amount of sample present study. So, this is something which is again it differs from many other techniques. So, because we do not destroy the sample. It is a non-destructive method you can actually preserve the quantification, you can measure what is amount of the sample is present and this is very very useful for many applications, for example, when we see what is called as Metabolomics? Where we look at quantity of sample a given mixture? We would like to know what is the different amounts relative amount present and this is where NMR spectroscopy is very useful and again stands out as a unique among other techniques.

We can study all different states of matter such as solids, liquids and gases and this is again a very important thing point because many of the many of the samples like we look at polymers if you look at differents materials they are in solid form you cannot make them in liquid form. So, you can use solid state NMR spectroscopy to study that. Liquids is again the solution state which is what ninety percent or most of the people do for using when they use NMR spectroscopy. So, solution state NMR is the standard approach and of course in some very rare cases you can also look at gases, but our focus in this course will be mainly in liquids where we will focus on solution state and a few example, if required we look at also solid state NMR spectroscopy.

So, NMR can be also useful for studying dynamics and this is very nice techniques because in many in different method such as X-ray crystallography. What happens is you look at this sample in a solid form, you look in the crystal form and therefore, you are frozen the sample in time and what you can get only the structural information but in NMR what you are doing is you are looking at the sample in a solution form. So, in a solution form sample is in it is native state. It is native state and therefore, it is has all the properties what you would expect in a real scenario and therefore, dynamics is one important property of a molecule.

Most of the molecules in solutions are very dynamic. You can never; they are never static as we see in pictures. So, therefore understanding dynamics is a very important part of understanding the whole function of the molecule and this is some, this is where NMR spectroscopy stands out and it helps us to look at dynamics at various times scales. You can go from as small as short as Pico seconds to go as few seconds.

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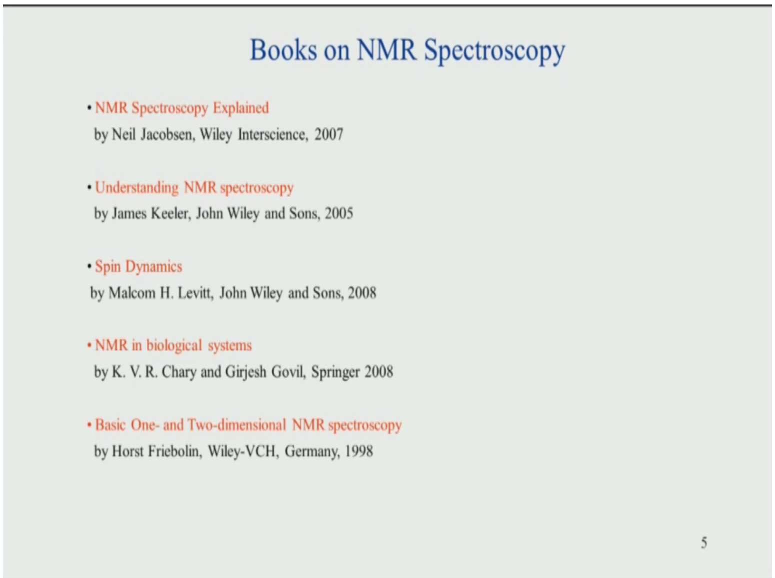

So, you can see there is a wide time scales of dynamic processes that can be studied by NMR. So, we will also look at in this course. So, before we go on to the details of the course I would like to suggest some books which you can actually refer to during this course. there is very nice book by called NMR spectroscopy explained by Neil Jacobsen and some of them are available online and you can have look at them.

James Keeler's book which is understanding NMR spectroscopy is the most popular book today among students and this is something which is very nice and can we looked at. If you are interested in biological systems which we will go to focus a little bit in this course. this book by Professor Chary and Govil is a very very important book. And so while all these books are going to be used in some way of the other, will cover all the details. So, these books will serve as mainly as reference materials or for more deeper this insight if you would like to have in the course.

So, let us then begin with this course. so, before we start NMR spectroscopy as the word stands out, it is a spectroscopic technique. So, it is like one of the other spectrum, many other spectroscopic techniques. So, we need to first understand, what is spectroscopy? In general, what does spectroscopy mean? What do we actually do in a spectroscopy?

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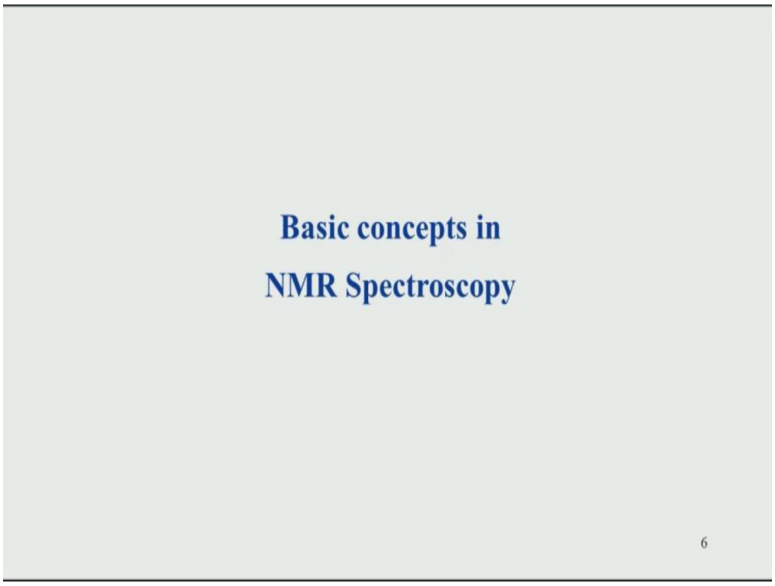

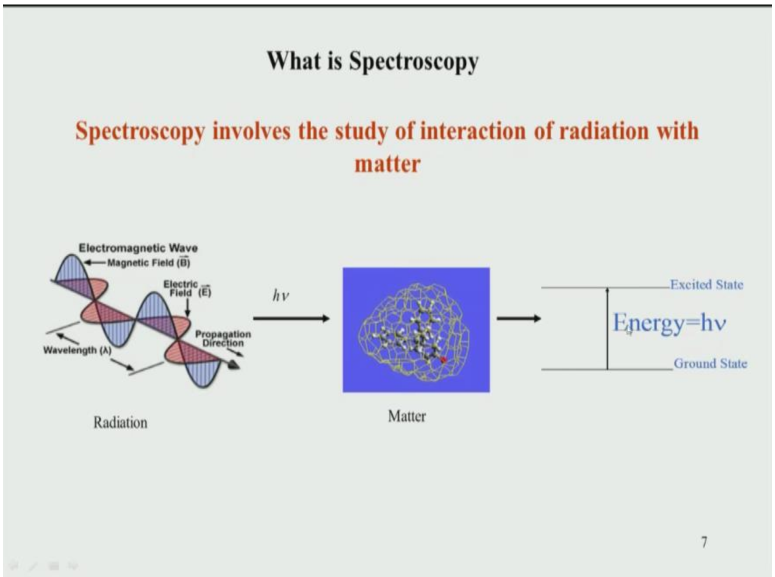

So, let us basic concepts. in spectroscopy, first and then before you move on to NMR. so, when you talk about spectroscopy. Spectroscopy involves the study of interaction of radiation with matter. So, when we say radiation, is basically we are referring to what is called electromagnetic radiation and this is a light. So, like for example, sunlight. Sunlight consists of electromagnetic radiation of different wavelengths. So, on the you can see here, there is this picture of a wave which is schematic drawing and it shows that there are two components of a electromagnetic wave.

One is called the electric field that the blue color and other is the red color and other is a magnetic field and these two fields are oriented perpendicular to each other. So, this is a standard picture of of a wave electromagnetic wave and what and we can see there is what characterizes the wave is the wavelength, that is the number of waves which are present per meter or per second. So, when we talk about number of wave per second, we talk about what is called frequency.

So, frequency is how many waves are traveling per second and wavelength is the length of the wave. So, these are basically two properties which will be very much using throughout our course because this is a basic aspect of an spectroscopy. So, essentially what happens in spectroscopy is you take this kind of magnetic electromagnetic field or a radiation or a wave and you put, shine it on a sample.

So, let us say that, this what you are seeing in the center is let us say a sample. So, we call it as matter or you can call it as your molecule or compound. So, when you shine light on this molecule. Now, the molecule starts responding has to the in respond to the incoming light. So, this incoming light we write it as this h Nu. This is a photon. So, one photon of energy is energy of one photon is h into Nu. Where h is the what is called as planks constant and Nu is the frequency of the wave.

So, whenever there is a electromagnetic wave it is energy is given by h Nu. So, this energy hv is now is shown on the sample and the sample then responds to this wave. So, it can do a several things number one, it can absorb that wave, it can absorb that energy in to the system or it can simply scatter that energy, it just reflects the energy or it just does not do anything and the wave simply passes through. So, when it simply passes through that means the matter or the sample is transparent. It is invisible to the to the light but when it absorbs when it absorbs the energy and part of it is absorbed part of it is radiated back or reflect transmitted out or part of it is scattered.

So, now what is that makes a matter or a compound absorb the energy or react to the energy. So, if you this come in for this we have to little bit go into what is called quantum mechanics in physics, we say that the matter at a like a microscopic at an atomic level does not have continuum of energy levels. So, it has what is called discrete energy level that means any sample will have any compound will have as discrete energy level and this is a called quantized system.

So, we have a quantized energy levels. And now, the energy which is shown upon this matter sample will only be absorbed by the matter if their energy matches the difference as shown here, difference between the two energy levels. Let us say that we have it is, this is very hypothetical system. Let us say we have what is called a ground state and we have what is called an excited state. So, this is basically different energy levels in a matter in the sample or in the compound molecule.

So, the energy which we are supplying that is h Nu if it matches this gap between the ground state and energy excited state, then this energy is absorbed by the sample and taken up by then molecules go from the ground energy level to an excited energy level. So, this is the qualitative way of explaining. For the deeper understanding I would suggest to also to look at the different books which I referred to. But in a qualitative manner, one can understand that when you shine a light on this matter, the matter which has different energy levels in it is molecule.

The molecules will absorbs that energies if there is difference in the energy level matches the energy which we are supplying. So, this is basically the general idea of a spectroscopy. In spectroscopy, that you shine light on the matter, the matter either absorbs, reflects or transmits the light and based on that we can find out what is happening in the, what is a nature of this matter.

So, the nature of the matter is hidden in a way in the way it responds to light. So, based on the response to the light which is shown on the matter. We can figure out what type of matter, what is the inside of this is system consist of. And we can also look at different types of properties such as the structure of the molecule, the dynamics of the molecule, the vibrations, the rotations and so on.

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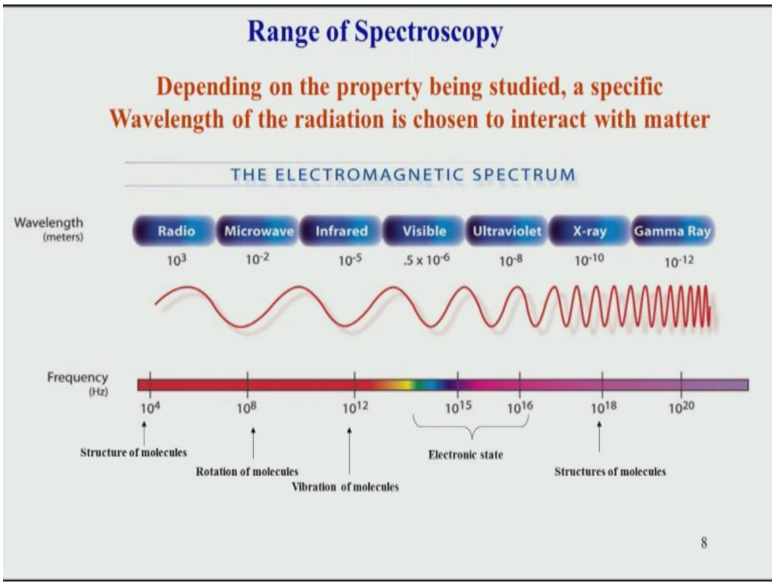

So, there are different spectroscopic techniques if you slide, here you see that depending on the property which you want to study, for example, Let us say you are looking at the dynamics of the molecule or rotation of the molecules or vibrations, you can see there are different energies involved. So, that means based on what type of energy I apply to the sample the different properties of the sample will respond to that kind of a spectrum to that can that light. So, this is called the electromagnetic spectrum where you can see the whole spectrum is now divided into different regions based on the frequency.

So, remember frequency is measured in hertz. So, we are going to use this hertz units throughout the course. So, if you start from the lowest frequency that is a lowest longest wavelength, remember wavelength and frequency are inversely related. So, if you go to the longest wavelength or shortest or lowest frequency, we use that word radio waves. So, this region is called radio waves. Then the next, higher little higher energy is called microwave and here, the microwave energy if you supplied to the sample or molecule, the rotation of the molecules are affected or respond to this particular range of frequency and this is called microwave spectroscopy.

Then the next energy is the infrared which this is responded, which depends on the vibrations of the molecule. The vibrations in the molecules respond to infrared radiation uh which is of, if it is applied to the sample. Then comes the electronic transitions that means the electrons in the molecule respond to these energies which is called ultra violet visible, Visible light ultra violet. And visible is essentially as the visible words means it is essentially you can see.

This is that range of frequency ultra violet is in the higher energy. Then as you go further the very high energy rays electromagnetic radiation they are called X-ray. An x-ray is where x-ray radiations are used for different applications and one of the very famous application is X-ray crystallography where we look at structures of molecules. And further a higher energy is Gama rays. So, this is the different energy ranges which to which the molecules can respond and depending on its property, depending on its composition, constitution and structure.

So, in NMR spectroscopy we will focus on this lowest energy that is radio waves. So, the whole of NMR spectroscopy technique is focused on these particular region and the radio waves.

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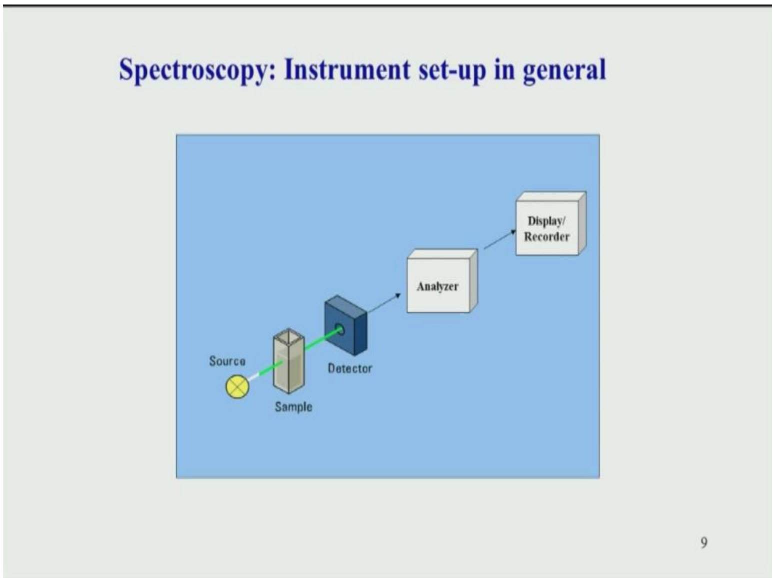

So, you can see what is the basic technique, what is the basic apparatus or setup in any spectroscopic in general. Remember NMR spectroscopy is also a spectroscopic technique. So, many of the things are indeed common with other spectroscopic technique but the details will vary. But generally what you need is a source. You have a source of radiation and this is what you shine on the sample. So, this is a sample, this is a just very schematic qualitative figure.

So, you take the radiation which you put it on the sample and the sample will absorbed radiation or transmit and whatever is transmitted is detected by physically a detector which is electronic component and then you analyze it and finally what is displayed is what is called as spectrum.

The Spectrum is essentially a plot of frequencies which are of the light, which are absorbed by the radiation. So, the radiation is basically is a broad source, can be a broad of radiation and different sources of radiations can be, is absorbed or transmitted by the sample and that is what we record and display. So, for in terms of the perspective of NMR spectroscopy, this source will basically be a electro radio wave source, the Source of radio waves, as we saw that NMR relies on radio frequency.

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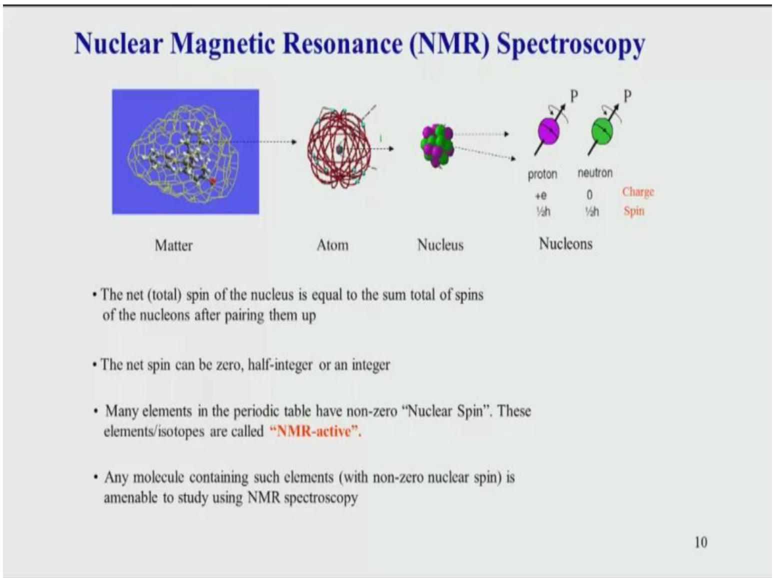

So, now let us go to NMR spectroscopy having understood what general spectroscopy means. So, let us go back to this picture where we see that there is a what is called a matter in other words it is a sample or a molecule or a compound. So, we see every molecule as we know from our basic chemistry and physics, it is made up of atoms. So, every molecule consists of atoms.

Now, if you look at atom more closely atom consists of nucleus which is at the center of the atom and it is surrounded by a electron cloud or electrons. Now, further if you zoom into this nucleus, you will see nucleus is made up of protons and neutrons and we use the word nucleons which for both protons and neutrons. Now, neutrons and protons have two properties associated associated with them which we will we use in NMR. One of the properties is called charge. So, all of us know that protons are positively charged and neutrons have charge zero, they are neutral. But what is one more important property they have is known as spin.

So, spin is essentially an abstract quantity, it is a quantum mechanical quantity. So, although it is not literally that they are spinning but we can always associate all the properties of motion with this quantity called spin. So, I would suggest that in the back of your mind, to keep in mind that is not literally rotation of spin which is from nucleus which is going on but at the same time we associate a property called spin which has, will be very useful. So, the spin of a proton and neutron are same, they are called, they are fermions and use the word half h cross. This h is a plank's constant and half h cross h bar we use that is h upon 2 Pi.

So, both protons and neutrons have spin but the charge is only for proton, and neutron does not have a charge. But if you see now the overall nucleus has a positive charge and that is again a well-known thing because the neutrons are positively, nucleus is positively charged and electrons are negative. So, this whole positively charged nucleus now has a spin associated with it because the protons and neutrons inside the nucleus have spins along with them.

So, now what happens is that the protons and neutrons now start pairing up with each other. So, this is what happens, Suppose, I have one proton and one neutron. They can pair up with each other such that one proton will have a positive half and other nucleon will have a negative half and they cancel with each other.

So, pairing essentially cancels out the nucleus the spin of the) particles. So, that means the nucleus after pairing the protons and neutrons together you can say that the total nucleus has a net spin value. The net spin value is the value which you come arrive at after pairing up all the protons and neutrons. So, these net spin can have any value as we will see shortly, the net spin value can be determined based on the number of protons and neutrons or basically based on the atomic mass and atomic number. But what is important is that the whole NMR spectra, the whole technique of spectrosco NMR spectroscopy relies on the nucleus having a net spin value which is not zero.

That means if all the protons and neutrons pair with each other exactly then the total spin value of the nucleus will become zero because the positive spin will cancel with a negative spin of the nucleons. And therefore, if the nets total spin is zero then NMR cannot be carried out on that particular molecule. So, the atom, so that means this atom or element should have what is called a non-zero spin. It cannot have a zero spin if it is zero, then it cannot be studied by NMR.

So, we will see shortly then what are the conditions which are important for getting spin not equal to zero, total spin of nucleus or equal to half integer or integer. So, this concept that an a nucleus is, we are, it is possible to study a nucleus NMR or element we use the word NMR active. So, we will say this particular NMR is nucleus, is NMR active or this particular nucleus is not active. So, we will say NMR inactive.

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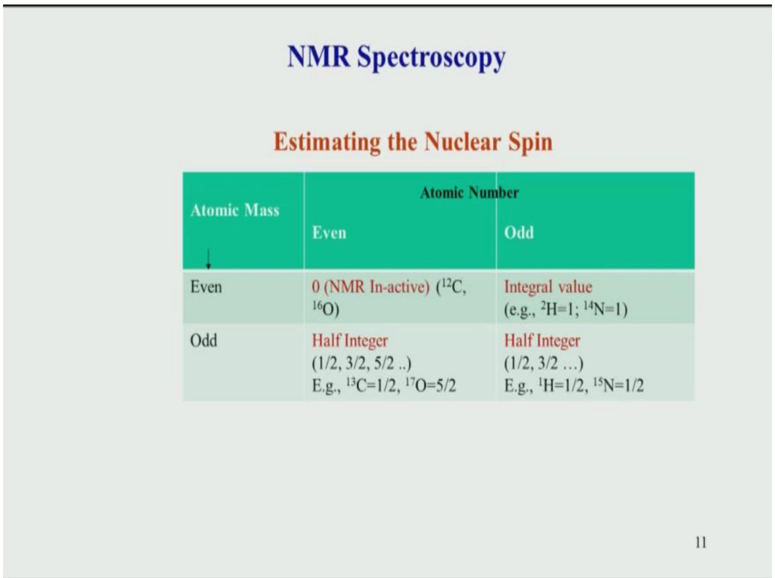

So, you can see so let us look at how a one can actually qualitatively determine whether a particular nucleus can be studied by NMR or cannot be studied. So, you can see at this table here, which shows how we can estimate. So, let us say on this column here is atomic mass. So, we can have any atom having a even atomic mass or it can be atomic, odd atomic mass. Similarly, an atomic number of that atom can be either even or odd depending on the number of protons.

So, number of protons will decide whether the atomic number is even or odd and the total number of atoms that is total number of nucleons, that is proton plus neutrons determine whether the atomic mass is even or odd. So, this is a very standard basic concept which you, we learn in chemistry and physics. So, let us say we take a particular nucleus carbon 12. It has 6 at a, 6 protons and 6 neutrons.

So, therefore it is even atomic number and even atomic mass and therefore, the spin of this is zero, that means the total all the protons and neutrons pair with each other and the total net value of the spin for that nucleus is equal to zero. So, therefore we cannot study 12C by

NMR. Similarly, oxygen 16 has 8 protons and 8 neutrons. So, it belongs to this even-even category and therefore it cannot be studied by NMR.

Now, let us look an example, where you have odd atomic number and even atomic mass. So, this is the case where example Deuterium, Deuteron this is an isotope of hydrogen and there is Nitrogen 14. So, if you look at this they have 7 protons. Nitrogen14 has 7 protons, 7 neutrons. So, it has total even atomic mass but atomic number is odd. So, such kind of element, a atom or a nuclei we will have what is called integral value of spin, the spin will be the integer multiple multiples of one and so on. Okay.

Now, let us look at another the third case where you have even atomic number and odd atomic mass and such examples for that could be is 13C carbon Oxygen 17. So, 13C is a very popular nuclei for NMR spectroscopy. So, here if you see we have 6 protons, that is even atomic number but we have odd atomic mass. So, this therefore the spin of this is half integer and it could be half three by two, five by two and so on, that is, oxygen 17 which is 5 by 2 and for carbon 13 it is half.

So, there are various rules by which you can determine the exact value of spin but the general qualitatively how we can get the idea is based on this spin. And the last case is essentially odd-odd combination, for example, it is a very most popular nucleus is proton. Of course the, there is no neutrons for hydrogen atom, so, it is called proton. So, it is has one proton and it is the nucleus is therefore odd atomic number also odd atomic weight, mass and that is why it is spin half integer. A very another important nucleus for NMR is N15 which has 7 protons and 8 neutrons. So, it makes as an odd-odd combination and this spin value is half.

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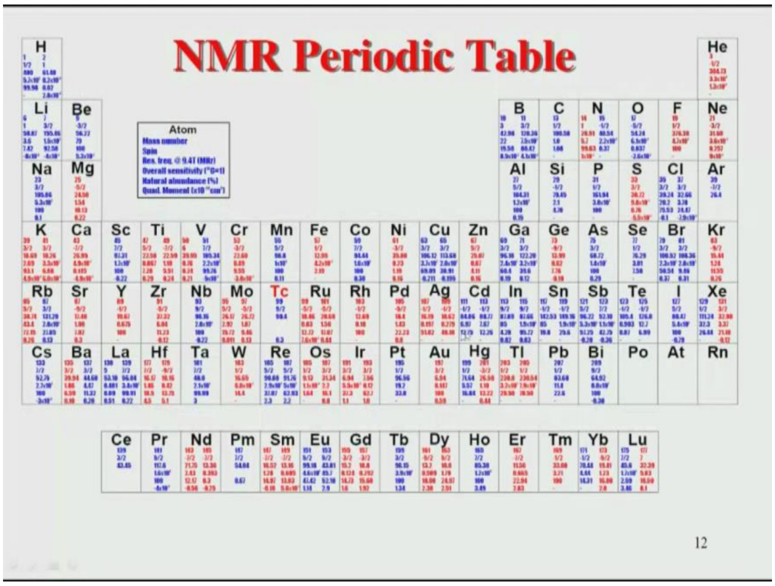

So, therefore these are the basically the different conditions by which we can study the different NMR properties and we will be focusing on this. So, in similar in this manner you can actually extends this idea to the entire periodic table. although , it is not very clear each of these numbers here but the idea the take home, take home from the slide is that the many elements in the whole of periodic table which can be studied by NMR spectroscopy, because they do not have, they have non-zero spin. Okay. So, either the compound, the element itself can be studied in the natural abundance or its isotope will be less abundant.

So, abundance is something which will be very much referring to again and again in this course. The abundance basically means in a given natural state what is the percentage of a given isotope which is present in that molecule. For example, Carbon12 is naturally abundant ninety-nine percent. Ninety-nine percent of the atoms in a molecule containing carbon is carbon12, one percent is carbon13. So, for us NMR, as far the NMR is concerned, only that one percent which is present contributes to the signal. The remaining ninety-nine percent is not, it just blind or transparent to NMR because it has spin zero. So, one percent is abundance.

So, you can see it is the very, the sensitivity is low because only out of hundred atoms carbon, one carbon atom will be C13. The similarly, the abundant nuclei will vary and based on the sensitivity is determined. So, as we go on, we look at different reason or property, so sensitivity how does it depend on the atom and the nucleus other properties and so on. So, from this onwards we will take up in the in the next class how actually we can look at the spin value and then how does NMR spectroscopy depend on this particular spin and how we take it forward. Thank you.