NMR Spectroscopy for Chemists and Biologists
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Introduction to Chemical Shifts
Lecture No. 05

Good morning. So my name is Ashutosh Kumar and in this section we will be teaching about high resolution NMR spectra of various molecules. So in the previous section we had looked at NMR is all about nuclear phenomena. There has to be a nucleus which has to have a nuclear moment and one has to put that nucleus in magnetic field and then there will be energy like energy state is created, you assign an radio frequency which will take the nuclei or nucleons from ground state to excited state and once they come back they give some kind of signal and that is recorded in NMR spectroscopy, briefly that we discussed in the previous section.

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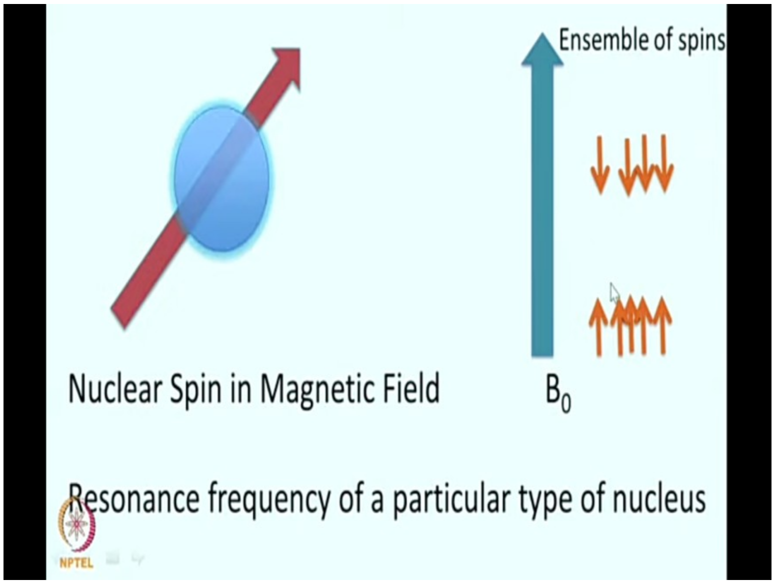

Ensemble of spins

Nuclear Spin in Magnetic Field

B_0

Resonance frequency of a particular type of nucleus

Now we will move how to get the high resolution spectrum from all these nuclei. So as we see that in the magnetic field there is a nucleus which is shown here. Now once we put that nuclear in magnetic field so there is an ensemble of a spin because NMR is not a particular nuclear phenomena but however it is about an ensemble of spins. So, if you it, put more will be aligned along the field and slightly less will be aligned against the field and this differential alignment of different spins create the energy state and then that is excited, goes to excited state comes back and gives signals. So whatever the resonance frequency of these particular nuclei is there, that is absorbed in NMR spectrum.

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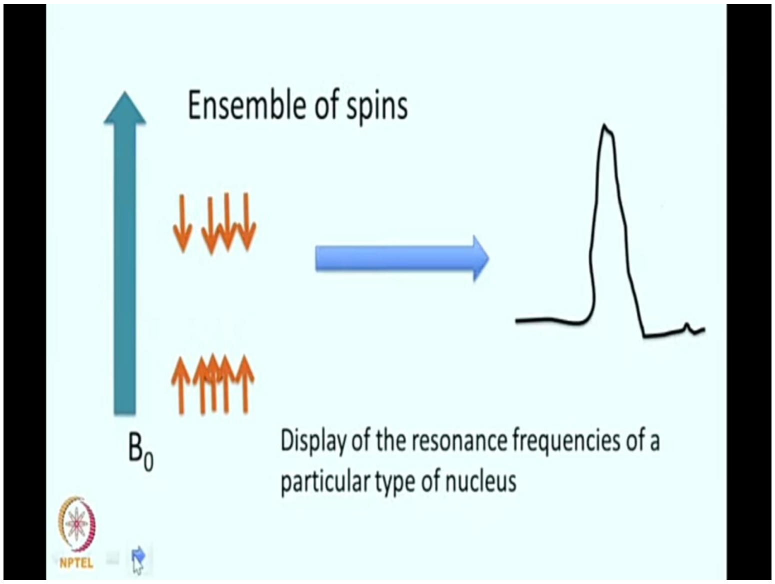

Ensemble of spins

Display of the resonance frequencies of a particular type of nucleus

Now that is what we are saying, so the energy states are created, then you assign an light which is perpendicular direction that is called *B_0* oscillating field and because of that you get some resonance frequency and that display of this particular resonance frequency of a particular type of nuclei is giving NMR spectrum. If you show different kind of nuclei gives different kind of resonance frequency that means hydrogen will have one frequency, carbon 13 will have another frequency, nitrogen 15 will have another frequency so and so forth.

Therefore in the NMR spectrum even in a given sample where there are different kind of nuclei like a proton, Carbon 13, Nitrogen 15, Phosphorous 31 we get a different resonance frequency for each of these nuclei and that are non-overlapping, therefore the information that can be extracted is phenomenal because of differential absorption line of different nuclei.

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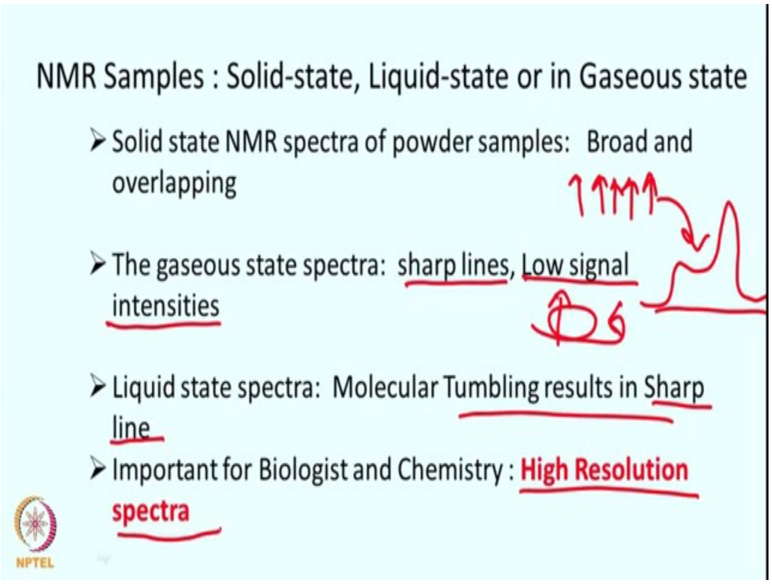

NMR Samples : Solid-state, Liquid-state or in Gaseous state

- Solid state NMR spectra of powder samples: Broad and overlapping
- The gaseous state spectra: sharp lines, Low signal intensities
- Liquid state spectra: Molecular Tumbling results in Sharp line
- Important for Biologist and Chemistry : **High Resolution spectra**

Now, so what kind of sample we can look at in NMR spectrum? So all sorts of samples ranging from the solid state, liquid states or gaseous state sample. Eventually theoretically we can look at all these samples, solid, liquid or gas.

In solid what happens solid spectrum of powder sample generally gives broad and overlapping line, this is because the spins in solid are essentially arranged very close to each other and that give rise to different kind of spectrum. So suppose here we have a spins, those are like this very closely placed and each of these will have a resonance frequency so resultant resonance frequency will be something like this, a very broad line. And that gives some limited information so therefore solid samples have a different way to do NMR spectroscopy and we in this course we are not going to particularly deal too much about solid state we will give some idea about how to do solid state NMR but for subsequent course we can take up this as exclusively.

On the other hand gaseous samples here molecules spin tumble fast but actually in a sample the actual number of spins are very less. So because of fast tumbling there are sharp lines, however the abundance of spins present in the gaseous samples is less and therefore they have a low signal intensity, so this is also not very preferred way of doing NMR spectroscopy.

Third case, liquid state sample, now in liquid the spins tumbles very fast and because of that we have a sharp line and like a solid there are not closely space so therefore also this signal overlap is not that much. Therefore these liquid state samples gives the high resolution spectrum and for biologists and chemistry this is very important because they can get high resolution spectrum where they can study property of each of the spins.

So in this course we are mostly going to deal with what kind of liquid state spectrum we get and how this is high resolution. Now let us look at what kind of nuclei gives the signal.

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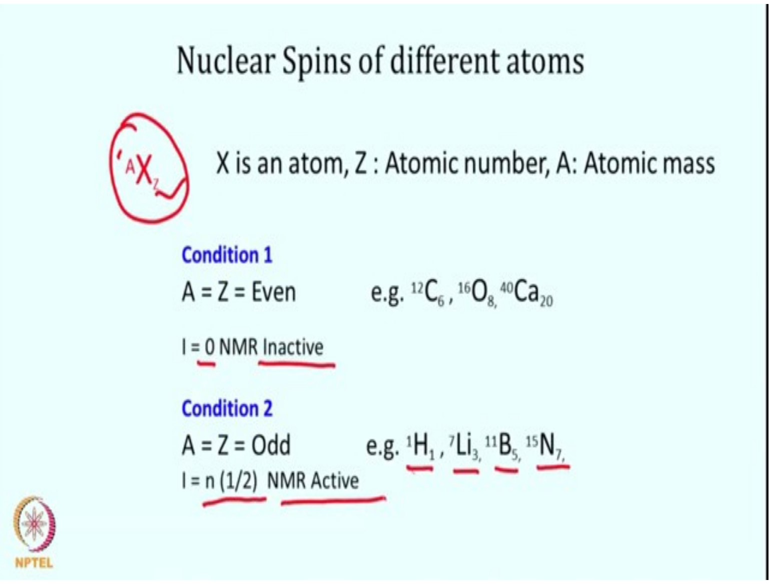

Nuclear Spins of different atoms

$^{A}X_{Z}$ X is an atom, Z : Atomic number, A: Atomic mass

Condition 1

A = Z = Even e.g. $^{12}C_{6}$, $^{16}O_{8,}$ $^{40}Ca_{20}$

I = 0 NMR Inactive

Condition 2

A = Z = Odd e.g. $^{1}H_{1}$, $^{7}Li_{3,}$ $^{11}B_{5,}$ $^{15}N_{7,}$

I = n (1/2) NMR Active

So let us consider an atom, the symbol of this atom is *X* and we have an atomic number which is *Z* and atomic mass which is *A*. So now let us consider few conditions. So, if *A* that is atomic mass and *Z* both are even, what I mean to say that *Z* is atomic number that means number of protons and number of electron, atomic mass consists of number of proton and number of neutron. So if *A* atomic mass and *Z* atomic number is are even. For an example, carbon 6, 12; oxygen 8, 16; calcium, these kind of nuclei they have spin quantum number as zero and therefore these nuclei's are NMR inactive nuclei, therefore there is no signal from such nuclei.

Let us consider condition two, in the same formula *Z* is odd and *A* atomic mass is also odd. So for example H(hydrogen) 1 which is most abundant nuclei or lithium or boron or nitrogen 15 which is the isotope of naturally occurring nitrogen 14. So all this nuclei where *A* and *Z* both are odd, their spin quantum number is half integral, n multiplied with half and these nuclei's are NMR active. So we can do NMR of these nuclei.

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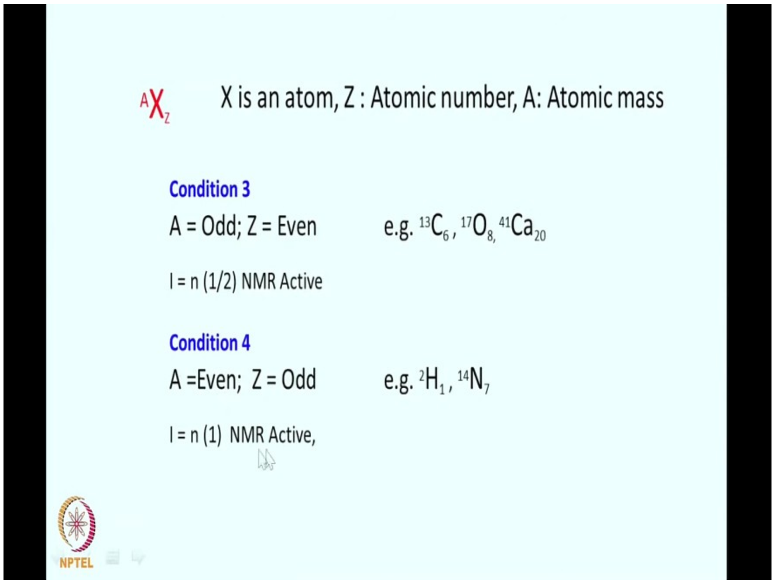

$^{A}X_{Z}$ X is an atom, Z : Atomic number, A: Atomic mass

Condition 3

A = Odd; Z = Even e.g. $^{13}C_{6}$, $^{17}O_{8}$, $^{41}Ca_{20}$

I = n (1/2) NMR Active

Condition 4

A =Even; Z = Odd e.g. $^{2}H_{1}$, $^{14}N_{7}$

I = n (1) NMR Active,

Let us consider third condition where atomic mass is odd and atomic number is even, like for an example Carbon 13, 6; Oxygen 17, or Calcium 41.So these nuclei again are half integral nuclei and they are NMR active, therefore these nuclei's are also amenable for high resolution NMR spectrum.

Consider condition four, where *A* atomic mass is even, atomic number *Z* is odd. For an example deuterium, this is an isotope of proton naturally occurring, so deuterium or N 14 this is a natural isotope of nitrogen. All these in all these case this spin quantum number is integral and they are also NMR active but their NMR requires little bit tweaking and we discuss how to do NMR of quadrupolar nuclei later.

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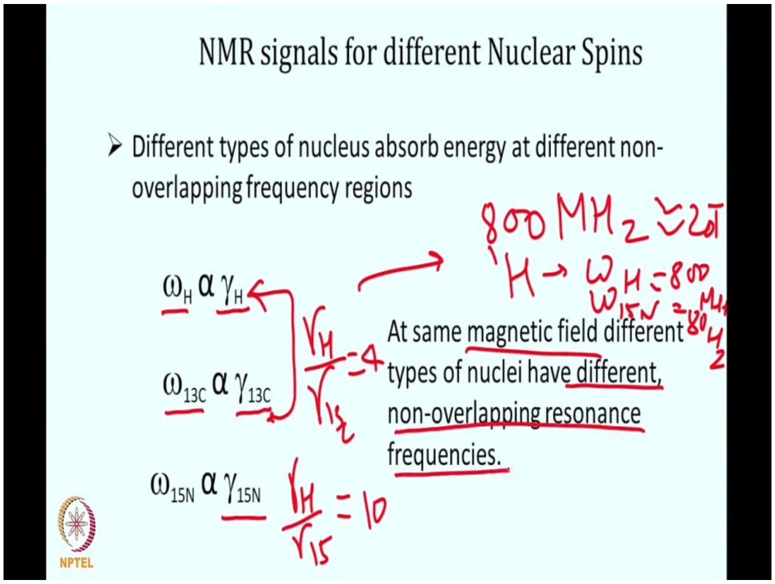

NMR signals for different Nuclear Spins

- Different types of nucleus absorb energy at different non-overlapping frequency regions

$\omega_H \; \alpha \; \gamma_H$

$\omega_{13C} \; \alpha \; \gamma_{13C}$

$\omega_{15N} \; \alpha \; \gamma_{15N}$

At same magnetic field different types of nuclei have different, non-overlapping resonance frequencies.

$800\ MHz \simeq 20T$

${}^1H \rightarrow \omega_H = 800\ MHz$

$\omega_{15N} = 80\ MHz$

$\frac{\gamma_H}{\gamma_{13C}} = 4$

$\frac{\gamma_H}{\gamma_{15}} = 10$

If you look at all these nuclei essentially in the periodic table there are so many nuclei which are amenable for an NMR spectroscopy. Therefore that is what we mean that NMR is amenable for all sorts of sample, biological or material science or physics related sample. Now NMR gives signal for different nuclei, different spins gives different signal and their signal depends upon something call gyromagnetic ratio. So that means proton resonance frequency will depend upon the proton gyromagnetic ratio at the same magnetic field. However, carbon 13 resonance frequency will depends upon carbon 13 gyromagnetic ratio. And if you have seen in the previous class there is a relation between ${}^{13}C$

$$\frac{\gamma_H}{\gamma_{13C}} = 4$$

So on the same magnetic field proton resonance frequency will be roughly four times more than the carbon 13 resonance frequency.

$$\frac{\gamma_H}{\gamma_{15N}} = 10$$

So on the same magnetic field the resonance frequency for N 15 will be 10 times less than the resonance frequency. So for an example we are recording an spectrum on 800 Megahertz which corresponds to say 20 Tesla, proton will resonate at 800 Megahertz whereas nitrogen will resonate at 80 Megahertz right, so that is what we mean at the same magnetic field different type of nuclei will have different resonance frequency and those are non-overlapping resonance frequency because they are too far.

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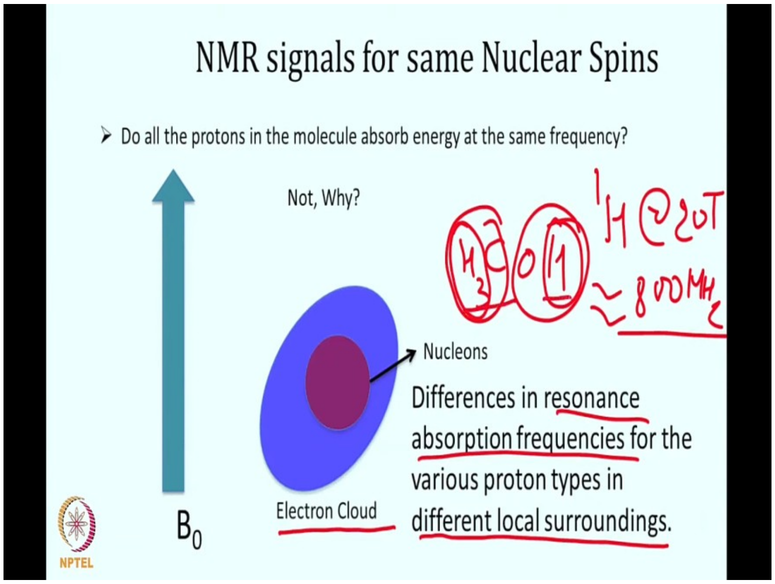

NMR signals for same Nuclear Spins

- Do all the protons in the molecule absorb energy at the same frequency?

Not, Why?

Differences in resonance absorption frequencies for the various proton types in different local surroundings.

So now what happens for a same kind of nuclei? Like for an example proton. Now for proton so do all proton in a molecule absorb same resonance frequency? No, they will not. In the last slide we saw that for a resonance frequency of proton at say 20 Tesla magnet is roughly equivalent to 800 Megahertz. But do all protons resonate at 800 Megahertz? No, the reason behind this because these proton nuclei are not bare nuclei, there is something around it, and what is that? It is the electric cloud.

Now this electronic cloud is also having spin if you put that in magnetic field, this electronic spin will interact with the magnetic field and they can generate a local field and that local field will be different for different kind of proton nuclei and that gives rise to a phenomenon which is called chemical shift. So the difference in the resonance absorption frequency for particular kind of nuclei depends upon the local surrounding. So if I take an molecule say let us take an methanol, here we have one proton here and one proton here but local environment for this proton and this proton is different therefore their resonance frequencies should be something different.

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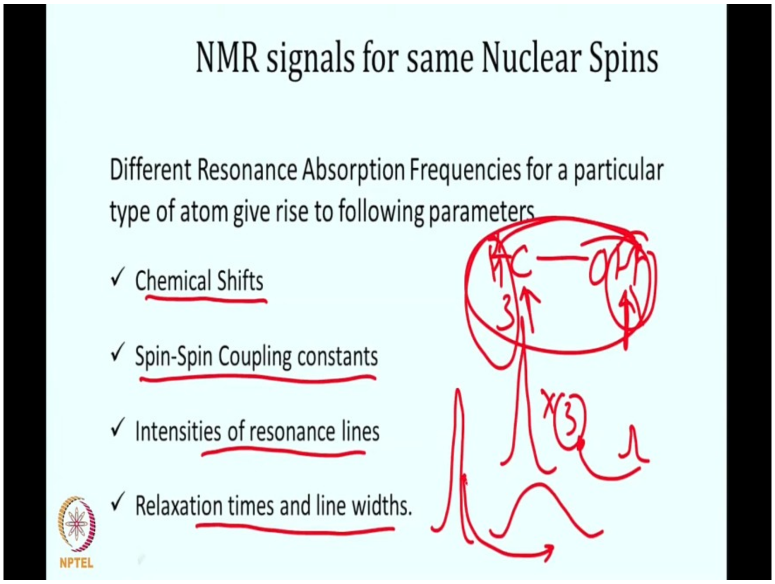

NMR signals for same Nuclear Spins

Different Resonance Absorption Frequencies for a particular type of atom give rise to following parameters

- ✓ Chemical Shifts
- ✓ Spin-Spin Coupling constants
- ✓ Intensities of resonance lines
- ✓ Relaxation times and line widths.

Now so if this is the different what kind of information that we can deduce because of different resonance frequencies for a particular type of atom and that gives various interesting phenomena, one is called chemical shift. This chemical shift arise because of the different chemical environment for a particular kind of nuclei at the same magnetic field, like for an example we give CH_3 resonance frequency is going to be different than OH resonance frequency in methanol. The another that we are going to discuss in this chapter is spin-spin coupling, so these two protons are also somehow coupled through bond and they will affect the resonance frequency, this is called spin-spin coupling that will come later.

The next thing if you see here we have three proton and here we have one proton, now this here three protons are contributing to signal and which is different than one protons contributing to signal. So intensity of CH_3 protons should be three times larger, than the OH proton and the intensity of resonance line gives enormous information about the number of proton that contribute to particular kind of signal.

The fourth one that we looked in the previous chapter is relaxation time and line width. So line width for some proton can be very sharp, for some proton can be broad and this gives information about the dynamics at a local site and these are, all these four important information can be deduced to understand the structure, the chemical environment, their neighboring effect and also the relaxation property of a particular kind of nuclei.

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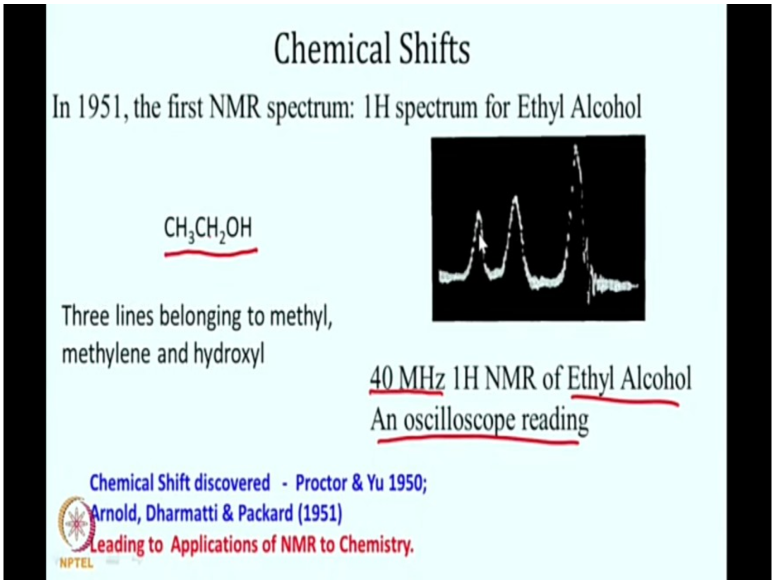

Chemical Shifts

In 1951, the first NMR spectrum: 1H spectrum for Ethyl Alcohol

CH_3CH_2OH

Three lines belonging to methyl, methylene and hydroxyl

40 MHz 1H NMR of Ethyl Alcohol
An oscilloscope reading

Chemical Shift discovered - Proctor & Yu 1950;
Arnold, Dharmatti & Packard (1951)
Leading to Applications of NMR to Chemistry.

Now so the chemical shift which is the first one, the difference in the resonance frequency because of the differential chemical environment was first discovered in 1951. Actually the first time what they did, they took ethyl alcohol, ethyl alcohol if you remember your chemistry it is a CH_3CH_2OH. They took this ethyl alcohol, put this in a 40 Megahertz NMR spectrum and then they recorded the spectrum of that and that time recording was done using Oscilloscope. So what they found that instead of one line they are getting three lines and these three lines triggered that there is a chemical environment different in protons of ethanol, ethyl alcohol. So here look at three lines, one corresponds to methyl, methylene and OH proton. So this is for methyl one here then this is for methylene and this is for OH.

And if you look at carefully, this intensity ratio of this looks three times more than this and methylene is two times more than OH. So clearly using this spectrum we can assess essentially quickly, we can recognize that here the contribution is coming from three protons, here the contribution is coming from two protons and here it is one proton. Now looking at their resonance frequency and looking at the intensity we can identify that this corresponds to CH_3, this corresponds to CH_2 and, this corresponds to OH. So that was first discovered by Arnold, Dharmatti and Packard.

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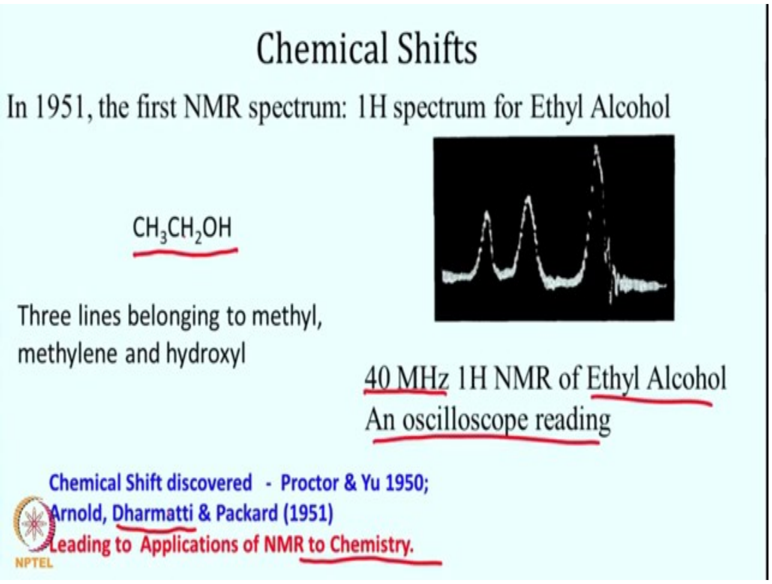
Inauguration of NMR facilities at TIFR, Mumbai, 1961



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Professor S. S. Dharmatti who has Post-doc when discovered this; this discovery opened its application of NMR spectroscopy in Chemistry. Now each and every molecule that chemistry people synthesize they record an NMR spectroscopy to find the number of proton contributing to signal and also what is the chemical environment and what is the coupling pattern and that gives a high resolution spectrum for a chemical moiety.

Now I was mentioning about Professor Dharmatti, so he was a Post-doc in 1951 when he discovered it, he came back to India and then early like early sixties in sixty one basically, actually with a generous support from the government of India they established the NMR facilities in TIFR Mumbai. If you look at Indian NMR has a long history which started right after the discovery of important phenomena like chemical shift and nuclear movement. And since then NMR legacy is carried over in India and many people are doing high quality research in this field. Here you can see Professor Dharmatti and Jawaharlal Nehru inaugurating the NMR facility in TIFR.

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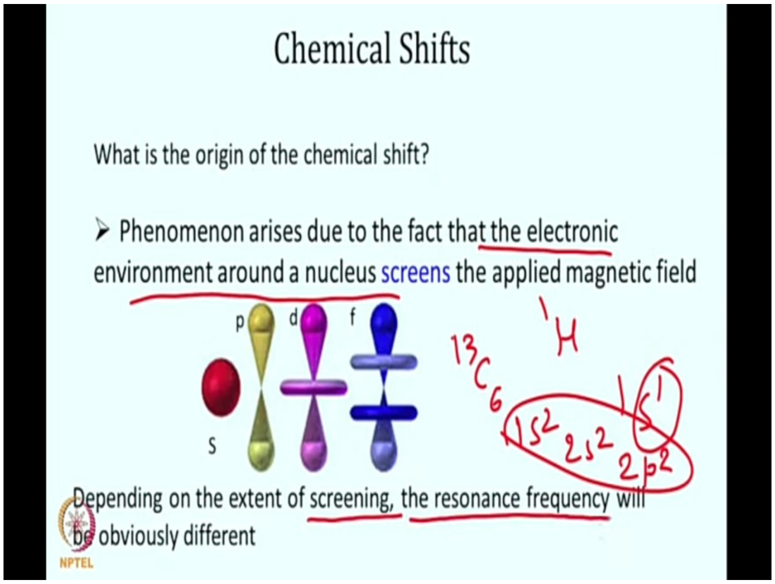

Chemical Shifts

What is the origin of the chemical shift?

- Phenomenon arises due to the fact that the electronic environment around a nucleus screens the applied magnetic field

Depending on the extent of screening, the resonance frequency will be obviously different

Now, more about chemical shift. So what is the origin of chemical shift? As I said electronic cloud around the nucleus causes these shifts in the resonance frequency for a particular nuclei. So this electronic cloud as we see that, as we see that there are different kind of electronic cloud around a particular nuclei. If you look at the twelfth class chemistry we know that there are various kinds of nuclei like, if you know electronic configuration for say proton it is 1s1, so S kind of orbiter is there. If you look at carbon we have $1s^2$ $2s^2$ and $2p^2$ right, so that is the electronic configuration around carbon 12, So here contribution is coming from s orbital as well as pre orbital and p orbital has a different shape.

Now electronic cloud around s orbital created by s orbital and pre orbital can be very different. Similarly if you look at the *d* orbital it is a dumble shaped and here is electronic cloud is very different than the *s* and *p* and similarly *f* is more complicated. So different kind of orbitals create a different kind of shielding effect and extent of screening will be different that will change the resonance frequency and that will be obviously different for different nuclei.

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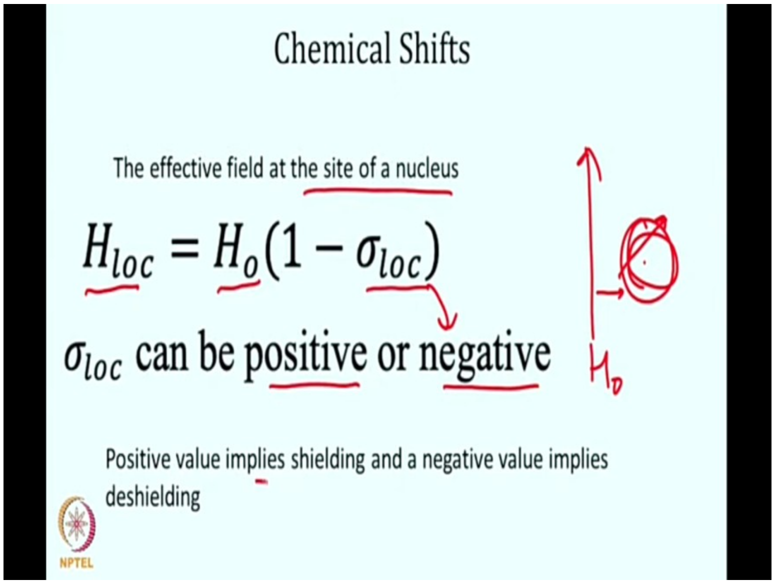

Chemical Shifts

The effective field at the site of a nucleus

$$H_{loc} = H_0(1 - \sigma_{loc})$$

σ_{loc} can be positive or negative

Positive value implies shielding and a negative value implies deshielding

So this effective field around that is different so local site like, if you have a magnetic field and here my nuclei and here is electronic cloud, so electronic cloud can be dense or sparse, and that is going to affect what is the local magnetic field absorbed by a particular nuclei so that will be different from H_0, H_0 is our main magnetic field. However, experienced magnetic field is different than the magnetic field because of something called local effect and this is called actually screening constant or shielding constant because this is creating kind of a screen that main magnetic field can absorb.

$$H_{loc} = H_0(1 - \sigma_{loc})$$

Now this sigma local can either be positive or negative depending upon how they are arranged, so positive value implies that this is creating a shielding and negative value implies this is creating a deshielding. So that means the main magnetic field in either case shielding or deshielding it is going to be different than the main magnetic field which is H_0 and the experience will be H_{loc}.

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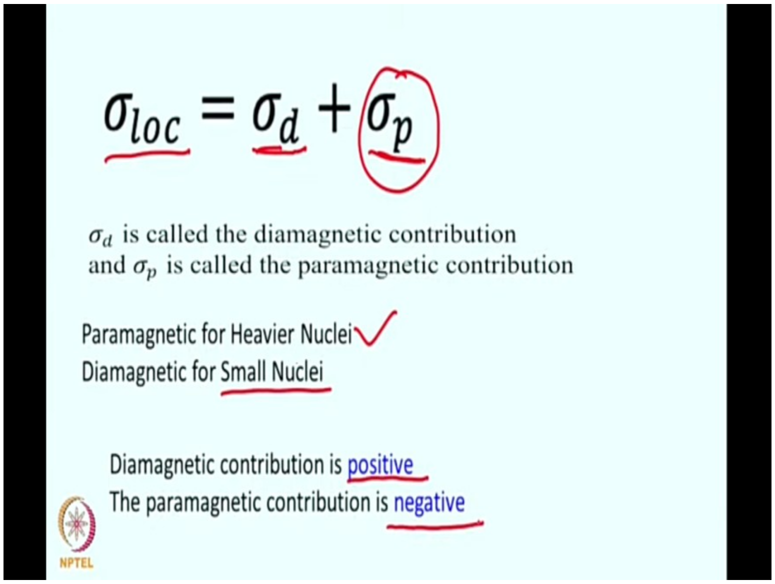

$$\sigma_{loc} = \sigma_d + \sigma_p$$

σ_d is called the diamagnetic contribution
and σ_p is called the paramagnetic contribution

Paramagnetic for Heavier Nuclei ✓
Diamagnetic for Small Nuclei

Diamagnetic contribution is positive
The paramagnetic contribution is negative

So this shielding constant which is essentially local, gives the contribution from the diamagnetic as well as paramagnetic. What are diamagnetic?

$\sigma_{loc}=\sigma_d+\sigma_p$

So if you look at heavier nuclei, heavier nuclei has a complex orbital. If you look at any of the heavy metal nuclei or f orbital nuclei their *s, p, d, f* and their mixing of orbital, because of this mixing orbital the electronic cloud are in particular direction and directionality of the electronic cloud. So that creates basically different kind of contribution of this shielding constant for heavier nuclei.

For a smaller nuclei if you look at like proton there is like a diamagnetic effect and for a smaller nuclei like carbon 13 or proton this is diamagnetic contribution which contributes mostly to the local magnetic field. Now diamagnetic contribution is generally positive and paramagnetic contribution is negative. So if you look at if you go back and try to analyze this, here we are saying this is positive. So if this is positive for diamagnetic nuclei, that means the local magnetic field absorbed by the proton is going to be less than the main magnetic field. However if this is negative then local magnetic field absorbed by the proton is going to be more than the magnetic field. Okay

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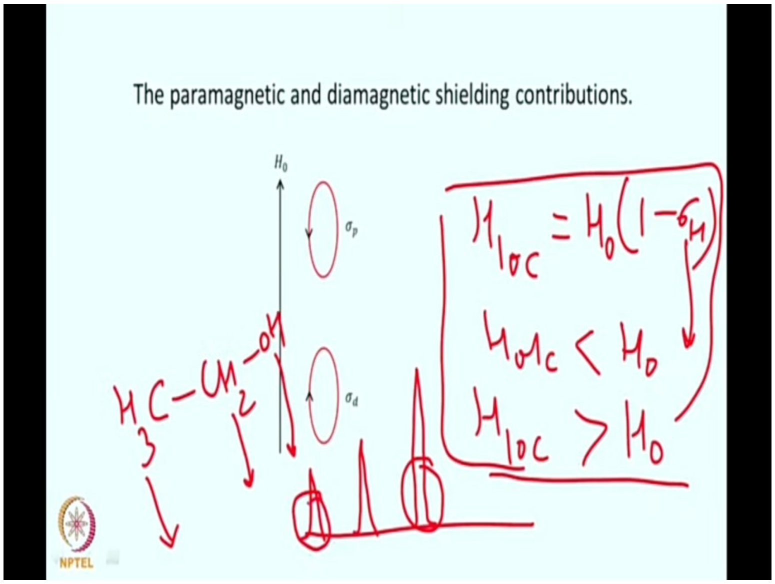

The paramagnetic and diamagnetic shielding contributions.

So if you look at the paramagnetic diamagnetic contribution that is what we are saying here the diamagnetic field is spinning along the magnetic field and therefore it is adding up and here paramagnetic is against it, because of that, now their resonance frequency is very different than the local magnetic field. So H local for proton will be H0 1 minus 6 H and that this is positive so H local will be less than H0. However for paramagnetic thing H local will be more than H0. So, so far so good, now we know that what kind of shielding and screening is created by different kind of nuclei.

Again go back to example of CH_3CH_2 and OH, mostly here we are dealing with a proton chemical shift, so all three are proton now we are saying, all of them have a diamagnetic contribution to the chemical shift. However the contribution because of CH 3 is different than the CH_2 and different than the OH. Therefore, their resonance frequency whatever we saw and that was seen by Professor Dharmatti is different.

So here it is more shielded, therefore the resonance frequency is here. OH is connected to an electronegative group it is deshielded and therefore you have resonance frequency which is coming here.

So till now we looked at different kind of nuclei will have a different kind of frequency. What I mean, that proton will have a one kind of resonance frequency, carbon 13 will have another kind of resonance frequency and 15 will have another kind of resonance frequency. Therefore they give non-overlapping spectra.

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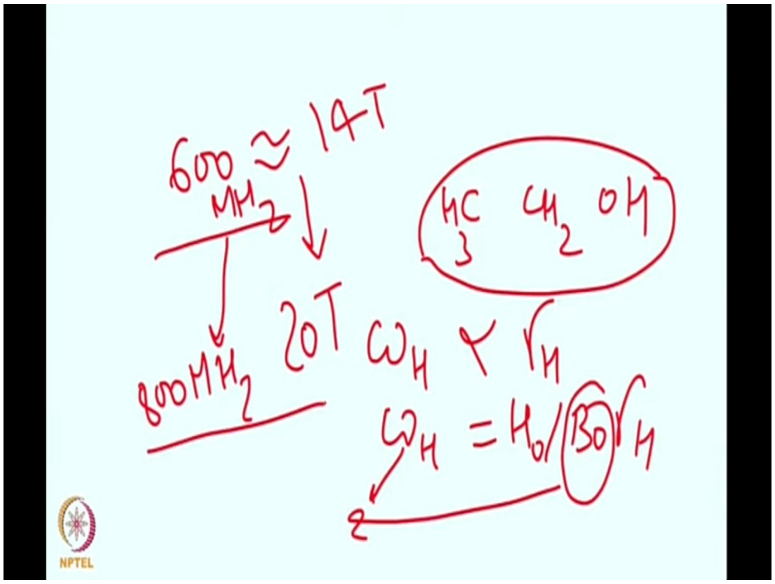

Next, we look at even in the proton their chemical environment they can have a different resonance frequency. So and we also looked at their resonance frequency

$$\omega_H \propto \gamma_H$$

$$\omega_H = \frac{H_0}{B_0 \gamma_H}$$

So that means at one field we are going to have one resonance frequency for proton, if we change the field there will be one resonance frequency for the proton.

So like on 600 Megahertz or that correspond to 14 Tesla, we have a resonance frequency for proton which corresponds to 600 Mz. If we change the field we make it 20 Tesla, we have a resonance frequency which is 800 Megahertz. But this is problematic, why because all the time we have to write on what magnetic field one recorded the spectrum and that that is going to be very problematic. So what people thought why we don't devise a strategy where we get rid of this field dependent in representing the chemical shift? And that give rise to a concept which is called the ppm value.

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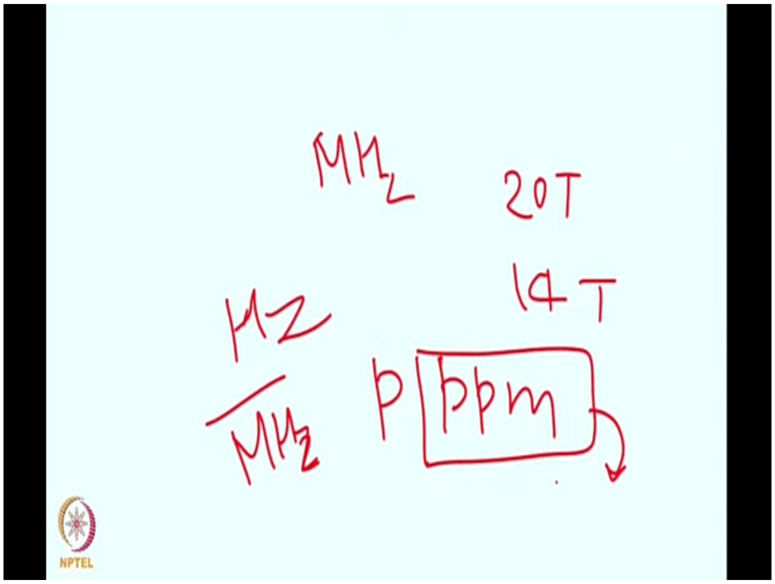

So ppm value actually get rids of the chemical shift on what field it was recorded whether it is 20 Tesla or 14 Tesla. So essentially what it says is that it is a difference from the main magnetic field and then you divide by the main magnetic field value, so the value so the resonance frequency we get in megahertz, difference in the CH_3 and CH_2 and OH resonance frequency that is in Hertz. So if you divide Hertz by Megahertz that comes to be it comes to be part per million, ppm. And now chemical shift is explained or is denoted in part per million, ppm. That gives, get rid of the field dependence representation of chemical shift.

So I will take the next class in explaining in detail, the how the ppm value has derived, how the contribution is coming, the contribution of the field is getting neglected when we define in ppm value. So I will stop it here and I will look forward for your question in the next class and in looking to have an interactive session where you ask lots of questions and we try to answer all of those. Thank you very much.