Principles and Applications of NMR spectroscopy
Professor Hanudatta S. Atreya
NMR Research Centre
Indian Institute of Science Bangalore
Module 1
Lecture No 05

Welcome back! So in the last class we looked at the different hardware components of NMR spectrometer and what are the different systems which are there. So, now given a (system) NMR spectrometer once we record the data we will now see from hence now on how do you actually analyze the data. How do you interpret the data, so this is typically from a chemist point of view because NMR spectroscopy is a very important tool for chemistry and therefore will now look at some of the chemicals or simple NMR experiments which can be done on chemistry in chemical compounds and we can look at how to analyze them?

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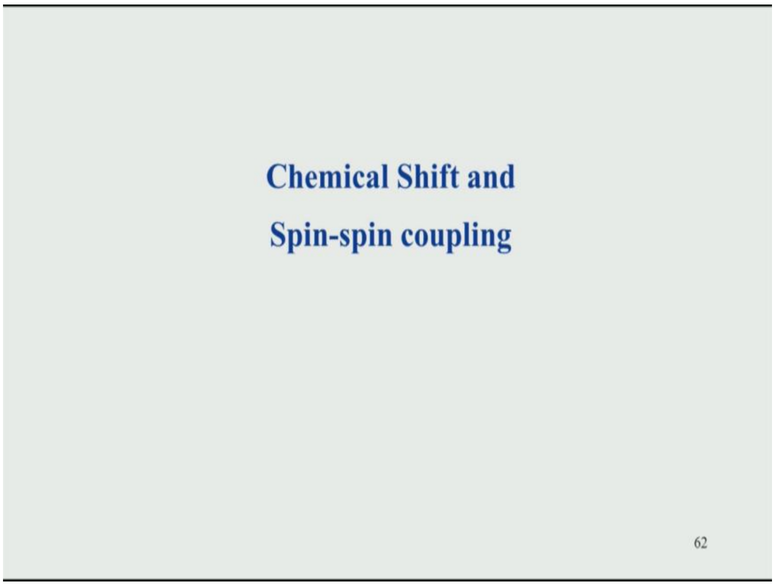
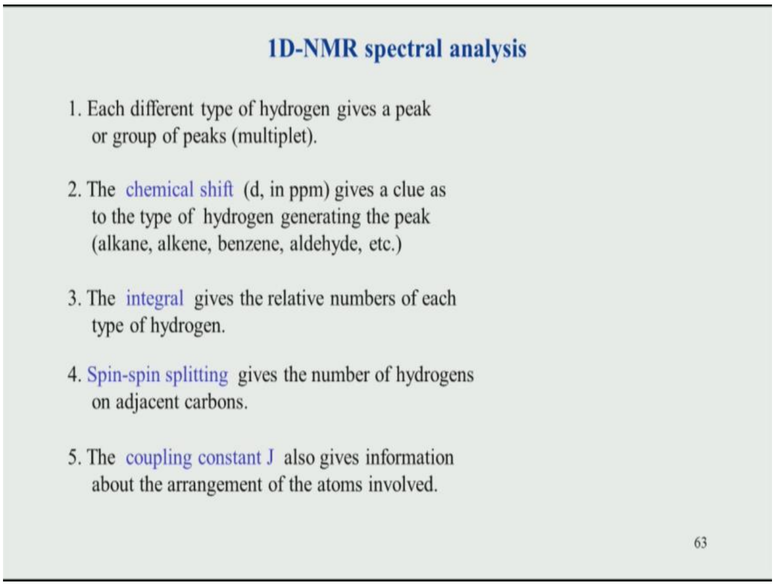


Chemical Shift and
Spin-spin coupling



1D-NMR spectral analysis

1. Each different type of hydrogen gives a peak or group of peaks (multiplet).
2. The chemical shift (d, in ppm) gives a clue as to the type of hydrogen generating the peak (alkane, alkene, benzene, aldehyde, etc.)
3. The integral gives the relative numbers of each type of hydrogen.
4. Spin-spin splitting gives the number of hydrogens on adjacent carbons.
5. The coupling constant J also gives information about the arrangement of the atoms involved.

So, this next few class is going to focus on chemical shifts and what is called spin-spin coupling or J-coupling. So, let us start from chemical shifts so the typically the NMR spectrum consist of the following four parameters, so if we record any NMR experiments. Any NMR spectrum of any compound so these are the four things which will have to analyze. The first thing what we look at is what chemical shifts.

This is the heart of the NMR experiment spectroscopy and chemical shifts is directly related to the structure of the molecule and also it gives the information about the different functional groups such as Alkane, Alkene, Benzene, Aldehyde. So, this is how chemical shifts are a very-very important and they are measured in the units called ppm. we will shortly will see, how this ppm scale is generated. The next parameter very important for NMR is the integral that is the area of the peak.

So, if you recollect we mentioned in the few class back that the area of the peak is directly related to the number of hydrogen atoms or number of protons giving under that peak. So, therefore integral is very important if you want to quantify measure the number of hydrogens and that is very useful parameter the another parameter is called spin-spin splitting which in the common jargon. We also , is known as J coupling of scalar coupling.

So, J coupling is basically in between hydrogens which are which interact with each other through bond. So, we will look at this thing shortly and this spin-spin coupling As, I said coupling constant is also gives the information about the arrangement of atoms so therefore is very important to know the value of spin-spin splitting not only the pattern. So, the pattern is obtained by just looking at the peak structure but the value of the coupling constant gives you information about the structure of the molecule.

So, therefore we have to analyze these four things suggest chemical shifts, integral, the pattern of spin-spin curl splitting and the value of the coupling constant. So, let us look at what is the chemical shifts, from where does actually the chemical shift originate. So, what happens is every any few take it any molecule, so if you look at any molecule as mentioned. Any molecule has basically atoms, which have nucleus now this nucleus is surrounded by electron cloud.

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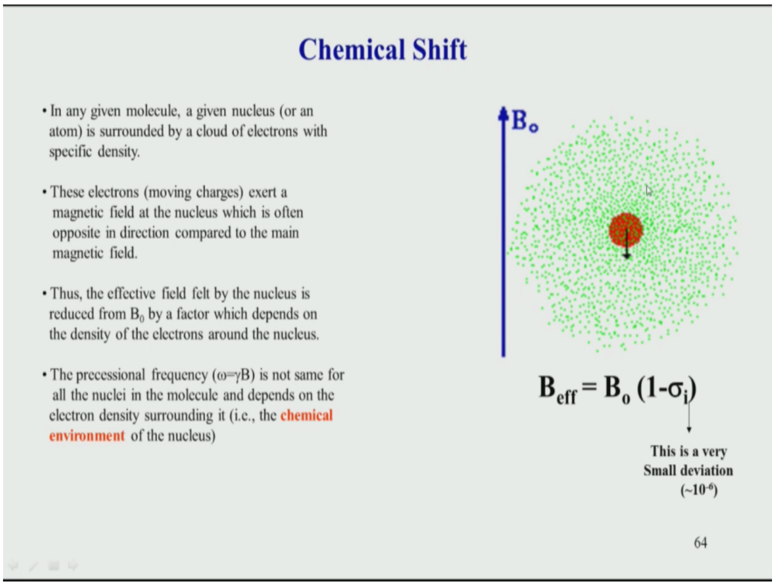

Chemical Shift

- In any given molecule, a given nucleus (or an atom) is surrounded by a cloud of electrons with specific density.
- These electrons (moving charges) exert a magnetic field at the nucleus which is often opposite in direction compared to the main magnetic field.
- Thus, the effective field felt by the nucleus is reduced from B_0 by a factor which depends on the density of the electrons around the nucleus.
- The precessional frequency ($\omega = \gamma B$) is not same for all the nuclei in the molecule and depends on the electron density surrounding it (i.e., the **chemical environment** of the nucleus)

$$B_{eff} = B_o\,(1-\sigma_i)$$

This is a very Small deviation ($\sim 10^{-6}$)

So, these are not number of electrons here is shown as electron cloud. So electron, now these electron cloud which is around this nucleus is generates a small magnetic field opposite to the main magnetic field so we see this is a main magnetic field which is the Tesla, The 9 tesla and so on which we discussed, but because of the motion of electrons. So, if you remember electrons are also charge particles.

They are negatively charged so when they are moving around they generate a magnetic field similar to what the magnetic field this is generated. So, if we remember in the last class we saw then this magnetic comes from the super conducting coils. Because of the current flowing in the coil it generates this field. Similarly the electrons which are also moving around they generate a magnetic field, which is in the opposite direction compare to the main magnetic field or it can be in the similar direction but, there is a magnetic field which is additional to the main magnetic field and that particular negative they opposite is represent.

So, like this so what we are looking at is the main actual the magnetic field around this nucleus, this red colour nucleus is no longer B0 but it is subtracted a small subtraction because of this negative field and this is called shielding. Shielding means protected so is essentially saying that, that these particular electrons which are around this nucleus. They are trying to protect this nucleus or shield, this nucleus from the main magnetic field and that is why we use the word shielding and it is basically denoted these parameter called Sigma.

So, we can see now what happens is that there is not the B0 is not this nucleus does not experience B0. It experiences an effective magnetic field and that effective magnetic field is given like this, where is this is a main magnetic field and this is the shielding factor so suppose shielding is zero, there is no shielding then this term becomes zero...

And, it is B effective it becomes equal to B0 so, basically that is! What is the main magnetic field? So, therefore the shielding is trying to reduce the effect of the main magnetic field at the nucleus side. A And this This shielding is a very small number is a very small deviation it is order of 10 to the power of -6 and if you now see the connection. The ppm typically comes from there because it is the very small deviation from the main magnetic field.

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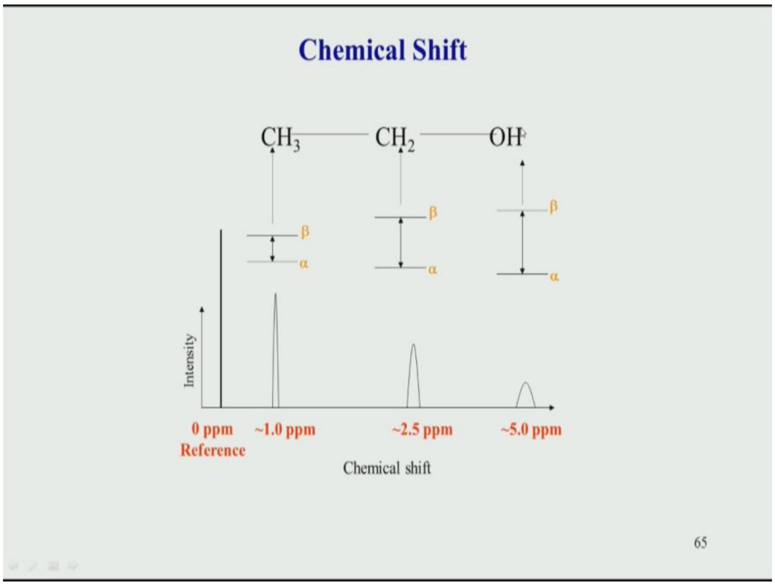

Chemical Shift

So, let us see you from how? How does this chemical shifts vary for different atoms. So, if you look at this structure now CH3, CH2, OH this is ethanol standard compound. So, what happens is the shielding so, for this nucleus that is proton these proton here is slightly different from the shielding caused by the electrons around this hydrogen and which is again different from the shielding caused around the nucleus of this hydrogen.

So, how does the shielding? Why does the shielding differ from here to here and there that those factors we look at shortly but the point understand here is that this nucleus which is hydrogen. The proton is now having a different affect different shielding by the electrons around it electron cloud around it compared to this hydrogen and compared to this hydrogen and because of that each of this hydrogens have a slightly different resonance frequency.

So, if you recollect the previous slides that resonance frequency Omega is equal to Gamma into B0. So, if if B0 that is affective field is different from here and here and here, The Omega also starts changing and Omega is basically the frequency of Lamar precession or the energy difference. So, we can that this particular CH3 has a different resonance frequency compared to this particular atom hydrogen and compared to this.

So, we can see there is a gradual change of the energy and therefore the resonance. What is here is not same as resonance here is not same as here and when you start exciting the protons this particular will have a different frequency of excitation which is converted into a ppm scale. Will see shortly and that is why this peak differs is comes at the different location compare to these peak. Because this peak has a different resonance frequency and therefore it has a different frequency of excitation and similarly this OH will have a different frequency of excitation.

So, you see the point in this slide is to see that, that each type of hydrogen has a different local magnetic field. So, when we talk about shielding you say that the local magnetic field why local? Because the local the main magnetic field is same for all of them the B0 but, locally around this nucleus, there is a shielding which is different from here and here so each of them has a different local magnetic field and if the local magnetic field if the magnetic field changes if the Omega changes.

That is the processional energy of excitation changes and therefore they come at different positions. So, this is how at the chemical shifts can be exploited to let look at the structure of molecules because, if all these hydrogens if all of them had no shielding then all of them would look alike and there would a single peak and that would make NMR very in uninteresting and of no us. The reason why NMR is so popular today is because, that local magnetic field changes between one atom to one other hydrogens and that change is the small change is good enough to separate them into different positions and that gives the information about the structure of the molecule.

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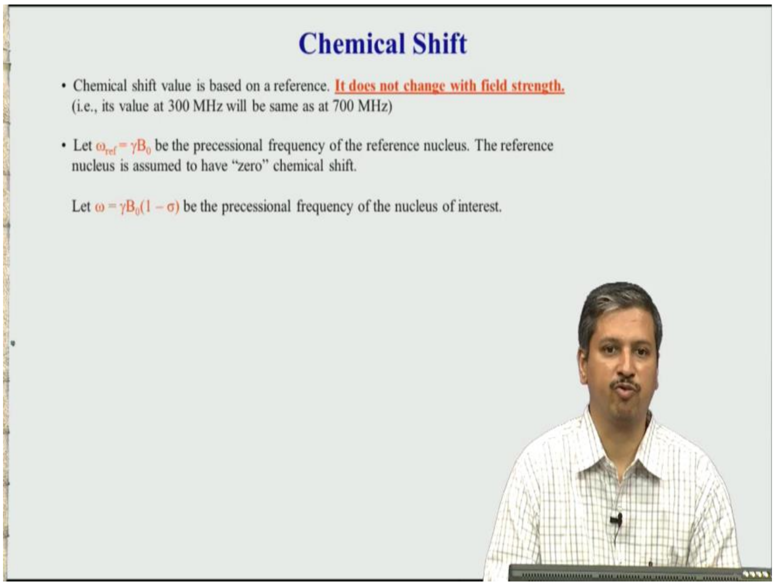

Chemical Shift

- Chemical shift value is based on a reference. **It does not change with field strength.** (i.e., its value at 300 MHz will be same as at 700 MHz)
- Let $\omega_{ref} = \gamma B_0$ be the precessional frequency of the reference nucleus. The reference nucleus is assumed to have "zero" chemical shift.

  Let $\omega = \gamma B_0(1 - \sigma)$ be the precessional frequency of the nucleus of interest.

So, now let us see how this ppm scale is calculated. So peak for understanding this one should keep in mind that chemical shifts when we say is always based on a reference so, ok so that why do we use the word reference the reference is use because, if I record an NMR spectrum at some particular spectrometer. Let us say I have a 300 megahertz in my laboratory and I take the same molecule and I go to 700 megahertz in some other laboratory then, I should not get a change in chemical shifts.

Because chemical shifts is inherently the related to the structure of the molecule and if the structure cannot change from here to here then, chemical shifts should also not change so therefore, to preserve this uniformity of chemical shifts we use a reference and with respect to reference always the chemical shifts are calibrated the chemical shifts values and therefore this is a very important point that chemical shifts does not change with the field strength meaning this does not change the spectrometer and that is the.

So, now see let us see how it is calculated? So, now look at this equation here this is a standard NMR equation we say that Omega is equal to GammaB0. So let suppose we have a reference molecule we can right now let us not look at what are the molecules use can be used for a reference but, suppose you have some molecule hypothetical molecule which is having we call it as a reference and that particular molecule will have a peak which has Omega.

A chemical shift or Omega frequency and that Omega frequency is precessional frequency is related to this. Ok! So, this reference now will assume as zero chemical shifts which means that that sigma. If you recollect the sigma parameter in the previous line the sigma is no longer their so we considered this as hypothetically zero chemical shifts this is how it look like. Now you will in your molecule of interest the molecule which we are trying to study will have not zero value.

It has some chemical shifts shielding factor and that shielding factor is denoted like this. So, you have now Omega equal to Gamma B0 into 1 minus Sigma and this is the processional frequency of the nucleus of our interest which we are trying to study. So, what we do is we then subtract if you subtract these two equations. So, if you simply do a simple mathematical operation of subtracting and you rearrange to get the Sigma you see the Sigma can be written like this.

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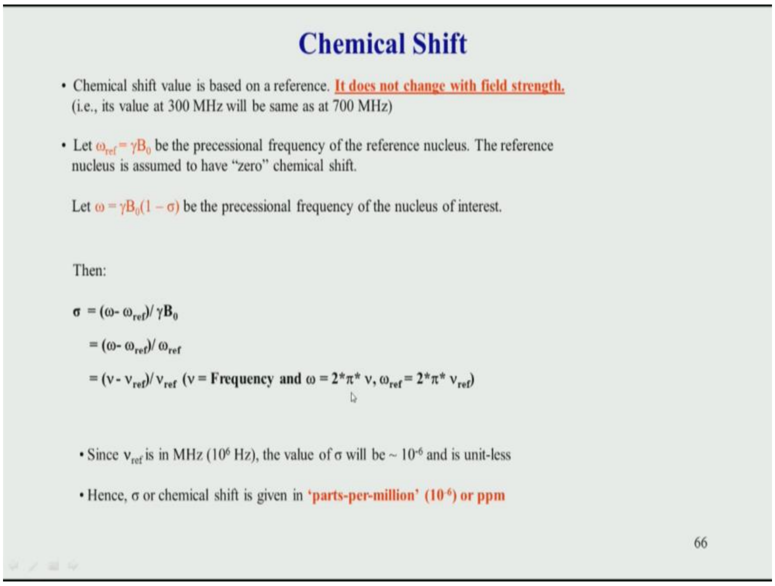

Chemical Shift

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- Let $\omega_{ref} = \gamma B_0$ be the precessional frequency of the reference nucleus. The reference nucleus is assumed to have "zero" chemical shift.

Let $\omega = \gamma B_0(1 - \sigma)$ be the precessional frequency of the nucleus of interest.

Then:

$$\sigma = (\omega - \omega_{ref}) / \gamma B_0$$
$$= (\omega - \omega_{ref}) / \omega_{ref}$$
$$= (\nu - \nu_{ref}) / \nu_{ref} \quad (\nu = \text{Frequency and } \omega = 2*\pi*\nu, \ \omega_{ref} = 2*\pi*\nu_{ref})$$

- Since ν_{ref} is in MHz (10^6 Hz), the value of σ will be ~ 10^{-6} and is unit-less
- Hence, σ or chemical shift is given in **'parts-per-million' (10^{-6}) or ppm**

So, you just have to simply subtract and then rearrange a little bit then you get like this. So, then if you look at this Omega, remember Omega is given by 2 pie mu. This is the formula for Omega this is in radians per second, this is in hertz per second this is per it is hertz per second. So, when you convert a hertz in to radians per second this is the multiplication factor 2 pie. So, when you convert it to frequency units into frequency you will get like this.

So, now you will see the frequency the reference frequency what we are using is in Mega Hertz? Why is it in Mega Hertz? Because this comes from here this according to our very beginning in the few first class we saw that the magnetic field is measured in Tesla and for a given Tesla 9 Tesla, 10 Tesla or 15 Tesla, any Tesla number you take magnetic field and you multiply with this Gamma this frequency will come out in megahertz.

So, that is why we talk about the 600 Mega Hertz and 300 Mega Hertz and so on. So, similarly the view new ref which is nothing but 2 pie. Omega by 2 pie is also in Mega Hertz, so when you put these value here in Mega Hertz you will see that the Sigma this Sigma this value will be of the order of 10 to the power of - 6 ok and then it is unit less. This is very important because you what if you see from these formula equation I am subtracting two frequencies this is for this unit is also in frequency units and I am dividing by another frequency.

So, when you divide the two units which have the same same units you get the parameter which is unit less. So, the shielding factor is parts per million we use the words because it is in this of that of 10 to the power of - 6.

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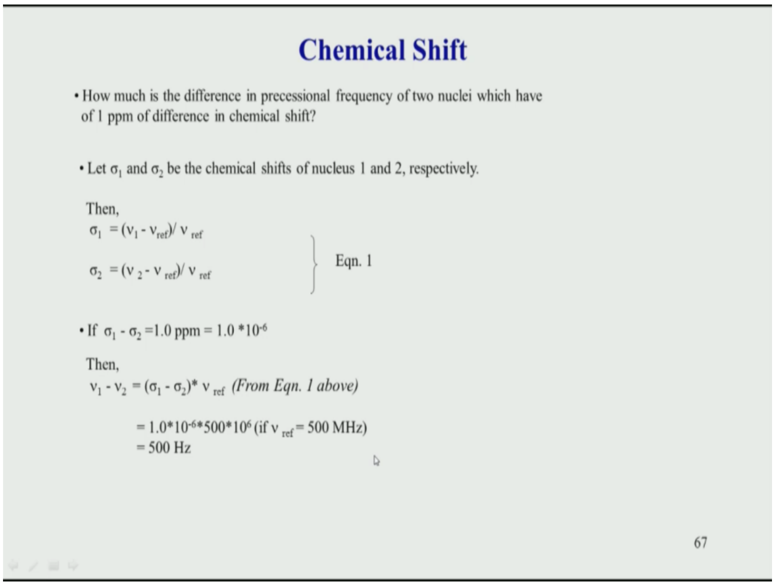

Chemical Shift

- How much is the difference in precessional frequency of two nuclei which have of 1 ppm of difference in chemical shift?

- Let σ_1 and σ_2 be the chemical shifts of nucleus 1 and 2, respectively.

Then,

$$\left.\begin{aligned}\sigma_1 &= (\nu_1 - \nu_{ref})/\,\nu_{ref}\\ \sigma_2 &= (\nu_2 - \nu_{ref})/\,\nu_{ref}\end{aligned}\right\}\ \text{Eqn. 1}$$

- If $\sigma_1 - \sigma_2 = 1.0\ \text{ppm} = 1.0 * 10^{-6}$

Then,

$$\nu_1 - \nu_2 = (\sigma_1 - \sigma_2) * \nu_{ref}\ \textit{(From Eqn. 1 above)}$$

$$= 1.0*10^{-6}*500*10^{6}\ (\text{if } \nu_{ref} = 500\ \text{MHz})$$
$$= 500\ \text{Hz}$$

So, we can say now let us say that we want do a simple calculation let us say we ask that question that suppose I have a difference in chemical shift of two nuclei which are one ppm difference.

Ok! So, how can we calculate in terms of Hertz so, this is a very important conversion we do routinely in NMR is that we convert ppm scale into hertz scale and vice versa. So, how do you convert a ppm into hertz is very simple. We can go through this algebra and what basically you happens is the following so let us sat you have two chemical shifts two peaks which differ in chemical shifts by one ppm, so one ppm means one into 10 to the power - 6 parts per million.

So, then if you want to convert this into frequency difference remember ppm and frequency are not same ppm units are different from frequency units. So, we want to convert into the frequency so, if you look at this equation here above what we have written this is coming from the previous line the last line which I showed you how we get the chemical shifts. So, all we want to do is we want to simply subtract these two.

So, if you subtract these two you will get one ppm there is what we have and that you can convert it into these difference in Hertz and remember this new ref is known to us let, suppose that is say we are doing at 500 Mega Hertz and the 500 Mega Hertz is 500 into 10 to the power 6 and this is unit is in 10 to the power one ppm one into 10 to the power – 6, when you multiply you finally get only 500 Hertz.

So, what it means are you suppose I have a spectrometer which I have a 500 Mega Hertz in my laboratory and I record a spectrum and I get two peaks which are separated by one ppm. Then the separation between those two peaks in Hertz scale in the Hertz value is 500 Hertz. So, all what we have done essentially here it was a rule of thumb. I mean a simple calculation we are simply multiplying the spectrometer frequency 500 into one.

So, therefore let us say we have two ppm difference. If the difference is two ppm then two ppm this will become 1000 Hertz, so is simply take the spectrometer frequency in Mega Hertz that is 500 or 600 Mega Hertz 600 you take multiply bye the difference in the ppm value and that will give you in the difference in the Hertz scale and this is very important point because, if you see now you notice. This number is coming from the spectrometer and that will not be the same.

So, if you write go through 300 Mega Hertz I will have 300 as the value here. If I go to 700 Mega Hertz this will become 700. So, you see when I change my frequency of spectrometer I will change this value and if I change this value this value is also change. So, what it means is that the ppm difference does not change which between two peaks the ppm value does not change. If you go from 300 to 400 to 500 or any spectrometer however the difference in Hertz depends very much on the spectrometer.

So, if I go to 700 Mega Hertz by peaks now will be separated in the difference separation is now equal to 700 Hertz. If, I go to one Giga Hertz 1000 Mega Hertz then it will be 1000 Hertz if, you see the peak separation varies with the spectrometer, when you calculate in Hertz value but it does not change when you calculate in ppm value. So, this is very important point because as I mentioned once we take with the reference respect to reference we do not want the ppm value to change from one spectrometer to other spectrometer.

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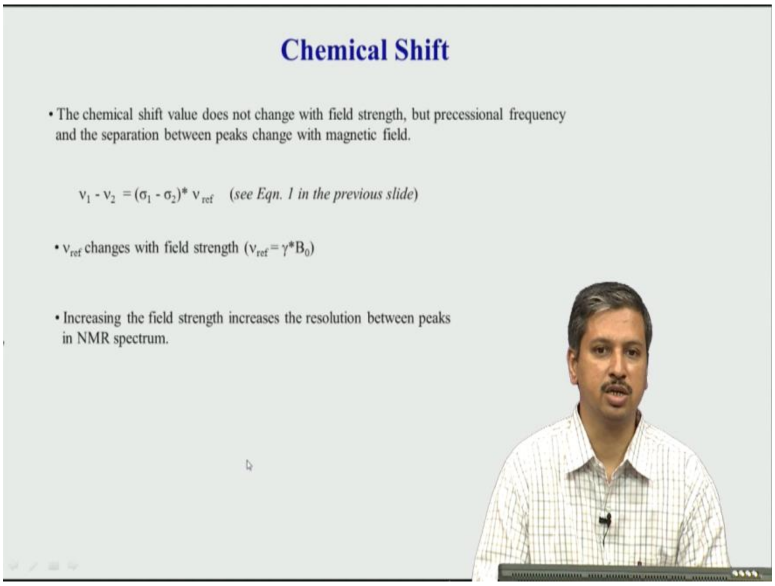

Chemical Shift

- The chemical shift value does not change with field strength, but precessional frequency and the separation between peaks change with magnetic field.

$\nu_1 - \nu_2 = (\sigma_1 - \sigma_2)^* \nu_{ref}$ *(see Eqn. 1 in the previous slide)*

- ν_{ref} changes with field strength ($\nu_{ref} = \gamma^* B_0$)

- Increasing the field strength increases the resolution between peaks in NMR spectrum.

But the separation of the peaks in Hertz value will change because depends on the field strength. This separation is actually what you helps you in giving a resolution. So, this is what you shown in this slide if you see that this what we calculated that the chemical shift value does not change with the field strength but the precessional frequency changes between two peaks changes with magnetic field.

So, this is what is shown in the last slight the same thing is repeated here is that the chemical shift value may not change in ppm. But, when I multiply with my spectrometer frequency the reference we can see of the spectrometer that changes with recorder and that will change. So, why is that so important? Then that is the point here is that when I change increase the separation between the increase when I increase the separation between the peaks basically what I am doing is I am increase the resolution between the peaks that means I am separating the peaks more and more.

If, I go to higher and higher magnetic field so, if a peak is separated by 10 Hertz or (**or**) 100 Hertz or a 300 Mega Hertz machine. If I go to 600 Mega Hertz machine the peaks will be separated by 200 Hertz double and that doubling of the separation is basically saying that the peaks are well resolved form each other and what that says is that it has resolution of the spectrum has increase and this for this is one of the reason why a lot of time and resource is spent on increasing the the frequency of the spectrometer.

If you go from 300 to 400 to 500 to 600 you keep increasing the resolution in between the peaks and therefore this very-very important to go to higher field. In case your resolution is not sufficient for you at the lower field. typically for chemist like organic chemist and those working in the area of peptide NMR upto 600 Mega Hertz is more than sufficient for their practical for the practical prospective is sufficient.

But when you go to biomolecule such as proteins and nucleic acids and carbohydrates and so on there you need to increase the resolution you need a lot of resolution and that is why those working in the area of biomolecule area NMR they prefer to use a high magnetic field like 800 Mega Hertz or one Giga Hertz and so on compared to the organic chemist who you are for them 600 and below is sufficient for most practical purposes.

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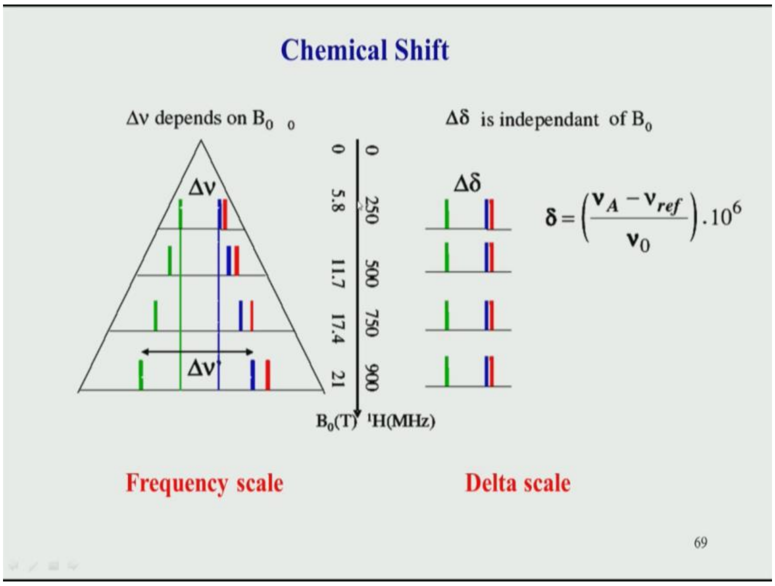

Chemical Shift

$\Delta\nu$ depends on B_0 o

$\Delta\delta$ is independant of B_0

$$\delta = \left(\frac{\nu_A - \nu_{ref}}{\nu_0}\right).10^6$$

Frequency scale

Delta scale

So, this is what is shown in this slide is that the Delta mu again remember this Delta mu in the difference in the separation between the peaks that varies with the value view the spectrometer. So, if I start from 0 Mega Hertz off course zero is zero frequency separation. If I go increase the spectrometer frequency I go to 250 Mega Hertz, 500 Mega Hertz, 750 Mega Hertz, 900 Mega Hertz you see the peaks are all changing.

Because, they are separated by the Hertz the resolution in Hertz is changing and they are they are we getting well more and more separate but however, if I convert this into this using this formula into ppm scale. In the ppm scale remember we subtract the frequency and divide by again a frequency and this takes care of this separation. What you see increasing is again when you divide by the increasing frequency it gets nullified and finally what you get is at the peak separation does not change in the ppm scale in the Delta scale.

So, this is very important point in a Delta scale in the frequency is remains the same that is the ppm value remains the same. Whether you go to 250 Mega Hertz you get the spectrum or you look at 900 Mega Hertz you get the same ppm value but, if you look at the Hertz scale the ppm the frequency is changing as an more and more, when you go down to higher magnetic field so, this is the important concept which has to be kept in mind.

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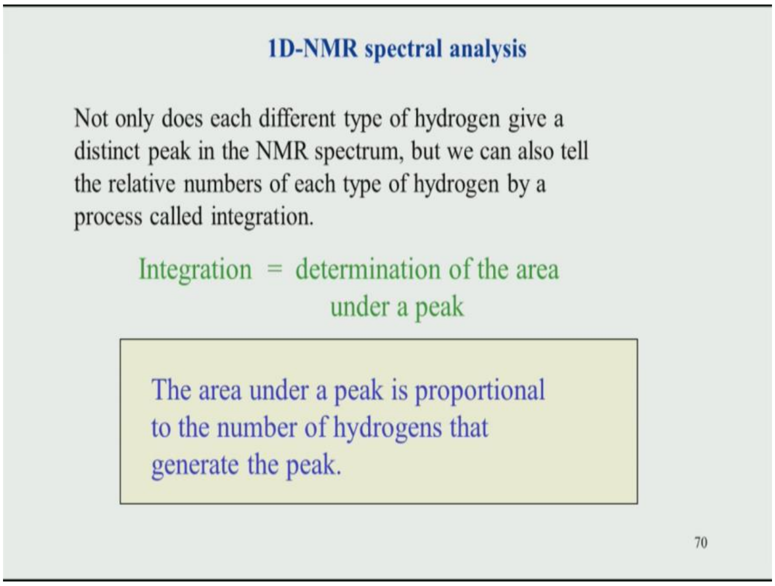

1D-NMR spectral analysis

Not only does each different type of hydrogen give a distinct peak in the NMR spectrum, but we can also tell the relative numbers of each type of hydrogen by a process called integration.

Integration = determination of the area under a peak

The area under a peak is proportional to the number of hydrogens that generate the peak.

So, the second point which in NMR spectrum which we saw is the area of the peak. So, that this what is shown here? Is that the area is very determines the number of protons so, the area under the peak is proportional to the number of hydrogens that generates the peak. So, this is what is called as integration? So, we integrate a peak to determine the area under the peak. So, this is the shown in this particular diagram here.

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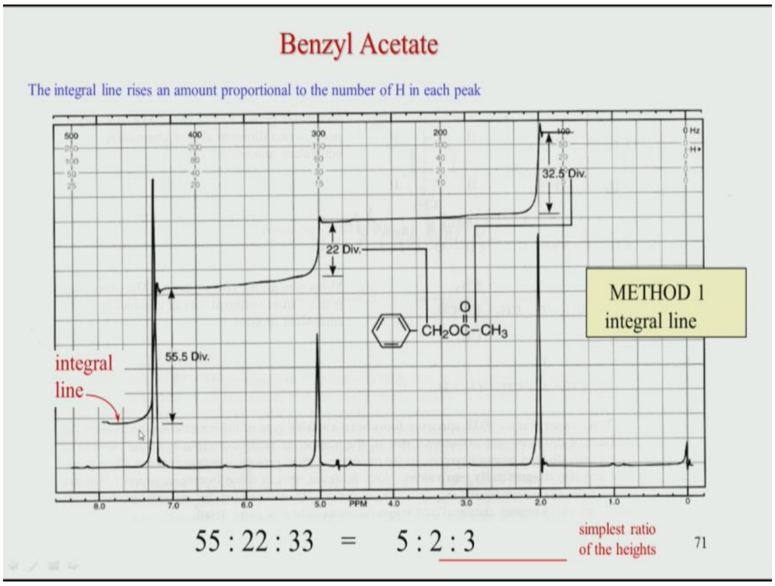

Benzyl Acetate

The integral line rises an amount proportional to the number of H in each peak

55 : 22 : 33 = 5 : 2 : 3

simplest ratio of the heights

This is taken from a book called a spectroscopy by Pavia here you can see that see a peak which if a this particular molecule so, will look at more closely at different why the peaks are coming here and so on that that comes under the chemical shifts analysis. But, in this slide the point to take home is that that number of protons. Here it is three here it is zero there is no protons so, off course you do not get any peak from this in the protons spectrum but this proton is number two, two number of hydrogens.

There are 3 hydrogens here and there are five hydogens in these aromatic systems. So, therefore the aromatic whatever peak you get in aromatic portion should be five times compared to the peak what you get in this five is to two compared to what you get in the CH2 and what you get in the CH3 should be three. So, therefore you should have this ratio five is to two is to three. So, this is the simplest ratio of the heights.

So, what typically we do if we measure the area under this peak and this area's ratio is taken and the ratio is what is presented in the spectrum. So, ratio is very important to know because we then come to know from the ratio how many hydrogens are there in this particular peak without which is very difficult to analyze the structure. Which will use in these later slides so the J coupling shifts and this area is very important.

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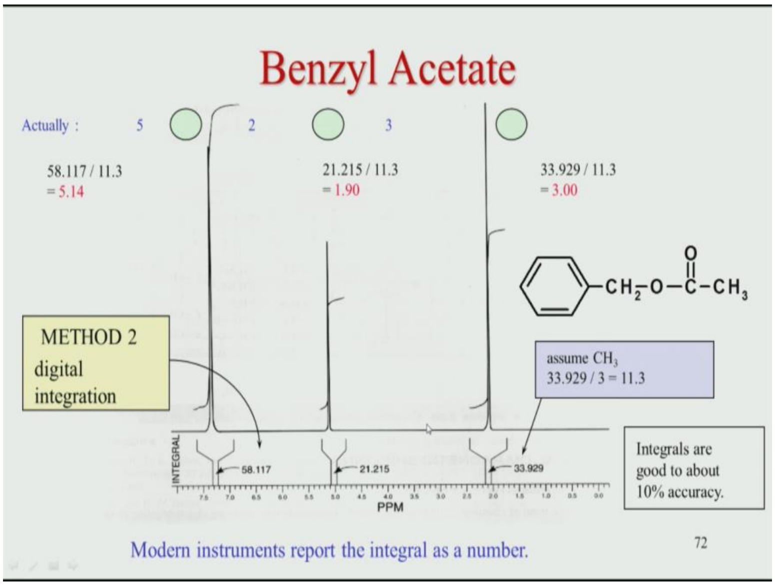

Benzyl Acetate

Actually : 5 2 3

58.117 / 11.3 = 5.14

21.215 / 11.3 = 1.90

33.929 / 11.3 = 3.00

METHOD 2
digital
integration

assume CH_3
33.929 / 3 = 11.3

Integrals are
good to about
10% accuracy.

Modern instruments report the integral as a number.

This is again shown in this particular slight this is for benzyl acetate and the same molecule that we get the height in the ratio of five is to two is to three because of the different type of protons.

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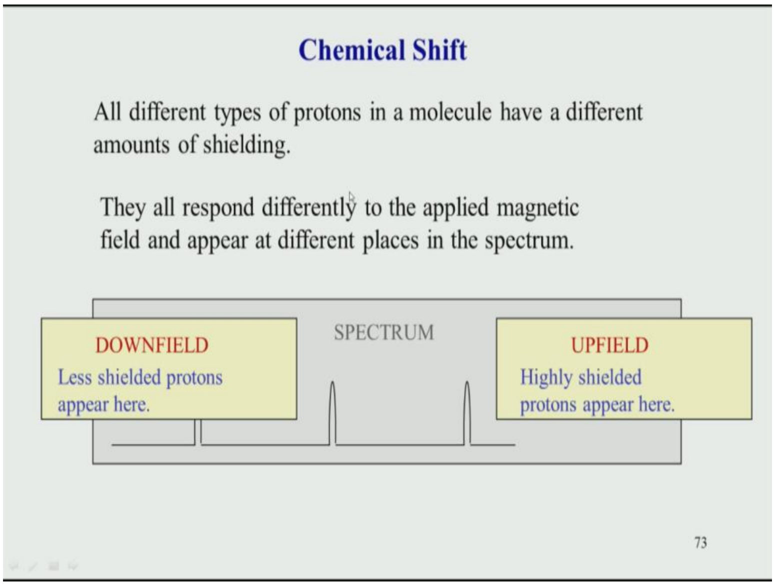

Chemical Shift

All different types of protons in a molecule have a different amounts of shielding.

They all respond differently to the applied magnetic field and appear at different places in the spectrum.

DOWNFIELD
Less shielded protons appear here.

UPFIELD
Highly shielded protons appear here.

So, will continue in the chemical shifts now and just have a look at how the chemical shift is determined by various factors. So, what is important to know here is that we typically when you look at a spectrum in NMR we use the word down field and up field.

So, this is some terminology which we are introducing now we should you keep in mind as we go further in what does an up field and down field mean this is a relative a way of looking at the peaks. So, typically the spectrum the reference is always at the right hand side of the spectrum. So, the reference molecule if you will see in the next few slides the reference is always kept at the right hand side that is at the high field.

We use the word shielded the shielded up field when you set it means it is coming towards the right hand of the spectrum any peak which comes at the right side we say it is up field shifted. Respect to the center or center is somewhere here so, the center is very important in NMR we usually keep all our many of the reference frequency of the center and with respect to the center you say that these peaks are up field shifted on the right side and if the peaks appear on the side of the spectrum we use the word down field.

So, down field is less shielded or de-shielded so the de-shielded ones come on the left side means their ppm value goes slow. If, you look at the spectrum the ppm starts zero somewhere here and they go from left to right. So, ppm scale always goes like this so, this is higher ppm value and as I am going to the right side is lower ppm value. So, the lower ppm value is denoted as up field shifted and the higher ppm value is denoted as the down field shifted.

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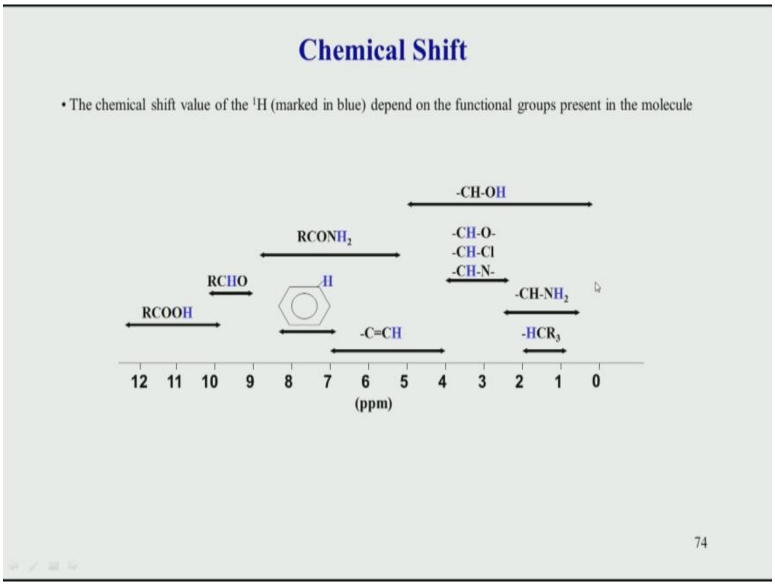

Chemical Shift

- The chemical shift value of the ^1H (marked in blue) depend on the functional groups present in the molecule

So, this chemical shifts typically see for different functional groups comes in this particular zone. So, when this is a hydrogen spectrum a proton spectrum. So, we can say here it is a marked, the scale is marked from zero onwards towards the positive side and typically this is what happens in all organic compounds we choose zero ppm as a reference.

Will talk about reference a little bit later so, that is a reference is always we set it to 0 and all the typical compounds which is studying in the organic chemistry they all fall on the higher down field side compared to the 0. So, that means anything more than the 0 is shifted down field with respect to the reference and you can see here there different compound functional groups are shown for example Amides.

Amides hydrogen in the amides region become typically between 6 to 8 ppm. The aldehyde comes very much down field the aromatics comes somewhere between 7 to 8. So, what we are looking at you have to keep eyes on these proton here you have to focus on the protons we are looking at the chemical shift of this proton this proton the one shown in blue colour. So, will see those which are attached to chlorine or nitrogen or oxygen they come here and typically when you look at methyls.

Methyls are CH3 groups they are always down field up field shifted to somewhere between zeros to two. So, methyl peaks are very dominant very prominent in NMR spectroscopy they can very easily recognize because they always comes somewhere between 0 to 2, 2.2, 2.5 ppm so, methyl’s peaks are very typical the zone is here and you will see in the different the analysis we will see why they come here and how they can be used.

But the reason for these hydrogrns to come at so, different positions is what is going to be the focus in the next class we will see what are the factors which affects the chemical shifts of different hydrogen and how we can figure out that what the functional group is present based on the chemical shift value and that we will have focus in the next class. Thank you!