Spectroscopic Techniques for Pharmaceutical and Biopharmaceutical Industries

Professor Shashank Deep
Department of Chemistry
Indian Institute of Technology Delhi

Lecture 31

NMR data processing and Chemical shift

Hello students, welcome back to lecture 31 of this course. In the last lecture, I started discussing about NMR and its principle. In this lecture, I will discuss about how to process the NMR data, then we will discuss about chemical shift and the factors affecting chemical shift.

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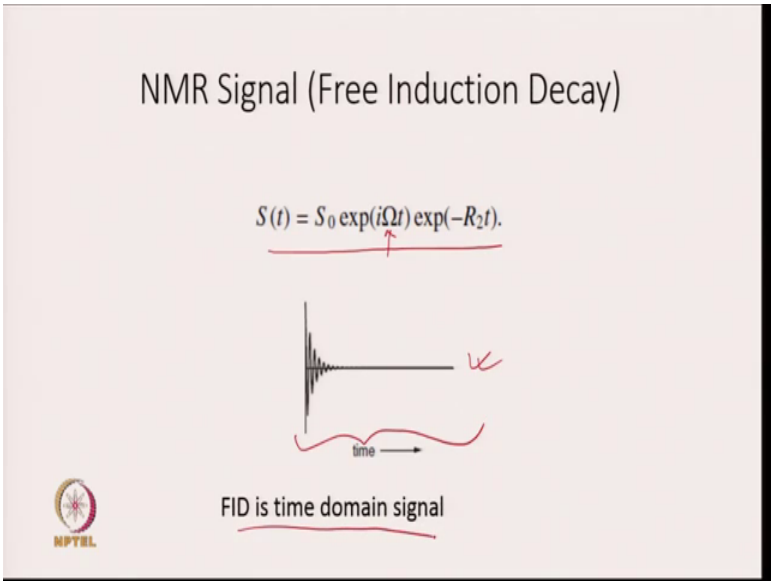

In the last lecture I showed you that signal in NMR is given by this equation S at a given time is equal to S naught multiplied by exponential i and this is capital omega, which is basically chemical shift multiplied by time, multiplied by exponential minus R2 into t, R2 into t. So, this signal is called Free Induction Decay and it will look like this, if I plot S versus t it will look like this, this is known as Free Induction Decay or FID and it is basically a time domain signal, time domains signal.

So, in NMR we generally start with time domain signal because that way we can maximize the sensitivity, we must remember that NMR is low sensitive technique because the gap between ground state and excited state is very low and that means that population difference between into states are quite small, population difference between

two state is quite a small and hence the sensitivity is low. So, one of the thing which we do is to get the signal in time domain and then do Fourier transformation.

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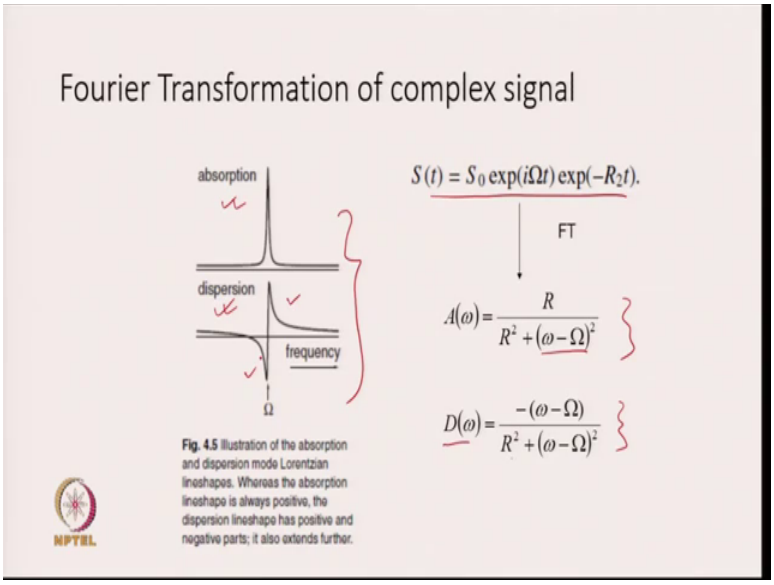

So, if we do Fourier transformation of the FID, what we are going to get is two different peaks, which is one is called absorption and the second is dispersion. So, if we take Fourier transformation of this signal what we are going to get is A which is a function of omega is equal to R divided by R square plus omega minus capital omega square whereas, D omega is given by minus omega minus capital omega divided by R square plus omega minus capital omega square. So, this is your absorption and this is your dispersion and they have Lorentzian lineshape, absorption lineshape is always positive, whereas dispersion linesshapes has positive and negative part, positive and negative part.

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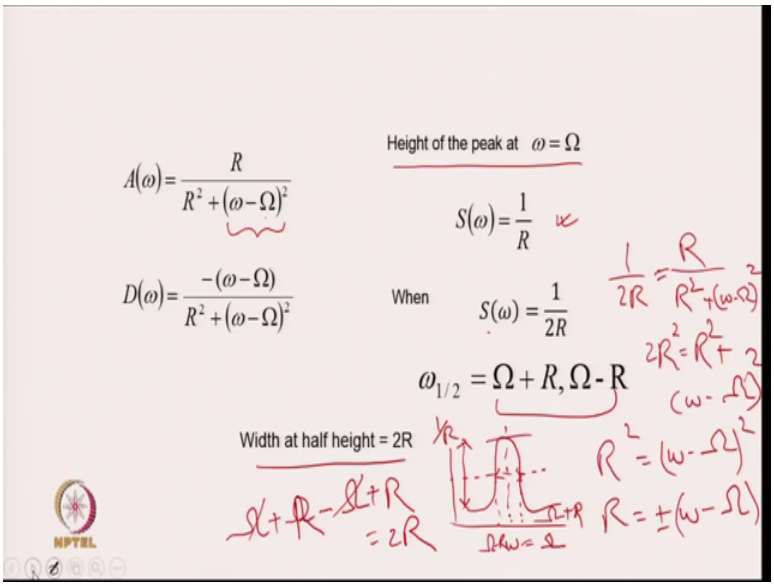

Now, some important facts about absorption and dispersion peaks that if you take height at height of the peak at omega is equal to capital omega, then S omega will be 1 by R and you can do heat here. So, if I take omega is capital omega then this part is going to be 0 and so, A omega is R divided by R square, which is 1 by R. And the second thing is if I take S omega is equal to 1 by 2R, so in that case A omega is 1 by 2R and this is equal to R divided by R square plus omega minus capital omega square and so what that means is 2R square is equal to R square plus omega minus capital omega square.

So, R square is equal to omega minus capital omega square. So, R is equal to plus minus omega minus capital omega and what does that mean is omega will be equal to, omega will be equal to capital omega plus R and or capital omega minus R, and so the difference so, if you remember that peak looks like this. So this height is 1 by R and that is what is written 1 by R because at this point omega is equal to capital omega.

And if I take this omega half it means the half height where signal is equal to 1 by 2R so, this is 1 by R so, this will be 1 by 2R so, this value is given as omega minus R and this value will be capital omega plus R and so difference between this is 2R. So, width at half height, half height means when signal is 1 by 2R, then width at half height is, this width is this plus omega plus R minus this minus minus plus R. So this cancels out so, this so is equal to 2R, so equal to 2R. So this is characteristic of absorption and dispersion peak.

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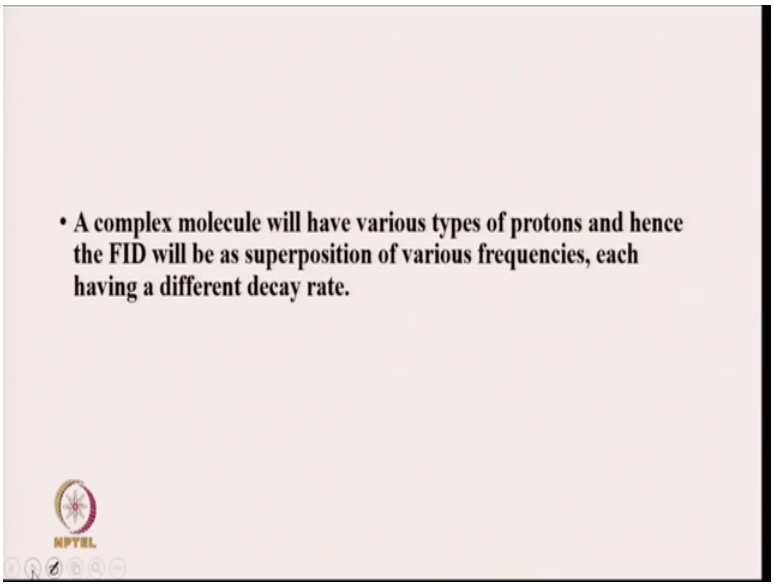

Now, let us think about that if we take a some molecule which is a complex molecule, it is going to have various types of protons. Hence, FID will be super position of various frequency and each having a different decay rate. So, now question is we have to apply pulse at one time.

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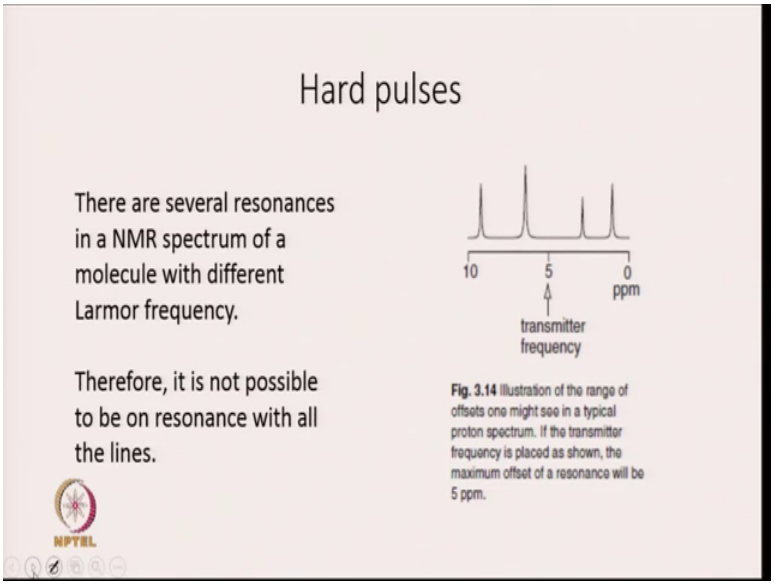

Suppose I am applying 90 degree pulse to get a 1D spectra then which kind of pulse we should use? Because there are several resonances in an NMR spectrum of a molecule

with different Larmor frequency and it is not possible to be on resonance with all the lines. So, we have to choose a transmitter frequency such that we are affecting all the resonance at the same time.

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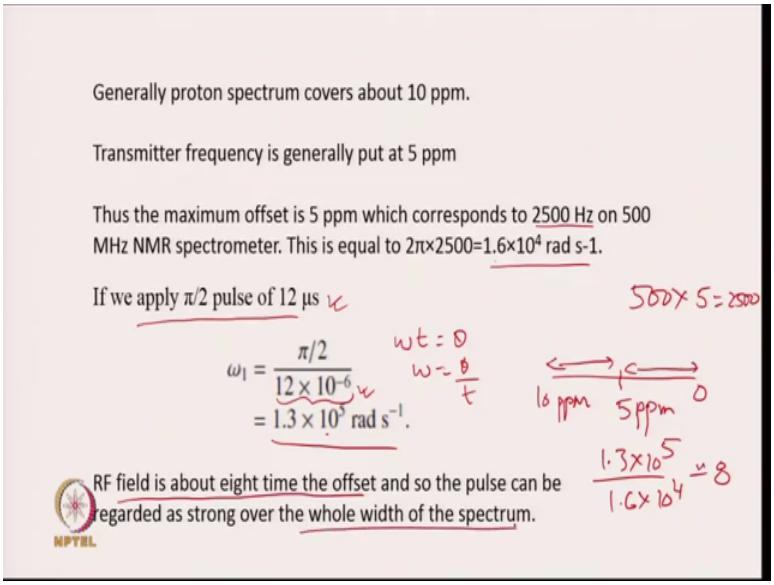

So, if you are looking at proton spectrum, it covers about 10 ppm, it goes from 0 to 10 ppm, so in that case transmitter frequency is generally put at 5 ppm. Thus the maximum of offset is 5 ppm on both sides, so 0 to 10 ppm suppose, this is your NMR spectrum region. So I generally put my transmitter frequency at 5 ppm so offset on both side is 5 ppm which corresponds to 2500 hertz on 500 megahertz NMR spectrometer.

So 500 into 5 is equal to 2500, this is equal to 1.6 into 10 to the power 4 radian per second. So, 2 pi into 2500 is equal to 1.6 in to 10 power 4 radian per second and for that generally we apply 90 degree pulse of around 12 microseconds, of around 12 microsecond. In that case omega will be pi by 2 divided by your time of the pulse, since, so, basically omega t is equal to theta and so omega is equal to theta by t, omega is equal to theta by t,

So, theta is pi by 2 and t is your 12 into 10 to power minus 6. So, which is around 1.3 into 10 to the power 5 radian per second. So 12 microsecond pulse is so chosen such that RF field is about eight time the offset, so offset is look offset here 1.6 into 10 power 4 radian

per second, whereas omega 1 chosen is 1.3 into 10 power 5 radian per second. And so RF field which is proportional to omega 1 is about eight times.

So 1.3 into 10 to the power 5 divided by 1.6 into 10 to the power 4 it is around eight times, eight times so RF field is about eight times the offset and so the pulse can be regarded strong over the whole width of the spectrum, whole width of the spectrum. So, if you want this pulse should be taking care of all the offset then you have to choose hard pulse, which has a small time, which is around 12 microseconds.

And that kind of pulse is known as hard pulse, that kind of pulse is hard pulse because it is affecting the resonance from 0 ppm to 10 ppm, 0 ppm to 10 ppm and it is almost around you know it will vary from 90 degree to 80 degree, for a 90 degree to the resonance which is at 5 ppm and around 80 degree to the resonance which is at supposed 10 ppm.

So, we have to choose a hard pulse such that we can get maximum intensity out of the spectrum, soft pulse are of higher time, of higher length, time length and they are chosen when the one to, excite only one resonance, one specific resonance then we choose the soft pulse because soft pulse RF field is very small. Whereas, for hard pulse RF field is quite large than offset and so they affect whole width of this spectrum strongly.

So, RF field is about eight times the offset and so the pulse can be regarded as a strong over whole width of the spectrum, so you want that whole width of the spectrum should get affected as strongly as possible, as strongly means the pulse your theta should be around pi by 2 for a peaks at 10 ppm or 0 ppm.

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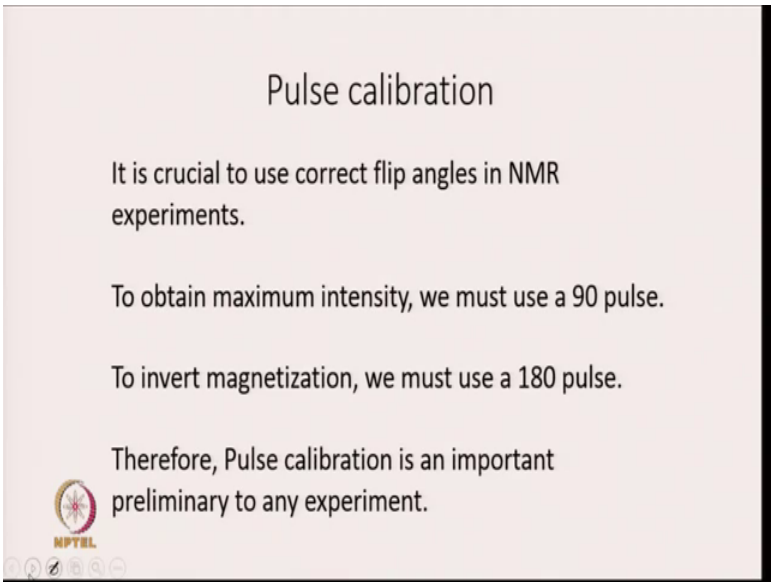

So, second important thing if you want to get maximum signal so it is crucial to use correct flip angles in NMR experiments, you must remember that NMR is low sensitive techniques. So, we want to get maximum signal from each scan and so it is crucial to use correct flip angle. And to obtain maximum intensity we must use a 90 degree pulse, and to invert magnetization we must use a 180 degree pulse.

And so correct length of pulse should be maintained. And therefore, pulse calibration is an important preliminary to any experiments. So, before starting any experiment, you must do pulse calibration, because if you are not using exact 90 degree pulse, you are going to get a small signal.

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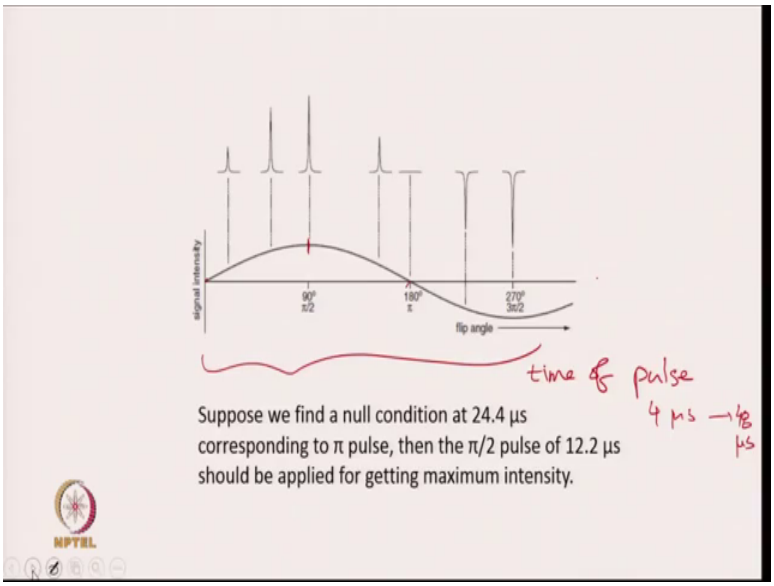

And the way we do is that we start (doing) taking a spectra by varying the time, time of the pulse, varying the time of the pulse. So for example, we start from 4 microsecond to suppose 48 microsecond and then get the intensity, get the intensity. So, initially when it is around 0 degree pulse the time corresponding to 0 degree pulse, you will get no signal.

But, as the time increases, your intensity will increase and at 90 degree pulse you should get maximum signal that is what you expect at 180 degree you should get 0 signal and again 360 you should get a 0 signal. So, what do you do that at a particular? You apply, so for example, you apply at 4 microsecond, then 5, 6, 7, 8 till 48 microsecond and then try to see a null condition.

Suppose, we find a null condition at 24.4 microsecond corresponding to pi pulse then, the pi by 2 pulse will be 12.2 microsecond and that should be chooses to get the maximum intensity. So, what, in the calibration what you are doing is you are applying, you are applying pulse of different time length, pulse of different time length and trying to find out a null condition and as you know that null condition can be found at 180 degree, 360 degree.

So, once you know that, that this is the time length which corresponds to your null condition, then you know where is 180 degree, where is 360 degree. And once you know

time corresponding to 180 degree, you just divide by 2 and then you get the pulse length for 90 degree, pulse length for a 90 degree pulse. So, if we find a null condition at 24.4 microsecond it means 90 degree pulse length should be 12.2 microsecond.

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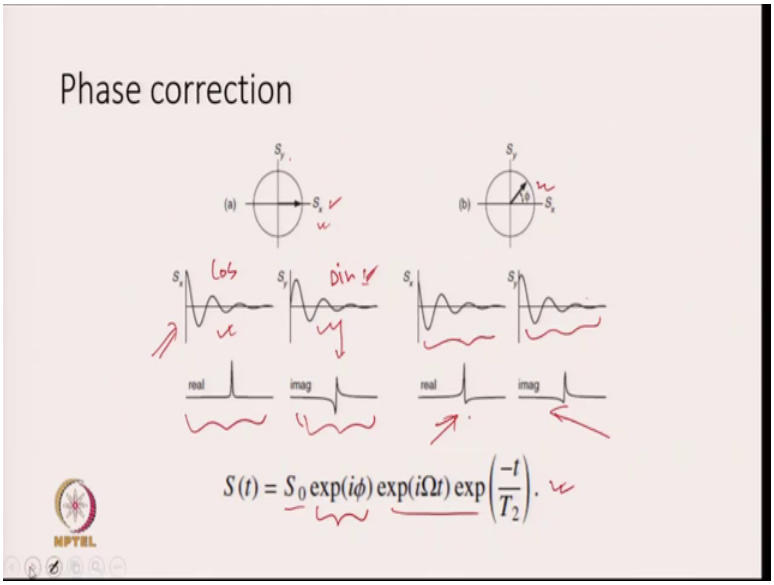

Now, let us go to how to process the data, how to process the data, NMR data it is very important and again because it is related to enhancing the signal and resolution. So, after you get your FID, you need to process the data before doing Fourier transformation. So, what you can do is for example, look at that, we are looking at, we are trying to look at the signal either in X direction or Y direction.

Suppose, we are looking at X direction and if then, I told you that you can get signal at X, you can get signal at Y, (1 will be your) X signal will be your cos modulated whereas, Y signal will be sin modulated so, that is what you get so, signal if at X versus time will look like this, signal at Y versus time is look like this because this is sin modulated, this is cos modulated and when you do Fourier transformation this will give you a real signal and this one will give you imaginary signal, imaginary signal.

But suppose our spectra is or our signal is making or suppose our 90 degree pulse is not exactly 90 degrees. So, what we will get is signal which has a phase, signal which has a phase with the X and Y axis. In that case your signal will not exactly like this one and this

one. It will have slight phase shift and your real image will look like this, your imaginary part will look like this.

So, Fourier transformation of this signal will look like this, Fourier transformation of this signal will look like this. And in terms of your signal it is basically the signal which we expect multiplied by exponential i phi, if this angle, this signal makes phi angle with the signal in X axis. Now, first thing you need to do is you have to do phase correction for this.

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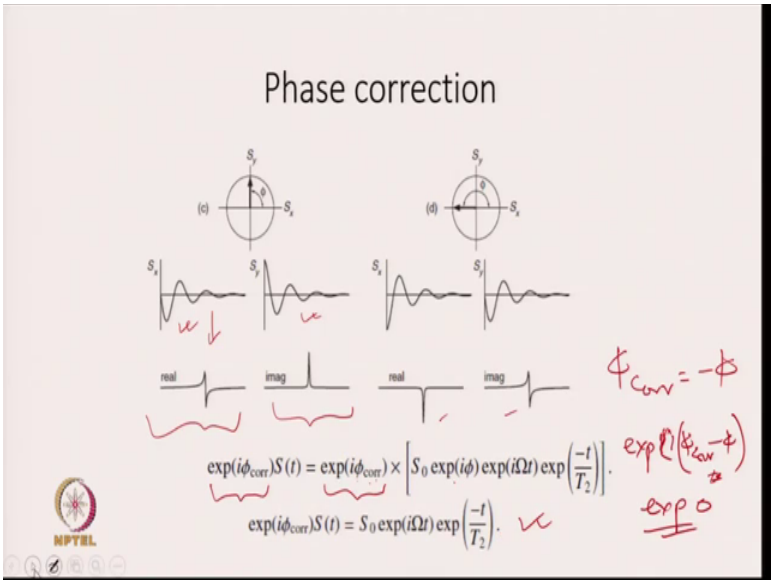

And it is not very difficult to understand what we need to do is, we need to multiply the whole signal by exponential i phi correction. So, what do you do that you multiply your signal by this factor and when this phi corrected is equal to minus 5, is equal to minus 5, you will get a signal which has been phase corrected. So, look at this if there is 90 degree the phase this phi value, then what you will get?

This will become kind of sin modulated, this will become kind of cos modulated and then Fourier transformation of this will (give) look like dispersion and Fourier transformation of this signal will look like an imaginary, the imaginary part will look like your absorption and if you have a phi is equal to 180 degree then real and imaginary will look like this.

So, only thing is that the way we do phase correction is that you multiply it by a correction factor which is equal to exponential i phi correct and when you do that, then you can see the change in the shape of the real and imaginary part, real and imaginary part and when this phi is equal to, for example, in this case phi is equal to your 90 degree, then minus 90 degrees, then what you will get is this exponential i phi correct into exponential i phi will be equal to 1, will be equal to 1 and it will be equal to 1 and then your exponential i phi correct minus phi this will be equal to 0 and so exponential 0 is 1 and so your signal is now phase correct. So this is the way you do phase correction and this is called for first order phase correction.

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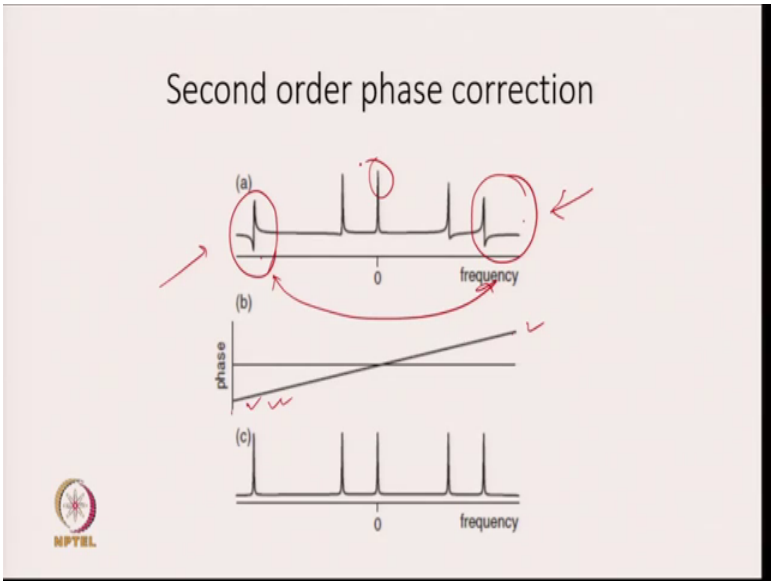

You also need to do second order phase correction, sometime what happens that if you look at the Fourier transformation, you will get your signal at the last part of the spectrum in one end of the spectrum like this and two other part it will look like this, it will look like this. So, if you notice here, this here, notice here that phase changes are totally in different direction to the two ends of the spectrum, two ends of the spectrum.

And for that, you cannot multiply by a constant exponential phase function, you have to multiply something like this, multiply phase something like this and when you do that, that is called second order phase correction. So, here is the negative part, here is the positive part. So, since you had the phase positive, phase is positive and here phase in

negative, so, positive part is multiplied by negative, whereas negative part is multiplied by positive.

So, that now effective effect is, effect is, so basically the correction is done at this phase phi is equal to 0 you see, there is no correction needed phi is equal to, for this resonance, no correction is made, for this resonance no correction is needed. So, this is the way you do second order phase correction.

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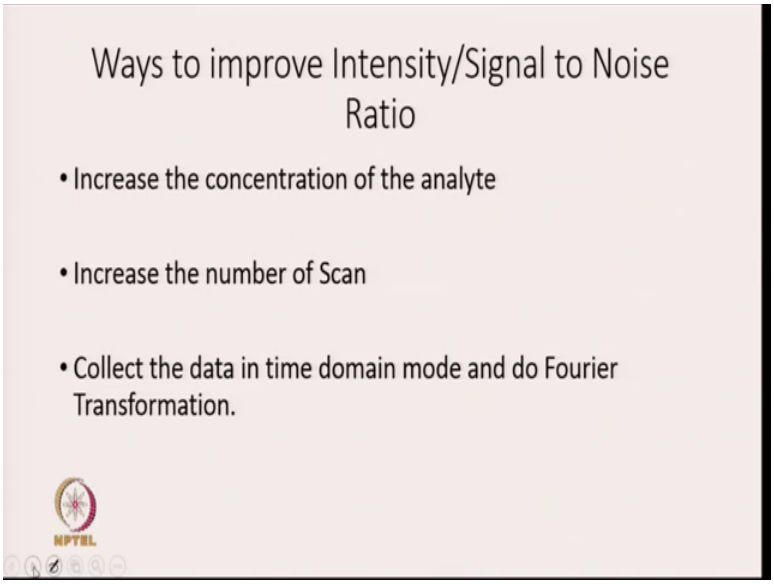

And as I discussed in the initial lectures that NMR is low sensitive technique and so we need to find a method to increase the intensity or signal to noise ratio and the way we do is increase the concentration of analyte. So, it is not possible every time, so second way is to increase the number of scan, increase the number of a scan. Collect the data in time domain mode and do Fourier transformation. These are the some of the way in which you can improve the signal to noise ratio.

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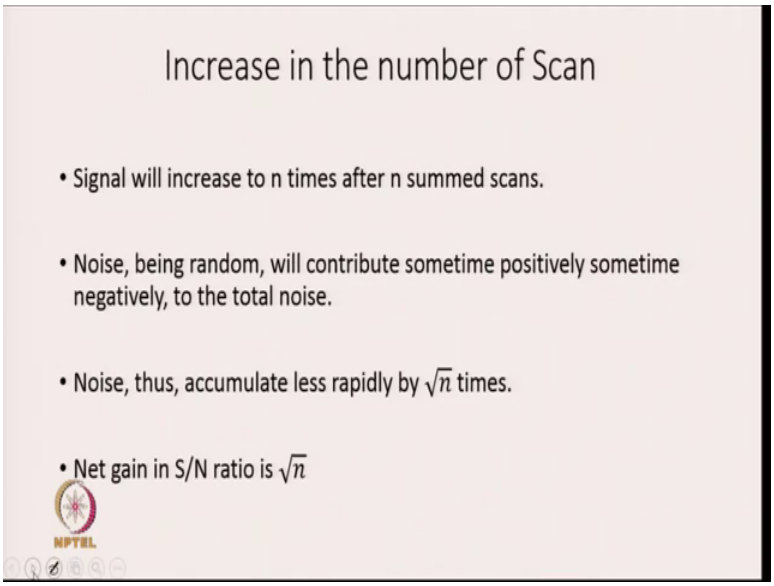

If I take n scan, then signal will increase to n times after n summed scans, noise being random will contribute sometime positively, sometime negatively to the total noise and so accumulation will be less rapid by root n times. So, if I take n scans then signal to noise ratio gain by root n times, this we have already discussed, so this is before acquiring the signal.

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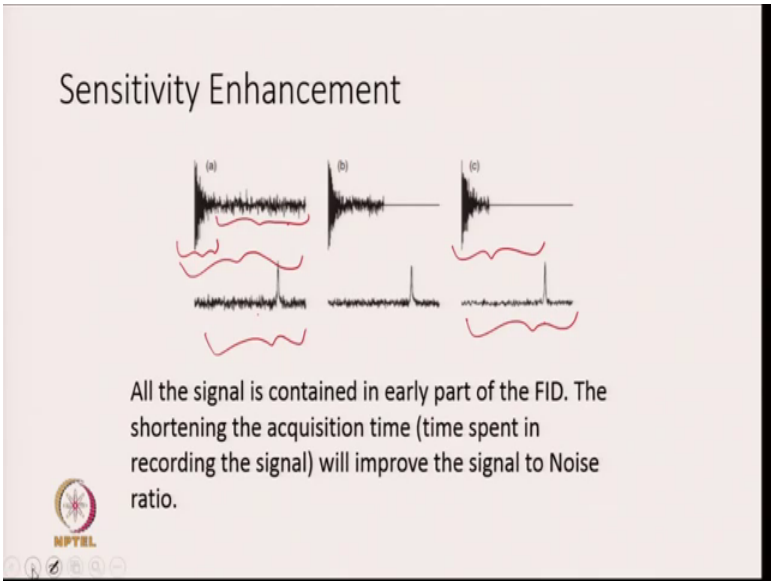

Now, what we need to do is we can also do sensitivity enhancement when we are processing the data. So these things need to be kept in mind when you are processing NMR signal and you are processing NMR signal. So, here is the three cases, here you see FID has been collected for a longer time, when and here the FID collected for less time. If I do Fourier transformation of this spectrum, this FID, I will get signal like this and if I Fourier transform this spectrum we will get signal like this.

So, if you look at this, there is more noise, whereas here is less noise. So, if you look at this NMR spectrum, what you will see that this part is quite long, this part is quite long, what does that mean is basically you are collecting more noise because if you notice the signal is mostly contained in very small part or what do you say that all the signal is contained in early part of the FID.

So, shortening the acquisition time will improve the signal to noise ratio, there is no need to collect all this FID, because the later part does not give any information. It basically increases the noise in the sample, increases the noise in the signal. And so if I want to improve the signal to noise ratio, then I need to shorten the acquisition time, which is basically time spent in recording the signal. So this is one of the way for sensitivity enhancement.

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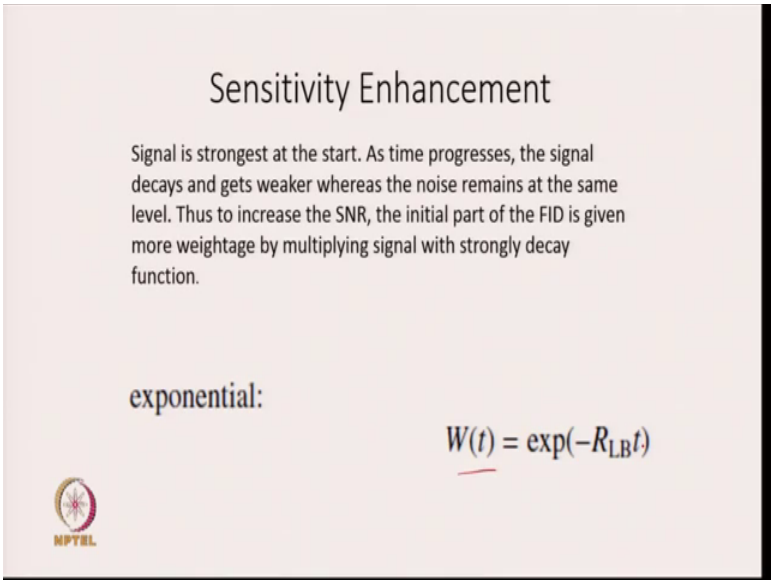

Now, we know that signal is strongest at the start. And as the time progresses the signal decays and gets weaker, whereas noise remains at the same level. So, there are another thing which we can do for increasing the SNR or signal to noise ratio that we should give more weightage to initial part of the FID and so, and the way we can do is it that we multiply by a function which strongly decay with time, strongly decay with time, so these functions are called window functions.

And for sensitivity enhancement we should use a window function which strongly decay function, what it does it is that it will give more weightage to initial part of the FID and very less weightage to the later part of the FID. And one of such function is exponential function and window function for that is exponential minus RLB into t, exponential minus RLB into t.

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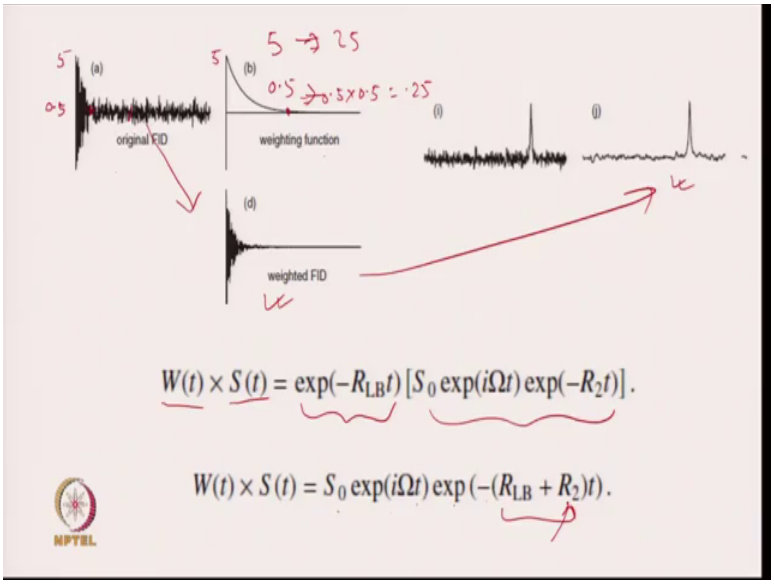

So, when you multiply by this window function, what it does is given in this scheme. So this is the original FID, if I multiply it by this function, what will it do? You see this function after this part is 0. So, if you multiply after this it will become 0 and again weighing is weight of this. Suppose this is 5 and this is 5, so basically if you multiply this it will be 25.

And if suppose you are here and here suppose signal is around 0.5 and suppose here this one is 0.5, so 0.5 multiplied by 0.5 and so that can be given by 0.25. So, you see 5 is going to 25, whereas 0.5 going to 0.25, so 5, 5 is getting enhanced where 0.5 is getting reduced. And so what you basically did is that you gave more weightage to initial signals, whereas you are giving less weightage to the signal and the end.

And if you do that, you will get this signal and so weightage FID will be if you multiply this by this you will get this, this is weighted FID and now you can see that how does this look like, here almost all noise is 0 and when you do your Fourier transformation of this FID you will get a very nice looking peak, you will get a very nice looking peak which has less noise, which has less noise.

So, in mathematic sense what you are doing is, you are multiplying this signal by weighing function and this is your weighing function and this is your signal. When you do that, then S naught into exponential epsilon t exponential minus RLB plus R2 into t. So, what you are able to do is now your decay will going to B not only by R2, it will be sum of RLB plus R2. So, signal will decay very fast, signal will decay very fast. So, initially your signal decay by rate constant R2 where as now, when you applied the weighing function now signal is decaying by RLB plus R2 and thus it leads to the signal to noise enhancement.

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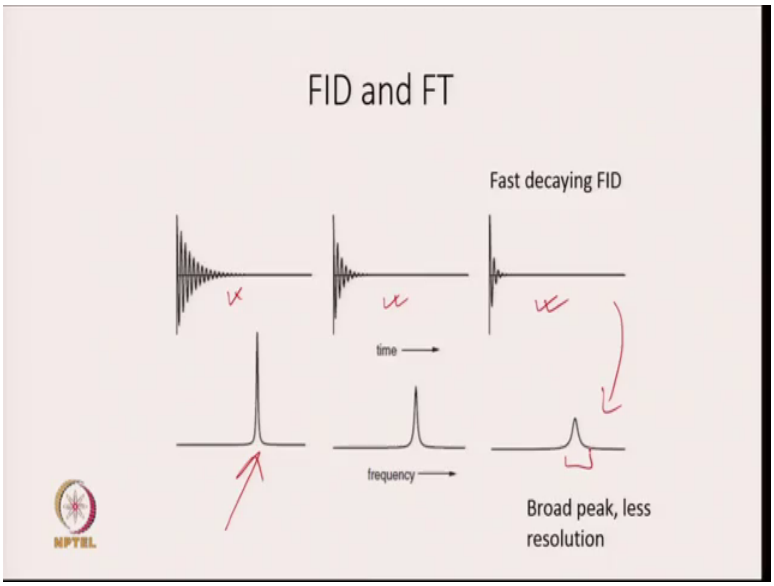

Now, you are able to enhance the signal, you are able to enhance the signal, but that has a problem that basically compromises with the resolution and now I will explain how. So, this is your FID which is decaying slow and this is a FID which is decaying faster than the first FID and this decay very fast.

If you do Fourier transformation you will get very sharp line for FID which is decaying slow. Whereas, a fast Fourier transformation or fast decaying FID will give a broad peak and that I have already explained in my initial lecture, why that happens and that has to do with Heisenberg uncertainty principle. So, the broad peak is obtained for fast decaying FID, whereas a sharp peak is obtained for slow decaying FID.

So for enhancement of signal to noise ratio, what we did that we made our FID, our FID a fast decaying, but that leads to broadening of peak, so sensitivity and resolution go in opposite way. So, if I increase the sensitivity the resolution get lost, when I increase the resolution your sensitivity get lost, a fast decaying FID has a less resolution.

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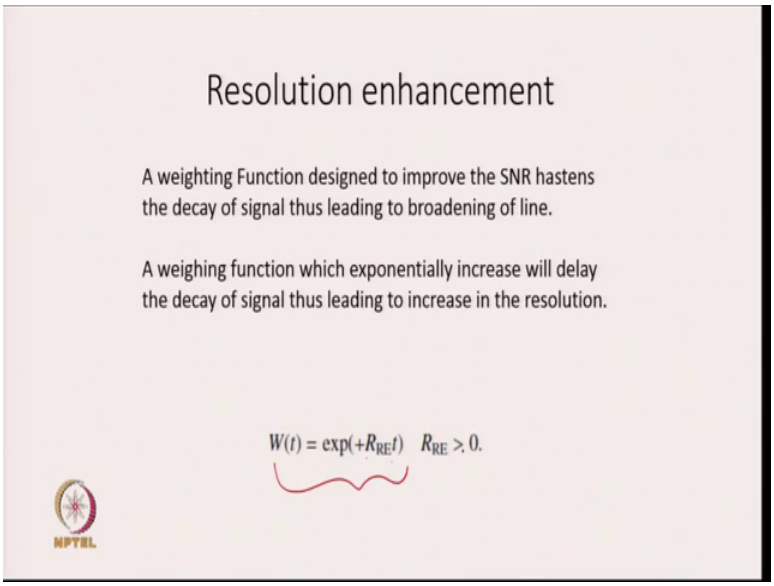

So, now question is, if suppose I want to do resolution enhancement, what we need to do? We know that a weighing function designed to improve the SNR hastens the decay of signal does leading to broadening of line. So, for increasing the resolution we need to multiply signal by a weighing function which exponentially increase, which exponentially increase and thus delay the decay of the signal.

And for that, we use this weighing function and now you can see this is exponentially increasing weighing function. So, weighing function is equal to exponential plus R RE into t, where RE is greater than 0.

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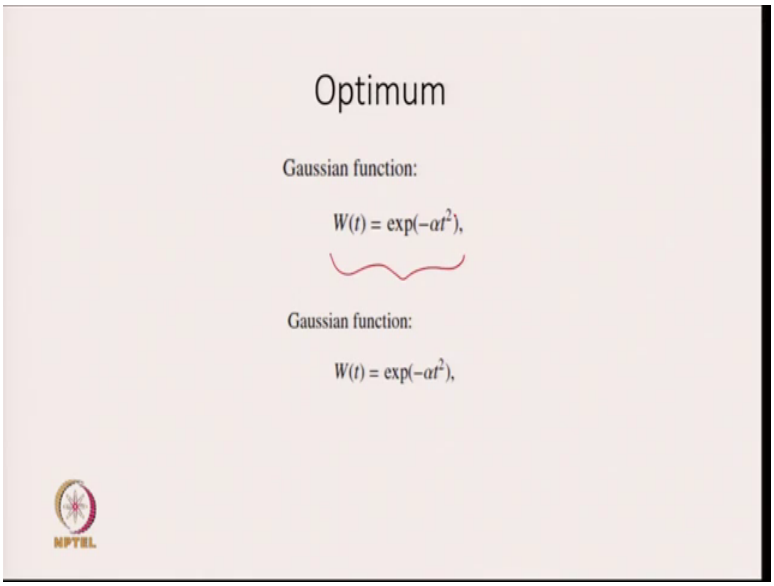

So, now, we looked at how to increase the signal to noise ratio, how to increase the resolution, but if you apply a window function which increases SNR, it leads to your decrease in resolution. So, we need to find out an optimum where we can have best of both. For that people design several different thing, one of the function which has been designed is Gaussian function where weighing function is exponential minus alpha t square, Reynolds exponential minus alpha t, it is exponential minus alpha t square.

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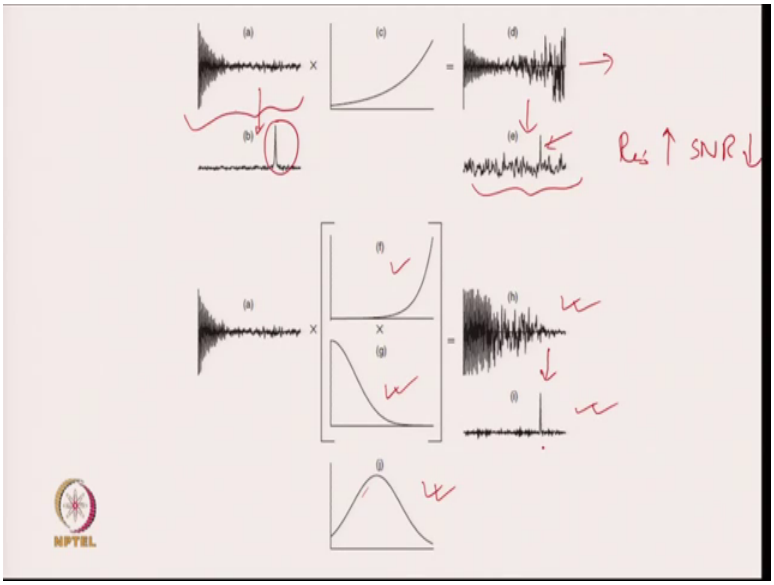

Let us look at this here is your FID and it is Fourier transformation, if I multiply by this function which is exponentially increasing function, then FID will look like this and its Fourier transformation will give this kind of peak. Here signal to noise ratio is bad, bad resolution is better, more sharp peak compared to this one. But you can see that here resolution increases but SNR decreases.

So, another way is to multiply by this function and then multiply by this function, so one is increasing and another is decreasing function and that looks like here. So, initially it is, so initial part as it is increasing, where later part it is decreasing and what it does is that it gives you this kind of FID and that leads to this kind of frequency domain signal.

So, here you have got an optimum level the signal and resolution, signal and resolution. So these kind of things you need to keep in mind when you are trying to process your data. For example, if your peaks are quite separate, quite separate in that case, you need maximum intensity and so you apply a window function which gives you maximum sensitivity.

If you can take higher concentration of the (())(36:00) and peaks are quite nearby to each other, in that case it is easier to get a signal to noise ratio, but you need a better resolution in that case you apply a window function which enhances the resolution. But, if suppose our spectra needs both sensitivity and resolution enhancement, then you need to multiply by weighing function which has this kind of feature.

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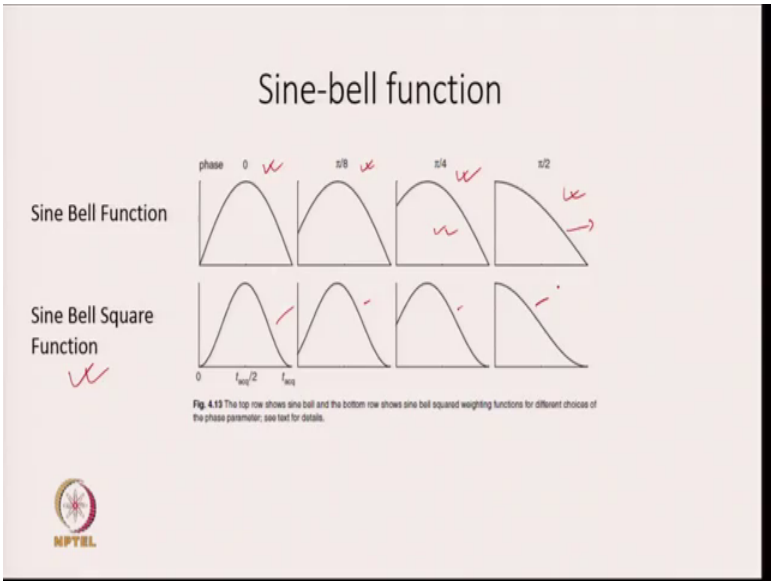

So, to get an optimum window function, sometimes people use Sine Bell function, Sine Bell function can be of different type, here is 0 degrees Sine Bell function with 0 phase, whereas this is Sine Bell function with pi by 8 phase. This is with pi by 4 phase and this is with pi 2 face. And again this can be applied keeping our, keeping our use in mind, keeping our use in mind.

So, for example here this kind of function 90 degree phase shift Sine Bell function is needed when we want to get, we want to get higher intensity, higher signal to noise ratio, whereas this kind of function will be used if we want to take both signal to noise and resolution to optimum, if we want optimum signal to noise ratio and signal to noise ratio and resolution, then we apply pi by 4 shifted Sine Bell function.

Sometime we also apply Sine Bell square function and the shape looks like this. So, I hope now you understand how to apply the window function, it is very important because it can affect your spectra, you can get better resolution or you can get better signal to noise ratio.

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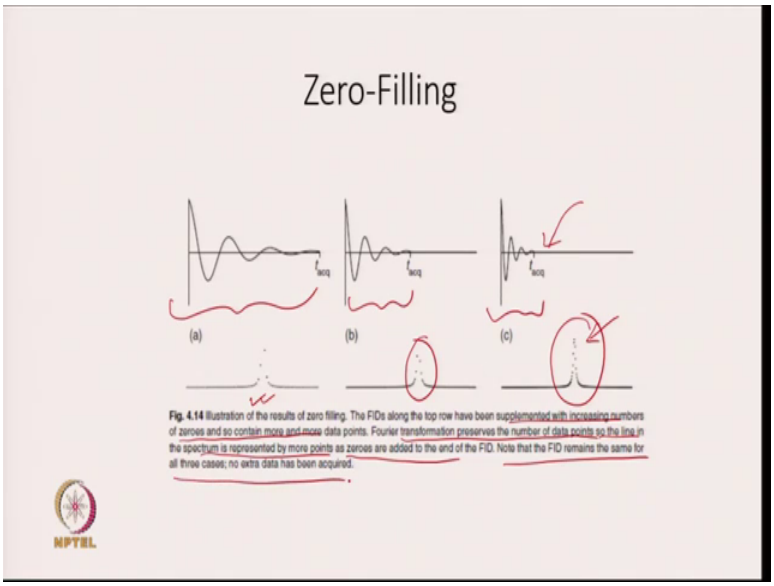

The another thing which is important in processing of NMR data is what is known as Zero-filling. So, if suppose I take your NMR data for this many time for this acquisition period so basically you are taking these many data. If I take time acquisition and keep the number of data same, then you will get more number of data at this point and if your t acquisition is small, and then we can get more number of data in this region.

So, what Zero-filling does is that if you look at here, the FID along the top row has been supplemented with increasing number of 0 so contain more and more data points. So here you can take more number of 0. And thus you can have more and more data point. The Fourier transformation preserves the number of data points. So the line in the spectrum is represented by more points as 0 are added to the end of the FID.

So if you take NMR spectra for this much time only and then add 0, then basically you are taking more number of data points in this period, more number of data point in this period and which leads to more number of data in your peak. So, in this FID remains the same for all three cases no extra data has been acquired.

So, this is about processing of data. So, three important things we discussed, how to correct the phase. The second thing we did, how to improve the signal to noise ratio, how to improve the resolution and if we want optimum resolution or optimum signal to noise

ratio then which kind of window function we need to use. And last is your Zero-filling. Last is your Zero-filling and I told you what is the importance of this.

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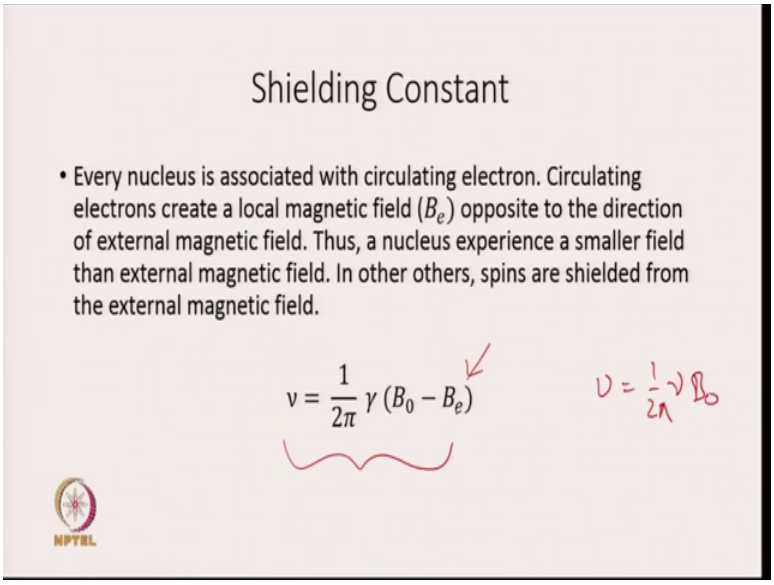

Now, NMR spectrum has chemical shift. So, if we are suppose, we are applying pulse at a particular ppm so, all proton will be excited, now question is if all protons are excited other chemical shift is going to be same. So, it is not so, because every nucleus is associated with circulating electron and circulating electrons create a local magnetic field opposite to the direction of external magnetic field thus a nuclei experience a smaller field than external magnetic field in other words, spins are shielded from the external magnetic field.

So, generally what you expect that nu is equal to 1 by 2 pi gamma B naught, but what happens because every nucleus is associated with circulating electrons. So, these electrons will create a local magnetic field Be opposite to the direction of external magnetic fields. So, what you are looking at is this frequency. So, spins are shielded from the external magnetic field by this magnetic field and so nu will be different for different nucleus, since Be will be different, since Be will be different for each nucleus.

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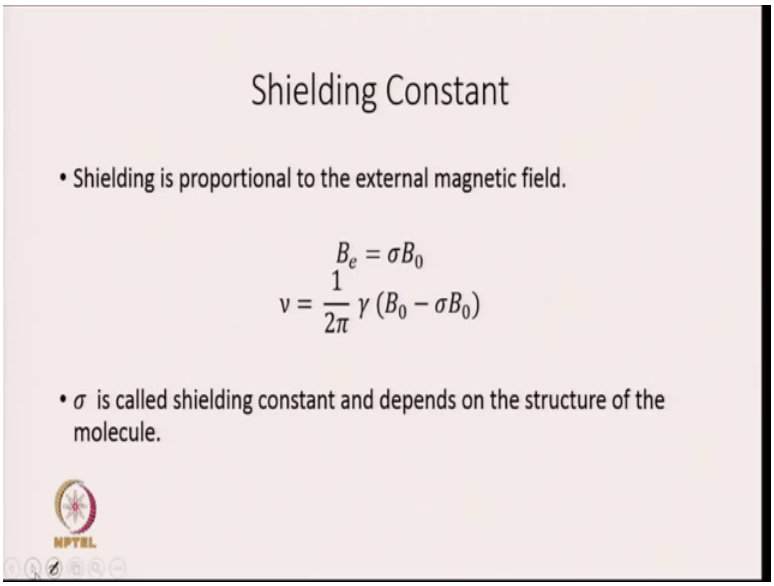

Shielding is proportional to external magnetic field, so shielding is not constant, it also depends on external magnetic field and Be is given by sigma into B naught and then frequency is 1 by 2 pi, this gyromagnetic magnetic ratio multiplied B naught minus sigma B naught, where sigma is called shielding constant and depends on the structure of the molecule. And since sigma is different and so different protons or protons in different environment will resonate at different frequency and that can be used further getting information about the structure of the molecule.

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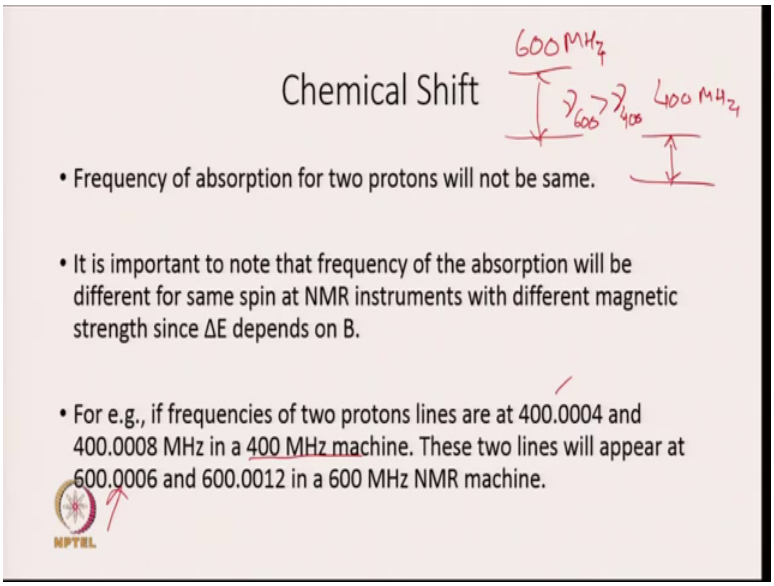

So, chemical shift, what we come to know is frequency of absorption for two protons will not be same. It is important to note that frequency of the absorption will be different for same spin at NMR instruments with different magnetic strength since delta E depends on B. So, one another thing which need to be keep in mind that frequency of absorption is going to be different for same spin when if you are looking at one spin, the frequency will be, frequency of absorption will be different for that spin at NMR instruments with different magnetic field.

For example, frequency will be different at 400 megahertz NMR machine and frequency of that same proton will be different at 600 megahertz NMR machine since Delta E depends on B and Delta E will increase if I increase B and so nu will be going to be greater. For example, a frequency of two protons lines at 400.0004 and 400.008 megahertz.

So, if suppose two protons are there, one resonate 400.004 and another resonate at 400.008 megahertz in 400 megahertz NMR machine. Then these two lines will appear at 600.006 megahertz and 600.0012 megahertz in a 600 megahertz NMR machine. So, the spin which resonate at 400.0004 in a 400 megahertz machine will now resonate at 600.0006 megahertz in 600 megahertz NMR machine. It is simple because in 400 megahertz machine this gap will be smaller and then 600 megahertz NMR machine since

this gap is now bigger and so nu and 600 will be greater than nu at 400 megahertz NMR machine.

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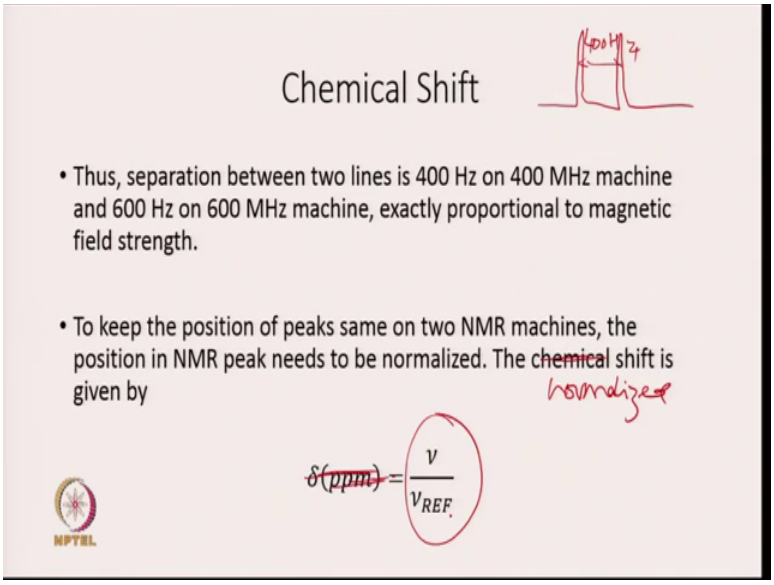

So, the separation between two lines is 400 hertz on 400 megahertz machine and 600 hertz on 600 megahertz machine, so this signal there is two resonances and the difference between these two is 400 hertz and 400 megahertz machine, whereas 600 hertz on 600 megahertz machine, exactly proportional to the magnetic field strength, exactly proportional to magnetic field strength.

So to keep the position of peaks same onto NMR machine, what we need to do? We need to normalize the position in NMR peaks. So, what we can do is we can simply divide this by nu, so just keep this thing, the normalized shift, that is right normalized shift is given by nu by nu REF. So, if you do that, then we will get one value.

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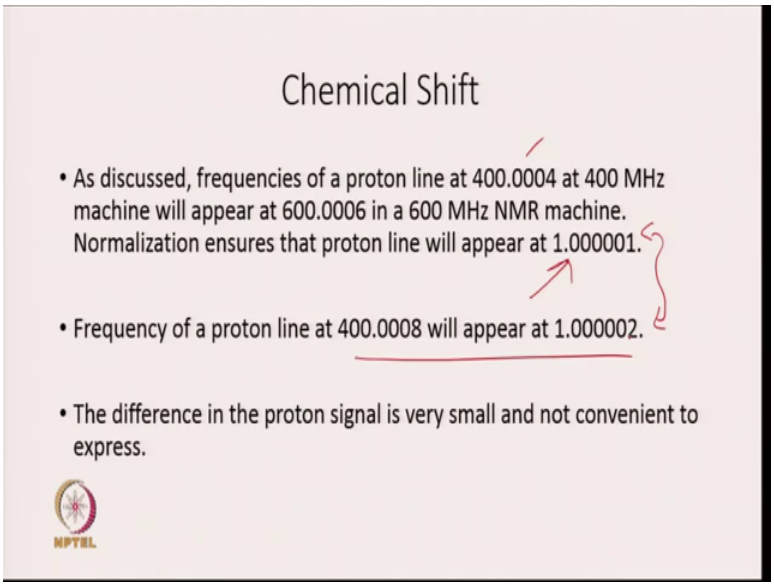

So let us do it, when we do that then normalization ensures that a peak which is at 400.0004 hertz at 400 megahertz machine and 600.0006 in 600 megahertz machine will appear at the value 1.000001 value, frequency of a proton line at 400 point this will appear at 1.000002. And so you see the difference between these two is very small 0.000001 hertz, not hertz it is unit less. So 0.000001. So difference in proton signal is very small and not convenient to express.

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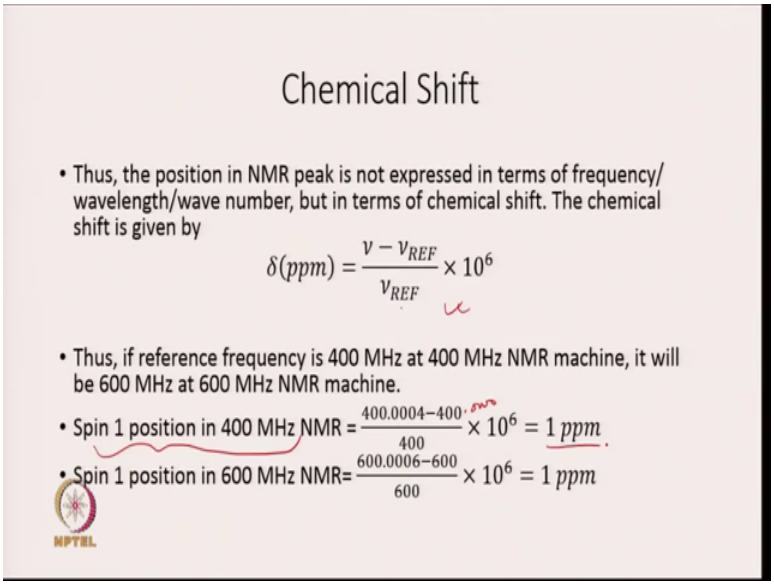

And thus, what we do is the position in NMR peak is not expressed in terms of frequency wavelength, wave number, but in terms of chemical shift and chemical shift is given by this value nu minus nu REF divided by nu of the reference material into 10 to the power 6, nu REF is because nu of reference material into 10 to the power 6. Now, if we calculate a spin 1 position in 400 megahertz machine, this is this minus 400.0000 divided by 400 and this will be 0.004 divided by 400. And if I multiply by 10 to the power 6 I will get 1 ppm, I will get 1 ppm. And so this value is convenient to express and that is why in the NMR spectrum we take X axis in ppm, not in hertz, as we do for other spectroscopy.

So first thing is that here the frequency is dependent on, frequency is dependent on external magnetic field and that is why it needs to be normalized. Again, the difference is very small, difference is very small and so we need to get our X axis in terms of a number, which is easier to express and that is why chemical shift has been devised, a scale of chemical shift has been devised.

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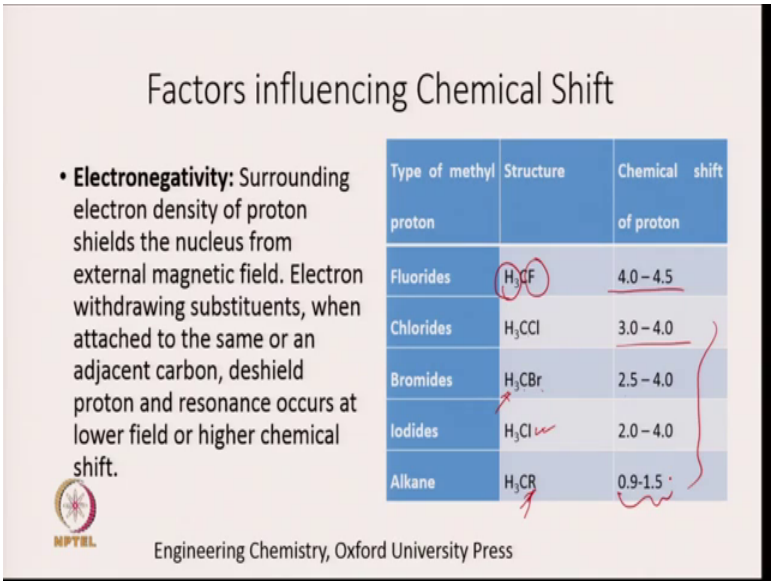

There are a lot of factors which affect the chemical shift, few of them I am going to discuss in this, the first factor is electronegativity, the surrounding electron density of protons shields the nucleus from external magnetic field, electron withdrawing substituents, when attached to the same or an adjacent carbon, deshield proton and

resonance occurs at lower field or higher chemicals shift or higher chemical shift. So this is one thing which you need to remember that electron withdrawing substituent deshields proton and resonance occur at lower field or higher chemical shift value.

So you see here this is F, this C, this l, Br, Cl, this is I so, CS3I, CSR and when electro negativity increases what it means that it is draws electron from carbon and just deshields this hydrogen and chemical shift is at higher position or lower field, higher position, higher chemical shift value or lower field. Chloride 3.0 to 4.0, if chloride is attached CH3 proton will resonate between 3.0 to 4.0.

If carbon is attached to Bromine these protons will resonate between 2.5 to 4.0. And similarly, the case here you see R is your Alkyl group in that case your chemical shift value is going to be small. So, electro negativity is a factor which plays a role.

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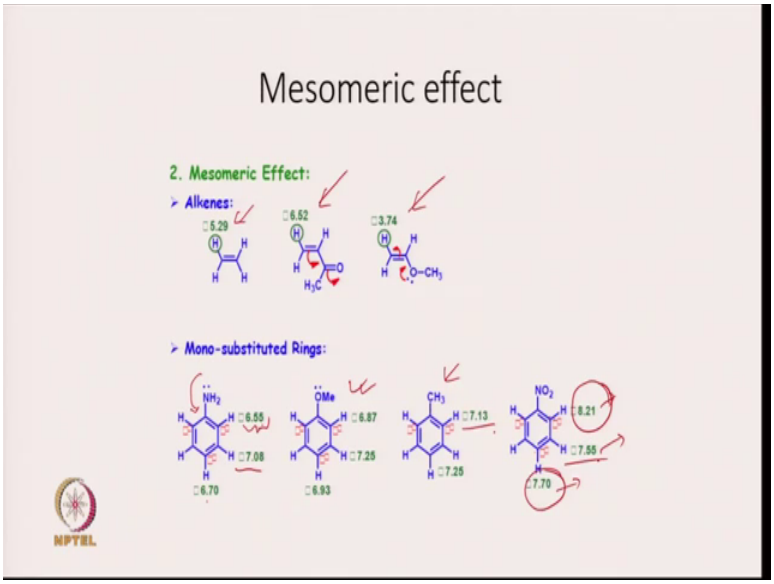

The second factor is Mesomeric effect. So, here you see this is your hydrogen attached to a double bond, its chemical shift is 5.29, chemical shift is 5.29 and when it is in resonance or Mesomeric effect with the C double bond O then its chemical shift value increases. So, what it does? It is taking away electron from this hydrogen, hydrogen and that is why chemical shift is increasing. When the conjugation is in such a way that

electrons is moving towards hydrogen in that thing, your chemical shift will be smaller, for substituted rings again same way you can think of.

So, a proton which is attached here it comes at 6.55 because this electron is getting donated, and the effect will be more at this position. And at ortho position or para position if you take Mesomeric effect into account, and so these two protons has a smaller chemical shift than this proton at meta position, same thing you can see when the substituent group is OME or OCH3, if I have CH3, which is electron donating group, and then you can see that this value if you compare between this and this, this value is higher NO2 is electron withdrawing group.

And now you see that this one has 8.21, this is 7.55, this is chemical shift is 8.21, 7.55 and 7.70, electron withdrawing group affects this ortho position more compared to meta position and para position, the higher value of chemical shift will be for ortho and para, whereas, for this meta the effect will be lowest and so its comical shift is smallest, chemical shift is smallest. So, today I will stop here we will see the effect of other factors on chemical shift in the next lecture. Thank you very much.