Principles and Applications of NMR spectroscopy
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Module 3
Lecture No 13

So in this class now we will continue with the Acquisition parameter that is the different parameter which is required for setting up an NMR experiment. we saw in the last class a few important ones such as Relaxation Delay between the scans, the 90 degree pulse width, Locking, Shimming etcetera and that is before the pulse is applied. Now what we will see now is what are the parameter to be looked at after the pulse that 1 D pulse is applied.

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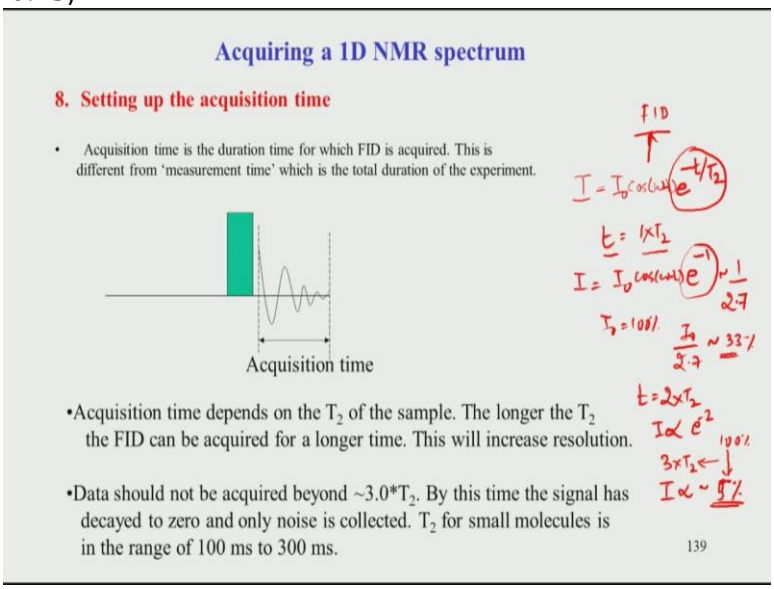

So this shown here this is the 90 degree pulse which is shown as a typical pulse program. S, we saw this of before this what all we have to do up to this point. Now after the pulse is applied then we have the signal which is called FID. This is induction current EMF which is induced in the coil and that is detected as an FID. So now if you recollect this different on the T2 with the T2 is short, the signal goes down very fast, if the T2 is long the signal will stay longer.

That means this oscillation oscillation depends how long the oscillation, oscillation happen forever but it is reduced in amplitude because of this T2 Relaxation and T2 Relaxation reduces the intensity and at some point, it goes to zero. So the Acquisition time, how long do you record a NMR signals depends on what is this T2 of the sample. So in the previous class we saw that we

should have a rough idea with T1 because we need to setup the Relaxation Delay in this particular case we need to know the T2 of the sample.

So we need to know what of T1 & T2 of a sample for recording any NMR experiment. Again remember T2 is something which you may and T1 & T2 will not know exactly for you sample. it may be a new com. every time you record. So you should get a rough idea. So again based on the size of the molecule typically the T2 values can be predicted. So how long then this should be the time taken this is typically the time taken about 3 times a T2. Because after 3 times the T2 the signal has gone completely to zero.

So this is, if this can be mathematically shown like this which I will show you, which I will derived now here. So the intensity of this FID is equal to I zero into cosine the frequency that is oscillation in time dependent into e to the power - t by T2. So this is the FID. This is the form of the FID, this is what we saw in the last class. Now if you look at this parameter here this is causing the decrease in the intensity with respect to time. So if i take t equal to one times T2 then your I is basically I zero.

Let us ignore this term say cosine some some frequency that does not depend that is an oscillation. But here you see it becomes - 1 because T isequal T2, now this is one over e. So 1 over e is 1 over 2 . 7 this is 1 over 2 . 7 e. So this is about 30 so it has reduced to about 33%. So you see it was earlier, this is if you consider I zero as 100%. Now I zero by 2 . 7 is roughly 33%. So signal has decade by 33% in one times T2.

Now if I take t equal to 2 times T2 then it will be here if you look at here. It will be I equal to proportional to e rest to - 2 e square which is now further gone down by square of this, which about 90%. So in about 3 times T2 the I will be almost equal to 90 only 1% or 5%. That means the signal has come down from 100% in the when it is here, when t is equal to 0 to 5%. So this reduction has happened because of using three times T2. So by three times T2 the signal has reduced by 95% and that is what we we have to keep it in mind that the when you setup this time delay Acquisition time it has to be three times T2 and not more.

If you give more than three times T2 if you look at this here it will further go down to the 1% lesser than that but then signal is not present but the noise will start coming because noise is present always remember. So noise is which is present coming from the electronics of the system

the hardware and that comes it is picks of from various sources down this line and all the noises also are accumulated are collected in FID. So FID is a combination of signal and noise.

So if signal is gone to zero but the noise level goes to zero, it is constantly always present. So at some point your signal is zero but the noise is present. So Signal to Noise is zero because signal is zero. So this is what is the problem that if you record this experiment for too long a time then you accumulate a noise but there is no signal and the Signal to Noise goes down and therefore this is one of the practical reasons, why many times people come up with the argument saying that they are not getting any signal in the sample.

The reason being they used a very wrong Acquisition time and they would have used probably a 10 times T2 without realising. Because T2 is something we should have a rough idea and if you wrongly estimate T2, then you will end up with a if you over estimate T2 you will end up with using a very long time and what happens is the signal has gone to zero but the noise has accumulated.

So Signal to Noise goes down, if you use a very long Acquisition time. So Acquisition time has to be optimized based on the T2 value. Again based on the experience if you look at typically the organic molecules, which we study in the laboratory or if you consider Bio-molecules we let look in this range 100 millisecond to 300 millisecond. So in NMR spectroscopy one has to keep in mind this different time values.

This is very important because as we go on it is one should have a feel what are the different time scales we are using. For example when we say pulse width, you should know it is in the order of microseconds. If you look at Relaxation Delay you should know it is in the order of seconds and if you talk about Acquisition Time, one should know that it is in the order of 100 milliseconds. So these are the different time scale.

one if one makes mistake in this time scale remembering then the Acquisition and the spectrum becomes pretty comes out bad.

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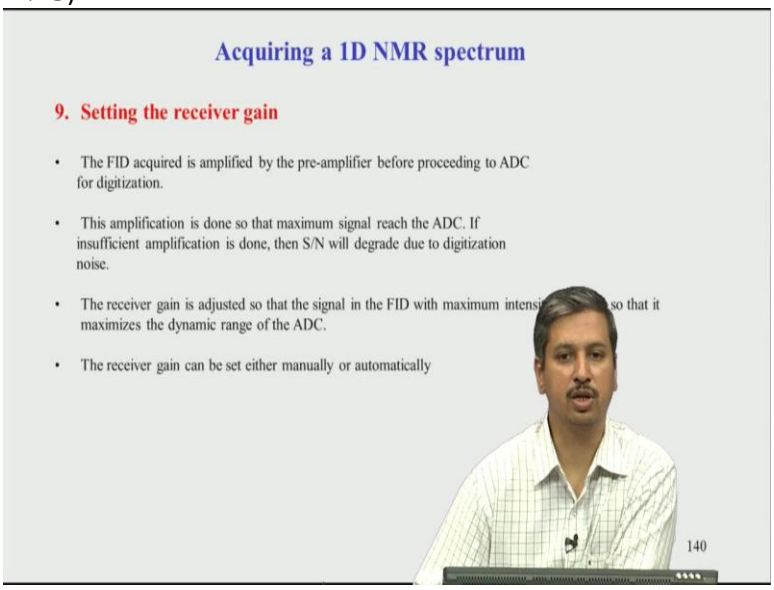

Now the next step is again after you when you are recording the signal there is hardware component called as a receiver. Again as the word receiver suggests it is basically receiving the signal. So if you want to receive the signal you want to receive at the maximum amplitude. So therefore you want to the increase intensity the amplitude of the FID it is amplified by then adjusting the gain in the receiver. So this is what is done, this is amplification is done so that maximum signal which is the ADC. What is an ADC? A ADC is called analog to digital conversion, convertor.

So ADC is what is the next step after we record a signal we will see that and that signal to maximum signal to which ADC one has to therefore increase the receiver gain and receiver gain is simply what is doing is, it is simply amplifying this are scaling up the signal. But remember noise is always there in the signal. So the noise also gets amplified.

So receiver gain is basically what it is doing, it is amplifying signal as well as noise. So both signal and noise are amplified and then that is fed to the ADC. So this is where is the dynamic range issue comes into the picture. What happens is this ADC has a limited capacity to receive signal. It cannot arbitrarily receive any large amount of signal that you give. So let us say you have a solvent signal in your sample and solvent if mentioned in the last class the solvent suppression is very important.

You have a huge a signal to noise dynamic range and therefore if the signal solvent signal exceeds, the dynamic exceeds the range of ADC then we end up what is called ADC over flow. You can think of ADC over flow in the similar manner like a let us say you have a water tank. The water tank fills some amount of water, if you feed more than that it will start over flowing. So similarly ADC has a limited capacity to hold the signal strength and if you have more signal strength then what it can take it will start over flowing.

So what is over flowing? It will simply subtract or it simply omit the signal ok. So this second important step.

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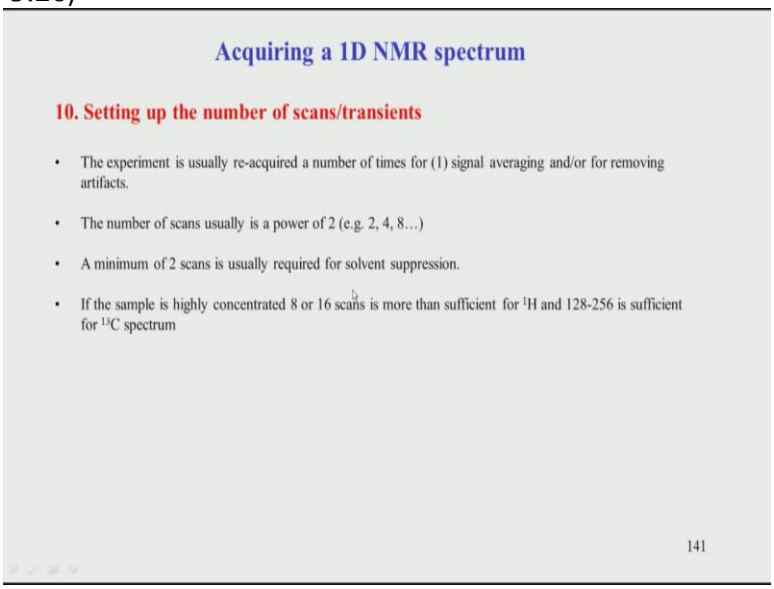

Then what we come to the more important practical point is the scans. So this is what we also use the word transients in NMR. The number of scan is basically how many times you want to average. So if you remember in the in one of the classes we mentioned that in 1D NMR you record the signal many number of times, you do not record it only once. You record it many number of times and this many number of times each time signal is added and then what you get is a final addition of course the noise also gets added.

But remember we said that noise does not increase as much as signal increases, when you add because noise is random wide noise. So it starts subtracting itself. So it goes slowly. So if you do it for a suppose you do as tools scan when we do a tools scan the signal will go by twice but the noise will not go by twice, it will go by square root of two.

So because of that the signal to noise overall increase by square root of the number of scans. So if you use 16 scans your signal to noise compare to one scan. If you use 16 scans then signal to noise will go up by a factor of square root of 16, which is 4. So depending on signal to noise one has to basically give a large number of scans. So again coming to a from a practical stand point in organic chemistry and in standard biomolecule peptides which we use, which we record typically about 8 to 16 scans are given.

But again remember this is for a 1 milli molar sample. The sample concentration where it is around 1 milli molar or more we can record good data with 8 or 16 scans. But if you go to carbon 13 remember carbon 13 will see later that it is very insensitive compared to hydrogen. Why? Because the gyromagnetic ratio is less 4 times and the natural abundance of carbon 13 is also less.

So because of that you need more scans and as written here if you have a good concentration typically let us say, you have a 5 milli molar sample or 10 milli molar sample then you need to record, you can record a good data set with what about 256 scans ok.So carbon 13 is remember very less sensitive.

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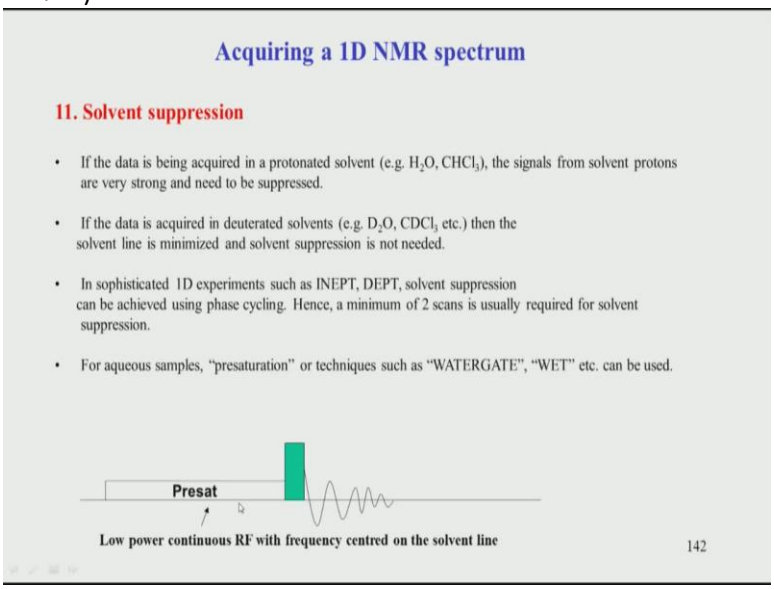

So this is the Solvent suppression part which we had brief look at in last class. we will not go in detail but I just like to tell you about what a very most basic type of solvent saturation which is used very routinely that is called as Presaturation. So Presaturation is shown, the picture is shown here. So this is called the pulse sequence again.

So remember before before we apply the 90 degree pulse this is the 90 degree pulse we apply what is called as Presat. This is a nothing but a low power continuous RF with radio frequency not a pulse now. Because pulse means it is in the micro second. But look at this delay this is about remember this is called Relaxation Delay and this is of the order of few seconds.

So for the entire Relaxation Delay period what is done, is a weak RF is applied on the solvent at the frequency of the solvent. So when you apply a weak RF on a solvent frequency what happen is, you irradiate the solvent, this is called irradiation and you equalise the population you equalise the population of the alpha and beta state of the solvent. So if you have to you recollect that we talked about two energy levels in NMR alpha and beta for spin half nuclei.

So water for example has proton and proton has alpha and beta states but selectively if I apply a continuous RF only on the water protons then the water proton populations between alpha and beta gets equalised after a long time after certain time and that is called saturation. we will not be able to go in details of how that particular saturation happens. But the idea is very simple that

you take the solvent the signal the chemical shift value of the water protons and you apply at that frequency weak weak continuous RF weak means is a really weak signals.

This is the strength of the signal is typically about 50 to 100 hertz. remember in NMR when we talk about strength of radiation irradiation we talk in terms of hertz. So this is typically kilo hertz the 90 degree pulse width but this is typically 100 hertz 50 to 100 hertz. So at that frequency the 100 hertz is the range of the amplitude of the irradiation but it is applied on the water resonance or the water proton, the solvent proton.

So because of that, the solvent proton population gets equalised and remember when the populations are equal the population difference is zero. So when population difference is zero no NMR can be done. That means what this process is doing, it is trying to make the solvent signal become zero by equalising the population. So you can select a weak irradiation; we also use the word saturation and hence the word Presaturation comes, Presaturation because this is applied before 90 degree.

Then you apply a 90 degree pulse in for a general for all items, for all nuclei all spins in that molecule. So what will happen when you record the signal here there will be no contribution to the FID from the solvent, because solvent has been already killed or eliminated from the spectrum while irradiating. So the oscillations that is chemical shift what you see, will only come from the solve com. peaks and a solvent peak signal are oscillation is gone.

So when you do or when you do a Fourier transform do as finally get a spectrum, your spectrum will not contain any solvent peak. So this is one way to do the Solvent suppression. But this is a very crude approach a brute force approach, we used the word brute force, the reason being we are simply doing a just applying a hard radiation, irradiation at weak radiation at the solvent line. So this is not really the best solution many times and therefore Presaturation although is used is the most common approach is not the best approach. The best approach, approaches are more advance called WATERGATE, WET etcetera, we will see that not now but in the third or fourth half part of the course, where we look at more advance topics.

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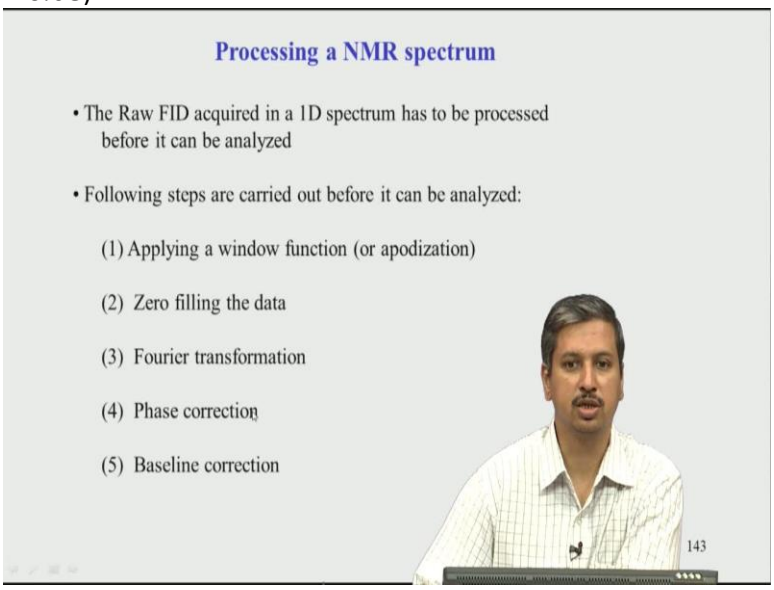

So once the data of NMR, NMR data is recorded, the next step is to do what is called the processing the data. So remember the word data comes out in NMR is called FID. FID is basically the raw data which is recorded by physically by by a signal by the spectrometer. But that is not what is a spectrum, the spectrum still has to be obtain by massaging thus the raw data and the raw data is massaged by doing many things. Then it is converted into a spectrum by Fourier transform.

This is a mathematical technic, which is why the word FTNMR is used. But even after Fourier transforms your the data is not completely ready to analyse because there are certain things which we have to follow further operate do on the NMR spectrum and that finally after all the steps is what you we say the spectrum is ready for analysis. So we will see the steps now one by one. So the first thing is called applying a window function or this is Greek word Apodization.

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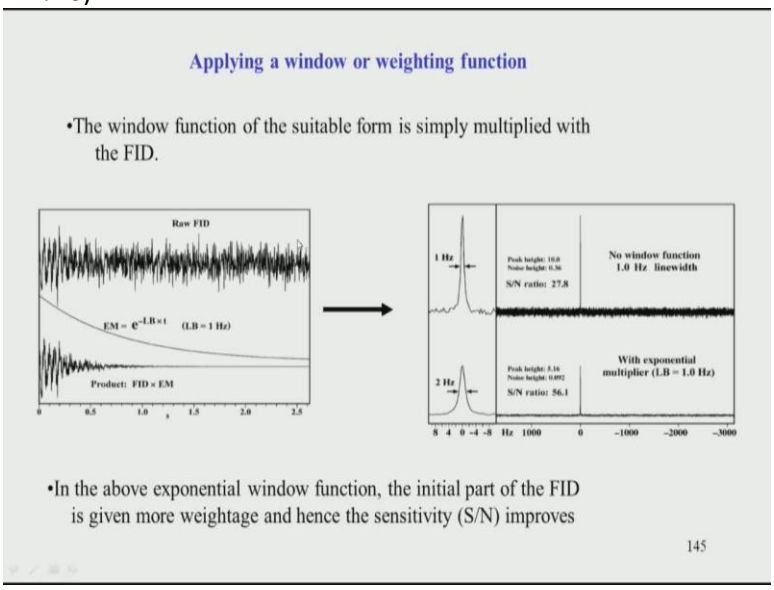

So what is this thing, what do we do in window function is the following. That if you remember in this the FID, let us go little further to see this FID and we will come back to the previous slide. So this is an FID, this is this is an FID. sorry this side this is an FID. So you see this is called a raw FID. Raw means it is the crude FID, which is coming out of the sample and this is what is captured by the primed and then this is stored in the computer in a digital form, this is what is digitised. Now we have to convert this into a spectrum.

So before doing that we do what is called Apodization. Apodization is like multiplying this whole signal with a exponential decay. So if I multiply this line, what you're saying line here if I multiply with this FID here, I will get this is the answer, this is output. So you see the signal is going down, down, down slowly and finally going to zero. Why this happened, because this is coming out this is exponential decay. Because of the decay, the signal has gone to zero.

So you see what we have done is, we have forced the signal go to zero. The signal was not going to zero, there was of course signal is already zero somewhere here and all these things, what you are seeing is noise. So this is how the noises look like. So the signal is present only you can see clearly the signal here. That is FID is more clearly only this side. His this part of the FID is only noise or signal little amount of signal with noise.

So therefore we do not want to have this portion. Because this portion if I include in my processing my signal to noise will go down. Why? Because the noise is only present and signal is

absent. So therefore we try to filter, filter means we try to stop the data here and what we do is we only take this part of the data and this part is anyways zero. So this does not contribute to, So you can see this is what happens

So this is your signal, if I do not apply any window function means if take directly this, if you look at this picture here if I directly take a Fourier transform of this FID, I will get a spectrum like this, peak like this and signal to noise has been shown to be 27 . 8. This is from the book a by this whole thing has been taken from this book by Killar and Pavia. So one of the books all this book explains so the picture is from taken from there and then you can see the signal to noise has to be calculated to 27 . 8.

That is the height of the signal divided by the height of the noise. But now when I do an Apodization I smoothen the signal I mean I apply truncation this is called a window function. And you you basically make the signal go to the zero and when I do a Fourier transform, I will get a peak like this. So if you look at compare these 2 pictures here you see this picture looks much better in the second one, because the noise level has gone down.

But what has also happened is your signal height is also gone down ok. So why why the signal height has gone down? The signal height has gone down because this peak has become wide, peak has become broad. It was earlier 1 hertz was the line width here, it has become 2 hertz. So obviously the height will go down.

Remember in NMR the area is always constant. Because the area under the peak gives you the number of hydrogens. So for the same samples, whatever I do my area under the peak should not change because that is the important point because that is what I need for quantification. So the area of the peak does not changed, then if I increase the line width I have to compensate by reducing the height. So automatically the height is reduced.

So that the area is kept constant. But here the noise has gone down also very high, now down a lot. Therefore when I take a signal to noise value I will get better value compared to this is almost double. So you see by doing this exercise of window function I can improve by signal to noise tremendously. Of course there is not always the factor of two it can vary from any way. But typically it can go from 1 . 5 to a factor of 2 . 5. So that is why it is very important do to window function.

Again in nowadays spectrometers these are done automatically by typing some (())(21:41). So most of the users, students not aware of what is happening behind the screen. So therefore this slide is basically meant to show you that what happens when you process the data.

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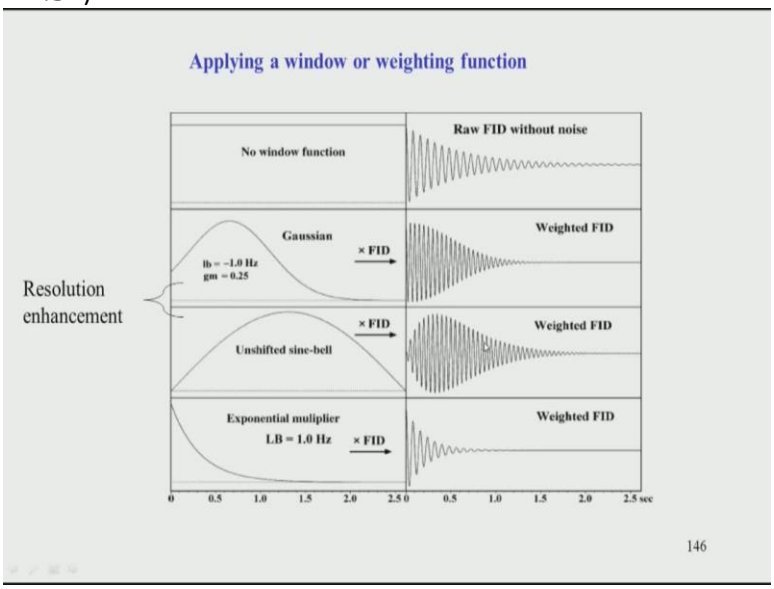

So there are different ways to Apodize the signal. You can take only the first part of this signal you can multiply with different functions. These are called window function and based on that you have to we can do the processing. So one thing important to note here is the resolution and this is a very important point in NMR. So one thing theory of NMR which we will not go into the detail but it comes from the Fourier transform that the signal the signal is basically present in the beginning.

If you look at the FID here what is happening is, the signal is present in the beginning. As the time proceeds the signal goes down to zero. So that means your sensitivity is all focused in this side. So if you want to get a very good signal to noise like we saw in the last slide then your emphasis that means your focus should be on this part. That mean you should not give too much importance to this side. When you say too much importance it means you multiply with a function with takes this part to zero and what is more important is this part.

But let us say you are not interested in signal to noise improving. You have a very good signal to noise anyway and your interest lies in the resolution means you want to separate a peak. If you want to do suppression of peaks better then this portion of the spectrum plays a role the second

later part of the FID. How do you rationalise this, how do you understand this? This can be understand, understood by simple analogy.

Let us say we have two trains moving at let us say, one moving at 100 kilometres per hour, another is moving at 102 kilometres per hour. That means there is only a difference of 2 kilometres per hour between the two trains. Now I start the race, the two train start at the same time. But initially they are moving at the same speed same distance. The distance suppression between them is not going to be visibly different.

Only when you wait for a long time then the train would have really separated in real time, separated in time space by a large distance. So therefore if you have two signals means two frequencies which are very close. That is like the speed of the train. Then you need to wait a long time in FID for them to be really separated from each other. In the beginning the two frequencies will look very much alike.

We will not be able to say there are two frequencies here. But only if you wait for a long time toward this side then you will you start seeing there are two frequencies. What it means that your resolution that is suppression between the peaks appears on this side is more than what appears on this side. So that means if I you want get a better resolution my emphasis or weightage or importance should be given to this part of the spectrum rather than going to this part and that is again done by using different mathematical functions that is the window functions and you can look at this kind of function here.

What is happening here? If you see it is giving very less weightage to this part. So this is multiply with the FID. This is raw a FID. If I multiply with this, this what shown here into FID. I will see that the initial portion is giving going to zero. But I am giving more importance to this part. So therefore my signal, suppression will become better and better.

But the signal to noise will go down and why is that because my spectrum is given less importance here. Which is where, the sensitivity is there. So the signal to noise goes down but the sensitivity resolution improves. So therefore in NMR, that is the main standard thing which we say that if you want to gain sensitivity, you have to lose resolution. Why? Because if i want this part of the spectrum this part section I have to sacrifice.

That is where the resolution is there. But if I want to get resolution then sensitivity I have to sacrifice because resolution is another side, second half the sensitivity is not so much there. So the resolution and sensitivity are kind of complimentary and to gain one you have to lose or trade off the other.

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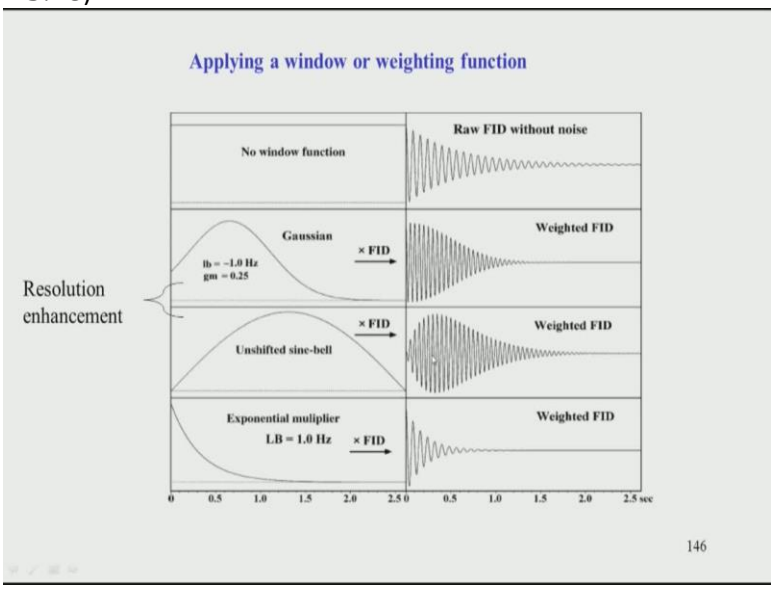

So this is what I shown in this slide also that if you want look at this here, the emphasis is given at this part of the signal and therefore the signal, the line looks broad. But if I want to get a better resolution enhancement then I will give less weightage to here and I will give more weightage this side. So you can see here, that lines are now you can see some resolution means the lines are getting sharper and there is a suppression. Here you cannot make out how many lines are there, peaks are there?

But here you can starts seeing from the peaks. But of course it is not shown here but the sensitivity goes down because of less weightage given to the beginning.

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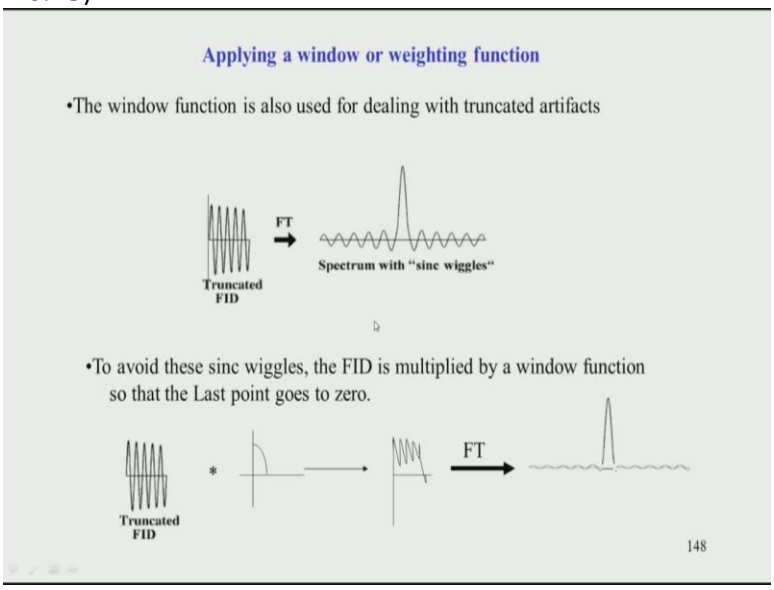

So the next step is to then to Fourier transform their spectrum and by applying the Apodization and you get a peak. This portion this slide basically talks about what is called truncated FID. What it means is that suppose you have an FID, which you do not complete. Remember we mentioned in the last class that the Acquisition has to be or in this class. In the beginning we saw, the Acquisition Time has to be three times a T2.

But that does not always happen; there is decrease in the signal to noise if you do not record and when you truncate the FID this is called truncation you will get bad spectrum because of what is called as wiggles and to improve that we apply this window function and do Fourier transform.

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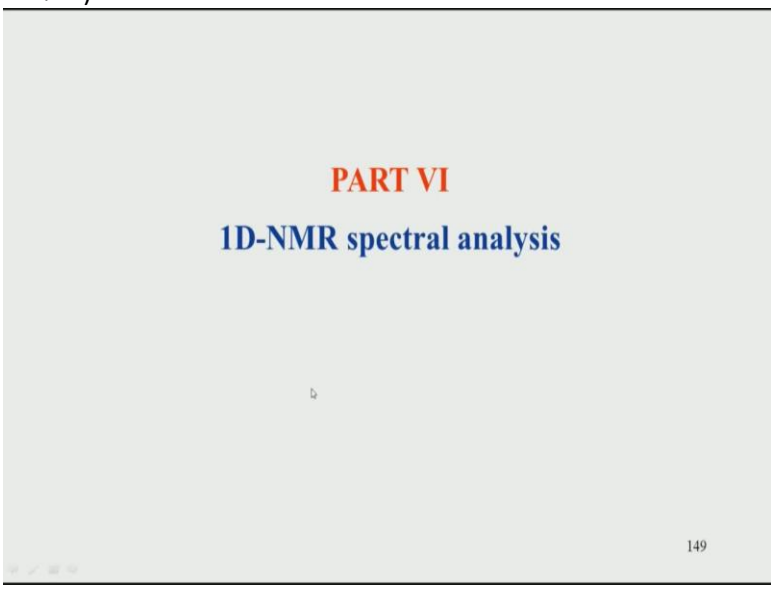

So we will come to this end of the class today in the next part, we look at how the signals spectra now once we have recorded the spectrum in different and processed the data we will now start looking at how it can be analysed and the data can be interpreted.