NMR spectroscopy for Structural Biology NS
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Lecture: 07
Fine Structure in NMR Spectra

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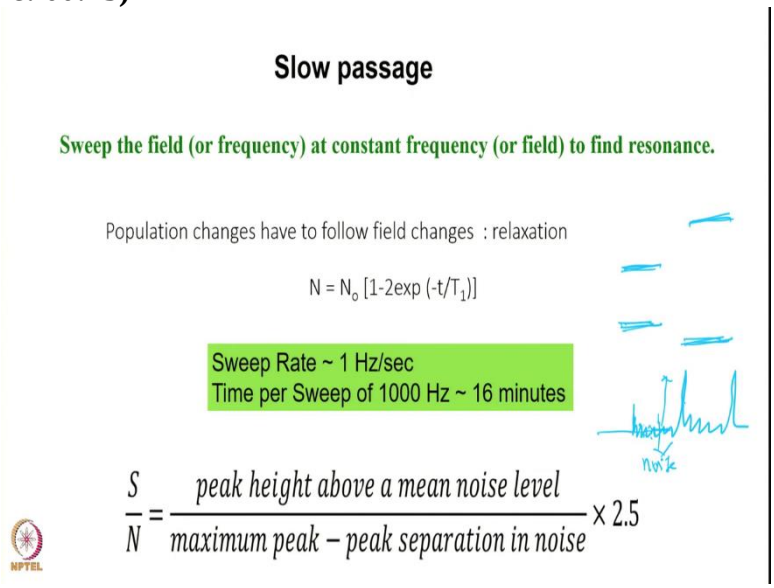

So, far we are considering 2 spins. So, we said can we consider the spins A and B there are 2 spins both are of I = ½ we are always talking about I = ½. So, each one of them in the absence of any coupling it has one line and this is and then when there is a coupling we generally represent this in this manner that this line is also split this line is also split and the separation is J in both cases this is ν_A and this is ν_B both are separated in the 2 cases.

But then of course molecules you will have more than 2 protons you will have several protons and alcohol is one example. So, there are so many molecules which are there then if there are 3 let us say if you have 3 protons 3 protons and I will represent them as A, M, X as some alphabets A, M, X. Now, there can be couplings between these 2 depending upon the structure of the molecule you can have different kinds of combinations of couplings.

How it is suppose I have a linear system like this A, M, X that is a is coupled to M, M is coupled to X as well but a is not coupled to X there is no coupling from a to a X or I can also have a situation like this A proton is here A proton is M is here and X is here. So, in this case all the 3 all the protons are coupled to each other how does such a spectrum look like. So, if you follow the simple arguments let us say I have here the; a chemical shift the M chemical shift and the X chemical shift.

I have this here I represent them as one line each. So, this is how I can consider how the spectrum will behave. Now in the case of the linear system if I have a linear system first case let us say I have a linear system and this is the linear and this is the triangular in this linear system what will happen A is coupled to M. So, therefore this will split into 2. Now, similarly M is coupled to A. So, this will also split into 2 and this coupling constant will be the same J_{AM} this is also J_{AM} and what about X?

X is coupled to M and this will also split into 2 and this is J_{MX} this is the coupling but now M is coupled to X as well. So, we extend this argument in the same manner and we will say each of these line will then get split further into 2 and this is because of the J_{MX} coupling. So, what will happen I will get a doublet here mean I will get a doublet of a doublet here and I will get a doublet here again. So, this is for the linear system.

Now if I take the triangular system. So, again I will write this is triangular A, M, X this will be split into 2 J_{AM} there is also A X coupling. So, therefore this will also be split into 2 and similarly here as well all of them will be split into 2 and ok. So, am and this is J_{AX} this is also J_{AX} and this one is J_{AM}, J_{MX}, J_{MX} and here you have J let us say J_{MX} here and this will be J_{AX} and J_{AX}.

So, all of them are doublets of doublets. So, therefore depending upon the nature of the coupling you will have different patterns and this is easy to understand on the same principles as I showed you in the previous case. So, typically one draws understands this in terms of the energy level diagram energy level diagrams can be drawn in a simple way. So, let me draw the energy level diagram here for you.

So, for 2 spins I will write this αβ, αα, βα, ββ these are the states and then I will have this energy draw the transitions like this transitions like this there are 4 transitions possible for αα, 2 of βα and we label them as 4 different transitions as in the case of we indicated there. Similarly for the 3 spin systems we can draw energy level diagrams where you have 2 sets of 2 spin systems here we will have the transition from here to here, here to here, here to here, here to here I use a different colour now.

Then I will have a transition here to here, here to here then here to here, here to here all these 4 lines I am indicating for the triangular system whenever there is a coupling is 0 of course you may not see the other one. Now these are the other transitions. So, let me use another colour here. So, I will have a transition here, here, here and here. So, you see you will have either 8 I mean there is a number of transitions will depend upon which coupling is zero which coupling is non-zero.

This is how we draw the energy level diagrams and such a kind of analysis is called as first order analysis. You can simply draw the splitting patterns of the individual nuclei if there are 4 couplings of course you will draw more couplings. And classic example of this is seen in the next before that we will say suppose any of these coupling constants are equal then what happens is these lines will merge.

So, in the case of let us say triangular one. So, if I have this if these coupling constants let us say $J_{AM} = J_{AX}$. So, what will happen these 2 lines will merge. Similarly if in this case if the MX coupling is equal to the AM coupling then these 2 central lines will merge therefore this will have the twice the intensity compared to the outer ones and like that if they are not equal then of course you will find doublets of doublets.

If the 2 lines are couplings are equal then you will have the center line which is twice the intensity compared to the other one then you will get a different intensity patterns triplet patterns etc.

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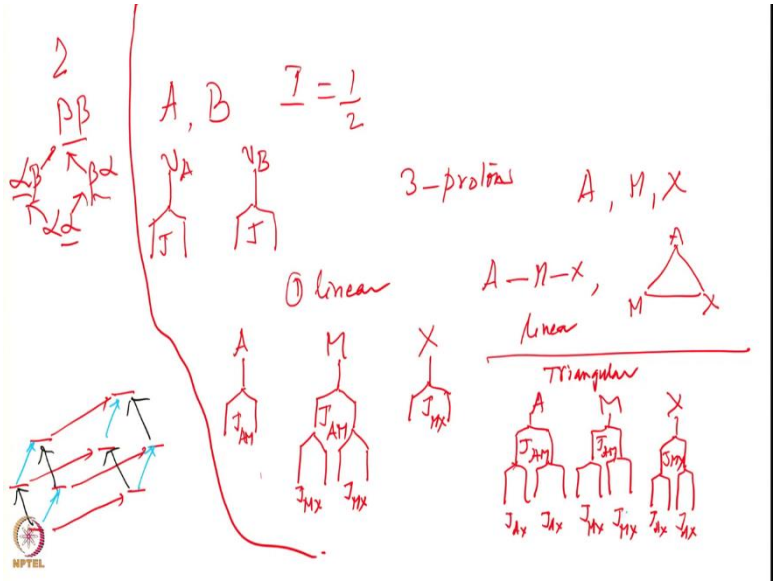

And that is what is actually seen in the case of this spectrum of alcohol. So, what how many protons are here you have the protons CH_3CH_2OH there are 3 protons here these 3 protons they are all chemically equivalent because of the same chemical shift and these 3 protons are coupled to these 2 protons right. So, therefore how many couplings are there here. So, these protons have 2 couplings.

So, therefore 2 couplings means it will produce a line which is a triplet and why is it triplet because the central 2 lines are merging because of the coupling constant to the 2 methylene protons is the same and therefore it will be a triplet. Now what about this CH_2 the CH_2 is coupled for let us ignore the OH coupling at the moment because sometimes we may see may not see it CH_2 if it is coupled to the CH_3.

How many couplings these protons are these are chemically equivalent in the first place and these ones are coupled to the CH_3. So, there are 3 couplings there. So, when you draw this pattern which I showed you earlier there then you will get for these 8 lines because there are 3 couplings the 3 couple this will result and the considering the merging of the peaks you will generate a pattern which is like this.

You will generate a 1:3:3:1 this is 1:2:1 this is 1:3:3:1intensity pattern and OH will be a triplet again and if it is when it is then of course in principle CH_2OH coupling also should show up here in that case of course it will be another doublet corresponding to doublet of a quartet that is how it will appear. And the OH will appear as a triplet if you consider this coupling otherwise if the coupling is not there then you will see a single line there.

And this will this can be further split due to OH coupling can split further. So, in the absence of the wedge coupling you will have this, this is the waste coupling is due to the CH coupling to the CH_2 this may not be there many times if you have the very rapid exchange happening then you may not see this OH coupling to the other ones it will exchange out and you will see that we will see afterwards dynamic range dynamic effects in the NMR spectra and that will cause vanishing of the spin multiplex that is the dynamic effect.

So, dynamic effects can cause because of the exchange process it can lead to removal of the coupling constants and then if you have such situation then you may not see this will have particularly happen in the case of OH protons or the NH protons if they are exchangeable with

water and they exchange between water and the OH group and on the alcohol then they will not be able to see the coupling see the spin states of the individual protons to the CH_2 protons then they may not show the splitting.

When that happens the CH_2 protons also will not be able to see the OH protons very clearly then that coupling also will not happen in that situation you will have a single line. So, let me draw that situation here as well when you do not see the coupling then it will be a single line here and this will be this will be the situation in the absence of the coupling to the OH protons here this is absence of coupling to OH .

So, that is how it will the spectrum will look like. Now but however you see I have drawn that if you look at the original spectrometer the CH_3 proton has intensity much more what is intensity ratios. So, here 3:2:1 because there are 3 protons here there are 2 protons here the intensity will approach to the number of protons. Therefore the total intensity when you take the integral that is I take the integral like this integral of this it will be proportional to the total of the number of protons.

There will be 3 protons here the intensity will be 3 times if you take this as one this is 3 this is 2 and this is how the intensities and the coupling patterns are useful to understand the chemical shifts and the spectra of molecules. So, that is the important feature of the NMR spectra which one needs to understand to be able to analyze all kinds of spectra.

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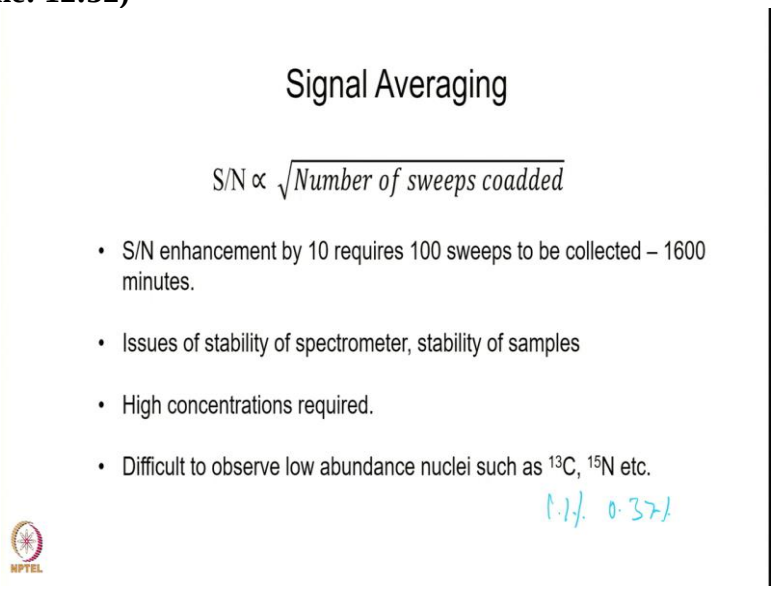

So, these were the kind of old spectrometers which were used to record the spectra and was one of the very early spectrometer where the electromagnet. So, here you have an electromagnet here and this is the kind of a console and sort of a power supplies and things like that but of course today's magnets are not like that.

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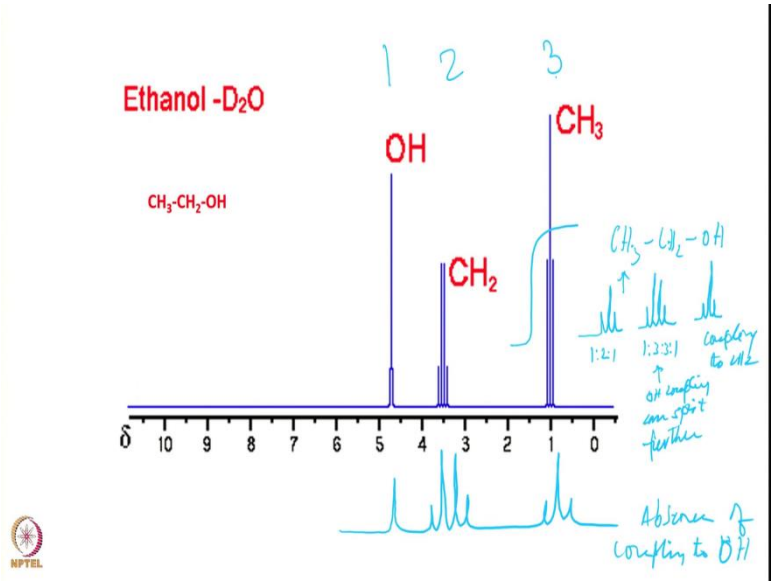

Now we turn to the next question. So, far we understood what are the principles of or what are the NMR parameters in the NMR spectra. We also understood the principles of NMR and we understood how the spectra will look like how many lines are there and what is the source of all of these lines. Now how do we measure it how do we know? So, in the early days I showed the spectrometer the electromagnet what people were doing was to sweep the field sweep the field at constant frequency.

Or you can sweep the frequency at constant field either way one can do it. Either way one can because as you change the field you match the frequency of resonance for a particular nucleus at one at a time. Slowly you start matching the frequency if you keep the field constant then you have to keep changing the frequency as you scan through it then you will change the match the resonance condition at some time and you will see the resonance condition and you will observe the signal.

Now convenient thing was to change the field in earlier days because you had the electromagnets and the changing the field meaning you will have to simply change the current in the electromagnet because this is the coil going around the iron rod. So, iron system and if you change the current the field will change therefore it is easy to change the current therefore people were sweeping the field.

But when you sweep the field what happens the energy levels will change. So, if I have a particular field here and if I change the field the field will become energy levels will become like this and the populations which are present here will have to readjust to these populations and that is dependent on what is called as the spin lattice relaxation time and this is the slow process. Therefore the population changes have to follow the field changes.

The population changes have to follow the field changes here this is the slow process this is depend on the relaxation time T_1 and that is given by this equation the population difference is given by n_0 1 -2 $e^{-\frac{t}{T_1}}$. Now if the T_1 is of the order of a second then you will have to wait for at least 4 to 5 T_1 values before you can actually make a measurement. So, if you before you make a change in the field you will have to wait for that long.

So, therefore it is an extremely slow process. Suppose I keep the sweep rate as one hertz per second and now suppose I have 100 megahertz spectrometer 100 megahertz 10 ppm will be

1000 hertz. So, for time for sweep will be 1000 seconds 1000 seconds this is approximately 16.5 minutes and that is a huge amount of time for scanning through the spectrum once it is enormously time consuming. Now as we know these population differences are not very large in the radio frequency regions are not very large.

Therefore the signal intensities are very very low as such therefore if you have to measure then what you have to do is you sweep through it several times and add the signals that is signal averaging. Now how we define the signal to noise ratio? The signal to noise ratio if I have a spectrum like this then I have the noise here this is noise and I have the signal here.

So, what you do is you take peak height above a mean noise level. So, here let us say you take a separation between these 2 points away mean noise level and then you take the maximum peak to peak separation in noise. You have this signal height signal above the mean noise this is the signal height and then you have the maximum peak to peak separation within the noise and that is the denominator.

Denominator maximum peak to peak separation in noise and the numerator is maximum peak height above the mean noise level and then you multiply factor 2.5 this comes as a result of stochastic theories. So, this is defined as the signal to noise ratio and this is extremely small in the case of NMR because what the signal depends upon? The signal depends upon the population differences and if that is very small the signal intensity will be very small. Therefore what people thought that this requires what is called as signal averaging.

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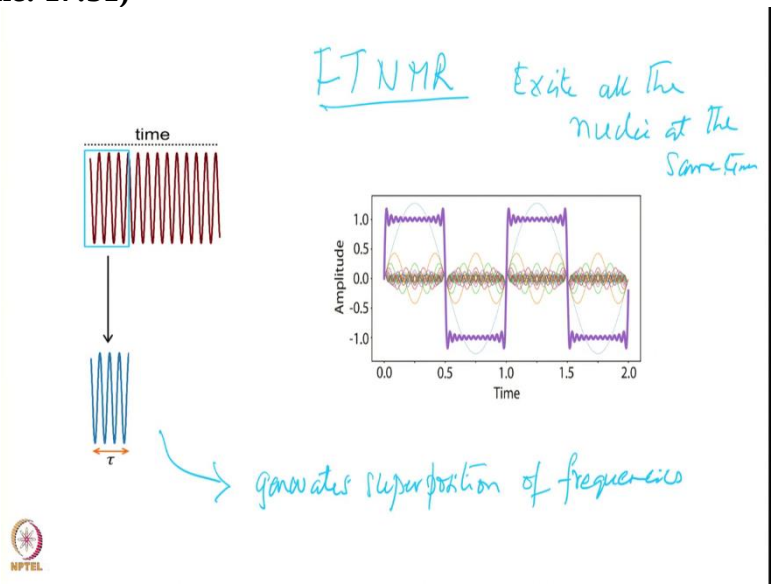

So, signal averaging would mean this. You sweep through the spectrum several times and add them when you add them signal to noise enhance it goes the signal to noise ratio goes as the number of sweeps squared as the square root of the number of sweeps go added. So, if you want to increase your signal to noise ratio by a factor of 10 then you will have to collect 100 sweeps and then you have the signal downward enhancement of 10.

And this creates a lot of issues with regard to the stability of the spectrometer stability of the samples you notice previously I told you for one scan it took about 16.5 minutes if you want to do it 100 times. So, you see 1600 minutes and there is a huge amount of time and what pressure it puts on the spectrometer your magnetic field has to be stable for that long the temperature has to be stable the room temperature has to be stable.

Current has to be stable all kinds of demands will be there and therefore the huge demand on the spectrometer. So, therefore and then you do not want to do it for that long you want to get a good spectrum then you will have to use high concentrations of the sample. So, that your signal intensity is inherently higher. So, obviously one had to use like half a molar sample or sometimes one molar sample.

But if we have a low solubility then of course such samples cannot be studied things which are not soluble and in the solvent it becomes very difficult to study those ones and difficult to observe low abundance nuclei such as ^{13}C and ^{15}N etc, what is the abundance of ^{13}C is 1.1% and see nitrogen 15 (^{15}N) is 0.37%. Therefore such nuclei is very difficult to observe this is 1.1% 0.37%.

So, it is very difficult to absorb such nuclei and therefore what people thought this may not be a very useful technique. Therefore people said NMR is not a very useful technique this was the kind of a conclusion people arrived at because you cannot apply too many systems and that is where a new revolution was required and that came up as a result of what is called as Fourier transform NMR. What do we do here the Fourier transform NMR is the principle is like this.

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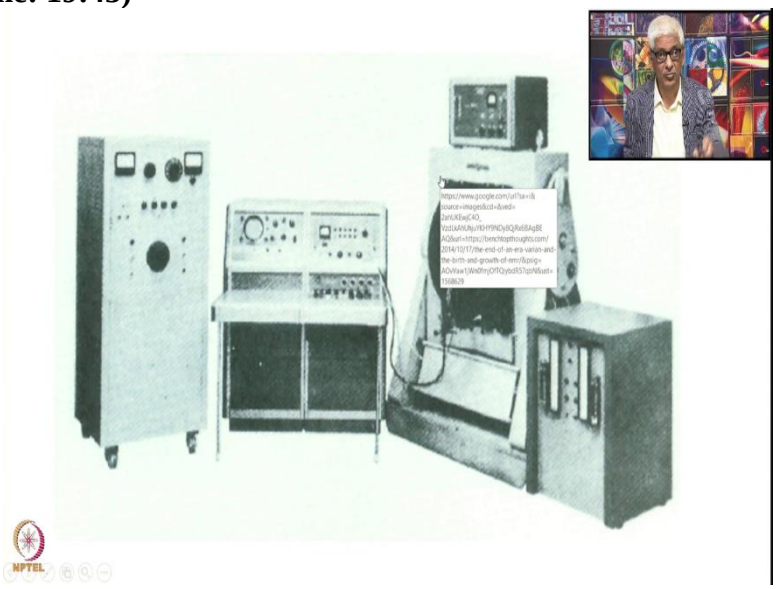

The principle is you want to excite all the nuclei at the same time all the nuclei at the same time. How do we do it? This is the strategy here. You notice your RF, RF is a continuous wave going on like this in this indication here the RF is this a cosine wave or a sine wave which you are applying which continuously go like this in the case of normal CW NMR. Now what you do is instead of applying the RF continuously like this you apply the RF for a short period like this.

A truncated you start here and immediately end within a few micro seconds within a micro. So, therefore your time profile of the RF is starts at zero and goes up like this a few cycles then it stops suddenly comes to zero what is the meaning of this? Now what is on the right hand side which is shown here you consider various kinds of frequencies various frequencies going as a function of time.

So, what I have shown here this is the oscillation right this is the one single frequency what is drawn here is the various frequencies present here and superposition of all of these frequencies

is shown on the this one dark blue what you see here this is the superposition of all these frequencies. There are so many frequencies present here the superposition of all of these frequencies creates a profile which is like this.

So, this looks very similar to this here right except that I start at a particular point only one frequency is going here I stop it and then truncate it. So, it is going then going for a short period and then truncated come to zero goes up RF goes up goes for some time then comes to 0. So, you produce a pattern which is like this but I choose only one particular portion of this and this is a small amount of time.

So, in other words the superposition of all of these frequencies presence is present in a time domain profile like this. So, generates superposition of frequencies and will they all have similar amplitudes no they need not have similar amplitudes. So, they will generate with the different amplitudes and that is indicated here.

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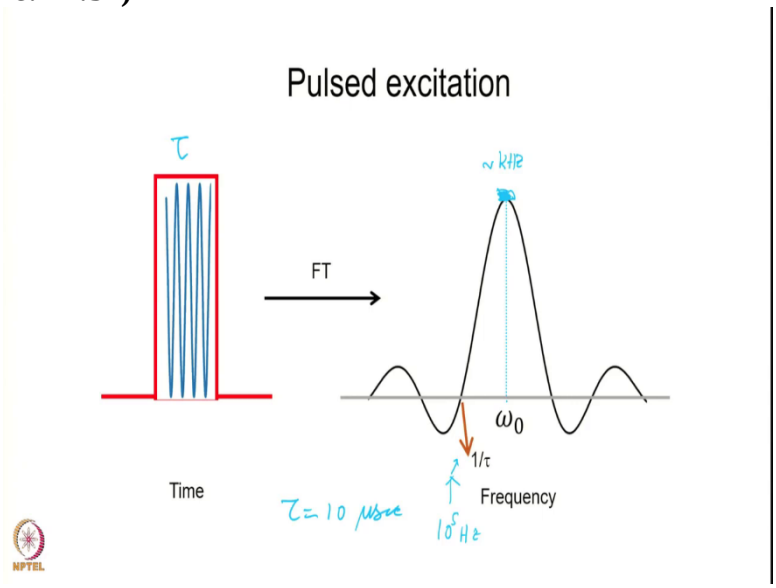

See, if you apply a time domain profile which is looking like this and now it will generate all of these the superposition meaning what superposition meaning it is a kind of a Fourier transform superposition of the frequencies is the Fourier transform. So, this Fourier transform provides the frequency profile like this and this is called as a pulsed excitation. Because the RF is applied for a short period that means it is a pulse I apply take it out start take it out.

So, that is a pulse applied for a short period and this is applied for a short period of something like about a few micro seconds. So, when I do this I generate a frequency profile like this I showed you there earlier right support position how does it lead to different kinds of time domain profile. All along this axis I have the various frequencies present there and what are their intensities the amplitudes this is the implied intensity at $\frac{1}{\tau}$ this actually is zero.

What is $\frac{1}{\tau}$? τ is the time for which this is applied. So, this is this is the time tau. So, for the time τ if I apply it then at one over tau this comes to zero and it is a maximum at its individual frequency which is ω_0 that is this frequency ω_0 it has a maximum here and it decays as different frequencies. So, electronics this process of starting and truncating the RF as a particular point in time generates the frequency domain profile which is like this. And they all different

frequencies with the different amplitudes and you have this sort of approach this is called as a sink function.

Now at $\frac{1}{\tau}$ suppose τ is of the order of let us say 10 micro seconds suppose τ = 10 micro seconds and what will be this then this point will be 10^5, $\frac{1}{\tau}$. So, this is 10^5 hertz that is 100 kilohertz, 10^5 hertz that is this point right. So, therefore and similarly here 10^5, so, it generates a wide range of frequencies.

So you do not have to scan through the frequencies by applying the RF pulse for a short time tau of 10 micro seconds you generate it with various frequencies with at various different amplitudes. However if the amplitude is different it will also mean a different power it mean a different power for the excitation. We do not want different powers we want to have a same power want to have a same power for all of these. So, therefore what one does is this is the wide range and typically our ranges are of the order of few kilohertz.

Now if I choose a small range here from here to here if we choose a small range this is the few kilohertz and that is enough for me. So, you how many as I said 100 megahertz 10 ppm is 10 kilohertz 1 kilohertz 1000 hertz, 1000 hertz is one kilohertz if I take 500 megahertz 5000 hertz is 5 kilohertz. So, that is quite small. So, I need to only if I select these many frequencies and that is enough for me but however how do I select that.

Do I need to select it? No, we will see that filtering is automatically done by the done by the recording procedure the detection procedure automatically provides that. So, if I have this sort of thing these are kind of filters which will eliminate all the other frequencies I have only a small range of frequencies which are present here and this will give me the excitation of all the frequencies at the same time.

Now how much time it took for me it only took 10 microseconds. 10 microseconds has allowed me to excite all the frequencies. Now if this $\omega_0$is closer to your spectral range where you are having your peaks in your spectrum around that area if I have this 5 kilohertz of excitation and then I have all the frequencies excited. Now but of course there are others there may be frequencies which do not belong to your resonance absorption frequency also.

So, how do you filter that out? But that filters out comes out as a result of your detection process that automatically the detection part is the filtering process . So, that will happen in the in the following manner but this will take a little time. So, I think we will stop here we will go into this in the next class. Just one more one point I would like to mention that the application of the RF will excite all the frequencies it will also result in the rotation of the magnetization outside of the out of the Z-axis.

And that is we have to talk about the flip angle and all of those over there. We have to go into the reading frame we talked about the rotating frame earlier we have to go back into the rotating frame to understand these phenomena and when we do that then of course we will see that the magnetization will tilt away from the Z-axis and that is the beginning of the detection process. I think we will stop here and we will go into the next one from here to the next class.