NMR spectroscopy for Structural Biology NS
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Lecture: 10
Practical Aspects of FT-NMR-II

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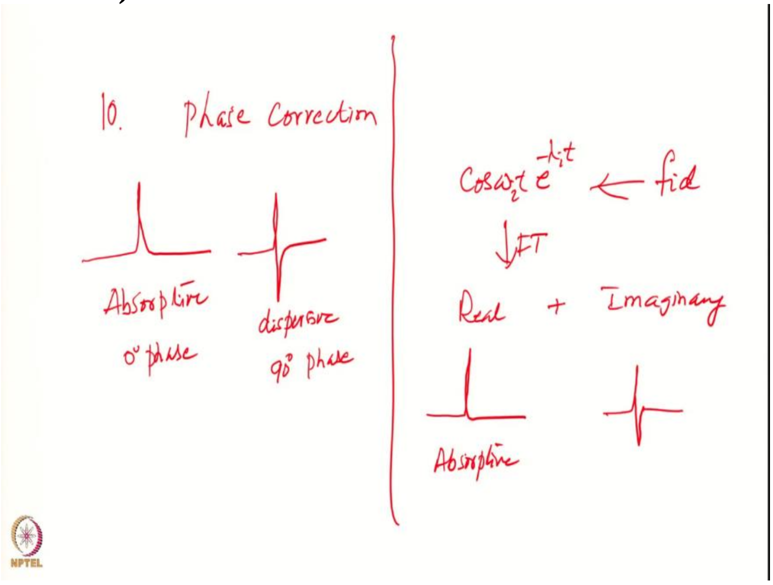

So, we have been discussing about features which are exclusive to Fourier transform NMR. We talked about various important parts in that one. So, we will continue with that discussion with regard to the special features with our exclusive for FT-NMR. In that one, we now come to the 10th point which is phase correction. Typically we know that NMR signals are of 2 types, one we have a signal which is like this.

And this we call as absorptive signal or you may call this also as a spectrum with a zero phase or the other this is the dispersive signal which goes like this and this is a dispersive signal and this we may call it as 90° phase. In FT-NMR we collect the data as let us say we collect the data is $\cos \omega_i\, t\, e^{-\lambda_i t}$. Suppose we have the signal which is like this when you Fourier transform this.

It generates 2 components one is the real component and then you also have the imaginary component. Time domain function this is my FID. If FID is like this the real part will have a pure phase absorptive signal this is absorptive and the imaginary part will have the dispersive component.

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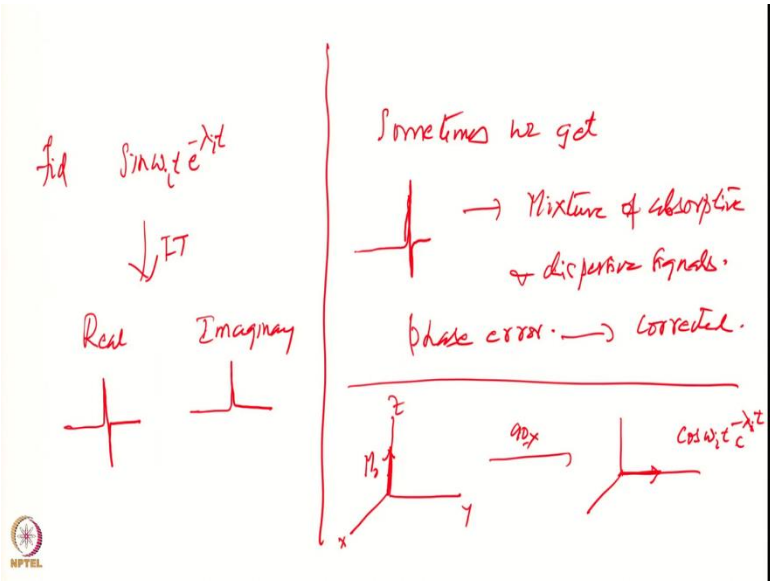

Now similarly if I have if my FID is of the type $\sin \omega_i\, t\, e^{-\lambda_i t}$ if I have this sort of a function FID this is my this is my FID then after Fourier transformation I will get again a real part and an imaginary part. And the real part will have the dispersive signal and the imaginary part will have the absorptive signal. So, therefore we have to see what is it that we are going to get in FT-NMR spectrum.

Now often we find that your signal is not exactly disperse you or absorb you sometimes you get sometimes we get signals which are like this. So, this is a mixture of absorptive and dispersive signals. So, therefore this is known as phase error. So, this is called as phase error and this has to be corrected. So, now what is the source of the phase error what causes the phase error this we have to understand.

Now as I explained to you suppose I have an experiment done like this I have the initial magnetization here this is the M_0 initial magnetization which is here if I apply a norm like 90° pulse along the X axis which is exactly correct 90x then my magnetization will come along there let us say the Y axis whether it is 90x or -x if it comes exactly along the Y axis. Then my signal will be $\cos \omega_i\, t\, e^{-\lambda_i t}$ this will be my signal.

If I collect the signal starting from the exactly magnetization aligned along the Y axis. But often it so happens that this is not achieved this is not achieved.

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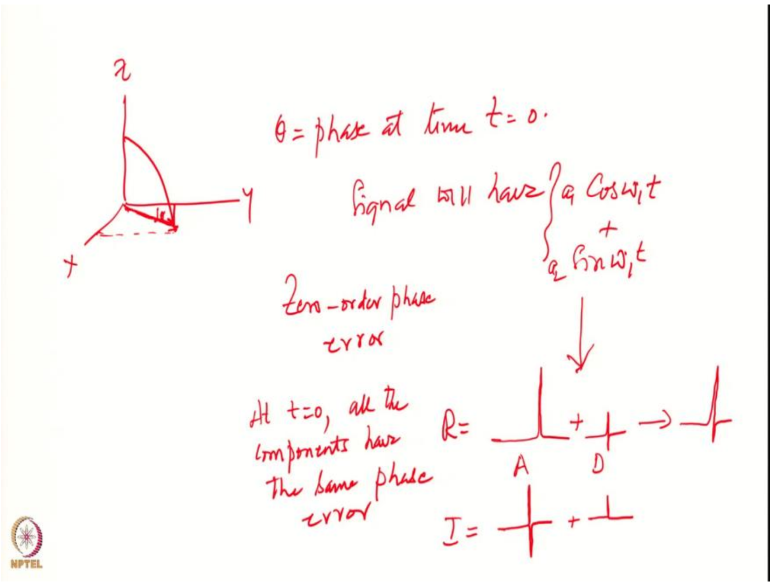

And what happens is you get the magnetization which was initially rotating to somewhere like this to some other position. So, then it will be oriented here in the transverse plane. If this is the case then you have the x, y, and z. So, the signal which you are going to collect will have the com contributions coming from this as well as from this right. So, if it is not zero phase this is let us say this angle is θ then θ is the phase at time t = 0.

So, in such a situation your signal will have real component which is the cosine component $\cos\omega_i\, t$ and also $\sin\omega_i\, t\, e^{-\lambda_i t}$. So, signal will have both the components. So, if a Fourier transform this what i should get of course this will have a certain amplitude here there is a coefficient let us call this as a 1 and call this as a 2 there are 2 coefficients depending upon what is the error here theta error depending upon the components along the Y and the X axis will be different.

Whatever is along the Y axis will have a $\cos\omega_i\, t$ whatever is along the X axis will be the $\sin\omega_i\, t$. Now if I do a Fourier transformation of this sum together which is collected here I will have a mixture of in the real part it is also true in the imaginary part also this is the absorptive component. And then I will also have a component which is coming from the x part which is the dispersion component this plus this will give me a signal which is like this.

So, this is because of the error in the initial pulse application the pulse is not applied exactly along the X axis but slightly deviated from the X axis. So, RF phase is not exactly zero but slightly away and that is the theta therefore when the 90° rotation happens it does not come exactly along the y axis but it comes somewhere in the xy plane and that if the angle is θ there will be certain mixture of these two things will happen.

So, this is this is this has to be corrected and this is called as a zero order phase error because this is not dependent on any frequency entire magnetization is rotated. So, at time t = 0 all the magnetization components have the same angle. At t = 0 all the all the components have the same phase error and in the this is in the real part. The imaginary part similarly will have this portion will have pure dispersion component and also a small absorptive component.

So, the trick in correcting the phase will be to mix these 2 appropriately. So, that the dispersive component from the absorptive signal will vanish and likewise the absorptive component from the imaginary part will vanish. So, that is the idea. Now there is another source of phase error and that is the following.

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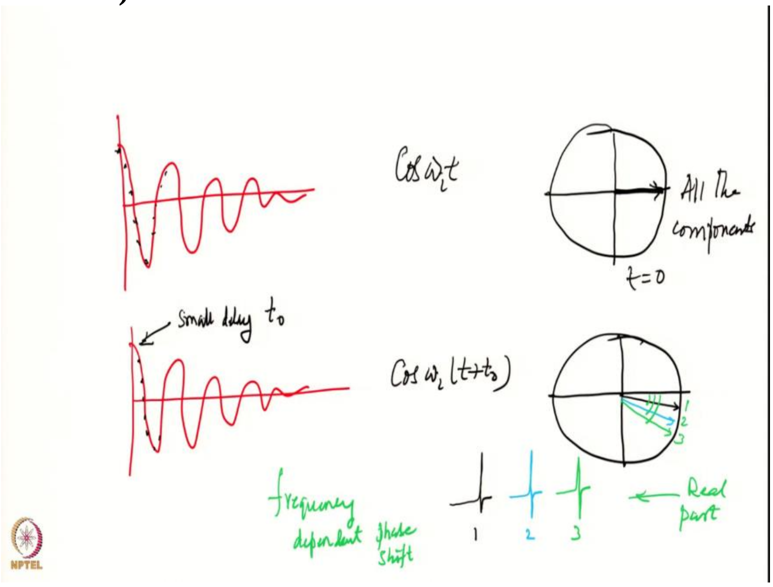

Suppose my FID is supposed to be like this right and my data points are should be like this here, here, here, here, here should be like this. So, therefore at time t is equal to 0 the magnetization is entirely along the Y axis or whatever that is see. In this case the magnetization is entirely along the Y axis if I am collecting the y components. But suppose I cause a small delay in the acquisition of the time. So, in this situation where will the magnetization be?

All the components of magnetization will be along here all the components. So, this will be all the components will be here in this case in the normal case this is but instead suppose I do not start collecting the data from time t = 0 this is at t = 0. Now if I collect the data this is my FID but I do not start collecting the data from the first point but I start collecting from here I give some delay for various practical reasons.

I do not collect them. So, this can be a small time also it can be a few hundred nanoseconds that is it has nothing to do with the dwell time it has some hardware limitations. So, you may want to give a small delay here this is a small delay which may call it as t_0. So, actually data's collection starts from t_0. So, therefore your signal here will be $\cos \omega_i\, t + t_0$. So, what does it mean in if I were to represent this in the transverse plane like this the magnetization where it will be to start with?

To start with we are because the t_0 is the delay during this period time the different components will have precessed to different extents right. So, during the time keynote the various components would have precessed although I applied a 90° pulse correctly that it came to the Y axis. The magnetization came to the Y axis by the I did not start collecting the magnetization from that point.

I started collecting when the different components have moved to different extents that is t_0. So, let us say one component has moved here and another component has moved here and the third component mode here these are different spins. Different lines in your spectrum they are corresponding to different spins. So, they all have moved to different extents let us call this as 1 2 3 and all of these now have different θ’s right.

They all have different thetas compared to the previous one it is a zero order phase. Now in this case because of the delay in the data acquisition the different frequency components in your magnetization have moved to different extents. Therefore the first line would have a mixture something like this let me call this as 1. And the second one may have had some more here.

And the third one would have even more third and so on. So, different ones will have different mixtures therefore this is what we call as frequency dependent phase shift and this is what we are talking about these are all in the real part of the spectrum. Similar thing will happen in the imaginary part of the spectrum. So, in order to correct for this here as well as in the previous case the strategy is to mix the real and the imaginary components.

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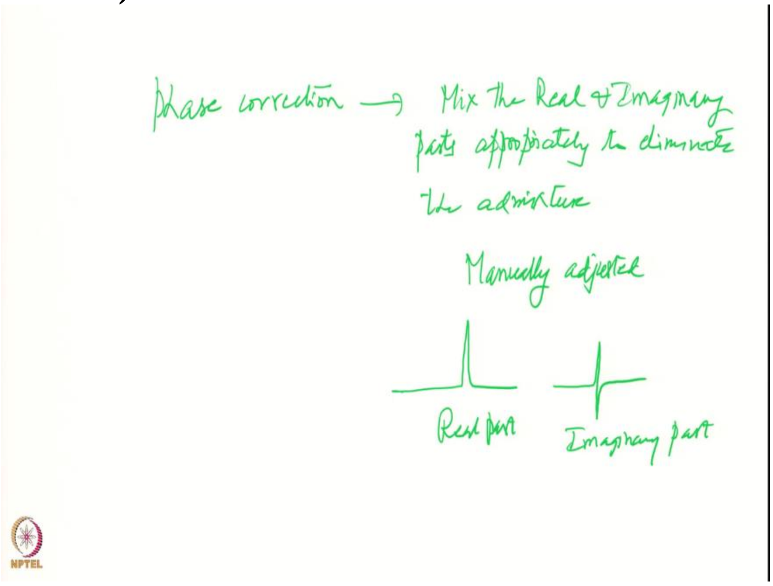

So, therefore phase correction would imply mix the real and imaginary parts appropriately to eliminate the admixture. The computer does this there are various kinds of knobs over there. So, this you just keep moving the knobs and in real time actually you can monitor on the screen how the phases are changing. So, these are the algorithms are there this is the mathematics behind this all that is all programmed into the computer it is all programmed.

How to mix the real and the imaginary parts and what should be the coefficients for the mixing all that is then you can manually adjust it. So, this can be manually adjusted to obtain the phase correction then we will have after you have done that you get pure absorptive line for the real part and pure dispersive part for the imaginary part. You generally do not use the imaginary part for other purposes in when you want to represent your spectrum you only plot the real part you only plot the absorptive spectrum right.

Imaginary part stays there in the computer but you do not you do not really look at that. However it is used for phase correction. So, that is the important part of the Fourier transform NMR. And this does not exist in case of conventional spectroscopy. So, now moving on to the next important concept and that is dynamic range.

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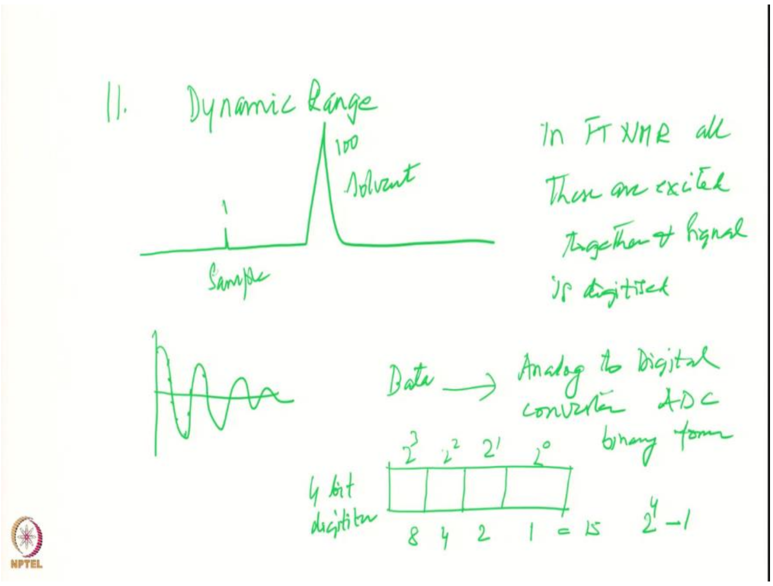
11. Dynamic Range

In FT NMR all These are excited together & signal is digitized

Data → Analog to Digital converter ADC binary form

So, what is dynamic range? Let us say we have a small signal and a huge signal like this. This may be a solvent this may be from your sample and you notice in Fourier terms of NMR we excite all of them together. In FT NMR all these are excited together and signal is digitized. So, when I collect the data in the digitized form it will be superposition of the contributions from all of those. Suppose this signal has intensity of one this signal has intensity what of hundred be collecting will be 101.

If this is 5 and this is 200 or it will be collecting will be 205. So, that is what will be representing in my in my FID. So, this is what happens in the FID. The FID which you have this you have the various points at various places and in every point we will have this summation represented. Therefore in every point the data has to be represented correctly this sum has to be represented correctly.

How it is stored? How it is stored in the data the data is digitized right. As I told you data is digitized and there is this device which is called as analog to digital converter also called as ADC. This collects data in the binary form. So, it is collect data in the binary form because that is what the computer understands right. And let us say we have analyzed digital converter which has a resolution of about let us say four.

So, there are 4 bits there are 4 bits in and the analog digital converter. What is this will be 2^0, 2^1, 2^2, 2^3. So, what is the largest number that can be stored here 2^0 is 1, 2^1 is 2, 2^2 is 4 and 2^3 is 8 this is equal to 15 this is the 4 bit 4 bit to digitizer. Each one of them is called as a bit the 4 bit digitizer the largest number is 15 that is 2^4 -1.

This of course is a very small number typically our intensity ranges will not be. So, small they will be much larger than this we would like to as a large a range as possible.

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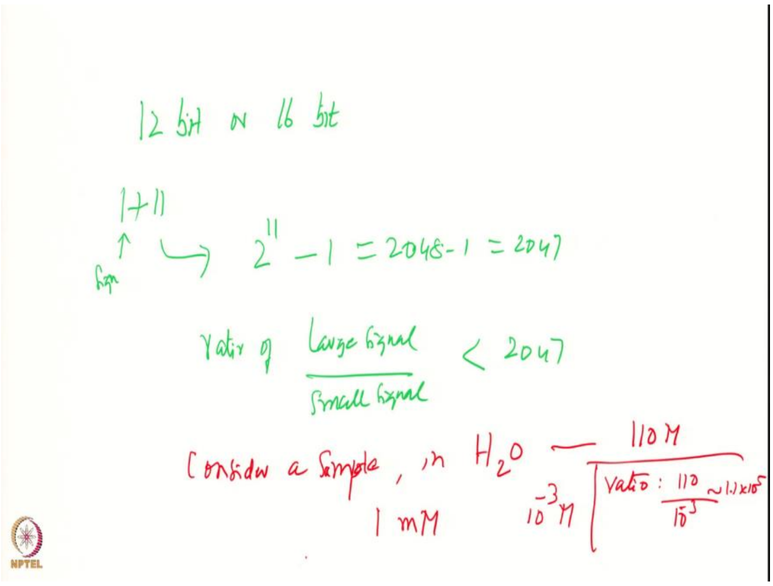

12 bit or 16 bit

1+11

↑ sign ↳ $2^{11} - 1 = 2048 - 1 = 2047$

ratio of $\frac{\text{Large Signal}}{\text{Small Signal}} < 2047$

Consider a Sample, in H_2O — 110 M

1 mM 10^{-3} M ratio : $\frac{110}{10^{-3}} \sim 1.1\times10^{5}$

So, therefore typically one uses what are called as 12-bit digitizer 12-bit or 16-bit, 16-bit digitizer. So, in out of these they also have to make account for the sign one bit is kept for the sign plus or minus one bit is kept for the sign. So, therefore what will be the number in this a 12 bit digitizer? In a 12 bit digitizer 1 + 11 this is for the sign and 11 bits what it will give you $2^{11} - 1$.

2^{11} is 2048 -1 that is equal to 2047. So, this is the largest number that you can store. Therefore in your FID the digital point should you when you add up the intensities of the small signal and the large signal it should be below 2047 or let us say if you scale it for some reason you have a possibility of scaling it down I multiply everything by scaling a factor like $\frac{1}{5}$ or $\frac{1}{10}$ or something that is a scaling factor even so that scaled number should be less than this.

In other words the ratio of large signal to small signal should be less than 2047 then the whole thing will be represented. And this is where the problem comes. Let us consider that you are collecting a signal in water. Consider a sample in water and we are recording the proton spectrum. Proton spectrum what is the concentration of proton here in water. So, this is approximately 110 molar water concept proton is 110 molar.

And if we have a sample which is 1 millimolar what is this concentration this is 10^{-3} molar and therefore what is the ratio? The ratio is is equal to $\frac{110}{10^{-3}}$ this is approximately $1.1 * 10^{5}$. So, therefore it is impossible to represent this number in this 12-bit digitizer and this is called as the dynamic range.

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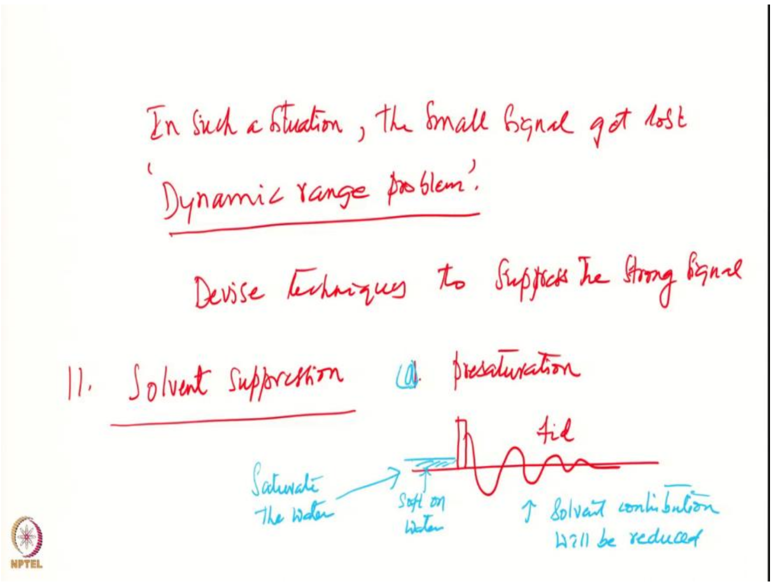

In such a situation, the small signal got lost

'Dynamic range problem'.

Devise techniques to suppress the strong signal

II. Solvent suppression (a) presaturation

In such a situation the small signal gets lost only the big signal is represented and the small signal is not represented this is called as dynamic range problem this is a problem. So, what should we do to avoid this we have to devise techniques to suppress the strong signal. Most of this most often the strong signal is from your solvent it is not of much use for you to collect your water signals solvent signal.

Therefore you can suppress that strong signal and. So, that you can observe the your actual signals and there are various strategies to do this. So, therefore you come to the next topic which is called as the solvent suppression. There are various strategies to do this I will take you the some of the very simple ones there and of course there are very elegant procedures which are there we will see use them.

The simplest thing is first the pre-saturation. So, what is the strategy here let me represent my FT-NMR experiment in this manner this is my 90 degree pulse and i am actually collecting the signal here as FID this is my FID. So, what I will do is before I apply the 90 degree pulse I apply a soft RF another RF I do not use the same RF which is here I will use the soft RF here on water that is the solvent signal.

That means it will excite only the water it will not excite anything else. So, this will saturate the water signal this will saturate the water and it will not affect anything else because it is soft. So, it is applied exclusively to the water signal therefore it will only saturate the water. Now before that water saturated water returns back to equilibrium before it comes back you apply 90 degree pulse here or whatever the flip angle you want to use and then you direct the signal you finish your experiment before the water comes back.

So, therefore in this area the solvent contribution will be reduced. So, this is the simplest of the experiments and there are other methods we will see what is called as the inversion recovery will see the next experiment which is called the inversion recovery let me call this as “a”.

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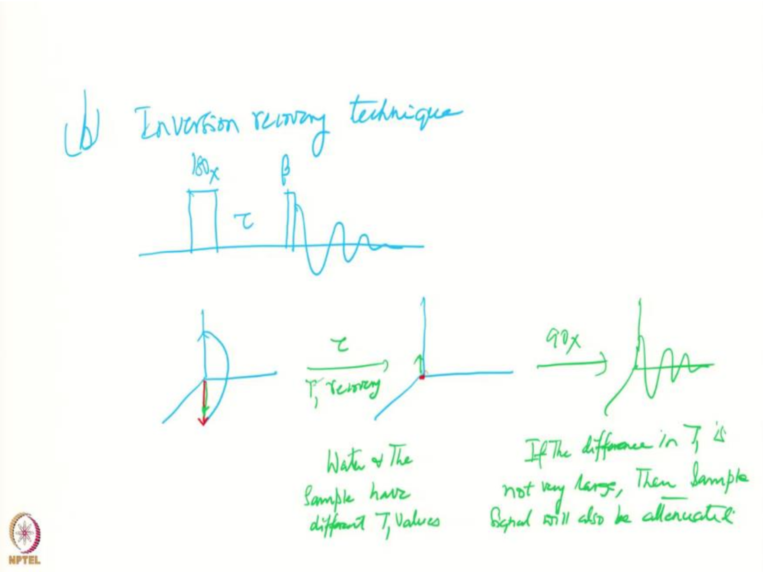

Then I will say “b”, b is inversion recovery technique. So, what we do here is, it actually uses more than one pulse here. So, strategy will be like this you apply 180° pulse a hard pulse you a time tau wait for a time τ and then you apply a 90° pulse or flip angle whatever flip angle you want to give and you collect the FID here. How does this work?

Let us try to understand this in a vectorial manner vectorial picture inversion recovery means 180°x+ meaning what the magnetization is rotated from here to here right. Initially magnetization was here and that gets rotated to this point when you have a 180° degree pulse. So, let me draw that as here. Now this magnetization contains contributions not only from the water but also from your other signals for your other samples as well.

Let me represent them with a different colour. So, let us say I have another one here for 2 is enough for purposes of understanding. Now, both these will try to recover back to equilibrium. Now what you do is suppose my red is water and the green one is my sample. So, in that situation what I will do is I will adjust tau such that water has come here because it is recovering. As it is recovering water has come here at zero point and but the green one has come here.

So, which means they have different relaxation times the red water and the sample water and the sample have different T_1 values because this is T_1 relaxation right this is a longitudinal magnetization longitudinal magnetization is what is happening here. This is here what is happening here is T_1 recovery during the time τ there is a T_1 based recovery. So, the if the 2 have different T_1 values I adjust the time tau in such a way that the red signal which has come down to zero but the green one has gone up or either down each other either way it is possible it may not have reached there or it has crossed that.

Now after this if I apply a 90x pulse I will not get any signal from the water ideally I will get only signal from the sample. However in practice this does not happen some signal will always come because the recovery is not all that very precise adjustment is not so precise. So, this is the strategy this of course requires that have distinctly different values things which are very distinctly different values only then you can suppress the water.

If they have similar relaxation times then this will not work because if the water has relaxed to zero your sample also relax to zero. So, then you may lose it and then of course if the difference is not very large in T_1 is not very large then your sample also will get will get attenuated sample signal will also be attenuated. So, this is an important technique. Now then we will stop here and in the next one; we will continue with this and take more.