**NMR Spectroscopy for Chemist and Biologist**
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Lecture 18
Spin Echo and Water Suppression

So let us continue the discussion with regard to dynamic range problem in FTNMR, we said last time when you had a huge solvent signal and a small sample signal, because of the limited ADC resolution the solvent signal overrides the ADC and then you will not be able to detect the small signal at all, the small signal will not get representation in the ADC and therefore no amount of signal averaging will help in recovering the sample signals. So therefore what one needs to do is to suppress the solvent peak and that is what we are going to discuss today.

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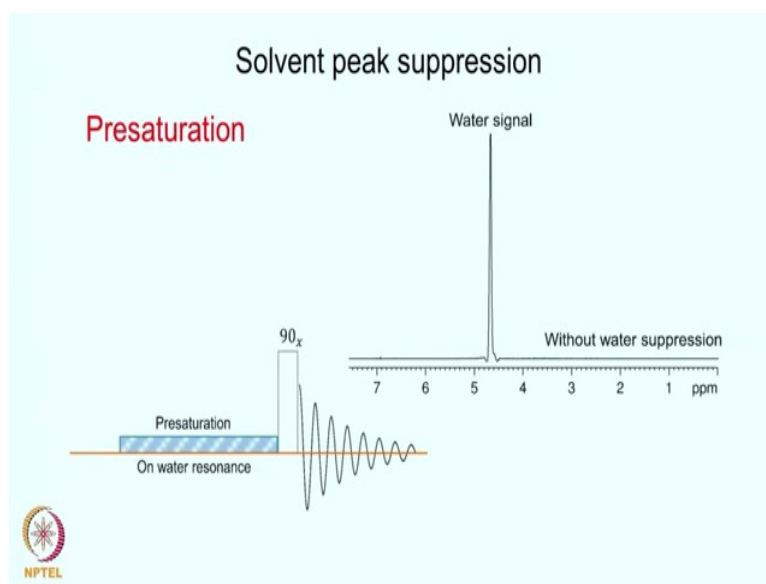
You look here this is the spectrum of a sample which is in water and you have a huge water signal and this is the spectrum without water suppression and you will see only the signal of the water you do not see anything of your sample set at all.

So one way to suppress the solvent signal is to do what is called as a kind of double resonance experiment where you use a second *RF* and eradiate the solvent resonance and the second *RF* frequency matches exactly with the water resonance therefore you put that RF here with the high power and well reasonably high power so that it only effects the energy levels corresponding to the water and all the other signals which are present here are not effected to a reasonable approximation, therefore the signals from here will get saturated, the

water signal will get saturated, all the other signals will not be perturbed up, this is achieved in the following manner the like this.

So, you have a pre saturation this is called as pre saturation period where you put second RF on the water resonance following that you apply 90°pulse and you have to collect your FID a usual, so we are at this point here the water resonance is reduced to very significant extent and once you apply the 90° pulse along with the other signals you detect the FID and this will not overload the receiver or it will not cross the dynamic range problem.

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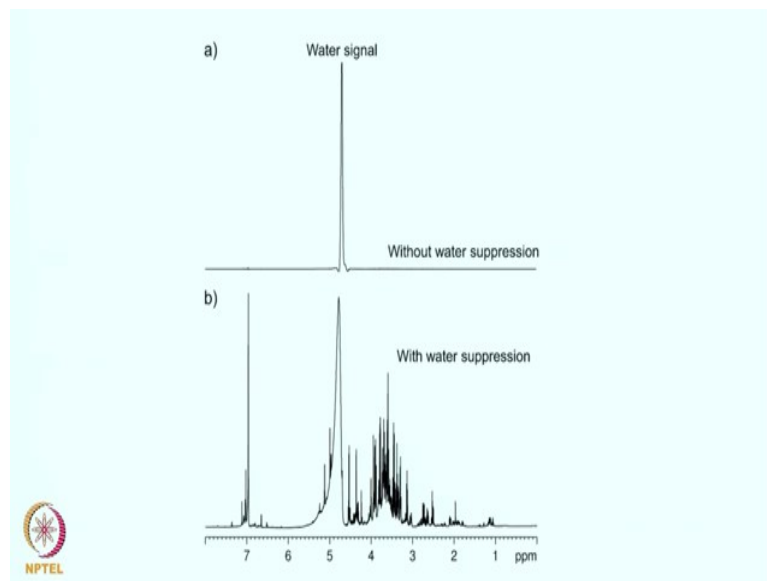
And this is indicated here this is the same spectrum what you showed in the previous slide and this is with water suppression by pre saturation here to see the water signal is substantially reduced and you are able to see the other signals of the sample this is the spectrum of the protein and you are able to see the other signals of the protein here pretty with good intensities. So this is one technique to suppress the water.
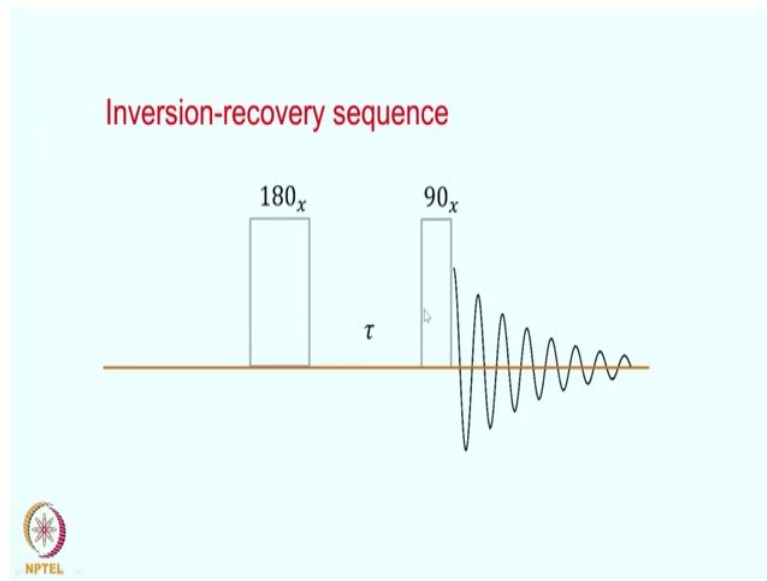
There are other techniques to suppress the water signal and one of the popular one is the inversion recovery sequence, what you do here is you apply a 180° to the entire magnetization which includes the water as well as the other sample signals and you wait for a time tau following that you apply 90° pulse along the X axis and then you detect your FID as usual.

What is to be done here? Here the trick is to optimize the value of tau so that in the end you do not get enough water magnetization here and your other magnetization are not perturbed up as much, so you get your solvent suppression effectively and you get your sample signals in a reasonable good intensity, how does his work?
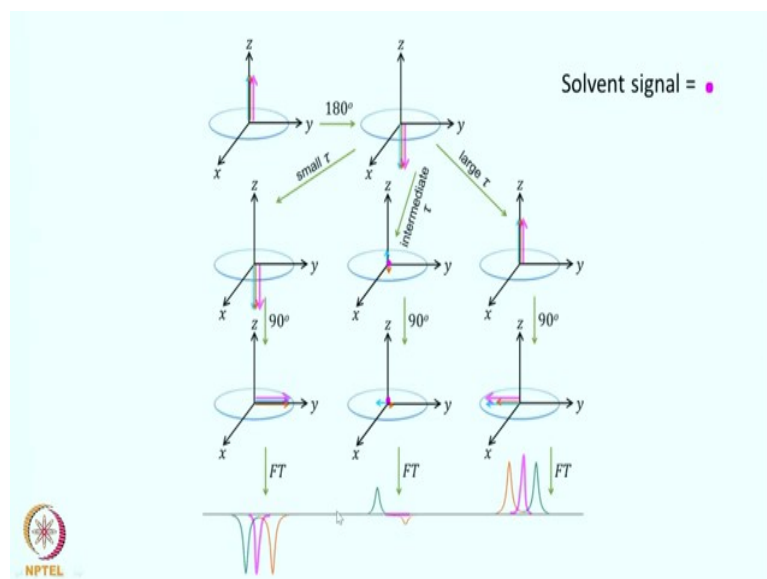
So, let us look at that with the following vector diagram. Here the magnetization is represented by this arrows here, initially at equilibrium all the magnetization is along the Z axis, the pink one represents that of the water and the other ones are of the sample signals although this is very huge for the schematic purposes we have drawn them with the same height, although this pink arrow has to be very large compare to the other ones.

Now what happens if you apply 180˚ pulse the entire magnetization rotates from the Z axis to the minus Z axis here, now this is the non equilibrium state, after the 180˚ pulse you allow the system to relax back, what it will do? It will relax back to the Z axis and this will be determine by the spin lattice relaxation time of the individual spins, different once will relax differently depending upon their relaxation times which are the characteristic of the individual spins.

For a small tau if this is extremely small the entire magnetization is still down here and if you apply a 90˚pulse to this situation then you have magnetization rotated into the transfers plane the Y axis and this is the negative Y axis and then we get a signal here the negative magnetization which is the three signals, these two are the sample signals and the pink one is your water signal.

For a very small value of tau, now suppose you adjust your tau in such way that the solvent signal during the recovery has come to 0 here and the blue line has gone on the positive axis and the other line is still on the negative axis but it is quite substantially reduced because it has recovered long the Z axis.

So, now we apply a 90˚ pulse to this situation your magnetization is rotated in the terms of plane and this fellow will be here, this blue one is here, the water is at 0 and your other signal is still on the lower side so if you do a Fourier transformation you will get this signal which is recovered we will have this sign because this opposite sign compare to this sign here. So, this will be on positive side and this will be on the negative side.

If this, we apply for a very long τ then of course all of them will recover back to equilibrium here and if you measure the apply the 90˚ pulse then you will see all of them positive in the X axis. So therefore the trick for what the suppression is to choose your tau such that the water has come down to 0 here and your other signals have recovered completely or partially, they are along the positive z axis or the negative Z axis but the trick is that your water signal is at 0 therefore when you detect it after the FID after applying the 90˚ pulse the FID thus get a

representation of this signals. So this is the technique called as the inversion recovery technique which you also for other purposes which we will see.

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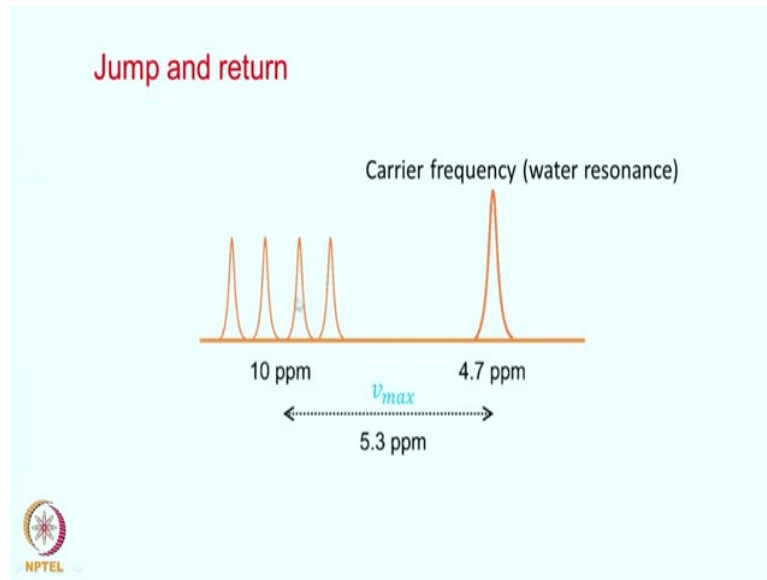
The other technique which has been commonly used is called the jump and return sequence, the idea is here is the following, here you have the strong water signal let us say it is 4.7 ppm if you are recording the water and the spectrum typically the water appears to around to 4.7 to 4.8 ppm and you have many others signals to which are little far away from there somewhere around 10 ppm.

Now let us assume that we are interested in this resonances and not any other which are closed by here anything like that, so therefore we want to maximize the signal intensity of this once, minimize the water intensity, we do not care so much about the once which are in the close vicinity of the water signal here. So therefore we want to get the maximum intensity of these signals because we are interested in this, assume that this distance from here to here in terms of frequency is nu max, if this is 10 ppm, 4.7 ppm this difference is 5.3 ppm, so we use that trick so that we get maximum intensity for this resonances for other you may get reduced intensity but that is not a concern to us.
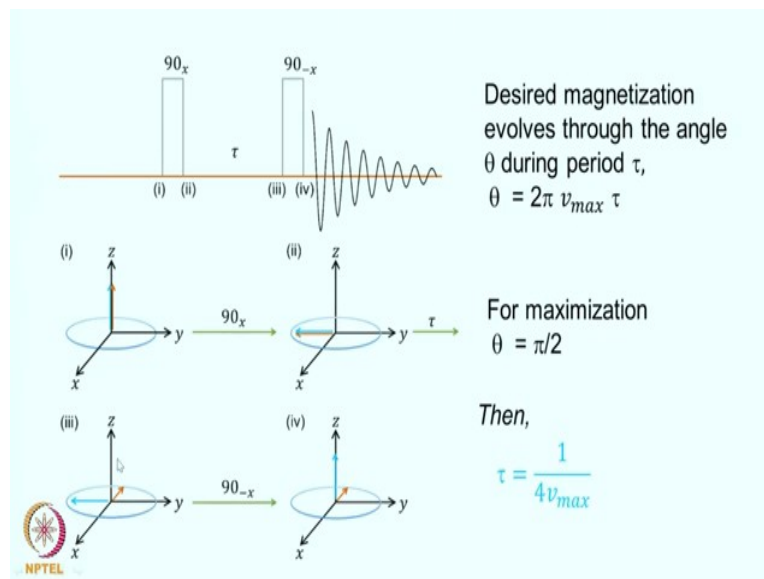
Because we are interested in this particular peaks. So, how does this experiment work? The pulse sequence for this experiment is like this, you have a first 90° pulse which is applied along the X axis, then you wait for a time tau then you apply a another 90° pulse but now with a opposite phase, this is with minus X axis, if it is applied along X this is along -X and following they have to detect your FID and Fourier transform it to get your spectrum.

So, let us look at it from the vector picture, how does the magnetization rotate, so here are two arrows drawn one is for the water and the other one is for your interested signal, and you put the carrier on water which means the carrier at 0 frequency, all your other frequencies are away from the water and they will have precessional frequencies, the water will not have the precessional frequency because you put offset on water which means that is 0 frequency with respect to the carrier.

Now when you apply 90° pulse along the x, so let us say the magnetization is rotated on to the minus Y axis so both the magnetization is here. now what happens during this period tau after that the individual components will precessing with their characteristic frequencies. Now after the time tau they would have precess to different extent but the water would have remained there because water is at 0 frequency which means it has no precessional frequency, it stays there and you sample which is of interest you would have moves.

Now you wait for such a time then this magnetization which is this orange magnetization here which is of interest you, has rotated by 90°, so for maximization you allow the magnetization to rotate by 90° so that it has come here and that is dependent on your tau period.

So, adjust your tau period so that the designed magnetization has rotated through 90 degrees, so what is the angle of rotation here how do we calculate that? now if v_{max} is you frequency of your interest then the angle of rotation of this transfers magnetization is $\theta = 2\pi v_{max} \tau$, τ is the period for which the magnetization is precessing, Now if wanted to be rotated by $\frac{\pi}{2}$, with the 90° then you say the $\theta = \frac{\pi}{2}$, if you put this equation in this equation here then you get

$$\tau = \frac{1}{4 v_{max}}.$$

So if you adjust your τ to this value then your designed magnetization has rotated by 90° and is along the x axis, now you apply a 90 -x, 90 -x meaning you apply it along the other axis here, here this magnetization is rotated here along the -X axis if you apply so this magnetization the orange magnetization does not rotate because it is along the -X axis itself, it stays there only but the water magnetization which was here now rotates back to the Z axis it comes here.

So the water magnetization has come back to the Z axis your desired magnetization has remained in the transfers plain, now if you detect the signal you will get the signals from the desired magnetization only, therefore this is the other way of suppressing the solvent signal and detecting the signal of interest, this is called as jump and return. Notice one thing here we have kept the carrier on the water, now there are signals which are on the left side of the water and on the right side of the water as well,
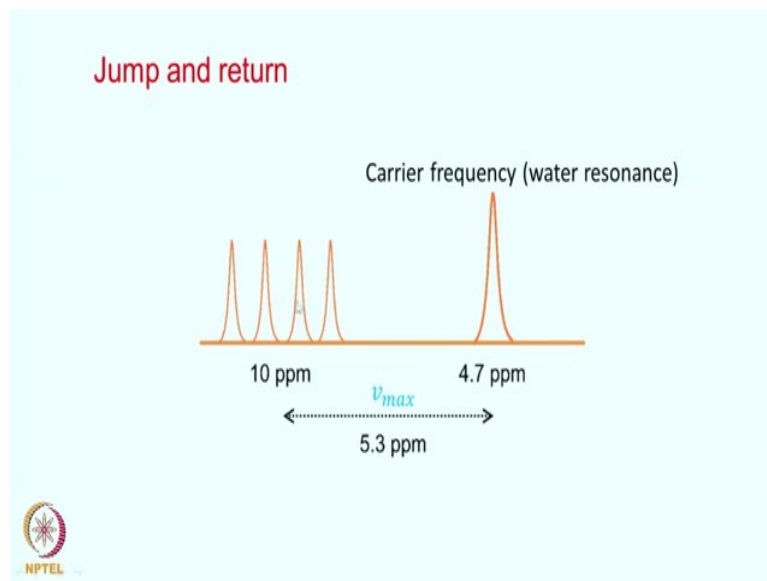
So, here we put the signals which the water is here and we are interested in the signals which are at this ppm at just 5.3 ppm away. Now if you go 5.3 ppm on this side there are the signals from this side as well. So with the respect to this if this frequency are the positive frequencies this will be negative frequencies. So whatever applying to this 5.3 ppm here will also apply to the 5.3 ppm on this side and those will also get excited, how will that show up in your spectrum.

That is I will not show on it here but one can visualize this how it could have happened? What would happen to those? these magnetization the orange has moved this direction during this period tau the other ones which are at negative frequency they would have moved in this direction, they will come like this, and at 5.3ppm they would have aligned along this axis here, the opposite axis here the once which are showed with orange they are here the other would have been here, therefore this two have opposite signs.

Therefore if your Fourier transform this FID which you collect from here the signals which are here will show would with one sign and he signals which are here will show up with the opposite sign therefore in your spectrum finally you will get on a one side of a water signal you will have peaks like this and the other side of the water signal you will have peaks like this, in the center you have the water there are peaks on this side, peaks on this side, as the result of the water suppression and maximizing at 5.3ppm peaks on this side of the water will go like this and the peaks on this side will go like this and that is of course is not a very big

advantage you get maximum signals none the less and one can select only the one portion of the spectrum for your analysis depending upon which one is interested on interested in.

So, there are other techniques for water suppressing we will come back to it later, but before we actually do that we have to consider one other important concept in multiple experiments or in the Fourier transform NMR or various other experiments and that is called as the spin echo.

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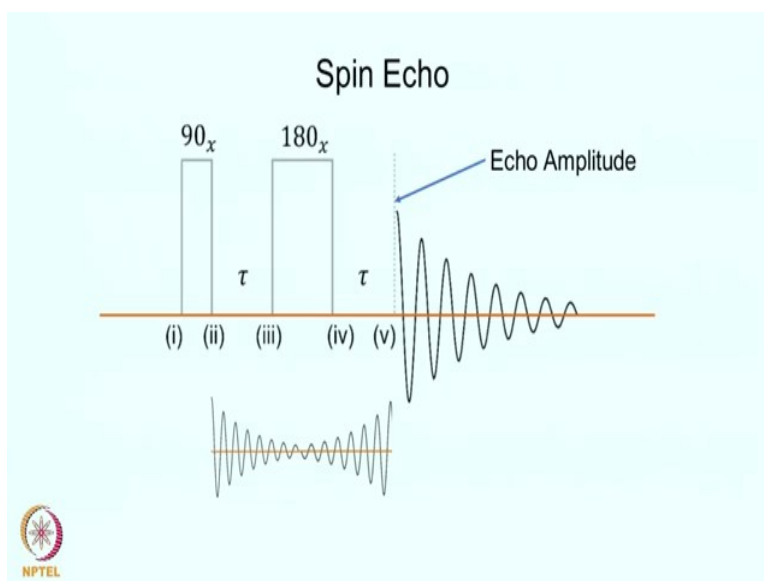
We will now consider another important invention namely the spin echo, this was made by Arvind Han in 1950 and it turned to be such an important technique and that was a period when many many important techniques were important concepts were being developed so this was almost worth the noble prize but however Arvind Han missed that one but we all realized how important this invention was, it now appears in all kinds of multiple experiments.

It is an integral part of many multiple experiments and therefore this is very crucial to understand the principles of this spin echo and see how it can be used in various experiments. It is a very simple experiment technique that it now consists of two pulses a $90°x$ pulse ad $180°$ pulse it could be $90x$ $180y$ or $180x$ or does not matter you apply a $90x$ to a spin system, wait for a period tau then you apply $180x$ pulse to the spin system and wait for a time tau.

It turns out that the signal which is actually decaying here, for the immediately after the $90°$ pulse we have seen there will be an FID there will be free percession and the magetization

decays in the following manner as it is indicated here, it turns out that application of $180x$ pulse reverses this decay of the magnetization and starts building it up.

So, as a result at the end of this period tau which is exactly equal to this tau there will be a refocusing of the entire magnetization and this is called as the echo and the magnetization here is called as the echo amplitude and following that of course it starts decaying once more which is the normal FID and the data is actually collected from here onwards.

You do not actually collect the data during this period we collect the date from here onwards, the important point to note here is this echo amplitude is essentially a recovery of the magnetization which has decayed during the first tau period and the 180 pulses reverses it's decay and you get this echo of the main magnetization. How does it work?

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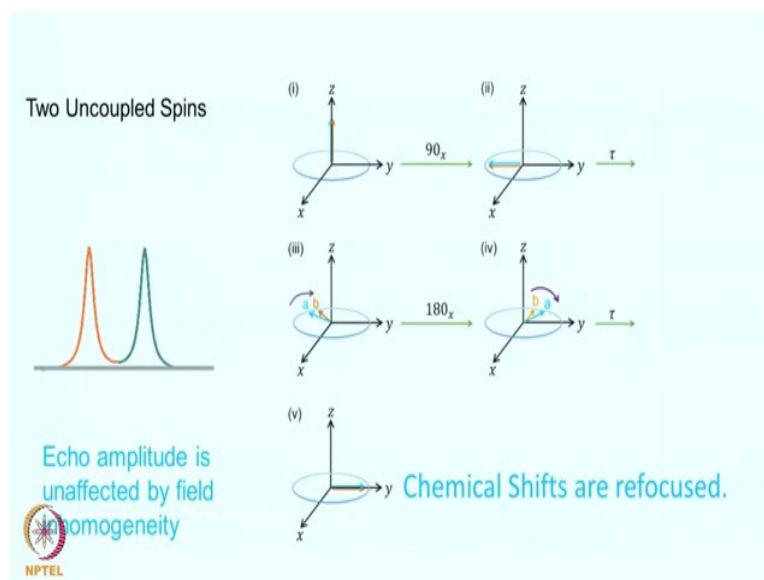
So let us look at that and try and understand how does it works, first let us consider two uncoupled spins and these two have two different frequencies there is one frequency here and there is another frequency here, and initially the magnetization of the two spins is along the Z axis when I apply $90x$ pulse to this magnetization this is rotated into the xy plane comes along the minus Y axis if it is a $90x$ pulse.

Now, during the period τ the two spins will start precessing with the respective frequencies, the one which is here is a higher frequency, which is here the lower frequency therefore this orange one has moved more than this sign 1 therefore this are going in this direction the precessional direction and the B has moved further than A in the transfers plane.

Now we apply 180° pulse to this which is along the X axis now the magnetization rotates out of the plane and comes back here to this position, so the A which is here comes here and the B which is here comes here, the sense of rotation remain the same, so they will continue to move in the same direction therefore B will cover a wider angle than A and eventually after the time tau here they will come back to the same Y axis, so this is called as the refocusing.

So, no matter how much was the difference between the A and B in this motion they will come back to the same position in phase at the end of this period tau and that is called as refocusing. So the chemical shifts we say are refocused because this is the regardless of what the frequency difference between A and B is, essentially this is the chemical shift difference, so therefore the regardless of what this differences are hey are coming back to the same point at the end of this period τ, so 180 pulse actually causes a time reversal and we get the refocusing of the magnetization at the end of the period to 2τ.

Here we consider this two spins are two independent spins or two separate spins, however it can also be that these are different frequencies of the same spin located in different portion of the sample if there is the inhomogeneity in your magnetic field the magnetic field is inhomogeneous one portion of the sample sees one field another portion sees another field and that may result in two different frequencies.

For example water, water at one portion of the sample sees one frequency another portion of the sample sees another frequency and they may be two different frequency because of the inhomogeneity in the magnetic field, but then as a result of the echo they come back to the same position that is what we say that the echo amplitude is unaffected by field inhomogeneity's, this is an important idea important concept and we will see how this will be use later for measurement of the transverse relaxation time.

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Two Coupled spins A,X
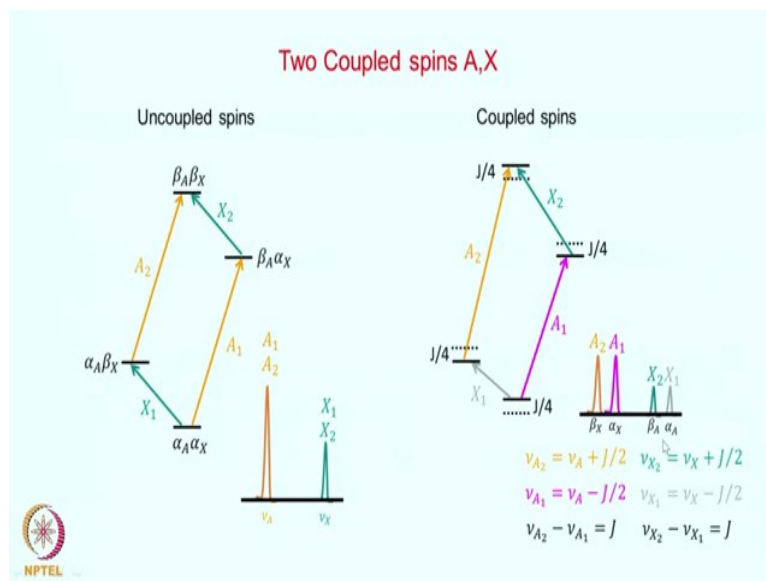
Now let us take two couple spins, we saw in the case of uncoupled spins the chemical shifts were refocused to look at the coupled spins we have to look at the energy level diagram and understand the spins system little bit better, so let us draw the energy level diagram for two spins.

So if there are two spins there are four energy states so these are α_A and α_X α_A, β_X β_A, α_X and β_A β_X. Now if you look at the transition from here to here which spin is flipping it is X spin which is flipping α_X goes to β_X α_A remains the same. So therefore this we call it as a X_1 transition here also it is the same thing α_X spin flips to the β_X transition we call this as X_2.

If you look at the transition between these two levels then here α_A spin flips to β_A and therefore this we call as the A transition and label it as A_1 and this is again the A transition and we label this as A_2 if there is no coupling between the spins then these two energy differences are identical and therefore the A_1 and A_2 frequencies will be identical. Similarly, the X_1 and the X_2 frequency will also be identical.

So if you look at the NMR spectrum of this that there will be one line at the chemical shift on nu A which will have the two transitions A_1 and A_2 likewise for the X spin also there will be two transitions X_1 and X_2 will be degenerate and there will be at the same position which we call it as the chemical shift of X so this is v_X and this is v_A.

Now what happens if the spins are coupled if the spins are coupled these energy levels get changed for example this energy level which was here will now go up in energy by an amount J by 4 this energy level which was here will come down by an amount $\frac{J}{4}$, J is the

coupling constant between the 2 spins, this energy level which was here comes down again by an amount $\frac{J}{4}$ and likewise this energy level which was here will now go up by an amount $\frac{J}{4}$, now what is the consequence of this? now you look at the A_1 transition, the A_1 transition has reduced in energy by an amount $\frac{J}{2}$, $\frac{J}{4}$here and $\frac{J}{4}$here therefore the energy is reduced by $\frac{J}{2}$.

So, we say v_{A1} is reduced to $v_A - \frac{J}{2}$, likewise the A_2 transition which was from here to here has now gone up by the same amount $\frac{J}{2}$., so the $v_{A2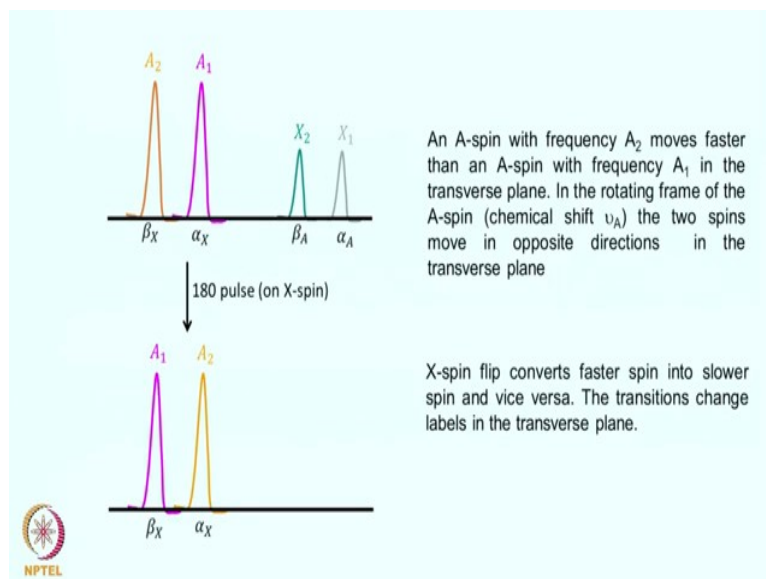}$is now $v_A + \frac{J}{2}$, so A_2 is always taken to be higher frequency compare to the A_1, that is a convention we will label them because we could have label them the other way around as well and therefore the separation between this two transition is J, $v_{A2} - v_{A1} = J$ and this separation is J. Similarly, the X_2 transition has the frequency $v_X + \frac{J}{2}$, and the X_1 transition has the frequency $v_A - \frac{J}{2}$, and $v_{X2} - v_{X1} = J$.

Now notice here one more thing that is a higher frequency A_2 transition has the X spin in the β orientation in the beta polarization state, and the lower frequency which is A_1 which has X spin in the α orientation, sometimes you also called them as faster spin and the slower spin rather if the energy is increasing in this manner we called this as the faster spin faster moving spin and the slower moving spin.

If you sit in the rotating frame of the v_A here for example then this one is too the left this is to the right, this is the higher frequency and this is lower frequency, sometimes you also refer to this as a faster moving spin and this one as a slower moving spin, same applies to this situation as well, for the X_2 transition the A spin is in the beta polarization and for the X_1 transition the A spin in the α polarization, okay what happens next?

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An A-spin with frequency A_2 moves faster than an A-spin with frequency A_1 in the transverse plane. In the rotating frame of the A-spin (chemical shift υ_A) the two spins move in opposite directions in the transverse plane

180 pulse (on X-spin)

X-spin flip converts faster spin into slower spin and vice versa. The transitions change labels in the transverse plane.

In the case of the spin echo experiment we have this 90 pulse then followed by a 180 pulse after the certain evolution period and what is the consequence of that? let us look at that once more here, here you remember from the previous slide the A_2 transition has the beta orientation beta polarization of the X spin and the A_1 transition has the alpha orientation of the X spin.

Now the A spin with the frequency A_2 moves faster than a A spin with the frequency A_1 in the transfers plane, after you apply the 90° pulse the magnetization corresponding to this two transitions are in the transfers plane and as it start precessing in the transfers plane this one moves faster compare to this one, this is what we are trying to say in the rotating frame of the A spin there is a chemical shift v_A that is in the middle here, this is the v_A position, the two spins moves in the opposite direction in the transfers plane, because this in the transfers plane once we put the magnetization in the transfers plane the magnetization corresponding to this two spins move in the opposite directions.

We treat the faster moving spin as moving in the anticlockwise direction and the slower moving one in the clockwise direction, now what happens when they apply 180 pulses on the X spin? X spin when I do I flip the X spin, X spin often goes to the β and β goes to the α, therefore the one which was moving the faster has to become slower, the one which was going slower now becomes faster, so the X spin flip converts the faster spin into a slower spin and vice versa, that means the transition change labels in the transfers plane.

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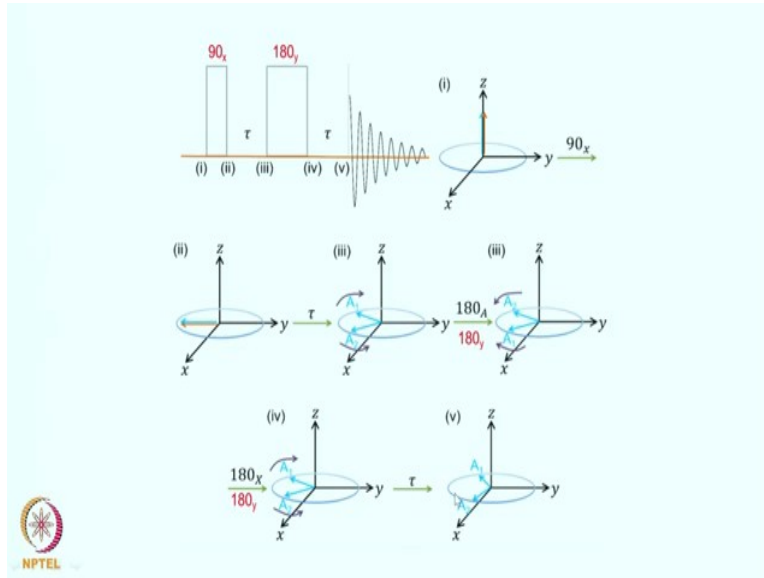
Let us look at that exclusively here, we have the same diagram as before in the case of uncoupled spins except that we are looking at the A spins in the rotating frame of the spin A, so this is initial rotation here and we are looking at the A_1 and the A_2 transitions, until the at this point then the two transitions have moved in the opposite directions, we said the A_2 goes in the anticlockwise direction and A_1 goes in clockwise direction therefore this goes in this manner, this goes in this direction.

If I apply 180y pulse then this one will gets rotated on to this, this one gets rotated here, so A_2 goes here, A_1 comes here and they continue to move in those directions A_2 goes here that will continue to go here and A_1 which one was going like this is moved here and that will move in the opposite direction A_2 goes here A_1 comes here, A_2 goes there and A_1 comes here but the sense of rotation remains the same, after the 180-degree y pulse.

If I apply 180x pulse now, then what happens we set the labels will get change the faster one will become slower one, slower one becomes the faster, so this comes back here, the A_1 comes back to A_2 and this A_2 goes back to A_1 and this will continue to go in this manner, this will continue to go in this manner as before because this is the faster moving transition and then you will see during the next τ period this ones have find out more than before, instead of refocusing they have actually moved apart away from each other as a result of this evolution during the τ period.

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For J-coupled spins, evolution due to J-coupling is not refocused.

Angle between the two lines A1 and A2 at the time of echo = 4πJτ
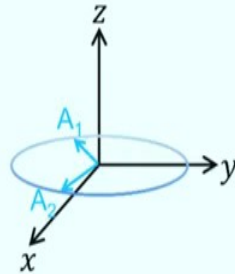
Therefore, what we say is at that for the J couple spins evolutions due to J coupling is not refocused. Angle between the two lines A_1 and A_2 at the time of the echo is given by $4\pi J\tau$, this is easily possible to calculate and because of the separation between the two transition is J and during the time J they covered the angle $J\tau$ and multiply by $J.2\tau$ and multiply by 2π to calculate that in terms of radius.

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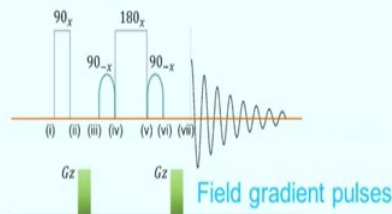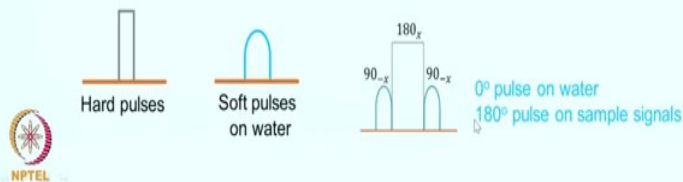
Now, so the spin echo has become an extremely important technique and it is also used for water suppression which we discussed in the previous class and that experiment is called as watergate. To use spin echo for water suppression he pulse sequence for that is given here, you have a $90°x$ pulse which is hard pulse which is applied on all the spins and then you give a certain time τ during which you apply a field gradient pulses.

This is applied for a small period of approximately 1 millisecond also and following that you have a soft pulse on water, soft pulse this this indicates this is the soft pulse on the water which is 90 -*x* on water, 180 pulse this is the hard pulse again, this is applied in all the spins and followed by again a soft pulse on water again 90 -*x* and the carrier is placed on the water therefore the water is at 0 frequency.

Now what is the consequence here of this pulse, let us look at this pulse more explicitly, this is very important pulse, it essentially tells you on water there is effectively no pulse,0 degree pulse on water, 90 -*x*, 90 -*x* this is 180 -*x* and this is 180 *x* together it means there is essentially there is 0 degree pulse on water, whereas hard 180 pulse is applied on your sample signals. How does it help in water suppression?

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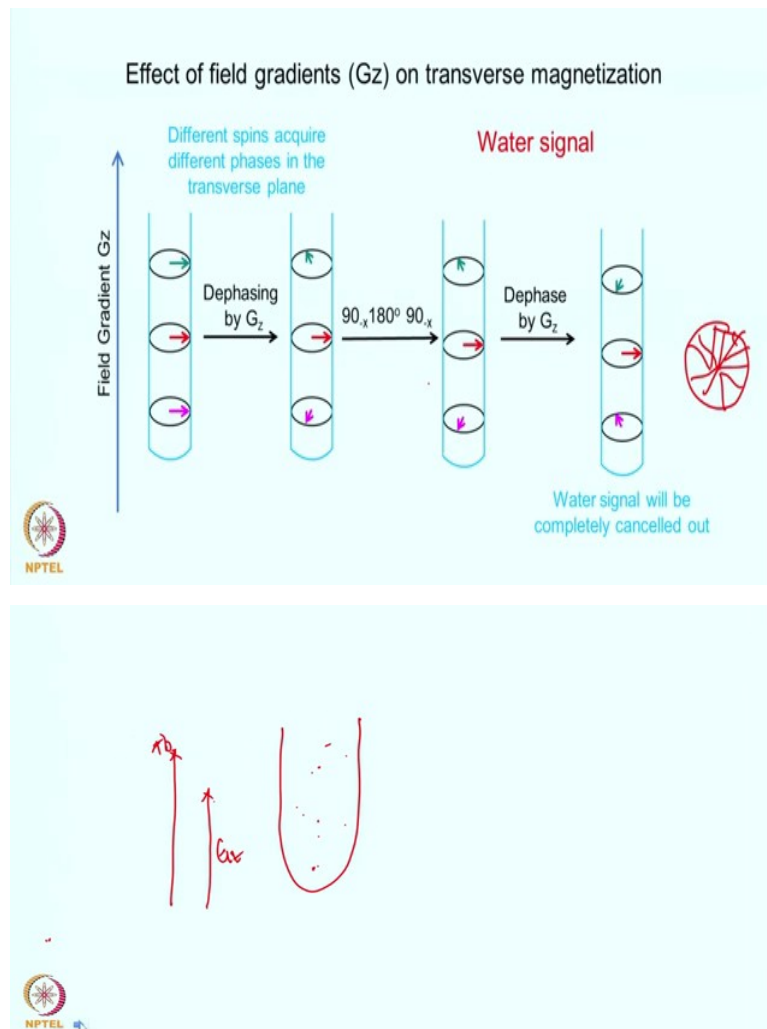
This we will see in this slide, the field gradients as I mentioned, this are the field gradients are applied along the *Z* axis, so if you have the main field H_0 like this, B_0 or H_0 we called it and we apply a field gradient along the same axis called as G_z, so if I have a my sample here

various spins at this point they now see different fields at different positions, for example the water here will see one field, water here will see another field, water here will see another field, likewise the sample spins as well. So they will see different kinds of fields which will manifests itself in their precessional frequencies.

So let us first of all look at how this effects the precession of the sample signals. So to begin with you have three different positions and all of them have the same frequencies the sample frequency is the same, the magnetization is in the transverse plane here oriented in this direction.

Now during the field gradient pulse which is for an approximately 1 millisecond his is something which one is optimized with reference to this we keep this as constant, these two would have moved to different extent because the fields have changed, because the field here and the field there are different, therefore they move by the different extent, let us assume that this moves here with the 180˚ rotation and this has moved in this direction by 120˚ and this is can be simply calculated by the field strength, how much they are moved.

Now if I apply 180˚ pulse to this, what happens? This spin moves over to here and it comes here and this spin moves over to here and it comes here, now I apply another field gradient pulse the same G_z which is identical to this, what it will do it will continue to introduce a phase angle to this they will continue to move it because of the field change and this will move to the same extent as it moved here from here to here it will come back to this position.

Similarly, this one which none was moving in this direction it was moving in this direction here it will continue to move like this and it will come back to the same position, this is our reference this has remain the same with respect to this all have come back to the same place, so the sample signals has refocused because you remember we are applying 180 pulse on the sample signals, the other two pulses were soft pulses they were not applied on the sample signals, they are only applied on to the water.

So, therefore the sample signals have refocused here at the end of the water gate sequence, so they are all in phase therefore you produce a good signal from the sample, what happens to the water?

Until the point it is the same, this are the water positions here now it is a water signal it is not the sample signal this is the water signal, here I have applied a 0 degree pulse, 90 -x, 180, 90

-x was applied a 0 degree pulse, essentially no pulse, so at the end of this will stay here only and this will stay here only.

So, when I apply the next gradient pulse G_z, so it will called further dephasing f this magnetization so this one which has moved here it will continue to move like this and it will come here to the exactly it will make the same amount of rotation it will come here and this will make a same amount of rotation and it will come here, so therefore this are not refocused to now and we have taken few particular position here and the illustrations.

So, typically what happens there are spins all over the place and none of them will actually refocused therefore you will get the end of this, if you consider as water signals from all over the place then you will have in the transverse plane I have water signals here, here, here, here, here, here, here everywhere there is a water signal and therefore they all cancel each other and that is why we say the water signal will completely cancelled out as the result of this pulse sequence, initially it causes the dephasing here and we don't apply any pulse to the water and apply gradient pulse here is called is further dephasing therefore when you consider an ensemble of the water signal from all over the place there will be complete cancellation of the transverse magnetization due to the water.

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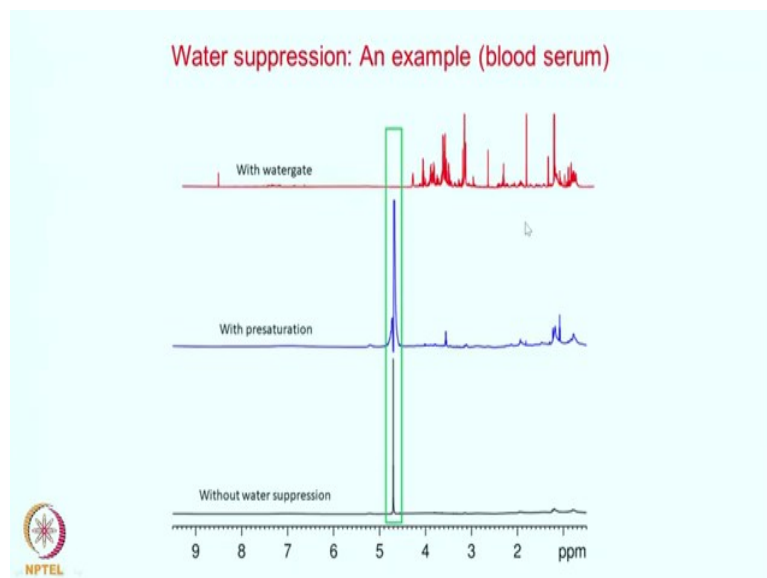
So this is what you wanted to achieve and that is what is happened here and we see the result in the experimental spectrum, so this is the result without water separation, see this is huge signal and nothing when other things cannot be seen at all and here water separation with pre saturation this is what we discussed in the very beginning and you do pre saturation, saturate

the water here and then you reduce the into certain extent alright and you start seeing some signals which are present here.

Now you see with a watergate you will see so many more signals and you are getting all the sample signals with good intensities in this experimental example, this is of a blood serum and you have so many signals here some with higher intensity some with small intensity and all of them can be clearly seen and another important point to note here is in a case of water pre-saturation the signals which are under the water will also get saturated and we will lose them.

In this case that is unlikely it will happen and it is only the water that is diphase and the other signals are not diphase and therefore you will get all the you will recover all these signals. With that we stop here and we will continue the discussion on multiple experiments in the next classes.