Advanced NMR Techniques in Solution and Solid-State

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Module-35
Measurement of T1 and T2
Lecture – 35

Welcome back all of you. In the last class we discussed a lot about relaxation; in fact last couple of class I would say we discussed a lot about relaxation phenomena. We understood what is spin lattice relaxation, we understood what is spin-spin relaxation and we knew that spin lattice relaxation is the exchange of energy or transfer of energy from spins to the lattice, spin-spin interaction is an exchange phenomena between two spins and the decoherence in the XY plane. And also we understood the spin lattice relaxation is the growth along Z axis the magnetization growth, spin-spin relaxation is decaying the XY plane. For all these things to happen there are various phenomenon and there must be some local fields generated at the site of the nucleus. These local field comes because of various motions. It could be diffusion or it could be chaotic motion, tumbling motion like that; and this motional frequency we can express in terms of spectral density function and that must be at the Larmor frequency only; then we can see that the spins gives energy to the lattice; of course same thing for the spin-spin relaxation but no energy is given to lattice, also we discussed there must be local field and then it also depends upon the correlation time the J(W); we discussed that.

And correlation time in turn depends upon the size of the molecule; and what is the correlation time we discussed, it is a rotation of the molecule by one radian per second. We also understood what is a correlation time and it is generally of the order of nanosecond. We have brought some conditions like omega 0 resonating frequency into rate tau c, if it is very much greater than 1; what happens it is equal to 1, what happens in intermediate case and when it is very much less than what happens. And how does the spectral density function varies; we have solved all those things; we understood quite a bit. Finally, the conclusion was the relaxation is a radiationless transition, it does not create any magnetization in the XY plane for detection. The transitions of the spins from upper state to lower state as some of the spins from the lattice is going from lower to higher; all these are happening.

But they are radiationless transitions. So, all these things we understood quite a bit. And as far as the spin lattice relaxation is concerned there are various mechanisms which aids for the

spins to give energy to the lattice like dipole-dipole interaction, chemical shift anisotropy, spin rotation and quadrupolar relaxation; various phenomenon we saw and we also understood when there is a quadrupole relaxation.

Sometimes it so happens some lines may get very broad also because the resident time of the spins in the upper state or excited state is very small time. It relaxes very fast, that is because of Heisenberg's uncertainty principle which we discuss in the earlier class or even in the previous course also; those who have attended fine they would have understood that.

So, nevertheless in some examples where the quadrupole coupling constitutes much more smaller, quadrupole moment is much smaller in some nuclei like deuterium or NH4 we saw the example, or BF4 where there is short distribution more like spherical; then it so happens we could see the coupling of quadrupolar spin half nuclei like CDCl3; we have got three lines of equal intensity for the carbon 13 because of coupling of deuterium which has spin one.

Same thing for BF4 and also for NH4 we saw those things. We understood a lot about various phenomenon and everything; Of course the concept of relaxation is fairly a tough topic. We tried our best to understand and I tried to make it as simple as possible so that the concept should be understood clearly; but fine we also understood after that we wanted to know how to measure the relaxation time, that is important.

Now we came to the real measurement of spin lattice relaxation time. There are several methods I have mentioned what is called as inversion recovery, progressive saturation, saturation recovery varieties of methods are there; or null method like that. But the most popular method in solution state is the inversion recovery method that is what we started discussing.

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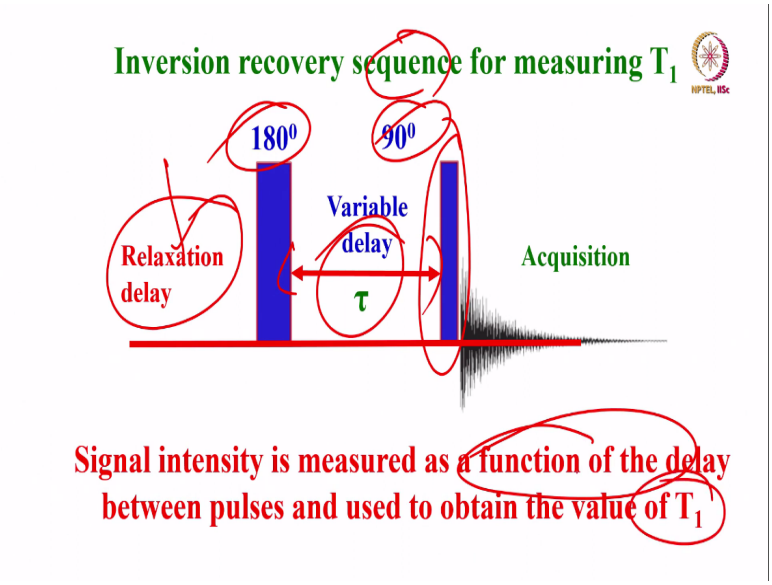

So, now we will come back to the inversion recovery method today I already showed this pulse sequence in the last class. It is a simple two pulse sequence start with the 180 degree pulse and then give a delayed tau and then apply a 90 degree pulse; afterwards start collecting the signal. And before that apply 180 pulse ensure that spins attain thermal equilibrium; enormous relaxation delay should be given so that spin should be in thermal equilibrium for the magnetization in this place.

And then you apply 180 pulse what is going to happen? we already know what happens to 180 pulse; the magnetization will be tilted from Z axis to – Z axis that is fine; from here it comes to – Z axis, but then with a delay it will start going back to the Z axis. Now you vary this delay then it take some time for the magnetization to go back to Z axis as a function of different time delay, we want to measure the signal.

For the detection of the signal we apply another 90 degree pulse after the tau delay, and collect the signal and start doing the Fourier transformation; and measure the signal intensity. This is the simplest experiment that one can do for the inversion recovery; that is commonly practiced. So, signal intensity is measured as a function of the delay between the pulses used to obtain a value of T1.

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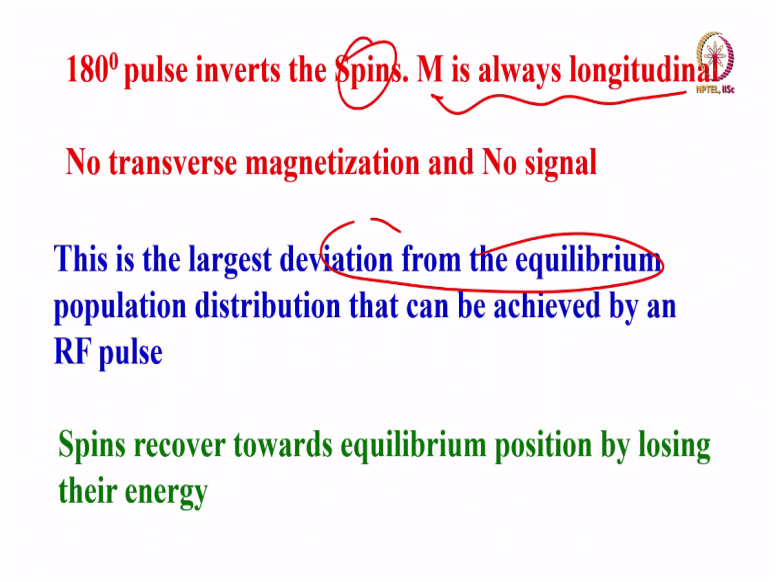

Now what does 180 pulse do? As I told you it inverts the spins and magnetization is always along longitudinal axis along Z axis and there is no transverse magnetization and no signal when 180 pulse inverts this and this is a largest deviation from the equilibrium population distribution that can be achieved by RF pulse. Anything less than that is only like 90 pulse etcetera there will be a magnetization in the transverse plane.

The deviation from the equilibrium is not much. Deviation from the equilibrium is maximum with a 180 pulse. This is the new information which I wanted to tell you. So, now what will happen, the spins recover towards equilibrium position by lowering their energy. This is what we wanted to, how it loses the energy, we also discussed.

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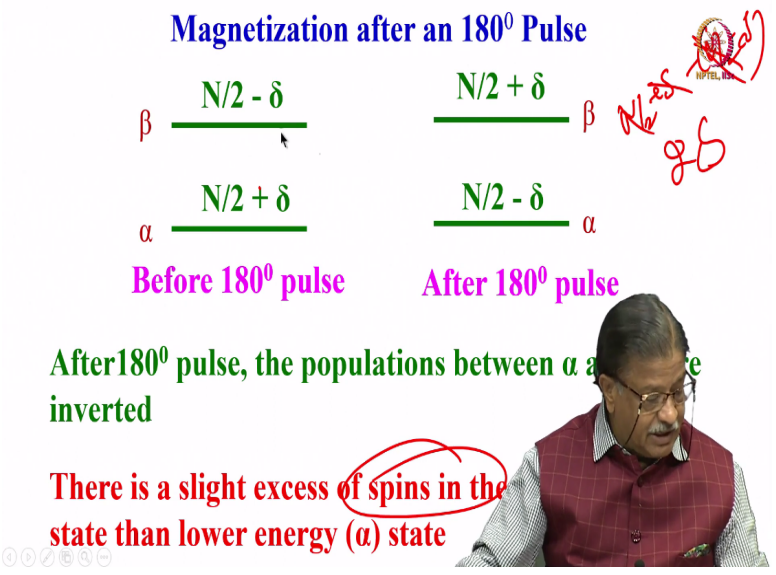

Let us see diagrammatically what is happening. Let us look at magnetization after 180 degree pulse. Now consider a situation like this, I have alpha state and beta state; there are more spins in the alpha state than in beta state; some number we have chosen; you do not have to worry about the accuracy of this number; just for calculation purpose; I will say because N is a total number of spin populations.

I divide by N by 2 here; and add some number which is more and here that much number is less here that is all. So, there is a population difference between alpha and beta states this is before 180 pulse. Now, we will apply 180 pulse when I apply 180 pulse what is going to happen? the population gets inverted; see beta state is here, alpha state is here. Now N + delta comes here; and N – delta comes here; population gets inverted.

This is the interesting thing which is going to happen. So, population gets inverted and there is excess of spin population in the upper energy state now. This is quite unusual, because normally we know population will be excess in the lower energy state. Now because this is 180 degree pulse alpha and beta spin state population got interchanged, now we have more spins, excess spins in the upper energy state; or in the beta state. That is the situation.

Now we have to understand what is population difference delta P. I already told you population difference is always obtained as the population difference from lower energy state to higher energy state. In this case for example if you want to consider what is the population difference you will see that the population difference is N / 2 + delta – of N / 2 – delta. So, this gets cancelled out and it will become 2 delta. So, delta P is 2 delta after 180 pulse that is a thing, which you should know.

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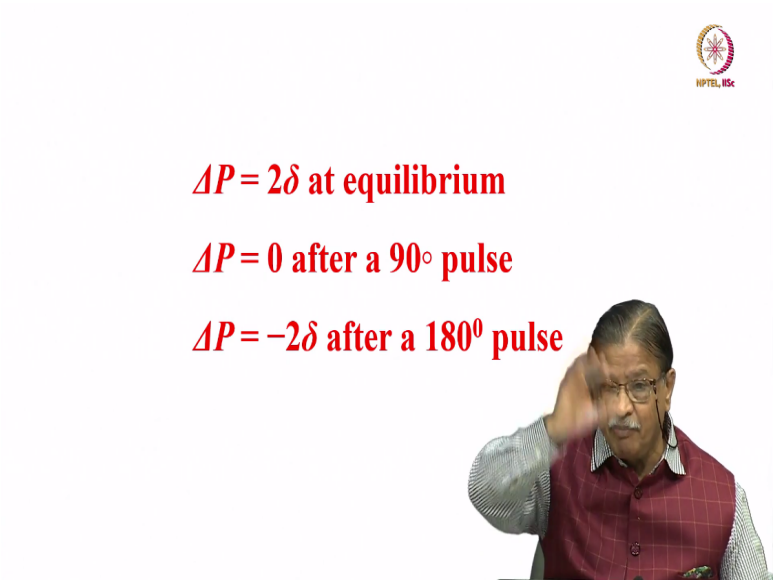

Delta P will be 2 delta before 180 pulse. Now after a 90 degree pulse what happens delta P = 0 exactly because if you remember apply a 90 degree pulse is going to saturating the spin states. You are equalizing the population between two energy states so it is delta P = 0, but 180 pulse makes this delta P = - 2 delta that is what we have to understand.

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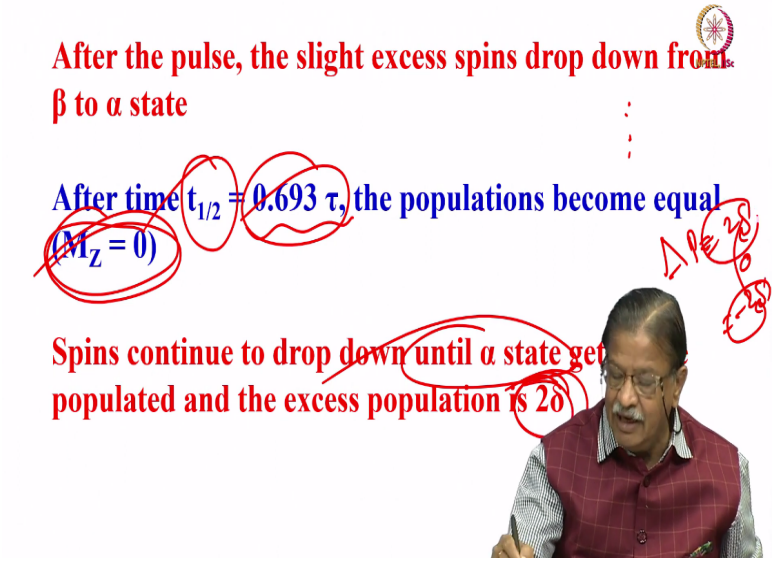

Now after the pulse there is a slight excess spins and these excess spins in the beta state cannot stay in the excited state for a long time, it has to come down. The spins which are in the excited states start dropping down slowly like balls falling down from the upper state. It starts falling down this is called spin drop. Spin drops from beta to alpha state and remember it does not create any rotation, it does not create precession, it does not create any magnetization.

From beta state to alpha state spin simply starts dropping down; that is what it is going to happen. At time now T half which is equal to 0.693 of tau, then you can see that from the equation Mz = M0 into 1 – e to the power of – t / T1 the population will become equal and Mz = 0; that is the situation, that is a null point. At exactly half of this period tau then 0.693 of this thing if you take the population become equal of the two states and it is Mz = 0.

But from higher energy state spin started coming down; after sometime population difference became 0, but that is not enough; it has to come to thermal equilibrium. In the thermal equilibrium there was excess population in the alpha state. So it continues to drop further until alpha state gets more populated and excess population again becomes 2 delta; that is what I said in the previous slide.

Before 180 pulse it is delta P = 2 delta; and then 0; and after 180, I said – 2 delta. Now from – 2 delta it has to come back to + 2 delta it goes through 0 and then come back to 2 delta and it comes to original state of thermal equilibrium; where the spin population will be excess in the alpha state and difference in the spin population is now again + 2 delta; that is what happens.

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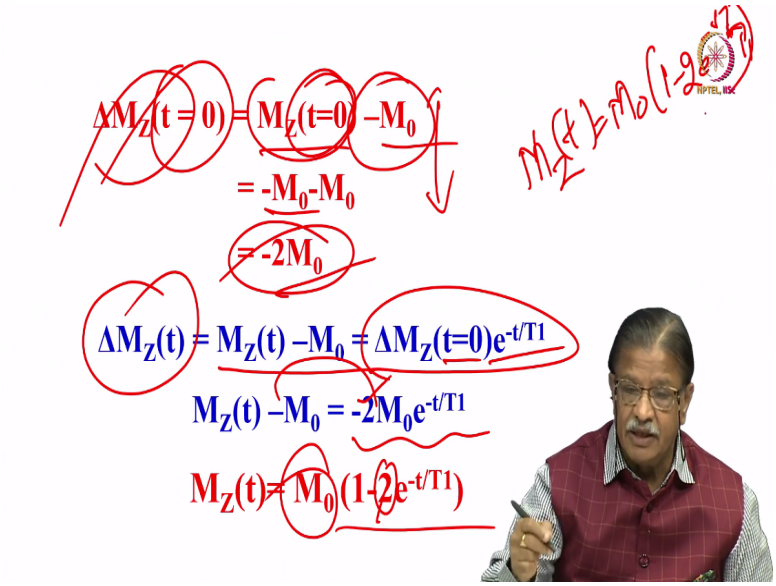

And you can work out; simple mathematics delta Mz at t = 0 is equal to Mz at t = 0, which is – of M0; that is a simple equation you know that; Mz at t = 0 difference between population; magnetization difference at t = 0 is magnetization t = 0 – M0, that is equilibrium population. This Mz at t = 0 is nothing, but M0; you substitute and it becomes – 2 M0. Now continue further delta Mz to t = Mz of t – M0; that is what I said.

Now delta Mz this is already from the previous equation you know, which at t = 0 is e to the power of – t / T1. We are in this equation; now M z of t – M0; it will become – of 2 M0 e to the power of – t /T1 because we know what is Mz of t here. We can substitute that with – 2 M0; so it will become – 2 M0 e to the power – t / T1. All I did is simple substitution, little arithmetic from our previous knowledge which we discussed here.

So, what if we do, if we rearrange these terms; now Mz t becomes M0; we bring it this side and take the M0 as a common factor outside. It will be equal to M0 into 1 – e to the power of – 2 into e to the power of – t / T1. This is the equation which we have been discussing and of course 2 was not there earlier and just for the spin lattice relaxation I said without 2 Mz between M0 into 1 – e to the power of – t / T1; I said. Now the 2 has come because of this experimental method what we are adopting, 180 tau 90 sequence for inversion recovery.

So, this is a common equation; this thing for inversion recovery Mz = M0 into 1 – 2 to the power of e to the power of – t / T1. This is the important equation, we remember.

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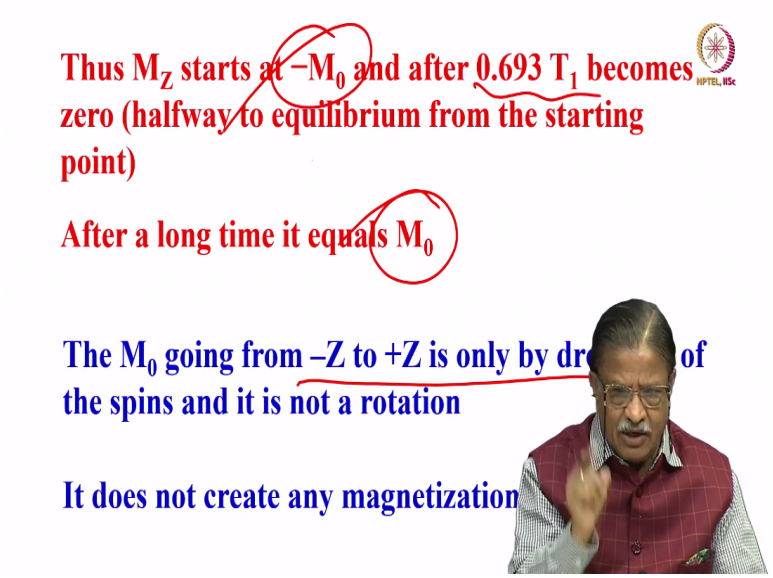

So, what we do is Mz starts at – M0 and that 0.693 T1 becomes 0; half way through that equilibrium; and after a long time it will become M0; that is the process. So, Mz going from – Z to + Z is only by dropping of the spins. I told you it is not a rotation it is not a precession and there is no magnetization created because of this relaxation. I have been telling you, it is a radiationless transition.

There is no magnetization created, you cannot detect any signal it is only a spin drop phenomena which is going on from – Z to + Z it goes by dropping the spins which goes through 0.

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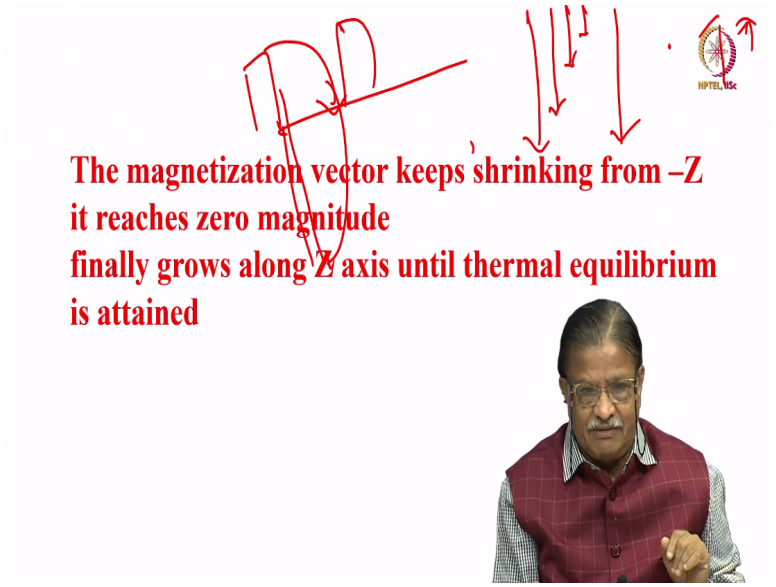

So, the magnetization vector what happens? initially it will be very large along – Z axis. So, let us say we apply 180 pulse in the magnetization this thing, and then apply a 90 pulse and when it bring to magnetization to- Z axis, it starts going back. Slowly let us say – Z axis this is magnetization vector. Next, after the time delay this is the inteisty, let us say increase delay, it is little bit less, little bit less, little bit less, you started growing and then go through 0 and they start going positive this is what happens.

The size of the magnetization vector keeps shrinking from – Z reaches 0 magnitude and then start going towards the positive Z axis; up to what; till that reaches thermal equilibrium; this is going on. This is important thing which happens, we will see that later.

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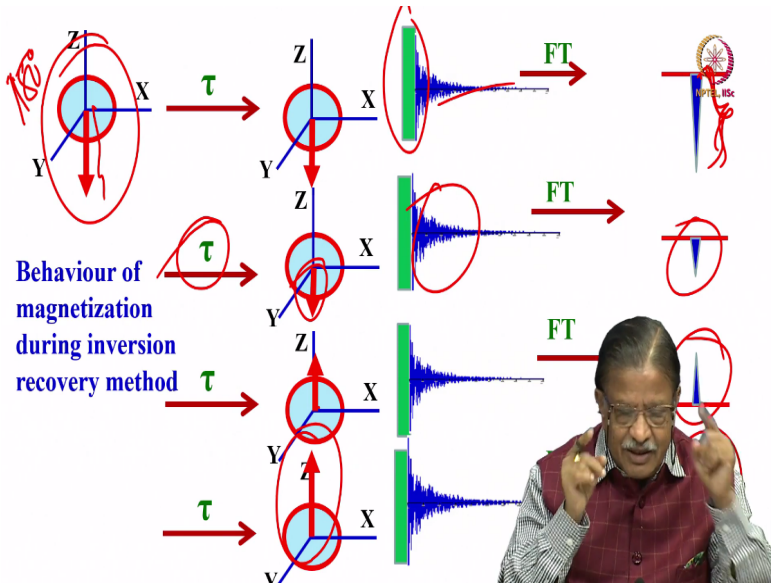

And this is experimentally what has been done; and this is what we are seeing. Now, with a 180 pulse I have brought the magnetization to – Z axis from + Z; I have applied 180 pulse here already. Give some delay; now with some delay magnetization length reduced, it started shrinking and then what you do, apply 90 pulse and collect the signal and magnetization still not reachdc the XY plane, which is still in the negative Z axis; slowly it is coming up.

So, intensity is negative like this and keeps on increasing with the tau delay. Now you have given enough time for the spins to come back to Z axis. It has not fully come back but slowly coming now the intensity reduced here. Now collect the signal now this intensity reduced now it went towards the positive and signal became positive and after some time enormous tau value I have given, sufficiently long delay between 180 pulse and 90 pulse. Then what happens the magnetization is completely recovered to what it was along the Z axis; before the application of 90 pulse. This is the phenomena what is happening. Magnetization initially we bring it to – Z it starts shrinking as a function of time slowly and then go back to Z axis. This is happening because of spin dropping, dropping of the spins and that does not create any rotation.

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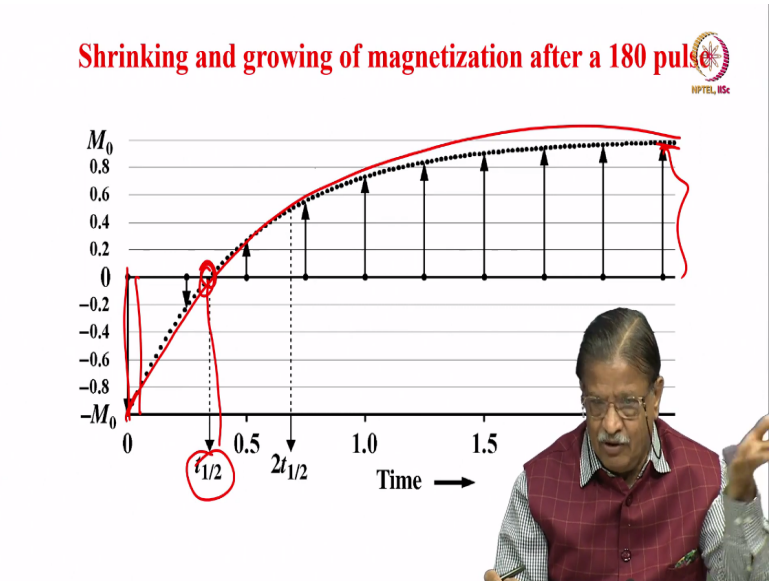

This is what you understand, magnetization is less here, less here, less here whatever I showed in the stick plot, it exactly if you plot it goes like this; starts from negative slowly starts going and finally it reaches the maximum. And that is thermal equilibrium; beyond that it would not increase. There is a limit and this should become equal to this, then it has come back to thermal equilibrium.

But there is a point here at this point it is tau half; that is the null point; I think that is the point I told you 0.693 times T1; you will get the T1 value also.

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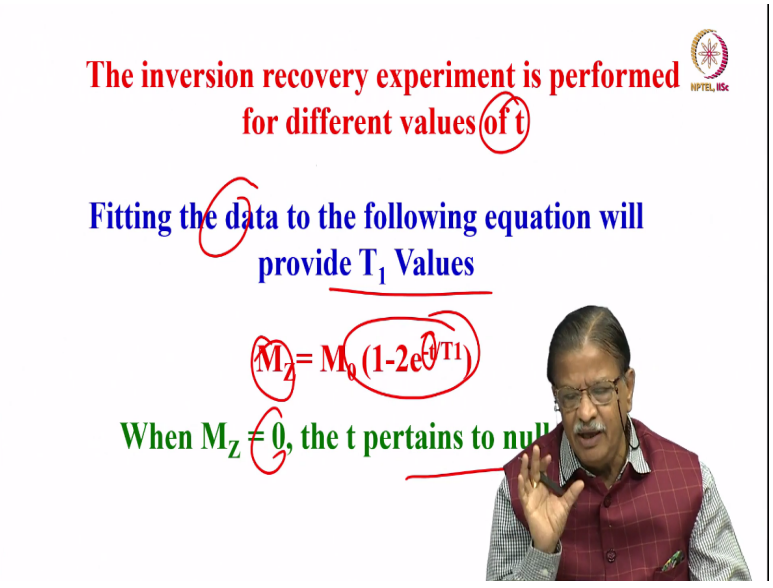

So, the inversion recovery experiment is performed for different values of tau or T delay between the 180 and 90 pulse; the delay you have to keep on varying. Every time when we vary and apply 90 pulse collect the signal; measure the intensity of the signal when any of the

peaks or all the peaks which are interested to get T1 values and make a plot, plot of tau values as a function of intensity of the signal; and then do the curve fitting of that for the equation which is provided for T1 values.

What is that equation? Mz = M0 into 1 – 2 into e to the power of 2 into – t / T1; we have to do the curve fit this intensity value what you get for different tau values to this equation, and for Mz = 0 we get a null signal; no problem do the fitting for all the intensity values you get; that is the Mz for different t values; and when you do curve fitting for each signal whatever may be number of peaks in the sample, does not matter. For each individual peaks you get the corresponding T1 value just by doing curve fitting of this.

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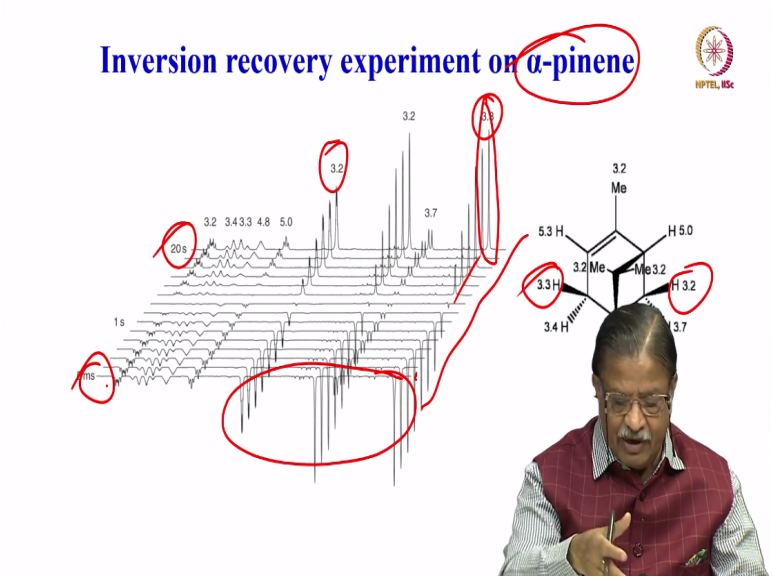

This is an example to show inversion recovery of a molecule alpha pinene. You see that now at 3.3 there is a peak which is coming here; at 3.2 is this proton and see. Now what is happening as a function of this one; as delay is kept on varying from 5 millisecond, 1 second finally go up to 20 seconds, that is a large value. Initially 5 millisecond between 180 and 90 then all the signals are negative, inverted and then starts growing backwards; spin dropping starts, this signal has started growing towards Z axis.

And after sufficiently long time it reaches to original value. This is what the experiment looks like many number of points you have to take, depending upon how accurate you want the values depending upon the time available for you, you can take as many points as you like.

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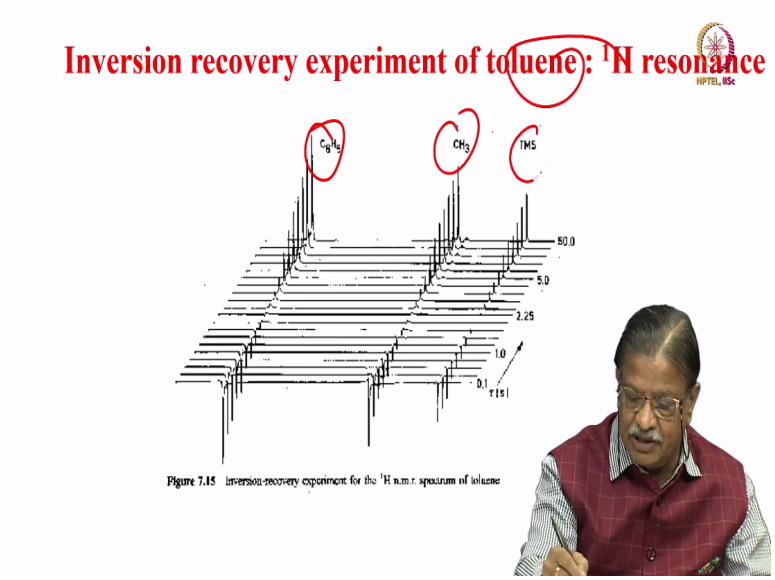

So, this is what is happening for another molecule like toluene; three different protons are there aromic proton, CH3 proton and of course this is for TMS reference.

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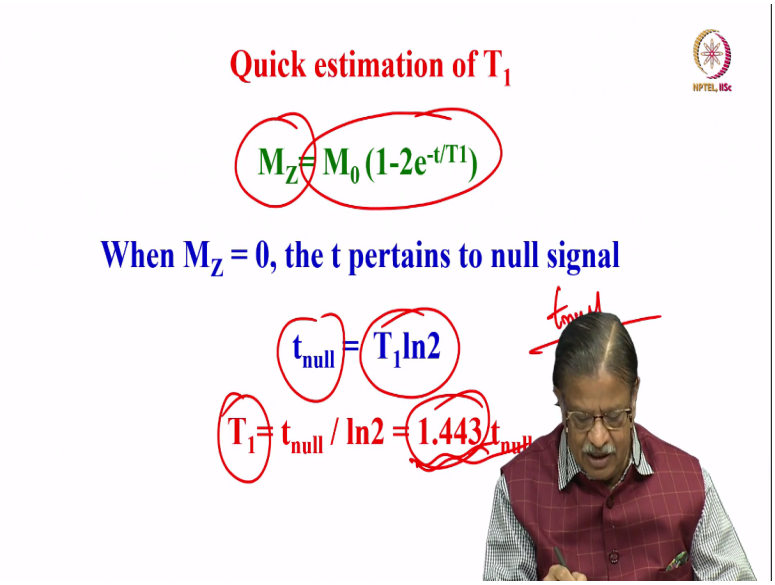

So, a quick estimation; you can do this experiment; it is a time consuming experiment. You have to give five times the delay between each acquisition. Remember you have to make sure that delay is sufficiently longer so that spins come back to thermal equilibrium. So, it is really a time consuming experiment; you have to do for lot of tau values and do the curve fitting.

Now you may ask me a question is there any way I can quickly estimate the T1 value? is it possible? of course it is possible; simply look at this equation Mz = M0 into 1 – 2 into e to the power of – t /T1; this is equation. Now Mz = 0 we put it then rearrange the terms; then tau

null when it is 0, this will become T1 into ln of 2. We have to put simplly natural logarithm value of this, which you know that is lan of 2 is 1.443.

So, T1 value we can get. It is nothing but the delay that is used for getting the null signal; nullifying the signal divided by ln of 2, the ln of 2 is 1.443. So, then you can find out what is T1 value very easily. So, it is a rough estimate, of course, fairly accurate. So, if you want to get more precise values you can do the curve fitting and get the accurate value, but it is a quick estimate of T1 value for any peak if you go through the point at which magnetization is 0 in the inversion recovery; the null point gives you quick estimate of the T1.

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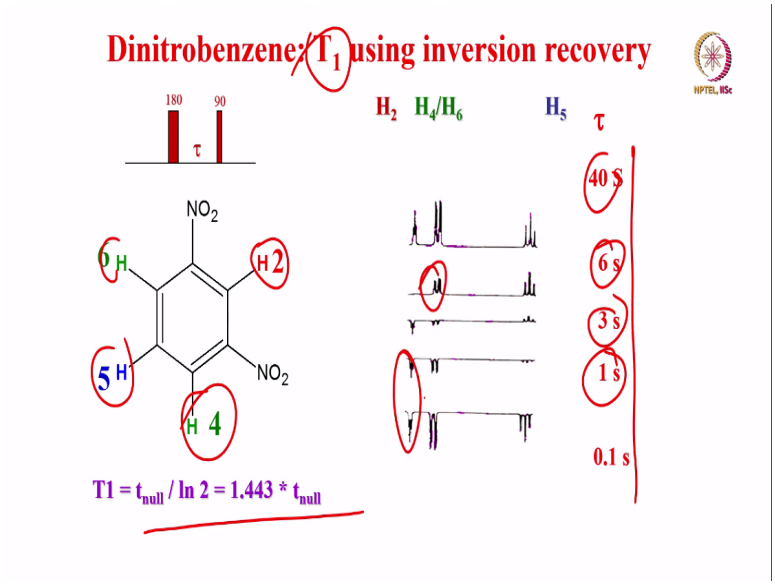

This is what it is for the different molecule T1 inversion recovery you can get for different spins in the sample. If you have different spins you can find out what is T1 value of this proton, this proton, this proton, this proton; each proton individually you can calculate. And as you can see different protons can have a different T2; you look at this one it is already recovered, but this has not.

So, different peaks, different spins of the sample may have a different T1 values. You can use that to measure it. There is no point in going further; now this is a null point which I wanted to say for example this one you can get the precise value there.

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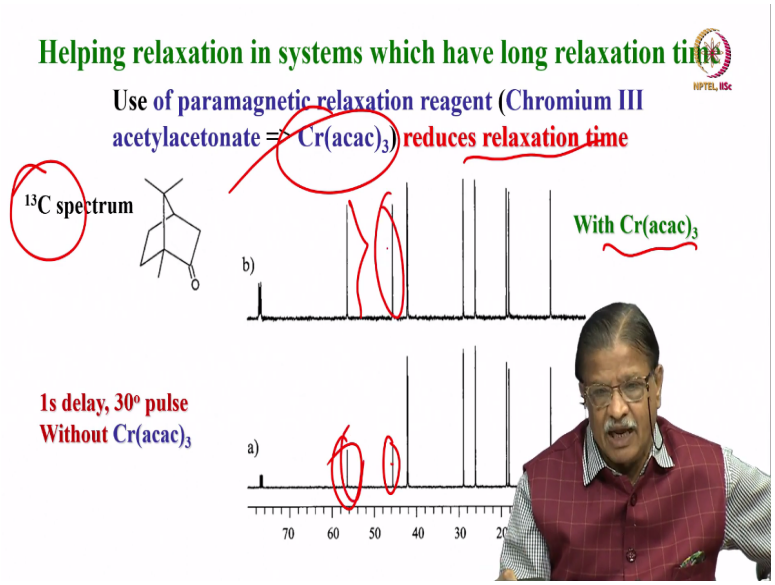

Now I was telling you something about relaxing agents to help this spins relax faster. Let us say in this system we have many proton which has a very, very long relaxation. If I want to do the inversion recovery time experiment it will take lot of time for me especially if you look at the carbon 13, we have long relaxation time; is much longer than protons. So, there are certain paramagnetic relaxation agents.

I told you one of them is a Cr acac is a Chromium acetylacetonate, if we add microgram quantity a very, very quantity of that it helps in reducing the relaxation and then as a consequence you can see you will get the signal much, much faster. Look at this one; this is much smaller look at this one see with the Cr acac the relaxations have become faster it relaxes so fast, see these two signals which are really weak in intensity here without Cr acac has picked up here with Cr acac; this is where signal intensity keeps building up.

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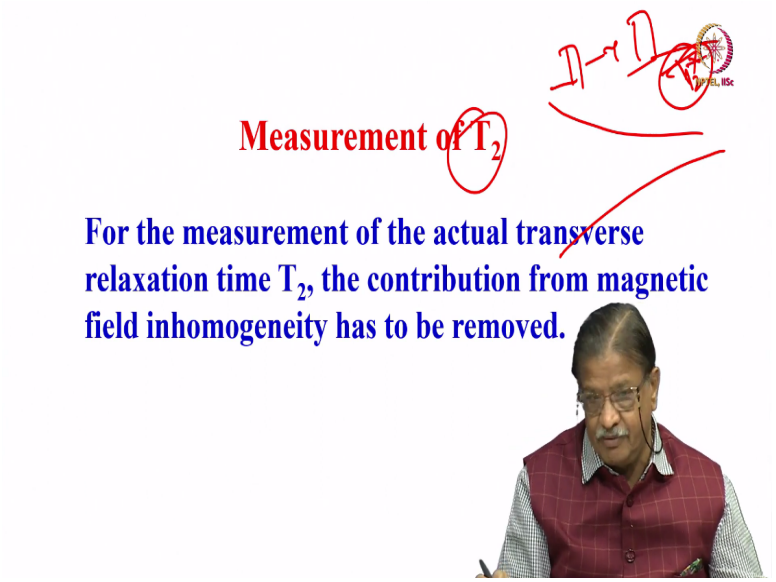

So, this is what basically the rough estimate rough idea I wanted to give you about how to measure the T1 with inversion recovery sequence. When the T1 is very, very large you have certain other experiments like progressive saturation or saturation recovery. First saturated signal and see how much time it takes to recover as a function of time; and then I can do the curve fitting and may get the T1 value. There are various other experiment which are now touching upon, because inversion recovery the one which is very important I discussed a lot. So, actually measurement of T1 we always use this.

And now with T2 we have to do this thing; that is a different method. Now T1 we understood how we will you do this T2 measurement? actual T2 we have to measure, we discussed about the spin echo, we apply instead of 180, 90 it is tau 90, tau 180 we can use spin echo sequence.

So, what is going to happen is we saw that as the echo starts decaying; for a natural decay, natural decay, it is T2. But we also say, rarely we get the actual T2 value because there are other contributions because of the inhomogeneity of the magnetic field etcetera. So, what we are going to measure is the T2*. To get the actual T2 we have to remove the homogeneity contribution of the magnetic field.

We discussed all those things at a stretch. So, we have to measure the T2 and then we have to ensure that inhomogeneity contribution of the magnetic field is removed.

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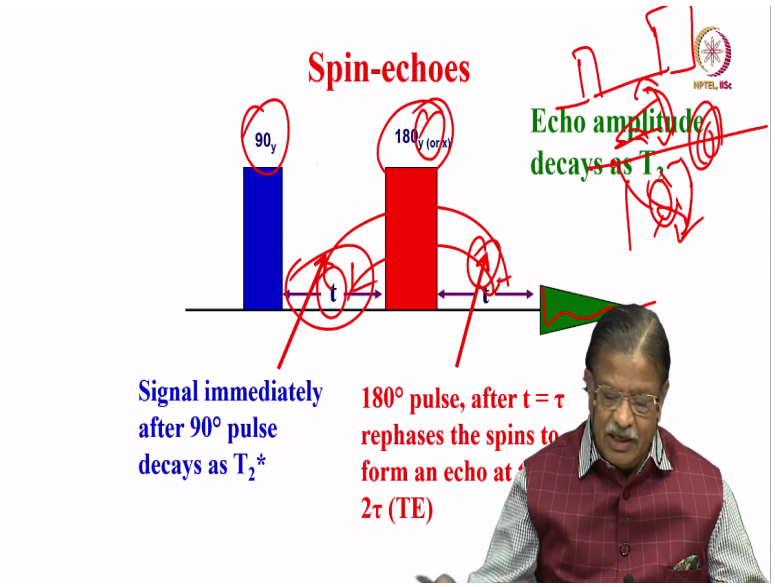

So, what we use is spin echo sequence. The spin echo sequence as I told you is first 90 then tau 180. It could be on X or Y axis; it does not matter, give the identical delay here and then start collecting the signal, and do the Fourier transformation. Very interesting thing happens; during this time singal immediately 90 degree pulse decays according to T2* because inhomogeneity contribution is already present.

And then after 180 pulse here and after certain time exactly equal to this t which is equal to tau the spin starts rephasing this is what is called spin echo. We discussed this in the previous course. I do not know whether in this course I did discuss or not, but remember this is the echo sequence. Echo is something very interesting; apply a 90 pulse here and then what happens? the spin start de-phasing.

After applying 180 pulse depending upon whether you apply along X axis or Y axis it could rotate the spins, you can make the spins by 90 degree then what happen this spins which let us say are the fast moving component, these are slow moving components. If you start rotating, inverting that, then again they start moving in the opposite directions and again refocus along a particular axis.

It can be X axis or Y axis depending upon where you are going to apply pulse; X or Y. So, spins exactly after the identical time given it rephases and forms an echo. This is called spin echo it forms an echo. So, echo amplitude now what happens, start decaying as a function of time; this is what is going to happen. So, this is echo amplitude then echo amplitude starts decaying and that decay again you have to fit into the equation.

What is that equation e to the power of – t / T2 we discussed that. So, you have to do the curve fitting similar to what you did for T1 measurement.

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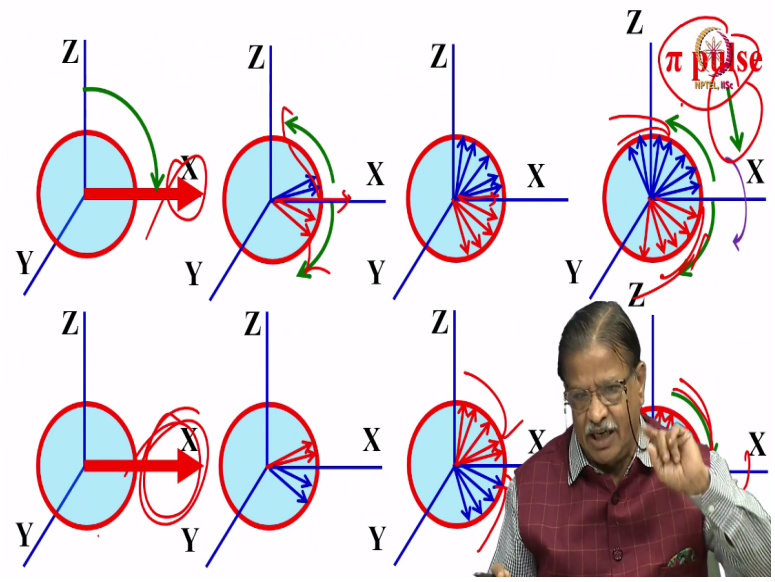

So, pictorially what is happening is shown here. See what happens as soon as we apply 90 degree pulse the magnetization is brought to X axis and then spins start de-phasing. Some spins go in this direction some goes in this direction; which we call as fast moving components and slow moving components; and then give some time the de-phasing keeps on going.

And if you give enormous amount of time there will be complete de-phasing and there is no magnetization at all in the XY plane. This is a totally zero signal ;now it is a maximum signal here, I did not write here signal it comes down comes down here like that. The vector addition of these components which are present here, if you take vector addition the total magnetization start decreasing here, it starts shrinking here.

So, finally it will become 0, but we will not go for that; somewhere when we are de-phasing like this, half way through what we do is we apply a pi pulse here; what does pi pulse do? Pi pulse will rotate these things along this axis; it makes the spin to rotate by 180 degree then what happens; these red spins which are moving like this will go like this.

And then blue spins which are moving like this will come here and then what is going to happen they start moving in the opposite direction. The spins which are moving like this now start moving in the opposite direction and you have inverted the spins in the X axis, continue

like that now they start coming backwards. So, they were actually dispersing, they were going outside. Now they started coming backwards like this; and finally after some time again they will come back to the same place where they started. It is a spin echo; it is like telling like, make 10 people standing in the row, ask them to run initially, they keep on running, different people have different running speeds and after some time l will ask them to stop where they are, let them turn back visually. Same amount of time if you give you them maintain the same speed all of them will come back to the starting point, exactly like that similar analogy, the spins whihc started de-phasing will all come back and start rephasing along the X axis to the same original state. This is what is going to happen; this is a spin echo.

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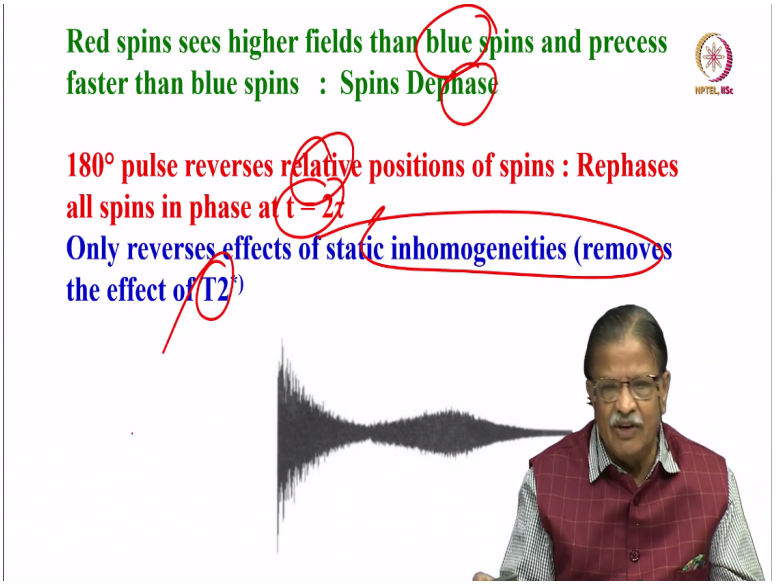

So red spins which were higher field than blue spins this is as faster and they dephase also faster; 180 pulse started reversing then they started going in the opposite direction and then came back and became echo. So, only t delay in the spin echo reverses the effect of strict static inhomogeneity; if they are decaying that is fine, but what is happening in homogeneity contribution that gets reversed because reversing effects get reversed inhomogeneity contribution will be removed. So, effectively effect of T2* when we remove you can measure actual T2 with this.

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During the echo time period, there is some loss of phase coherence by natural transverse relaxation

This is not refocused by the spin-echo since, in effect, there is no phase memory associated with this process to be undone.

During echo, the intensity of the observed magnetisation will have decayed according to the natural T2 time constant, independent of field inhomogeneity.

And during the echo time there is some loss of phase, because of natural transverse relaxation; naturally spins are de-phasing apart from inhomogeneity contribution which we remove by echo sequence. But the natural decay is not refocused inihomogeneity contribution is refocused, but natural decay is not. So, in effect then is a very loss of signal; there is memory loss for the coherence with time; and this process has to be undone.

So, this is what happened during echo and they will try to come back and get refocused, but during that process the intensity has reduced because there is a natural decay T2 and this is now devoid of contribution from the field inhomogeneity; there is no inhomogeneity contribution and start measuring the T2 fit into the curve.

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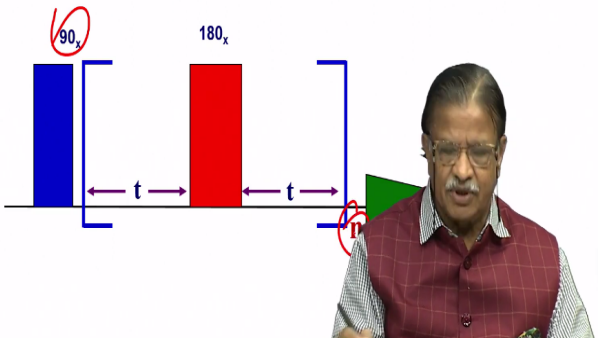
Carr-Purcell sequence

Multiple echo approach minimises the loss of magnetization during the echo sequence, also overcomes the effect of diffusion, within a single experiment using a short T



But sometimes due to pulse inhomogeneity 180 pulse may not be exactly 180 178, 179 or 181 like that; similarly 90 pulse also there maybe pulse imperfections; refocusing may not be efficient. For that there is experiment called Car Purcell sequence which goes N number of times.

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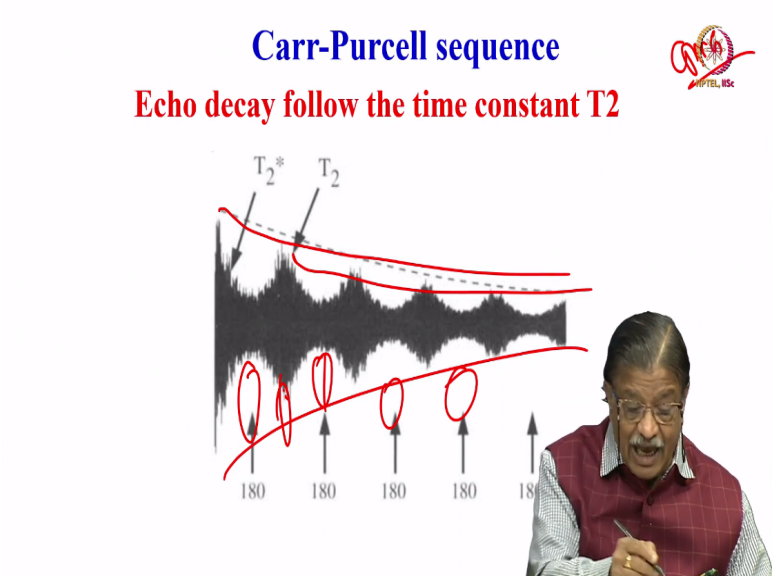

It is a Meiboom-Gill modification; CPMG sequence it is called; and then what we will do is we apply number of such pulses; see the echo start decaying like this slowly; and see there is a echo here and maximum echo is here and then start decaying; exponential decay is there. Here at each intervals in between you are applying the 180 pulse to reverse the spins. So, number of times you make the spins to go from one axis to other axis by 180 pulse.

Keep on changing asking the spins to go back from this place, fast moving to slow moving c, slow to fast; keep on interchanging by 180 pulse. In the process inhomogeneity contribution can be drastically reduced and there is a natural decay here; that you collect.

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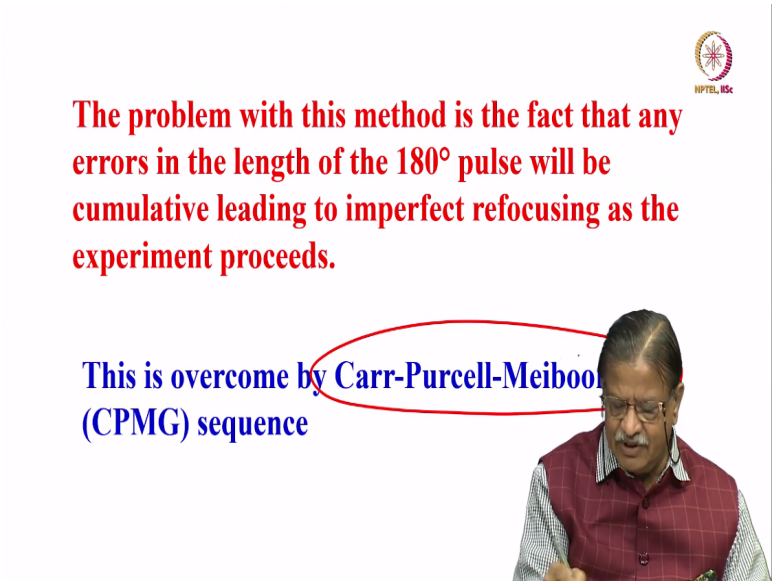

And then you can do this by curve fitting. Of course; as I told you still there are errors in 180 pulse we can use this by what is called Meiboom-Gill modification sequence where number of echoes become more.

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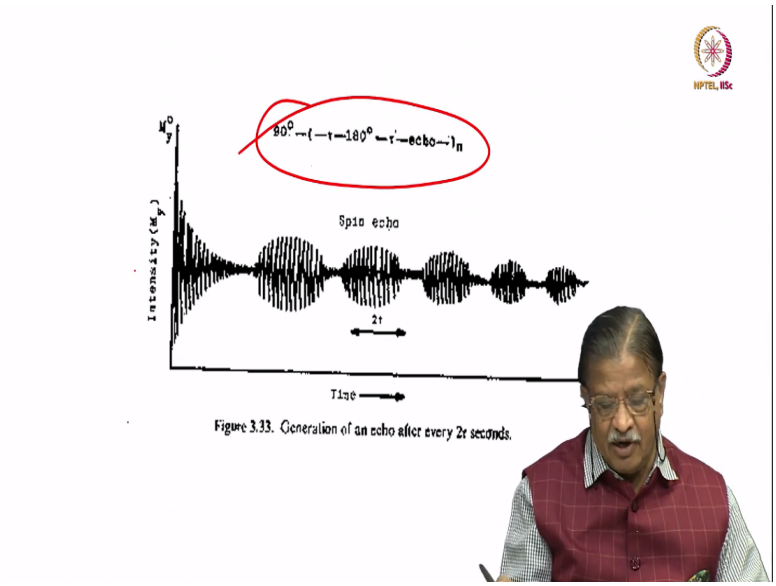

Of course that is the Carr Purcell Meiboom-Gill modification with more number of echoes; and this is how actually in an experiment it has decayed.

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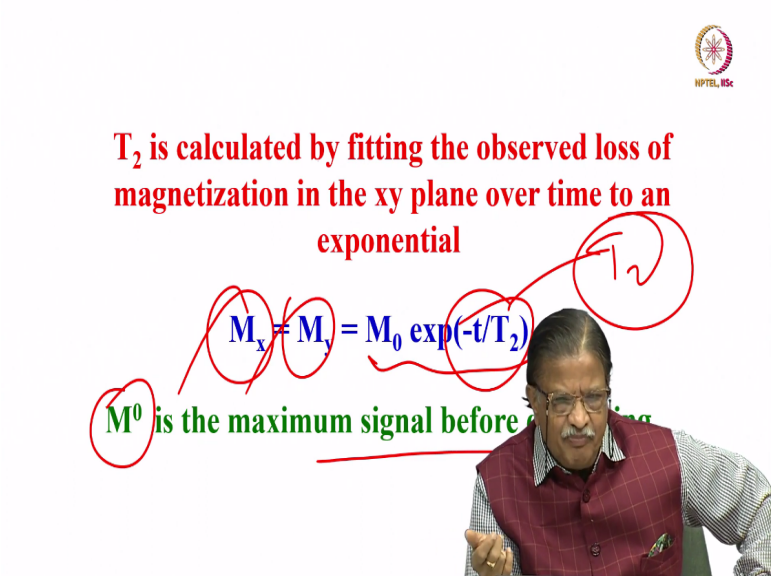

You measure the intensity of the peak as a function of spin echoes; there is a reduction in intensity due to natural de-phasing phenomenon, calculate the intensities and then do the curve fitting. The intensity what you are going to measure in the XY plane could be Mx or My; does not matter; you fit it into this equation M0 into exponential – t / T2; this is also exponentially decaying.

So, when you do this of course M0 is a maximum signal before de-phasing and Mx and My is what is the signal after de-phasing; fit into this curve; you get what is called T2. Very easier way of measuring the T2. So, this is what I wanted to tell you. With this, we discussed a lot about relaxation phenomena or relaxation concepts; the process that involve in relaxation; what do you mean by relaxation, what is the way spin systems give energy to the lattice, spin lattice relaxation; the mode of interaction, mode of energy transfer, how the heat get disperse varieties of things we discussed. Similarly what happen to spin lock, what happened to the spin relaxation T2, how these two differ; lot of things we understood; and of course this class we have also understood how we can measure T1 and T2.

The common experimental methodology that is adopted for the measurement of T1 is inversion recovery, which is 180 tau 90 tau sequence. Apply 180 pulse, bring the magnetization to – Z axis with a tau delay they start going backwards; then apply it 90 pulse and start collecting the signal and do the Fourier transformation, measure the intensity of the signal at different values of tau.

Do the curve fitting to the equation M z = M 0 into 1 – 2 into e to the power of – t / T1 that will give you value of T1 for different peaks. Same way spin echo experiment we can do; one echo or two echo the N number of echoes takes care of all pulse imperfections and everything. One single echo is Carr Purcell method, more number of echoes in Carr Purcell Meiboom-Gill modification method; so many things are present. So many echoes are there which takes care of inhomogeneity contribution of the magnetic field and also pulse imperfections. Finally measure the intensity in the X axis. This pulse sequence is reverse of the T1 measurement; where T1 is 180 tau 90. Here it is 90 tau 180, this is for T 2, but in this case there is an echo after 180; there is an equal amount of time is given. And then collect the signal and measure the intensity, echo amplitude; how it is decaying naturally de-phasing and fit into this curve to measure the T2. So, this is what I wanted to tell you about concept of T1 and T2; how we measure everything in this class. So, we have covered a lot, extensively for the relaxation phenomena and this being the advance course. In the previous course I did not teach much about relaxation. This being an advance course I touched a lot about these things and discussed as much as possible in a basic and conceptual way. I hope you got some point; this is very useful for your research activity later. So, with this I am going to stop now. We will continue with other topic later. Thank you.