NMR spectroscopy for Structural Biology NS

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Lecture: 14
Polarization Transfer Technique - 2

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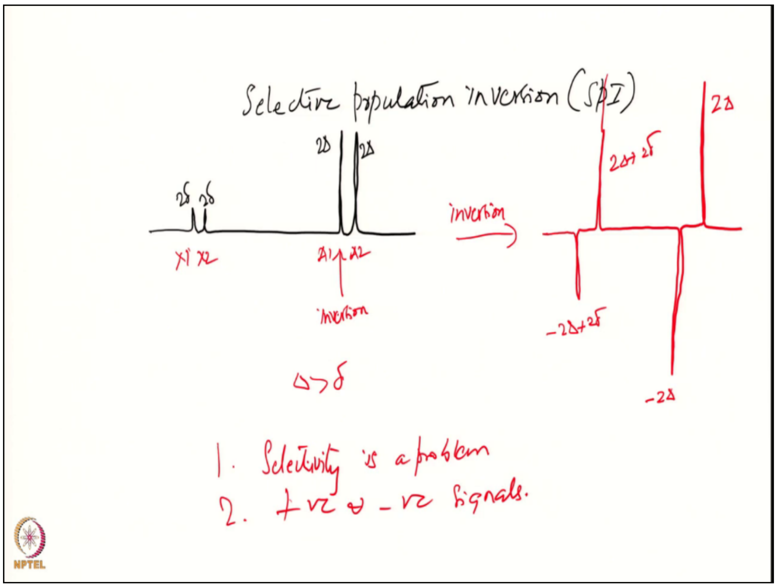

We discussed last time a technique called selective population inversion. The idea was to do a polarization transfer from a more sensitive nucleus to an insensitive nucleus. So, for example if I have a 2 spin system we have 2 lines here and then I have another like this and this is more sensitive. So, this is more intensity and this we saw let us say we have this intensity of these 2 lines as 2δ, 2δ and this one has 2Δ, 2Δ these are more in sensitive.

Then we saw that if I do an inversion on one of these transitions let us say I do an inversion here inversion means I apply a selective 180° pulse on this particular transition. So, when I do this transition. So, as a result of this inversion I will get spectra where the intensity of these 2 weak lines they get enhanced. So, let us say this one is where X_1 X_2 and this is these ones are A_1 A_2 and if A_1 is inverted then what is kind of a spectrum I will get?

We saw that we will get a huge enhancement in the intensity of these signals. So, what we saw was this will become a get a signal like this for X_1 and a signal like this for X_2 and for A_1 it will be like this and A_2 also it will be like this. So, because we have inverted this A_1 transition so, this will have the intensity of -2Δ and this will have the intensity of +2δ this signal has an intensity of -2Δ+2δ and this one has the intensity of 2Δ +2δ of course this one should go longer compared to this there is no space there.

So, this can you can extend it below about there. So, therefore you see this there is a substantial gain in the intensity of this signals and here of course Δ >> δ. So, this was a population transfer as a result of the population transfer from the A spin one of the transitions then we get a significant enhancement in the intensities of the X transitions.

However we had this problem that we have positive negative signals here and selectivity is a quite an issue so, applying a selective pulse was an issue and therefore we had some difficulties here. That is what were the difficulties; selectivity was an issue selectivity is a problem and we cannot do it in crowded spectra then we also have the positive negative signals so, in order to get over this problem. So, there was a new technique which came up which is called as INEPT.

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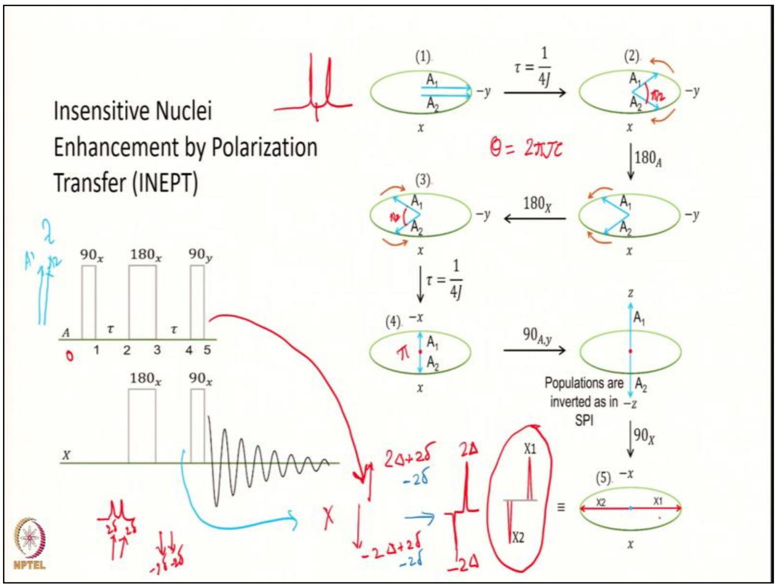

Insensitive nuclei enhancement by polarization transfer so, the experiment is quite well described here I will go through it step wise and we will use you will see that we will use here the principle of SPI also here at this point we will see where I will explain to you where we will use this pulse sequence goes like this. So, this is called insensitive nuclear enhancement by polarization transfer or in short INEPT.

Experiment starts like this. So, you have the A spin here and the X spin there and the X spin is supposed to be the less sensitive one and the A spin is supposed to be the more sensitive nucleus. So, now we apply a hard pulse to a hard pulse means it is not selective anymore. So, you invert you apply a hard pulse that is called as a hard pulse all the transitions of A spin are excited.

So, we apply a 90x pulse to the A spin wait for a period to τ and then you apply simultaneously 180 degree pulses to both the A spin and the X spin and this is particularly easy if the A spin is for example a proton and X spin is like a carbon and then it is very easy to do it because these are 2 different channels and to apply pulses on 2 different channels is not a problem at all because independently they work.

So, therefore you can apply without any issue of interference between the 2. Then you wait for the same time τ again then you apply a selective and not selected hard again 90° pulse on along the Y axis here and the 90x pulse here it does not matter which one you take but more importantly this has to be a Y if this is X this has to be a Y. So, therefore you make this sort of a pulse sequence and then you will see what happens that you will get a substantial enhancement of the X magnetization and that is what you will detect here.

Now let us try and analyze this. So, how does this work basically if you recall the spin echo experiment we had this spin echo from this is $90 - \tau - 180 - \tau$ this is the spin echo sequence at the end of this 2τ we have the echo appearing. And then you apply the 90° pulse on both the channels the A channel and the X channel to transfer magnetization from the A spin to the X spin.

How does that work let us see here let us assume that we sit on the rotating frame of the A spin and this is these are the time points indicated here 1 2 3 4 5. So, at before this 90 degree pulse what is the magnetization the magnetization is along the Z axis at this point the magnetization this if I call this time point is zero the magnetization is along the Z axis if I say here if the time point is zero here the magnetization here is along the Z axis.

Now when I apply a 90° pulse the magnetization comes on to the -Y axis. Now notice I am applying my pulse only to the A spin therefore I need to consider only the spin here. Now if it is coupling between A and X this will be transition there will be 2 transitions A_1 and A_2 and both they are both magnetizations are along the -Y axis. So, I just still represent that as A_1 and A_2 these are the Y magnetizations corresponding to the A_1 and the A_2 transitions.

Now what I do during the period $\frac{1}{4J}$ during period one by 4 J what will happen these ones will start precessing. Now as I said I am sitting in the middle of the A_1 and the A_2 transitions when I see this is exactly the spin echo sequence the spin echo sequence meaning. So, I have this A_1 and the A_2 transitions here I am sitting here if I am sitting there.

So, therefore the one of them goes faster other one goes slower therefore these ones will move in the 2 opposite directions. So, these A_1 and A_2 will move in opposite the ones will move like this other one will move like this because I am sitting in the middle. Now it will move away a certain angle and what is that angle that angle is given by what is the frequency difference between the 2.

And the delay you give because this separation between them is J and therefore $2\pi J$ and the τ we give that is the angle with which it will rotate. So, the angle with which will rotate is let me write here angle θ what it will rotate is $2\pi J\tau$. So, J is the frequency difference between the 2 therefore the separation between them will be $2\pi J\tau$. So, if I say $\tau = \frac{1}{4J}$ how much will be the angle?

If it is 4J then it will be $\frac{\pi}{2}$. So, angle between these 2 will be $\frac{\pi}{2}$ at this point it is $\frac{\pi}{2}$. So, now I am applying A 180° pulse on the A spin. So, A spin apply 180 pulse this A_2 transition will come here and the A_1 transition will come here that is what is indicated here. And they will continue to move in the same direction this will continue to move in the same direction like this A_1 will go like this and the A_2 will go like that.

Now the trick is here I apply also 180° pulse to the X spin as well remember what happens if I apply 180 pulse to the X spin it will invert the X and the it will invert the alpha in the beta states of the X spin convert alpha to the beta this 180° pulse converts α to the β of the X spin. So, when that happens the 2 these 2 transitions will get interchanged A_1 becomes A_2, A_2 becomes A_1.

Therefore there when you have this if this will become A_1 here, this will go in the opposite direction this A_2 this will go in this direction. Now this one because the slower becomes faster the faster becomes slower. So, therefore now this will go like this and this will go like this. So, now during the next $\frac{1}{4J}$ what will happen they will again move away from each other and another $\frac{\pi}{2}$ phase angle will be introduced.

So, this was $\frac{\pi}{2}$here and this is also $\frac{\pi}{2}$ this is also π but we did not do anything here this was also $\frac{\pi}{2}$ but when it moves. Now it is π during the next one another $\frac{\pi}{2}$ is added there. So, therefore now they become opposite to each other. So, they are along the. Now the X and the -Y axis X in the -Y axis right. So, now we apply a 90°y pulse here on to the a transition. So, that means I am applying a pulse along the Y axis.

So, what will happen to this these 2 which are along the X and the Y they will now go to the Z-axis on the positive and the negative Z-axis they will come along this. Now we compare this situation with what we had here in the beginning in the beginning the 2 transitions were at time δpoint zero at time point zero where they were at time point zero these 2 transitions were both along the Z axis right let me use that same colour at time point 0 the both the transitions of A_1 and A_2 magnetizations were along the Z-axis.

This is the Z-axis A_1 and A_2. Magnetizations corresponding to this they were along the positive Z axis. Now what I have achieved here at the at this point I have done inverting one of these inverting the A_2 transition here the A_1 is kept up there like that only nothing has happened to the A_1 and the A_2 is inverted. This is like SPI populations are inverted as in SPI you see one transition is inverted.

So, therefore I get therefore I get the same benefits as I will get from the SPI how much do I get now. So, if you look back what did we have? In this in this one I had $-2\Delta+2\delta$ and $2\Delta +2\delta$ the same thing I will, have there also and this will be $-2\Delta+2\delta$ that will be present there. So, here therefore if when you apply 90ypulse to the next pulse if I apply the transitions of the A spin have gone on to the positive and the negative Z axis.

And the X now X spin this is the first time applying a 90 pulse therefore the x magnetization will. Now come on to the transverse plane will now come into the transverse plane. See before this point at this time point 4 these 2 transitions the intensities of these are along the Z axis only right these added is along the Z axis. So, at this point the there is no transverse magnetization of the X spin it has got the populations of the A spin it has gone at this point at this point the X spin to use a different color the X spin have both the transitions along the Z axis but one of them is like this other one is like this along Z axis part of this has come from the a spin transition how much has come from the spin from the a s pin as we said if it is 2Δ -2δ to begin with it was 2Δ. So, let us say this was 2Δ+2δand this was -2Δ+2δ this is at this point after the 90 degree pulse this will be at this point. Now notice here but I also applied a 180 degree X onto the X spin. So, therefore what was 2δ here.

So, here there was the 2 transitions of the magnetizations corresponding to the X spin where like this like this and these were 2Δ and 2δ and but this is. Now inverted to this 2Δ, 2δ, -2Δ, -2δ delta this is great coming directly from the excitation of the X spin this is coming from the excitation of the X spin at this point the x magnetization along the Z axis was corresponding to 2 small delta 2 small delta as it in this way.

Now this 180x pulse makes this to -2Δ,-2δ with regard to the populations this will carry forward therefore I have to add that here whatever is coming here will be added here. So, in that case what I will get I will get -2Δ let me add with a different colour. So, then I will have to add here -2δ to this and -2δ to this. So, therefore what I got I got 2Δand -2δ as a result.

As a result I get I will use the same colour here. I get this is 2Δ and this is -2δ that is what I have shown here that is what I have shown here X_1 and X_2 as they are coming these are identical to this. So, let me put a circle here to explain this to you. So, this is the same. So, when I do this the initial magnetization is along the populations are corresponding to this and when I apply a 90x to 90x here this magnetization gets transferred onto the onto the transverse plane.

So, then I measure it then it will be 2 signals of equal amplitude but along the positive and the negative Z axis. So, how much is the enhancement here what was δ earlier is now Δ right. So, therefore the enhancement is. So, therefore the let me write. So, I have.

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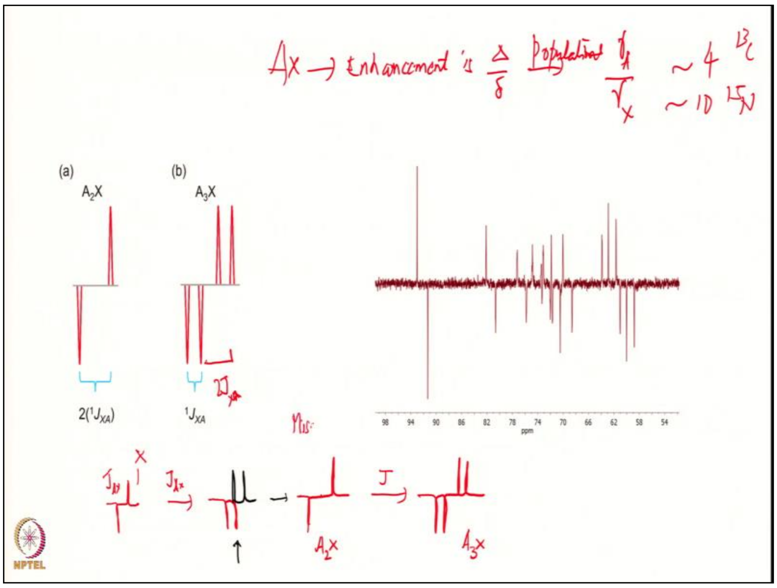

So, therefore in the AX case the enhancement is by factor Δ by δ and these ones are dependent on the populations or the sensitivity therefore this will be proper will be proportional to $\frac{\gamma_A}{\gamma_X}$. So, this is the kind of an enhancement you will get for a simple 2x spin system and this will be for example this will be like a factor of 4 for carbon 13 and factor of 10 for nitrogen 15. So, this is a substantial enhancement.

So, now what happens but however we still have this problem of positive negative lines and that continues. So, we are not gotten over that problem but we have gotten over the selectivity issue we do, do not need to apply selective pulse anymore we are applying a hard pulse by applying the heart pulse we have got the same result intensity enhancement and similar intensities for both the lines. Earlier remember in SPI we did not have similar intensities for the 2 lines they were slightly different.

Now here we get similar intensities for the 2 lines in the X spin. Now what happens suppose I consider an A_2X system and this is the experimental spectrum here which shows exactly that point here see these are identical lines with similar intensities similarly here similarly here. And of course we have more complex spin systems here because this is only not only a X but also you have A_2X, A_3X and things like that. Now of course in real life because you not only have an AX not only have a CH CH group we also have CH_2 groups and CH_3 groups and things like that.

So, what happens in the case of a CH_2 group and that is indicated by this A_2X A_2X spin system the a 2X indicates like a CH_2X is the C and A_2 is the H_2 but the transfer is considering we consider the transfer from the individual protons at a time individual protons at a time. So, therefore how will we analyze this A_2X system consider the X consider the X to analyze this A_2X system.

Consider the X now this one is. Now split because of one coupling one transfer into 2 lines like this equal intensity. So, when I apply when there is a next coupling there is a this is due to the one J_{AX} coupling one J_{AX} coupling the second coupling what it will do the second J_X coupling will split these individual lines into 2 again it will split these individual lines into but. Now this will be inphase splitting this will split like this.

And the other one will also split and that will happen use a different colour here and that will happen like this the second line. So, so this line has split into these 2 and this line has split into these 2 because of another coupling the second coupling because there are 2 a's there the first a has resulted in this antiphase separation this is called as antiphase separation and this. Now is split further due to another coupling of the X to the second proton here and those results in splitting.

Now what happens these 2 central lines will cancel this will cancel. So, then I will have only 2 lines. Now this will result in this will result in one line here and. Now there is a gap and there is another line here. So, then the center there is nothing that is what will happen in the case of A to X. If the central portion has got Zero this separation is. Now $2J_{AX}$ from here to here is not J_X but 2 J_X the center line which is there here it was J_{AX} here it was in the single case there was J_{AX}.

Now this central line has vanished therefore in this case I get a 2 J_{AX}. So, that is explanation for that. Now you can consider further for suppose that is the A_3X if there is A_3X these 2 will split again these 2 will split again. So, if you have another J then these 2 will again split as like this and then other one will also split. Now it will come closer because it will go on to this side this is what will happen for an A_3X.

So, that A_3X. So, therefore this will be the A_3X and this will be the A_2X we still have the positive negative signals and the total separation from here to here is of course 3 J_X separation from here to here because there are 3 couplings therefore that has to be 3 J_X from here to here should be 3 J_{AX}. So, and that is this is $1J_X$ and from here to here is 2 J_{AX} these are one bond couplings remember X A.

So, these are one, one coupling therefore there is a total of 3 bonds. So, therefore A_3X spin system will appear in this manner A_2X will appear like this and this is again a disadvantage for the INEPT sequence because the middle sequence is gone. Now you may tend to think that if I get a spectrum like this, this is actually an AX with the coupling constant which is twice that of the normal coupling.

Now one bone coupling which is not the case it is actually an A_2X but this is because of the sequence you will get a separation which is twice that and the central line has vanished. So, this is while you have circumvented the problem of sensitivity I mean the the problem of selectivity problem you have still got the antiphase problems and you have some missing signals in the middle. So, that will be a kind of a disadvantage.

So, therefore here we will have missing signals missing central line. So, this is what I indicated there with regard to the INEPT sequence how it will increase the sensitivity.

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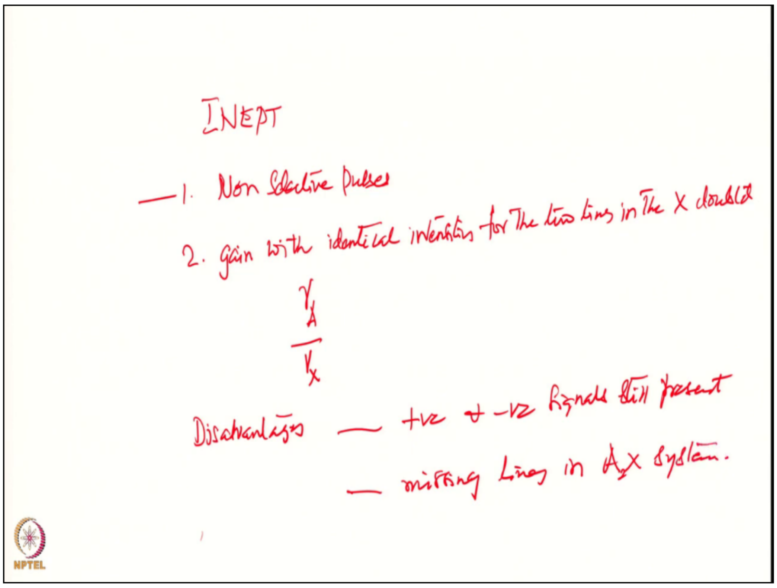

So, therefore the INEPT use non selective pulses. So, you have gain with identical intensities for the 2 lines in the X doublet and the gain is by the fact is $\frac{\gamma_A}{\gamma_X}$. So, this much gain we will get in the case of INEPT. Now the disadvantage of course will be are still disadvantages are. So, you have the positive negative signals still present still present but missing line in A_2X systems.

So, to get over this problem there was one more technique which came into existence and this is called as the refocused INEPT.

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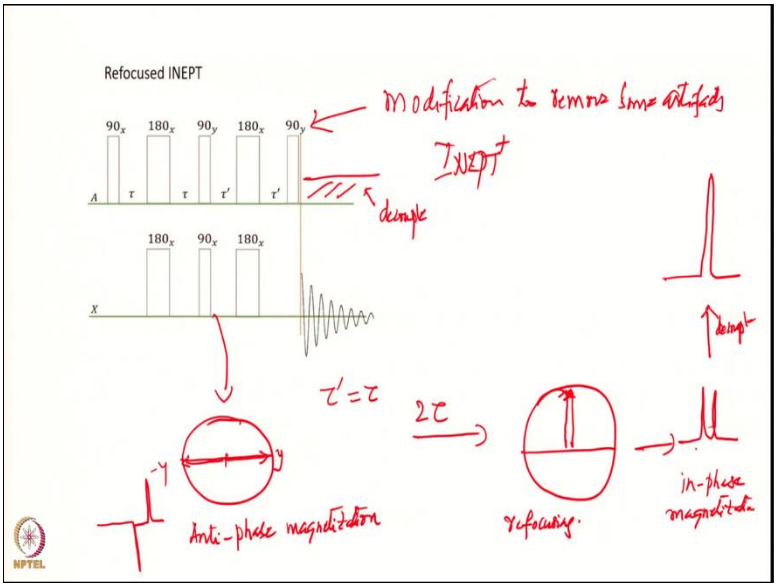

Refocused INEPT what it does it is until here it is the same as the INEPT until here it is the same as the INEPT and then we have introduced another block here another block here which is tau it can be τ’ or it can be τ_r also in the simplest case it is tau and tau. So, then what happens here. So, at this point at this point if I draw the X transitions if you recall the X transitions where along the X axis or the Y axis whatever that is.

So, if this is -y this is -y and the 2 transitions were oriented like this and such a signal is called as antiphase magnetization this is called as antiphase magnetization because 2 transitions have opposite phases. Now what we have done is you continued this spin echo you continued the spin echo in an identical manner here the τ and τ is supposed to τ’ = τ.

So, that is equal to you are simply repeating that same sequence therefore as a result of this during the next 2τ period what will happen this one will again clear another 180° phase difference and then they will become oriented like this they will refocus. So, they will come here along they will refocus the separation 190° phase difference which was there that would be gone because it resists by another 180° another 100°.

Meaning they will come back in phase this that is this is called the ref refocusing. So, when the refocus now, if you now take a spectrum of this you will get in phase lines because the magnetizations are not along the opposite directions right and this magnetization here was like this and like this and when you do a refocusing. So, you will get magnetization along this axis here and we will get pure phase after use 2 phase correction etcetera you will get in phase magnetization this is called as in phase magnetization.

Now of course you notice here of course the tau dash is a variable tau dash can be optimized for certain purposes we will not go into those details here and there is also one other pulse which is applied as a modification to a refocused INEPT is called as INEPT plus and this is a modification to remove some artefacts. And this actually pulse sequence is called as INEPT plus with that modification it is called as INEPT plus.

But we will not go into those details here that requires more of theoretical calculations and but this is enough to understand that we can actually do a refocusing. If you refocus what is the advantage we collect the data here we collect the data then magnetization is now in phase therefore we can decouple it also. So, now if you do decoupling you can do decoupling here you can decouple they can get a one line.

You can get one line which has the sum of the intensities of these 2. So, decouple. So, this is possible this deep coupling can be introduced here this is decoupling. So, the proton can be decoupled while you are observing the X when you observe the X. Now you get a very huge gain in the. Now the splitting is gone you do not need this splitting this is one bond coupling one bond coupling is not of much use.

So, therefore you can remove that coupling then you will get a single line for the particular carbon particular carbon or the nitrogen or whatever you are talking about. So, you will have a substantial gain in the signal to noise ratio and this is the benefit of this refocused INEPT. Every time you want to decouple you have to remove this interface character bring them into in phase in phase means they both go in the same direction like this.

So, therefore then you can collapse them there if they are like this. So, you know decouple they become zero but if they are going like this if they are 2 like this if you decouple they will become like that and of course they will add on intensity. So, there will be substantial gain in the intensity because of that.

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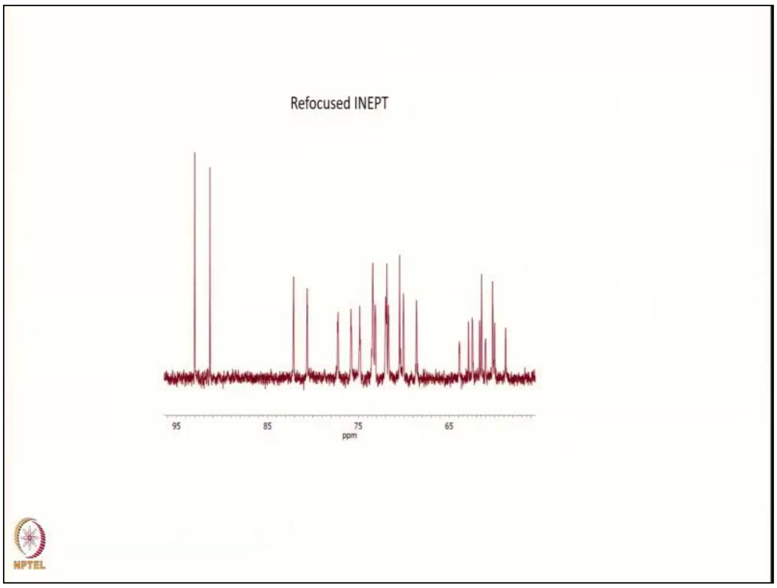

So, and here is an example of such a spectrum is a practical example you can see all in phase lines and with much higher signal to noise ratios intensities and all multiplied structures will get retained. So, you will not have any problems with these missing peaks and all the things will be retained. So, this is the ideal technique one can use and most of the time this is what one uses all multi-dimensional NMR experiments and I think we will stop here with the polarization transfer.