NMR Spectroscopy for Chemists and Biologists
Professor Ramkrishna Hosur
Department of Biosciences and Bioengineering
Indian Institute of Technology Bombay
Lecture 44 – Two Dimensional Separation of Interaction in NMR

So we have been discussing two dimensional separation experiments in NMR and in the last class we talked about 2D heteronuclear separation experiments where the chemical shifts and the coupling constants were separated around the 2D dimensional 2D experiment. So we are going to extend this discussion to homonuclear experiments, homonuclear 2D (JRES) experiments.

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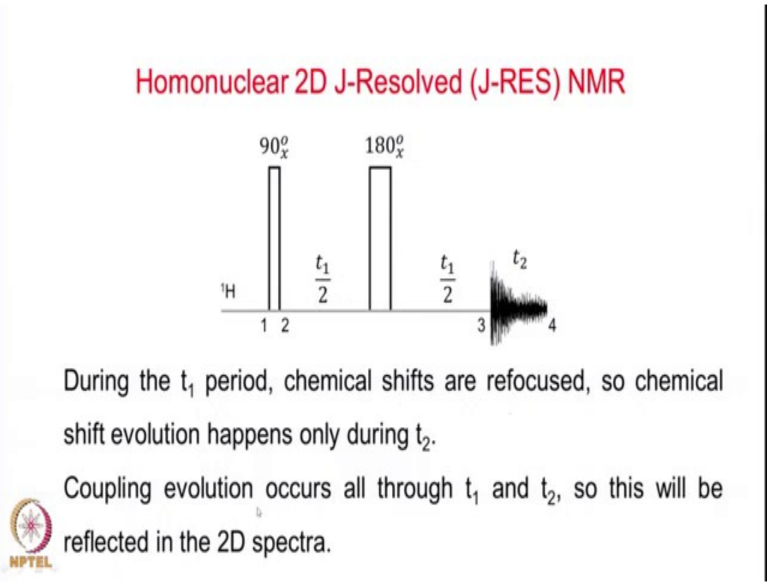

Homonuclear 2D J-Resolved (J-RES) NMR

During the t_1 period, chemical shifts are refocused, so chemical shift evolution happens only during t_2.

Coupling evolution occurs all through t_1 and t_2, so this will be reflected in the 2D spectra.

So this is generally represented as (J-RES) NMR or 2D J-Resolved NMR. So the difference here will be because of the homonuclear coupling constant that will be present. In the hetronuclear experiments which we discussed earlier ^{13}C was the heteronuclear and the proton was the other nucleus. So we are talking about the coupling constants between carbon and proton.

And we did not talk about the homonuclear carbon-carbon couplings because we are working at natural abundance and therefore the chances of carbon-carbon coupling occurring is very very small. But in homonuclear proton experiments there is always will be proton-proton couplings. So therefore homonuclear 2D J-Resolved experiments with protons actually leads to some additional complications and we are going to discuss that today.

So the pulse sequence is very similar to the previous ones except that of course you only have 1 channel here. So we have the proton channel, so the experimental sequence is like this sequence as before, it starts with a $90^{\circ}x$ pulse followed by the $\frac{t_1}{2}$period and then $180x$ pulse followed by another $\frac{t_1}{2}$ period. These two together constitute the evolution period.

The 180 pulse is put in the middle of the evolution period and then the detection happens during the t_2 period here. So this is the detection period. Now of course I put here the numbers 1, 2, 3, 4 to indicate the time points and these will be used when we actually calculate the product operators at the different time points. Notice here during the t_1 period chemical shifts are refocused, this we have seen before when we discussed the spin echo, these chemical shifts are refocused during this spin echo.

There is spin echo happens at this point, so chemical shift evolution happens only during t_2. On the other hand coupling evolution occurs all through t_1 and t_2. So this will be reflected in the 2D spectra.

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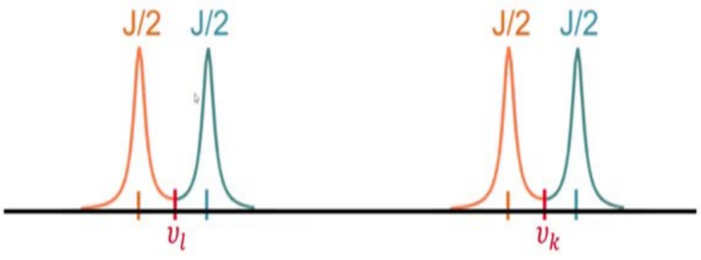
A weakly coupled two-spin system, k and l



So consider a two spin system for understanding the experiment it weakly coupled two spin system k and l. So each one will produce the doublet. So if this is the chemical shift of k spin it has doublet with 2 lines separated by coupling constant J. Similarly the l spin also is a doublet with the lines separated by the coupling constant J.

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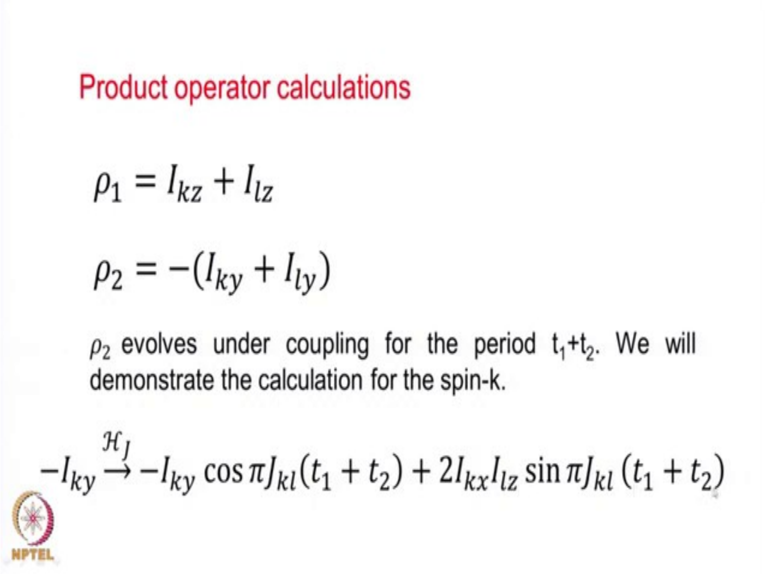

Product operator calculations

$$\rho_1 = I_{kz} + I_{lz}$$

$$\rho_2 = -(I_{ky} + I_{ly})$$

ρ_2 evolves under coupling for the period t_1+t_2. We will demonstrate the calculation for the spin-k.

$$-I_{ky} \overset{\mathcal{H}_J}{\rightarrow} -I_{ky} \cos \pi J_{kl}(t_1 + t_2) + 2I_{kx}I_{lz} \sin \pi J_{kl}(t_1 + t_2)$$

Let us try and understand this experiment using product operator calculations. At time point 1 the density operator is

$$\rho_1 = I_{kz} + I_{lz}$$

the magnetization is along the Z axis and this is the density operator for the magnetization at time point 1. And when you apply $90x$ pulse now we apply to both the spins and this for this will result in y magnetization of the both the spins. So

$$\rho_2 = -(I_{ky} + I_{ly})$$

Now this row 2 evolves under coupling for the period $t_1 + t_2$, you remember we can calculate the evolutions of the spins under the influence of the chemical shifts or the coupling constants independently, it does not matter which one we calculate first and which one we calculate later. So in this case we will first calculate the coupling evolution and this happens for the whole period $t_1 + t_2$, let us consider for one of the spins, the same applies to the second spin as well.

So we consider the calculation for the spin k. So therefore this

$$-I_{ky} \overset{\mathcal{H}_J}{\rightarrow} -I_{ky} \cos \pi J_{kl}(t_1 + t_2) + 2I_{kx}I_{lz} \sin \pi J_{kl}(t_1 + t_2)$$

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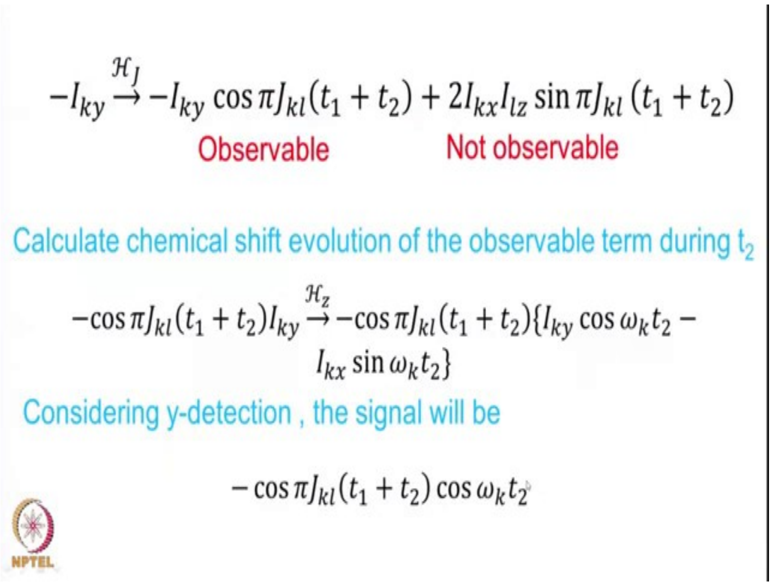

$$-I_{ky} \xrightarrow{\mathcal{H}_J} -I_{ky}\cos\pi J_{kl}(t_1+t_2) + 2I_{kx}I_{lz}\sin\pi J_{kl}\,(t_1+t_2)$$

Observable Not observable

Calculate chemical shift evolution of the observable term during t_2

$$-\cos\pi J_{kl}(t_1+t_2)I_{ky} \xrightarrow{\mathcal{H}_z} -\cos\pi J_{kl}(t_1+t_2)\{I_{ky}\cos\omega_k t_2 - I_{kx}\sin\omega_k t_2\}$$

Considering y-detection , the signal will be

$$-\cos\pi J_{kl}(t_1+t_2)\cos\omega_k t_2$$

Among these you also recall our previous discussion that this anti phase term is not observable because the trace of this with whether you take with I_{kx} or I_{ly} it goes to 0 and therefore this is not an observable term. Whereas this is, it represents in-phase magnetization of *k* transitions and therefore this will be an observable term and therefore we have only this term to consider for further calculations.

Now what we do, we take this term and calculate chemical shift evolution of the observable term during t_2. So this gives me

$$-\cos\pi J_{kl}(t_1+t_2)I_{ky} \xrightarrow{\mathcal{H}_z} -\cos\pi J_{kl}(t_1+t_2)\{I_{ky}\cos\omega_k t_2 - I_{kx}\sin\omega_k t_2\}$$

Now let us assume that detect the *y* magnetization which is without loss of any generality. This we can do this to understand the experiment and the principles behind it.

Therefore the signal here will be

$$-\cos\pi J_{kl}(t_1+t_2)\cos\omega_k t_2$$

Of course notice here this sign appears because we applied *x* pulse or it could be considered without this minus sign as well. If you apply the 90 -*x* in the beginning then you would have only the I_{ky} term. So then ofcourse this minus sign is of not much consequence for the understanding of the experiment just now.

So therefore the signal that we will detect will be just this: $\cos\sin\omega_k t_2, \cos\pi J_{kl} t_1 + t_2$. Notice here that the coupling constant evolution modulates the detected signal and it has both the t_1 and the t_2 dependence, therefore this is the very interesting situation. What is the consequence of this, we will see.

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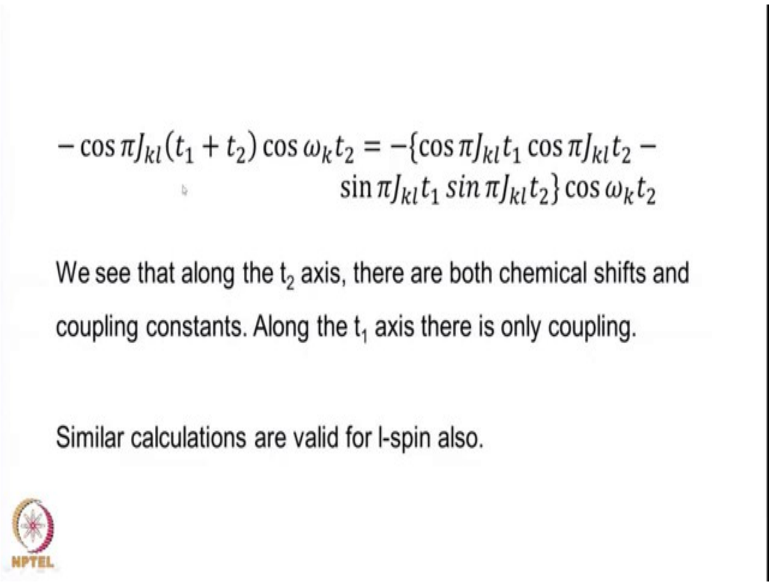

$$-\cos\pi J_{kl}(t_1+t_2)\cos\omega_k t_2 = -\{\cos\pi J_{kl} t_1 \cos\pi J_{kl} t_2 - \sin\pi J_{kl} t_1\, sin\,\pi J_{kl} t_2\}\cos\omega_k t_2$$

We see that along the t_2 axis, there are both chemical shifts and coupling constants. Along the t_1 axis there is only coupling.

Similar calculations are valid for I-spin also.

So now let us expand this,

$$-\cos\pi J_{kl}(t_1+t_2)\cos\omega_k t_2 = -\{\cos\pi J_{kl} t_1 \cos\pi J_{kl} t_2 - \sin\pi J_{kl} t_1\, sin\,\pi J_{kl} t_2\}\cos\omega_k t_2$$

that comes from the expansion of this term and $\cos\omega_k t_2$. So we see here that along the t_2 axis there are both chemical shifts and coupling constants, you see the chemical shift is here and the coupling constant is here.

Whereas along the t_1 axis there is only coupling and similar calculations are valid for the l spin also. So we consider here for the k spin and the same kind of terms will appear for the l spin except that here you will have ω_l instead of ω_k.

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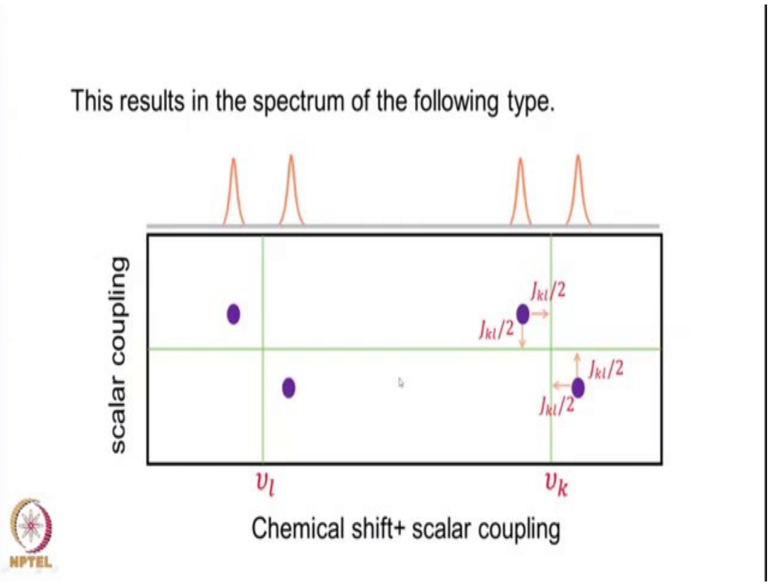

This results in the spectrum of the following type.

Now let us look at this in somewhat greater detail. What sort of spectrum it will produce? Now to understand this let us try and expand the term which I wrote in more explicit detail.

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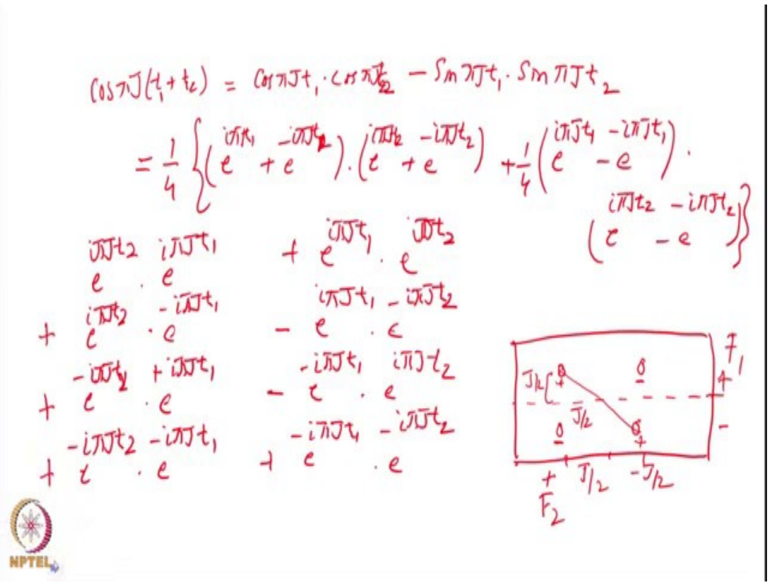

$$\cos\pi J(t_1+t_2) = \cos\pi J t_1 \cdot \cos\pi J t_2 - \sin\pi J t_1 \cdot \sin\pi J t_2$$

$$= \frac{1}{4}\Big\{\left(e^{i\pi J t_1} + e^{-i\pi J t_2}\right)\cdot\left(e^{i\pi J t_2} + e^{-i\pi J t_2}\right) + \frac{1}{4}\left(e^{i\pi J t_1} - e^{-i\pi J t_1}\right)\cdot\left(e^{i\pi J t_2} - e^{-i\pi J t_2}\right)\Big\}$$

$$e^{i\pi J t_2}\cdot e^{i\pi J t_1} \qquad + e^{i\pi J t_1}\cdot e^{i\pi J t_2}$$

$$+ e^{i\pi J t_2}\cdot e^{-i\pi J t_1} \qquad - e^{i\pi J t_1}\cdot e^{-i\pi J t_2}$$

$$+ e^{-i\pi J t_2}\cdot e^{+i\pi J t_1} \qquad - e^{-i\pi J t_1}\cdot e^{i\pi J t_2}$$

$$+ e^{-i\pi J t_2}\cdot e^{-i\pi J t_1} \qquad + e^{-i\pi J t_1}\cdot e^{-i\pi J t_2}$$

Let us now calculate explicitly the appearance of the 2D *J*- resolved spectrum by expanding this the density of operator what we got in explicit terms. We can assume without loss of generality that the chemical shift of the *k* spin is 0. So in which case the density operator what I have is cosine by the signal what I have is $\cos\pi J(t_1+t_2)$ and I am not writing the J_{kl} here because it is implicit that it is between the two spins *k* and *l*.

So this I have to expand, this gives me

$$\cos\pi J(t_1+t_2) = \cos\pi J t_1 . \cos\pi J t_2 - \sin\pi J t_1 . \sin\pi J t_2$$

Now this is equal to

$$¿\frac{1}{4}\left\{\left(e^{i\pi J t_1}+e^{-i\pi J t_1}\right).\left(e^{i\pi J t_2}+e^{-i\pi J t_2}\right)+\frac{1}{4}\left(e^{i\pi J t_1}-e^{-i\pi J t_1}\right).\left(e^{i\pi J t_2}-e^{-i\pi J t_2}\right)\right\}$$

So therefore this expansion will have total of 8 terms. Let us write these 8 terms explicitly

$$1.e^{i\pi J t_2}.e^{i\pi J t_1},2.e^{i\pi J t_2}.e^{-i\pi J t_1},3.e^{-i\pi J t_2}.e^{i\pi J t_1},4.e^{-i\pi J t_2}.e^{-i\pi J t_1},5.e^{i\pi J t_1}.e^{i\pi J t_2},6.e^{-i\pi J t_1}.e^{-i\pi J t_2},7.e^{-i\pi J t_1}.e^{i\pi J t_2},8.e^{i\pi J t_1}.e$$

So these are the 8 terms we have here and let us see what sort of spectrum this will produce. So we have here, we have assumed that the chemical shift is 0, so assume the chemical shift 0 frequency is here, then along the F_2 axis, this is the F_2 axis, this is the F_1 axis, I have the J information here and let us assume that the peaks that doublet the peaks will be shifted by $\frac{J}{2}$, this will be $\frac{J}{2}$ and this will be $\frac{J}{2}$again.

This is on the plus axis, this is the plus sign and this is the minus sign. So the 0 frequency if it is here is 0 and on this axis I will have the $+J$ and on this side I will have the $-J$. So because they will be shifted from the centre one side we take it as a positive frequency, other side will be the negative frequency. We take this side as plus and let us say we take this side as minus here, then I have 0 line at this point and now let us see what each term gives me.

The first term gives me along the t_2 axis $\frac{+J}{2}$ that is at this point and it will produce a peak at $\frac{+J}{2}$ on F_1 axis, so it will produce me a peak here. The second term will produce me at the same point but on the $\frac{-J}{2}$ here and that will produce a peak here, the third term will produce at $\frac{-J}{2}$ and at $\frac{+J}{2}$ on the t_1 axis and that will be a peak here and the fourth term will be at $\frac{-J}{2}$ along F_2 $\frac{-J}{2}$ along F_1 and that will be peak here.

Now the fifth term is $\frac{+J}{2}$ along the t_1 and $\frac{+J}{2}$ along this, so they have still this will be at the same place here. So I will add here to that, so there is a fifth term produces the addition at this point and the sixth term will be $e^{-i\pi J t_1}.e^{-i\pi J t_2}$ this will be $\frac{-J}{2}$ along this axis and $\frac{+J}{2}$ along this. So that will be here, so this will be a minus here because this will be subtraction here and the seventh term this will be $\frac{-J}{2}$along the F_1 and that is here and $\frac{+J}{2}$ along the F_2 and that will be here and this is again a minus sign.

So that will, this will subtract here and the last term is $\frac{-J}{2}$along the F_2 and $\frac{-J}{2}$along the F_1 and that will be here this will add, so therefore this will be plus. So now what happens, therefore these two will cancel and these two will co add, therefore in the end you will see that in the spectrum these peaks are not present and will only have peaks here. Now these peaks are if you want to look at it in this manner they are actually tilted with respect this by an angle which is 45 degrees because this is $\frac{J}{2}$and this also $\frac{J}{2}$.

So therefore this is an isosceles triangle. So this angle is 45 degrees. So this is the way the spectrum will appear and see in the explicitly in this schematically I have shown this in the next slide.

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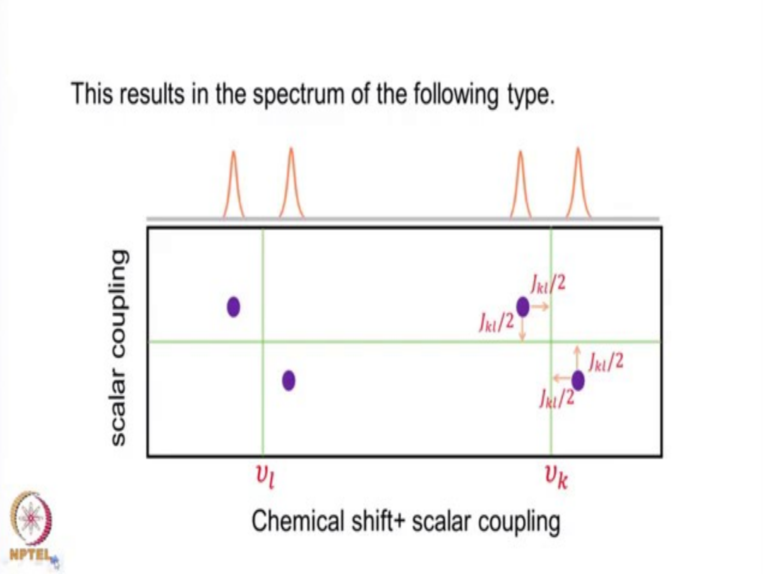
This results in the spectrum of the following type.

So this is how the schematically the spectrum will appear and we have actually calculated why this comes in this manner. We have shown it for *k* spin assuming the *k* chemical shift is 0 but that does not matter that is only for convenience, no matter what the chemical shift is the same thing will be valid and therefore you will have here this tilted pattern here and we will have for both the spins we have the 2 lines which are now shifted along the F_1 axis at this particular angle.

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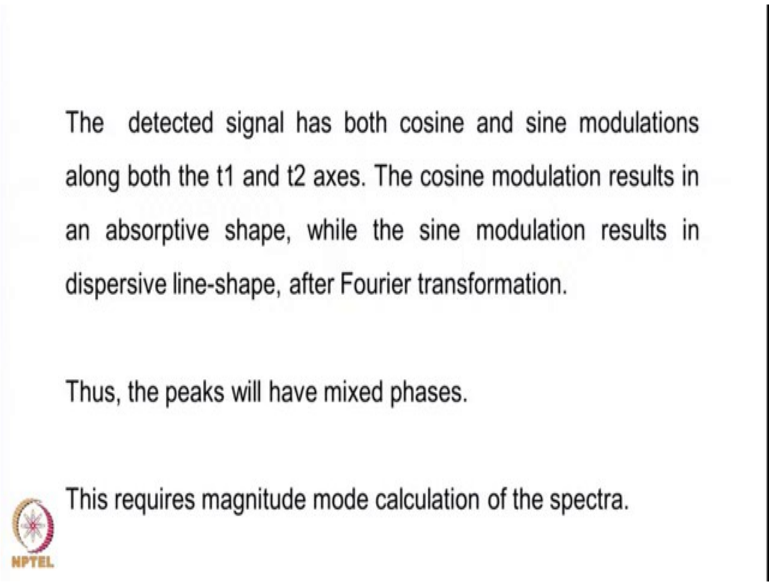

The detected signal has both cosine and sine modulations along both the t1 and t2 axes. The cosine modulation results in an absorptive shape, while the sine modulation results in dispersive line-shape, after Fourier transformation.

Thus, the peaks will have mixed phases.

This requires magnitude mode calculation of the spectra.

So the detected signal has both cosine and sine modulations as we saw before along both the t_1 and the t_2 axes. The cosine modulation results in the absorptive line shape and the sine modulation results in dispersive line shape after Fourier transformation. We actually looked at it explicitly taking the as $e^{i\pi J t_1}$ and things like that, you notice from the previous discussion that when you have such a kind of phase modulation $e^{i\pi J t_1}$, it already implies that we are going to get mixed phases. Therefore here along both the axes F_1 and F_2 axes we will have mixed phases, mixed line shapes and therefore we cannot do a phase sensitive experiment in this situation and we have to do a magnitude mode calculation. That means the square of the absorptive signal and the square of the dispersive signal take the sum and then take the square root and that is called as the magnitude mode calculation of the spectra.

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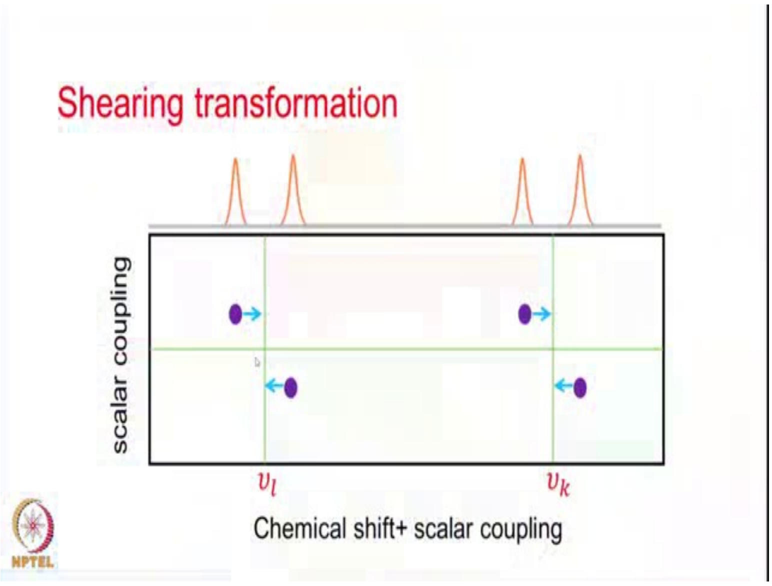

So when we did that of course you get a spectrum which is like this. However we can do a further manipulation of the spectrum by what is called as shearing transformation. We do not want this to be oriented like this. If we were to take a projection of the spectrum like this it will produce me the same one dimensional spectrum as before but we want to remove tis coupling information along this axis, keep it only along the F_1 axis so that you have complete separation of the chemical shift and the coupling constants along the two orthogonal axes.

So what we do, we can do a manipulation of the spectrum after data collection after Fourier transformation, this is called the shearing transformation. So we move this peak to this point and move this peak to this point. Similarly we move this peak to this point, move this peak to this point, this is artificially done.

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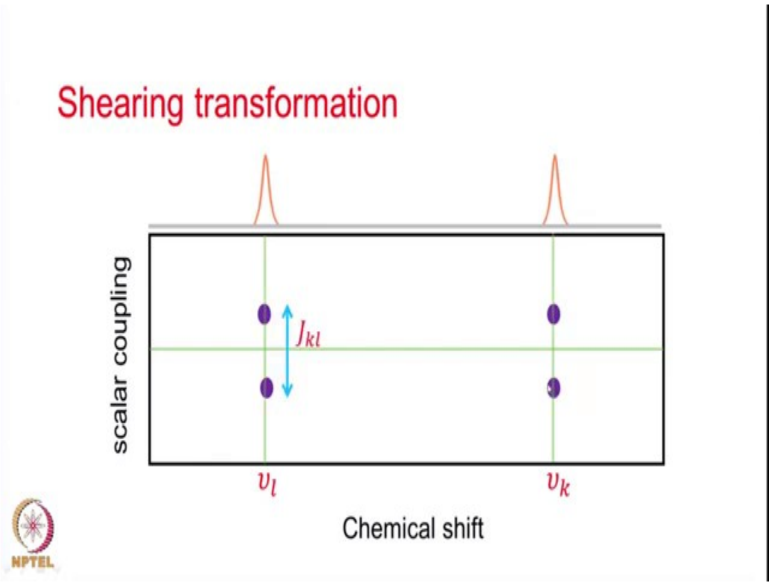

So when you do that you get peaks like this, this separation is J_{kl} and we have removed the coupling information along the F_1 axis completely. So therefore if we were to take a projection now so we have only one line, here you also have only one line and you have the coupling constant present along the F_1 axis. So this is the clear separation of the chemical shift and the coupling constants along the two orthogonal axes.

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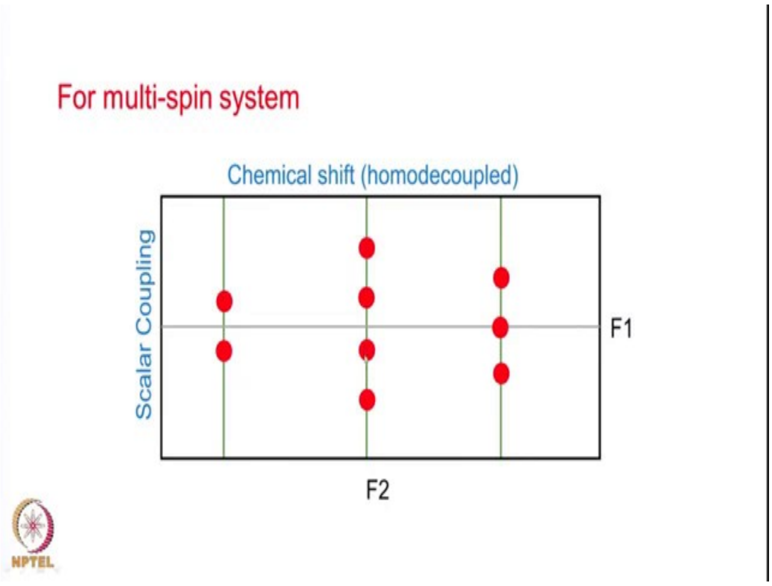

For multi-spin system

You can extend this calculation to more complex spin systems, we did it for two spins now but you can have more, you can have 2 spins 3 spins coupled and then you will see you will multi plates appearing like this. This will be the doublet as we saw before and here you will have doublet of the doublet and here you will have the triplet and so depending upon the nature of the spin system we will different fine structures here and that is what will be helpful to measure the coupling constants in accurate manner.

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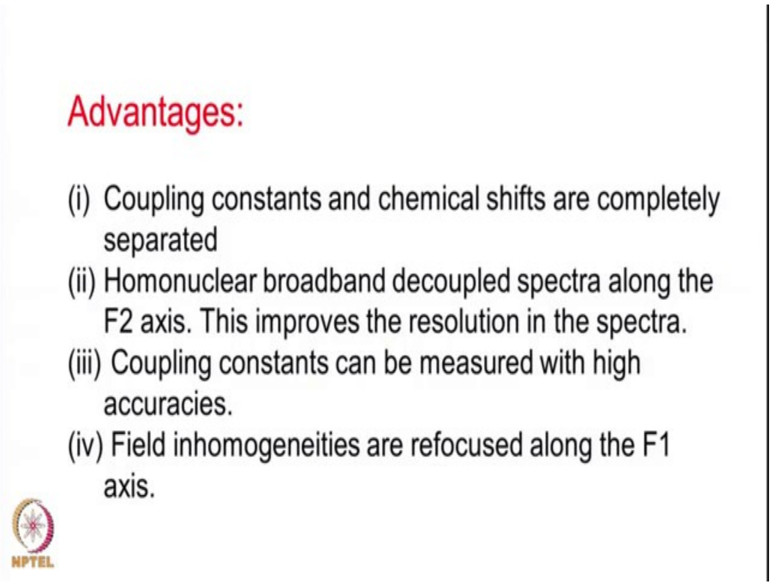

Advantages:

(i) Coupling constants and chemical shifts are completely separated

(ii) Homonuclear broadband decoupled spectra along the F2 axis. This improves the resolution in the spectra.

(iii) Coupling constants can be measured with high accuracies.

(iv) Field inhomogeneities are refocused along the F1 axis.

So what are the advantages of this? So we can measure the coupling constants in the chemical shifts they are clearly separated, the homonuclear broadband decoupled spectra along the F_2 axis, this is the unique feature, it is extremely difficult to obtain a homonuclear decoupling, homonuclear broadband decoupling. We have discussed earlier how to selectively decouple one spin from the other but here we are decoupling every spin from every other spin.

So therefore along the F_2 axis you have homonuclear broadband decoupled spectra and this improves the resolution in the spectra because along one axis you do not have coupling information at all and then coupling constants can be measured with high accuracy and notice also that because we used the spin echo, the homogeneity is refocused along the F_1 axis and this is extremely useful feature and you will have better line shapes and accuracy of the measurements can be high. Although of course we lose some of this because of the mixed phases, because of the magnitude mode calculations some resolution will be lost.

However along the t_1 axis now you see the spectral width is extremely small, it is only determined by the J coupling information and the J values are not too large, the homonuclear couplings are of the order of 8 to10 hertz. So even if you go like doublet of a doublet or doublet of doublet of doublets, then it will go not more than 40-50 hertz. Therefore your increment along the t_1 axis will be extremely large compared to what you have when you have the full chemical shift information along the F_1 axis.

Therefore with in a small number of experiments you can have very high acquisition time along the t_1 domain and that will produce very high resolution in the J-Resolved spectrum.

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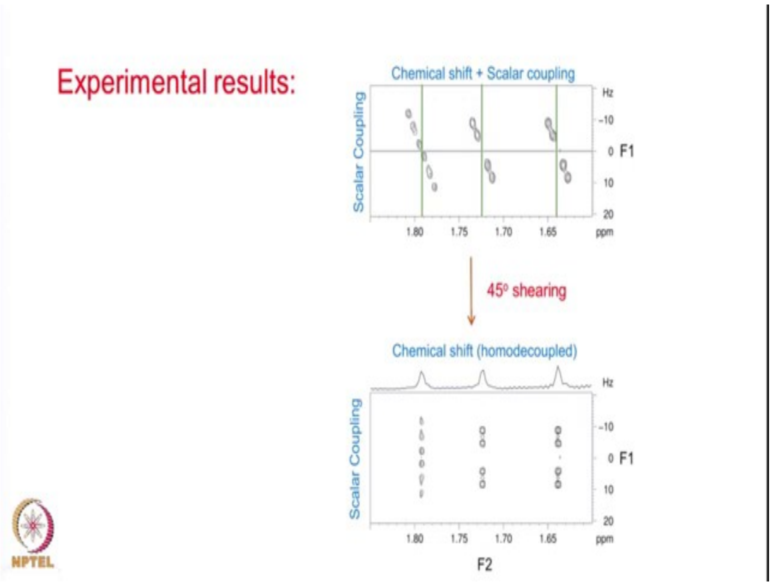

Experimental results:

Now let us look at some experimental data here, so here is an experimental spectrum of some particular molecule it does not matter what it is. You can see here very nicely these all peaks and here you have a doublet of a doublet and doublet of a doublet and this is more complex spin system here. Now you do 45 degree shearing that means you move all of these peaks but to this line. So move all of this to this line, notice of course the extent to which you move will depend upon the peak where it is appearing, how much it is tilted away from here.

So you do shearing transformation of all of this, all of this will move along this axis, this will move here along this axis. So you get now all of them along one line here, one line here and one line here. So if you take a projection you will have a fully decoupled spectrum and if you take cross-sections here then you get the fine structures of the individual spins and it will allow you to determine what is coupling constants and sort of nucleus it is, to how many protons it is coupled and so on. So we stop here and continue with other methods in the next classes.