NMR Spectroscopy for Chemists and Biologists
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Lecture 51
Scaling in 2D NMR

We are now going to discuss a new concept in 2 dimensional NMR. So far we have discussed different types of 2D experiments, 2D separation of interactions, namely the chemical shifts and the coupling constants, along the 2 dimensions of the 2D NMR spectra. We discuss correlation experiments, where spins which are coupled in one way or the other, they can be understood by looking at the correlations in the 2 dimensional spectra.

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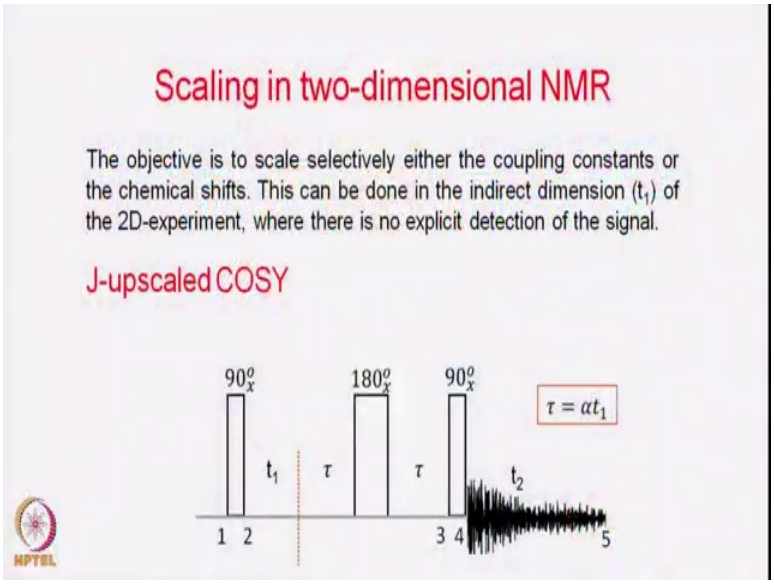

The cross peaks in the 2D correlation spectra reflect the correlations or the interactions between the spins. And we also looked at how to improve the line shapes and the resolution in the spectra by choosing appropriate coherence transfer pathways. So these are new concepts. So we are going to introduce now another important concept namely scaling in 2 dimensional NMR. What do we scale? We scale the two parameters which appear in the spectra namely the coupling constants or the chemical shifts.

We can do that at will. It is not that the chemical shifts of the coupling are actually changed for the sample, it is just that they will appear as modified in your spectrum. It will have its advantages with regard to the suppression of the peaks with regard to the resolution in the peaks, with regard to the intensities of the peaks and so on and so forth. And therefore that is a concept which are going to discuss now.

So, as we see here, the objective is to scale selectively either the coupling constants or the chemical shifts, this can be done in the indirect dimension of the 2D experiment where there is explicitly no detection of the signal. During the T_2 period when the signal is actually detected the receiver is on, your will be limited with regard to the manipulations you can perform. Therefore, it is rather hard to do such manipulations of the coupling constants or the chemical shifts during the detection period.

Because obviously, if you want them to appear differently in your spectrum, you will have to do some manipulations in the evolution and that is a little harder. Whereas in the T_1 period which is the before the detection period where there is no actual detection of the signal, we can play around with the pulse sequence and get some information of the type you want. We did the same thing in constant time COSY where we are decoupled along the F_1 axis.

Here, we are now going to discuss how we can make the J coupling constants changed. In this particular case, we will see that the J's will appear up-scaled, okay and the pulse sequence is as follows. You have the 90° pulse and this will be followed by the T_1 evolution period and that will be followed by an extension of the evolution period which consists of τ –180 –τ and then you have the detection pulse the 90°x pulse as in the COSY.

So this part of the pulse sequence which is τ –180 –τ, this is like a spin echo sequence, right. So and therefore, this is a kind of an extension of the evolution period. Notice also here that $\tau \propto t_1$, $\tau = \alpha t_1$, α is a positive number and is obviously greater than 0 and therefore, you have an extended evolution period here. And as we perform the experiment as t1 is getting incremented, this period is also getting incremented. Therefore, the separation between these 2 pulses will go on increasing as we increase the given period.

Of course that is same as in COSY as well, as we increase the t_1 period this separation go on increasing and also this 180° pulse keeps moving further and further as we increase the t_1 value because it is equal to αt_1 and alpha is a constant, okay.

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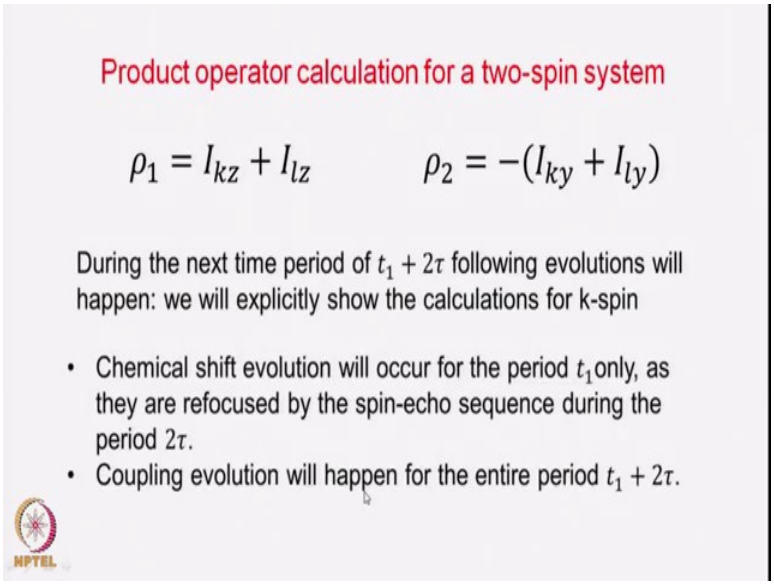

Let us see how it works, let us try and understand this. We will do a product operator calculation for 2 spins system as usual, considered 2 spins k and l.

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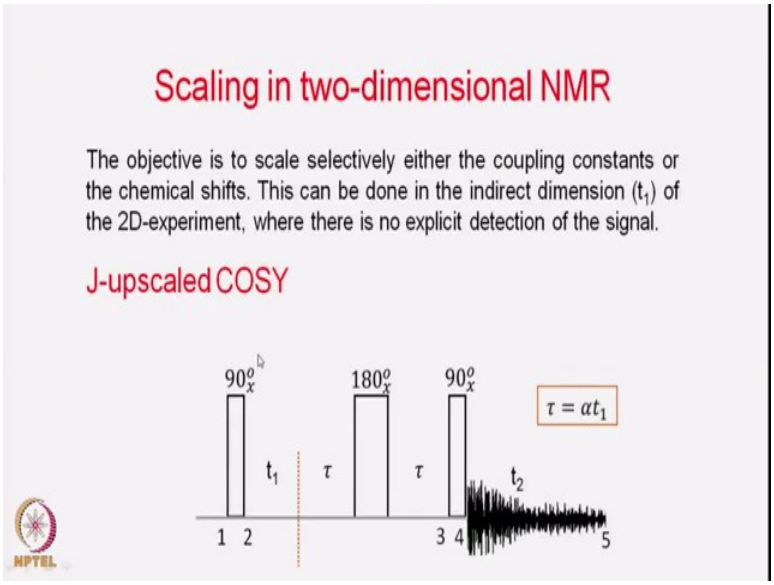

And these are the density operator time points where we will actually look at the density operator.

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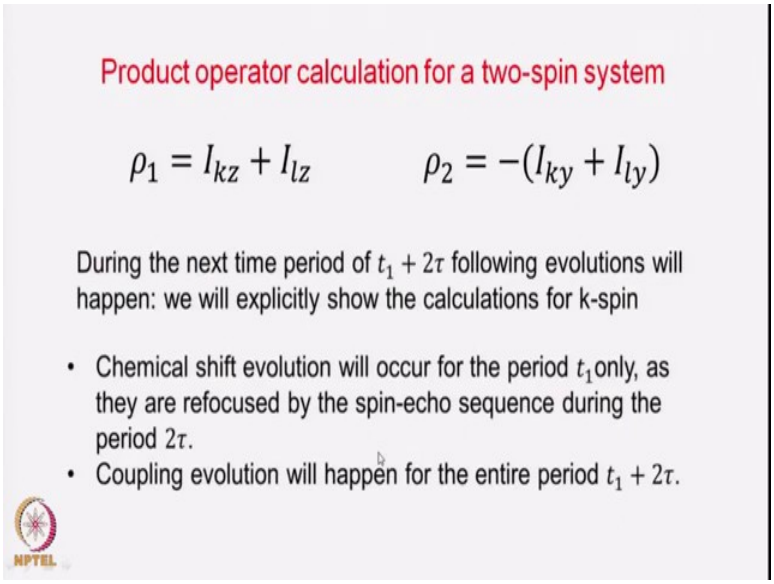

At time point 1 so it is basically *z* magnetization of the 2 spins, the $I_{kz} + I_{lz}$ and when you apply the 90˚ pulse on *X* axis, you get $-(I_{ky} + I_{ly})$, these are independent spins. So they will evolve independently during the next evolution time period. For our purpose, we will only illustrate evolution of the *k* spin and the same thing will apply for the *l* spin as well. During the next period $t_1+2\tau$, what happens? What kind of evolutions happened?

Notice the chemical shift evolution will occur for the period t_1 only. Because they are refocused by the spin echo sequence during the period 2τ, as you remember the spin echo refocus is the chemical shifts, but it does not affect the coupling constants, it does not affect the coupling evolution. Therefore, coupling evolution will continue to happen during the 2τ period. Therefore, the total coupling evolution period will be $t_1+2\tau$. The chemical shift evolution will be restricted to t_1 whereas the coupling evolution will be for the period $t_1+2\tau$, okay.

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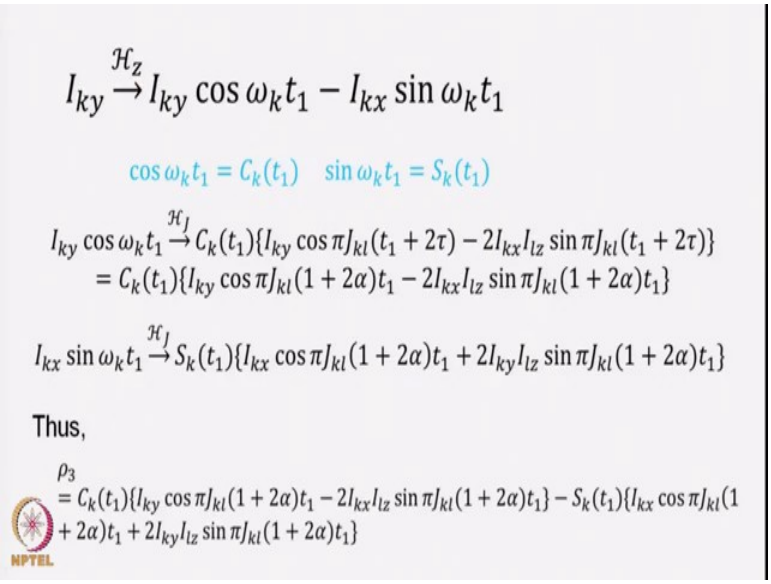

So let us write these evolutions here, consider

$I_{ky} \rightarrow I_{ky}\cos\omega_k t_1 - I_{kx}\sin\omega_k t_1$

Now let us use this abbreviations here $\cos\omega_k t_1$is written as a $C_k(t_1)$, $\sin\omega_k t_1$ is written as $S_k(t_1)$ if you write that, then ofcourse you will write this expression once more here and evolve further under the influence of the coupling constant.

$$I_{ky}\cos\omega_k t_1 \rightarrow C_k(t_1)\left\{I_{ky}cos\pi J_{kl}(t_1+2\tau) - 2I_{kx}I_{lz}sin\pi J_{kl}(t_1+2\tau)\right\} = C_k(t_1)\{I_{ky}cos\pi J_{kl}(t_1+2\alpha)t_1 - 2I_{kx}I_{lz}sin\pi J_{kl}(t_1+$$

Similarly, for the second term here

$$I_{kx}\sin\omega_k t_1 \rightarrow S_k(t_1)\left\{I_{kx}cos\pi J_{kl}(t_1+2\alpha)t_1 + 2I_{ky}I_{lz}sin\pi J_{kl}(t_1+2\alpha)t_1\right\}$$

Therefore were total density operator ρ_3, okay. At the end of the period 2τ is the sum of these 2 evolutions and that is

$$¿C_k(t_1)\left\{I_{ky}cos\pi J_{kl}(t_1+2\alpha)t_1 - 2I_{kx}I_{lz}sin\pi J_{kl}(t_1+2\alpha)t_1\right\} - S_k(t_1)\left\{I_{kx}cos\pi J_{kl}(t_1+2\alpha)t_1 + 2I_{ky}I_{lz}sin\pi J_{kl}(t_1+2\alpha)t_1\right\}$$

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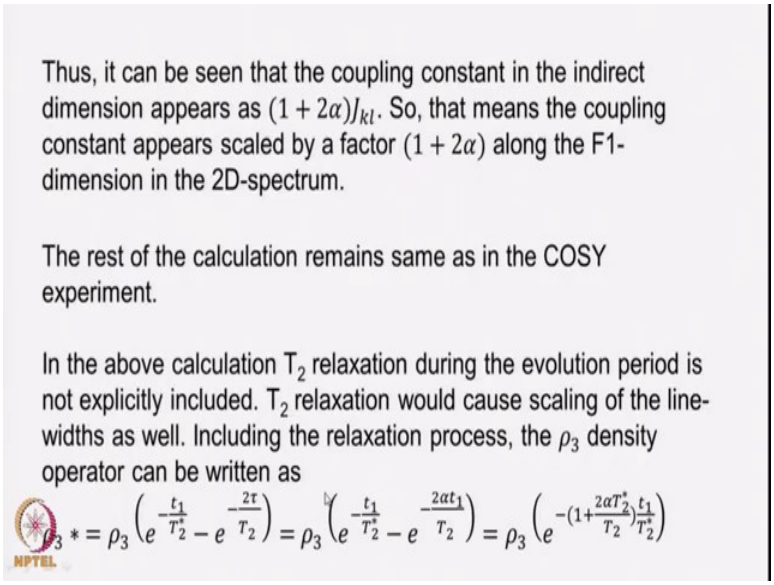

So, now therefore, you see the coupling term is multiplied by the factor $(1+2\alpha)$, okay. So therefore, when you Fourier transform this total thing along the F_1 dimension, the coupling concept will therefore appear as $(1+2\alpha)J$,. This is the coupling constant appears scale by a factor $(1+2\alpha)$, along the F_1 axis in the 2D spectrum. We will not go through the rest of the calculation along the t_2 evolution and things like that, that remains the same as in the COSY and we do not want to repeat that here.

The idea here was to show that during the t_1 period, the coupling constant appears scale by the factor $(1+2\alpha)$, and this is because of that introduction of the spin echo sequence $\tau -180 -\tau$. Now, in this calculation what we have done, we actually have not considered the relaxation that happens during the evolution period. We did not consider that in the previous cases as well, but here it is more important to consider that because there is a spin echo sequence in the evolution period. So what happens during this period?

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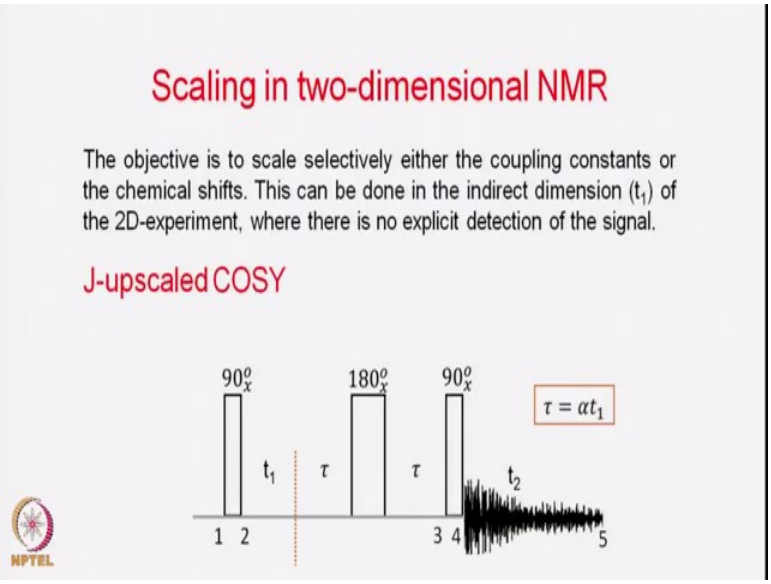

So if you look at this sequence here, during this spin echo, if you recall the previous discussions, the field inhomogeneity effects will also be refocused. The chemical shift refocusing means the field inhomogeneity will be refocus as well. Therefore, during this period the transverse relaxation happens with the time constant which is $t_2^{'}$which includes t_2 plus the contribution from the field inhomogeneity, whereas here during this period, the relaxation will be dictated by t_2 alone, there will be no field inhomogeneity contributions.

Therefore, the signal will decay in the following manner, up till here, it will decay with the time constant of$t_2^{'}$ star and from here to here it will decay with the time constant of t_2. Therefore, we have to calculate that explicitly and that is what we will show here.

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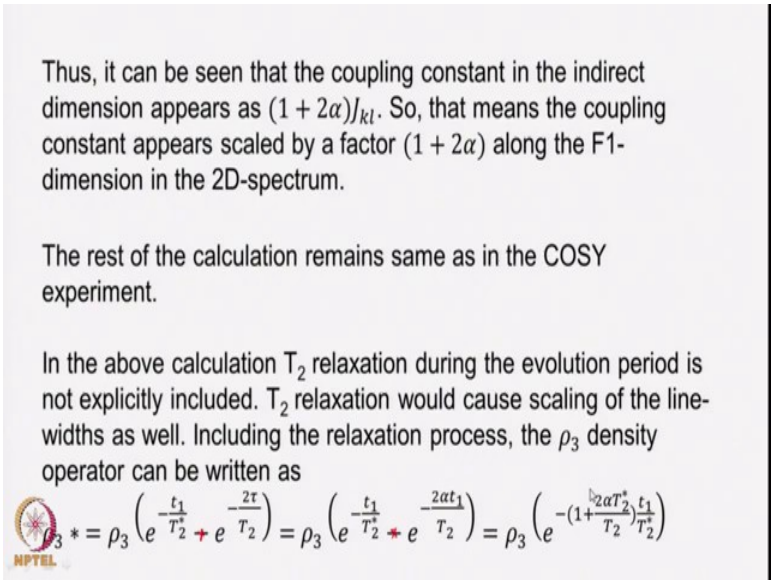

So this t_2 relaxation would cause the scaling of the line-width as well. So, that is what we would like to show what is the effect of T_2 relaxation during this whole period. Now, if I want to write the density operator as $\rho_3^{¿}$for this and I have this density operator ρ_3 which is without the relaxation considered and then I will have to multiply this by the relaxation factors. So, if during the t_1 period the time constant of relaxation is $t_2^{¿}$therefore I have this

$$\rho_3^{¿} = \rho_3\left(e^{\frac{t_1}{T_2^{¿}}} \times e^{\frac{-2\tau}{T_2}}\right) = \rho_3\left(e^{\frac{t_1}{T_2^{¿}}} \times e^{\frac{-2\alpha t_1}{T_2}}\right)$$

Because this total is the relaxation factor, this is the relaxation factor, this is the first relaxation during the time t_1 and this is the relaxation with the time constantly t_2. So, when you put this together then of course you will get

$$¿\ \rho_3\left(e^{-\left(1+\frac{2\alpha T_2^{¿}}{T_2}\right)\frac{t_1}{T_2^{¿}}}\right)$$

This is the same as this and this one ofcourse is coming from this term here. So therefore if I were to call this as a factor.

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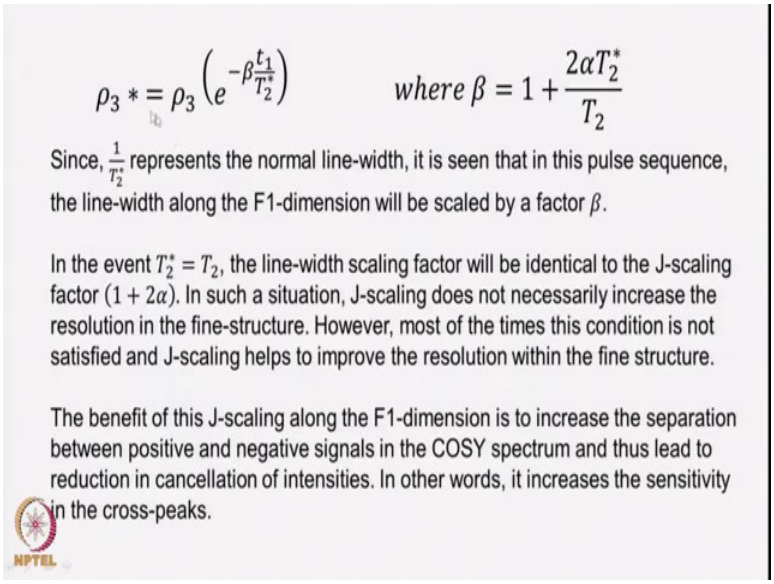

So,

$\rho_3^{¿} = \rho_3 \left(e^{-\beta \frac{t_1}{T_2^{¿}}}\right)$

Then where $\beta = 1 + \frac{2\alpha T_2^{¿}}{T_2}$

So therefore, the relaxation causes a modulation of the line-width as well, what is the line-width?

The line-width is $\frac{t_1}{T_2^{¿}}$ if we had simply e to the $\frac{-t_1}{T_2^{¿}}$, then $\frac{t_1}{T_2^{¿}}$ corresponds to the line-width $\frac{t_1}{T_2^{¿}}$ represents the normal line-width. Now, that is now multiplied by a factor beta, therefore the line-width will appear modified and along the F_1 dimension therefore, the line-width will be scaled by a factor beta, okay, that is this one here.

Now, in the event $T_2^{¿} = T_2$, the line-width scaling factor will be identical to the J scaling factor $(1+2\alpha)$ that is if $T_2^{¿} = T_2$ this will cancel and then you have $(1+2\alpha)$ here. So, the J-scaling then does not necessarily increase the resolution in the fine structure of the peaks, the individual components in the cross peak of the diagonal peaks will have a line-width, right and we are trying to increase the separation between those components by scaling the J values and therefore they appear more resolved.

However, if the line-width also increased by the same factor, then you do not achieve a significant improvement in the resolution. The *J*-scaling does not necessarily increase the resolution the fine however, in most of the cases, this condition is not satisfied and $T_2^{i} \neq T_2$. So therefore the relaxation time constants in the two cases are different. Therefore, this will be less than a T_2, $T_2^{i} < T_2$.

Therefore, this factor is the less than 1 therefore, you will have the line-width will be scale by a smaller factor than the J's and then you will get the benefit of up-scaling of the *J* values and then it will improve the resolution in the spectrum. The benefit of the *J* scaling along the F_1 dimension is to increase the separation between positive and negative signals in the COSY spectrum and does lead to reduction in the cancellation of the intensities.

Remember in the COSY, the cross peaks will have plus-minus character in the cross peaks and they would tend to cancel their intensities in the event of insufficient resolution. Therefore, if you are able to increase the separation then cancellation will be reduced and that increase the sensitivity of the cross peaks.

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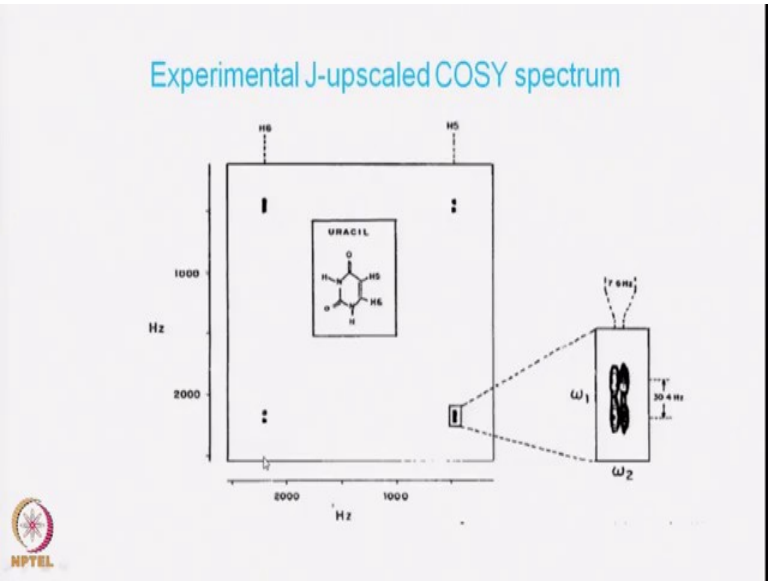

And here is an experimental example, this is a small molecule Uracil. It has 2 protons here and these 2 protons are J-coupled and the coupling constant between them is about 7.3 hertz, okay and this coupling constant appears in the F_2 dimension because we have done nothing in the F2 dimension during the T_2 period the evolution happens as with the normal COSY and it will

appear in the fine structure as well. So, there is 1 cross peak here and the diagonal peak here and this cross peak for example or this cross peak or this cross peak is actually blown up here in this 1.

We can see along the ω_2 dimension or the same as F_2 dimension this coupling constant remains the same this is 7.3 hertz and the same one now appears scaled along the F_1 dimension near the scaling factor is 4, $1+2\alpha=4$. Therefore, this appears multiplied by a factor 4, okay. So, this is a clear indication of the enhancement of the resolution and you can see the positive and negative components here very clearly, although there is the same color is used here but these positive negative components all the 4 components can be seen very clearly in this spectrum.

So, therefore this helps you to improve the resolution in the spectra and enable measurement of the coupling constants wherever they are not sufficiently well resolved in your spectra, okay.

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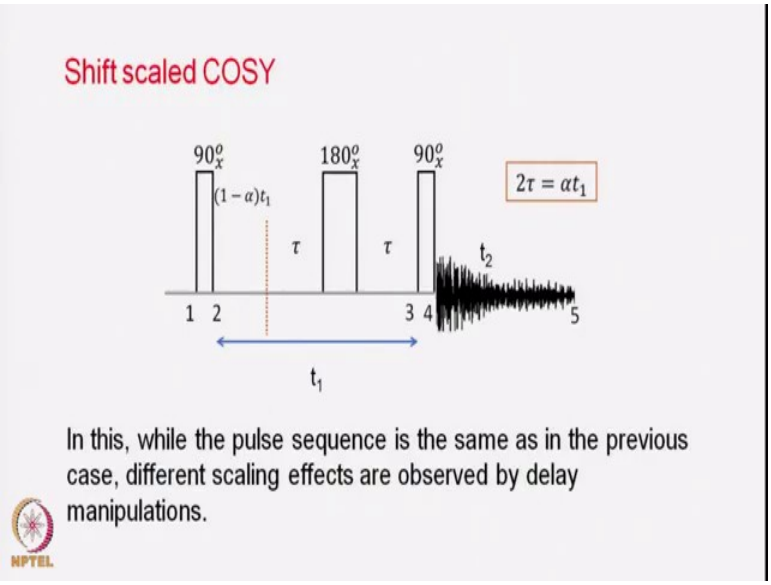

Now we see the next scaling experiment and it is a simple modification of the previous one. So this is called shift scale COSY. In the earlier case, we scaled the coupling constant number. Now we will scale the chemical shifts, what do we do? Is simple modification here, now the t_1 period is all the way from here to here. Earlier t_1 period was from here to here and this was additional, and we put this is alpha t1. So the whole period was $1+2\alpha t_1$, okay.

So now what we are doing is the, we are keeping this whole period from here to here as t1 and in between we introduced this, okay. So for $\tau - 180 - \tau$ this remains the same and $2\tau = \alpha t_1$ here, just a small change in the representation. So this whole period is called αt_1. So therefore, the chemical shifts will now evolve only for this period from here to here right and that is $t_1 - \alpha t_1$.

That is $1 - \alpha t_1$ and the coupling constants will evolve for the whole period t_1 whereas the chemical shifts will evolve for the period $t_1 - \alpha t_1$, okay. So the pulse sequence the same, we are just played around with the, the timing, how you adjust the timings of the various pulses, okay. Now this produces a different scaling effects absorbed by delay manipulations.

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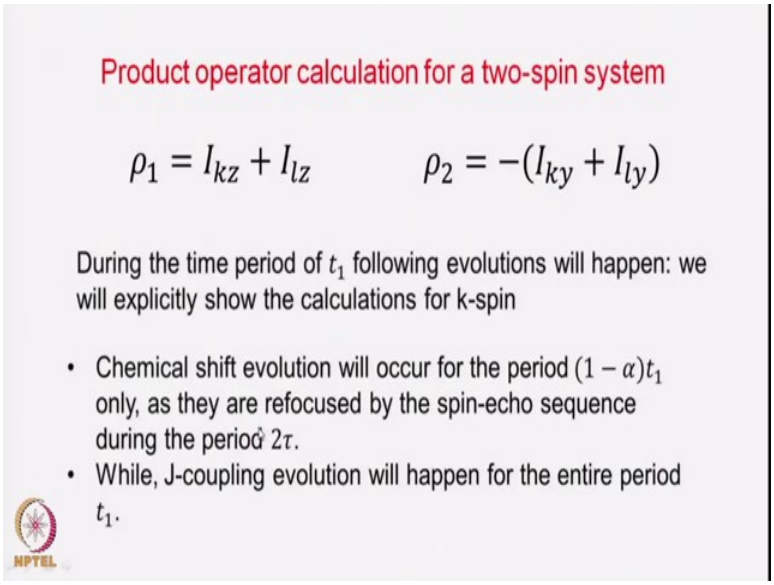

So what is the consequence here? We just do the same calculation once more. So during the time period t1 the following evolutions will happen. The again we show only for the *k* spin, the chemical shift evolution we occur for the period $1 - \alpha t_1$only. As they are refocused by the spin echo sequence during the period 2τ. While *J* coupling evolution will happen for the entire period t_1, okay.

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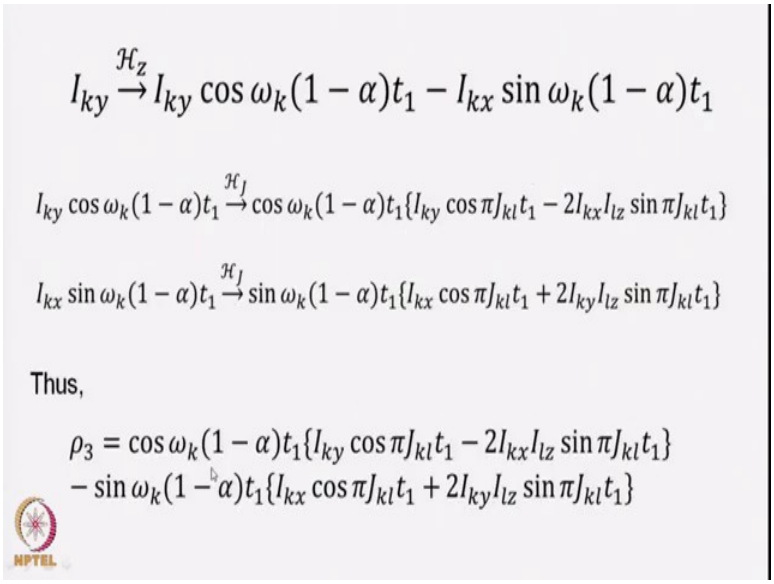

So we do this calculation

$$I_{ky} \xrightarrow{\mathcal{H}_z} I_{ky}\cos\omega_k(1-\alpha)t_1 - I_{kx}\sin\omega_k(1-\alpha)t_1$$

Now you all these under the coupling

$$I_{ky}\cos\omega_k(1-\alpha)t_1 \xrightarrow{\mathcal{H}_J} \cos\omega_k(1-\alpha)t_1\{I_{ky}\cos\pi J_{kl}t_1 - 2I_{kx}I_{lz}\sin\pi J_{kl}t_1\}$$

$$I_{kx}\sin\omega_k(1-\alpha)t_1 \xrightarrow{\mathcal{H}_J} \sin\omega_k(1-\alpha)t_1\{I_{kx}\cos\pi J_{kl}t_1 + 2I_{ky}I_{lz}\sin\pi J_{kl}t_1\}$$

Therefore

$$\rho_3 = \cos\omega_k(1-\alpha)t_1\{I_{ky}\cos\pi J_{kl}t_1 - 2I_{kx}I_{lz}\sin\pi J_{kl}t_1\}$$
$$-\sin\omega_k(1-\alpha)t_1\{I_{kx}\cos\pi J_{kl}t_1 + 2I_{ky}I_{lz}\sin\pi J_{kl}t_1\}$$

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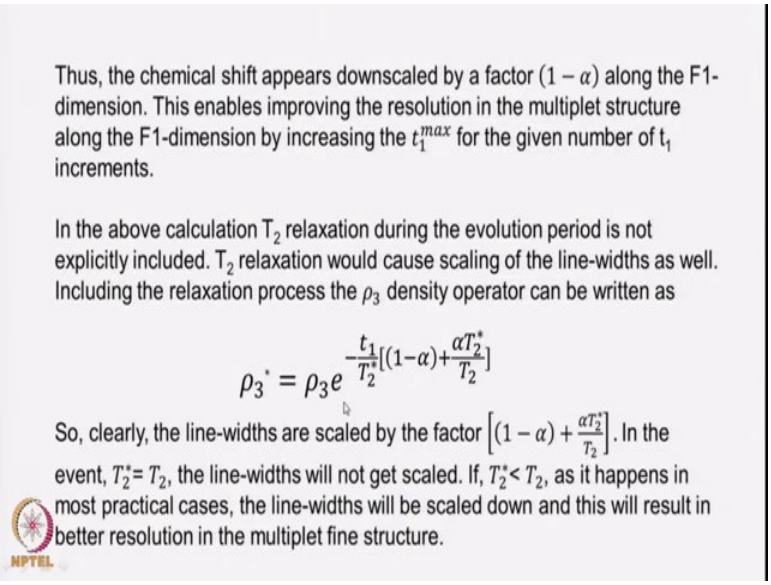

Okay, so therefore, the chemical shift appears a downscale by factor $1-\alpha$ along the F_1 dimension. Now this enables improve the resolution in the multiplet structured along the F_1 dimension by increasing the t_1^{max} for the given number of t_1 increments. How does that happen? Because now since you have scaled down the chemical shifts suppose you have the chemical shift range of 5000 hertz then you scale it by a factor of half then you will have if alpha is equal to half okay then it will be 1 minus half, so therefore it is 0.5.

So if you have that one then your chemical spectral range will be 2500 hertz, in which case your increment from experiment to experiment in the t1 will be twice that in the normal COSY where you had 5000 hertz. Therefore, for the same number of increments you are t_1^{max} will go up and therefore, you are inherent resolution in the spectrum will increase because you remember, it will depend upon what is the acquisition time, resolution will depend upon the acquisition time.

So this will be the acquisition time and this will get increased because you have change the chemical shift, okay, spectral range. So therefore, again you will get an improvement in the resolution, but however, the separation between the peaks overall that will get reduced. So as, so long as you can afford that you can do this and so long as you can do it in such a way that the

peaks do not overlap on each other you can do this and you can achieve enhanced resolution in the fine structure of the cross peaks or the diagonal peaks.

Now once again here, we have not included the relaxation effects, we can include the relaxation effects here true relaxation during the period is not explicitly included. Now if we include this it will cause a scaling of the line-widths as in the case of J scaling. Now if we include the relaxation effects we will get the rho 3 star explicit calculation will go in the same manner as was done for the J scaling effect. So you get a rho 3 into $e^{¿}¿$, $1-\alpha$, this is from the chemical shift and this is from the coupling of $\frac{\alpha T_2^{¿}}{T_2}$, okay.

So therefore, clearly if the line-widths are scaled by the factor, see this whole thing if I take away T_2, $\frac{T_1}{T_2^{¿}}$ this whole factor is the so called beta here and that is $1-\alpha+\frac{\alpha T_2^{¿}}{T_2}$. In the event $T_2^{¿}=T_2$, the line-widths will not get scale because this will become alpha and this $1-\alpha+\alpha$ will cancel and this will remain as $\frac{T_1}{T_2^{¿}}$ in that case it will not appear scaled, okay. But if $T_2^{¿}<T_2$ as it happens in most practical cases the line-widths will be scaled down and this will result in better resolution in the multiplet fine structure, okay. And this condition will always be satisfied.

The $T_2^{¿}<T_2$, you get improvement when you have this condition satisfied, the fine structure will appear much better when you have downscale the chemical shifts.

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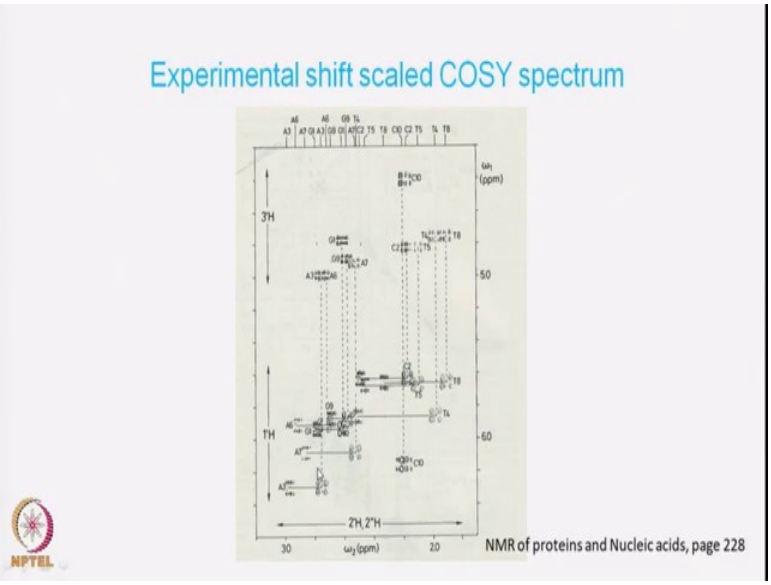

Okay, so here is an experimental example, practical example although you cannot see the fine structures here but this was used to improve the resolution in the fine structure of the peak. This is a spectrum taken from Wuthrick's book NMR of proteins and nucleic acids and this is a spectrum of a DNA molecule.

You have so many cross peaks here, what is shown here is under the cross peak region of a DNA segment, along this axis you have particular portion of the protons and this axis you have certain segments, these are basically 2 double prime protons in the DNA and this is the 1 prime protons in the DNA in the sugar ring. And these are the 3' protons in the sugar ring.

And you can see in every case, the fine structure here is improved along the omega 1 axis and one can actually use this to measure these individual coupling constants and then when you do that you can obtain information about the geometry of the sugar ring in the DNA segments. And that is the application why this was done, okay. So with this application, you could actually determine the coupling constants in the sugar ring and that will allow you to determine the geometry of the sugar ring in the DNA segment, okay.

So therefore, in summary, I have shown you here today a new concept that is how to manipulate the parameters in your spectrum, the parameters are the coupling constants and the chemical shifts, we have considered the up-scaling of the coupling constants, which will allow you to improve the sensitivity in the spectra and improve the separation between the multiplets in the

fine structure and thereby you can measure the coupling constants from their separations in the cross peaks.

And I have shown you the downscaling of the chemical shifts, you can afford to do this if the peaks are not too close by in the 2D spectrum, so long as they do not overlap with each other by downscaling you can do it and that will improve the acquisition time along the F_1 dimension. The t_1 dimension and that will improve the resolution along the F_1 dimension or the $\omega_1$dimension and as is indicated here. In the early days one used to use call as ω_1 and ω_2, but often people use your F_1 and F_2 as well.

So this is the application and we will see later how to increase the chemical shifts appearance of in the spectrum, the can we make the chemical shifts and hands of the scale down. So we will see that later and so with that we will stop here.