One and Two Dimensional NMR Spectroscopy for Chemists

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Lecture - 60

NMR Data Processing

Welcome back all of you. Since last two or three classes, we started discussing about some of the practical considerations that we need to look into while recording the NMR spectrum. We discussed a lot about how to choose the spectral width, how to choose the offset, what is single channel detection, what is quadrature detection, how do you tune the magnetic field, how do you shim the magnetic field, what type of sample you require, how do you choose the spectral width, how do you caliber a 90 degree pulse, all those things, we discussed in depth. Now let me assume you have understood everything, and you are now in a position to record the spectrum. You have collected the time domain data and now you are in a position to do the Fourier transformation and start processing the data. Of course, I already showed the spectra and I said you can do the Fourier transformation and you got the spectrum. But little bit deeper we can go now and see how we can process the data. This is our focus on NMR data processing.

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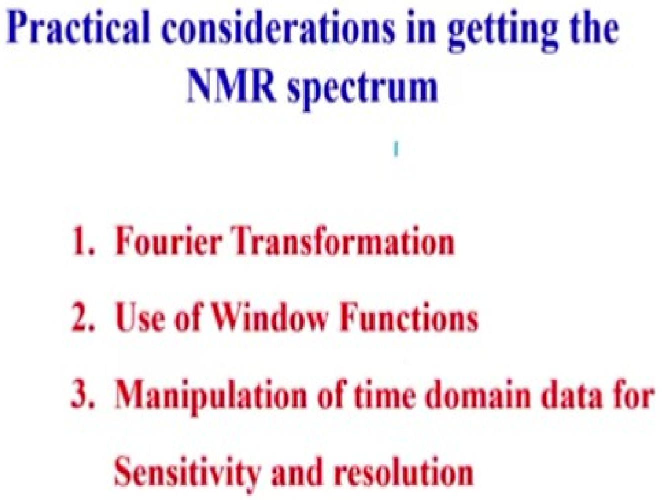

Practical considerations in getting the NMR spectrum

1. Fourier Transformation
2. Use of Window Functions
3. Manipulation of time domain data for Sensitivity and resolution

There are certain practical considerations in getting the spectrum and while processing the data. Of course, we should know we have to do the Fourier transformation and after getting the spectrum, sometimes we use window functions. What are these window functions, why are they used, we will discuss. And we can manipulate time domain data either to achieve sensitivity or the resolution or both, we can manipulate this. So these are some of the points we need to discuss.

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A Fourier transformation is a mathematical operation which converts the time domain signal into a frequency spectrum.

$$F(\omega) = \int_{-\infty}^{\infty} f(t) e^{-i\omega t} dt$$

$$f(t) = \frac{1}{2\pi} \int_{-\infty}^{\infty} F(\omega) e^{i\omega t} d\omega$$

In NMR the FID collected as a function of time, is Fourier transformed to obtain a frequency spectrum.

Let us start with Fourier transformation. Of course, we have been discussing even from the early classes, first, second or third class itself, we discussed that time domain signal can be converted to frequency domain by a mathematical operation called Fourier transformation. Then I said time and frequency are Fourier pairs. So this is the formula for converting the time domain into frequency domain. You collect the signal as a function of time, and do this mathematics and then you will get all the frequencies present in that.

Of course, you can do the reverse also. If I have a frequency domain signal, I can do inverse Fourier transformation and get the time domain signal. These are interchangeable. These are called Fourier pairs. Exactly what we do in NMR, is same. As I have been telling you, in NMR we collect the signal in time domain, which I referred to as free induction decay. You apply a radiofrequency pulse and then in the absence of the pulse, magnetization starts processing freely. While processing, it induces EMF in the receiver coil, which you collect and it is a damped oscillation, the signal starts decaying, which we said is free induction decay. And we collect this as a function of time, which we saw even in the last class, and several other classes and we do the Fourier transformation, now we are going to get a frequency domain spectrum.

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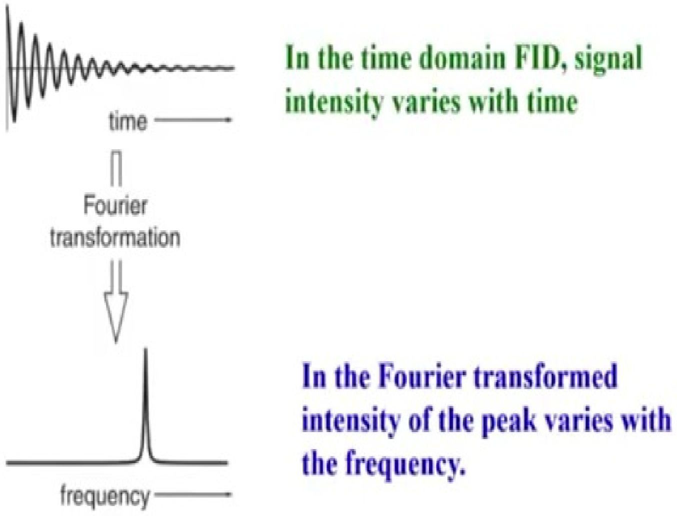

In the time domain FID, signal intensity varies with time

In the Fourier transformed intensity of the peak varies with the frequency.

This is what it is, all about NMR. You collected the signal in the time domain like this, what happens in the time domain is the signal intensity varies with time as a function of time, it is a damped oscillation. Now the Fourier transformation of this gives you peaks with varied intensities. This may have an intensity like this, other may intensity like this, other may have a small intensity, does not matter.

Finally, what happens in the time domain, it is the variation of the intensity of signal as a function of time. Here it is the variation of the intensity of the peaks in the frequency domain. So that is what is all about. So basically, you do not know anything. All you do is simply collect the signal and give a simple command called Fourier transformation in the instrument, and computer is going to give you the spectrum like this. This is what you finally require for doing the analysis.

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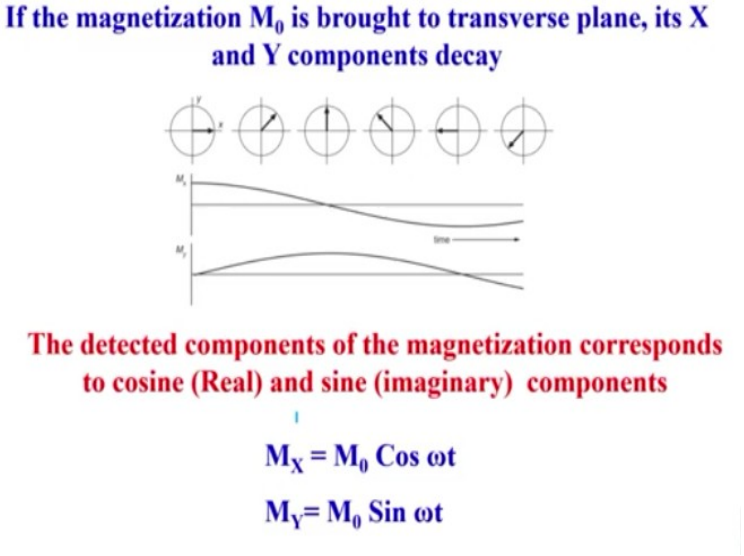

If the magnetization M_0 is brought to transverse plane, its X and Y components decay

The detected components of the magnetization corresponds to cosine (Real) and sine (imaginary) components

$$M_X = M_0 \text{ Cos } \omega t$$

$$M_Y = M_0 \text{ Sin } \omega t$$

Now let us see, what happens to the magnetization. We will discuss little bit about components of the magnetization. Now if the magnetization M_0 is brought to the transverse plane by applying a 90 degree pulse, which we already knew, how to calculate 90 degree pulse. I am applying a 90 degree pulse, brought the magnetization to xy plane. Let us say, I am going to bring it in this x axis. Now we have x and y components of this.

Remember this magnetization is a vector. We can resolve into two components. Look at this one here, the precision, how it is undergoing precision. The magnetization is here, starts going like this, starts going like this, starts going like this. So you can resolve them into two components, Mx component and My component, that is the cosine and the sine component. We also discussed this in the previous class. We call it as real and imaginary and after Fourier transformation. I said it is an absorptive and dispersive type peaks. So, the detected signal here, the time domain signal, we can resolve them into two components cosine and sine, real and imaginary components. This we can represent by a simple equation like this. This we already knew earlier also. An oscillating wave with an amplitude and frequency is always represented like this.

Now Mx component of this, I represent as M_0Cos ωt. What is M_0? M_0 is our magnetization, which is in thermal equilibrium. The magnetization before application of the pulse, which was along z axis. That is considered as M_0. Now this M_0 has two components, MX correspond to cosine component, which is written as M_0Cos ωt; where omega is the frequency. My = M_0Sin ωt, this is simple. I think you all know; we can resolve this magnetization in two components.

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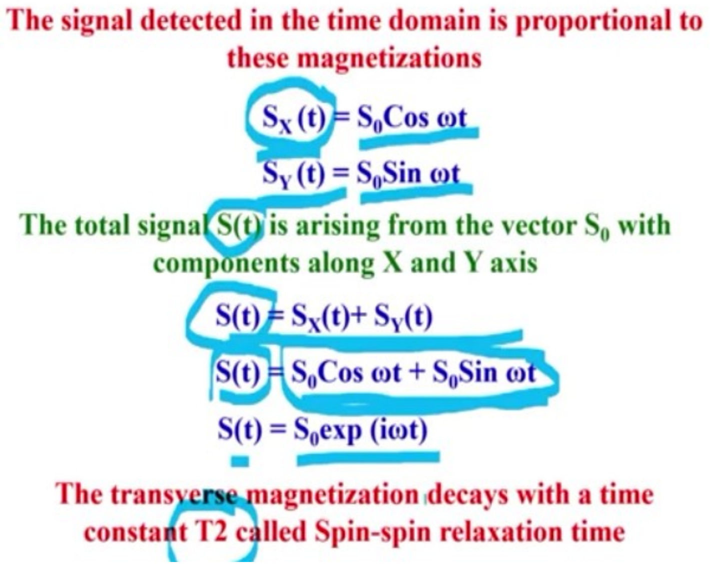

The signal detected in the time domain is proportional to these magnetizations

$$S_X(t) = S_0 Cos\ \omega t$$

$$S_Y(t) = S_0 Sin\ \omega t$$

The total signal S(t) is arising from the vector S_0 with components along X and Y axis

$$S(t) = S_X(t) + S_Y(t)$$

$$S(t) = S_0 Cos\ \omega t + S_0 Sin\ \omega t$$

$$S(t) = S_0 exp\ (i\omega t)$$

The transverse magnetization decays with a time constant T2 called Spin-spin relaxation time

Now the signal that is detected in the time domain is proportional to this magnetization. What is the amount of magnetization present in the x axis or in the y axis, or the components of the magnetization? That is what the signal we are going to detect. This we saw earlier also. Let us say, the Sx is the signal in the x axis. I call it as S_0Cos ωt.

M_0 which is total magnetization. Now I am taking signal in the time domain; Sx of t corresponds to S_0Cos ωt. The same y component signal in the y axis, as a function of time is S_0Sos ωt. But the total signal St is the sum of Sx(t) + Sy(t), like M0 = Mx(t) + My(t); earlier in the previous slide we saw.

Now what I am telling is when the signal which we are collecting the time domain, S(t) can be resolved into two components, signal along x axis and signal along y axis collected as a function of time. So total signal is sum of Sx(t) + Sy(t). This is the total signal that we are collecting. This is FID. Now we know what are Sx and Sy. The Sx = S_0Cos ωt and Sy = S_0Sos ωt. Plug in these terms here. The total signal is written as S(t) is equal to this thing. Now you can express this as an exponential. This is the basic trigonometry; you would have studied in the early days.

Now combining these two, we can write as St = S_0 exp(iωt). So, the transverse magnetization decays with the time constant T2. It is the signal, which we are collecting, but how are we collecting it. It is also decaying, you know that. I said there is decay constant; it is damped oscillation; and we already discussed earlier that decay of the magnetization in the transverse plane is called T2; spin-spin relaxation time.

We already discussed this. It is a relaxation time in the transverse plane. That is the total dephasing time in the xy plane, the transverse plane. So this has to happen, it is a decaying function. So plug it in.

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Since decaying NMR signal is exponential, the frequency spectrum is a Lorentzian

The real part of the spectrum is called absorptive Lorentzian and the imaginary part is called dispersive Lorentzian

$$S(t) = S_0 \exp(i\omega t) \exp(-t/T_2)$$

If the time constant T_2 is shorter, then the signal decays more rapidly

Since it is a decaying signal, of course when you do the Fourier transformation, you are going to get a Lorentzian, that is the first part. Now when you do the Fourier transformation, you get absorptive Lorentzian as a real part and imaginary part is called dispersive Lorentzian. Apart from that now first you plug in the decaying component, the exponential function, this we knew. We are collecting the signal; you plug in $e^{(-t/T2)}$. This we are plugging in because we know signal is decaying as a function of T2.

Now what it is going to tell us? What happens if T2 is shorter? Signal decays very rapidly. If the T2 is long, longer the T2, the signal takes more time to decay. That is the physical explanation here. Look at this term. So this decay term depends upon T2. So you can talk about the decay of the signal in terms of T2.

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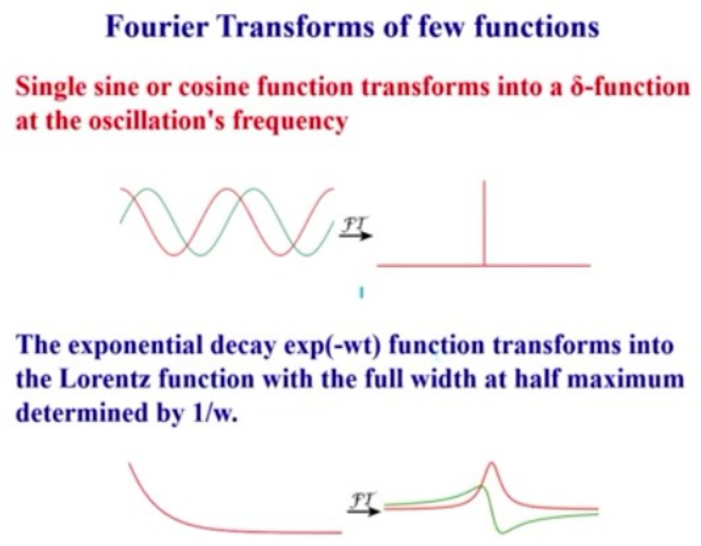

Fourier Transforms of few functions

Single sine or cosine function transforms into a δ-function at the oscillation's frequency

The exponential decay exp(-wt) function transforms into the Lorentz function with the full width at half maximum determined by 1/w.

Before you proceed further, since we are doing Fourier transformation, I want to tell you few functions. You just remember, you can work out, because we knew the equation for Fourier transformation, I told you know. So I have given you already the equation of the Fourier transformation. You can use that, like f of omega is equal to integral of minus infinity to plus infinity f(t) $e^{i\omega t}$ dt, that you wrote already. I showed you in 2-3 slides before.

You can plug all these functions, whatever you want. If you know what is the time domain function and if you know the boundary conditions of the function, you can work out and find out the Fourier transformation. You can do the Fourier transformation of all the functions. Let us say, I have a single sine or cosine function. Of course, this is a cosine function; and this is the sine function, starting from the origin.

If I do the Fourier transformation of these functions individually, we get a delta function. Where does it come? you can calculate the frequency from the separation. This is lambda, you know that, inverse of that is the frequency. So you can find out, the oscillation frequency and it is a delta function. I am just giving you the information. Please remember, because we will be using Lorentzian, Gaussian, etc., after the Fourier transformation.

On the other hand, let us say, I take an exponential decay function. If it is exponential decay, like we saw in the previous slide or signal it is decaying exponentially, if you do the Fourier transformation, you must get the Lorentzian. This is the important thing, and this Lorentzian function is a spectrum like this, you get. Now you can calculate the full width at half maxima, this is one. This is given by 1/ω.

If it is exponential omega t, this full width at half maxima is given by 1/ω. This is an exponential function. NMR signal, what we detect in the solution state is an exponentially decaying function. So the frequency domain spectrum is a Lorentzian.

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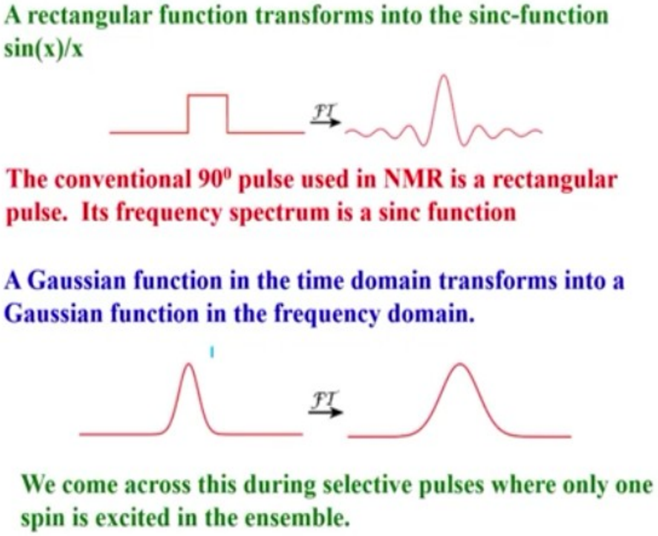

A rectangular function transforms into the sinc-function sin(x)/x

The conventional 90^0 pulse used in NMR is a rectangular pulse. Its frequency spectrum is a sinc function

A Gaussian function in the time domain transforms into a Gaussian function in the frequency domain.

We come across this during selective pulses where only one spin is excited in the ensemble.

Of course, you should know; this we come across very often. We already discussed in the previous class while discussing something about FID truncation. So this is like a square pulse or a rectangular pulse. Depending upon the width of this, if you do the Fourier transformation, of course, you get a sinc function. This is called a sinc function and the zero crossing here is inversely proportional to this width. If this width is t, the zero crossing is equal to here -1/t, here +1/t. See this is Fourier transformation of a rectangular function. Conventional 90 degree pulse, what you use in NMR is a rectangular pulse depending on the width or you can call it a square pulse, but the frequency spectrum is a sinc function. Please remember, these are some of the things, which you must remember; you can work out, by mathematics. I do not want to work out each and every function, do the Fourier transformation and show you what it is. But I am just giving you what is the starting function in time domain; what you get in the frequency domain? If I take a Gaussian, that is Gaussian function and do the Fourier transformation, interestingly you get in the frequency domain also a Gaussian function. We come across this very often when we do the selective pulses, where only a single spin is selectively excited in the ensemble of spins, I can selectively excite a particular spin or band of frequencies. Then, I tell you we come across a thing that is why I am giving you this information. If I say it is a Gaussian function, the Fourier transformation of that is also a Gaussian.

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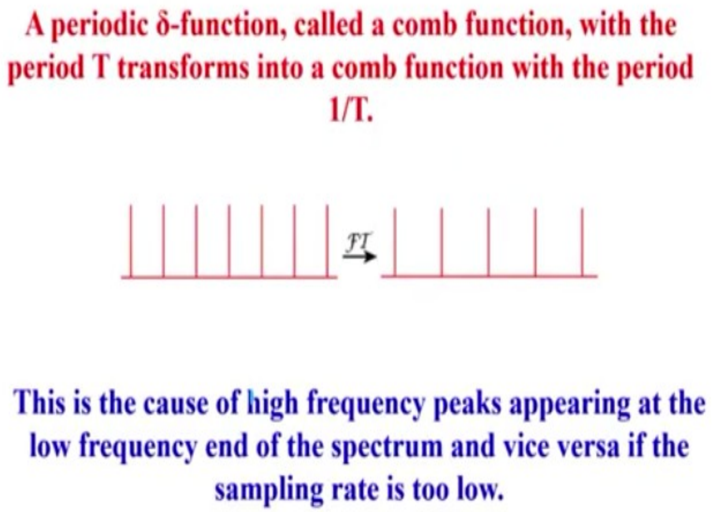
A periodic δ-function, called a comb function, with the period T transforms into a comb function with the period 1/T.

This is the cause of high frequency peaks appearing at the low frequency end of the spectrum and vice versa if the sampling rate is too low.

We also come across what is called as periodic delta function. What is delta function? It has no width and it has infinite intensity at particular frequency 0, and its value is 0 on anywhere at any point outside the 0, this is a delta function. And many such periodic delta functions is called a comb function. And this comb function, how often it repeats is given by T, and interestingly, the Fourier transformation of this is also a comb function, with period 1/T.

This is what the reason for high frequency peaks appearing with low frequency end of the spectrum and vice versa if we do not obey Nyquist theorem. You understand. Remember what is the periodic function? It is a comb function, and it transforms into a comb function with period is equal to 1/T.

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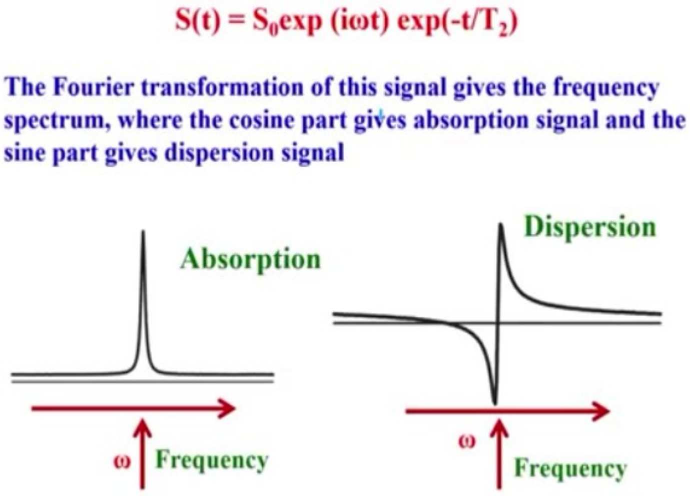

$S(t) = S_0 \exp(i\omega t) \exp(-t/T_2)$

The Fourier transformation of this signal gives the frequency spectrum, where the cosine part gives absorption signal and the sine part gives dispersion signal

With that, we will come back to our expression, S(T) we detect in the xy plane, is like this. Both x and y axis combined together, expressed as an exponential function, and we have incorporated the decay function because of T2 damping oscillation. Now Fourier transformation of this signal gives the frequency spectrum, with cosine part as pure absorptive and sine part as pure dispersive. Look at it. This is called absorptive spectrum.

This is called dispersive spectrum, and at the center of this, you are going to get frequency of the oscillation. And at the center of this here, at the 0 crossing point, you get the frequency from the dispersive spectrum. Simply remember, a decaying function, which you collect in NMR, when you do the Fourier transformation, you get two types of peak. This is called the real part. This is called the imaginary part. It is called absorptive signal. It is a dispersive signal.

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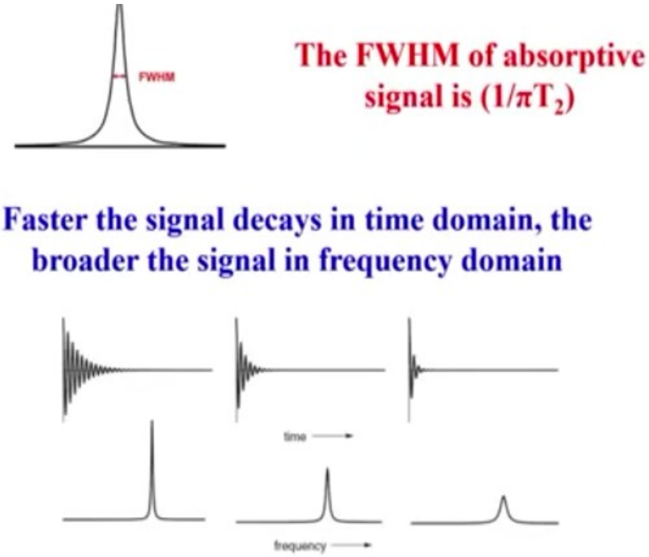

The FWHM of absorptive signal is $(1/\pi T_2)$

Faster the signal decays in time domain, the broader the signal in frequency domain

Now I said, this has a function exponentially $e^{(-t/T2)}$. Now I also said in the Lorentzian it depends upon full width at half maximum, which is equal to $1/\omega$ I said. Remember, in one of the slides I said $e^{(-i\omega t)}$, somewhere here I said, you know. Then the transverse magnetization decays with a time constant T2, not here, somewhere I said. It is here, its full width at half maxima is determined by inverse of that frequency.

So now we are seeing here, $e^{(-t/T2)}$, so we can get the inverse of that line width. If I know what this line width is, I know what is the decay constant. This T2 I can get it. The full width at half maxima of this absorptive signal is given by $1/\pi T2$. So if I know this, I know approximately or more or less precisely what is T2. What does it mean? I can approximately calculate this spin-spin relaxation time. If I have a broader signal, what does it mean? If I have a broader signal, signal is decaying very fast. If I have a sharper signal like this, if the full width at half maxima is very small, we get sharp signal. That means the signal will be decaying for a long time. Look at here, FID is dying down very fast. As a consequence, if you collect for a short time and do the Fourier transformation, you have a larger line width. So the width of this spectrum, full width at half maximum, inverse of that will give you T2. That is spin-spin relaxation time.

Remember this, if you do not understand anything, at least please remember faster the signal decays in the time domain, broader the signal the frequency domain. If decay is very fast, it gives a broad signal. If decay is very slow here, it gives a sharp signal. So I told you, in 1 or 2 classes while doing the shimming, you have to ensure that signal decays very fast. You get a lock signal; you have to use the lock signal to tune the homogeneity. When you are shimming using the shim coils, finally you get a better homogeneity. Essentially you will see that free induction decay will be decaying for a longer time. Longer the time it decays, sharper is the signal in the frequency domain. But remember whatever may be that, signal may be broad or sharp, do not get confused, nothing is happening. The area of the peak remains same. The area will not change, whatever happens, the area of the remains same. So you can still integrate the signal and know how many protons are present in each peak.

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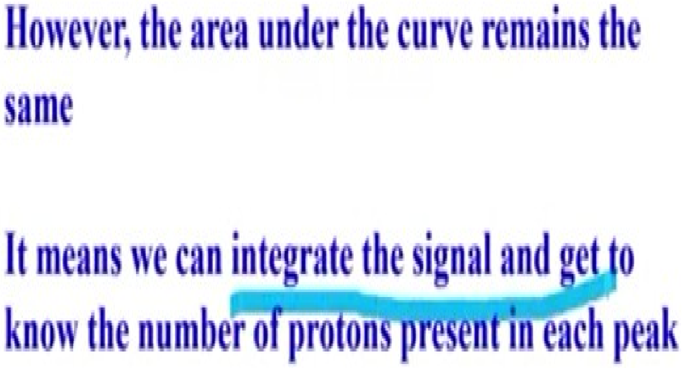

However, the area under the curve remains the same

It means we can integrate the signal and get to know the number of protons present in each peak

Remember while analysis we discussed this. The area of the peaks will tell us the number of protons present in that peak, you can compare relatively with some other peak. If I know the peak, which has only one proton, relative to that you find out the area of the other peak, then you find out how many protons are present.

Remember, I gave the example of proton count with integral intensity with a couple of examples earlier. So that means, you can still continue to do the integration irrespective of the fact whether the peak is sharp or broad, area remains same. It will not change; and still you can find out the number of protons present in each of these peaks.

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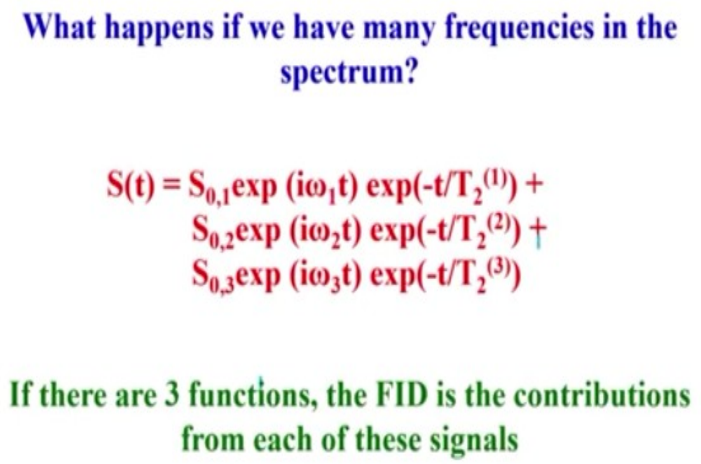

What happens if we have many frequencies in the spectrum?

$$
\begin{aligned}
S(t) = {} & S_{0,1}\exp(i\omega_1 t)\exp(-t/T_2^{(1)}) + \\
& S_{0,2}\exp(i\omega_2 t)\exp(-t/T_2^{(2)}) + \\
& S_{0,3}\exp(i\omega_3 t)\exp(-t/T_2^{(3)})
\end{aligned}
$$

If there are 3 functions, the FID is the contributions from each of these signals

Let us now start questioning a bit more. So far, I took only one signal, one decaying frequency to understand different components of it, etc. In reality, the NMR spectrum only one signal will not be there. FID may not contain a single frequency. There may be so many peaks in the spectrum. There will be a lot of frequencies. Many frequencies will be there. Then the question is what happens if you have many frequencies in the spectrum? How does it work?

Let us say, we have three frequencies in time domain. We have worked out this example. Signal S0 as a function of St as a function of T2, it is decaying, we wrote this expression. Now let us say, this is for frequency 1. I have another time domain signal with a frequency omega 2, one more peak is there; and one more peak is there. Thus, let us say, three different types of time domain signals, each of them with a different frequency and having different T2. They are decaying.

If I have a single frequency, we saw a single oscillatory signal. When all the 3 are, there could be many more, then you are going to see the time domain signal as an interferogram. Now the question is, if there are many such FIDs, many such time domain signals, how does each of the signals contribute to FID.

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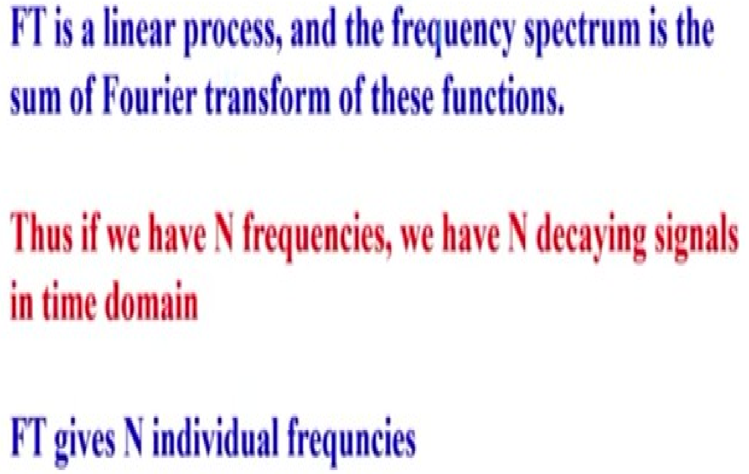

FT is a linear process, and the frequency spectrum is the sum of Fourier transform of these functions.

Thus if we have N frequencies, we have N decaying signals in time domain

FT gives N individual frequncies

Also, one of the important point to remember is Fourier transformation is a linear process and the frequency spectrum is the sum of Fourier transformation of individual time domain functions. There are 3 FIDs in the time domain corresponding to 3 frequencies, which were present. Do the Fourier transformation, we will get 3 frequencies. Now in the realistic NMR spectrum, there will be N frequencies present. You have N decaying signals, each with a different T2, no problems.

Each of them would have a different T2; they are all decaying signals. Collect them. It is an interferogram, you do not know. It is a mixture. Do the Fourier transformation, you will get individual frequencies; N individual frequencies. So it does not matter. The explanation which was given for a single FID holds good, even if the time domain signal has a superposition of several frequencies.

Finally, do the Fourier transformation. That being a linear process, you are going to get frequency domain spectrum with as many number of frequencies, which are present in the time domain signal.

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Phase Correction in the Frequency Domain

Now we have to do what is called phase correction in the frequency domain. We discussed about pulse phases and phases of the signal. In the previous class and earlier also, we discussed this 2 or 3 times.

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The appearance of the spectrum depends on the position of the signal at time zero

This depends on the starting point of a sinusoidal function is called its "phase (φ)"

$$S(t) = S_0 \exp(i\varphi)\exp(i\omega t)\exp(-t/T_2)$$

A phase term gets introduced to the signal

Now let us see the appearance of the spectrum, how it depends? Remember, I am going to apply a radiofrequency pulse and bring the magnetization here. Immediate or instantaneously, the spin vectors all of them are in coherence. I have to start collecting the signal immediately, without any time lapse. If there is a time lapse, what happens? Some problems of phases will come into picture. So the appearance of the spectrum depends on the position of the signal at time T = 0.

Immediately after the pulse, the time is equal to 0, you have to start collecting the signal. If pulse is applied here; give some delay and start collecting the signal here. We get into phase problem. So you have to literally or in principle start collecting the signal immediately after the application of the pulse at time T = 0. If the starting point is different, we have what is called the phase errors in the sinusoidal function. Now we will incorporate this one here.

This is this thing, we are going to get as the time domain signal. This I knew, I told you already and if you incorporate this for a decaying function. Now let us say, there is a phase error, that I incorporate here. This is the phase component. The phase term I am going to introduce here to the signal. So the total signal is; this is because of the phase term which is introduced, and the decaying signal as a function of T2; and this is signal collected from the NMR sample. So this is the total signal, including the phase error, that is already present.

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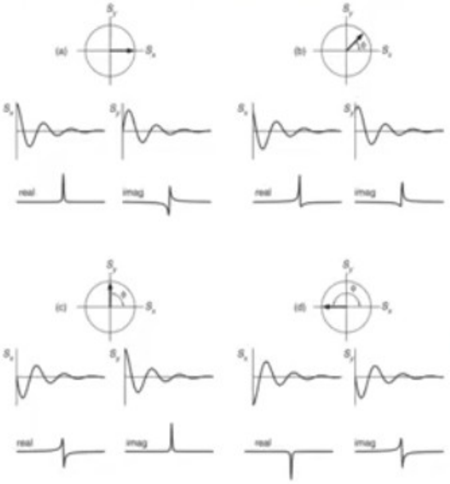
The real spectrum depends on the position of the signal at time zero, that is on the phase of the signal at time zero



Now you know why the phase error comes. I will not go into this in detail, because we discussed this earlier also. Now if my signal is detector is here, if the signal is along x axis, I told you, we have a real part, which is absorptive, imaginary part, which is dispersive. Both of them are out exactly out of phase by 90 degree. You collect a real absorptive spectrum without any phase error. See there is no phase error.

The angle difference between cosine and the sine part must be exactly 90 degrees. In case, if the signal is not along x axis, it is somewhere at a different place. Then what happens? There is a mixture of phases here. See the real component is not pure absorptive and the imaginary part is not pure dispersive. You get into a mixture of phases in the spectrum. This is what happened, and we saw what happens if the magnetization is along y axis or –x axis. You can even see if it is along –x axis, real and imaginary components gets interchanged. When your detector is along x axis, signal is along –x axis, we will get a real absorptive peak, which is negative maximum. So this is how you should know about the phases of the signal depending upon the pulses.

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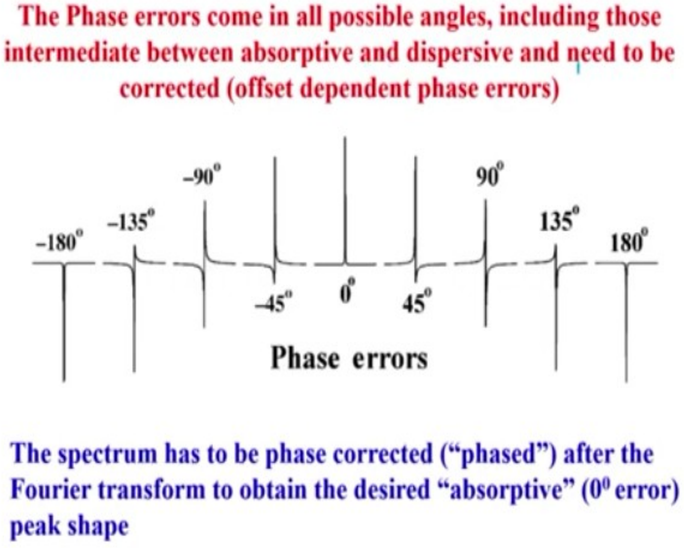

The Phase errors come in all possible angles, including those intermediate between absorptive and dispersive and need to be corrected (offset dependent phase errors)

The spectrum has to be phase corrected ("phased") after the Fourier transform to obtain the desired "absorptive" (0° error) peak shape

So let us say, there is no error, but generally there will be error. So phase error comes in all possible angles, including intermediate between absorptive and dispersive; that is what I said. At 45 degrees, you have a mixture. It need not be 45 degree, it can be any angle. If they are not exactly out of this by 90 degree, let us say, there is a phase difference of 85 degree, 80 degree, 70 degree, different angles will be there between cosine and sine.

Accordingly, our signal intensity, signal if you look at it, they have different phases. Let us say, this is a perfect signal, on resonance. There is no difference. There is no phase error, and cos and sin components are exactly out of phase by 90 degree. The phase error is 0. You can go on either side. I showed you the signal it can be in the first quadrant or the fourth quadrant. So you can have phases, both positive and negative.

It can go like this, if the phase error is 45, 90 degree, 135. If the phase error is exactly 180 degree, you can get a negative signal, that is what we saw. Our detector was along x axis, the magnetization was along –x axis. I said real and imaginary gets interchanged. Real part is a negative absorption; that is what I said. Same thing happens, if you go on other side. So the phase errors can come in all possible angles. This is a very important thing.

Now how do you correct the phase. This is a phase error. And it can be frequency dependent and frequency independent, both types of phase errors are possible. We will discuss that now. But how do you correct it? The phase correction is nothing but your real component and imaginary component, you have to adjust it, electronically or you can do in the instrument, in such a way to ensure, that these 2 components are exactly out of phase by 90 degree. In which case you will get pure absorptive spectrum like this without any phase error. So what do you mean by phase correction? It is you are going to ensure that real and absorptive part of the signal have exact the phase difference of 90 degrees. That is what is all about phase correction. We can discuss more about phase corrections and manipulation of the time domain data, how we apply window functions, everything in the next class.

Right now, I am going to stop here. Today, of course, I started telling you about the processing, I introduced to you Fourier transformation. I explained to you what happens when there are many signals in the time domain, many time domain signals overlapped, what happens if you do Fourier transformation. I said you will get that many number of frequencies in the frequency domain, because Fourier transformation is a linear process.

And also gave many examples of how phase errors can come and what will happen to Fourier transformation for different types of functions. Many, many things we discussed today. We will continue our discussion tomorrow on processing of the data.