One and Two Dimensional NMR Spectroscopy for Chemists
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Lecture - 59
Practical Considerations of 1D NMR

Welcome back. So we will continue our discussion on some of the important practical considerations while acquiring the NMR spectrum, and how do we process that? How do we acquire the data and how do we process it? We were discussing varieties of points about acquiring the time domain signal. How do we acquire the signal? Of course, we will discuss about the processing later. So now we will continue further. Last week, we discussed about signal to noise ratio and varieties of things. Now, today I will discuss something more about pulse phase and signal phase.

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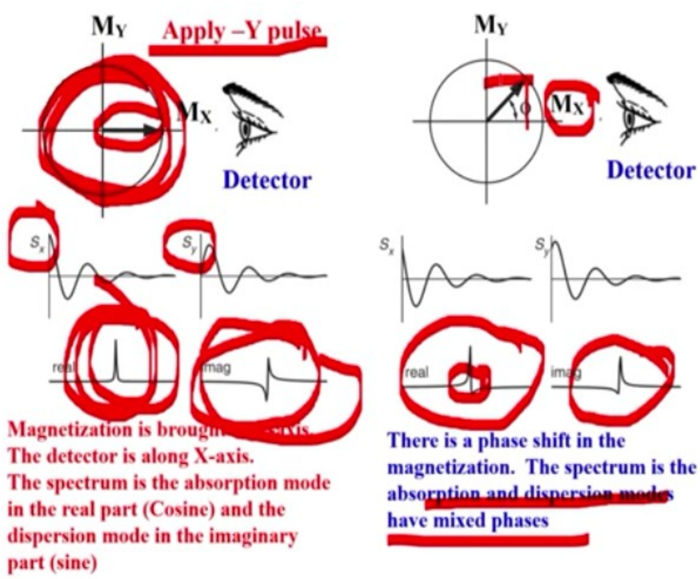

Remember we discussed this at some stretch earlier. This is very important while acquiring the signal. Now let us say, I am going to apply a pulse along some axis and bring the magnetization. It is initially under thermal equilibrium along z axis, to x axis. My detector, let us say, is along x axis. As soon as I bring the magnetization immediately after the pulse, that is you bring the magnetization to x axis; they are all in coherence.

All these spin vectors are in coherence. We will get maximum signal here, that is what I said when I was discussing T2, then afterwards there is a decoherence. So there is a coherence statistically, we will say, there is a coherence of all the nuclear spins along this axis. All the magnetic moments come there. Now if I do the Fourier transformation, remember, I am going to get real part and imaginary part. The Fourier transformation contains the real part and the imaginary part.

Real part is a cosine part. You see it starts like cosine function, decaying and oscillatory. Let us look at this one; and when you do the Fourier transformation, I get a signal, which is in phase like this. This is called absorptive signal. On the other hand, because Mx can be resolved into two components, I call this signal Sx, that is X component of singal S, or Sy, the Y component. This is the signal here. Sx is a cosine component, that is called the real part of the spectrum or absorptive peak. And Sy is the imaginary part of the signal, after the Fourier transformation, you will get a dispersive signal like this. Real part is always absorptive and imaginary part is dispersive. And now the signal is along x axis. My detector is also along this axis. Now I am going to see this type of signal. In the frequency domain the real part is like this absorptive and the imaginary part is dispersive. Let us say, I will bring the magnetization, instead of x axis, somewhere in between x and y axis.

Let us say from z axis, I will bring it somewhere here, which makes an angle φ with respect to x axis. This is my detector. Now the magnetization Mx is not maximum, like what you expected when all the signals instantaneously, all these spin packets, vectors were brought to this axis, creating phase coherence. Here we have brought it at 45 degrees, let us say, some angle phi. Now you have to resolve them into two components. You have two components here.

Cosine and sine component both are present here. Now it is not a pure phase, unlike here, where you were getting absorptive signal in the real part and dispersive signal in the imaginary part. Now there is a mixed phase here. So because of this mixture of absorptive and dispersive, phase is not exactly like along x axis, all the spins are not along x axis. Now there is a mixture of absorption-dispersion modes. We would get mixed phases. Look at it. This is the type of signal you are going to collect.

Then after Fourier transformation, the real part is not exactly absorptive. There is some phase mixture of imaginary and real parts, both are present. As a consequence, there is a phase shift. Same way in the imaginary part, you see the dispersive part also, dispersive signal is also not exactly like what you would have expected. Remember, here I am applying a pulse along some axis minus y brought the magnetization here.

You can apply any angle of the pulse, which brings the magnetization to x axis or y axis from z axis, is called 90 degree pulse. I can continue further like this to 180, go continue like this, it is 270 and go like this to 360. I can keep on making the magnetization to rotate along this axis, along this plane, continuously it can rotate. On the other hand, I can bring it to any axis, then I can have mixed phases like this.

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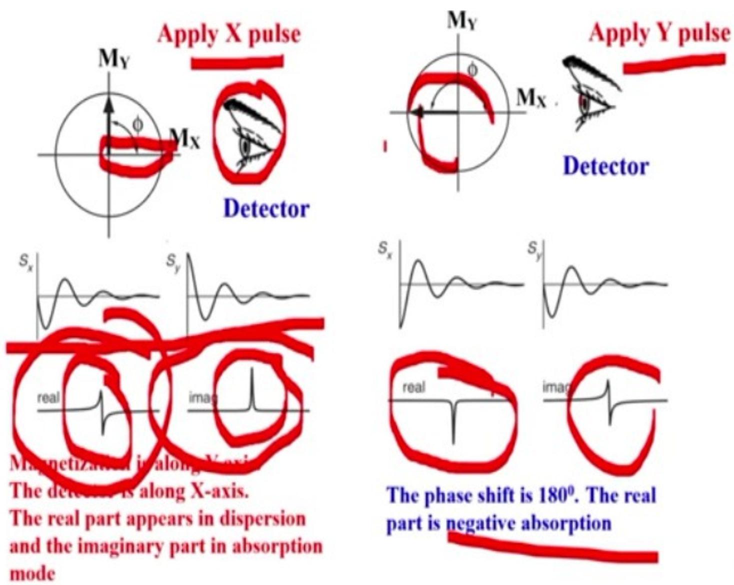

Now I am looking at this one. Some pulse I am applying. I have just written x pulse; you can find out if it is right or wrong. I told you how we flip the magnetization. Magnetization is flipped in a particular axis. You can apply right hand thumb rule and find out which direction it is flipping. If I apply a magnetization along x axis, magnetization is along z axis, let us say this is considered to be x axis and this is y axis. Now the magnetization has come to y axis.

So which direction you have to apply the pulse, because y axis is this side, x is along this axis. Magnetization is along this axis. Imagine a three dimensional axis. Now our detector is here, and magnetization is along y axis. So, what did I do? Apply a x pulse, magnetization along z axis curly fingers you see, it came to y axis. So now my detector is here. What is the type of signal I am going to see for real and imaginary? Remember, now the real part, which was here has gone here. That means real part appears like dispersive and imaginary part appears like absorptive. Interesting, right. So just because of 90 degree phase shift, you shifted the magnetization from x axis to y axis, but your detector is here. Now when you do the Fourier transformation, look at the frequency spectrum, we will find, now the real and imaginary parts have not changed, they are out of phase by 90 degrees. The real part is now dispersive and imaginary is absorptive.

On the other hand, I will do one thing. I will apply a pulse along y axis, no problem. You can find out whether it is right or wrong, by application of right hand thumb rule and bring the magnetization to, let us say, minus x axis. What is going to happen? My detector is here. Now it is seeing the signal in this direction, opposite.

It is actually the real part is a negative absorption and similarly imaginary part compared to this one is reverse one. In the previous example, you would have seen it. So this real part is negative absorption. That is the important point you are seeing here. On the other hand, if it had come here, that would have been a different thing. You can work out. So this is the way, with the application of a particular pulse or particular angle, in a particular direction, you can find out the phase of the signal.

After Fourier transformation, how does the signal behave? Whether real is absorptive component or imaginary, is it dispersive or vice versa, or is there a mixed phase. This is how you can play with the pulses, play with the direction in which you are going to apply the pulse and manipulate the direction in which you can flip the magnetization. That is a very, very important concept, remember. This is what we people do, when we design the pulse sequences.

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Setting Carrier Offset

Next another point, I wanted to tell you is, we need to set the carrier offset. Remember, we discussed about the rotating film, etc. I need to keep the carrier exactly on resonance, then frequency on the right side is the faster component, positive frequency and the left side of the carrier frequency, these frequencies are negative frequency. So these are all positive and negative frequencies I would say. But I need to have a receiver. The receiver, if I keep at one place, let us say, I will show here.

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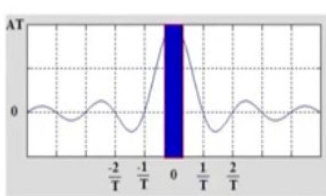
The carrier offset has to be chosen such that there is uniform excitation of all the spins.

The spectral widths normally chosen of 10 ppm for protons, corresponds to 5000 Hz at 500 MHz

Thus our carrier offset should be at Larmor frequency (ω_0). This is the centre of the power spectrum



If the carrier offset is far away from this region, (e.g. at zero crossings) all the spins are not excited uniformly.

I have to choose the carrier offset in such a way, first thing, I have to get uniform excitation. You know we are applying a square pulse or a rectangular pulse, we can consider, depending upon the width. If you take the power spectrum of that, through the Fourier transformation of that, this is the type of spectrum you are going to get. This is called sinc function and you see if T is by pulse width, then the 0 crossing of the synch function at -1/ T and +1/ T.

Now let us say, I have to cover a 5000 Hz per spectral width at 500 MHz, where do I take this spectral width? My spectral width will be defined from the carrier frequency. Let us say this is my carrier frequency. I sit here or I sit here and cover 5000 Hz. That is not good for me, because it is not the uniform excitation here; and some places here you know, there would not be excitation at all. We have to ensure all the frequencies, all the spins, which are chemical inequivalent spins, which give frequencies between 0 to 10 ppm, should be uniformly excited.

In which case, we have to choose a spectral width and carrier offset in such a way to focus on this region. When you focus on this region, this is quite a bit, in fact we calculated earlier for a 10 microsecond or 5 microsecond pulse width of proton if I take, this is about 100,000 Hz, this is about 100,000 Hz, 200,000 Hz or 200 KHz will be there. And this is region, this is very small. All I require is only 5000 Hz, very easy and this 5000 Hz at the center is of uniform power.

See the power is almost uniform and I can say horizontal, unlike here and here, which is 0 crossing here. For example, power is much less, here is much less. So I choose this region, and take the spectral width in this region, so that all these spins are uniformly excited. And for that I need a carrier frequency 5000 Hz from where. I have to set a carrier offset, I can put at one end of the spectrum here or here, wherever I want.

Let us say, this is my spectral width. I can put my carrier offset here, or here on one end of the spectrum; right or left, does not matter. And then, from that place, from that carrier frequency, I can cover this 5000 Hz spectral width. This is called carrier offset, which we have to do. If the carrier offset is far away, for example at 0 crossing or something, you will not have uniform excitation. That is very important to remember.

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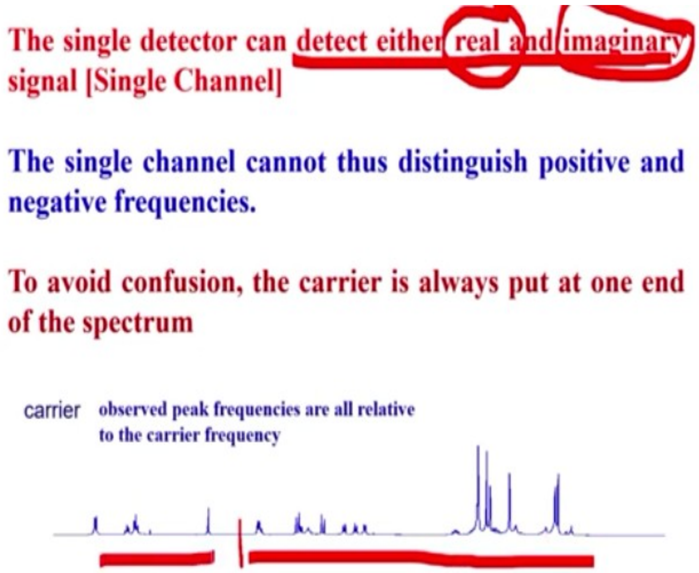

So if I have to do that, I use a single detector. See we have both real and imaginary component after Fourier transformation of a time domain signal. It can detect either of them. We have a single detector, because real and imaginary, I saw the phase of the signal, just now I showed you. Sometimes, they will be out of phase by 90 degrees. If they are exactly out of phase by 90 degrees, real and imaginary, one is absorptive, other is dispersive.

A single detector if I have, it can either detect real signal or imaginary signal. It cannot detect both. In which case, let us say, if my carrier is somewhere here, I cannot distinguish whether it is a positive side signal or negative side signal. In otherwords positive frequency or negative frequency, I cannot differentiate at all. All right, does not matter is, I keep at one end, then also I can get only one sign, whether it could be positive or negative, I cannot distinguish.

So in any case, to avoid confusion, always in the single channel, when there is only one detector, the carrier is put at one end of the spectrum. Somewhere here or here we put and collect the signal. But the only disadvantage is you cannot distinguish between positive and negative frequencies.

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If we need to detect both real and imaginary, then two receivers that are opposite in phase by 90^0 are required [Quadrature Channel]

But it is better to detect both real and imaginary. There are lots of advantages in deciding how the magnetization behaves to decide your pulse sequence everything. You need to detect both real and imaginary signal, it is advantageous. Also there are various reasons, why. First thing, you can distinguish between positive and negative signal; that is one thing. At the same time, there is a lot of gain in the signal to noise ratio also.

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Quadrature detection

If we have two receivers and collect both Mx and MY components separately and ensure they are properly out of phase by 90^0 then we can distinguish between positive and negative signals.

The carrier is put at the centre of the spectrum, and those towards the right of it are positive frequencies and those towards its left are negative frequencies

For example, if I go here, although I am putting my receiver here, remember my Nyquist frequency. There are certain frequencies here on this side. It may be noise. Noise has higher frequency than these. This is my region of interest. This noise will fold back here. The entire noise will fold back into the spectrum, is it not? The noise beyond this carrier offset has to fold back into the spectrum. So, the noise will be there.

On the other hand, if I have two receivers, let us say, one to detect real component, one to detect imaginary component. One to detect cosine signal, one to detect sine signal, which are out of phase by 90 degree. If I have two receivers, which are out of phase by 90 degree, then it is better. I can distinguish between cosine part and sine part, in which case I can distinguish peaks with positive sign and negative sign. How can I do that? What I will do is, if I have to distinguish these things, I am going to put my carrier at the center of the spectrum.

If I have a spectral width here, I will put my carrier here. And positive, negative signal I can understand by having two receivers. I will detect Mx component in one receiver, and My component in another receiver. That is Mx is the cosine part, My part is the sine part. I can detect them individually in two receivers. But in reality there are no two receivers. It is only manipulation of the Euler equation, you know that $e^{-i\theta} + e^{i\theta}$. Cosθ+isinθ, so we do mathematical manipulation in such a way by adding and subtracting the signal, we can detect only one component. That is possible. This is called quadrature detection.

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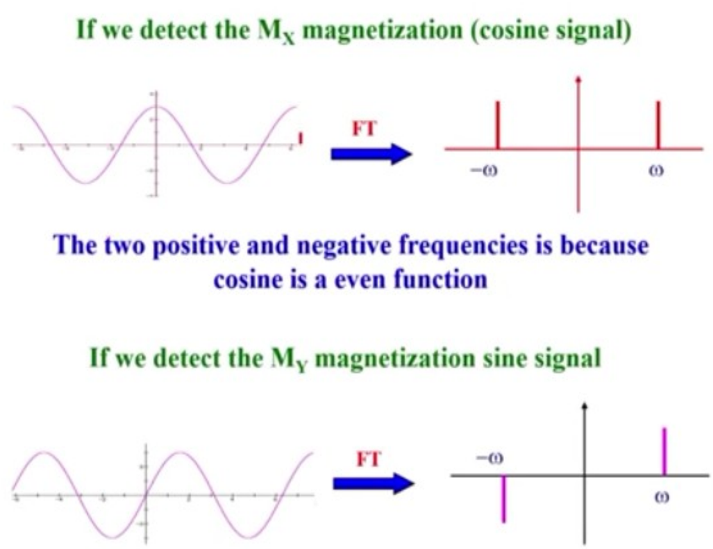

And before that, I want to show you how the signal detection sine and cosine part works. For example, if I detect Mx component, cosine signal and do the Fourier transformation, I get minus omega and plus omega, two frequencies. Both negative and positive frequencies are there, because cosine is an even function, it cannot distinguish between positive and negative frequency. Cosφ= cos (–φ), that you know in trigonometry. If I detect My component, sine signal and do the Fourier transformation, there will be one positive component, this one, and the negative is out of phase by 90 degree, an inverted signal we get. Now what I will do with this?

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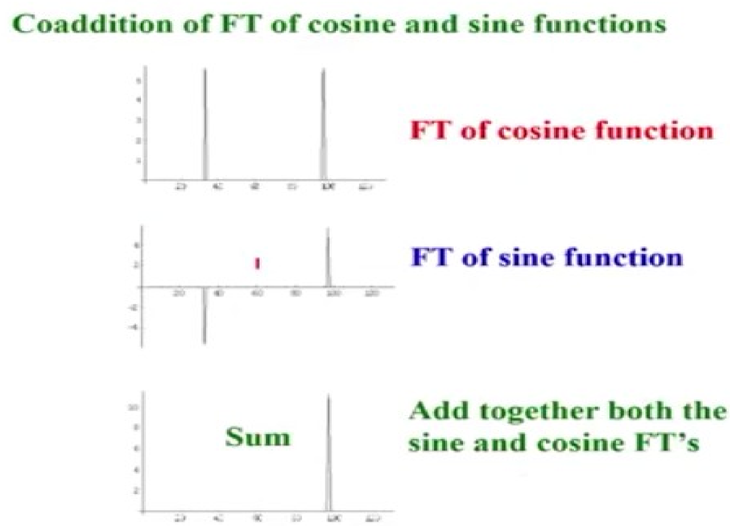

Now what I will do is, I will co-add these things. I will take the Fourier transformation of cosine function and sine function. I do the co-addition; I get this one. I know, if my carrier is here. I know what is the signal here; and if I subtract, let us say, I can get only this component, then I know what is the frequency here. So I can manipulate the cosine and sine functions in such a way I can add or subtract and detect either cosine component and sine component. This is what it is.

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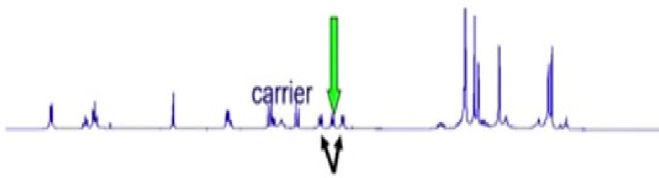
In quadrature detection the carrier frequency is set at the centre of the spectrum



In the quadrature detection, the carrier frequency is set at the center of the spectrum. Now these are all signals with positive sign, these are all with negative frequency. These are positive frequencies and these are negative frequencies.

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Calibration of 90^0 pulse

So that is why quadrature detection is what you are going to routinely utilize. Of course, there are lot of difficulties with both sine and cosine components, two receivers if they are not exactly out of phase by 90 degree, what happens? If let us say, they differ by 5 degrees, not exactly 90, if 85 degrees difference is there, because cosine and sine function should be exactly out of phase by 90 degree. The phase angle is differing, we will have a mixed phase.

But in such type of quadrature detection, what happens is you are going to get some additional peaks, some extra leakage, that is called quadrature images. And of course in the electronics, we need to have a gain for the receiver; one for the cosine and one for the sine. We should have equal gains. In the electronics, it would be such that both receivers should have equal gains. If the gain is not equal, let us say cosine component has a better gain than sine component by some degree, little bit of difference may be there. That can also cause, what are called quadrature images, which cannot be corrected. They have a different phase altogether.

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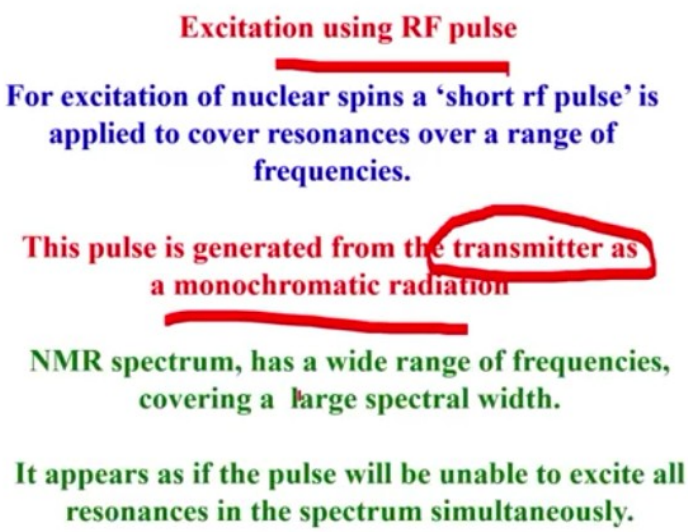

So these are the things, which we should know, but I am not going into the details of the quadrature detection; and there are lots of problems in that and lots of advantages are there. If it is not very good, it gives problem. If you have perfect quadrature, it is very advantageous and we use that in very large number of experiments. But anyway without going more into the details, I told you what is the advantages of quadrature detection, what is the advantages of single channel detection.

Now let us discuss more about the radiofrequency pulse. Now up to this, you have done something, but you need to now start collecting the signal. How do you start collecting the signal? You have to excite the nuclear spins by application of radiofrequency pulse in a direction perpendicular to the magnetic field, which is along Z direction. Magnetization is here, I have to apply in a direction perpendicular to it and flip the magnetization to transverse plane.

Now the question is what type of RF pulse you are using? What is the width of it? What is the power of it? This is another thing we should know. Another interesting thing is, the frequency generated is single frequency. We are having a single frequency RF. It is monochromatic radiation. The transmitter which generates frequency is only one frequency. But what is happening in our spectrum? We have so many frequencies, so many peaks are there in the 0 to 10 ppm range. There may be 50 peaks. How do we excite them, because this frequency has to match the energy separation, to get into a resonance condition. But we are having a monochromatic radiation. So it should match only one of the frequencies or between one of the two energy levels. How does it work? and remember NMR spectrum has a wide range of frequencies, covering large spectral width. So in the first instant, you will guess, you cannot excite all the frequencies by a monochromatic radiation. The single frequency radiofrequency pulse, but it is not true.

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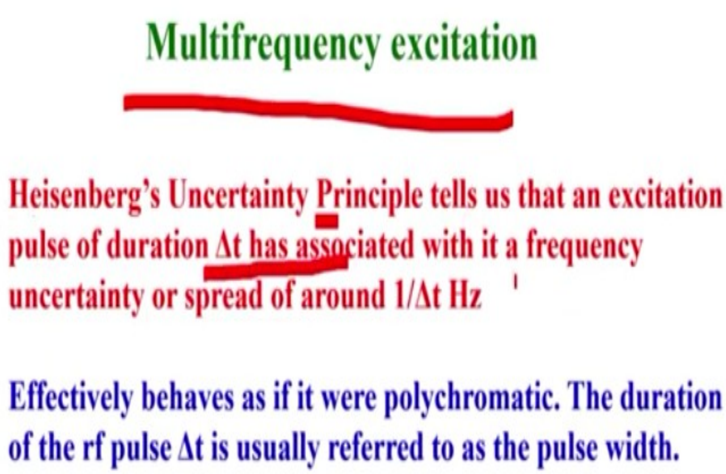

What is happening is, you have a multifrequency excitation. The reason is we have to take into account Heisenberg's uncertainty principle, which tells us that an excitation pulse of duration Δt has associated with it uncertainty of frequency spread like this $1/\Delta t$. You understand; I have a pulse duration, it is, let us say, 5 microsecond of a single frequency, no problem and then there is a spread in the frequency 1 /5 microsecond, imagine, It will cover a wide range. It will cover 200 KHz. This is the reason. Although you apply monochromatic single frequency RF, but the excitation is of all the peaks of the entire spectra width, entire range. This you can understand more by uncertainty principle. There are lots of books, which can explain these things. You go to the electrical engineering book about radiofrequency; it will tell you everything, all those things you can understand. Even a couple of very good NMR books will discuss this. Effectively, the RF pulse behaves as if it is a polychromatic pulse. Pulse of multifrequencies.

And now what I said Δt is called duration of the pulse, very important, you should know that. Δt is referred to the duration of the pulse. The pulse width can be manipulated with the duration and RF power, that is the together with the power of the pulse, you can iterate in such a way you can manipulate the magnetization; the way you want to flip. It can flip by 90 degree, 180 degree, 45 degree by manipulating this RF pulse width and the RF power. Now you know that.

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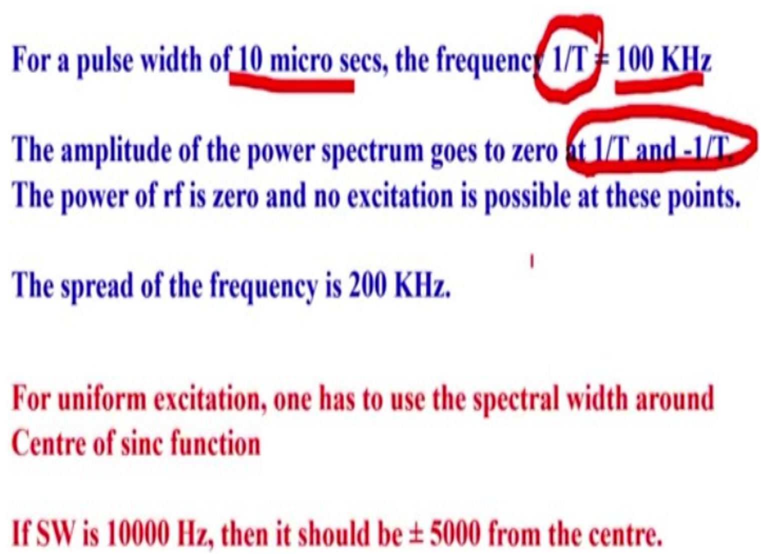

Supposing, I want to have a pulse width of 10 microsecond, what is my frequency spread according to uncertainty principle? It is 1/ T = 100 KHz. So the amplitude of the power spectrum goes from 1/T to -1/T. When the RF power is 0, in the sinc function I showed you, at the 0 crossing, there is no excitation possible. So your spread of the frequency should be such that, it should be as I told you, it should be uniform at the center of the power spectrum. So if a spectral width is, let us say, 10,000 it should plus or minus 5000 from the center of this power spectrum, then you will get uniform excitation.

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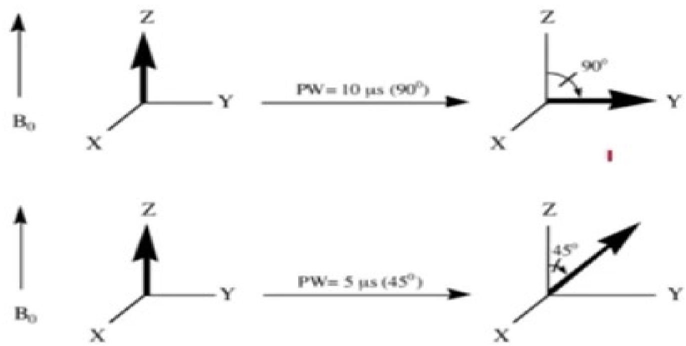
Pulse widths and flip angles



$$\varphi_t = \gamma * t_p * B_1$$

And now the question is how do I deal with the power and the pulse? I can get the 90 degree pulse or 180 pulse of my liking, of my choosing, whatever I want, I should choose. Remember, mathematically you can calculate like this. The angle of the pulse, the angle at which you can tilt the magnetization from z axis to any of the other axis, is governed by an equation called γ * tp * B1. This is the RF power. Gamma is the gyromagnetic ratio, tp is the pulse width.

If you have to tilt by 90 degree, this angle is known, gamma is known. You have to play with these two. Pulse width and the power. That is what I said. You can play with the pulse width and the power, so that you can design the pulse of 90 degree, 45 degree, 180 degree or whatever it is. So remember, important thing, how do you calculate the pulse, which angle of the pulse, the flip angle of the pulse is given by γ * tp * B1. This equation is very important to calculate the flip angle.

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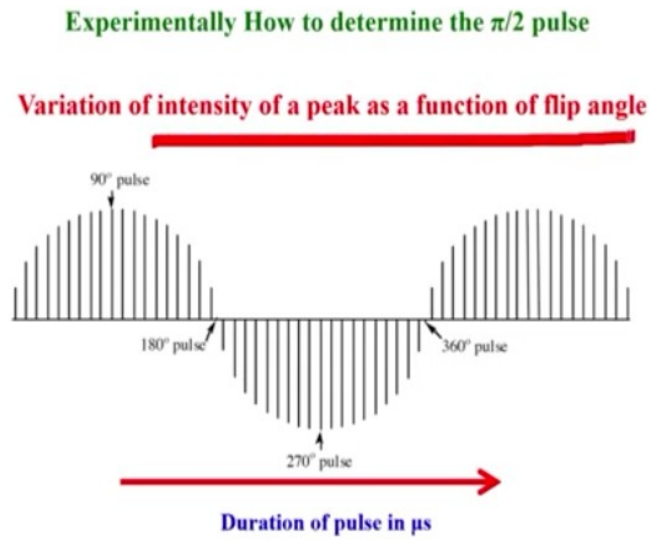

Now experimentally, how it is done? The variation of the intensity of the peak as a function of flip angle is monitored. How it is monitored? Look at this. I have to collect the signal. I take some standard sample. Let us say one which gives me a single peak. I do not know, what is my 180 pulse. I do not know what is my 90 pulse, but I have to calibrate. What I will do is, I will put the sample in the magnetic field, set the pulse width at 2 microsecond or 1 microsecond.

Start with a small value, collect the signal. Do the Fourier transformation; get the frequency spectrum, and measure the intensity. Now vary the pulse width; 2 microsecond, 3 microsecond, 4 microseconds, all in microseconds. Pulse width cannot be very long. And of course this pulse width of few microsecond, you can convert it into frequency. How much is the power of the pulse can be expressed in frequency. It is of the order of 25-30 KHz normally for the excitation pulse.

So keep on varying the pulse width without changing any other conditions. Power remains same, vary the pulse width. Then, you keep on measuring the intensity. See the intensity follows a sine curve like this. And reaches maximum here; and then keeps decreasing. It becomes 0, goes back, goes back, goes back, like this. So what is happening? I remember I told you, the magnetization can be flipped to any axis, by any degree.

Magnetization was along z axis, it was there. This is at thermal equilibrium. This is a bulk magnetization. I applied 2 degree, comes here, 5 degree, 10 degree, 20 degree; and I want to bring it to x axis. I am applying in some particular direction; perpendicular to z axis. I brought it to 90. I can take it, slowly increase the width, increase the width, now angle becomes 95, 100, 120, 180. It keeps increasing 200, 250, 270, then come back. So you can make the spins to oscillate.

The magnetization keeps on rotating in a particular plane. So if I have a detector somewhere here, let us say, I bring the magnetization to x axis. My detector is along x axis, that is what I discussed about the phase of the pulse. Then I get maximum signal. Now I do not change my receiver place. I receive in the same axis. When it goes to 180 degree, when the signal is from the z axis comes to minus z axis, my receiver is here you will not get any signal. It is 0; and at 270 degree you get negative signal, again 360, it is 0. So it keeps oscillating. So you find out what is the maximum intensity, where you get; that is the 90 degree pulse. If you have a difficulty, you can look at this one for example, all are more or less of equal intensity and you do not know which one to select as your 90 degree pulse. That difficulty will be there. What I suggest is, look for the 180 degree pulse, signal has to be 0.

You know what is 180 degree pulse, you measure in microseconds, take 50% of that, half of that, that is 90 degree pulse. If 180 pulse is, let us say, 10 microsecond, take half of that and it is 5 microsecond. Now do another experiment by setting width to be 5 microsecond, you will get maximum signal, that is your 90 pulse. That is the way you have to calibrate the pulse, 90 degree pulse, not only 90, any angle. I give an example how to calibrate the 90 degree pulse.

What you have to do, is to vary the duration of the pulse. As a function of duration of the pulse collect the signal, measure the intensity, make it to go through one complete oscillation, find out at which pulse width the intensity will be 0; at a particular place, that angle corresponds to 180 degree. Take half of that as 90 degree pulse. Then rest of the things is a simple manipulation. This is the thing, which I wanted to tell you as far as the data acquisition is concerned. We discussed lot of points.

Lot of things we discussed right from the sample preparation to data acquisition. Now you have done an experiment with various ways by calibrating pulse width, setting spectral width, and then carrier offset, quadrature, single channel, everything you know, shimmed the magnet for better resolution. You understood what the line shape is; everything you have done. But now you have to process the time domain signal.

Of course, here itself you have already done by calibration, you have already processed the signal, does not matter. I will tell you more about processing. This is because I have to tell you 90 degree pulse, then you have already Fourier transform; and you have already done that processing. But much more can be done during processing. We will discuss how do we process the time domain signal in the next class.