One and Two Dimensional NMR Spectroscopy for Chemists

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Lecture – 49
Introduction to 2D NMR

Welcome back all of you in this class I will start disusing about 2 dimensional NMR. An additional dimension for NMR is added to simplify the spectral complexity and to derive information in a better way. This 2 dimension has been extend into 3 dimensions, 4 dimensions, 5 dimensions, so there is N dimension NMR possible. So, in the next couple of classes left, I will not able to completely cover all 3D, 4D etc.,

Even 2D is a vast subject in 2 dimensions NMR, I will not be able to cover, several hundreds of 2 dimensional experiments possible; which can be designed for specific to your needs. So, the goal of this class is not to make you to understand every pulse sequence available in the literature. But in this class or next 1 or 2 classes about 2 dimensional NMR, what I am going to say is; what is basic 2D NMR, what are the basic experiment which you are required to do, when you want to analyse the spectrum of a simple molecule.

So, I will start with a requirement; how to do a 2D, what is a 2D, how to interpret 2D spectrum; we will take examples, we will go like that, okay, And of course in the 2 dimensional NMR, the question comes to all your mind, what is a dimensionality in NMR, we are talking 2 dimensions, then you must know something about dimension. Of course, you dont get confused with the physical dimensions, we have always X, Y, and Z dimensions that is okay, that is different. The dimensionality in NMR is something different, which we will discuss as we go ahead.

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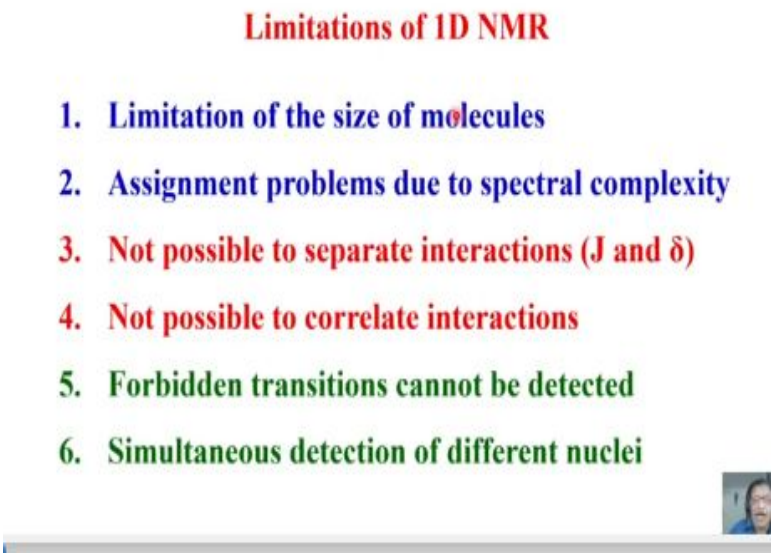

First of all, the question one can ask, why we require 2D NMR? what is a need for a 2D NMR? cannot we solve our problem using 1D NMR, since so many classes we have been discussing about varieties of 1D sequences and 1D experiments, we can do. We can even enhance the signal intensity, analyse the spectrum. We observed lots of things, we analysed in fact many proton spectra and hetero nuclear spectra. We discussed in depth about the carbon 13 spectra and we discussed about the editing of the carbon 13 spectra.

We understood how to enhance the signal intensity by polarisation transfer, lots of things we understood. Then the question comes; why we require 2D? what is the use of that? for that we have to understand some limitations of 1 dimensional NMR. What is the limitation of 1 dimensional NMR? First of all, the limitation of the 1D NMR comes because of the size of the molecule that we are interested.

For example, you take a simple molecule, ethyl alcohol. What you will do; how many peaks you are going to get; CH3, CH2, OH, okay that is possible; now we know how to analyse those things; that is done. We know where is CH3, CH2, OH, etc. How did we make the assignment? based on the chemical shift information using our knowledge of shielding, deshielding and also we understood lot about scalar couplings, looking at the multiplicity pattern, we made the assignment of which is CH3 peak, which is CH2 peak, which is OH; done, no problem about it.

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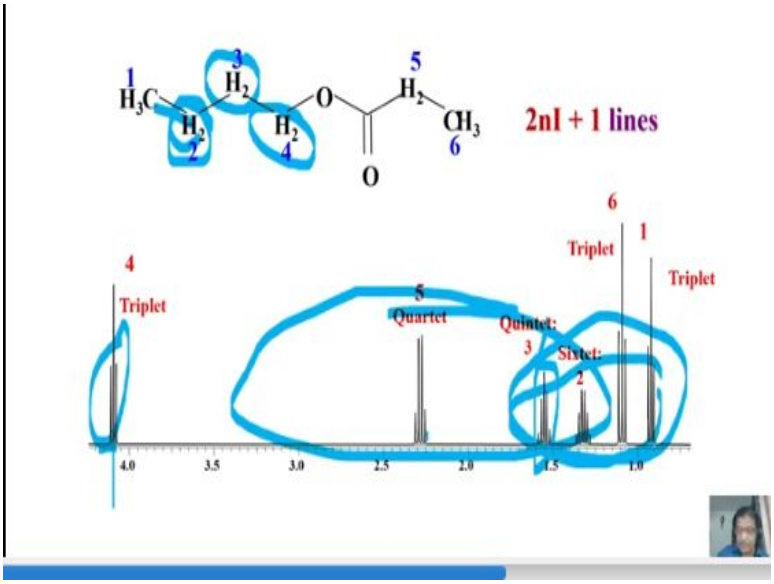

We also analysed this molecule, what is this molecule; a simple molecule we started analysing by looking at the multicity pattern, triplet, triplet, sixtet, quintet, quartet, triplet. Again what is the idea we used; we used an idea of coupling between one spin to another spin; For example this CH3 was coupled to CH2 and there is no other long range coupling, so we interpreted this, remember in the interpretation of 1H NMR spectra I took this as an example.

So, see this CH3 is a triplet because of the CH2 proton, as a consequence this has to be a quartet because of this, you understood this. Very clearly we could interpret and of course, we interpreted sixtet, quintet and triplet based on the coupling with the neighbour, which will be the coupled neighbour for each group. For example, for this CH3 the only coupled neighbour is CH2 proton. And if you assume it is no long range coupling, interaction do not extend in the longer range, then we could interpret this, because this has to be triplet because of this, that is correct. You understood all those things, this has to be a triplet and all those things we know, what it is. But when we come to this place, we understood one proton is coupled to 2 protons, one to the left of it, one to the right of it; okay we used the logic, this will become triplet of triplet because of these 2 couplings, and then we understood, this will be a quintet and we know this is a quartet of triplet because it is coupled to one CH2 to the left, one CH2 to the right. So we understood this, a fairly simple molecule. Now, what happens, if I give a very big molecule, big molecule in the sense, I give a big protein, containing let us say 40, 50 amino acids, what you will do; remember it is not a simple spectrum like this.

For example, sometimes each amino acid could be a small molecule of this order, where spectrum can be as complex as this. Now, if I have a protein with 50 amino acids like this, each of them can give, C-alpha, C-beta, C-gamma and NH peak like this. In the entire range of this spectrum, some 0 to 10 ppm in the proton spectrum, all 50 amino acids will give proton signals. What you will do? how you will analyse?

Analysis is next, first look at a complexity of a spectrum. It becomes so complex, resolution is a big problem, you will be not be able to analyse beyond a certain molecular size, just by 1D NMR. You will simply not be able to identify which peak belongs to which, because of severe overlap, severe complexity puts a restriction on the size of the molecule. So, as a consequence, you will be not able to analyse the spectrum beyond a small molecular weight size of the order of few hundreds or sometimes maximum 1000 or like that.

Beyond that if you want to analyse the spectrum of a molecule just by using the 1D NMR, it is will be a very difficult task. So, first thing I wanted you to tell you was, the limitation of the size of the molecule; the size of the molecule as it becomes bigger and bigger and bigger, there is severe limitation for the use of 1D NMR. I hope you are all with me, right. Now, assignment problem is also due to severe complexity.

Remember, look at this one, here I was able to analyse easily, here it is not a big problem, life is simple, I simply know this is coupled to this and simply I start making assignment; because you know if you have to get the information about the molecule of your interest, complete molecular structure or confirmation, first and the foremost thing is you have to assign each and every peak individually. Every multiplet pattern has to be assigned to a particular proton. And if you know why this multiplet is coming, then you know what is it, what are the couple partners for it on either side.

That is a very important information. So what will happen, this spectral complexity becomes more and more; as I said if the molecule size becomes enormously big, you will be not able to analyse so easily, okay. That is a big point, assignment is very difficult due to spectral

complexity. Second, another important problem comes is the resolution, here the peaks are well resolved, I know this is a triplet very beautifully I can identify, you see.

If you look at this, if I take the highlighter I will show you, this is the very beautiful triplet, this is a triplet, well resolved, it is a quartet, I know it is well resolved. If all of them come let us say within this region, all these peaks within this region, triplet, quartet, quintet, sixtet and if you have to identify the peaks, when there is severe overlap, how you will do? It is not possible, the resolution is one of the important criteria. There is a way we can enhance the resolution in 2 dimensional NMR by using some tricks, okay.

So, 2 dimensional NMR in fact resolves some information in 2 or 3 dimensions or 3 dimensional NMR also, generally the multi-dimensional NMR has an ability to separate the interaction parameters or resolve the interaction parameters or correlate the information in different dimensions making your assignments very simpler. That is the reason 1D NMR has a limitation, it cannot be done beyond certain limit because of enormous spectral complexity.

Another thing, let us look at this one, here take an example, this is sixtet, this is coming because of proton 2. Why this is triplet? why this is sixtet? because it is a quartet because of this CH3 and each line of this quartet is spilt in to a triplet because of the CH2. Depending upon the coupling strength, it is a large quartet and each line of the quartet is a triplet. As a consequence due to severe overlap, you have got this pattern. If this is the case, this triplet coupling is larger, then it is going to be triplet of quartet, then the analysis is you have to do in different style and this is the triplet and each line of this triplet is going to get quartet like this, fine. So this is a spectrum; if this coupling is larger, CH2 coupling is larger, so it is triplet of quartetx, otherwise it is quartets of triplet that is fine. Now my question is; here when one coupling is larger, I know this is a triplet coupling between this proton and this proton. I know this is a quartet. This separation gives me the coupling between this proton to this proton; because you have been analysing the proton spectrum since several weeks, we know that. But if there is no resolution like this, they are not resolved like this, it is overlap like this, now how do you know which is a triplet, which is a quartet and how do you know what is a coupling? it is not possible; okay, in this case; we can still do it the simple molecule.

Let us say this is also coupled to another CH3 or another CH2, then what you will do? The spectral complexity will be so much because of the multiplicity pattern they overlap. Then additional problem comes; in addition to identifying which proton is this, we should extract the J coupling of this proton with the remaining protons which is coupled to, the extraction of the coupling information is a challenging task.

So, what you will do for that? Let us think of a possibility; if somehow I can separate out the coupling and chemical shift by some way, I get chemical shift here; and get the J coupling here in this direction. And let us think of 2 directions, in this direction here, I am going to have chemical shift, in this direction, let us say, I am going to have only the scalar coupling information. If I can separate it out, here I will get only J coupling; here I get only chemical shift here. I can make a plot like this. Here I get J couplings and here I get only chemical shifts, what does that mean? I am able to separate out the interactions, Does it not simplify my job? exactly, that is what happens.

This sixtet can be simplified, if I separate the interactions. We cannot do this by 1D NMR. That is one of the biggest limitations. 1D NMR cannot permit you to do the step of separation of interaction, you get the point? That is one thing; thus the separation of J and delta is a difficult task; and it is not possible to correlate interactions, that is another interesting thing. Here, in this case what we do is, I know it is a simple molecule, this is coupled to this, I know this is coupled to this based on the multicity pattern.

In general, in a given molecule, there are many things overlapped here, let us say all these 4 overlapped, assume all 4 multiplet patterns the bunch of peaks come here. Everything is overlapped. Now if you want to identify which is coupled to which, how you will do that? There is no way you can directly do it; but in the earlier days, there used to be what is called a selective decoupling.

What I will do is; go and do this decoupling by hitting at this proton. When I collapse this make the energy states of these protons saturate, then what happens? this proton will break the coupling with some of the protons. For example this is the proton 4, if I saturate this, it breaks

the coupling with proton 3, somewhere here, then this complexity gets reduced, then I know this is coupled to this.

So, this is a way we can find out which are the couple partners, which proton is sitting next to which proton; the proximity of the proton which is coupled to that one, if you want identify, to the nearest neighbour coupled proton, and if there is enormous spectral complexity, what we have do is, only selective decoupling; which is a very difficult task. And also enormous time consuming. It takes, if a big molecule is there, let us say there are 500 lines or 300 lines present in the entire range of the spectrum, If I want to get a molecular structure, for finding out which is coupled to which, I have to do several hundreds and hundreds of such decoupling experiments, selective decoupling experiments, then I know this is coupled to this, this is coupled to this. And then I can get the structure. This is not possible easily in a one dimensional spectrum. It is a very, very difficult job. So, we have to correlate which proton is coupled to which proton; that is called the correlation of interaction to establish, the connectivity pattern; for that, we cannot do by 1D, we need to go to 2D. These are the limitations of 1D.

And then forbidden transitions cannot be detected; that is a very interesting thing I told you in NMR. Remember in the very first class or one of the classes, as I said this selection rule for NMR is that you find out the magnetic quantum number for each energy state, and find out the change in magnetic quantum number between 2 energy states. It should be either +1 or -1, only then, I said transitions are allowed. That means, we have one spins can flip at a time, of course there is a combination of spin, 3 can flip at a time that is a combination transition but still the selection role is one quantum of energy is changed. When spin flows from alpha to beta or beta to alpha, the detected energy is only a one quantum that is called single quantum NMR. The conventional spectra what you have seeing in NMR spectrum; that is conventionally one dimensional NMR spectrum, is single quantum, okay, conventional one dimensional NMR spectrum is single quantum NMR.

But I showed you in a 2 spin case example, we have an alpha, alpha state, beta, beta state, alpha beta, beta alpha; all 4 energy states are there. I said this magnetic quantum number, total magnetic quantum number of the energy states, beta, beta state is minus 1, alpha, alpha state is

plus 1. Can there be a transition between these 2 states, alpha, alpha to beta, beta? It is forbidden transition; because it requires double quantum, 2 quantum of the energy, single quantum requires only 1 quanta of energy, because only one spin can flip alpha to beta or beta to alpha; but here it requires 2 quantum of energy.

Or if a 3 coupled spin system, I can, can I get the transition from this lowest energy level, alpha, alpha, alpha to beta, beta, beta. If I want to see that transition between 2 energy states in the 3 coupled spin system, 3 quantum transition is not possible, they are all forbidden transitions. These are called multiple quantum transitions; these are all forbidden, you cannot detect in the conventional one dimensional NMR.

But of course in 2 dimensional, indirectly there is a way to detect, we can do that, it is not difficult at all. So, this is the biggest advantage, and what is the use of detecting such forbidden transitions, multiple quantum transitions; enormous advantage is there which I am going to tell you, many information can be derived, first we can simplify the spectrum, we can analyse the spectrum in the easy way, lots of advantages are there, we can do spin system filtering, lot of things we can do, by detecting multiple quantum spectrum. That is one of the limitations here in 1D NMR.

So, 1D NMR has several limitations. Another limitation is simultaneous detection of 2 different nucleus is possible, see in one dimension NMR, I can see proton NMR, I can detect proton signal, send RF pulse, 90 pulse on proton, bring the magnetisation to xy plane, start collecting the free induction decay and get the NMR spectrum, fine, it is the proton spectrum. If I want you do the carbon 13, okay we can do that, I will apply 90 pulse on the carbon channel, bring the magnetisation to xy plane, start decoupling simultaneously, immediately after the pulse and start collecting signal, you will get decoupled carbon 13 spectrum.

But in one experiment if I want to get carbon chemical shifts and also proton chemical shifts, simultaneously, it is not possible I cannot detect it. But on the other hand, if we go to 2 dimensional NMR, that is a big advantage, I can do a heteronuclear 2 dimensional experiment, a carbon chemical shift in this direction, proton chemical shift in this direction. I can get hetero

nuclear coupling in this direction, I can get here the chemical shift of proton or carbon in this direction.

So, all such interesting things we can do in 2D NMR and in 1D NMR all these things are limitations; that is the reason why 1D NMR cannot be applied for many things especially, as a the size of the molecule keeps increasing and for various things problems which I suggested here, 1D NMR spectrum has lot of limitations. Are you all with me, I hope you are all with me, right, okay, this we discussed.

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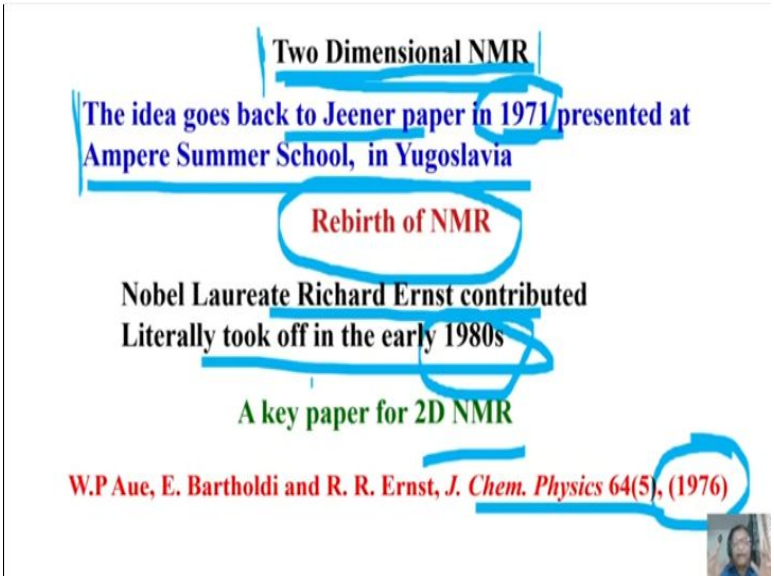

Now, as a consequence very interesting discovery took place, thanks to all our seniors, pioneers in NMR spectroscopy who made a breakthroughs in this research; as a consequence, you can utilise the NMR the way we want today. This is because of many, many stalwart, pioneers who made a significant contribution by doing lot of discoveries, and this 2 dimensional NMR discovery. The idea of this was given in 1971 in a Ampere Summer school, in Yugoslavia by Jeener.

Jeener presented a paper in summer school in 1971 where he gave the idea of 2 dimensional NMR. What is 2 dimensional NMR? and we will come to that later. And that was called rebirth of NMR. Till 1970, from the year it was discovered, first observed in 1945, NMR was used to

some extent, after the discovery of chemical shift, then J couplings, and then then NMR is used to some extent.

But application of the NMR for a very big molecule like bio-molecule was greatly restricted, largely it was restricted; till 1970, because again, as I said we have to use only 1D NMR which has several restrictions, lot of constraints were there. But in 1971 because of the advent of the 2 dimensional NMR spectroscopy; which was introduced, we call it as the rebirth of NMR, NMR spectroscopy took another birth.

Thanks to Professor Richard Ernst, a Nobel Laureate who contributed for this; who is the one who is responsible in early 80s and early 76, the first paper in 1976 was published in the journal chemical physics. And it is the key paper for 2D NMR, afterwards literally it took off in the early 80s. It was published in 1976, first paper and the idea of 2 dimensional NMR. Literally after 80s, it has just exploded like anything; and lots and lots and lots of pulse sequences were designed and lots of 2 dimension NMR experiments, were designed and modified to derive specific information.

And literally the application of NMR by the utilisation of 2 dimensional NMR, then extended for the multi dimensions, like 3 dimensions, 4 dimensions, everything began. And that is where the application, utility of NMR and the idea of NMR, development of NMR, everything exploded during that time; and that is why NMR has become very powerful now because we can use it in a number of possible ways, okay.

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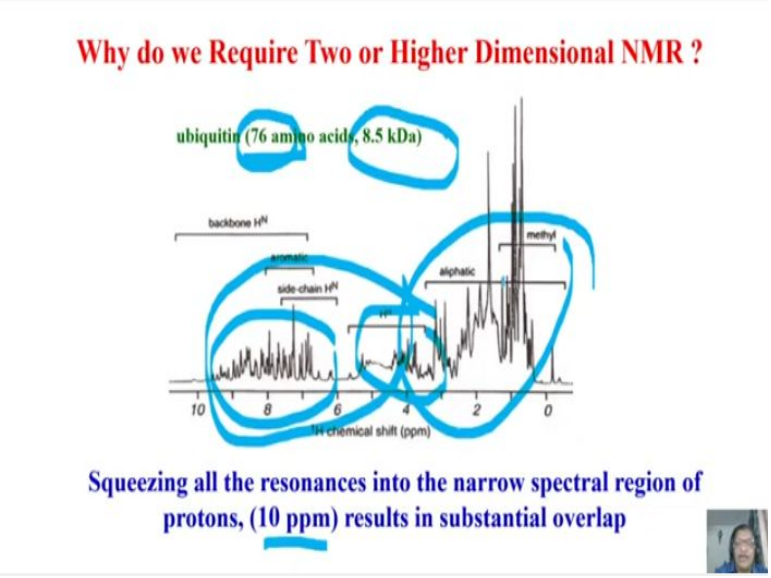

Now, with this now you may ask another thing, fine what do you mean by this 2 dimension NMR, why is it really required? whether we really require 2 dimension, higher dimensions, how do I know? We may say there is a limitation of 1D NMR; but there are many higher dimensional experiments; 2 dimension, 3 dimension. You may ask me a question which one I have to use, what experiment I should do for my molecule.

That is a judicious choice. Look at this spectrum like this, it is ubiquitin, 76 amino acids, 8.5 kilo Dalton molecular weight and look at this range, this is very old spectrum, nowadays in high frequency, we may get a better spectrum. Look at this region, you can only assign side chains here, methyl here, aliphatic here, C-alpha here, there is no resolution. This is a big problem, more than thousands of peaks here, you know. All resonances have been squeezed within 10 ppm, that is the range of chemical shift of protons.

The protons chemical dispersion is in the range of 0 to 10 ppm, so all 76 amino acids, each of them can be considered as spin system, 76 spin systems with so many protons present, all the peaks are hidden here. Just look at the spectrum of your molecule depending upon the molecular size, then you have to decide which one to use, as a consequence depending on the problem, we require to go to higher and higher dimension. Now, what is the higher dimension? what do you mean by dimensionality? and which molecule requires how many dimensions? are the next questions. Let us answer, I hope you are all with me right.

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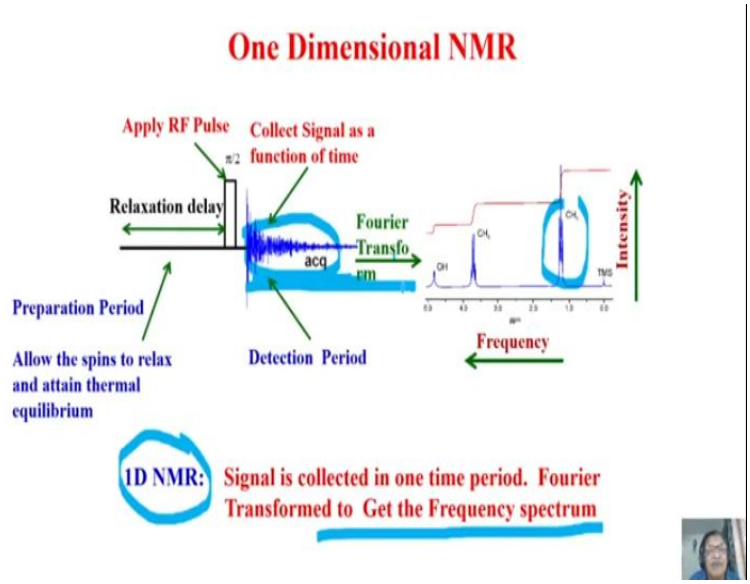

Now, what is this NMR pulse sequence that I have written here. All of you must be knowing by now. It is a conventional one dimensional spectrum, apply radio frequency pulse may be let us say I am looking at proton, apply a pulse on proton channel, start collecting the signal. Of course this called the dead time delay, do not worry, it can be given, it need not be given, in principle without delay, you have to start, so that there would not be any phase problems in the NMR spectrum.

So, you have to immediately start collecting the signals from the zero point, okay does not matter. This is the one dimensional pulse sequence where you apply a $\pi/2$ pulse, the 90 degree pulse brings the magnetization to xy plane; and start collecting the signal; your detector and receiver is here, fine. What is happening here now, if you look at it, you are collecting the signal as a function of time here, okay. You are collecting the signal as a function of time and what you are going to do is the Fourier transformation.

Remember, I said when you collect the signal in time domain and do the Fourier transformation, time and frequency are Fourier pairs, I said. Mathematically, time and frequency are Fourier pairs, now what is happening is you are converting the time domain signal to frequency domain signal. What you are going to get is the spectrum like this, so, this our one dimensional NMR spectrum; where we assign all the peaks, and get the area of peaks, multiplicity, etc.

Now, here we can divide this into some time periods. One dimensional spectrum, in reality, is 2 dimensional, you know why? In this dimension we get frequency, in this dimension we get intensity. But we do not bother so much about it, we always talk dimensionality in the terms of time; here we are collecting the signal as a function of time. How many time domains are there here? Only one time domain, in a single time domain, we are collecting the free induction decay, and doing the Fourier transformation. This is called one dimensional NMR; because single time period is there. Single Fourier transformation gives you frequency spectrum like this, okay. I will call this as the relaxation delay; this is called preparation period, okay; and this is where spins are allowed to relax and attain thermal equilibrium; and afterwards you apply RF pulse, collect the signal here and I called this as a detection period, okay.

So, in 1D NMR what is happening? we have only one time period; of course, two time periods I would say, one is the preparation period where you do not do anything, you simply keep quiet. Put the sample in a magnetic field and keep quiet, allow this spins to attain thermal equilibrium, there is a build-up of bulk magnetisation, simply keep quiet for some time till it builds up that is all; that is called preparation period. This time period is also called relaxation delay, for the spins to attain equilibrium.

Then apply pulse and collect signal, this is the real time domain dimension signal where we are collecting the signal and do the Fourier transformation, this is the way I get in the real signal from the sample; okay, signal is collected in one-time period here; and do single Fourier transformation to get a frequency spectrum; that is why it is called one dimensional NMR, 1D NMR, you understand.

What is a dimensionality? Dimensionality refers to time, time domain, how many time domain periods are there for collecting signal, you are collecting signal in one time, one-time domain, it is the single dimension, if I collect in 2 time domains, it is a 2 dimensions, if we collect signal in 3 dimension is 3 dimension, we will see that later.

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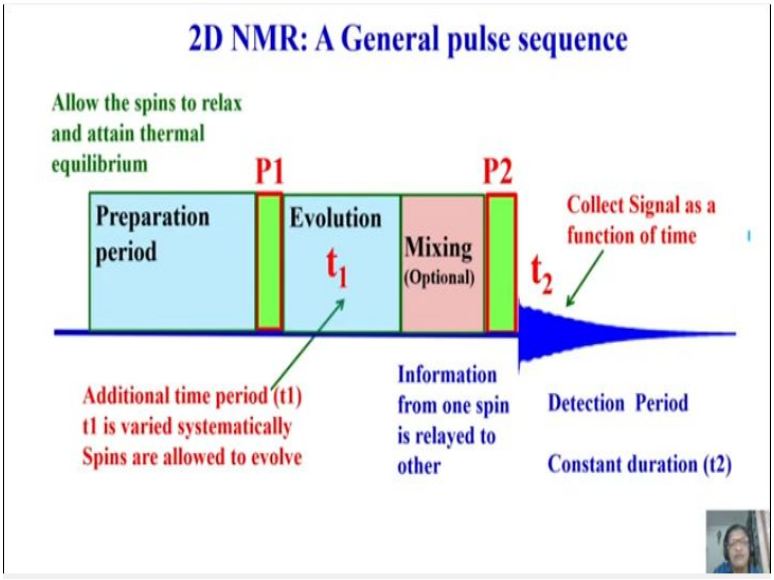

And general pulse sequence for a 2 dimensional NMR consists of 4 time periods. Okay. What are the 4 time periods; first is a preparation period like this, you understand, we have one pulse here, we apply a pulse here okay, pulse we can talk later. There are 4 time periods we talk; preparation period, evolution period, mixing period and this called a detection period, it is a real period at which you collect the signal for doing the Fourier transformation.

In principle, we have 2 time domains here; this is one-time domain where you allow the spins to evolve; this is another time domain where you collect the signal for a time constant time. In this time domain you collect signal, and it is not varied. But in this time domain, this time is varied. Now you collect the signal here and do the Fourier transformation here. Indirectly here also we collect the signal, similar to FID; I will tell you how we do it later.

Here, we do something for the spins in the preparation period, allow the spins to evolve in this period, how it evolves? Evolution take place; like human evolution like evolution in the life, whatever you do for the spin system here, you do something with the pulse sequence, bring it here and it start evolving with time, depending upon type of interaction, it could be J coupling, chemical shift, homo nuclear, hetero nuclear J coupling; whatever it is. Some parameter is responsible between 2 spins or 2 coupled spins or interacting spins. As a consequence theespins will start evolving with time, their behaviour pattern changes with time here; okay, crudely. And then whatever happens here is reflected here; we are going to collect the signal in this time

domain and do the Fourier transformation; and we collect signal here and collect signal here, 2-time domain signal.

If I have one time domain signal, I will do one Fourier transform. If I have a 2 time domain signal here, I can do two Fourier transformations, double Fourier transformation I can do; and get the frequency spectrum in 2 dimensions. The 2 dimensional time domain signal, gives you two dimensional frequency domain signal; that is double Fourier transformation.

So, we will come to that how we do that later and for the day, I will stop here, I wanted to introduce generally the pulse sequence for a 2D NMR; we have not completed yet, we will start from this again tomorrow and continue further.