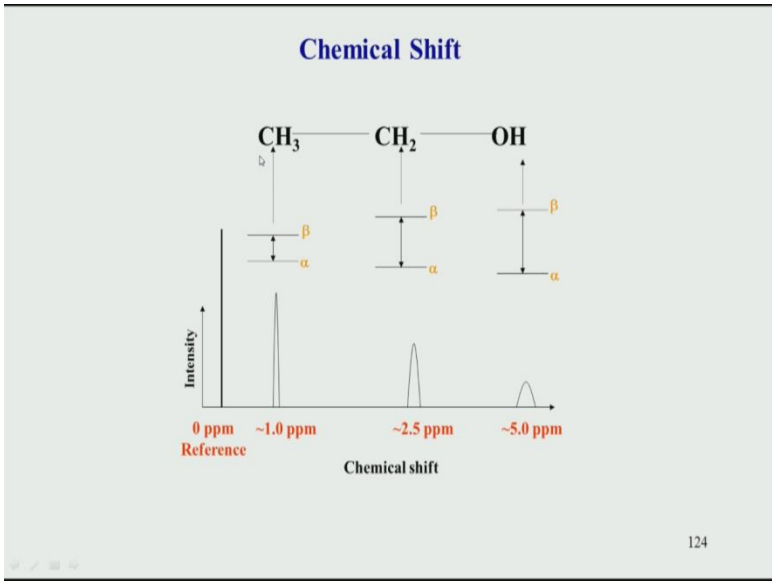
Principles and Applications of NMR spectroscopy
Professor Hanudatta S. Atreya
NMR Research Centre
Indian Institute of Science Bangalore
Module 3
Lecture No 11

Welcome back, this is a second part of this course we will now look at how we record NMR spectrum? How we analyse the 1 D spectrum? So, this is just a brief quick look at to the chemical shifts.

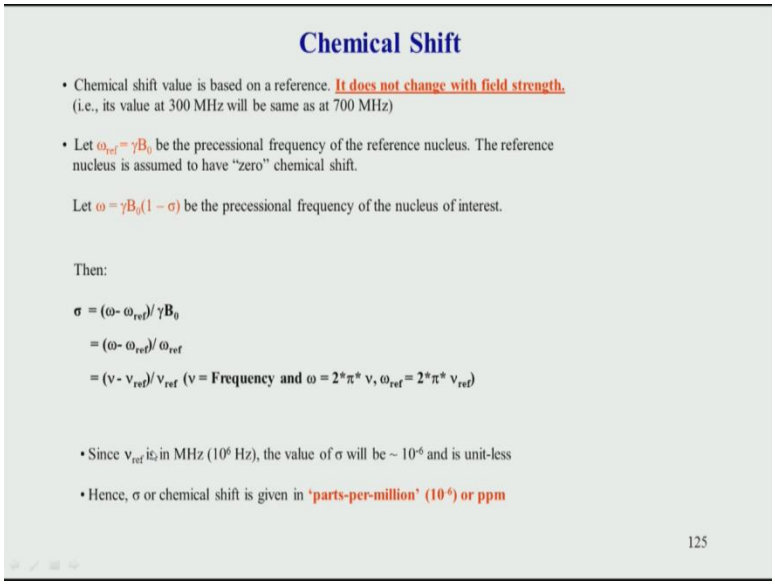
(Refer Slide Time: 0:34)



So, this is what I shown here, that in a given molecule let us say we take example of ethanol we saw that the different hydrogen atoms they are having different energies because they are different chemical shift value that is coming because of electron density around these hydrogens.

They are different compared to each other and this difference in that energy the electron density translates or results in a difference in the different energy levels. Remember omega is gamma B0. So, if the B value changes the omega changes and that is converted into a chemical shift which shown here. This is schematic drawing here of ethanol.

(Refer Slide Time: 1:16)



But let us look at mathematically what we saw that that if this how we calculate the chemical shift value that when we have an omega. Now, let us take some reference compound as you may reference can be any particular compound, we will see that latter and reference we assumed remember as an assumption we force it to a having a zero chemical shift that means the sigma value, we will say for reference molecule let us say zero in that case that omega of that reference is gammaB0.

But in reality in for other the actual molecule what we are looking at where the sigma we cannot considered as zero because that has some final sigma value with respect to the reference and that is why this effective magnetic field around that nucleus is not gammaB0 but is used because of this sigma. So, this is a shielding effect. Shielding factor.

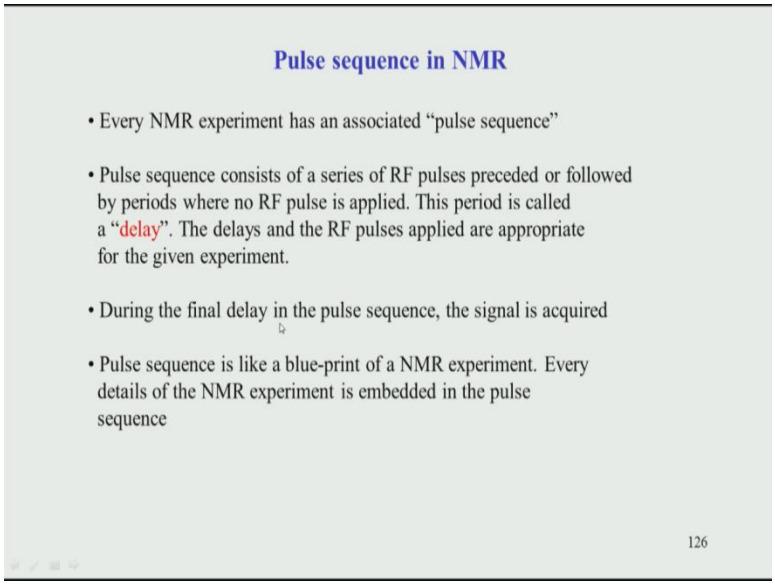
So, now if I do this math what I will do is I will subtract this two and then rearrange the equation to get the sigma. So, this is what we shown here and we can see here now based on this how we can this the chemical shift. So, if I take that the chemical shift of the reference of course reference in terms of the difference of ())(2:22) from that reference divided by gammaB0. So, what basically the chemical shifts are always calculated by taking the difference between the your molecule the hydrogen which you are looking at from the reference reference hydrogen and then divided by the main magnetic field value which is what is in MHz.

So, if you do that calculation because Vref is in MHz we basically get the value in MHz. So, that is why it is if you take the denominator 10 to the power 6 and (you) it becomes 10 to the power minus 6 when comes to numerator and therefore we use a word million, parts per million. 10 to the power six in million. It is per million. So, the sigma value typically what we get is in parts per million.

So, this is how a chemical shifts are calculated a nutshell, it basically means we take a reference compound call it as zero frequency and zero chemical shift and take the difference of that come up that peak in many other peak and that frequency is converted into this sigma value by dividing it by the main magnetic field and that is how we come to ppm value.

So, this is what it means is this is something we saw again in the last few slides or classes that the chemical shift value does not change with field strength that is because they can care by dividing it by the magnetic field. So, in another in some other magnetic field it will basically be 600 MHz. This will be 600 MHz, if the 700 to the 700 but because the frequencies are also scale according to the (fre) chemical shift. The omega also depends on the frequency on B0. The B0 and B0 are cancelled out and essentially it becomes independent of the magnetic field. It is also we saw in the previous class.

(Refer Slide Time: 4:06)



Now, let us see how we actually experimentally record the NMR data. What are the practical aspects? this will be useful if you are setting up an NMR experiment on NMR spectrometer and therefore you should know what are the different the parameters involved and how a spectrum you can see. So, the NMR spectroscopy one thing one has to understand is very critically dependent on parameters.

So, these base many of the spectrometers which you have commercially available. They have all these parameters set by default. So, many of the users, many of the people, students and users who record NMR spectrum they never actually get to see these value. They never get a feel of these values because they are all set by default but as an NMR expert or spectroscopy if you want to understand spectroscopy in more detail and actually use it in your research, it is very important that you understand the different parameters which are used in setting up an NMR experiment.

So, we are not going to go in details of this we are only going to look at few the practical values, practical parameters which are very important because if a wrong value of this delay, suppose somebody comes and changes this value and the default value changes, it may give a completely bad spectrum and you may think the sample is a problem, you may think there something happen to the spectrometer but actually what would have happen is a simply the delay value suddenly got changed because some miss-setting or miss-calibration and that resulted in a bad spectrum.

So, therefore to interpret NMR spectrum also one has to know what how the spectrum was recorded, how it was acquired and that is where the practical aspects helps in NMR (())(5:47). So, these are in NMR experiment typically we use a word pulse sequence. So, we will use this word more often. So, what is a pulse sequence? pulse sequence is nothing but a sequence of pulses.

So, (if you) Now as we go further into NMR details like 1D NMR or if you go to 2D NMR you will see that is not limited to one pulse if you see in the last class we mentioned that single pulse is what is applied and that is called 90 degree twice or you call it as 180 and so on, but in reality other than 1D simple 1D NMR if we go to more complicated experiments you apply a series of pulses means series of the RF radiations very burst quick pulses which are microsecond typically long and there are series one or two other we also use a word train of pulses.

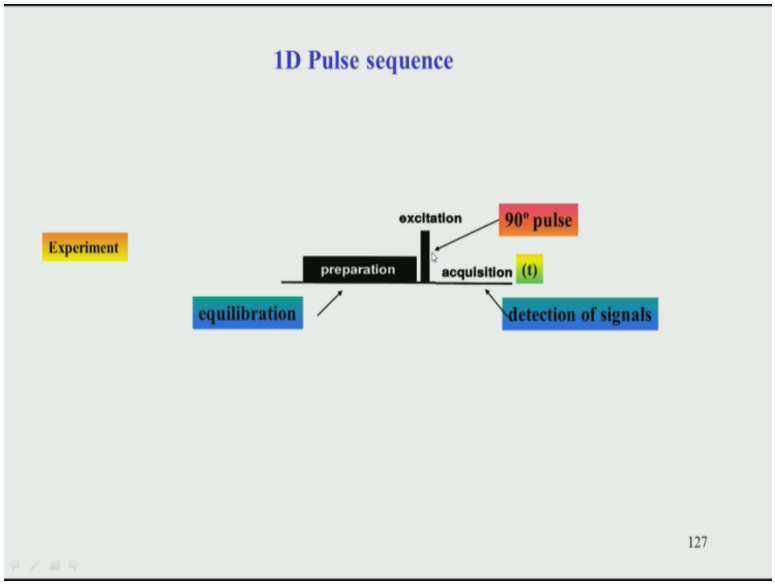
So, this is this train of pulses are applied in a particular sequence you do not apply it arbitrarily. It is not a random sequence. It is well defined a way you depending on what experiment you want to do and between any two pulses there is a gap and that gap is we called as a delay and gap is typically a short gap it could be a millisecond long gap, it could be a few microseconds and depending on again that on depend experiments but always remember there always a gap a few between two pulses and that is called a delay.

So, basically NMR experiment is nothing but series of RF pulses (())(07:12) pulse with delays. So, we have pulse delay pulse delay or delay pulse delay pulse and so on. So, this are basically how NMR experiments are performed you applied a series of pulse and delay in between and after the last pulse is over that is after the final delay in the pulse program in the sequence then you start recording the signal and remember we saw that is called FID.

So, the FID is which is recorded that sample is essentially after the last pulse in the sequence. Okay. So, the pulse sequence therefore is like a blue-print. So, let us say you want to a construct a building you give a blue paint to the architecture give blue print out to the contractor. The contractor start building and essentially he needs a blue prints he needs how these particular experiment has to be performed. Also a pulse sequence is telling an NMR expert basically that is how the experiment we perform and the results what you get all depends on how this pulse sequence was constructed.

So, for as we go into more advanced level NMR spectroscopy this pulse sequence becomes more and more complicated. So, in this particular course you are going to restrict to early a simple 1D experiment and a few 2D experiment. So, here you will see that in 2D experiment already the pulse sequence starts getting more complicated. But, let us start from simple 1D NMR experiment how what do you pulse sequence looks like.

(Refer Slide Time: 8:36)

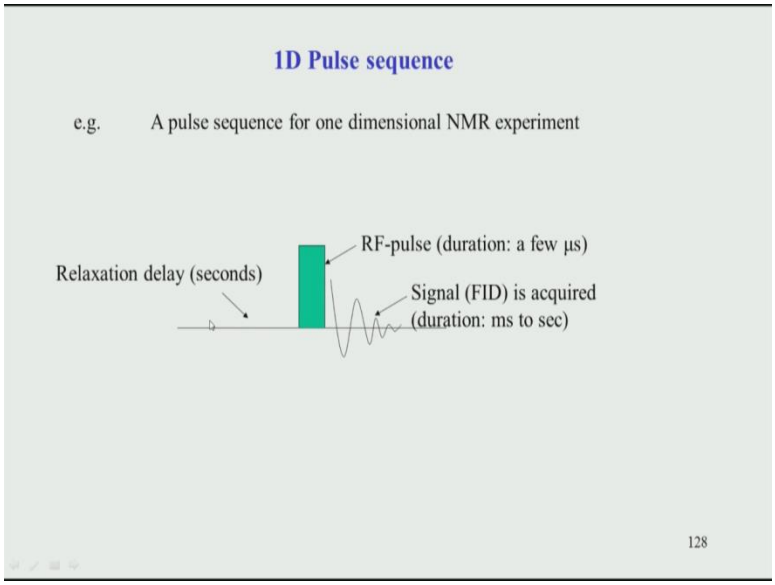


So, it is looks like this. So, you have basically a pulse an excitation pulse. A pulse is there and you apply let us say 90 degree pulse. When you apply a pulse after immediately after the pulse the signals starts coming and the relaxation starts again, this is what we saw in the previous class and that is how we detect the signals but what happens before the pulse. Before the pulse we use the word preparation. Preparation is basically preparing the sample.

So, as soon as we put the sample in the magnet in the spectrometer you will have to wait for some duration and that duration is called a preparation delay that is bond to establish an equilibrium situation. I remember again according to the Boltzmann law the populations have to be distributed between the ground state and the upper state, but that is before you apply the magnetic field that is not the case and population there is no energy difference of the two states and they are all degenerate and population had randomly oriented, but when the moment you apply a magnetic field you need to wait for some time for this population to the redistributed between the two states that is the preparation period.

Once that happens the system is in equilibrium you apply a pulse that is called excitation, so the (gra) molecules get excited and they go to the non-equilibrium excited state and they start coming back because of the relaxation and during that period you acquire or you capture the signal in the form of FID free induction decay. Once that is over you need to sometime go back to do the experiment.

(Refer Slide Time: 10:13)



So, this is what we shown here we look at the times in little more in a few seconds. Let us look at this picture first. So, we have an preparation period we you also use the word relaxation delay. So, this we apply a pulse and then you have the signals which is called FID. Then once the FID is over we need to go back to the this position again wait for this preparation delay relaxation delay and again repeat this pulse sequence.

So, this is how a 1D NMR experiment is done by repeating a number of times this particular sequence that is why we use the word pulse sequence. This is a sequence of delays and pulses. You have first simple relaxation delay then we apply excitation pulse this is now a 90 degree pulse then you record the signals then again you go back. So, this is called when you say number of scans. So, this is what the word we use when you go back and do repeat this n number of time. Each time the FID is stored sub and added to together. So, this is each the cycle is called as scan.

So, if you do this cycle to the n number of times we use the word n scans. So, now let us look at the values of these delays. So, typically this relaxation delay is of the order of seconds and this depends on the t1of the sample of the molecule. So, if you know a t1 so some many times of course you may not know the t1 of your sample. let us say that you have rough estimate experiment because base of the molecular size one can roughly estimate the t1 value.

So, typically for small molecules if we draw few seconds one or two seconds. So, you have to wait about three to four times the value of the t1. So, therefore suppose let us say your t1 of sample is one second then you will have to wait about three to four seconds or ideally this a five times the t1. So, five times the t1 is five seconds. Practically typically we wait about three times a t1. So, about three to five seconds you wait for that particular sample then you apply this pulse and this pulse remember is a very short duration and few microseconds.

So, this is called RF pulse and burst of RF and after this few microseconds are over this magnetization would now be the y-axis or x-axis depending on the direction of the pulse and this is what we saw in the last class, the how the excitation brings magnetization to the x-y direction. Now, once you remove the pulse the magnetization goes back towards that axis and is de-phases in the xy-plane and that is what we called as t1 and t2 relaxation and during this time when it is in the xy-direction it is start de-phasing.

The de-phasing is what is captured as a signal (())(12:56) what you can see here and the oscillation the magnetization is rotating and going back to z-axis that is the oscillation is coming because of the chemical shift and that is captured as a frequency of this lane. So, this is FID what is stored in the computer but if you do it n number of times the repetition and each time very it is added together. Suppose, you record ten times the signal is added ten times and why it is recorded so many time the reason is to get better signal to noise ratio.

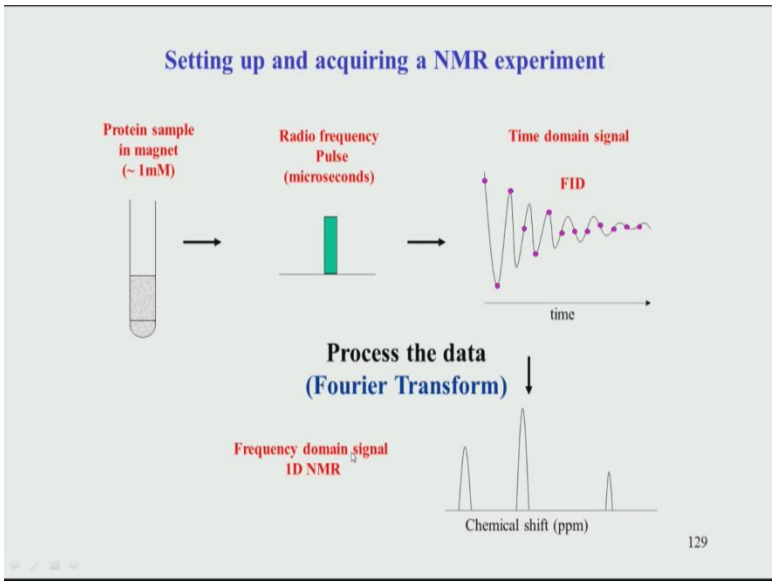
So, if you remember what happens is in a noise. The noise is random wide noise. So, the noise starts cancelling even each time we add each other but it does not cancel to zero it cancel it add sub slower then the signals. So, signal if it is x and we multiply ten time repeat ten time the signal will become ten x ten times x. So, but the noise does not as ten times noise into the square root of ten times noise. So, if you go as the root of ten. So, when you take a signal to noise you are basically dividing ten by square root of ten. So, the signal to noise has gone up of 10 by 10 square root of 10 which is roughly three times.

So, if I repeat it in my experiment 10 times now signal to noise goes on the factor of roughly three times. So, this is what is idea behind repeating this experiment n number of times and therefore we use the word scans. So, how many scans was spectrums recorded. There is typically

the questions you will asked and for practically and that depends on your sample sensitivity. So, depends on how good your sample is?

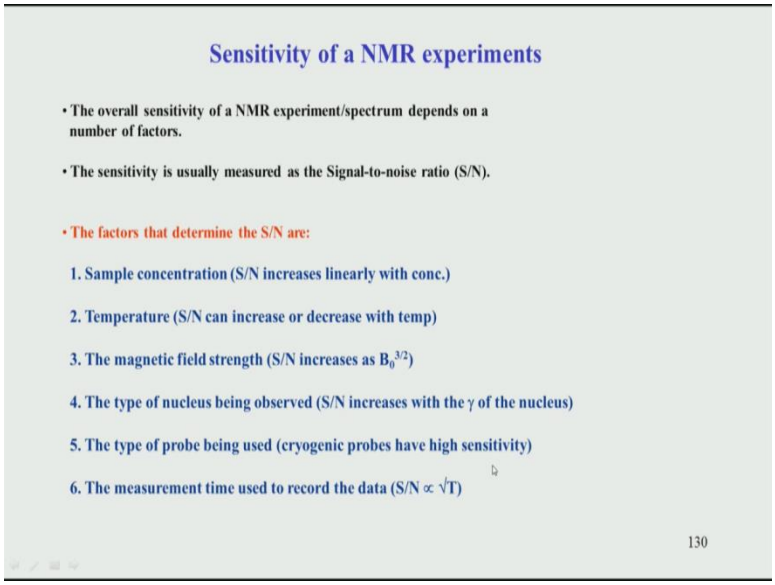
If your sample is very high in sensitivity, in concentration and so other factors you can, you do not need very high number of scans but if your sample let us say very dilute and it very dilute sample or if a very less amount samples we cannot make very concentrated sample then you have to use more number of scans because your signal to noise has to be increased.

(Refer Slide Time: 14:59)



So, this is typically the process we saw in the NMR but you apply read a frequency pulse to a sample and then you record and acquire. This is physically acquiring a spectrum and FID in the time domain and upon Fourier transform you get a spectrum.

(Refer Slide Time: 15:16)



So, we look at this number of scans. So, scans basically means what is signal to noise the sensitivity. So, this is where we have to keep in mind what are the factors which determine the sensitivity of a NMR spectrum or experiment. So, this is what is a general list is given here. so, we can see that the first most important thing is a concentration of the sample.

The signal to noise increases linearly with concentration. So, suppose I have a 10 milimolar sample of my molecule and I record with 20 milimolar sample another spectrum. So, the 20 milimolar sample will be two times the sensitivity signal to noise compared to the 10 milimolar provided all other conditions are kept same.

So, the means we should that we are comparing with the same spectrometer same number of scan, same temperature etcetera, if you keep all this parameter same then the sample concentration is directly proportional to the signal to noise. So, that means if you double the concentration the signal to noise will go on. So, what is a typical concentration that people should use in NMR is typically about milimolar or so. Again depends on spectrometer frequency depend on the type of sample for large molecules we need more concentration in because large molecules are less sensitive.

We will see that as we go on. So, sample concentration is important parameter and for the sensitivity. Temperature is the another parameter. So, whether temperature can increase or

decrease. So, what happens is if you look at theory in a theoretical way if you remember the Boltzmann law. The Boltzmann law says that if you increase the temperature the population between the upper and the ground state difference will reduce which means that more and more molecules will now be in the upper state relative to ground state, that this comes from Boltzmann law. I would suggest you to look at the Boltzmann law again and figure it out from there how the temperature determines the population ratio.

So, if you let us say you go to a highest infinity temperature is very high then the population will be equal between the two states. So, remember when population is equal upper and lower state the difference is zero then you do not take any NMR spectrum. Similarly, if we go to the other extreme let us say we go zero kelvin, absolute zero, in that situation they will be all the spins will be in the ground state nothing will be in a upper state that is a maximum sensitivity but this is all theoretical limits.

Practically, you will not go at that extreme temperatures because your sample will anyway not be stable. So, typically we do not go below the freezing point. We will look at the solution. So, for if you take a water sample. The typical water sample will be you will not go below the ice temp freezing temperature which is zero degree Celsius. So and so, typically operating temperature is somewhere from pi degree Celsius to let us say 40 degree Celsius depending on their sample. So, it that under goes range of temperature the (sam) signal to noise scan increase or decrease.

So, there is no particular reason because it depends on the molecular structure and so on. So, if we increase the temperature sometimes it improves because it can now there are variety of factors will go into that later the t2 value increases. If t2 increases at high temperature the lines are very sharp and if the lines are very sharp the signal to noise is good. Remember we saw in the last class that the line width that is the width of line depends on the t2 value.

So, temperature is scan increase or decrease again in sample specific that theoretically, in theory if you go down in lower a temperature we saw the population difference improves and it can increase but practically it may depend on other factors also. So, now let us look at magnetic field and here is very clear that the magnetic field direct signal to noise directly increases based on this (param) factor B to the power 3 by 2 . This is very important because if we go from 500

MHz to let us say 1GHzs 1000 MHz and this signal to noise will go more than double if you go by this factor.

So, therefore it is very important to increase the (())(19:24), you have to go to higher and higher magnetic fields and very important parameter of the sensitivity is depending on the type of nucleus because remember again in the Boltzmann law, we saw it depends on the gamma value. So gamma of a nucleus. So, if you have hydrogen which has highest gamma will have highest sensitivity whereas if you go to carbon into this gamma is low. Similarly, the detection sensitivity also depends on the gamma value.

How sensitivity is detection and that also depends on omega, omega depends on gamma. So, gamma is very important parameters. So, therefore one has to be careful when you looking at we cannot expect the hydrogen spectrum to be as sensitive as carbon because of the lower similarly (())(20:09) and so on. Remember there is one more parameter which is the natural abundance which is not listed in this particular list and there what is natural abundance is that that particular isotope, how abundant is it. So, in case of hydrogen the abundance is 100 percent but in case of carbon the abundance is only 1 percent.

So, carbon is already ninety-nine 100 times weaker compared to the, carbon is 100 times weaker compare to proton because of the abundance on top of that and on further this gamma value also adds to the reduction in sensitivity of carbon. So, there is a carbon to proton remember ratio the proton to carbon the aromatically shown is four. So, carbon is four times less gamma value compared to proton. So, carbon will be four times in fact if you detect carbon ad observe carbon it is about 16 times less. So, these parameters natural abundance and gamma further contribute to the signal to noise. So, signal to noise increases with increasing the gamma.

Now, the important the in the todays spectrometer technology is very important what type of probe you are using. So, remember we discussed two types of probes. One is called a gyro magnet as Sorry, RF probe which is cryogenic probes and other is basically a room temperature probe. So, the cryogenic probes are three times remember three times more sensitive roughly which are operating at liquid helium gas temperatures that is 25 kelvin.

So, there are different types of cryogenic probes. But typically a cryogenic probe means that the probe the electronics, The RF coils and the pre-amplifier and other electronic parts are at a very

low temperature. So, when you reduce the temperature in electronics a thermal noise reduces. So, when we look at this an S by N, the N will reduce the sample is a same. You are comparing sam same sample between around normal probe and cryogenic probe the sample is same but the noise will reduce in a cryogenic probe.

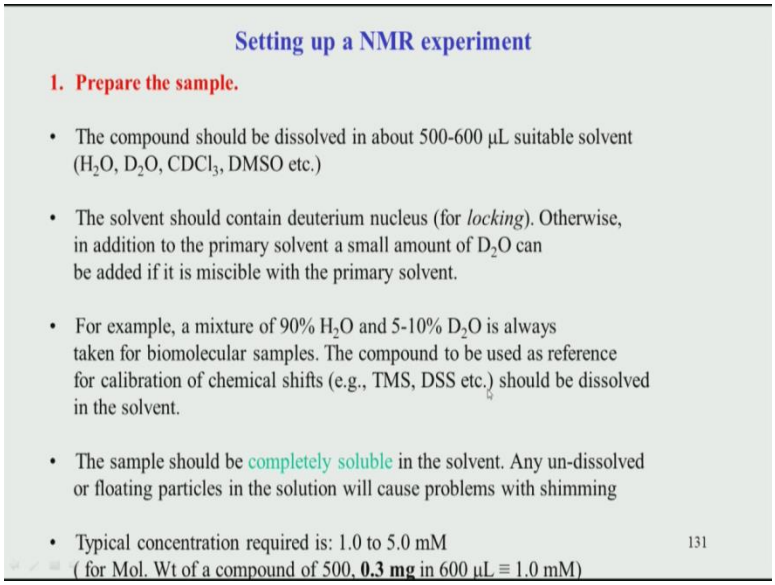
So, as a result the signal to noise goes on. So, signal to noise can be further can be increase by using this special cryogenic probes and this is typically used for biomolecules. When you look at biomolecule we look at very high magnetic field. In biomolecules sensitivity is a major (import) critical issue. So, anything which boost the sensitivity is welcome in a cryogenic probe or in a bimolecular study the NMR. So, in bio molecule when we study we typically the studies involved using cryogenic probe in combination with high magnetic fields.

So, this is the combination used high (())(22:43) the biomolecules and that determines the sensitivity the increase. So, now the most important again parameter from spectroscopy's practical point of view is a measurement time that means if you record a NMR experiment twice the measurement time how much there is a sensitivity improve and signal to noise improve.

The signal to noise is proportional to the square root of measurement time and this square root is coming because of this what I discuss in a previous slide that when you increase the number of scans your signal increases proportionately but the noise increases only by square root of scans. So, when you take the ratio of signal to noise this is proportional to square root of the scans. Square root of scans meaning square root of time because if I double the scans double the time if I increase the scans ten times I increase the time by ten times.

So, time and scans are kind of indirectly proportional. So, if I increase the measurement time by factor of x, now signal to noise improve by factor square root of x. There is a very important parameter because that was determines if you want to double a sensitivity, let us say you are getting a signal to noise of 5 for a given sample and you are not happy with it. Let say you want to improve the signal to noise with 10 that means you want to double the (noi) the signal to noise but then you measurement time what you record as to increase 4 times because only if we increase the time by four (your fact) signal to noise will improve by factor of two based on this equation. So, you can see the time is very important because sensitivity depends on time very critically.

(Refer Slide Time: 24:21)



So, typically this is how we prepare the sample for NMR experiment you start from basically a molecular sample which is of this volume. To standard NMR spectrometers if you are using what is called as 5 mm probe. The slandered spectrometer has this is the volume required for preparing a particular sample and that sample has they has to be prepared in different in a given solvent. So, based on the type of your molecule, solubility and so on the solvent will vary.

So, typically if you are looking at bio molecules like proteins, carbohydrates and nucleic acids we use the naturally the standard solvent that is acquivious medium which is H2O and one times you put it also an D2O heavy water H2O which is an isotope of proton and sometimes we required per certain reasons we will see that later.

So, this are the two both are acquivious kind of solvents. Now, for organic compounds which are not soluble in water you can use different other solvents such as chloroform, deuterated chloroform, CDCl3 which is a most popular solvent in organic chemistry or you can use DMSO. DMSO is another organic solvent which is used for the looking at molecule which are not soluble in water.

So, similarly there are many varieties of other solvents like methanol and so on and all depends on the types of experiment and the solvent one important point which will see little later on is that is important to have the Deuterium nucleus in your samples if you remember we looked at

the hardware aspects there we saw that the magnetic field a continuously drifts means it reduces in the the frequency.

this something which we cannot avoid. This is a basic aspects this is inherent in the magnetic field and therefore, during the course of the experiment as long as you are doing experiment. You do not want you want a correction for the drift, you do not want to allow the drift and drift will continue to happen but you want the system to not see the drift and if you want to do that you have to do a correction and that is done by this concept of locking.

In a locking basically compensates for this drift. so, what is done is typically that that you take a nucleus which is kind of inert or which in the background and that you add a small amount of Deuterium. It not be D2O remember it can be also D here. You can see in CDCl3 also you have a deuterium. So, you just need a presence of deuterium nucleus in a your sample not in your molecule, it must be in the solvent can be in a solvent because the molecule then we will have different frequency of D.

So, typically the solvent deuterium is taken and that what you do is you adjust the frequency of the spectrometer by continuously monitoring the Deuterium. Deuterium signal starts moving remember you do not see the deuterium signal yourself, it is happening in the background. So, that deuterium signal is continuously monitor by the hardware and as it as the molecule as a drift take place the deuterium value is corrected and when signal is corrected based on a signal of deuterium and the magnetic field is continuously kept at the same value at the as a saw as a sample experiment proceeds.

So, therefore the locking is a very important concept and one has to have a deuterium. So, it is called deuterium locking. So, typically if you look at the biomolecules and when you look at biological systems what we do? We typically solvent in 90 percent of hydro H2O and few five 95 typically nowadays we use 95 percent and about 5 percent D2O is added and so that the D2O which is in that sample can be in the in a solvent can be used for this locking.

The next important point is that you have to use a reference compound. The reference compound again depends on the sample. So, for organic molecules when we take the CDCl3, the standard compounds is reference is used as teramethylesilane. It is called TMS. this is what we use. Remember the chemical shift is for referencing only and referencing in NMR is sort of an

arbitrary thing because of you just force a peak to the zero that if you record a spectrum of TMS we force at to zero and the with respect to that all the other molecules are reference.

For biological molecules we use another compound for reference in known as DSS. So, there are about three or four standard reference molecules which are used for variety of studies and very important point here is that the molecule should be completely soluble in the solvent. We should not have particulate matter floating around in the sample because that distorts disturbs the magnetic field around the sample molecule. So, therefore it is very very important that your molecule is completely soluble in water.

So, with this we will go to the next class where we look at how the experiments are practically recorded and what are the different parameters we should choose to that 1D NMR spectrum.