NMR Spectroscopy for Chemists and Biologists
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Lecture 12
Dynamic effects in the NMR spectra

So welcome to today's class, today again we will be discussing some aspects of high resolution NMR spectra of molecules and here after today's lecture we will end this chapter and we will move to next chapter.

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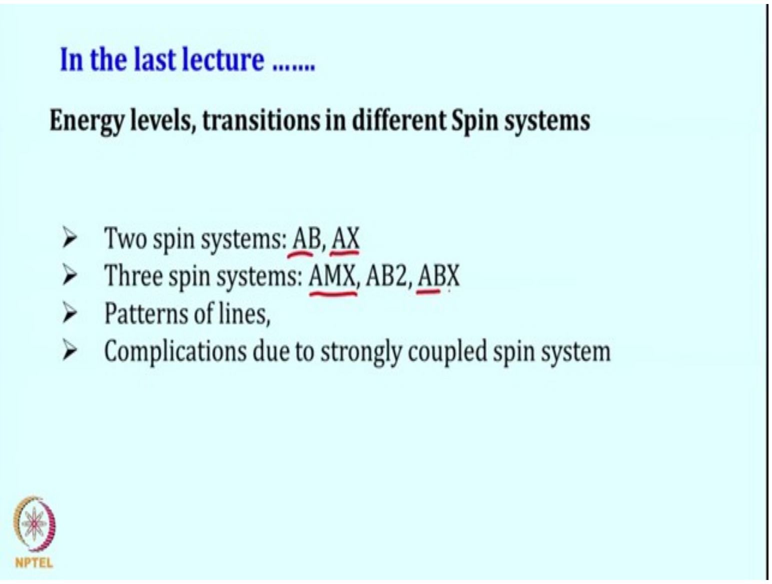

In the last lecture …….

Energy levels, transitions in different Spin systems

- Two spin systems: AB, AX
- Three spin systems: AMX, AB2, ABX
- Patterns of lines,
- Complications due to strongly coupled spin system

So in the last class, we discussed about energy levels and transition that happens in various spins systems, so we derived using quantum mechanical formalism the different energy levels and even we looked at water could be transition probability, so we derived for two spin system, strongly couple system and weakly couple system, strongly couple system as *AB* and weakly couple system as *AX*.

So in detail we looked at what could be the energy level for that and how the intensity will vary for these if they are weakly coupled or strongly coupled, then we move ahead and looked at the three spin system, again for like a *AMX* system which will be all three spins are weakly coupled or *AB* system or *ABX* system, so here *AB* strongly coupled and they are weakly coupled with *X*.

So looking at that we went ahead and looked at the patterns of lines so as we are saying that, for strongly coupled system the line depends upon that factor $\sin 2\theta$,and that change is the patterns of the line and the intensity of line, so that we looked at how it will be for three spin system ABX system, and then we also looked at the splitting pattern and complication that could arise because of strongly coupled spin system, so there we ended it.

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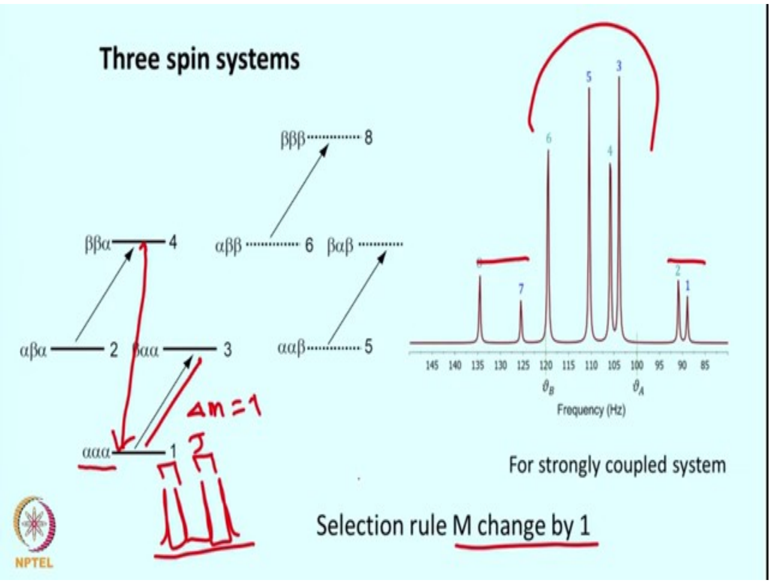

Three spin systems

For strongly coupled system

Selection rule M change by 1

And today we look at some of the interesting phenomena, so just to give you some links, that three spin couple system that we talking about energy states will be something like that, so energy states here in three spin coupled system like all three will be here α, α, α state or two will be an α state and here in these two cases and here two in β states for there will be three states like this and the final one will be β, β, β states.

So we look at the transitions in such cases will be single quantum that means here $\Delta m = \pm 1$, so these kind of transition that we have looked 1 → 3 or 2 → 4 or 5 → 6 or 6 → 8, all those will be single quantum transitions and here we are looking first spin flipping to beta, so because of that if they are weakly coupled system we will get like all equal intensity P_x and depending upon what is coupling constant they will be splitted and they will be distributed in equal intensity ratio like this, this will be J.

But for strongly coupled system as we discussed the line will be unequal intensity and the central lines will be having more intensity than the outer lines, so here if we look at the intensity here is less compare to the central line that is here and that as we looked at this is coming because of the strong coupling system. So but in all these cases one thing will be true that Δm will change by 1, so single quantum transition will happen. As we looked at there is possibility but there is forbidden in NMR that this is this kind of transition is called double quantum transition, this can be achieved but the moment we will stick to mostly single quantum transition.

So in this case either *AB* system or *AX* system or *ABX* system mostly we are looking at single quantum transitions and the lines that arises because of this transitions.

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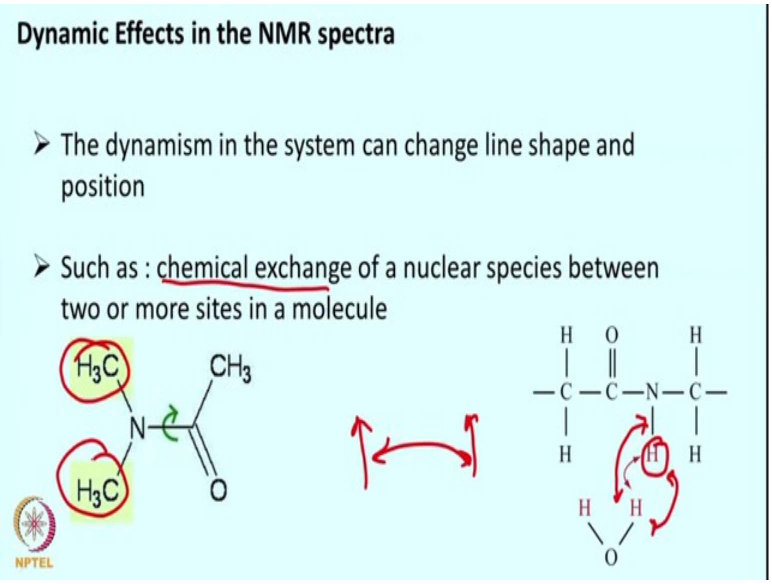

Dynamic Effects in the NMR spectra

- The dynamism in the system can change line shape and position
- Such as : chemical exchange of a nuclear species between two or more sites in a molecule

So we will move ahead and today we will discuss some interesting aspect of the NMR that can arise because of the dynamic factor. So as we know that molecules in solution are not static they have various kind of dynamics, and this dynamism in system can influence the line set and the position.

So what kind of dynamics have one of those that we are going to today look at is something called chemical exchange. So chemical exchange of a nuclear species between two and more sites in molecule that can give rise to dynamic effect in NMR spectrum. So what we mean by chemical exchange of nuclear spin, so say spin is hoping two stets so if it is hoping to states then that is called exchange between the two spins, so if a spin is here or it is here so sometime if it is hoping between them, or for an example here I show here a might bond NH is exchanging with water so this spin is hoping between bulk water and the bound NH. So such kind of phenomena is called chemical exchange.

Or for an example here the bond rotates H-C-N bond rotates this CH_3 and this CH_3 that position can change, and these effects in NMR will give various dynamic effects, and there spectrum can change. So that is what today we are going to most the focused on.

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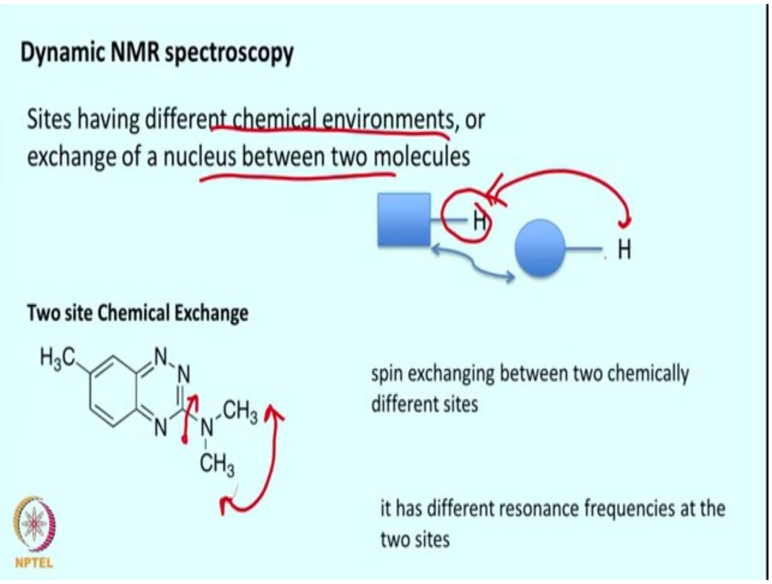

Dynamic NMR spectroscopy

Sites having different chemical environments, or exchange of a nucleus between two molecules

Two site Chemical Exchange

spin exchanging between two chemically different sites

it has different resonance frequencies at the two sites

That we called it dynamic NMR spectroscopy it is not for a molecule, which shows only on kind of transition, but there is change and there is hoping of the spins and that give rise to various line set or the effect resonance frequency.

So suppose site having different chemical environment and or the exchange of nuclei between two molecules is like happening like here, suppose this H is exchanging between two conformation, one say is bound to this conformation another bound to this conformation. So if the proton is exchanging between these two environment, so what is happing here that chemical environment is changing and that is what we are saying chemical environment change of the nucleus between two molecules or here we can say two site exchange, like the previous example here, if you look at this CH_3 because of rotation of this bond can be rotating into states.

So now because of this rotations the spin will be exchanging between two chemically different site, so one may be here the chemical environment is different than here if this proton is exchanging that means they are exchanging at two different site. And therefore as we know that, the resonance frequency for any proton depends upon the chemical environment, now this spin is swapping between two states, so therefore their resonance frequency can also be different, now this proton is swapping between two different frequencies.

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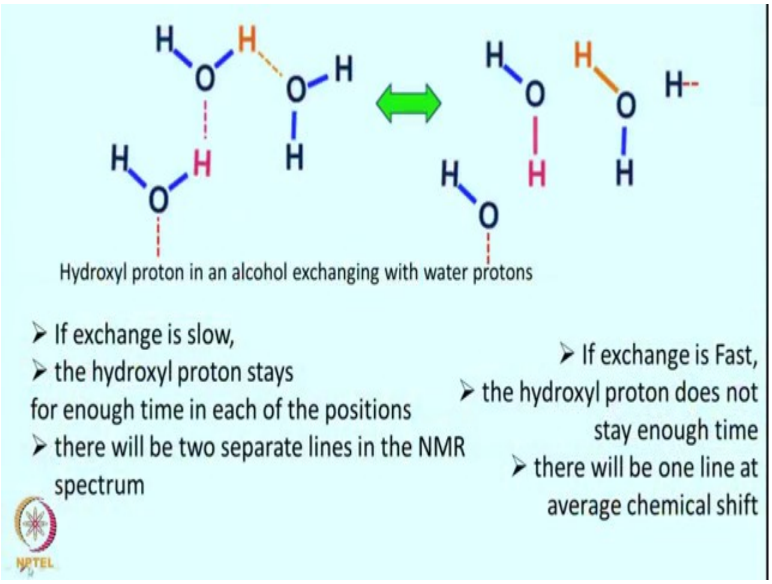

Hydroxyl proton in an alcohol exchanging with water protons

- If exchange is slow,
- the hydroxyl proton stays
for enough time in each of the positions
- there will be two separate lines in the NMR spectrum

- If exchange is Fast,
- the hydroxyl proton does not stay enough time
- there will be one line at average chemical shift

Okay, or for an example let us take hydroxyl ion in alcohol, so OH can exchange between bulk water, so now this OH being labile can go to the bulk water, and again it come back so something like here, here is H_2O molecule and suppose in some of those case like here OH can exchange with bulk water, so hydroxyl proton in alcohol exchanging with bulk water such kind of phenomena also comes under purview of chemical exchange.

Now if the exchange is happing slow, that means this spin is able to see two different frequency, right?

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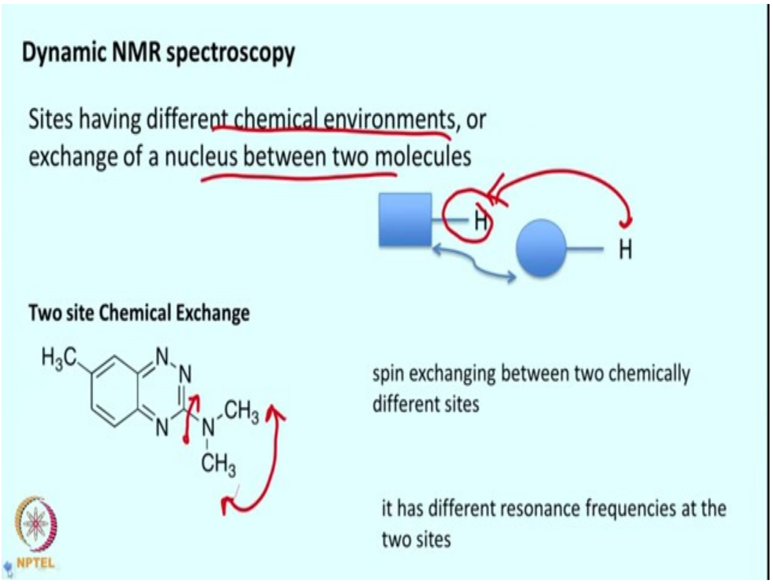

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So because one chemical environment like we had seen in previous slides, so one chemical environment that also spins easier, and the another chemical environment is seen from here.

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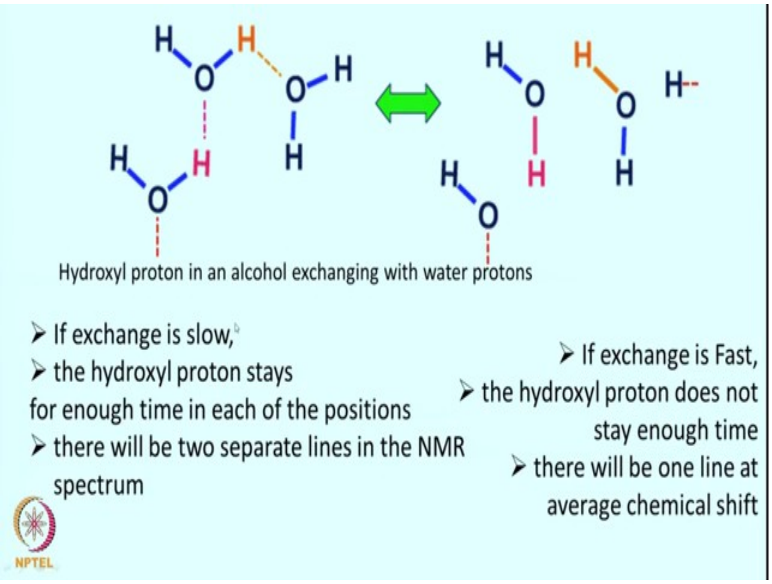

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So if the exchange is slow the here for case of ethyl alcohol hydroxyl proton has enough time at each position, so there if we two peaks, one at the position number one, another at the position number two. So because now two chemical environment are distinctly absorbed by this hydroxyl proton in slow exchange, therefore there will be two separate lines for two chemical environment, so say chemical environment number one, chemical environment number A and chemical environment number B and now if they are slow exchange, so now this proton has enough time to see both of these and therefore there will be two separate peak, but if they are swapping fast the exchange is fast, so now this proton does not have enough time to see both the chemical shift distinctly, therefore there will be result in chemical shift which will be average of these two chemical shift, so rather than A B to distinct we have an average which will be average of A state as well as B state.

So this dynamic phenomena also depends upon how the exchange is happening whether it is fast or slow or intermediate time scale. So those can be classified at various NMR time scale.

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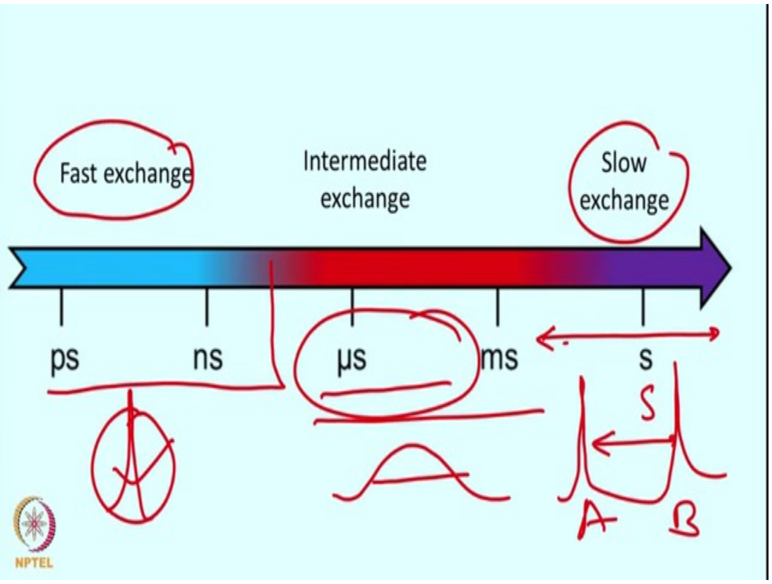

So something fast exchange we call at if it is happening the exchange between those two proton happening fast if the time scale is somewhere in this domain like nanosecond to picosecond time scale, and slow will be somewhere here millisecond to second time scale. So we can call this as slow exchange and this will be fast exchange, so in fast exchange as we saw that there is average peak here, and in slow exchange because now proton can see or this spin can see two distinct chemical environment A and B there will be two peaks. Here, now in this case this is average peak.

What will happen to intermediate time scale? That is the interesting phenomena to see, so intermediate time scale what is happening, now it is not fast enough to have a unique sharp peak at intermediate state, as not slow enough to see two distinct. So therefore it will be at average position but lines will generally be broad and that is actually comes because of this intermediate exchange, those intermediate time scale comes in the regime of micro second at the NMR time scale, so microsecond scale at compare to like NMR phenomena this is called intermediate time region domain.

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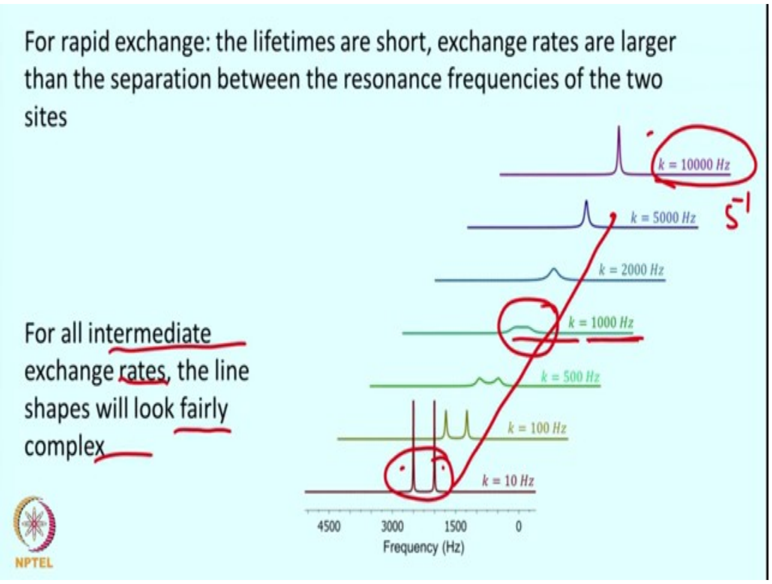

For rapid exchange: the lifetimes are short, exchange rates are larger than the separation between the resonance frequencies of the two sites

For all intermediate exchange rates, the line shapes will look fairly complex

So now so as we say if the exchange is fast we are seeing here the one average peak, so exchange rate say *k* is exchange rate is ten thousand hertz means like if you see ten thousand per second hertz is per second, so if something is exchanging very very rapidly with speed of ten thousand per second that will be fast exchange, and if it is ten hertz so it can see both of these states and that is slow exchange.

As we see here in slow exchange regime we have a two distinct peak and as we move to fast exchange regime we have only one peak but interesting phenomena is happening here, so as the motion exchange increases between these two states, we see line intensity get decreased and at some position like here 1000 hertz gives a broad line, and then it becomes sharp and intensity increases. So for all intermediate exchange rates the line will look fairly complex.

And that's what here we see, so this is kind of fairly complex line because of intermediate exchange regime.

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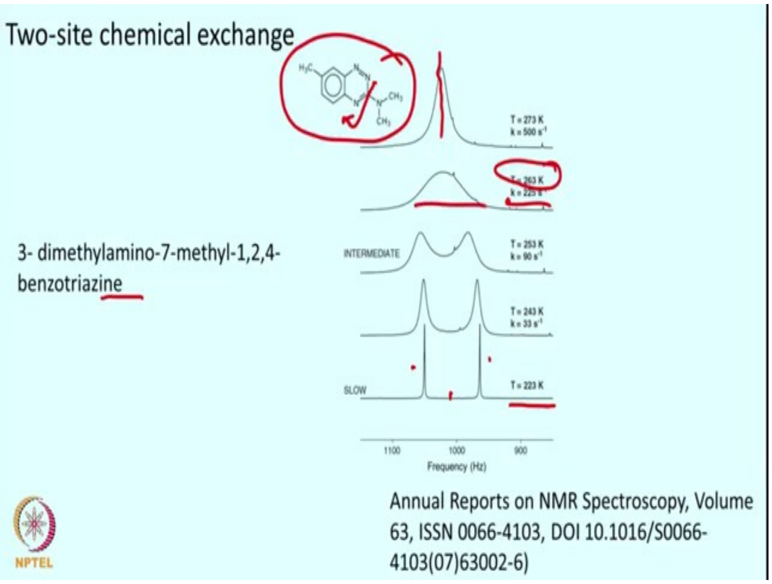

Two-site chemical exchange

3- dimethylamino-7-methyl-1,2,4-benzotriazine

Annual Reports on NMR Spectroscopy, Volume 63, ISSN 0066-4103, DOI 10.1016/S0066-4103(07)63002-6)

Now suppose if we take this molecule, 3-methylamino-7-methyl-1,2,4-benzotriazine this molecule like this, here and here the NMR spectrum recorded for at different time scale motion. So this what happens this bond as we saw this can rotate C-N bond, and because of this rotation the peaks for CH_3 can come differently.

So what happens now it is slowly very slowly rotating and that we can achieve by lowering the temperature so at the lower temperature, actually at 223 K we see distinct two peaks, but as we go increasing like here temperature getting increased and therefore rate is also changing. So at some temperature like minus 10 K to 63 K the rate is 225 per second and now one can see a very broad line at the central position of these two, and as we increase come at the 0 degree rates of the exchange is 500 per second and we have an average peak like this.

So two site chemical exchange as we saw it depends upon the rates of exchange and at the slow exchange rate we have a two distinct line, at intermediate we have a complex line, and at fast exchange we have sharp relatively sharp line but at average chemical shift.

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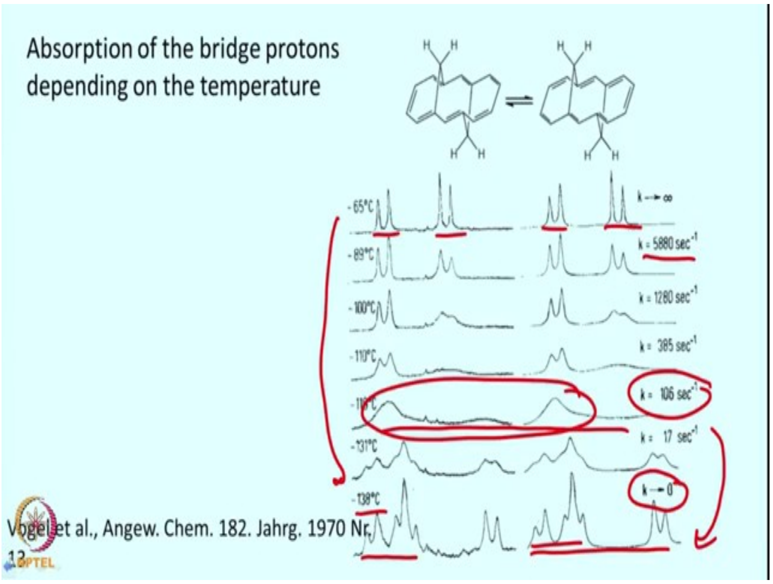

Absorption of the bridge protons depending on the temperature

Vogel et al., Angew. Chem. 182. Jahrg. 1970 Nr. 13

Now let us take some another example of like absorption of the bridge proton that also depends upon the temperature, so if we like start here, if you see here $k \rightarrow \infty$, one can see the spectrum for protons are extremely sharp so here you get doublet, doublet for each of these two protons.

So four peaks doublet because of the coupling but as the speed like as the exchange rate increases at the different temperature, so here we are reducing temperature the exchange is getting slower down and at same like at some of the rates like 106 per second at -119˚ C one can see broad line, so this is intermediate exchange regime, as we go down and if the rate like exchange rate is very very slow at very low temperature like -138˚ C, again you see the lines are broad but now it has become more complicated because now motion is restricted and as you see more complicated like some of those here looks doublet of doublet because now proton can see other proton for very long time, and that is why some couplings are active. So temperature plays a role in case of exchange.

So at the lower temperature exchange can be reduced. So this kind of phenomena is used for many of the experiment where the proton is exchanging between two sides to record a sharp spectrum. And many of the organic molecules where one see that exchange chemical exchange is happening very fast, experiments are typically record at lower temperature to see if this proton is really exchanging between two sides also or so by lowering temperature scientists can identify the protons that are in exchange. So temperature dependence experiment in such case can be very useful.

Now same thing can be also seen if we go for a slightly bigger molecule say if you are talking about proteins, so proteins are also very very dynamic molecule.

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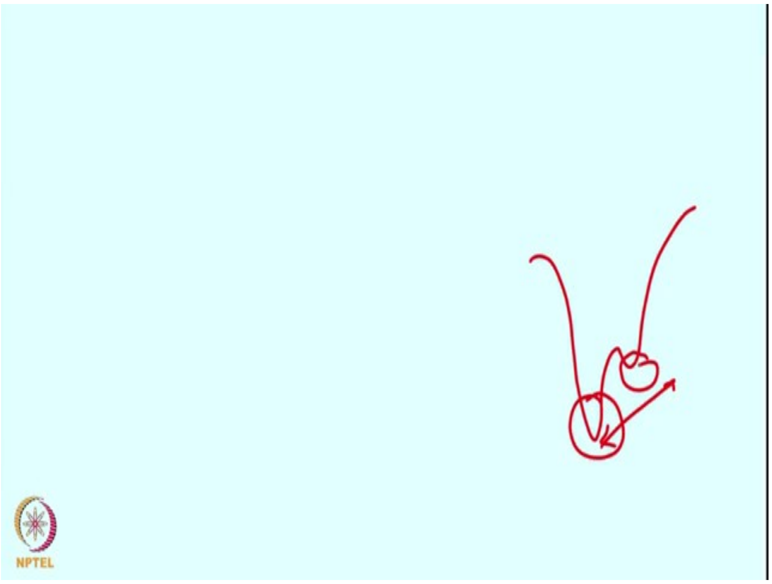

And proteins are also very dynamic molecule and say if we want to study some phenomena where proteins is exchanging between two conformation and so protein conformation say if you like landscape of a protein here there is one conformation of protein here that another conformation of protein. If protein is exchanging between these two states exchanging this slightly conformation is changing or maybe a loop is opening closing something like happening but it is not enough to be that lowly populated states actually by changing the landscape by lowering the temperature one can capture such states.

So that is what people do. They either populate this state or lower the temperature to find it out the alternate conformation that protein can exchange. So this kind of phenomena is very helpful in understanding the exchange between two sides or protein conformation exchange and so on and so forth. Now coming to the next chapter if exchange is happening what happens to multiplet pattern as we see that because of the decoupling we see a spectra are getting split and that we looked at in detail how they split.

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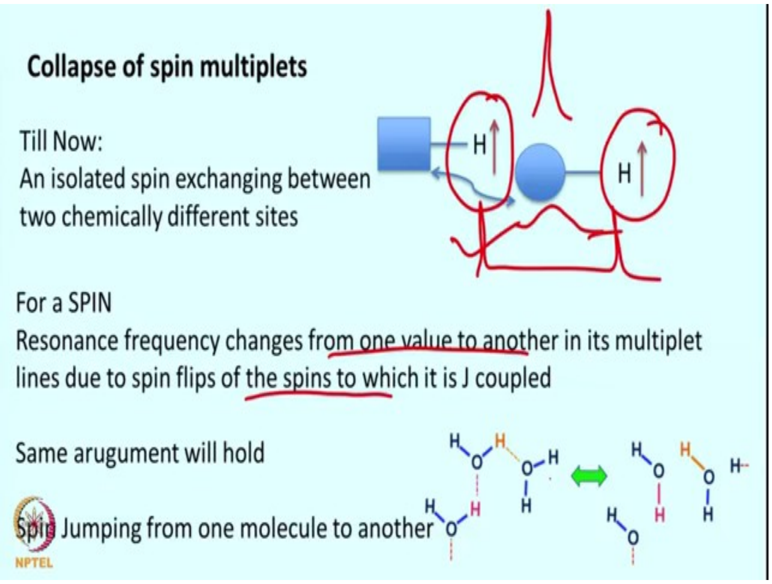

Collapse of spin multiplets

Till Now:
An isolated spin exchanging between
two chemically different sites

For a SPIN
Resonance frequency changes from one value to another in its multiplet
lines due to spin flips of the spins to which it is J coupled

Same arugument will hold

Spin Jumping from one molecule to another

So what happens if those protons that causes the splitting are exchanged, so say here same example we take here the proton in one conformation and here protons are in other conformation these two have two distinct chemical site, Okay? So that is what we looked at because of these two chemical site shift they are exchanging exchange is slow we see two lines, but if we exchange is fast enough we see an average line and if it is intermediate we see a broad line for two different chemical site, but for a spin the resonance frequency changes for one value to another value in its multiple state structure and the lines due to spin flip of the other spin which is *J* coupled what happens to these splitting pattern that's what we want to ask.

Let me rephrase this. So say this spin is coupled with this spin, both have some coupling. Now what happens if the spin is flipping what happens coupling with this? So actually the same argument can hold or whatever we just looked at. The flipping rate or the exchanging rate or exchanging rate of the spin is how much? Is it fast, slow or intermediate. Like for an example we can look at that again the same molecules or OH proton from the alcohol is extending with water.

What happens if it is fast exchanging what happens if slow exchanging or what happens intermediate exchanging.

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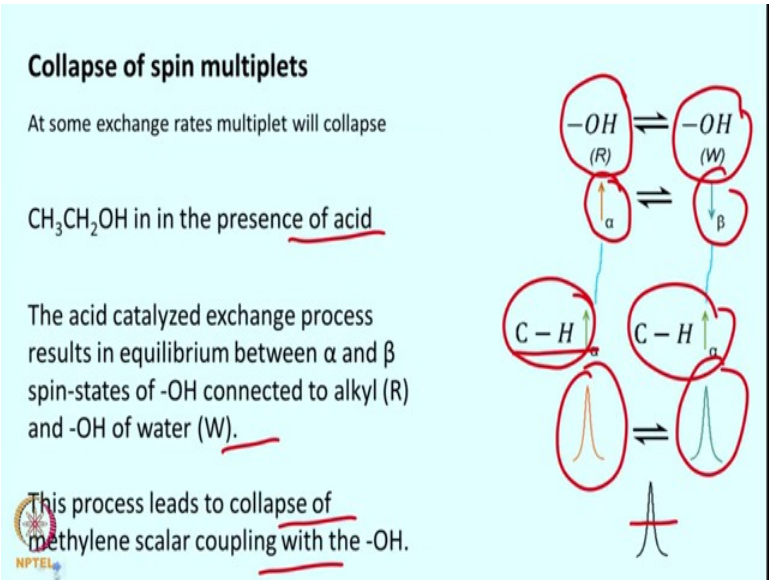

Collapse of spin multiplets

At some exchange rates multiplet will collapse

CH_3CH_2OH in in the presence of acid

The acid catalyzed exchange process results in equilibrium between α and β spin-states of -OH connected to alkyl (R) and -OH of water (W).

This process leads to collapse of methylene scalar coupling with the -OH.

So same thing one can hold if it is fast exchanging you see a generally soft peak but a on a splitted peak, when it is slow exchanging you will see the two peaks just for like here an example I will give collapse of multiplet spectrum what is happening here say this OH group is exchanging with the water group. So now for this these is a alpha state and here is a beta state for this.

Now what happens of this water proton how does it effects of the methylene proton in alcohol. So as we are seeing here it is alpha state and in water it is beta state. So now the chemical shift if it is α is like this and if it is in β state it is the other chemical shift. Now if the exchange is happening very fast then one can see an average chemical shift at this state. So that is what we are saying in ethyl alcohol if there is a little bit of acid.

So because of this acid the proton OH proton can exchange and then it that because this acid can catalyze the exchange process. So results in equilibrium between α state and β for OH proton. Now this OH proton is connected to the alkyl R are proton and water proton. So this leads to the collapse of the methylene scalar coupling with OH and that will see in the next slide.

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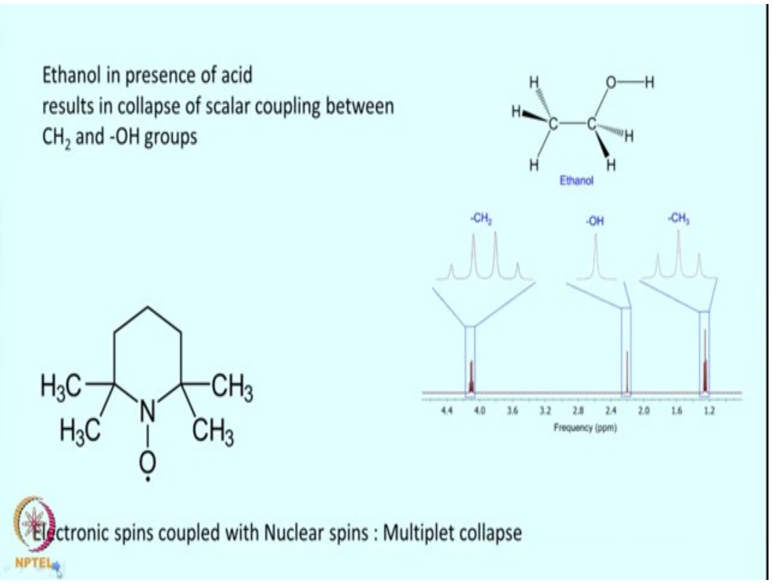
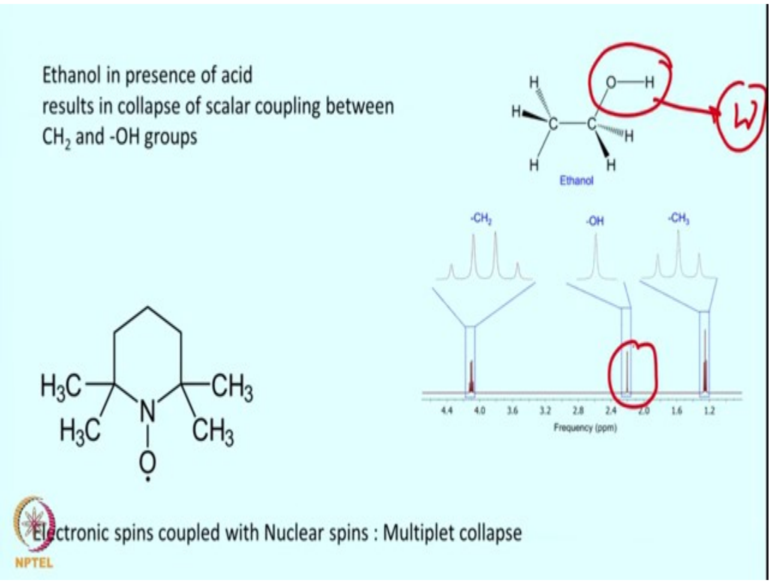


Ethanol in presence of acid
results in collapse of scalar coupling between
CH_2 and -OH groups

Electronic spins coupled with Nuclear spins : Multiplet collapse



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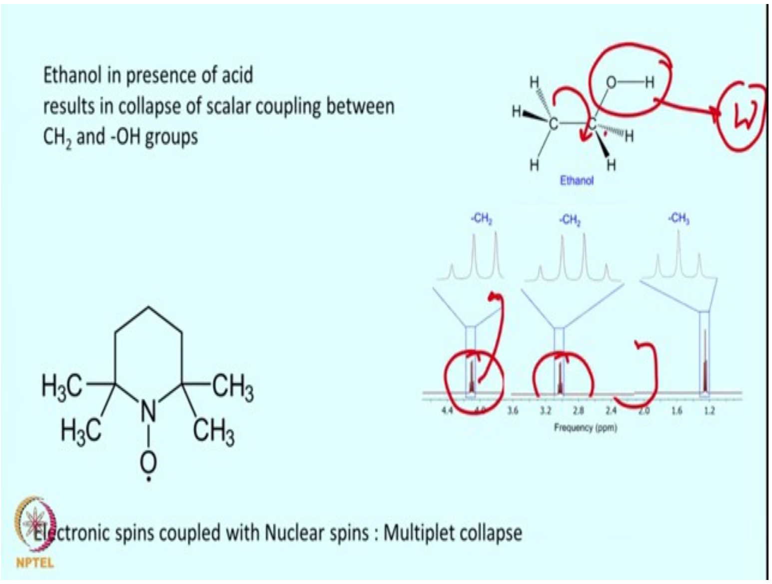

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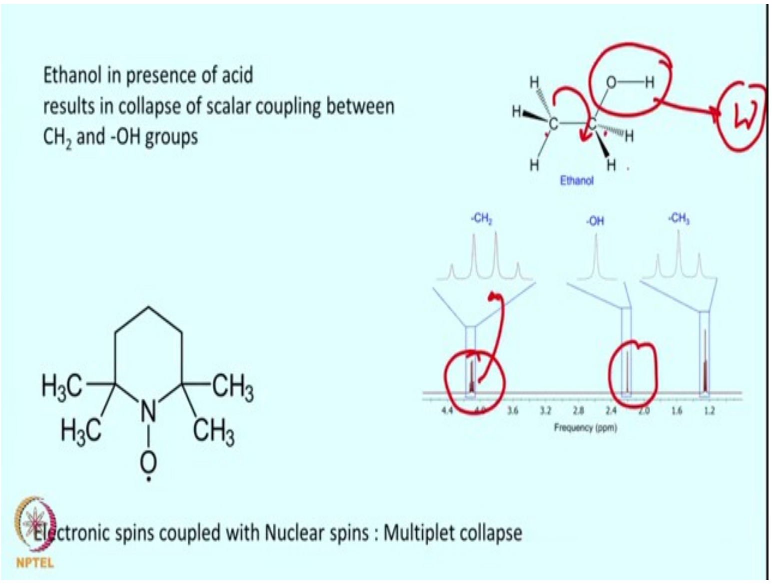
Electronic spins coupled with Nuclear spins : Multiplet collapse



Ethanol in presence of acid
results in collapse of scalar coupling between
CH_2 and -OH groups

Electronic spins coupled with Nuclear spins : Multiplet collapse

So this this what we are talking about so now here is our OH proton which is exchanging this OH proton is exchanging with water proton. So because of exchanging it will have a singlet peak otherwise it could have also had a triplet peak because of this splitting of this CH_2 proton. Now this exchange also affects these splitting of CH_2 because now OH proton is not causing any splitting to this only splitting will come from CH_3 which will be triplet.

So this methylene proton will have triplet splitting because of this CH_3 not because OH because now OH is exchanging, okay? So that's what we say collapse of multiplet if it is in exchange. The another phenomenon that can cause the collapse of multiplicity is if there is a electron unpaired electron present with the nuclear spin. So in this case suppose this in a radical which is called tempo radical if here is an unpaired electron so what will happen to these protons.

Now these protons had actually OH this proton has a electron free electron, now this free electron cause a fast relaxation of these protons here these protons and because of that they will not see the *J* coupling and they will dissolve into collapse of multiplet structure because of the fast dynamics that is happening of this electron proton coupling.

So electronic spin also can cause the collapse of multiplet structure. So if that is the case then what happens to the *J* value of such exchanging protons. So as we saw that in case of multiplet collapse the rapid exchange happening, so if protons are exchanging between two states this rapid exchange can cause the collapse of multi plate structure, so that can also cause the averaging of the *J* value, so like for an example I will try to explain it here.

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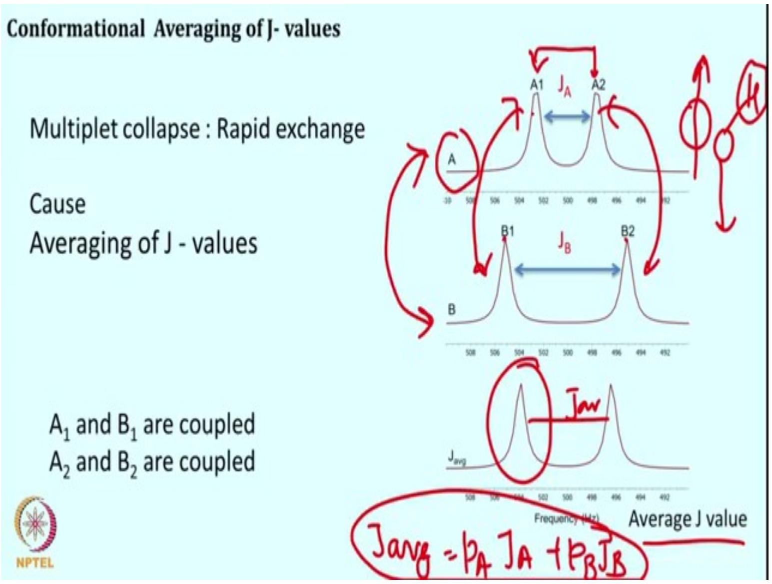

Conformational Averaging of J- values

Multiplet collapse : Rapid exchange

Cause

Averaging of J - values

A_1 and B_1 are coupled

A_2 and B_2 are coupled

Average J value

$J_{avg} = P_A J_A + P_B J_B$

So suppose this A molecule because of the coupling there are like two resonances for this *A1* and *A2* under that coupled by *J* states. So this is support this is a proton or whatever attached OH this has a coupling because of something is pretending to do, now the same proton goes to B state and here the chemical shift is different and there will be two transitions *B1* and *B2* because of the different chemical environment the J_{AB} is there.

So now what happens if the A state and B state exchanges with each other at some time scale, then if you look at now A, so here A and B are coupled and that is why we are seeing two splitting here and here *A2* and *B2* are a coupled that that's why we see split here. So because of coupling we see splitting and because of exchange what will happen that they will give *J* average here so this will be *J* average So that will be they will cause the averaging of the chemical shift average *J* value because A site and B site is exchanging with each other.

Okay. So that one can give it the weighted average of with A site and B site depending upon how much population it spends in each state how much population it spends in B state and that will be contribution coming from the both states, so this will be

$$J_{Avg} = P_A J_A + P_B J_B$$

So in this chapter we started from the chemical shift, we looked at the chemical shift depends upon the environment of the molecule, then we went ahead and explained the another important parameter which is called coupling constant, coupling constant and chemical shift together is very helpful in determining the chemical moiety structures and the small molecules can be identified using these two parameters.

Then we went ahead looked at little more complication of the spin spin coupling if their weakly coupled or strongly coupled on how the lines set patterns, sorry how the intensity of lines change or how the transitions how they are moving

There and here, then we looked at the dynamic phenomena of the NMR because of chemical change if the protons are exchanging between those two sites and then we looked at what could be the parameter that will be affecting the exchange like the temperature each one and if it is exchanging between two sites the electron presence of electronic spin can influence the exchange process, then we looked at the averaging of the *J* value. So we will end up here for this chapter and then we continue with the next chapter the next week, thank you very much.