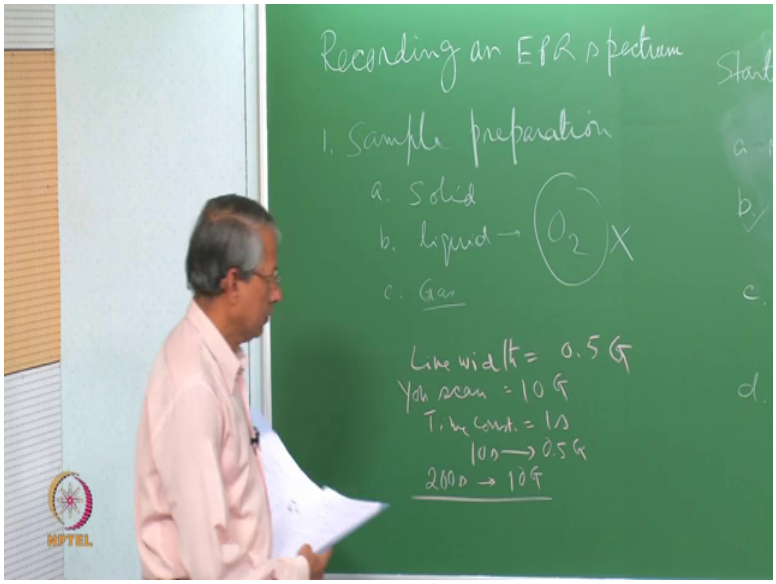
Principles and Applications of Electron Paramagnetic Resonance Spectroscopy

Prof. Ranjan Das
Department of Chemical Sciences
Tata Institute of Fundamental Research, Mumbai

Lecture - 17
How to Record EPR Spectra

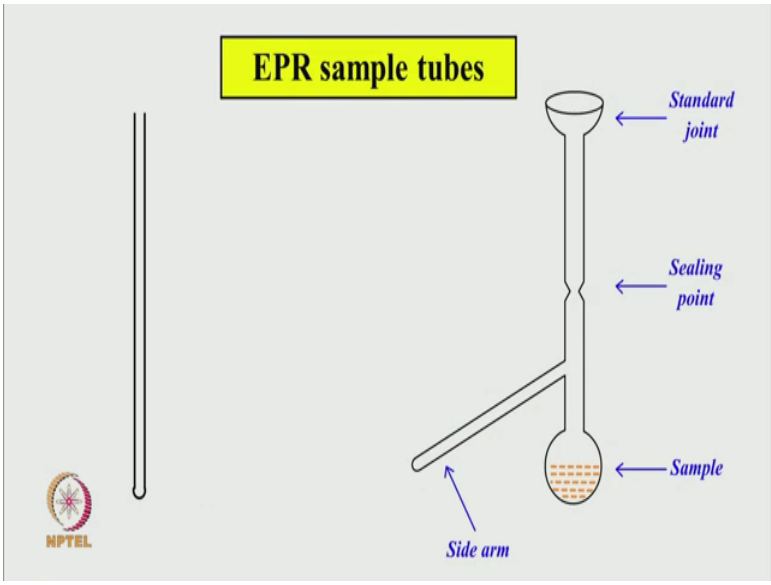
Hello there, today we are going to learn how to record an EPR spectrum.

(Refer Slide Time: 00:27)



And what are the ways that we can get the best quality spectrum. To start with of course, we need to prepare our sample. And sample could be a various kind: it could be solid, it could be liquid, it could be even gaseous samples. So, for different types of samples, we need to have sample tubes.

(Refer Slide Time: 01:25)

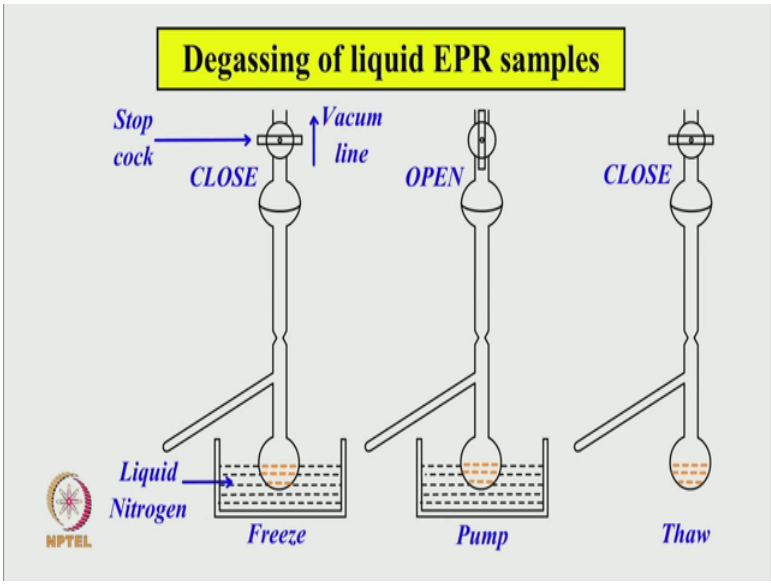


So, if it is solid or liquid, one could use tube of this kind, it is a narrow tube typically in a dimension of maybe 2 to 3 millimetre and keep the solid here or fill the liquid maybe up to 2-3 centimetre, and insert it inside the cavity. Now, often in liquid sample most of the solvents dissolve some oxygen and you all know that oxygen is a paramagnetic species.

So, this presence of oxygen increases the spin lattice relaxation rate of the sap paramagnetic species that is we are studying. So, effectively it causes line broadening. So, also it can even kill the pre radical that is present there if oxygen reacts with that. So, oxygen is something undesirable, you do not want oxygen in the sample. So, how to remove oxygen from the liquid sample, so what we can do is to use a sample tube that is shown here, this kind of tube.

Now, here we use a vacuum line to remove the dissolved air it, so that will remove both oxygen and nitrogen. So, this type of tube which has a small vessel here where the liquid sample is kept, and the sidearm is similar to this standard EPR tube which can be actually inserted in the cavity. Now, this standard joint here is used to connect this to a vacuum line; and after degassing this is the place where this could be sealed off and permanently kept under vacuum. Now, how do I remove the dissolved air from this spot that these are the steps?

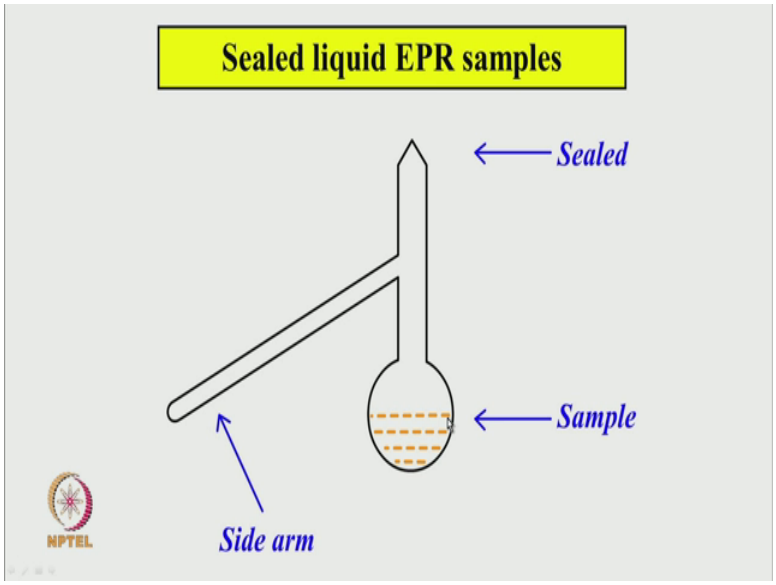
(Refer Slide Time: 03:26)



So, that is called degassing of liquid EPR samples. So, first we connect this is our EPR sample tube after placing the sample here and connect to the vacuum line. And you keep it closed first, and this is the stopcock which can either connect to the vacuum line or disconnect it. So, first you freeze this sample by placing it in a liquid nitrogen dewar. So, freeze the sample first. And then once the sample is frozen we connect this sample tube to vacuum line by opening the stop cock and remove the air which is trapped there. Once this is evacuated substantially well again close the stopcock and then remove the liquid nitrogen from the sample and allow it to thaw to room temperature. As it thaws this frozen liquid melts and because the pressure is low here the dissolved air comes out of this and remains here.

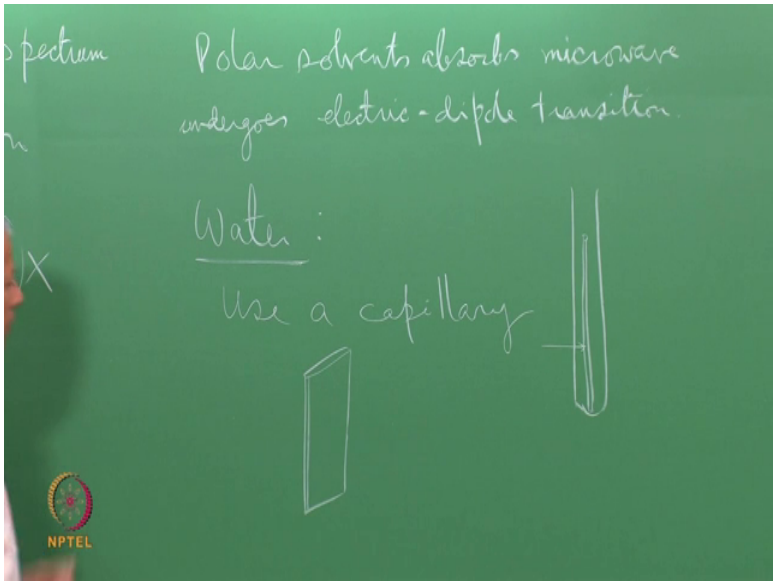
So, again we come back to this step freeze it, keeping the stopcock close; and once it is frozen I pump the released air from the sample here and wait for some time till the vacuum is established. Again close the vacuum line thaw it, and when it thaws whatever residual gas was there or air was there that will again be released so that way if you keep on doing it 3-4 times and if the vacuum line gives reasonably good vacuum let us say 10 to the power minus 4 or minus 5 torr. Then this solvent will almost be free from dissolved air and that is the sample we like to work on. And then with after having reached the desired level of freeze pump and thaw and the degassing of the sample you can seal it here.

(Refer Slide Time: 05:41)



So, this is the way the sealed sample tube this place is sealed. So, for doing the experiment, this liquid is transferred here and then that could be kept inside the cavity. Now, liquid then of course, we can use sample tube of this kind provided this liquid is not very polar.

(Refer Slide Time: 06:04)



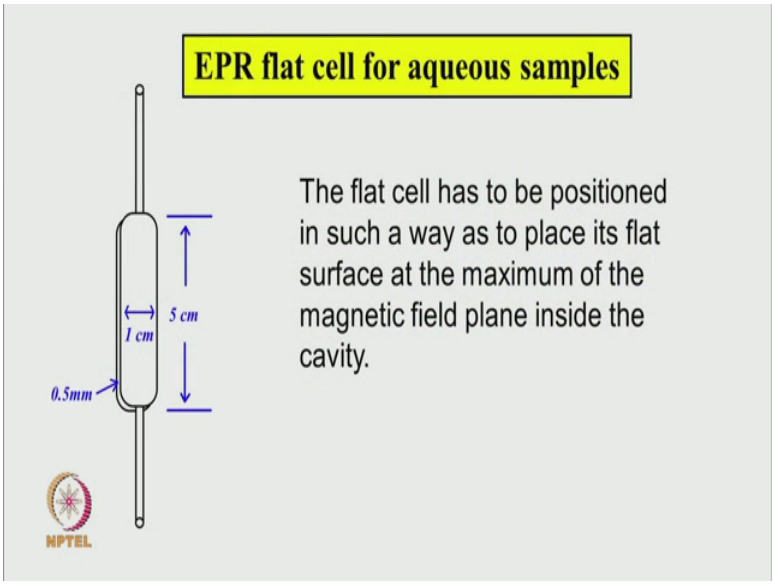
We have seen earlier that polar solvent absorbs microwave and undergoes electric dipole transition that is nothing but the rotational transitions and that is bad we do not want that to happen. So, if the sample is reasonably polar then this sort of sample to you this

sidearm here or here, here they may be substantially larger in dimension, so that the microwave electric field might be influencing the sample here and we can get this sort of transition and that will make the whole thing very insensitive. We do not want this to happen, all we want is the magnetic dipole transition to take place. So, one of the common solvents of a biological experiment is there water, water we all know is highly polar. So, experiment that needs water as a solvent cannot be done in this type of sample tubes that I have shown just now.

So, for that one can get around in two ways, one is of course use a capillary. So, the dimension of this tube here instead of 2 to 3 millimetre diameter to reduce to let us say 1 millimetre diameter and then it may be reasonably acceptable for recording the EPR spectrum, but then very difficult to handle this capillary tubes. So, what normal does is to use the standard EPR tube like this, and then insert the capillary inside there. So, this is the capillary which contains the aqueous sample, then one could possibly record the EPR spectrum of this sample, kept inside the capillary.

But this is not quite ideal because the amount of sample that is present here is very small, and like any other spectroscopic technique, the intensity of the signal will depend on how much sample I put in. So, the smaller the amount less will be the signal. So, unless the sample concentration is very high which is very difficult to get decent signal using a capillary tube; so there is another way of doing it that is use different type of sample tube and that is shown here.

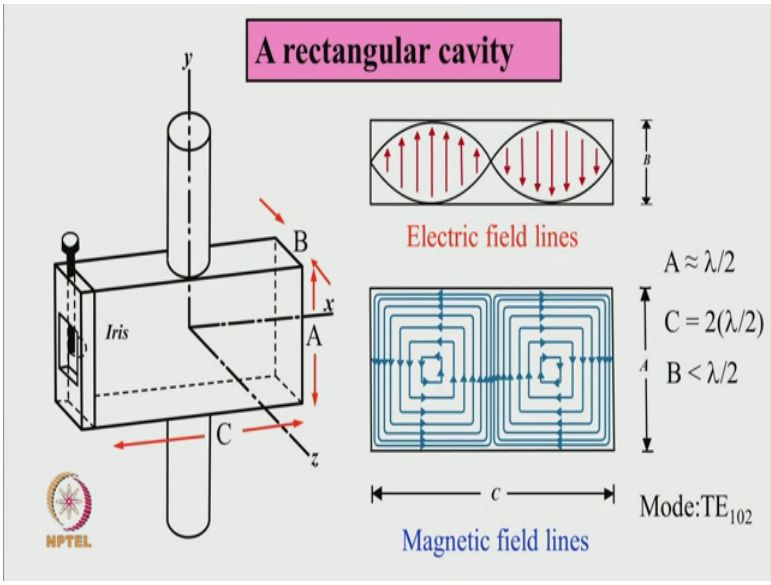
(Refer Slide Time: 09:07)



This is the sample tube, which is called flat cell. This flat cell is a rectangular chamber of approximately this dimension 5-centimetre long, and 1-centimetre wide and this thickness of this is about 0.5 millimetre. So, it is like a rectangular box and these two tubes are to hold the sample tube inside the cavity now the design is such that this will be a flat surface and the rectangular dimension and the thin region is only 0.5 millimetre.

So, if we take a say cross section of this kind, so I can ensure that the electric field actually sees only this much of the sample, but the majority of you can see this much of the sample. So, that way I can minimize the interaction of the electric field with the sample. so that it has to be placed in a cavity such a way that that condition is satisfied. So, the wider dimension of this should be facing the maximum of the magnetic field, the thinner dimension same time will be at the minimum of the electric field, so that is not very difficult to understand where to place it.

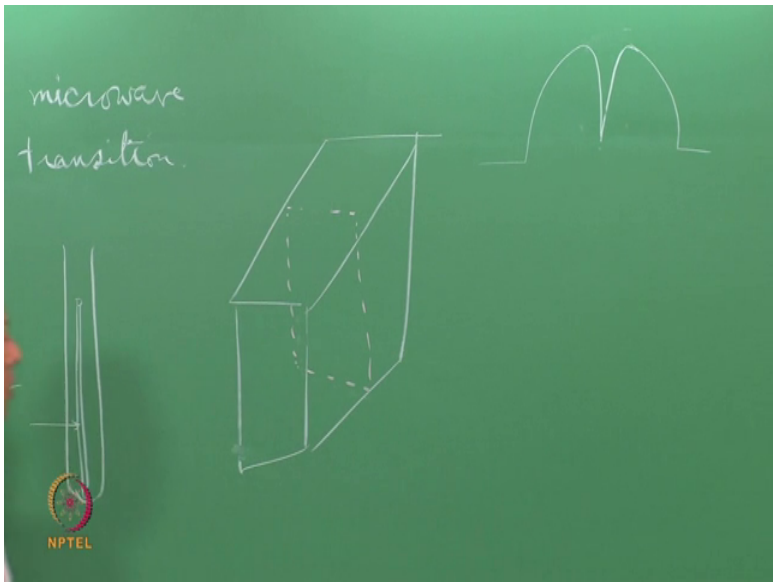
(Refer Slide Time: 10:43)



This is the cavity rectangular cavity of TE 102 mode and we know that at this place exactly here this plane the magnetic field is maximum and electric field is minimum. So, all I need to do is at this plane I must position this flat cell, so that I achieve the requirement that is the sample we will see where will the electric field and maximum of the magnetic field.

Now, for holding the sample in this cavity there of course, holders are available which are mounted here and here and for any of these tubes their corresponding holders for holding the EPR sample this flat cell also special holders are available. Because it is important to realize that it is not only that the flat cell has to be exactly in the centre of the cavity it also has to orient it properly. So, what I mean by that is that?

(Refer Slide Time: 12:03)



Suppose this is the cavity, and the centre of this is a centre of this a place of maximum magnetic field is somewhere here. So, I need that this plane must be aligned here, but it also has to be aligned such a way that it in need perpendicular to this surface. If there is a little bit of mismatch of this kind then the sample from the edges will see the electric field and that is not good. So, it not only it will be a centre also has to be exactly made parallel to this. And in this lateral direction front and back also it has to be exactly centre of the cavity. How does that position that it is not very difficult to do that if we see the cavity mode by reflect modulating the microwave frequency?

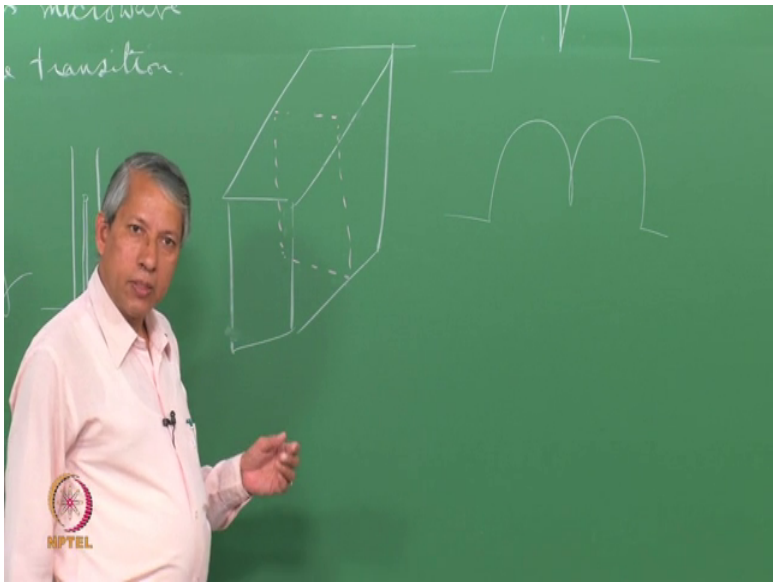
Now, this is the let us say klystron mode and the cavity mode is just superimposed on that. So, this is the cavity mode. When we insert this one the frequency of the cavities of course, are going to change. So, to start with we take the flat cell and approximately insert in this position, so that the front surface of the flat cell is almost parallel to this that is the way we start with, and they get the cavity mode in the middle of this. Now, see that because of the rectangular nature, it has certain symmetry. If you see it once again here its symmetric with respect to this plane on both sides, similarly this is also symmetric with respect to this plane at the centre.

So, having approximately started with this cavity and the flat cell here in this position, if I now gently orient it in this direction, then this is going to shift in one direction, it will reach an extreme position again go back in other direction why is that so. Because

exactly when the flat cell is parallel to this surface that is what the frequency is going to be extreme now. If it goes this way or that way, the shift in frequency will be same, so that we can see this is going this way if you keep turning in one direction and then after some time will come out and come back to this opposite direction. So, that way I can find out the optimum placement of this cavity in the flat cell to get the extremum of this. Assumption in that you must start with the initial placement that this is approximately parallel to the desired plane that the first part.

Second part is to how to ensure that this is indeed centre by adjusting this back and forth here the holders at the top and bottom of the cavity allows one to have that moment also. Say once again because the cavity has this sort of a symmetry plane with us here and see if I the flat cell from the centre if it comes this way or that way that change of frequency will be in a similar direction. So, again exactly at the centre of the flat cell and it is parallel to this plane, this placement twice appearance of this will be or the extremum that is the way one decides the correct position of the flat cell.

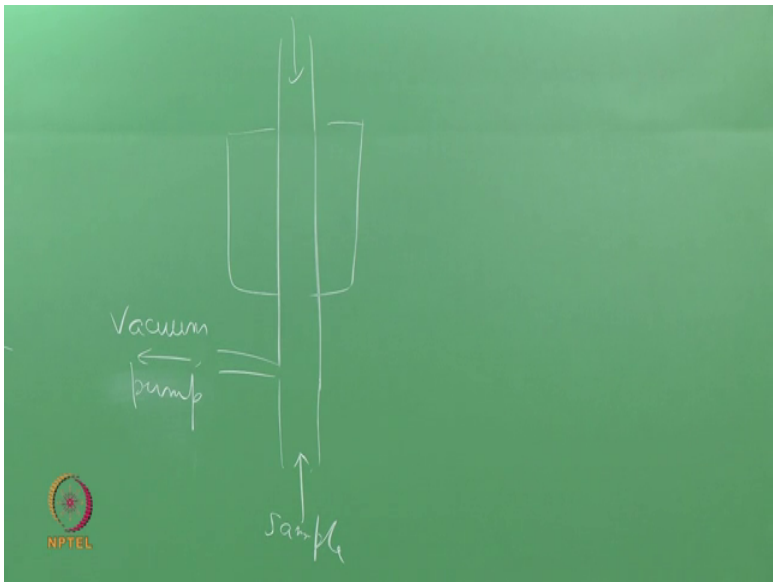
(Refer Slide Time: 16:17)



For cylindrical cavity there is your cylindrical symmetry, so it is not very easy to use similar argument, but the aim is that even in the cylindrical and cavity this is the dip let say. So, when the electrical interaction is minimum, this dip will be maximum. So, I must orient the cap flat cell inside the cylindrical cavity such that I maximize this dip. Sore electric field is seen by the sample poorer will be the Q, and this will become

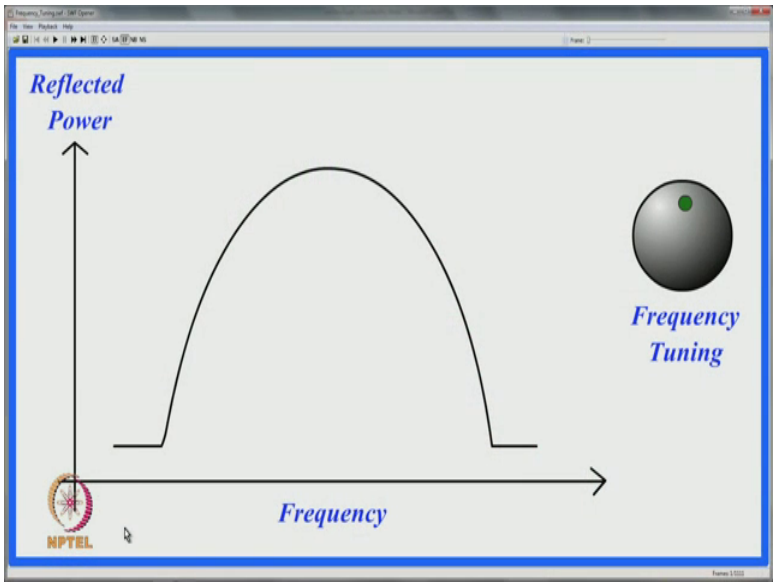
poorer. So, I can either rotate it in this axis or see that this becomes deeper and deeper I try to reach the extreme position and then also move it back and forth to maximize this that is the way to place the sample.

(Refer Slide Time: 17:13)



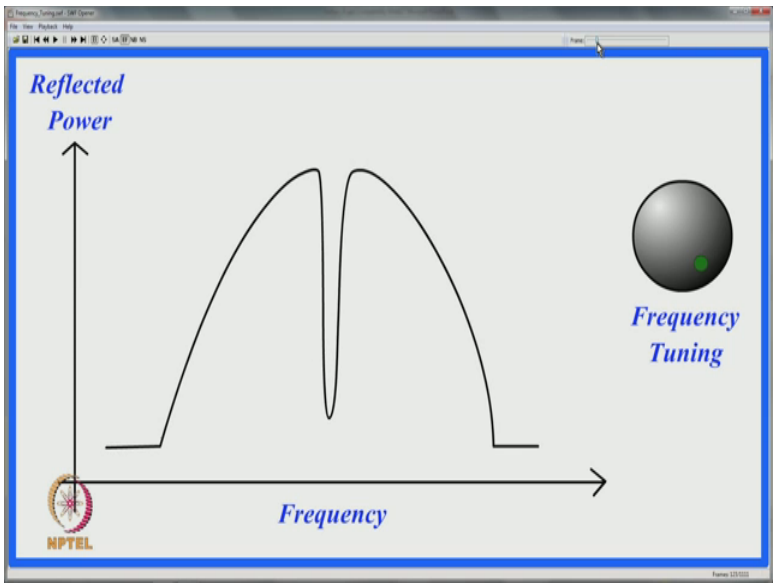
If the sample happens to be a gas then it is very simple all we need to do have a just a tube and which is insert kept inside the cavity and let the gas be inside. But then these paramagnetic molecules when they collide with each other they change their angle momentum directions, so that way lines become broad. So, to minimize the collision at the same time to get a decent signal to noise ratio one has to work at a moderate pressure. So, this has to be connected to a vacuum line or a pump let us say pump. Here the sample goes in, so that way one can do the experiment. Here we inserted the sample and next is to turn on the micro frequency and match the micro frequency to the cavities on characteristic frequency.

(Refer Slide Time: 18:19)



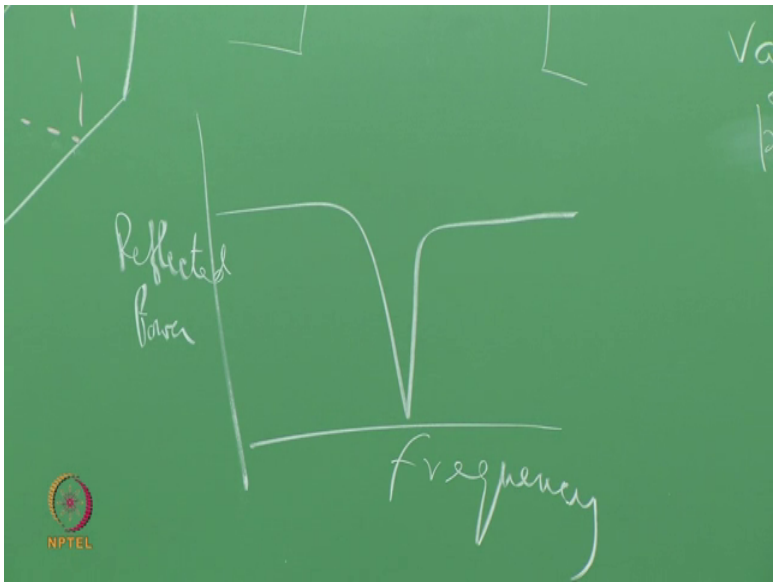
For that, we modulate the microwave power and see where the cavity dip is. See in general we may not see any dip, so we tune the micro frequency using the appropriate knob here and then we should be able to see the dip here it started appearing, we want this frequency to be at the maximum of the micro power that is emitted by the klystron. So, this is the klystron mode and this the cavity dip which is coming there.

(Refer Slide Time: 18:49)



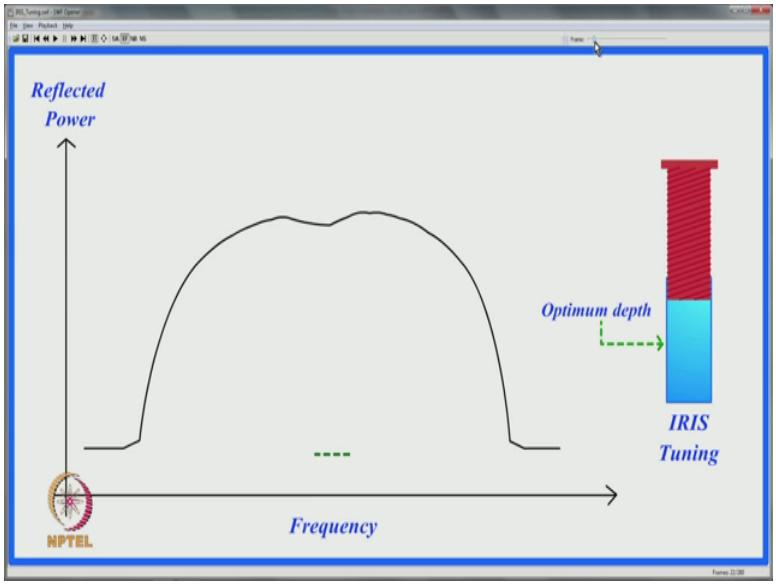
So, tune the frequency. So, more or less this is the correct place. Now, if it is a, if the microwave source is a solid state source gone oscillator then that mode will not look like this, but will look like.

(Refer Slide Time: 19:07)



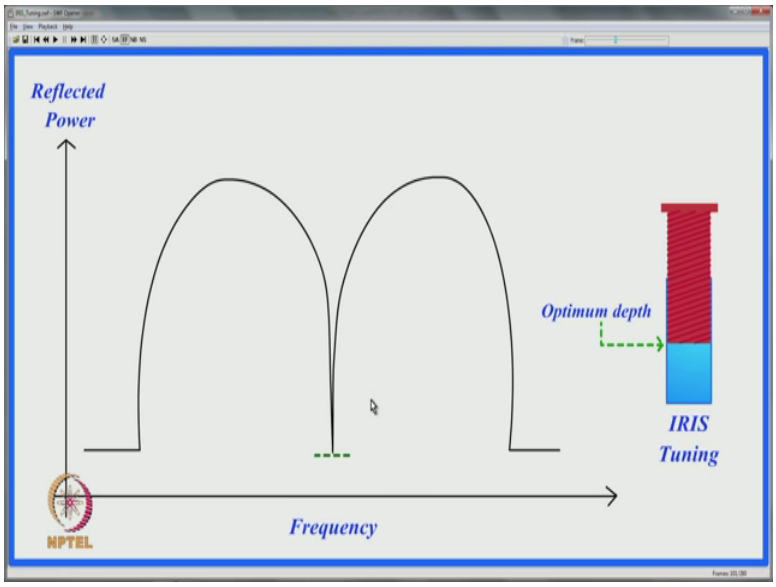
This sort of thing, this is the frequency axis and this is the power. So, this is flat that is the only difference; otherwise it is the same thing. So, we get the micro frequency to the maximum the klystron mode and then we have to optimize the coupling there is this should be very nearly the critical couple condition.

(Refer Slide Time: 19:50)



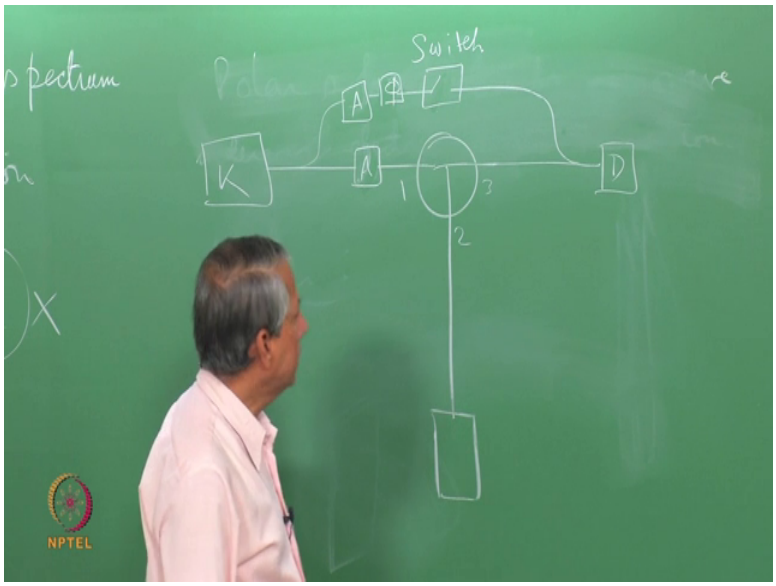
For that so we have to adjust the iris tuning screw which is here. So, we adjust the iris tuning screw, so that this dip becomes maximum, but we should not make it over coupled. So, that is just to turn the screw.

(Refer Slide Time: 20:10)



This is screw is going inside and the dip is increasing and increasing, increasing keep tuning then dip is increasing. Now this is almost critically coupled. So, you go beyond that it is started going on up again. So, this has become over coupled we do not want that go back here. So, this is almost critically coupled, we stop here. So, having obtained the nearly critically coupled condition, we can now switch off the modulation and turn on the automatic frequency control. But here one important point to be kept in mind is that the bias power that is used to bias the detector is present or not while we are doing this tuning up.

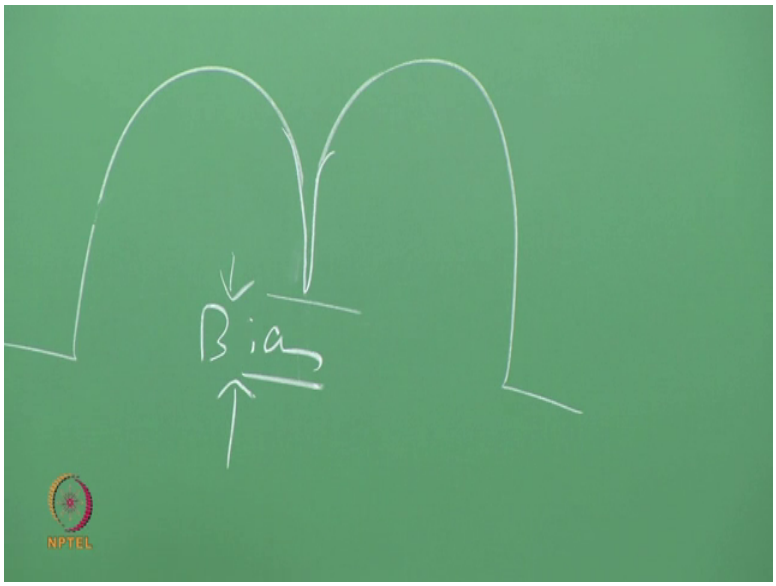
(Refer Slide Time: 21:17)



So, if you remember let us recall our design of the spectrometer this is the simplest arrangement, this is the source of microwave comes here goes to a circulator, this is the cavity is kept here and this is the detector, let us call it D. And we have a bias for coming from here and through this again mixing there you know what appropriate attenuator and at phase it attenuator phase sector. So, if the bias power is present the way I have drawn here then detector sees that power all the time. So, in this tuning this will not reach to the bottom of this one, because that will give a constant micro power there that has to be kept in mind. Some spectrometers allows you to switch off this bias bar during the tuning period.

So, there could be some sort of less symbolically speaking there to be some switch here to either disconnect this or connect this. So, if that provision is there it is better to switch off the bias power, and do the tuning. In that case there will not be any confusion when the critically coupled condition is reached, but if we this provision is not there then once you try to bring it to bring it to as low as possible and then watch that this does not go up again.

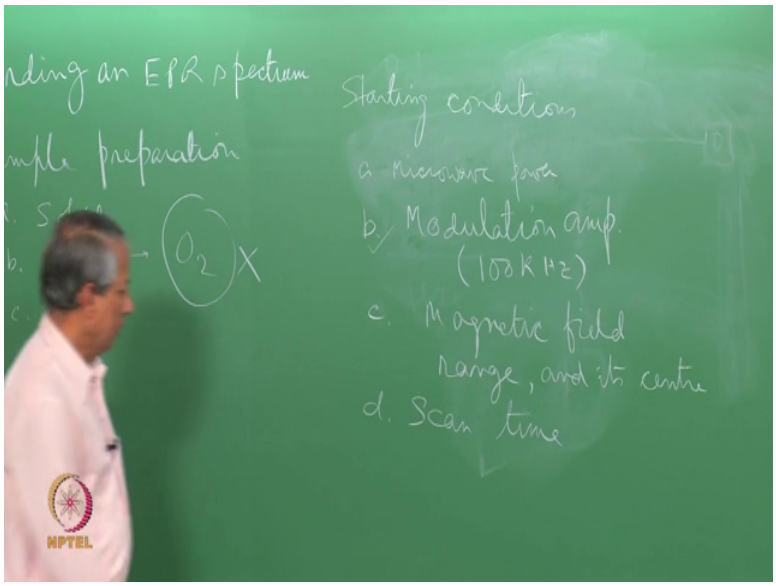
(Refer Slide Time: 22:53)



So, this dip will probably go down here. So, this may be the bias power. So, you will not be able to bring it below that one. So, then again it will go up. This is also assumes that the phase of this is same as this one; otherwise these two power will try to cancel each other if the phase is opposite. So, one has to also adjust the phase of the bias power such that I get maximum signal here and I can see the maximum here also that way also one can start with the bias power to have the appropriate and correct phase with respect to the micro power that is going there.

Now having done that, we now switch on the AFC and then ready to start recording the spectrum.

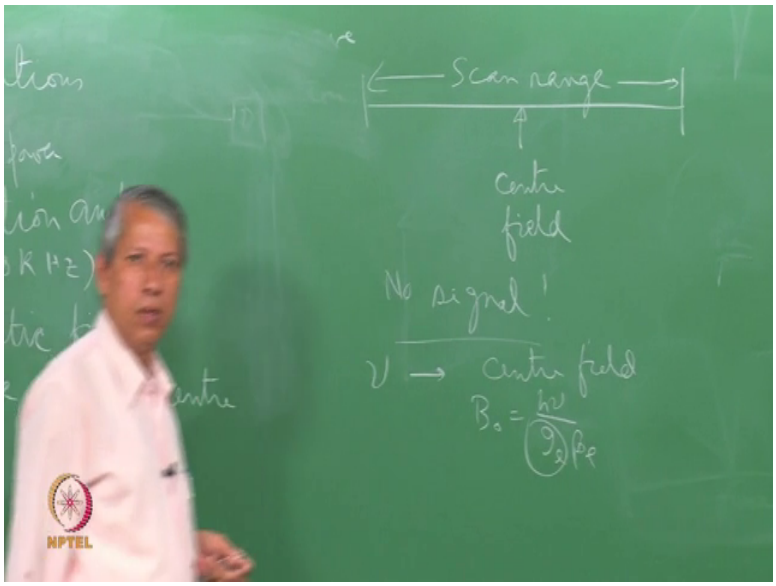
(Refer Slide Time: 23:58)



First, we need to use certain starting conditions. One is microwave power how much microwave power to use to see the spectrum, this is something one does not know a priori, but if you have some idea the sample relaxation time is fairly long and it saturates very easily then one uses low absorbing microwave power. But if the sample does not saturate very easily, one uses high microwave power. So, one start see some intermediate value of the microwave power.

Next, the magnetic field modulation amplitude typically 100 kilohertz. So, here again one has to decide how much modulation amplitude is to use. So, we start with some intermediate value to start with, then the magnetic field range to where the signal is likely to appear here and its centre. So, these two parameters mean that the region of interest where I expect the signal to appear.

(Refer Slide Time: 25:44)



So, let us say this much is a magnetic field, I want to scan and search for signal and this is the centre field this is the I call it scan range, these two parameters need to be adjusted. So, how does one decide that? Again if one has no idea what sort of g value is the sample has then one really does not know what centre field to use and how much scan range to use. So, one usually starts with a reasonably large scan range and assume that g is equal to 2 or so then keep the centre field approximately there and the c f signal is appearing.

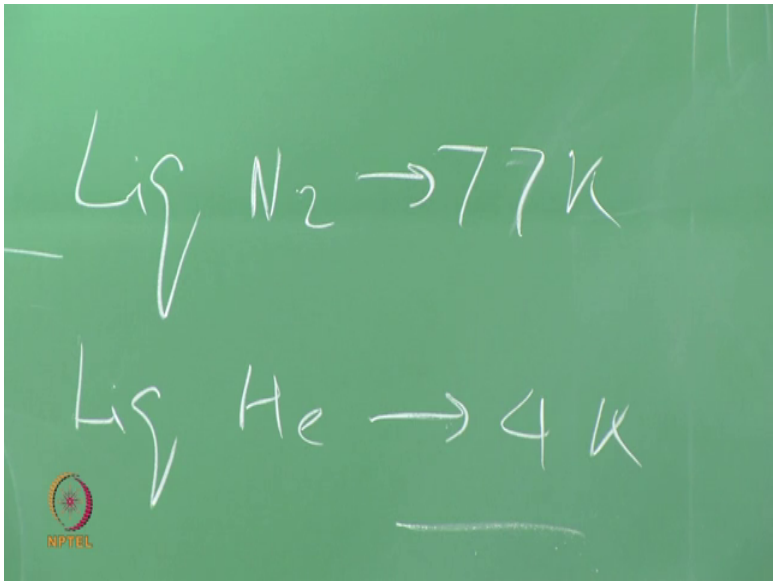
Having setup these conditions, if we look for signal and scan the magnetic field and find that there is no signal appearing there. Then what happens what could have gone wrong or can we really change some of the settings and look for signal. One possibility is that the range of this magnetic field is not right maybe the g value is higher it is somewhere else. So, if one suspects that g value is equal to 2 or any other known values then from the micro frequency nu you can find out the centre field by this formula.

So, you have some idea what this is then you keep the centre field approximately the value that you calculate from the micro frequency and put it here, and then have a large scan range then they try again see if the signal appears there. Or it is possible that a that I may be scanning too little or then we can increase the scan range or it is possible that even then the spectrum is somewhere else that is I can, but that is a assume g value is not what is right. So, I can go somewhere else then change next scan centre will somewhere else again scan it. It is possible then that the one modulation amplitude was not

appropriate enough maybe it was given to a small signal we will see later that it may even give broadening of line if the modulating amplitude is very high. So, you can change this parameter and see if the signal comes or not maybe the magnetic power was either too high which is causing saturation, and this signal was not appearing or it may be is too little. So, the signal also was not coming. So, keep on adjusting this and look for signal.

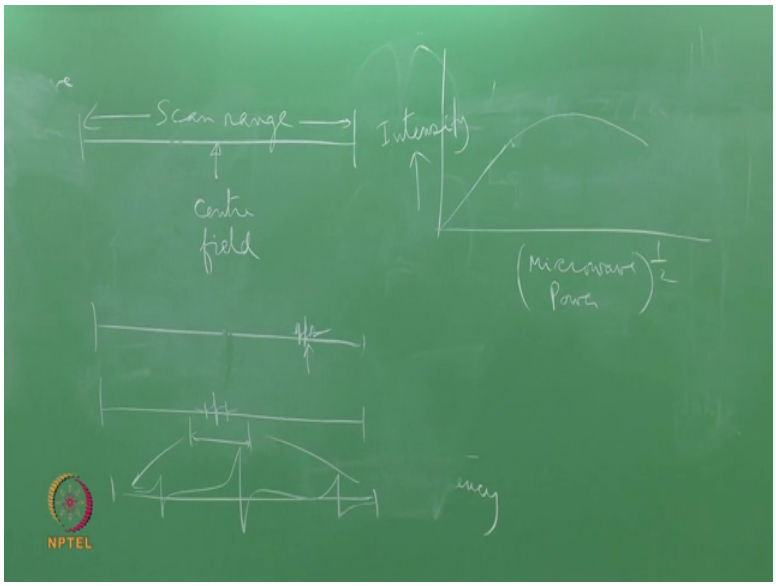
So, in spite of all this suppose the signal really is not coming then what can one conclude. One obvious conclusion will be the sample is not paramagnetic either all the preparation of samples and whatever you have done to bring the sample to the cavity you mean may have just died that is too bad you have to start all over again. On the other hand if there is strong reason to believe that it is indeed paramagnetic and still the sample is not giving a pure signal the way you have done it and what I of course, implicitly assume that we are doing it at room temperature it is possible. Therefore, that the relaxation time is so fast or that it does not give signal at a room temperature then one has to go to lower temperature.

(Refer Slide Time: 29:41)



Maybe, liquid nitrogen temperature, which is 77 Kelvin or even liquid helium which corresponds to 4 Kelvin; so maybe we will get some signal that time. Now, having done all this let us say we have seen some signal now. What are the adjustments we can do to optimize the quality of the spectrum. First thing is to do is to adjust this.

(Refer Slide Time: 30:26)



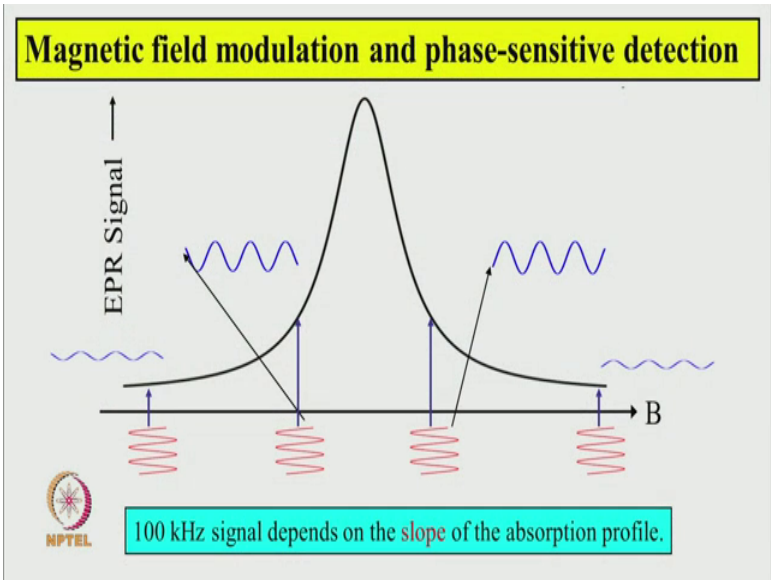
Suppose our signal has appeared in this fashion this is the range of magnetic field scan and it is a appearing of some sort of signal appears here this sort of thing. So, first I do is to it is obvious that the scan range was too much, and also there it is not the centre of the spectrum. So, I first change the centre field this was the centre field earlier I know bring the centre field here. So, centre field is brought here then again I scan the magnetic field get the spectrum. This time it will look like this, it will look like this type of thing

So, now it is this much a magnetic field is not doing anything its simply compressing the spectrum here. So, to do proper measurement there is a primary coupling constant align position, in other words to get a more resolved spectrum we do not need to scan this region. So, reduce the scan range, instead of this now new scan range could be let us say this much new scan range, again you do record the spectrum. So, this time this may look like is a new scan range which has been expand this fashion it may look like this type of this is much better now we can do some measurement. But is that all or we can improve it further as I said earlier now we can try to increase the microwave power and see if we can get better quality spectrum, microwave power will cause more efficient transition and signal intensity will go up. Provided recall our discussion earlier of the saturation of the spin system and the relaxation mechanism which are present there.

So, these two will also and work such a way that you cannot increase the microwave power too much without bringing in saturation. So, the way it will behave is that EPR

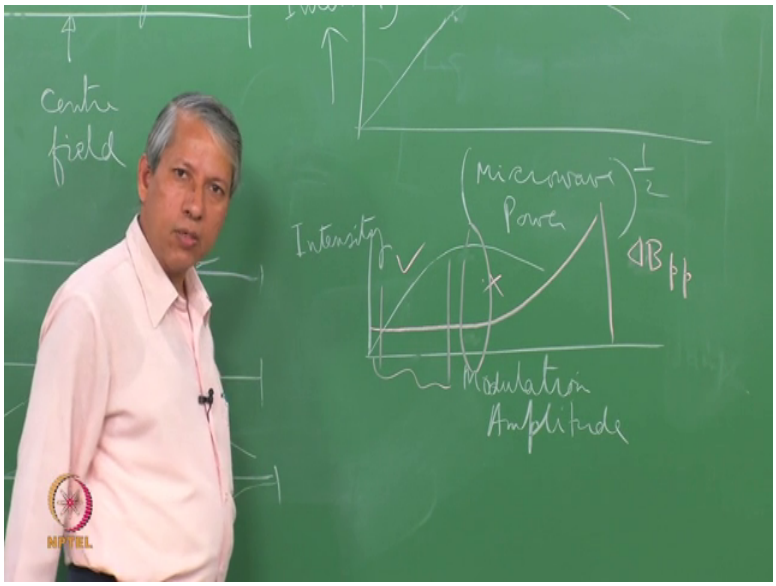
signal intensities and the microwave power, if it is the square root of that this will increase linearly first. Increase, the microwave power the signal height will go up and open up in the squat in this fashion and then it will start showing some sort of saturation and goes down here. So, in this place for the relaxation mechanism is not able to maintain the population difference. So, we cannot use this much power and lose the signal height intensity of signal. So, we have to decide that somewhere here we can stop and not increase the microwave power. So, we get bigger signal in the process. Next is this modulation amplitude, how to optimize that.

(Refer Slide Time: 33:40)



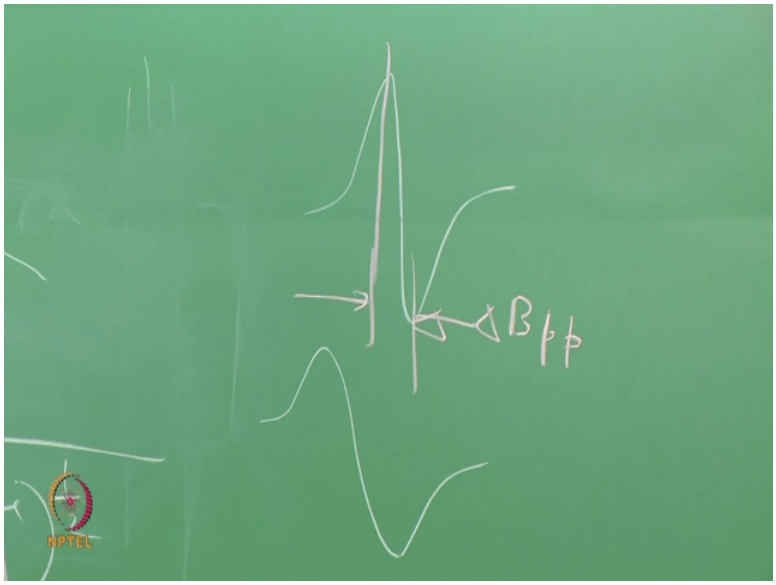
This is the principle of magnetic field modulation and phase sensitive detection. So, here we have come across this slide earlier, but let us recall once again this is the amplitude of the magnetic field modulation, and this is the corresponding response of the sample to the modulated magnetic field. So, it is obvious that not only this amplitude depends on the position of the magnetic field and that is the reason of course, for the derivative presentation of the EPR signal, amplitude of this also depends on how big this is if I increase this amplitude, this will also increase this if this is increased this will also increase. So, the EPR signal is going to increase with the increase of the modulation amplitude, but to get a true line shape that is line shape is exactly the derivative of this absorption profile this should not be too much. So, the signal will have this sort of dependence on the magnetic field modulation amplitude.

(Refer Slide Time: 34:50)



So, this way then it will go down here. By the way, by intensity I mean here the height of the signal not the area of this, height of this signal here. So, this is linearly increasing and then it will start going down when the modulation amplitude becomes comparable to the line width. So, when that happens the intrinsic line width of this, this here, this is the intrinsic line width and correspondingly there will be derivative line width that will start. So, in distortion and the EPR signal will become broad.

(Refer Slide Time: 35:59)



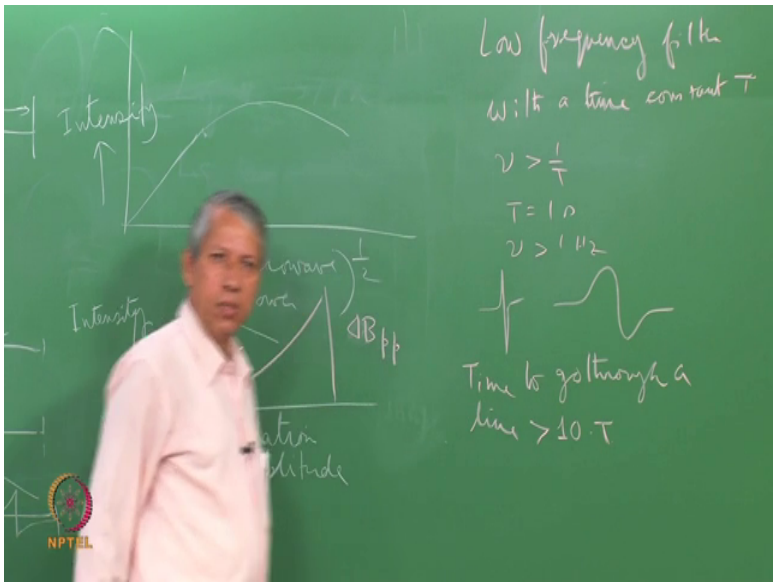
So, if suppose this is the EPR signal at a moderate amplitude of the modulation, this will height will go up and up as you increase the modulation amplitude, but then when this modulation amplitude becomes comparable to these width this will so broadening of this kind, so that is not desirable. So, one has to make some compromise. The way this is another axis here this is a width here, this delta B peak to peak is defined to be this delta B peak to peak there is that width of the derivative line. So, that will have this sort of dependence on the modulation amplitude it will remain almost constant then we starts going up.

So, the width remains constant so long as the amplitude of modulation is much smaller than its own width here, so that is the true line width of the sample, but as signal goes up this also up to remains constant. So, this is a desirable region of operation these are the high acceptor this is ok, but some are here now and this is going down and width is going up is not acceptable, but this gives distortion.

So, that way one decides how much magnetic field modulation is to be used here. Sometimes one can sacrifice the line shape and get the EPR signal, because we are trying to struggling to find if at all there is EPR line or not you really do not care the shape is correct or not. So, then once work somewhere here this region, where there is some sort of distortion of the width, but nevertheless signal is somewhat bigger than what is supposed to be if it was not if one was using smaller amplitude of modulation. So, some distortion, but slightly higher intensity is preferable or even acceptable if one interested only in detecting the presence of the radical and getting some set of EPR signal there.

And then after adjusting this next parameter to be adjusted how much time I should use to scan this magnetic field range, this is called the scan time. The scan time how many minutes or how many seconds depends on how quickly the magnetic field is going through these lines. If the lines are very sharp then one should spend enough time for spectrometer to respond sufficiently quickly to the changing signal here. Now, how does one decide that quantitatively?

(Refer Slide Time: 39:27)



After the phase (Refer Time: 39:18) detection and the derivative signal has been recorded, one usually puts a what is called the low frequency filter time constant. Low frequency filter with a time constant let say T that means, any signal or noise whatsoever oscillations whose frequency is faster than 1 by T will not be given out, but will be filtered out. For example, if T is equal to 1 second time constant that means, any oscillations or instability which is more than 1 hertz, 1 hertz will be effectively reduced. So, depending on what time constant I use here, I must allow several times this time constant for the magnetic field to go through this line.

So, the narrower the line let us say this line is this narrow other is broad. So, it has to go through sufficiently slowly through this one compared to this time constant. So, that this is faithfully reproduce. So, typically time to go through a line should be less or what greater than 10 times the time constant, this is a time constant which is used to filter out the signal. So, that the spectrometer has sufficient time to slowly go through this. So, that depends on the how broad and how narrow is it is very narrow then it will take it should once will allow longer time; if it is broad then one does not need to have that much time.

So, here is a small guide number. Suppose the line width is 0.5 gauss and you scan 10 gauss and time constant 1 second. So, I should give 10 seconds to scan 0.5 gauss 10 times time constants to go through this one so that means, to scan 10 gauss which is the range from this to this, I have to give a time of 200 second to scan 10 gauss that is the

way one decides the scan time. And a little bit more of final adjustment is sometimes necessary not always if the phase of the modulation frequency which is usually set up by the manufacturer and some often one does not change that very often. But if the one can see things have got disturbed or not one can just tweak the reference phase of this one to maximize the signal. And also the bias power phase which we have said that we could optimize by looking at the cavity mode which may also need to adjust a little bit to maximize the EPR signal. So, these are the various parameters which need to be optimized to get the best quality signal to noise ratio.

With this, we come to an end of this session.