

This chapter is an introduction to the basic theory and practice of EPR spectroscopy. It gives you sufficient background to understand the following chapters. In addition, we strongly encourage the new user to explore some of the texts and articles at the end of this chapter. You can then fully benefit from your particular EPR application or think of new ones.

Basic EPR Theory

2.1

Introduction to Spectroscopy

2.1.1

During the early part of the 20th century, when scientists began to apply the principles of quantum mechanics to describe atoms or molecules, they found that a molecule or atom has discrete (or separate) states, each with a corresponding energy. Spectroscopy is the measurement and interpretation of the energy differences between the atomic or molecular states. With knowledge of these energy differences, you gain insight into the identity, structure, and dynamics of the sample under study.

We can measure these energy differences, ΔE , because of an important relationship between ΔE and the absorption of electromagnetic radiation. According to Planck's law, electromagnetic radiation will be absorbed if:

$$\Delta E = h\nu, \quad [2-1]$$

where h is Planck's constant and ν is the frequency of the radiation.

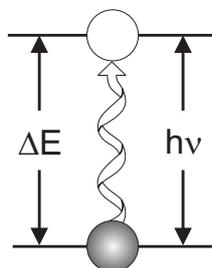


Figure 2-1 Transition associated with the absorption of electromagnetic energy.

The absorption of energy causes a transition from the lower energy state to the higher energy state. (See Figure 2-1.) In conventional spectroscopy, ν is varied or swept and the frequencies at which absorption occurs correspond to the energy differences of the states. (We shall see later that EPR differs slightly.) This record is called a spectrum. (See Figure 2-2.) Typically, the frequencies vary from the megahertz range for NMR (Nuclear Magnetic Resonance) (AM, FM, and TV transmissions use electromagnetic radiation at these frequencies), through visible light, to ultraviolet light. Radiation in the gigahertz range (the same as in your microwave oven) is used for EPR experiments.

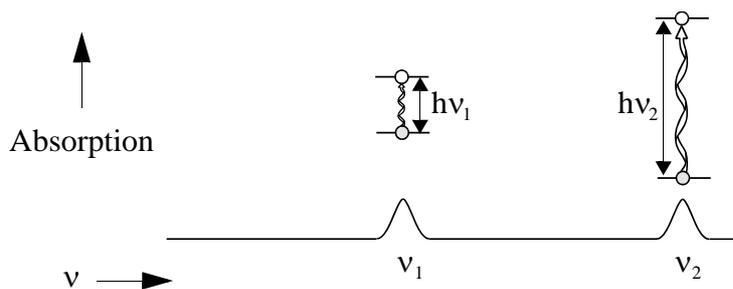
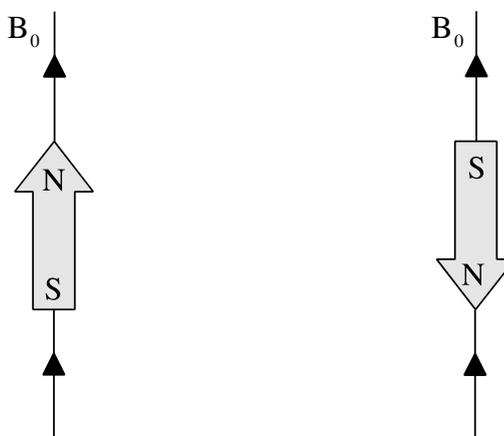


Figure 2-2 A spectrum.

The Zeeman Effect

2.1.2

The energy differences we study in EPR spectroscopy are predominately due to the interaction of unpaired electrons in the sample with a magnetic field produced by a magnet in the laboratory. This effect is called the Zeeman effect. Because the electron has a magnetic moment, it acts like a compass or a bar magnet when you place it in a magnetic field, B_0 . It will have a state of lowest energy when the moment of the electron, μ , is aligned with the magnetic field and a state of highest energy when μ is aligned against the magnetic field. (See Figure 2-3.) The two states are labelled by the projection of the electron spin, M_s , on the direction of the magnetic field. Because the electron is a spin $1/2$ particle, the parallel state is designated as $M_s = -1/2$ and the antiparallel state is $M_s = +1/2$.

Figure 2-3 Minimum and maximum energy orientations of μ with respect to the magnetic field B_0 .

From quantum mechanics, we obtain the most basic equations of EPR:

$$E = g \mu_B B_0 M_s = \pm \frac{1}{2} g \mu_B B_0 \quad [2-2]$$

and

$$\Delta E = h\nu = g \mu_B B_0. \quad [2-3]$$

g is the g -factor, which is a proportionality constant approximately equal to 2 for most samples, but varies depending on the electronic configuration of the radical or ion. μ_B is the Bohr magneton, which is the natural unit of electronic magnetic moment.

Two facts are apparent from equations Equation [2-2] and Equation [2-3] and its graph in Figure 2-4.

- The two spin states have the same energy in the absence of a magnetic field.
- The energies of the spin states diverge linearly as the magnetic field increases. For $g = 2$, the slope is 2.8 MHz/G.

These two facts have important consequences for spectroscopy.

- Without a magnetic field, there is no energy difference to measure.
- The measured energy difference depends linearly on the magnetic field.

Because we can change the energy differences between the two spin states by varying the magnetic field strength, we have an alternative means to obtain spectra. We could apply a constant magnetic field and scan the frequency of the electromagnetic radiation as in conventional spectroscopy. Alternatively, we could keep the electromagnetic radiation frequency constant and scan the magnetic field. (See Figure 2-4.) A peak in the absorption will occur when the magnetic field “tunes” the two spin states so that their energy difference matches the energy of the radiation. This field is called the “field for resonance”. Owing to the limitations of microwave electronics, the latter method offers superior performance. This technique is used in all Bruker EPR spectrometers.

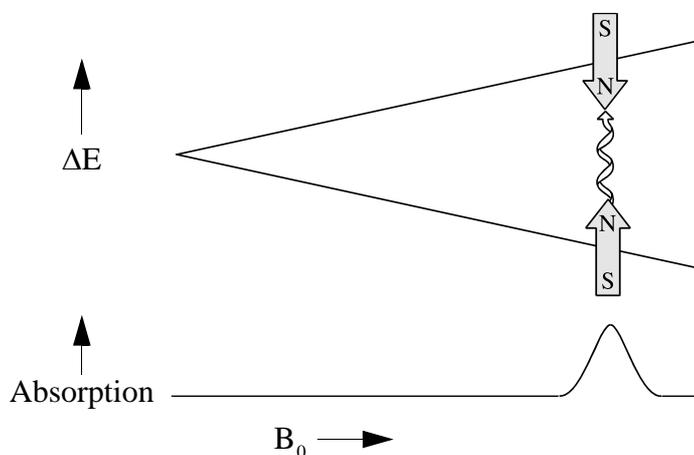


Figure 2-4 Variation of the spin state energies as a function of the applied magnetic field.

The field for resonance is not a unique “fingerprint” for identification of a compound because spectra can be acquired at several different frequencies. The g -factor,

$$g = \frac{h\nu}{\mu_B B_0} \quad [2-4]$$

being independent of the microwave frequency, is much better for that purpose. Notice that high values of g occur at low magnetic fields and vice versa. A list of fields for resonance for a $g = 2$ signal at microwave frequencies commonly available in EPR spectrometers is presented in Table 2-1.

Microwave Band	Frequency (GHz)	B_{res} (G)
L	1.1	390
S	4.0	1430
X	9.75	3480
Q	34.0	12100
W	94.0	33500

Table 2-1 Field for resonance, B_{res} , for a $g = 2$ signal at selected microwave frequencies.

Hyperfine Interactions

2.1.3

Measurement of g -factors can give us some useful information; however, it does not tell us much about the molecular structure of our sample. Fortunately, the unpaired electron, which gives us the EPR spectrum, is very sensitive to its local surroundings. The nuclei of the atoms in a molecule or complex often have a magnetic moment, which produces a local magnetic field at the electron. The interaction between the electron and the nuclei is called the hyperfine interaction. It gives us a wealth of information about our sample such as the identity and number of atoms which make up a radical or complex as well as their distances from the unpaired electron.

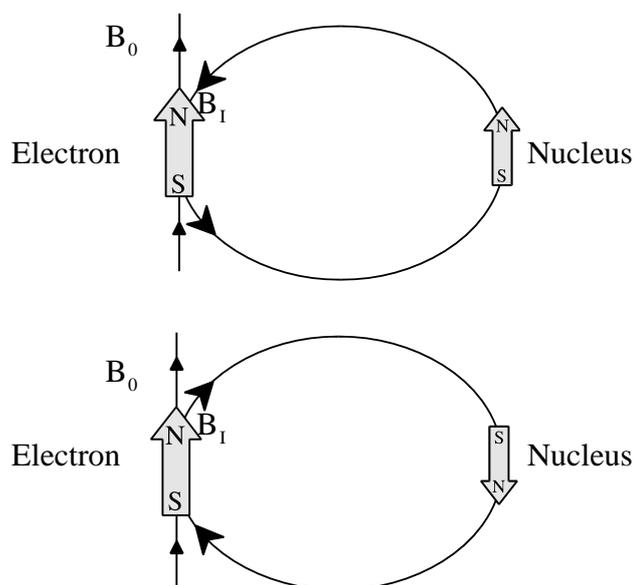


Figure 2-5 Local magnetic field at the electron, B_I , due to a nearby nucleus.

Figure 2-5 depicts the origin of the hyperfine interaction. The magnetic moment of the nucleus acts like a bar magnet (albeit a weaker magnet than the electron) and produces a magnetic field at the electron, B_I . This magnetic

field opposes or adds to the magnetic field from the laboratory magnet, depending on the alignment of the moment of the nucleus. When B_I adds to the magnetic field, we need less magnetic field from our laboratory magnet and therefore the field for resonance is lowered by B_I . The opposite is true when B_I opposes the laboratory field.

For a spin $1/2$ nucleus such as a hydrogen nucleus (proton), we observe that our single EPR absorption signal splits into two signals which are each B_I away from the original signal. (See Figure 2-6.)

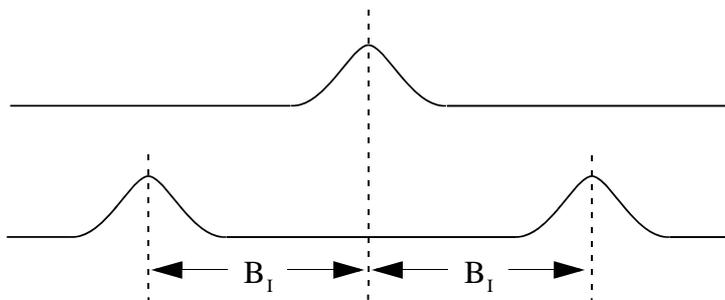


Figure 2-6 Splitting in an EPR signal due to the local magnetic field of a nearby nucleus.

For nuclei with spins other than $1/2$, the number of lines equals:

$$\text{Number of Lines} = 2I + 1 \quad [2-5]$$

where I is the spin quantum number of the nucleus.

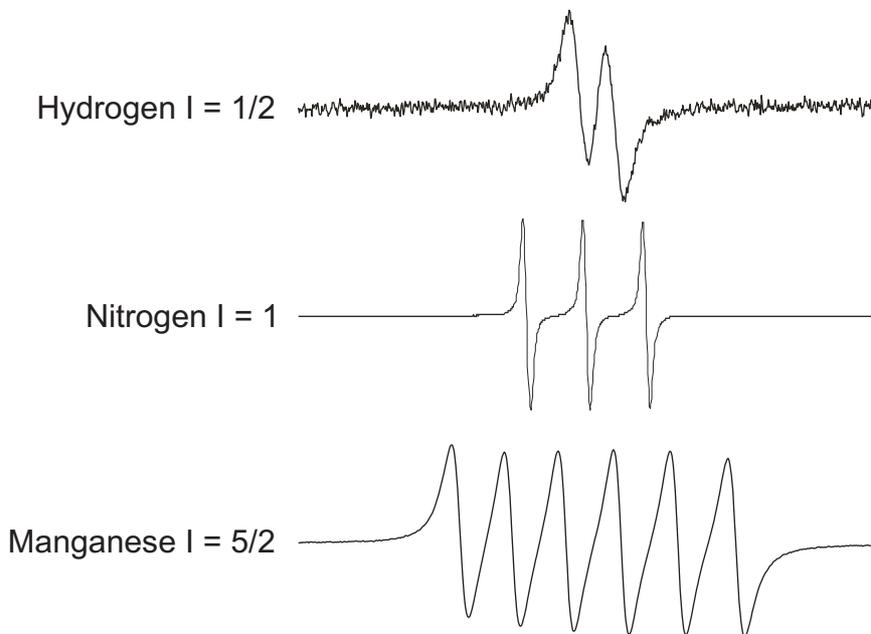


Figure 2-7 The number of lines from hyperfine interactions increases as $2I+1$ with the nuclear spin quantum number, I .

If there are two spin $1/2$ nuclei with the same hyperfine coupling, each of the two lines is further split into two lines. Because of the equal hyperfine cou-

pling two of the EPR signals will overlap, giving a triplet with an intensity distribution of 1:2:1.

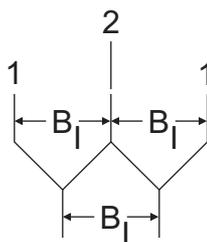


Figure 2-8 A 1:2:1 triplet resulting from the hyperfine interactions of two equivalent spin 1/2 nuclei.

For n spin 1/2 nuclei with equal hyperfine couplings, the number of lines is given by:

$$\text{Number of Lines} = 2n + 1 \quad [2-6]$$

with each of the lines separated by the hyperfine coupling. The relative intensities are given by:

$$\binom{n}{k} = \frac{n!}{k!(n-k)!} \quad 0 \leq k \leq n \quad [2-7]$$

which are related to Pascal's triangle and polynomial coefficients.

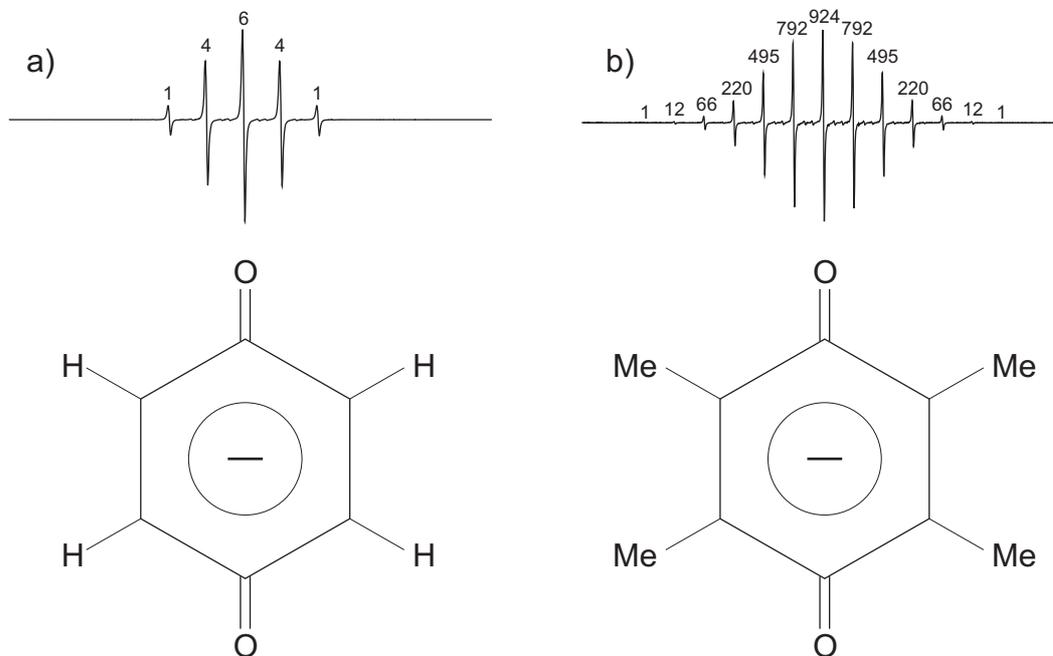


Figure 2-9 Relative intensities for benzosemiquinone (a) and durosemiquinone (b) radical anions in alkaline DMSO. The number of lines is given by Equation [2-6] and the relative intensities by Equation [2-7].

The situation for nuclei with different hyperfine couplings is similar to equal hyperfine couplings, except that there is no overlap between the lines leading to the Pascal triangle intensity distribution. Each of the lines is split by the additional hyperfine couplings.

As an example, it is possible to make the durosemiquinone radical cation. This is similar to the anion shown in Figure 2-9 except that the oxygens are protonated, thus producing further hyperfine splittings. We start off on the left side of Figure 2-10 with the 13 line pattern to be expected from the 12 equivalent methyl protons. Then there is the additional splittings from the hydrogens bound to the oxygens. Since the two protons are equivalent, we have a 1:2:1 triplet from them. If we split each of the 13 lines from the methyl proton splittings with this 1:2:1 triplet, we see that we can nicely reproduce the complicated experimental EPR spectrum of the durosemiquinone radical cation in sulfuric acid.

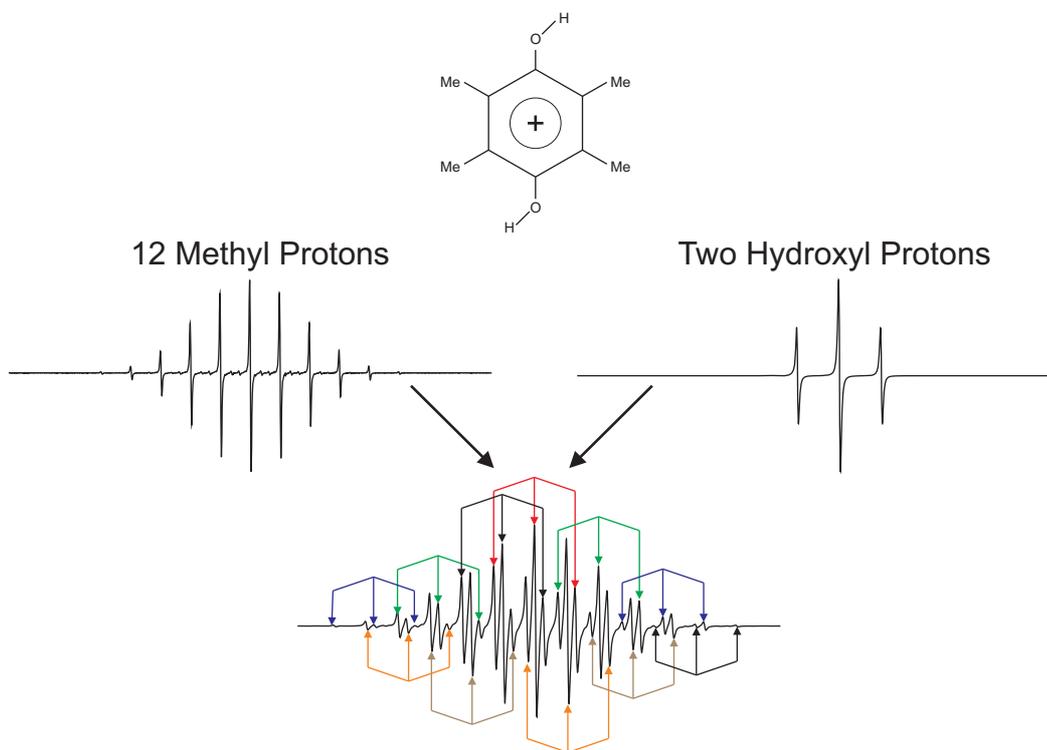


Figure 2-10 Estimating the EPR spectrum of the durosemiquinone radical cation in sulfuric acid and reduced with sodium dithionite by splitting each of the EPR lines due to the 12 methyl protons by a 1:2:1 triplet from the hydroxyl protons.

For N spin $1/2$ nuclei, we will generally observe 2^N EPR signals. As the number of nuclei gets larger, the number of signals increases exponentially. Sometimes there are so many signals that they overlap and we only observe one broad signal, resulting in what is called a gaussian lineshape.

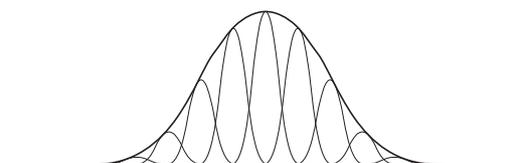


Figure 2-11 A large number of nuclei produces a gaussian lineshape.

Signal Intensity

2.1.4

So far, we have concerned ourselves with the field for resonance of the EPR signal, but the size of the EPR signal is also important if we want to measure the concentration of the EPR active species in our sample. In the language of spectroscopy, the size of a signal is defined as the integrated intensity, *i.e.*, the area beneath the absorption curve. (See Figure 2-12.) The integrated intensity of an EPR signal is proportional to the concentration.

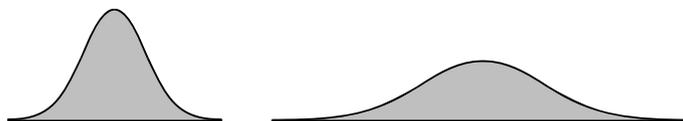


Figure 2-12 Integrated intensity of absorption signals. Both signals have the same intensity.

Signal intensities do not depend solely on concentrations. They also depend on the microwave power. If you do not use too much microwave power, the signal intensity grows as the square root of the power. At higher power levels, the signal diminishes as well as broadens with increasing microwave power levels. This effect is called saturation. If you want to measure accurate linewidths, lineshapes, and closely spaced hyperfine splittings, you should avoid saturation by using low microwave power. A quick means of checking for the absence of saturation is to decrease the microwave power and verify that the signal intensity also decreases by the square root of the microwave power.

Some of these topics are covered in greater detail in Section 2.7.

Basic EPR Practice

2.2

Introduction to Spectrometers

2.2.1

In the first half of this chapter, we discussed the theory of EPR spectroscopy. Now we need to consider the practical aspects of EPR spectroscopy. Theory and practice have always been strongly interdependent in the development and growth of EPR. A good example of this point is the first detection of an EPR signal by Zavoisky in 1945. The Zeeman effect had been known in optical spectroscopy for many years, but the first direct detection of EPR had to wait until the development of radar during World War II. Only then, did scientists have the necessary components to build sufficiently sensitive spectrometers (scientific instruments designed to acquire spectra). The same is true today with the development of advanced techniques in EPR such as Fourier Transform and high frequency EPR.

The simplest possible spectrometer has three essential components: a source of electromagnetic radiation, a sample, and a detector. (See Figure 2-13.) To acquire a spectrum, we change the frequency of the electromagnetic radiation and measure the amount of radiation which passes through the sample with a detector to observe the spectroscopic absorptions. Despite the apparent complexities of any spectrometer you may encounter, it can always be simplified to the block diagram shown in Figure 2-13.

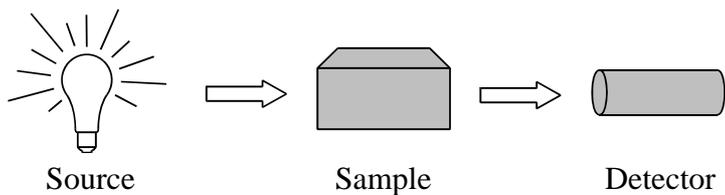


Figure 2-13 The simplest spectrometer.

Figure 2-14 shows the general layout of a Bruker EPR spectrometer. The electromagnetic radiation source and the detector are in a box called the “microwave bridge”. The sample is in a microwave resonator (or cavity), which is a metal box that helps to amplify weak signals from the sample. As mentioned in Section 2.1.2, there is a magnet to “tune” the electronic energy levels. In addition, we have a console, which contains signal processing and control electronics. There is a computer used for analyzing data as well as coordinating all the units for acquiring a spectrum. In the following sections you will become acquainted with how these different parts of the spectrometer function and interact.

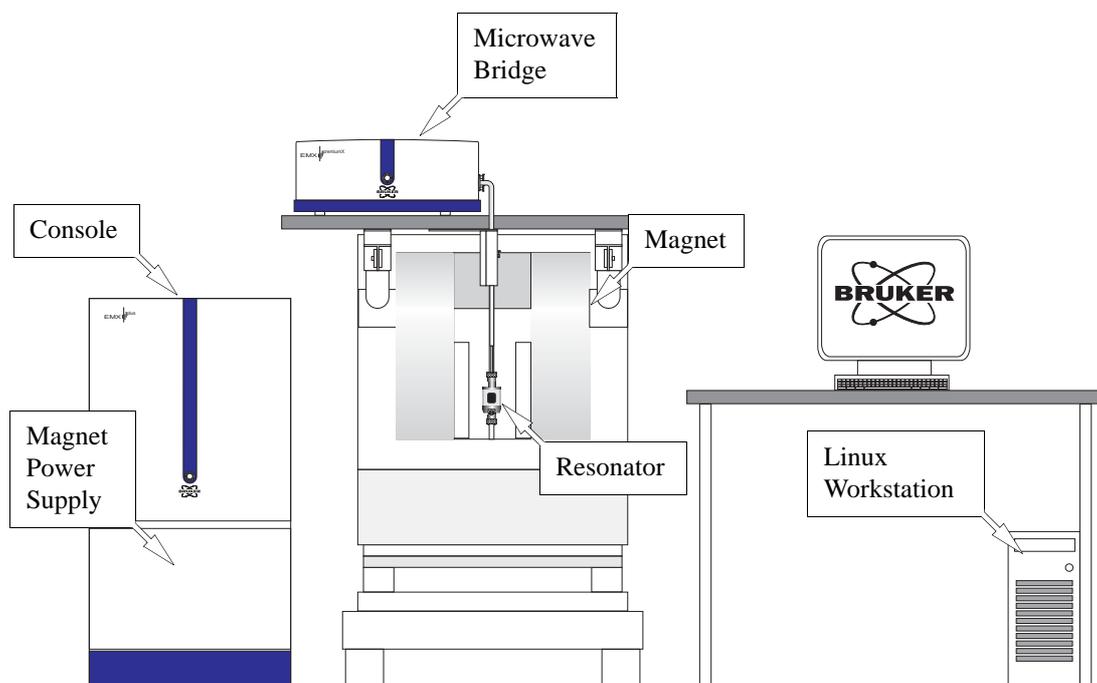


Figure 2-14 The modules and components of the EMXplus spectrometer.

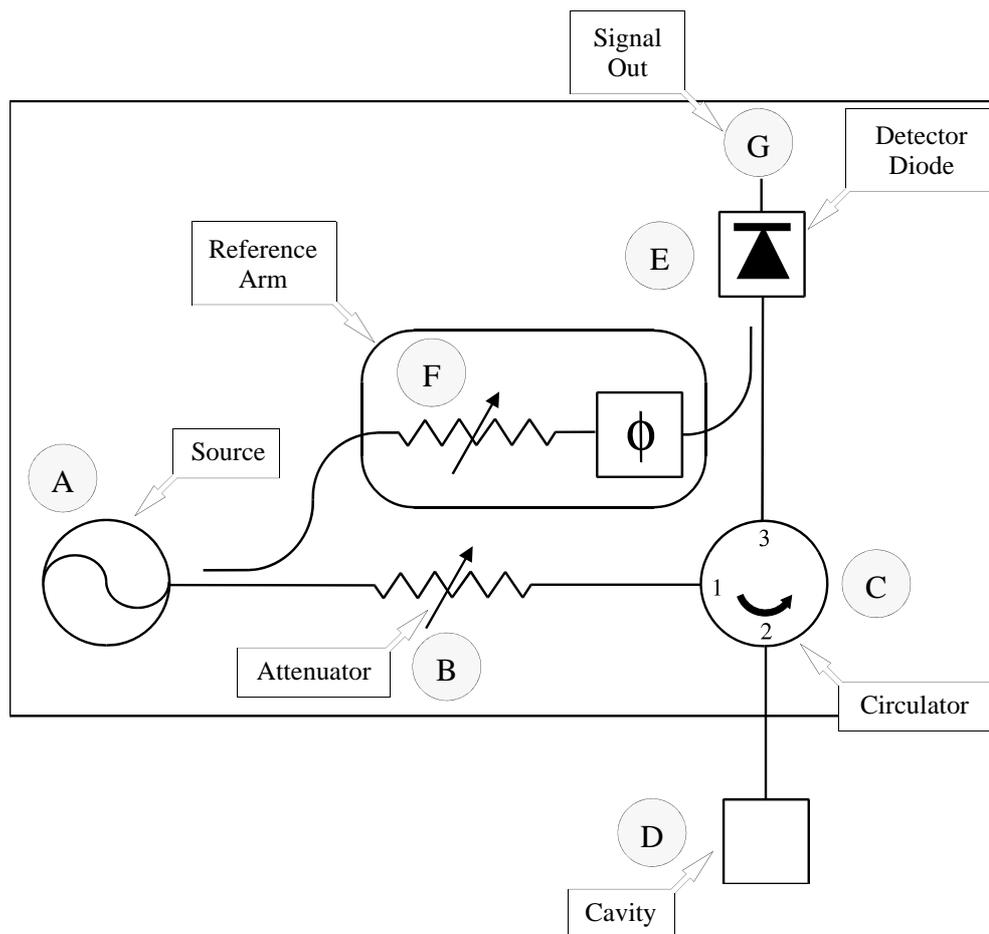


Figure 2-15 Block diagram of a microwave bridge.

The Microwave Bridge

2.2.2

The microwave bridge houses the microwave source and the detector. There are more parts in a bridge than shown in Figure 2-15, but most of them are control, power supply, and security electronics and are not necessary for understanding the basic operation of the bridge. We shall now follow the path of the microwaves from the source to the detector.

We start our tour of the microwave bridge at point A, the microwave source. The output power of the microwave source cannot be varied easily, however in our discussion of signal intensity, we stressed the importance of changing the power level. Therefore, the next component, at point B, after the microwave source is a variable attenuator, a device which blocks the flow of microwave radiation. With the attenuator, we can precisely and accurately control the microwave power which the sample sees.

Bruker EPR spectrometers operate slightly differently than the simple spectrometer shown in the block diagram, Figure 2-13. The diagram depicts a transmission spectrometer (It measures the amount of radiation transmitted through the sample.) and most EPR spectrometers are reflection spectrometers. They measure the changes (due to spectroscopic transitions) in the amount of radiation reflected back from the microwave cavity containing the sample (point D in the Figure 2-15). We therefore want our detector to see only the microwave radiation coming back from the cavity. The circulator at

point C is a microwave device which allows us to do this. Microwaves coming in port 1 of the circulator only go to the cavity through port 2 and not directly to the detector through port 3. Reflected microwaves are directed only to the detector and not back to the microwave source.

We use a detector diode to detect the reflected microwaves (point E in Figure 2-15). It converts the microwave power to an electrical current. At low power levels, (less than 1 microwatt) the diode current is proportional to the microwave power and the detector is called a square law detector. (Remember that electrical power is proportional to the square of the voltage or current.) At higher power levels, (greater than 1 milliwatt) the diode current is proportional to the square root of the microwave power and the detector is called a linear detector. The transition between the two regions is very gradual.

For quantitative signal intensity measurements as well as optimal sensitivity, the diode should operate in the linear region. The best results are attained with a detector current of approximately 200 microamperes. To insure that the detector operates at that level, there is a reference arm (point F in the Figure 2-15) which supplies the detector with some extra microwave power or "bias". Some of the source power is tapped off into the reference arm, where a second attenuator controls the power level (and consequently the diode current) for optimal performance. There is also a phase shifter to insure that the reference arm microwaves are in phase with the reflected signal microwaves when the two signals combine at the detector diode.

The detector diodes are very sensitive to damage from excessive microwave power and will slowly lose their sensitivity. To prevent this from happening, there is protection circuitry in the bridge which monitors the current from the diode. When the current exceeds 400 microamperes, the bridge automatically protects the diode by lowering the microwave power level. This reduces the risk of damage due to accidents or improper operating procedures. However, it is good lab practice to follow correct procedures and not rely on the protection circuitry.

The EPR Cavity

2.2.3

In this section, we shall discuss the properties of microwave (EPR) cavities and how changes in these properties due to absorption result in an EPR signal. We use microwave cavities to amplify weak signals from the sample. A microwave cavity is simply a metal box with a rectangular or cylindrical shape which resonates with microwaves much as an organ pipe resonates with sound waves. Resonance means that the cavity stores the microwave energy; therefore, at the resonance frequency of the cavity, no microwaves will be reflected back, but will remain inside the cavity. (See Figure 2-16.)

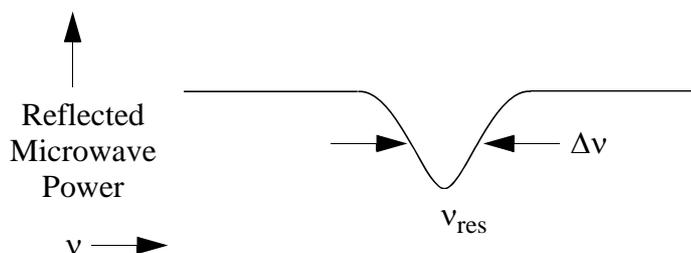


Figure 2-16 Reflected microwave power from a resonant cavity.

Cavities are characterized by their Q or quality factor, which indicates how efficiently the cavity stores microwave energy. As Q increases, the sensitivity of the spectrometer increases. The Q factor is defined as

$$Q = \frac{2\pi (\text{energy stored})}{\text{energy dissipated per cycle}} \quad [2-8]$$

where the energy dissipated per cycle is the amount of energy lost during one microwave period. Energy can be lost to the side walls of the cavity because the microwaves generate electrical currents in the side walls of the cavity which in turn generates heat. We can measure Q factors easily because there is another way of expressing Q:

$$Q = \frac{\nu_{\text{res}}}{\Delta\nu}, \quad [2-9]$$

where ν_{res} is the resonant frequency of the cavity and $\Delta\nu$ is the width at half height of the resonance.

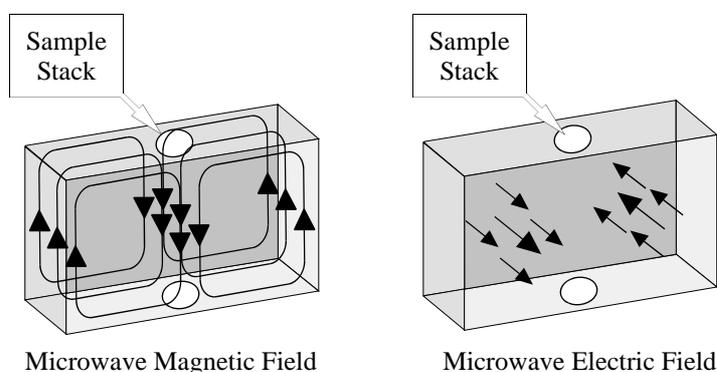


Figure 2-17 Magnetic and electric field patterns in a standard EPR cavity.

A consequence of resonance is that there will be a standing wave inside the cavity. Standing electromagnetic waves have their electric and magnetic field components exactly out of phase, *i.e.* where the magnetic field is maximum, the electric field is minimum and *vice versa*. The spatial distribution of the amplitudes of the electric and magnetic fields in a commonly used EPR cavity is shown in Figure 2-17. We can use the spatial separation of the electric and magnetic fields in a cavity to great advantage. Most samples have non-resonant absorption of the microwaves via the electric field (this is how a microwave oven works) and the Q will be degraded by an increase in the dissipated energy. It is the microwave magnetic field that drives the absorption in EPR. Therefore, if we place our sample in the electric field minimum and the magnetic field maximum, we obtain the biggest signals and the highest sensitivity. The cavities are designed for optimal placement of the sample.

We couple the microwaves into the cavity via a hole called an iris. The size of the iris controls the amount of microwaves which will be reflected back from the cavity and how much will enter the cavity. The iris accomplishes this by carefully matching or transforming the impedances (the resistance to the waves) of the cavity and the waveguide (a rectangular pipe used to carry microwaves). There is an iris screw in front of the iris which allows us to adjust the “matching”. This adjustment can be visualized by noting that as the screw moves up and down, it effectively changes the size of the iris. (See

Figure 2-18.) When the iris screw properly matches the cavity impedance (also called critical coupling), no microwaves are reflected back from the cavity.

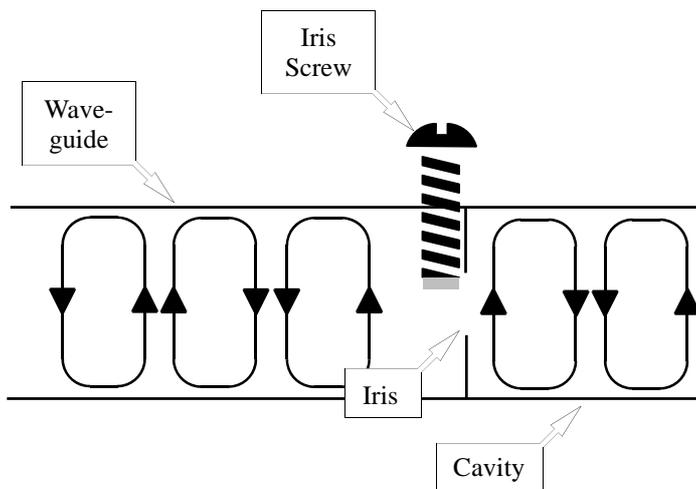


Figure 2-18 The matching of a microwave cavity to waveguide.

How do all of these properties of a cavity give rise to an EPR signal? When the sample absorbs the microwave energy, the Q is lowered because of the increased losses and the coupling changes because the absorbing sample changes the impedance of the cavity. The cavity is therefore no longer critically coupled and microwave will be reflected back to the bridge, resulting in an EPR signal.

The Signal Channel

2.2.4

EPR spectroscopists use a technique known as phase sensitive detection to enhance the sensitivity of the spectrometer. The advantages include less noise from the detection diode and the elimination of baseline instabilities due to the drift in DC electronics. A further advantage is that it encodes the EPR signals to make it distinguishable from sources of noise or interference which are almost always present in a laboratory. The signal channel, a unit which fits in the spectrometer console, contains the required electronics for the phase sensitive detection.

The detection scheme works as follows. The magnetic field strength which the sample sees is modulated (varied) sinusoidally at the modulation frequency. If there is an EPR signal, the field modulation quickly sweeps through part of the signal and the microwaves reflected from the cavity are amplitude modulated at the same frequency. For an EPR signal which is approximately linear over an interval as wide as the modulation amplitude, the EPR signal is transformed into a sine wave with an amplitude proportional to the slope of the signal (See Figure 2-19.)

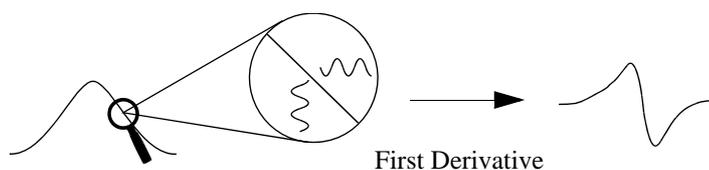


Figure 2-19 Field modulation and phase sensitive detection.

The signal channel (more commonly known as a lock-in amplifier or phase sensitive detector) produces a DC signal proportional to the amplitude of the modulated EPR signal. It compares the modulated signal with a reference signal having the same frequency as the field modulation and it is only sensitive to signals which have the same frequency and phase as the field modulation. Any signals which do not fulfill these requirements (*i.e.*, noise and electrical interference) are suppressed. To further improve the sensitivity, a time constant is used to filter out more of the noise.

Phase sensitive detection with magnetic field modulation can increase our sensitivity by several orders of magnitude; however, we must be careful in choosing the appropriate modulation amplitude, frequency, and time constant. All three variables can distort our EPR signals and make interpretation of our results difficult.

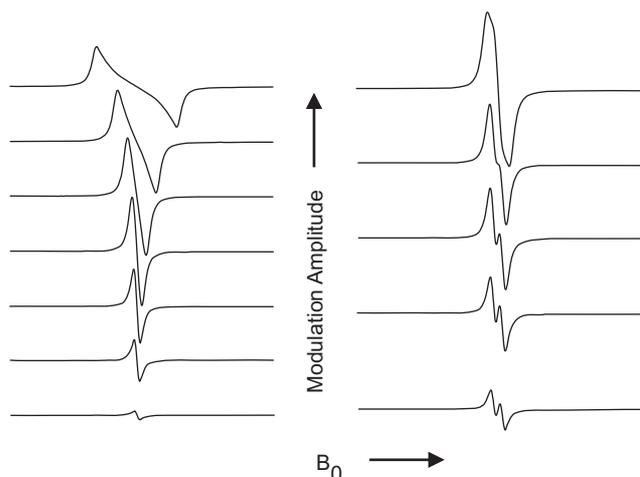


Figure 2-20 Signal distortions due to excessive field modulation.

As we apply more magnetic field modulation, the intensity of the detected EPR signals increases; however, if the modulation amplitude is too large (larger than the linewidths of the EPR signal), the detected EPR signal broadens and becomes distorted. (See Figure 2-20.) A good compromise between signal intensity and signal distortion occurs when the amplitude of the magnetic field modulation is equal to the width of the EPR signal. Also, if we use a modulation amplitude greater than the splitting between two EPR signals, we can no longer resolve the two signals.

Time constants filter out noise by slowing down the response time of the spectrometer. As the time constant is increased, the noise levels will drop. If we choose a time constant which is too long for the rate at which we scan the magnetic field, we can distort or even filter out the very signal which we are trying to extract from the noise. Also, the apparent field for resonance will shift. Figure 2-21 shows the distortion and disappearance of a signal as the time constant is increased. If you need to use a long time constant to see a weak signal, you must use a slower scan rate. A safe rule of thumb is to make sure that the time needed to scan through a single EPR signal should be ten times greater than the length of the time constant.

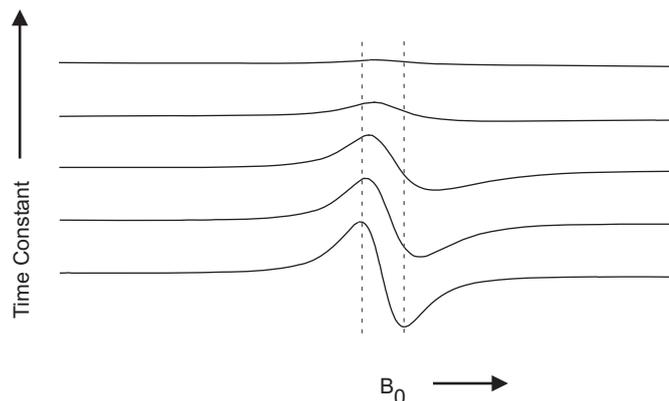


Figure 2-21 Signal distortion and shift due to excessive time constants.

For samples with very narrow or closely spaced EPR signals, (~ 50 milli-gauss. This usually only happens for organic radicals in dilute solutions.) we can get a broadening of the signals if our modulation frequency is too high (See Figure 2-22.)

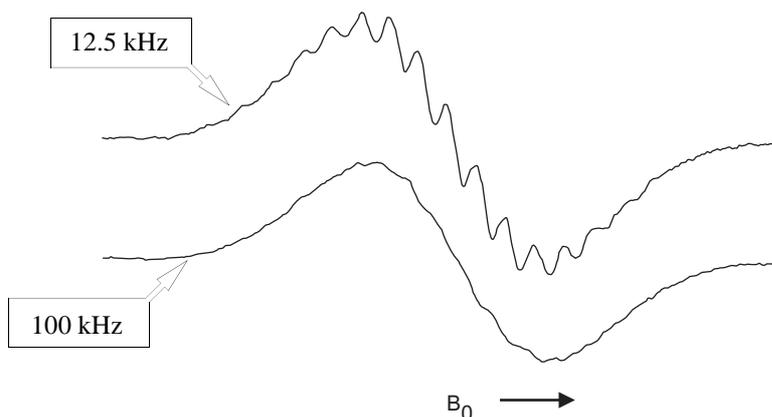


Figure 2-22 Loss of resolution due to high modulation frequency.

The Magnetic Field Controller

2.2.5

The magnetic field controller allows us to sweep the magnetic field in a controlled and precise manner for our EPR experiment. It consists of two parts; a part which sets the field values and the timing of the field sweep and a part which regulates the current in the windings of the magnet to attain the requested magnetic field value.

The magnetic field values and the timing of the magnetic field sweep are controlled by a microprocessor in the controller. A field sweep is divided into a maximum of 256,000 discrete steps (128,000 for the EMXmicro) called sweep addresses. At each step, a reference voltage corresponding to the magnetic field value is sent to the part of the controller that regulates the magnetic field. The sweep rate is controlled by varying the conversion time (waiting time at each step of the individual steps during which the signal channel digitizes the EPR signal).

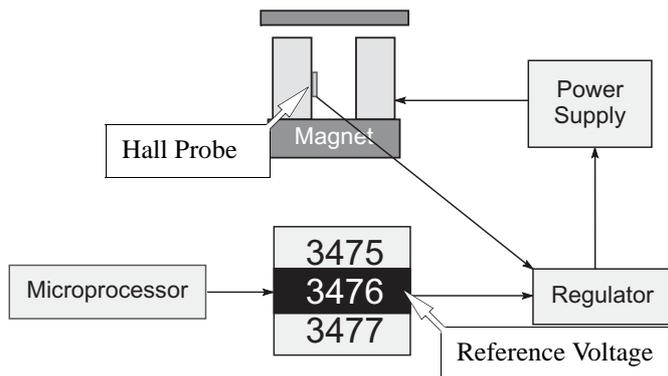


Figure 2-23 A block diagram of the field controller and associated components.

The magnetic field regulation occurs via a Hall probe placed in the gap of the magnet. It produces a voltage which is dependent on the magnetic field perpendicular to the probe. The relationship is not linear and the voltage changes with temperature; however, this is easily compensated for by keeping the probe at a constant temperature slightly above room temperature and characterizing the nonlinearities so that the microprocessor in the controller can make the appropriate corrections. Regulation is accomplished by comparing the voltage from the Hall probe with the reference voltage given by the other part of the controller. When there is a difference between the two voltages, a correction voltage is sent to the magnet power supply which changes the amount of current flowing through the magnet windings and hence the magnetic field. Eventually the error voltage drops to zero and the field is “stable” or “locked”. This occurs at each discrete step of a magnetic field scan.

The Spectrum

2.2.6

We have seen how the individual components of the spectrometer work. Figure 2-24 shows how they work together to produce a spectrum.

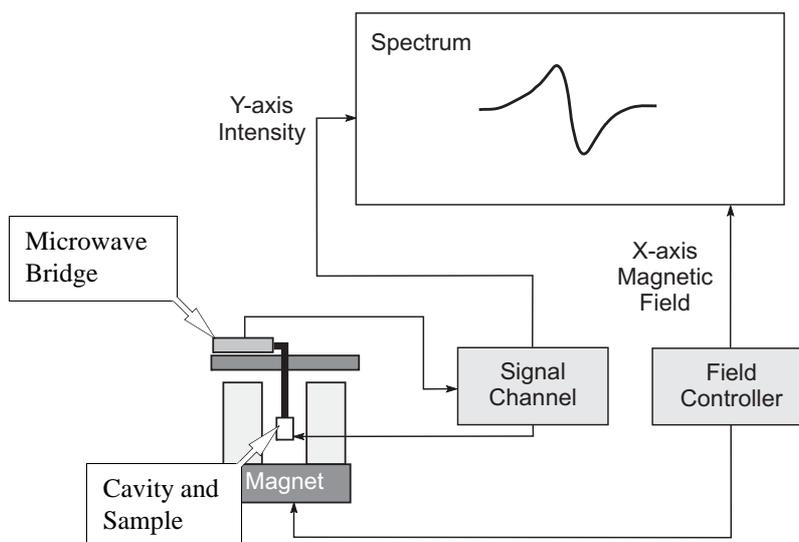


Figure 2-24 Block diagram of an EPR spectrometer.

Automated Parameter Adjustments

2.3

Traditionally, acquisition parameters such as number of points, conversion time and time constant were adjusted separately and it was assumed that the user knew how to optimize all the parameters. It turns out that many of the parameters are inter-related and some linking of parameters can be used to ease and simplify the optimization process. This section explains how some parameters are set automatically and which ones have priority in Xenon.

Effect of Mod. Amp. on the Number of Points

2.3.1

The parameter that most influences the choice of other parameters is the modulation amplitude. As can be seen in Figure 2-25, the apparent linewidth that is observed is dependent on the modulation amplitude. It is difficult to resolve features narrower than the modulation amplitude owing to the modulation broadening or smearing out of the EPR lines. Also one typically sets the modulation amplitude to a value approximately less than or equal to the EPR linewidth.

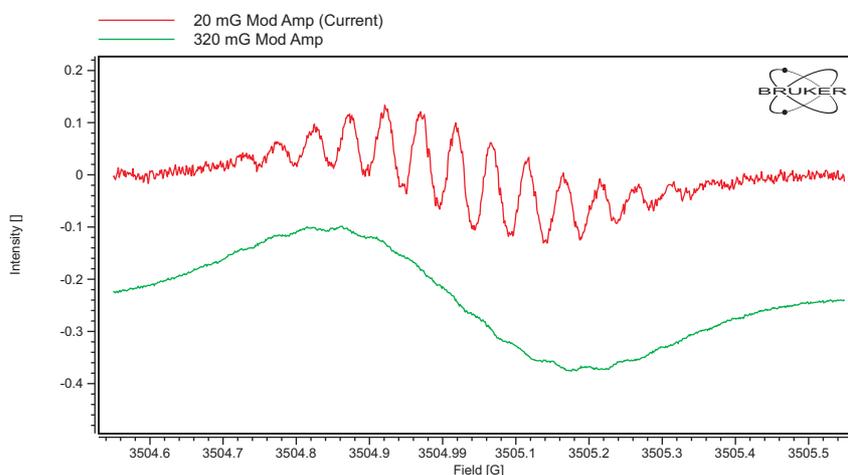


Figure 2-25 Reduction in resolution owing to excessive modulation amplitude.

EPR spectra are recorded by stepping the magnetic field in discrete steps and digitizing the EPR signal at each of these field steps. The size of these discrete steps must be sufficiently small that the EPR lineshape is characterized well. (See Figure 2-26.) Because the resolution cannot greatly exceed the modulation amplitude, this sets a limit on the number of points required to characterize an EPR signal. This is expressed by the parameter Pts / Mod. Amp. The Pts / Mod. Amp. parameter has an influence on how well the EPR lineshape is characterized. A value of 1 produces a very poor representation of the EPR lineshape. Increasing the value yields an increasingly faithful representation of the EPR lineshape.

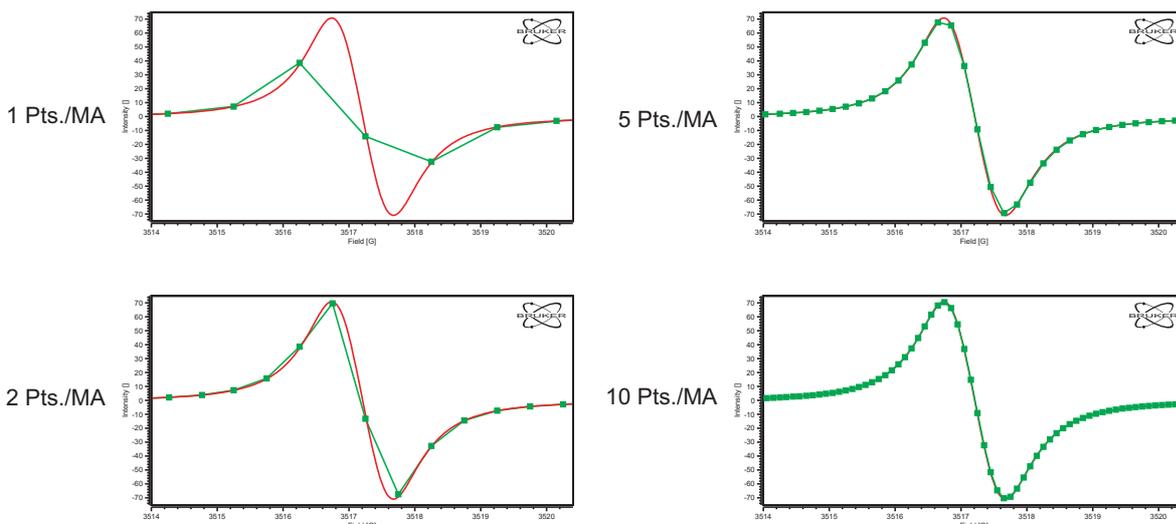


Figure 2-26 Increasing the Pts / Mod. Amp. gives a more faithful representation of the EPR line-shape.

The Pts / Mod. Amp. parameter always takes priority in setting other acquisition parameters. Its value remains constant unless you intentionally change its value. The other linked (or automatically adjusted) parameters are adjusted according to its value.

The resultant Number of Points in the EPR spectrum is set to:

$$\text{Number of Points} = \text{Pts / Mod. Amp.} \times \frac{\text{Sweep Width}}{\text{Mod. Amp.}} \quad [2-10]$$

This relation is the first automatic parameter linkage in Xenon. As can be seen in Figure 2-27, 1 G Mod. Amp. and 10 Pts / Mod. Amp. and a Sweep Width of 100 G yield 1000 for Number of Points.



The Number of Points is automatically calculated and cannot be directly changed by the user. To change its value, change the value of Pts / Mod. Amp.

Magnetic Field

Center Field [G]: 3480.00

Sweep Width [G]: 100.0

Sweep Time [s]: 2.62

Set Field to g Factor

Sample g-Factor: 2.000000

Signal Channel

Receiver Gain [dB]: 30

Mod. Amp. [G]: 1.00

Number Of Scans: 1

Offset [%]: 0.0

Magnetic Field

Number Of Points: 1000

Field Settling: Wait LED off

Settling Delay [s]: 0.0

Sweep Direction: Up

Field Flyback: On

Signal Channel

Calibrated:

Modul. Freq. [kHz]: 100.00

Modulation Phase: 0.0

Conv. Time [ms]: 2.62

Time Constant [ms]: 0.01

Dual Detection: OFF

Pts / Mod. Amp.: 10.00

Figure 2-27 Number of Points for a given Mod. Amp. and Sweep Width.

Effect of Sweep Width on Sweep Time

2.3.2



The Conversion Time is automatically calculated and cannot be directly changed by the user. To change its value, change the value of Sweep Time.

The next thing to consider is the time it takes to sweep the field. The Conversion Time is the amount of time the magnetic field remains at the individual discrete field steps and the EPR intensity is digitized. The Sweep Time then equals:

$$\text{Sweep Time} = \text{Number of Points} \times \text{Conversion Time} \quad [2-11]$$

From Equation [2-10] we see that if we increase the Sweep Width by a factor of two, the Number of Points is increased by a factor of two. Xenon keeps the Conversion Time constant when automatically adjusting parameters for different Sweep Widths, therefore, the Sweep Time is increased by a factor of two. This is the second automatic parameter linkage in Xenon. You can of course change the value of the Sweep Time manually should you wish to do so.

Effect of Mod. Amp. on Conversion Time and Number of Points

2.3.3

The third automatic parameter linkage in Xenon is the effect of changing the Modulation Amplitude. The Pts / Mod. Amp. remains constant and therefore the Number of Points changes. The Sweep Time remains constant and Xenon adjusts the Conversion Time to maintain the original Sweep Time. Therefore if the Modulation Amplitude is increased by a factor of two, the Number of Points is halved and the Conversion Time is doubled.

Summary of Automated Parameter Setting Rules

2.3.4

Initial Values	The Number of Points is determined by Pts/Mod. Amp., Mod. Amp., and Sweep Width. (See Equation [2-10].)
Pts/Mod. Amp.	This parameter always has priority and remains constant unless you intentionally change its value.
Change Sweep Width	The Conversion Time and Pts/Mod. Amp. parameters have priority and remain constant. The Sweep Time is automatically adjusted to accommodate the new Number of Points. (See Equation [2-11].)
Change Mod. Amp.	The Sweep Time and Pts/Mod. Amp. parameters have priority and remain constant. The Conversion Time is automatically adjusted to accommodate the new Number of Points. (See Equation [2-11].)
Changing Parameters	With the exceptions of Number of Points and Conversion Time, if the automatically adjusted parameters are not appropriate for your sample, you can always change their values to the desired values.

Some Fine Points Regarding Modulation Amplitude

2.3.5

There are a few fine points that you should be aware of regarding the modulation amplitude. If you undermodulate (Mod. Amp. \ll linewidth), then the Number of Points tends to be much larger than needed. If you overmodulate (Mod. Amp. \gg linewidth), then the Number of Points tends to be much smaller than needed.

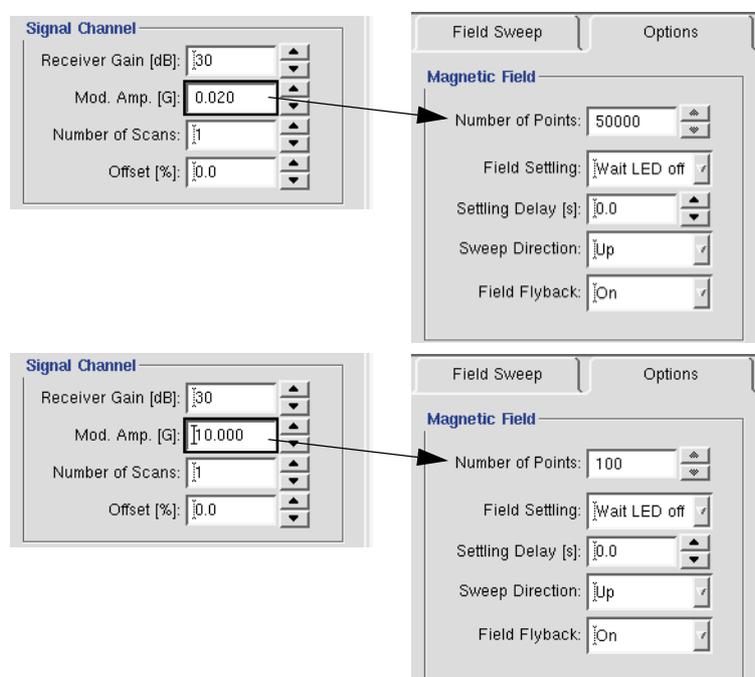


Figure 2-28 Over and under modulation can result in too many or too few points in the spectrum.

Another thing you may notice is that not all values of Mod. Amp. are allowed because:

$$\text{Number of Points} = \text{Integer Value} = \frac{\text{Pts}}{\text{Mod. Amp.}} \times \frac{\text{Sweep Width}}{\text{Mod. Amp.}} \quad [2-12]$$

You cannot have fractional number of data points in your dataset. You may ensure that you have the exact Mod. Amp. you want by setting the Sweep Width such that Equation [2-12] has an integer value.

Time Constants and Digital Filtering

2.3.6

As we saw in Section 2.2.4, the time constant of the signal channel is used to filter out noise and thereby attain a higher S/N (Signal to Noise ratio). The assumption is that the signal has mostly low frequency components and the noise will have components at all frequencies. By filtering out the high frequencies components in the signal channel output with the time constant, we are suppressing the noise in the spectrum. If we sweep too quickly for a given time constant, we can start to filter out the EPR signal as well. Figure 2-21 showed that increasing the time constant too far leads to a significant distortion of the lineshape and also of the line position.

Another approach to improving the S/N ratio is to suppress noise by using digital filtering techniques after the data has been acquired instead of using long time constants. In Xenon a binomial smoothing technique is used. This technique replaces the intensity value for a particular point in the EPR spectrum by a weighted average of the surrounding data points. The important parameter for the filtering is n , the Number of Points of the Digital Filter.

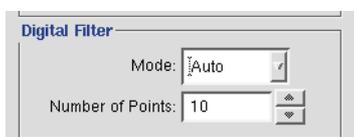


Figure 2-29 The Digital Filter parameters.

For a given n , the intensities of the n points before and the n points after the data point as well as the intensity of the data point itself are used in the weighted average comprised of $2n+1$ points. (These data points are the green points labeled I_{-2} to I_2 in Figure 2-30.) The weighting coefficients are given by the binomial coefficients that are the polynomial coefficients when $(1+x)^n$ is expanded. For $n = 2$, this is 1:4:6:4:1. After filtering, the filtered intensity at the center (the blue point in Figure 2-30) is given by:

$$I_0^{\text{filtered}} = (1 \cdot I_{-2} + 4 \cdot I_{-1} + 6 \cdot I_0 + 4 \cdot I_1 + 1 \cdot I_2) / 16, \quad [2-13]$$

where the factor of 16 is required for normalization. This filtering procedure is then repeated for each individual data point of the spectrum.

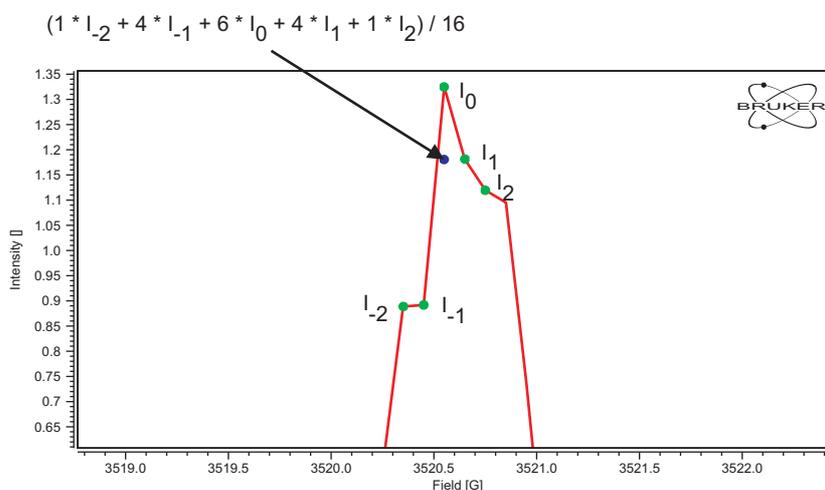


Figure 2-30 Binomial smoothing of the EPR data.

Figure 2-31 shows a comparison of the two noise reduction techniques. The unfiltered spectrum acquired with the minimum time constant is noisy. Increasingly longer time constants filter out more noise in the data. Increasingly larger Number of Points in the Digital Filter also filter out more noise in the data. Both techniques introduce distortions in the EPR lineshape for excessive parameter values, most notably a broadening of the peak-to-peak width and a diminishing of the peak-to-peak amplitude. The advantage of the digital filtering technique is that the EPR signal remains symmetric and does not exhibit a field shift as an excessively long analog time constant does.

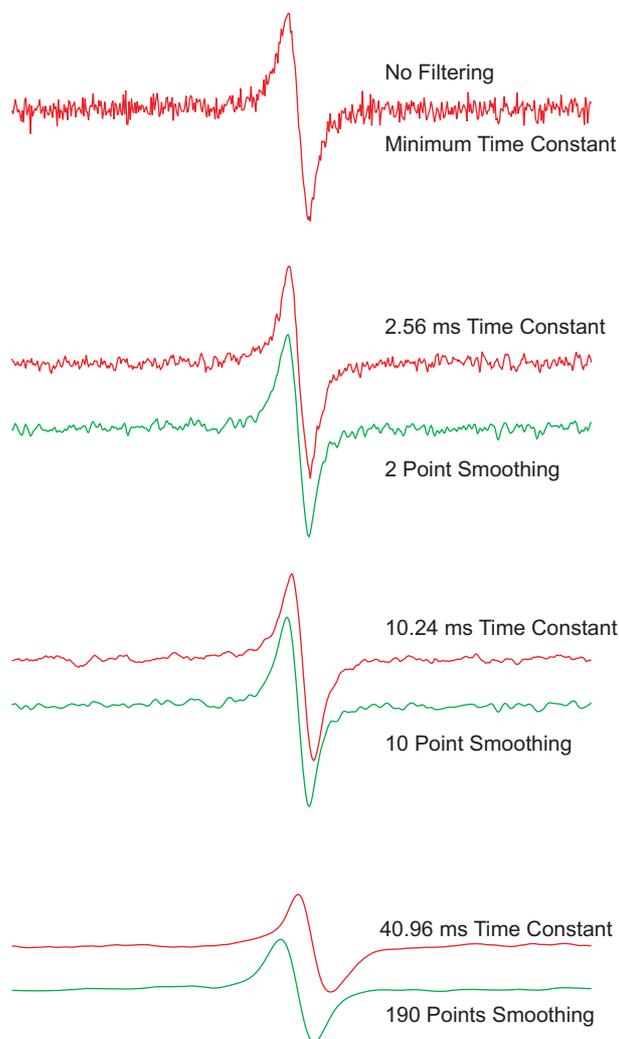


Figure 2-31 Comparison of spectra acquired using different time constants with spectra acquired using the minimum constant and digital filtering with different numbers of points.

By default, Xenon sets the Time Constant to the minimum value. The Digital Filter Mode is set to Auto mode. In this mode the Number of Points for the Digital Filter is set to the Pts/Mod. Amp. parameter value.

Default Xenon Parameters

2.4

In order to aid in finding EPR signals and optimizing them there are three sets of default parameters available in the Xenon software for different types of samples. It should be emphasized that these default parameter sets are only starting points. The first step for parameter optimization is to have an observable EPR spectrum (even though it may not be pretty) that you can optimize and the default parameter sets should give you an EPR spectra with which to start in most cases. Section 2.5 describes methods to further optimize the acquisition parameters.

Initial Default Parameters

2.4.1

When the software is first started there is a general default parameter set that is an appropriate starting point for finding EPR signals from almost any organic radical sample with a strong EPR signal such as calibration samples.



Figure 2-32 Initial default parameters.

Organic Radicals Default Parameters

2.4.2

The Organic Radicals default parameter set is a good starting point to find EPR signals from organic radicals. Organic radicals tend to be $g=2$ and exhibit transitions over a fairly narrow field range. Therefore the Center Field is set to the $g=2$ value for the present Microwave Frequency and the Sweep Width is set to 200 G. Linewidths or spectral features tend to vary typically from 0.1 G to 15 G which makes the Mod. Amp. of 1 G appropriate for signal detection. For most samples, 2 mW of microwave power will not saturate the EPR signal too much. These parameters are not optimum for all of your samples, but it is a good starting point to optimize the acquisition parameters.

The figure displays two screenshots of the Bruker EPR software interface, showing default parameters for Organic Radicals. The left screenshot shows the 'Field Sweep' and 'Options' tabs, and the right screenshot shows the 'Options' tab.

Left Screenshot (Field Sweep and Options):

- Magnetic Field:** Center Field [G]: 3524.30, Sweep Width [G]: 200.0, Sweep Time [s]: 30.00, Sample g-Factor: 2.000000.
- Signal Channel:** Receiver Gain [dB]: 30, Mod. Amp. [G]: 1.00, Number Of Scans: 1, Offset [%]: 0.0.
- Microwave:** Attenuation [dB]: 20.0, Power [mW]: 2.000.
- Digital Filter:** Mode: Auto, Number of Points: 10.
- Default Values:** Organic Radicals, Transition Metals.

Right Screenshot (Options):

- Magnetic Field:** Number Of Points: 2000, Field Settling: Wait LED off, Settling Delay [s]: 0.0, Sweep Direction: Up, Field Flyback: On.
- Signal Channel:** Calibrated: , Modul. Freq. [kHz]: 100.00, Modulation Phase: 0.0, Conv. Time [ms]: 15.00, Time Constant [ms]: 0.01, Dual Detection: OFF, Pts / Mod. Amp.: 10.00.
- Scan:** Fine Tune Each Scan: On Off, Auto Scaling: On Off, Replace Mode: On Off, Auto Offset: On Off, External Trigger: .

Figure 2-33 Default spectrometer parameters for organic radical samples.

Transition Metals

2.4.3

The Transition Metals default parameter set is a good starting point to find EPR signals from paramagnetic metals. Transition metals tend to exhibit transitions over a fairly broad field range. Therefore the **Center Field** is set to 3200 G and the **Sweep Width** is set to 6000 G in order not to miss any EPR signals. Linewidths or spectral features tend to be broader than for organic radicals thereby requiring a higher **Mod. Amp.** of 4 G. For most samples, 2 mW of microwave power will not saturate the EPR signal too much. These parameters are not optimum for all of your samples, but it is a good starting point to optimize the acquisition parameters.



Figure 2-34 Default spectrometer parameters for transition metal samples.

Parameters That Are Not Changed

2.4.4

The following parameters are not changed or reset when either the Organic Radicals or Transition Metals default parameters are chosen.

Receiver Gain	All Magnetic Field Parameters in Options
Time Constant	Modulation Phase
Dual Detection	All Scan Parameters
Digital Filter Mode	Digital Filter Number of Points

Table 2-2 Unchanged parameters.

Parameter Optimization

2.5

Once an EPR signal has been found, the acquisition parameters must be optimized for the sample under study. This section offers advice on how to optimize these parameters.

Microwave Power

2.5.1

The intensity of an EPR signal increases with the square root of the microwave power (dashed line in Figure 2-35) in the absence of saturation effects. When saturation sets in, the signals broaden and peak-peak amplitude decreases. The first thing that is obvious is that more microwave power helps to increase the signal strength until it starts decreasing. Also, if you are measuring spin concentrations, you want to work in the linear region.

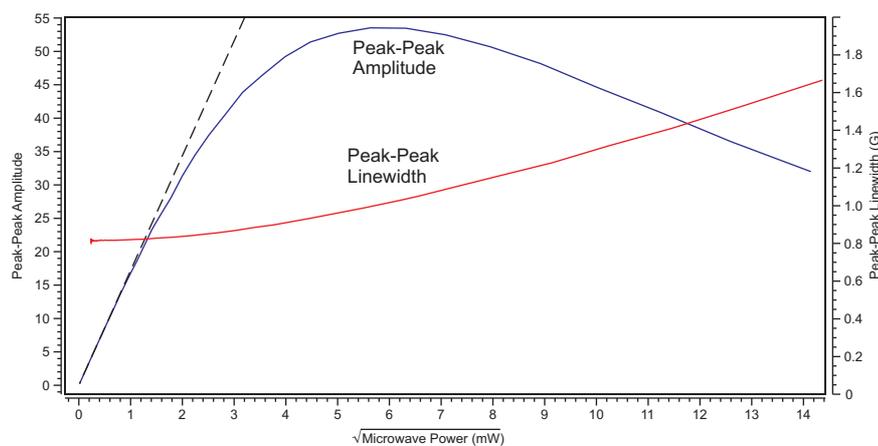


Figure 2-35 Experimental microwave power dependence data for a BDPA (Bis Diphenyl Allyl) point sample. The system is homogeneously broadened with $T_1 \sim T_2 \sim 100$ ns.

Though, the peak-peak intensity may decrease at higher microwave power, the integrated intensity of the EPR signal continues to grow. The increase in linewidth offsets the decrease in peak-peak intensity.

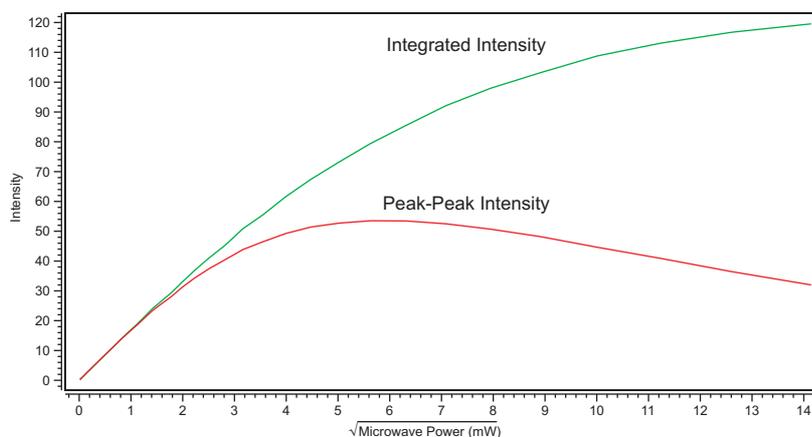


Figure 2-36 Comparison of the peak-peak intensity and integrated intensity for a BDPA (Bis Diphenyl Allyl) point sample as a function of the square root of the microwave power.

Even though the signal intensity may not change greatly with microwave power, EPR signals with very narrow lines (linewidth < 100 mG) are particularly susceptible to distortion because of excessive microwave power broadening.

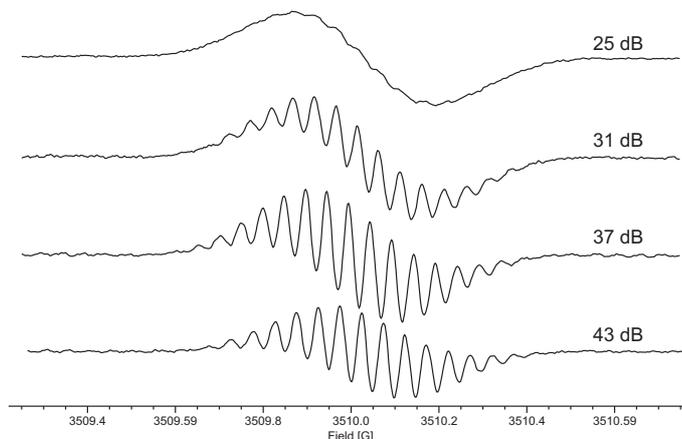


Figure 2-37 Galvinoxyl in heptane at different microwave attenuations.

You should try several microwave power levels to find the optimal microwave power for your sample. A convenient way to find the optimum power is to use the 2D_Field_Power experiment routine described in Section 8.2.

A general “rule of thumb” is that samples saturate more readily as the temperature decreases. Systems with greater orbital angular momentum tend to saturate less readily. Therefore organic radicals that usually have their orbital angular momentum quenched saturate readily. Transition metals and rare earth ions in particular have a great deal of orbital angular momentum and therefore do not saturate readily. The exceptions to this rule are S state ions such as Mn^{+2} and Gd^{+3} that do not have orbital angular momentum and these ions will saturate more readily than other ions in the series.

Field Modulation

2.5.2

Excessive field modulation broadens the EPR lines and does not contribute to a more intense signal. Figure 2-38 shows the dependence of the peak-peak linewidth and amplitude on the modulation amplitude.

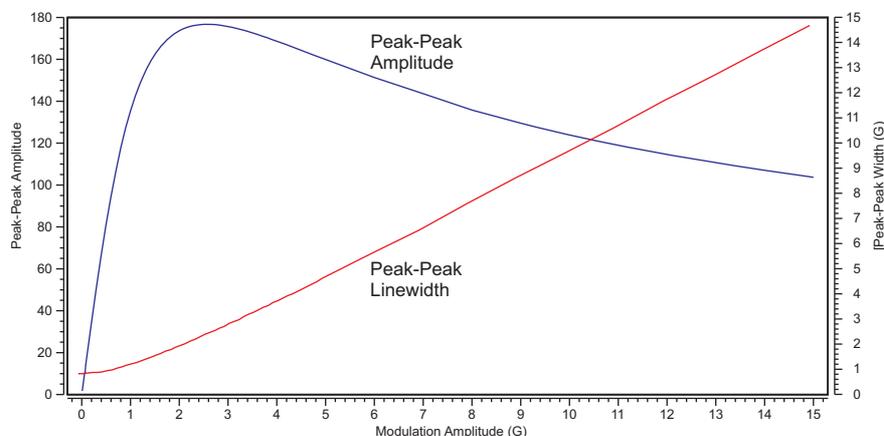


Figure 2-38 Experimental Modulation Amplitude data for a BDPA (Bis Diphenyl Allyl) point sample.

In contrast to the peak-peak intensity, the integrated intensity (or double integral) of the EPR signal maintains a linear dependence with respect to the Modulation Amplitude owing to the modulation broadening.

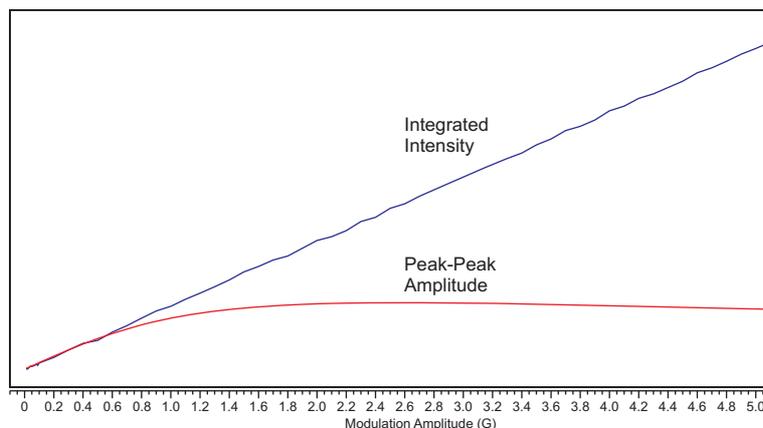


Figure 2-39 Experimental Modulation Amplitude integrated intensity data for a BDPA (Bis Diphenyl Allyl) point sample.

A good “rule of thumb” is to use a field modulation amplitude that is approximately one quarter the width of the narrowest EPR line you are trying to resolve. Keep in mind that there is always a compromise that must be made between resolving narrow lines and increasing your signal to noise ratio. If you have a very weak signal, you may need to sacrifice resolution (*i.e.*, by using a higher field modulation) in order to even detect the signal. However, if you have a high signal to noise ratio, you may choose to use a much lower field modulation amplitude in order to maximize resolution. For small splittings in EPR spectra, excessive Modulation Amplitude can mask small splittings as shown below.

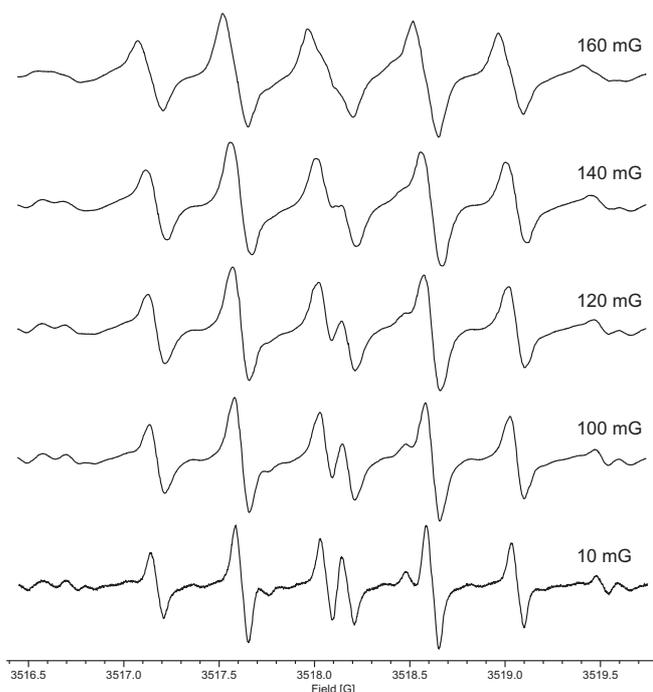


Figure 2-40 Experimental data for perylene radical cations in H₂SO₄ at different modulation amplitudes.

The best sensitivity is usually attained with 100 kHz field modulation, but the Modulation Frequency can also affect the resolution or linewidth of the EPR signal if the signals are very narrow (< 50 mG). The 100 kHz field modulation produces 35 mG sidebands that can broaden the linewidth. Figure 2-41 shows the effect of the Modulation Frequency on the linewidth of a very narrow EPR line from nitrogen in C₆₀.

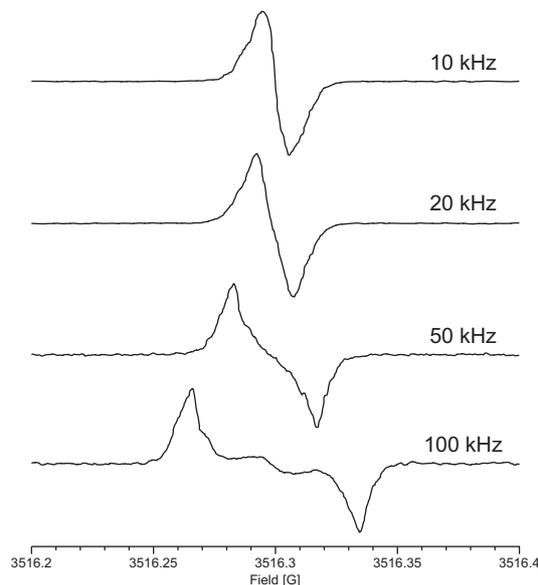


Figure 2-41 Experimental Modulation Frequency data for a N-C₆₀ sample in CS₂ sample.

J.S Hyde found a nice example of how a higher modulation frequency can cause problems sometimes in the interpretation of EPR spectra with very narrow lines and small hyperfine splittings. The galvinoxyl radical has very small hyperfine splittings (~ 50 mG) from the two adjacent t-butyl groups producing a multiplet of 37 lines from the 18 equivalent protons. Figure 2-43 shows the results with 100 kHz and 10 kHz modulation. The 10 kHz spectrum appears to be much better resolved than the 100 kHz spectrum. Owing to the closeness of the hyperfine splittings and the 100 kHz sidebands, the integral of the 100 kHz first harmonic spectrum shows immediately and incorrectly an even number of EPR lines in the multiplet. The 10 kHz integrated signal shows correctly an odd number of lines in the multiplet.

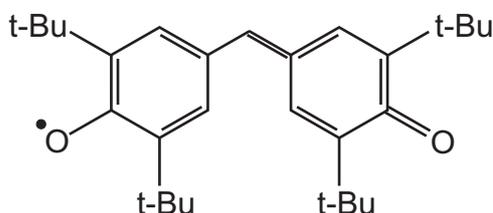


Figure 2-42 The structure of the galvinoxyl radical.

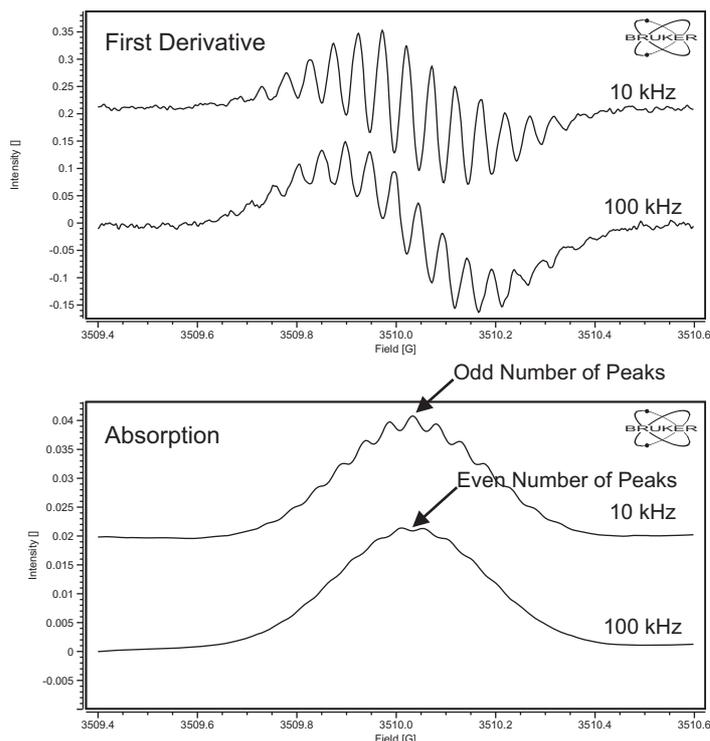


Figure 2-43 Galvinoxyl radical in heptane spectra acquired at 100 and 10 kHz Modulation Frequency.

Second Harmonic Detection

2.5.3

There is an option in the software for detecting the second harmonic of the field modulated EPR signal. The second harmonic signal represent the second derivative of the EPR absorption signal. It is usually smaller (and therefore less sensitive) than the first harmonic (derivative) signal, but it has one big advantage; it can give better resolution for overlapping lines.

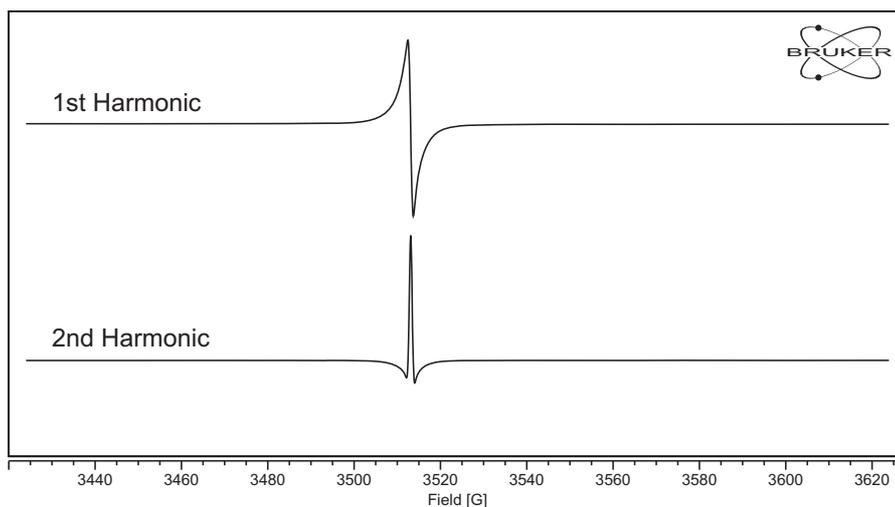


Figure 2-44 First and second harmonic EPR signals.

Below is an example of the superior resolution of the second harmonic signal compared to the first harmonic signal. The nitroxide TEMPOL exhibits a nitrogen hyperfine splitting (the three line triplet) and each of the lines is further split by hyperfine splittings from the six methyl group protons. The first harmonic exhibits some wiggles that may hint at splittings. The second harmonic nicely shows the seven expected EPR lines with their predicted intensities.

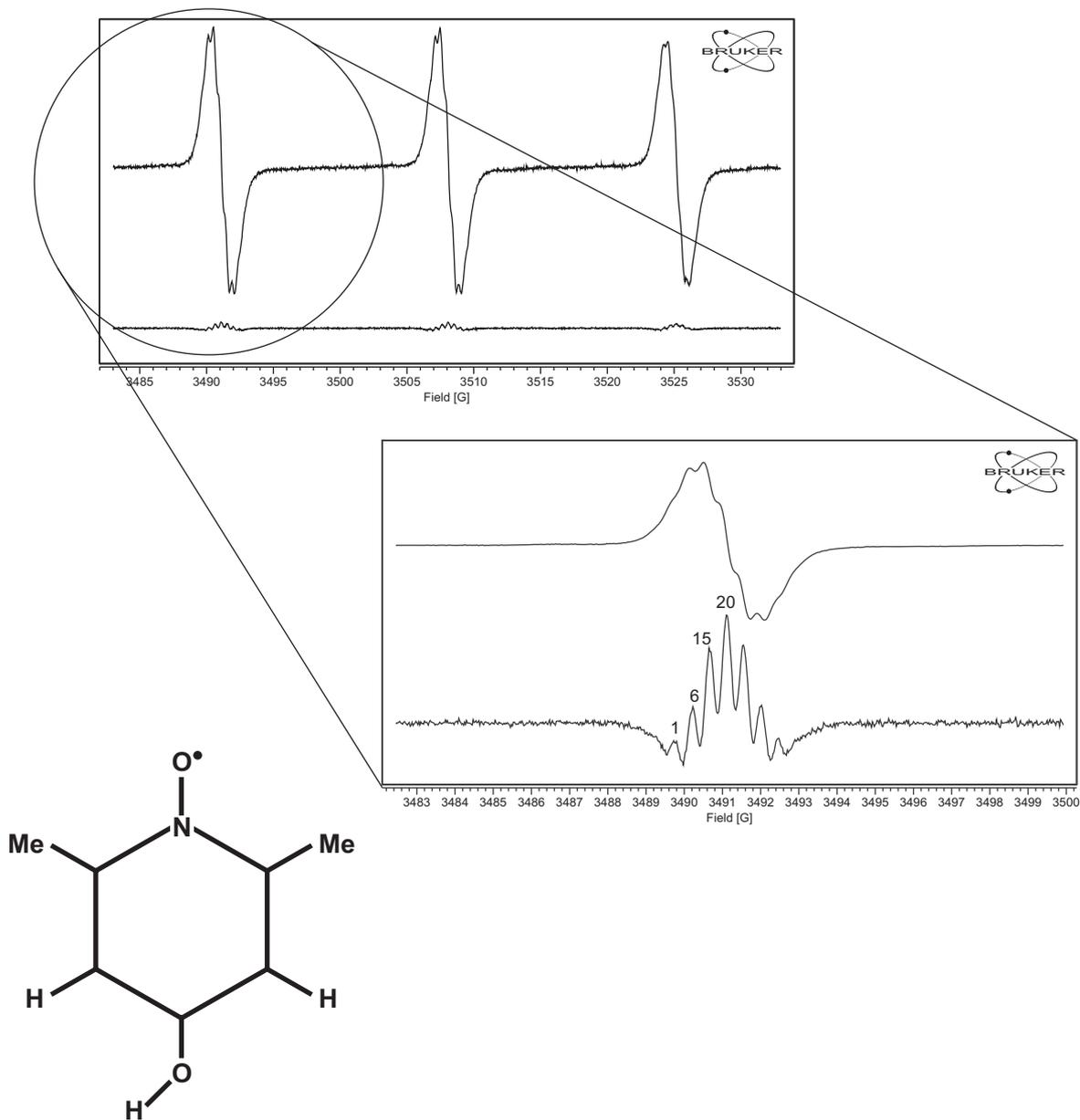


Figure 2-45 Superior resolution from the use of the second harmonic when looking at the small methyl group proton hyperfine splittings in the nitroxide TEMPOL.

Measurement Time

2.5.4

We have seen in Section 2.3.6 that time constants and digital filtering can be used to increase the S/N (Signal to Noise ratio). Another means of improving S/N is to use signal averaging. The field sweep is repeated for a specified number of times, n . The result of the n acquisitions is then averaged. The signal grows linearly with n , but the noise increases more slowly, as \sqrt{n} , because of the random nature of the noise. Therefore the S/N increases as \sqrt{n} .

Another alternative is to increase the **Sweep Time**, thereby automatically increasing the **Conversion Time**. This in effect is also signal averaging because the digitizer can digitize or average more times at each individual point of the field sweep as the **Conversion Time** increases. As we can see in Figure 2-46, a single 2.62 s field sweep produces a rather noisy signal. Averaging the field sweep 100 times produces a 10 fold increase in S/N. Increasing the **Conversion Time** by a factor of 100 and only acquiring once produces the same improvement. The S/N improvement comes with a price:

$$S/N \propto \sqrt{\text{Total Acquisition Time}} \quad [2-14]$$

If you have a very weak signal, each doubling of S/N requires increasing the acquisition time by a factor of four. This is the reason why it is important to optimize all the parameters for weak and noisy spectra.

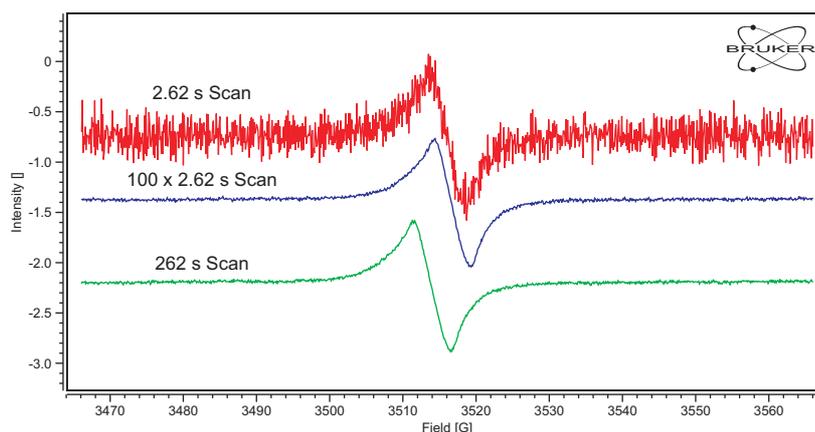


Figure 2-46 Improving the S/N by signal averaging or increasing the Conversion Time.

Given that both methods, signal averaging and increasing the **Conversion Time**, increase the S/N with the same dependence on the total acquisition time, why would we choose one over the other? With a perfectly stable laboratory environment and spectrometer, signal averaging and increasing the **Conversion Time** are equivalent. Unfortunately, perfect stability is usually impossible to attain and slow variations can result in considerable baseline drifts when measuring very weak signals. A common cause of such variations are room temperature changes or air drafts around the cavity. For the slow single scan, the variations cause broad features to appear in the spectrum as is shown in spectrum b of Figure 2-47. You can achieve the same sensitivity without baseline distortion by using signal averaging. For example, if you were to signal average the EPR spectrum using a scan time that was significantly shorter than the variation time, these baseline features could be averaged out. In this case, the baseline drift will cause only a DC offset in each of

the scanned spectra. Spectrum a shows the improvement in baseline stability through the use of short time scans with signal averaging when the laboratory environment is not stable.

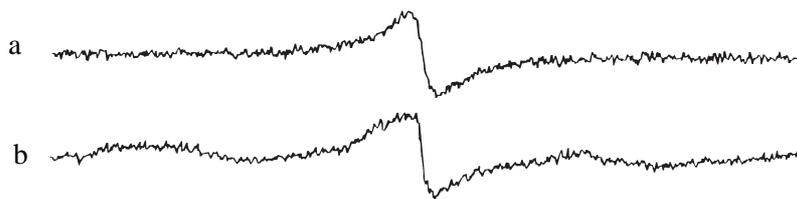


Figure 2-47 a) Signal with signal averaging with a short Sweep Time.
b) Signal with a long Sweep Time.

Receiver Gain

2.5.5

Improvements in the dynamic range of the ADC (Analog to Digital Converter) in the signal channel make the optimization of the Receiver Gain much less critical than with previous generations of spectrometers. The remaining problem is to keep the Receiver Gain sufficiently low to prevent the signals from clipping. In Figure 2-48 we can see an example of clipping: the lines are suddenly cut off at a certain amplitude. This can sometimes result in all of the lines appearing to have the same amplitude.



Figure 2-48 The effect of using gain settings that are either (a) optimal or (b) too high on an EPR spectrum.

Adjust the Receiver Gain to prevent clipping. You may also monitor the Receiver Level while acquiring the spectrum to verify that the absolute value of the Receiver Level stays less than 100%.

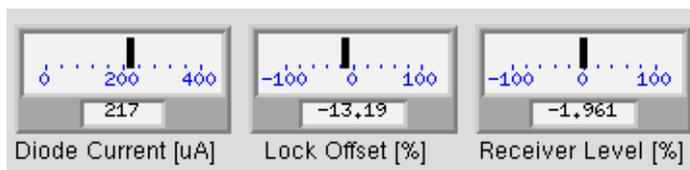


Figure 2-49 Monitoring clipping using the Receiver Level indicator.

Magnetic Field Parameters Optimization

2.6

The g -values and hyperfine splittings of samples give you valuable insight into the electronic structure of the species you are studying with EPR. In order to measure these parameters accurately, you need to be careful in your measurements. In this section we discuss some of the possible pitfalls that can cause problems with accurate field measurement.

Field Offsets

2.6.1

The Hall probe used to control and measure the magnetic field and the EPR sample are not in the same place in the magnet airgap. A great deal of effort is used to manufacture a magnet with the highest magnetic field homogeneity (a measure of how constant the magnetic field is at all places in the magnet airgap). However, there are difference in the magnetic fields at the two previously mentioned positions. The difference is typically 3-4 G at $g=2$ for X-band.

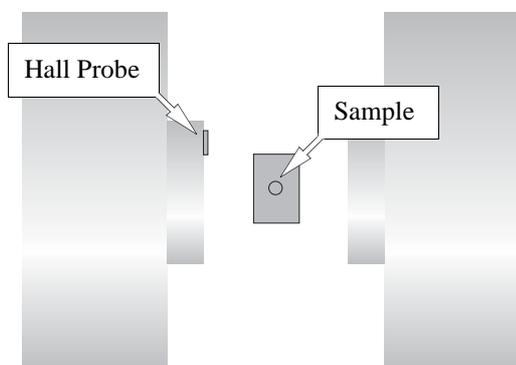


Figure 2-50 Differences in positions of the Hall probe and the EPR sample leads to offsets in the measured field for resonance of an EPR line.

Section 10.2 describes some strategies for measuring and correcting for these field offsets.

Field Sweep Rates

2.6.2

The signal averaging with fast field sweeping has some advantages in terms of baseline, but you need to be a bit careful to not sweep more quickly than the magnet and field controller can follow.

Ideally the magnetic field sweep should be linear and the indicated magnetic field values in the spectrum correspond to the actual magnetic field values. There are a few situations in which this may not be possible. The first case is when you have a very rapid field sweep. The inductance of the magnet combined with the rapidly changing current will lead to a nonlinear sweep with magnetic field offset at sweep rates greater than 30 G/s. Note the difference between the magnetic field for the EPR signals of the two traces in Figure 2-51 is not constant over the sweep width. Initially the field lags and then catches up towards the end of the sweep.

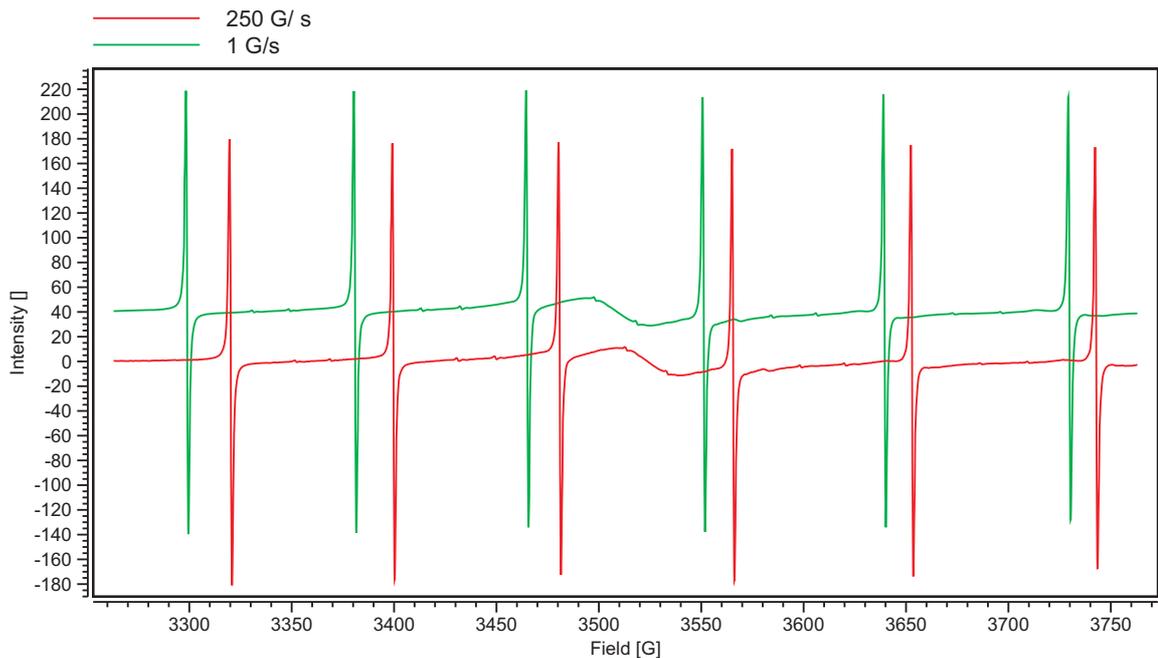


Figure 2-51 Field offsets from sweeping too rapidly. The green trace was swept at 1 G/s and accurately reflects the correct magnetic field values. The red trace was swept at an excessive rate (250 G/s) to exaggerate the offset effects.

For slower sweep rates than 30 G/s, the field sweep may be linear, but there still may be a constant magnetic field offset. If you need very precise magnetic field measurements, it is best to use a sweep rate of 1-2 G/s.

Another situation in which you may encounter these offset effects is setting the static field for time sweep experiments from the EPR spectrum using the Position Level tool. (See Section 8.1.) Because of the field offset, the level indicator will indicate the EPR maximum at a lower field than the peak in the spectrum trace when sweeping too quickly. It is best to acquire your EPR spectrum for setting the static field at a sufficiently slow rate.

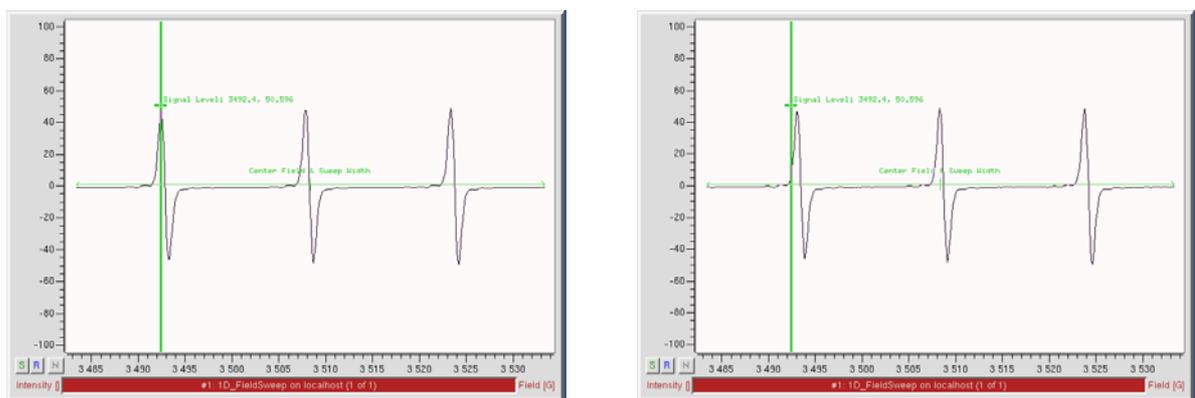


Figure 2-52 Field offsets from sweeping too rapidly. The left figure shows the Position Level tool maximum agrees with the EPR spectrum acquired at 1 G/s. The right figure shows the Position Level tool maximum happens at a lower magnetic field with the EPR spectrum acquired at 10 G/s.

Field Settling after Flyback

2.6.3

A magnetic field sweep is comprised of three parts. The first is the magnetic field sweep in which the field is swept slowly and then followed by the flyback consisting of rapid return to the initial magnetic field value. The third part is a period of time in which the field needs to be stabilized or settled to the desired initial magnetic field value of the field sweep.

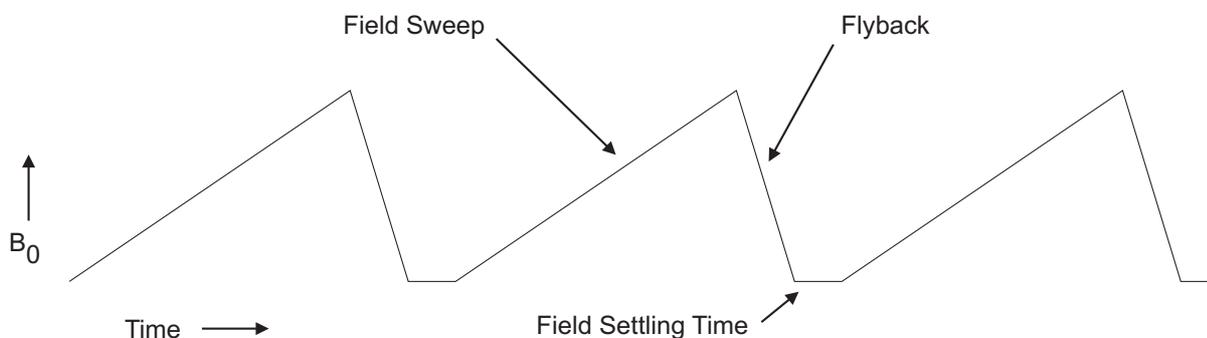


Figure 2-53 The three parts of a magnetic field sweep when signal averaging.

Typically problems with field settling appear at the left edge of averaged spectra. There are four options for controlling the field settling time:

Do Not Wait There may be cases where you need to average the EPR spectra quickly because of unstable species in the sample. By selecting this option, you eliminate the overhead associated with the field settling time. You need to be careful though as the field linearity of the field sweep may suffer, thereby preventing you from obtaining precise field for resonance value from your EPR spectrum. This option should never be used for precise magnetic field measurements when measuring g -values and hyperfine splitting constants.

Figure 2-54 shows what can happen when averaging. The first scan is correct because the magnetic field starts in a stable or settled condition. The second scan is different because the starting magnetic field is unstable because of the lack of settling time after the flyback. There is an extra line from the flyback. The first non-flyback line is also shifted to the right. When we sum the two scans and divide by two, we see that some extra peaks in the averaged spectrum have appeared. Also we can see that the magnetic field has finally caught up by the middle line of the nitroxide spectrum.

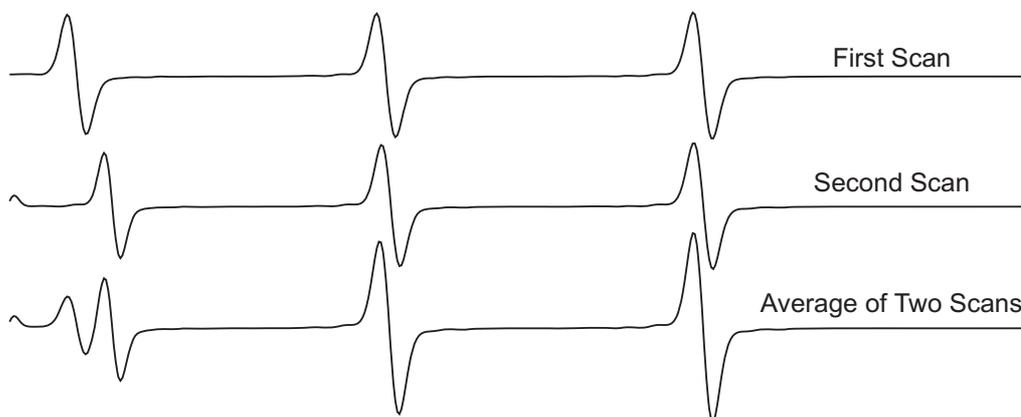


Figure 2-54 Extra lines caused by not waiting for the field to settle with the Do Not Wait option.

Wait LED Off This is the default option. The magnetic field value is measured at regular time intervals and once the measured field value is equal (within a certain tolerance) of the desired initial magnetic field value, the field sweep starts. It usually works well for most spectra. For fast averaging and with an EPR line at the left edge of the sweep, this option may still not be sufficient. Early peaks in the spectrum may be distorted as shown in Figure 2-55.

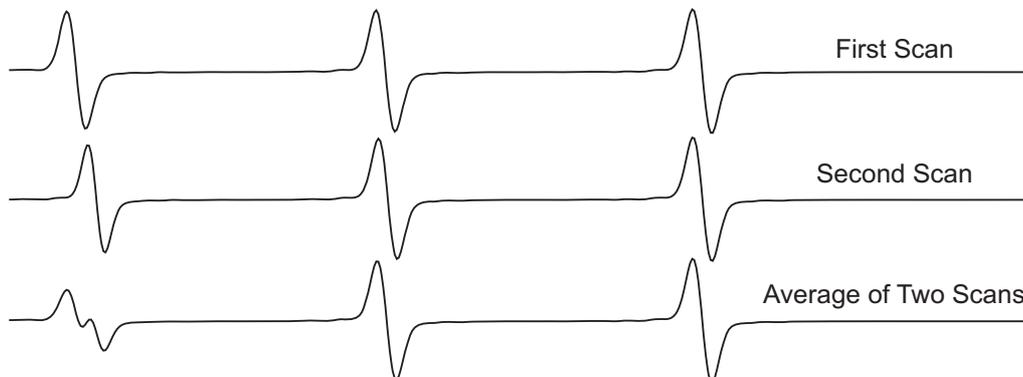


Figure 2-55 Distorted peaks caused by the magnetic field not being completely settled with the Wait LED Off option.

Wait Stable For wide field sweeps and field sweeps going close to zero magnetic field, the default option may not work as well as desired. After the flyback, the magnetic field may oscillate a bit resulting in a false start of the field sweep. With the Wait stable option, the field sweep starts after three consecutive readings of the magnetic field match the desired initial magnetic field value. This more stringent criterion helps minimize the effect of the oscillations and results in better sweep linearity and reproducibility. For signal averaging, it will also add a bit of overhead to the measurement time. Figure 2-56 shows that this option works fairly well, even for this problematic case. The first line is very slightly distorted by a slight field shift.

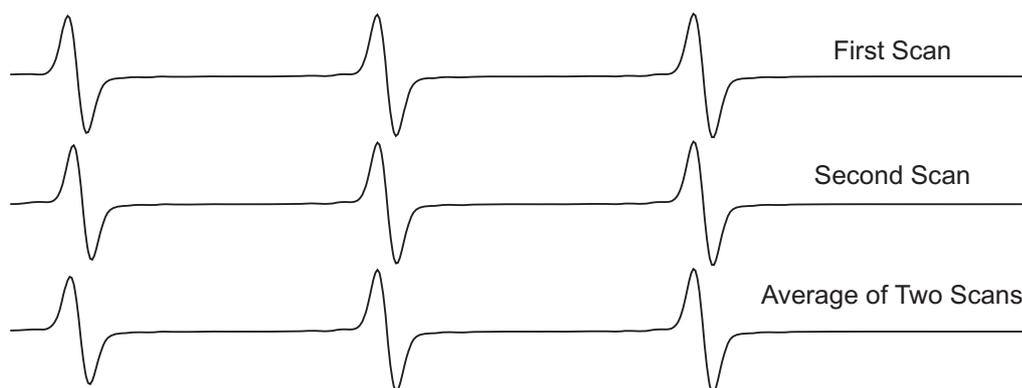


Figure 2-56 Almost undistorted averaged EPR spectrum using the Wait Stable option.

Given Delay For particularly wide field sweeps or if you need to go all the way to zero field, the best option is **Given delay**. The field sweep only starts after the time interval specified in the **Settling Delay** parameter.

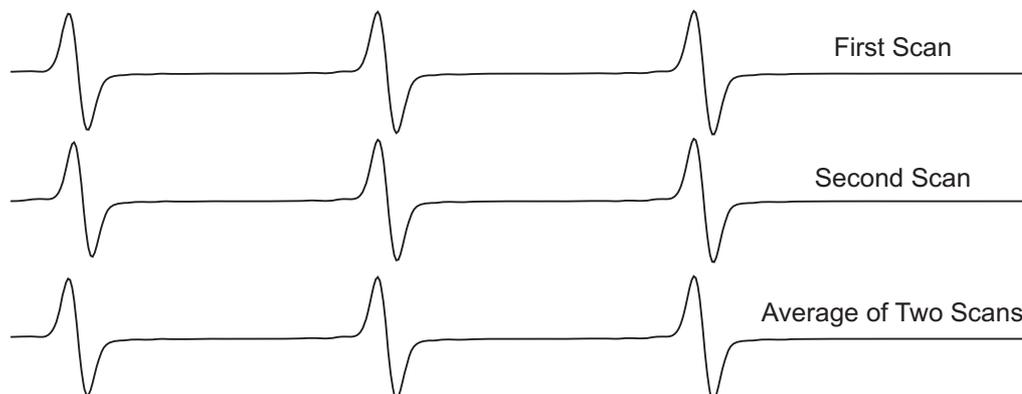


Figure 2-57 Undistorted averaged EPR spectrum by using a three second delay for field settling.

Which option you should use depends on what experiment you are doing. If you need to average quickly, **Do Not Wait** is the required option. If you need very precise field measurement or you have very wide sweeps, the **Wait Stable** and **Given delay** options are the best. The default **Wait LED Off** option is a good compromise; it gives reasonable precision and does not greatly increase acquisition time.

Spin Quantitation

2.7

Often you need to answer the question, “How many radicals or spins are in my sample?”. There are two approaches to answering this question: relative measurements in which the unknown sample is compared to a sample of known concentration or absolute measurements in which the absolute EPR signal intensity is directly converted to a concentration without the need of a reference sample.

Signal Integration

2.7.1

Both approaches require the integrated intensity of the EPR absorption signal, *i.e.* the area under the absorption curve. Because we are using field modulation and demodulation, we obtain a first derivative of the EPR absorption signal. Therefore, in order to obtain the integrated intensity, we need to integrate the EPR signal twice. Figure 2-58 shows the expected shapes of the first and second integral of an EPR signal. The first integral rises and then falls back to zero at the end of the spectrum. The second integral starts flat, rises, and then maintains a steady level after the rise. The value of the last point of the double integral is equal to the area of the EPR absorption.

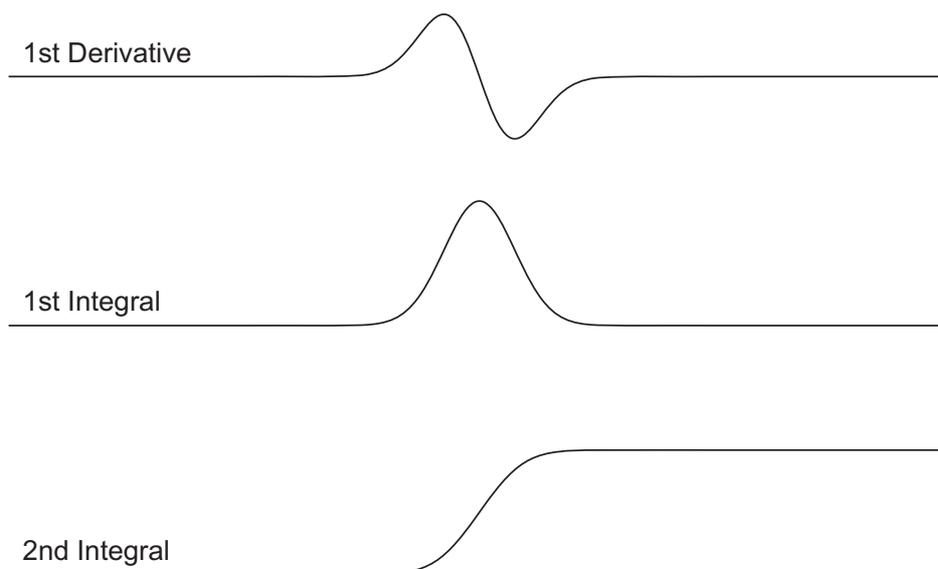


Figure 2-58 Integrated EPR signals with no offsets or backgrounds.

Things get a little complicated if there are any background signals or offsets. Figure 2-59 shows the integrals of an EPR signal that has a tiny DC offset in the signal level. In such a case, the first integral exhibits a linear sloping baseline. This is to be expected because the integral of a constant term is a sloped line. The second integral shows even more dramatic effects because the integral of a sloped line is a quadratic or parabola. We can see that the second integral not only has information regarding the EPR integrated intensity but also a large contribution from the DC offset. The value of the last point of the double integral is no longer equal to the area of the EPR absorption. The situation is even worse if there is a linear background, as the double integral of a sloped line is a cubic polynomial. Often there may be a broad almost unseen underlying signal such as a signal from metal ions in the sample that can contribute to the double integral.

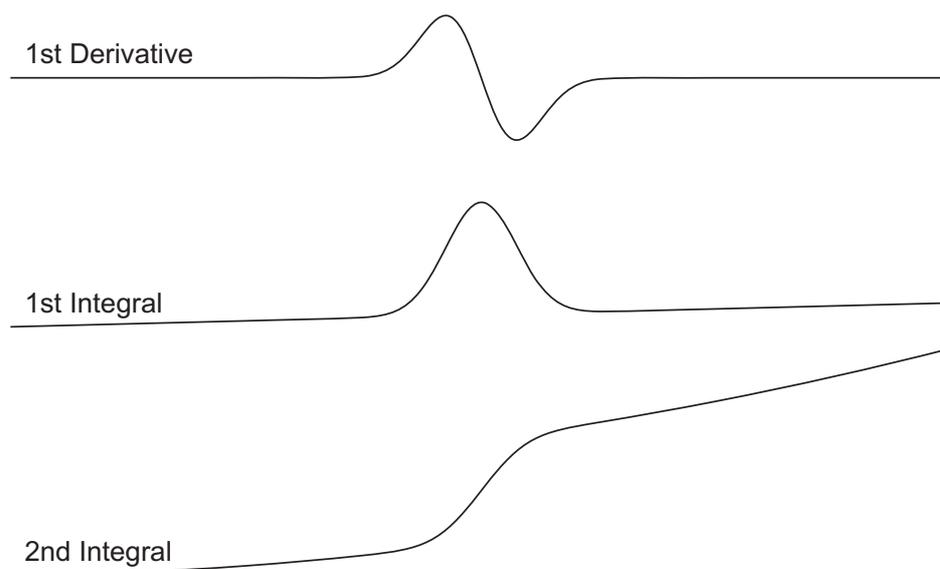


Figure 2-59 Integrated EPR signals with a DC offset or backgrounds.

Because the double integral is so sensitive to offsets and backgrounds, it is very important to perform a careful background subtraction in order to perform a successful double integration.

There are a few tricks that can be used to overcome some of these difficulties or to obtain a quick estimate of the integrated intensity. One means of estimating the relative integrated intensity if the different EPR signals have the same linewidth and lineshape is to compare the peak to peak amplitude of the first derivative signals. Background effects are suppressed when subtracting the peak and trough values. Note that the EPR signals must have the same linewidth for such a comparison to be made.

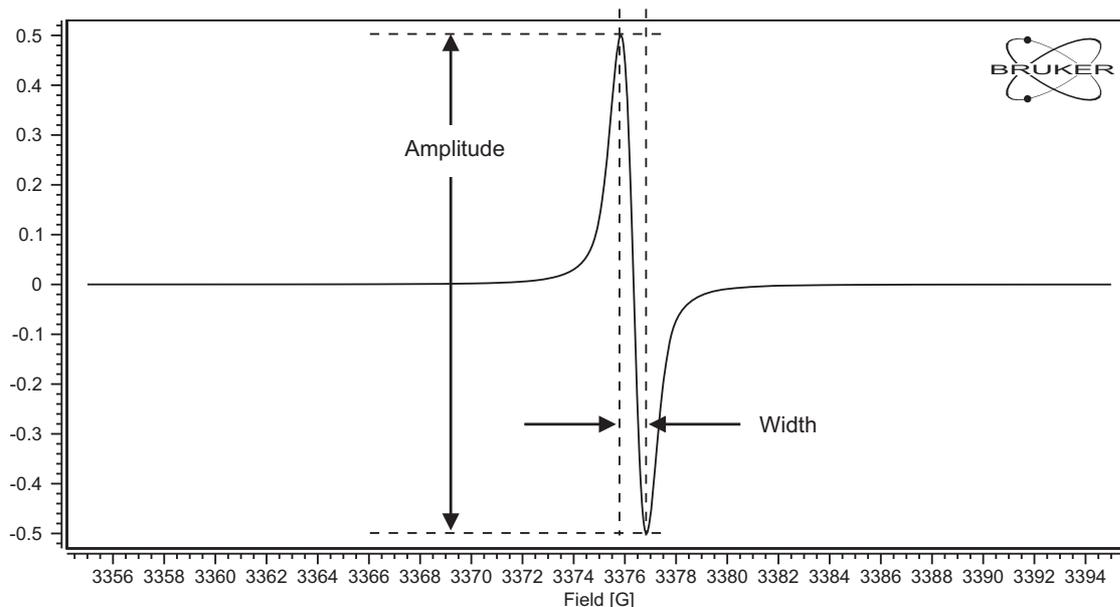


Figure 2-60 Peak to peak amplitude and linewidth of an EPR line.

A better estimate can be made by including the linewidth as well as the amplitude. The double integral of an EPR signal can be approximated by

$$\text{Double Integral} \propto \text{Amplitude} \cdot \text{Width}^2 \quad [2-15]$$

The best means of integration is to simulate the EPR spectrum with a program such as SpinFit as described in Section 7.6 and Section 8.3.5 followed by integrating the simulated spectrum.

Relative Measurements

2.7.2

Traditionally, spin quantitation has been accomplished by comparing the integrated intensity of an unknown sample with the integrated intensity of a sample of known concentration, commonly called the standard sample. This technique is known as a relative measurement.

The double integral (DI) of an EPR spectrum can be expressed as:

$$\text{DI} = \frac{c}{f(B_1, B_m)} \times \{G_R \times C_t \times n\} \times \{\sqrt{P} \times B_m \times Q \times n_B \times S \times (S + 1)\} \times n_S \quad [2-16]$$

where

c	point sample calibration factor
B_1	microwave magnetic field
B_m	modulation amplitude
$f(B_1, B_m)$	spatial distribution of B_1 and B_m
G_R	receiver gain
C_t	conversion time
n	number of averages
\sqrt{P}	square root of the microwave power
Q	quality factor of the resonator
n_B	Boltzmann factor to correct for temperature
S	electronic spin
n_S	number of spins

Table 2-3 Parameter definitions for Equation [2-16].

The number of spins can be expressed as:

$$n_S = \frac{DI}{\frac{c}{f(B_1, B_m)} \times \{G_R \times C_t \times n\} \times \{\sqrt{P} \times B_m \times Q \times n_B \times S \times (S + 1)\}} \quad [2-17]$$

So if we had values for all these parameters, we could directly calculate the number of spins. (See Section 2.7.3.) In the past, these values have not been easily accessible. If we keep all these parameters identical for the standard and unknown sample, these terms cancel out if we take the ratio of the double integrals:

$$\frac{n_{S \text{ unknown}}}{n_{S \text{ standard}}} = \frac{DI_{\text{unknown}}}{DI_{\text{standard}}} \quad [2-18]$$

Alas keeping all parameters and conditions identical for the standard and unknown sample is not always possible or desirable.

We still can make corrections for the parameters that are different and have known values by entering their values into Equation [2-17] when we calculate the ratio. Easy corrections for some of the experimental parameters may be made. The Xenon software accounts for these easy corrections by normalizing (or dividing) the EPR amplitude by the normalization constant, N:

$$N = \text{Conversion Time(ms)} \times \text{Number of Scans} \times 20 \times 10^{\text{Receiver Gain(dB)/20}} \quad [2-19]$$

Note that this corresponds to the second term in Equation [2-16].

The third term can often be accounted for in a relatively straightforward manner. As we saw in Section 2.5.1, the EPR signal grows with the square root of the applied microwave power in the absence of saturation. Therefore in order to make a proper comparison of two EPR spectra, it is important that the two spectra have been acquired in a non-saturating microwave power. Another factor is the quality factor of the resonator, Q . Strictly speaking, the EPR intensity is proportional to the microwave magnetic field, B_1 , in the resonator and the efficiency of the resonator in converting the EPR absorption into a measurable signal. By recording the microwave power and the Q value, these two factors can be accounted for.

As was shown in Figure 2-39, the integrated intensity of an EPR spectrum is proportional to the modulation amplitude. By recording this parameter, this factor can also be accounted for.

The Boltzmann factor can be accounted for by recording the sample temperature at which the EPR spectrum was acquired. In the high temperature limit (satisfied under most EPR experimental conditions) this factor is:

$$n_B \approx \frac{h\nu}{2k_B T} \quad [2-20]$$

where h is Planck's constant, ν the microwave frequency, k_B the Boltzmann constant, and T the sample temperature in Kelvin. For the last term in the third term of Equation [2-16], we need information regarding the spin state of the paramagnetic species in the samples.

Once all this information is known, the integrated intensities can be normalized (or divided) by the third term of Equation [2-16].

The first term of Equation [2-16] is perhaps the most difficult term required to compare unknown and standard samples. The point sample correction factor accounts for response of the EPR detector, electronic gains, and resonator properties. This should not be a problem if the same spectrometer is used to measure the unknown and standard sample but must be accounted for if measured on different spectrometers. $f(B_1, B_m)$, the spatial distribution of the microwave magnetic field and modulation amplitude corrects for the fact that not all parts of a sample give the same signal amplitude owing to its position in the resonator. Figure 2-61 shows the dependence of the signal intensity as a function of the vertical distance from the center of the resonator. The maximum intensity is at the center and this defines the point sample correction factor that has been previously mentioned. The signal then drops off and finally disappears as the distance from the center increases.

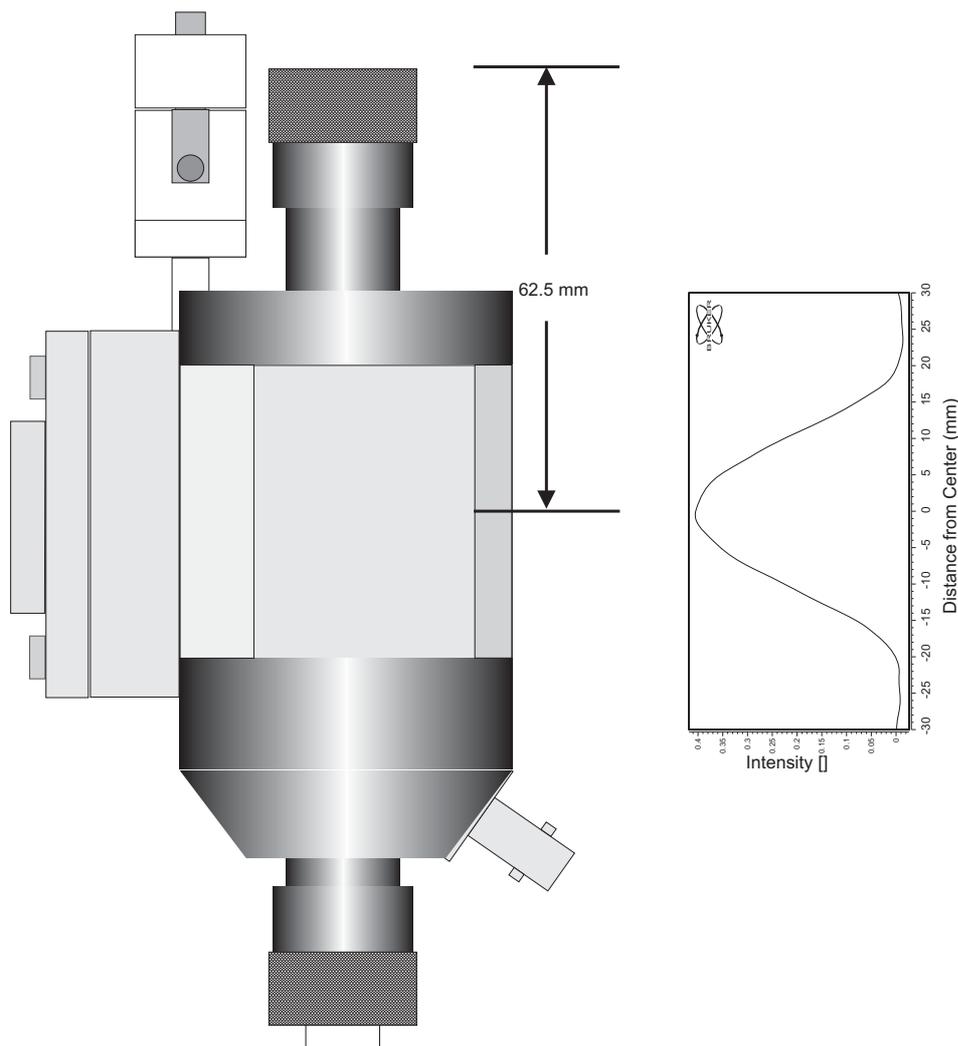


Figure 2-61 The signal intensity distribution in an ER 4119HS resonator.

The signal intensity is proportional to the intensity distribution curve integrated over the length of the sample. Therefore to compare samples of different lengths, the signal intensity needs to be normalized by the integrated intensity distribution shown in Figure 2-62.

Because of the signal variation, it is important to center samples shorter than the length of resonator for maximum signal. As can be seen from Figure 2-62a and b, a sample that is not centered will produce less signal than the sample centered in the resonator. Also a longer centered sample as shown in Figure 2-62c will have a greater signal intensity than a shorter sample shown in Figure 2-62b.

A convenient way to eliminate this dependency on sample length and positioning is to prepare samples that are longer than the resonator length. In the case of the ER 4119HS resonator, this is 40 mm. Provided the spin concentration is homogeneous throughout the sample volume, there should be no large change in signal intensity as the sample is moved up or down.

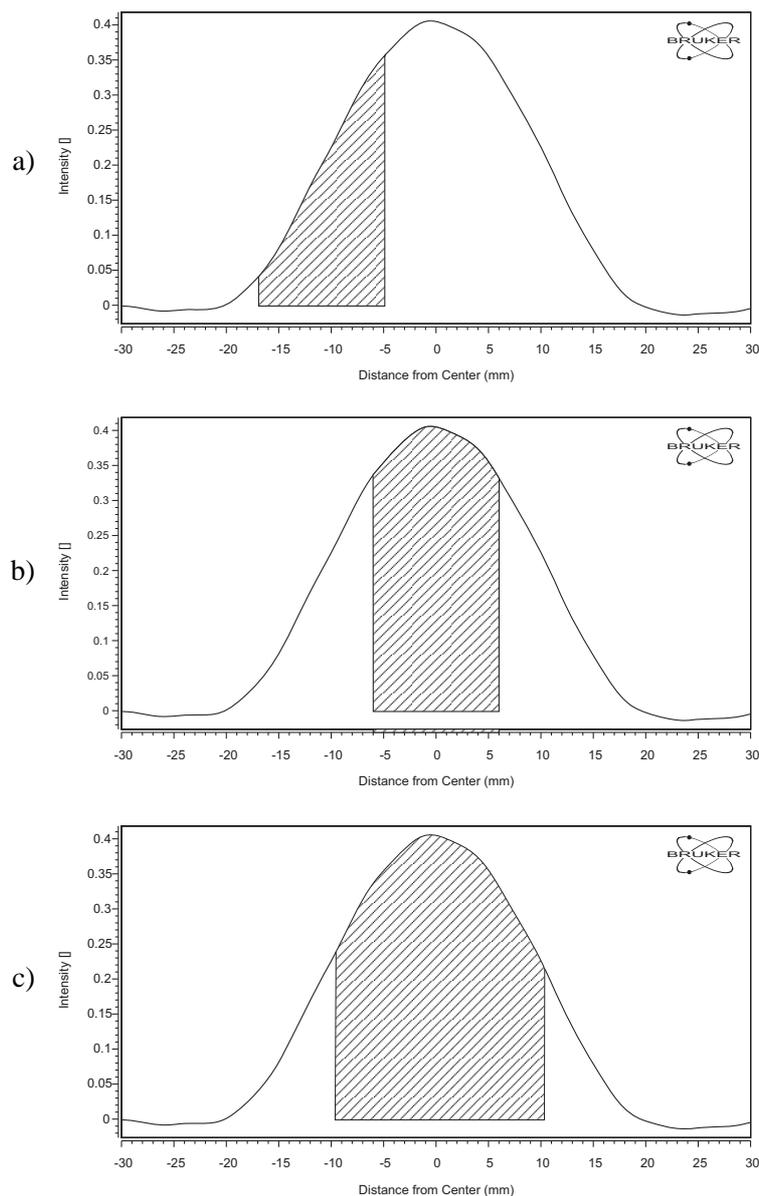


Figure 2-62 Integrated intensity distribution for different sample lengths and positions. a) shows a short sample that has been inserted too low into the resonator. b) shows the same sample but centered in the resonator. c) shows a longer centered sample.

The accuracy of the spin quantitation depends strongly on the number of known parameter values, how accurate those values are, and how identical the unknown parameters are. With care and attention to carefully controlling these parameters, the relative measurement or comparison with a standard sample can yield accurate spin counts. It is also very important to do all the bookkeeping and record all the relevant parameters for the spectra.

Absolute Measurement

2.7.3

It would be wonderful to quantitate the number of spins without the need for a reference standard. In principle, Equation [2-17] can be used directly if we have values for all the parameters. As can be seen however, there is quite a bit of record keeping and calculations required to accomplish this goal.

Xenon can perform the calculations for you automatically and give you accurate results provided you are careful in setting up the experiment. As in any quantitative work, the microwave power must be kept below saturation. As can be seen in Figure 2-62, centering of the sample in the resonator is important.

The Q can be measured from the tuning mode curve. Owing to the nonlinear response of the microwave detector, it is important to measure the Q only after the resonator and bridge are properly tuned and at a specific microwave attenuation (33 dB).

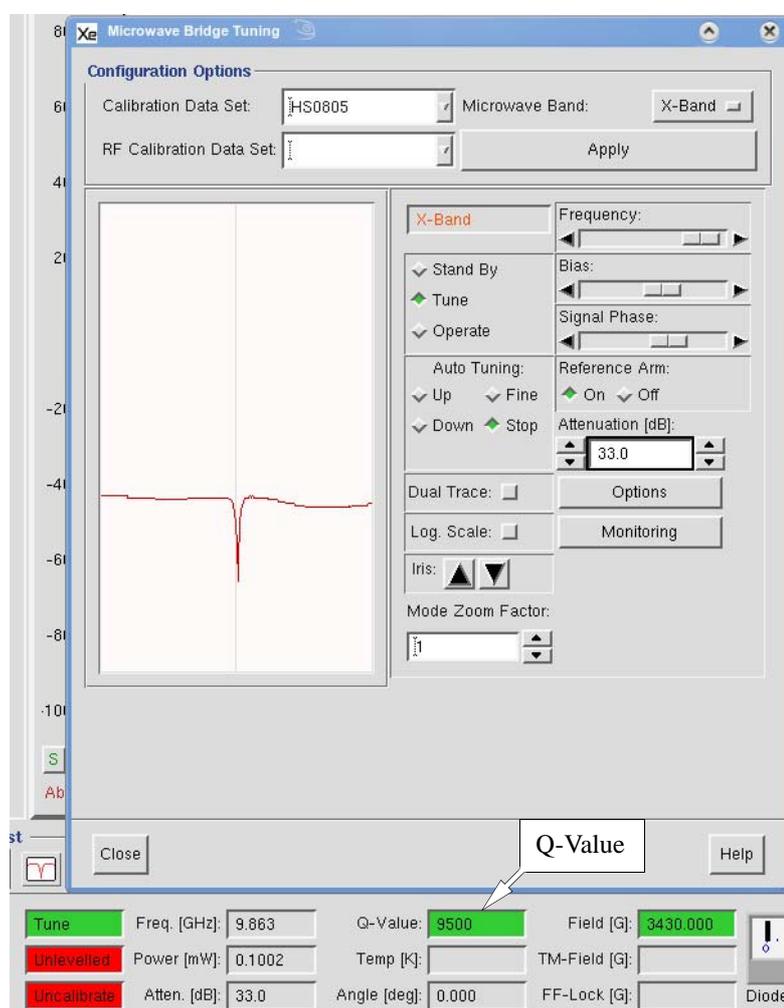
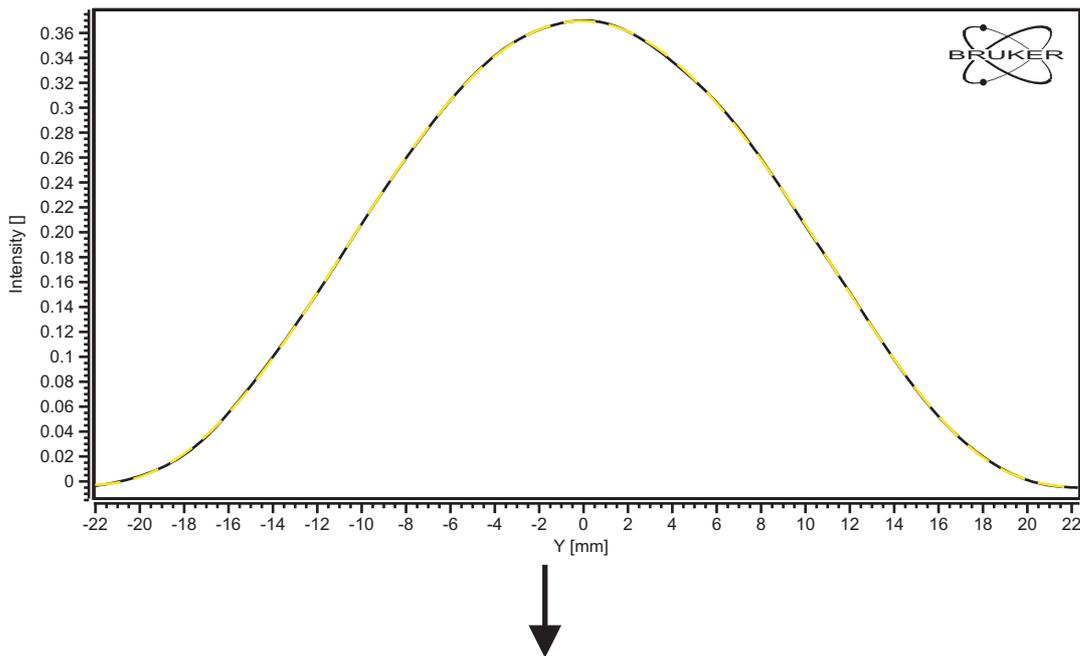


Figure 2-63 Measuring the Q-value in tune mode at 33 dB.

The spatial distribution $f(B_1, B_m)$ (See Figure 2-64.) and c (point sample calibration factor) have been characterized at the factory. An eighth order polynomial is fitted to the spatial distribution along the axis of the resonator. All of the other factors in Equation [2-16] are recorded by the software except for the electronic spin state of the sample, S .



Spectrometer Configuration

Field Misc. Signal Channel Resonator ENDOR

Edit and View Resonator Type

To Resonator: [] From Resonator: ER4119HS

Save Values To Resonator Load Values From Resonator

Resonator Properties

Freq. Empty [GHz]: 9.85

Freq. Dewar [GHz]: 9.30

Conversion Fctr.: 9.271e-09

Resonator Length [mm]: 40

Resonator Center [mm]: 62.5

AFC AC Gain: 60

AFC Modulation: 60

AFC DC Gain: 1.10

AFC Fine Adjustment: 0

AFC Time Constant: Medium Low

Polynom Coefficients

	X	Y	Z
0	0	0.99652	0
1	0	0.00737177	0
2	0	-0.00559614	0
3	0	-2.88221e-05	0
4	0	1.00404e-05	0
5	0	3.43695e-08	0
6	0	-5.0404e-09	0
7	0	-1.4783e-11	0
8	0	-1.29132e-12	0

Close Apply Reset Load Save Help

Figure 2-64 Fitting a ninth order polynomial to the intensity distribution along the axis of a resonator.

What remains to be done is to acquire an EPR signal with good sensitivity and enough baseline on either side of the signal to ensure a good background subtraction for the double integration. Poor background subtraction is often the limiting factor in the success of quantitative EPR. (See Figure 2-59.) Once integrated, clicking the **Calculate** button opens a dialog box asking for more information regarding the sample and resonator. The first is the sample diameter (not including the sample tube), the distance to the center of the sample from the top sample collet (62.5 mm for the ER 4119HS resonator when the sample is centered in the resonator), the length of the sample, and the electronic spin state.

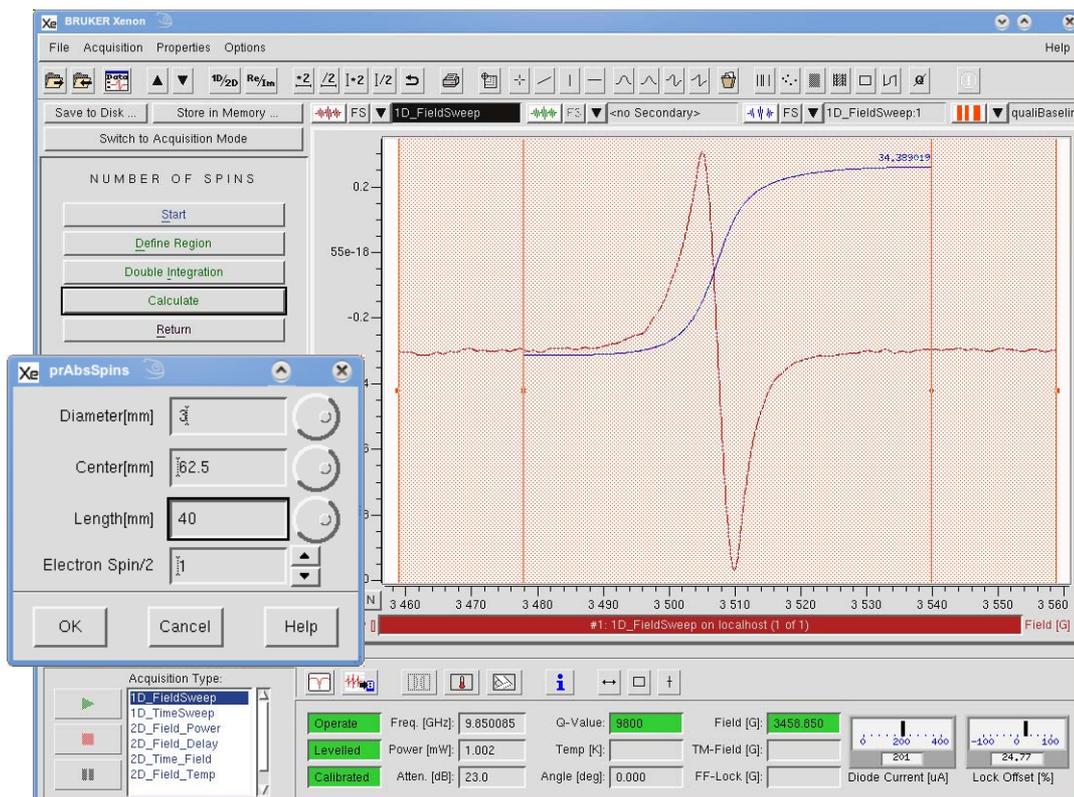


Figure 2-65 Calculating the number of spins and spin concentration.

Xenon then calculates the spin concentration in spins/mm³ and M as well as the total number of spins in the sample.

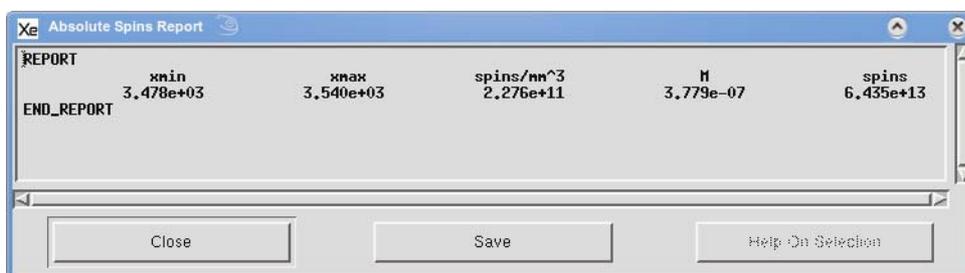


Figure 2-66 The results of a spin number and concentration calculation.

Suggested Reading

2.8

This chapter is a brief overview of the basic theory and practice of EPR spectroscopy. If you would like to learn more, there are many good books and articles that have been written on these subjects. We recommend the following:

Instrumentation: Poole, C. *Electron Spin Resonance a Comprehensive Treatise on Experimental Techniques, Editions 1,2*: Interscience Publishers, New York, (1967), (1983).

Feher, G. *Sensitivity Considerations in Microwave Paramagnetic Resonance Absorption Techniques*: Bell System Tech. J. 36, 449 (1957).

Theory: Knowles, P.F., D. Marsh and H.W.E. Rattle. *Magnetic Resonance of Biomolecules*: J. Wiley, New York, (1976).

Weil, John A., J.R. Bolton, and Wertz, J.E., *Electron Paramagnetic Resonance, Elementary Theory and Practical Applications*: Wiley-Interscience, New York, (1994).

A more extensive bibliography is found in last chapter of this manual.