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Quantitative EPR

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Foreword

There is a growing need in both industrial and academic research to obtain accurate quantitative results from continuous wave (CW) electron paramagnetic resonance (EPR) experiments. This book describes various sample-related, instrument-related and software-related aspects of obtaining quantitative results from EPR experiments. Some specific items to be discussed include: selection of a reference standard, resonator considerations (Q, B_1, B_m), power saturation, sample positioning, and finally, the blending of all the factors together to provide a calculation model for obtaining an accurate spin concentration of a sample.

This book might, at first glance, appear to be a step back from some of the more advanced pulsed methods discussed in recent EPR texts, but actually quantitative "routine CW EPR" is a challenging technique, and requires a thorough understanding of the spectrometer and the spin system. Quantitation of CW EPR can be subdivided into two main categories: (1) intensity and (2) magnetic field/microwave frequency measurement. Intensity is important for spin counting. Both relative intensity quantitation of EPR samples and their absolute spin concentration of samples are often of interest. This information is important for kinetics, mechanism elucidation, and commercial applications where EPR serves as a detection system for free radicals produced in an industrial process. It is also important for the study of magnetic properties. Magnetic field/microwave frequency is important for g and nuclear hyperfine coupling measurements that reflect the electronic structure of the radicals or metal ions.

The book contains sufficient background to make it useful to scientists who are new to the field, and who strive to obtain the best possible results from their spectrometers. Information is presented with sufficient rigor so that the book should also be valuable to experienced users who seek to give their work a solid quantitative basis. More detailed discussions and advanced topics are provided in the appendices. Suggestions for further reading guide the reader to additional aspects of EPR. The objective of the authors is to convey this topic to industrial and academic scientists at different stages of their careers.

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Contents

Chapter 1
Basics of Continuous Wave EPR

This chapter is an introduction to the basic theory, practice, and instrumentation for EPR spectroscopy. It is designed to give someone who is new to EPR sufficient background to understand the following chapters. A reader who is already well acquainted with the basics of EPR theory and instrumentation, may skip this chapter. Topics in several sections of this chapter are presented in greater detail in subsequent chapters. This introduction, and much of the book, focuses primarily on molecules with a single unpaired electron, which is denoted as having a spin state of $S = 1/2$.

1.1 The Zeeman Effect

The energy differences studied in EPR spectroscopy are due predominately to the interaction of an unpaired electron in the sample with a magnetic field, B_0, 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 it is placed in a magnetic field. Since energies are quantized, a single unpaired electron has only two allowed energy states. It has a state of lower energy when the moment of the electron, μ, is aligned with the magnetic field and a higher energy state when μ is aligned against the magnetic field (see Fig. 1.1). The two states are designated 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 has $m_s = -1/2$ and the antiparallel state has $m_s = +1/2$. (Note that because of the charge of the electron, the energies of the ±1/2 states are opposite to those for the proton.) The difference between the energies of these two states, caused by the interaction between the electron spin and the magnetic field, is shown in (1.1).

$$\Delta E = g\ \mu_B\, B_0\, \Delta m_s = g\ \mu_B B_0, \tag{1.1}$$

where g is the g-factor, which is discussed below, μ_B is the Bohr magneton, which is the natural unit of the electron's magnetic moment, and the change in spin state is

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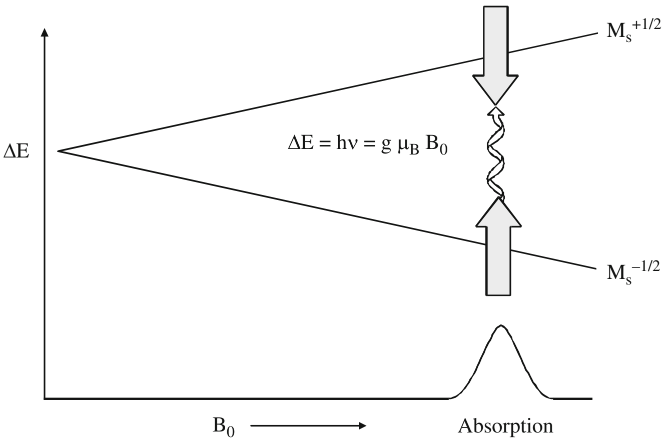

Fig. 1.1 When the magnetic field is scanned, the energies of the two spin states of an unpaired electron diverge. At the magnetic field for which the energy difference between the two electron spin states is equal to hυ for the spectrometer, there is absorption of energy by the spins, which is called resonance

$\Delta m_s = \pm 1$. The energy, $\Delta E = h\nu$, that is required to cause a transition between the two spin states is given by (1.2),

$$\Delta E = h\ \nu = g\ \ \mu_B B_0 \quad \text{and} \quad \mu_B = g_e\ \beta/2. \tag{1.2}$$

Two facts are apparent from (1.1) and (1.2) and its graph in Fig. 1.1.

- The two spin states have the same energy in the absence of a magnetic field, so without a magnetic field, there is no energy difference to measure.
- The energies of the spin states diverge linearly as the magnetic field increases and the energy difference depends linearly on the magnetic field.

Because the energy difference between the two spin states can be varied by changing the magnetic field strength, there are two potential approaches to obtaining spectra. The magnetic field could be held constant while the frequency of the electromagnetic radiation is scanned, which would be analogous to UV-VIS spectroscopy. Alternatively, the electromagnetic radiation frequency could be held constant while the magnetic field is scanned as shown in Fig. 1.1. Absorption of energy occurs when the magnetic field "tunes" the two spin states such that the energy difference matches the energy of the applied radiation. This field is called the "field for resonance". Because of difficulties in scanning microwave frequencies and because of the use of a resonant cavity for signal detection, most EPR spectrometers operate at constant microwave frequency and scan the magnetic field.

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

$$g = h\ \nu /\ \mu_B B_0 \tag{1.3}$$

Table 1.1 Field for resonance for a g = 2 sample at various microwave frequencies

Microwave band	Microwave frequency (GHz)	B_0 (for g = 2) gauss
L	1	390
S	3	1070
X	9	3380
K	24	8560
Q	35	12,480
W	94	33,600

is independent of the microwave frequency, so the g-factor is a better way to characterize signals. Note 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 commercial EPR spectrometers is presented in Table 1.1. Spectrometers are labeled with a letter as shown in the table that is based on the designation that engineers used for microwaves in that frequency range.

The g-factor helps to distinguish and identify types of samples. Carbon-centered radicals have g close to the “free electron value,” which is 2.0023. Heteroatoms shift the g-factor. For example benzosemiquinones, which have significant spin density on oxygens, have g $\sim$ 2.004 and nitroxide radicals which have spin density on a nitrogen and oxygen have g $\sim$ 2.006. Metal ions have very different g-factors. For example, the vanadyl ion has g $\sim$ 1.96 while the g-factor for Cu^{2+} may be as large as 2.3, depending on the geometry of the complex. In immobilized samples the anisotropy of g may be observed (i.e. it may depend on the orientation of the molecule with respect to B_0).

1.2 Hyperfine Interactions

Additional information about the species that contains the unpaired electron can be obtained from nuclear hyperfine interactions. The nuclei of the atoms in a molecule or complex often have magnetic moments, which produce a local magnetic field at the electron. The interaction between the electron and the nuclei is called the hyperfine interaction. It gives a wealth of information about the sample such as the identity and number of atoms that make up a molecule or complex; as well as the electron spin density at nuclei that have magnetic moments.

Figure 1.2 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, less magnetic field is needed from the 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 an electron spin interacting with a spin 1/2 nucleus such as hydrogen, the EPR absorption signal splits into two signals that are each B_I away from the original

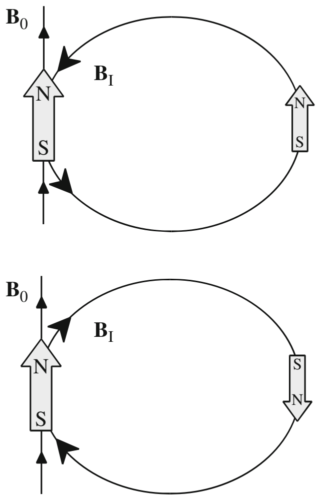

Fig. 1.2 Influence of the magnetic field of a nucleus on the field experienced by an electron spin

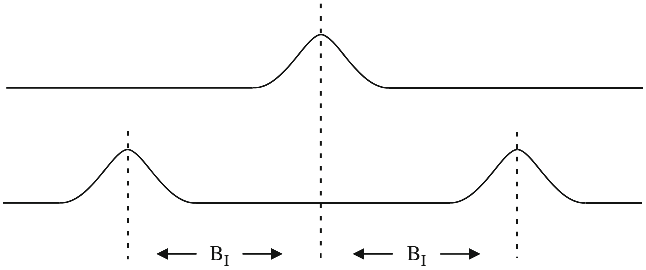

Fig. 1.3 Splitting of an EPR signal due to the local field of a neighboring spin = 1/2 nucleus

signal (see Fig. 1.3). The hyperfine splitting constant, a_H, is equal to the spacing between the two lines, which is $2B_I$.

If there is a second nucleus with $I = 1/2$, each of the signals is further split into a pair, resulting in four signals. For n spin 1/2 nuclei, there are 2^n EPR signals, provided that the splitting is large relative to the linewidth. The general rule is that the number of hyperfine lines is equal to $2nI + 1$, where n is the number of symmetry equivalent nuclei and I is the nuclear spin. Hyperfine splittings can be very useful in identifying radical species. For example, ^{14}N has $I = 1$, so coupling to one nitrogen gives three lines with equal intensity and Mn has $I = 5/2$, so coupling to one manganese ion gives six hyperfine lines. As the number of nuclei gets larger, the number of signals increases rapidly. Sometimes there are so many signals that they overlap and a smaller number of broad signals is observed. The magnitude of B_I, and hence the splitting, depends on the type of nucleus, as well as the electron spin density at that nucleus.

1.3 Signal Intensity

So far, only the position of the EPR signal has been discussed. However, the size of the EPR signal is what is important for measurement of the concentration of the EPR active species in the 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 Fig. 1.4). The integrated intensity of an EPR signal is proportional to the concentration of unpaired electrons in the sample, provided that appropriate operating conditions are used.

Signal intensities do not depend solely on concentrations. They also depend on the microwave power. If low enough microwave power is used, the signal intensity grows as the square root of the power. At higher power levels, the signal grows more slowly, or even diminishes, as well as broadens with increasing microwave power levels. This behavior is called saturation. To measure accurate intensities, linewidths, lineshapes, and closely spaced hyperfine splittings, saturation must be avoided. A quick means of checking for the absence of saturation is to decrease the microwave power and verify that the signal intensity decreases proportional to the square root of the microwave power (for more information on power saturation see Chap. 4.11).

1.4 Introduction to Typical CW EPR Spectrometers

The first half of this chapter provided an introduction to the principles of EPR spectroscopy. Now it is important to consider practical aspects of EPR spectroscopy. Theory and practice have 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 and Gorter had searched for magnetic resonance with a less sensitive method, but the first direct detection of EPR had to wait for improvements in instrumentation. The development of radar during World War II provided technology for rapid development of applications of EPR. Only then, did scientists have the necessary components to build sufficiently sensitive spectrometers. Even today, the availability of improved electronic components has made possible the continued development of advanced techniques in EPR such as Fourier Transform and high frequency EPR. The history of the development of EPR is described in (Eaton et al. 1998).

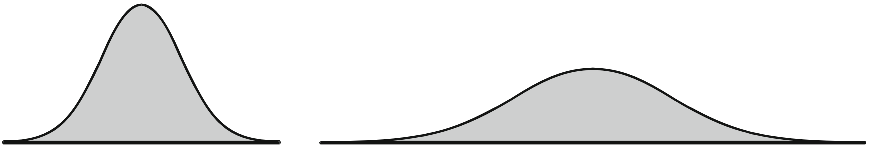

Fig. 1.4 The integrated intensity of the two absorption signals is the same

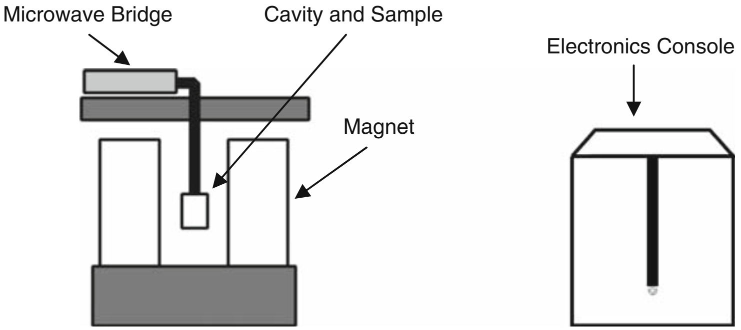

Fig. 1.5 General outlay for an EPR spectrometer

Figure 1.5 shows the general layout of a typical CW EPR spectrometer. The electromagnetic radiation source and the detector are in a box called the “microwave bridge.” The sample is in a cavity, which is a metal box that helps to amplify weak signals from the sample. As mentioned in Sect. 1.1, there is a magnet to “tune” the electronic energy levels. In addition, there is a console, which contains signal processing and control electronics and a computer. The computer is used for analyzing data as well as coordinating all the units for acquiring a spectrum. The following sections explain how these parts of the spectrometer function and interact.

1.5 The Microwave Bridge

The microwave bridge houses the microwave source and the detector. There are more parts in a bridge than shown in Fig. 1.6, but most of them are control, power supply, and security electronics that are not necessary to understand the basic operation of the bridge. The following discussion traces the path of the microwaves from the source to the detector.

The tour of the microwave bridge starts at point A, the microwave source. In older spectrometers the source was a vacuum tube called a klystron. Newer spectrometers use a solid state source called a Gunn diode. The output power of the microwave source cannot be varied easily. However the discussion of signal intensity stresses the importance of changing the power level to avoid saturation. Therefore, the next component, at point B, after the microwave source is a variable attenuator: a device that controls the flow of microwave radiation. With the attenuator, the microwave power that gets to the sample can be precisely and accurately controlled.

Most EPR spectrometers are reflection spectrometers. That is, 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). Therefore the detector should only see the microwave radiation coming back from the cavity.

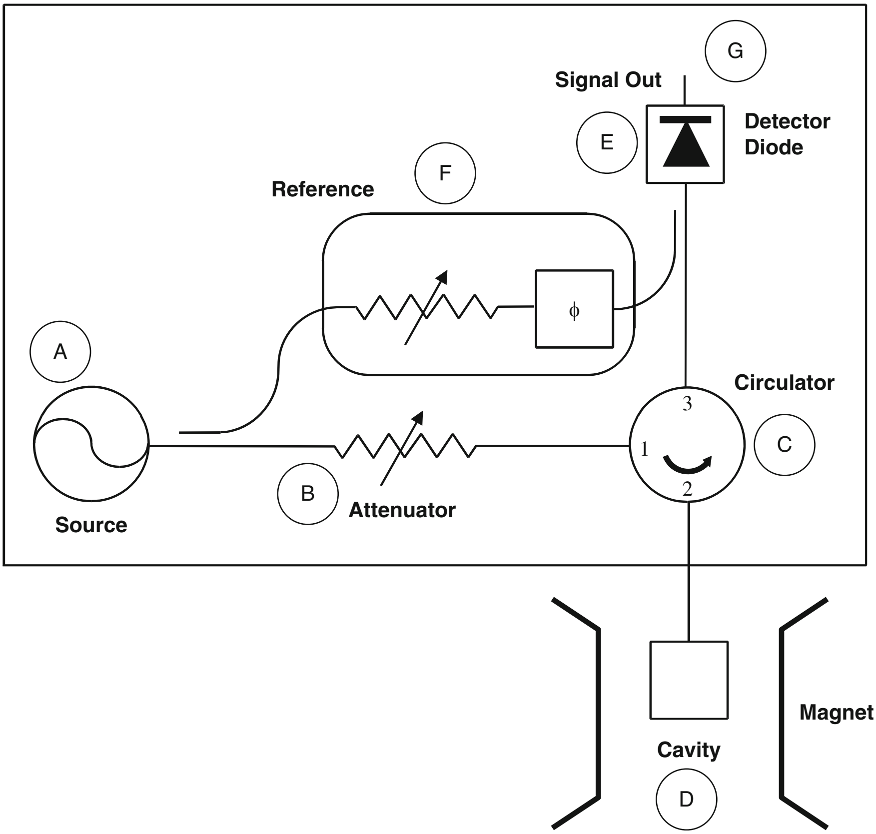

Fig. 1.6 Block diagram for a microwave bridge

The circulator at point C is a microwave device that makes this possible. Microwaves coming in port 1 of the circulator only go to the cavity through port 2 and not to the detector, which is attached to port 3. Reflected microwaves are directed only to the detector (port 3) and not back to the microwave source.

Bruker spectrometers use a Schottky barrier diode to detect the reflected microwaves (point E in Fig. 1.6). The diode converts the microwave power to an electrical current. At low power levels (less than 1 μW), 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 mW), 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 μA. To insure that the detector operates at that level, there is a reference arm (point F in Fig. 1.6) that supplies the detector with 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 slowly lose their sensitivity. To prevent this from happening, there is protection circuitry in the bridge that monitors the current from the diode. When the current exceeds 400 μA, 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 as described later in this book and not rely on the protection circuitry.

1.6 The EPR Cavity

This section discusses the properties of microwave (EPR) cavities and how changes in these properties due to absorption result in an EPR signal. Resonators are used to amplify weak signals from the sample. There is no loss of generality if descriptions are based on a microwave cavity, which is simply a metal box with a rectangular or cylindrical shape that resonates with microwaves much as an organ pipe resonates with sound waves. Many other resonant devices have been used in EPR spectrometers (Poole 1967). Resonance means that the cavity stores the microwave energy. Therefore, at the resonance frequency of the cavity, microwaves remain inside the cavity and are not reflected back (see Fig. 1.7).

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:

$$Q = \frac{2\pi(\textit{energy stored})}{\text{energy dissipated per cycle}}, \tag{1.4}$$

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. Q factors can be measured easily because there is another way of expressing Q:

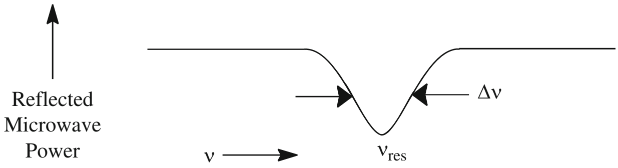

Fig. 1.7 Reflected microwave power from a resonant cavity