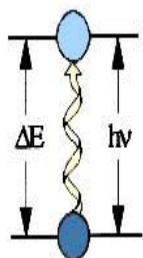


Basic EPR Theory

This section is an introduction to the basic theory of continuous wave EPR spectroscopy. It gives you sufficient understanding of the properties which Bruker's EPR spectrometers read and translate into statistical data.

Introduction to spectroscopy

During the early part of this 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 electro-magnetic radiation.

According to Planck's law, electromagnetic radiation

will be absorbed if: $E = h\nu$, where h is Planck's constant and ν is the frequency of the radiation. The absorption of energy causes a transition from the lower energy state to the higher energy state. 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. 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.

The Zeeman Effect

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. 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 $M_s = +1/2$.

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

$$E = g\mu_B B_0 M_s = \pm \frac{1}{2} g\mu_B B_0 \text{ and } \Delta E = h\nu = g\mu_B B_0$$

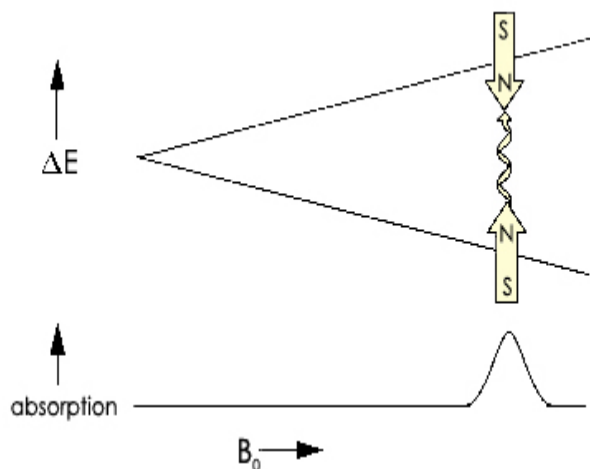
g is the g -factor, which is a proportionality constant approximately equal to 2 for most samples, but which varies depending on the electronic configuration of the radical or ion. μ_B is the Bohr magneton, which is the natural unit of electronic magnetic moment. Two facts are apparent from the above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

- * 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.

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. 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.



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, $g = hv / (\mu_B B_0)$, 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.

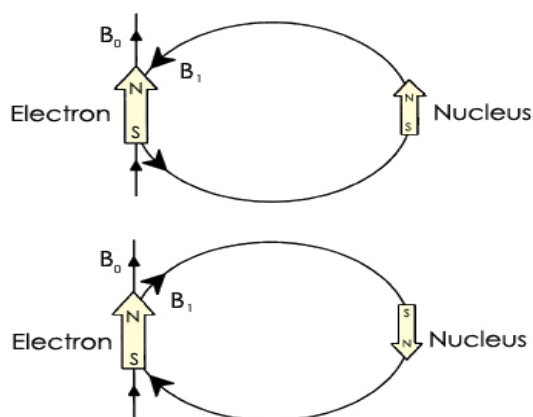
A list of fields for resonance for a $g = 2$ signal at microwave frequencies commonly available in EPR spectrometers is presented below: Field for resonance, B_{res} , for a $g = 2$ signal at selected microwave frequencies.

Microwave Band	Frequency (GHz)	B_{res} (G)
L	1.1	392
S	3.0	1070
X	9.75	3480
Q	34.0	12000
W	94.0	34000

Hyperfine interactions

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 that make up a molecule or complex as well as their distances from the unpaired electron.

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_1 . 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_1 adds to the magnetic field, we need less magnetic field from our laboratory magnet and therefore the field for resonance is lowered by B_1 . The opposite is true when B_1 opposes the laboratory field.



For a spin $1/2$ nucleus such as a hydrogen nucleus, we observe that our single EPR absorption signal splits into two signals which are each B_1 away from the original signal. If there is a second nucleus, each of the signals is further split into a pair, resulting in four signals. For N spin $1/2$ nuclei, we will generally observe $2N$ 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 the one broad signal.

Signal intensity

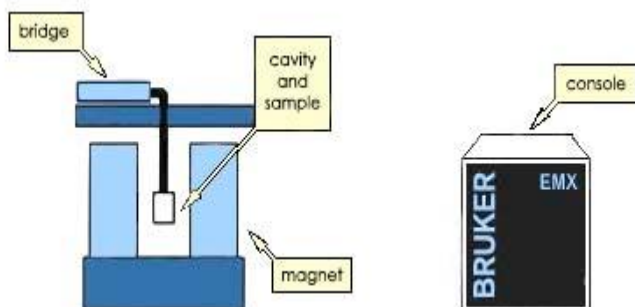
So far, we have concerned ourselves with where the EPR signal is, but the size of the EPR signal is also important if we want to measure the concentration of the EPR active species in our sample. In the language of spectroscopy, the size of a signal is defined as the integrated intensity, i.e., the area beneath the absorption curve. The integrated intensity of an EPR signal is proportional to the concentration. 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, line shapes, and closely spaced hyperfine splitting, 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.

Experimental EPR

In the previous section, we discussed the theory of continuous wave 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.

Spectrometers

The simplest possible spectrometer has three essential components: a source of electromagnetic radiation, a sample, and a detector. 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 a



block diagram. The electromagnetic radiation source and the detector are in a box called the **microwave bridge**. The sample is in a microwave cavity, which is a metal box that helps to amplify weak signals from the sample. As mentioned in the epr theory section, there is a magnet to **tune** the electronic energy levels. In addition, we have 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.

Useful Links/information

US Research groups

Advanced Magnetic Resonance of Metalloproteins: EPR and ENDOR research group, Chemistry Department, Northwestern University, Evanston,
Biomedical EPR facility, Department of Biological Sciences, University of Essex, Essex,
Britt Research Group, University of California, Davis,
The Brudvig Lab, Department of Chemistry, Yale University, New Haven,
The Cafiso Lab Group, Department of Chemistry, University of Virginia, Charlottesville,
EPR Lab, Department of Chemistry and Biochemistry, Miami University, Ohio,
EPR Center, Biomedical Technology Research Center, Dartmouth College, Hanover,
EPR Facility, Department of Chemistry, University of Arizona, Tucson,
EPR Groups, National Institute of Environmental Health Sciences (NIEHS)/ National Institute of Health (NIH), Research Triangle Park, North Carolina,
Center for Ecogenetics and Environmental Health (CEEH), University of Washington, Washington,
Free Radical Research Center, Biophysics Research Institute, Medical College of Wisconsin, Milwaukee,
National Biomedical EPR Center, Biophysics Research Institute, Medical College of Wisconsin, Milwaukee,
Home page of Electron Magnetic Resonance Laboratory, Department of Chemistry, University of Houston, Houston,
The Illinois EPR Research Center (IERC), University of Illinois, Urbana - Champaign, Illinois,
Minnesota Muscle Laboratory, Department of Biochemistry, Molecular Biology and Biophysics, University of Minnesota, Minneapolis,
National High Magnetic Field Laboratory (NHFML), (second page) Florida State University, Tallahassee,
EPR Spectroscopy, National High Magnetic Field Laboratory (NHFML), Florida State University, Tallahassee,
NMR Spectroscopy and Imaging Program, National High Magnetic Field Laboratory (NHFML), Florida State University, Tallahassee,
Advanced ESR Technology Research Center (ACERT), Cornell University, Department of Physiology and Biophysics, Albert Einstein College of Medicine, New York,
Department of Chemistry, Illinois State University,
Jack H. Freed, Physical Chemistry, Cornell University
Lawrence J. Berliner's Home Page, University of Denver, Denver,

Home page of Prof. Gareth R. Eaton, Department of Chemistry and Biochemistry, University of Denver, Denver,
Home page of Prof. Sandra S. Eaton, Department of Chemistry and Biochemistry, University of Denver, Denver,
The Peroxynitrite Page,
Professor Britt's Research Group, University of California, Berkeley,
The Shin Group, Department of Chemistry Faculty, University of California, Berkeley,
Department of Chemistry and Biochemistry, Montana State University, Bozeman,
Biophysics - prof. William Berhard, University of Rochester, New York, Imaging Science Faculty, Carlson Center for Imaging Science, dr Joseph P. Hornak, Rochester Institute of Technology, New York,
EPR Group of dr Roger Lloyd, Department of Chemistry, University of Memphis,
The Biophysics Research Group, East Tennessee State University, Alvin L. Kwiram, Department of Chemistry, University of Washington, Dr LoBrutto's Research, Arizona State University,
The EPR Laboratory, Biophysics Laboratory, Department of Physics and Astronomy, California State University, Northridge,
EPR Laboratory, Department of Physics and Astronomy, University of North Carolina, Chapel Hill,
EPR Laboratory, National Institute of Standards and Technology (NIST), Gaithersburg, MD,
NMR Laboratory, Department of Chemistry, The University of Chicago, Chicago, IL,
High Field Magnetic Resonance Facility, The William R. Wiley Environmental Molecular Sciences Laboratory (EMSL), U.S. Department of Energy national scientific user facility, Pacific Northwest National Laboratory, Richland, Washington,
TAMU NMR Facility, Department of Chemistry, Texas A&M University, Texas,
Department of Chemistry, MIT,
Department of Chemistry, Northwestern University, Evanston, IL,
Biophysics Research Program, Department of Physics, Astronomy and Geology, East Tennessee State University,

• **Societies/Organizations**

Society for Free Radical Research (SFRR)
The Federation of European Biochemical Societies
Asia-Pacific EPR/ESR Society
International Zeolite Association (IZA)
The Society of Biological Inorganic Chemistry
Groupement Ampere
The Nordic EPR Society (NES)

Berkeley Spectroscopy Club

Gruppo Italiano di Risonanza di Spin Elettronico (GIRSE), Italy

The International EPR(ESR) Society

International Society of Magnetic Resonance (ISMAR)

International Society for Magnetic Resonance in Medicine

Magnetic Resonance Discussion Group of the German Chemical Society

Society for Free Radical Biology and Medicine

Oxygen Club of California

SENTINEL

• **Software**

Xemr program home page, EPR Server at University of Jyvaskyla, Finland

World-Wide-Web site for the program EPR-NMR

Scientific Software Services: EPRWare, EWWIN, EWPlot, EWSIM,

EWVoigt, EWImage, EPR software

Dr John Boswell's EPR Simulation Program (Igor Pro),

• **Scientific Databases**

The RCSB Protein Data Bank

Swiss PDB Viewer Deep View

MDL-ISIS Draw

WebSPIRS

The EPR Newsletter

LIPIDAT Database

NDRL Radiation Chemistry Data Center

• **Further reading**

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).

Commercial sources of EPR and related supplies

Magnettech GmbH

E-I-A GmbH

Institute of Solid State Physics, Russian Academy of Science, Chernogolovka, Russia

Bruker WWW Server (USA)

KyoSpin: High Frequency EPR Instruments

Oxford Instruments
Rototec-Spintec
Cambridge Isotope Laboratories, Inc.
Cryomagnetics Inc.
JEOL USA, Inc.
Millimeter-Wave Oscillator Company (MMWOC)
Resonance Instruments, Inc.
Varian (USA)
Wilmad
Summit Technology