This article is about the experimental application of Electron Paramagnetic Resonance spectroscopy (EPR). For theoretical background on EPR, see alternate article EPR: Theory. The most basic application of Electron Paramagnetic Resonance spectroscopy requires the use of a microwave radiation source, an electromagnet, a resonator, and a detector.

Background

The experimental procedure is to place a paramagnetic sample in the resonator, which is designed to resonate at a specific microwave frequency. The resonator is positioned between two electromagnets, which vary in magnetic field strength depending on how much current is run through them. Electromagnetic radiation in the microwave frequency range is produced using a klystron and channeled into the resonator. While the microwave radiation is kept at a constant frequency, the strength of the applied magnetic field will be swept. Resonance is detected by a decrease in the amount of microwave radiation that is being reflected out of the resonator. Reflected microwaves are channeled specifically into the detector by a special component called a circulator. Resonance will be detected at the field strength that corresponds to the energy splitting of the spin states of the unpaired electron.

Figure 1: Basic experimental setup of an EPR spectrometer.

These are the most basic elements in an experimental setup for the observation of electron paramagnetic resonance. This article first elaborates on each of these basic elements of EPR Instrumentation and introduces other advanced elements necessary for collecting more sophisticated spectra, which are used to characterize an unpaired electron and its the environment. The experimental applications of this spectroscopy are then briefly reviewed.
Instrumentation

History of EPR

EPR was first detected in 1945 by Yevgeny Zaboisky at Kazan State University in the Soviet Union. The instrumentation required for EPR was greatly benefited by the development of RADAR during WWII, which required the use of reliable and tunable microwave sources. After the war, the necessary components for an EPR spectrometer were cheap and available. Initially, the field was advanced with the production of home-built EPR spectrometers. In the 1980s, commercially built EPR spectrometers became available. The German company Bruker leads the field in the production of commercially available EPR spectrometers, as well as NMR spectrometers and MRI detectors. Today, EPR spectroscopy is done at a wide range of frequencies and fields.

Microwave radiation source

A microwave radiation source is necessary for any EPR spectrometer, because this the energy that corresponds with a detectable splitting of the electronic spin states. Microwave radiation is typically produced and amplified by a klystron, which is capable of tuning waves to a precise frequency, amplitude, and phase. The microwaves are then channeled into the resonant cavity by use of either waveguides or coaxial cables. Waveguides are the most common method of microwave propagation. Waveguides are essentially open air, brass rectangular channels with dimensions that correspond to the wavelength of the radiation that is to be propagated. However, for low power, low frequency microwave radiation, like that often used in EPR, special coaxial cables have been found to be just as effective.

In order to observe resonance, the frequency of the microwaves must correspond to the splitting of the spin states of the electron, which is determined by the strength of the magnetic field. Thus, the strength of the magnetic field required is dependent on the frequency of radiation used, and vise versa. In addition, the dimensions of the waveguides and the resonator are specific to the microwave frequency. In this light, an experimental setup requires a corresponding combination of microwave energy, magnetic field strength, and resonator/waveguide dimensions. Experimental setups are classified by the frequency of microwave energy utilized, by the table below:

Table 1: Letter designations of various microwave frequency ranges.

<table>
<thead>
<tr>
<th>Letter Designation</th>
<th>Frequency Range</th>
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<tbody>
<tr>
<td>L Band</td>
<td>1-2 GHz</td>
</tr>
<tr>
<td>S Band</td>
<td>2-4 GHz</td>
</tr>
<tr>
<td>C Band</td>
<td>4-8 GHz</td>
</tr>
<tr>
<td>X Band</td>
<td>8-12 GHz</td>
</tr>
<tr>
<td>Ku Band</td>
<td>12-18 GHz</td>
</tr>
<tr>
<td>K Band</td>
<td>18-26.5 GHz</td>
</tr>
</tbody>
</table>
This naming system is a vestigial organ of WW2 RADAR use. The letters assigned to a given range are random and were used as code to prevent the axis powers from knowing which frequency was being used to detect incoming bombers.

The first observation of electron paramagnetic resonance was seen at S-band. The majority of EPR spectrometers operate at X-band, a mid-range frequency, due to the fact that equipment from this range was most easily available after WW2. In addition, this is a suitable range to observe many EPR transitions and does not require and exceptionally high magnetic field strength. However, in the past few decades, spectrometers operating at higher frequency ranges have become widely used, thus requiring greater applied field strengths. Different energy level experimental setups allow the observation of different transitions that are hidden at one applied field strength, but clearly visible at another. This is because at a higher magnetic field strength, the spin states will be split further, spreading apart transitions that are close in energy.

<table>
<thead>
<tr>
<th>Band</th>
<th>Frequency Range</th>
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<tbody>
<tr>
<td>Ka</td>
<td>26.5-40 GHz</td>
</tr>
<tr>
<td>Q</td>
<td>30-50 GHz</td>
</tr>
<tr>
<td>U</td>
<td>40-60 GHz</td>
</tr>
<tr>
<td>V</td>
<td>50-75 GHz</td>
</tr>
<tr>
<td>E</td>
<td>60-90 GHz</td>
</tr>
<tr>
<td>W</td>
<td>75-110 GHz</td>
</tr>
<tr>
<td>F</td>
<td>90-140 GHz</td>
</tr>
<tr>
<td>D</td>
<td>110-170 GHz</td>
</tr>
</tbody>
</table>

**Resonator**

A resonant cavity is the most common form of resonator used in EPR spectroscopy. The resonant cavity of an EPR spectrometer is designed with dimensions corresponding to the specific wavelength of microwave radiation that is to be used. For example, at X-band frequencies (~9.5 GHz), the wavelength of the electromagnetic wave is ~3 cm. In this case, the resonant cavity has dimensions of ~3 cm. This allows the microwaves to resonate in the cavity. In a resonant cavity, the level of microwave energy is 1000s of times greater than that in the waveguide. The sample is located in the cavity at a location that allows maximum absorption of magnetic energy from the microwaves, and minimum absorption of electric energy. This is because it is the magnetic field component of the microwave energy that excites and EPR transition, while the electric field component will be absorbed in a non-resonant manner. Typically, the sample is placed in a central location in the cavity, where it will absorb the greatest level of energy from the magnetic field of the microwaves.
To fine-tune the range and level of resonating microwaves, different procedures are used. The most common technique is by controlling the amount of microwaves allowed to enter the cavity with an iris screw. The iris is the hole by which the microwaves enter the cavity and a screw is used to precisely vary the size of this whole. "Tuning" the microwaves to resonate in the cavity must be done prior to each experiment. This is done by first finding the frequency that results in the least amount of reflection out of the cavity, and then fine-tuning the cavity to the optimum range or resonance desired for the experiment. The frequency and coupling required will change depending on sample and its position (this changes the dimensions of the cavity), and hence, must be done whenever either of these parameters is changed.

An optional characteristic of a resonance cavity is the ability to operate in either "parallel mode," or "perpendicular mode." In this case, the dimensions of the cavity will vary just slightly, so that a slightly different frequency of radiation will resonate parallel to the magnetic field as compared with that which resonates parallel to the magnetic field. Hence, the sample will absorb energy from the electromagnetic radiation either parallel or perpendicular to its induced magnetic dipole moment. The purpose of this component is not to change the frequency of radiation, but to change the direction that the electromagnetic radiation approaches the sample. This changes the number and nature of transitions that are allowed by quantum mechanical rules. These different transitions produce spectra that can provide different structural insights into the electronic nature of the molecule. Typically, a spectrometer operates in perpendicular mode.

Figure 2: An X-band resonant cavity with waveguide attached. Note the dimensions of ~3 cm.
Figure 3: the resonator of a high field EPR spectrometer.

This is a custom built resonator designed to resonate microwaves at a frequency of 130 GHz. This corresponds to a wavelength of ~2mm. Thus, the size of the cavity in this resonator has those dimensions. The probe that this resonator is attached to has coaxial cables to propagate radio frequency waves for ENDOR experiments (see optional section below), as well as a fiber optic cable to illuminate light-sensitive samples and observe changes in their spectra. When in the spectrometer, then resonant cavity sits directly on top of a waveguide, which propagates the microwaves into the cavity. This resonator has specially designed components that allow one to change the dimensions and orientation of the cavity, in order to tune the cavity to resonate.

Detection Methods

Various detection methods exist for EPR spectroscopy. The majority of EPR spectrometers are reflection spectrometers, meaning they measure the amount of radiation that is reflected back out of the resonator. Changes in the level of reflected microwave energy at various field strengths allow the observation of spectroscopic transitions. A special channeling device called a "circulator" is used to insure that radiation from the microwave source is sent only into the cavity and that reflected radiation is sent only to the detector. A diode is used to measure the power of reflected energy. To achieve an accurate quantitative measurement of absorbed microwave energy, the diode must sometimes be "biased," or supplied with a steady level of current which insures that the diode is reading in the correct linear range.

CW spectrometers (those which have been described up to this point) make use of a "field modulator," which creates an oscillating magnetic field around the sample, in addition to the applied magnetic field created by the electromagnet. This serves to fix the range of electromagnetic energy that reaches the detector, increasing resolution. Because the absorption of microwave energy is measured relative to this the modulating field, CW EPR spectra show up as a derivative line shape relative to actual energy absorption.

Electromagnet

An electromagnet is used in an EPR experiment because it allows one to vary the strength of the applied magnetic field precisely, quickly, and consistently. As mentioned previously, in any experimental setup, the strength of the applied
magnetic field must correspond to the frequency of the electromagnetic radiation being applied to a sample. For example, if the experiment is being performed at X-band (~9.5 GHz), the field strength should be swept from a range of 0-4000 gauss (or 0-0.4 Tesla) in order to see all transitions. At higher frequencies, the field strength must be higher to see the same frequencies, because the degenerate spin states are be split more.

In some experiments, the magnetic field is kept constant for continuous measurement of a single transition. In spin labeling experiments, where the paramagnetic species are bound to specific positions on a molecule (usually, a protein), the field strength of the electromagnet only needs to be swept a couple hundred Gauss. This small sweep range makes EP spectrometers specially designed for spin labeling experiments much smaller than a traditional EPR spectrometer. Spin-label specific EPR spectrometers can sit on a lab bench, while a typical EPR spectrometer will take up a good 25 square feet of lab space including computer and power source.

As the field is swept, a significant amount of current is run through the electromagnetic coils. This creates a large amount of heat. For this reason, an EPR spectrometer must have some kind of cooling apparatus to keep it from getting hot. This is usually accomplished by continuously flowing water around the electromagnet.

![Figure 4: Ka-band EPR spectrometer.](image)

The two copper-colored discs in the Ka-band spectrometer pictured in figure 4 are the electromagnet. The sample cavity sits directly in the center of these two discs, as the center of the magnetic field. This is the conventional design for the applied magnetic field of an EPR spectrometer. The pictured magnet can be swept from 0-1.5 Tesla, allowing observation of EPR transitions excited with 31 GHz microwave energy.
Figure 5: D-band spectrometer.

Figure 5 pictures an alternate experimental setup for the applied magnetic field of an EPR spectrometer. This instrument utilizes a superconducting electromagnet that is kept at a constant temperature of ~4 Kelvin by a closed-loop liquid helium pump. This setup contains 2 magnetic coils, and can sweep an applied field of 0-8 Tesla. Powerful superconducting magnets like this are necessary to split paramagnetic spin states when high frequency microwaves are to be used to excite the transitions. This instrument operates at D-band frequencies of 130 GHz.

Optional Components

Though optional, the following components are commonly used in EPR experimental setups:

1. A cryogenic cooling device is used to cool samples down to ultra-cold temperatures. This leads to a greater population difference in the split spin states, increasing the signal level. Many transitions can only be seen at ultra-cold temperatures, especially in biological samples. A temperature as low as 4 Kelvin is often necessary to gain a resolved spectrum. Liquid helium must be used to maintain these ultra-cold temperatures. Two types of cryogenic cooling setups are common: A) A reservoir dewar, which maintain a constant level of liquid helium and/or liquid nitrogen surrounding the sample, or B) A continuously flowing liquid helium pump, which continuously pumps liquid helium into the space surrounding the sample cavity. The latter is the setup commonly utilized on commercially available spectrometers. Naturally, these setups require the use of high-powered vacuum pumps to evacuate the areas insulating the lines and reservoirs that transfer and contain the cryogen.

2. Most EPR experimental setups do not only operate in the "continuous wave" (CW) format described above, during which the sample is continuously bathed in microwave energy. Instead, advanced EPR experiments utilize pulsed microwave energy to selectively excite different transitions of a paramagnetic sample. These experiments have the same basic components as previously described, but require different amplifying and detecting equipment specifically designed for pulse creation and detection. Experiments that utilize pulse microwaves include Electron Spin Echo Envelope Modulation (ESEEM) and Hyperfine Sublevel Correlation Spectroscopy (HYSCORE).

3. Many EPR experimental setups contain a radio frequency amplifier, which is used to excite transitions between the
spin states of magnetic moments of nuclei surrounding the paramagnetic nuclei. These advanced EPR experiments are called Electron Nuclear Double Resonance (ENDOR) experiments, and provide valuable information about nuclei in the environment surrounding an unpaired electron.

CalEPR Center

The CalEPR center on campus contains 5 EPR experimental setups, located in the basement of the Chemistry building. These were assembled and are maintained and used by the lab Dr. David R. Britt. The range of frequencies and the overall experimental capability makes the CalEPR center a truly world-class EPR lab. Of the 5 spectrometers, 3 are home-built by Dr. Britt and his graduate students, and 2 are commercially built by Bruker.

![Figure 6: X-band CW spectrometer.](image)

Figure 6 pictures the ECS-106, a Bruker instrument. This is an X-band instrument configured only for CW EPR at ~9.5 GHz. The resonant cavity of this instrument is capable of performing in parallel or perpendicular mode. The sample cavity of this instrument is cooled with continuous flowing liquid helium, pumped straight from the helium dewar.

The power supply is sitting to the left of the instrument, which can be identified by the large circular electromagnets. To the right of the instrument is the console, which contains the necessary electrical components. Above the magnet is the cryostat and bridge, which contains important components like the microwave amplifier, attenuators, and the detector.
Figure 7: Home-built pulsed X-band spectrometer.

Figure 7 pictures the oldest instrument in the lab, a home-built spectrometer capable of a wide frequency range (8-18 GHz). It is configured for pulsed EPR spectroscopy, including ESEEM and ENDOR. The sample of cavity of this spectrometer is cooled with a reservoir dewar which must be filled with liquid helium and/or nitrogen. This instrument is currently not in use because it is going through some necessary upgrades to increase its speed (as of March 2009).

Figure 8: X-band and Q-band CW and pulse spectrometer.

Figure 8 pictures the other commercially built spectrometer, the E560 ELEXYS EPR system built by Bruker. This spectrometer is capable of doing CW and pulsed EPR at both X-band (~9.5 GHz) and Q-band frequencies (~35 GHz). The sample cavity of this instrument is cooled with continuous flowing liquid helium, pumped straight from the helium
The line to the helium dewar flowing into the sample cavity can be seen in this picture.

Figure 9: Home-built pulse Ka-band spectrometer.

Figure 9 pictures the home-built Ka-Band spectrometer, which performs experiments at ~31 GHz using a 100 W traveling wave tube amplifier. This spectrometer is configured for pulsed EPR spectroscopy, and has primarily been used for ESEEM studies. The sample of cavity of this spectrometer is cooled with a reservoir dewar which must be filled with liquid helium and/or nitrogen. This spectrometer was built by current Britt lab group member Michelle Dicus and former Britt lab group member Gregory Yeagle.

Figure 10: Home-built pulse D-band spectrometer.

Figure 10 pictures the home-built pulsed D-band spectrometer, which performs high-field pulsed EPR experiments at ~130 GHz. It utilizes a superconducting electromagnet to produce fields of up to 8 Tesla. The magnet is constantly kept at ~4 Kelvin by a closed helium loop pump system. This spectrometer is configured for ESEEM and ENDOR studies. This spectrometer was built and is maintained by current Britt lab group member Alex Gunn.
Experimental Applications

EPR spectroscopy can be applied to any sample that contains a paramagnetic electron. This includes a range of samples from basic organic and inorganic compounds to complex biomolecules such as proteins. Spectra are used to identify molecules within a sample and more importantly, to characterize the environment of the unpaired electron. EPR spectroscopy has advantages in that it is very sensitive (1000x more sensitive than NMR) and in that it has very good specificity (it looks only at the region that contains the unpaired electron). These characteristics make EPR especially useful for the study of metallic cofactor-containing proteins, which perform the most important enzymatic reactions on the planet (photosynthesis, nitrogenase, etc.). How substrates bind to the metallic cofactors in these enzymes can be elucidated best using EPR.

CW EPR

Continuous Wave EPR (CW) is an experiment during which a sample is continuously illuminated with microwave radiation at a fixed frequency and the strength of the applied magnetic field is swept, observing changes in microwave absorption. CW EPR is the most basic experiment performed. The instrumentation section of this article primarily describes the instrumental setup utilized to obtain CW spectra. Figure 11 displays a sample CW spectrum taken at X-band. This is a sample of isolated photosystem II, a complex of proteins involved in photosynthesis. Note that the X-axis displays magnetic field strength in Gauss. Also note that the spectrum displays the derivative of absorption of microwaves. The Y-axis displays the derivative of units of absorption in arbitrary units.

![Figure 11: Sample CW EPR spectrum](image)

Each peak of this spectrum represents a transition between the spin states of the unpaired electron at a specific energy. In a system as complex as PSII, each transition corresponds to an entirely different molecule, be it a metallic cofactor or a biomolecular cofactor such as chlorophyll. This spectrum is interpreted in light of theory which describes how the unpaired electron's spin states are affected by its environment, most importantly, coupling to the surrounding nuclei. A thorough interpretation of this spectrum would require comparisons with spectra of similar samples. Such an analysis can be used to determine precisely the orientation of the surrounding nuclei and the unpaired spin density on each.
Pulsed EPR

Pulse EPR varies from CW in that microwaves are applied to the sample as a series of nanoseconds-long pulses. The magnetic field strength is kept constant, and the sample is pulsed with microwave energy. The strategic pattern of pulse length and spacing allows the creation of a spin echo. The parameters of the pulse pattern can be changed and utilized in such a way that the hidden features of a transition observed at a specific field strength in a CW EPR spectrum can be observed. For example, figure 12 presents an ESEEM (electron spin echo envelope modulation) spectrum taken on the same PSII sample see in figure 11. The ESEEM spectrum is taken at the field strength corresponding to the largest (in amplitude) transition in figure 11 (~3450 G).

![Figure 12: This sample ESEEM spectrum demonstrates the advanced capabilities of pulse EPR.](image)

ESEEM spectra provide direct information about the frequencies that make up the less energetic transitions of an EPR transition. While CW EPR more bluntly shows the transition between one spin state to another. Pulse EPR spectra have the capability of showing the transitions that occur within a spin state, which are caused by the interaction of the unpaired electron with the surrounding nuclei. The peaks seen in figure 12 correspond to specific quantitative parameters that describe the coupling of the electron and the nucleus, thus corresponding to the distance between the nuclei and the electron. In short, pulse EPR allows the observation of transitions within the transitions seen in CS spectra.

ENDOR

Electron nuclear double resonance spectroscopy (ENDOR) further advances pulsed EPR techniques by including pulses in the radiofrequency region to the pulse patterns utilized to produce spin echoes. The result is the ability to measure the resonance energies of the specific nuclei that are coupled to the unpaired electron. Most importantly, it allows one to observe how these resonance energies are perturbed with respect to the coupling of the nuclear and electronic magnetic moments. The direct measurement of these coupling parameters allows understanding of physical relationship of the electron with its surrounding nuclei.
Spin-Spin Labeling Experiments

An entirely different application of EPR spectroscopy is done with spin-spin labeling experiments. In these, a protein is tagged with paramagnetic groups (usually NO$_2$) that can be easily detected by EPR. The distance between parts of the molecule which contain the paramagnetic groups can observed by the interaction of their respective transitions in spectra. The most valuable information is gained from these experiments by altering the molecule and observing the whether the paramagnetic groups interact with each other. For example, this technique has been utilized to determine whether membrane-bound ion channels open or close in response to a stimulus.

References


Outside links

- Brit lab homepage contains more information about CalEPR center and EPR research at UCD.
- The National High Magnetic Field Laboratory in Tallahassee conducts EPR research at even higher frequencies and fields.
- Wikipedia on EPR spectroscopy contains useful background information.
- Bruker’s EPR Product Lines contain information about commercially available EPR spectrometers.

Problems

1. Approximately what size waveguide would be appropriate if you were operating in mid-range S-band frequencies?
2. Would S-band frequencies require higher magnetic field strength or lower? Based on magnetic field ranges described in this article, what would expected the magnetic field range of an S-band spectrometer to be?

Contributors

- Alan Wilder