EPR Applications

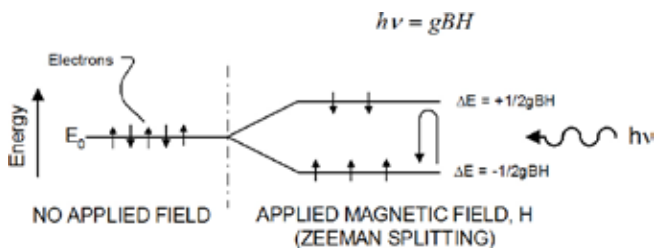
Electron Paramagnetic Resonance (EPR) measures the concentration and composition of free radicals in a sample. Samples can be either liquid, solid or gas. Free radicals are atomic or molecular species with unpaired electrons that can be highly reactive. There are also many stable free radicals, such as melanin in hair and ultramarine blue pigment. Many transition and rare earth metals have unpaired electrons, and are EPR active. Some minerals (eg amethyst, smoky quartz and fluorite) receive their color from unpaired electrons, and are also EPR active.

EPR, also known as Electron Spin Resonance (ESR), is a form of magnetic resonance spectroscopy, along with NMR and MRI. In NMR and MRI, the atomic nucleus interacts with electromagnetic radiation (EMR); with EPR, the reaction is with one or more unpaired electrons.

Even though not all nuclei are NMR active, most compounds have an NMR signal. This is not true of EPR. It is the magnetic component of EMR that interacts with the atomic nucleus or electron's magnetic moment in all forms of magnetic resonance. Spin-paired electrons have a net magnetic moment of zero, and are therefore EPR silent.

In an EPR spectrometer, the sample is loaded into a high frequency resonant cavity in a slowly varying, uniform magnetic field. When irradiated with microwave radiation at a fixed frequency, the unpaired electrons undergo resonant transitions between spin 'up' and spin 'down' state in a particular magnetic field, governed by the equation in Figure 1. The magnetic field at resonance is a function of the g-factor, and the amplitude of the resonant peak is determined by the concentration of the radical in the sample.

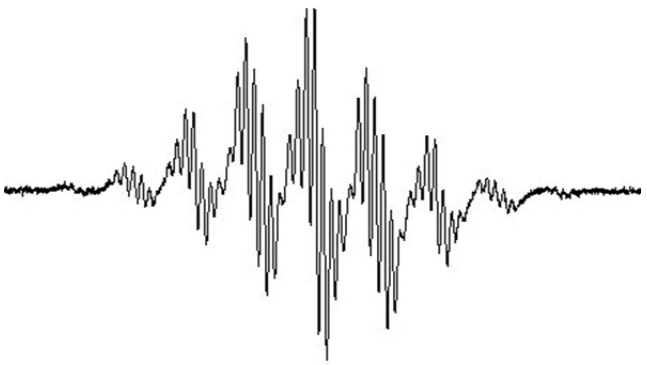
Figure 1



Electron transitions stimulated by incident microwave energy.
h = Planck's constant; B = Bohr Magneton; ν = resonant frequency; H = applied magnetic field; g = a characteristic of the radical (the "g-factor," an empirically determined number, typically around 2 for organic radicals)

The EPR effect was first measured in 1945. Historically, EPR spectrometers consist of large water-cooled electromagnets that generate a variable magnetic field. They often have a similar design to older NMR spectrometers. This causes significant portability issues; the electromagnet assembly weighs upwards of 200 kg and requires several kW of power. Bruker MicroESR™ spectrometers overcome this problem with a small, strong rare-earth magnet and a low power electromagnetic coil. The sample is placed in a high-Q resonant cavity, which has a large 'fill factor' compared to a conventional system. This reduces the size of the instrument by a factor of 100, without compromising high sensitivity and excellent resolution. The spectrum of perylene radical cation in Figure 2 is an example of the resolution that can be achieved with benchtop EPR spectrometers.

Figure 2



EPR spectrum of perylene radical cation, taken with the Bruker MicroESR.

Additional fundamental innovations in the design of the microwave bridge and receiver, which now use low-cost integrated components similar to those in wireless communications devices, further reduce the cost and size compared to conventional EPR spectrometers. These innovations have resulted in a fundamental shift away from large centralized EPR systems towards small, portable, versatile instruments that can even be used in the field.

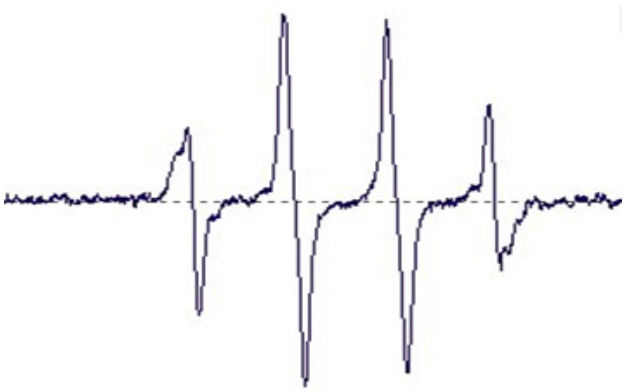
EPR Applications

Spin Trapping

Spin trapping is a common technique that detects very short-lived radicals such as hydroxyl, superoxide and thiyl, which cannot be directly observed by EPR because their short lifetimes. A spin trap is a compound, often containing nitrone or nitroso, which reacts quickly with a radical to form a stable nitroxide that is EPR active. This is known as a spin adduct.

BMPO, 5-*tert*-butoxycarbonyl-5-methyl-1-pyrroline-N-oxide, is a fairly new cyclic nitrone spin trap that produces long-lived spin adducts with hydroxyl, superoxide and glutathiyl radicals. The advantage is that hydroxyl and glutathiyl radicals are distinguishable.

Figure 3



BMPO-OH spin adduct.

$Fe^{2+} + H_2O_2 \quad Fe^{3+} + \bullet OH + OH^-$

$\bullet OH + BMPO \quad \bullet OH\text{-}BMPO$

Fenton Reaction used to produce hydroxyl radicals

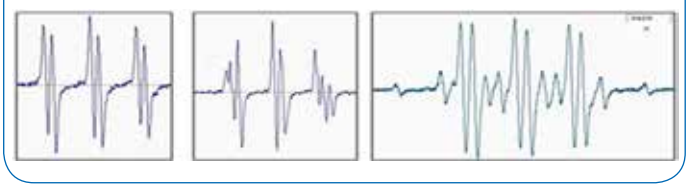
Active Spectrum offers BMPO for only $250 for 50 mg

EPR is also a very useful for elucidating reaction mechanisms involving free radicals. Some radicals can be observed directly, but often they have short lifetimes, so a spin trap is necessary. The rate at which the radicals are produced can be calculated by monitoring the spin adduct's increasing signal.

Another method involves using a stable radical such as the nitroxide TEMPOL, which is EPR active. Radicals are produced, which react with the nitroxide. The rate at which the nitroxide signal disappears can be monitored easily with EPR.

Depending on the species involved, EPR provides a vast amount of information about the species involved in reaction mechanisms, as well as insights into competing and changing reactions.

Figure 4



Fenton reaction in DMSO with PBN as a spin trap over time.

Figure 4 is a Fenton-type reaction using DMSO as the solvent. The spin trap is t-butyl-α-phenyl nitrone (PBN). Initially the PBN reacts with methoxyl radicals, and then methyl. Eventually, the PBN is broken down into benzaldehyde and MNP (2-methyl-2nitrosopropane). MNP is a spin trap itself, and reacts with methyl radicals to form a stable spin adduct. The final spectrum is shown in Figure 3.

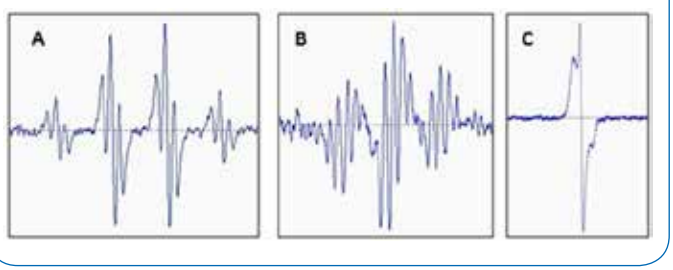
Kinetics

Kinetics experiments are very easy to set up, execute and process with Active Spectrum's user-friendly acquisition and processing software.

Process Additives

Monitor process additives such as antioxidants in lubricants and other fluids. Peroxy and other radicals formed as thermal degradation products react with antioxidants, often forming stable radicals which are visible by EPR. Radical adducts can help to elucidate reaction mechanisms.

Figure 5

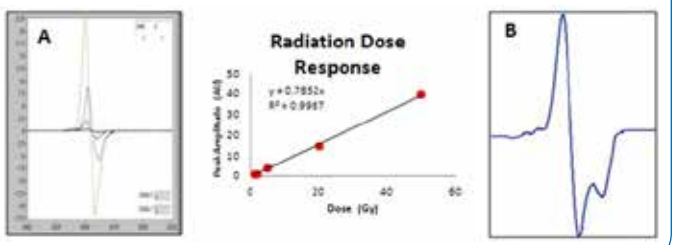


EPR spectra of common antioxidant additives. A: BHT (butylated hydroxyl toluene); B: 4,4-methylenebis(2,6-Di-tert-butylphenol); C: APANA (alkylated pheny-α-napthylamine.

Dosimetry

Dosimetry is another application that suits EPR perfectly. After being irradiated, many substances contain stable free radicals and are therefore EPR active. Good dosimetry samples have an EPR signal with an amplitude proportional to the dose.

Figure 6

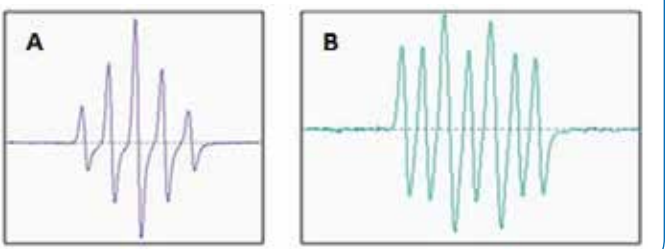


EPR spectra of $Ca(HCOO)_2$ irradiated with various doses of ^{60}Co. The graph in the center plots radiation dose vs. peak amplitude. B: A typical irradiated signal.

Nitric Oxide

Nitric oxide (NO•) is another biologically and environmentally important free radical that can be studied easily by EPR. Unlike hydroxyl, superoxide and atomic radicals, nitric oxide is reasonably stable and can be directly observed by EPR. The signal is, however, quite broad, so this radical is often observed using a spin trap. PTIO (α-phenyltetramethylnitronyl nitroxide) works well for trapping nitric oxide radicals. PTIO is a stable radical itself, so is EPR active; however, the EPR signature of the PTIO-NO spin adduct is very different (Figure 7).

Figure 7



A: PTIO; B: PTIO-NO spin adduct.

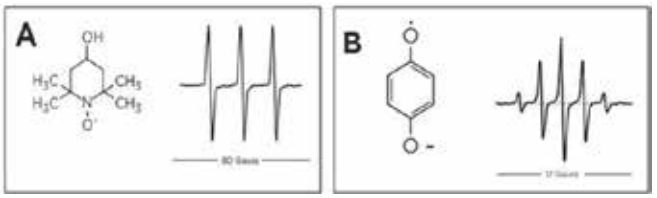
Unpaired Electron Spin Density

Properties such as unpaired electron spin density can be determined by EPR. Studying hyperfine splitting patterns and coupling constants provides information about the electronic structure of molecules. For example, compare the spectrum of TEMPOL (4-hydroxy-2,2,6,6-tetramethylpiperidin-1-oxyl) to that of the the p-benzosemiquinone radical anions; TEMPOL has a single unpaired electron that is localized on the oxygen and nitrogen atom. The unpaired electron interacts with the ^{14}N atom, which has a spin quantum number equal to I=1, so the spectrum is a triplet with all lines of equal intensity (Figure 7A). The benzosemiquinone radical anion (Fig. 7B) also has a single unpaired electron, but that electron is a delocalized pi electron. Even though the unpaired electron is in a pi orbital,

it still overlaps with the four equivalent proton s orbitals, as shown by the equally spaced five-line EPR spectrum (Fig 7B). Each of the four equivalent 1H atoms has a spin quantum number I=½; therefore there are five lines with a 1:4:6:4:1 ratio. If the electron were localized on the oxygen atom, the EPR spectrum would contain only a single line. Although the electron does have density at the oxygen atoms, we see no hyperfine splitting from the oxygen because ^{16}O has spin quantum number I =0.

Other non-intuitive properties that can be studies with EPR include rotational correlation times, spin orbit coupling, zero field splitting, electron spin-spin exchange, relaxation times and forbidden transitions.

Figure 8

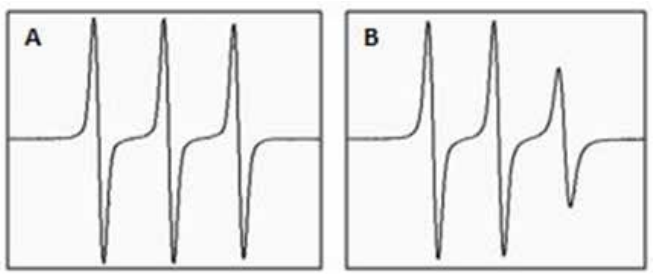


A: TEMPOL; B: benzosemiquinone radical anion.

Spin Labeling

Spin labeling is another important application, and is important when studying protein structure and dynamics. The shape of the nitroxide spectrum changes dramatically as rotational motion is restricted. Nitroxide compounds, referred to as spin labels, can be incorporated into proteins, similarly to how ^{13}C, ^{15}N, and ^{2}H labels can be for NMR. Protein grown with the spin label can then be studied with EPR. Figure 9 is a spectrum of TEMPOL in water with no hindered motion (A), and the same concentration of TEMPOL in a viscous solution in which rotational motion is hindered (B).

Figure 9

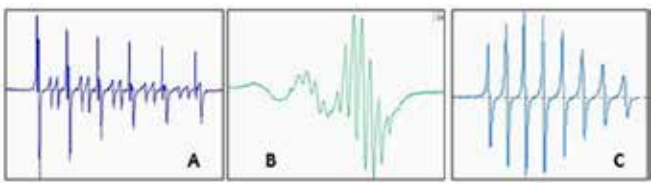


Spectrum A is of a nitroxide with no hindered motion. Spectrum B is of the same nitroxide in a viscous solvent which hinders free rotation.

Coordination Chemistry and Crystal Defects

Coordination chemistry and crystal defects such as nitrogen vacancies in diamond and F-centers in minerals can be studied by EPR. Many transition metals have unpaired electrons so are EPR active. A great deal can be learned about the structure of these compounds, both in solid state and solution. Transition metals often have large zero field splitting and strong spin orbit coupling, so can have g-factors far from 2. Studying these types of compounds requires a wide sweep width. Figure 9 depicts EPR spectra of several transition metal compounds in solution.

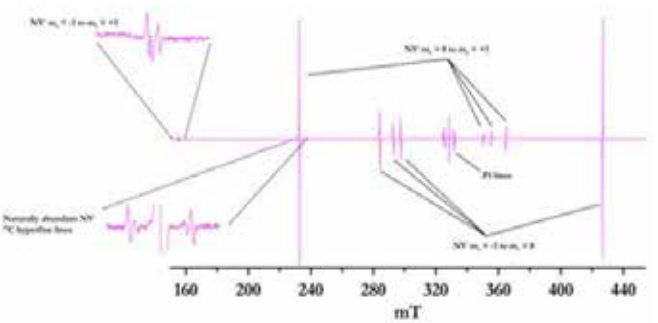
Figure 10



A: Mn(II) in plasticene; B: Cu(II)TPP; C: $VO(acac)_2$

Figure 10 is an example of an EPR spectrum of nitrogen vacancies and P1 center in diamond. The sample was irradiated with a Nd:YAG laser (532 nm) to induce optical polarization in the NV centers. All Active Spectrum EPR spectrometers can be equipped for easy sample irradiation easily.

Figure 11

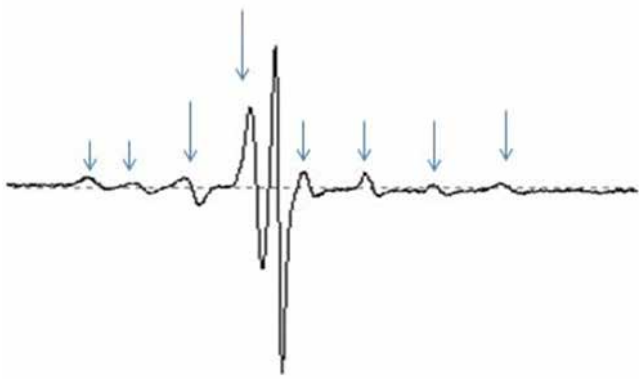


NV and P1 centers in diamond. The sample was irradiated with a Nd:YAG laser at 532 nm. Data courtesy of Eric Scott, Jeff Reimer's Lab, UC Berkeley

Crude Oil

Asphaltenes, a nuisance for refineries everywhere, have a large organic radical signal. They tend to flocculate unpredictably, clogging the refinery and causing expensive shut-downs. Asphaltenes are solubility class compounds, and contain vanadyl and nickel porphyrins as well as aromatic hydrocarbons. Vanadylprphyrin is EPR active, and although tetrahedral Ni(II) is EPR active, the square planar nickel porphyrin is not. Figure 11 is the EPR spectrum of an asphaltene containing a large amount of vanadium. The large, narrow, unmarked peak is due to organic radicals, and the other peaks are from vanadium. Despite causing numerous problems in the petroleum industry, asphaltenes are fascinating chromophores that lend themselves to many forms of spectroscopy, including fluorescence.

Figure 12



EPR spectrum of an asphaltene sample with a large vanadyl porphyrin signal. The arrows point to the vanadium signal. The large, unmarked narrow signal comes from organic radicals.

Humic and Fulvic Acids

Humic and fulvic acids are, like asphaltenes, a class of molecules rather than an entity with a single specific structure. Humic and fulvic acids are decay products of organic matter, and also have EPR signals. These materials are important in agriculture as fertilizers. EPR is useful for studying this class of compounds.

Conclusion

These examples are just a few of the many applications of EPR spectroscopy. With the advent of affordable benchtop spectrometers, the EPR industry will continue to grow and become more mainstream. New applications will continue to emerge as EPR has become a straight-forward, user friendly spectroscopic technique.

EPR References

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