



microESR

- General Purpose ESR / Industrial Analysis / Education

Electron Spin Resonance Spectroscopy

An electron spin resonance (ESR) spectrometer detects the concentration and composition of free radicals present in a sample.

The sample can be a liquid, solid or gas. Free radicals are atomic or molecular species with unpaired electrons which can be highly reactive. There are also many stable free radicals such as melanin in hair or the pigment ultramarine blue. Many transition and rare earth metals have unpaired electrons, and have ESR signals. Some minerals such as amethyst, smoky quartz, and fluorite receive their color from unpaired electrons, and also have ESR signals.

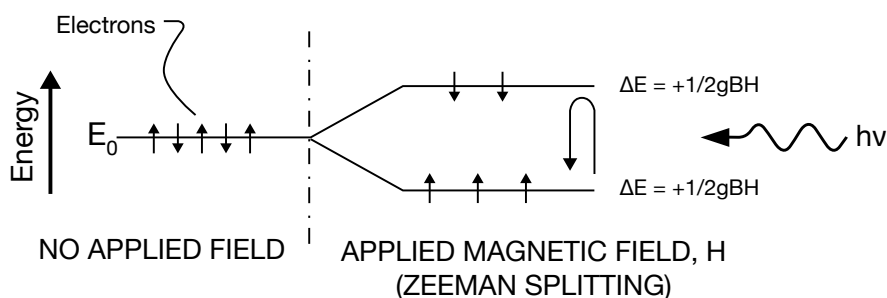
Electron spin resonance (ESR), known also as electron paramagnetic resonance (EPR), NMR, and MRI are all forms of magnetic resonance spectroscopy. In NMR and MRI it is the atomic nucleus that interacts with the electromagnetic radiation (EMR); in ESR/EPR, it is one or more unpaired electrons. Even though not all nuclei can be observed by NMR, most all compounds will have an NMR signal, but this is not true of ESR. In all forms of magnetic resonance, it is the magnetic component of the EMR that interacts with the magnetic moment of the atomic nucleus or electron. Spin-paired electrons have a net magnetic moment of zero; therefore, have no ESR signal.

In a typical ESR spectrometer, the sample is loaded into a high frequency resonant cavity in a slowly varying uniform magnetic field. Unpaired electrons irradiated with microwave radiation at a fixed frequency will undergo resonant transitions between the spin 'up' and spin 'down' state at a characteristic magnetic field governed by the equation $E = h\nu = gBH$, and shown conceptually in the drawing below:

Here, h is Planck's constant, B is the Bohr Magneton, ν is the resonant frequency, H is the applied magnetic field, and g is a characteristic of the radical (the "g-factor," an empirically determined number, which is close to two for organic radicals). 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.

Historically, since the ESR effect was first experimentally measured in 1945, ESR spectrometers have been designed using large water cooled electromagnets to generate a variable magnetic field. Conventional ESR spectrometers use a similar arrangement to that found in older nuclear magnetic resonance (NMR) spectrometers. This design has posed a significant hindrance in terms of portability, since the electromagnet assembly weighs upwards of 200 kg and requires several kW of power in operation. Our microESR™ spectrometers have circumvented this problem by using a small, strong rare-earth magnet assembly with a low power electromagnet coil. The sample is contained in a high-Q resonant cavity which has a large 'fill factor' relative to a conventional ESR. Thus high sensitivity and excellent resolution can be obtained but the size of the entire device is reduced by a factor of 100.

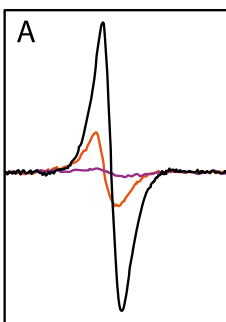
Additional fundamental innovations in the design of the microwave bridge and receiver, which now use modern low-cost integrated components similar to those used in wireless communications devices, offer further cost and size reductions compared to conventional ESR spectrometers. Our innovations have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



● Introduction to ESR Spectroscopy

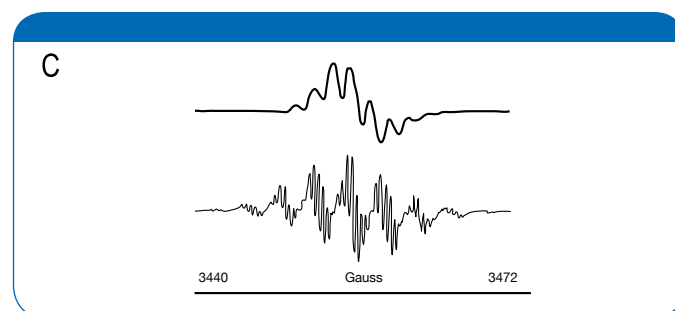
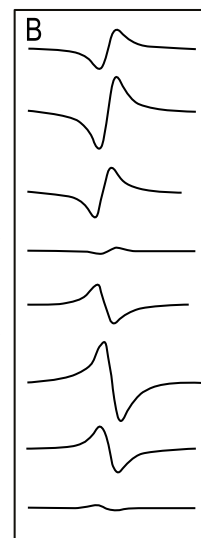
- Introduces students to the microESR spectrometer, and gives them hands on experience running the instrument.
- The spectrometer is very easy to use; however, still gives students access to many important parameters.
- Most parameters are set in the factory and do not need to be adjusted, but are easily accessible so that students can experiment with them.
- There are no user settings that can damage the spectrometer.

1 Students run spectra on common items found in their everyday environment that contain free radicals such as hair, pine needles, tea, and blue make-up. Melanin, the pigment in hair, contains free radicals. The darker the hair, the larger the concentration of free radicals. Figure A is an overlay of ESR spectra from black, orange, and white cat hair. The mass of hair was the same for all samples. Theoretically, white hair should have no signal; however, the cat that the white hair came from is not albino, the hair came from a white patch, so there is a small residual signal.



2 Students will also learn what effect acquisition parameters such as modulation amplitude, modulation phase, RF power, sweep rate, gain, and resolution have on their spectra, and how to optimize those parameters. Although the maximum sweep range is fixed at 3480 +/- 250 Gauss, any range less than the maximum can be selected. The number of points and sweep rate can also be adjusted. The modulation phase is another important parameter that can be adjusted. Figure B shows the effect of changing the modulation phase in 45 degree increments using a solid DPPH sample. This parameter should be adjusted such that the signal amplitude is the largest and in phase.

Both the RF power and modulation amplitude need to be adjusted for a given type of sample. If the RF power is too high, the sample will saturate, and signal to noise will be lost. The modulation amplitude will also need to be adjusted depending on the sample's natural linewidth. The modulation amplitude needs to be less than the natural linewidth. Figure C shows the spectrum of perylene radical cation with the modulation amplitude set to 2 Gauss (top), and 0.02 Gauss (bottom). Note the change in resolution as the modulation amplitude is decreased. Unfortunately, decreasing the modulation amplitude also decreases the signal to noise, so there is, as always, a trade off.

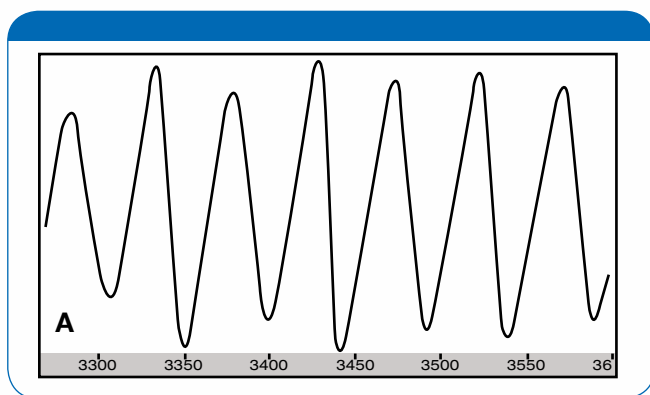


Double Integral values are calculated by the Processing and Analysis software, and plotted as a function of scan number. The time stamp on the data file is saved in the .csv output file.

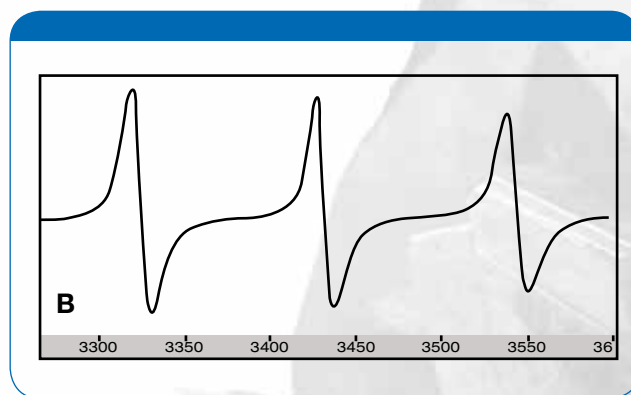
3 Parameter Arrays: A number of acquisition parameters can be easily arrayed, so multiple data sets can be acquired with a single start. For example, it is important to know the optimal RF power to use on a given sample type. The microESR Processing and Analysis software allows the user specify any number of RF power values, and the spectrometer will tune and acquire and save a spectrum using each requested RF power value. The array feature is available for RF power, modulation amplitude, digital gain, and d1 delay. The parameter array feature allows for up to two parameters to be arrayed at a time.

● Transition Metals and Hyperfine Splitting

- Introduces students to hyperfine splitting caused by atomic nuclei
- In this lab, several coordination compounds are synthesized, and the ESR spectra are analyzed.
- Students learn that not only the transition metal nuclei cause hyperfine splitting in these compounds, but other atomic nuclei can also contribute to hyperfine splitting
- Nuclei with spin quantum number zero ($I = 0$) are also discussed.



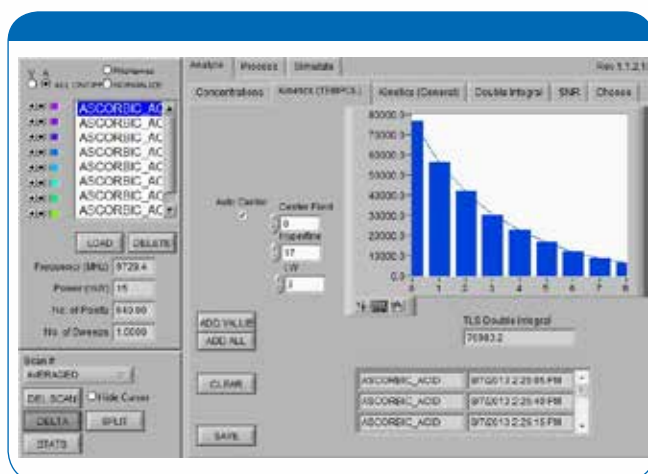
A. ESR spectrum of bis(*O,O'*-diethyldithiophosphato) oxovanadium (IV)



B. ESR spectrum of vanadyl acetylacetonate

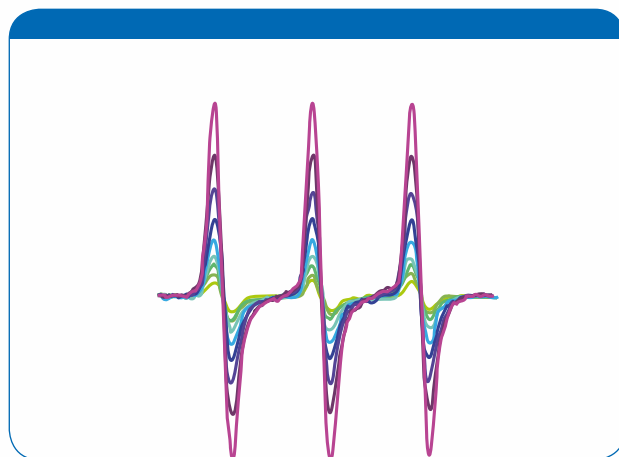


● Kinetics



Double Integral values are calculated by the Processing and Analysis software, and plotted as a function of scan number. The time stamp on the data file is saved in the .csv output file.

- Introduces students to the use of ESR to monitor reactions.
- This lab uses the stable nitroxide radical, TEMPOL (2,2,6,6-tetramethyl-piperidine-N-oxyl-4-ol), to measure the antioxidant properties of fruit juice.
- The microESR's interactive acquisition software allows students to set up kinetics experiments. The students can specify the number of scans for each run, the number of runs, and the time between runs.
- The spectrometer displays the spectrum after each scan.
- The data acquired on the ESR spectrometer can then be further analyzed using the microESR's Analysis and Processing Software.
- All data are saved as a .csv file, so can be loaded into any spreadsheet program for numerical processing.
- The experiment is run in water, so melting point capillaries placed inside a 5mm quartz tube can be used for each student's run. The 5mm tubes can be reused many times.

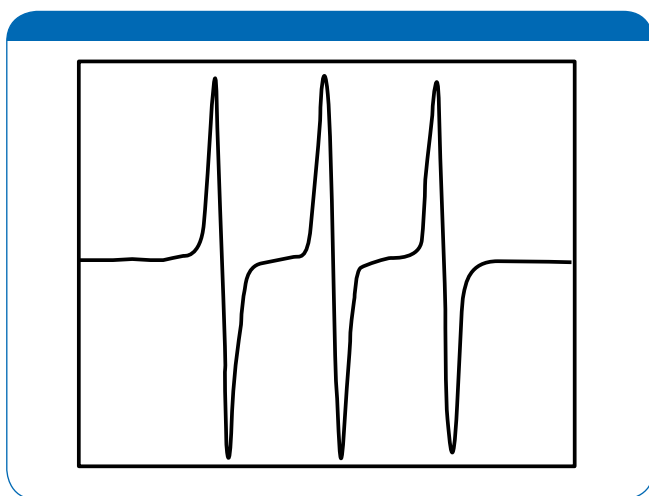


This plot shows the decrease in TEMPOL signal amplitude over time. The kinetics processing software can return a value for each spectrum in either peak to peak amplitude or double integral.

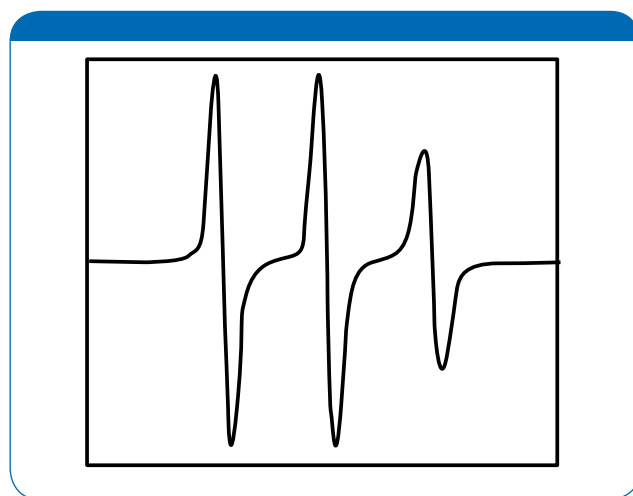


● The Shape and Width of ESR Spectral Lines

- This lab looks at a number of phenomena that affect the shape and width of ESR spectral lines.
- Explores the effect of viscosity on TEMPOL spectra. This is a direct application for measuring rotational correlation times.
- Looks at the effect of solvents on linewidth, and how the presence of molecular oxygen provides an effective relaxation pathway for electrons.
- Looks at the effect of concentration on linewidth, and introduces the concept of spin-spin exchange.
- Introduces the concept of spin labeling.



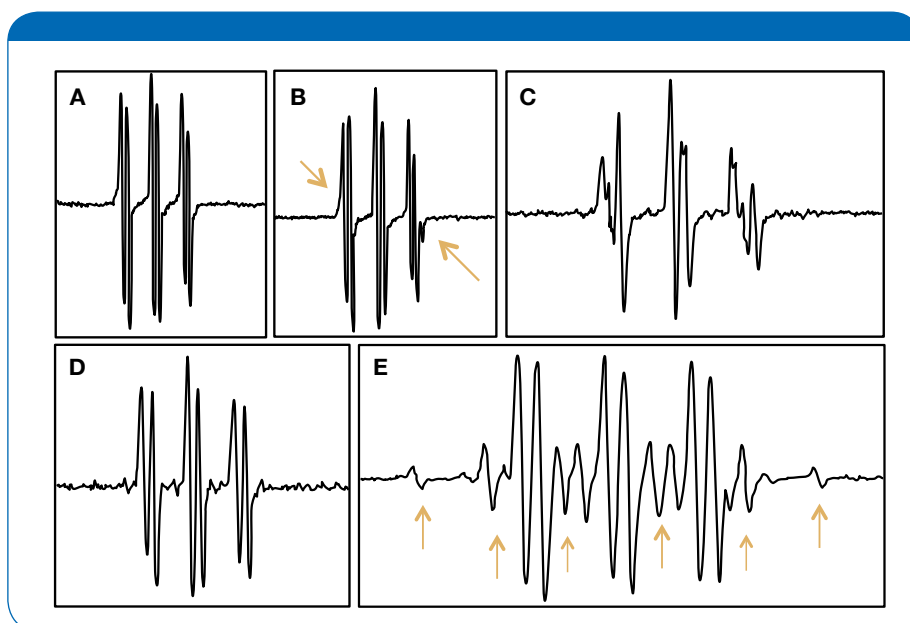
A. Spectrum of TEMPOL in water



B. Spectrum of TEMPOL in 25% water and 75% glycerol

● Spin Traps and Spin Adducts

- Although there are many radicals that are stable enough to observe directly with ESR, some very important radicals, such as hydroxyl and superoxide, are too short lived to be directly observed. Many important intermediates in organic reactions also fall into this category.
 - Very short lived radicals can be detected by ESR with a technique called spin trapping.
 - A spin trap, often a nitron or nitroso, is a compound that reacts readily with reactive radicals to form a much more stable radical that can be observed by ESR. This product is referred to as a spin adduct.
 - The ESR signature from the spin adduct provides only implicit information about the trapped radical. The hyperfine splitting from the nitrogen atom, splitting from other magnetic heteroatoms, and α hydrogen atoms all give information about the structure of the trapped radical.
 - Care must be taken when choosing a spin trap. Understanding the chemistry involved, including the role the solvent may play is very important. It is possible for side reactions to create unexpected, stable spin adducts- one of the pitfalls of spin trapping.
- In this lab, students will use PBN (t-butyl- α -phenyl nitron) to trap short lived radicals. They will be able to watch the initial spin adduct turn into a second spin adduct, and then yet a third spin adduct will appear. The third spin adduct is quite long lived, and can be left to run overnight. The third spin adduct can be attributed to side reactions, and demonstrates this pitfall well. The solvent is also involved in this reaction.
 - The best spin trap to use for hydroxyl or superoxide is BMPO, 5-tert-butoxycarbonyl-5-methyl-1-pyrroline-N-oxide. Unfortunately, the cost of this spin trap makes it impractical to use in an undergraduate chemistry lab. The PBN-OH spin adduct has a half life of only seven seconds, so also cannot be directly observed by ESR. The reaction used in this lab is a bit complex. The chemistry, however, is well understood, and this lab does a good job of demonstrating the technique of spin trapping.



Spectrum **A** is the first spin adduct formed.

Spectrum **B** shows the second spin adduct forming.

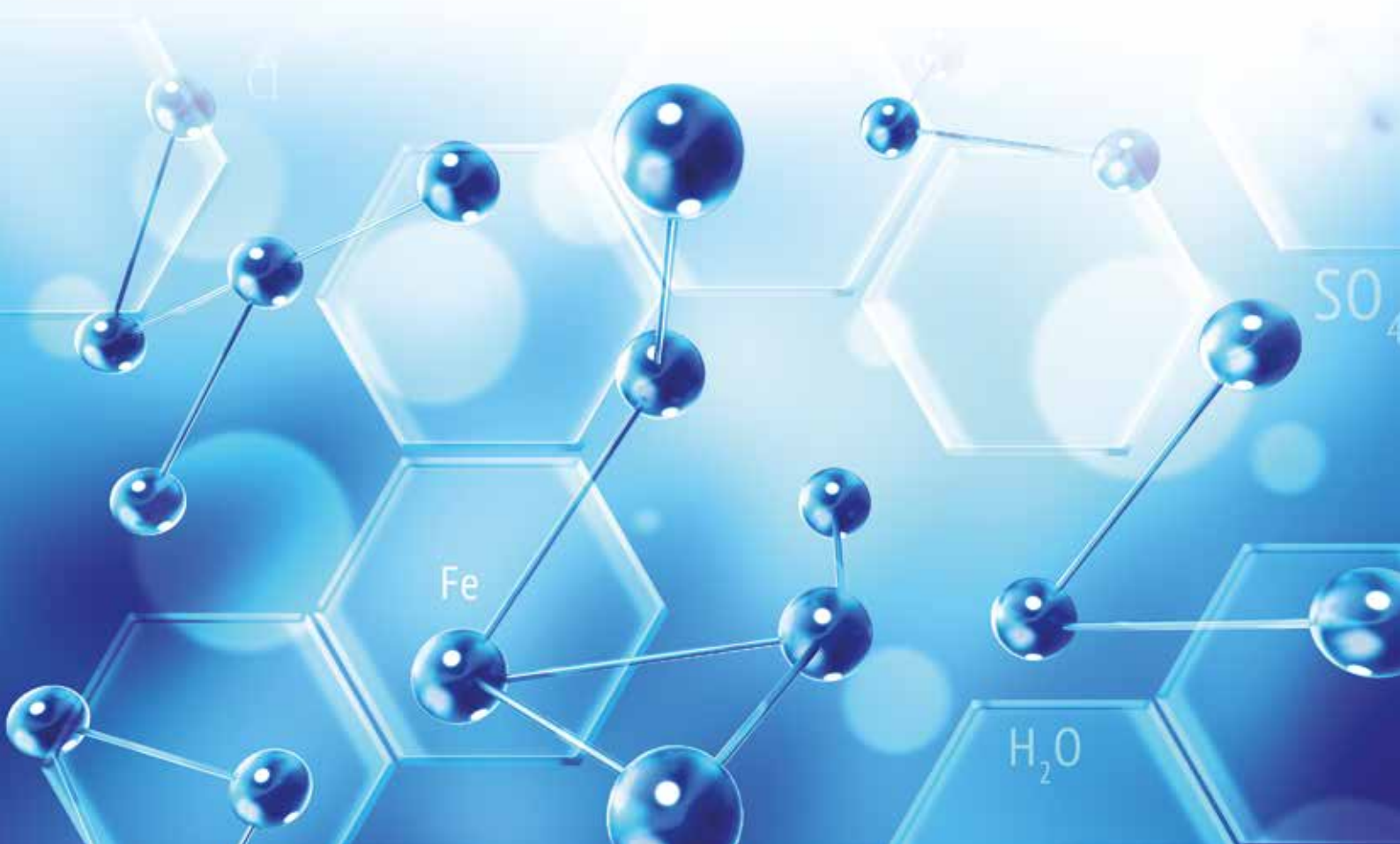
Spectrum **C** is a combination of both the first and second spin adduct.

Spectrum **D** is the second spin adduct. The third spin adduct is already forming here.

Spectrum **E** is an overlap of the second and third spin adduct. The arrows point to the lines which belong to the third spin adduct.

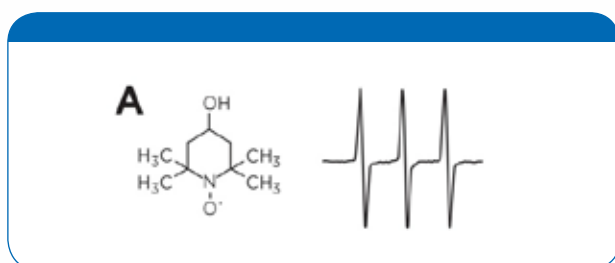
● Determining Concentrations by ESR

- Students use ESR to determine the concentration of a transition metal complex
- This is an excellent lab for analytical chemistry, especially to compare with other methods of determining concentration such as UV/VIS, titration, and gravimetric.
- This lab does require the students to create a calibration curve
- The microESR's Processing and Analysis software allows students to compare the use of peak to peak amplitudes and double integral values to determine concentrations. Which method is more precise?
- When making quantitative ESR measurements, sample alignment and orientation play an important role. Even though the samples looked at in this lab are liquid, students are asked to compare measurements using a borosilicate capillary in a 5mm quartz tube to measurements using a 2mm quartz capillary

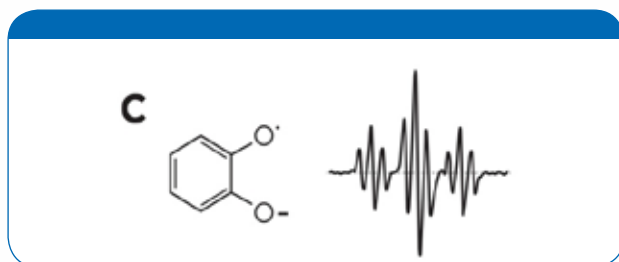


● Electron Density

- ESR is an excellent tool for understanding electron density, a non-intuitive concept
- Students will make several semiquinone radical anions, and look at their respective ESR spectra. Students will also look at the ESR spectrum of the stable nitroxide radical, TEMPOL



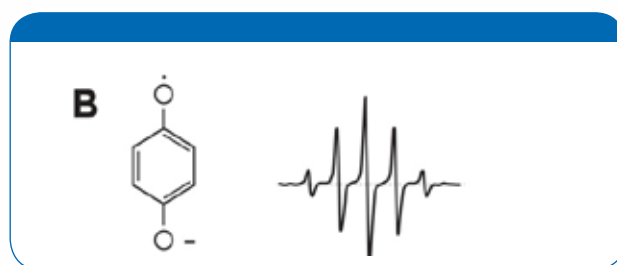
Compound A is TEMPOL with its corresponding ESR spectrum. Note the spectrum is a triplet. The only hyperfine splitting is from the ^{14}N atom ($I=1$).



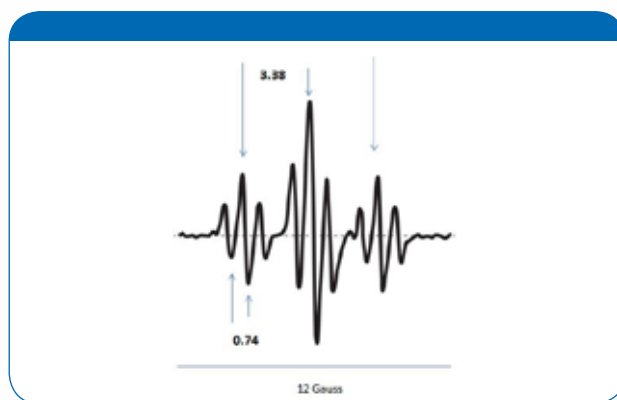
Compound C is the catechol (1,2-dihydroxybenzene) radical anion which has two sets of two equivalent protons. Its corresponding ESR spectrum is a triplet of triplets. For each set of equivalent protons the unpaired electron can "see" both protons as spin-up (α , α), one spin-up and one spin-down (α , β) or (β , α), or both spin-down, (β , β). Each one of these states corresponds to a different energy; hence, the three lines. The lines are in a 1:2:1 pattern because there are twice as many ways to have the (α , β) energy state. In addition, note that there are two distinctly different hyperfine coupling constants for each set of equivalent protons. This means that one set of equivalent protons has greater electron density than the other set. The two hyperfine splitting constants are 3.38 and 0.74 Gauss. There will also be electron density on the oxygen atoms, but since ^{16}O has a spin quantum number, $I=0$, there will be no hyperfine splitting from the oxygen atoms.

Note that although there are two equivalent protons on the TEMPOL ring, we do not see any hyperfine splitting from these protons. This is because the unpaired electron is localized on the oxygen and nitrogen atoms, and not delocalized like in the aromatic semiquinone radical anions.

- Although all compounds are rings, the nitroxide's unpaired electron is localized on the oxygen and nitrogen atoms; whereas the semiquinone radical anions have a delocalized π electron. The hyperfine splitting from protons observed in the semiquinone radical anions illustrate where the unpaired electron spends its time. Even though TEMPOL has 2 equivalent protons on its ring, we do not observe any hyperfine splitting from them,



Compound B is hydroquinone (1,4-dihydroxybenzene) radical anion. Its ESR spectrum contains five equally spaced lines in a 1:4:6:4:1 pattern. This hyperfine splitting comes from the four equivalent protons on the aromatic ring. ^1H has $I = \frac{1}{2}$. The line intensities come from the probability of a proton's spin being "up" (α) or "down" (β). All protons are α (1 way), one proton is α , and three are β (4 ways), two are α and two are β (6 ways), three are α and one is β (4 ways), and all four are β (1 way).



Catechol Radical Anion

● About The microESR



Benchtop Micro ESR

The microESR uses a small, 0.348 Tesla, rare-earth magnet. The magnet assembly contains a low power electromagnet coil used to vary the field. The microESR is a strictly CW instrument with a sweep range of over 500 Gauss. The field is centered near the free electron spin g-factor. The spectrometer uses a linear voltage controlled oscillator as a microwave source, and can generate between 0.5 and 70 mW RF power at 9.7 GHz. The microESR uses quadrature lock-in detection, and the lock-in amplifier is internal to the system.

The resonator is a cylindrical dielectric $TM_{0,11}$ cavity with a maximum volume of $\sim 400 \mu\text{L}$ (5 mm diameter by 20 mm length). The field is homogeneous across the resonator within 0.1 Gauss. The entire microESR has a small footprint ($30.5 \times 30.5 \times 30.5 \text{ cm}^3$) and a mass of only 10 kg, so can be easily moved. The microESR requires no special installation and no special maintenance making it accessible to any undergraduate laboratory.

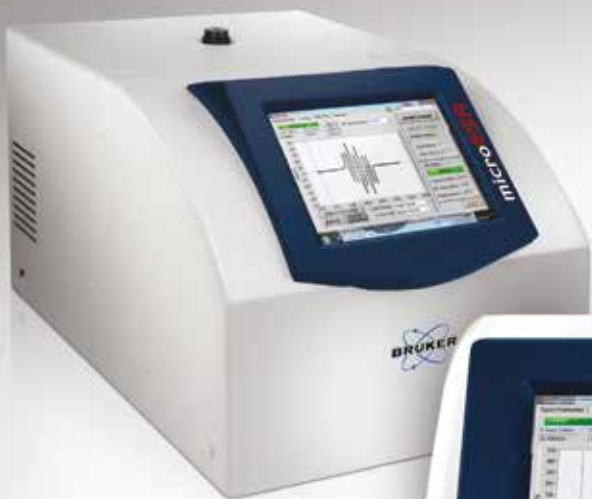
● Lab Accessory Kit

- 1g Stable Nitroxide Radical (TEMPOL, TEMPO)
- 1g PBN (N-tert-Butyl- α -phenylnitron)
- 10 ea - 5 mm quartz ESR Tubes
- 1 ea - 4 mm quartz ESR Tube
- 2 ea - 2 mm quartz ESR Tubes
- 1 ea - 2 mm Tube Alignment Guide
- 2 ea - 100 pks. 1.7 mm Capillary Tubes
- 1 ea - Pkg Sealing Clay
- 10 ea - 12 Ga Blunt Loading Needles
- 1 ea - Pkg Misc. O-rings
- 1 ea - Blue Make-up Sample (S3-●)



microESR Education Package

- microESR Spectrometer
- microESR Experiments Manual
- microESR Instructor's Guide
- Lab Accessory Kit
- microESR User's Manual
- microESR Analysis and Processing Software with Manual



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