Experiment #2B: Electron Spin Resonance Spectroscopy

I. Introduction

Electron spin resonance (ESR)[1] has developed over the past several decades as a technique to provide information on the electronic structure of organic, inorganic, biological, solid state, and surface molecular species. These include organic free radicals,[2] biradicals, triplet excited states, and most transition metal and rare earth species. Biological applications, in addition to studies of these organic and inorganic species in biological systems, include the use of "spin labels" as probes of molecular environment in enzyme active sites and membranes. Point defects in solids and, increasingly, active chemical species on surfaces of catalysts are also investigated using ESR. This experiment will provide an introduction to the theoretical principles and experimental techniques used in ESR investigations. Assuming that you are more familiar with NMR techniques, a brief comparison between NMR and ESR will be beneficial.

First, NMR studies mostly diamagnetic compounds (all electrons are paired); ESR is an important spectroscopic technique for the study of paramagnetic species (unpaired electrons) such as free radicals, metal complexes, or triplet excited states of diamagnetic molecules.

Second, the NMR "chemical shift" is analogous to the "g factor" in ESR. For carbon-centered organic free radicals, the g-factor is always close to the free electron value of 2.0036 (therefore diagnostically less important). For metal complexes, the g factor changes substantially and can be used to give information about the electronic structure of the metal ion.

Third, the NMR coupling constant (J, expressed in Hz), corresponds to the hyperfine coupling (or hyperfine splitting) constant in ESR, with the symbol a, expressed in gauss or MHz.

In this experiment you will record the ESR spectra of $Mn(CH_3COO)_2$, perylene radical cation and several 2-alkyl substituted 1,4-benzosemiquinone. From the spectra you will extract the **hyperfine coupling constants** (**hfc**)[3] of the electron with hydrogen nuclei. The second stage comprises the computational segment at **Density Functional Theory (DFT)**[4] level of theory to acquire the hfc for hydrogen atoms in semiquinones.

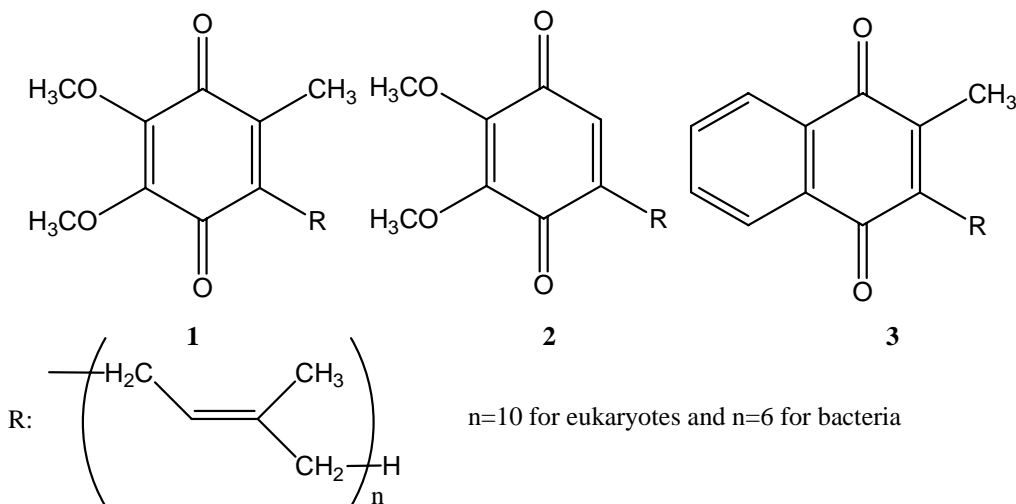[1] The first ESR experiment was carried out by E. Zavoisky, *J. Phys. USSR* **1945**, *9*, 245.

[2] The earliest report of an organic free radical (triphenylmethyl radical): Gomberg, M. *Ber. Dtsch. Chem. Ges*. **1900**, *33*, 3150. Gombert, M., *J. Am. Chem. Soc*. **1900**, *22*, 757.

[3] In monoradicals, interactions between the spin of an unpaired electron and the spins of magnetic nuclei are referred to as **hyperfine coupling**. In polyradicals, the interaction between two or more unpaired electrons is referred to as **fine coupling**.

[4] Foresman, J. B.; Frisch, A. *Exploring Chemistry with Electronic Structure Methods*, Gaussian, Inc.: Pittsburg, PA 1996, p. 272 (QD461.F72; is also available in the computation room, 4-472). Hehre, W. J.; Lou, L. *A Guide to Density Functional Theory*, Wavefunction, Inc., Irvine, CA, 1997 (available upon request from Dr. Gheorghiu)

Why study semiquinones using ESR? The answer is easily understood from the following facts. Quinones play an important role in nature. There are about more than 80 naturally occurring quinones. Three quinones: Coenzyme Q (**1**, ubiquinone), plastoquinone (**2**) and menaquinone (**3**) are among the most important biological electron carriers.

Semiquinone anion radicals are directly involved in the electron transfer reactions in photosynthesis and respiration. Higher plants, algae and some bacteria perform photosynthesis, one of the most fundamental processes of life on earth. During photosynthesis, the initial electron transfer produces an oxidized chlorophyll cation radical and a reduced semiquinone anion radical.

For a full electronic structure interpretation of the ESR data, accurate theoretical prediction of the hyperfine coupling is a valuable aid to the assignment of the spectral features. DFT methods, in particular the B3LYP method with the basis set EPR-II, has been shown in recent years to provide an accurate value of the hfc. The Gaussian 98W output files provide Gauss-Fermi isotropic hyperfine coupling constants.

II. The Essence of ESR

In most atoms and molecules electrons are paired. The paired electrons do not give an ESR signal. Atoms and molecules with unpaired electron give an ESR signal. Electrons and nuclei possess spin angular momentum and an accompanying magnetic moment. The electron's characteristics are given Table 1.

Table 1: Electron Properties (all in SI units)

Rest mass of electron	m_e	$9.109\ 389\ 7 \times 10^{-31}$ kg
Electron charge	e	$-1.602\ 177\ 33 \times 10^{-19}$ C
Angular momentum* (intrinsic angular momentum)	**S**	$\hbar/2 = h/4\pi$; $h = 6.626\ 076 \times 10^{-34}$ J s
Magnetic dipole moment (Bohr magneton)	$\boldsymbol{\mu_S}$	$\boldsymbol{\mu_S} = -g_e\mu_e\mathbf{S}$ $g_e = 2.002\ 322$ (dimensionless)** $\mu_e = (e\hbar)/(2m_e) = 9.274\ 015 \times 10^{-24}$ J T^{-1}

* Bold face symbols denote vectors.
** Landé g factor. All fundamental particles with spin are characterized by a magnetic momentum and a g-factor. The g-factor is a proportionality constant between the magnetic dipole moment and the angular momentum.

The electron angular momentum is quantized in units of $\hbar = h/2\pi$. The magnitudes of the spin angular momentum **S** and S_z (the z component of S) are given by:

$$S = \sqrt{s(s+1)}\ \hbar = \frac{\sqrt{3}}{2}\hbar \quad \text{(because s=1/2)} \tag{1}$$

$$S_z = m_s\ \hbar \quad\quad m_s = \pm\frac{1}{2} \tag{2}$$

In eq. 1, *s* is the **electron spin quantum number** (or simply the spin) and has the value 1/2. The quantum number m_s for $\mathbf{S_z}$ has two possible eigenvalues $\pm 1/2$, or in spectroscopic jargon "spin up" and "spin down."

For a nucleus, the total **nuclear spin angular momentum** is represented by **I**, the spin quantum number by I, and the z component of the nuclear spin by I_z. The eigenvalue of $\mathbf{I^2}$ is $I(I+1)\hbar^2$. The magnitudes of the spin angular momentum |**I**| and the eigenvalue of I_z are given by:

$$|\mathbf{I}| = [I(I+1)]^{1/2}\hbar \tag{3}$$

$$I_z = m_I\hbar \quad\quad m_I = -I, -I+1, \ldots\ldots, I-1, I \tag{4}$$

For nuclei, spin I = 0 for ^{4}He, ^{12}C, ^{16}O, etc.; I=1/2 for ^{1}H, ^{13}C, ^{15}N, ^{19}F, etc.; I=1 for ^{2}H, ^{14}N, etc.; and so on.

For nuclei, the magnetic dipole moment is proportional to the spin angular momentum:

$$\boldsymbol{\mu}_I = -g_N\mu_N\mathbf{I}/\hbar \tag{5}$$

where g_N is the nuclear g-value and μ_N is the nuclear magneton. The quantity $g_N\mu_N$ is often referred to as the gyromagnetic ratio, γ_N, because it is the proportionality constant relating the magnitude of a spin-angular momentum to the resulting magnetic moment.

Table 2: Proton Properties

mass	m_P	$1.672\ 623\ 1 \times 10^{-27}$ kg
Charge	Ze $(Z=1)$	$+\ 1.602\ 177\ 33 \times 10^{-19}$ C
Angular momentum*	$\mathbf{I}$	$\hbar/2 = h/4\pi$; $h=6.626076 \times 10^{-34}$ J s
Nuclear magnetic moment (nuclear magneton)	$\boldsymbol{\mu}$	$\boldsymbol{\mu} = -g_N\mu_N\mathbf{I}/\hbar$ $g_N = 5.5856$ (dimensionless)** $\mu_N = e\hbar/2m_N = 5.050\ 787 \times 10^{-27}$ J T^{-1}

* Bold face symbols denote vectors.
** Lande g factor. All fundamental particles with spin are characterized by a magnetic momentum and a g-factor. The g-factor is a proportionality constant between the magnetic momentum and the angular momentum Note that $g_N \neq g_e$

For systems with more than one electron, the total electron spin angular momentum can be as high as the sum of the spin angular momenta for each electron. Since spin has a direction, it is a vector quantity. The total spin is the vector sum of the individual spins, and therefore can be oriented so that they cancel each other. In fact, the lowest energy condition for most of the electrons in molecular species is the **paired** state with a total spin of zero. In practice one only need be concerned with systems where the total spin is a small integral multiple of 1/2:

- $S=1/2$ (radicals and some transition metal species)
- $S=1$ (biradicals, triplets, and some transition metals)
- $S>1$ (some other transition metals).

The projection of the total angular momentum along a given spatial direction is also quantized in units of $\hbar$. The quantum number for a given spatial component of angular momentum, m_I, can only take on values $m_I = |I|, |I|-1,...,-|I|+1,-|I|$; $(2I+1)$ values in all,

where I is the quantum number for total angular momentum. For the electron, $S = 1/2$, $m_S = \pm 1/2$, and $(2S+1) = 2$, so that molecules with a single unpaired electron are in a doublet state, i.e. there are <u>two</u> m_s levels. In the absence of external magnetic fields, all the m_I states for a given system are degenerate (have the same energy).
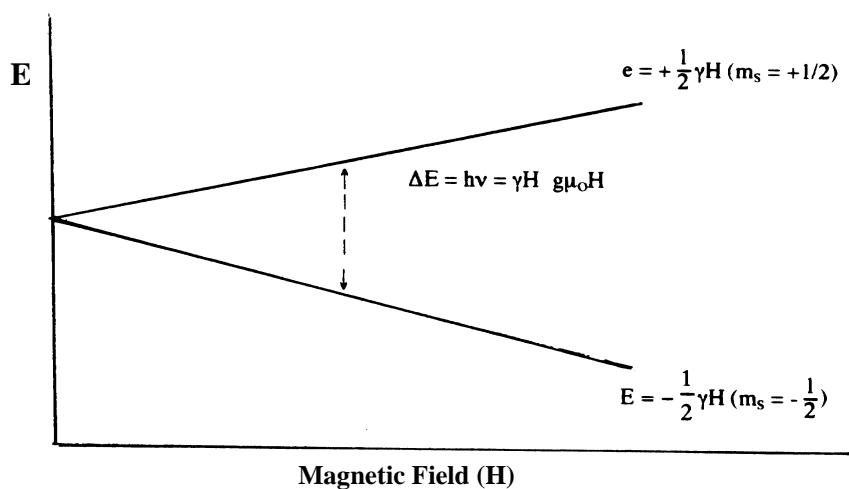
The energy of interaction of a magnetic moment (μ) with a magnetic field (**B**, or sometimes **H**), the "Zeeman" energy, is given by:

$$E = -\mu \cdot \mathbf{B} \tag{6}$$

So, for an unpaired electron, with B along the z-axis:

$$E = -\mu_Z B = g_e \mu_e m_S B = \gamma_e B S_z \tag{7}$$

Thus, we have the field dependent energy levels shown in Figure 1.

$e = +\frac{1}{2}\gamma H \ (m_s = +1/2)$

$\Delta E = h\nu = \gamma H \quad g\mu_o H$

$E = -\frac{1}{2}\gamma H \ (m_s = -\frac{1}{2})$

Magnetic Field (H)

Magnetic field dependence of the energy levels of an S=1/2 system.

Figure 1

Transitions can take place between the $m = 1/2$ and $m = -1/2$ spin levels split by a magnetic field. Typical values of the transition frequencies are:

Particle	ν@10 KG	Typical Experimental Conditions
proton (^{1}H), I=1/2	42.577 MHz	60 MHz @ 14 KG
carbon (^{13}C), I=1/2	10.705 MHz	14 MHz @ 14 KG
electron, S=1/2	28,026 MHZ (28 GHz)	9.5 GHz @ 3400 G

ESR spectroscopy can be applied to study any species with a non-zero total electron spin: S=1/2, 1, 3/2, 2,.... . The principal aspect of electron spin resonance which is of interest to chemists is that the apparent electron spin energies are sensitive to paramagnetic nuclei in the molecular environments, particularly hydrogen atoms. That is, the total energy is composed of three parts, namely,

E = electron Zeeman energy + nuclear Zeeman energy + electron-nuclear coupling

$$= g_e\mu_e M_S B + \sum_i \; g_{Ni}\mu_{Ni}M_{Ii}(1-\sigma_i)B + \sum_i a_i\, M_S M_{Ii} \tag{8}$$

M_S is the quantum number that measures the component of the spin angular moment along the field direction (z). The a_i stand for the hyperfine coupling constant which denotes the interaction between the nucleus and the electron. The nuclear Zeeman term in eq. 8 does not change for levels involved in an ESR transition ($\Delta M_S = \pm 1$) and will be omitted in the following. For a hydrogen atom with I=1/2, I_Z (or m_I)=$\pm 1/2$. We thus obtain the energy levels shown in Figure 2.
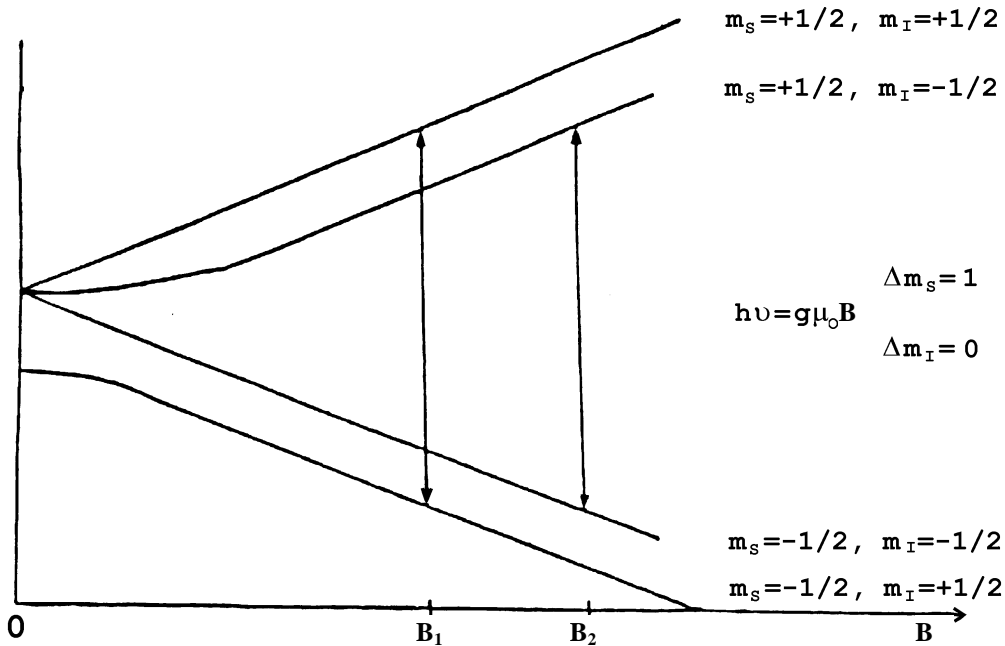

Figure 2. Magnetic field dependence of the energy levels of an S=1/2 electron spin system interacting with an I=1/2 nuclear spin.

If a constant rf field at frequency ν is applied to this system and the magnetic field is swept, there will be two resonances at when the rf frequency matches the energy level splitting, $h\nu = \Delta E$. The two resonances will appear at the magnetic field values B_1 and B_2 pictured by the arrows in Figure 1.
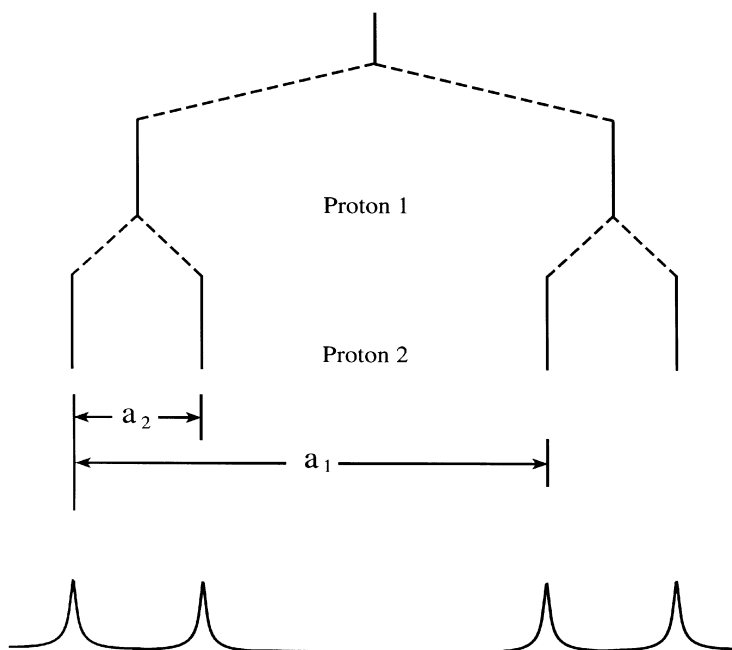
$$B_1 = \frac{h\nu - a/2}{g\mu_e} \quad \text{and} \quad B_2 = \frac{h\nu + a/2}{g\mu_e} \tag{9}$$

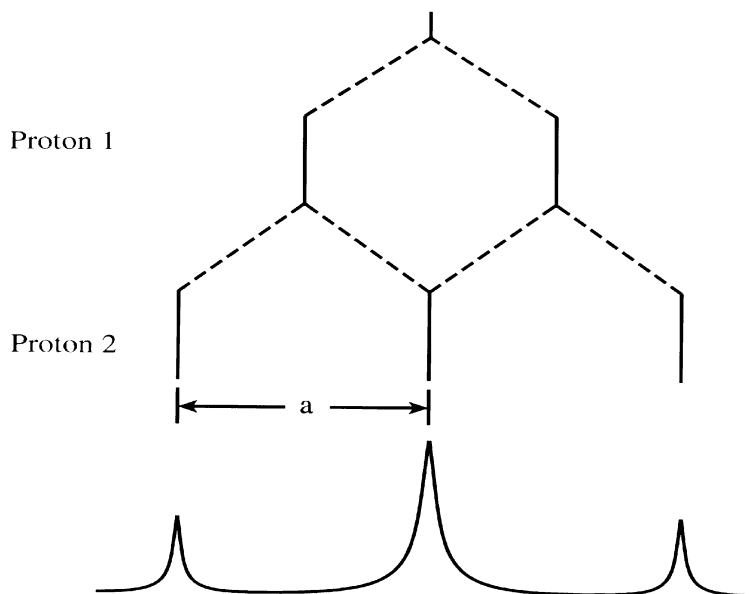The splitting constant a is then obtained from:

$$\Delta B = B_2 - B_1 = \frac{h\nu + a/2}{g\mu_e} - \frac{h\nu - a/2}{g\mu_e} \tag{10}$$

Thus, $a = g\mu_e\Delta B$; but note that a is usually reported directly in gauss. This splitting is known as <u>hyperfine structure</u> (hfs), and a as the <u>hyperfine constant (**hfc**)</u>.

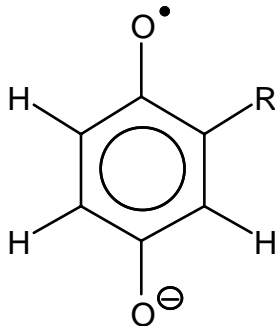One proton gives a **hfc** of two lines. Two inequivalent protons give a pair of doublets.

Two <u>equivalent</u> protons give the following pattern (since $a_1 = a_2$):

In general *n* equivalent protons give a spectrum of *n+1* hyperfine lines.   The spacing between each two adjacent lines is the hfs constant *a*. The intensities are given by a binomial expansion.

$$I(M) = \frac{n!}{(\frac{n}{2} + M)! \, (\frac{n}{2} - M)!} \qquad \text{where} \qquad M = \frac{-n}{2}, \frac{-n}{2} + 1, ..., \frac{n}{2} + 1, \frac{n}{2} \ . \qquad (11)$$

Consider the hyperfine structure of the semiquinone radical anion

The delocalized unpaired electron is distributed over the entire molecular frame consisting of 6 C atoms and 2 O atoms. With R = H, by symmetry, it is clear that the four protons are all equivalent; hence, five hyperfine lines, with relative intensities 1 : 4 : 6 : 4 : 1, are expected in the **ESR** spectrum of the unsubstituted para-benzosemiquinone radical anion. By contrast with R ≠ H none of the three protons are related by symmetry, and thus all may be expected to possess different **hfs** constants. For example, a hyperfine structure pattern of eight unequally spaced lines of equal intensity is expected for R=t-butyl.

The **hfc** is the sum of the **isotropic hfc** (Fermi contact) and **anisotropic hfc** (dipole-dipole interactions). The magnitude of the anisotropic coupling depends on the orientation of the molecule relative to the external magnetic field. In isotropic solutions the dipolar interactions are averaged out, and therefore only the orientation-independent isotropic coupling is observed.

Interpretation of the observed **hfc** in ESR spectra depends on understanding the origin of the hyperfine interaction and its relation to other molecular properties. For a free hydrogen atom, the magnitude of *a* depends upon the electron density at the nucleus ("Fermi Contact Interaction"):

$$a = (8\pi/3)g_e \, \mu_e \, g_H \, \mu_H \, \rho(0) \qquad (12)$$

where $\rho(0) = |\psi(0)|^2$ is the unpaired electron density at the H nucleus. For a "pure" s orbital on a H atom, the value for *a* in gauss is 507. For the radicals that you will study, *a* is ca. 2-4 gauss; i.e. 5-7 % of the limiting value of 507 Gauss. In this experiment you will calculate the spin densities (see Appendix 1). The sign of the calculated *a*'s may be either negative or positive.

II. References

1. John E. Wertz and J. R. Bolton, *Electron Spin Resonance: Elementary Theory and Practical Applications,* McGraw-Hill, New York, 1972.

2. D.J. Pasto, C.R. Johnson, and M.J. Miller, *Experiments and Techniques in Organic Chemistry*, Prentice-Hall, Englewood Cliffs, N.J., 1992.

3. A. Carrington and A. D. McLachlan, *Introduction to Magnetic Resonance*, Harper and Row, New York, 1967, pp. 1-23, 72-175.

III. Experimental Section

This experiment includes two parts:

(A) A brief examination of the ESR spectra of $Mn(OAc)_2$ and perylene, examples of transition metal species and organic free radicals, respectively. These will also provide an opportunity to become familiar with operation of the spectrometer.

(B) An investigation of the **ESR** spectra of several substituted benzosemiquinone radical species, *viz*., unsubstituted benzosemiquinone radical and two of the following derivatives: methyl-, isopropyl- and *t*-butyl-benzosemiquinone. This will include interpretation of spectra using molecular orbital calculations (see Appendix 1), elucidation of splitting constants from complex spectra, and examination of substituent effects in **ESR** spectra.

This experiment will be done in groups of three students. The **Bruker EMX EPR** instrument is located in Instrumentation Facility, Room 18-082 (http://web.mit.edu/speclab/www/). Lab partners should attend the lecture-demonstration on use of the instrument and learn to operate the instrument to record spectra of satisfactory resolution. Stock solutions should be prepared in advance so that efficient use of available instrument time can be made.

A. Manganese Acetate, Perylene, and ESR Spectrometry Procedures

Obtain spectra of perylene in H_2SO_4 (solution available from TA) and 1×10^{-2} M $Mn(OAc)_2$ in H_2O. Use the following procedure for all samples run in this experiment. Degas the stock solution by bubbling nitrogen through it for several minutes if required. (For some spectra it is important to remove dissolved O_2 from the solution because the presence of significant concentrations of this paramagnetic species produces broadening of the sample spectra.) Fill a melting point capillary at least halfway with the solution to be studied; there should be no bubbles. A syringe or disposable pipette drawn out to have a long thin tip can be used for this; consult the TA. The needle or pipette tip should reach the bottom of the capillary to avoid getting air bubbles when the tube is filled. The filled capillary tube is placed in a regular quartz or pyrex ESR tube and capped. The TA will demonstrate how to insert the ESR tube into the cavity. Signals for both $Mn(OAc)_2$ and perylene should appear at about 3400 gauss. Center the spectrum, choose a suitable scan width, adjust microwave

power, receiver gain (about 10^5), and modulation amplitude (about 1.0) for optimum resolution and/or signal-to-noise. In using the ESR instrument, note that the scan range control setting corresponds to the full width of the chart paper (the range markings on the paper are correct). Using the perylene sample, note especially how to get optimum resolution since it will be necessary to do this quickly with some of the samples in Part B which require good resolution to distinguish the structure, but which have only a limited lifetime once prepared. Half-width-at-half maximum (HWHM) of 0.1 gauss for perylene and 20 gauss for $Mn(OAc)_2$ should be attainable, see Figure 3.
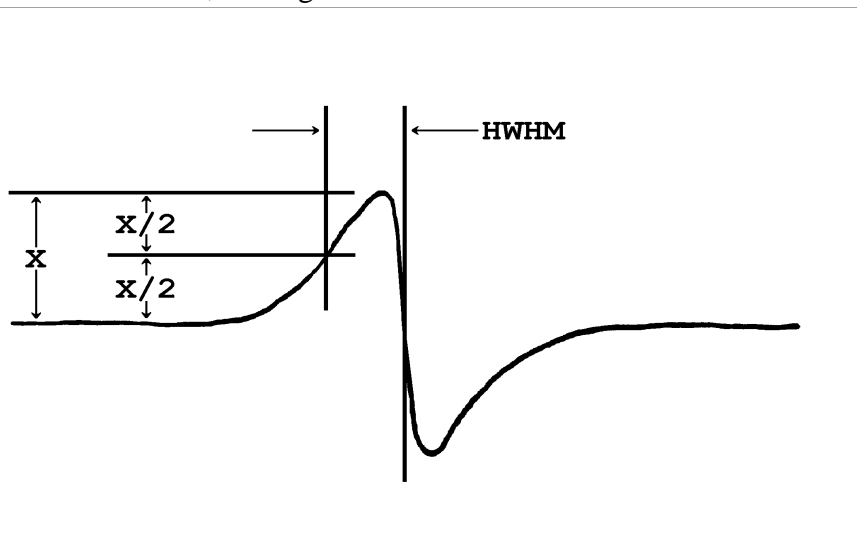

Figure 3. Measurement of HWHM for an ESR line

B. Substituted Benzosemiquinones

Commercial samples of the unsubstituted, *t*-butyl-, isopropyl-, and methyl-hydroquinones are available in the lab. If further purification of these is required (consult TA), recrystallize a small portion (or obtain a recrystallized sample from the TA or another group). You are encouraged to turn in excess supplies of specially purified materials to the TA so that they may be made available to other students.

Semiquinone radicals are produced through base-induced oxidation of the hydroquinone by molecular oxygen. It is thus presumably not necessary to bubble nitrogen through the solution to remove oxygen. However, excess O_2 could still cause broadening of the spectrum, and too little O_2 could still result in unnecessarily slow build up of the radical product, so some experimentation with solution oxygen is useful. Radical concentration will increase over a period of time as the oxidation reaction proceeds, and then decay as radical-radical reactions and other processes destroy the radicals. Impurity radical species may tend to build up with time. Rates for these processes will depend on concentration of the hydroquinone in the alkaline methanol, ethanol or acetonitrile medium, temperature, and other parameters.

Prepare 1 M solutions of the hydroquinones in methanol or acetonitrile (ask your TA which hydroquinones and solvent you will work with; different groups will carry out measurements on different sets of samples). Make 1 mL each of unsubstituted, *t*-butyl, *i*-propyl, and/or methyl hydroquinone solutions. Pulverize a dry KOH pellet. Add a tiny

amount directly to the t-butyl and i-propyl solutions and about 1/4 pellet to the methyl and unsubstituted hydroquinone solutions. Before recording the ESR spectrum, wait until the solution begins to turn yellow and then transfer liquid (leaving solid KOH residue at bottom of test tube) into ESR tube. If it turns dark red or black, you either added too much KOH or waited too long. The *tert*-butyl semiquinone is produced very rapidly on mixing of the solutions; mix immediately before use. The methyl semiquinone radicals are also produced very rapidly, but have been found to be stable in solution for up to 2 hours. The unsubstituted semiquinone takes about 15 to 30 minutes to reach maximum concentration. The ESR signals for all of the semiquinone radical anions should appear in the range 3370 to 3390 gauss.
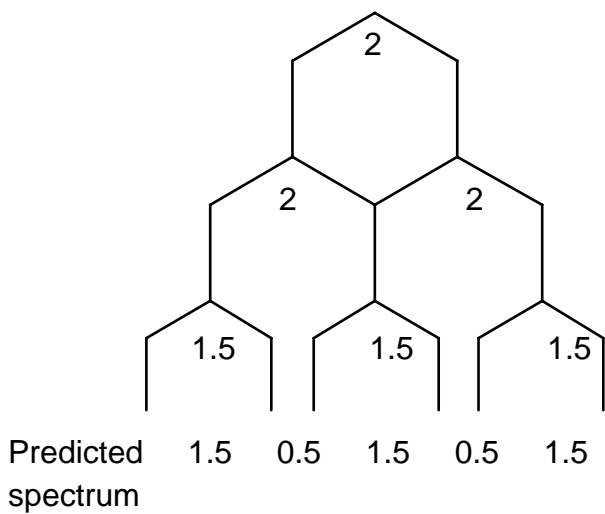
Obtain spectra for the semiquinone radical anions you are working with. All the expected lines in the unsubstituted and *t*-butyl spectra should be clearly resolved. In the methyl spectrum, it may prove impossible to obtain a recording showing all 32 expected lines clearly separated, but at least 20 separate lines should be readily distinguishable.
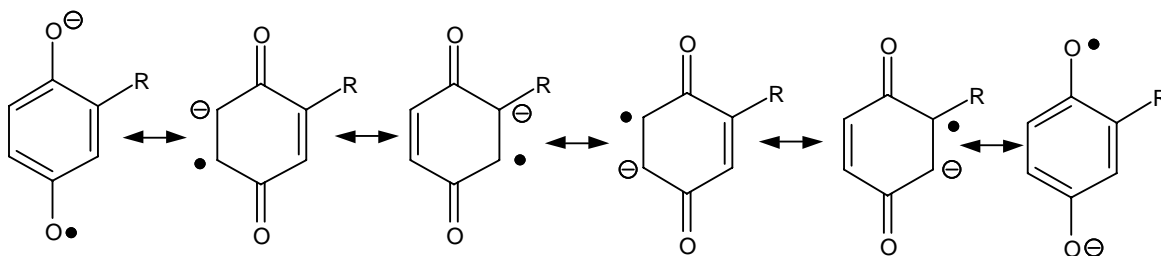
IV. Calculations

Each lab partner should do the calculations and discussion for *all* parts of this experiment.

A. Mn(OAc)$_2$ and Perylene. Measure and record the splitting in the Mn(OAc)$_2$ spectrum; is it uniform across the set of lines? (Should it be?)

B. Substituted Benzosemiquinones. Analyze the spectrum of the of unsubstituted, *t*-butyl, and isopropyl benzosemiquinone radicals to obtain the hyperfine splitting constants, a_H. (Use the data you collected and obtain any additional spectra you need from another group, giving the source of the data.) In the case of the methyl derivative, carry out the calculation described below and attempt to reconstruct the ESR spectrum from the calculated hfs constants. Note that the separation between the two outermost lines in the methyl hydroquinone spectrum is given by a simple linear combination of the constants. Construct a predicted stick-spectrum derived from your constants. For example, given two hydrogens with splitting constant of 2 plus one hydrogen with splitting constant of 1.5, you would draw

Predicted   1.5   0.5   1.5   0.5   1.5
spectrum

Now compare your stick spectrum to your actual ESR spectrum. In the analysis of the ESR spectra of this series of related radical species, we wish in part to focus on the identification of systematic effects of varying the substituent through the series -H, -CH$_3$, -CH(CH$_3$)$_2$-and -C(CH$_3$)$_3$. It is desirable in such an analysis to have a theoretical model to use in trying to understand the mechanism underlying the observed effects. In this case we need to be able to describe the distribution of spin density, i.e. the average occupancy of the unpaired electron on each atomic center. A possible model for describing such aspects of the electronic structure is the valence bond model in which the distribution is viewed as the weighted average over a series of possible resonance structures:

Note that there may also be resonance structures in which the radical and/or anion site is located in the substituent R.

In the computational part of the experiment, you will carry out a molecular orbital calculation of electron spin densities in the unsubstituted, methyl-substituted, isopropyl-substituted benzosemiquinones.

VI.   Notebook and Discussion

Record all observations and procedures in your laboratory notebook as they occur in the lab. The oral report of each lab partner should be complete and independent; indicate clearly who did what in the lab. Only one complete set of spectra need be produced. These should be referred to and "incorporated" in the notebook; <u>all calculations and analysis of the spectra should be done directly in the notebook.</u> If you find they are too messy, add a summary section to organize the significant results.

Your report should include the following:

1. Interpreted ESR spectra of Mn(OAc)$_2$ and Perylene.

2. ESR spectra of unsubsituted benzosemiquinone (in acetonitrile and in methanol) and of the three alkyl-substituted benzosemiquinones. *Clearly indicate which spectra were recorded by your group and which were provided by another group* (give source).

3. Comparison of BSQ spectra in acetonitrile and methanol. What is the magnitude of the solvent effect on the hfs constant? How do you interpret this solvent shift?

4. Analysis of isopropyl and *t*-butyl benzosemiquinone spectra, and derived values of hfs constants.

5. Theoretical computation of hfs constants for all systems studied. *Clearly indicate which calculations you did and which were provided by another group* (give source).