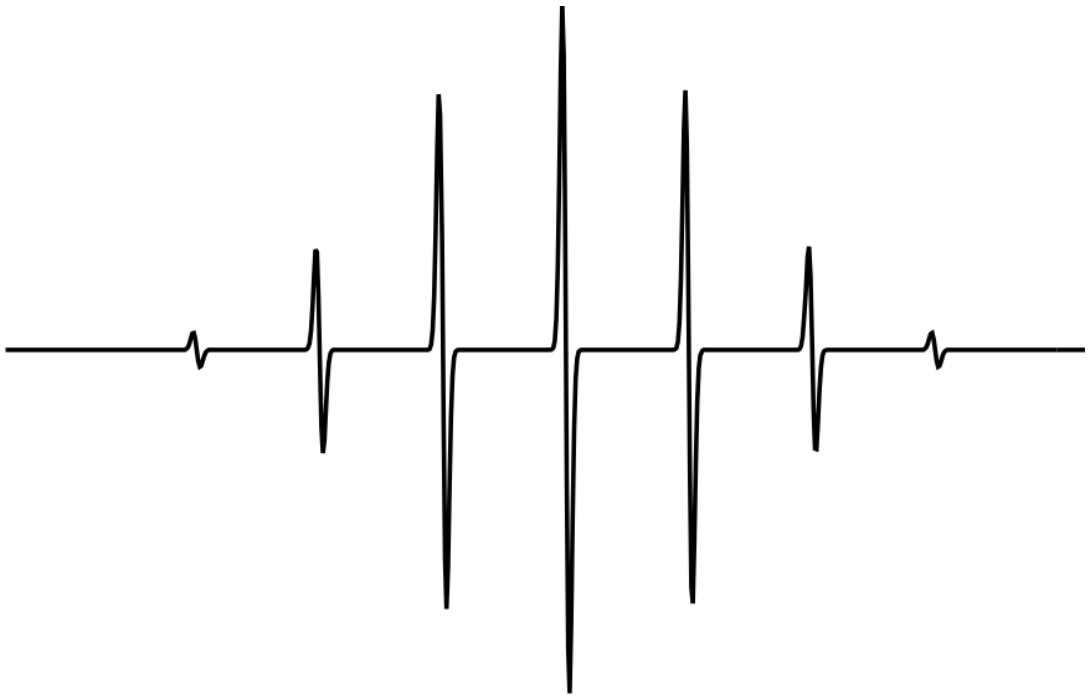
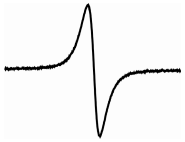
Electron Spin Resonance Spectroscopy





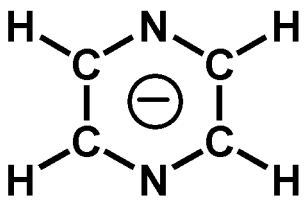
ESR Spectroscopy

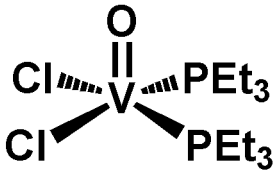
- Electron Spin Resonance Spectroscopy
- Also called EPR Spectroscopy
  - Electron Paramagnetic Resonance Spectroscopy
- Non-destructive technique

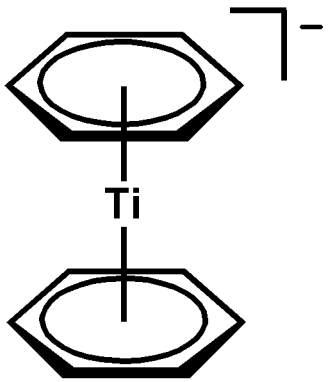
- Applications
  - Oxidation and reduction processes
  - Reaction kinetics
  - Examining the active sites of metalloproteins

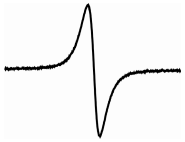
What compounds can you analyze?

- Applicable for species with one or more unpaired electrons
  - Free radicals
  - Transition metal compounds
- Useful for unstable paramagnetic compounds generated *in situ*
  - Electrochemical oxidation or reduction





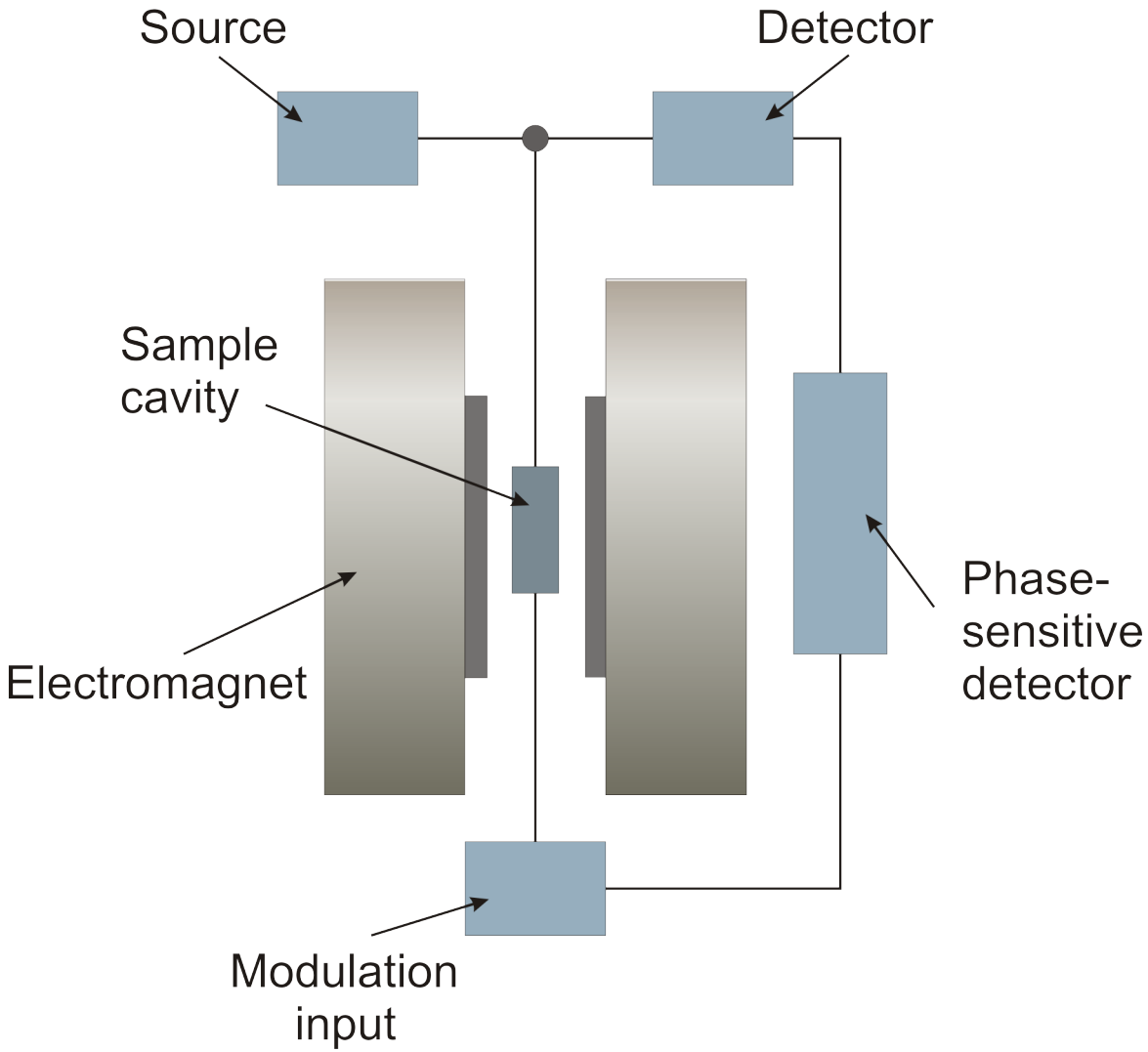


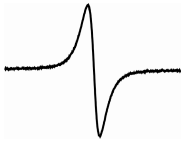


Energy Transitions

- ESR measures the transition between the electron spin energy levels
  - Transition induced by the appropriate frequency radiation
- Required frequency of radiation dependent upon strength of magnetic field
  - Common field strength 0.34 and 1.24 T
  - 9.5 and 35 GHz
  - Microwave region

How does the spectrometer work?





Describing the energy levels

- Based upon the spin of an electron and its associated magnetic moment
- For a molecule with one unpaired electron
  - In the presence of a magnetic field, the two electron spin energy levels are:

$$E = g\mu_B B_0 M_S$$

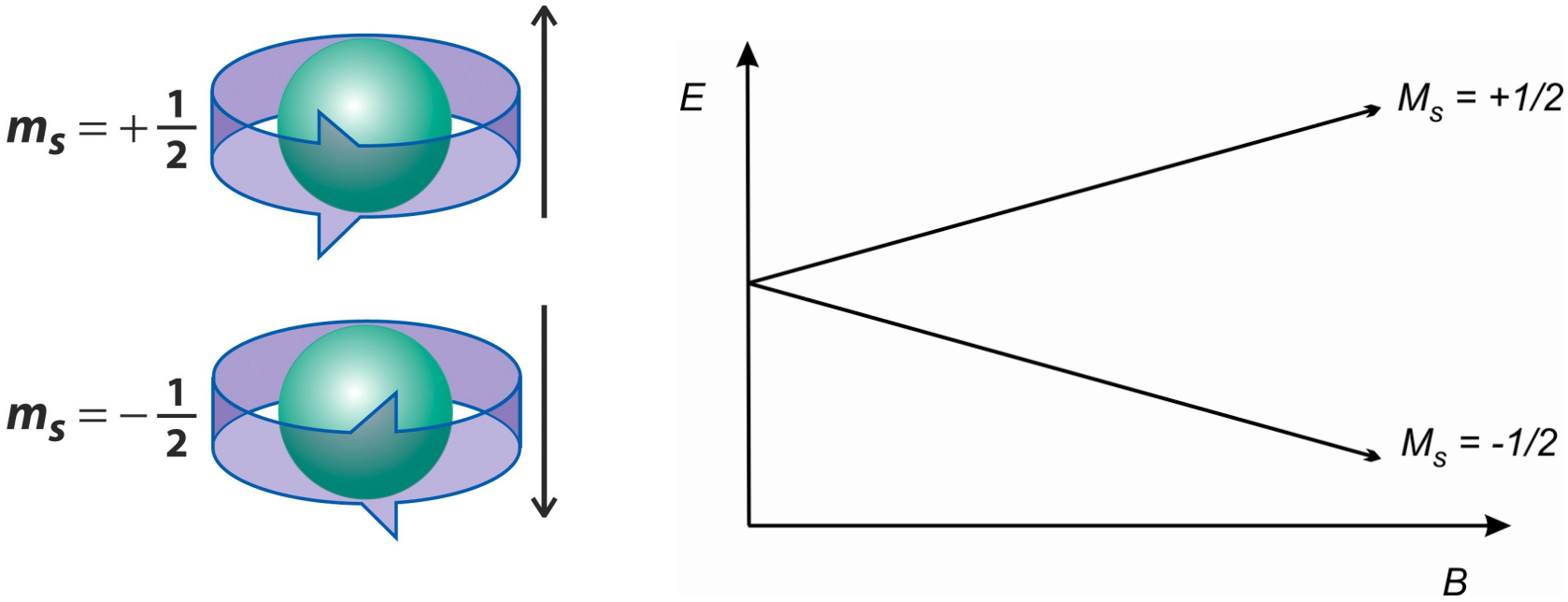
g = proportionality factor

μ_B = Bohr magneton

M_S = electron spin quantum number (+½ or -½)

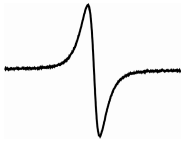
B_0 = Magnetic field

What causes the energy levels?



Resulting energy levels of an electron in a magnetic field

Ebsworth, E. A. V.; Rankin, David W. H.; Cradock, Stephen *Structural Methods in Inorganic Chemistry*; CRC Press: Boca Raton, 1987.

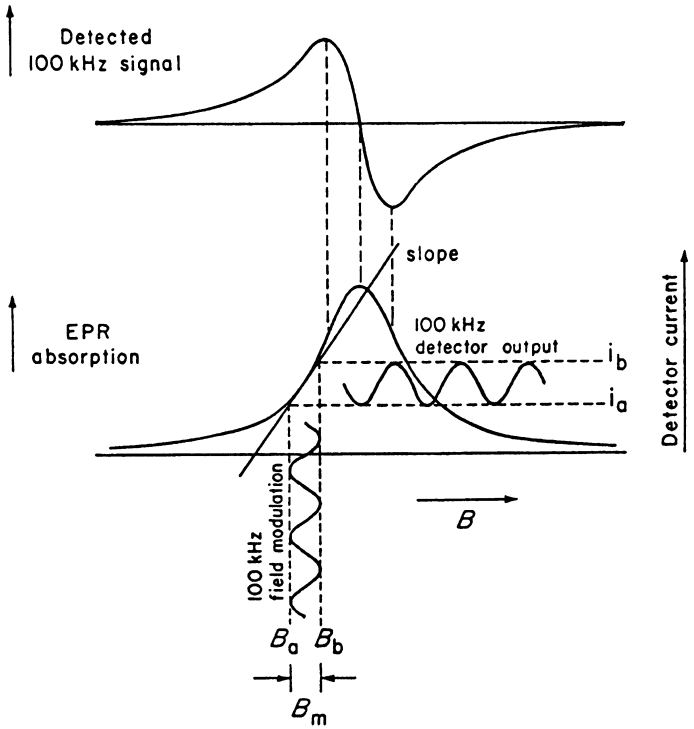


The EPR spectrum

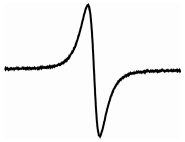
- A 1st derivative spectrum is obtained from the unpaired electron
- $h\nu = gB\beta_0$
- g is a characteristic of the chemical environment of the unpaired electron; for free radicals it is near 2.00; can vary widely for transition metal centers
- Complicated/enhanced by hyperfine interactions with nuclei with non-zero spin

The EPR spectrometer

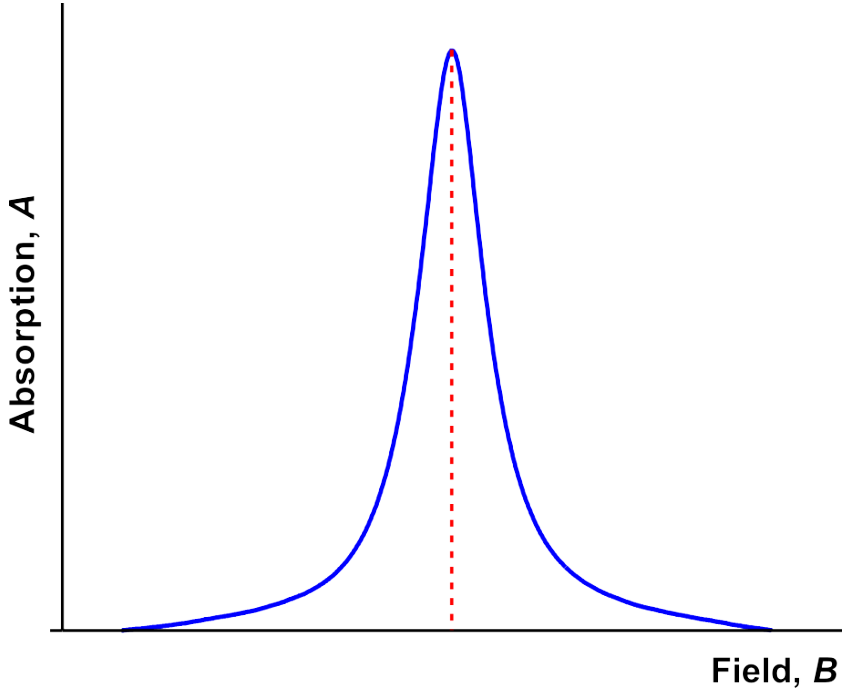
- Electromagnet
- Microwave source and detector (typically X band, ~9.5 GHz)
- Modulation of magnetic field and phase-sensitive detection
- Spectrum 1st derivative

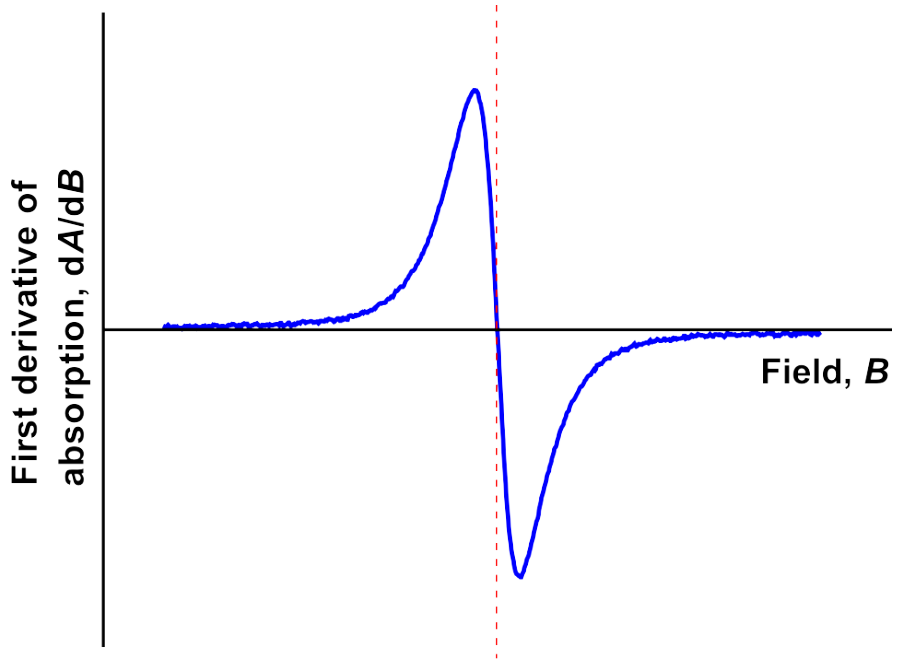


Weil, Bolton, and Wertz, 1994, "Electron Paramagnetic Resonance"



Spectra

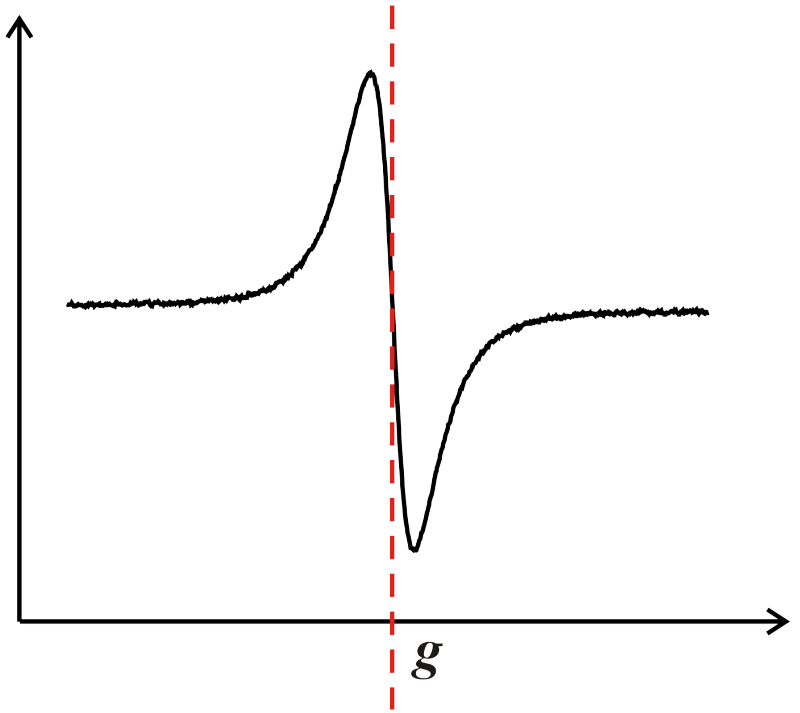


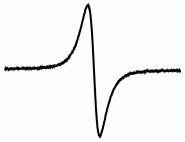


When phase-sensitive detection is used, the signal is the first derivative of the absorption intensity

Proportionality Factor

- Measured from the center of the signal
- For a free electron
  - 2.00232
- For organic radicals
  - Typically close to free-electron value
  - 1.99-2.01
- For transition metal compounds
  - Large variations due to spin-orbit coupling and zero-field splitting
  - 1.4-3.0





Proportionality Factor

$MoO(SCN)_5^{2-}$	1.935
$VO(acac)_2$	1.968
e^-	2.0023
CH_3	2.0026
$C_{14}H_{10}$ (anthracene) cation	2.0028
$C_{14}H_{10}$ (anthracene) anion	2.0029
$Cu(acac)_2$	2.13

Atherton, N. M. *Principles of Electron Spin Resonance*; Ellis Horwood: Chichester, 1993.

Hyperfine Interactions

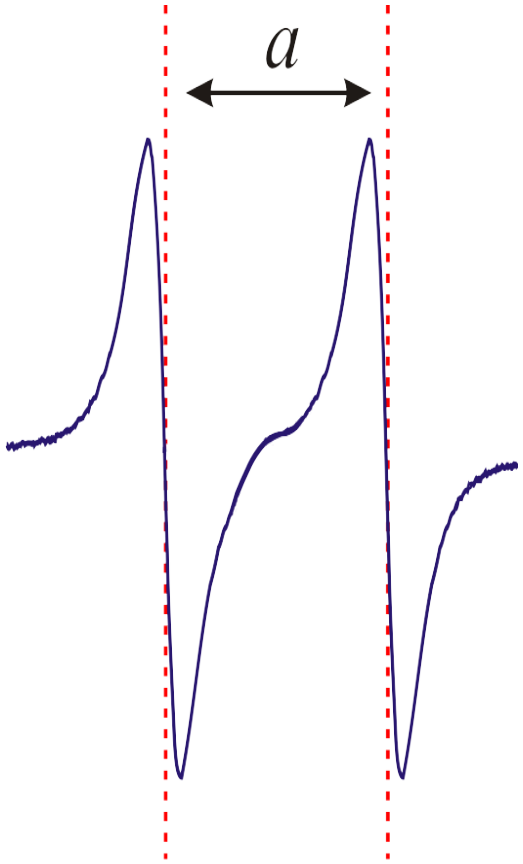
- EPR signal is ‘split’ by neighboring nuclei
  - Called hyperfine interactions
- Can be used to provide information
  - Number and identity of nuclei
  - Distance from unpaired electron
- Interactions with neighboring nuclei

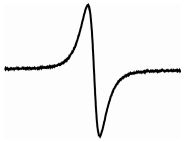
$$E = g\mu_B B_0 M_S + a M_s m_I$$

a = hyperfine coupling constant

m_I = nuclear spin quantum number

- Measured as the distance between the centers of two signals

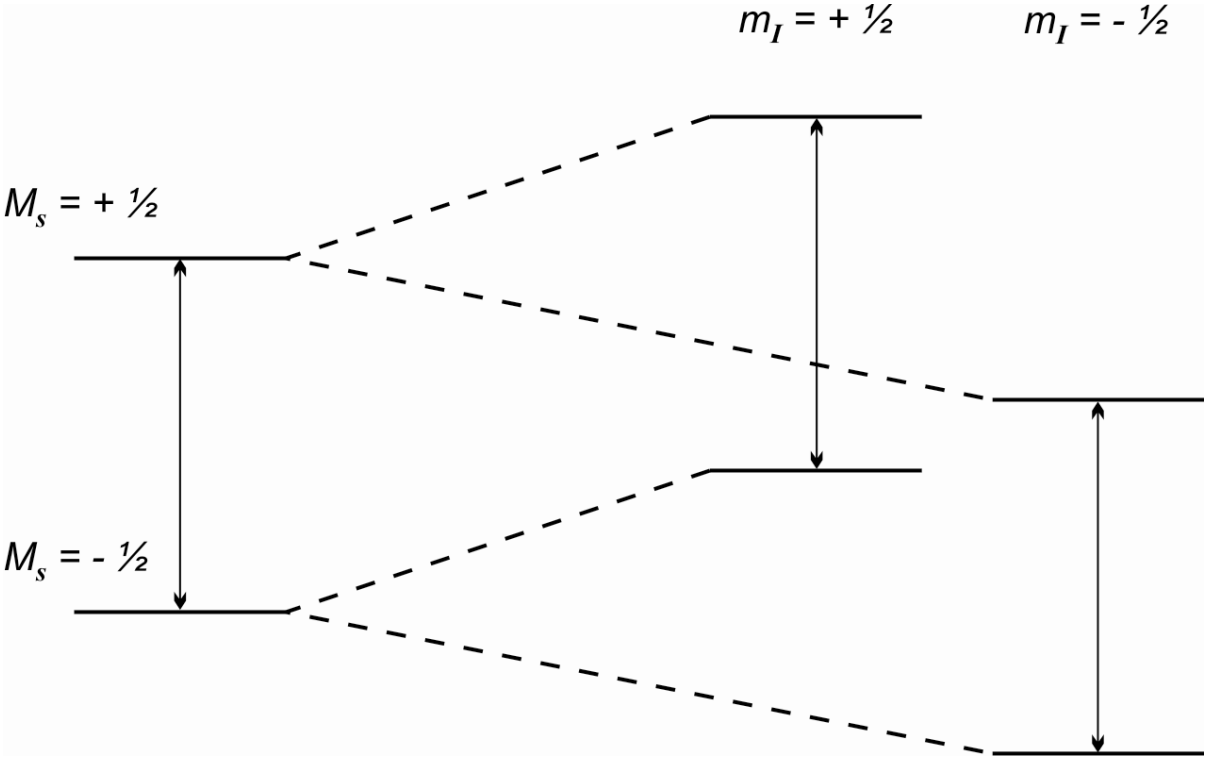




Which nuclei will interact?

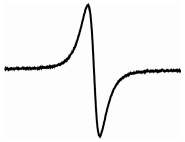
- Selection rules same as for NMR
- Every isotope of every element has a ground state nuclear spin quantum number, I
  - has value of $n/2$, n is an integer
- Isotopes with even atomic number and even mass number have $I = 0$, and have no EPR spectra
  - ^{12}C, ^{28}Si, ^{56}Fe, …
- Isotopes with odd atomic number and even mass number have n even
  - ^{2}H, ^{10}B, ^{14}N, …
- Isotopes with odd mass number have n odd
  - ^{1}H, ^{13}C, ^{19}F, ^{55}Mn, …

Hyperfine Interactions



Interaction with a single nucleus of spin ½

Ebsworth, E. A. V.; Rankin, David W. H.; Cradock, Stephen *Structural Methods in Inorganic Chemistry*; CRC Press: Boca Raton, 1987.



Hyperfine Interactions

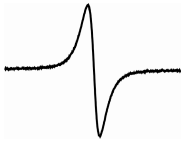
- Coupling patterns same as in NMR
- More common to see coupling to nuclei with spins greater than ½
- The number of lines:

$$2NI + 1$$

N = number of equivalent nuclei

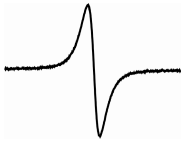
I = spin

- Only determines the number of lines--not the intensities



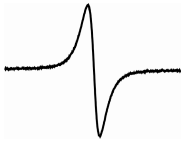
Hyperfine Interactions

- Relative intensities determined by the number of interacting nuclei
- If only one nucleus interacting
  - All lines have equal intensity
- If multiple nuclei interacting
  - Distributions derived based upon spin
  - For spin ½ (most common), intensities follow binomial distribution

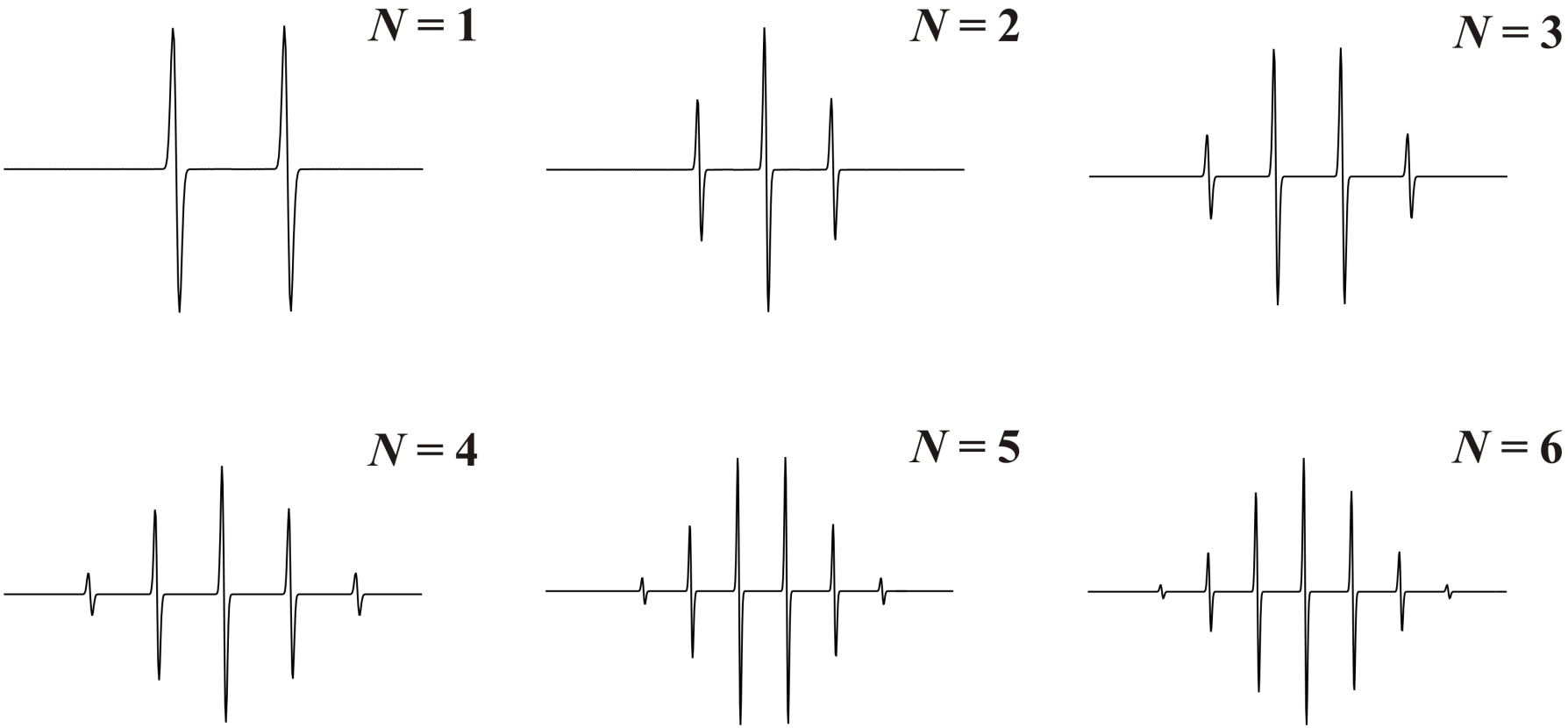


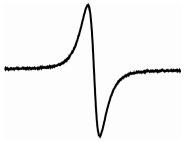
Relative Intensities for I = ½

N	Relative Intensities
0	1
1	1 : 1
2	1 : 2 : 1
3	1 : 3 : 3 : 1
4	1 : 4 : 6 : 4 : 1
5	1 : 5 : 10 : 10 : 5 : 1
6	1 : 6 : 15 : 20 : 15 : 6 : 1



Relative Intensities for $I = ½$

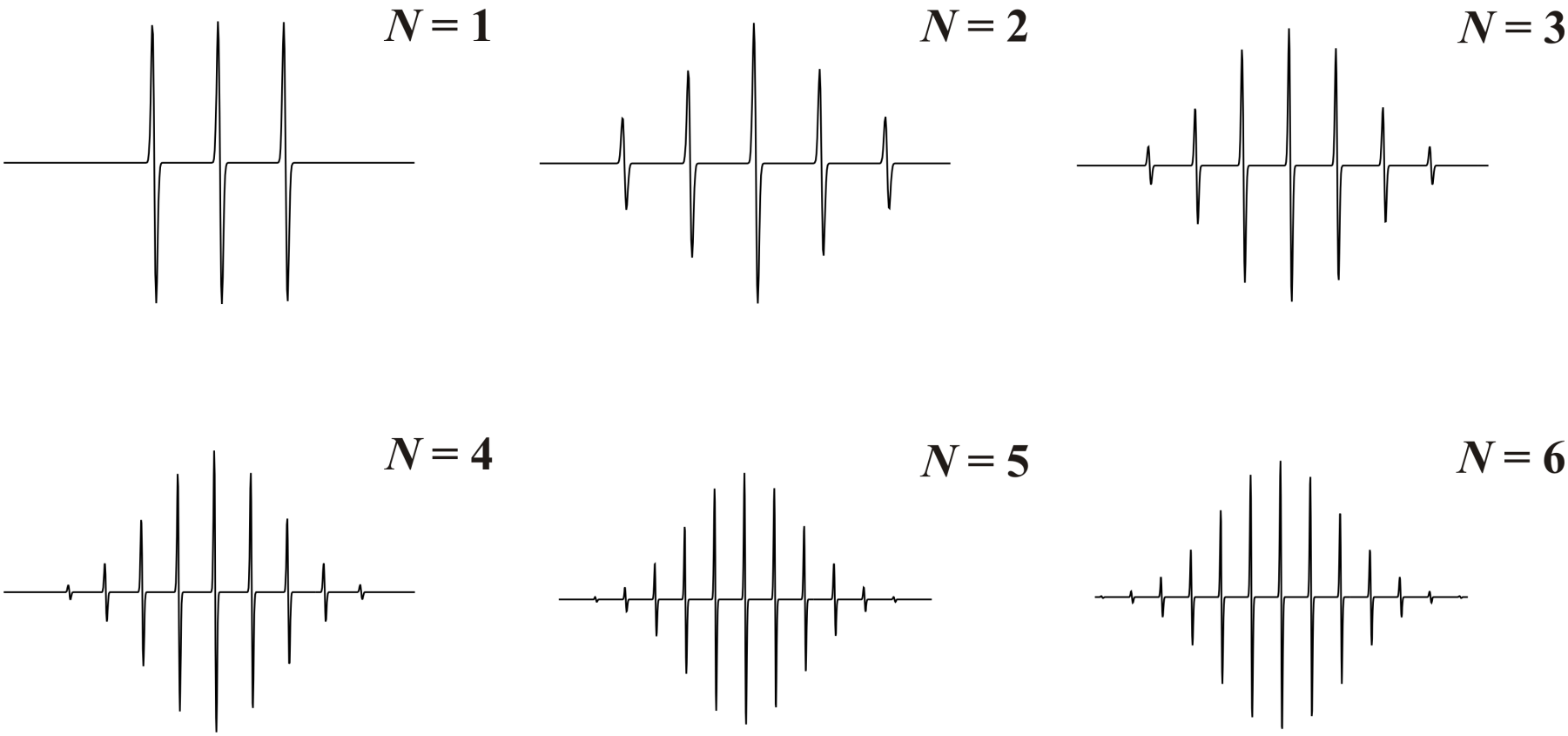


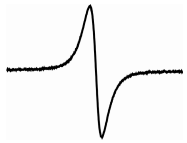


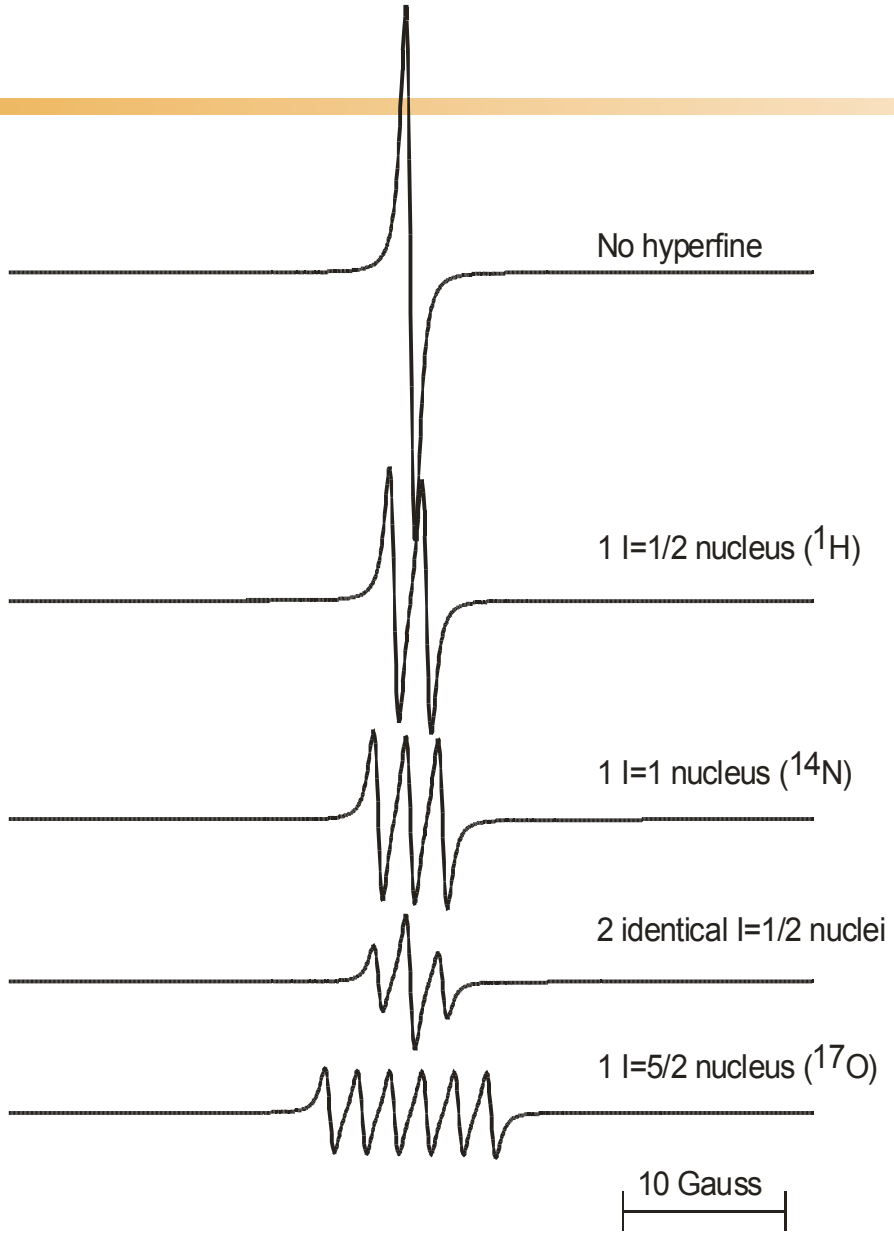
Relative Intensities for $I = 1$

N	Relative Intensities
0	1
1	1 : 1 : 1
2	1 : 2 : 3 : 2 : 1
3	1 : 3 : 6 : 7 : 6 : 3 : 1
4	1 : 4 : 10 : 16 : 19 : 16 : 10 : 4 : 1
5	1 : 5 : 15 : 20 : 45 : 51 : 45 : 20 : 15 : 5 : 1
6	1 : 6 : 21 : 40 : 80 : 116 : 141 : 116 : 80 : 40 : 21 : 6 : 1

Relative Intensities for $I = 1$

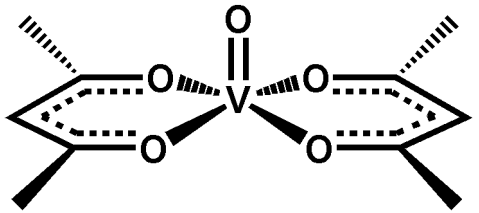





No hyperfine
1 I=1/2 nucleus (^{1}H)
1 I=1 nucleus (^{14}N)
2 identical I=1/2 nuclei
1 I=5/2 nucleus (^{17}O)
10 Gauss

Hyperfine Interactions

- Example:
  - $VO(acac)_2$
  - Interaction with vanadium nucleus

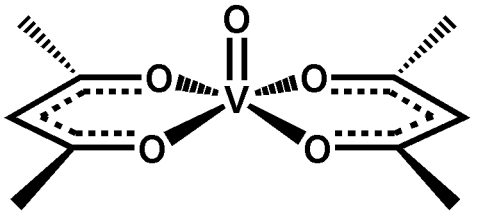


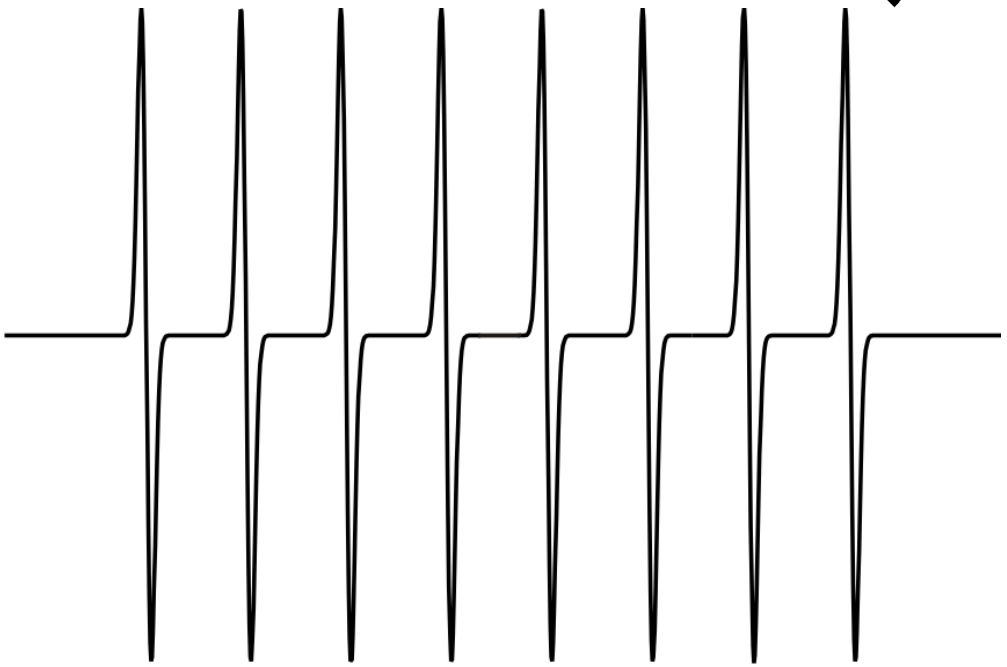
  - For vanadium, $\boldsymbol{I} = 7/2$
  - So,

$$2\boldsymbol{NI} + 1 = 2(1)(7/2) + 1 = 8$$

  - You would expect to see 8 lines of equal intensity

Hyperfine Interactions

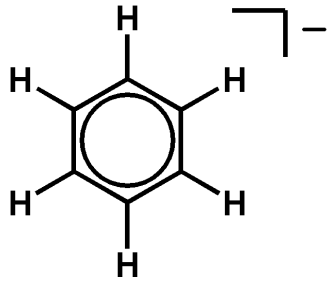




EPR spectrum of vanadyl acetylacetonate

Hyperfine Interactions

- Example:
  - Radical anion of benzene $[C_6H_6]^-$

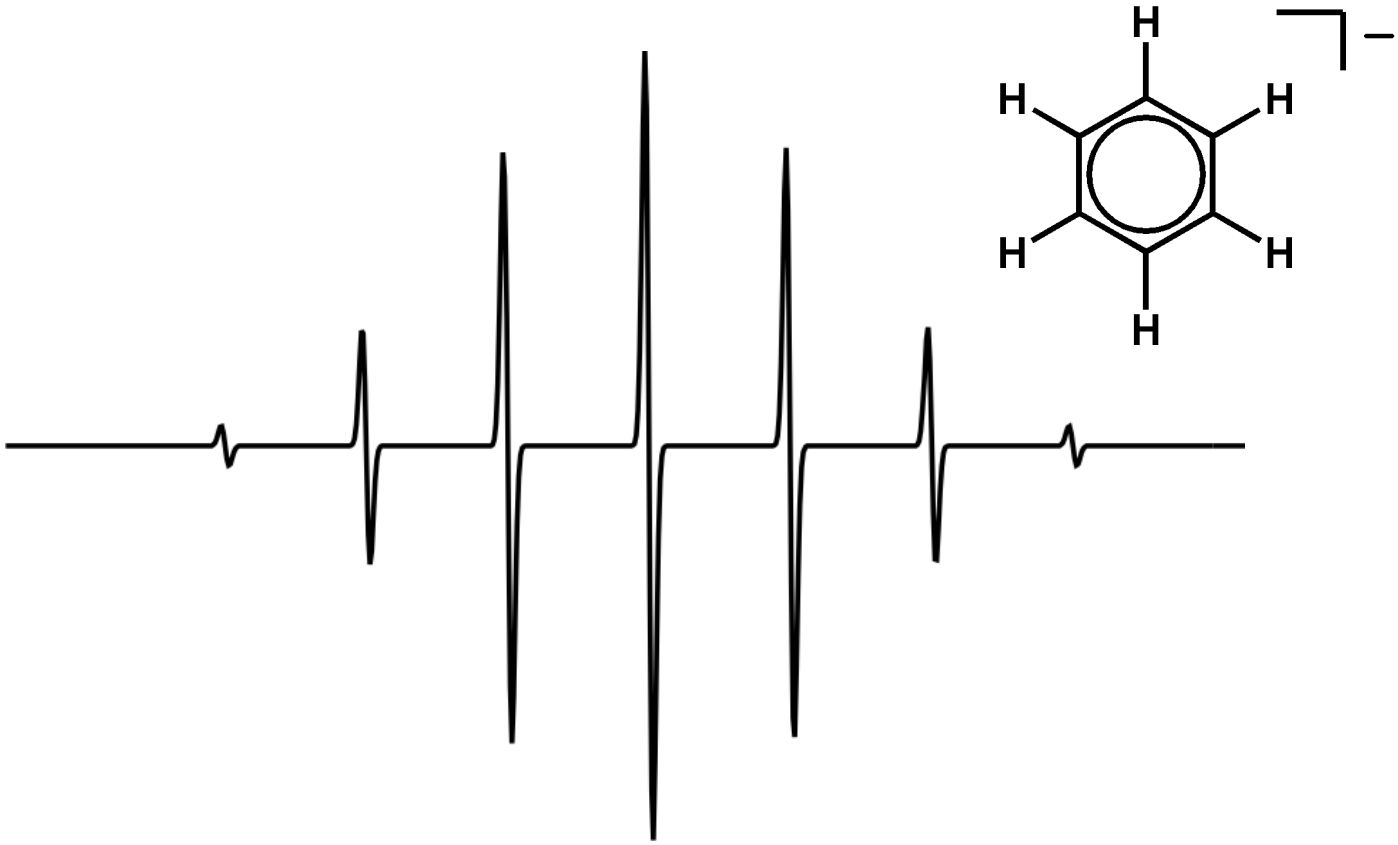


  - Electron is delocalized over all six carbon atoms
    - Exhibits coupling to six equivalent hydrogen atoms
  - So,

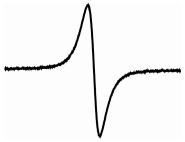
$$2NI + 1 = 2(6)(1/2) + 1 = 7$$

  - So spectrum should be seven lines with relative intensities 1:6:15:20:15:6:1

Hyperfine Interactions



EPR spectrum of benzene radical anion

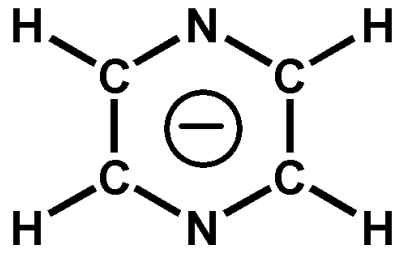


Hyperfine Interactions

- Coupling to several sets of nuclei
  - First couple to the nearest set of nuclei
    - Largest a value
  - Split each of those lines by the coupling to the next closest nuclei
    - Next largest a value
  - Continue 2-3 bonds away from location of unpaired electron

Hyperfine Interactions

- Example:
  - Pyrazine anion
  - Electron delocalized over ring



    - Exhibits coupling to two equivalent N (I = 1)

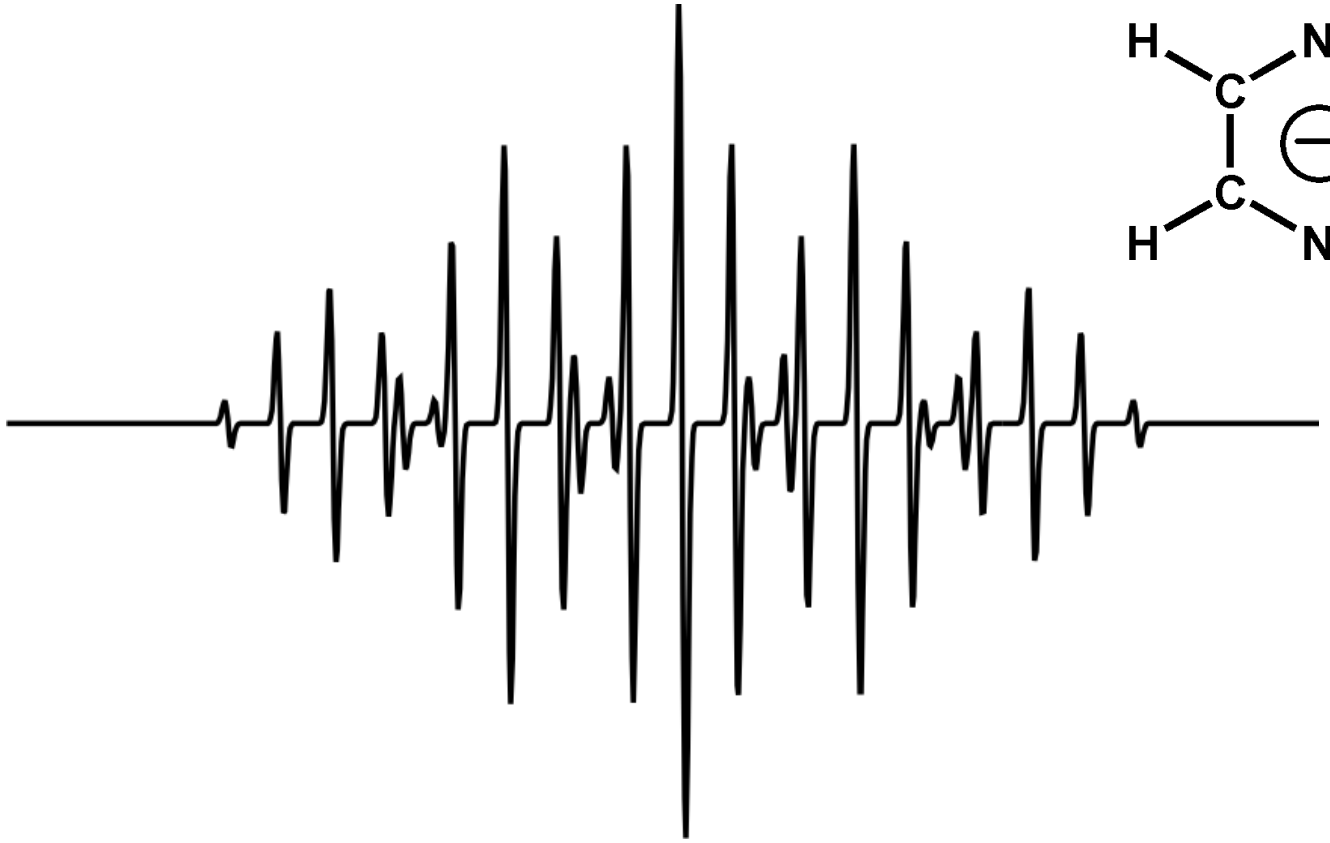
$$2NI + 1 = 2(2)(1) + 1 = 5$$

    - Then couples to four equivalent H (I = ½)

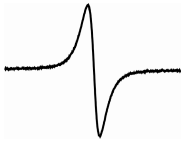
$$2NI + 1 = 2(4)(1/2) + 1 = 5$$

  - So spectrum should be a quintet with intensities 1:2:3:2:1 and each of those lines should be split into quintets with intensities 1:4:6:4:1

Hyperfine Interactions

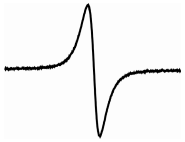


EPR spectrum of pyrazine radical anion



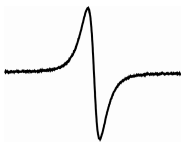
Conclusions

- Analysis of paramagnetic compounds
  - Compliment to NMR
- Examination of proportionality factors
  - Indicate location of unpaired electron
    - On transition metal or adjacent ligand
- Examination of hyperfine interactions
  - Provides information on number and type of nuclei coupled to the electrons
  - Indicates the extent to which the unpaired electrons are delocalized



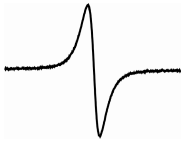
Direct EPR analysis of a radical

- Radical cannot be diatomic
- Radical must be available at a detectable concentration
  - At least metastable
  - Frozen solution to greatly decrease radical decay
    - Can greatly complicate the spectrum due to anisotropy
  - Continuous formation inside resonator
    - Enzymatic radical formation
    - Flow experiment
- Radical characterized by hyperfine analysis



Spin trapping: when direct EPR is not convenient or possible

- Unstable free radical reacts with diamagnetic molecule (the spin trap) to form a relatively stable free radical
- The vast majority of spin traps form radical adducts through the addition of the radical to the trap to form a nitroxide radical
- 2 major classes of traps: nitrones and nitroso compounds

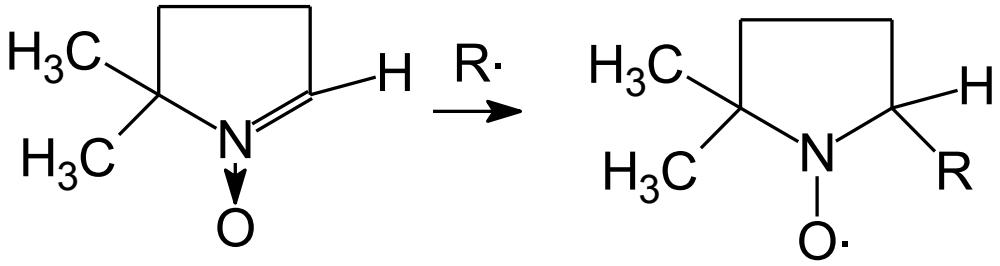


Advantages of the nitrones

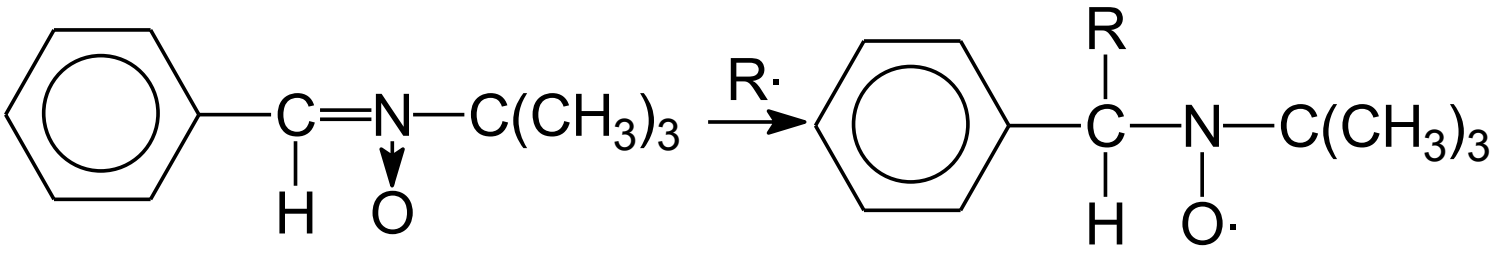
- React with a variety of different free radicals to form nitroxide adducts
  - $RC^{\cdot}$, $RO^{\cdot}$, $RS^{\cdot}$, in some cases $RN^{\cdot}$
- Adducts are often quite stable
- Not terribly toxic so amenable to *in vivo/ex vivo* spin trapping

Nitrone spin traps

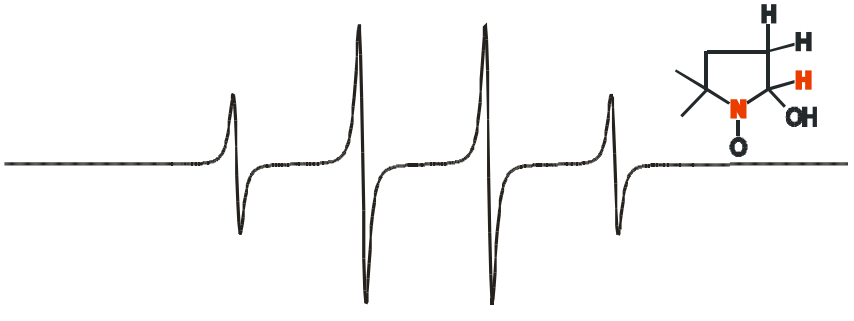
- DMPO, 5,5-dimethylpyrroline *N*-oxide

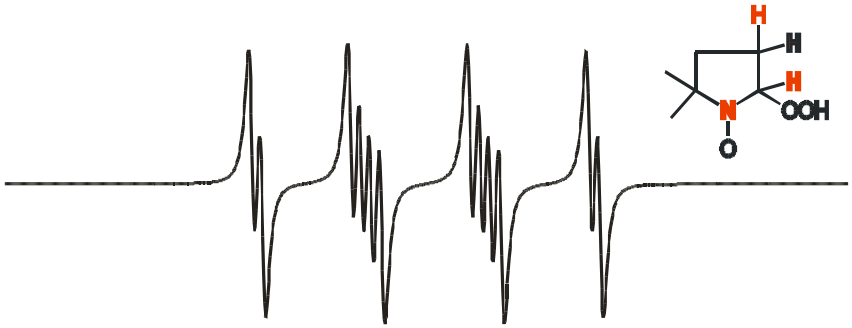


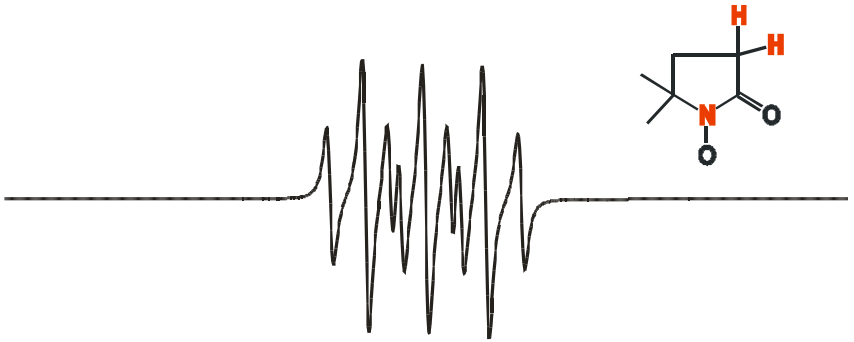
- PBN/4-POBN, phenyl-N-*t*-butylnitrone

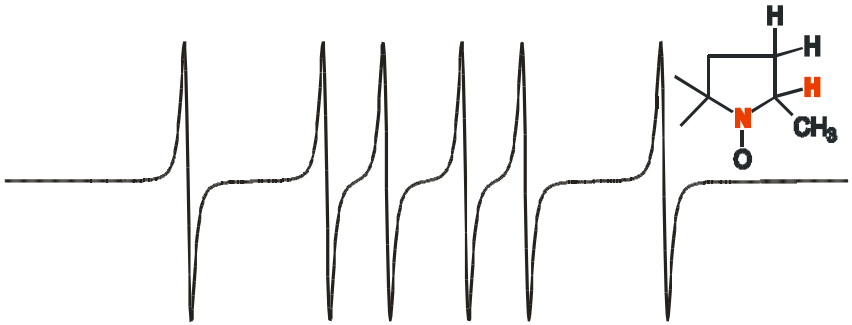


EPR spectra from DMPO adducts

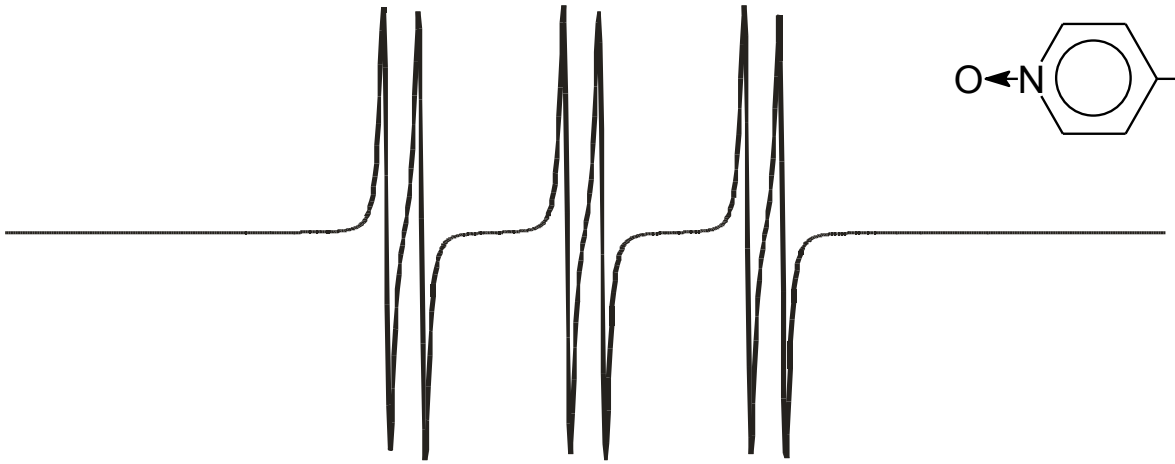


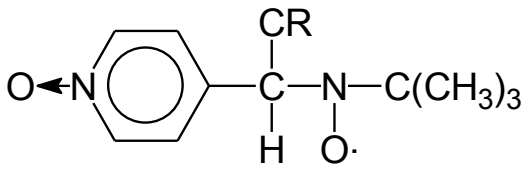


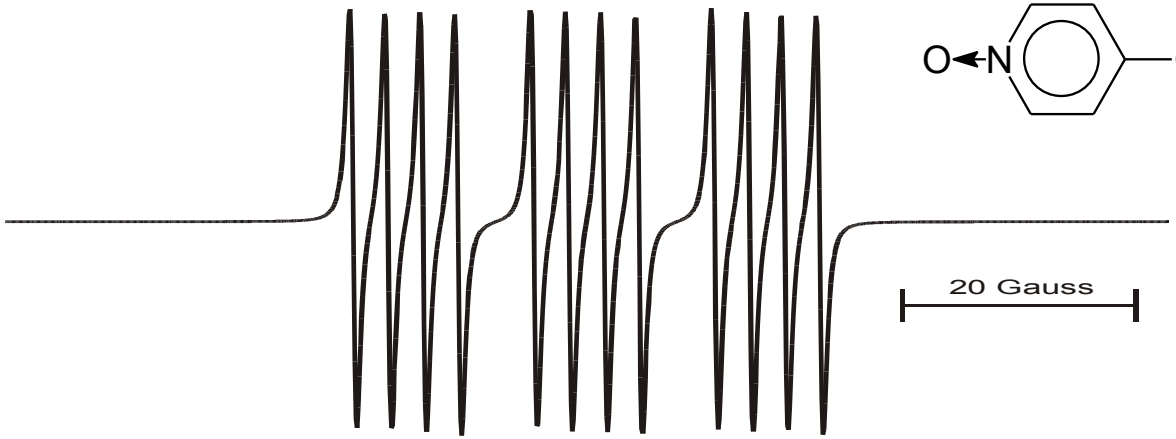


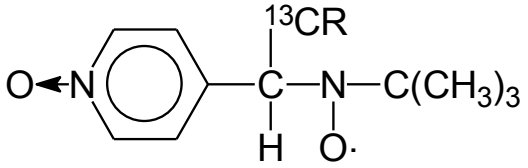


EPR spectra from 4-POBN adducts



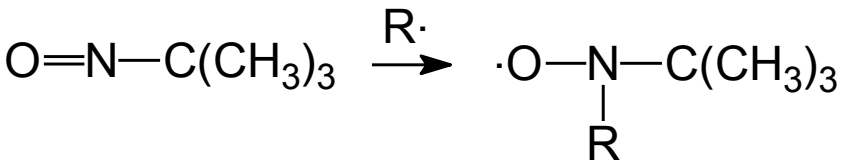




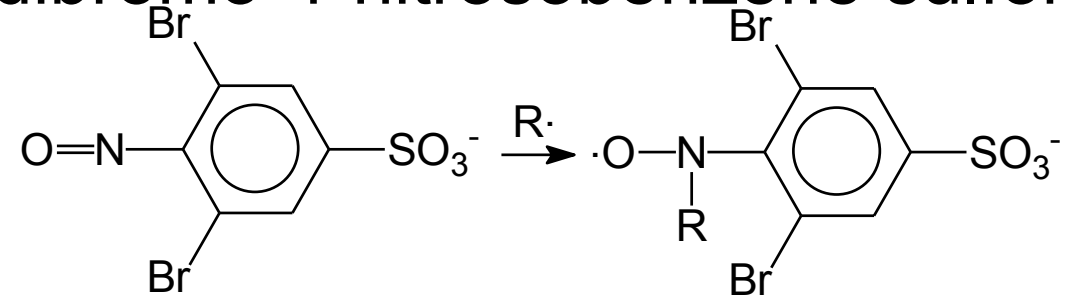


Nitroso spin traps

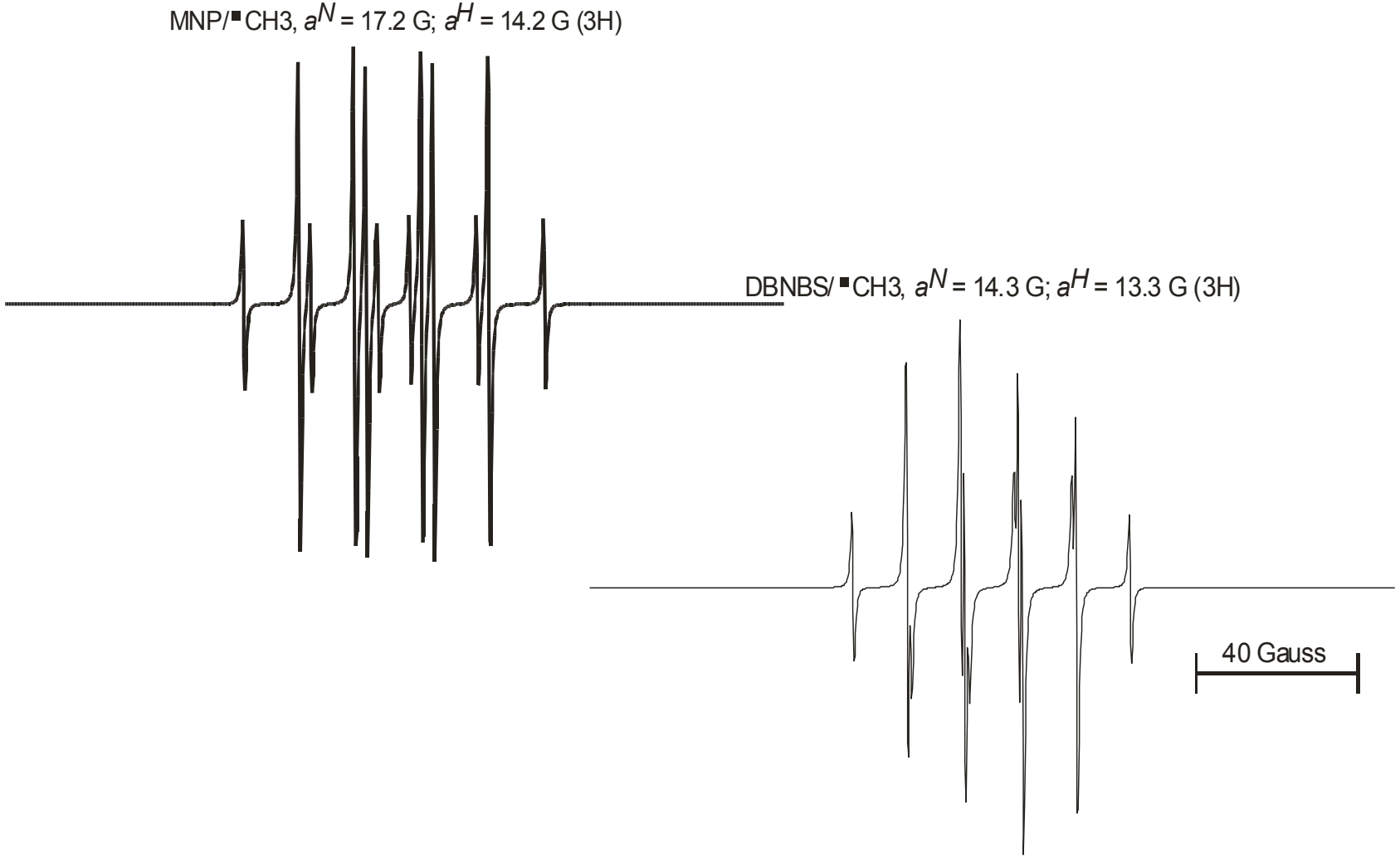
- Free radical adds to the nitrogen atom of a C-nitroso compound
- 2-methyl-2-nitrosopropane, MNP



- 3,5-dibromo-4-nitrosobenzene sulfonate

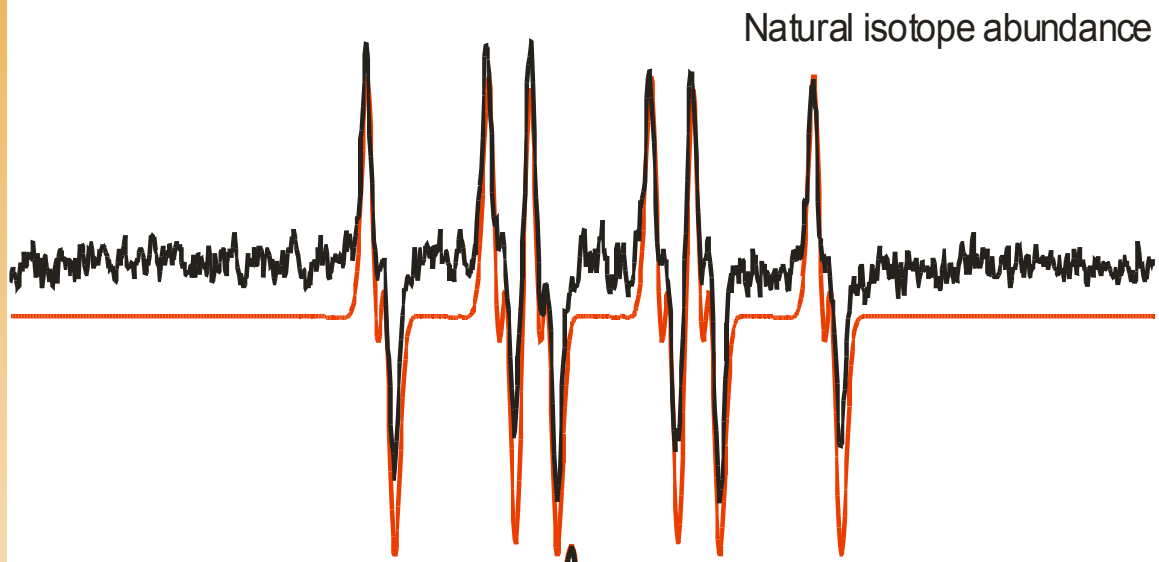


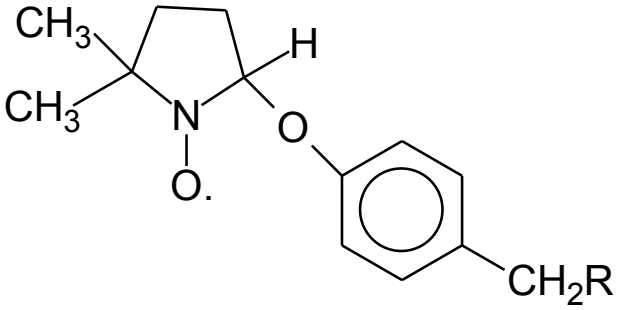
EPR spectra from methyl radical adducts of nitroso traps

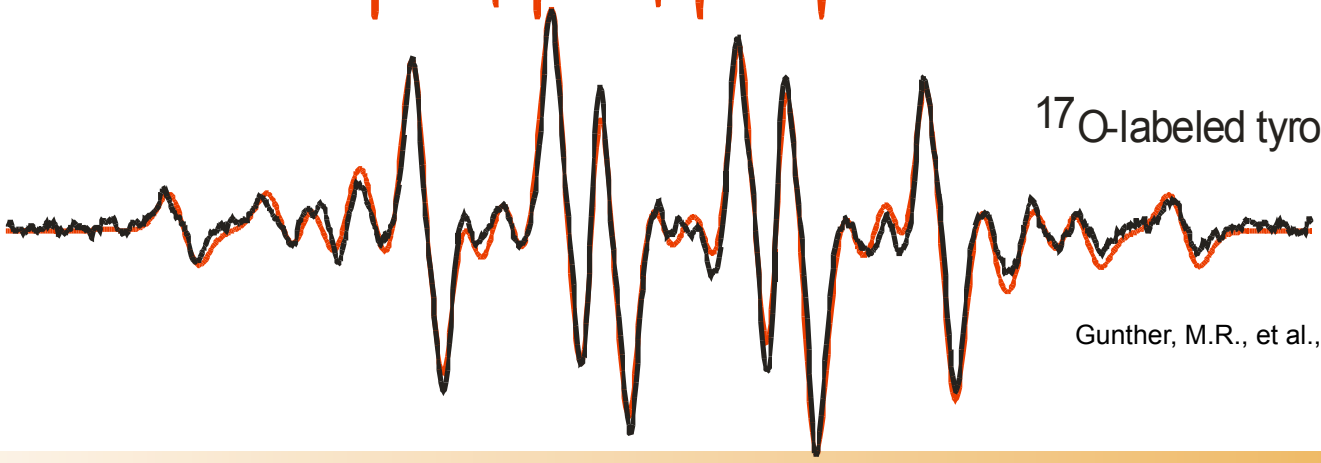


DMPO-trapping the tyrosyl radical

- Oxidize tyrosine with HRP/H_2O_2



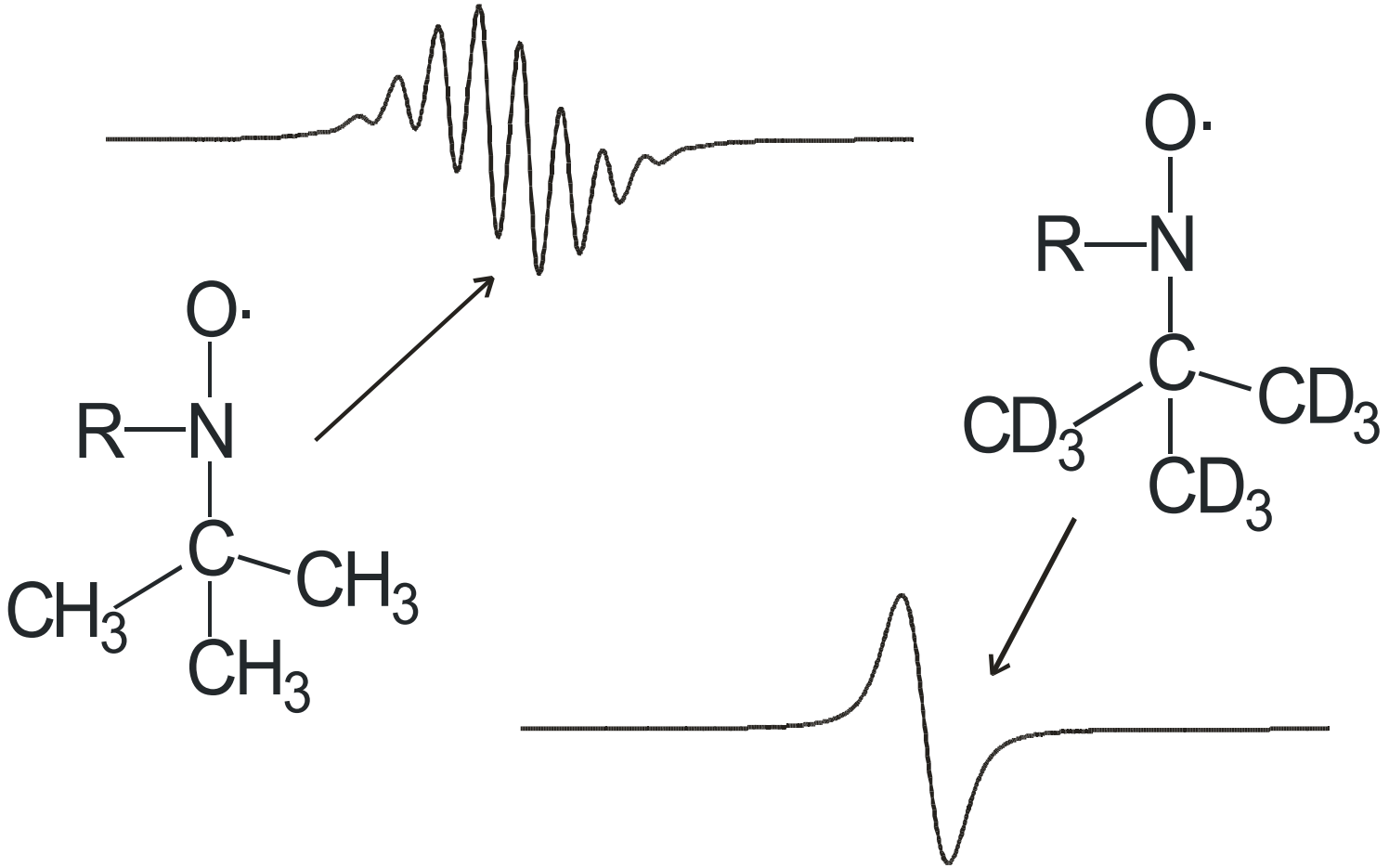




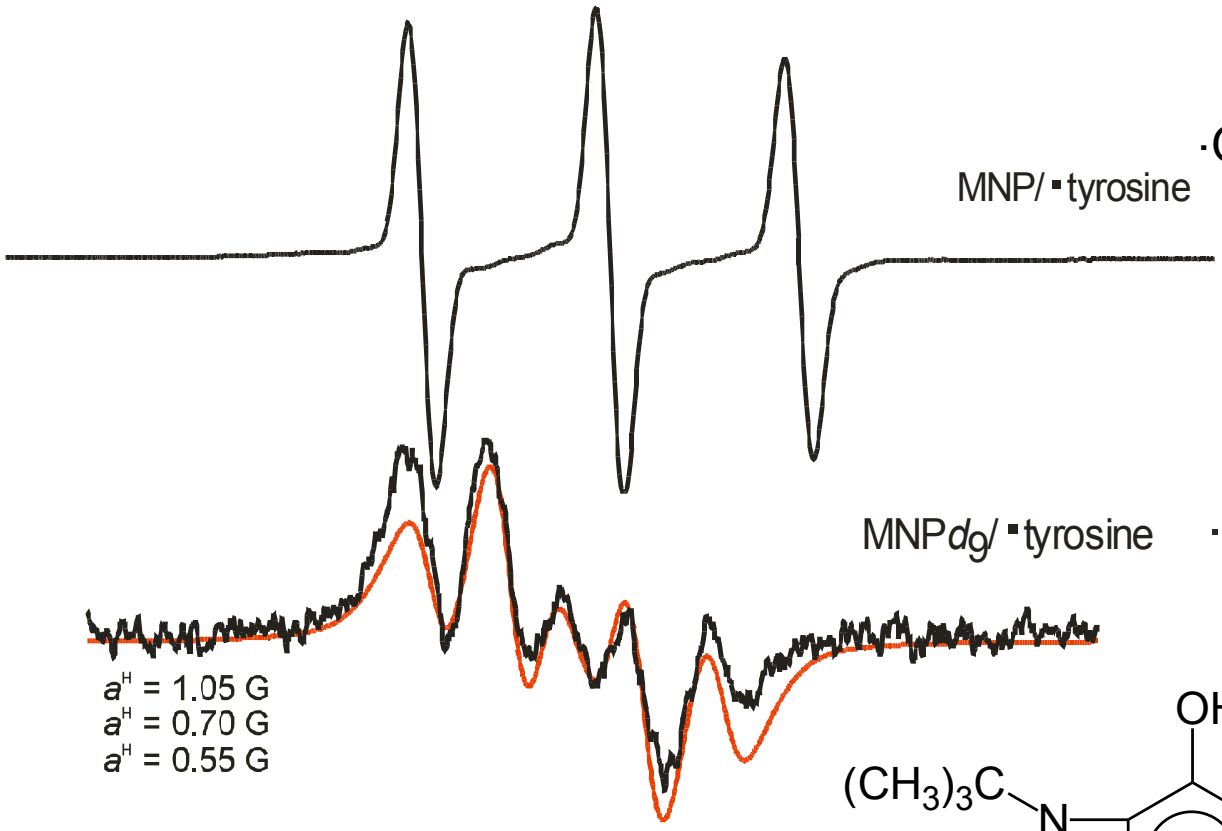
Gunther, M.R., et al., Biochem. J. 330:1293-1299, 1998.

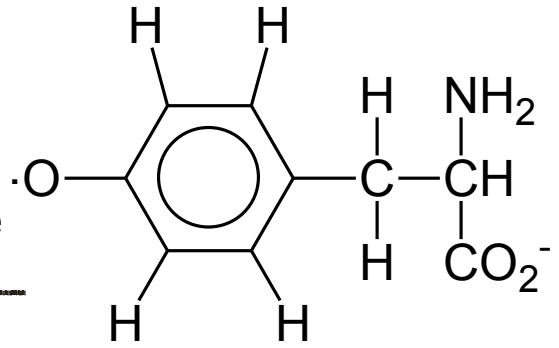
Spin trap-derived hyperfine from MNP and MNP-d_9

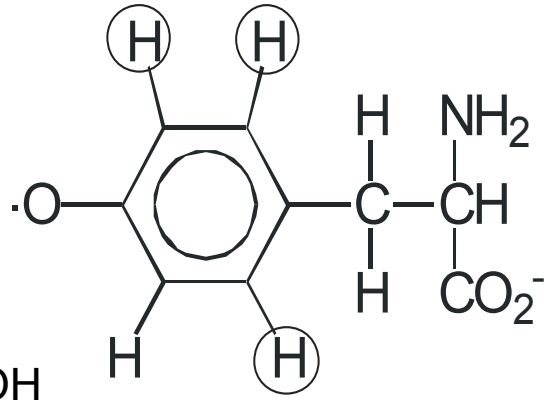
- **Each** line in the EPR spectra from MNP adducts is broadened by hyperfine from the 9 equivalent protons on the spin trap

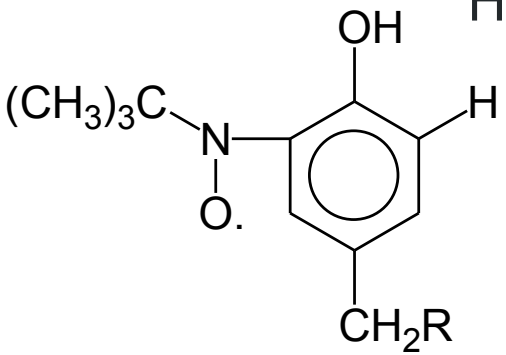


MNP-trapping the tyrosyl radical





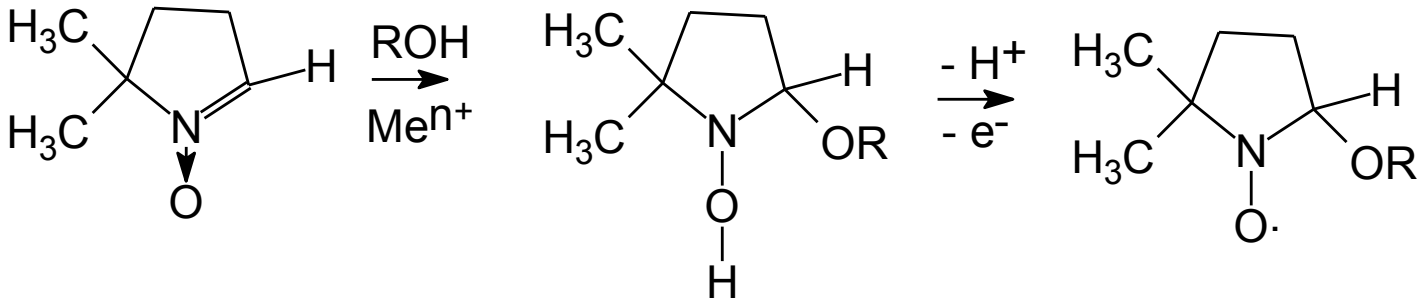




- Gunther, M.R., et al., Biochem. J. 330:1293-1299, 1998.

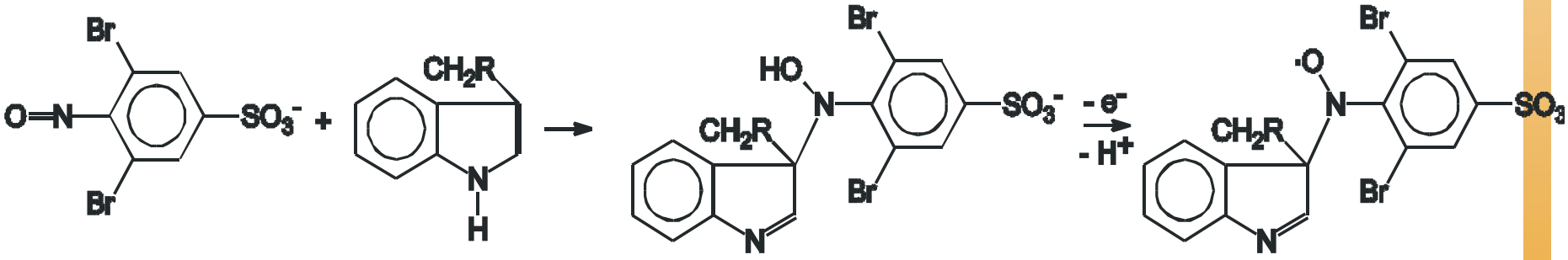
Why not spin trap?

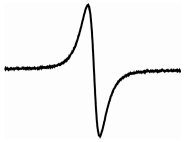
- Nitrone spin traps, especially DMPO
  - Adducts can interconvert, i.e., DMPO/·OOH decays to form DMPO/·OH
  - Subject to ***rare*** nucleophilic addition across their double bonds
  - Yields an EPR silent hydroxylamine which can be facilely oxidized up to the nitroxide



Why not spin trap?

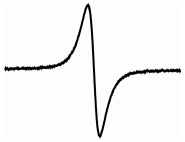
- Nitroso spin traps MNP and DBNBS
  - Often acutely toxic so can't use *in vivo*
  - The C-nitroso group critical to their function is highly reactive
  - Tend to directly add across unsaturated systems giving EPR-silent hydroxylamines that are readily oxidized to the corresponding nitroxides





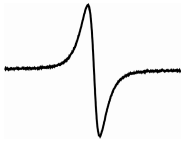
Summary

- The main feature of EPR spectra that is useful for assignment to a particular free radical structure is hyperfine splitting
- Direct EPR spectra can provide a wealth of structural information
- Highly unstable free radicals can, in many cases, be stabilized for EPR characterization by spin trapping
  - The increased stability of the detected free radical comes with a loss of structural information
  - The adduct may undergo chemistry between formation and detection
  - Adduct assignment is assisted by selective isotope labeling and EPR analysis of an independent preparation of the suspected adduct
  - The performance of appropriate controls is essential

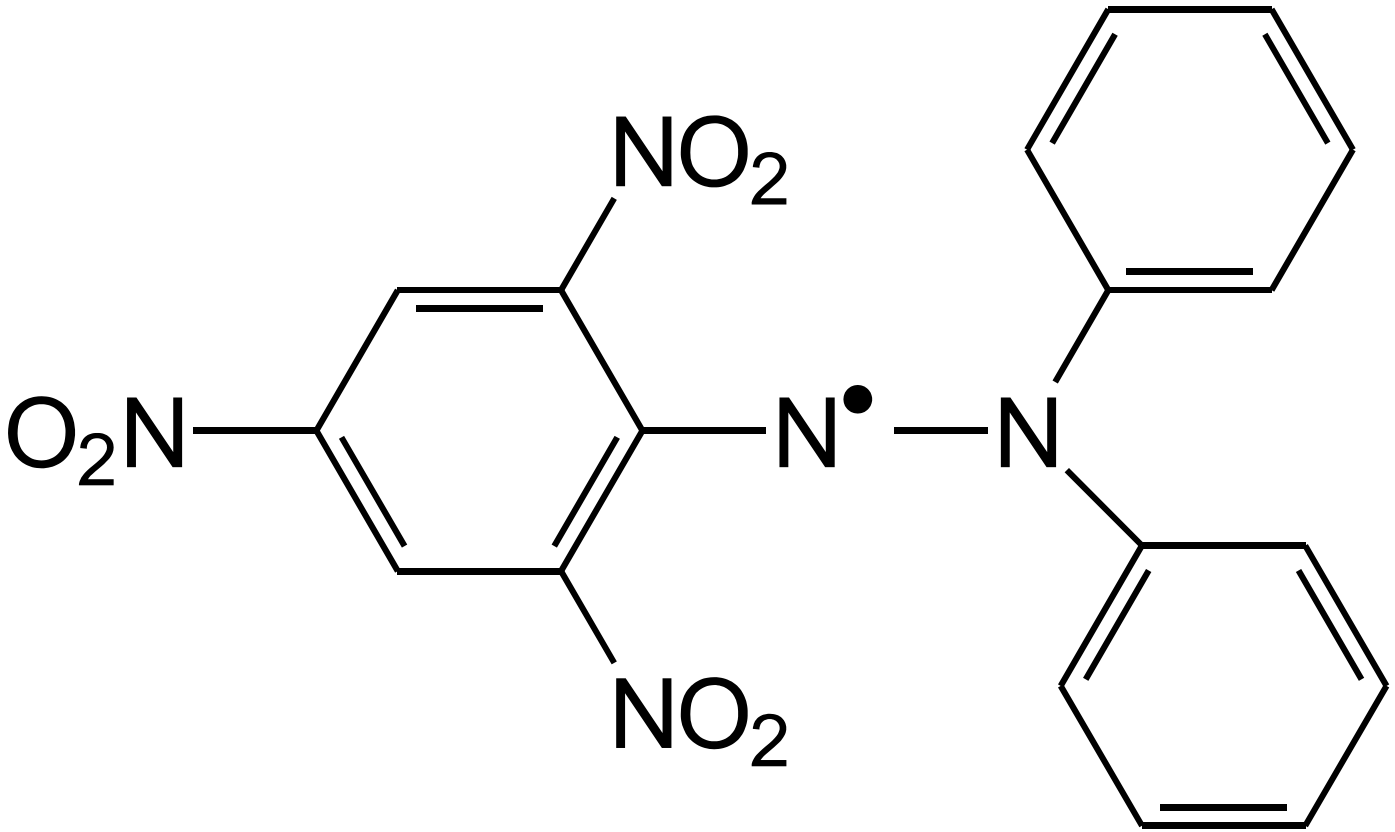


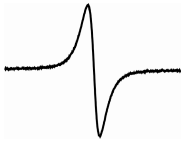
Applications of EPR

- Role of vitamin E and phenolic compounds in the antioxidant capacity, measured by ESR, of virgin olive, olive and sunflower oils after frying Food Chemistry 76 (2002) 461–468
- Use of ESR and HPLC to follow the anaerobic reaction catalysed by lipoxygenases Food Chemistry 168 (2015) 311–320
- Application of electron spin resonance spectrometry in nutraceutical and food research Mol. Nutr. Food Res. 2008, 52 ,62–78



DPPH





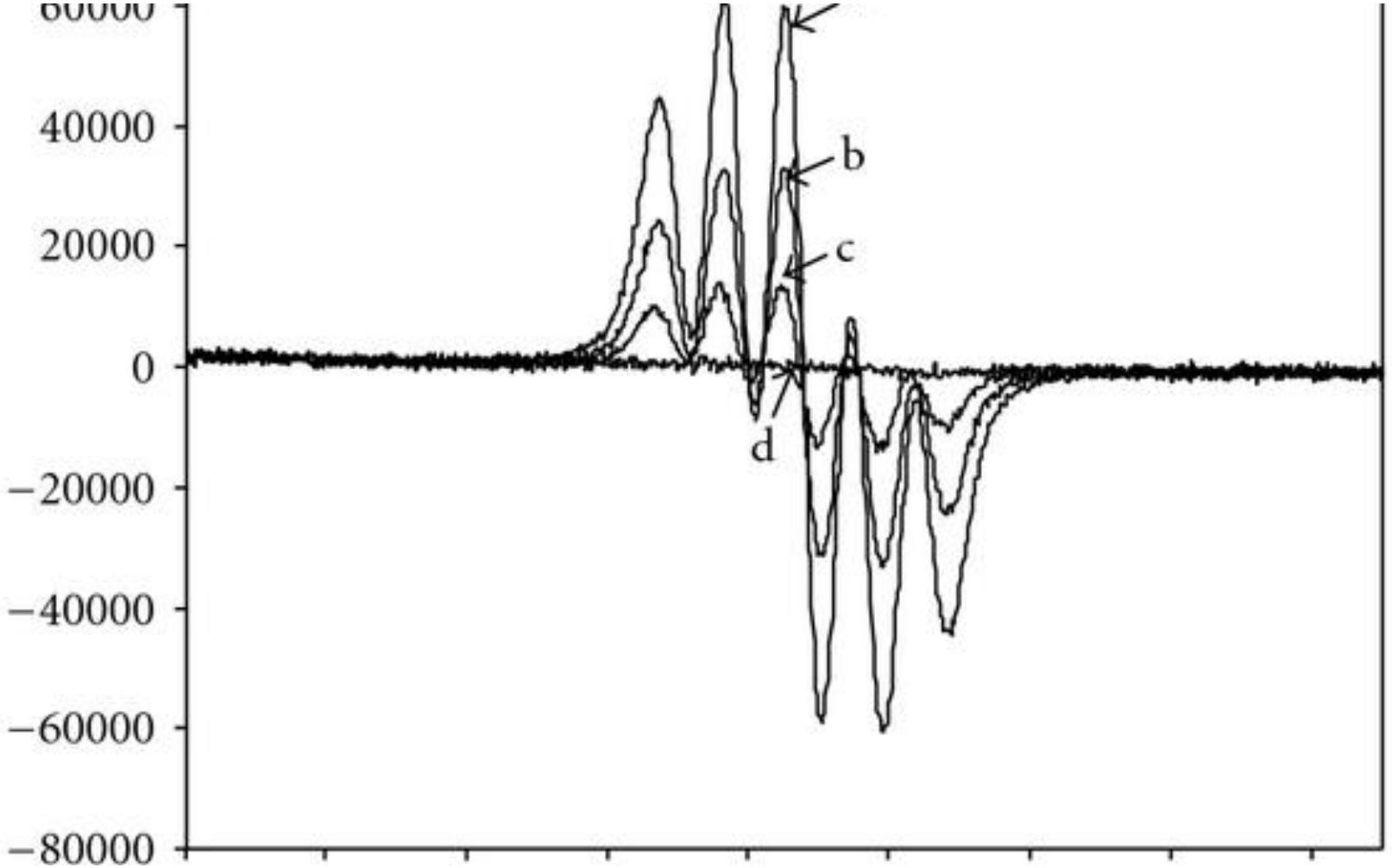
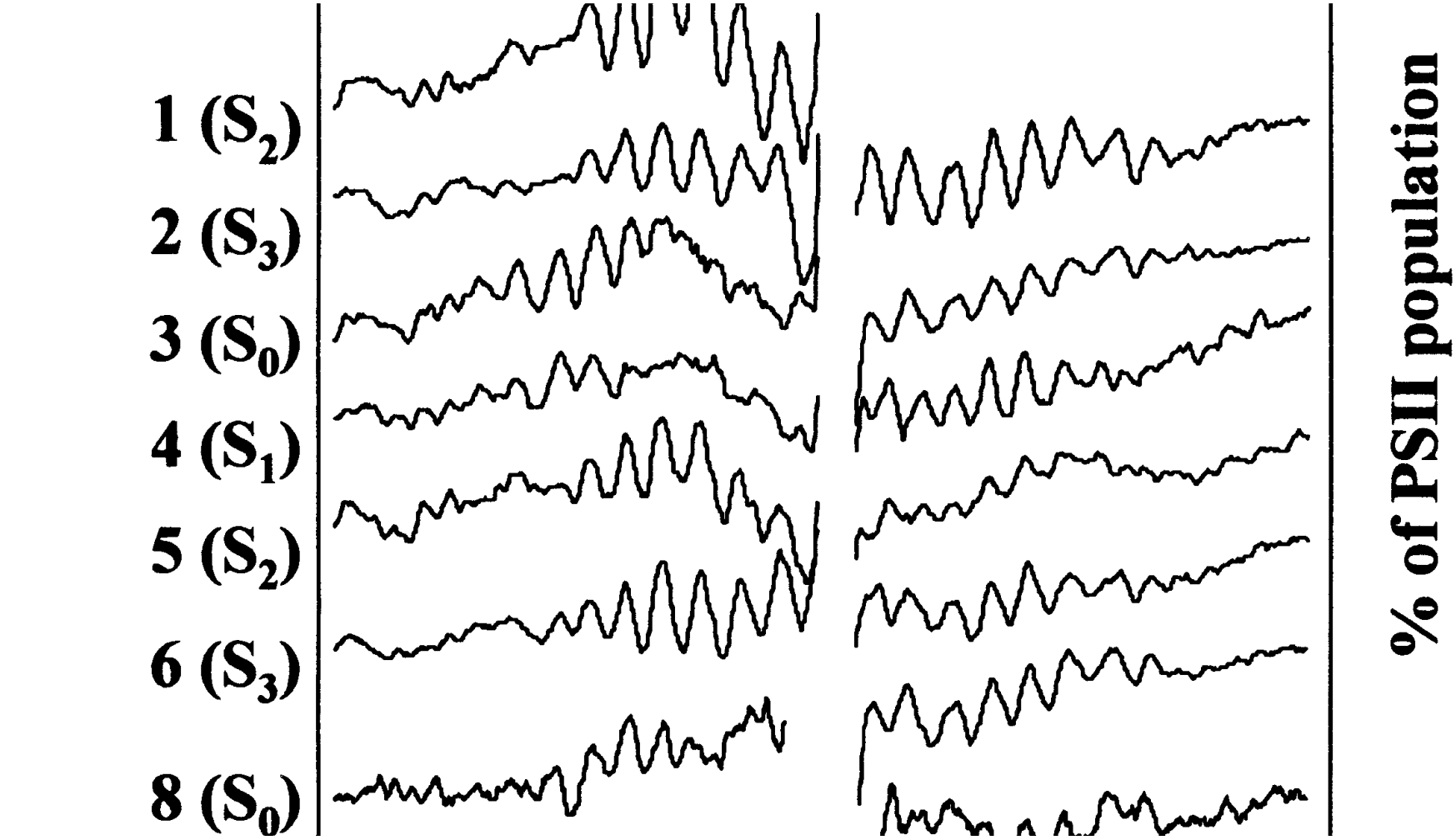

60000
40000
20000
0
−20000
−40000
−60000
−80000
b
c
d

Table 1. Bio transition metal nuclear spins and EPR hyperfine patterns

Metal	Valency	Isotope	Spin (abundance)	EPR lines
V	IV	51	7/2	8
Mn	II	55	5/2	6
Fe	III	54, 56, *57*, 58	0 + *1/2* (2%)	1 + 2 (1%)
Co	II	59	7/2	8
Ni	III,I	58, 60, *61*, 62, 64	0 + *3/2* (1%)	1 + 4 (0.25%)
Cu	II	63, 65	3/2	4
Mo	V	92, 94, *95*, 96, *97*, 98, 100	0 + *5/2* (25%)	1 + 6 (4%)
W	V	180, 182, *183*, 184, 186	0 + *1/2* (14%)	1 + 2 (7%)


1 (S2)
2 (S3)
3 (S0)
4 (S1)
5 (S2)
6 (S3)
8 (S0)
% of PSII population