EPR Spectrometer

E500 CW-Series
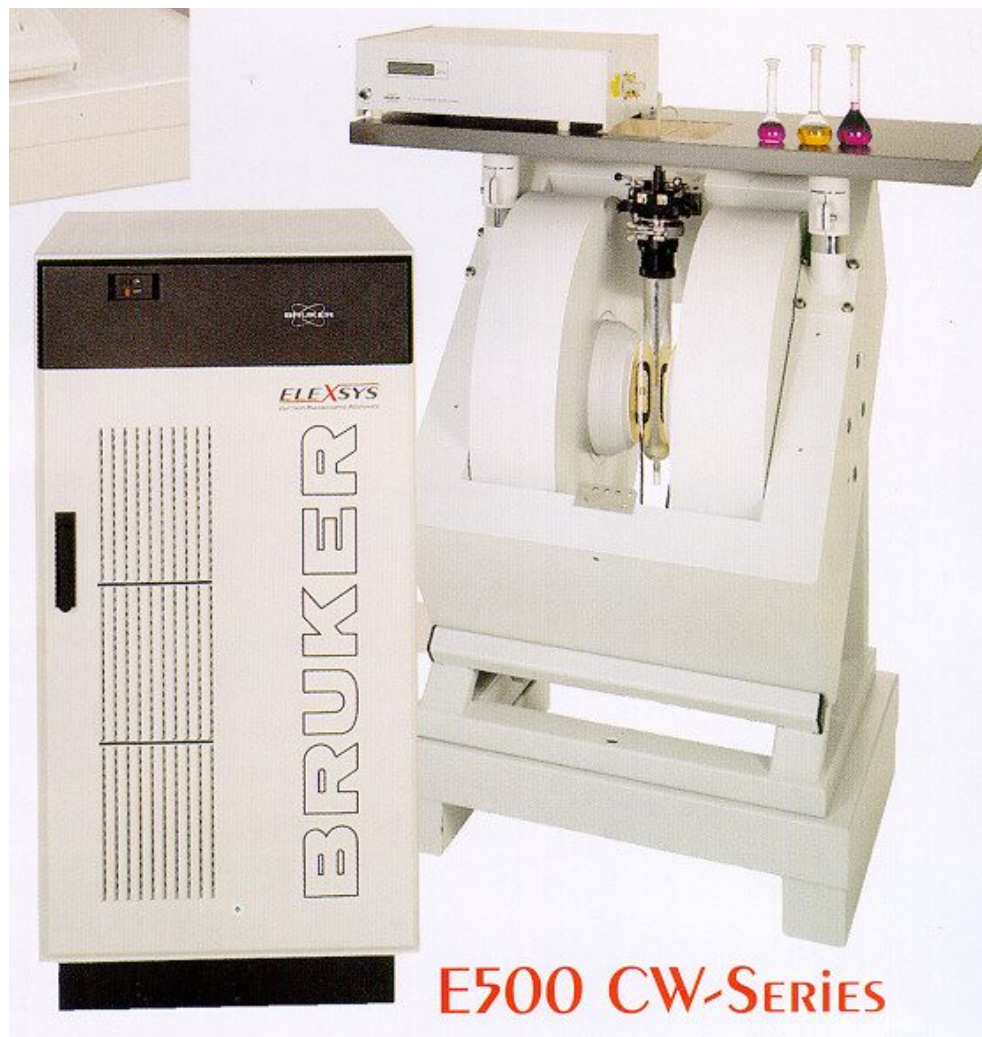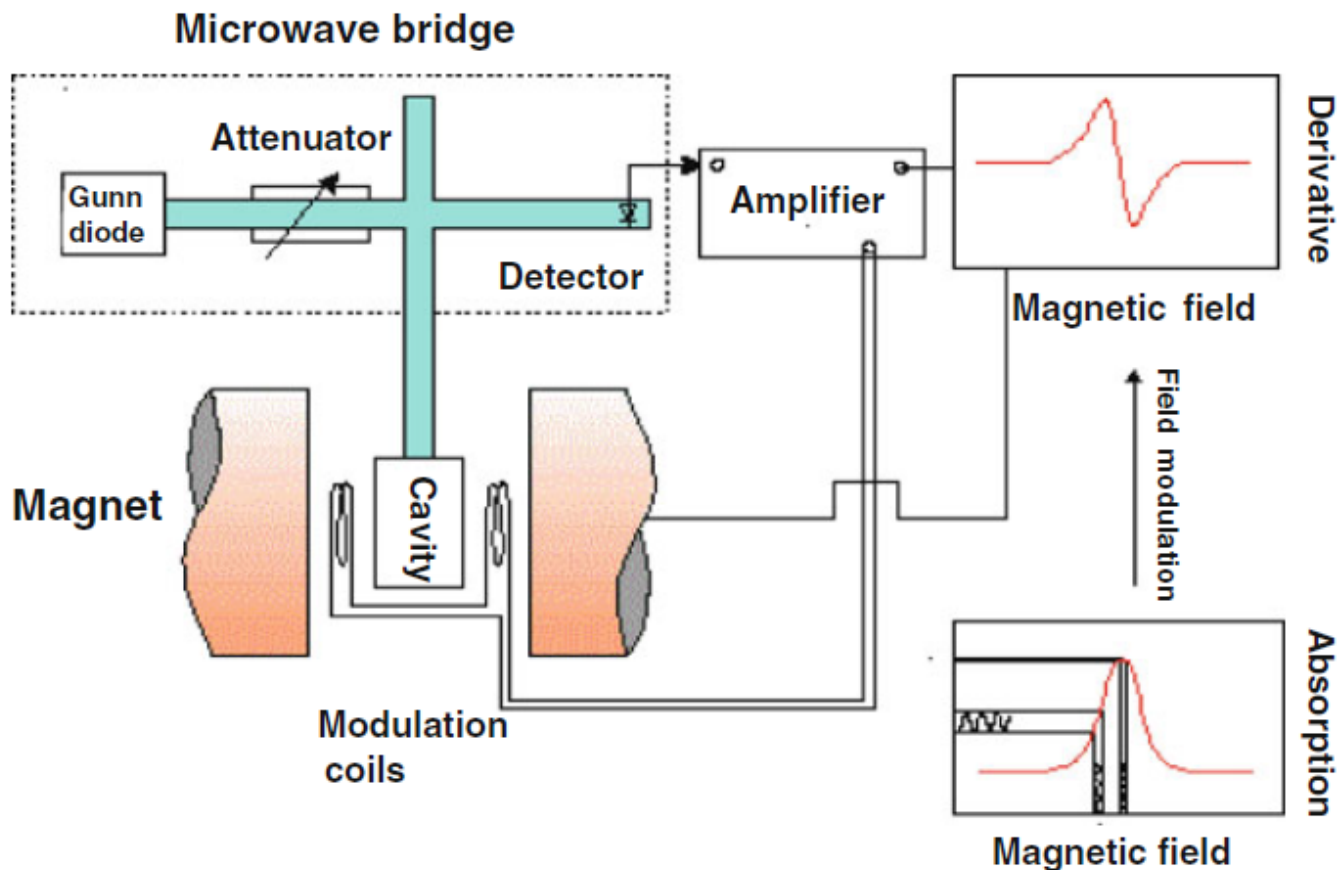
Instrumentation

The highest reported sensitivity of commercial instruments is ca. $109 \cdot B$ spins, where
B is the width of the signal in mT. This applies to instruments operating at the
normal "X-band" wavelength, $\lambda = 3$ cm. As mentioned the sensitivity increases
at higher frequencies, but the sample volume decreases, and the instrument also
14 1 Principles of ESR becomes more difficult to use. The commercial instruments for relative measurements
of concentrations that have been developed, e.g. as a means to measure
radiation doses usually employ X-band, while higher frequencies like in W-band
spectrometers (1011 Hz) find applications in structural studies.

CW-EPR Spectrum
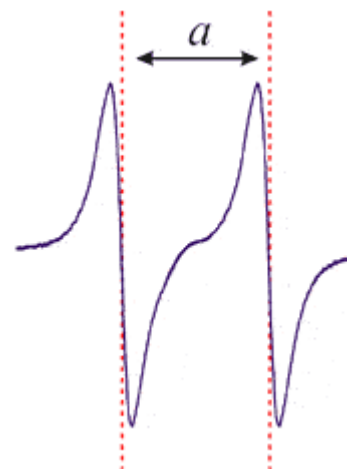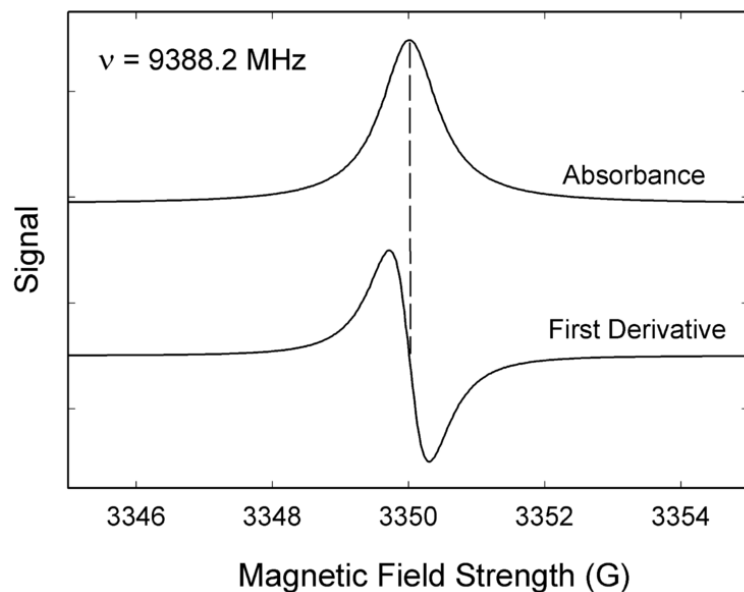
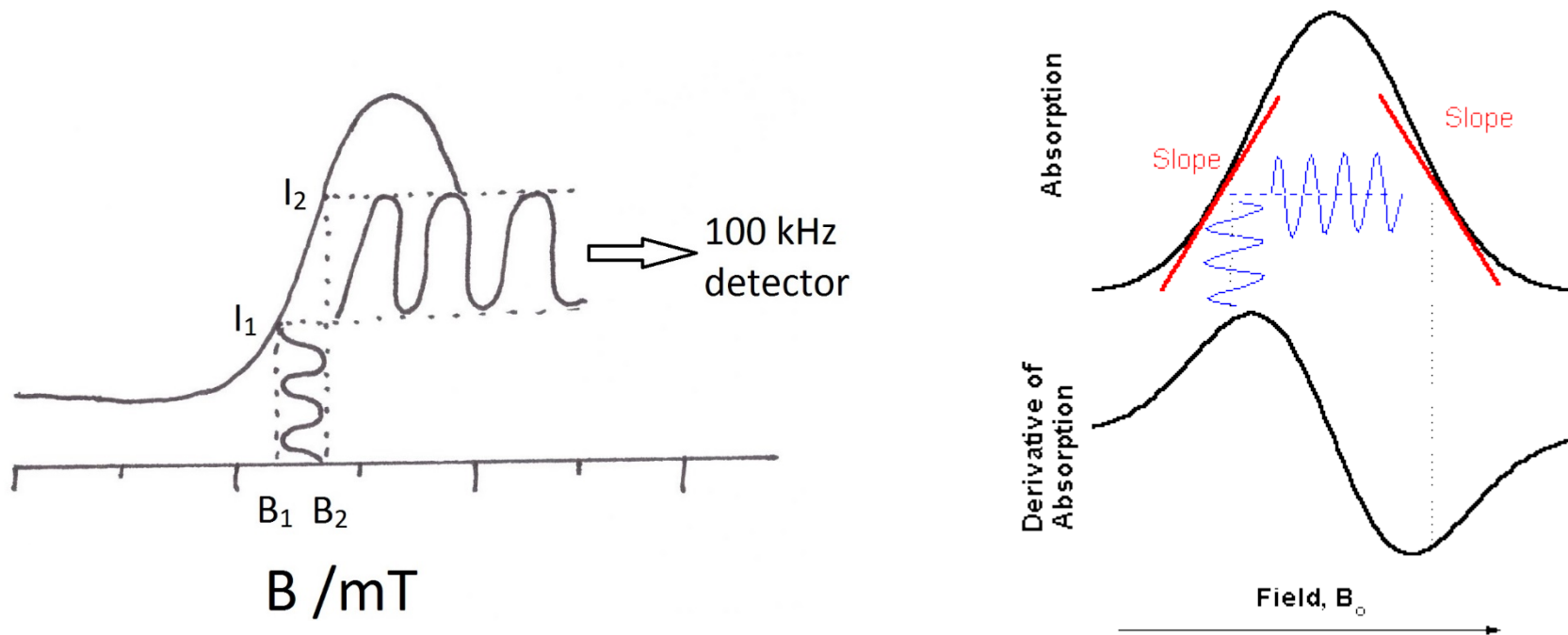$$\hbar\omega_0 = g\beta_e B_0.$$

Left.Idealized absorbance data (top), converted to an EPR spectrum by taking the first derivative (below).
Right. Hyperfine Splitting, Peaks multiplicity
Bohr magneton β_e. ω_0= Instr. Frequency

1ˢᵗ Derivative, Modulation

As previously mentioned an EPR spectra is usually directly measured as the first derivative of the absorption. This is accomplished by using field modulation. A small additional oscillating magnetic field is applied to the external magnetic field at a typical frequency of 100 kHz.[4] By detecting the peak to peak amplitude the first derivative of the absorption is measured. By using phase sensitive detection only signals with the same modulation (100 kHz) are detected. This results in higher signal to noise ratios. Note field modulation is unique to continuous wave EPR measurements and spectra resulting from pulsed experiments are presented as absorption profiles.

Radicals
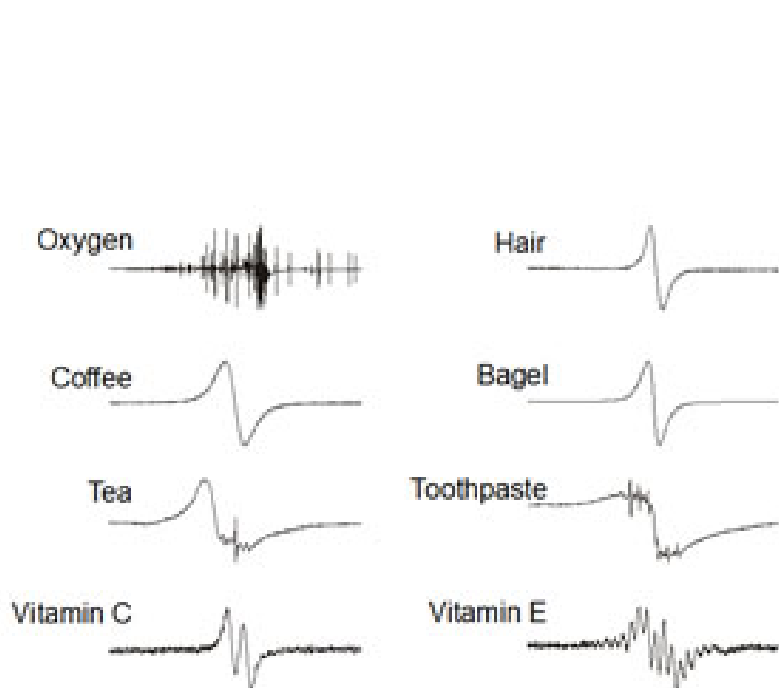
Free radicals occur quite frequently in nature due to one electron transfer reactions. Transition metal ions are often paramagnetic. Here are a few examples of EPR spectra of paramagnetic substances you frequently encounter in everyday life:
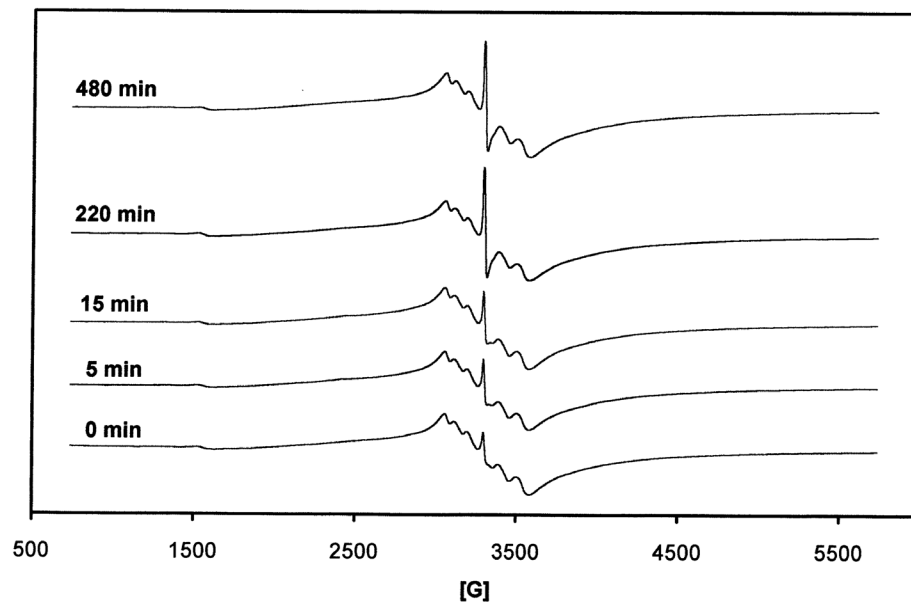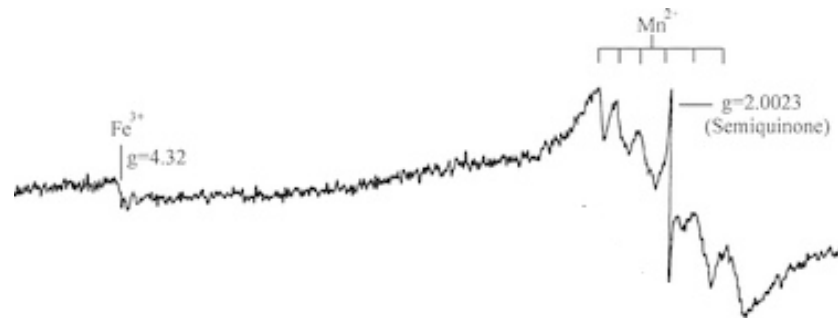
EPR spectra of illuminated RLB tea by xenon lamp at different time intervals: (A) after 0 min; (B) after 5 min; (C) after 15 min; (D) after 220 min; and (E) after 480 min
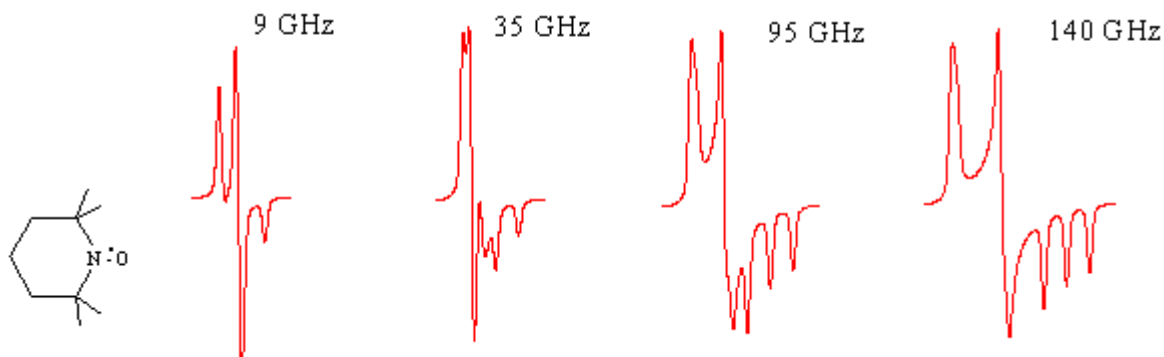
High Frequency

Table 1.2 Band designation of commonly employed ESR-spectrometers

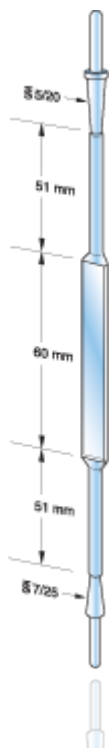| Band	Frequency (GHz)	Wavelength (cm)	Field (T), ($g = 2$)
S	3.0	10.0	0.107
X	9.5	3.15	0.339
K	23	1.30	0.82
Q	35	0.86	1.25
W	95	0.315	3.39

$$\Delta B = B_2 - B_1 = \frac{h}{\mu_B} \cdot \frac{g_1 - g_2}{g_1 \cdot g_2} \cdot v$$

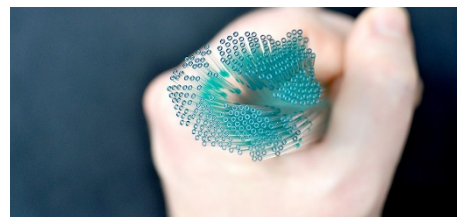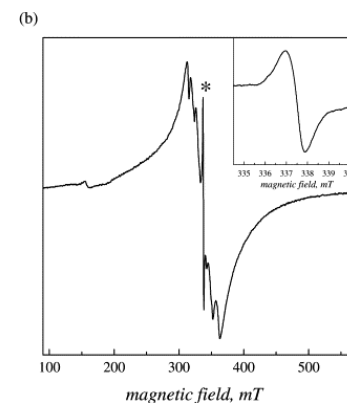The resonances of two species with different *g*-factors, g_1 and g_2 are separated by

Cavity

8 S/20
51 mm
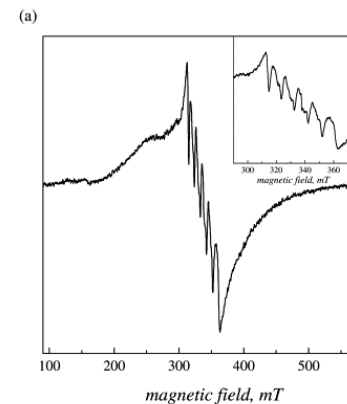80 mm
51 mm
8 7/25

**Quartz EPR tube
2-10 mm OD**

Aqueous Cells

Melting point tubes

**Samples could be solids, liquids, gases, frozen liquids glacial at
temperatures low as 4 K**

(a)

magnetic field, mT

(b)

magnetic field, mT

The Hamiltonian

$$\hat{H}_{\text{EPR}} = \hat{H}_{\text{EZ}} + \hat{H}_{\text{ZFS}} + \hat{H}_{\text{NZ,m}} + \hat{H}_{\text{NQI}} + \hat{H}_{\text{HFI,m}}$$

$$= \frac{\mu_{\text{B}}}{\hbar} \mathbf{B}_0{}^* \mathbf{g}\hat{\mathbf{S}} + \hat{\mathbf{S}}^* \mathbf{D}\hat{\mathbf{S}} - \gamma_{I_{\text{m}}} \mathbf{B}_0{}^* \hat{\mathbf{I}}_{\text{m}} + \hat{\mathbf{I}}_{\text{m}}{}^* \mathbf{Q}_{\text{m}} \hat{\mathbf{I}}_{\text{m}} + \hat{\mathbf{S}}^* \mathbf{A}_{\text{m}} \hat{\mathbf{I}}_{\text{m}}$$

electron Zeeman (EZ)
Zero Field Splitting (ZFS)
nuclear Zeeman (NZ)
nuclear quadrupole interaction (NQI)
Hyperhine interactions (HFI)
. The index 'm' denotes the high-spin metal nucleus;
μ_B is the Bohr magneton,
$\gamma_{I\mu}$ is the (metal) nuclear gyromagnetic ratio,
g, A, D, and Q are the EZ, HFI, ZFS, and NQI tensors, respectively
B_0 is the external magnetic field vector pointing in the z-direction
Sˆ and Iˆm are the electron and (metal) nuclear spin operator vectors

Electron Magnetism

$$\mu = -\frac{e}{2m_e}L$$

$$Na^+ : 1s^2 2s^2 2p^6 3s^0 \quad Cl^- : 1s^2 2s^2 2p^6 3s^2 3p^6$$
$$Na : 1s^2 2s^2 2p^6 3s^1 \quad Cl : 1s^2 2s^2 2p^6 3s^2 3p^5$$

$$m_S = \pm \tfrac{1}{2}.$$

$$m_l = \pm 1, 0.$$

$$S = \frac{h}{2\pi}\sqrt{s(s+1)}$$
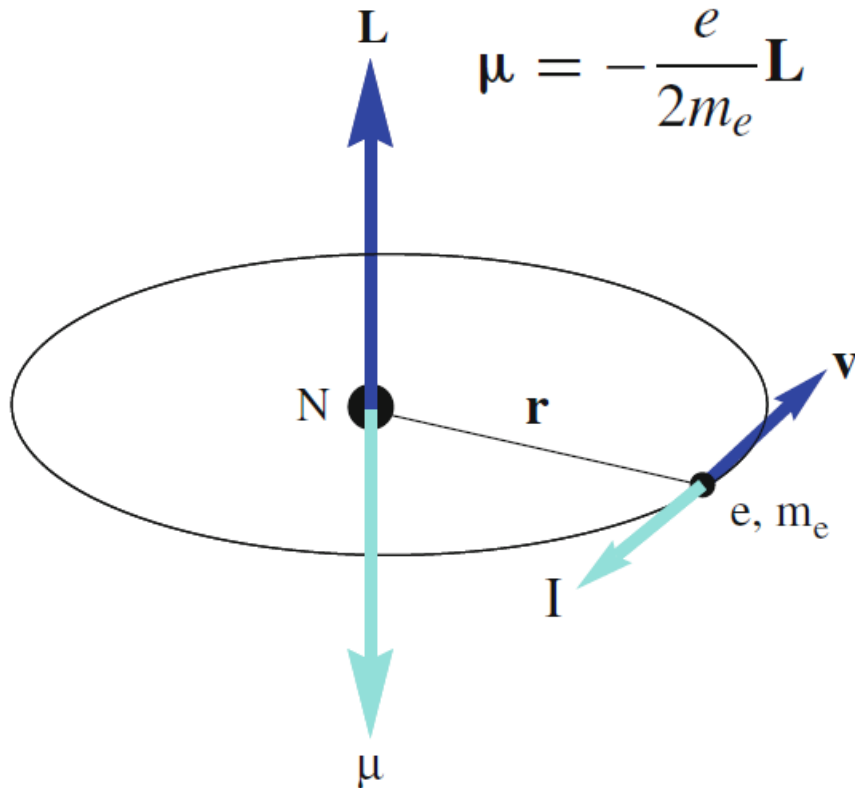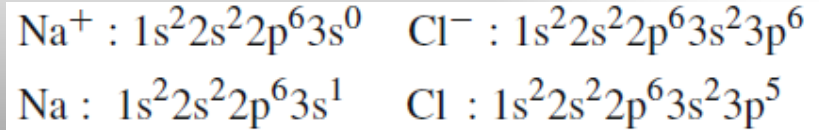
$$E_m = g\mu_B B m_s, \quad m_s = \pm 1/2$$

Fig. 1.1 Classical model illustrating relationship between angular momentum $L = m_e \cdot v \cdot r$ of electron, e, moving around a nucleus N and magnetic moment μ.
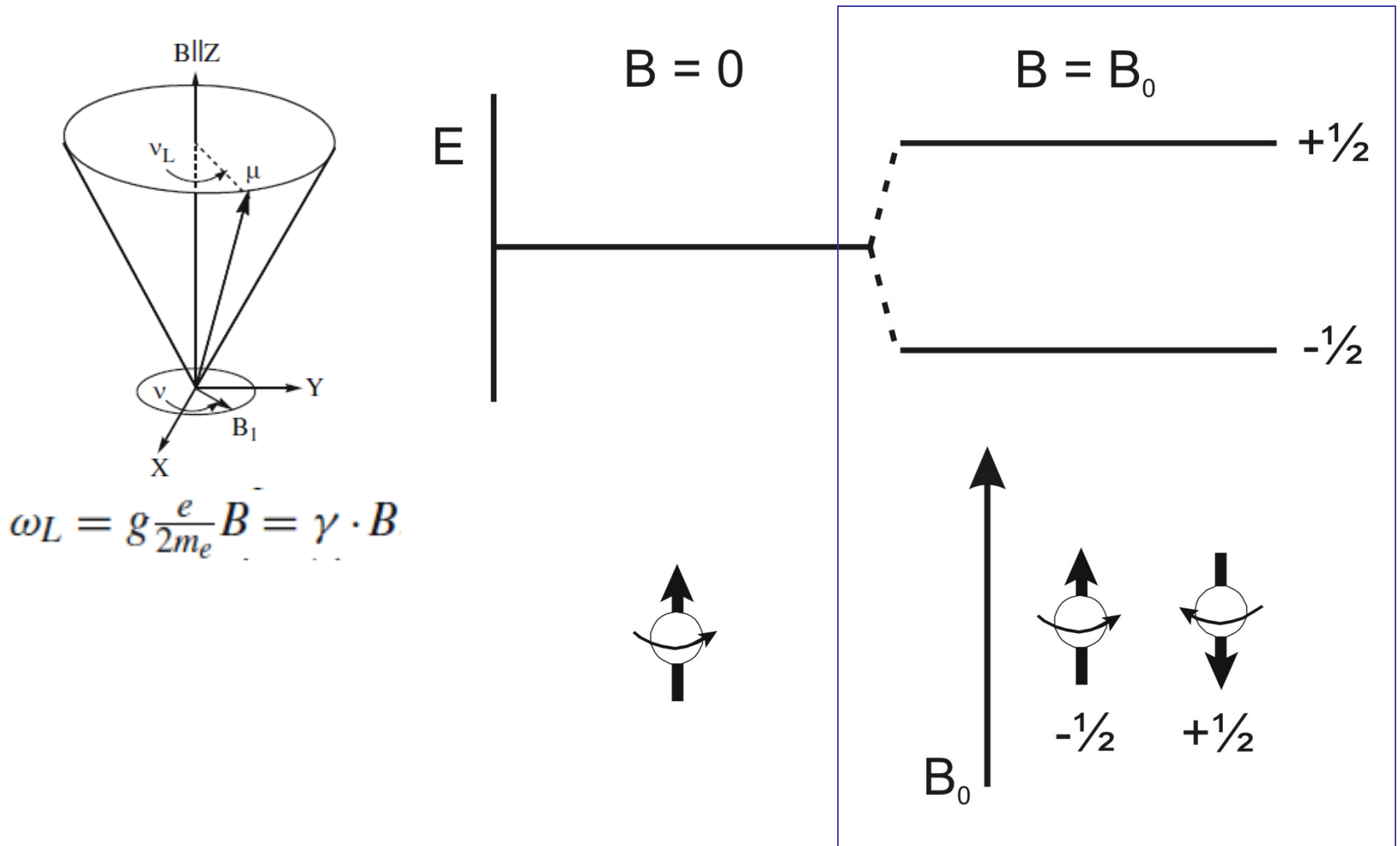
$$L = \frac{h}{2\pi}\sqrt{l(l+1)},$$

$$h\nu = E(m_s = 1/2) - E(m_s = -1/2) = g\mu_B B$$

A Free Electron in a Magnetic Field

$$\omega_L = g\frac{e}{2m_e}B = \gamma \cdot B$$

B = 0

B = B$_0$

E

+½

-½

+½

-½

B$_0$

A Free Electron in a Magnetic Field

B_0

$+\frac{1}{2}$: $E = +\frac{1}{2}g_e\beta B_0$

ΔE

$\Delta E = g_e\beta B_0$

$-\frac{1}{2}$: $E = -\frac{1}{2}g_e\beta B_0$

Absorption

β: Bohr magenton
B_0: magnetic field
g_e: g value

1st Derivative

For a free electron:
$g = g_e = 2.00232$

Powder Spectrum

$$g = g_e + \delta g \qquad g' = g_e + \delta g'$$
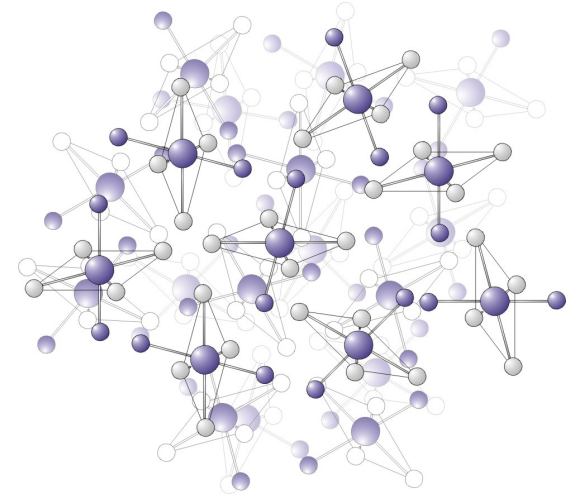
B_0

$g \qquad g'$
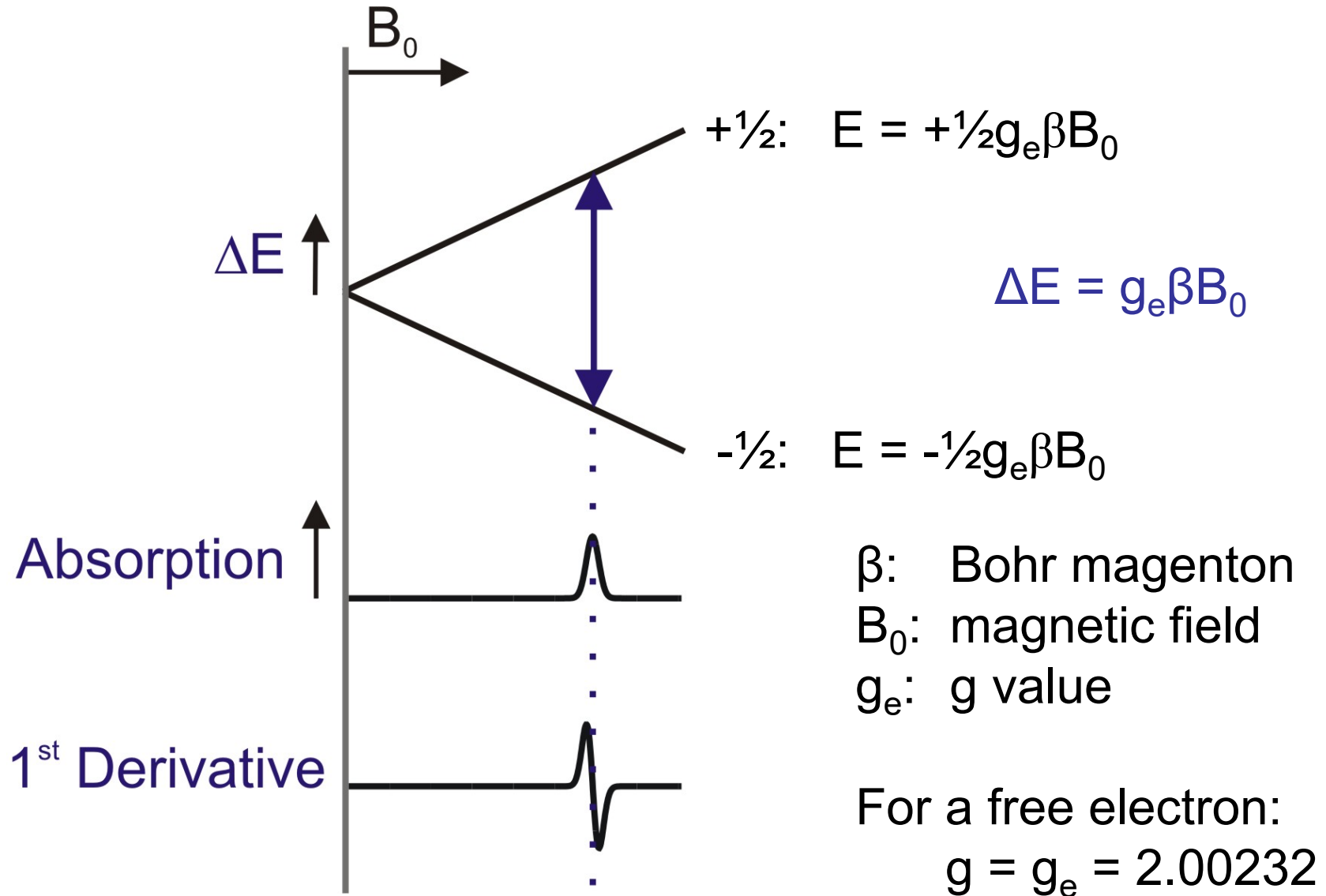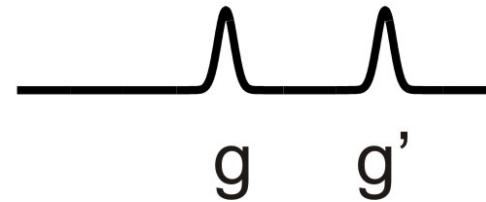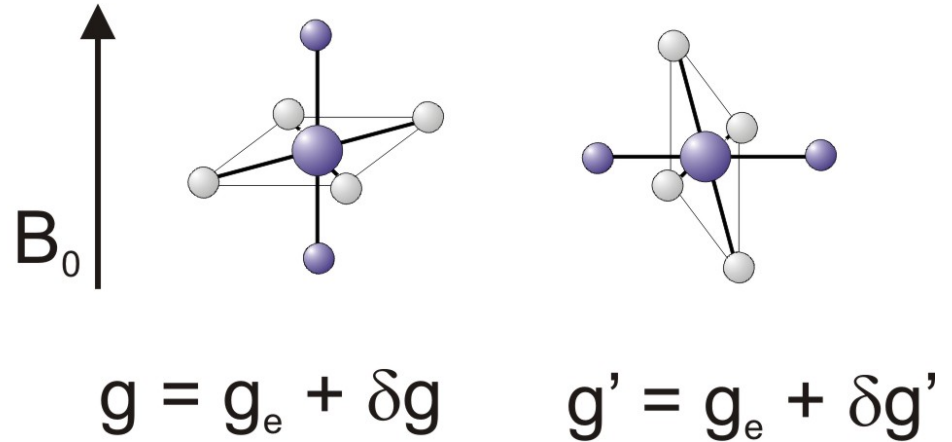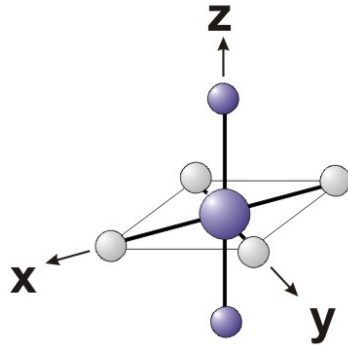
Example: compound with axial
paramagnetic anisotropy.
This will have a different δg value
for different orientations dependent
on the alignment of B along the *z*
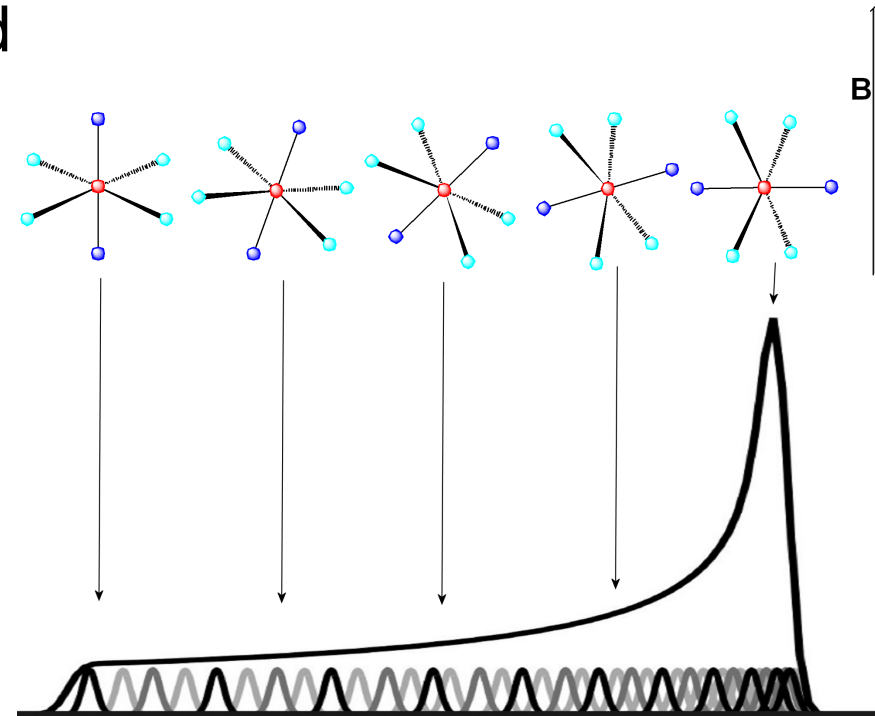axis or the *y* or *x* axes.

Powder Spectrum

A sample of realistic size consists of randomly oriented molecules, resulting in a so-called 'powder spectrum'.
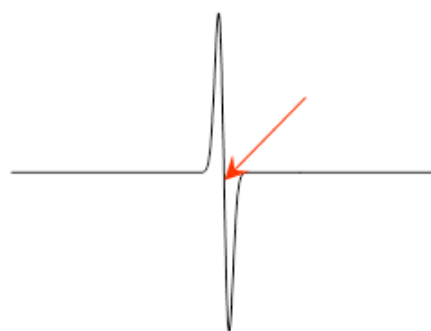
In the example of the compound with axial paramagnetic anisotropy, the spectrum has axial EPR absorption.

(Higher chance of having the *B* vector anywhere in the *xy* plane than parallel to the *z* axis.)
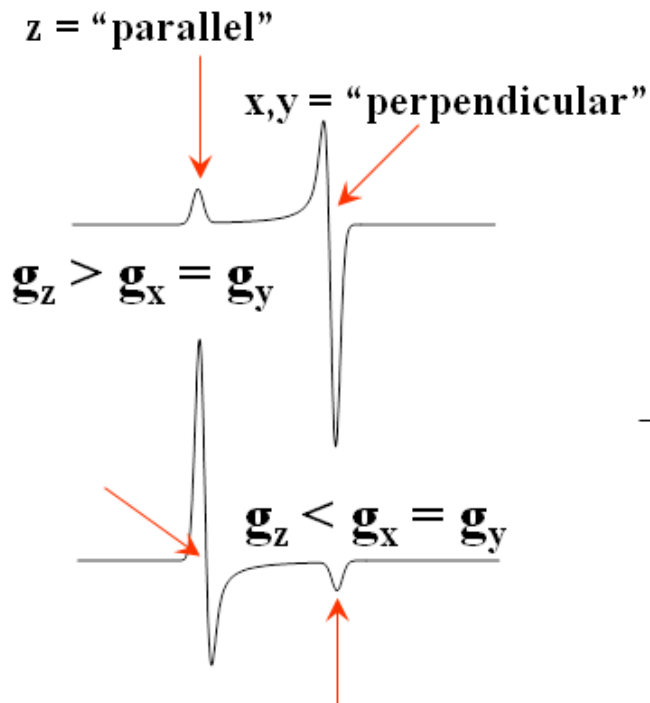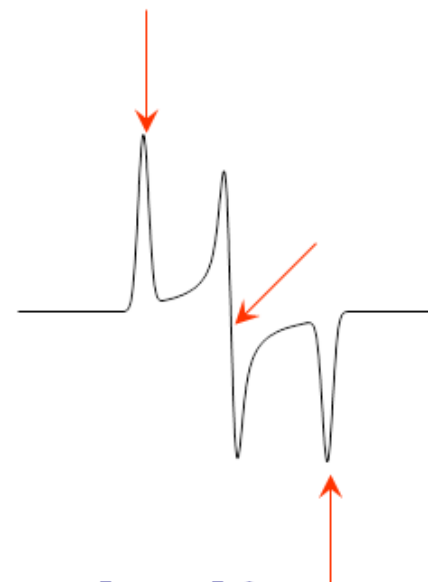
Line Shape of EPR Spectra

z = "parallel"

x,y = "perpendicular"

$g_z > g_x = g_y$

$g_z < g_x = g_y$

isotropic

$g_x = g_y = g_z$
e.g. O_h, T_d

axial
e.g. D_{4h}, C_{4v}, D_{3h}, etc

rhombic

$g_x \neq g_y \neq g_z$
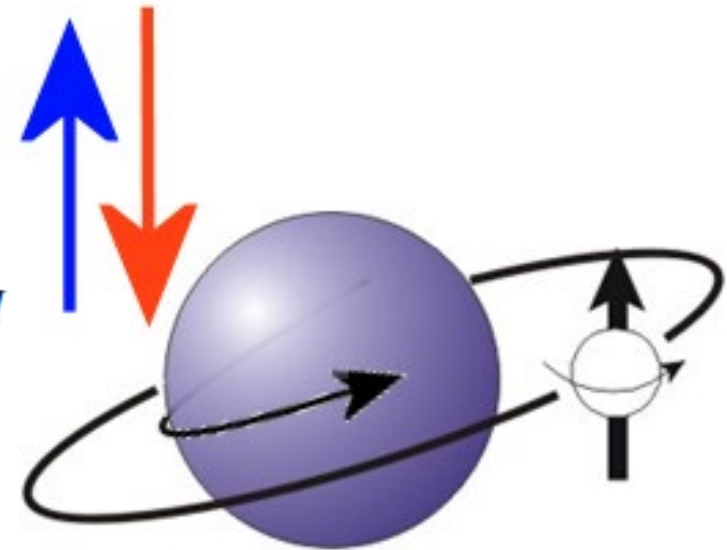e.g. C_{2v}, D_{2h}, C_{2h}, etc

Hyperfine Interactions

$$E(m_S, m_I) = g\mu_B B m_S + a m_I m_S - g_N \mu_N B m_I$$

$\mu_N = eh/4\pi m_p$ nuclear magneton

a is the hyperfine coupling constant

$\Delta m_S = 1$, $\Delta m_I = 0$ selection rules:

Resonance condition: $\Delta E = h\nu = g_e\beta B_0$

When the electron is bound to one, or more nuclei, then a virtual observer on the electron would experience the nucleus (nuclei) as an orbiting positive charge producing a second magnetic field, δB, at the electron.
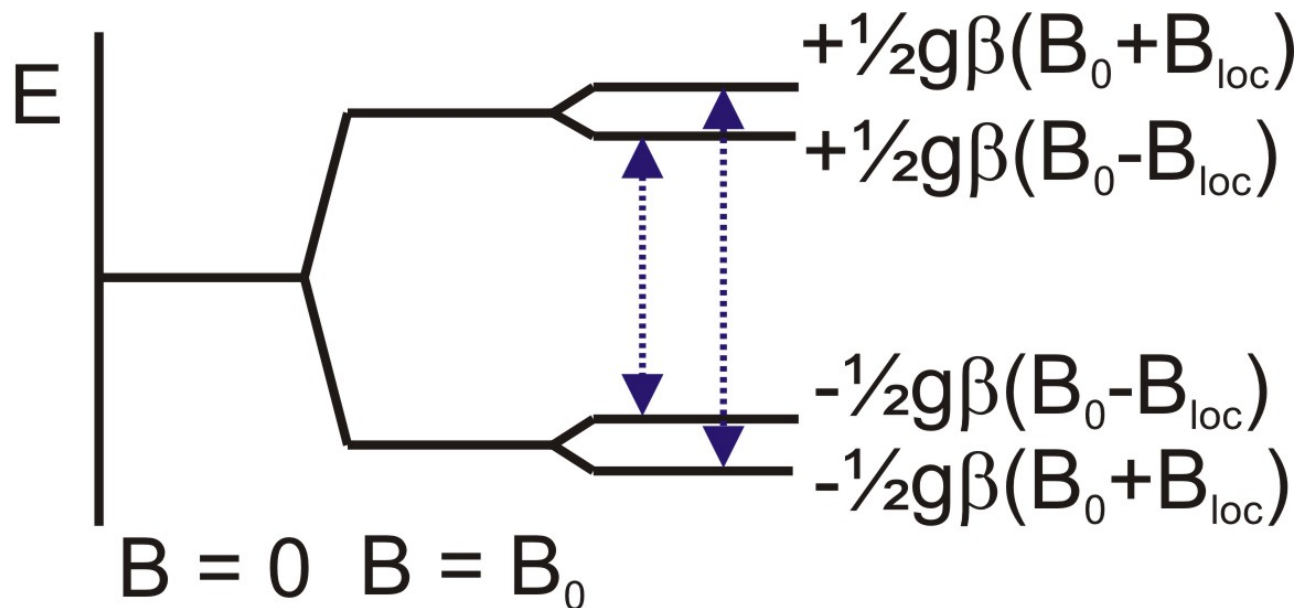
$$h\nu = g_e\beta(B_e + \delta B)$$

Since only the spectrometer value of B is known:

$$h\nu = (g_{e\,+}\,\delta g)\beta B = g\beta B$$

The quantity $g_e + \delta g$ contains the chemical information on the nature of the bond between the electron and the molecule, the electronic structure of the molecule.

Hyperfine Interactions

E

$+\frac{1}{2}g\beta(B_0+B_{loc})$

$+\frac{1}{2}g\beta(B_0-B_{loc})$

$-\frac{1}{2}g\beta(B_0-B_{loc})$

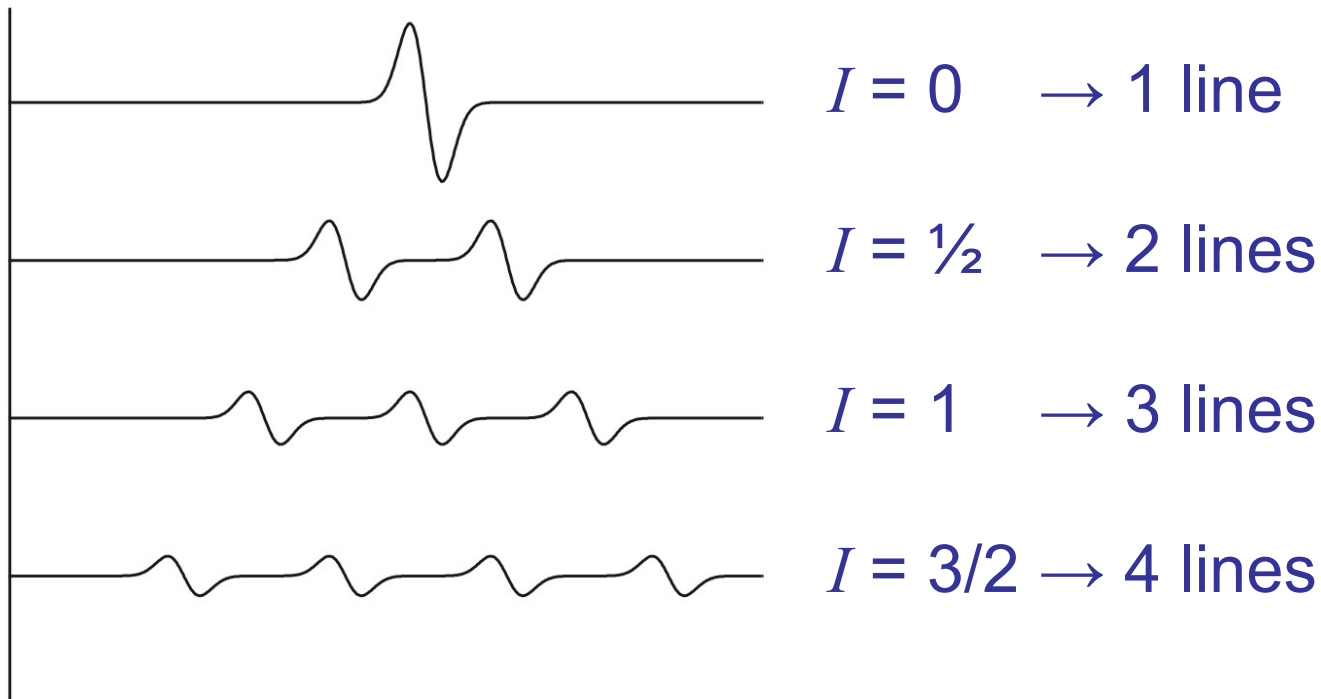$-\frac{1}{2}g\beta(B_0+B_{loc})$

B = 0   B = B_0

Interactions of the electron spin with the nuclear spin of the metal ion nucleus or first coordinate sphere ligands nuclei or other electron spins within 10 Å distance cause additional splitting.

Hyperfine Interactions

$$\Delta E = g\beta B_0$$

B_0

ΔE

Absorption

1st Derivative
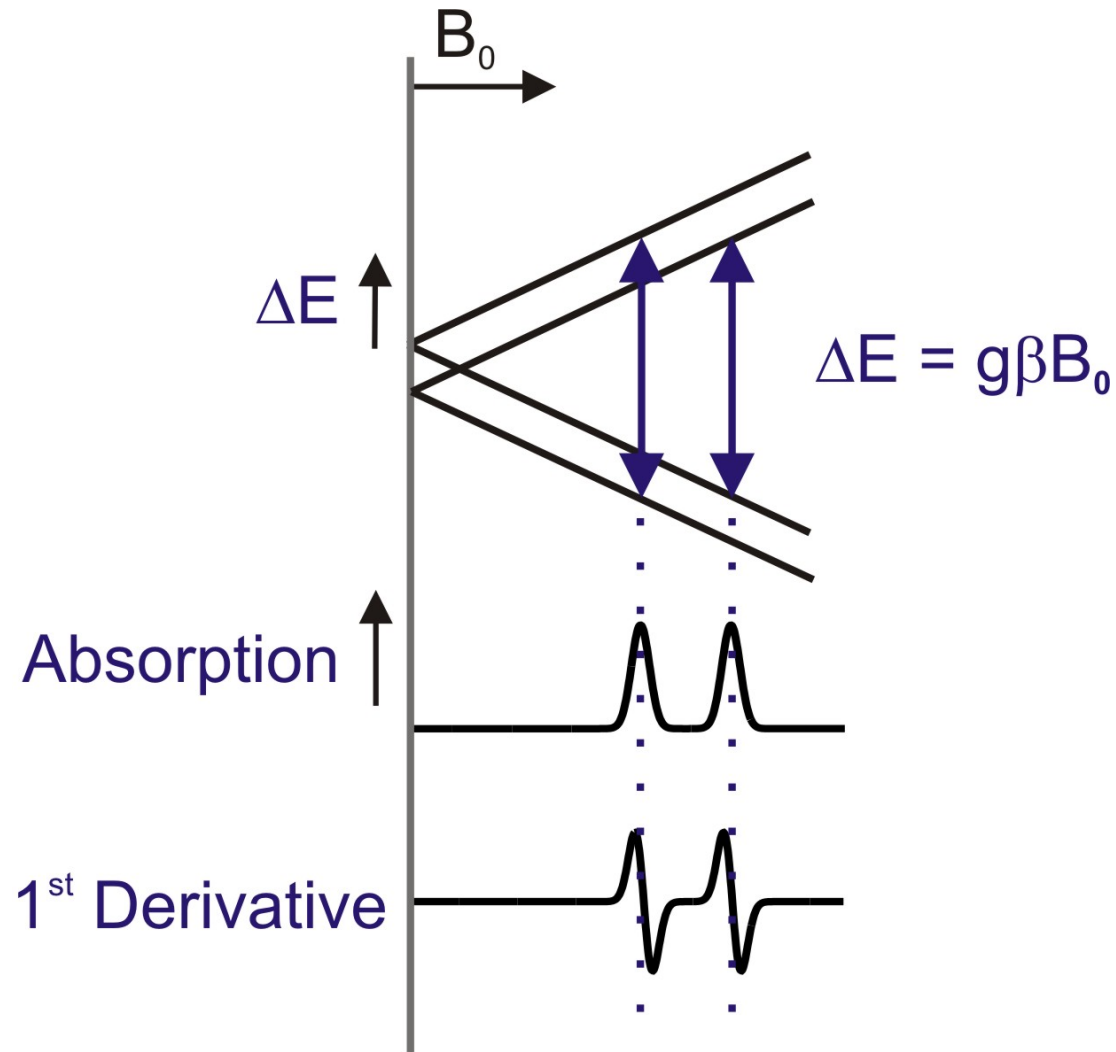
Hyperfine Interactions

- Bio transition metal nuclear spins (I): $2I + 1$ EPR lines
    (*Called hyperfine structure*)

$I = 0 \quad \rightarrow 1$ line

$I = \frac{1}{2} \quad \rightarrow 2$ lines

$I = 1 \quad \rightarrow 3$ lines

$I = 3/2 \rightarrow 4$ lines
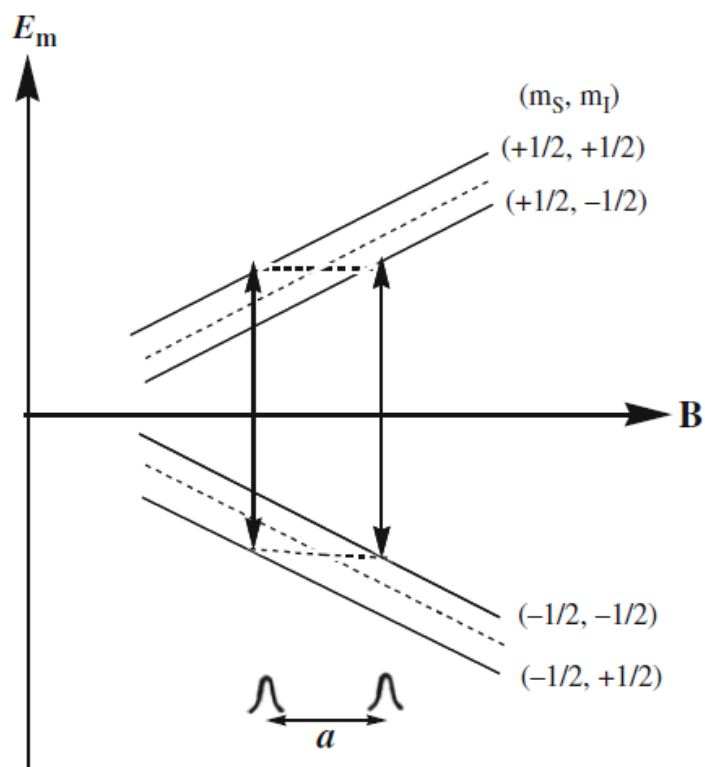
Hyperfine Interactions

Fig. 1.5 Energy levels for an H atom with $I = \frac{1}{2}$. The *arrows* show the allowed $\Delta m_I = 0$ transitions induced by the microwave field radiation
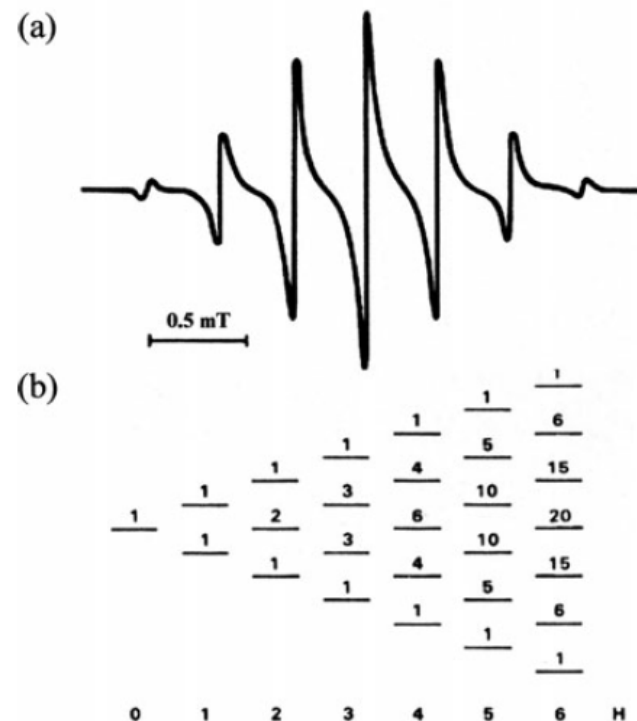

Fig. 1.6 ESR 1st derivative spectrum of the benzene anion. The relative intensities of the seven lines due to six equivalent H nuclei are obtained from the Pascal triangle

Isotropic couplings

$$a = \frac{2}{3}\mu_0 g\mu_B g_N \mu_N \rho(X_N)$$

$\rho(X_N)$ is the unpaired electron density at a nucleus located at X_N.

Table 1.1 Hyperfine couplings of some nuclei with the unpaired electron completely localized to an s-orbital (a_0) or to a p-orbital (B_0)

Nucleus	Spin (I)	Natural abundance (%)	a_0 (MHz)	B_0 (MHz)
^{1}H	1/2	99.985	1,420	
^{2}H	1	0.015	218	
^{13}C	1/2	1.11	3,110	91
^{14}N	1	99.63	1,800	48
^{19}F	1/2	100.0	52,870	see [1]
^{31}P	1/2	100.0	13,300	see [1]

Isotropic couplings occur for s-orbitals, while p-, d- and f-orbitals are characterised by a zero density at the nucleus (Fig. 1.9) and thus give no contribution to a_0. The values of a_0 and B_0 in the table are obtained when the unpaired electron is entirely localised to an s-orbital and a p-orbital, respectively.

Electron densities
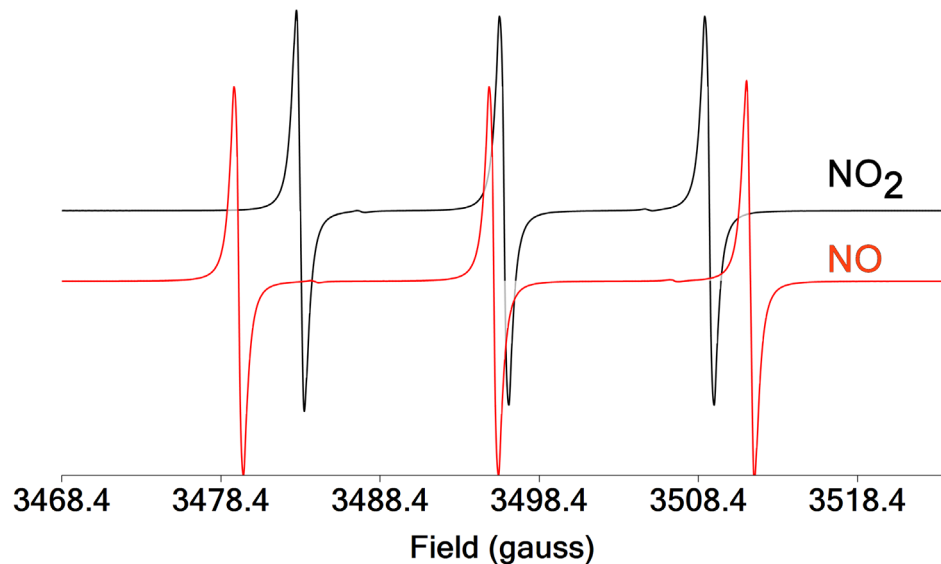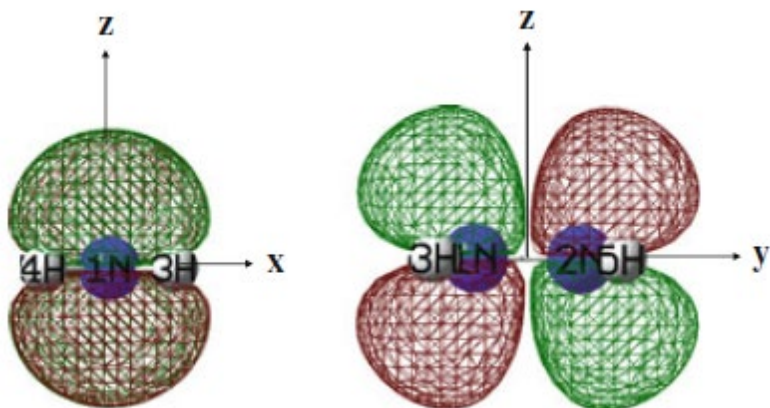
$$A_{iso, H} = Q_H \rho_\pi$$

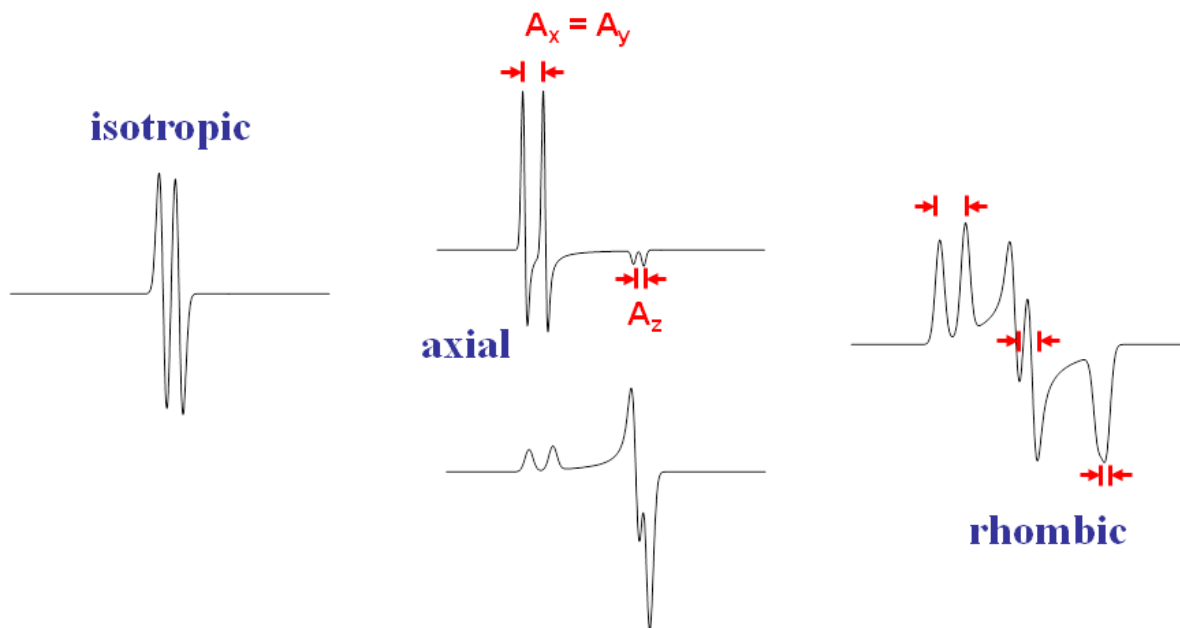$$\rho_N 1(2p_z) = \rho N2(2p_z) = 23.3/48 = 0.49.$$

$$\rho_{N1}(2s) = \rho_{N2}(2s) = 32.2/1,800 = 0.018$$

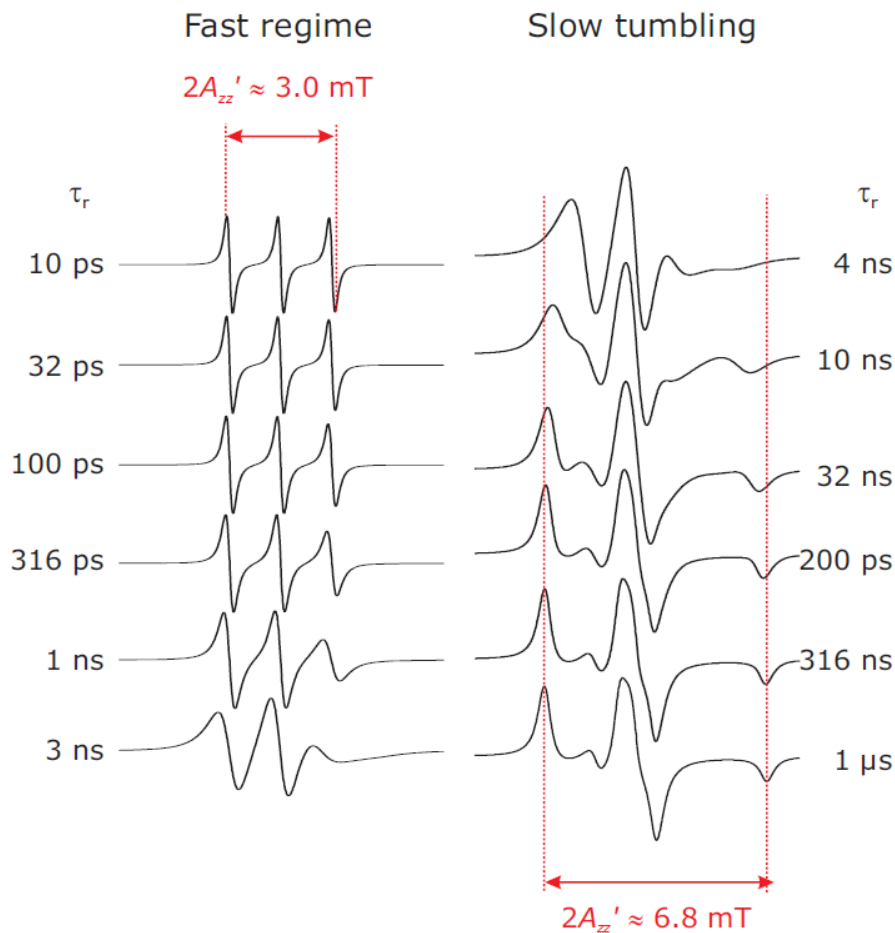$$\rho_{H1}(1s) = \rho_{H2}(1s) = 30.8/1,420 = 0.022$$

the probability for the unpaired electron to be located at each of the nitrogen atoms
pπ is the spin density at the adjacent atom and Q_H hyperfine coupling
constants

Anisotropic Coupling

isotropic

axial

$A_x = A_y$

A_z

rhombic

If the unpaired electron is localised at a p-, d- or f-orbital of an atom, the hyperfine coupling is anisotropic, that is measurements on a species in a single crystal give different values depending on the orientation of the magnetic field

Fast regime

$2A_{zz}' \approx 3.0$ mT

Slow tumbling

τ_r

τ_r

$$\tau_r = \frac{\pi\sqrt{3}}{b}\left[\frac{b}{8} - \frac{4\Delta\gamma B_0}{15}\right]^{-1}\frac{g_{iso}\mu_B}{h}\Delta B_{pp}(0)\left[\sqrt{\frac{I(0)}{I(-1)}} - 1\right]$$
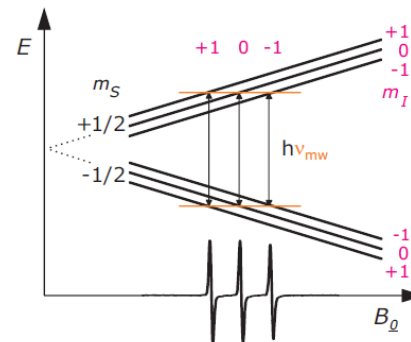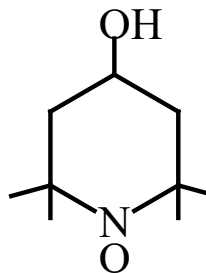
$\Delta B0$ is the peak-to-peak linewidth of the central line
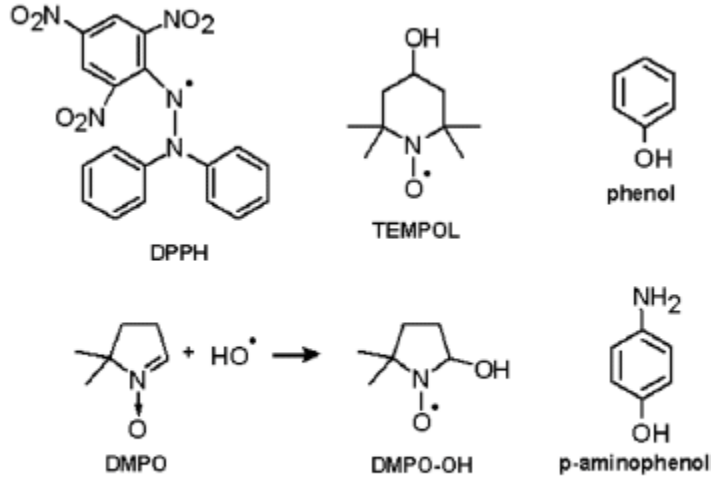peak-to-peak line amplitudes $I(m_l)$

hyperfine anisotropy parameter

$$b = \frac{4\pi}{3}\left[A_{zz} - \frac{A_{xx} + A_{yy}}{2}\right]$$

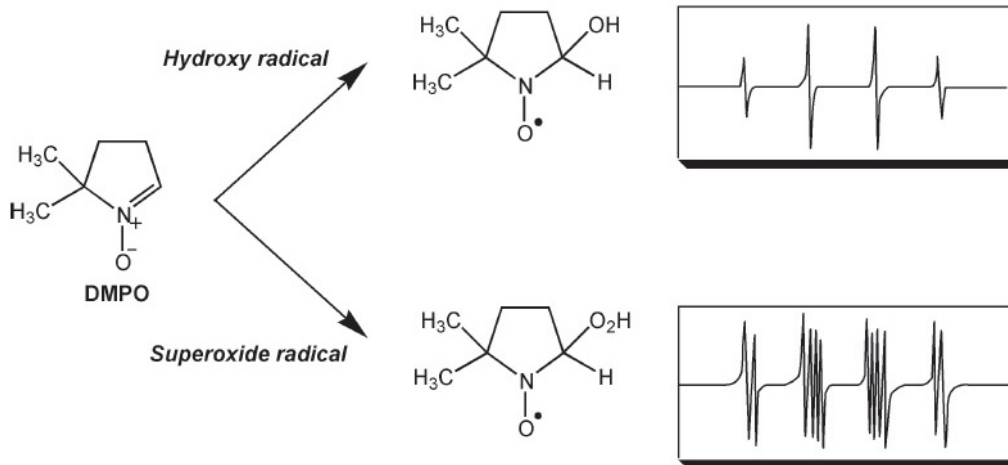electron Zeeman anisotropy parameter $\Delta\gamma$

$$\Delta\gamma = \frac{2\pi\mu_B}{h}\left[g_{zz} - \frac{g_{xx} + g_{yy}}{2}\right]$$

10 ps → 4 ns
32 ps → 10 ns
100 ps → 32 ns
316 ps → 200 ps
1 ns → 316 ns
3 ns → 1 µs

$2A_{zz}' \approx 6.8$ mT

Dependence of the Tempol cw EPR spectrum on the rotational correlation time τr.

Scheme 1. Structural formula of free radicals DPPH and TEMPOL; DMPO and respective spin adduct DMPO-OH; phenol and *p*-aminophenol.

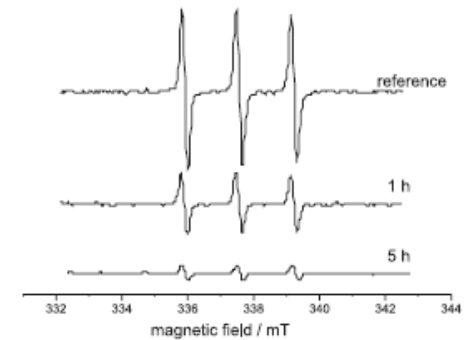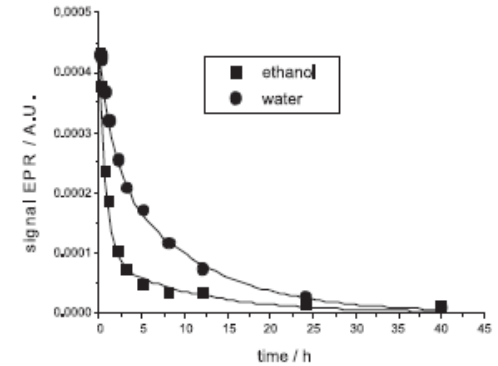ESR spectra of DMPO adducts

Antioxidant activity

Figure 1. Above, the TEMPOL EPR signal decay, in the presence of *Crysophyllum inornatum* extract solubilized in water and ethanol; and its respective fit biexponential decay. Below, EPR spectra of TEMPOL with *Crysophyllum inornatum* extracts after 1 h, 5 h and reference.
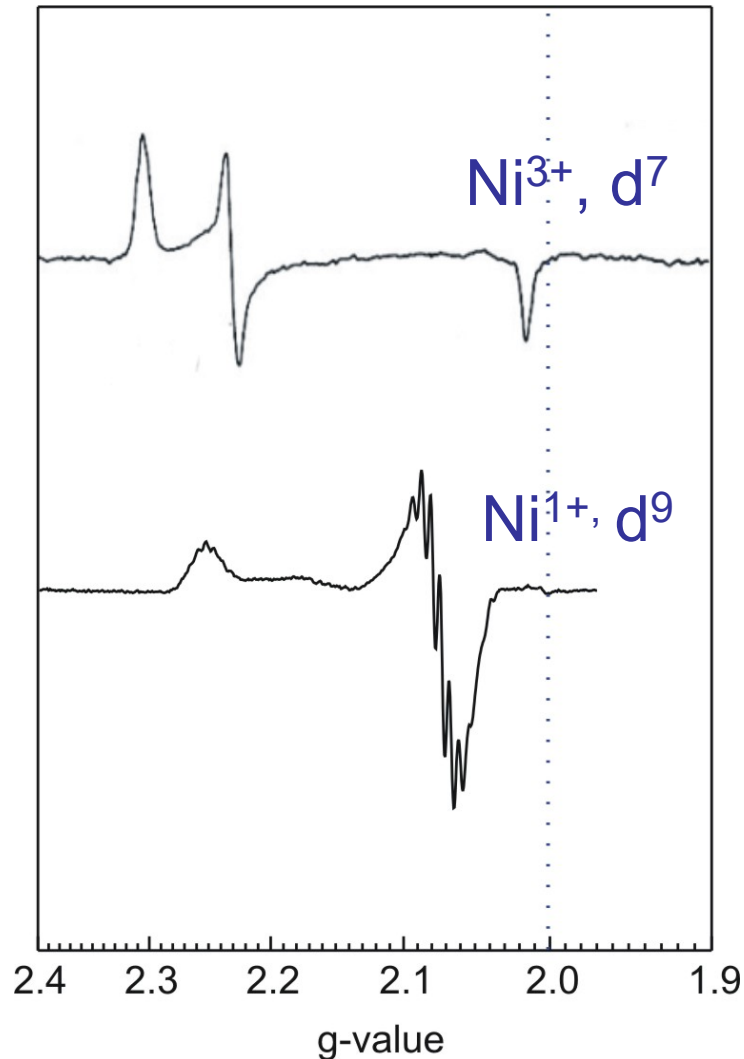
Type Identification - Metals

- Bio transition metal nuclear spins

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%)

- The spin-orbit coupling parameter is positive ($g<g_e$) for systems with less than half filled outer shells and negative ($g>g_e$) for those with more than half filled shells (Generally!)
- With redox state is EPR active?
- How many unpaired electrons present?
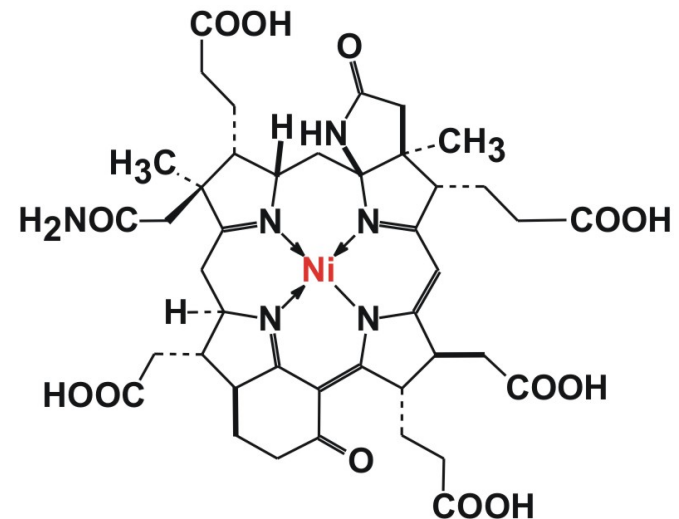
Type Identification - Metals

Ni^{3+}, d^7

Ni^{1+}, d^9

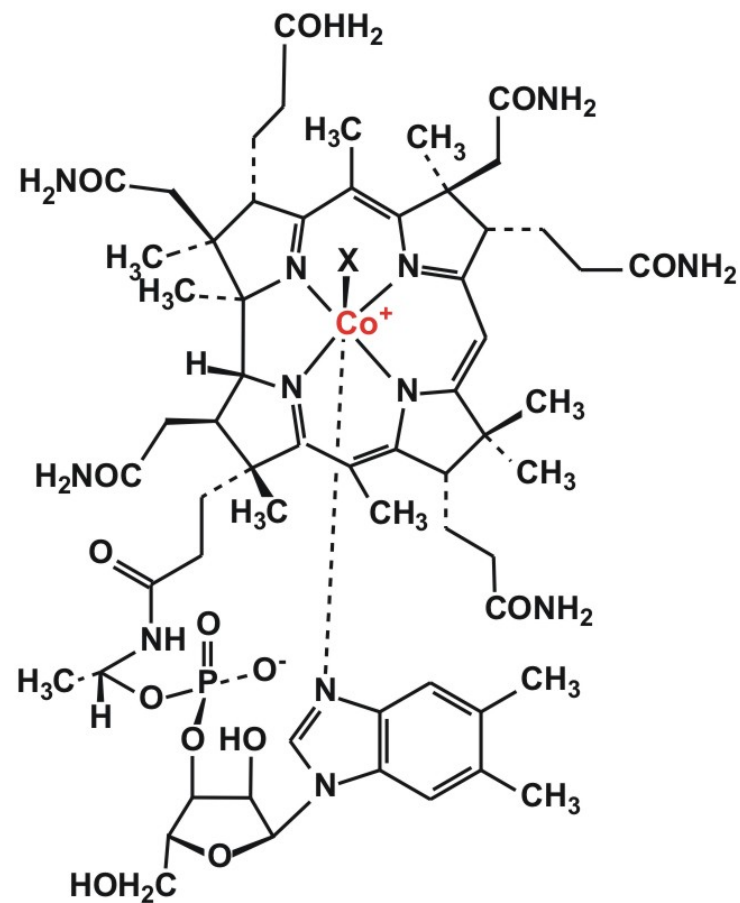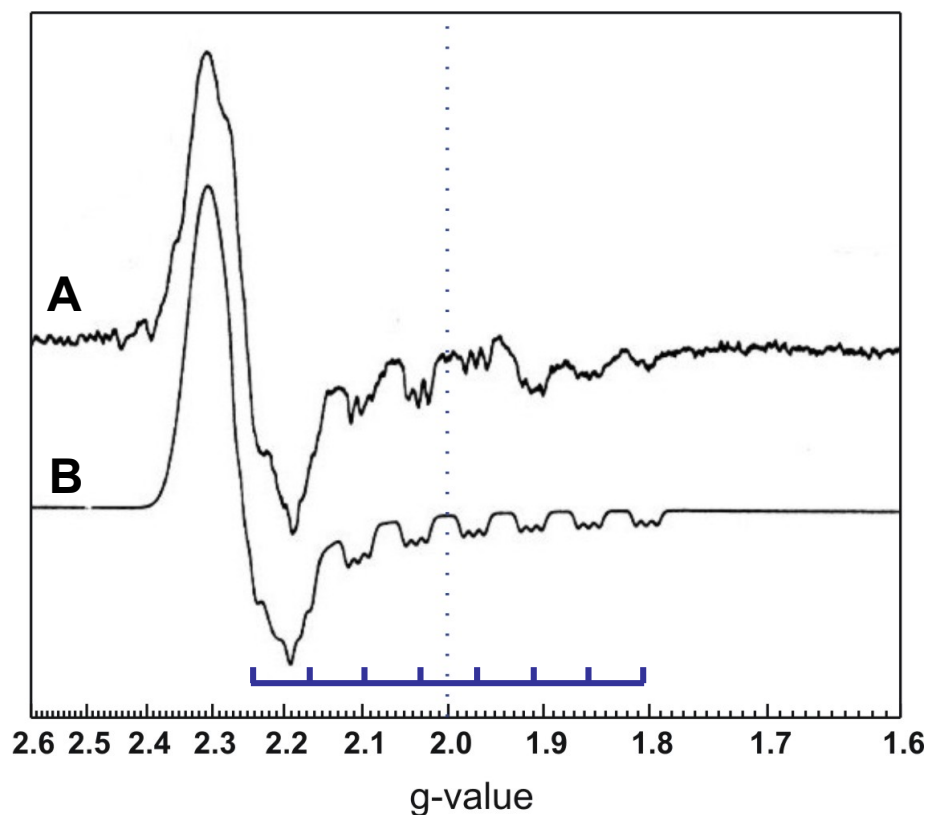Hydrogenase – g_{xyz} = 2.32, 2.24, 2.01
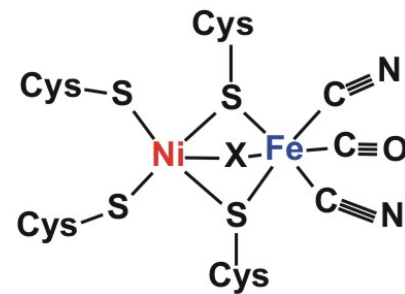
Methyl-coenzyme-M reductase
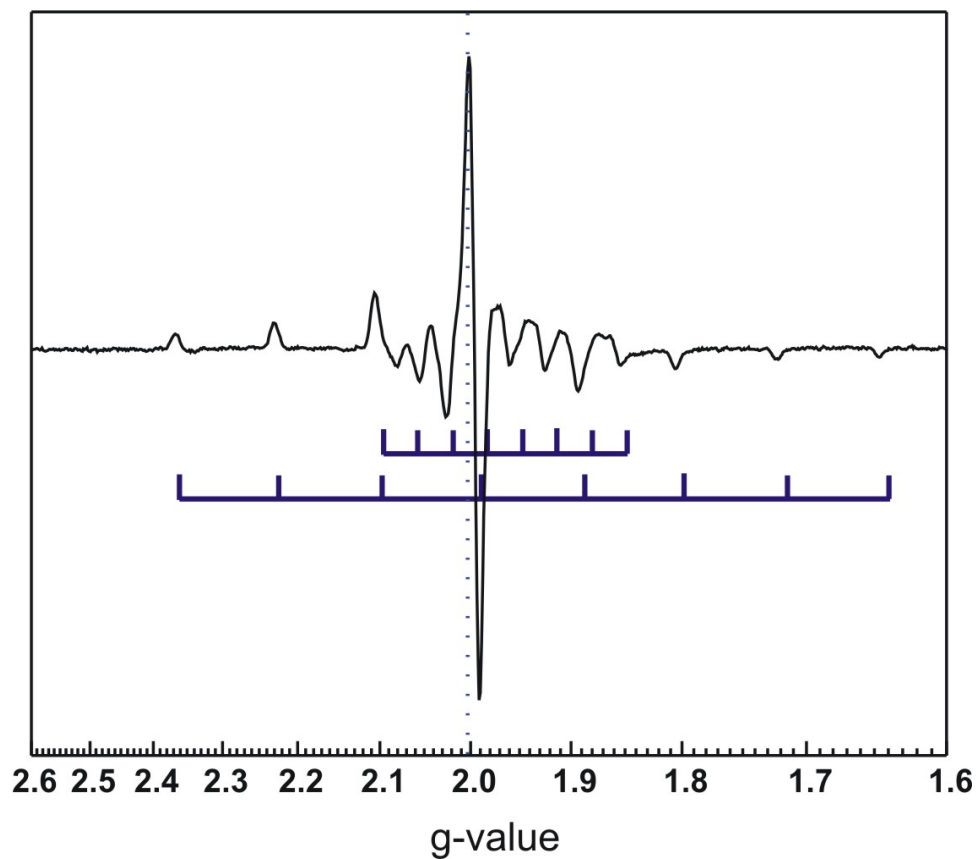g_{xyz} = 2.252, 2.073, 2.064

Type Identification - Metals

Methyltransferase from *M. marburgensis*. (**A**) Protein as isolated. (**B**) Computer simulation. g_{xyz} = 2.2591, 2.2530, 2.00659
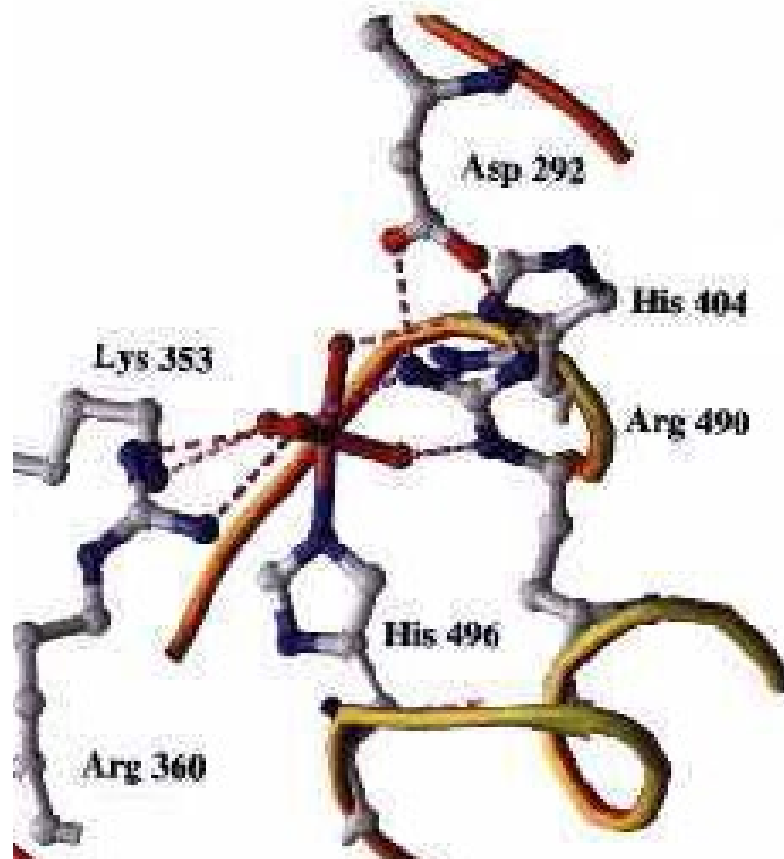
Co^+, d^8, $I = 7/2$

Type Identification - Metals
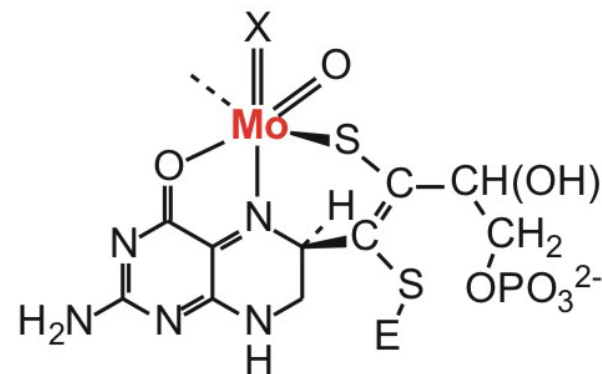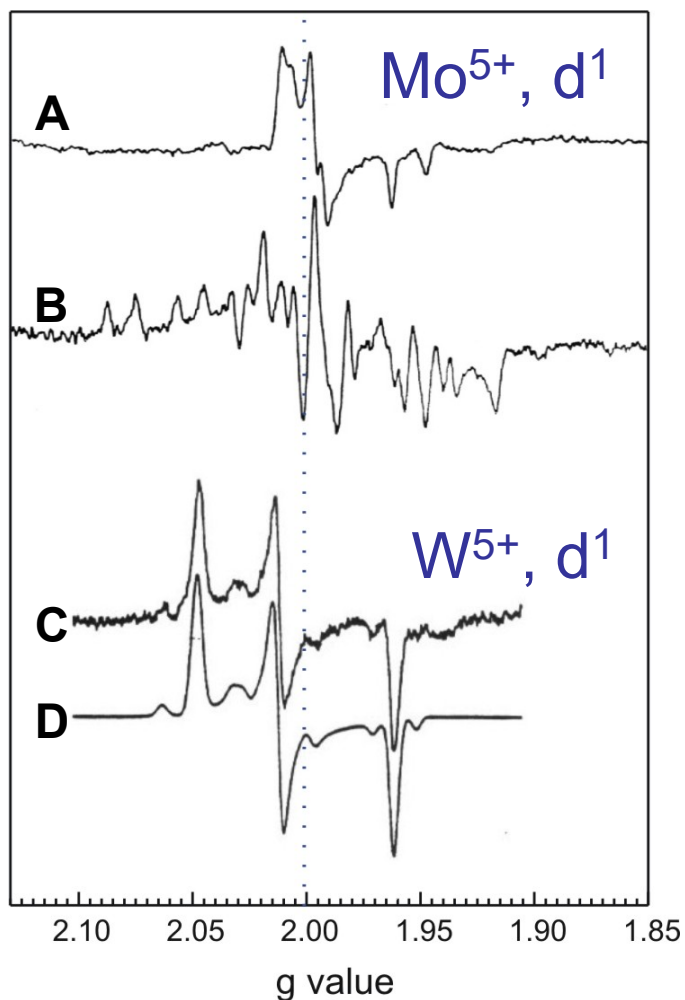
Vanadium-containing chloroperoxidase from the fungus *Curvularia inaqualis*

V^{4+}, d^1, $I = 7/2$
($g_{//} = 1.95$ and $g_{\perp} = 1.98$)

Type Identification - Metals

Mo^{5+}, d^1

W^{5+}, d^1

Methanobacterium wolfei formyl-methanofuran dehydrogenase (FDH I) isolated from cells grown on molybdate
  (A) Two signals with g_{xyz} = 2.003, 1.989, 1.955 and g_{xyz} = 2.00, 1.984, 1.941
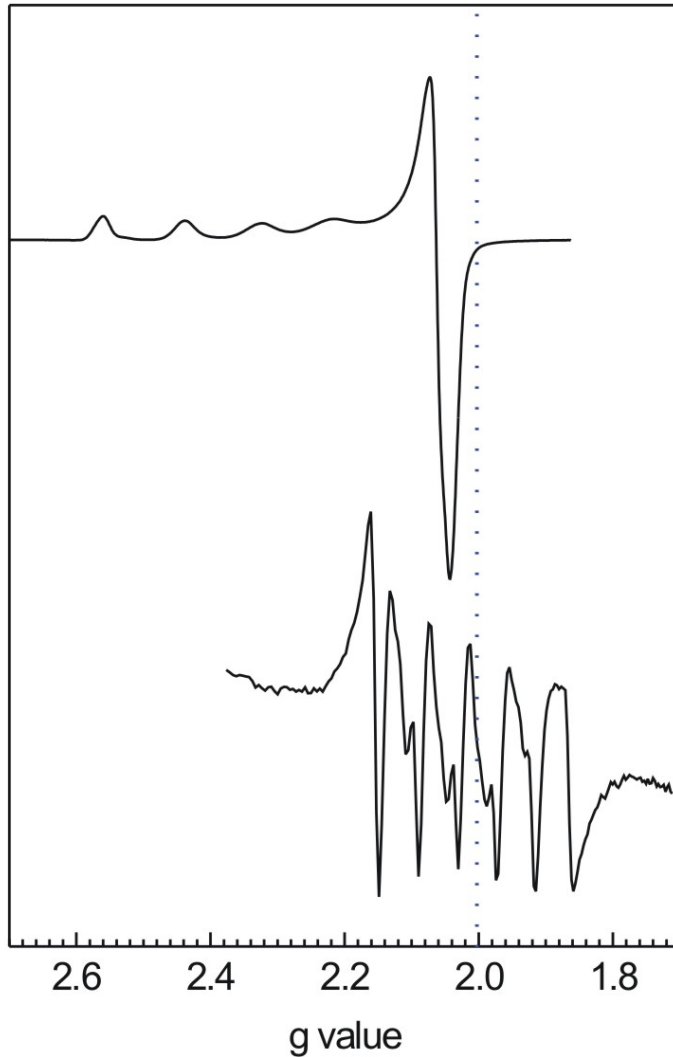 (B) Cells grown in the presence of ^{97}Mo-molybdate (I = 5/2).
FDH II from cells grown on tungstate.
  (C) g_{xyz} = 2.0488, 2.0122, 1.9635.
  (D) Simulation of C based on the natural abundance of the tungsten isotopes: I = 0: ^{180}W, 0.14%; ^{182}W, 26.4%; ^{184}W, 28.4% and I = 1/2: ^{183}W, 14.4%.
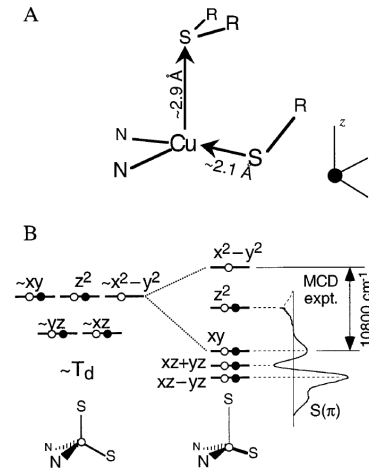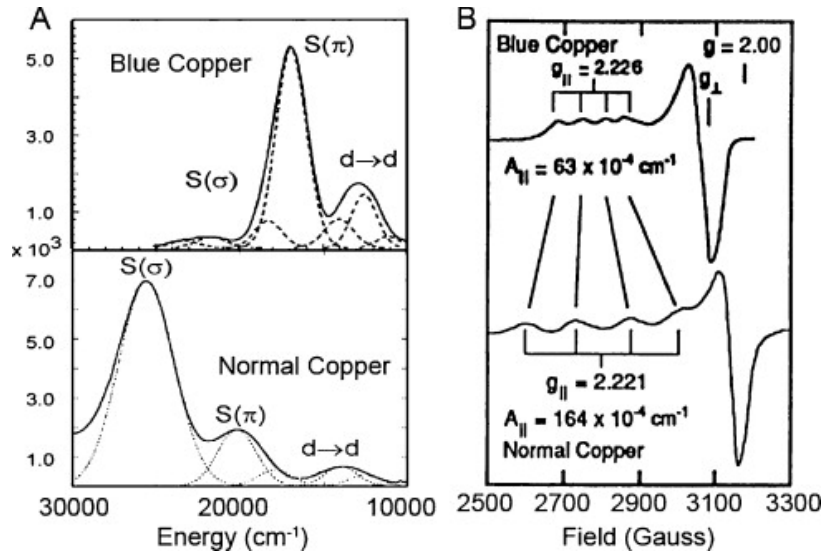
Type Identification - Metals

Cu^{2+}, d^9, I = 3/2

Mn^{2+}, d^5, I = 5/2
(S = 5/2)
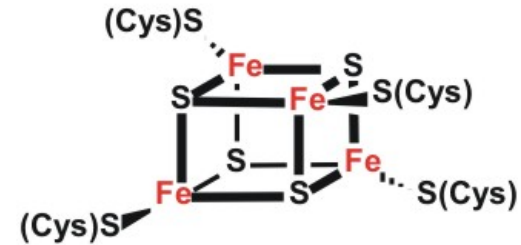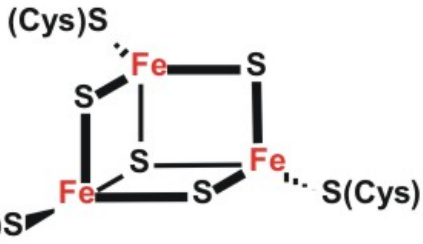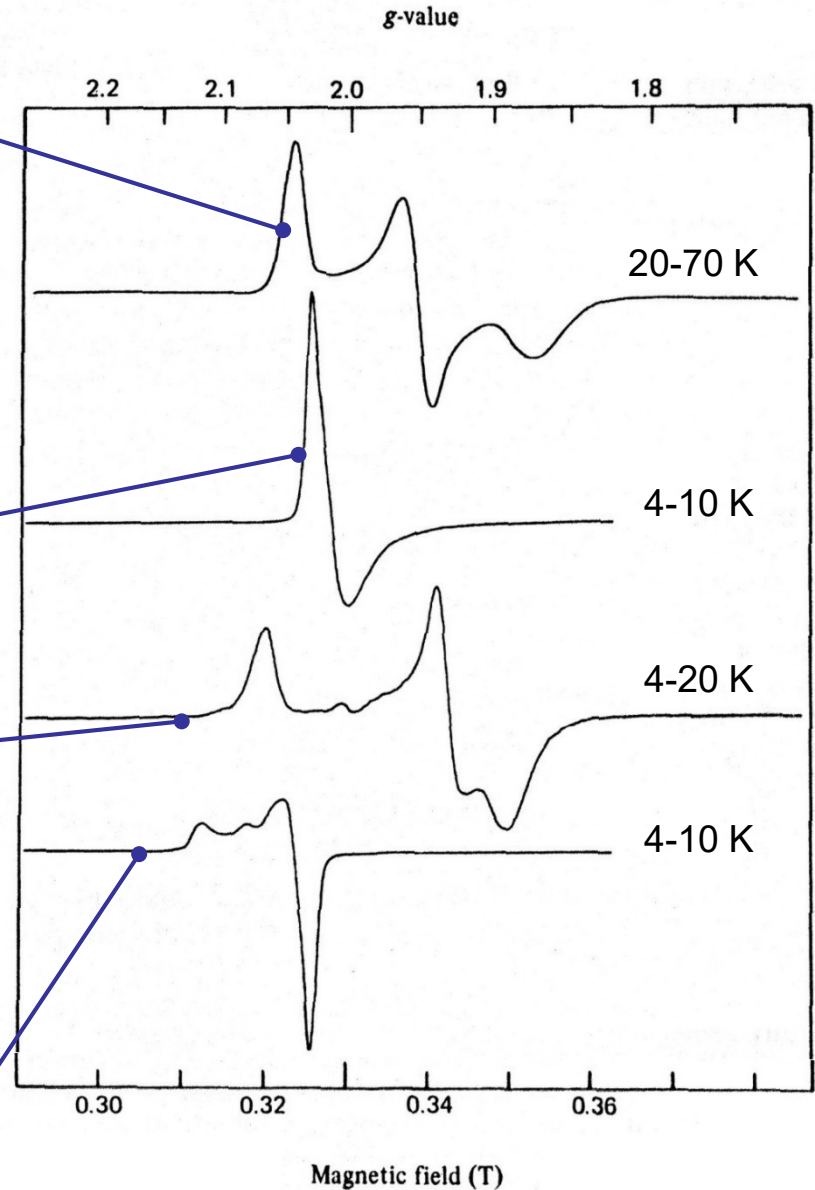
- (A) Comparison of blue Cu and normal Cu absorption spectra. (B) X-band EPR spectra of blue Cu (top) and normal Cu (bottom). (The normal Cu absorption spectrum is represented by nitrosocyanin where the large change in ligand field relative to plastocyanin described in leads to a bonded thiolate to Cu2+).

Axial ligand effects on the electronic structure of blue Cu sites. (A) Thiolate charge donation compensates for decreased axial thioether donor interaction . (B) Elimination of Jahn–Teller distortions in blue Cu sites: geometric distortion in (A) generates an electronic structure where $x^2 - y^2$ and xy splitting is $10\,800$ cm^{-1} from low temperature MCD.

Type Identification – Iron-sulfur Clusters

$[2Fe-2S]^{1+}$ S = ½

$[2Fe-2S]^{2+}$ S = 0

$[3Fe-4S]^{0}$ S = 2

$[3Fe-4S]^{1+}$ S = ½

$[4Fe-4S]^{1+}$ S = ½

$[4Fe-4S]^{2+}$ S = 0

(HiPIP)
$[4Fe-4S]^{2+}$ S = 0

$[4Fe-4S]^{3+}$ S = ½

g-value

20-70 K

4-10 K

4-20 K

4-10 K

Magnetic field (T)

Identification of Ligands

Bio ligand atom nuclear spins and their EPR *superhyperfine* patterns

Ligand	Isotope	Spin (abundance)	EPR lines
H	1, *2*	1/2 + 1 (0.015%)	2 + 3
C	12, *13*	0 + *1/2* (1.1%)	1 + 2
N	14, *15*	1 + *1/2* (0.4%)	3 + 2
O	16, *17*, 18	0 + *5/2* (0.04%)	1 + 6
F	19	1/2	2
P	31	1/2	2
S	32, *33*, 34	0 + *3/2* (0.8%)	1 + 4
Cl	35, 37	3/2	4
As	75	3/2	4
Se	76, *77*, 78, 80, 82	0 + *1/2* (7.6%)	1 + 4
Br	79, 81	3/2	4
I	127	5/2	6

Identification of Ligands

No interaction

   1 x S=1/2

   2 x S=1

   3 x S=3/2

4 x S=4
2 x 4 + 1 =9 peaks
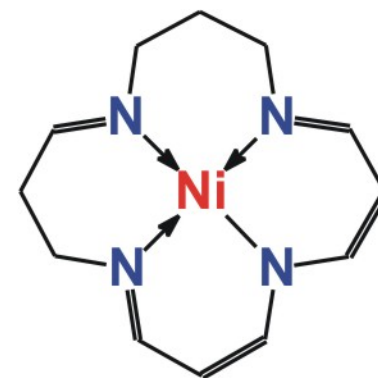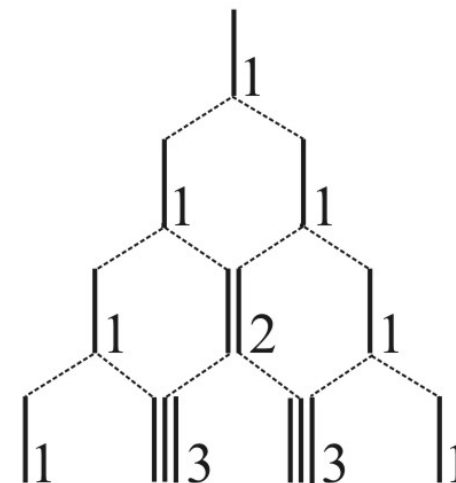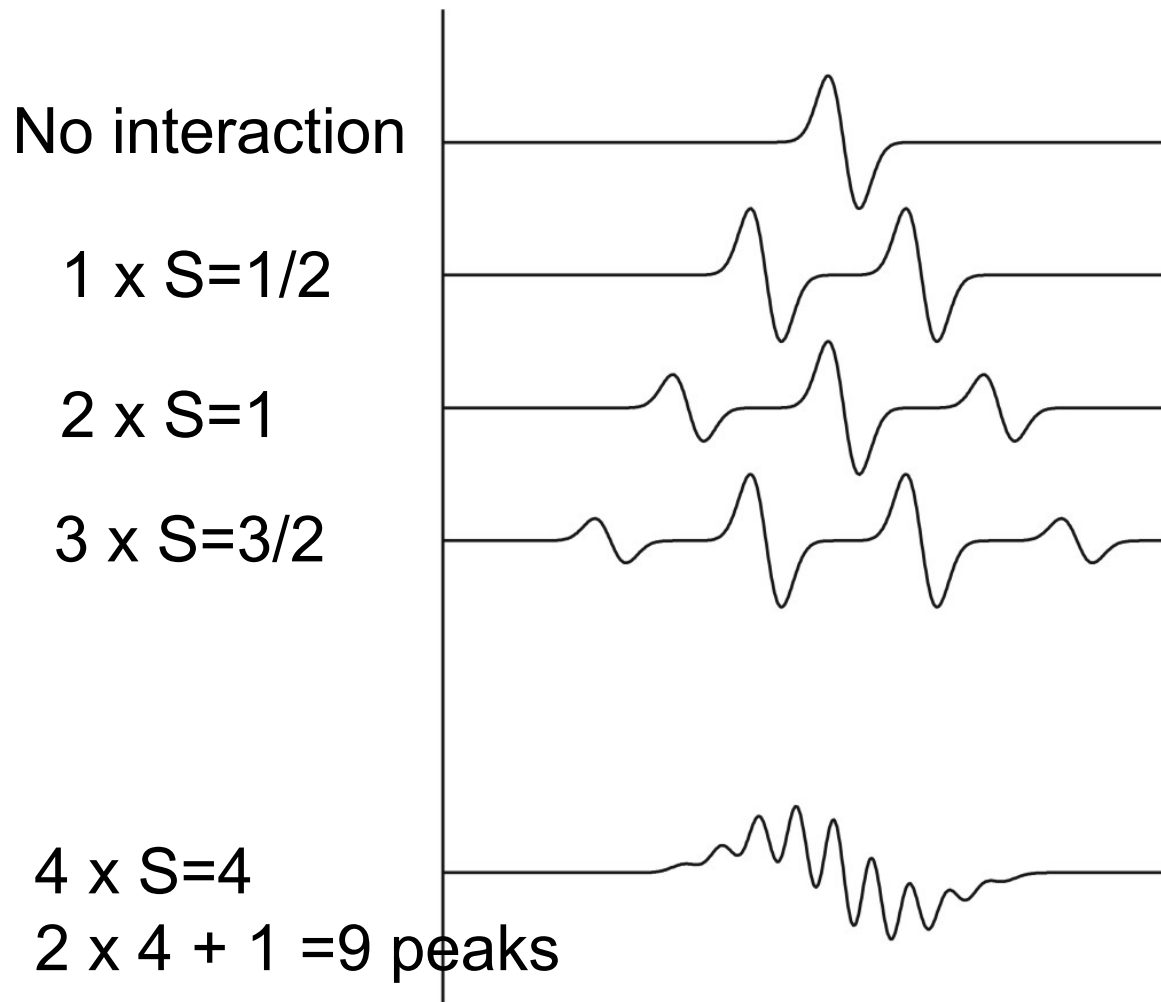
Identification of Ligands
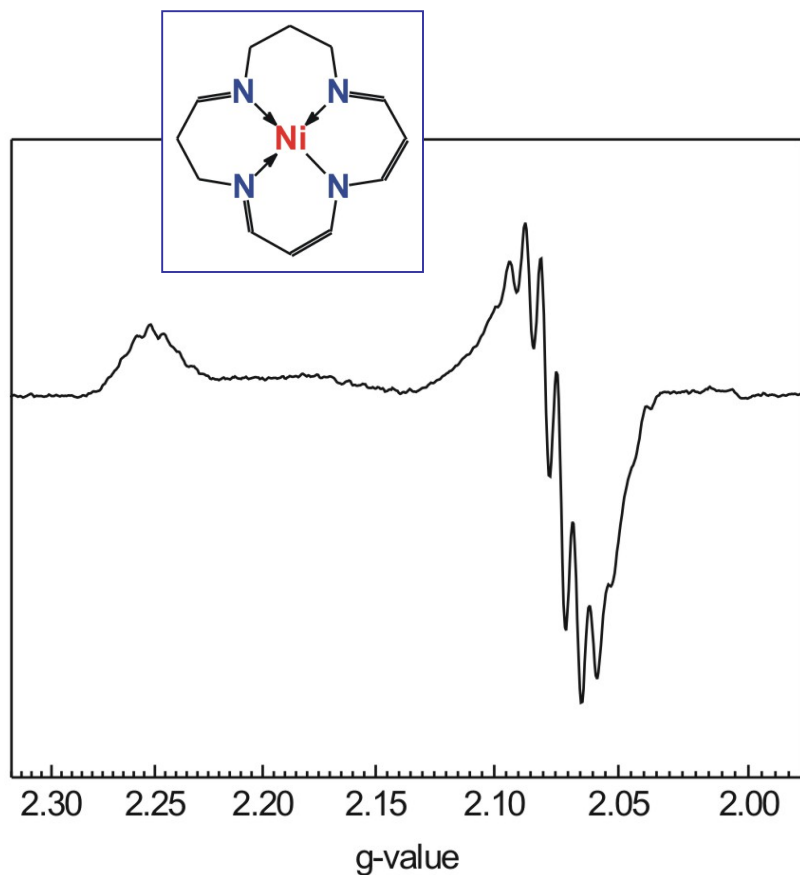
Free electron in $d_{x^2-y^2}$ orbital
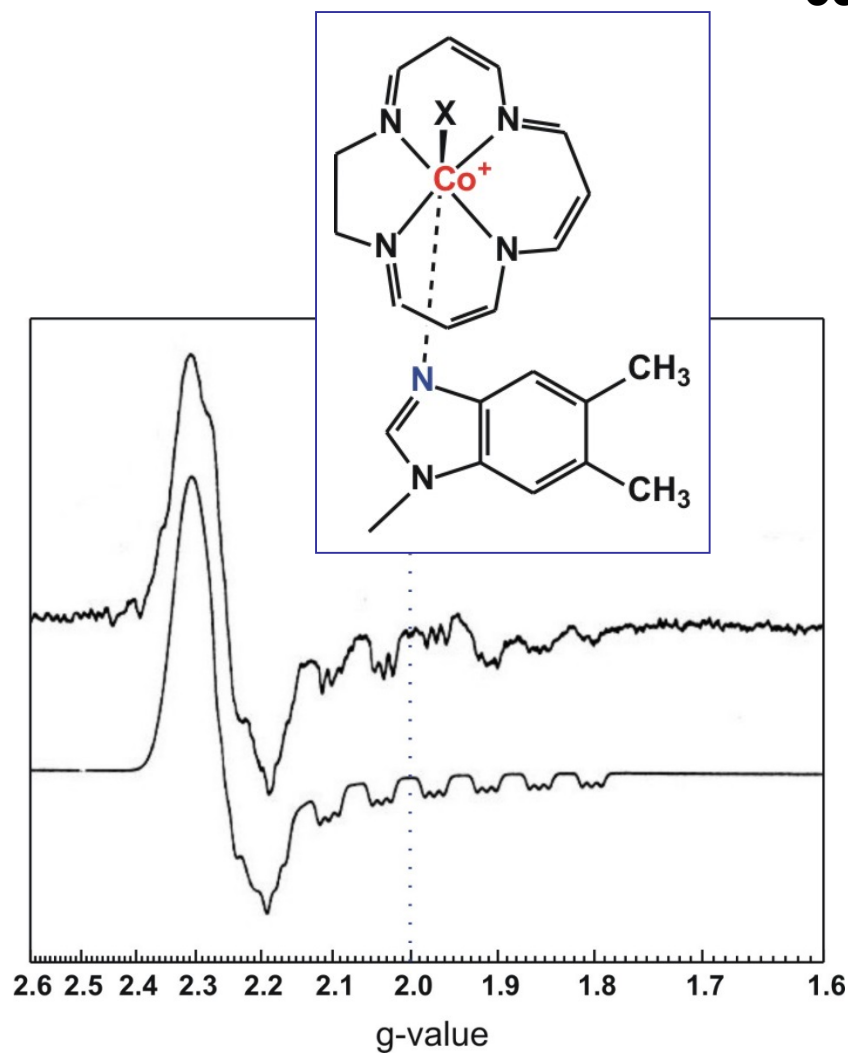
Free electron in d_{z^2} orbital

http://www.easyspin.org/
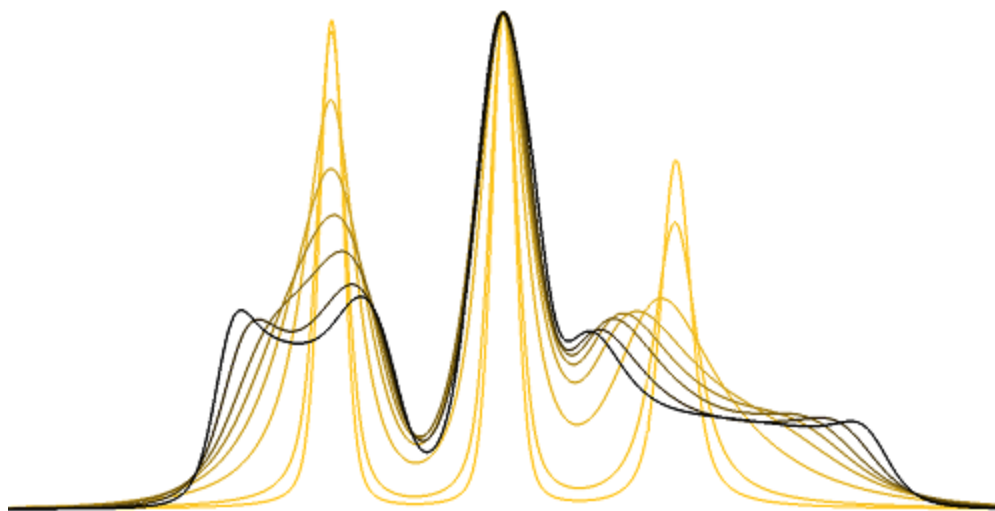
EasySpin is a MATLAB toolbox for simulating and fitting a wide range of Electron Paramagnetic Resonance (EPR) spectra. It supplements the numerical and visualization power of MATLAB with the best computational methods devised by EPR spectroscopists.

EasySpin runs on Windows, Linux and Mac, and is available free of charge.

Problems

1.If there is one unpaired electron in Cu^{2+} (I=3/2) and the copper ion is coordinated by one nitrogen atom (I=1) and one OH- (I=1/2), how many lines can be expected in the EPR spectrum? ((2*1*3/2+1)*(2*1*1+1)*(2*1*1/2+1)=24)

2.For a radical, the magnetic field is 3810 G, the frequency of the microwave is 9600 MHz. What is the value of its g-factor?

g=(71.4484)(9600x10−3)3810x10−1=1.800

3. Calculate the time correlation for
$g_x = 2.0094$, $g_y = 2.0059$, $g_z = 2.0023$

$A_x = 2\pi\ 18\text{x}10^6$, $A_y = 2\pi\ 22.5\text{x}10^6$, $A_z = 2\pi\ 103\text{x}10^6$ rad/s

$I(+1) = 13.5$, $I(0) = 16.4$, $I(-1) = 3.4$ (arbitrary units)

$\Delta B_o = 3.52$ Gauss $\beta = 9.274\text{x}10^{-21}$ erg/G
$\nu = 9.2449\text{x}10^9$ s^{-1} h=6.626x10^{-27} erg s
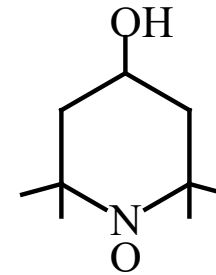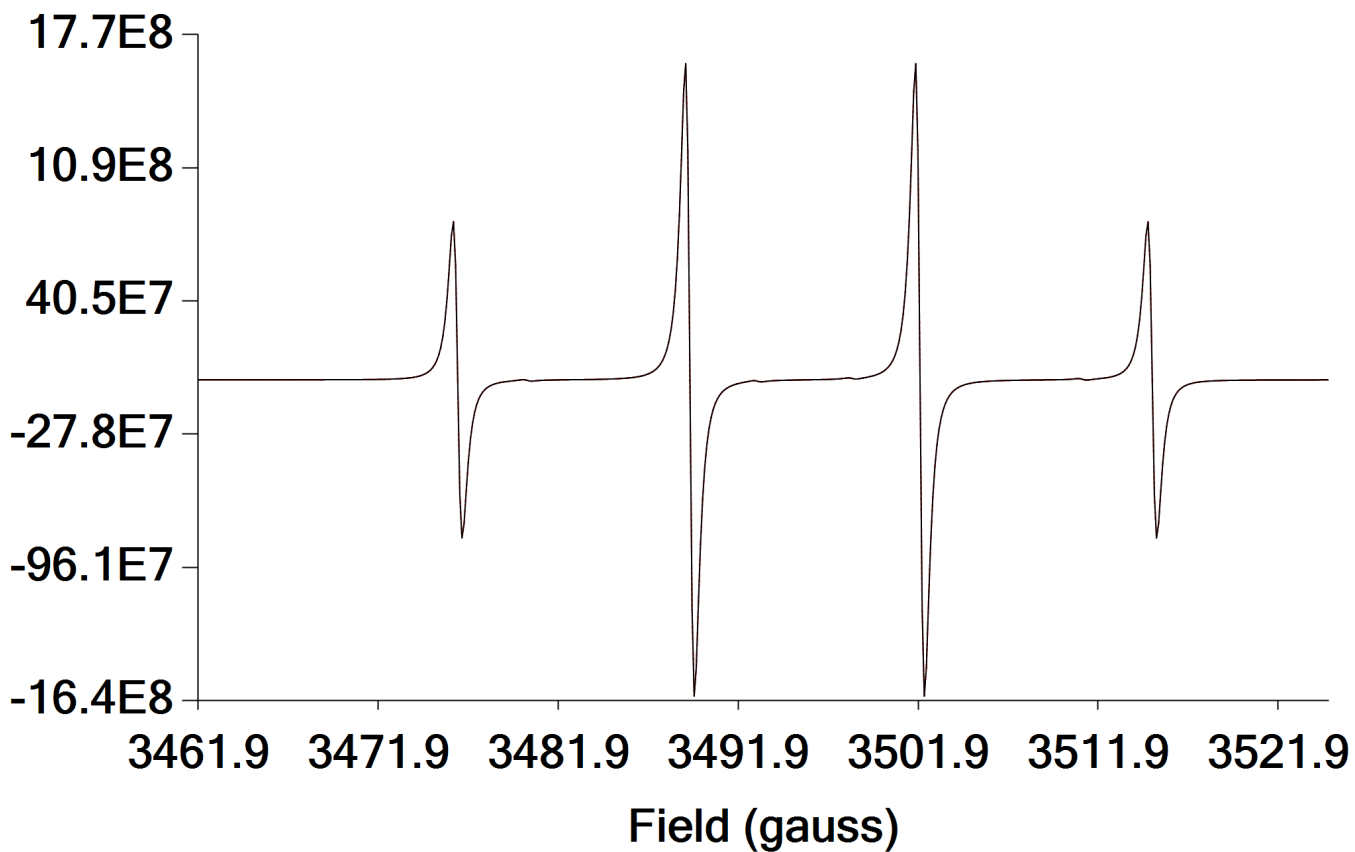
4. The above isotropic spectrum is from a radical that is coupled with one hydrogen and one nitrogen. Calculate the coupling constant of the electron with both nuclei.
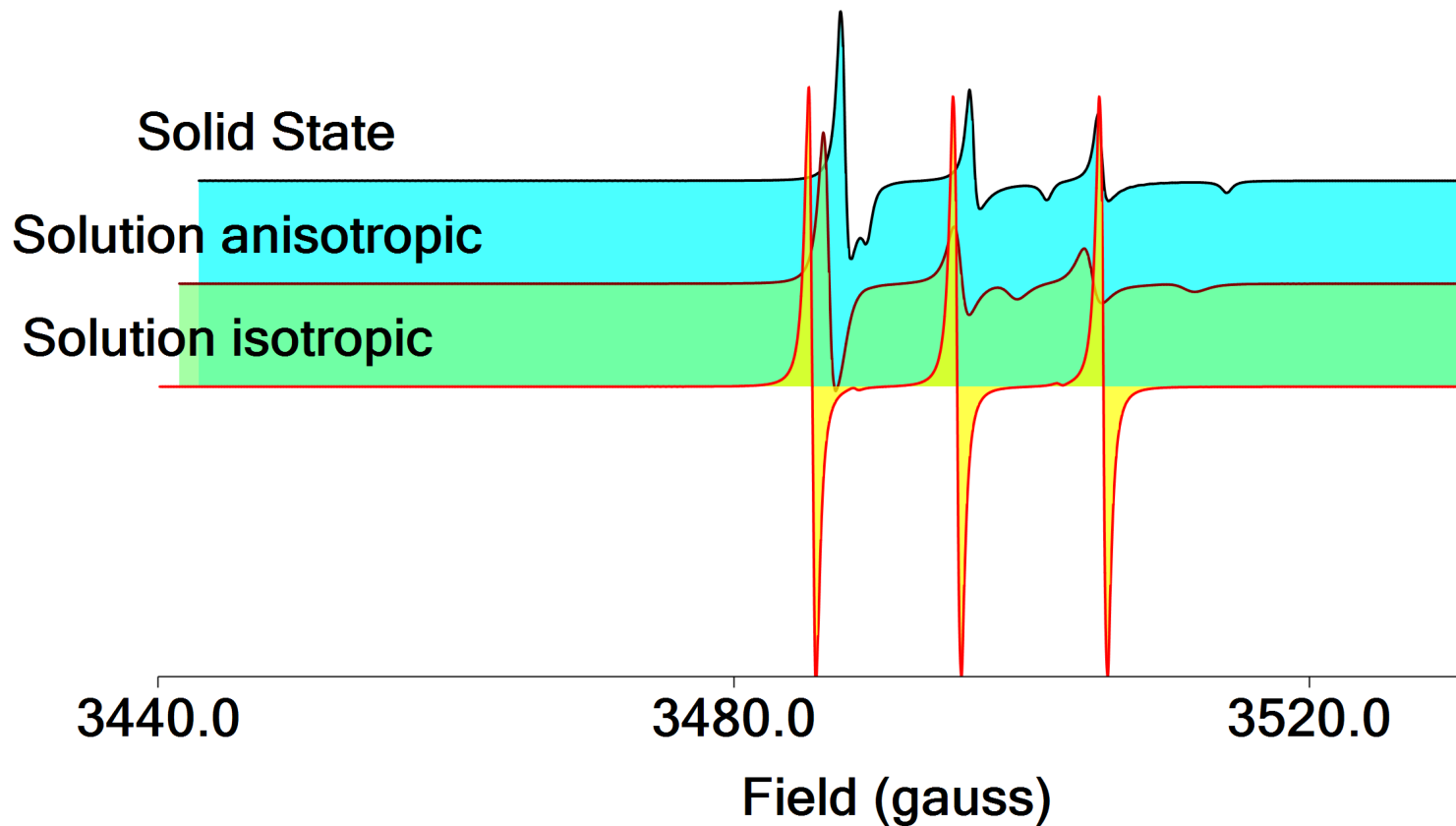
5. The above spectra are from the solution and solid state of a radical coupled with N atom. Calculate the isotropic and anisotropic *g* and *A* values. The frequency of the experiment is 9.8 GHz.

2nd Integral

3200   3400   3600

Field (gauss)
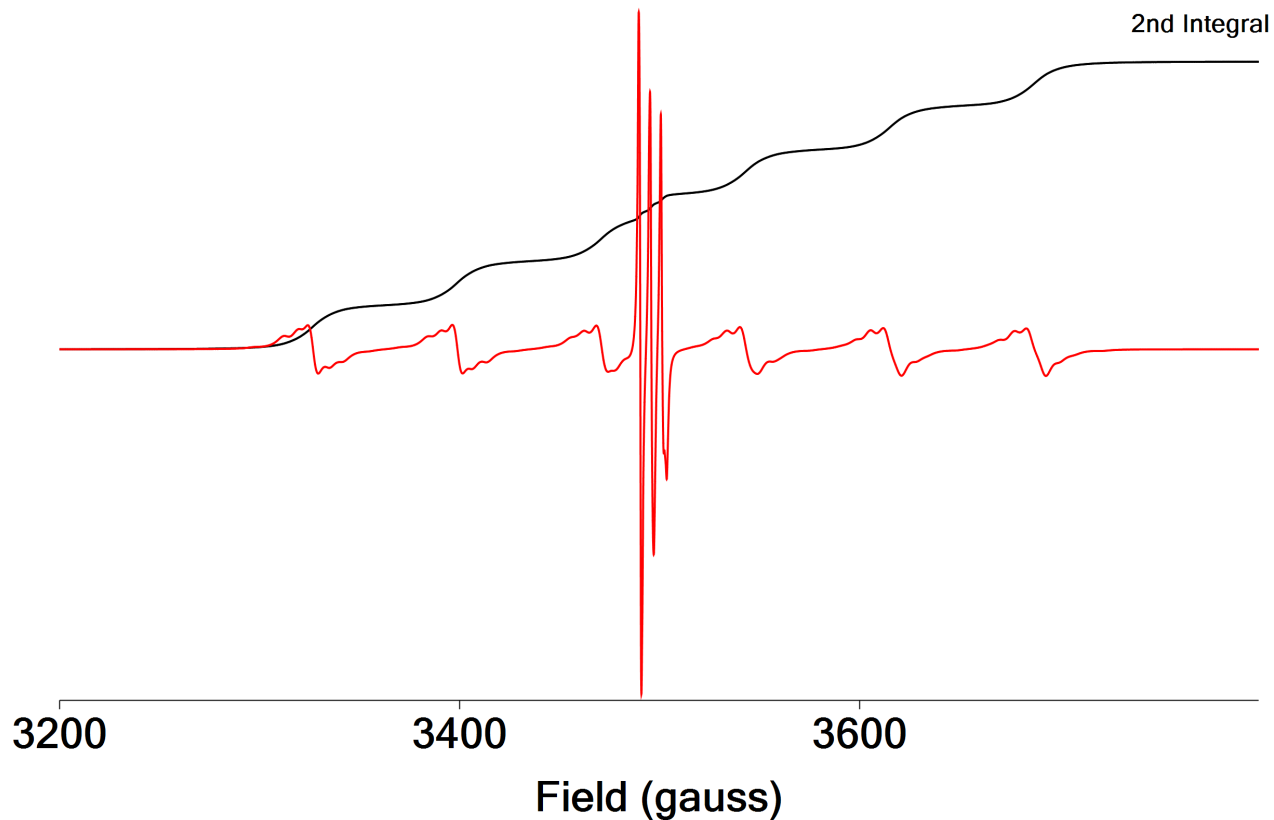
6. The above spectra show the solid state spectrum of a mixture of Mn^{2+} and a N centred radical. Determine the *g* and *A* for both paramagnetic species. From the double integral of the spectrum calculate their relative quantities. The frequency of the experiment is 9.8 GHz.