Electron Paramagnetic (Spin) Resonance

References: Jardetzky & Jardetzky, *Meth. Biochem. Anal.* **9**, 235.
Wertz & Bolton, **Electron Spin Resonance**
Poole, **Electron Spin Resonance...**
Abragam & Bleaney, **EPR of Transition Ions**
Atherton, **Electron Paramagnetic Resonance**
Palmer, in **Physical Methods in Bioinorganic Chemistry**

EPR is a probe of the oxidation state and chemical environment of:

1) **organic free radicals** (*e.g.*, semiquinones and spin-labels)
2) **transition metals** (*e.g.*, Fe^{3+}, Mo^{5+}, Cu^{2+}, Mn^{2+}, Co^{2+}, Ni^{3+} and multinuclear centers having half-integral electron spin)

Differences between EPR and NMR

1. A major reason for the several qualitative differences between electron and proton magnetic resonance is the difference between the particle masses:

$$m_{p^+}/m_{e^-} = 1838$$

The energy of a magnet in a magnetic field is: $E = -\boldsymbol{\mu}\cdot\mathbf{B} = -\mu\cos\theta\cdot B$
$= -\mu_Z\cdot B$ *for B in the z direction*

For a quantum mechanical system:

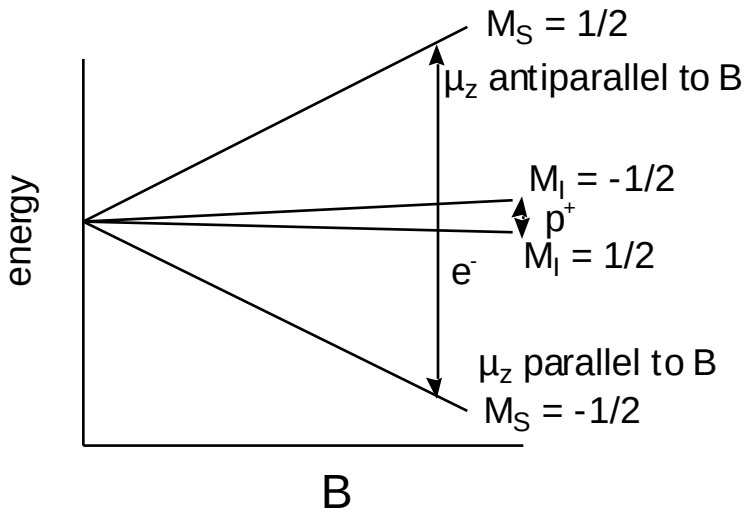
$$\mu_Z = (\hbar q)/(2mc)\cdot M_X = -g\beta_B M_S \text{ for } e^- \text{ and } +\beta_N M_I \text{ for } p^+$$

where: μ_Z = Z component of the magnetic moment
$\hbar$ = Planck's constant in units of cycles per second ($h/2\pi$)
q = charge of the particle (including the sign of the charge)
m = mass of the particle
c = speed of light
M_X = spin quantum number [$\pm 1/2$ for an e^- (M_S), or p^+ (M_I)]
g = a constant of proportionality for a given (electron) system
β = particle magneton (β_B for electron, β_N for proton)

For the electron, the solution E to the spin Hamiltonian describing the energy of the quantum mechanical system ($H\Psi = E\Psi$) becomes $E = g\beta_B B M_S$.

A magnetic field splits the $M_S = \pm 1/2$ spin states into two energy levels, separated by $\Delta E = g\beta B$. Because of the difference in mass of p^+ and e^-, a given field **B** will split the electron states about 2000-fold further than the proton states.

Zeeman Diagram



with: $E = g\beta BM_S$ and $\Delta E = g\beta B$

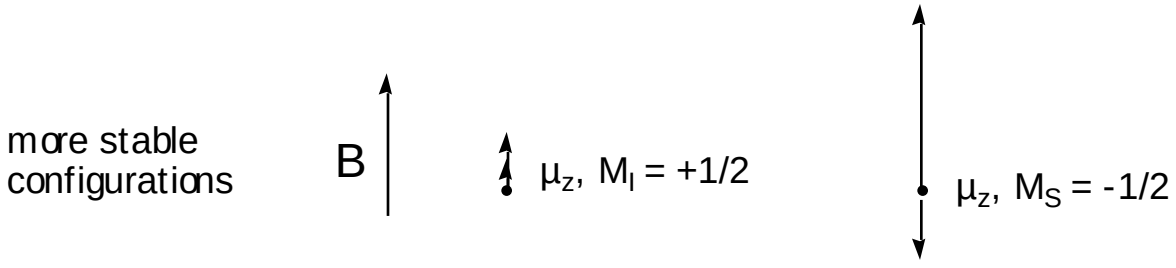
Consequences:

a. $\Delta E = h\upsilon$ The frequency is GHz for EPR and MHz for NMR: the EPR timescale is 1/GHz = 10^{-9} s while the NMR timescale is 1/MHz = 10^{-6} s

b. The thermal distribution of spins between the two Zeeman states is greater for e^- than p^+ (Boltzmann: $N_{ex}/N_g = \exp(-\Delta E/kT)$. Since the signal intensity magnetic resonance techniques is directly proportional to the difference in the two populations, EPR is intrinsically more sensitive than NMR (other things being equal).

ΔE is ~ 1cal/mol in an EPR experiment (1/2000 of that for an NMR experiment), much smaller than kT (~1 kcal/mol) and the excited state is substantially occupied thermally. The ratio of excited to ground state populations N_{ex}/N_g is about 0.999, meaning there is only about one extra spin in the ground state for every 2,000 electrons.

2. Because the charges are opposite for e^- and p^+, the spin quantum numbers associated with the lower-lying Zeeman state, *i.e.* that having μ_Z parallel to B, will be opposite in sign (recall $\mu_Z = qg\beta M_X$):



3. The **proportionality constant g** in the equation $\mu_z = qg\beta M_x$ is invariant for p^+, since the only angular momentum for proton is spin angular momentum. Electrons constrained in atomic or molecular orbitals, however, have orbital as well as spin angular momentum. The extent to which these couple will vary from system to system, and the observed value of g will vary from that of 2.0023 for the free electron. *On the other hand*, unpaired electrons are invariably confined to the outermost orbitals of molecules and ions, and there is no EPR equivalent to NMR chemical shift (the result of partial shielding of the proton magnetic moment from the external magnetic field by electrons in molecular orbitals).

For most organic free radicals, spin and orbital angular momenta are weakly coupled, and as a result g is ~ 2. With most transition metal complexes, on the other hand, spin-orbit coupling is significant, and g may vary between 1 and 9. Also, for an asymmetric molecule the extent of spin-orbit coupling will depend on the orientation of the molecule in the magnetic field. For a randomly oriented sample, up to three g values, corresponding to the three principal axes of an orthorhombic coordinate system, may be required to describe the observed EPR signal (referred to as g_z, g_y and g_x or g_1, g_2 and g_3, from low field to high). In such cases it is appropriate to speak of a tensor **g** that relates the magnetic moment μ to the spin quantum number. The **g** coordinate system is usually (but not necessarily) related to structural symmetry in the signal-giving species.

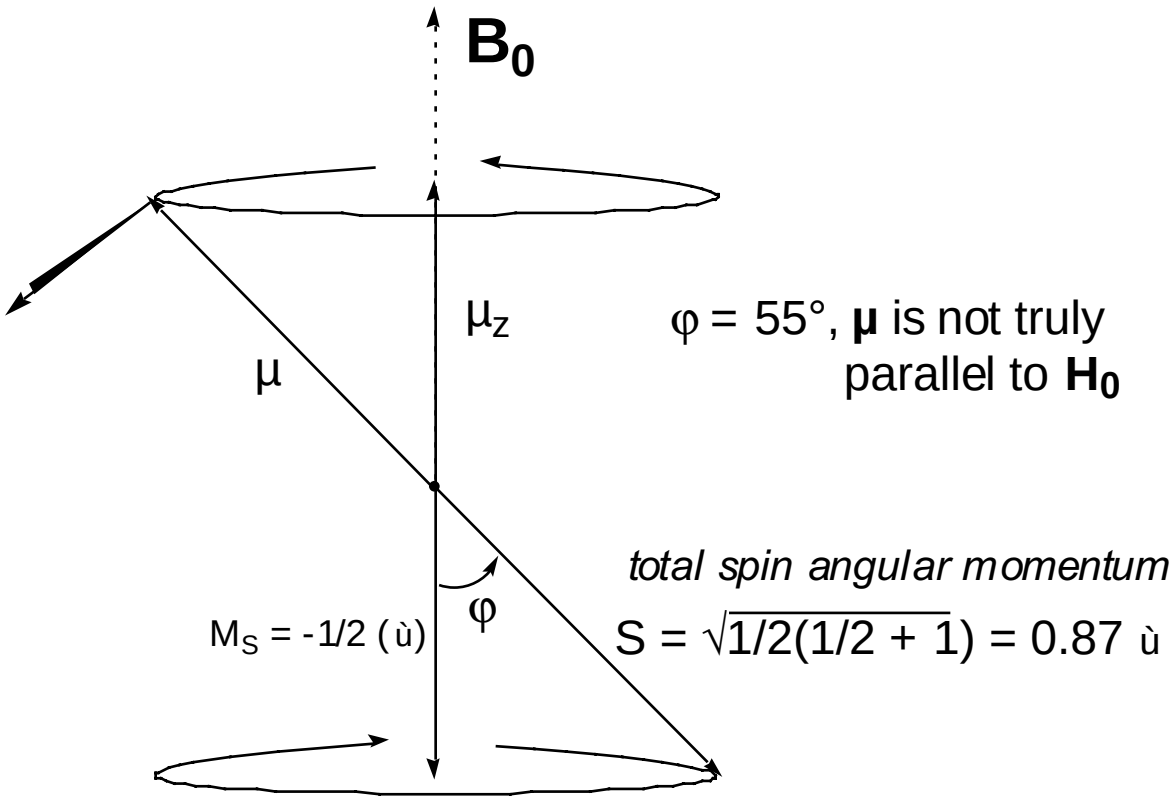
4. Relaxation from the resonance condition (below) is faster for electrons than protons. Since time and energy are related by the Heisenberg uncertainty principle ($\Delta E \cdot \Delta t \geq$ ù), EPR lines are broader than NMR lines. This is especially true in systems exhibiting spin-orbit coupling, because the coupling tends to increase the rate of relaxation from the resonance condition. The relaxation time may be slowed, sharpening the lines, by lowering the temperature.

Power saturation is the loss of signal at high levels of incident light (lots of energy being deposited in the system per unit time). It is due to the loss of the Boltzmann population disparity between the Zeeman energy states under conditions where the spin system is unable to relax from the resonance condition sufficiently rapidly to dissipate the energy being deposited into it (power = energy per unit time). Power saturation becomes an increasing problem at low temperatures where relaxation is relatively slow.

The trade-off between a suitably low temperature for reasonable EPR linewidths and a sufficiently high temperature to avoid power saturation is a significantly more serious consideration with EPR compared to NMR. Many EPR experiments are performed well below ambient temperatures (2 - 150 K).

The Resonance Phenomenon

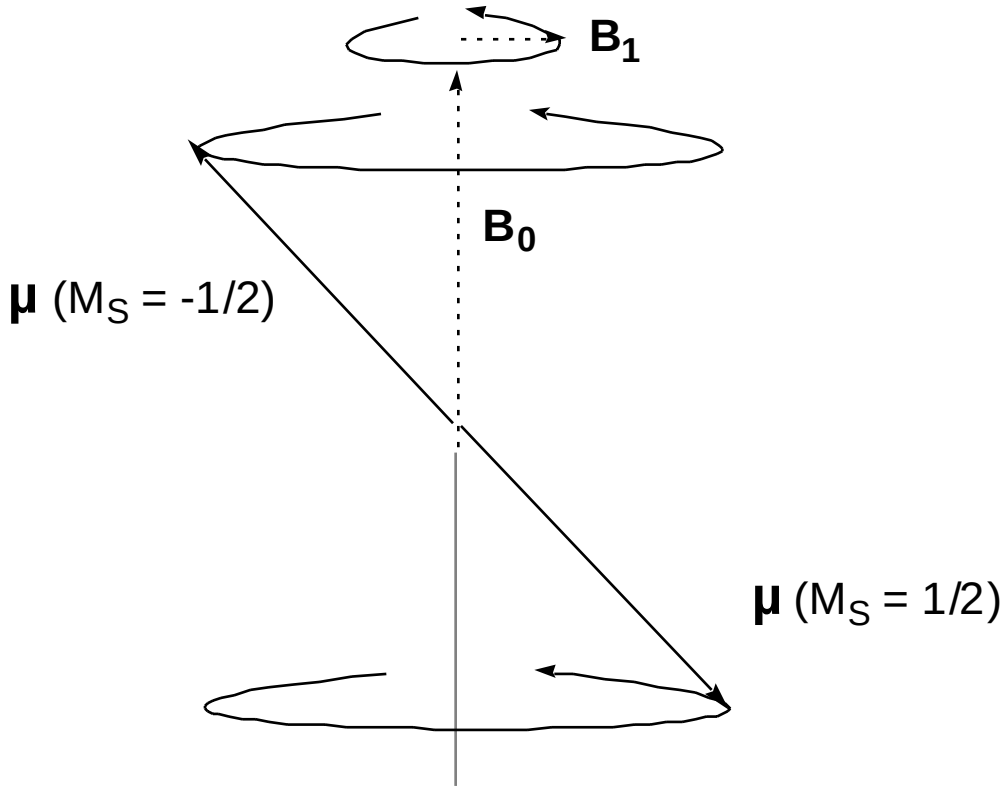
In its effort to align with the external magnetic field $\mathbf{B_0}$, the magnetic moment $\boldsymbol{\mu}$ of an unpaired electron is subjected to a torque perpendicular to both $\mathbf{B_0}$ and $\boldsymbol{\mu}$ that forces $\boldsymbol{\mu}$ to precess about $\mathbf{B_0}$:



The angular frequency of the precession, called the Larmour frequency, is related to the external magnetic field strength B_0, by the gyromagnetic ratio γ (= -gβ/ ù):

$$\omega_0 = \gamma B_0$$

Consider the presence of a second, much smaller magnetic field $\mathbf{B_1}$. If $\mathbf{B_1}$ is stationary, its orientation relative to **μ** is constantly changing due to the precession, and only the component of $\mathbf{B_1}$ parallel to $\mathbf{B_0}$ does not average to zero over time. The net effective magnetic field sensed by **μ** is $\mathbf{B_{eff}} = \mathbf{B_0} + \mathbf{B_1}$, approx. $\mathbf{B_0}$. If, however, $\mathbf{B_1}$ is spinning about $\mathbf{B_0}$ at the Larmour frequency of precession, it is stationary with respect to **μ** and can no longer be ignored, even though $\mathbf{B_1} << \mathbf{B_0}$. Under these conditions, **μ** will precess about $\mathbf{B_1}$ with a frequency $w_1 = gH_1 << w_0$. The resulting motion is that of a tight spiral from the **μ** orientation parallel to $\mathbf{B_0}$ to that antiparallel and back again. This motion is termed **resonance** .



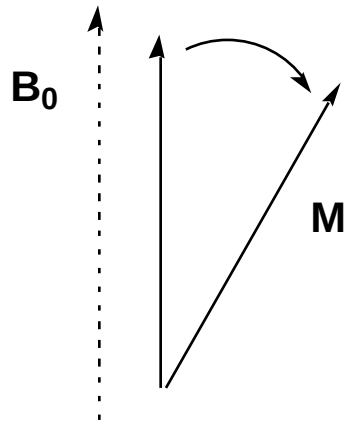
Experimentally, in an EPR experiment the spinning magnetic field $\mathbf{B_1}$ is provided by the oscillating magnetic wave vector of the right circularly polarized component (+) of linearly polarized microwave frequency light (The left, or - component rotates in the opposite direction to the precession of **μ** about $\mathbf{B_0}$ and its effect on the motion of μ time-averages to zero. It is the left circularly polarized component, however, that precess in synchrony with a proton's precessing magnetic moment.).

The reorientation of **μ** (and therefore also M_S) in the strong magnetic field $\mathbf{B_0}$ requires energy, which is provided by the absorption of a quantum of the microwave frequency light. At resonance, $\Delta E = h\upsilon_0 = g\beta B_0$.

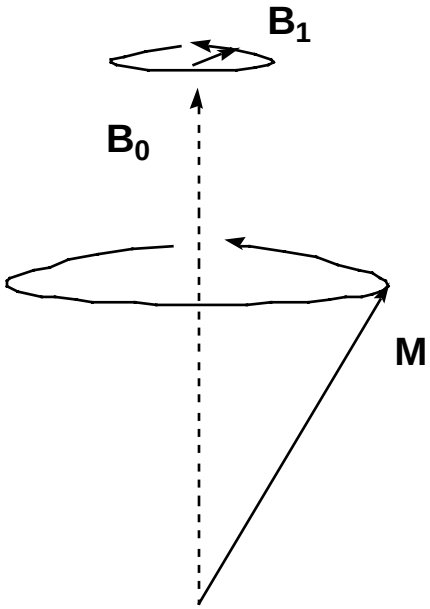
For an ensemble of particles...

... with a Boltzmann distribution between the two Zeeman energy states, those electrons with M_S = -1/2 at the outset of resonance absorb energy, while those with M_S = 1/2 emit energy. It is only because the two populations are not equal that there is a net absorption at resonance. Further, all components of the uncanceled **μ**'s in the M_S = -1/2 state that are in the x-y plane also cancel, since their precessions about $\mathbf{B_0}$ are not in phase. This cancellation leaves only a *bulk magnetization vector* **M** that is truly parallel to $\mathbf{B_0}$.

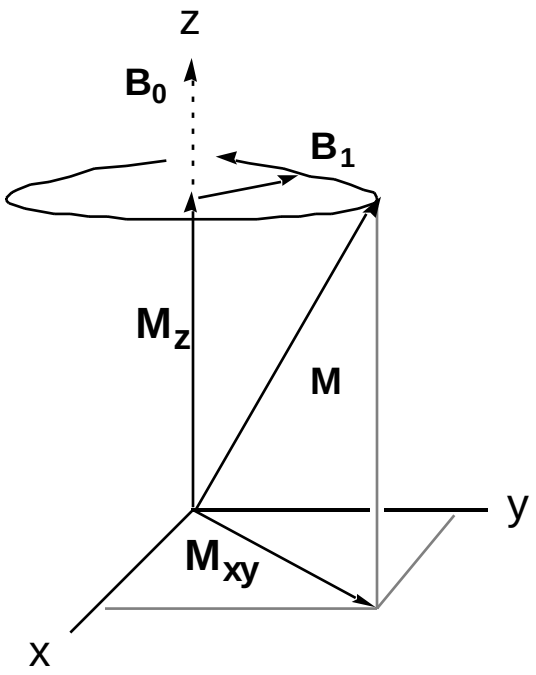
The net effect of $\mathbf{B_1}$ is to tip **M** out of parallel with $\mathbf{B_0}$. Now out of parallel, **M** precesses about $\mathbf{B_0}$ with frequency ω_0 while relaxing to its equilibrium position parallel to $\mathbf{B_0}$.



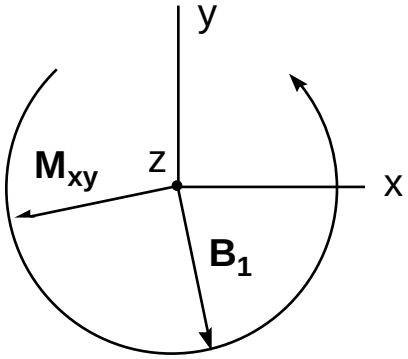
In a pulse experiment, **M** is transiently tipped out of parallel with $\mathbf{B_0}$ by a high-power square-wave pulse. The rate at which **M** spirals back to its initial position is characterized by the *longitudinal relaxation time* τ_1 (or T_1) of the particular system observed. It is this rate that (in part) determines the linewidth of the EPR spectrum and the sensitivity of the system to power saturation. τ_1 is also referred to as the *spin-lattice relaxation time*, because the energy absorbed by the system in tipping **M** out of parallel with $\mathbf{B_0}$ is dissipated to the surroundings (*i.e.*, the lattice) in the course of **M** returning to its initial position.



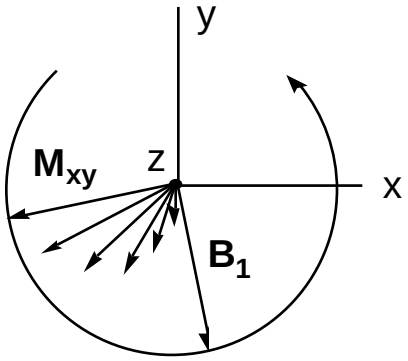
In a continuous-wave experiment, the scan rate is slow, with a low-power source. At resonance a steady-state is attained where the absorption and dissipation of microwave energy balance one another. The result that **M** sits at a constant angle with respect to $\mathbf{B_0}$ and now has components $\mathbf{M_z}$ along the z axis and $\mathbf{M_{xy}}$ in the x-y plane. At resonance, $\mathbf{M_{xy}}$ is lags 90° behind $\mathbf{B_1}$ in precessing about the z axis, and precesses about $\mathbf{B_1}$ (the motion of **M** is comparable for that of **μ** for a single particle). $\mathbf{M_z}$ and $\mathbf{M_{zy}}$ need not relax at the same rates, and the *transverse relaxation time* τ_2 (or T_2) characterizes the latter.



Perspective looking back down the z axis at resonance. Because $\mathbf{M_{xy}}$ is stationary with respect to $\mathbf{B_1}$, it precesses about it as the two precess about the z axis.



In the course of a sweep, as the resonant frequency is approached, **M** tips out of parallel with $\mathbf{B_0}$ and $\mathbf{M_{xy}}$ grows in. Initially it is approximately in-phase with $\mathbf{B_1}$, but becomes increasingly out of phase as the resonant frequency is approached. As soon as $\mathbf{M_{xy}}$ becomes finite, it begins to precess about $\mathbf{B_1}$ according at $\omega_1 = \gamma B_1$.

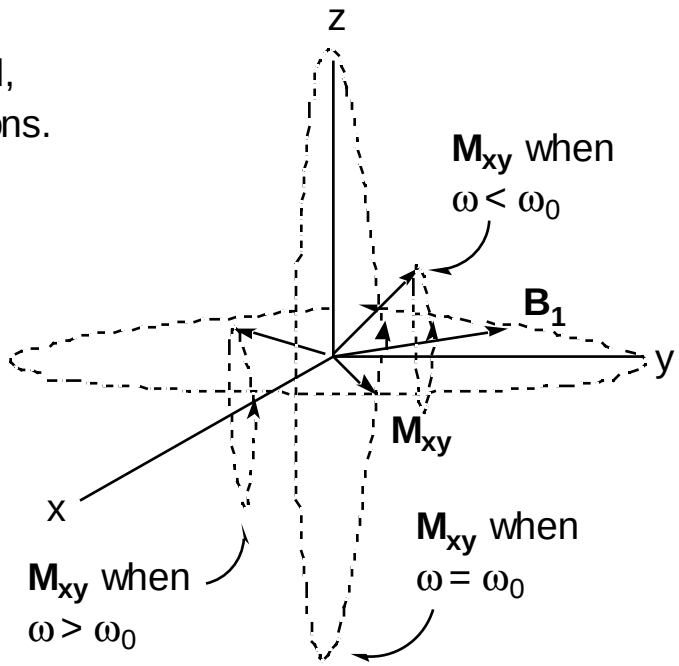


The motion of $\mathbf{M_{xy}}$ in the course of a scan through the region around ω_0 is complicated, but given quantitatively by the Bloch equations. Under steady-state conditions these are:

$$\mathbf{M_x} = \mathbf{M_0}\gamma T_2 \frac{T_2(\omega_0-\omega)B_1\cos\omega t + B_1\sin\omega t}{1 + T_2^2(\omega_0-\omega)^2 + \gamma^2 B_1 T_1 T_2}$$

$$\mathbf{M_y} = \mathbf{M_0}\gamma T_2 \frac{H_1\cos\omega t - T_2(\omega_0-\omega)B_1\sin\omega t}{1 + T_2^2(\omega_0-\omega)^2 + \gamma^2 B_1 T_1 T_2}$$

$$\mathbf{M_z} = \mathbf{M_0} \frac{1 + T_2^2(\omega_0-\omega)^2}{1 + T_2^2(\omega_0-\omega)^2 + \gamma^2 B_1 T_1 T_2}$$

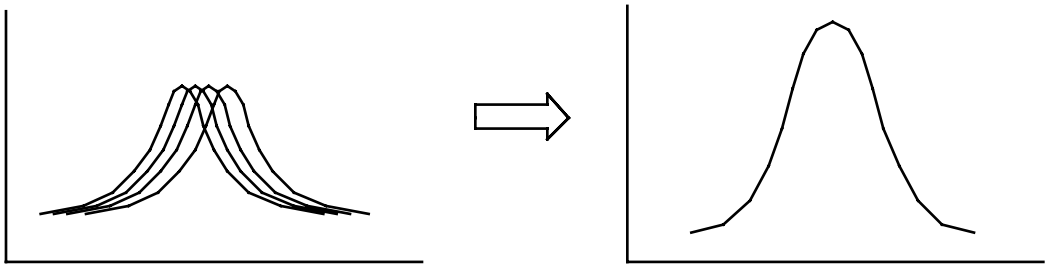


The bulk magnetization vector can be understood in terms of *magnetic susceptibilities* (χ) along the z axis and in the xy plane, each given the equation $\mathbf{M} = \chi\mathbf{B}$. Because of the time dependence of the direction of $\mathbf{M_{xy}}$ as it rotates about the z axis) two magnetic susceptibilities are required to describe its motion, an in-phase χ' and an out-of-phase χ''. An examination of the figure above provides a justification for the absorption of energy being a function of the out-of-phase χ''.

It can be shown that the amount of energy absorbed per period of $\mathbf{B_1}$ at any frequency is proportional to χ'', as given by:

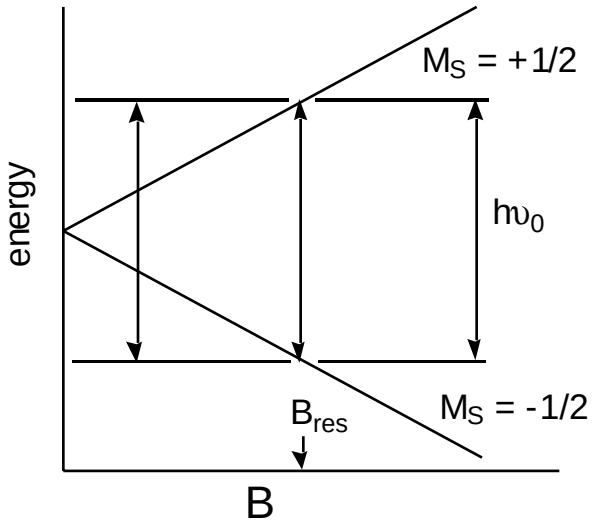
$$E(\nu) = 2\omega B_1^2\chi'' = \frac{2\omega B_1^2\chi_0\omega_0 T_2}{1 + 4\pi^2 T_2^2(\nu_0 - \nu)^2 + \gamma^2 B_1^2 T_1 T_2}$$

The lineshape function $2T_2/(1 + 4\pi^2T_2^2(\nu_0 - \nu)^2 + \gamma^2B_1^2T_1T_2)$ simplifies to a Lorentzian form when $B_1 << 1/\gamma T_1 T_2$: $2T_2/(1 + 4\pi^2T_2^2(\nu_0 - \nu)^2)$. Most frozen biological samples give a distribution of such lines, in which case the signal is said to be inhomogenously broadened. Such composite signals are typically *Gaussian* rather than *Lorentzian* in shape.



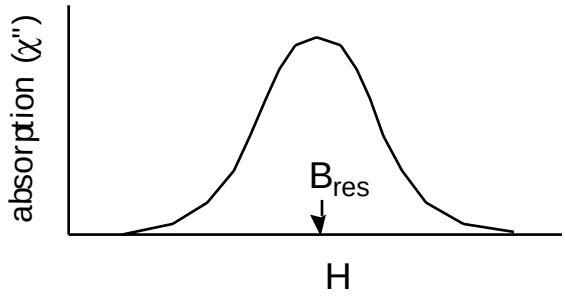
EPR Spectra

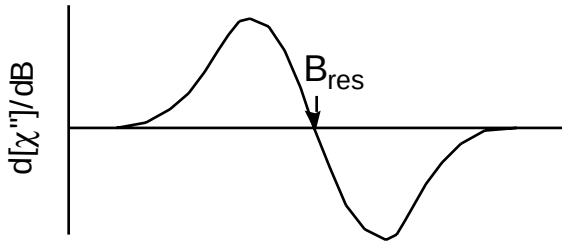
The selection rule for an allowed EPR transition is $\Delta M_S = \pm 1$. (Recall that $E = g\beta B M_S$ for the electron.) For technical reasons, it is more convenient to vary the magnetic field strength H_0 than the microwave frequency. The effect is to vary the energy separating the Zeeman energy states rather than the excitation energy itself.

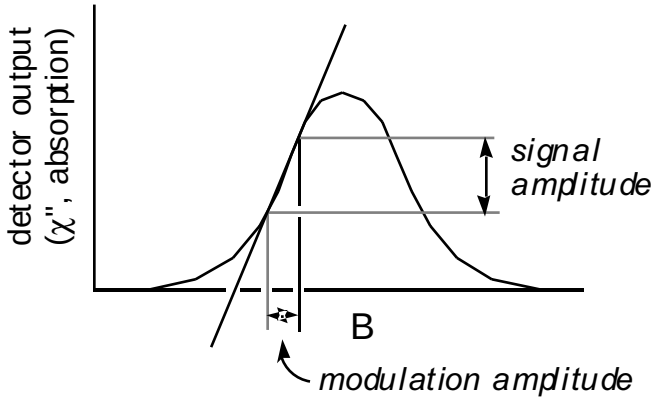


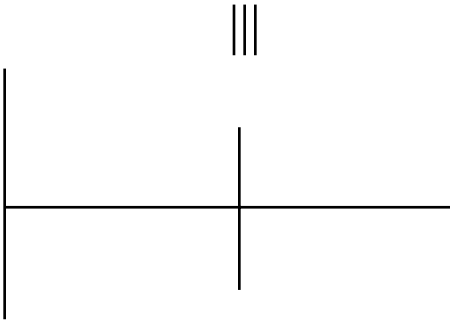
$$B_{res} \text{ (in gauss)} = \frac{h\upsilon}{g\beta} = \frac{714.484 \times \upsilon \text{ (in GHz)}}{g}$$

Also, to improve the signal-to-noise of the spectrometer, the magnetic field is modulated (typically at ~100 kHz) so that noise can be filtered out. The result is that the slope of the absporption envelope is obtained, rather than the absorption envelope itself.





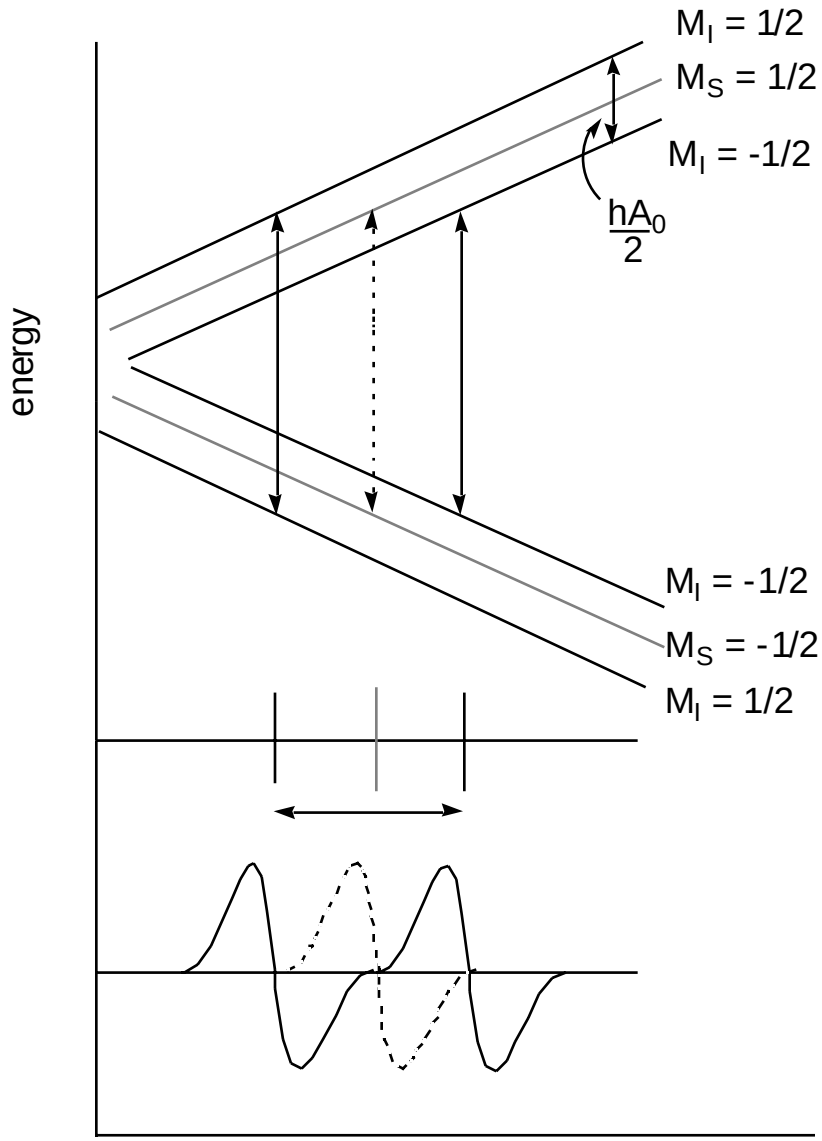




The amplitude of the field modulation (in Gauss) has significant effect on the observed spectrum. Too small a modulation and the signal is weak, too large a modulation and the shape of the spectrum is distorted. A maximum safe modulation of half the linewidth of the narrowest observed feature is a good rule of thumb.

In the presence of a proton, there are two possible orientations for M_I for each M_S state. The resulting Zeeman diagram has a pair of doublets.

The solution to the Hamiltonian gives: $E = g\beta BM_S + hA_0M_SM_I$ where A_0 is the hyperfine coupling constant, generally expressed in units of MHz (or occasionally cm^{-1}). The selection rule in this case is $\Delta M_S = \pm 1$, $\Delta M_I = 0$.



The resulting spectrum is a doublet, just as in NMR, with the two new lines centered on the original line and separated by a, the hyperfine splitting:

$$a \text{ (in gauss)} = \frac{0.714484 \times A_0 \text{ (in MHz)}}{g}$$

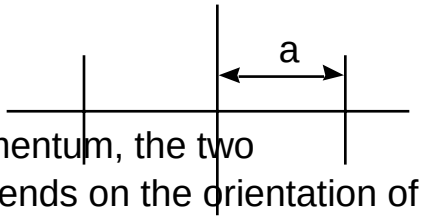
A big hairy equation: $$A_0 = \frac{{}^8/_3\pi \times \gamma_e\beta_B\beta_N \times \varphi^2}{h}$$

where φ^2 is the probability of finding the lone electron at the coupled nucleus.

The point is that A_0 is proportional to β_N, and thus will vary from one element to another, and from one isotope of an element to another in a predictable fashion (e.g., $^{14}N \rightarrow {}^{15}N$).

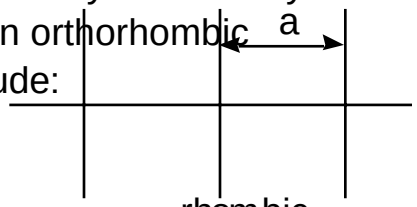
The term "hyperfine" is by convention used when the magnetically coupled nucleus is host to the lone electron. Otherwise, "superhyperfine" is used.

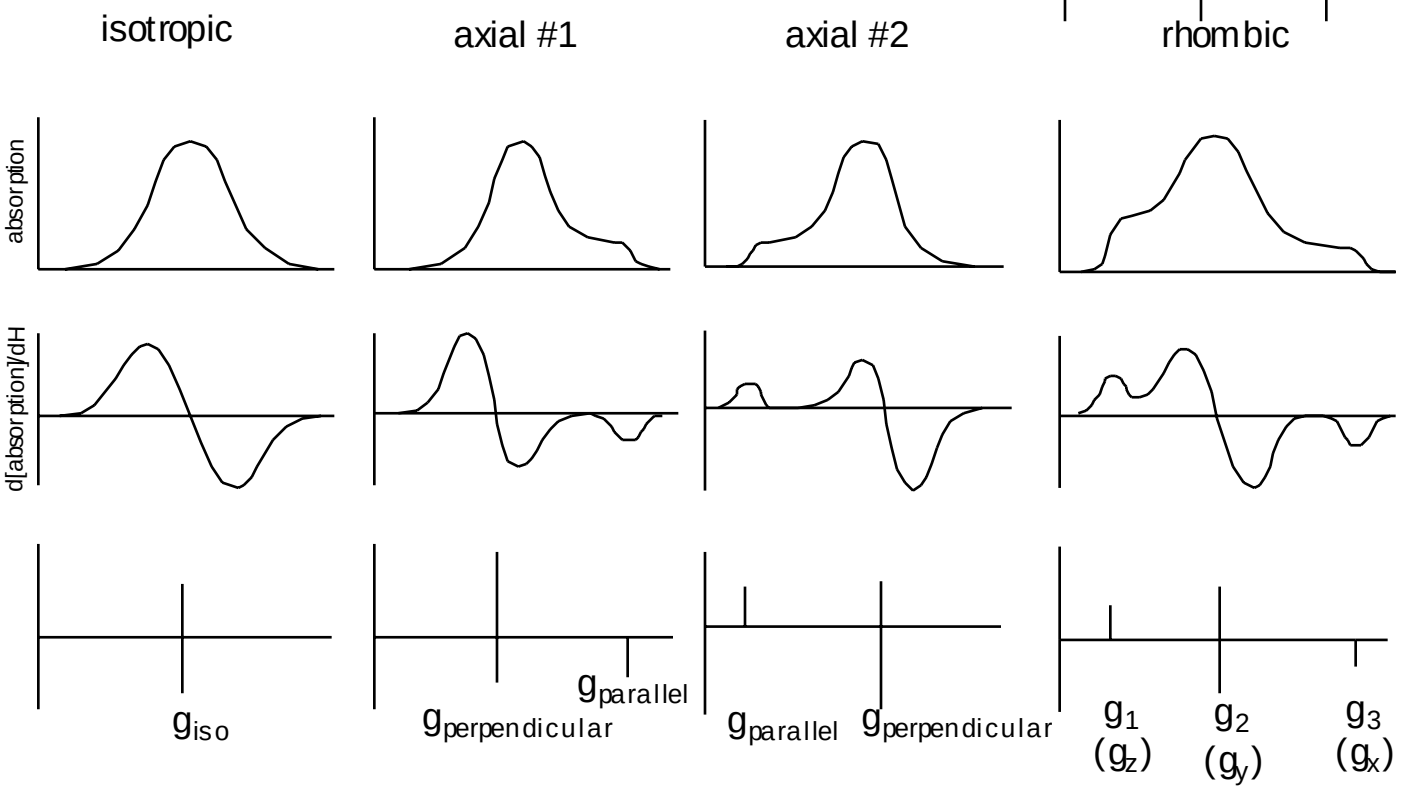
For two magnetically equivalent protons interacting identically with the lone electron, a 1:2:1 triplet is observed.



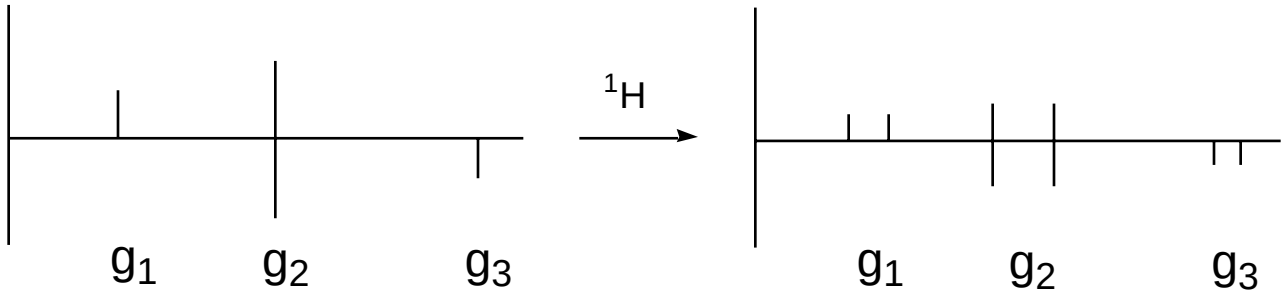
For systems possessing orbital as well as spin angular momentum, the two associated magnetic moments couple in a manner that depends on the orientation of the molecule in the magnetic field $\mathbf{B_0}$. The result is that there may be as many as three independent g values (corresponding to the axes of an orthorhombic coordinate system designated **g**). The possible results include:

For a nucleus with I = 1 (*e.g.*, ^{14}N), the Zeeman states are split into triplets, and the spectrum gives a 1:1:1 triplet.





In the presence of hyperfine or superfine coupling, each feature in an anisotropic EPR signal will be split. For a rhombic signal:



The hyperfine coupling constant need not be the same for each g value, although this is frequently found to be the case.

From the solution to the spin Hamiltonian for an anisotropic system exhibiting (super)hyperfine coupling:

$$E_{1,2,3} = g_{1,2,3}\beta B + hA_0M_SM_I$$

Only the first term for the principal g values contains B; the second term is independent of B for a given M_SM_I pair. this means that, to first order, the hyperfine splitting will not change in going from one microwave frequency to another. Thus a doublet separated by 10 G at X band (approximately 9 GHz) will still be separated by 10 G at Q band (approximately 35 GHz) or S band (3 GHz). This pair of doublets will vary in distance from features associated with other g values with microwave frequency, however: