Case Studies of Questions Addressed by EPR at Various Frequencies

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Outline

- Introduction
  - When or Why Would One Use a Frequency Other Than X-band?
  - How Many Frequencies Do You Need?
- Spectral Dispersion – Multiple Species
- Hyperfine Interactions
  - Multifrequency EPR of Copper
    - Distributions of $a$ and $g$
    - The Importance of S-band
  - Nuclei in the Vicinity of Electron Spins
    - ESEEM
- Copper-Copper Sites in Proteins
- Motion of Molecules – Tumbling Correlation Time Effects on CW Spectra
  - Copper
  - Nitroxy radicals
- Distances Between Electron Spins
- In Vivo EPR
  - Nitroxy clearance from mouse
  - Oximetry
- Zero-Field EPR
  - Mn(II)
  - ESEEM - Integer-Spin Diferrous Cluster in Proteins
- Even-Electron Systems
  - $S = 3/2, 5/2, \ldots$
    - Mn(II)
    - High-Spin Fe(III)
- Time-Resolved EPR
- Exchange Narrowing
- Nuclear Quadrupole Interactions
- References

Figures are labeled both with the number as in the original paper, and with a sequential number in this document. Within the text and figures, references are cited by author name and year. Full citations are labeled alphabetically at the end of this document.

When or Why Would One Use a Frequency Other Than X-Band?

In the following table we denote g-strain and a-strain as $\sigma g$ and $\sigma a$, and other features contributing to line width as $\Delta B$, for conciseness.
### Table 1 – Spin Phenomena that Require Various EPR Frequencies

<table>
<thead>
<tr>
<th>Phenomenon to be studied</th>
<th>General relations to be fulfilled</th>
<th>Special cases or conditions</th>
<th>Comments on field/frequency selection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Measure g-values</td>
<td></td>
<td></td>
<td>need accurate field and frequency</td>
</tr>
<tr>
<td>Multiple species?</td>
<td>$\Delta g &gt; \text{linewidth}$</td>
<td>free radicals</td>
<td>X, Q, W</td>
</tr>
<tr>
<td>Measure hyperfine</td>
<td>$h\nu &gt; a$; homogeneous field</td>
<td>second-order effects</td>
<td>trade-off between more first-order spectra and $\sigma g$ and $\sigma a$</td>
</tr>
<tr>
<td>Nuclear neighbors? CW</td>
<td>$a &gt; \sigma a$, $\Delta B$, Eq. 1</td>
<td>L, S for CW Cu(II)</td>
<td>select for exact cancellation in $^{14}$N ESEEM</td>
</tr>
<tr>
<td></td>
<td>$a = 2\nu_N$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Measure intermediate motion?</td>
<td>tumbling correlation time comparable to g and a anisotropies that are averaged by motion</td>
<td>wide range of frequencies needed</td>
<td>each transition and correlation time has an optimal frequency</td>
</tr>
<tr>
<td>Distances between electron spins</td>
<td>lower for half-field, higher for g=2</td>
<td></td>
<td>S, X, Q, W, and higher; fit at multiple frequencies</td>
</tr>
<tr>
<td>In vivo or large samples</td>
<td>depth penetration, large resonators</td>
<td>physiological motion</td>
<td>VHF or L-band</td>
</tr>
<tr>
<td>$S = 1/2, 3/2, 5/2$</td>
<td>$\pm 1/2$ transition always observable</td>
<td>$h\nu &gt; \text{ZFS}$</td>
<td>multiple frequencies</td>
</tr>
<tr>
<td>$S = 1, 2$</td>
<td>sometimes observable in parallel mode; usually need high field</td>
<td>$h\nu &gt; \text{ZFS}$</td>
<td>multiple frequencies</td>
</tr>
</tbody>
</table>

Eq. 1: $M_T\sigma A_\parallel = -\frac{h\nu_0}{g_\parallel^2\beta_e} \sigma g_\parallel$ (from Froncisz and Hyde, 1982)

- In general, if the sample is limited and not lossy, better S/N in principle could be obtained at frequencies above X-band.
- If the sample is not limited, but is lossy, better S/N in principle could be achieved at frequencies below X-band.
- For integer-spin systems, most measurements have to be done at frequencies higher than X-band, independent of whether the sample is limited or lossy.

There are practical limitations or opportunities relating to available power, quality of the available spectrometers, and line widths as a function of frequency.
How Many Frequencies Do You Need?

How many frequencies are needed depends on the purpose of the study. If the question is simply whether there is an \( S = \frac{1}{2} \) ground state free radical present, a CW X-band spectrum should answer the question. If the question is whether there is an \( S = 1 \) species present, a much more extensive, multifrequency study may be needed. For some problems one needs a particular frequency. For example, there could be a need to match a particular energy separation. In general, there are so many contributions to an EPR spectrum, some dependent on magnetic field (e.g., Zeeman splittings) and some independent of magnetic field (e.g., hyperfine couplings and dipolar interactions), that more than one magnetic field is helpful to assign the spectrum. Hyde (personal communication) said that in general 3 EPR frequencies are needed. However, there is a large body of information that has been accumulated during the first half-century of EPR, on which we can rely to “know” some of the assignments so that we can simulate X-band spectra without each time also acquiring an S-band or Q-band spectrum. This is the case for many organic radicals – e.g., most people recognize the “3-line” pattern of a nitroxyl radical or the “6-line” pattern for Mn(II). However, if the “3-line” pattern is actually a superposition, then Q-band or W-band might also be required. Similarly, the Mn(II) spectrum is inherently more complicated than the simple “6-line” pattern most commonly seen, and multiple frequencies, usually higher than X-band, will be needed to learn much about the electronic structure of the Mn(II) species.

Some of the applications of selected and/or multiple frequencies include:
- Separating g-value and hyperfine contributions
- Resolving spectra from multiple species
- Number of N bound to Cu by CW or ESEEM
- \( S > \frac{1}{2} \) species
- Distances between spins
- Even-spin species
- Measurement of rotational correlation times

Some of the problems that occur as one changes from the well-engineered commercial spectrometers to other frequencies include:
- Reduction in S/N, even at higher frequencies
- Reduced magnet field homogeneity and accuracy
- Resonator background signals

Some sample-related "problems" cause changes in spectra as a function of frequency. Spectra at multiple frequencies can be used to study these effects and to separate variables:
- g-strain
- increased validity of first-order approximations at higher frequency
- smaller samples at higher frequency
- some relaxation properties are field-dependent
- energy level separations equal to \( h\nu \) at various fields

Whether resolution of a particular feature in a spectrum will improve at higher frequency depends on what determines the line width (e.g., unresolved hyperfine, relaxation, g-strain). As
shown below, the optimum frequency for resolution of hyperfine splitting in immobilized Cu(II) spectra is in the S band. In other cases, g-strain may not dominate until a much higher magnetic field. There are, of course, situations in which a particular microwave frequency is disadvantageous because of the accidental overlap of spectral features.” (Hyde and Froncisz 1982.) A general introduction written several years ago by Belford and coworkers (1987) remains a good starting point for the person desiring a quick overview.

**Spectral Dispersion**

Lebedev and coworkers showed that a major advantage of high-field EPR is enhanced g factor resolution. Spectra of very similar radicals, such as different nitroxyl radicals, that overlap considerably at X-band can be separated at 95 GHz. Spectra are somewhat broader at 95 GHz than at 9.5 GHz, because of enhanced contribution at 95 GHz of rotational modulation of g anisotropy. Figure 1 (from Smirnov et al., 1997) shows that at X-band only a shoulder on the high-field line indicates the presence of two radicals in the mixture of phenyl-PBN and trichloromethyl-PBN ($\Delta g \approx 1.2 \times 10^{-4}$). However, the presence of the two radicals is clear at 95 GHz.

*Figure 1 (from Smirnov et al., 1997)*

![Figure 1](image_url)

*Figure 3. (A) X-band (9.5 GHz) EPR spectrum from a mixture of phenyl-PBN and trichloromethyl-PBN adducts in benzene at 24°C. The arrows points to some extra broadening on the line shoulder, which is caused by the presence of two species. (B,C,D) Experimental W-band (95 GHz) spectrum of the mixture and the corresponding least-squares simulated spectra of phenyl-PBN and trichloromethyl-PBN adducts, respectively. (E) Residual: difference between experimental (B) and simulated spectra (C,D).*
Enhanced g-value resolution at 95 GHz facilitates measurement of the g-anisotropy in “powder” spectra, so long as the magnetic field and frequency can be measured accurately. Trade offs between g resolution and g-strain are best made by comparing spectra at both 9.5 and 95 GHz.

The g-anisotropy of semiquinone radicals yields only a slight splitting of the X-band CW EPR spectrum, but the three principal values can be read off the W-band spectrum (Burghaus et al., 1992).

**Figure 2 (from Burghaus et al., 1992)**

285 GHz EPR resolved two peaks of the gx component of the tyrosyl radical of ribonucleotide reductase, demonstrating conformational heterogeneity in the environment of the tyrosyl radical. The spectrum changed upon thawing and refreezing the sample (Liu et al., 2000).

**HYPERFINE INTERACTIONS**

**MULTIFREQUENCY EPR OF COPPER**

Hyde and Froncisz (1982) provided, in the context of EPR of Cu(II) proteins, a general introduction to the uses of multiple frequencies in EPR. The three main themes they identify are

- State mixing, which depends on the relative values of electron Zeeman interaction, nuclear Zeeman, nuclear hyperfine, and nuclear quadrupole interactions.
- Line broadening that results from variations in the geometry of the site of the spin, resulting in variations in g and in hyperfine. These are some times called g-strain and a-strain.
- The shape of spectra in fluid solution depends on rotational correlation time relative to g anisotropy.
Distributions of a and g (a-strain and g-strain)

In many Cu(II) complexes there is a correlation between $A_{||}$ and $g_{||}$, such that the g-strain and a-strain partly cancel in low-field components, decreasing the line width. The high-field line widths are increased. The $m_I = -3/2$ line has minimum width near 6 GHz, and the $m_I = -1/2$ line, which will have the narrowest width, has a minimum width near 2 GHz.

Figure 3 (from Hyde and Froncisz, 1982)

This line width effect is especially important for resolution of $^{14}$N hyperfine splitting, and hence for the determination of the number of N coordinated to Cu(II) in some proteins. The confirmation of these predictions for common Cu(II) parameters, has been a stimulus for increased interest in S-band EPR over the past two decades. Coupling to a single N in monocupric transferrin, illustrating the relative resolution of $m_I$ components as a function of frequency is shown in Figure 4 (Hyde and Froncisz, 1982).
Even in the “well-known” azurin, multifrequency EPR was needed to obtain accurate estimates of the Cu hyperfine (Antholine et al., 1993; Froncisz and Hyde, 1980). Ligand hyperfine structure was not resolved in the X-band spectra, but was resolved in 1.1 and 2.4 GHz spectra. At the low frequencies, ligand hyperfine coupling was observed in the $g_{\perp}$ but not in the $g_{\parallel}$ region. From x-ray and Q-band ENDOR it was known that two imidazole N are coordinated to the Cu. Even with this knowledge, simulation of the second derivative CW spectra at both L-band and S-band was needed to achieve self-consistent estimates of the $g$- and hyperfine parameters. The value of $A_x^{Cu}$ was about half that obtained by previous methods.

**Nuclei in the Vicinity of Electron Spins**

**ESEEM**

At X-band, state mixing of nuclear and electron states can occur, and ESEEM, which depends on mixing of states, reveals the remote N in imidazole, but the directly-bound N experiences much too large a hyperfine field from the Cu(II) unpaired electron for significant
electron-nuclear state mixing. At higher EPR frequency, there is more significant mixing of states with the bound N, which then becomes observable in the ESEEM measurement. The major consideration is the relative values of the electron Zeeman field and the hyperfine field from the electron at the nucleus (Flanagan and Singel, 1987; Gerfen and Singel, 1990). See Hyde and Froncisz, 1982 for an introduction to the state mixing concepts relevant to the ESEEM applications of multifrequency EPR.

Raitsimring and coworkers have shown the power of selecting the microwave frequency to optimize selected features of ESEEM and HYSCORE. See Arnold Raitsimring’s web site (http://128.196.184.6/epr/). Figures 5-7 (from Astashkin et al., 1999) show that the proton sum combination band was well-resolved at the low-field g-value ($g_z$) in the X-band spectrum, and at the $g_x$ position in the S-band spectrum.

**Figure 5 and 6 (from Astashkin et al., 1999)**

![Figure 5 and 6](image-url)
Copper-Copper Sites in Proteins

S-band EPR was used to show the nature of copper sites in cytochrome c oxidase (CcO or COX) and nitrous oxide reductase long before the nature of these sites was shown by other biophysical techniques. The spectra of these two proteins are rather similar, suggesting similar copper environments in both proteins. The first observation of well-resolved hyperfine structure in the copper lines was at S-band (Froncisz et al., 1979). Interpretation of the structure remained elusive, even after it was seen in deuterated CcO at X-band, at which time it was hypothesized that the CuA site was due to a Cu-S radical center (Stevens et al., 1982). In 1988, Antholine and coworkers proposed a mixed valence [Cu(1.5)…Cu(1.5)] state for the CuA center in CcO and nitrous oxide reductase (Kroneck et al., 1988). The number of lines (10 to 14) resolved in 2.7, 4.6 and 9.2 GHz spectra is consistent with a mixed valence [Cu(1.5)…Cu(1.5)] S = ½ site. This was not immediately accepted (see Kroneck et al., 1989), but has withstood subsequent examination. Spectra obtained at L, S, C, X, and Q bands are consistently interpreted in terms of this model. The C-band spectra were particularly important, because different parts of the CcO spectrum are better resolved at different resonant frequency (Kroneck et al., 1990). Two Cu would yield 7 lines, and overlap of g_x, g_y, and g_z could lead to complicated patterns (Antholine et al., 1992). The development of these concepts, emphasizing the role of multifrequency EPR, was reviewed by Antholine (1997).
Low-frequency EPR was used analogously to investigate the copper sites in particulate methane monooxygenase (pMMO) (Yuan et al., 1997). A suggestion that there was a mixed-valence site was shown not to be consistent with the S-band spectrum, in which the hyperfine structure on the $m_S = -1/2$ line was consistent with coupling to 3 or 4 nitrogens. Use of $^{15}$N and $^{63}$Cu improved the resolution in both the X-band band and S-band spectra, and provide greater confidence that there were 4 approximately equivalent nitrogen donor atoms (Yuan et al., 1999). A figure from Yuan et al. (1999) illustrating these points is in the Introduction by Ralph Weber.

A Cu$_A$ site engineered into azurin exhibited a well-resolved 7-line EPR spectrum at X-, C-, and S-band (Hay et al., 1998). The spectra are similar to those observed for cytochrome c oxidase and nitrous oxide reductase. There are sufficient ambiguities in the interpretation of any one spectrum that the set of multifrequency spectra are needed for confident interpretation, even though these are better resolved than prior spectra of mixed-valence copper proteins.
Figure 10 (from Hay et al., 1998)

An Engineered Purple Cu₄ Center in Azurin

Figure 5. X-band (9.2 GHz), C-band (4.5 GHz), and S-band (3.4 GHz) EPR spectra aligned about \( g_\perp \). Vertical lines mark the center of a seven-line pattern, i.e. \( g_\perp \) and the seven-line pattern with relative intensities 1:2:3:4:3:2:1 for a single electron delocalized over two equivalent coppers. The concentration of purple Cu₄ azurin was 0.8 mM.

Figure 6. First harmonics of spectra in Figure 5 aligned along \( g_\parallel \).
Motion of Molecules

Cu(II) complexes in fluid solution

Room temperature aqueous solution spectra of various Cu(II) complexes are better resolved at lower frequency (see Hyde and Froncisz (1982)). Rotational correlation times derived from Kivelson theory were in the range ca. 30-150 ps.

Figure 11 (from Hyde and Froncisz, 1982)

Simulations of Cu(II) spectra in fluid solution, especially for longer correlation times, must include nonsecular terms (Pasenkiewicz-Gierula, et al., 1997). (Nonsecular transitions arise from terms containing the operators $S_\pm$, and the different types of combinations are $S_\pm I_{\pm\pm}$, $S_\pm I_{\pm}$, or $S_\pm I_{\pm+}$. The selection rules are $\Delta m_S=\pm 1$ and $\Delta m_I=0, \pm 1$, or +/-1, ...).
Using the same physical phenomena, but exploring from the opposite extreme, faster motion is required to fully average $g$ anisotropies at higher frequency so faster motions can be detected at higher frequencies than at lower frequencies. Freed has used 250 GHz CW EPR of nitroxyl radicals to observe faster motion than can be seen at X-band, thus extending significantly the range of motions whose mechanism can be studied. X-band EPR is more sensitive to slower motions, such as overall motion of a macromolecule, and 250 GHz EPR is more sensitive to faster motions, such as local motion of a spin label on a macromolecule (Liang and J. H. Freed, 1999; Pilar et al, 2000).
**Figure 13 (from Pilar et al., 2000)**

Figure 1. X-band ESR spectra of the copolymer SL–ST–AA in toluene at the given temperatures (experimental spectra, full lines; simulated spectra, dotted lines).

**Figure 2.** Far-IR ESR spectra of the copolymer SL–ST–AA in toluene at the given temperatures (experimental spectra, full lines; simulated spectra, dotted lines).
Distances Between Spins

Dipolar coupling between pairs of electron spins, as in proteins containing two nitroxy1 spin labels, increases the number of lines in the CW EPR spectrum. The definition of the relative orientations of the magnetic axes of the two spins adds several parameters that need to be determined to achieve simulation of the spectrum. Hustedt et al., (1997) showed that a global analysis of spectra obtained at three frequencies (X-, Q-, and W-band) provided a unique fit for spin labeled tetrameric glyceraldehyde-3-phosphate dehydrogenase. Figure 14 shows spectra at low enough spin label concentration that there is no nitroxy1-nitroxy1 interaction. Figure 15 shows the comparable spectra at nearly stoichiometric spin label concentration, exhibiting dipolar interaction between nitroxy1 radicals.

**Figure 14 and 15 (Hustedt et al., 1997)**
A semiquinone biradical formed in the photosynthetic reaction center was studied at 9.6, 35, and 94 GHz (Calvo et al., 2000). There are 13 independent parameters to be fit, plus the line width. From fitting the EPR spectra, the distance between the spins was found to be $17.2 \pm 0.2$ Å, in agreement with the distance of $17.4 \pm 0.3$ Å determined by X-ray crystallography.

**Figure 16 (from Calvo et al., 2000)**

![EPR spectra](image)

The spectra of Q<sup>-</sup> are not a superposition of the individual Q<sub>A</sub> and Q<sub>B</sub> spectra, reflecting the magnetic interaction between Q<sup>-</sup> and Q<sub>B</sub>.

**In Vivo EPR**

The first in vivo EPR study of which we are aware was by Piette and coworkers (Feldman et al., 1975). This was done at X-band by implanting a gold-coated brass helix ca. 1.5-2 cm long in the liver of a rat. Injected tempol was detected with a sensitivity of $10^{-5}$ M.

Most in vivo EPR has been done at L-band, following the initial studies of Stanislaw J. Lukiewicz at 1.1-1.3 GHz (ca. 1983). Halpern selected 250 MHz in order to get deeper penetration in animal tissue. Several labs have performed in vivo spectroscopy and/or imaging at VHF or L-band, including the labs of Lawrence Berliner (Ohio State), Bernard Gallez (Bruxelles), Howard Halpern (Chicago), Hitoshi Kamada (Yamagata), Murali Krishna (NIH NCI), David Lurie (Aberdeen), Antonello Sotgiu (L’Aquila), Harold Swartz (Dartmouth), and Jay L. Zweier (Johns Hopkins).

MRI has been using increasingly higher frequencies, in spite of the penetration problems, with attendant phase and relative intensity complexities, to get better S/N and spectral dispersion, just as for high-resolution NMR. The state of the art is represented by the recent set of papers by Robitaille and coworkers (1999), and by Vaughan and coworkers (2001).

Clearance of carbamoyl-proxyl from the abdomen of living mice was slowed after the liver was damaged by CCl<sub>4</sub>. As shown in Figure 17, 3D images were acquired at 700 MHz using a LGR 41 mm diameter and 10 mm long (Togashi et al, 1998).
Using 250 MHz EPR, it was found that the microviscosity sensed by a small nitroxyl radical was significantly lower (1.8 ± 0.1 centipoise) in a murine fibrosarcoma than in normal leg tissue of a living animal (2.9 ± 0.3 centipoise) (Halpern et al., 1999).
Zero-Field EPR

An external magnetic field is required for EPR only for those systems for which the internal fields are not sufficient to split the electron states. In principle, there is a resonance at zero external magnetic field for any multi-spin system. Bramley and coworkers have exploited zero-field EPR for measurement of the magnetic parameters of transition metals (Bramley and Strach 1983; Strach and Bramley 1983; Delfs and Bramley, 1997). The frequency is swept. Interpretation of zero-field EPR is simplified somewhat by the fact that the g matrix does not appear in the Hamiltonian. Copper carboxylate dimers have large exchange interactions (-2J ≈ 300 cm⁻¹). Zero field EPR can be used to measure the D and E parameters fairly directly. Although called “zero-field,” usually Zeeman (magnetic) field modulation is used to improve sensitivity.

An example of a zero-field spectrum of Mn(II) in NH₄Cl is in the Bramley and Strach 1983 review. Transitions between the mₛ = 3/2 and 5/2 levels occur at 4D + M₁A (A is the hyperfine coupling), so spectra look like a six-line first-order EPR spectrum. Transitions between mₛ = 1/2 and 3/2 levels result in 11 lines as shown in Figure 19.
Figure 19 (from Bramley and Strach, 1983)

(a) Schematic energy level diagram for $S = \frac{5}{2}, I = \frac{5}{2}$ with dominant $D \gg A$ (isotropic hyperfine interaction). (b) Resulting ZFR transitions (transitions in the region of $\nu = 5A$ are not shown). (c) Observed ZFR spectrum for 0.1 mol % Mn$^{2+}$ doped in NH$_4$Cl at 295 K which could be simulated with $D = -4508, a + \frac{2}{3}g = 21, A_1 = -247, A_2 = -254$ MHz. The powdered sample was contained in a coaxial line sample cell and the spectrometer was used in transmission mode. Magnetic field modulation (perpendicular to the microwave field) of ±33 G was used. The spectrum is the ‘2D’ group of lines, slightly different from the pattern in (b) because of anisotropy of $A$. Sharp features are spurious effects of the reflection spectrometer.

ESEEM at Zero Field

Methane monooxygenase has a diferrous form that reacts with O$_2$. Hoffman and coworkers have obtained ESEEM from this non-Kramers system (Sturgeon et al., 1997). The echo is largest at zero applied field, but a magnetic field of only a few gauss applied parallel to the microwave field introduced echo modulation, the depth of which increases rapidly with increasing field.
Even-Electron Systems

To a first approximation, X-band EPR is selective for half-integer spin systems \( (S = \frac{1}{2}, \frac{3}{2}, \ldots) \). Relatively few integer spin systems \( (S = 1, 2, \ldots) \) have transitions that can be observed at X-band. The resonance condition for integer-spin systems is

\[
(hv)^2 = (g_{\text{eff}} BB)^2 + \Delta^2
\]

where \( \Delta \) is the zero field splitting. Observation of EPR signals requires \( hv > \Delta \). Since there are two unknowns in the resonance equation, \( g_{\text{eff}} \) and \( \Delta \), two EPR frequencies are needed to determine both of them. Further, components of \( B_1 \) parallel to \( z \) contribute to the signal intensity, in contrast to half-integer systems which require \( B_1 \) perpendicular to \( z \). The direction of the quantization axis is determined by the molecule rather than be the applied Zeeman field.
S = 2 Ferrous Sites
The species for which Q-band spectra are shown in Figure 21 has two high-spin S = 2 ferrous sites bridged by a water molecule (Petasis and Hendrich, 1999).

Figure 21 (from Petasis and Hendrich, 1999)

Prior study of this species observed X-band resonances with B\textsubscript{1} both perpendicular and parallel to z, and Q-band with B\textsubscript{1} perpendicular to z (Hendrich et al. 1991).

S = 1 V(III) d\textsuperscript{2}
Vanadium(III) is a d\textsuperscript{2} cation. CW EPR spectra of single crystal and powdered samples with V(III) doped into CsGa(SO\textsubscript{4})\textsubscript{2}·12H\textsubscript{2}O, and the deuteriated analogs, obtained at 95, 190, and 285 GHz, enabled precise determination of the spin-Hamiltonian parameters for the $^3A_g$ ($S_\alpha$) ground state (Tregenna-Piggott et al., 1999).
The lowest triplet level for $V^{3+}$ ($S = 1$) in corundum ($\alpha$-Al$_2$O$_3$) is split by spin-orbit coupling into a ground state singlet and an excited doublet. The $|\Delta M| = 2$ transition can be seen at "normal" frequencies (X-band, Q-band). A 37 GHz spectrum is shown and interpreted in Figures 24 and 25 from Zverev and Prokhorov, 1960.

**Figure 24 and 25 (from Zverev and Prokhorov, 1960)**

![Figure 24 and 25](image-url)

**Table 1. Spin-Hamiltonian Parameters for $[\text{V}($O$_x$)$_2$]$^{3+}$-Doped CuGa(SO$_4$)$_2$:12X,0 (X = D or H) Determined from Analysis of High-Field, High-Frequency EPR Data**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value 1</th>
<th>Value 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>$D$ (cm$^{-1}$)</td>
<td>4.7735 (3) [2]</td>
<td>4.8581 (4) [4]</td>
</tr>
<tr>
<td>$B_0$</td>
<td>1.9549 (4) [1]</td>
<td>1.9560 (6) [5]</td>
</tr>
<tr>
<td>$g_L$</td>
<td>1.8690 (5) [2]</td>
<td>1.8656 (4) [2]</td>
</tr>
<tr>
<td>$A_x$/10$^5$ cm$^{-1}$</td>
<td>99 (1) [1]</td>
<td>98 (2) [2]</td>
</tr>
<tr>
<td>$A_y$/10$^5$ cm$^{-1}$</td>
<td>78 (3) [2]</td>
<td>78 (3) [2]</td>
</tr>
</tbody>
</table>

*The accuracy with which the parameters are determined is given in curved brackets and the precision in squared brackets.
In weak magnetic fields, spins on nuclei close to an unpaired electron are quantized along the electron-nuclear axis, whereas at sufficiently strong magnetic fields, nuclear spins become quantized along the external magnetic field. Consequently, in strong fields the spectrum can become simplified due to the decreased intensity of forbidden transitions. See, for example, Figure 26 from Lebedev, 1990.

\textit{Figure 26 (from Lebedev, 1990)}

\begin{center}
\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig26}
\caption{ESR spectra of frozen methanol solution of VCl$_2$ ($3 \times 10^{-2}$ M) at 150 GHz (a) and 10 GHz (b). The high-frequency spectrum is resolved due to damping of forbidden transitions.}
\end{figure}
\end{center}

Ni(II) $d^8$

Ni(II) doped into tris(ethylenediamine)zinc dinitrate exhibits a broad spectrum at X-band, but a triplet powder pattern at 130 GHz (van Dam et al., 1998) as shown in Figure 27.
**Figure 27 (from van Dam et al., 1998)**

![Figure 27](image)

**FIG. 1.** X band (A), Q band (B), and D band (C) room temperature EPR spectra of Ni(II) in [Zn(en)$_2$][(NO$_3$)$_2$]. Experimental conditions were as follows. X band: microwave power, 15 mW; modulation amplitude, 0.5 mT; modulation frequency, 100 kHz. Q band: microwave power, 30 mW; modulation amplitude, 0.5 mT; modulation frequency, 100 kHz. D band: microwave power, 0.2 mW; modulation amplitude, 0.6 mT; modulation frequency, 20 kHz. The lower traces are simulations as described in the text using the parameters from Wilson et al. (12) ($D = 0.832$ cm$^{-1}$, $E = 0$ cm$^{-1}$, and $g_x = 2.156$, $g_y = 2.156$, $g_z = 2.181$).
Mn(III) $d^4$

Mn(III) is another common oxidation state that rarely can be investigated using X-band EPR. At 349 GHz the spectrum of a tris-chelate complex of Mn(III) had at least 11 features. The spectrum at 245 GHz exhibited only 9 features. Note that the relative intensities of the transitions were temperature dependent, because the Zeeman energy was similar to thermal energy at 15 K (Barra et al., 1997).

*Figure 28 and 29 (from Barra et al., 1997)*

![Figure 1](image1.png)

Figure 1. Experimental (bold) and simulated powder HF-EPR spectrum of polycrystalline [Mn(dmb)$_3$] at 349.3 GHz and 15 K. The free electron resonates at about 12.46 T.

![Figure 2](image2.png)

Figure 2. Experimental (bold) and simulated powder HF-EPR spectrum of polycrystalline [Mn(dmb)$_3$] at 245.0 GHz and 15 K. The narrow line at about 8.75 T is attributed to the standard (α,α-diphenyl-β-picrylhydrazyl, dpph).
Cr(II) d⁴

Cr(II) is also d⁴. In frozen solution, [Cr(H₂O)₆]²⁺ is a perfectly axial (tetragonally elongated) high-spin (S = 2) system with E = 0 (Telser et al., 1998).

**Figure 30 (from Telser et al., 1998)**

**High-Field EPR Spectroscopy of Aqueous Chromium(II)**

![Diagram](image)

**Figure 1.** HF-EPR spectrum of aqueous Cr²⁺ (~0.1–0.2 M) with a sulfate counterion: (A) experimental spectrum; (B) simulated spectrum. (A) Experimental conditions: temperature, 10 K; microwave frequency, 109.564 GHz; field modulation frequency, 8.7 kHz; modulation amplitude, 1.5 mT; time constant, 0.3 s; scan rate, 0.3 T/min. The sharp signal from an aqueous Cr³⁺ impurity at g = 2.00 is indicated. (B) Simulation parameters: S = 2, D = -2.2 cm⁻¹, E = 0; gₓ = gᵧ = 1.98; single-crystal EPR line width, 100 mT. Fully allowed EPR transitions with ΔMᵧ = 1 are assigned as to |Mₓ⟩ ground and excited states and magnetic field orientation (l = B₀ along z, = B₀ along x) as indicated on the simulated spectrum. The resonance corresponding to |−1⟩ → |0⟩ occurs at an off-axis extremum or "turning point" when B₀ is ~60° from z and is so identified by the label TP. A partially allowed transition with ΔMₓ = 2 is also indicated.
S = 3/2, 5/2, ...

S = 3/2 Cr$^{3+}$ d$^3$
More EPR lines were observed in the Q-band spectrum of chromium-doped LiScGeO$_4$ than in the X-band spectrum. Some lines were also observed at ca. 70 GHz. There were no transitions observed in parallel mode at frequencies up to 370 GHz, suggesting that there were no integer spin species present (Galeev et al., 2000).

S = 5/2 Mn$^{2+}$ d$^5$
All five allowed transitions for S = 5/2 Mn$^{2+}$ in complexes with $\gamma$-picoline and halides were observed at 250 GHz. The ZFS parameters determined by fitting the spectra correlate with halide, with D increasing in the order Cl < Br < I (Lynch et al., 1993). Note that in the figure caption $\eta = E/D = 0$.

Figure 31 (from Lynch et al., 1993)

![Graph](image)

**Figure 3.** 250-GHz ESR of Mn($\gamma$-picoline)$_2$Cl$_2$ (—) with simulation (—) using $g = 2.004$, $D = 0.186$ cm$^{-1}$, $\eta = 0$, and line width = 400 G.

High-Spin Fe$^{3+}$ d$^5$
From 1 to 285 GHz, there are changes in the spectrum of high-spin Fe(III) in metmyoglobin, which are attributed to distributions in $g$ (g-strain) and in the ZFS parameter D. The value of $g_\perp$ decreases as the Zeeman energy approaches D (van Kan et al., 1998).
Figure 32 (from van Kan et al., 1998)

Performing time-resolved EPR at Q-band has the advantage of higher spectral dispersion and shorter dead time due to resonator Q, relative to X-band. Furthermore, field-dependent phenomena are expected for biradicals. Photoreduction of acetone in 2-propanol yielded TREPR spectra that are more absorptive at Q-band than at X-band, which was attributed in part to longer relaxation times at higher frequencies (Forbes, 1992). No reference or formula was given for the statement about relaxation. Presumably it is assumed that very fast motion is occurring, and that a thermally activated process occurs. No distinction was made between $T_1$ and $T_2$.

Figure 33 (from Forbes, 1992)

Figure 1 TREPR spectra taken at a delay time of 1 μs during the laser flash photolysis of a 1:1 mixture of acetone in 2-propanol at 298 K: (A) X-band spectrum, (B) Q-band spectrum. The laser light intensity was the same during both experiments.
Exchange Narrowing

The line width of $K_2CrO_4$ decreases from 1 GHz to ca. 100 GHz, and then increases at higher frequency, consistent with the predictions of Anderson-Weiss model for the line width effect of exchange interactions (Cage et al., 1998).

Nuclear Quadrupolar Interactions

Cu(II) nuclear hyperfine and quadrupolar couplings are similar in magnitude to nuclear Zeeman interactions in a magnetic field of ca. 12 KG (Q-band EPR). The resultant state mixing results in forbidden transitions. Simulation of the spectra permits extraction of the quadrupolar interactions. This is easiest if the lines are sharp, as in single crystals, but methods have also been developed for interpretation of powder spectra. See references cited by Hyde and Froncisz (1982).

References


J. S. Hyde and W. Froncisz, The Loop-gap resonator in Medical Applications of ESR, Abstract, First Annual Meeting of the Society of Magnetic Resonance in Medicine, August 16-18 (1982), page 83. This abstract mentions in vivo studies including tails of mice and in four day old chick embryos.


