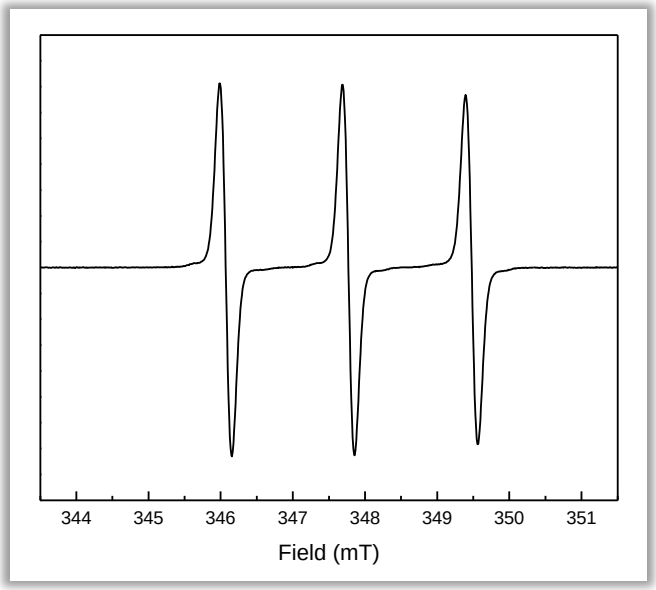
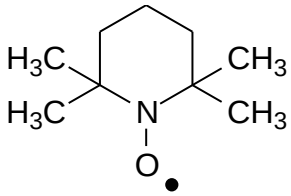
5 Selected samples

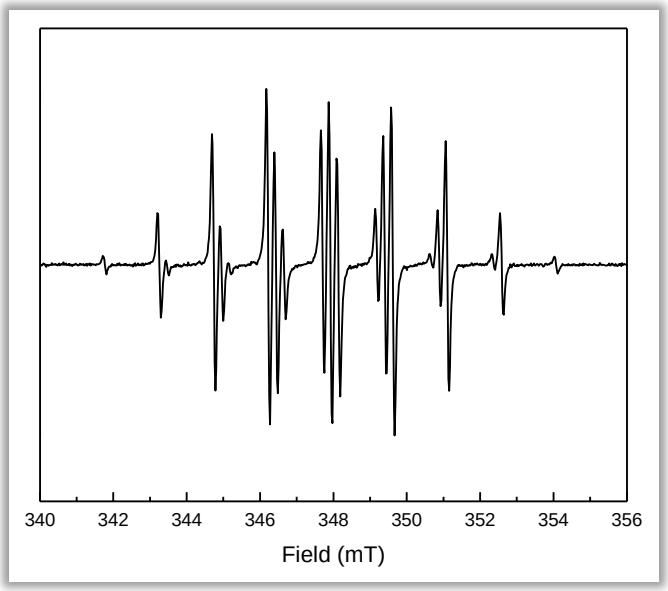
5.1 Organic Radicals in Solution

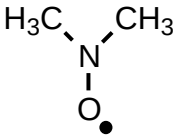
2,2,6,6-tetramethyl-1-piperidinyloxyl (TEMPO)





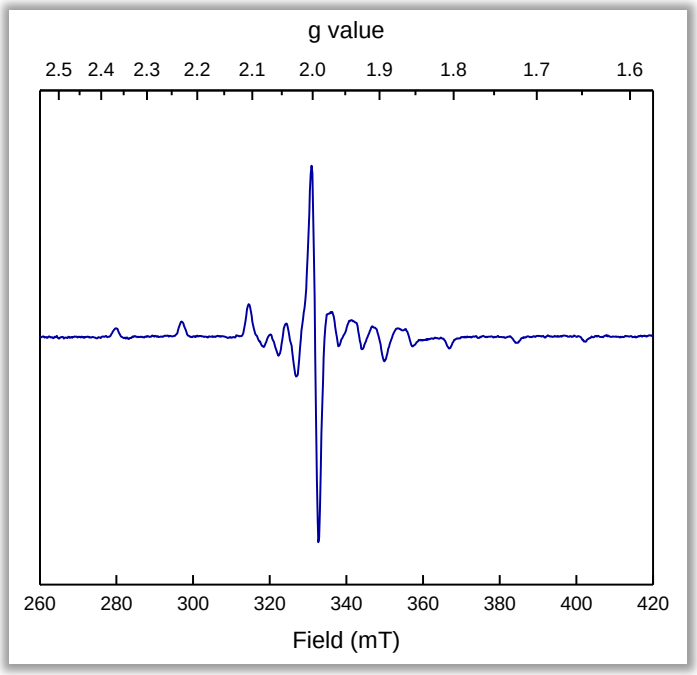
dimethylnitroxyl radical





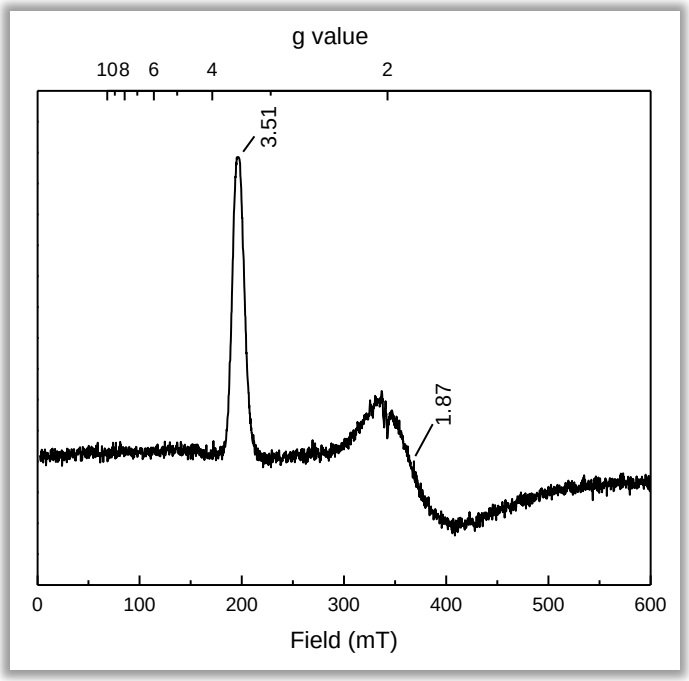
5.2 Single Metal Ions

V in chloroperoxidase



In the reduced form of the enzyme, the spectrum of V^{4+} (d^1) can be observed. Because vanadium has axial symmetry, its powder spectrum consists of two major peaks ($g_\perp$ = 1.95 and $g_{/\!/}$ = 1.98). Vanadium possesses one stable isotope ^{51}V with I = 7/2. Therefore each peak will be further split into eight ($2I + 1$) lines. Due to overlap, not all lines are observed. On top of that the Hyperfine Coupling Constant (A) is very large, causing the hyperfine lines of $g_\perp$ to pass the $g_{/\!/}$ peak, causing an effect termed ***overshoot***. The lines of the $g_\perp$ peak will have a different orientation when they are present on the site of the $g_{/\!/}$ peak opposite to that of the position of the $g_\perp$ peak. The same is true for the hyperfine lines of the $g_{/\!/}$ peak.

Fe in Met-Myoglobin with bound CN



S = ½

Large spin angular momentum

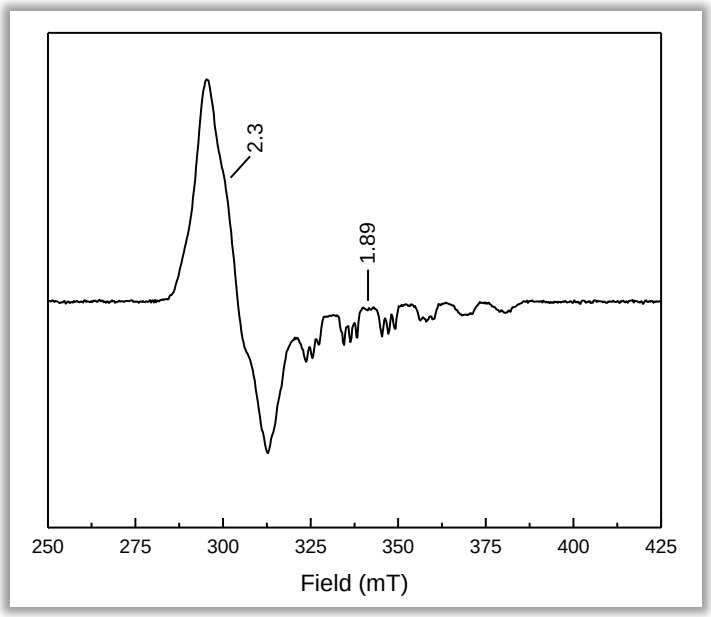
Degenerate orbitals

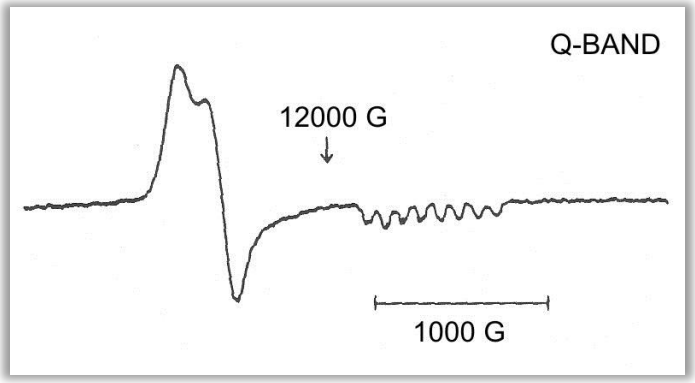
High symmetry

HALS-type (highly anisotropic low spin)

$g_x^2 + g_y^2 + g_z^2 = 16$; therefore $g_z = 0.9$

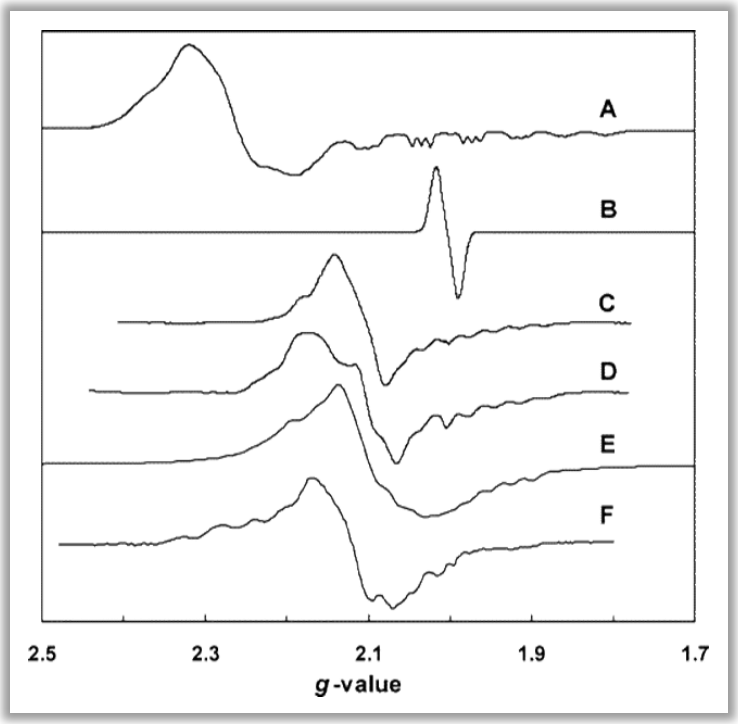
Co in B_{12}





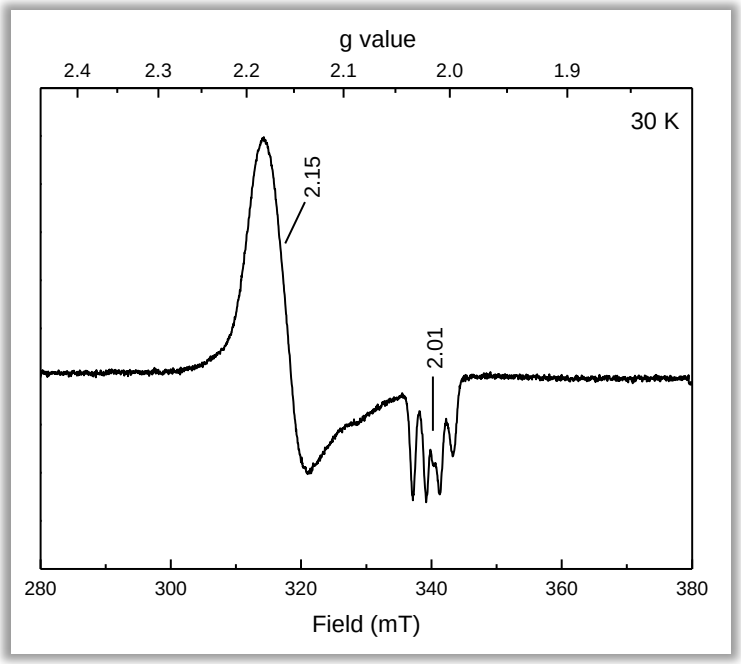
- $S = ½$
- Equally spaced hyperfine: $I = 7/2$; not V ($g_{av} < 2$), but Co^{2+} (d^7)
- Superhyperfine from N
- Vit B_{12}, nitrogen splitting from axial adenosyl

Examples of coupled cob(II)alamin in several different enzyme systems



EPR spectra of enzyme bound cob(II)alamins and radicals. (A) Uncoupled cob(II)alamin in methanol:coenzyme M methyltransferase *MtaC* subunit. (B) A simulated malonyl radical. (C) Glutamate mutase and (*S*)-glutamate. (D) 2-Methyleneglutarate mutase and 2-methyleneglutarate. (E) Methylmalonyl-CoA mutase and succinyl-CoA. (F) Ribonucleotide triphosphate reductase, reductant and dGTP. The spectra have been converted to a *g* value *x* axis to allow better comparison. (Taken from: Pierik et al. (2005) Biochemistry, 44, 10541–10551)

Nickel-containing rubredoxin



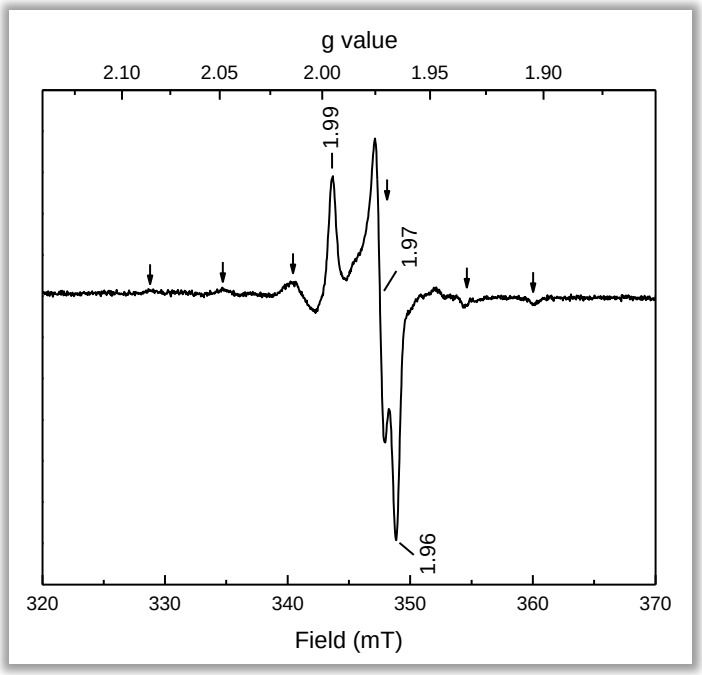
S = ½

Equally spaced hyperfine on $g_{//}$ (2.01): I = 3/2, the hyperfine of the $g_{\perp}$ (2.15) is not resolved

Ni(III) containing rubredoxin with CN^- bound

Sample was enriched in Ni^{61} (I = 3/2)

Mo in sulfite oxidase



S = ½

$g_{av} < 2$

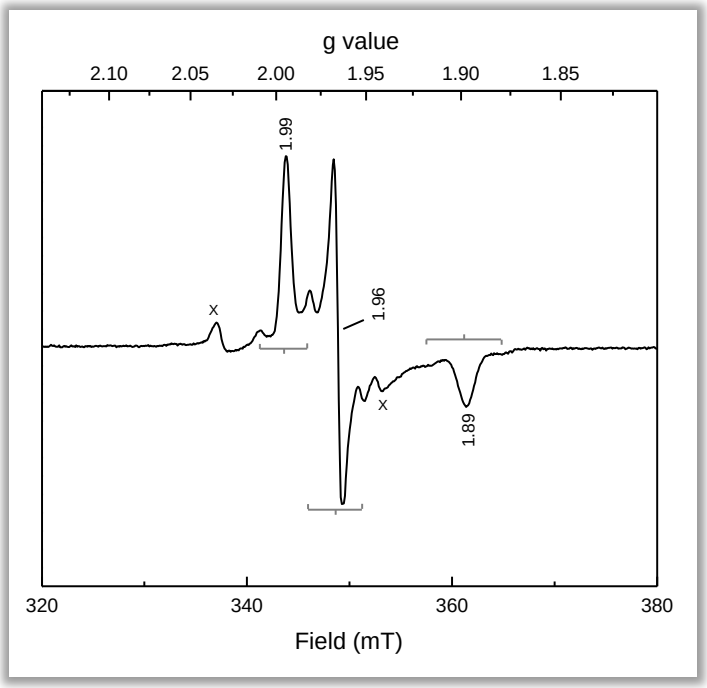
Mo(V) (d^1)

25% $Mo^{95/97}$ with I = 5/2

75 % Mo with I = 0

Each peak can give different hyperfine

W in aldehyde oxidoreductase



X = ignore
$S = ½$

$g_{av} < 2$

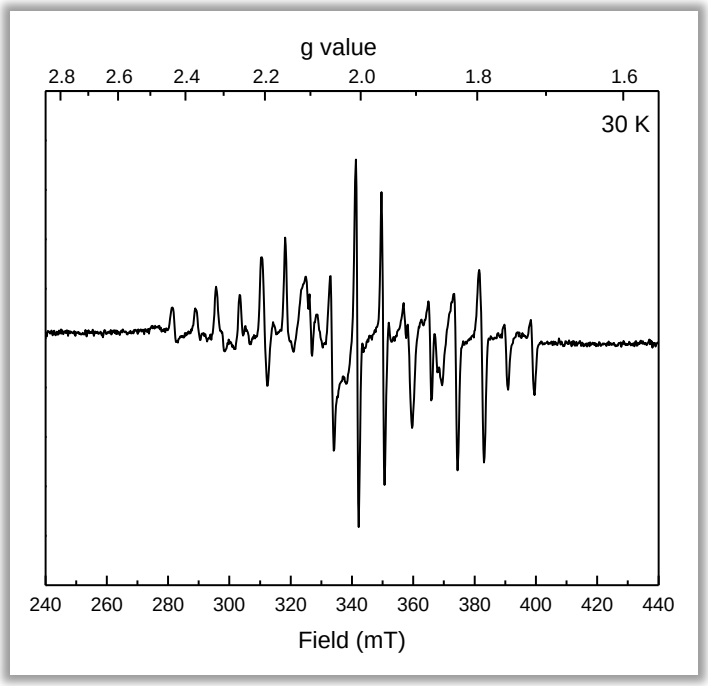
mayor component: $I = 0$

minor component $I = ½$

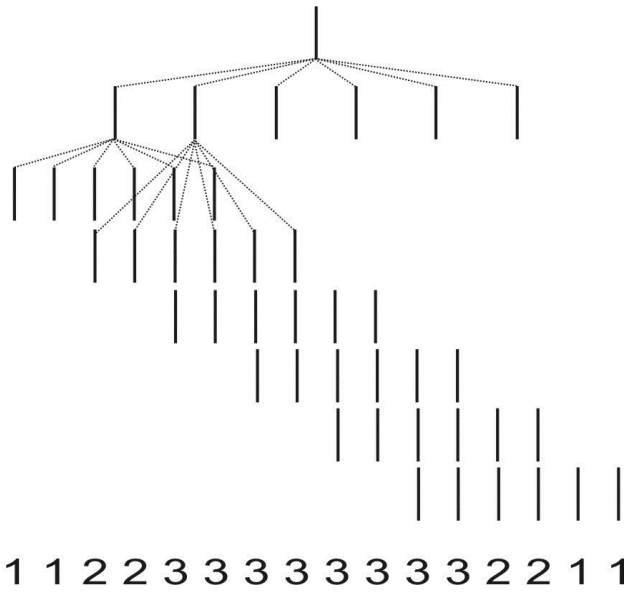
W: 87 %, $I = 0$; 13 % ^{183}W, $I = 1/2$

5.3 Multi-Metal Systems

2-Mn catalase

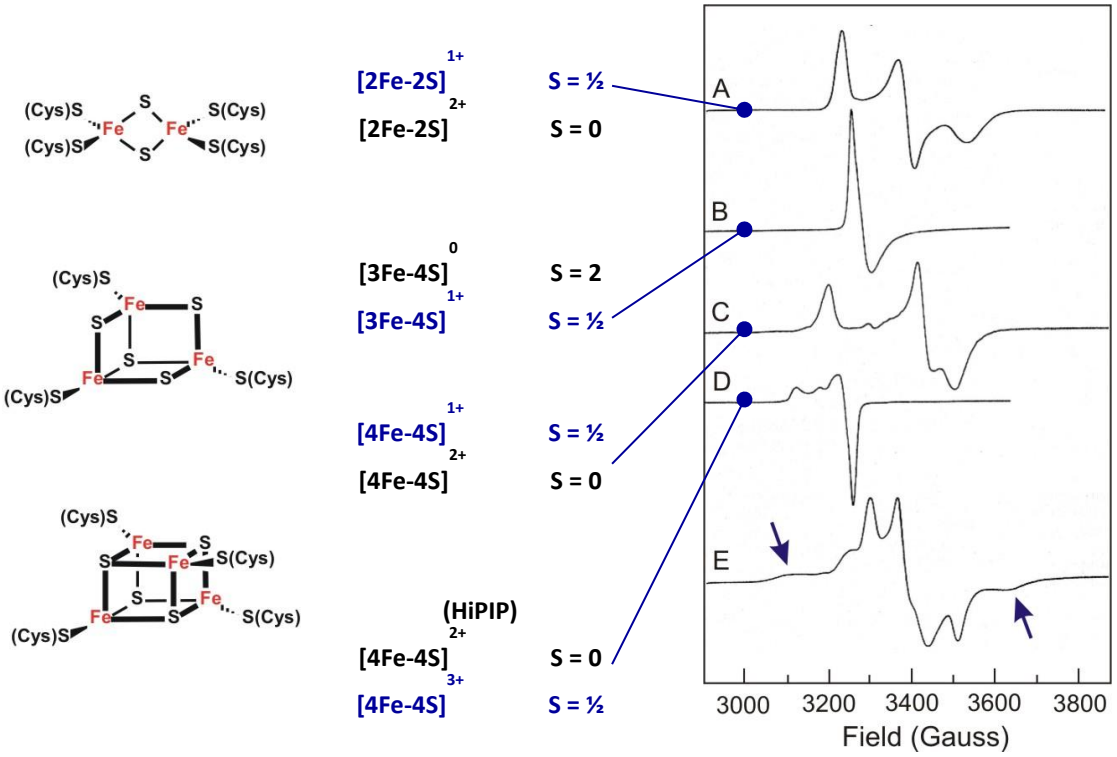


- S = ½
- Isotropic (derivative shapes)
- Hyperfine: equally spaced lines (80 G), only 16 lines
- Mn catalase: $Mn^{3+}Mn^{4+}$; I = 5/2
- Mn^{4+}, S = 2; Mn^{3+}, S = 3/2; AFC give S = ½



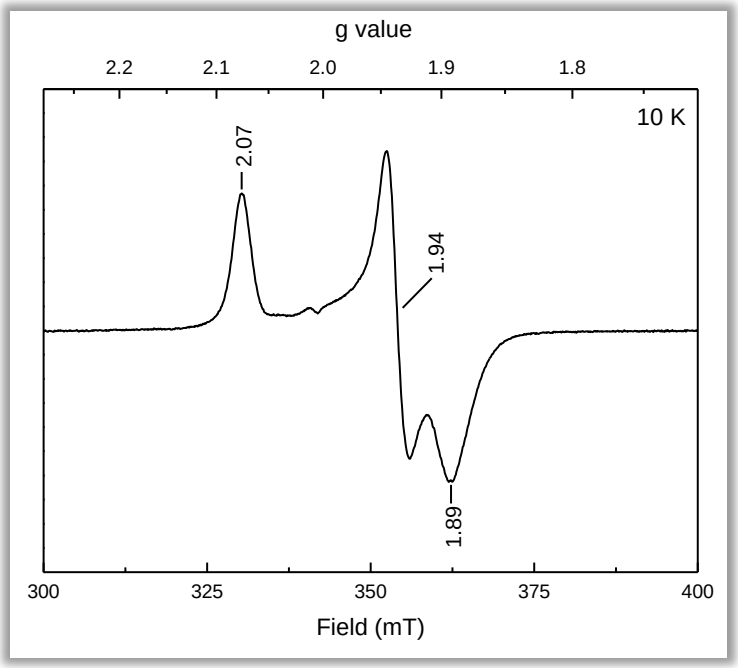
- Mn^{4+} stronger nuclear coupling
- Mn^{3+} : half times Mn^{4+} splitting
- Only 16 lines, could have been 36

5.4 Iron-Sulfur Clusters



EPR spectra of different types of iron-sulfur clusters. On the left the basic structure of the cluster types is shown. In the middle redox states and their respective spin states are indicated. The panel on the left show the EPR spectra of the iron-sulfur clusters in ferredoxins from *Mastigocladus laminosus* (A), *Desulfovibrio gigas* (B), *Bacillus stearothermophilus* (C), *Chromatium vinosum* high-potential iron-sulfur protein (HiPIP) (D), and *Clostridium pasteurianum* 8Fe ferredoxin (E).

[4Fe-4S]$^{+}$ (S = ½)



Rhombic *S* = ½

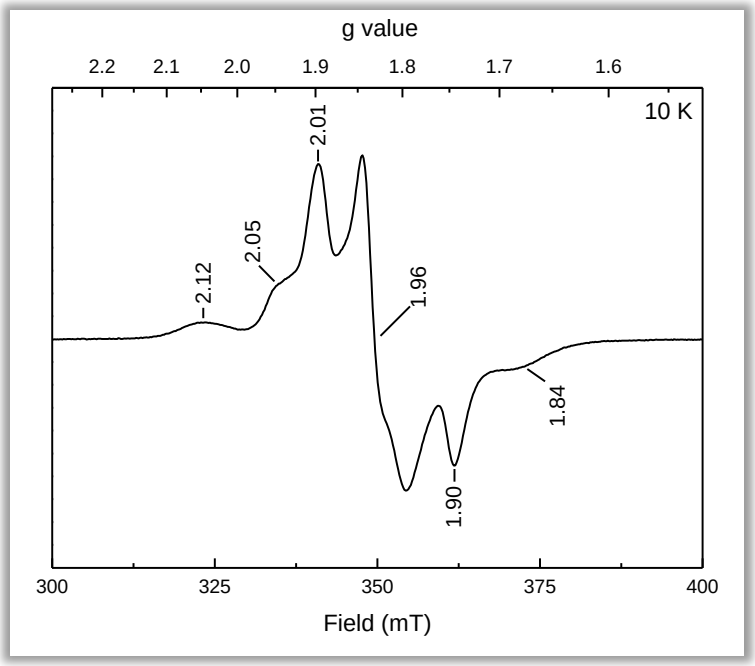
g values average out close to 2

g values below 4

Feature at *g* = 2.00 is due to a radical impurity

Signal measured at 10 K, not due to [2Fe-2S]. 4Fe clusters have low-lying excited states causing the signal to show fast relaxation.

Two interacting [4Fe-4S]$^{+}$ (S = ½)



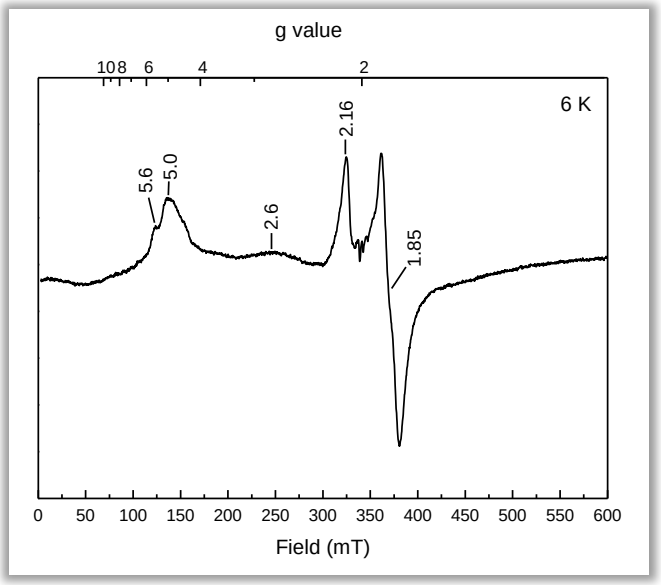
S = 1/2 (g_{av} close to 2)

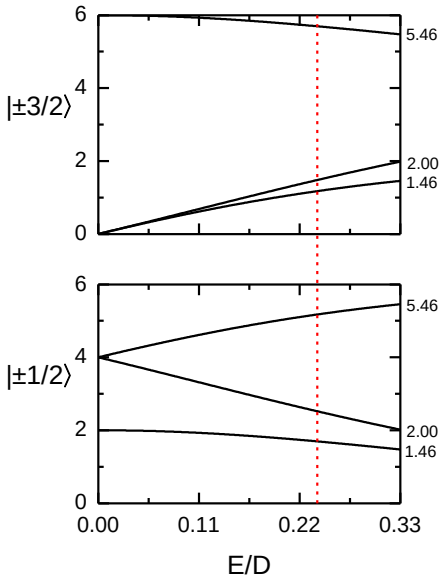
2 clusters?

Origin: 8Fe ferredoxin containing two [4Fe-4S]$^{+}$ 12 Å apart.

Next step would be to run different temperatures, powers etc.,
to do a simulation of the EPR signal,
to run the same sample at Q or S-band. (If this is an interacting species the *g* values will change.)

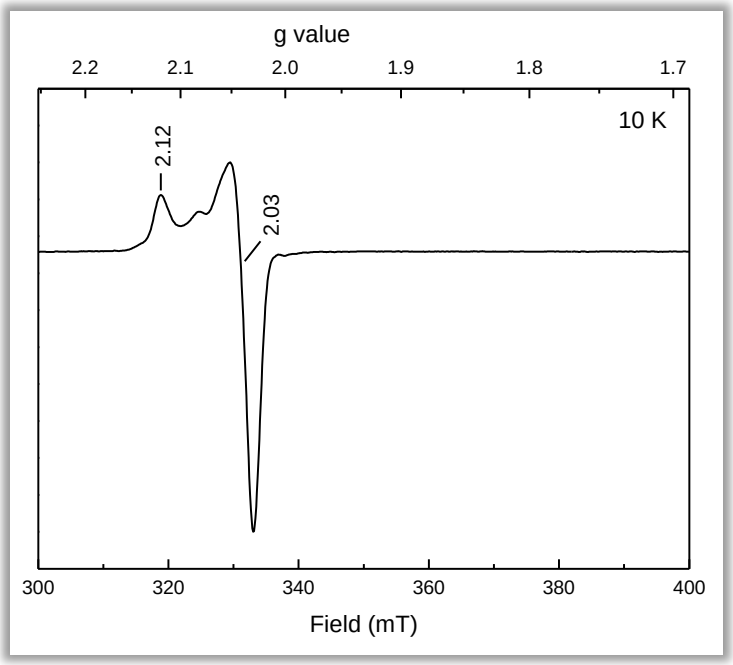
[4Fe-4S]$^{+}$ (S = 3/2)





- S = 3/2 (all g values below 6)
  20% S = ½, 80% S = 3/2
- S = ½ peaks detectable in the g = 2 region
- E/D ≈ 0.24
- D > 0

[4Fe-4S]$^{3+}$ (S = 1/2)



Rhombic S = ½

g values average out close to 2

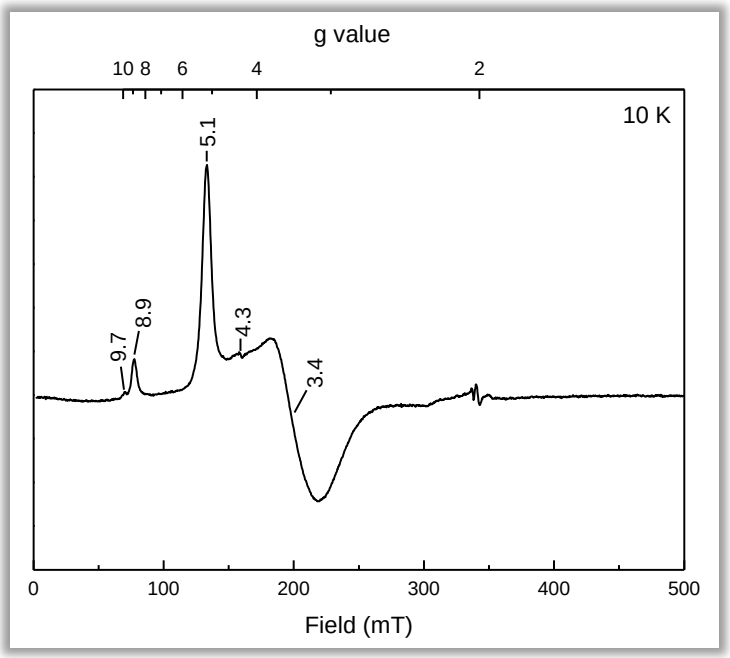
g values below 4

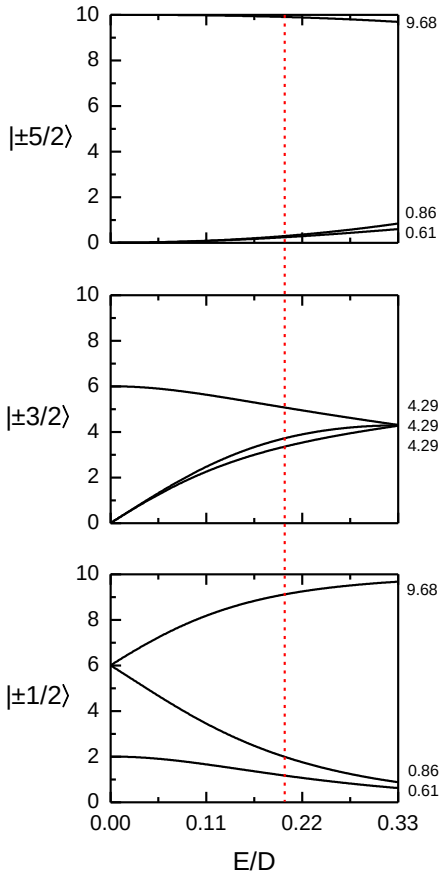
Origin: [4Fe-4S]$^{3+}$ (HiPIP). In general more than on EPR signal associated with one cluster due to valence isomers that consist in this type of cluster

Feature at g = 2.00 is due to a radical impurity

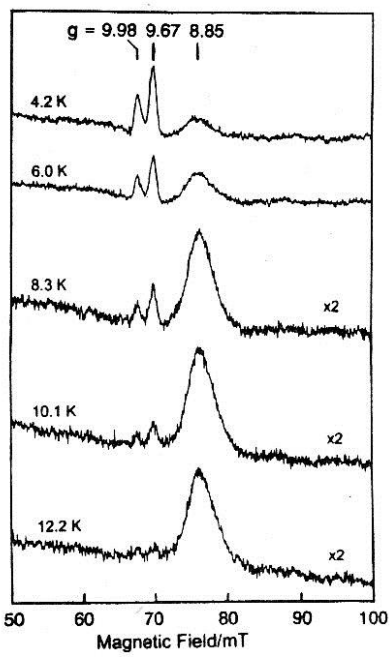
Signal measured at 10 K, not due to [2Fe-2S]. 4Fe clusters have low-lying excited states causing the signal to show fast relaxation.

[Zn-3Fe-4S]$^{+}$ cluster (S = 5/2)

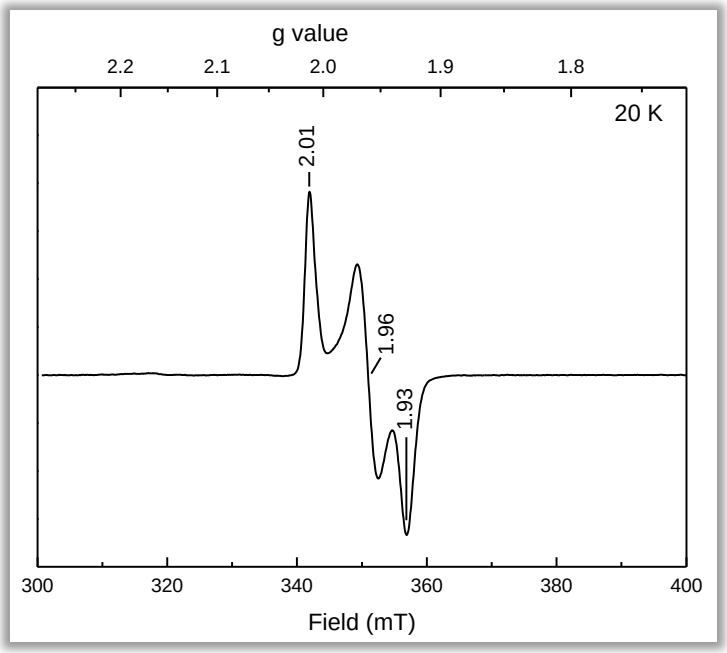




- S = 5/2 (all g values below 10)
- E/D ≈ 0.2 (g = 9.67 is due to a different species)
- D < 0 (determined by temperature studies)



[2Fe-2S]$^{+}$ (S = ½)

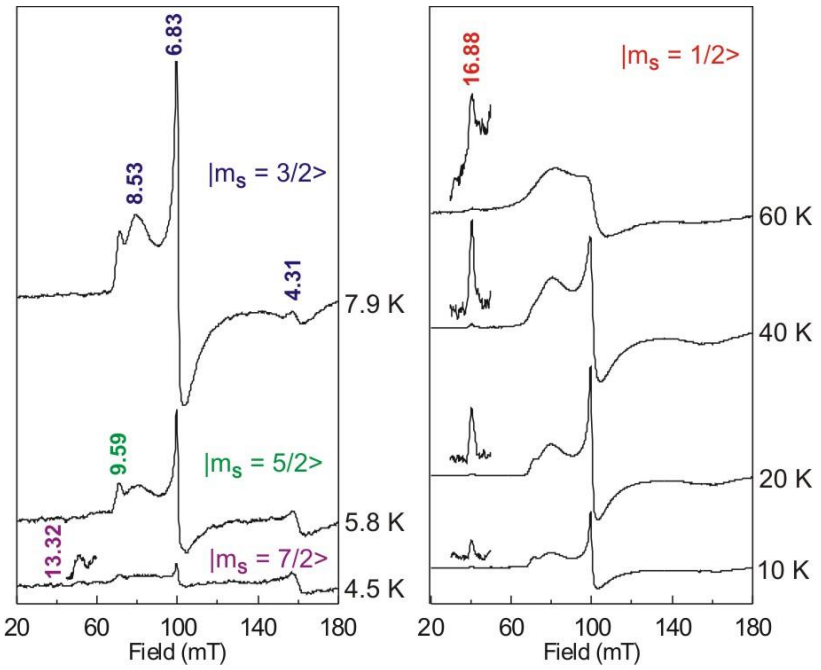


- Rhombic S = 1/2
- g values average out close to 2
- g values below 4

Origin: [2Fe-2S]$^{+}$ (*Clostridium pasteurianum* 2Fe Fd)

- Signal measured at 20 K, not due to [4Fe-4S]. 4Fe clusters have low-lying excited states causing the signal to show fast relaxation.

[2Fe-2S]$^{+}$ (S = 9/2)



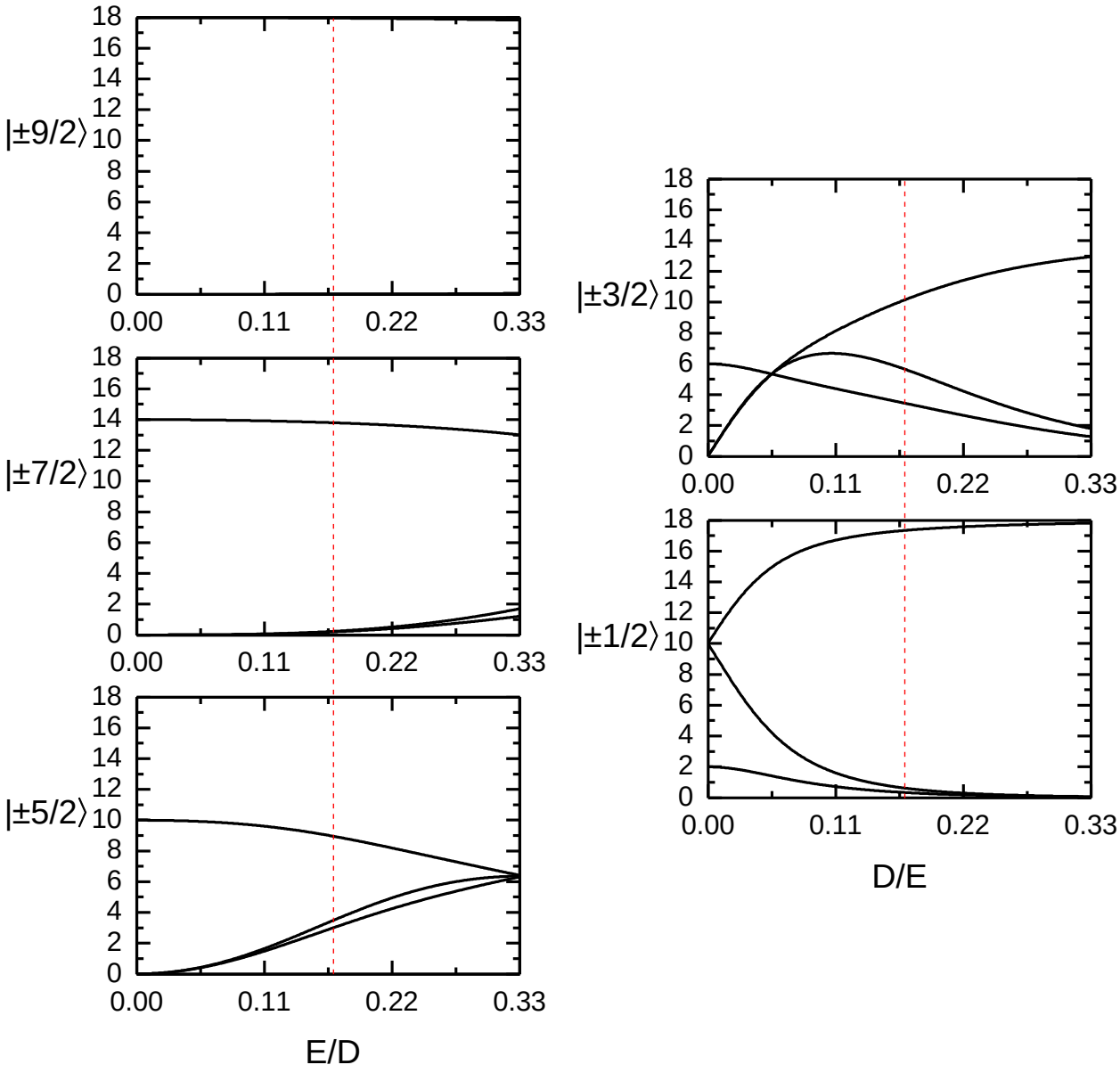
Sample is clearly a species with S > ½. Temperature studies needed to get correct spin state:

Origin [2Fe-2S]$^{+}$ cluster with S = 9/2 (Clostridium pasteurianum 2Fe Fd, C60S mutant)

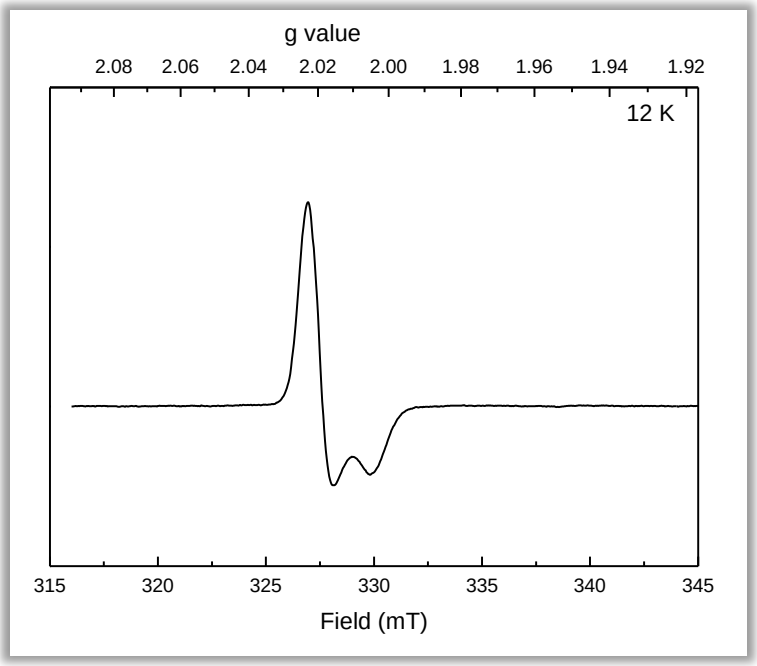
E/D = 0.17, *g* = 2.00						
| ± 1/2 ⟩	0.35		17.32		0.66	
| ± 3/2 ⟩	3.51	(3.70)	10.05	(10.05)	5.75	(5.92)
| ± 5/2 ⟩	9.00	(9.24)	2.91		3.37	
| ± 7/2 ⟩	13.80		0.19		0.22	
| ± 9/2 ⟩	17.96		0.003		0.003	

D = - 1.4 cm^{-1} (log [$I_{10.05}/I_{9.24}$] versus 1/T)

Rhombogram for S = 9/2



[3Fe-4S]$^{+}$ (S = ½)



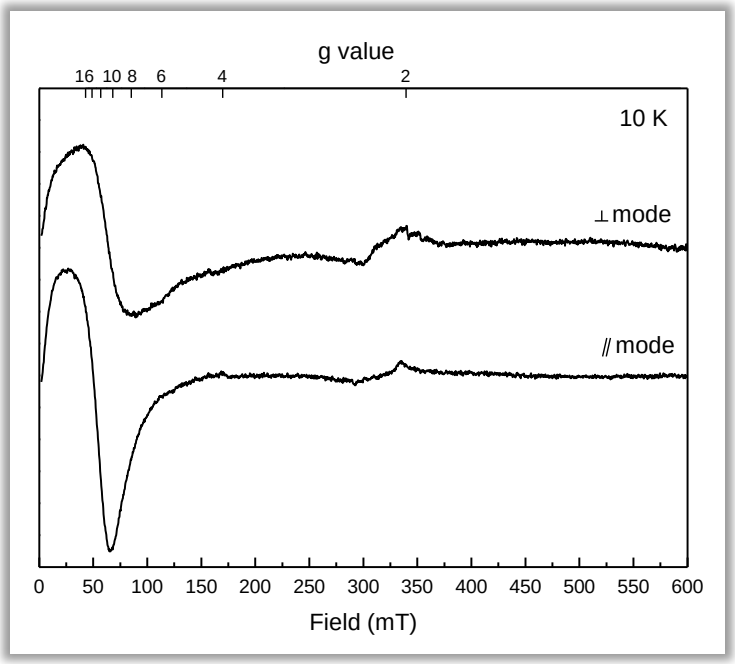
Axial *S* = ½

g values average out close to 2

g values below 4

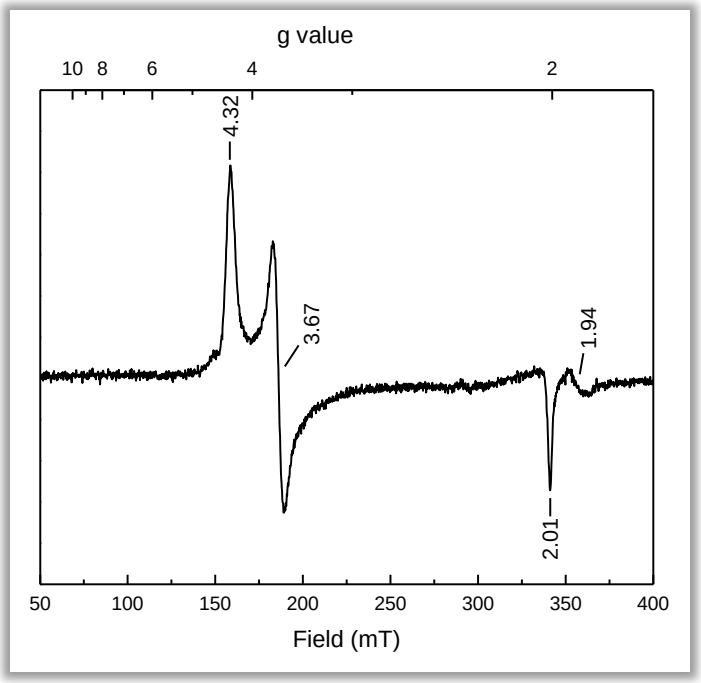
Origin: [3Fe-4S]$^{+}$ (*Allochromatium vinosum* Hydrogenase)

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



Origin: [3Fe-4S]^{0}, S = 2

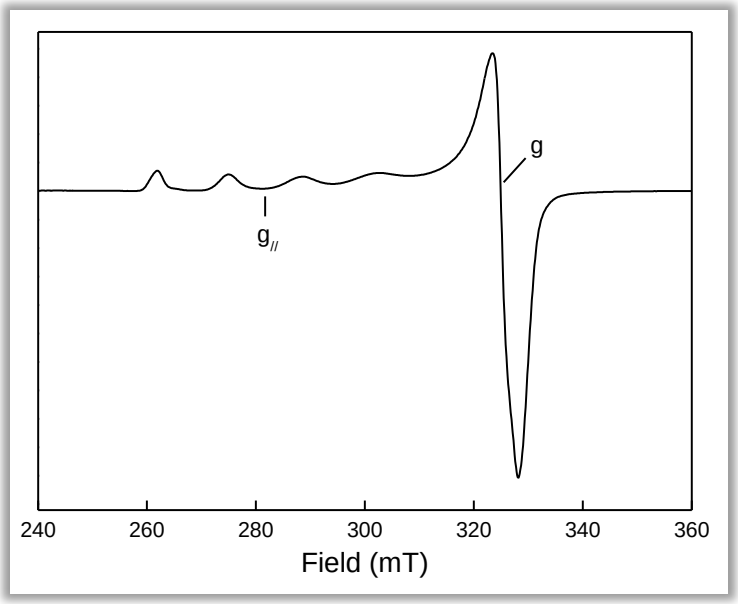
[7Fe-9S-Mo-homocitrate] cluster in nitrogenase MoFe protein



- $S = 3/2$
- E/D = 0.08, D < 0
- $g_{av} < 2$
- regular 4Fe signal present at 1.94
- $g = 6.0$ signal detectable at 20 K
- The nitrogenase MoFe protein contains the active site metallocluster called FeMo-cofactor [7Fe-9S-Mo-homocitrate] that exhibits an $S = 3/2$ EPR signal in the resting state

5.5 Inorganic Complexes

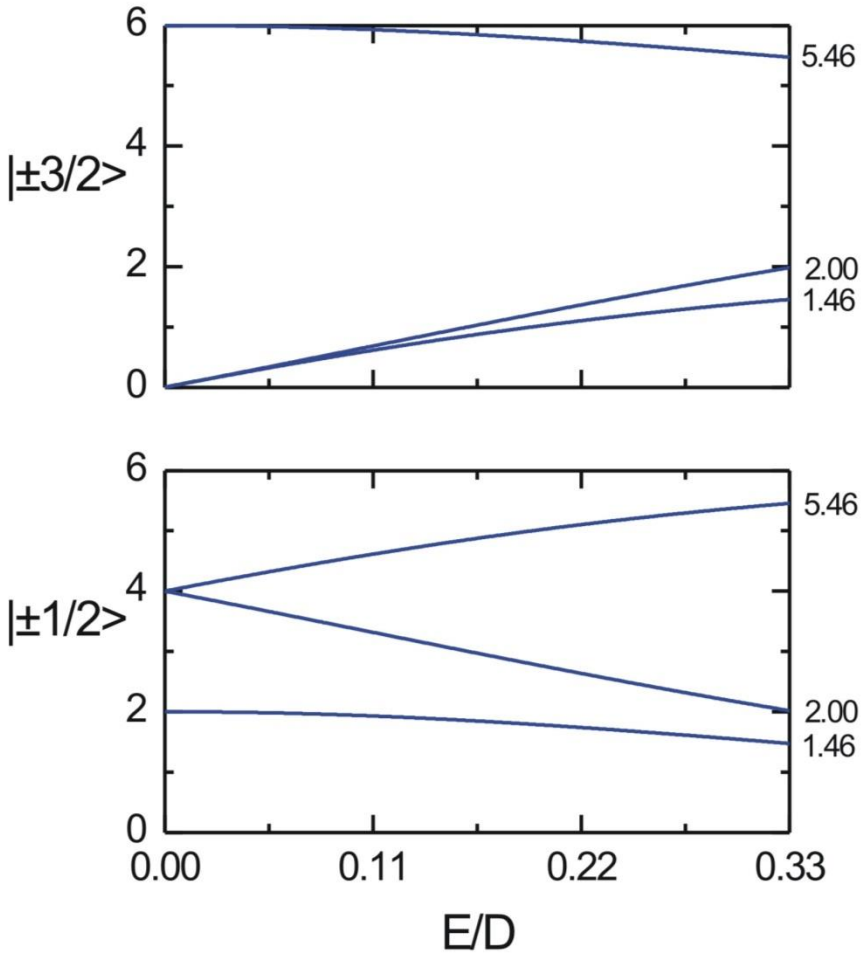
Cu^{2+} in $Cu(ClO_4)$



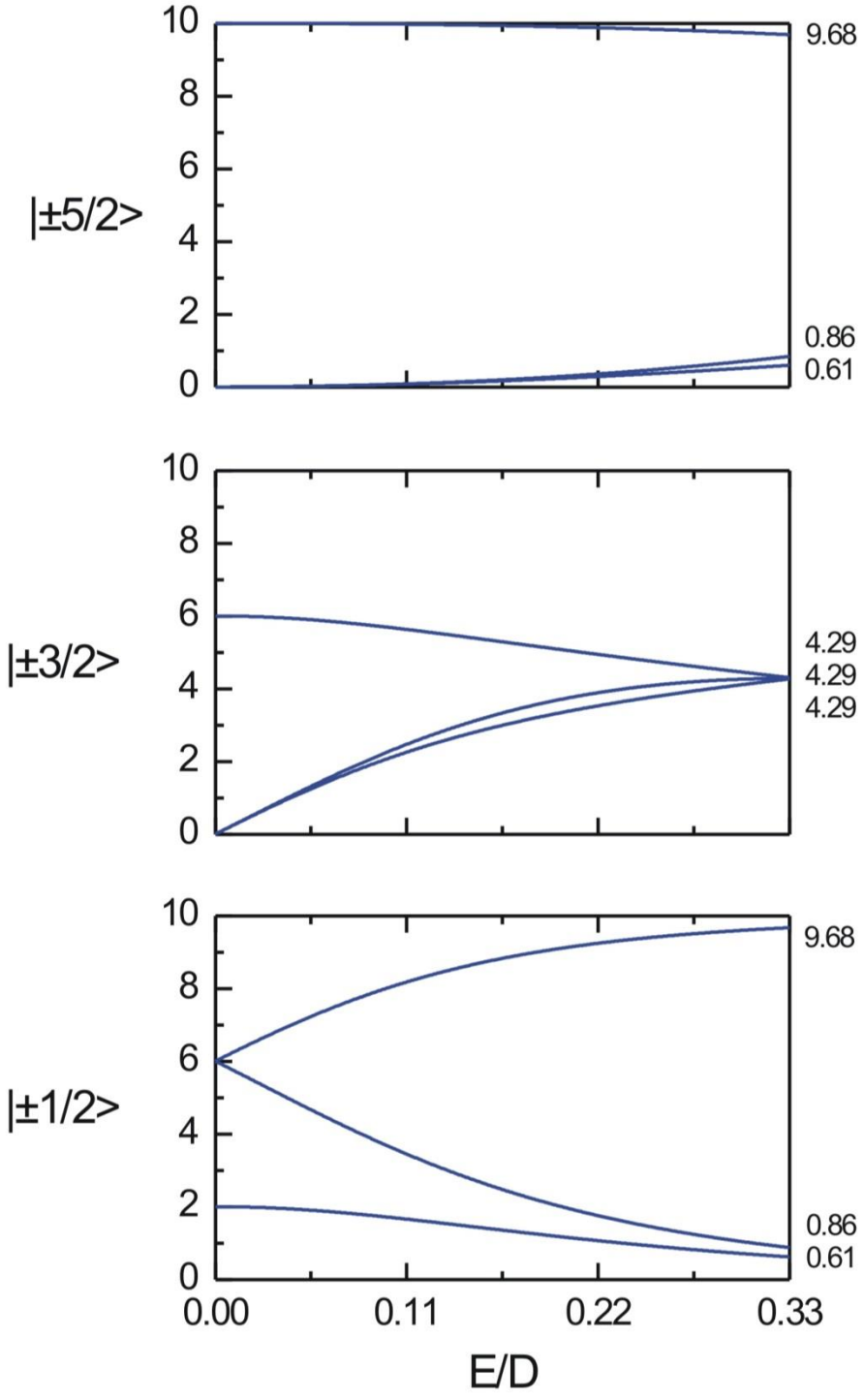
- Cu^{2+} (d^9) typically yields an axial EPR spectrum. The two principal isotopes of copper, ^{63}Cu and ^{65}Cu, both have nuclear spins of 3/2 so that the Zeeman line will be split into four lines (m_I = 3/2, 1/2, -1/2, -3/2). Since the magnetic moments of these two isotopes are very similar, the hyperfine couplings are nearly coincident. The hyperfine coupling along $g_{/\!/}$ for Cu^{2+} is always much greater than that along $g_{\perp}$, resulting in a large splitting of the $g_{/\!/}$ line with only minor (often unobservable) splitting of $g_{\perp}$.

5.5 Rhombograms

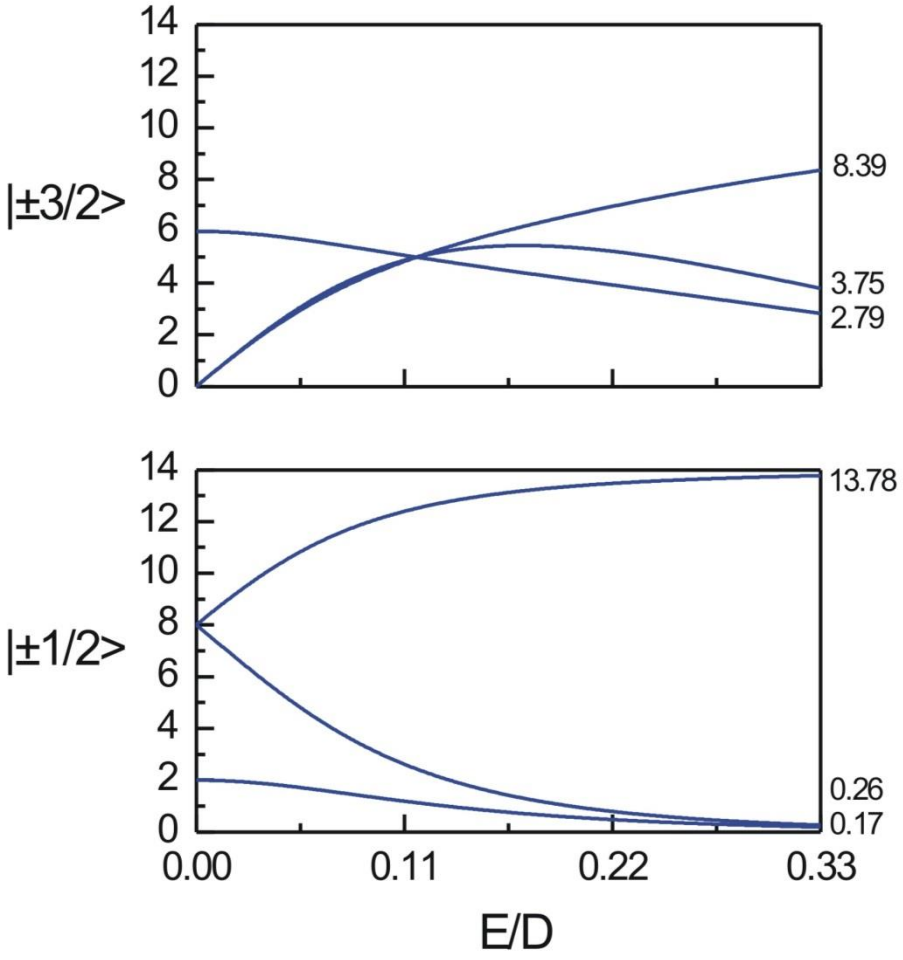
S = 3/2

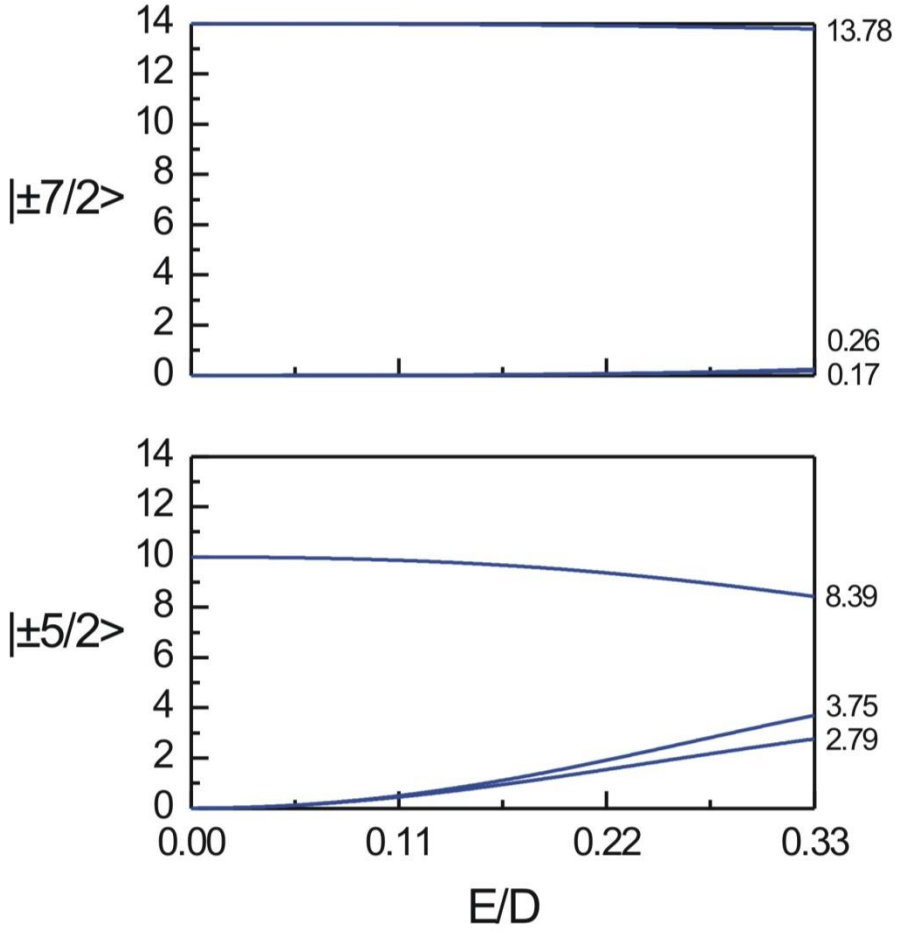


S = 5/2

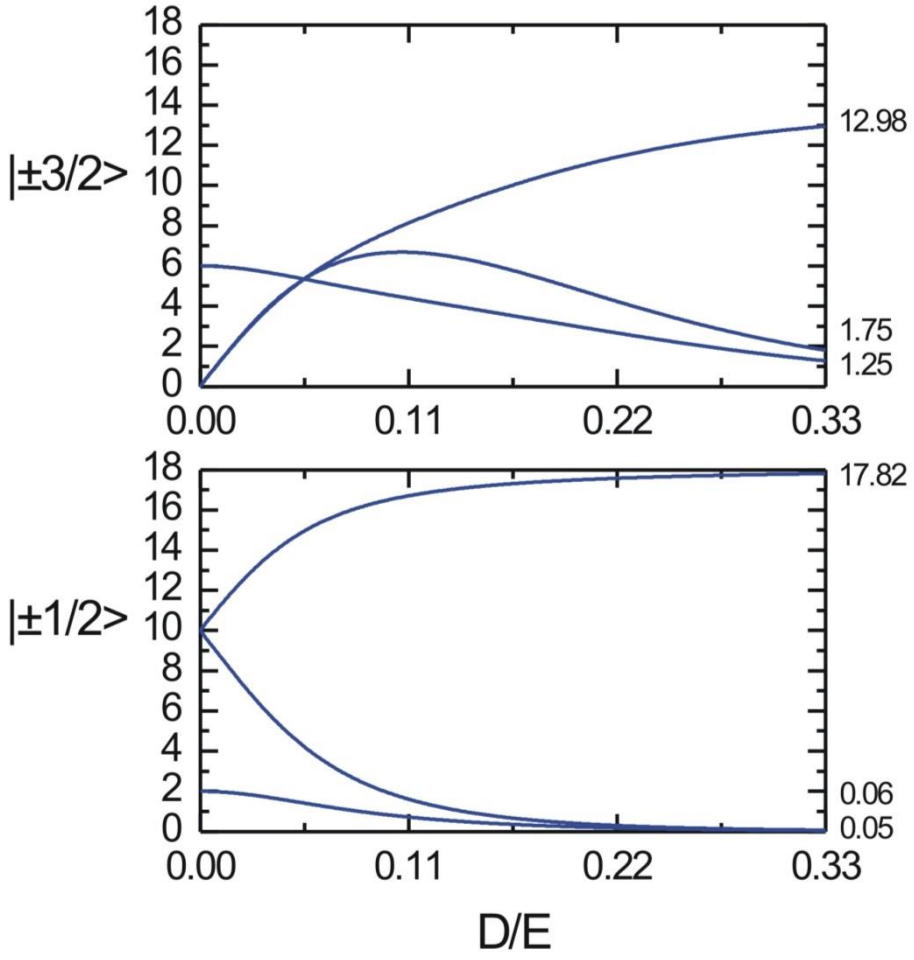


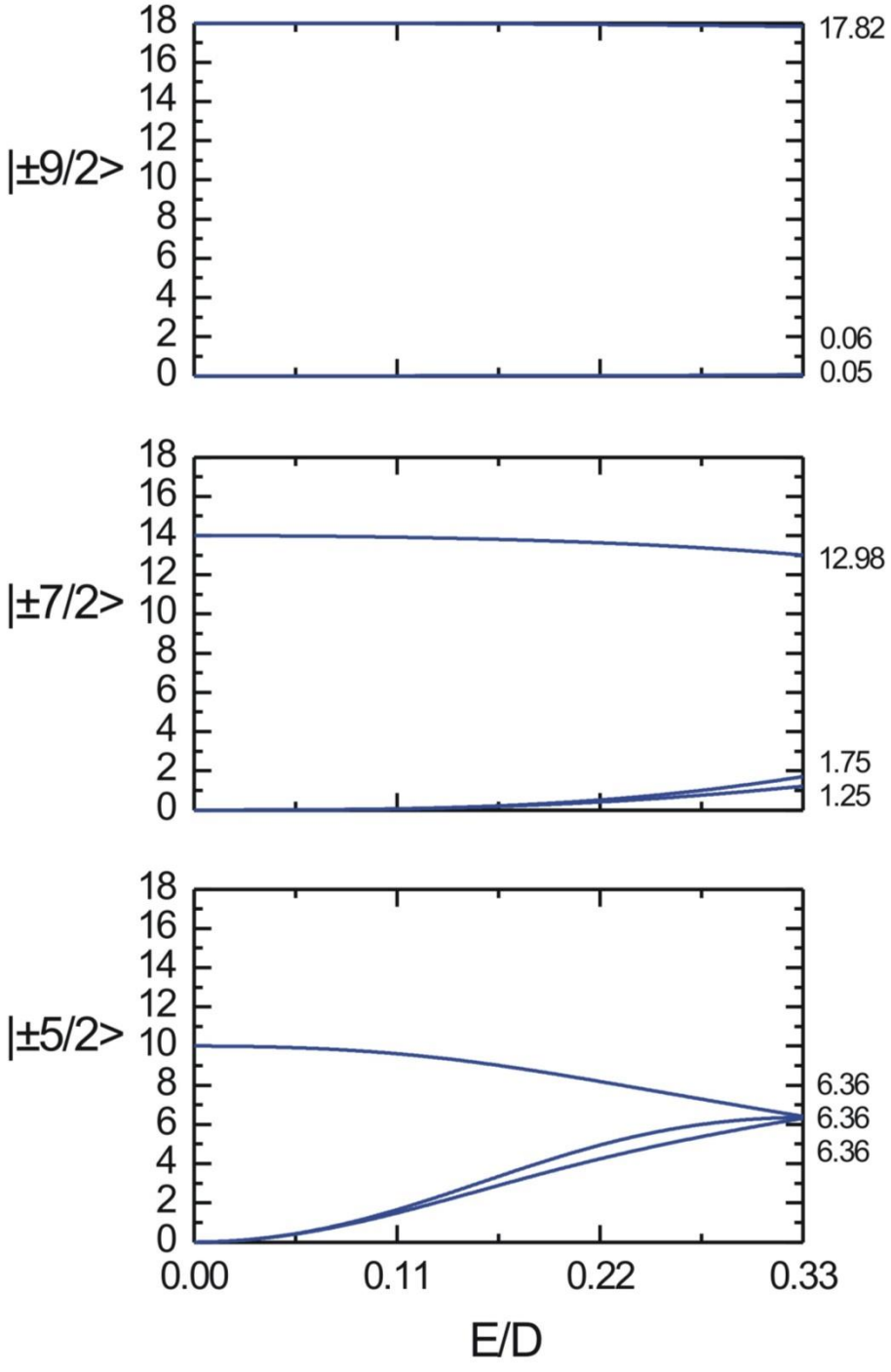
S = 7/2




14
12
10
8
6
4
2
0
|±7/2>
13.78
0.26
0.17
14
12
10
8
6
4
2
0
|±5/2>
8.39
3.75
2.79
0.00
0.11
0.22
0.33
E/D

S = 9/2




|±9/2>
|±7/2>
|±5/2>
18
16
14
12
10
8
6
4
2
0
17.82
0.06
0.05
12.98
1.75
1.25
6.36
6.36
6.36
0.00
0.11
0.22
0.33
E/D