

# Commentary

## When is a Radical not a Radical?

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It is not commonly realised that symmetrical Fe(III) complexes are expected to have EPR spectra dominated by a symmetrical narrow line close to the free-spin  $g$ -value. The aim of this note is to stress that such features can be mistakenly identified as the spectra for stable organic radicals. This situation is a particular problem in studies of animal or plant tissue samples.

### INTRODUCTION

In various lectures on 'free-radicals' one of us (MCRS) has often posed the question 'when is a free-radical not free?' Since most of his work on radicals, ever since the early days,<sup>1</sup> has been on trapped radicals, and since, in all this work, we never used the term 'free-radical' until encouraged by editors very recently, this seemed to be a good introduction. However, in rejecting a recent paper of ours, a referee told us categorically that the EPR ( $\equiv$  ESR) spectrum that we had assigned to an Fe<sup>III</sup> complex was, in fact, 'due to free-radicals, probably semi-quinones'. This, had in fact also been our initial view, but we are now sure that it is a ferric complex that is responsible. If this ref-

eree can make this mistake, despite the evidence put forward that 'shows' it really is iron, then so may many others. We feel that it is not generally appreciated by free-radical EPR spectroscopists working in biological areas, that this confusion is a real danger. So the answer to our question is 'when it is Fe<sup>III</sup>,' and the aim of this note is to explain why this can be the case.

### EPR SPECTRA OF $3d^5$ TRANSITION METAL COMPLEXES

It is generally assumed that if Fe(III) is high-spin (HS), it will have strong features at *ca.*  $g = 6$  (axial symmetry: e.g. haeme-iron) or  $g = 4.3$  (rhombic symmetry, e.g., lacto- or trans-ferrin). Features closer to  $g = 2$  are taken to establish the presence of low-spin (LS) iron, typical  $g$ -values being in the range 2.5–3.2 ( $g_x$ ), *ca.* 2.2 ( $g_y$ ) and 1.5–1.9 ( $g_z$ ). However, for the isoelectronic ion, Mn<sup>2+</sup> ( $3d^5$ ), high-spin is normal, and a  $g$ -value of *ca.* 2.00 is also normal. (This ion gives a well-defined sextet for

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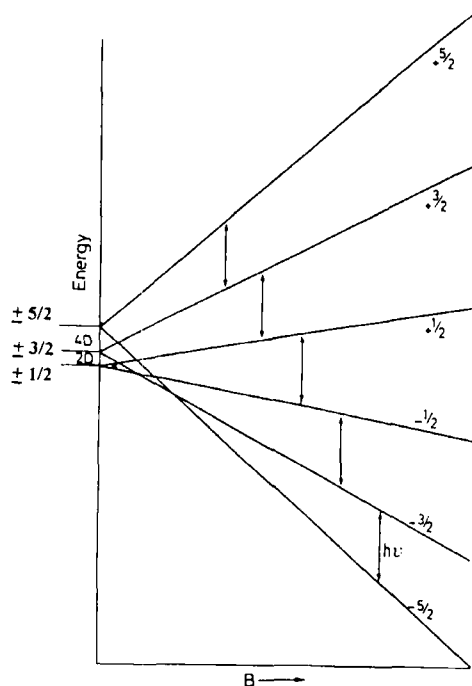


FIGURE 1 Divergence of levels with magnetic field for a Fe(III) complex having  $E = 0$ , and  $B$  parallel to the symmetry axis. As the orientation changes the  $D$  terms change sign and the outer four lines move whilst the centre line remains isotropic.

hyperfine coupling to  $^{55}\text{Mn}$  nuclei having  $I = 5/2$  so there is no potential confusion with radical signals). Hence it is clearly quite reasonable for Fe(III) complexes to give similar results, and indeed they do. Thus, for example,  $\text{Fe}^{3+}$  doped into the crystals  $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$ ,  $\text{ZnO}$  and  $\text{ZnWO}_4$  gave intense isotropic singlets at  $g$ -values of 2.0021, 2.0060 and 2.0019 respectively.<sup>2-4</sup> In several cases, there were also sets of lines on either side of the singlets, associated with transitions between the  $(\pm 5/2)$ ,  $(\pm 3/2)$  and  $(\pm 3/2)$ ,  $(\pm 1/2)$  electron-spin levels. These are shown, together with the dominating  $(+ 1/2)$ ,  $(- 1/2)$  transition in Figure 1. In a completely symmetrical ligand field all 5 transitions are superimposed ( $D = 0$ ). We now consider what happens as this symmetry is reduced to the limit of total loss of symmetry but with strong ligand bonding. Initially, the  $\pm 5/2$  and  $\pm 3/2$  levels move above or below the  $\pm 1/2$  levels as indicated. This generates two sets of asymmetric

transitions on either side of the  $\pm 1/2$  line, which remains isotropic. The zero-field splitting parameters ( $D$ ), which are a measure of these distortions, can be derived from the  $x$ ,  $y$  and  $z$  features of the side lines. As the distortion increases, the central line splits into a doublet and the wing lines move away asymmetrically. Beyond this stage there is no clear pattern, with many transitions of varying probability scattered through a large field range. The situation for high spin iron was nicely depicted by Dowsing and Gibson, some time ago,<sup>5</sup> and a range of computed spectra are given by Mabbs and Collinson.<sup>6</sup> Fortunately this situation quickly moves to two limiting sets of lines which are invariant for further increases in the zero-field splitting. These are the strong features at  $g \sim 6$  for axial systems such as haeme iron, and  $g = 4.3$  for rhombic systems – (we stress that these are not true  $g$ -values, but simply field-markers: the true  $g$ -values usually remain close to  $g = 2$ ). Further increase in the strength and asymmetry of ligand binding now causes a catastrophic switch, generally to give LS  $\text{Fe}^{\text{III}}$  with  $S = 1/2$ . (In principle, the intermediate spin-state,  $S = 3/2$  is possible, but this requires relatively subtle forms of bonding and is rare). Now there are three (true)  $g$ -features ( $x$ ,  $y$  and  $z$ ) which are initially well separated and may require very low temperatures for detection. Increasing the asymmetry still further shifts these values towards  $g = 2$ , and this value can, in principle, be reached, with  $g_x \approx g_y \approx g_z \approx 2.0$ , but we know of no examples of this extreme limit. This covers all possible structures (we think), and it is interesting that the multitude of potential transitions have moved in full circle from  $g = 2$  back to  $g = 2$ , with increase in ligand binding.

### IF $g = 2$ COULD IT BE IRON?

Yes, but it is usually possible to make a clear choice between an Fe signal and one for free radicals. In principle, the most characteristic features that would finger-print iron would be the doublet expected for  $^{57}\text{Fe}$  complexes. However, nature has

not been kind in this case, and the splitting is expected to be small, in the region of 10 G. Since the doublet satellites will be very weak relative to the main transitions, they will generally not be observable in powder spectra, unless it is possible to enrich – which is rarely the case.

However, the outer asymmetric transitions are usually detectable, and can be taken as diagnostic of Fe(III). If in doubt use a simulation method.<sup>6</sup> Also, radical transitions always saturate readily as the microwave power increases, but these Fe(III) features will not. So compare a typical radical signal with the unknown, with respect to power saturation.

## CONCLUSION

So our answer to the question is 'yes' it could be iron, and real care needs to be taken, especially if there are wing lines. We have found a number of published cases where Fe(III) is a real possibility – for example, in our ESR studies of human tumour tissues,<sup>7</sup> a feature in the  $g = 2$  region may

possibly have been from symmetrical iron, at least in part – but what convinced us of the reality of this problem was the referee's 'certainty' – so for once, a rejection was welcome!

We thank our colleagues for helpful discussion.

## References

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