Objective Analysis of EPR Spectra by Computer Methods

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Correlation methods and the maximum entropy method are used to provide objective analyses of EPR (ESR) spectra and accurate values of coupling constants.

Simple EPR spectra can often be interpreted visually or by comparison with a simulation, and coupling constants determined by direct measurement from the spectrum. Computer methods come into their own when the spectra are complex or weak, and to obtain accurate values of coupling constants. In this paper, we show how correlation methods provide reliable approaches to the objective analysis of complex and weak EPR spectra, and accurate values of coupling constants.

Results and Discussion

MATCH and SEEK.—MATCH is a correlation program[1] which starts with a pattern consisting of a Gaussian or Lorentzian derivative shape with an arbitrary linewidth, and stored as a one-dimensional array of numbers. This pattern is used to determine the position of the most intense line of the spectrum [line A for the 2,6-di-*tert*-butyl-4-methylphenoxyl radical[2] in Fig. 1(*a*)], and the linewidth is optimized to give the best fit with the experimental line [Fig. 1(*b*)]. This line is then taken as the provisional centre of the spectrum. The criterion of best fit is the product function, a modified cross-correlation coefficient, which for a particular experimental spectrum has a maximum value when the best simulation has been chosen, but the value of the product function (unlike the cross-correlation coefficient) increases with the intensity of the experimental spectrum. SEEK[1] then looks either for a line of equal intensity to the left or right of the central line to establish a doublet coupling [Fig. 1(*c*)], or for two lines equispaced to the left and the right of the central line to establish a triplet splitting [Fig. 1(*d*)]. In this instance, SEEK shows that the doublet splitting [Fig. 1(*c*)] gives the higher product function. MATCH determines that a better fit is given by a quartet [Fig. 1(*e*)] than by the doublet [Fig. 1(*c*)]. A new sweep by SEEK, using this quartet [Fig. 1(*e*)] as the basic pattern, determines that a new triplet corresponding to Fig. 1(*d*) provides a substantial improvement to the product function, and provides the pattern shown in Fig. 1(*f*). The parameters are refined using MATCH and a further pass of SEEK establishes that there is no further improvement in product function by the introduction of either a further doublet or a further triplet splitting, so the analysis shown by Fig. 1(*f*) is complete.

Maximum Entropy Method (MEM).—The MATCH and SEEK method of analysis is usually successful where the couplings are mainly triplets, but difficulties arise for spectra with numerous doublet couplings. For example the 3-thenyl radical[3] (Fig. 2) which has five different doublet couplings gives a 32-line spectrum, the lines of which are all of approximately equal intensity. Whichever peak MATCH chooses as the provisional centre of the spectrum, only five of the remaining 31 peaks will provide a correct doublet coupling constant if chosen as the next most intense line by SEEK, giving a less than 1 in 6 chance of success. In the particular case of the 3-thenyl radical [Fig. 2(*a*)], MATCH chooses the 19th line as the most intense,

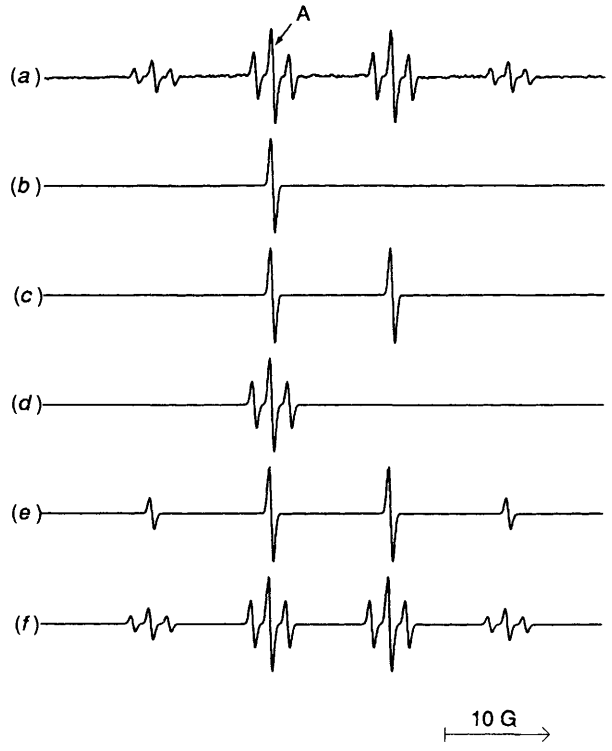

Fig. 1 MATCH and SEEK analysis of the EPR spectrum of the 2,6-di-*tert*-butyl-4-methylphenoxyl radical. See the text for stages in the analysis.

and SEEK chooses the 28th line, giving a false proposed coupling constant of 9.7 G which in fact corresponds to 16.7–8.8 + 1.8 G.

A good way of avoiding this problem is to use the maximum entropy method.[4,5] This method looks for a pattern (in the first instance a Gaussian or Lorentzian derivative pattern) in any number of positions in the experimental spectrum. Noise is ignored, and the output is constrained to be smooth and always positive. The result [Fig. 2(*b*)] is a presentation similar to the normal NMR absorption mode. This output is autocorrelated to produce possible values of the first coupling constant: the highest off-zero autocorrelation maximum, in this case 0.73 G, usually, but not always,[6] corresponds to a true coupling constant. A new pattern is chosen consisting of two lines separated by 0.73 G, and application of MEM gives the new output [Fig. 2(*c*)] consisting of 16 lines from which the 0.73 G coupling has been stripped out. This 16-line output is autocorrelated to produce new potential coupling constants, the highest value being 8.76 G. A new four line pattern is constructed using the two coupling constants 0.73 and 8.76 G, and this is used in a new pass of MEM to produce the eight-line output [Fig. 2(*d*)]. Three further passes of MEM remove coupling constants of 16.66, 1.83 and 16.23 G, respectively,

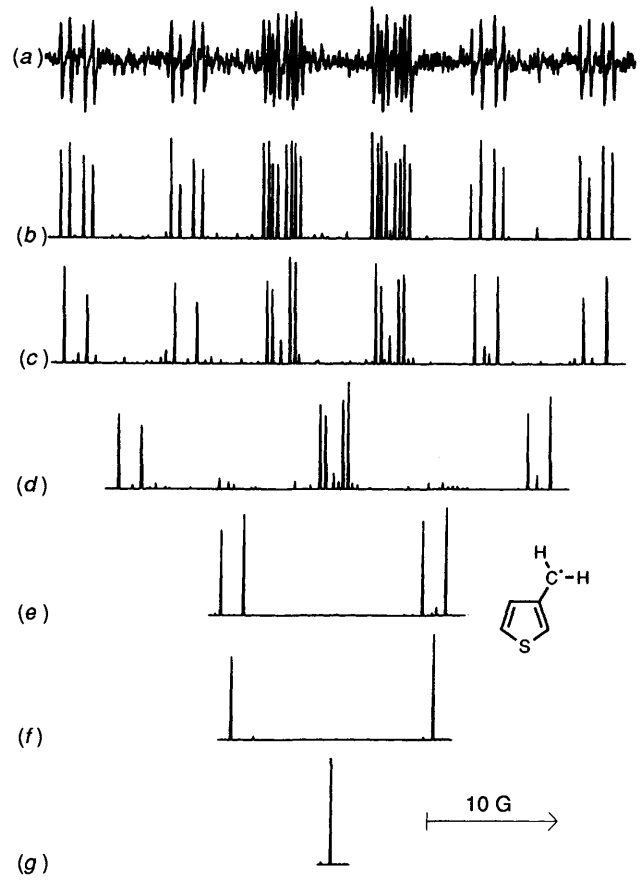

Fig. 2 MEM analysis of the EPR spectrum of the 3-thenyl radical: (*a*) raw spectrum; (*b*) after MEM processing, using a single Gaussian derivative shape as the pattern; (*c*) 0.73 G coupling removed; (*d*) 8.76 G coupling removed; (*e*) 16.66 G coupling removed; (*f*) 1.83 G coupling removed; (*g*) 16.23 G coupling removed

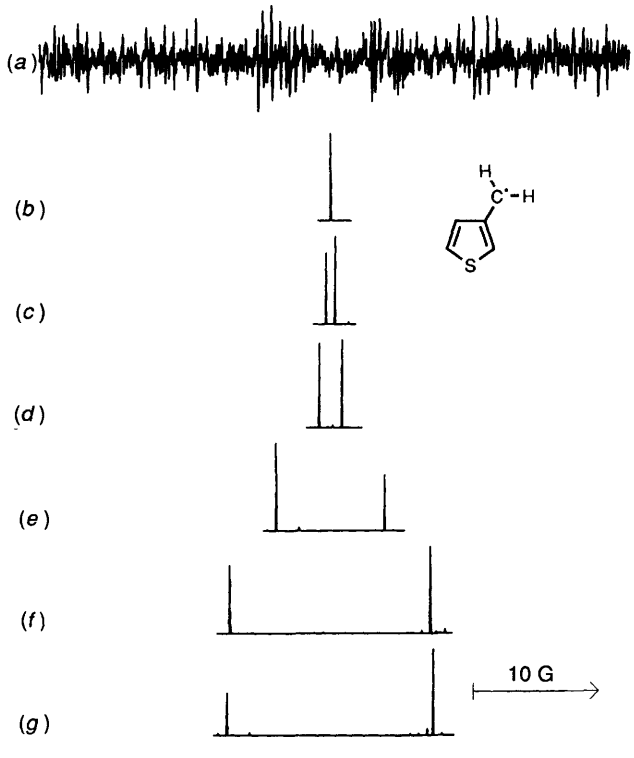

Fig. 3 MEM analysis of a weak EPR spectrum of the 3-thenyl radical: (*a*) raw spectrum; (*b*) after complete analysis; (*c*)–(*g*) input pattern contains all the coupling constants except (*c*) 0.73 G; (*d*) 1.83 G; (*e*) 8.76 G; (*f*) 16.23 G; (*g*) 16.66 G

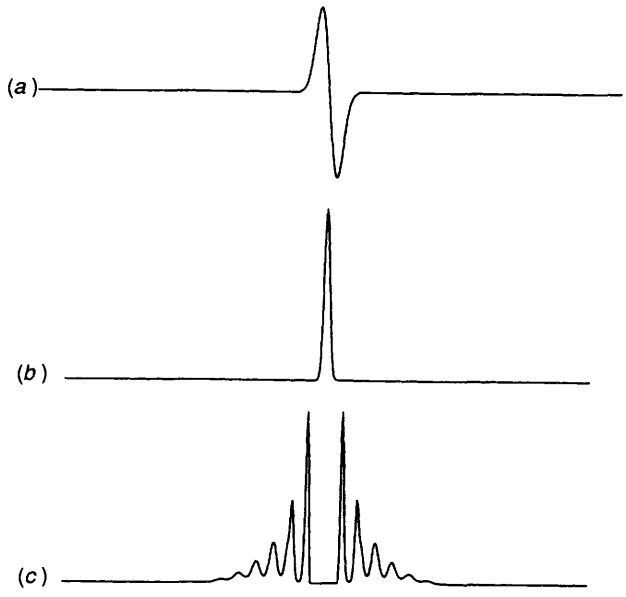

Fig. 4 Test of the maximum entropy method: (*a*) single Gaussian derivative peak; (*b*) output from MEM analysis, using a similar shape as the pattern; (*c*) output using similar shape, but inverted phase

reducing the output [Fig. 2(*g*)] to a single line, showing that the analysis is complete. The coupling constants obtained are finally refined by MATCH; the values agree well with the literature values of 0.75, 1.86, 8.94, 16.39 and 16.89 G found by Hudson *et al.*[3]

The 3-thenyl spectrum shown in Fig. 2 was obtained with an optimum modulation amplitude of 0.25 G. Fig. 3 shows the same sample, but with the modulation amplitude reduced to 0.063 G, giving a much noisier spectrum for which visual analysis would fail. However the maximum entropy method successfully analyses this spectrum by successively removing all the coupling constants to give the single line shown in Fig. 3(*b*). Figs. 3(*c*)–(*g*) show the output from passes of MEM using all but one of the couplings; each produces output consisting of two lines at the spacing of the coupling constant left out, confirming the analysis.

Caution should be used when applying MEM to weak spectra where it may not be obvious whether the EPR lines go up first or down first. Fig. 4(*a*) is a noise-free single Gaussian line. Application of the maximum entropy method using a similar lineshape as a pattern gave the output shown in Fig. 4(*b*). However, if a pattern of the wrong phase is inadvertently chosen, the result will be the complex output shown in Fig. 4(*c*). The two largest peaks represent the effort of the out-of-phase pattern to match the two lobes of the spectrum as two separate lines. The two smaller lines outside the large ones represent the effort of MEM to cancel the negative and positive residual lobes left by the initial matching, and further smaller peaks outside these attempt to cancel the new smaller residual lobes, and so on. MEM is a Bayesian method, utilizing prior information about the spectrum, in this case the lineshape and phase, in the analysis. If this prior information is incorrect, the method will fail spectacularly: garbage in, garbage out.

MULTIPEAK—An alternative to the maximum entropy method is to use MULTIPEAK.[7] This is a correlation program which determines all possible line positions by MATCH, using a single Gaussian or Lorentzian derivative shape as the pattern, and assigns (integral) intensities to these positions to a total appropriate for the radical, *e.g.*, 32 for the 3-thenyl radical with five protons. Assignments are initially made to the most intense apparent peaks, and the assignments are varied to give the best

Fig. 5 Very weak 3-thenyl radical EPR spectrum. For objective analysis using MULTIPEAK, see the text.

Fig. 6 EPR spectrum of the nitrobenzene radical anion. See the text for MULTIPEAK analysis.

possible match with the experimental spectrum. Coupling constants are now established one by one. The program looks for pairs of peaks, with a definite separation corresponding to a possible coupling constant; in the 3-thenyl example, integral intensities to a total of 16 are applied to possible positions so as to give the best overall match with the experimental spectrum. Possible couplings are chosen from autocorrelation maxima, or better by sweeping systematically over the entire range of likely coupling constants.

The coupling constant that gives the highest product function is taken as an established doublet coupling constant, and the procedure is now repeated using this (fixed) coupling and a new variable trial coupling to produce a four-line pattern. Integral intensities to a total of 8 are now applied to possible line positions to give the best fit. The procedure is repeated until all the couplings have been assigned; in practice, when the spectrum has been reduced to four lines, the remaining two couplings can normally be obtained by inspection of the positions of the four lines. This procedure works well on the 3-thenyl radical shown in Fig. 3(*a*); in spite of the noise in the spectrum, the 32 line positions are all correctly assigned in the first pass. MULTIPEAK determines that the 8.8 G coupling gives the best fit for 16 peaks, and, in fact, all five true couplings give better values than any others possible in the range 0–20 G. Two more passes of MULTIPEAK give couplings of 0.7 G and 1.8 G, respectively, and these three couplings establish a four-line pattern consisting of two pairs from which the remaining couplings of 16.2 and 16.7 G are established.

The spectrum shown in Fig. 5 was taken after the one shown in Fig. 3, with a modulation amplitude of 0.032 G, which will reduce the signal-to-noise in the sample by a factor of two, in addition to any decay in intensity of the radical with time. This is a very weak spectrum, and there are no obvious lines. The nine most intense autocorrelation maxima are at 18.8 > **16.6** > 10.8 > 16.9 > 5.4 > **0.7** > 9.8 > 15.4 > 10.5 G. Only two of these (in bold) correspond to true couplings, and the highest value (only 0.072) does not correspond to a true coupling. In its first pass, MULTIPEAK in fact assigns (with hindsight) only 15 of the 32 line positions correctly. However a sweep of all possible couplings in the range 0–20 G showed that a coupling of 0.7 G is preferred by a significant margin over the 18.8 G which gave the highest autocorrelation maximum, and gives 16 peaks, eight of which are in the correct positions. The next pass of MULTIPEAK gave 1.8 G as the best value for a second coupling constant (with the next best three values corresponding to the remaining true coupling constants), and seven out of the eight peak positions correctly located. The next pass produced a coupling of 8.8 G (with the next two values corresponding to the remaining coupling constants), giving four peak positions in two pairs in the correct positions for the two remaining coupling constants. Thus MULTIPEAK has provided a complete objective analysis of this very noisy spectrum.

MULTIPEAK can be applied to spectra containing splittings from $I > \frac{1}{2}$ nuclei, but there can be difficulties arising, for example, in systems containing nitrogen where it may be difficult to distinguish between a 1:2:1 and a 1:1:1 triplet, especially since nitrogen 1:1:1 triplets often show unequal intensities of the three lines.* Fig. 6 shows a spectrum of the nitrobenzene radical anion, made by reduction of nitrobenzene by glucose in aqueous methanolic sodium hydroxide solution. This spectrum is well resolved, but there is a pronounced reduction in height of the lines at the right of the spectrum. With 92 expected lines, MULTIPEAK was asked to produce the best 48 pairs of lines, or 32 nitrogen triplets, searching in each case over the entire range of 0–20 G. The best value found was for a doublet splitting of 3.40 G, ahead of a possible doublet splitting of 3.65 G, and with no suitable nitrogen splitting emerging. The 3.40 G doublet was incorporated, and the program instructed to look either for 24 positions involving another (variable) doublet, or 16 positions involving a (variable) nitrogen triplet. The best fit was for a second doublet splitting of 3.40 G, ahead of 3.65, 1.14 and the nitrogen splitting of 13.77 G. All these (true) values were better than any other values, even at this stage of the analysis. A third pass established a 3.65 G doublet splitting, ahead of the 1.14 G doublet and the 13.77 G nitrogen triplet in that order. The fourth pass, using the three established couplings, and looking for six positions involving another doublet splitting or four positions involving a nitrogen triplet showed a better fit for a doublet of 1.14 G compared with a nitrogen splitting of 13.77 G. The positions of the six lines in the former case were correct for a further doublet of 1.14 G and a nitrogen splitting of 13.77 G, thus completing the analysis. The assignment of these coupling constants is in good agreement with the values of 3.35 (*ortho*), 1.15 (*meta*), 3.6 (*para*) and 13.65 G (nitrogen) obtained by Ayscough and co-workers.[8]

Checks on the Validity of Analysis.—It is desirable, especially for the analysis of weak spectra, to have independent confirmation of the proposed analysis. The first check is that several different spectra should give concordant analyses. We have developed a method of determining the centre of a spectrum without carrying out a full analysis of the spectrum:[7] this centre should agree with that found by MATCH. If EPR spectra are available for radicals with and without a substituent such as *tert*-butyl[9] (which will effectively remove the coupling) or deuterium[10] (which will alter the coupling pattern in a predictable way), the coupling constant at that position can be obtained from the autocorrelograms of the two spectra without further analysis of the spectrum. This value can be compared with the coupling constant obtained by the complete analysis. Finally, the coupling constants obtained for the radical without input constraints should have values which are concordant with those of analogous known radicals.

* The spectrum in Fig. 6 analyses objectively in spite of the significant broadening of the high-field lines of the nitrogen triplet, but more severe distortions would, at some stage, undermine the viability of the analysis.

Accurate Values of Coupling Constants.—Use of MATCH allows the refinement of coupling constants. Slightly better results are obtained by using FTMATCH,[11] in which the theoretical spectrum is obtained by the fast Fourier transform method, which allows continuous variation of coupling constants and linewidths, in contrast with MATCH, where discrete values of these parameters are used. It is unnecessary to filter the experimental spectrum or apply a baseline correction. To obtain accurate values of coupling constants, we calibrate each session by running a manganese standard at room temperature,[12] and obtain the separation between the two central lines by autocorrelation, thereby giving the channel numbers equivalent to 84.0 G. Spectra are obtained on at least three separate occasions, giving reproducibility to within 0.013 G in a recent study on 3,5-disubstituted benzyl radicals.[11] Such accuracy is not always important, but is useful, for example, in studies of benzyl radical stabilization by substituents. The reduction in the $\alpha(CH_2)$ coupling constant caused by substituents has been suggested as the basis of the $\sigma\cdot_\alpha$ scale for radical substituent effects.[13] Since the total range of $\alpha(CH_2)$ values is only about 1.4 G, accurate values are essential for comparison with other measures of radical stabilization.[14]

Other Uses of Correlation Methods.—FTMATCH has been adapted to work with mixtures of two radicals. Coupling constants and linewidths can be varied individually for the two radicals, and the spectrum centres of each, and the relative intensities can also be varied. The simulation is optimized to give the best cross-correlation with the experimental spectrum.[2] The difference in position between the radical centres provides a convenient and accurate measure of *g* value differences between the two radicals, and the relative concentrations of the two radicals can be obtained from the ratio of the double-integration of the two radical simulations, done separately—this avoids problems of overlapping lines, noise and baseline drift which apply to double-integration of experimental spectra.

Experimental

EPR spectra were obtained on a Varian E104A EPR spectrometer, as described previously.[11] A mixture of 3-methylthiophene and *tert*-butyl peroxide (1:3 v/v) was photolysed at −40 °C; the reduction of nitrobenzene by glucose in aqueous methanol was carried out by gently heating the sample before transfer to the cavity of the spectrometer. The digitized spectra (approximately 4K points per spectrum) were transferred to a mainframe Solbourne computer for processing. The programs in general are rapid, but scanning a wide range of possible couplings using MULTIPEAK consumes substantial amounts of cpu time. The FORTRAN 77 programs described in this paper are available from the author (e-mail: R.A.Jackson@sussex.ac.uk) for non-commercial use.

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