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Electron Paramagnetic Resonance in Biochemistry

COMPUTER SIMULATION OF SPECTRA FROM FROZEN AQUEOUS SAMPLES

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Two computer programs are described; they can be used to simulate e.p.r. spectra of effective spin-$\frac{1}{2}$ systems in frozen aqueous samples. One program is written in BASIC and the other in FORTRAN IV. Both programs are deposited as a Supplementary Publication (SUP 50082; 27 pages) at the British Library Lending Division, Boston Spa, Wetherby, West Yorkshire LS23 7QB, U.K. References are given to the applications of these programs in biochemical work.

E.p.r. is of increasing use in biochemical studies (Swartz *et al.*, 1972). Unfortunately the spectra obtained are usually from a random spatial distribution of paramagnetic centres ('powder spectra'), as in frozen aqueous solutions, and the lineshapes may be complex. Thus the parameters of the spectra (such as *g*- and *A*-values), which contain structural information (Swartz *et al.*, 1972; Abragam & Bleaney, 1970) are often difficult to distinguish. In biochemical applications of e.p.r. it is frequently important to know how many chemical species are contributing to a spectrum; simulations can assist greatly in determining unambiguously whether one, two or more species are present. Thus accuracy of fit of simulated to experimental spectra can be a good criterion of purity. A related use is that simulation can help quantify individual species whose spectra overlap.

In the literature, simulation programs are often described only in terms of the quantum-mechanical approach used, and the programs themselves are not readily available (e.g. Aasa & Vänngard, 1975; Fritz *et al.*, 1971). Thus many users of biological e.p.r. have not used them to obtain the maximum information from their experimental spectra. The present paper should be more readily understood by biochemists.

This paper briefly describes two programs for simulating powder e.p.r. spectra; one is written in FORTRAN IV and the other in BASIC. The FORTRAN IV program incorporates more features than the BASIC program, it is faster, but uses much more core store. Both programs should be easily adaptable, with very few changes, to most computer systems having these languages available. They are deposited as a Supplementary Publication (SUP 50082) at the British Library Lending Division, Boston Spa, Wetherby, West Yorkshire LS23 7BQ, U.K., from whom copies can be obtained on the terms indicated in *Biochem. J.* (1978) **169**, 5.

These programs have so far been used by Fielden *et al.* (1974) to study the copper of superoxide dismutase, Vincent & Bray (1978) to study molybdenum in nitrate reductase, by Lowe *et al.* (1978) to study iron in nitrogenase, and by Bray *et al.* (1978) to study molybdenum in xanthine oxidase.

The present paper is intended to make the programs available, to demonstrate in detail the procedures used in the publications mentioned above, and also to collate the formulae used so that programmers with little experience of e.p.r. theory can easily write programs to suit their own particular application or computer system.

Description of Programs

E.p.r. spectra are usually displayed as the first derivative of the microwave absorption, and to simulate a powder spectrum it is necessary to integrate this derivative over all possible angles between the centre being observed and the applied magnetic field; thus the central problem is to calculate the contribution to the microwave absorption as a function of direction and of the input parameters (*g*-values, *A*-values, linewidths etc.). The function used in the two programs described first calculates the position of the e.p.r. absorption and then convolutes this with a lineshape function.

Function evaluated

The magnetic field (H_T) of the e.p.r. absorption (including a hyperfine interaction) in a field-swept experiment is given by:

$$H_{\mathrm{T}}=\frac{hv}{g\beta}-\frac{K}{g\beta}m \qquad (1)$$

where h is Planck's constant, β is the Bohr magneton, v is the microwave frequency, g and K are the values of the g-factor and hyperfine interaction constant in the field direction being considered, and m is the magnetic quantum number of the nucleus (with nuclear spin I) giving rise to the hyperfine interaction (m runs from $+I$ to $-I$). A hyperfine interaction described in this way is said to be evaluated to first order. These programs use spherical polar co-ordinates with equatorial angle ϕ and azimuthal angle θ, that is the z principal axis of the g-tensor has $\theta=0$, the x principal axis $\theta=\pi/2$, $\phi=0$, and the y principal axis $\theta=\pi/2$, $\phi=\pi/2$. With this co-ordinate system, g in the direction of the magnetic field is given by:

$$g^2=g_{xx}^2\sin^2\theta\cos^2\phi+g_{yy}^2\sin^2\theta\sin^2\phi+g_{zz}^2\cos^2\theta \quad (2)$$

where g_{xx}, g_{yy} and g_{zz} are the three principal g-values of the paramagnetic centre.

K is evaluated, not in the direction of the magnetic field, but in the direction of magnetization of the system, and so is a function of the g-values:

$$K^2=(A_{xx}^2g_{xx}^2\sin^2\theta\cos^2\phi+A_{yy}^2g_{yy}^2\sin^2\theta\sin^2\phi \\ +A_{zz}^2g_{zz}^2\cos^2\theta)/g^2 \quad (3)$$

Where A_{xx}, A_{yy} and A_{zz} are the three principal values of the hyperfine coupling, assumed to be parallel in these programs to g_{xx}, g_{yy} and g_{zz} respectively.

In the BASIC program these are the functions used, but three sets of hyperfine lines can be input. The FORTRAN IV program allows second-order hyperfine terms to be added to one hyperfine set, and these corrections are evaluated as described by Toy *et al.* (1971) (allowing for the typographical errors that occur in their paper). When added to eqn. (1) they give:

$$H_{\mathrm{T}}=\frac{hv}{g\beta}-\frac{K}{g\beta}m-\frac{(r_1^2+r_2^2+r_3^2)}{4(g\beta)^2}[I(I+1)-m^2] \\ -\frac{(r_4^2+r_5^2)m^2}{2(hv)^2} \quad (4)$$

where I is the nuclear spin being considered.

The r values are:

$$r_1=A_{zz}A_1/K$$
$$r_2=A_{xx}A_{yy}/A_1$$
$$r_3=[A_{zz}(A_{yy}^2-A_{xx}^2)g_{xx}g_{yy}g_{zz}\sin\phi\cos\phi\cos\theta]/KA_1g_1^2g$$
$$r_4=g_{zz}g_1(A_1^2-A_{xx}^2)\sin\theta\cos\theta/Kg^2$$
$$r_5=[(A_{yy}^2-A_{xx}^2)g_{xx}g_{yy}\sin\phi\cos\phi\sin\theta]/Kg_1g$$

where

$$g_1^2=g_{xx}^2\cos^2\phi g_{yy}^2\sin^2\phi$$

and

$$A_1^2g^2=A_{xx}^2g_{xx}^2\cos^2\phi+A_{yy}^2g_{yy}^2\sin^2\phi$$

Lineshape function

The individual transitions have a lineshape centred on the value of H_{T} calculated from eqns. (1) or (4) and this is taken as Gaussian:

$$\mathrm{G}(H)=-\frac{(H-H_{\mathrm{T}})}{\sigma^3}\exp\left[-\frac{(H-H_{\mathrm{T}})^2}{2\sigma^2}\right] \quad (5)$$

or Lorentzian:

$$\mathrm{L}(H)=-\frac{(H-H_{\mathrm{T}})\sigma}{[(H-H_{\mathrm{T}})^2+\sigma^2]^2} \quad (6)$$

Where $\mathrm{G}(H)$ and $\mathrm{L}(H)$ are the first-derivative lineshape functions suitably normalized so that the overall integrated intensity is independent of the linewidth parameter, σ. This parameter is assumed to vary as if it were due to unresolved hyperfine structure, i.e.:

$$\sigma^2=(\sigma_{xx}^2g_{xx}^2\sin^2\theta\cos^2\phi+\sigma_{yy}^2g_{yy}^2\sin^2\theta\sin^2\phi \\ +\sigma_{zz}^2g_{zz}^2\cos^2\theta)/g^2 \quad (7)$$

where σ_{xx}, σ_{yy} and σ_{zz} are the linewidths in the directions parallel to g_{xx}, g_{yy} and g_{zz}. The FORTRAN IV program gives a choice of these two lineshapes, whereas the BASIC program uses only a Gaussian lineshape function. Both programs have normalization so that the final integrated intensity is independent of the input parameters. It is also necessary to include the variation of transition probability with g-values. The multiplier used is given by Toy *et al.* (1971) with a correction to give the transition probability formula of Aasa & Vänngård (1975); it is:

$$[g_{xx}^2g_{yy}^2\sin^2\theta+g_{yy}^2g_{zz}^2(\sin^2\phi+\cos^2\theta\cos^2\phi) \\ +g_{zz}^2g_{xx}^2(\cos^2\phi+\cos^2\theta\sin^2\phi)]/2g^3 \quad (8)$$

Method of integration

Since the programs assumed rhombic symmetry it is only necessary to integrate over one octant of the θ, ϕ sphere, and the one chosen is $\theta=0$ to $\pi/2$, $\phi=0$ to $\pi/2$. The BASIC program performs a simple addition over a number of steps of θ and ϕ read with the input; the number of steps must be increased until the spectrum obtained does not vary. (The number of steps used is always greater than the input number since the step size is automatically varied in a predetermined way to minimize run times.) As an example of typical values for Mo(V), Fig. 1(*c′*) of Vincent & Bray (1978) uses input parameters: g-values, 1.987, 1.9805, 1.9612; half linewidths, 0.295, 0.265, 0.33 mT; microwave frequency, 9.3 GHz; number of steps of θ, 28; number of steps of ϕ, 28. The FORTRAN program uses a Simpson's-rule technique that automatically adjusts the step size to an optimum value; the technique used is a modified version of subroutine QSIMP supplied by the University of Sussex Computing Centre. Gaussian or Clenshaw–Curtis quadrature was found to produce inaccuracies. The FORTRAN program also produces a lineprinter plot of the spectrum calculated.

The programs can be extended to systems with monoclinic symmetry (S. A. Cockle & D. J. Lowe, unpublished work) as described by Pilbrow & Winfield (1973).

Details of use

In the references given above to the use of the programs, the FORTRAN IV program is run on the University of London Computer Centre CDC 7600 machine, and the BASIC program is run on a PDP 11/10 computer with 16k words of store and without a hardware floating-point unit. Typical run times for a 9GHz molybdenum(V) spectrum, with an anisotropic *g*-tensor and two protons interacting anisotropically, are 40s on the CDC 7600 and 4h on the PDP 11/10 computers. We have software available for immediate comparison of simulated and experimental spectra using the PDP 11/10 computer (D. J. Lowe, unpublished work). An assembly code version of the BASIC program has been written for the PDP 11/10 computer and this decreases the calculation time for the above simulation to 40min. Copies are available from Digital Equipment Computer Users Society, Maynard, MA 01754, U.S.A.

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