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Application of Fast Fourier Transforms to EPR Spectra of Free Radicals in Solution

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A method of reducing EPR spectra of free radicals in solution is presented in detail. This method is based on the use of the fast Fourier transform algorithm and curve fitting in the Fourier space by weighted least-squares minimization. Comparison with previous work is shown for EPR spectra of methyl viologen.

INTRODUCTION

To interpret the electron paramagnetic resonance spectra of free radicals, one must obtain a theoretical spectral fit which is unambiguous in its parameter values. Since the spectra can be quite complex, the methods for obtaining a good fit have become mathematically sophisticated. This paper deals with methods which use the fast Fourier transform (FFT) to solve this problem.

In most organic free radicals, the electron spin density is delocalized over the entire molecule via the π-bond system. This spin delocalization causes a lack of orbital angular momentum for the unpaired electron so that the EPR signals are very close to the free electron g value, 2.0023. It also causes magnetic hyperfine interactions between the electron spin and the nuclear spins, most notably the spins of the hydrogen and nitrogen nuclei. Since the hyperfine interactions are localized at their specific atoms, they reflect the directional anisotropies associated with the charge distribution around a bound atom. To remove these anisotropies, the EPR spectra are taken on liquid state samples, where rapid tumbling can average the directional anisotropies to zero so that the magnetic

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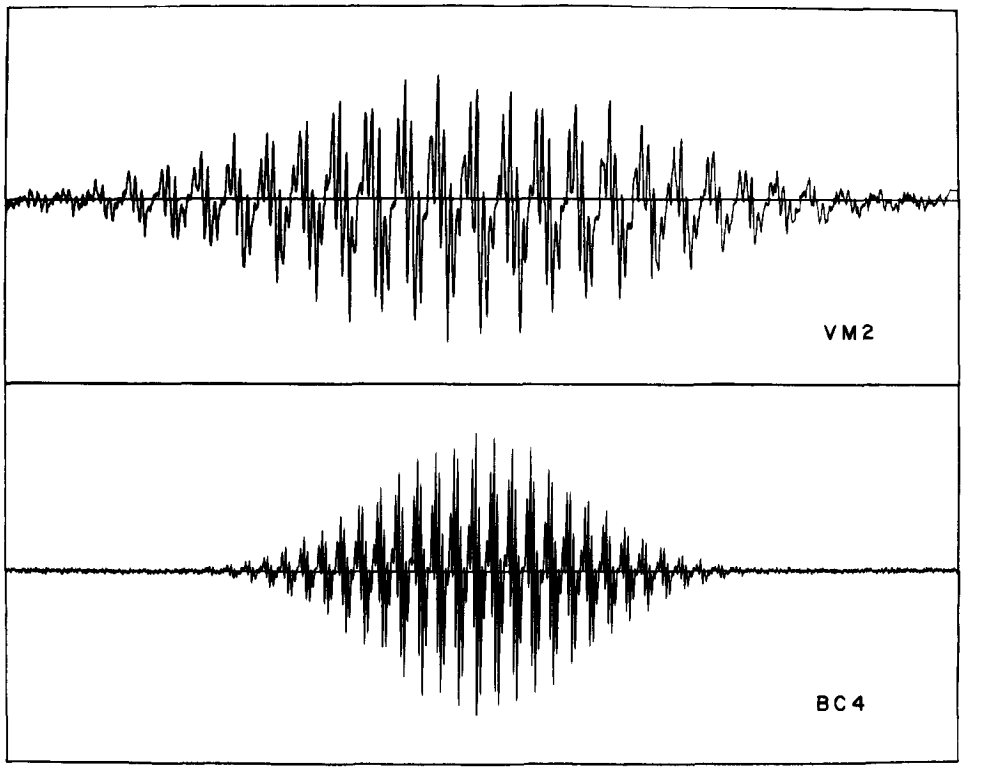

FIG. 1. Experimental X-band EPR spectra VM2 and BC4 of methyl viologen with scan ranges 38.6 and 70.0 G, respectively.

hyperfine interaction appears as a scalar interaction between the electron spin and the various nuclear spins in the molecule. Thus one can write the spin Hamiltonian for this idealized case as

$$\mathcal{H} = g\beta H S_z + \sum_{i=1}^{N} a_i I_{iz} S_z, \qquad [1]$$

where g is the electron g value, H is the applied magnetic field, S_z is the electron spin operator along the applied field, I_{iz} is the nuclear spin operator along the applied field for the ith nucleus, and a_i is the magnetic hyperfine constant of the ith magnetic hyperfine interaction. Here we assume that the a_i are very much smaller than $g\beta H$ so that this perturbation theory equation is accurate. As can be seen in Fig. 1, these spectra can be complicated if the number of nuclear spins is large.

All EPR spectra of samples with Eq. [1] as a Hamiltonian have some features in common which facilitate the use of Fourier transforms in their interpretation. These spectra are convolutions of: (1) the line patterns for each hyperfine interaction, (2) the lineshape function (usually the derivative of a Lorentzian or Gaussian lineshape), and (3) the central EPR resonance. Because the line patterns are

centrosymmetric and the lineshape is antisymmetric, the convolved EPR spectrum is antisymmetric about the central EPR resonance. Experimental results deviate from this ideal due to "slow tumbling," spectrometer baseline deviation, cavity contamination, noise, improper operation of the spectrometer (saturation, dispersion, overly long time constant, overly large field modulation), nonlinearity or asymmetric response in the signal detection circuitry, or nonlinear field sweep. A close inspection of Fig. 1 will reveal some lack of antisymmetry in the first methyl viologen spectrum which was presumably due to the digitization process. In the following, a method for deriving the a values from experimental EPR data which can be represented by Eq. [1] is described. Two EPR spectra of methyl viologen, which were obtained independently, are used to demonstrate the performance of the method on a well-characterized system.

EXPERIMENTAL METHODS AND PREPARATION

For the first spectrum (VM2), methyl viologen obtained from the Sigma Chemical Company was dissolved to 100 mM in dilute phosphate buffer containing 0.3 μM EDTA and 10 μM deazaflavin (3,10-dimethyl-5-deazaisoalloxazine). This solution was purged with nitrogen gas for approximately ½ hr, transferred to a quartz EPR flat cell (0.02 × 1 × 5 cm), stoppered tightly, and then illuminated with a high-intensity visible light source. The cell was kept in a water bath to prevent excessive heating. After approximately ½ hr of illumination, the blue color of the methyl viologen radical cation reached a maximum. This procedure is similar to that described by Johnson and Gutowsky (*1*) except that the deazaflavin increases the efficiency of the photoreduction. The EPR spectrum was run at room temperature with 10 mW microwave power and a modulation amplitude of 32 mG on a Varian E-112 EPR spectrometer. The resulting EPR spectrum was digitized (2048 points) manually using analog-to-digital curve-tracing equipment.

For the second spectrum (BC4), methyl viologen obtained from the Sigma Chemical Company was dissolved to 0.5 mM in a 100 mM Tris–HCl buffer (pH 9.0) at 23°C. The concentration was checked by measuring a diluted solution at 604 nm, using an extinction coefficient (*2*) of 13,600 M^{-1} cm^{-1}. The solution was made anaerobic by repeated cycles of evacuation and N_2 flushing. Thereafter, a twofold excess of a dithionite solution in the same buffer was added to reduce the methyl viologen solution. By working at pH 9.0, it is assured that full reduction is obtained and that the concentration of the methyl viologen dimer is negligibly small (*3*) since the pK dimer at pH 8.0 is 0.0086. After reduction, the solution was transferred to an N_2-flushed capillary of 0.7-mm inner diameter. The EPR spectrum was run at 23°C with 1.28 mW microwave power and a modulation amplitude of 32 mG on a Bruker 200-tt spectrometer. The Bruker spectrometer was directly coupled to a Data General NOVA-3 which was used to average (16 sweeps), record, and store the digitized data.

THEORY

The importance of the Fourier transform in EPR spectra has been published elsewhere (*4*, *5*). The basis of these methods has been to employ the convolution

theorem of Fourier transform theory; i.e., the Fourier transform of a convolution is the product of the Fourier transforms of the elements of the convolution. The theoretical model for the Fourier transform of an experimental EPR spectrum is, therefore, simply the product of the Fourier transforms of the hyperfine, lineshape, and the central EPR resonance components. The Fourier transform corresponding to the hyperfine component for a spin-½ nucleus, such as hydrogen, is

$$\cos(\pi ap/\Delta) \qquad [2]$$

while that for a spin-1 nucleus, such as nitrogen or deuterium, is

$$[1 + 2\cos(2\pi ap/\Delta)]/3. \qquad [3]$$

In these formulas, a is the a value in gauss, Δ is the scan range of the spectrum in gauss, and p is the argument of the Fourier space. These equations are adapted from the literature (*4*). The Fourier transform of the derivative of a Lorentzian lineshape is

$$\phi(p) = -ip \cdot \exp(2\pi\Gamma p/\Delta), \qquad [4]$$

where Γ is the half-width at half-height, in gauss. Ideally, this will be the lineshape in solution. The final component, the central EPR resonance, is simply a shift in the field domain and is represented in the Fourier space as

$$\exp(2\pi i\sigma p/\Delta), \qquad [5]$$

where σ is the distance, in gauss, from the spectral center to the left margin. Clearly, σ can be made zero by an appropriate shift in the field domain so that this component need not appear.

On the basis of the convolution theorem, the Fourier transform of an EPR spectrum can be written as

$$\exp(2\pi i\sigma p/\Delta) \cdot \phi(p) \cdot \prod_{i=1}^{m} \cos(\pi a_i p/\Delta) \cdot \prod_{j=1}^{n} [1 + 2\cos(2\pi a_j p/\Delta)]/3 \qquad [6]$$

when it contains m nuclei of spin ½ and n nuclei of spin 1, where m and n include the possible degeneracies; i.e., the a_i and a_j are not necessarily distinct. Note that if an appropriate shift is applied, then σ equals zero and the resulting theoretical model is pure imaginary and antisymmetric. Graphs of the hyperfine components with typical degeneracies are shown in Fig. 2.

From Eq. [6], it follows that if a zero occurs in one or more of the hyperfine components, the entire transform will be zero. Lebedev and Dobryakov (*4*) determine the a values by searching for the zeros in the Fourier space and then pattern-matching these zeros to the patterns of zeros implied by Eqs. [2] and [3] for a particular a value. On the basis of our experience, this approach is not feasible because the zeros of the Fourier transform are very poorly defined even when there are only a few distinct a values. Thus an alternative method based on least-squares fitting of the Fourier transform was developed.

METHOD

The first step in our method is to determine a baseline and center for the spectrum. The standard baseline is determined by least-squares fitting at the left and

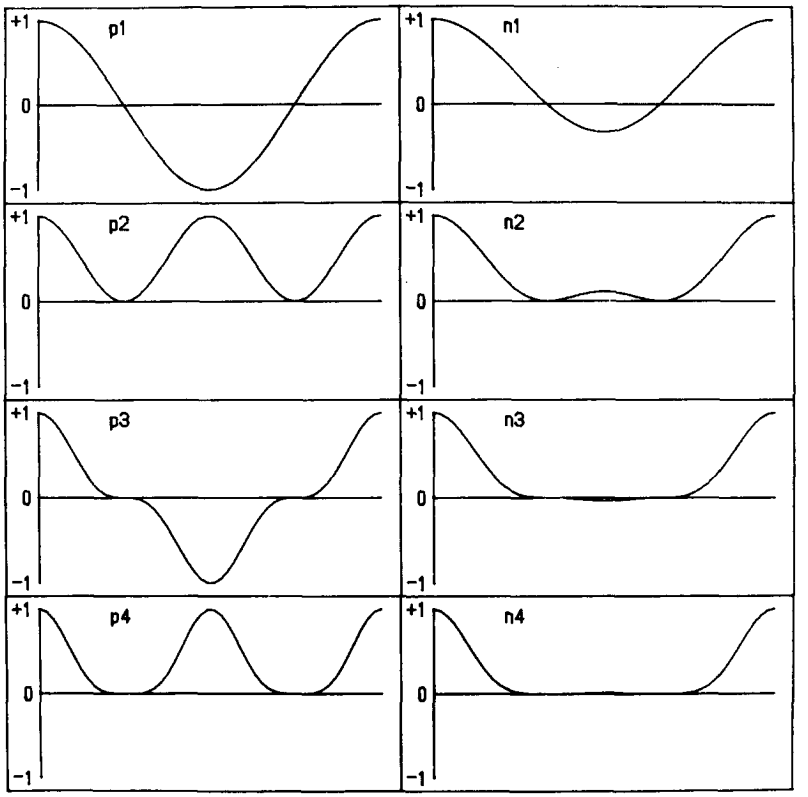

FIG. 2. A single period of the Fourier transforms of the hyperfine components corresponding to spin ½ (p_1, p_2, p_3, p_4) and spin 1 (n_1, n_2, n_3, n_4) with degeneracies 1 through 4.

right ends of the spectrum but can be altered using the interactive graphics facility (*6*) employed for this portion of our method. This capability was used to verify that the accuracy of the baseline does not significantly change the a values obtained by our fitting program. The center is also located using the interactive graphics facility. With the full spectrum displayed on the upper portion of the screen of a Tektronic 4010 and the two antisymmetric halves, reflected so that they should match, displayed on the lower portion of the screen, the vertical screen cursor is moved to the approximate center. The program converts this screen position to the nearest zero in the EPR spectrum by interpolation. Each change in the center which is made in this manner causes the screen to be appropriately updated. In addition to its simplicity, this technique provides visual assessment of the degree of antisymmetry and, therefore, the quality of the EPR data. To prepare the EPR data for the FFT algorithm, the baseline must be removed and the spectrum must be folded with respect to the center; i.e., the portion to the right of the center becomes the left "half" and the portion to the left of the center is reflected to become the right "half." This folding is necessary because the discrete Fourier transform is based on the interval $(0,2\pi)$ rather than $(-\pi,\pi)$. Because of this central shift, σ is approximately zero so that the cosine series

coefficients in the Fourier transform can be discarded. As a consequence, baseline error which is symmetric about the center will be eliminated also. The fitting procedure is applied to the transforms computed in this manner; however, before we discuss this procedure, it is instructive to consider some observations which dictated its design and which provide initial approximations to the a values.

Inspection of the transforms in Fig. 3 shows that they consist of a few significant peaks separated by long regions where the transform is approximately zero. These peaks correspond to those regions where all the hyperfine components are near an extreme value, while the flat regions correspond to those points where one or more of the hyperfine components is small. Flat regions dominate the transform space because the hyperfine components enter multiplicatively and are less than or equal to one. In contrast to the zeros, these peaks are well defined and easily located. Only the first few peaks will appear, however, because of the damping effect of the lineshape component.

The extrema of Eq. [2] occur when the argument of the cosine equals a multiple of π. The peaks in the transform for a proton splitting and the major peaks for a nitrogen splitting which correspond to a value a occur when $p = k\Delta/a$, $k = 1, 2, \ldots$. Thus, one can be fairly certain that the significant a values are approximate multiples of the a value obtained by letting p equal the abscissa of the first major peak of the Fourier transform of the spectrum, i.e., for $k = 1$,

$$a_{\text{com}} = \Delta/p. \qquad [7]$$

Finding this common factor in the a values is most effective for the larger a values, i.e., those which are larger than the linewidth. These a values give rise to the major features of the EPR spectrum and, therefore, dominate the spectral transform.

If the half-width of the EPR spectrum is defined as the distance, in gauss, between the center and the last zero crossing, one can write for this width in terms of the a values the expression

$$\text{width} = \sum a_i d_i t_i, \qquad [8]$$

where d_i is the degeneracy of the ith a splitting and t_i equals 1 for a spin-½ nucleus and 2 for a spin-1 nucleus. Thus, if $a_i = k_i a_{\text{com}}$, we obtain from Eq. [7] that

$$\sum k_i d_i t_i = 2 \text{ width}/a_{\text{com}}. \qquad [9]$$

The set of solutions k_i which satisfy Eq. [9] when the right-hand side is replaced by the closest even or odd integer (depending on the sign of the peak used to compute a_{com}) provides a complete set of possible a values which are compatible with the data. In some cases, this set is quite large, but additional information can be used to eliminate some of the possibilities. For example, if some of the a_i can be constrained to be within a specified range, the list can be considerably shortened. For the remainder of the set, one must test each computed spectrum against the experimental spectrum using a goodness-of-fit criterion.

A weighted least-squares criterion, which has proved successful in fitting the Fourier transforms of Mossbauer spectra (*7*), is employed. The weights are pro-

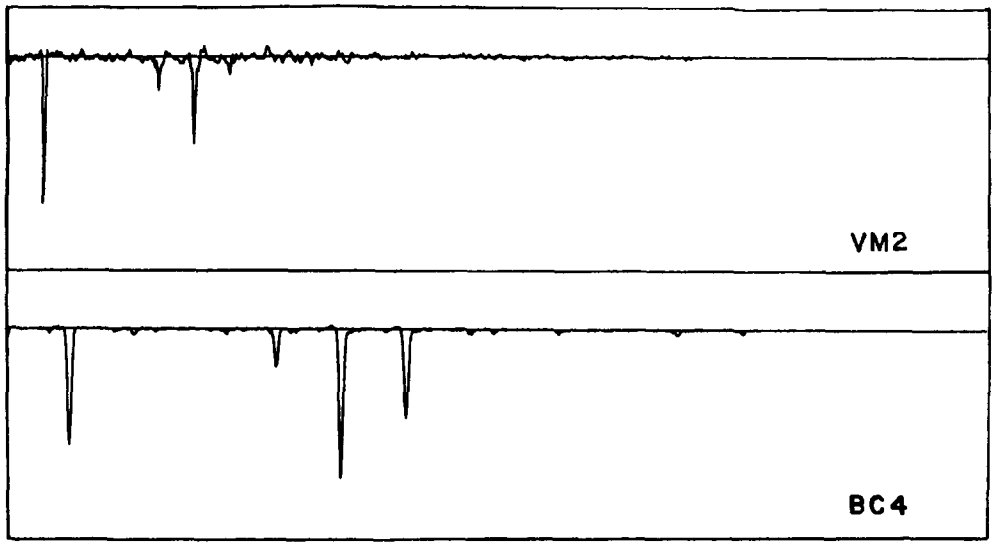

FIG. 3. Fourier transforms of the methyl viologen spectra VM2 and BC4 from 0 to 768 with scan ranges 38.6 and 70.0 G, respectively.

portional to the sum of the absolute values of the transforms of the experimental spectrum and the theoretical fit so that the peaks are properly emphasized. The fitting algorithm is based on Powell's (*8*) algorithm for minimization without derivatives and uses Brent's (*9*) combined quadratic–golden rule algorithm for one-dimensional minimization along the directions selected by Powell's algorithm.

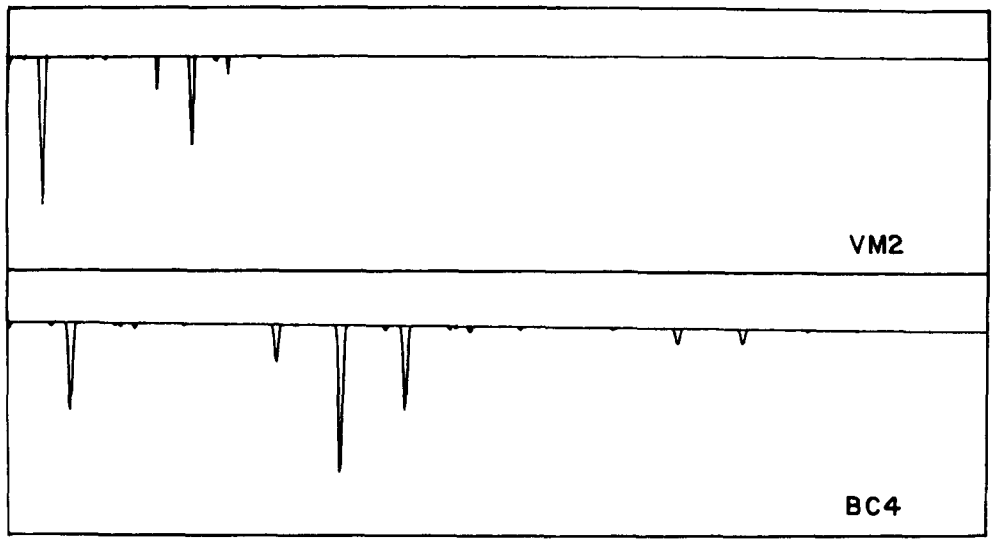

FIG. 4. Theoretical Fourier transforms corresponding to the best fits obtained for the EPR spectra VM2 and BC4 from 0 to 768 with scan ranges 38.6 and 70.0 G, respectively.

TABLE 1

VALUES FOR METHYL VIOLOGEN FROM THE LITERATURE AND THE BEST FITS OBTAINED FOR VM2 AND BC4 USING THE STANDARD BASELINE AND FOR BC4 USING THE "SKEWED" AND "CENTRAL" BASELINES

Type Degeneracy	Nitrogen 2	Proton 4	Proton 4	Proton 6	Lineshape
Johnson and Gutowsky (*1*)	4.23	1.57	1.33	3.99	
VM2, standard	4.212	1.602	1.336	3.980	0.148
BC4, standard	4.249	1.577	1.349	3.996	0.110
BC4, skewed	4.254	1.568	1.360	3.991	0.097
BC4, central	4.247	1.569	1.355	3.988	0.100

SOFTWARE AND TESTING

The software produced in connection with this research consists of four basic programs: (1) a program to determine the baseline, center, half-width, and common factor in the a values employing interactive graphics; (2) a program to enumerate the possible solutions based on the half-width and common factor; (3) a program to fit the Fourier transform of the experimental EPR spectrum; and (4) a program to plot the Fourier transform of the EPR spectrum corresponding to a specified set of a values and lineshape. This last program is also capable of producing numerical values for the EPR spectrum as if it had been digitized using a 12-bit analog-to-digital converter. Using this capability, numerous example spectra have been generated to test the procedure represented by the first three programs. Because of the rounding errors inherent in numerical computation, these artificially generated spectra contain noise, require nontrivial baselines, and have centers which are not at a mesh point; i.e., they are typical of experimental spectra. Indeed, although we anticipated inserting noise, etc., this proved unnecessary. In these tests, the a values were recovered correctly to the third digit to the right of the decimal point, i.e., the convergence limit incorporated in the fitting program.

RESULTS AND DISCUSSION

To verify the applicability of our method, we show the fits to the two experimental spectra, VM2 and BC4 (Figs. 3 and 4). The baseline computed by our software using a least-squares criterion is visually consistent with the data and is termed the "standard" baseline. To test the sensitivity of the a values with respect to the baseline, fits to BC4 for a "skewed" baseline and a "central" baseline are given also. These baselines were determined using the interactive graphics capability of our software to produce baselines which are visually inconsistent with the data. The "skewed" baseline was determined by selecting a point below the left end of the spectrum and a second point above the right end. The "central" baseline was determined by selecting two points within the central third of the spectrum which appeared as though they should be symmetric and then forcing the baseline through these points. These and the previously published results are presented in Table 1.

On the basis of our results, the computed a values are not overly sensitive to the baseline determination, so this potentially subjective portion of our method cannot be suspect. Our results for both the VM2 and BC4 spectra are entirely consistent with the previously published values.

Our method is quite efficient. Although the determination of the baseline, etc., can require 30 min of real time, the actual computer time is negligible. Similarly, the computer time required to enumerate the potential solutions based on the half-width and common factor is not significant. Our fitter automatically recognizes any parts of the theoretical transform which are fixed during a minimization step and performs these computations only once. Our experience indicates that the savings achieved in this manner are worth the programming effort. Typically, the fitter consumes about 180 sec of CPU time on an Amdahl 470V/7.

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